PLANT AND SOIL FEEDBACK RELATIONSHIPS IN MANITOBA TALLGRASS PRAIRIE

by

Sandi M. Faber

A Thesis submitted to the Faculty of Graduate Studies of

the University of Manitoba

in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Department of Botany

University of Manitoba

Winnipeg

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Thesis Abstract

Research on the mechanisms of plant community feedback, particularly the soil abiotic and biotic factors influencing local species, has been minimal in the northern tallgrass region, where less than 1% of the native community remains. Through field and greenhouse studies, I examined the nature of the plant and soil relationships within a tallgrass community in Manitoba. The main objectives were to: 1. determine the vegetation spatial dynamics; 2. characterize the abiotic soil conditions; 3. determine the effect of soil biota from dominant plant patches on dominant species; and 4. determine the effect of different soil communities on dominant and rare species. Results of field sampling showed that local patch structure exists for the dominant species, while abiotic properties differ across the patches. Greenhouse assays demonstrated a strong positive effect of soil biota on native plant performance, but there was a varying effect of the different soil communities on performance.

Acknowledgements

I thank all my field assistants Paul Mutch, Scott Routley, Michael Martin, and Erin Essery who were essential in helping me persevere through the challenges of tallgrass prairie field work. I also thank my partner Scott, and my close family and friends, who have all offered love, patience, and emotional support through both the best and trying times of my research.

I thank my advisor Dr. John Markham for his technical support and guidance on my research, along with my thesis committee Dr. Norm Kenkel and Dr. Richard Westwood for their input and advice. The Sustainable Development Innovations Fund (SDIF) with MB Conservation provided financial support for this study.

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Chapter 1: Literature Review

Introduction

A central focus of plant ecology is on the processes that regulate the composition and diversity of natural communties, and the factors influencing the distribution and abundance of species (Begon et al. 1996; Barbour et al. 1999; Hartnett and Wilson 2002). However, the precise mechanisms maintaining the composition and diversity of species are not well understood (Van der Heijden et al. 1998). In general, plant distribution and abundance is regulated by ecological factors (abiotic and biotic) in their immediate environment, which help to shape the structure and dynamics of the entire community (see various chapters in Grace and Tilman 1990; Begon et al. 1996; Barbour et al. 1999). These factors can occur in the environment as resources (e.g. sunlight, water, nutrients) and conditions (e.g. temperature, natural disturbance, herbivory, soil biotic community) (Barbour et al. 1999). The distribution of these are heterogenous in both space and time (Harper 1977). As such, the factors influencing species diversity vary across a community at any given time (Barbour et al. 1999).

Previous research on the factors controlling diversity in plant communities (with many examples from grasslands) has focused on 'traditional' abiotic and biotic factors. These factors are considered to be traditional because in the past they were accepted as exclusive determinants of plant species composition. Examples of the factors traditionally examined are abiotic natural disturbance, e.g. fire; biotic disturbance, such as grazing by large ungulates and herbivory by small animals and insects (Collins and Glenn 1990; Collins and Wallace 1990; Collins et al. 1998; Collins and Steinauer 1998; Knapp et al. 1998; Collins 2000); resource partitioning and cycling of macronutrients,

primarily nitrogen and phosphorus (Jeffrey 1987; Blair et al. 1998; Barbour et al. 1999; Vitousek 2004); and competition between plants, e.g. intraspecific and interspecific comptetion (Harper 1977; Grace and Tilman 1990).

Non-traditional factors commonly encompass belowground factors which influence the structure and dynamics of plant communities. The effects of the biotic soil community on the local plant species is a large component of this area of research (Wardle 2006). The significance of non-traditional factors was disregarded by ecologists in the past, but is now apparent. For example, the symbioses of soil micro-organisms with plant host species such as N-fixing bacteria, mycorrhizal fungi and endophytes (of grasses) has been demonstrated to influence plant competition, coexistence, and soil nutrient dynamics (Clay 2001).

Plant Community Structure

Perspective on the structure of individuals and populations is essential in understanding the dynamic processes occurring within- and between- other trophic levels in a particular plant community (Barbour et al. 1999; Wardle 2006). Analogous with their environment, plants are arranged unevenly along both spatial and temporal scales (Barbour et al. 1999). Distributions of plant species can be categorized as one of three types: localized, randomly-mixed, or regular. Localized populations, leading to a patch-like (clumped) species distribution, occur when the location of one plant increases the probability of finding another of the same species nearby. Randomly-mixed populations, leading to a spread-out distribution, occur when the presence of any one plant has no effect on the presence of another. Examples of localized populations in Manitoba are

Solidago and *Helianthus* spp. in tallgrass prairie, while *Andropogon gerardii* Vitman and *Poa pratensis* L. are examples of randomly-mixed populations (unpublished data). Regular populations, leading to an even distribution, occur when the presence of one plant decreases the probability of finding another nearby or far away (Barbour et al. 1999). A fruit orchard or trees planted in rows on a tree farm are examples (although extreme) of regular populations. Typically, plant species at the smallest spatial scale follow a local dispersal pattern, where seeds and fruit fall close to the parent plant, or vegetative reproduction (e.g. rhizomes) produces plants in the immediate vicinity of the same species, and this leads to increasing patchiness of vegetation and localized plant populations (Barbour et al. 1999). Although seed dispersal patterns can also occur over a much larger spatial scale (i.e., regions), via the action of wind or animals for example, local dispersal patterns are particularly important within an individual plant community.

It should also be pointed out that an ecological theory called the Unified neutral theory of biodiversity was recently proposed by Stephen Hubbell to explain the diversity and relative abundance of species in ecological communities and could be applied to understanding the structure of local plant communities (Hubbell 2001). Hubbell's theory, using mathematical models of biodiversity, assumes that the differences between members of an ecological community of trophically-similar species are "neutral," or irrelevant to their success. However, this runs counter-intuitive to the ideas of most plant community ecologists, as it suggests that the distribution of species does not require complex mechanisms of species interaction, and that plant species assemble in a community not as a result of niche-partitioning or competition, but due to chance alone.

Soil Community Structure

The value of the heterotrophic soil community in plant community structure and maintenance of diversity is a modern concept in ecology (Wardle 2006), and previously received little attention in the literature. Recently at the local level it has been reported that the biotic soil community is greater in biomass and more active in nutrient cycling in comparison to the associated plant community (Seastedt and Ramundo 1990; Wardle 2006). Furthermore, the local soil community is regarded as a key factor in the flow of energy between trophic levels and development of soil aggregate structure in individual plant communities (Ransom et al. 1998). Meanwhile (according to Rice et al. 1998), on a global scale carbon (energy) allocation and processing by soil organisms has particularly shaped the large accumulations of organic matter and nutrients defining the grassland regions of North America.

While many soil physio-chemical properties and abiotic processes have been characterized (Jeffrey 1987; Barbour et al. 1999), information on local distribution (both spatial and temporal) and taxonomy of soil microbes within individual plant communities is not as extensive (Rice et al. 1998). Primarily, it is believed that this is a result of widespread lack-of-understanding of belowground diversity, as dictated by the sheer complexity of species facing today's ecologists, and the diminishing number of taxonomists with necessary expertise to characterize this community (Wardle 2006). Additionally, difficulty in measuring and quantifying the density and frequency of a particular soil taxa, or that many microbial species have yet-to-be described or isolated, further complicates soil characterization (Bever et al. 1997; Hooper et al. 2000; Wolfe and Klironomos 2005; Wardle 2006). Over the last decade there have been a few research

programs established to intensively measure soil community structure, such as the Natural Environment Research Council's (NERC's) Soil Biodiversity (SB) program; the primary goal of which was to describe soil species diversity and relative functions in a single grassland in the United Kingdom (Usher 2004). Such projects, however, are often short-term (e.g. NERC's SB program began in 1997 and ended in 2004) and geographically-localized (e.g. NERC's SB program was based in the Sourhope upland hills in Scotland), and it is further acknowledged by the associated researchers that longer-term studies in differing soil types and other locations in the world are needed (NERC 2006; Usher 2004).

In general, it is known that many soil microbes form obligate or non-obligate associations with plants (Bever 2003). Examples include fungal endophytes (such as arbuscular mycorrhizal fungi and ectomycorrhizae with roots), nitrogen-fixing bacteria within root nodules, rhizobacteria, root-feeding nematodes, and opportunistic pathogens and herbivores of roots and seedlings (Jeffrey 1987; Rice et al. 1998; Ransom et al. 1998; Barbour et al. 1999). These relationships can be mutualistic, where the microbial symbiont and plant host both benefit from the association (e.g. N-fixation); or parasitic, where one member is negatively affected- typically the plant host, by association with the microbial species (e.g. damping-off pathogens or root-feeding nematodes). The microbial symbiont can individually be classified under two types: 1. host-specific, or 2. host non-specific; meaning respectively, that a particular microbial species will join exclusively with a particular host species (i.e., a specialist), or be unselective and associate with a number of plant host species (i.e., a generalist). However, a microbial species does not always strictly adhere to one of these categories, as they also can

concurrently exhibit characteristics of both. For example, a particular mutualism can be highly infective or non-specific in their choice of plant partner (i.e., a generalist), but may differ in their level of effectiveness or influence on growth of the individual host plants (i.e., a specialist) (Sanders 2003).

Soil Biotic Groups

The collective soil biota associating with plant hosts are tremendously complex (Bever 2003), but can be separated taxonomically and/or functionally into a number of key groups including: rhizosphere bacteria, mycorrhizal fungi, soil invertebrates, and pathogens (De Deyne and Van der Putten 2005). Rhizosphere bacteria or rhizobacteria are found in the soils adjacent to- and influenced by- plant roots and root exudates (Westover et al. 1997), that are high in moisture, rich in nitrogen and contain readily decomposable substrate, thus allowing for fast cycling of nutrients (Scheu et al. 2005). Rhizobacteria are free-living, which form associative relationships with plants, but physically do not form special organs or attach to the plant root system (Chanway et al. 1991). Roles of rhizobacteria include beneficial influence on plant performance, ecosystem productivity, and vegetation dynamics. These effects can occur through fixing nitrogen, increasing mineral solubilization, or reducing negative effects of other soil pathogens (Chanway et al. 1991; Jeffrey 1987; Bever et al. 1997; Westover et al. 1997; Brussaard et al. 2001). For example, a study by Westover and Bever (2001) found that rhizosphere bacteria can ameliorate the negative effects of pathogenic soil microbes (such as fungi) on plant performance.

Mychorrizal fungi are the main component of the root-soil interface, and have co-evolved mutualistically with a number of terrestrial plants (Eom et al. 2000; Brussaard et al. 2001; Brundrett 2002). Typically, two morphological types are well-recognized: ectomycorrhizae (EM) and arbuscular mychorrizae (AM) (Johnson et al. 1999; Brundrett 2002). EM fungi constitute a symbiosis in which most of the hyphae are found outside the root, in the inter-cellular spaces between the outer cortical and epidermal cells, but never directly penetrate the root cells themselves (Johnson et al. 1999). AM fungi penetrate within the cortical cells, forming intra-cellular organs such as arbuscles, vesicles, hyphae and/or coils. The principal role of mycorrhizae is in improving plant mineral nutrition (along with increasing surface area for absorption), through vastly improving the supply of available phosphate (Van der Heijden et al. 2006) and to a lesser extent assisting in assimilation of nitrogen (Jeffrey 1987). Secondary roles include increasing a plant host's resistance to disease (Newsham et al. 1995), improving water relations (Hartnett and Wilson 2002), and increasing soil structure and stability (Van der Heijden et al. 2006).

Soil pathogens are a diverse group of microbes that colonize roots, and comprise many species of bacteria, fungi, protozoa and nematodes (Bever et al. 1997). They can greatly reduce the survival, growth, and reproduction of plants through causing disease, reducing biomass, competitiveness, and beneficial mutualists, and interfering with root processes (Bever et al. 1997; Van der Putten and Peters 1997; Holah and Alexander 1999). Antagonistic plant pathogens (primarily bacteria and fungi, but also some microbe- and root-feeding nematodes and protozoa) are also hypothesized to be involved in the control mechanisms of plant species diversity and have garnered recent attention in

the literature (Bever 1994; Mills and Bever 1998; Holah and Alexander 1999; Brussaard et. al 2001). Research by Brussaard et al. (2001), for example, demonstrated that specific soil pathogens interfered with nutrient uptake and allocation within a plant species and competitively reduced the presence of mutualistic fungi and bacteria. This was suggested to prevent establishment or abundance of individual species, and/or shift the overall balance of competition and succession in similar communities. Furthermore, Holah and Alexander (1999) have shown that pathogenic microbes associated with an annual grassland species reduced the growth of the perennial *Andropogon gerardii,* and potentially resulting from a reduction in abundance of soil mutualists. This was speculated to be important in the persistence of the annual species with perennial neighbours.

Soil invertebrates are one of the most numerous and diverse groups of the soil micro-organisms (Seastedt et al. 1988). Invertebrates overlap with the pathogen group in encompassing microfauna such as nematodes and protozoa, along with rotifers and tardigrades. In addition, the macro-arthropods (e.g. insects, millipedes, arachnids, etc) and earthworms are members of the invertebrate group (Ransom et al. 1998; Coleman et al. 1999). Nematodes are considered to be the most abundant soil invertebrate and greatly exceed the macro-arthropods in number, but the macro-arthropods, based on their large body size, constitute a high proportion of the biomass in the invertebrate group (Ransom et al. 1998, Bardgett et al. 1999). The sheer diversity of soil invertebrates naturally leads to greater niche-partitioning, but the primary roles of the microfauna are in root decomposition, synergistic plant-disease effects with fungal pathogens, and predation of other soil biota (Riedel 1988; Ehrenfeld 2005). The macro-arthropods and

earthworms are involved in litter decomposition, nutrient mineralization, and aeration and homogenization of the physical soil structure (Brussaard et al. 2001; Ransom et al. 1998; Rice et al. 1998).

Plant-Soil Relationships

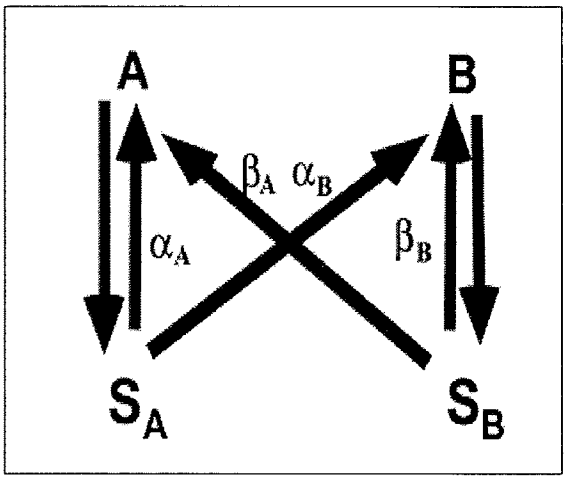
The interactions between plant and soil are recognized to be important in describing the characteristics of an individual community. Focus on studying the traditional ecological factors (see **Introduction**) and aboveground species diversity in this relationship provides limited understanding to the contribution of the soil component (Wardle 2006). However, as more emphasis is being placed on describing drivers of diversity, and providing information essential to the conservation of species and communities, research linking the aboveground and belowground systems is gaining significant attention (Hooper et al. 2000; Wolters et al. 2000; Klironomos 2002; De Deyn and Van der Putten 2005; Wardle 2006).

According to Hooper et al. (2000), determining connecting patterns between the aboveground plant- and belowground soil communities could guide approaches to protecting local soil species, and be functionally important in the maintenance of community processes. Van der Heijden et al. (1998) further advise that without greater attention, the loss of soil biodiversity may lead to large decreases in plant productivity and substantial increases in ecosystem instability. Perry et al. (1989) point out that while conserving microbial soil diversity is important, it is the functional role of these species groups- i.e. the positive reciprocal effect on plant host, which is more critical to maintain. Understanding the relationships between the plant and soil systems could also assist in

the conservation of rare and endangered species and reduction of invasive plant species (Klironomos 2002; Wolfe and Klironomos 2005). Ultimately, De Deyn and Van der Putten (2005) and Wolters et al. (2000) stress that linking these two systems is necessary for formulation of precise ecological predictions and management strategies, prevention or minimization of global destruction of above- and below-ground species diversity, and ensuring future ecosystem sustainability.

Feedback: a Conceptual Model

The reciprocal interaction between vegetation and soil, whereby plants influence the abiotic and biotic characteristics of a soil community and the soil community in turn shapes the local plant community is a type of "feedback" operating at the community level (Bever 1994; Bever et al. 1997; Bever 2003; Ehrenfeld et al. 2005). Through conceptualization in a mathematical model, feedbacks can be characterized as one of two types: positive or negative, depending on overall effect of the (abiotic and biotic) soil community on performance of the associated plant community (Bever et al. 1997; Fig. 1.1). In a negative feedback system, the effect of the plant on the soil community is such that the soil has a negative influence on plant performance (relative to the performance of a nearby species in the local plant community). This could occur through a plant having a positive effect on its own soil pathogens or a positive effect on a nearby species mutualists. A positive feedback system occurs when the soil community, as affected by the plant, has a positive influence on plant performance (relative to the performance of a nearby species). This could result from a plant having a negative effect on its own soil pathogens or a negative effect on a nearby species' mutualists. It should also be noted



INTERACTION COEFFICIENT (I_S):

$I_S > 0$ = positive (+) feedback
$I_S < 0$ = negative (-) feedback

Fig. 1.1 The potential feedback interactions between two plant species, A and B, and their soil communities, represented by S_A and S_B, respectively. The presence of plant A causes a change in its associated soil community that can then directly alter the growth of plant A, represented by the parameters α_A, or alter the growth of plant B indirectly, represented by the parameter α_B. Similarly, plant B can have direct feedback on its own growth, represented by β_B, as well as indirect feedback through changes in the growth of plant A, represented by the parameter β_A. The two plant species can also have direct density dependence on their own growth and competitive effects on each other's growth. Positive feedback results when the sum of the direct and indirect feedbacks is greater than 0, i.e. the interaction coefficient (I_S) is a plus, or + sign; negative feedback occurs when the total of the direct and indirect feedbacks is less than 0, i.e. the I_S is a minus, or – sign (adapted from Bever 2003).

that the above concept of feedback is species-specific, meaning that the effect of a particular biotic soil community on plant growth is highly host-dependent, and the components of the soil will shift according to changes in plant species (Bever et al. 1997).

Typically, feedback interactions between the plant and soil community occur on a local scale (in the vicinity of the root system), as plant species and soil microbes often have limited dispersal (Bever 2003). Soil microbes, as well, exert differential effects on the growth of local plants (Bever et al. 1997; Bever 2003). This can result in growth rate being directly influenced by a feedback, e.g. where a change in the composition of a soil community directly influences host-plant growth, or indirectly, e.g. where the growth of a nearby competing plant is altered (Bever 2003; Fig. 1.1). In a conceptual model, feedback is defined by the net (total) direct and indirect interactions between a plant species and its local soil community, as opposed to being characterized by the individual interaction alone (Bever et al. 1997; Bever 2003). The final direction (positive or negative) of the relationship thus depends upon the sum of the direct feedbacks on host growth and indirect feedbacks on competing plant, and is referred to as the "Interaction Coefficient" in feedback dynamics (Bever et al. 1997; Bever 2003; Fig. 1.1).

Feedback and Maintenance of Diversity

The theory of community feedback has been extended in recent literature to describe a larger role in predicting plant composition or species richness. In particular, feedback systems have been implicated in overall regulation and maintenance of biological diversity (Mills and Bever 1998; Bever et al. 1997; Bever 2002). Research by

Bever et al. (1997) suggests that in applying a conceptual model to a particular plant community, positive feedback will lead to the loss of diversity. This is based on the idea that positive feedback may strengthen the interaction between a plant and soil community, particularly for the highly competitive (or dominant) plant species, leading to an increase in the dominant's abundance and subsequent decrease in overall species richness of the community. In other words, the coexistence of locally competing plant species will be lower in communities regulated by positive feedback (Bever 2003). Over a larger spatial scale, however, the coexistence of species is still possible under positive feedback, as a diverse array of local communities with single dominant species can be found across a region (Bever et al. 1997). Meanwhile, negative feedback may create a reverse circumstance at a local scale, where an interaction between plant and soil decreases or restricts the abundance of a dominant species, thus allowing the persistence of rarer (or subordinate) species and leading to greater species richness within the community (Bever et al. 1997). As a result, the coexistence of local plant species will be greater in negative feedback-regulated systems (Bever 2003) and competing species will be uniformly distributed across the region (Bever et al. 1997).

The original principles of feedback established by Bever and colleagues is supported in a literature review by Ehrenfeld et al. (2005), which defines positive feedback in plant communities as directional, while negative feedback is stabilizing. A directional system is described as one where the original feedback event generates a cascade of effects, which are always increasing in magnitude and in a particular direction. A stabilizing system is a feedback circumstance where the cascade effects are gradually decreasing towards a threshold, and eventually become limited. In other words, positive

feedback results in an ever-increasing abundance of a common species, thereby maintaining low diversity, while negative feedback limits the abundance of any one species, thus promoting high diversity.

Pathways of Feedback

The precise mechanisms by which feedback interactions can operate in a community is thought to be either through changes in the soil biotic community associated with a host species, i.e. host-specificity (Bever et al. 1997; Eom et al. 2000; Bever 2002; Hartnett and Wilson 2002; Ehrenfeld et al. 2005); as a result of alterations to the abiotic physical, chemical or biogeochemical conditions of the soil environment (Bever et al. 1997; Hartnett and Wilson 2002; Clay 2001; Ehrenfeld et al. 2005), or through a combination of the above.

Recent work by a number of ecologists has greatly increased our understanding of these distinct pathways. Pertinent examples include Eom et al. (2000) and Bever (2002), who explored the host-specificity of microbes through two separate, but similar greenhouse studies. Specifically, each looked at the differentiation of an AM fungal community when planted with selected grassland species. Results from both studies illustrated that for an individual species of plant, changes to the compositional structure of the AM fungal community did occur. It was concluded that AM fungi are likely less-randomly distributed in nature than was previously assumed, as there is evidence that many AM-plant relationships have some degree of host-dependency (Bever et al. 2001).

Bever et al. (1997) also studied the mechanism of host-dependency in a grassland microcosm. They found that overall sporulation, often used as a measure of AM fungal

composition (Bever 2002), varied significantly depending on species of plant host, and for a given pair of plant species, one plant was determined to be a better host for a particular fungal species, but a worse host for another. The patterns of host-dependent sporulation observed in the greenhouse were also found to be weakly correlated with those from a similar experiment in the field, once again suggesting that spatial differentiation of AM fungi in nature conforms to that of the host plant (Bever 2003). It has been established that the above studies on microbial host-specificity (i.e. Bever et al. 1997; Eom 2002; Bever 2002) support current feedback models (which predict the regulatory effects of soil communities on aboveground structure) because plants respond differently to individual species of AM fungi (Bever 2002). On the other hand, experiments of this nature demonstrate only one half of the reciprocal relationship summarized by the feedback model, or that a plant species can have an effect on the composition of the soil community. In order for a true feedback system to occur (i.e., in nature), the microbial community must also influence the growth of the associated plant species. Demonstration of a true feedback system, though, is often very difficult to achieve.

Alternatively, alteration of physio-chemical soil properties as a mechanism of feedback was explored by Wolfe and Klironomos (2005), which reviewed the invasion of exotic plant species into natural systems. It was suggested that exotic plants or weeds release secondary compounds and exudates into the soil (e.g. allelochemicals) that are often novel to a particular soil community. These compounds may result in changes to soil composition, structure and function, and cause reduced growth effects on the native vegetation. In other words, exotic species can generate a positive feedback system.

Overall, this was proposed to be the basis for establishment and dominance of weedy species in many native plant communities.

In addition, Van der Heijden et al. (2006) illustrated that a combination of abiotic and biotic feedback pathways can operate in a community. This was demonstrated through examining the effects of certain soil micro-organisms on changes to the abiotic soil conditions and temporal shifts in host-specificity. Specifically, they assessed the impact of four different AM fungal taxa on soil structure, stability and nutrient acquisition, and influence on performance of local plant species in a grassland microcosm. Experimental results showed that the AM fungi promoted soil aggregate stability and improved plant nutrition via increased inorganic phosphate uptake, which created beneficial effects on plant species (i.e., indicating a positive feedback system). Growth responses of the plants to the different AM fungi were temporally-dependent and varied with host-plant species, where in the first growing season certain plant species (e.g. *Trifolium* sp. and *Lotus* sp.) performed best with one AM taxa, but grew maximally with several taxa in the second season. The growth of juvenile (or first season) plants also varied with the different fungal species, but these differences were not as evident in the second growing season after the plants had aged. It is thought that as the abiotic conditions of the soil change and plants develop, the relationship with AM fungi can change. The final conclusion was that the indirect effects on soil condition (e.g. aggregate stability and nutrient supply) and plant productivity (e.g. temporal growth); via microbes such as AM fungi, may play a key role in regulating structure and dynamics in natural plant systems.

Testing Feedback

The majority of empirical studies on plant-soil feedback systems and associated pathways have involved using artificial plant communities, evaluated under greenhouse conditions, but with a few examples from field studies (Bever et al. 1997; Mills and Bever 1998; Holah and Alexander 1999; Bever 2002; Klironomos 2002; Bever 2003). In most cases, simple protocols have been developed to evaluate the importance of the soil community with a particular plant species in pair-wise feedback assays (Bever et al. 1997). These feedback protocols involve two main steps: 1. the soil community selected for study, which is initially similar in composition, is grown with a number of different plant species and often repeated over a number of generations. The soil community per se, is then "cultured" by each of the different plant species and differentiates according to host plant (Bever et al. 1997). 2. Feedback effect is determined by measuring the growth of the plant species (from step 1) in pots inoculated with each cultured soil type. Control pots are also established to measure growth of the plant species in a non-inoculated (i.e. microbe-free or sterile) soil type. The direction (positive or negative) and magnitude of each feedback is evaluated by contrasting growth of a plant species in its own soil community against growth in each of the other plant species' soil communities, and also comparing inoculated growth results to the microbe-free control. This is also referred to as 'Home vs. Away' contrast (Bever et al. 1997).

The 'Home vs. Away' (or 'H-A') contrast method is not without pro and con aspects in design. Advantages of 'H-A' contrast are that it allows the complexity of abiotic and biotic interactions taking place in the soil to be treated as a whole system. This also permits the impact of host-specific changes (in soil composition) to be observed

exclusively on plant growth (Bever 2003). Moreover, as it has been established that soil composition is difficult to characterize, it eliminates the need to determine the precise soil species or groups responsible for the feedback (Bever et al. 1997).

Drawbacks of 'H-A' contrast are that results can be difficult to extrapolate from whole-soil systems, as testing feedback has been inconsistent across studies thus far (Bever 2003), while feedback relationships in nature are known to be far more complex (Casper and Castelli 2007). To illustrate, an individual plant community can vary in any of the following: i. linearity of plant-soil interactions, e.g. soil organisms may differ in their rates of host-specific changes and corresponding effects on host plant growth (Bever 2003), ii. synergistic processes taking place, e.g. plant competition, dispersal, and succession can all affect the feedback system (Hartnett et al. 1993; Bever 2003; Casper and Castelli 2007) and, iii. spatial and temporal structure, e.g. feedback influences can fluctuate depending on a given time (Van der Heijden et al. 2006) or location (Bever 2003). Additionally, situations may occur where knowledge of the precise components of the soil may be more desirable than overall feedback test results, e.g. where the density of a specific group of micro-organisms such as pathogens or mutualists differs according to plant host, and this information could potentially improve prediction of the community dynamics (Bever et al. 1997) and experimental design of future studies.

By and large, the assessment of feedback systems should involve artificial microcosms in complement with natural system studies to entirely understand the complexity of dynamics occurring (Casper and Castelli 2007). Moreover, because influential community processes (e.g. plant competition) are inseparable components of feedback models, they should be incorporated into experimental protocols (such as 'H-A'

contrast) (Van der Putten and Peters 1997; Bever 2003; Casper and Castelli 2007). Meanwhile, greater information on the spatial and temporal scales at work is necessary to better manage plant-soil relationships (Bever et al. 1997; Bever 2003). However, the methodology for evaluation at this magnitude of scale appears to be potentially complicated, laborious, and time-intensive for an individual researcher.

Examples of Feedback

There is evidence from research over the past two decades that both positive and negative feedback systems can influence plant growth and community structure. Illustrations of positive feedback systems are ample in the literature. The earliest suggestion of positive feedback in natural systems appears to be based on work by Perry et al. (1989), which proposed the concept of "Bootstrapping"; i.e., a particular community can pull itself up by its own bootstraps and create favourable conditions that allow it to persist. Under bootstrapping, a system is characterized by positive feedback when there are strong, self-reinforcing interactions between the individual components (i.e. plant and soil) which favour maintenance of the health and resilience of that community. This was first demonstrated by Perry and research associates in the high elevation forests of the pacific northwest- an environment previously characterized as harsh- where removal of the trees through clear-cutting resulted in loss of the soil community. It was speculated that the positive mutual link between the tree species and its soil microbial community was likely to have allowed this system and others like it to persist under extreme environments. Such limited success of plant species in establishment without their microbial symbionts is believed to be a direct testament to the

existence of positive feedback systems (Bever 2003). A positive feedback system is also speculated to occur in the invasion of exotic European species into North American plant communities, with the results of two studies suggesting that accumulation of mutualistic mycorrhizal fungi (and corresponding lack of host-specific pathogens [Callaway et al. 2004] or slower rate of accumulation of host-specific pathogens [Klironomos 2002]) in the soil community of the invaded range promotes growth of the weedy species, and potentially contributes to expansion of their range. Furthermore, plant species diversity and productivity was demonstrated to rise with increasing richness of AM fungal communities in research by Van der Heijden et al. (1998), suggesting a positive mutual relationship exists between plants and soil microbes in that system.

Alternatively, illustrations of negative feedback are also abundant in the literature. A negative system can be demonstrated through the results of a North American old-field community study, where the survival of *Krigia* sp. (a forb) and growth and flowering of other particular grasses was reduced when each was grown in a soil community cultured previously by that same species (Bever 1994). Similarly, Bever (2002) observed that growth of the forb *Plantago lanceolata* L. was lower in its own AM fungal soil community (or 'Home' soil) in comparison to growth in a soil fungal community cultured by the grass *Panicum sphaerocarpon* Elliot (or 'Away' soil). The accrual of host-specific soil pathogens was further shown to contribute to a negative feedback system, by reducing biomass and root to shoot ratios of two grassland species, *Danthonia* and *Panicum*, when each was grown in a soil community containing biota (i.e. pathogens) previously isolated from roots of the same species (Mills and Bever 1998). Using the

simple protocols of 'H-A' contrast, Bever et al. (1997) demonstrated negative feedback in seven of eleven pair-wise comparisons of specific grassland species.

Grasslands of North America

The native prairie grasslands, also known as the Great Plains, have been described as one of the largest units of terrestrial vegetation on the North American continent (Weaver 1954; Samson and Knopf 1994; Fig. 1.2), and prior to European settlement, covered approximately 162 million hectares of land (Samson and Knopf 1994). The majority of ecological research on prairie grasslands has historically encompassed the abiotic and biotic aboveground factors defining community structure, and recently, involved describing the simple, pair-wise feedback systems occurring within selected microcosms. In contrast, characterization of the belowground structure and composition of species in grassland communities and associated influence on plant biological diversity, particularly in communities of the tallgrass prairie region, has not been well documented.

Tallgrass prairie, once an abundant pre-settlement vegetation type in North America- occupying 60 million hectares, is an area of recent conservation efforts due to ever-increasing threats of habitat extinction (Samson and Knopf 1994). It is furthermore a plant community for which the factors guiding species diversity are not well known. However, a sense of urgency in understanding and preserving the ecology and diversity within this rapidly disappearing community has become apparent (Knapp and Seastedt 1998). This is particularly important as grasslands are a major storage for global carbon

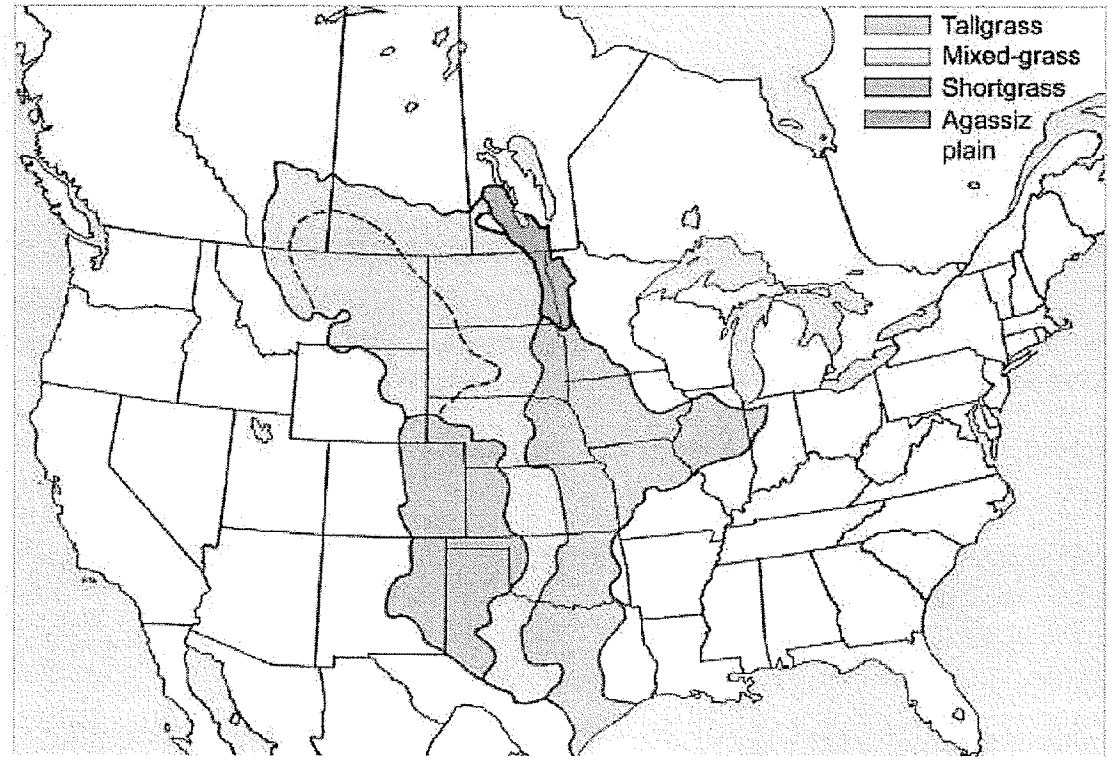


Fig. 1.2 Traditional subdivision of the Great Plains into tallgrass (green), mixed-grass (yellow), and shortgrass prairie (orange). Glacial Lake Agassiz plain (blue) is further segregated as the "northern tall grasslands" of the tallgrass subdivision which extends into Manitoba (adapted from Hamilton 2005).

Reserves and grass species provide the bulk of human nourishment (Samson and Knopf 1994).

Tallgrass Prairie Region

The tallgrass prairie region is a temperate, mesic grassland located centrally within the North American continent, and for which the historic range extends from south-eastern Manitoba, through mid-central Kansas, and into Texas (Samson and Knopf 1994; Fig. 1.2). The climate of the region is classified as continental, with an average annual precipitation of about 600 millimetres (mm) in the northern portion (south-central Canada) and 1000 mm in the southern portion (southern Texas). The temperature gradient in January ranges from -15˚C in the north to 15˚C in the south, and in July ranges from 20˚C to 30˚C north to south, respectively (Bragg 1995).

Tallgrass prairie, also known as "True prairie", is bordered by mixed-grass prairies to the west, and forested systems to the east and north (Weaver 1954; Knapp and Seastedt 1998). In general, it can be characterized as a highly productive system, occurring on rich, dark prairie soil (Bragg 1995), and encompassing a diverse array of tall perennial grasses (e.g. flowering shoots can be up to 2m in height), woody shrubs, and forbs (Weaver 1954; Barbour et al. 1999).

Research by Collins and Glenn (1990) tested the significance of certain community models in predicting the regional distribution and abundance of tallgrass species. The data indicated that the spatial and temporal dynamics of the selected grassland species across a particular region followed a bimodal pattern, as it was observed that many of the species were uncommon in all of the sites (i.e., regionally-

rare), while a fewer number occurred at most or all of the sites (i.e., regionally-dominant). It was concluded that these results conform to the predicted patterns of the Core-Satellite hypothesis (Hanski 1982) which states that within a region a large number of rare or 'satellite' species occur at only a few sites, and a distinct mode of dominant or 'core' species occur throughout the region. It was further suggested that Hanski's model could be applied at local scales to explain the patchy-distribution of species characterizing much of the greater tallgrass region, where numerous satellite species- often temporally variable in abundance- occur within a matrix of abundantly-stable, dominant species (Collins and Glenn 1990).

In the past, tallgrass prairie was habitat for a large number of native herbivores including the Plains bison and Prairie dog; a variety of animal species such as the Monarch butterfly, Swift fox and Ferruginous hawk; and a majority of the North American breeding songbirds (Samson and Knopf 1994; CWHP 1998). Today, this region is experiencing large-scale declines of many of those animal species, and is known for endangered plant species like the Western prairie fringed orchid, *Platanthera praeclara* Sheviak & Bowles, and Small white lady's slipper, *Cypripedium candidum* Muhl. ex Will. (CWHP 1998).

Declines in tallgrass species diversity at present are a consequence of the cultural practices employed by humans over the past 200 years (i.e., since European settlement), where 99% of the original native prairie has been converted to intensive agriculture and urban development, and natural ecological forces such as fire and ungulate grazing are all but removed from the landscape (Howe 1994). The alarming quantity of tallgrass habitat destroyed over the past two centuries is the largest reported for any plant community in

North America (Samson and Knopf 1994). The less than 1% that presently remains is found within small parcels of land or remnants that escaped cultivation because they are unsuitable for agriculture (CWHP 1998), and/or are protected under conservation efforts of government agencies, environmental groups, and individuals (Howe 1994). In addition, it is unknown whether the remaining habitat is characteristic of the 'true prairie' region that existed prior to European settlement, but it has been suggested that these remnant prairie areas are atypical communities (in structure and composition) of the original tallgrass region (Steinauer and Collins 1996).

Tallgrass Prairie Research

Past research on the factors involved in influencing species composition and distribution of tallgrass prairie have focused on the mid-central portion of its North American range, with much of the scientific literature being published by ecologists working in the Konza Prairie Research Natural Area near Manhattan, Kansas (Collins and Glenn 1990; Gibson and Hulbert 1987; Collins 2000; Collins et al. 1998; Briggs and Knapp 1995). Long term field experiments at the Konza Prairie site have looked at the effects on plant species diversity, abundance patterns, or aboveground biomass through grazing (Collins and Glenn 1990; Vinton and Hartnett 1992; Vinton et al. 1993; Collins et al. 1998), mowing (Collins et al. 1998), topography (Gibson and Hulbert 1987; Briggs and Knapp 1995), climatic variation (Gibson and Hulbert 1987; Briggs and Knapp 1995), soil moisture (Briggs and Knapp 1995), nitrogen enrichment (Collins et al. 1998), and fire frequency (Collins and Glenn 1990; Collins 2000; Gibson and Hulbert 1987; Briggs and Knapp 1995; Collins et al. 1998). This work is important to our current

understanding of the dynamics acting within tallgrass communities because historically, abiotic and biotic forces (e.g. namely fire and grazing) created spatial and temporal dynamics of nitrogen availability (Collins 2000). These forces are also suggested to control overall diversity and productivity on the prairies (Collins et al. 1998).

Experiments by Collins et al. (1998), which looked at bison-grazed, and/or frequently burned sites in the Konza Tallgrass area, showed that species richness and abundance was increased greatly by those particular disturbance regimes, but varied depending on treatment type, e.g. total species richness was significantly higher on grazed-alone and grazed and burned sites, than on annually-burned sites and un-manipulated (i.e., disturbance-free) sites. Furthermore, it was found that patterns of increasing dominance and decreasing richness are altered by natural disturbance regimes because the dominant species appears to be highly sensitive to the disturbance (Collins and Glenn 1990). Collins et al. (1998) point out that as the large-scale disturbances of the past occur less frequently, there has been a drastic decline in species diversity for the tallgrass region; concurrently, community structure on a local scale has become a function of interactions (e.g. competition and dispersal) among individual plants (Collins and Glenn 1990). As a result, the examination of the factors promoting community level interactions and the composition and distribution of species within the remaining prairie remnants, is more important now than ever.

In addition, it should be emphasized that the work in the Konza Prairie may not be applicable to all regions of the North American tallgrass range, particularly for the northern portion extending into Manitoba. This research may not be relevant to the Manitoba region for two main reasons: 1. The Konza site is located in central North

America (Kansas) and therefore quite geographically distant to Manitoba; 2. It is considered an area of low productivity- a result of the underlying shale rock layers in the Flint Hills region (Knapp et al. 1998), which is uncharacteristic of most prairie grasslands (Barbour et al. 1999).

Manitoba Tallgrass Prairie

In Manitoba, tallgrass prairie is an ecologically important community extending into the southeast portion of the province (Fig. 1.3), but of which little habitat remains. At present, less than 1% of the original 6,000 square kilometres remains in Manitoba (CWHP 1998). Only a fraction remains because it is located on rich, fertile Red River Valley soil, which is highly desirable for farming, and has therefore been extensively disturbed by cultivation and ranching.

In general, it has been observed that Manitoba tallgrass prairie is characterized by patch-like distributions of particular dominant plant species interspersed with a greater number of rare species. Species observed in Manitoba tallgrass prairie include perennial C4 grasses: *Andropogon gerardii* (Big bluestem), *Panicum virgatum* L. (Switchgrass), *Calamagrostis neglecta* Ehrh. (Narrow reedgrass) and *Spartina pectinata* Link (Prairie cordgrass); certain C3 grasses: *Poa Pratensis* (Kentucky bluegrass) and *Stipa spartea* Trin. (Porcupine grass); and an array of perennial dicot members: *Helianthus maximilianii* Schrad. (Narrow-leaved sunflower), *Aster ericoides* L. and *A. laevis* L. (Many-flowered and Purple aster, respectively), and *Solidago canadensis* L. (Canada goldenrod) (Sveinson 2003; Borkowsky 2006; Friesen 2007).

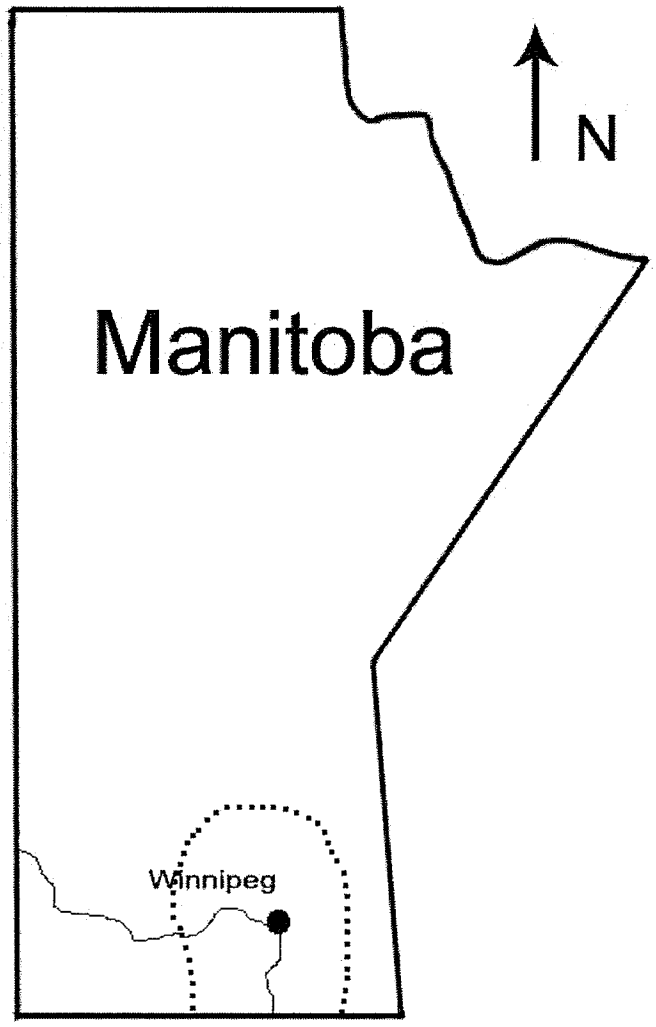


Fig. 1.3 A map of the tallgrass prairie area extent in Manitoba (dotted line) with the city of Winnipeg (black dot), and intersection of Assiniboine River (west to east) and Red River (north to south) at Winnipeg (adapted from Natural Resources Canada [online] 2002).

Thesis Research

Although many of the plant species occurring in Manitoba tallgrass prairie have been described taxonomically (Sveinson 2003; Borokowsky 2006; Friesen 2007), the individual abiotic and biotic factors promoting the abundance and distribution of these species are not well understood. In particular, the abiotic and biotic soil dynamics controlling biological plant diversity within this prairie community can be considered a prime candidate for examination within a thesis project. As such, I chose to examine the nature of the interactions that occur between plants and their soil community within tallgrass prairie in Manitoba, as a Master's of Science (Botany) candidate. The overall objective of my thesis research was to determine the patterns by which particular local tallgrass species affect the underlying (abiotic and biotic) soil community, and the soil community in turn influences the performance of the species within the relationship. In specific, I examined the magnitude and direction of interactions taking place between plants and soil.

As mentioned previously (see **Plant-Soil Relationships:** *Feedback and Maintenance of Diversity*), a number of studies have attempted to characterize the feedback interactions taking place within tallgrass prairie communities (Bever et al.1997, Bever 2003, Hartnett and Wilson 2002, Holah and Alexander 1999). The majority of this research has suggested the role of a negative feedback system in maintaining diversity, but often with inconclusive results. For example, Holah and Alexander (1999) hypothesized that a negative feedback system characterized the biotic soil communities associated with two co-existing prairie species, *Chamaecrista fasiculata* (Michx.) Greene (an annual legume) and *Andropogon gerardii*, but the individual growth results for each

species only partially supported this hypothesis. In particular, *C. fasiculata* biomass was no different in its own soil community compared to the soil community of A. *gerardii*, and seedling growth and survival was unaffected by the microwaved (control) or nonmicrowaved (NM) soil treatment, providing no evidence to support the hypothesis for a negative feedback system. Conversely, *A. gerardii* height was reduced in the NM soil of each species as compared to the sterile control, and effects were more pronounced in the *C. fasiculata* NM soil, suggesting a negative feedback effect occurred for *A. gerardii* due to biotic components in the *C. fasiculata* soil (e.g. soil pathogens). However, because the effects of NM *A. gerardii* soil on the growth of *C. fasiculata* were neutral, evidence for an overall negative feedback system could not be established.

Primary Research Objectives

In light of the inconclusive results of the studies mentioned above (e.g. Holah and Alexander 1999), I intend to determine whether the feedback relationships within a particular tallgrass prairie community can be characterized as positive or negative overall, and relate this to maintenance of diversity in the community. Furthermore, I intend to link the growth and abundance of selected tallgrass species in the prairie community with species-specific associations within the biotic soil community (e.g. mycorrhizal fungi).

Secondary Research Objectives

Along with the primary objectives, I have identified a number of secondary objectives to be investigated within this thesis project. The four objectives are to:

1. Determine the spatial dynamics of vegetation patches in tallgrass prairie.

2. Characterize the abiotic soil conditions of a particular tallgrass prairie community in Manitoba.

3. Determine the effect of soil biota from dominant plant patches on the dominant plant species.

4. Determine the extent of variation in the soil communities of other tallgrass prairies (including both remnant and restoration prairies) on plant performance.

Chapter 2: Temporal and spatial dynamics in Manitoba tallgrass prairie

Introduction

Plant communities are spatially and temporally variable in response to a variety of abiotic and biotic forces in nature (Collins 2000). Particularly variable are prairie grasslands, in which historically, fire and large-ungulate grazing were major determinants of species distribution and abundance (Collins and Glenn 1990; Steinaeur and Collins 1996; Collins and Steinaeur 1998; Collins et al. 1998; Knapp et al. 1998). As natural disturbances have all but been eliminated from the landscape through intensive agricultural practices and land-use change (Howe 1994; Samson and Knopf 1994), community structure is now a product of interactions among individual plant species with the local environment (Collins and Glenn 1990). The local environment in this case encompasses abiotic factors such as soil moisture, nutrients (especially nitrogen and phosphorus), pH, and organic matter (Barbour et al. 1999). Additionally, biotic factors such as insect and animal herbivory, plant competition, and the soil community (micro-organisms) are components of the local environment. The distributions of these abiotic and biotic factors are heterogeneous in both space and time (Harper 1977). Accordingly, the local environment influencing species distribution and abundance can vary across a plant community at any given time (Barbour et al. 1999).

The tallgrass or true prairie region comprises a large portion of the native grasslands- the Great Plains- present in North America, extending centrally from south-east Manitoba to southern Texas (Sampson and Knopf 1994; Fig. 1.2). Along this broad geographical range a gradient of climate exists, which is colder and drier in the northern section and warmer and wetter in the south (Bragg 1995). Thus, it is expected that the

abiotic and biotic factors influencing local plant communities will also vary from north to south across the entire tallgrass range.

Most work on spatial and temporal dynamics within tallgrass prairie communities though has been limited to the south-central US. In particular, long-term research at the Konza Prairie Research Natural Area in the Flint Hills of northeastern Kansas has established the importance of interacting abiotic and biotic forces such as climate, topography, soil moisture, nutrients, fire, and grazing on species diversity, abundance patterns, aboveground biomass, and overall community structure (Gibson and Hulbert 1987; Collins and Glenn 1990; Vinton and Hartnett 1992; Briggs and Knapp 1995; Collins et al. 1998; Collins 2000). Briggs and Knapp (1995), for example, found that inter-annual fluctuation in primary productivity was the product of spatial and temporal variability in a number of local abiotic factors such as light, water, and nutrients, in combination with the greater driving forces of topography, fire frequency, and climate, rather than the result of any one factor alone. Additionally, Vinton and Hartnett (1992) concluded, based on the study of bison grazing in unburned and burned prairie communities, that grazing, fire, and topographic position collectively influenced plant growth, and no two plant species responded the same to the fire and grazing interaction.

In contrast, the study of the spatial and temporal factors operating locally in Manitoba tallgrass communities is not well known and not particularly understood. This is especially important because Manitoba is at the northern extent of the tallgrass range and as described previously, rather geographically distant- and climatically distinct from the tallgrass areas in the south (Bragg 1995). Additionally, the physical topography and soil structure of Manitoba tallgrass communities, comprised of rich glacial-till deposits

overlain with heavy clay or silt soil with little topographic variation, is recognized to be considerably different from the southern tallgrass areas, particularly the Flint Hills region, where shallow soils of low organic content overlay prominent rolling hill topography and limestone and shale strata (Briggs and Knapp 1995).

For the purposes of this study, I examined the spatial and temporal dynamics occurring within a particular tallgrass prairie community in Manitoba. The objectives of this study were two-fold: 1. to determine the spatial dynamics of vegetation patches in the prairie, and 2. to characterize the abiotic soil conditions.

Materials and Methods

Study Area and Site Description

The study area was located in a 115.8 ha remnant tallgrass prairie community at Oak Hammock Marsh (OHM), Manitoba, Canada (N 50° 10' 40.383", W 97° 09' 36.966") (Fig. 2.1), approx. 25 km north of Winnipeg, Manitoba. Oak Hammock Marsh is part of the greater Glacial Lake Agassiz Plain, which falls within the Red River Valley region of southeast Manitoba (Joyce and Morgan 1989). The OHM community occurs in the 'northern tall grasslands' of the North American tallgrass range (Hamilton 2005; refer to Fig. 1.2), and occurs on land which was designated by Manitoba Conservation as a Wildlife Management Area (WMA) in 1973 (Suggett, pers. comm.). This is particularly important as a large portion of the remaining tallgrass area in Manitoba (<1% of the original 6000 km^2) is found within small parcels of land or remnants that are protected under conservation efforts of government agencies, environmental groups, and individuals (Howe 1994; CWHP 1998). Before the OHM tallgrass community was

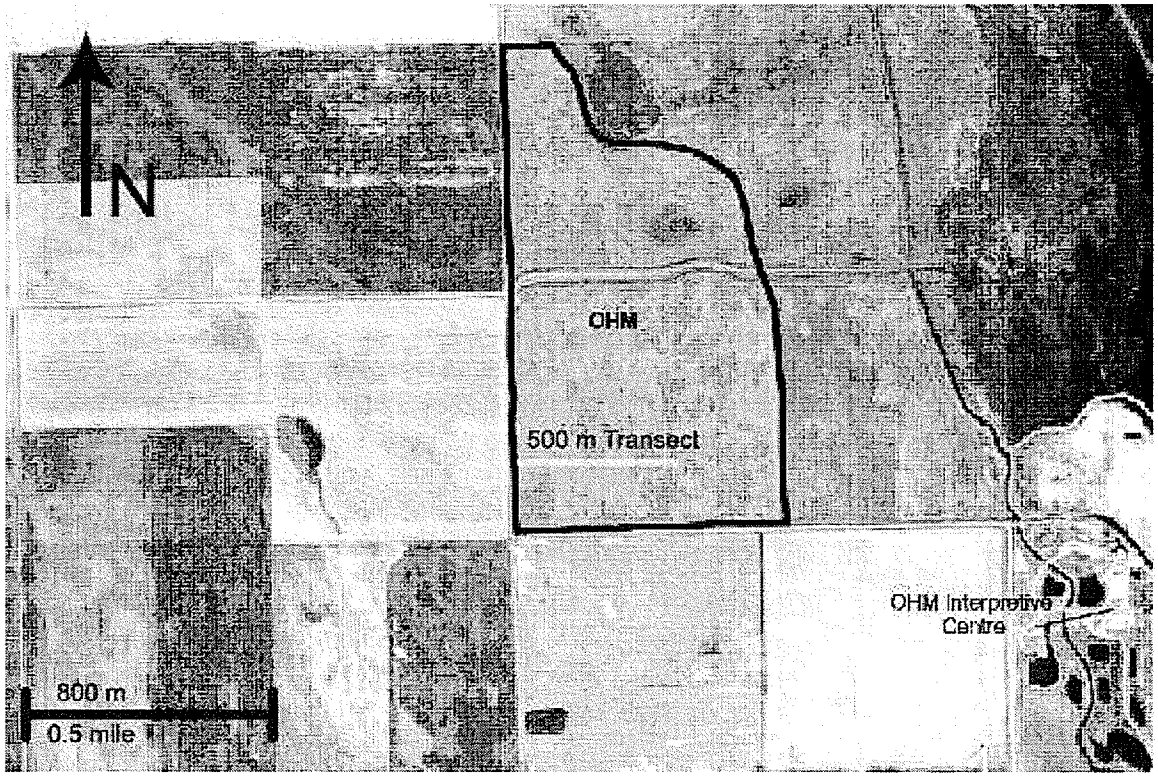


Fig. 2.1 A map of the Oak Hammock Marsh (OHM) tallgrass study site (N 50° 10' 40.383", W 97° 09' 36.966") with prairie boundary (black line) and 500 m permanent transect (yellow line) represented. The aerial photo was taken in 2005.

protected by the Manitoba government it was most likely privately-owned and used solely for agriculture, but it is unclear how characteristic the remnant is of the original tallgrass region, or what level of local (human) disturbance has occurred in the past. However, it is speculated to have been sectioned as pastureland and grazed by livestock (Suggett, pers comm.).

It is known that the OHM tallgrass community was once part of the extensive St. Andrews Bog in Manitoba and subject to periodic inundation with water preceding extensive drainage efforts that began in the early 1900s (Suggett, pers. comm.). Agricultural lands to the south and west, and wet, restored marsh areas to the east and north surround the prairie today. The physical topography is relatively flat, with fluctuations in upland and lowland areas occurring on a micro-scale (cm) range. Soil texture/classification is heavy clay in the *Mollisols* group or black chernozems; dark, moist, fertile soil characteristic of the deep glacial-till deposits in the Red River Valley basin (Joyce and Morgan 1989; Bragg 1995; Hamilton 2005). Natural disturbance regimes are minimal, except for controlled burns once every ~4 years in the WMA by staff at Manitoba Conservation (Hagglund, pers. comm.).

Patches of perennial grasses and forbs, and the occasional shrub species characterize the vegetation of the OHM prairie. Prior to historical drainage alterations, it is suggested to have been a wet meadow type of prairie, which gave way to greater species adapted to drier conditions after 1900 (Suggett, pers. comm.). Past species sampling has revealed that the OHM prairie is currently dominated by C4 grasses including *Andropogon gerardii* (Big bluestem), *Panicum virgatum* (Switchgrass) and *Spartina pectinata* (Prairie cordgrass), and interspersed with C3 grasses such as *Poa*

Pratensis (Kentucky bluegrass) and *Stipa spartea* (Porcupine grass), and perennial forbs including *Helianthus maximilianii* (Narrow-leaved sunflower), *Aster ericoides* (Many-flowered aster), *A. laevis* (Purple aster) and *Solidago* spp. (Goldenrods) (unpublished data).

The OHM area climate is continental with an average yearly temperature of 2.5°C recorded from 1971-2000 at the nearest weather station, Stony Mountain, MB (Environment Canada 2002). The average daily temperature in January and July for this time period was -18°C and 20°C, respectively. The average annual rainfall was 408 mm, occurring (mainly) between May and September, and the average annual snowfall was 102 cm, occurring (mainly) between November and January. During 2006 and 2007 when the bulk of the OHM study was conducted, the average daily temperature in January and July 2006 was -7.8°C and 21.7°C, respectively, and in 2007 was -15.2°C and 21.7°C, respectively. The annual rainfall and snowfall amount recorded in 2006 was 256 mm and 158 cm, respectively. The annual rainfall amount in 2007 was 487 mm, while the annual snowfall amount was missing from Environment Canada records. However, the total precipitation (rainfall plus snowfall) amount for the 11 months that were recorded in 2007 was 563 mm, which exceeded the 2006 total precipitation amount of 414 mm for the full 12 months recorded.

A 500 m permanent transect (see Fig. 2.1) was established in the OHM tallgrass prairie immediately following a spring controlled-burn by Manitoba Conservation in 2002 (Markham, pers comm.). Fifty, 1 x 1 m permanent sample plots (PSP) were located along the 500 m transect using stratified random sampling (i.e., 1 PSP was randomly located in each 10 m section along the transect).

Vegetation Sampling

Vegetation was sampled within each PSP in June 2002 using pin-frame sampling method (Markham, pers. comm.). The pin-frame held 10 pins, each 10 cm apart, which could be raised and lowered as needed. Species abundance was recorded in each PSP along 3 parallel-line positions from the transect- 25, 50 and 75 cm away (Fig. 2.2). Individual species were recorded for the number of times a pin hit a particular plant (or bare ground) divided by the total number of pin hits (a possible 30 per plot), and converted to percent total abundance. Given that species structure was regarded as overlapping for this sampling method, the total abundance of all species in the plot was greater than 100%.

The vegetation within each PSP was sampled by me in July 2007 using quadrat sampling method. It should be noted that the OHM prairie was expected to be burned in spring 2007 by Manitoba Conservation, but an unusually dry spring meant that any form of fire was regarded as potentially hazardous to the surrounding marsh and agricultural areas. Seeing that 5 years had passed since the previous burn, sampling the transect using the original pin-frame method was regarded as futile, if not impossible, with the thickness of thatch present. Using percent cover sampling, species abundance was evaluated within the area of a 1 x 1 m metal quadrat, positioned with the south edge of the quadrat on top of the PSP transect boundary (Fig. 2.2). Individual common species were recorded as a percent cover, or portion of the total area occupied within the quadrat. Rare species were excluded from the survey because they were too difficult to accurately assess, a result of the thickness of thatch present. Species structure was considered to be non-overlapping for this method, and the total cover of all species in the plot was equal to 100%.

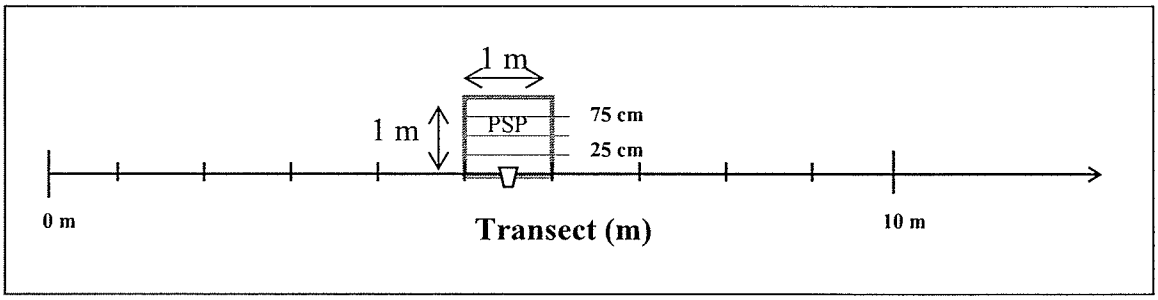


Fig. 2.2 An illustration of soil and vegetation sampling within a permanent sample plot (PSP), located using stratified random sampling method along a 500 m transect, in the Oak Hammock Marsh tallgrass community. Pin-frame sampling of plant species was conducted in 2002 along the 25, 50, and 75 cm line position within each 1 x 1 m PSP. Percent cover sampling was conducted in 2007 to assess the plant species within the total 1 x 1 m area of each PSP. Soil cores (▽) were collected in 2005, 2006 & 2007 from each PSP. Specifically, one core was collected from the centre of the 1 m boundary of each PSP (i.e., the boundary of the plot is positioned on the transect), for a total of 50 soil cores collected per sample year.

Soil Sampling

Soil cores were collected for nutrient analysis from the 50 PSPs in 2005 (October), 2006 (July) and 2007 (July) (i.e., 50 cores per sample year). A soil corer (2 cm by 30 cm depth) was used in 2005 and 2007, and a soil auger (3 cm by 30 cm depth) was used in 2006 to collect samples from the centre of the 1 m plot boundary positioned on top of the transect (Fig. 2.2). To estimate bulk density, an additional sample was collected in 2006 beside the auger core using a hand trowel (10 cm by 10 cm depth). The hole of trowel-excavated soil was replaced with an equivalent amount of sand, for which the volume was recorded. All soil samples were brought back to the lab and broken into small pieces, air-dried (the 2006 samples were weighed fresh before drying), and stored for future analysis.

A portion of the soil samples collected in all three survey years were analyzed for inorganic nitrogen (N) using the techniques of Microdiffusion (Mulvaney 1996), and inorganic phosphate (P) using Olsen's method (Kalra and Maynard 1991). Six other soil properties: pH, gravimetric water content (GWC), organic matter (OM), bulk density (BD), nitrogen mineralization (N Min.), and phosphorus mineralization (P Min.) were determined for the 2006 samples. Determination of N Min. and P Min. was performed in the field by incubation of soil cores for 30 days in plastic tubes (Ray Leach "Cone-tainers"™, 2.5 cm x 12 cm, Stuewe & Sons Inc., Corvallis, OR), which were covered with plastic-wrap to minimize moisture changes and to allow gas exchange (Robertson et al. 1999). Analysis of levels of N and P after incubation was then performed (Mulvaney 1996; Kalra and Maynard 1991), and the difference from the pre-incubation levels (determined previously as mentioned above) was divided by the incubation time to

calculate the mineralization rate per day. Methods for the remaining soil analyses followed standard protocols: pH – soil was mixed into a 1:2 paste of soil to 0.1 M calcium chloride solution and measured with a pH electrode (Kalra and Maynard 1991); GWC – soil was weighed fresh, oven-dried at 105˚C until a constant weight was achieved, and weighed dry (Jarrell et al. 1999); OM – oven-dried (105˚C) soil was pre-weighed in a ceramic crucible, ignited at 500˚C in a muffle furnace for 4-5 hours, and re-weighed (i.e. weight loss on ignition) (Harmon and Lajtha 1999); and BD – soil was oven-dried at 105˚C until a constant weight was achieved, weighed dry, and divided by the volume of sand required to fill the trowel-excavated hole in the field, as described previously (Elliot et al. 1999).

Using a surveyor's transit, microelevation (ME) was also determined along the OHM transect in 2007. The height (measured with metre sticks) of each PSP was recorded in reference to the known height of the transit, positioned every 100 m along the transect. The difference between the height of the transit and the height of the associated PSP was calculated to determine the positive and negative change in micro-scale (cm) topography. The lowest value determined along the transect was added to each plot value to position the starting elevation at zero.

Statistical Analysis

Linear correlation analysis (JMP-SE 6.0.3©) was utilized to establish relationships between the 9 soil variables: ME, OM, BD, GWC, pH, Inorganic N, N. Min., Inorganic P, and P Min., measured within the single survey year 2006 (with the exception of ME measured in 2007). Correlation of nutrient levels (N and P) between years was further

performed to look at possible relationships between 2005 and 2006, 2006 and 2007, and 2005 and 2007 in order to determine if plots had consistent nutrient levels through time. One-way ANOVA using time series analysis (followed by Tukey-Kramer post hoc) was performed to compare nutrient levels between the 3 survey years and test for overall trends through time.

Multivariate analysis of variance (MANOVA) was used to examine the interactions between the multiple variables collected along the OHM transect, and in particular to determine: a) a relationship between the species variables and soil (environment) variables, b) the strength of the relationship, and c) the important variables accounting for the relationships. All environment data except pH, which was measured on a log scale, was log-transformed prior to MANOVA to increase normality of frequency distributions. The species data had a value of '1' added (i.e., x + 1) to eliminate '0' values, undefined by logarithm, and to increase normality.

Principal Component Analysis (PCA) (SYN-TAX 2000©), a multivariate method of ordination, was chosen to best represent the broadly linear variance within the individual (species and environment) data sets (Kenkel 2006). The purpose of using ordination was to explore and summarize the underlying data structure and determine the importance of individual variables. Three separate PCA were performed on the: 1. 2002 species data (p = 21), 2. 2007 species data (p = 16) and 3. environment data (p = 7), with PCA performed using a covariance matrix for the 2002 and 2007 species data, and a correlation matrix for the environmental data.

Redundancy Analysis (RDA) (CANOCO 4.5©), a multivariate model of canonical analysis, was selected following ordination to best represent both the asymmetric

relationship between the species and environment data and the underlying linear structure (Kenkel 2006). The overall objective of using canonical analysis was to summarize the strength and redundancy of the relationship between the two sets of variables, and to determine the degree to which the environment could be used to predict species composition in the OHM community. Two separate RDA were performed to constrain each set of species 'response' variables ($p = 21$ and $p = 16$ for 2002 and 2007 species, respectively) by the set of environment 'predictor' variables ($p = 7$), with redundancy and r-square values reported to indicate the strength of the relationship. Monte Carlo Permutation was further utilized to determine if the proportion of the total variance reported for the first canonical axis (and subsequently all canonical axes) was statistically significant.

Results

Vegetation Sampling

A total of 40 species: 12 grasses, 26 forbs, 1 sedge, and 1 woody shrub were recorded along the OHM transect (Table 2.1). The majority were observed in 2002 and 2007, with the exception of those species considered weedy (according to Royer and Dickinson 1999) observed solely in 2007 along the transect in areas recently disturbed by burrowing mammals. Mean percent cover of the common species (i.e. >1% cover) compiled in each of the two years demonstrated that five grass species accounted for nearly three quarters of the total species abundance, with *Andropogon gerardii* having the highest abundance in both years, and the remaining quarter was attributable to a diverse number of forbs, with *Helianthus maximilianii* most abundant (Table 2.2). In 2002, *Poa*

Table 2.1 Scientific name, code, common name, and growth form for all species recorded along the OHM transect in June 2002 and July 2007 (* = present; ** = present mainly in disturbed-soil areas).

Scientific name	Code	Common name	Form	June 2002	July 2007
Achillea millefolium	ACHmil	Common yarrow	F	*	*
Agropyron trachycaulum	AGRtra	Slender wheatgrass	G	*	*
Ambrosia artemisifolia	AMBart	Ragweed	F	*	*
Andropogon gerardii	ANDger	Big bluestem	G	*	*
Antennaria sp.	ANT	Pussy-toes	F	*	
Asclepias ovalifolia	ASCova	Dwarf milkweed	F	*	*
Aster ericoides	ASTeri	Many-flowered aster	F	*	*
Aster laevis	ASTlae	Smooth aster	F	*	*
Aster spp.	ASTER	Aster	F	*	
Bromus inermis	BROMine	Smooth brome	G		**
Calamatagrostis neglecta	CALneg	Narrow reedgrass	G	*	*
Carex spp.	SEDGE	Sedge	S	*	*
Cirsium arvense	CIRarv	Canada thistle	F		**
Convovulus sp.	CONV	Bindweed	F	*	
Euthamia graminifolia	EUTgra	Flat-topped goldenrod	F	*	*
Galium boreale	GALbor	Northern bedstraw	F	*	*
Glycyrrhiza lepidota	GLYlep	Wild licorice	F	*	*
Helianthus maximilianii	HELmax	Narrow-leaved sunflower	F	*	*
Helianthus rhomboides	HELrhom	Rhombic-leaved sunflower	F	*	*
Hierochloe odorata	HIEodo	Sweet grass	G	*	
Heuchera richardsonii	HEUrich	Alum root	F	*	*
Hypoxis hirsuta	HYPhir	Yellow star grass	F	*	
Lappula sp.	LAPP	Bluebur	F		**
Liatrus ligulistylus	LIAlig	Meadow blazing star	F	*	*
Panicum capillare	PANcap	Witchgrass	G		**
Panicum leibergii	PANlei	Leiberg's panic grass	G	*	
Panicum virgatum	PANvir	Switchgrass	G	*	*
Petalostemum purpurem	PETpur	Purple prairie clover	F	*	
Phalaris sp.	PHA	Canary grass	G	*	*
Poa pratensis	POApra	Kentucky bluegrass	G	*	*
Preanthes recemosa	PRErec	Glaucous white lettuce	F	*	
Rosa arkansana	ROSark	Prairie rose	W	*	*
Solidago canadensis	SOLcan	Canada goldenrod	F	*	*
Solidago rigida	SOLrig	Stiff goldenrod	F	*	*
Spartina pectinata	SPARpec	Prairie cordgrass	G	*	*
Stipa spartea	STIspa	Porcupine grass	G	*	
Thalictricum sp.	THAL	Meadow-rue	F	*	*
Vicia sp.	VICIA	Vetch	F		*
Viola petatifida	VIOpet	Birdfoot violet	F	*	
Zizia aptera	ZIZapt	Heart-leaved alexanders	F	*	*

G = Grass, F = Forb, S = Sedge, W = Woody shrub

Table 2.2 Mean percent cover and percent frequency of 23 common plant species (≥1%) in the OHM tallgrass community. Species data was collected using pin-frame sampling in June 2002 and quadrat sampling in July 2007.

Species	2002 Mean Cover (%)	2007 Mean Cover (%)	2002 Frequency (%)	2007 Frequency (%)
Andropogon gerardii	30	28	70	56
Poa pratensis	17	10	84	26
Spartina pectinata	17	18	50	40
Calamagrostis neglecta	15	13	38	30
Panicum virgatum	13	8	58	40
Helianthus maximilianii	9	8	66	46
Hierochloe odorata	9	<1	30	0
Galium boreale	6	1	48	12
Bare ground	5	---	60	---
Solidago rigida	4	1	32	12
Rosa arkansana	4	<1	50	0
Carex spp.	4	1	38	2
Solidago canadensis	4	3	24	8
Helianthus rhomboides	3	2	14	8
Hypoxis hirsuta	2	---	28	---
Agropyron trachycaulum	2	<1	16	0
Glycyrrhiza lepidota	2	1	8	8
Preanthus recemosa	1	---	26	---
Aster ericoides	1	1	24	2
Zizia aptera	1	---	12	---
Aster laevis	1	<1	10	0
Phalaris sp.	<1	4	0	6
Asclepias ovalifolia	<1	1	0	8

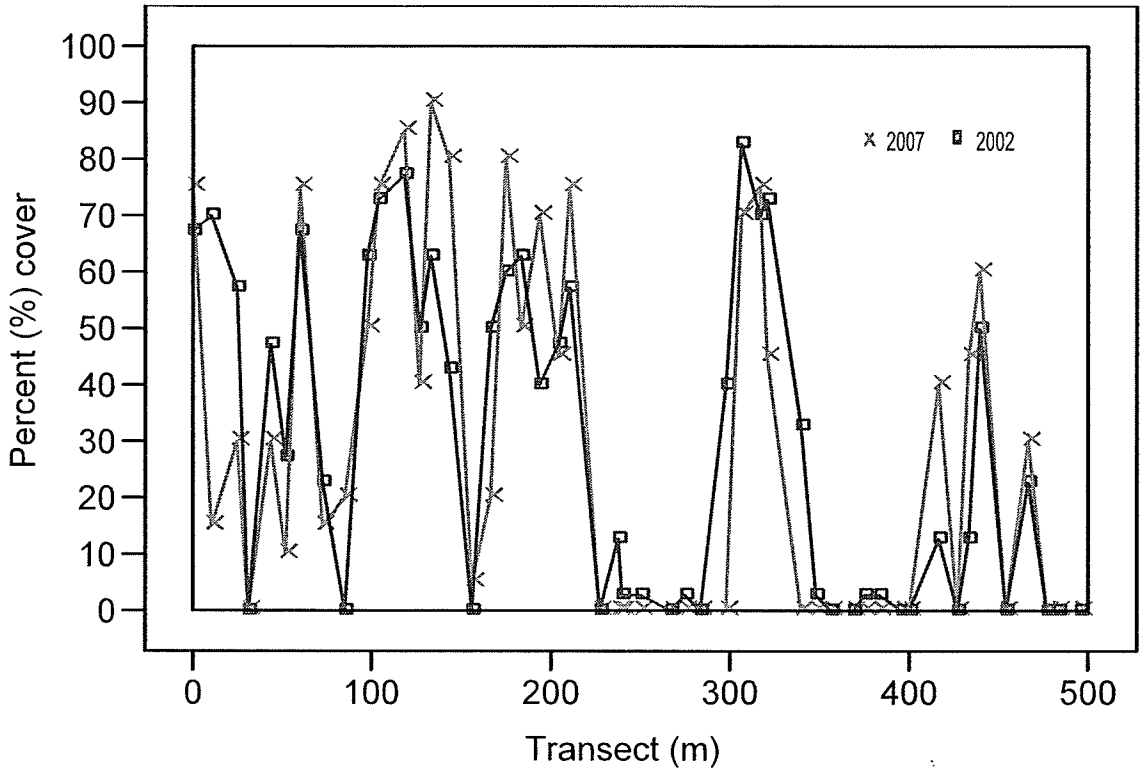
--- = not recorded

pratensis and *A. gerardii* occurred most frequently species along the transect, while *Aster laevis* and *Glycyrrhiza lepidota* (Nutt.) Pursh were least frequent (Table 2.2). *Andropogon gerardii* and *H. maximilianii* occurred most frequently in 2007, while Carex spp. and *Aster ericoides* were least frequent (Table 2.2).

Six common species (3 grasses and 3 forbs) comprising most of the cover were individually plotted by PSP and compared between both years (Fig 2.3). The variation observed along the transect generally showed that percent cover values were stable over the same five year period. However, it appeared that in areas where *A. gerardii* was rare in 2002 (e.g. plots 237 m to 275 m) it had disappeared in 2007 (Fig. 2.3a), while *Spartina pectinata* had disappeared altogether or was reduced in approximately three quarters of the plots where previously recorded in 2002 (Fig. 2.3b). *Solidago canadensis* and *S. rigida* also had disappeared from much of the transect by 2007, but those two species did seem to recur in greater abundance in the last 100 m (Fig. 2.3e and Fig. 2.3f, respectively). A tally of three uncommon species: *Aster ericoides*, *Glycyrrhiza lepidota* (Nutt.) Pursh, and *Asclepias ovalifolia* Dcne., recorded by presence or absence in the fifty plots along the transect, indicated little change in total abundance and plot location between the two survey years (data not shown). For example, *A. ericodes* was present in three (of the fifty plots) in 2007 and twelve plots in 2002, *G. lepidota* was present in six plots in 2007 and four plots in 2002, and *A. ovalifolia* was present in six plots in 2007 and four plots in 2002.

The difference in sampling methods between 2002 (i.e., pin-frame) and 2007 (i.e., percent cover) was not considered to decrease confidence in species comparisons between years because the total composition and abundance of common and rare species

Fig. 2.3
a) *Andropogon gerardii*



b) *Spartina pectinata*

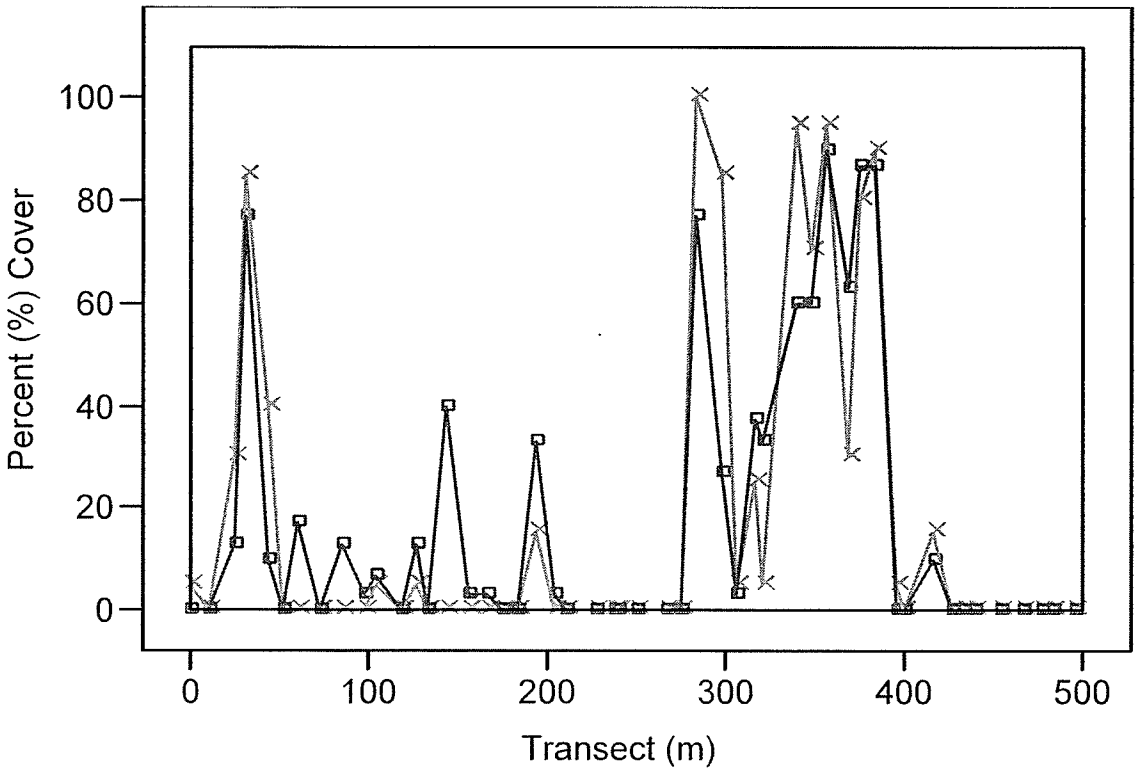
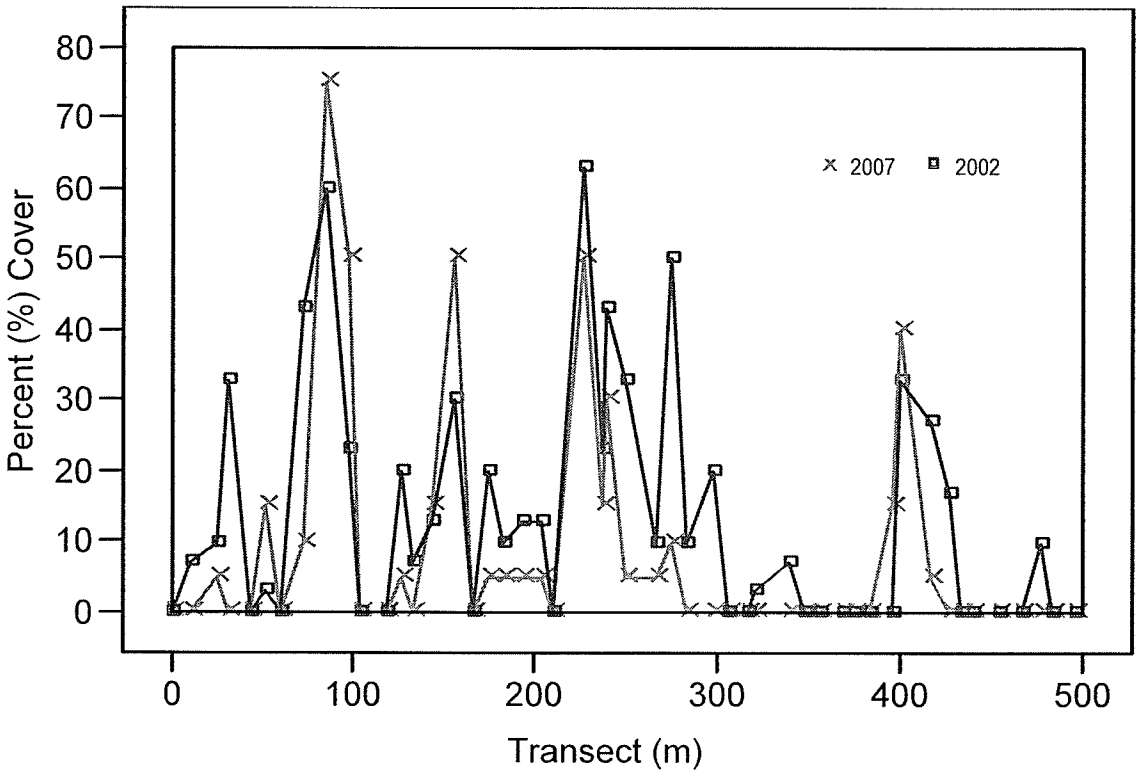


Fig. 2.3 cont'd
c) *Panicum virgatum*



d) *Helianthus maximilianii*

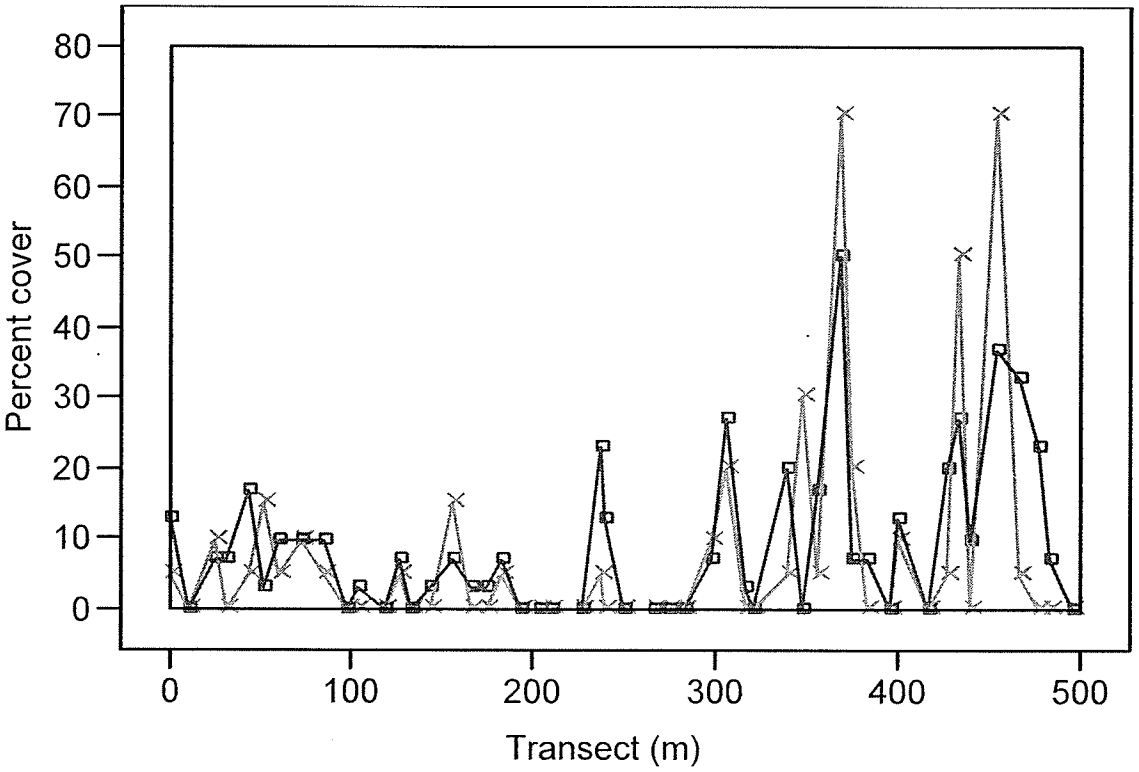
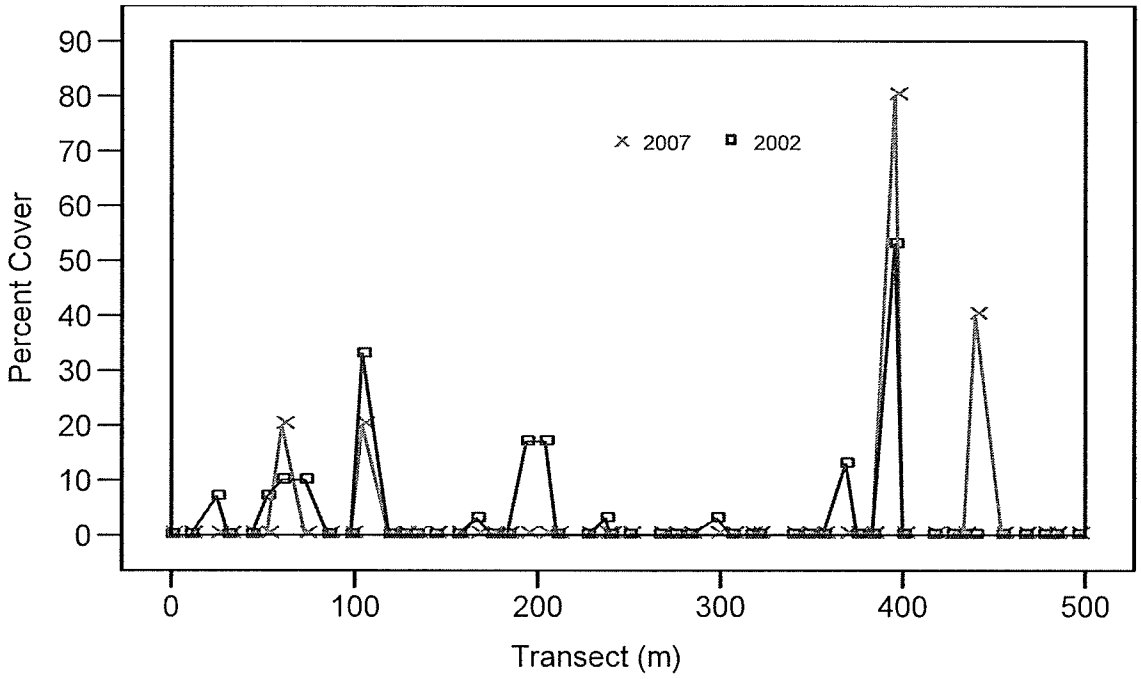


Fig. 2.3 cont'd
e) *Solidago canadensis*



f) *Solidago rigida*

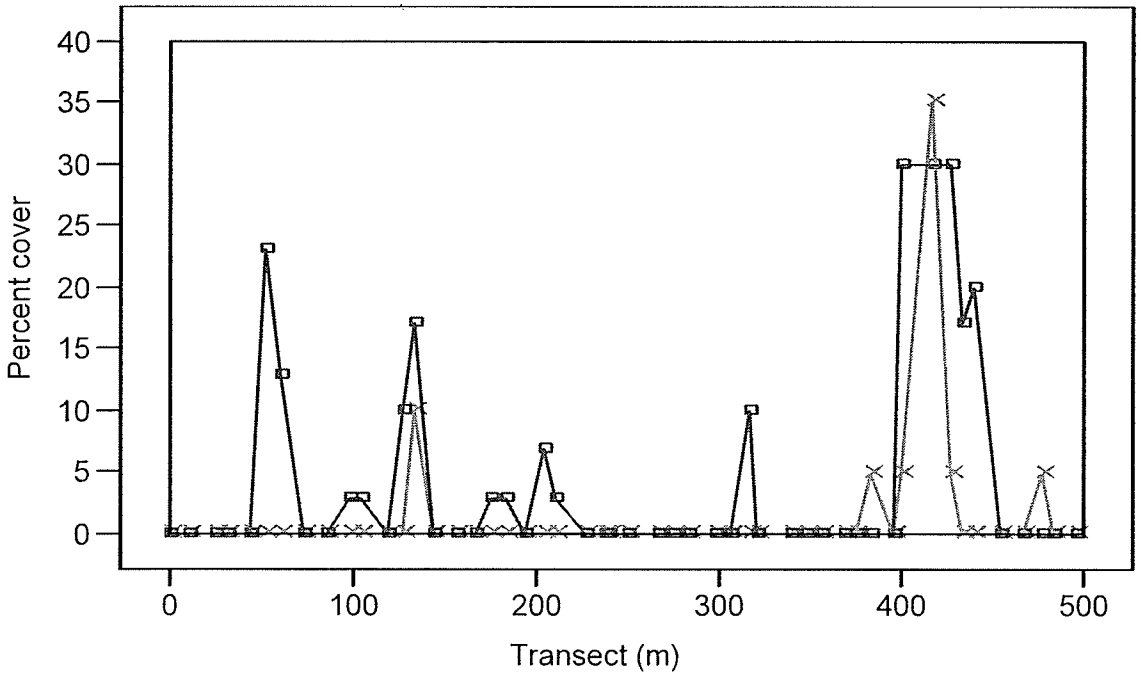


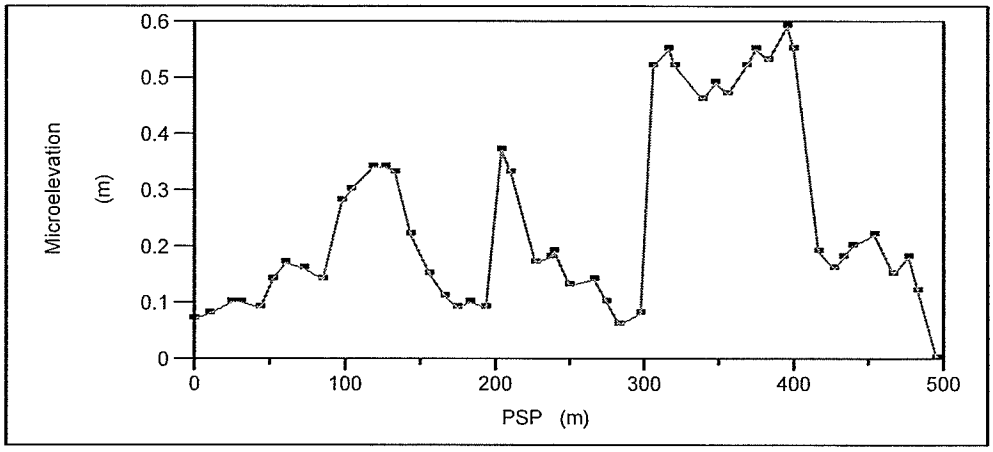
Fig 2.3 Percent (%) cover values of six dominant species in the OHM tallgrass community (3 grasses and 3 forbs, respectively): a) *Andropogon gerardii*, b) *Spartina pectinata*, c) *Panicum virgat*um, d) *Helianthus maximillianii*, e) *Solidago canadensis*, and f) *Solidago rigida* for two survey years. Data was collected by pin-frame sampling in 2002 (□), and quadrat sampling in 2007 (X).

in the 50 plots remained consistent. In addition, species present in lower abundance in particular plots in 2002 further decreased in abundance by 2007 (e.g. *A. gerardii* and *S. pectinata*) with the increase in amount of plot area sampled for percent cover method. An increase in abundance for low abundance species in 2007 would suggest that those particular species were missed by pin-frame method in 2002 due to the lower plot area sampled for that method.

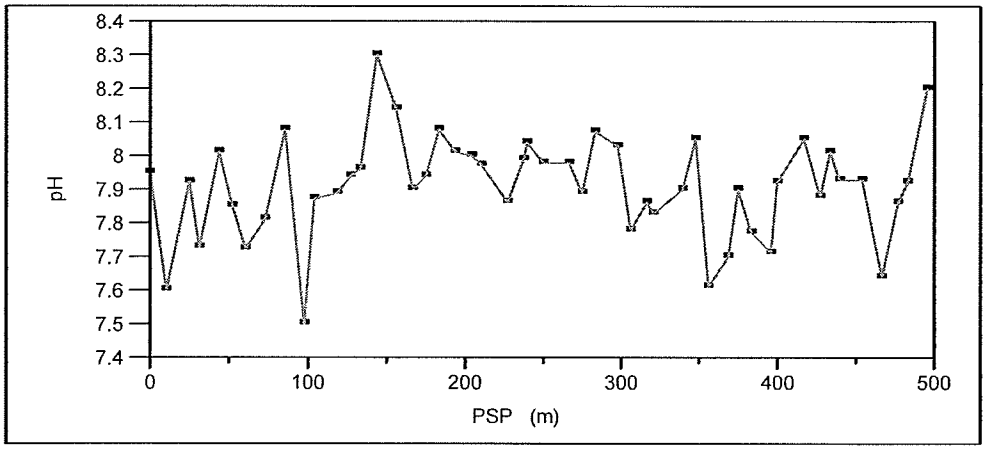
Soil Sampling

The soil data (9 variables) measured in July 2006 (microelevation was done in July 2007) demonstrated variation along the transect (Fig. 2.4). The highest variation occurred in inorganic N (2.7 ppm to 44.2 ppm) (Fig. 2.4c), inorganic N mineralization (-0.50 $ppm \cdot d^{-1}$ to 3.18 $ppm \cdot d^{-1}$) (Fig. 2.4e), and organic matter (5.8% to 38.4%) (Fig. 2.4i), while pH showed least variation (Fig. 2.4b) according to the coefficient of variation (CV) calculated for each variable (Table 2.3). Nutrient levels measured between 2005 and 2007 were generally decreasing, with inorganic N values (mean ± SE) highest in 2005 (20.4 ± 1.2 ppm), mid-range in 2006 (mean 15.7 ± 1.1 ppm), and lowest in 2007 (mean 12.1 ± 0.8 ppm). Inorganic P values were highest in 2005 (mean 2.3 ± 0.3 ppm), lowest in 2006 (mean 0.81 ± 0.07 ppm), and mid-range in 2007 (mean 1.2 ± 0.07 ppm) (Fig 2.5). Overall, there were significant differences for time series comparison of mean inorganic N and P levels (Table 2.4a and 2.4b) and a post hoc test indicated differences between the individual means (data not shown), but there were no significant correlations between the individual plots across the three survey years (Table 2.5a and 2.5b).

Fig. 2.4
a) Microelevation



b) pH



c) Inorganic N

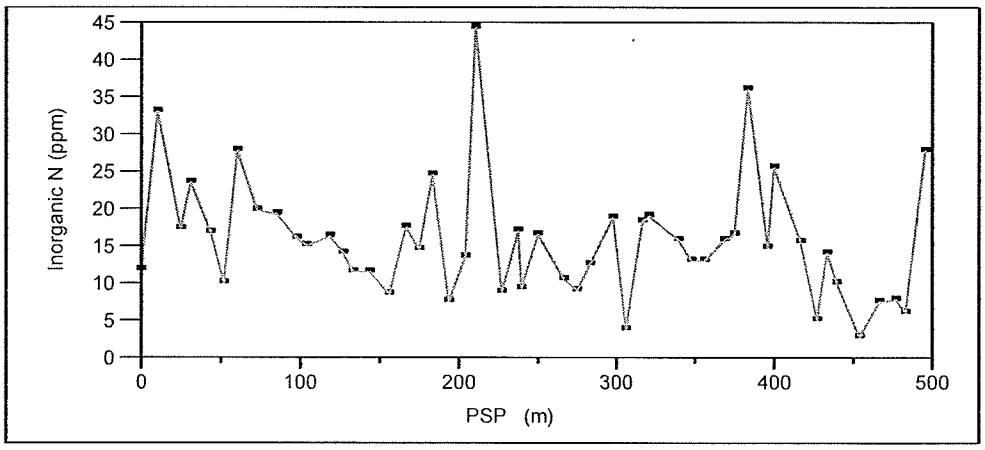
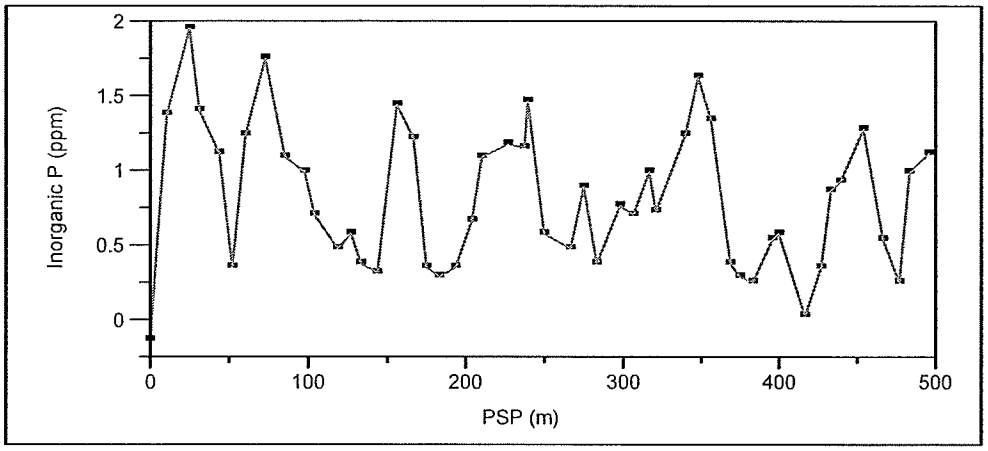
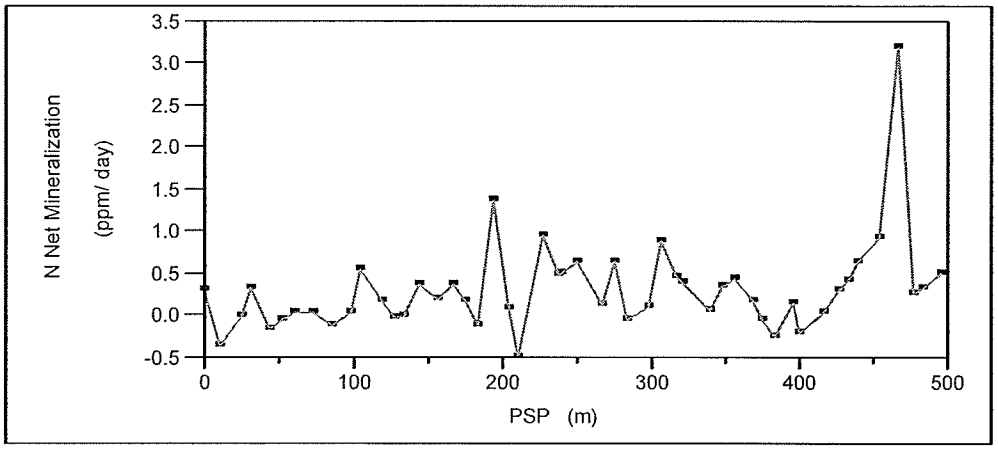


Fig. 2.4 Cont'd
d) Inorganic P



e) Mineralization N



f) Mineralization P

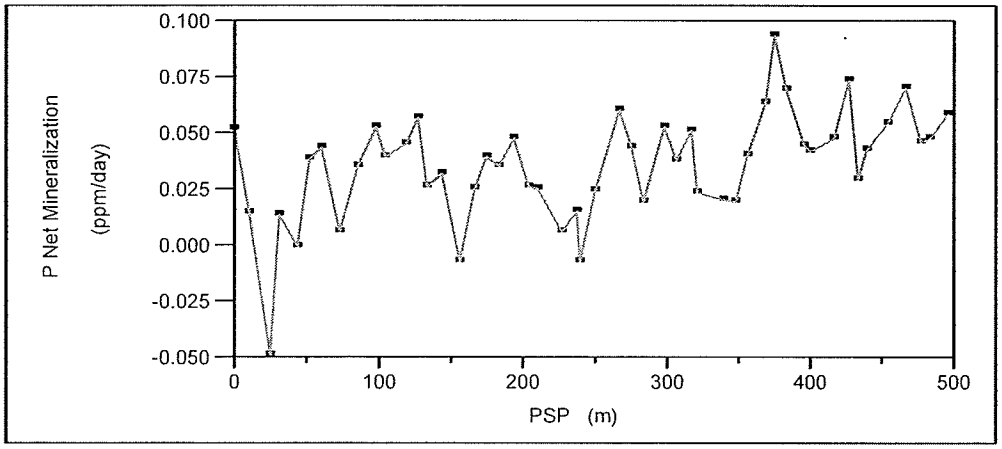
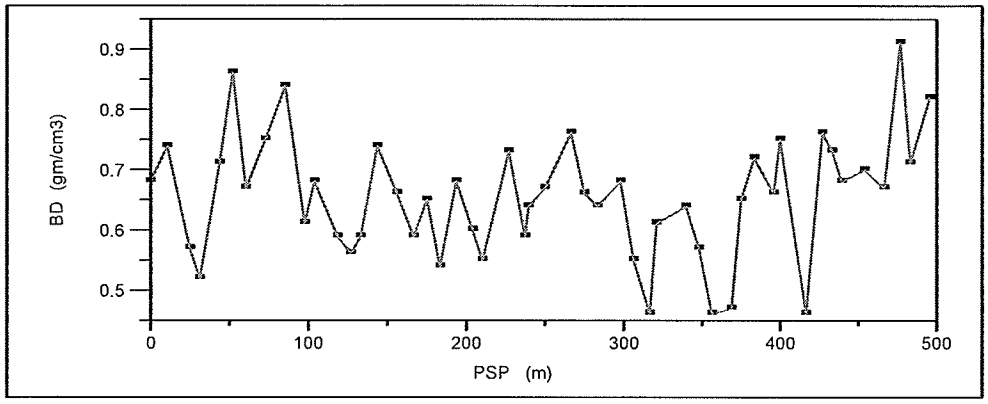
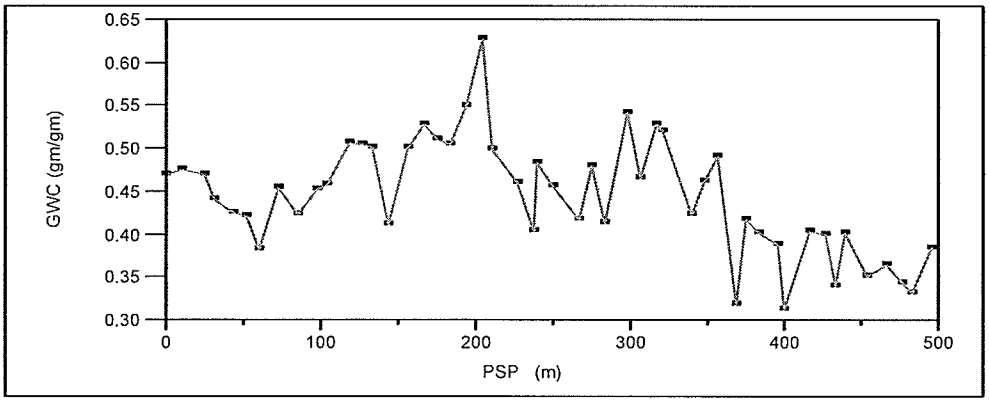


Fig. 2.4 Cont'd
g) Bulk Density



h) Gravimetric Water Content



i) Organic Matter

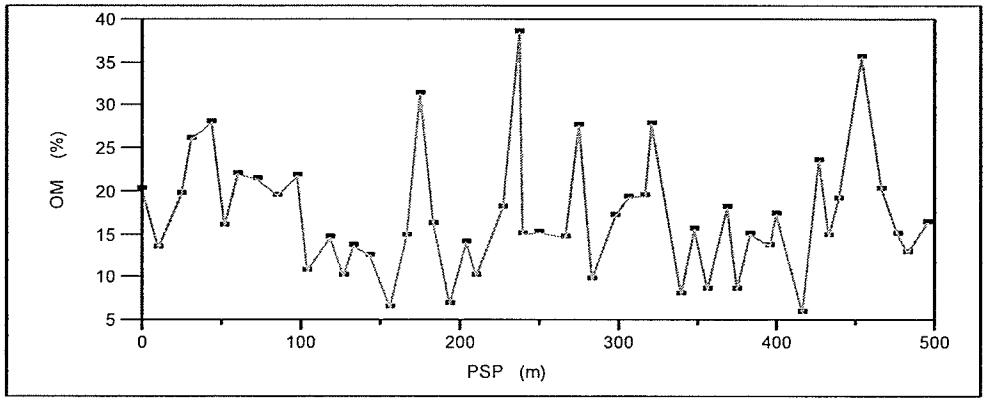
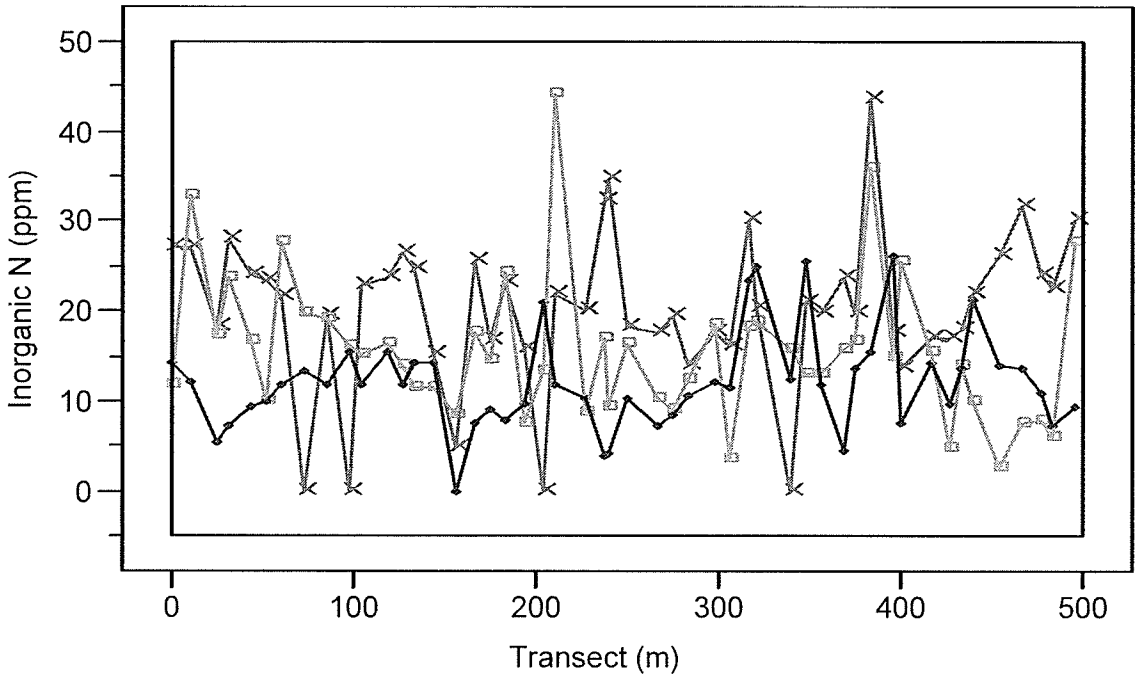


Fig. 2.4 Nine soil variables measured along the OHM transect (July 2006): a) microelevation (July 2007), all plots had the lowest value measured added to begin scale at 0), b) pH c) inorganic N, d) inorganic P, e) N mineralization rate, f) P mineralization rate, g) bulk density, h) gravimetric water content, and i) organic matter.

Table 2.3 Soil summary including mean, standard error (SE), and coefficient of variance (CV) for nine properties measured along the OHM transect in July 2006, with the exception of microelevation measured in July 2007.

Soil Parameter	Mean ± S.E.	C.V.
pH	7.91 ± 0.02	0.30
Bulk Density ($gm \cdot ml^{-1}$)	0.66 ± 0.014	1.56
Gravimetric Water Content ($gm \cdot gm^{-1}$)	0.44 ± 0.009	0.99
Organic Matter (%)	17.13 ± 1.00	294.57
Inorganic N (ppm)	15.65 ± 1.14	417.36
Inorganic P (ppm)	0.82 ± 0.077	26.60
Inorganic N Mineralization ($ppm \cdot d^{-1}$)	0.30 ± 0.08	100.89
Inorganic P Mineralization ($ppm \cdot d^{-1}$)	0.036 ± 0.003	1.65
Microelevation (m)	0.25 ± 0.024	11.58

a) Inorganic N



b) Inorganic P

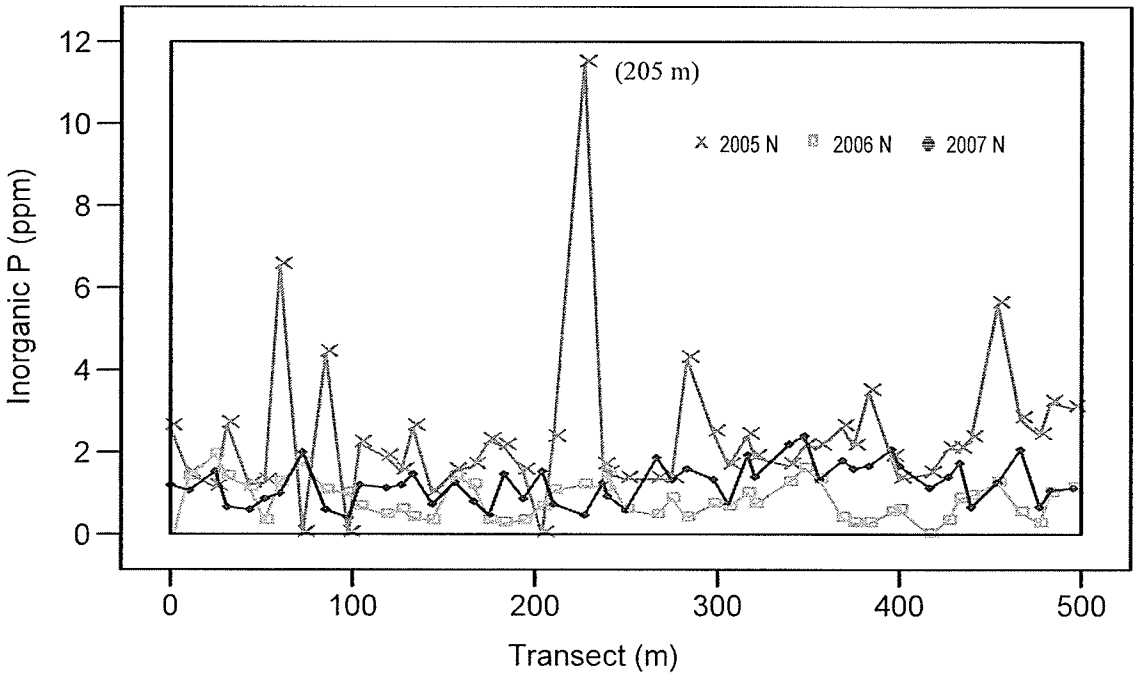


Fig. 2.5 Nutrient levels measured for: a) inorganic N and b) inorganic P along the OHM transect for 2005 (×), 2006 (□), and 2007 (♦). Sample 205 m was checked twice due to high value.

Table 2.4a ANOVA (time series) for comparisons of mean inorganic N levels between survey years and plots along the OHM transect. Degrees of freedom (df), F-statistic (F), Sum of Squares (SS), and p values are reported with significant values presented in bold ($p < 0.05$).

Source of Variance	SS	df	F	p
Year	1733.9	2	16.5	**<0.001**
Plot	3221.3	49	1.25	ns
Error	5143.9	98		
Total	10099.1	149		

ns = not significant ($p > 0.05$)

Table 2.4b ANOVA (times series) for comparisons of mean inorganic P levels between survey years and plots along the OHM transect. Degrees of freedom (df), F-statistic (F), Sum of Squares (SS), and p values are reported with significant values presented in bold ($p < 0.05$).

Source of Variance	SS	df	F	p
Year	58.8	2	23.8	**<0.001**
Plot	56.4	49	0.93	ns
Error	121.1	98		
Total	236.2	149		

ns = not significant ($p > 0.05$)

Table 2.5a Relationship between the individual plot N levels for three survey years along the OHM transect. r^2 values are presented.

	2005 N	2006 N
2006 N	0.041 ns	----
2007 N	0.003 ns	0.002 ns

ns = not significant ($p > 0.05$)

Table 2.5b Relationship between the individual plot P levels for three survey years along the OHM transect. r^2 values are presented.

	2005 P	2006 P
2006 P	0.006 ns	----
2007 P	0.031 ns	0.0009 ns

ns = not significant ($p > 0.05$)

Analysis of the paired soil variables revealed 6 significant correlations (Table 2.6): microelevation was negatively correlated with bulk density ($r^2 = 0.146$) (Fig. 2.6a) and pH ($r^2 = 0.103$) (Fig. 2.6b); gravimetric water content was negatively correlated with bulk density ($r^2 = 0.142$) (Fig. 2.6c) and P mineralization ($r^2 = 0.079$) (Fig. 2.6d); inorganic N was negatively correlated with N mineralization ($r^2 = 0.256$) (Fig. 2.6e); and inorganic P was negatively correlated with P mineralization ($r^2 = 0.420$) (Fig. 2.6f). The correlation of gravimetric water content and microelevation with more than one variable suggests the importance of those two variables in determining the abiotic conditions of the OHM plant community. Meanwhile, the negative correlation of nutrients with mineralization may indicate the mechanism of nutrient uptake in this community. A negative correlation between the rate of mineralization and the standing level of nutrients could indicate that when mineralization is high plant uptake is also high, leading to low standing levels of nutrients. Conversely, when mineralization is low plant uptake is also low, results in high standing levels of nutrients.

Table 2.6 Relationship between nine environmental variables measured along the OHM transect. r^2 values are reported with significant values in bold ($p < 0.05$). All significant relationships are negative. Codes for variables are defined below table.

	ME	OM	GWC	BD	pH	N	P	N Min.	P Min.
ME	---	0.032	0.0007	**0.146**	**0.103**	0.011	0.012	0.016	0.065
OM	---	---	0.018	0.0056	0.027	0.0054	0.041	0.027	0.0042
GWC	---	---	---	**0.142**	0.012	0.0048	0.011	0.0082	**0.079**
BD	---	---	---	---	0.033	0.0091	0.0081	$1.15e^{-5}$	0.0089
pH	---	---	---	---	---	0.010	0.012	0.019	0.038
N	---	---	---	---	---	---	0.022	**0.256***	0.0041
P	---	---	---	---	---	---	---	0.0005	**0.420***
N Min.	---	---	---	---	---	---	---	---	0.032
P Min.	---	---	---	---	---	---	---	---	---

* $p = 0.0001$

Key:
ME – Microelevation
OM – Organic matter
GWC – Gravimetric water content
BD – Bulk density
pH – pH
N – Inorganic nitrogen
P – Inorganic phosphorus
N Min. – N mineralization rate
P Min. – P mineralization rate

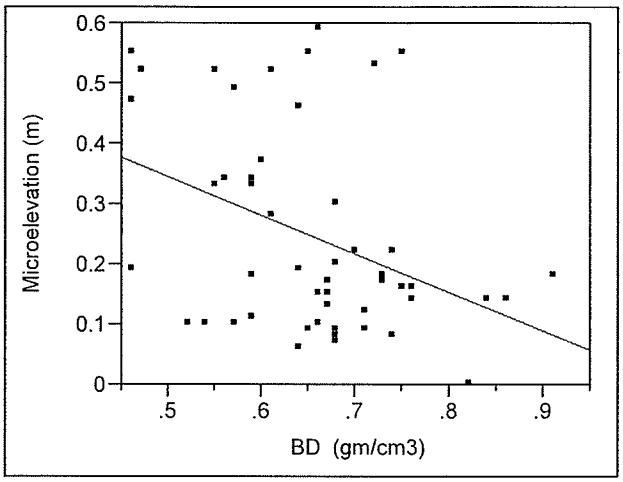


Fig. 2.6a Microelevation fit by bulk density measured along the OHM transect ($p = 0.006$; $r^2 = 0.146$; $y = -0.639x + 0.665$).

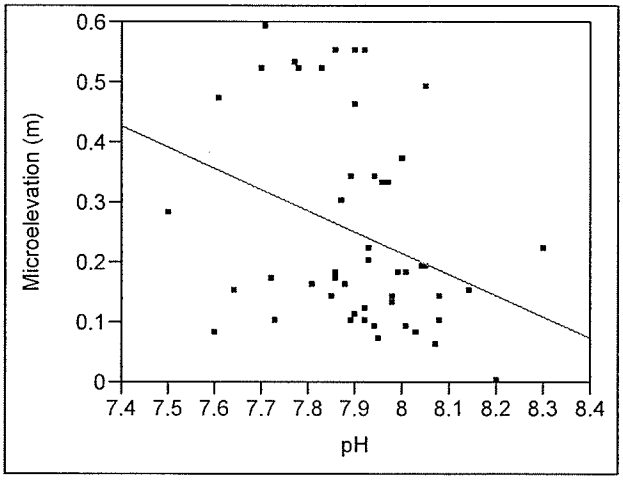


Fig. 2.6b Microelevation fit by pH measured along the OHM transect ($p = 0.02$; $r^2 = 0.103$; $y = -0.353x + 3.04$).

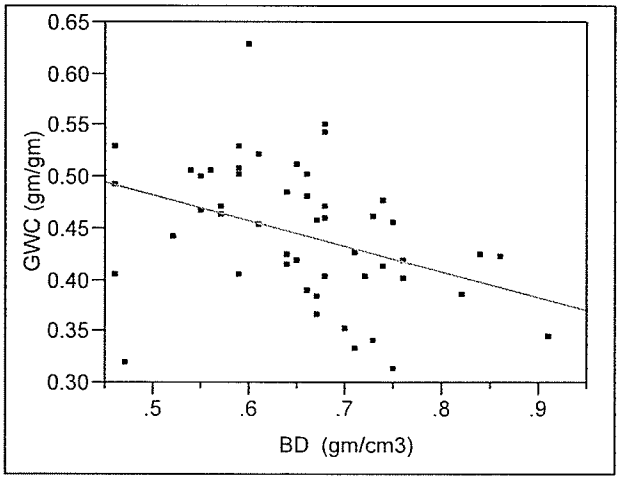


Fig. 2.6c Gravimetric water content fit by bulk density. measured along the OHM transect ($p = 0.007$; $r^2 = 0.142$; $y = -0.247x + 3.04$).

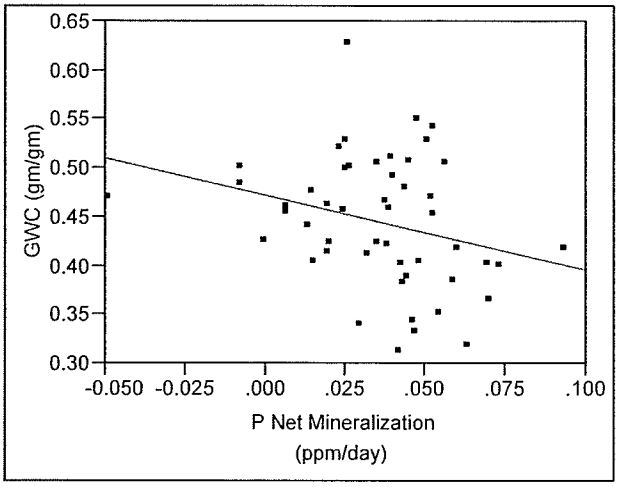


Fig. 2.6d Gravimetric water content fit by phosphorus mineralization measured along the OHM transect ($p = 0.05$; $r^2 = 0.079$; $y = -0.769x + 0.471$).

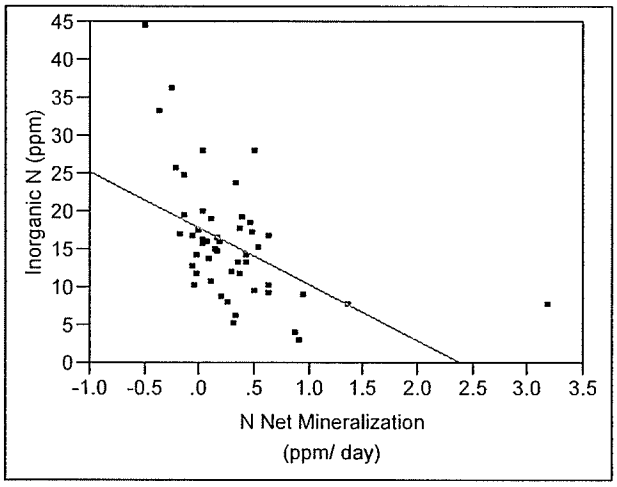


Fig. 2.6e Inorganic N fit by N mineralization measured along the OHM transect ($p = 0.0002$; $r^2 = 0.256$; $y = -7.49x + 17.9$).

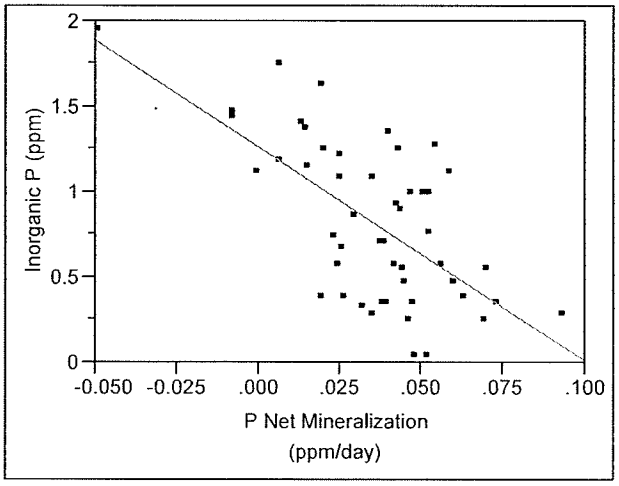
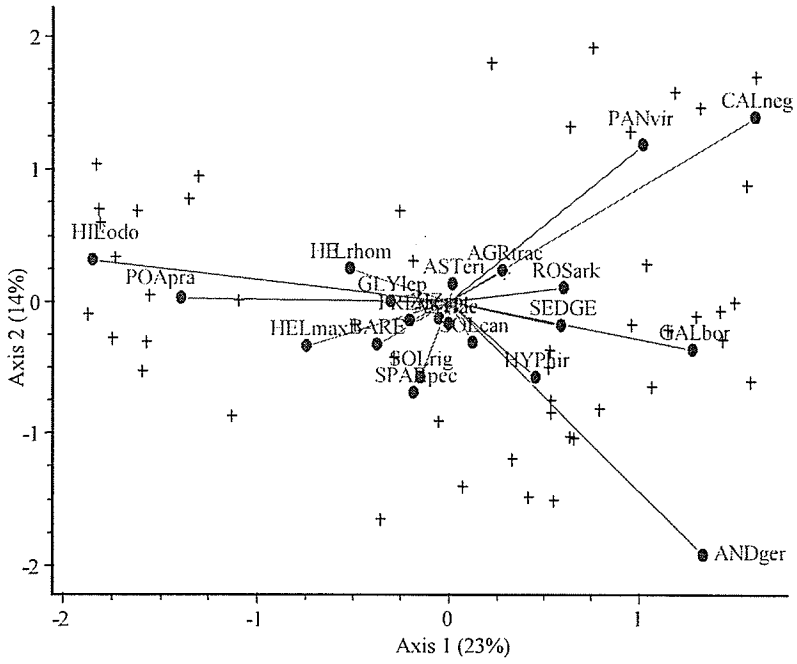


Fig. 2.6f Inorganic P fit by P mineralization measured along the OHM transect ($p < 0.0001$; $r^2 = 0.420$; $y = -12.5x + 1.26$).

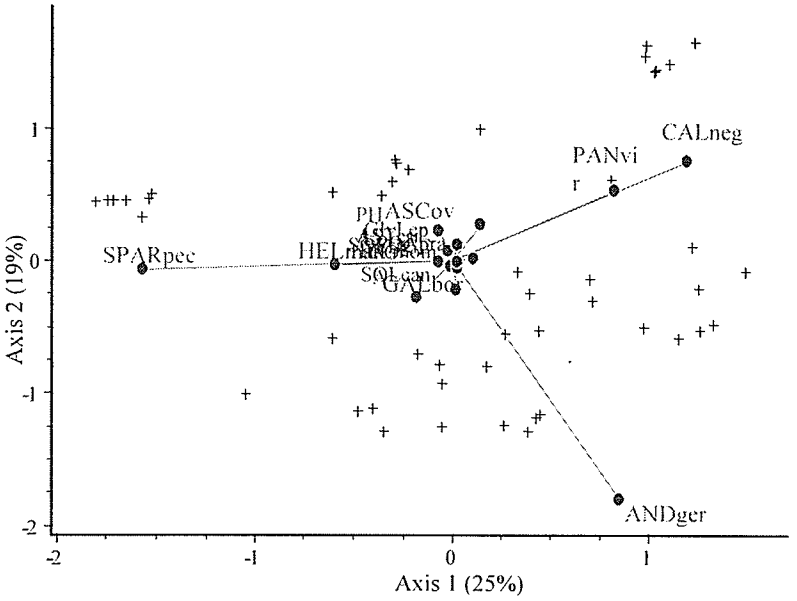
Multivariate Analyses

The results of the 2002 species PCA indicated that 6 species accounted for most of the variance on ordination axis 1 and 2: *Hierochloe odorata*, *Poa pratensis*, C*alamagrostis negelecta*, *Panicum virgatum*, *Galium boreale* and *Andropogon gerardii*, with both axes representing 37% of the original variation in the data (Fig. 2.7; Appendix 1). *Hierchloe odorata* and *P. pratensis* were negatively associated with axis 1- along with *C. negelecta*, *P. virgatum*, *G. boreale* and *A. gerardii,* while *A. gerardii* was also negatively associated with axis 2- and *C. negelecta* and *P. virgatum* positively associated (Fig. 2.7). Four species explained most of the variance in the 2007 species PCA: *Spartina pectinata*, *C. negelecta*, *P. virgatum*, and *A. gerardii*, with 44% of the original variation represented (Fig. 2.8; Appendix 1). *Spartina. pectinata* was negatively associated with axis 1- and *C. negelecta*, *A. gerardii* and *P. virgatum* positively associated, while *A. gerardii* was also negatively associated with axis 2 (Fig. 2.8). In general, plots dominated by the important species variables were found in discrete, marginal groups on the graphs (Fig. 2.7 and 2.8). A notable difference between the species PCA was that *H. odorata* was the most important variable on axis 1 determining plot grouping in 2002, but not in 2007 where *S. pectinata* was most important (Fig. 2.7 and 2.8). The environment PCA indicated that bulk density (BD), microelevation (ME), gravimetric water (GWC), pH, and organic matter (OM) were the most important variables, with 45% of the original variation represented (Fig. 2.9; Appendix 1). BD (positively associated) and ME (negatively associated) were the important variables on axis 1, while GWC and pH (positively associated) and OM (negatively associated) were important on axis 2 (Fig. 2.9).



Key:
AGRtra – *Agropyron trachycaulum*
ANDger – *Andropogon gerardii*
ASTeri – *Aster ericoides*
ASTlae – *Aster laevis*
BARE – Bare ground
CALneg – *Calamagrostis neglecta*
GALbor – *Galium boreale*
GLYlep - *Glycyrrhiza epidota*
HELmax – *Helianthus maximiliani*
HELrho - *Helianthus rhomboids*
HIEodo – *Hierchloe odorata*
HYPhir- *Hypoxis hirsuta*
PANvir – *Panicum virgatum*
POApra – *Poa pratensis*
PRErac – *Preanthus recemosa*
ROSark – *Rosa arkansana*
SEDGE – Sedge sp.
SOLcan – *Solidago canadensis*
SOLrig - *Solidago rigida*
SPARpec – *Spartina pectinata*
ZIZapt – *Zizia aptera*

Fig. 2.7 Principal component analysis showing the relationship between the 2002 species variables (see key on right) and permanent sample plots (+) (n = 50) measured along the OHM transect.



Key:
ANDger – *Andropogon gerardii*
ASCova - *Asclepias ovalifolia*
ASTeri – *Aster ericoides*
CALneg – *Calamagrostis neglecta*
GALbor – *Galium boreale*
GLYlep - *Glycyrrhiza epidota*
HELmax – *Helianthus maximiliani*
HELrho - *Helianthus rhomboids*
PANvir – *Panicum virgatum*
POApra – *Poa pratensis*
PHA – *Phalaris* sp.
SEDGE – Sedge sp.
SOLcan – *Solidago canadensis*
SOLrig - *Solidago rigida*
SPARpec – *Spartina pectinata*

Fig. 2.8 Principal component analysis showing the relationship between the 2007 species variables (see key on right) and permanent sample plots (+) (n = 50) measured along the OHM transect.

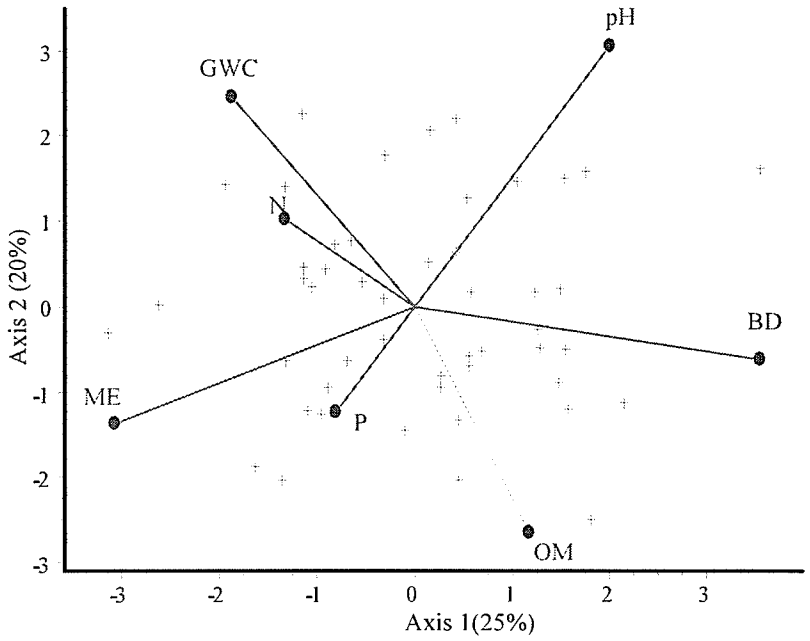
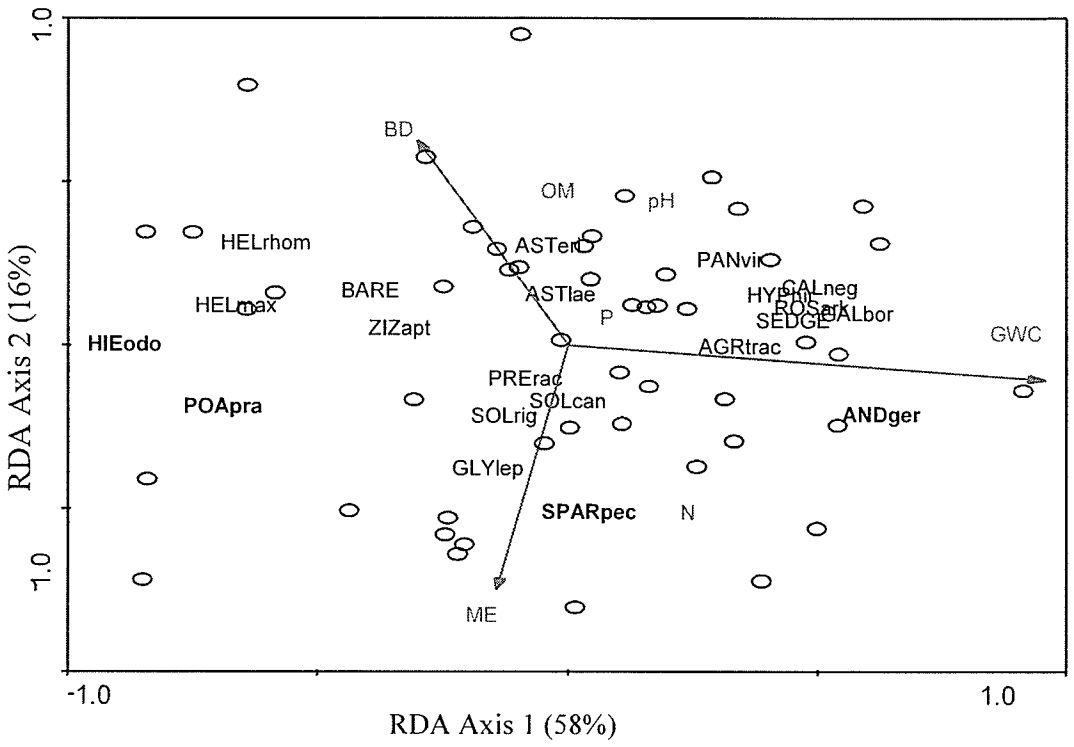


Fig. 2.9 Principal component analysis showing the relationship between the 2006 (ME: 2007) environmental variables (see key on right) and permanent sample plots (+) (n = 50) measured along the OHM transect.

The RDA for the 2002 species with the environment data indicated a strong correlation between the predictor (environment) and response (species) variables (r^2 = 83%; redundancy = 27%) (Appendix 2). Eigenvalues reported for RDA axis 1 and 2 were 0.154 and 0.042 respectively, with a Monte Carlo permutation test indicating axis 1 was significant and a second test of all canonical axes was significant (p = 0.002 for both tests) (Appendix 2). The amount of constrained variance accounted for by axis 1 was 58%, and 16% for axis 2 (Fig. 2.10). GWC, BD and ME were important predictor variables, with GWC positively correlated with axis 1 and negatively correlated with axis 2, BD negatively and positively correlated with axis 1 and 2 respectively, and ME negatively correlated with both axes (Fig. 2.10). *Hierchloe odorata*, *P. pratensis*, *S. pectinata*, and *A. gerardii* were important response variables, with *P. pratensis* negatively correlated with axis 1 and axis 2, *H. odorata* negatively and positively correlated with axis 1 and 2 respectively, and *A. gerardii* and *S. pectinata* positively correlated with axis 1 and negatively correlated with axis 2. In general, RDA axis 1 alone was highly significant (p = 0.002) (Appendix 2), and plots characterized by *H. odorata* and *P. pratensis* in association with low GWC were increasing toward the left on the triplot (i.e., species, environment, and plots) graph, while plots characterized by *A. gerardii* in association with high GWC were increasing toward the right (Fig. 2.10). Additionally, plots with high ME, low BD and characterized by *S. pectinata* were increasing toward the bottom of the graph and plots with high BD, low ME, and low-to-zero abundance of *S. pectinata* were increasing toward the top. Scatterplots of % cover *H. odorata* and *A. gerardii* (Y-axis) with % GWC (X-axis) demonstrated the main trends of RDA axis 1 (Fig. 2.11a; 2.11b).



Key: AGRtra – *Agropyron trachycaulum;* ANDger – *Andropogon gerardii;* ASTeri – *Aster ericoides* ASTlae – *Aster laevis;* BARE – Bare ground; CALneg – *Calamagrostis neglecta;* GALbor – *Galium boreale;* GLYlep - *Glycyrrhiza lepidota;* HELmax – *Helianthus maximilianii;* HELrho - *Helianthus rhomboides;* HIEodo – *Hierchloe odorata;* HYPhir- *Hypoxis hirsute;* PANvir – *Panicum virgatum;* POApra – *Poa pratensis;* PRErac – *Preanthus recemosa;* ROSark – *Rosa arkansana;* SEDGE – Sedge sp.; SOLcan – *Solidago canadensis;* SOLrig - *Solidago rigida;* SPARpec – *Spartina pectinata;* ZIZapt – *Zizia aptera*

Fig. 2.10 Redundancy analysis triplot showing the 2002 species (p = 21) constrained by the environmental variables (p = 7) for the OHM tallgrass community. The relationship between the 4 species 'predictor' variables (bold text), 3 environment 'response' variables (vectors), and permanent sample plots (open ovals) (n = 50) is represented. Codes for variables are defined in the key above.

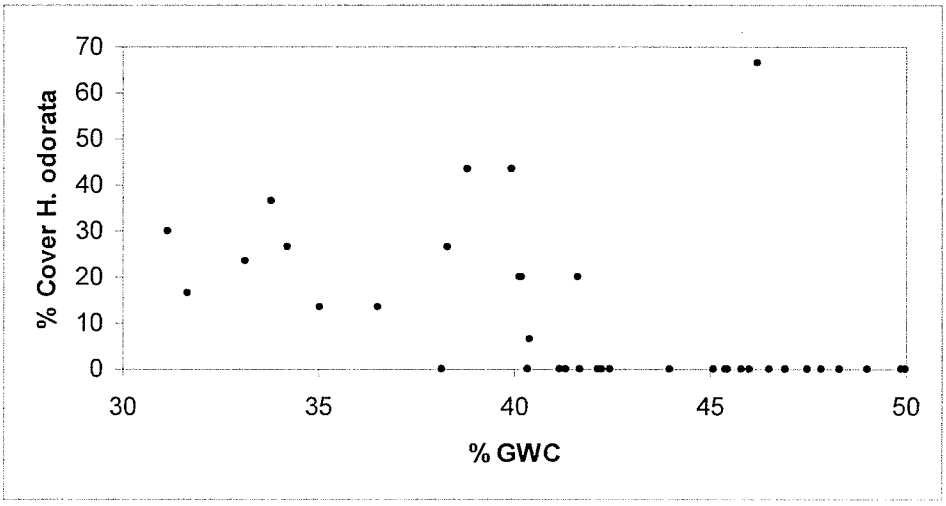


Fig. 2.11a Scatterplot graph showing the main 2002 species-environment trend of decreasing % cover *H. odorata* with increasing % gravimetric water content for the OHM tallgrass community ($r^2 = 0.27$).

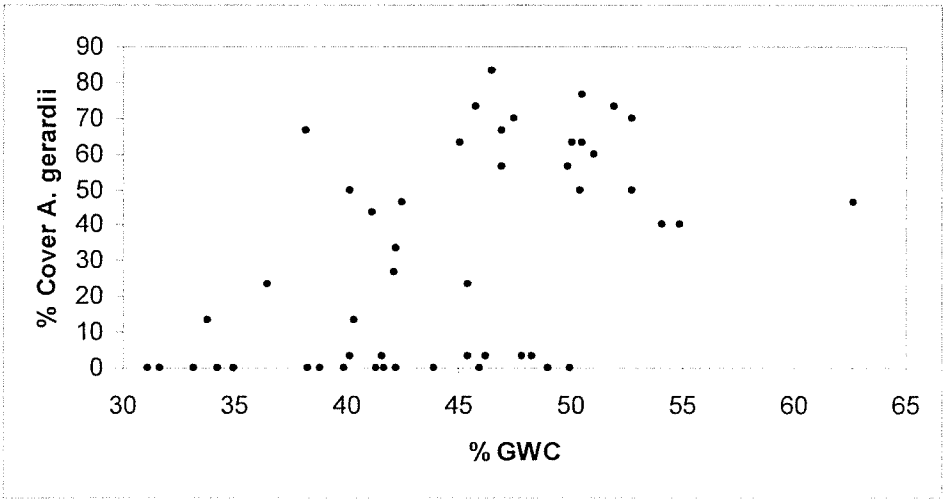
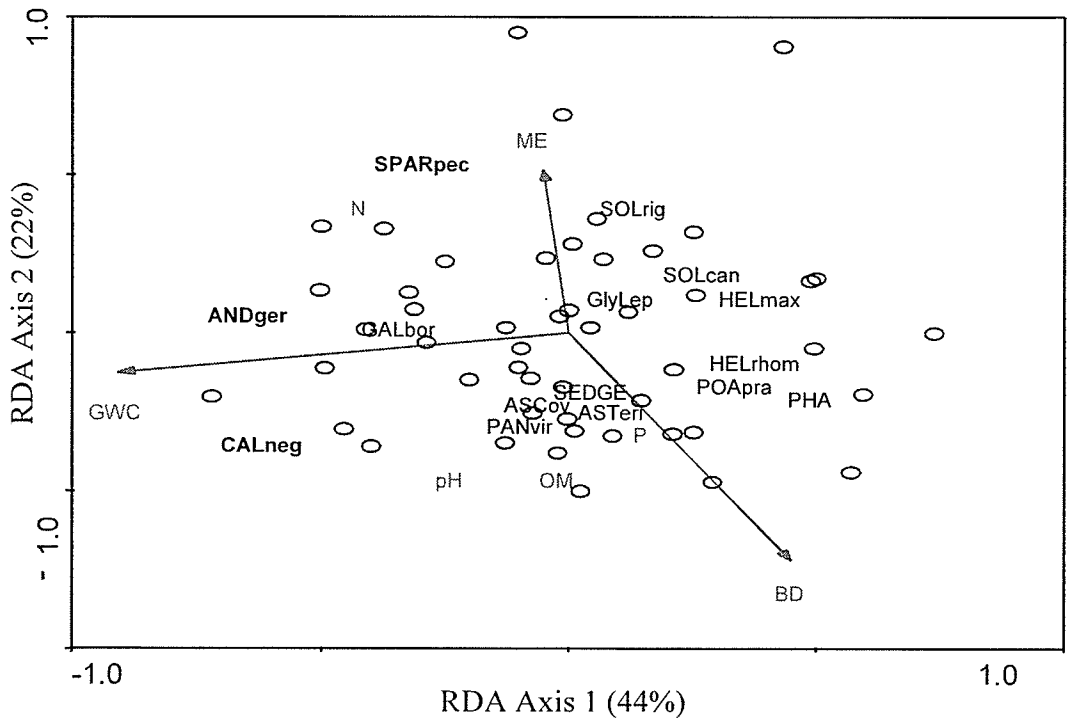


Fig. 2.11b Scatterplot graph showing the main 2002 species-environment trend of increasing % cover *A. gerardii* with increasing % gravimetric water content for the OHM tallgrass community ($r^2 = 0.26$).

There was a strong correlation between the predictor and response variables for the RDA of the 2007 species with the environment data, but not as high as for the 2002 species (r^2 = 77; redundancy = 22%) (Appendix 2). Eigenvalues reported for canonical axis 1 and 2 were 0.095 and 0.055, respectively (Appendix 2). A Monte Carlo Permutation test indicated axis 1 was significant, and a second test of all canonical axes was also significant (p = 0.026 and 0.008, respectively) (Appendix 2). The amount of constrained variance accounted for by axis 1 was 44% and 26% for axis 2 (Fig. 2.12). GWC, BD and ME were important predictor variables, with GWC negatively correlated with axis 1 and 2, BD was positively and negatively correlated with axis 1 and 2 respectively, and ME was negatively correlated with axis 1 and positively correlated with axis 2 (Fig. 2.12). *Spartina pectinata*, *A. gerardii*, and *C. neglecta* were significant response variables, with *A. gerardii* negatively correlated with axis 1 and positively correlated with axis 2, *C. neglecta* was negatively correlated with both axes, and *S. pectinata* was negatively and positively correlated, respectively, with axis 1 and 2. In general, RDA axis 1 was significant (p = 0.026) (Appendix 2), and plots characterized by *A. gerardii* and *C. neglecta* in association with high GWC were increasing toward the left periphery of the tri-plot graph, while plots with low-to-zero abundance of those 2 species and associated with low GWC were increasing toward the right (Fig. 2.12). Additionally, plots with high ME, low BD and characterized by *S. pectinata* were increasing toward the top edge of the graph and plots with high BD, low ME, and low-to-zero abundance of *S. pectinata* were increasing toward the bottom edge. A scatterplot of % cover *A. gerardii* (Y-axis) with % GWC (X-axis) demonstrated the main trend of RDA axis 1 (Fig. 2.13).



Key: ANDger – *Andropogon gerardii;* ASCova - *Asclepias ovalifolia;* ASTeri – *Aster ericoides;* CALneg – *Calamagrostis neglecta;* GALbor – *Galium boreale;* GLYlep – *Glycyrrhiza lepidota;* HELmax – *Helianthus maximilianii;* HELrho - *Helianthus rhomboides;* PANvir – *Panicum virgatum;* PHA – *Phalaris* sp.; POApra – *Poa pratensis;* SEDGE – Sedge sp.; SOLcan – *Solidago canadensis;* SOLrig - *Solidago rigida;* SPARpec – *Spartina pectinata;* ME – Microelevation; OM – Organic matter; GWC – Gravimetric water content; BD – Bulk density; pH – pH; N – Inorganic nitrogen; P – Inorganic phosphorus

Fig. 2.12 Redundancy analysis triplot showing the 2007 species (p = 15) constrained by the environmental variables (p = 7) for the OHM tallgrass community. The relationship between the 3 species 'predictor' variables (bold text), 3 environment 'response' variables (vectors), and permanent sample plots (open ovals) (n = 50) is represented. Codes for variables are defined in the key above.

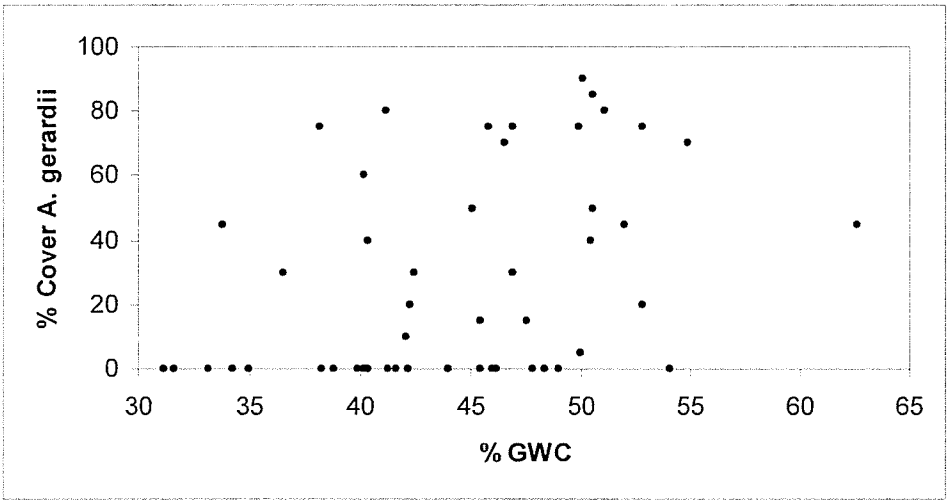


Fig. 2.13 Scatterplot graph showing the main 2007 species-environment trend of increasing % cover *A. gerardii* with increasing % gravimetric water content for the OHM tallgrass community ($r^2 = 0.14$).

According to the results of both RDA triplot graphs (Fig. 2.10 and 2.11), there appears to be a spatial gradient in existence in the OHM prairie, where species that prefer or tolerate wet soil conditions (e.g. *A. gerardii* and *C. neglecta*) are found distinctly separated from those species that prefer drier soil conditions (e.g. *P. pratensis*, *Helianthus maximilianii,* and *Helianthus rhomboides*). In summary, GWC (and BD and ME to a lesser extent) were the principal environmental variables predicting composition of the species in the 2002 and 2007 plots (Fig. 2.10 and 2.11), and there was a strong relationship between the environment and species data (r^2 = 83% and redundancy = 27% for the 2002 species RDA; r^2 = 77% and redundancy = 22% for the 2007 species RDA) (Appendix 2). Overall, a significantly high proportion of the total variance could be explained through constraining the species variables by the environment variables (RDA axis 1 = 58%, p = 0.002 for the 2002 species RDA; RDA axis 1 = 44%, p = 0.026 for the 2007 species RDA) (Fig. 2.10 and 2.11; Appendix 2).

Discussion

This study is unique because it characterizes the spatial and temporal dynamics of the vegetation structure and soil abiotic conditions within a tallgrass prairie community across a very small elevation change (± 30 cm) and at a local scale (116 ha prairie). Furthermore, to my knowledge no descriptive studies of spatial and temporal changes in local community structure (i.e., Manitoba) have been published in the literature, as this research was completed at the northern extent of the tallgrass region. The closest comparison in Manitoba is a tallgrass study characterizing the structure and composition of the plant community associated with the endangered western prairie fringed orchid (*Platanthera praeclara*) and the pollinator community of this orchid species (Friesen 2007). On the contrary, the spatial and temporal dynamics of particular southern tallgrass communities has been examined extensively, but much of the research has focused on variation at a greater regional- and topographic scale (Gibson and Hulbert 1987; Collins and Glenn 1991; Briggs and Knapp 1995; Collins et al. 1998; Collins 2000). For example, studies in the Konza Prairie Research Natural Area of northeast Kansas- an area of southern research concentration, occurred within a 3500 ha tallgrass prairie region, which fluctuated over 100 m in elevation and contained multiple watershed communities (100 m^2 sample blocks) with low numbers of permanent plots (max. 20) and large individual plot sizes (10 m^2) (Gibson and Hulbert 1987; Collins and Glenn 1991; Briggs and Knapp 1995; Collins et al. 1998; Collins 2000). Therefore, it can be generally stated that the OHM study is important in contributing to the understanding of spatial and temporal dynamics in communities within the northern extent (i.e., the northern gradient) of the tallgrass range.

Vegetation Patterns

Vegetation sampling within the OHM tallgrass community revealed local distribution of plants, which were spatially structured in patches of a few dominant species intermixed with a greater number of rare species. This appears to be the inherent structure of many North American grassland communities- a hierarchical system of persistent vegetation organized into lower level patch units (Collins 1987). Furthermore, grasslands like the OHM community are characteristically dominated by perennial plants which spread slowly via clonal roots (in contrast to the greater- and more rapid- seed dispersal mechanisms present in annual systems). According to Collins and Glenn (1990), patterns of species distribution and abundance in grassland communities are solely a function of small spatial-scale interactions, such as competition and dispersal, as a result of diminished frequency of natural disturbances at the regional scale. The diversity of species present within the OHM prairie is attributed to the large number of uncommon species, many of which were forbs, interspersed within a small number of dominant grasses including *Andropogon gerardii*, *Spartina pectinata* and *Panicum virgatum*. The most abundant grass species was *A. gerardii*, which is a typical and widespread (i.e., broad-niche) dominant species of the greater tallgrass region (Collins 1987; Collins and Glenn 1990; Hamilton 2005).

Percent cover estimates recorded at the start and end of a 5-year period (2002 to 2007) suggest that the OHM prairie is generally temporally-stable. That is, with the exception of minor fluctuation in location of vegetation patches, the overall community structure does not vary from year to year. It is suggested that fire is a critical component of the natural disturbance regime in tallgrass prairie (Bragg 1995) and that grasslands

exhibit increasing stability in structure of vegetation with increasing fire frequency (Collins 2000). The optimal fire frequency interval (based on historical data) is estimated to be once every 3-5 yr (Collins 2000). Given that the OHM prairie is managed within a 4-year fire frequency (with the exception of 2006, when the schedule was delayed following the 2002 burn due to drier-than-average conditions in the area), it is believed that fire plays a role in increasing patch stability within this plant community. There was some evidence from this tallgrass study that the two species of *Solidago*- which had disappeared from many of the plots by 2007, but re-appeared in plots in the last 100 m- may be spreading clonally. However, further research would be required to explain reasons for this movement of selected species patches (e.g. through competitive interactions between dominants), while vegetation sampling following the latest burn would be essential to fully assess the effects of fire on local temporal stability.

It should also be emphasized that because there were two different sampling methods used between 2002 and 2007 (i.e., pin-frame and percent cover sampling, respectively), abundance values were expected to differ. At the same time, total species (i.e., common and rare species) identified using pin-frame were also expected to be detected with percent cover sampling due to the increased amount of area sampled within each plot in 2007. An increase in rare species would imply that species present in low abundance along the transect were overlooked by pin-frame sampling and later detected by percent cover because of the increase in sample area. This was not the case, however, as rare species were found to decrease in abundance or disappear overall between 2002 and 2007, which suggested that rare species became more rare or were simply difficult to locate due to the thickness of thatch present in the plots following the lack of fire.

Soil Patterns

The small amount of variation in the 9 abiotic soil properties measured in the PSP in the single survey year (2006 or 2007) was not unexpected, and likely a result of the small spatial scale (i.e., 500 m transect), but it should be noted that high variation (i.e., a large CV) did occur in organic matter, inorganic N, and N mineralization. Although average nutrient levels differed significantly between the 3 survey years (2005, 2006, and 2007) and showed overall decrease with time, the lack of temporal correlation in PSP nutrient levels between years indicates that at the plot level, past nutrient levels do not predict future levels. It should additionally be pointed out that the measurement of soil nutrients was only initiated in 2005, which was 3 years beyond the previous OHM burn. Thus, larger changes in nutrient levels may have already occurred and would not be accounted for in this study.

The spatial and temporal variability in soil nutrients measured within the OHM community is a general characteristic of terrestrial plant communities (Barbour et al. 1999), including prairie grasslands (Collins 2000). The change in inorganic nitrogen (or soil N) levels – the nutrient often regarded to be most limiting to productivity- is particularly noteworthy. Soil N levels were at their highest in 2005 and lowest in 2007 suggesting that it was being depleted from the community faster than it could be replenished through decomposition. One possible explanation for this is that the spatial and temporal dynamics of nitrogen availability were historically driven by periodic disturbance events such as prairie wildfires (Collins et al. 1998; Knapp and Seastedt 1998). Frequent burning cycles (approx. once every 3-5 years) in grasslands volatilize organic N- previously tied up in the aboveground biomass, into nitrate- most of which is

lost to the atmosphere, and also increase community productivity within the initial few years (Gibson and Hulbert 1997). However, it is possible that fire may also result in the production of some inorganic N in the soil increasing soil fertility. A delayed burning cycle in excess of 5 years in the OHM prairie, due to drier than average conditions during the study, was possibly the cause of the 2005-2007 soil N declines.

Inorganic phosphorus (or soil P) levels- often the second-most limiting nutrient in plant communities- were somewhat random, peaking in 2005, dropping off in 2006, and climbing again in 2007 (but cresting below 2005 levels). As fire frequency is also found to increase soil P through breakdown of aboveground biomass and production of ash (Eisele et al. 1989), levels were expected to follow the same patterns as nitrogen and decrease as a result of the delayed burn cycle. No significant relationship, however, was found between the two nutrient variables. This could possibly be explained by a previous study (2002) which found that average soil P levels along the OHM transect were much higher (Markham, pers. comm.) than the values I found from 2005-2007. As mentioned earlier, larger changes in soil P may have occurred following the 2002 burn, which were not accounted for by my study, but may indicate that levels are generally decreasing with time similar to the patterns of soil N. Additionally, it should be noted that the extraction procedure for analyzing soil P in the laboratory is recognized to be problematic (Kalra and Maynard 1991) and does not always determine the total amount of phosphorus available to plants, which may be higher in the soil than calculated, or present in other mineral forms (Binkley and Vitousek 1989).

The negative correlations of particular soil variables with microlevation (e.g. ME with bulk density, ME with pH) and gravimetric water content (e.g. GWC with BD,

GWC with P mineralization) in the OHM community was not unexpected, as topographic position and soil moisture are suggested to be important environmental factors influencing primary productivity and community structure in tallgrass prairie in studies by Gibson and Hulbert (1987) and Briggs and Knapp (1995). However, the results of such studies are based on vegetation structure over a regional scale (e.g. multiple watershed communities) and may be impossible to extrapolate to a much finer, spatial scale (e.g. a local community) such as the OHM prairie. The lack of correlation between elevation and water content along the OHM transect was surprising, as upland areas typically have significantly lower soil moisture levels than lowland areas (Knapp et al. 1993; Turner et al. 1997). Additionally, the lack of any variable correlations with organic matter (OM) was surprising, particularly GWC and pH, which have been found associated with OM in other grassland systems (Markham et al. 2008). It should be noted that the significant inverse relationship ($p = 0.0001$) established between nutrient level (inorganic N or P) and associated mineralization rate (N Min. or P Min.) may reflect the mechanism of nutrient uptake across the plant community, such that the occurrence of high nutrient levels with low mineralization rate would suggest that there is a lack of uptake, which results in high standing levels, perhaps because the nutrient is in excess supply. Low nutrient levels found in association with high mineralization rate would suggest that there is high uptake, typically because plants have more active uptake in areas of high mineralization which results in low standing levels, possibly because the nutrient is in short supply.

Vegetation and Soil Relationships

The multivariate analyses suggests that a strong relationship exists between the soil and species variables within the OHM tallgrass community, which was expected given that community structure is recognized to be a product of interactions among individual plant species with the local environment (Collins and Glenn 1990). Gravimetric water content was the principal soil variable predicting species composition and abundance within the OHM tallgrass community, while bulk density and microelevation also played a role, which is particularly interesting because these three variables did not vary as much as the soil nutrient variables across the site. Nevertheless, these three variables are clearly correlated with the vegetation. Furthermore, although N and P are the most limiting nutrients in this community, it is likely that they change too rapidly in space and time for the species to respond. Local climatic variables (Vinton and Hartnett 1992; Briggs and Knapp 1995) and topographic position (Vinton and Hartnett 1992) particularly have been found to influence vegetation structure in other tallgrass communities in the southern range, but the environmental gradient within these studies occurs on a regional scale (e.g. multiple watersheds), with large changes in elevation (e.g. +100 m). Thus, the findings of the OHM study may be distinct in that they occur on a much finer scale. In general, I found that a spatial gradient exists within the OHM tallgrass community where patches of species that prefer or tolerate wet habitats such as *Andropogon gerardii* and *Calamagrostis neglecta* occur in areas with high soil moisture, while patches of species that prefer or tolerate dry locales including *Poa pratensis* and *Helianthus* spp. are found in areas with low soil moisture.

It should be noted that the multivariate analyses also produced results that were not anticipated. For one, microelevation was uncorrelated with gravimetric water content (see *Soil Patterns* above), indicating that a topographic gradient in opposition to the moisture gradient was not evident. Typically, high soil moisture levels occur in lowland areas of a plant community, while low moisture levels occur in uplands (Knapp et al. 1993; Turner et al. 1997). One explanation for this lack of correlation could be the presence of an artesian well in the vicinity of the OHM community (Suggett, pers. comm.), which had been previously drilled out, but might still be causing atypical flow of water beneath the prairie and upwelling into the upland areas through underground springs. Additionally, the alteration of drainage patterns in the wetlands encompassing the tallgrass area by historical settlers (Suggett, pers comm.) may be generating irregular soil moisture-topographic patterns within the prairie today. These irregular patterns could potentially explain the uncharacteristic distribution of certain species within the OHM prairie found in the redundancy analyses. For example, the triplot graphs for the 2002 and 2007 species showed positive association of *Spartina pectinata* with higher elevation. *S. pectinata* is described in most prairie plant guides as a ditch species, which prefers wet, lowland soil areas (Van Bruggen 1992). Consequently, because topographic position and soil moisture share no relationship, the distribution of habitat-specific species like *S. pectinata* may be random. On the other hand, it is also possible that the lack of correlation between these two variables could be a result of the minimal topographic variation measured in the OHM prairie (± 30 cm), which may simply be insignificant in overall influence on soil moisture.

Conclusion

In general, this is a descriptive study of the local spatial and temporal dynamics operating within a particular Manitoba tallgrass prairie. Soil nutrients (inorganic N and P) exhibited trends of temporal and spatial variation which were important in characterizing the abiotic conditions within the OHM community, but were not important in explaining the plant community structure. It was anticipated that they would play a greater role in characterizing the spatial dynamics of vegetation patches, both because they showed a high degree of variation across the site and are generally considered to limit plant performance. This lack of effect can perhaps be explained by the fact that grasslands are perennial systems with local dispersal patterns, resulting in minimal rates at which the vegetation patches can shift in spatial location over time within a prairie community like OHM. Since nutrient levels fluctuate on a much shorter temporal scale than species dispersal, plant patches are not able to move quickly enough in location to track the yearly change in nutrients. Additionally, although GWC, ME and BD had low degrees of variation across the site, they were still important environmental variable predicting structure of the vegetation. Thus, it can be concluded from this study that spatial variation occurs within the vegetation structure of the OHM community, while temporal fluctuation in soil nutrients (and minimal spatial variation in the important environmental variables) appears to be coupled with temporal stability in vegetation over a 5 year period. An empirical study looking at the effects of fire (i.e., natural disturbance) on the spatial and temporal dynamics of community structure, especially soil nutrients, in the OHM prairie would be a good complement to this study.

Chapter 3: Biotic and abiotic effects of soil patch type on the performance of Andropogon gerardii

Introduction

In terrestrial communities, plants share a close relationship with the soil in which they grow (Perry et al. 1989; Ehrenfeld et al. 2005). Plants can change the properties of the soil (top-down effects) and the soil can directly affect the performance of plants (bottom-up effects). The bottom-up and top-down effects are linked concurrently in a type of local feedback system (Bever et al. 1997; Bever 2003; De Deyn and Van Der Putten 2005; Ehrenfeld et al. 2005; Wardle 2006; Casper and Castelli 2007). Properties of the soil community which are shaped by plants can be abiotic or biotic. Abiotic properties include physical conditions: e.g. aggregate structure and temperature; and resources: e.g. moisture and nutrients (see various chapters in Grace and Tilman 1990; Ehrenfeld et al. 2005; Markham et al. 2008). Biotic properties encompass the microbial community composition (Bever 1994; Bever et al. 1997; Ehrenfeld et al. 2005; Wardle 2006), including microbe-plant mutualisms: e.g. arbuscular mycorrhizae and nitrogen-fixing bacteria in roots (Clay 2001; Bever 2002; Bever 2003; Ehrenfeld et al. 2005); and microbe-plant antagonisms: e.g. fungal pathogens and root-feeding nematodes in the rhizosphere (Mills and Bever 1998; Westover and Bever 2001; De Deyn and Van Der Putten 2005). The soil can in turn influence the performance of the plant community by affecting growth (Bever 1994; Bever et al. 1997), plant-plant competition: i.e., intra- or inter-specific (Bever 2003; Casper and Castelli 2007), and demography and distribution of community members: i.e., species composition, abundance and spatial structure

(Ehrenfeld et al. 2005). The suite of abiotic and biotic properties characterizing the soil community (as shaped by the plant-soil relationship) may be directly beneficial to the performance of a plant species relative to a nearby species, which further reinforces the relationship with the particular soil community and leads to a positive feedback system. Conversely, the soil properties may be disadvantageous to the performance of a plant species relative to another, which further weakens the relationship with the soil community and leads to a negative feedback system (Bever et. al 1997; Bever 2003, Ehrenfeld et al. 2005; see Fig. 1.1).

Positive and negative feedback systems have recently been implicated in the regulation and maintenance of local species diversity. This has been demonstrated through application of conceptual feedback models to characterize vegetation and soil relationships in particular plant communities (Bever 1994; Bever et al. 1997; Bever 2003). Models of positive feedback propose that strengthening the interaction between the plant and soil community can favour the persistence of highly competitive species (e.g. dominant species which out-compete rare species) and subsequent reduction of less-competitive species (e.g. rare species which are out-competed by dominant species), leading to a loss of community diversity (Bever et al. 1997). Models of negative feedback suggest that weakening the interaction between the plant and soil community decreases the competitiveness of particular species, thus allowing persistence of less-competitive species and leading to greater diversity within the community (Bever et al. 1997). In other words, the coexistence of locally competing species (e.g. dominant and rare species) will be lower in systems regulated by positive feedback and the distribution of species randomly-mixed throughout the plant community, while local species

coexistence will be greater in negative feedback-regulated systems and the competing species uniformly-distributed throughout the plant community (Bever et al. 1997; Bever 2003).

In general, the effect of the feedback depends on the degree of dispersal. For example, in grassland communities with local dispersal patterns, models of positive and negative feedback can only be applied to the individual plant community, as opposed to the larger grassland region. Additionally, the coexistence of species is still possible under positive feedback, but on a greater spatial scale, as demonstrated by the diverse array of local communities with single dominant species found across many regions (Bever et al. 1997). Nevertheless, on a small spatial-scale negative feedback systems are believed to be effective in many well-established North American grassland communities due to the coexistence of locally-competing species (Bever 1994; Bever et al. 1997; Casper and Castelli 2007).

Alternatively, positive feedback systems are thought to contribute to dominance by exotics in invasive communities (Callaway et al. 2004; Wolf and Klironomos 2005), where highly-competitive exotic species replace less-competitive native species in composition and abundance, and generate large mono-specific patches with few-to-no native species. The regulation of community productivity and the stability of plant-soil systems is also considered to operate under positive feedback, particularly through the concept of 'bootstrapping' by Perry et al. (1989), where positive feedback between the plant and soil (e.g. a tree species associated with root ectomycorrhizal fungi) creates self-reinforcing links which result in an increase in productivity, health, and resilience of the plant community. If key positive links are severed between the plant and soil community

such as through introduction of foreign disturbance (e.g. loss of soil ectomycorrhizae), a reverse effect resulting in loss of productivity, amplified stress, and rapid degradation of the plant community can arise.

Current research on feedback recognizes that the biotic interactions or biotic changes occurring in the local soil community, in response to a particular aboveground species, can significantly influence plant growth rate and the linkages within local feedback systems, particularly grassland communities (Bever et al. 1997; Bever 2002; Bever 2003, Ehrenfeld et al. 2005, Wolfe and Klironomos 2005, Wardle 2006; Casper and Castelli 2007). Examples from grasslands include plant-soil mutualisms: e.g. arbuscular mycorrhizal root colonization of specific grass hosts (Bever 2002; Casper and Castelli 2007), and plant-soil antagonisms: e.g. soil pathogen accumulation in the rhizosphere of particular grass and forb species (Mills and Bever 1998; Holah and Alexander 1999; Westover and Bever 2001). The complexity of the total interactions (abiotic and biotic) occurring within the soil community, however, necessitates the incorporation of a whole-system approach- as opposed to focusing on specific soil components- to accurately evaluate the effects on the associated plant community (Wolfe and Klironomos 2005). One example of the whole-soil approach is through integration of the soil type differentiated by mono-specific patches of particular grassland species (Markham et al. 2008).

In this study, I examined the biotic and abiotic soil effects of specific plant patch types on the performance of *Andropogon gerardii* from within a local tallgrass prairie community (Oak Hammock Marsh [OHM], Manitoba). The grass species was selected based on dominance in the OHM study area as described in **Chapter 2**. A greenhouse

growth assay was carried out to test the hypotheses that: a) first, there will be a patch effect on the performance of a dominant species caused by biotic and abiotic changes in the soil and b) second, the performance of a dominant species will be greater in a combined abiotic and biotic (inoculated) patch soil than in an abiotic (sterile) patch soil alone, due to an additional influence of biotic interactions.

Materials and Methods

Study Site and Soil Sampling

Soil cores were collected in October 2006 from the patches of three dominant species: *A. gerardii*, *P. virginicum*, and *S. pectinata* within the tallgrass prairie community at Oak Hammock Marsh, Manitoba (see **Chapter 2** for site description). The locations of patches sampled in the OHM community were approximately 50 metres away from- but parallel to- a 500 permanent transect. A commercial bulb planter was used to collect individual cores (6 cm diameter by 12 cm depth). Three individual patches of each patch type were sampled and 10 cores collected per patch (i.e., 30 cores per patch type), for a total of 90 cores collected. Distance between patches was approximately 10-25 metres to ensure that distinct patches of each species were sampled. The bulb planter was cleaned of excess soil during sampling to minimize soil carry-over from one patch to the next. Additionally, each core was placed in a polyethylene bag, the surrounding plastic wrapped tightly, and secured with an elastic band to ensure the soil remained intact and patch-specific. Cores were placed in cold-storage (4˚C) for approximately 4 weeks between collection and sterilization.

Experimental Design

The performance of *A. gerardii* in the soil of three dominant species (i.e., plant patch types) from within a single tallgrass community was assessed through a greenhouse growth assay. Seedlings of *A. gerardii* were planted in sterile soil cores from each of the three patch types: *A. gerardii*, *S. pectinata*, and *P. virgatum*. The abiotic and biotic effects of the soil were separated by growing plants in one of two inoculation treatments: 1. inoculation with a small amount of fresh soil (inoculation treatment), or 2. amendment with a small amount of sterile soil (control treatment). The source of the sterile amendment and inoculum soil used in the two treatments was a small plug of soil collected from each core prior to sterilizing.

The purpose of the sterile soil core in which all seedlings were initially planted was to create a background soil of abiotic properties for each patch type. The inoculation treatment was used to incorporate biotic properties with the background soil to assess the abiotic and biotic effect of each patch type. The control treatment was applied to generate the equivalent level of soil disturbance as the inoculation treatment, but still maintain the background soil properties for each patch type. It was predicted that the difference in performance of seedlings under the inoculation treatment (biotic plus abiotic soil properties) would be significantly greater than the seedlings under the control treatment (abiotic properties). This was expected to be due to the additional affect of soil biota, present in the fresh soil inocula, on plant performance (i.e., the effect of the biotic plus abiotic soil properties would be greater than the abiotic properties alone).

Soil Inoculum

A small plug of fresh soil (1 cm diameter by 3 cm depth) was removed from the centre of each soil core prior to sterilization, for a total of 90 plugs collected. All plugs were wrapped in aluminum foil, and half (45) were autoclaved at 120°C for 1 hr to prepare the control soil treatment, while the remaining half (45) were maintained fresh to prepare the inoculation treatment. All plugs (autoclaved and fresh) were stored at 4°C along with the sterile cores (i.e., background soil) for 1 week prior to planting.

Background Soil

All soil cores were wrapped in aluminum foil during sterilization and autoclaved at 120°C for 1 hr to prepare the background abiotic soil for each patch type. The cores were kept whole, i.e., not broken up and sieved, to preserve the physical soil structure for the growth assay.

Growth Assay

Seeds of *Andropogon gerardii* were collected from Oak Hammock Marsh, MB tallgrass community in fall 2006, air-dried, and stored in a freezer (-20°C). Trays of *A. gerardii* were seeded on Turface® (Profile Products, Buffalo Grove, IL) in a greenhouse, with no fertilizer added, and allowed to establish for 1-2 weeks before planting. Experimental pots (10 cm diameter by 9 cm depth) were set up with a core positioned vertically, or 'top' up, to emulate original orientation in the field. The surrounding space in the pot was packed with perlite to maintain the shape of the core. The upper foil covering each core (from autoclaving earlier) was removed to expose the soil surface, and

holes punctured underneath to allow for drainage. One seedling of *A. gerardii* was transplanted into an existing hole in each core, where a soil plug was removed previously during preparation of the soil inoculum. A plug of sterile soil or soil inoculum, depending on inoculation treatment, was added (corresponding to the core it originated from) to the hole with the seedling and lightly packed down. A total of 15 pots per combination of patch type and inoculation treatment were planted (i.e., 90 pots overall). All pots were arranged randomly by patch type and inoculation treatment on tables in the greenhouse. The seedlings were watered through a drip line system for approximately 45 seconds every other day, under natural light conditions and average growing season temperatures (ca. 24°C daytime, 18°C night-time). No fertilizer treatments were added. The experiment was maintained for 12 weeks, after which time plants were harvested.

Measurement of Growth and Soil Analysis

The roots and shoots of each plant were separated. Total root fresh mass was measured, along with total shoot fresh mass. Dead plants were noted and excluded from measurements because most mortality occurred within the first two weeks of planting. The roots for each plant were further separated into two halves: one half was collected in small plastic vials for mycorrhizal examination and placed in a -80°C freezer, while the root fresh mass of the remaining half was measured, then dried at 65°C and the dry mass measured. The total root dry mass was estimated for each plant by using the ratio of dry mass to fresh mass of the root subsample multiplied by total fresh mass. Total shoot dry mass was measured directly for each plant by drying the shoots at 65°C. Mean biomass was calculated for each treatment by obtaining the average of the root dry mass plus

shoot dry mass, while root to shoot ratio was determined from the average of the root dry mass divided by the shoot dry mass.

All soil cores were broken into small pieces and air-dried at room temperature. Random soil samples for approximately half of the replicates, or 45 samples, were analyzed for inorganic phosphate (P) through Olsen's method (Kalra and Maynard 1991), and inorganic nitrogen (N) using the Microdiffusion method (Mulvaney 1996). The mean values of N and P were calculated for each treatment.

Mycorrhizal Examination

One-third of the 90 root samples collected from the experiment were analyzed for percent arbuscular mycorrhizal (AM) colonization in June 2008. Specifically, the individual samples of one patch of each of three plant patch types were examined, for a total of 20 samples (note: 10 samples were not analyzed from the three particular patches as the plants died during the growth assay and no roots were collected). Root samples were cut into small lengths (~1 cm), cleared with 2.0 M KOH, acidified in 1% HCl, and stained in Trypan Blue in June 2008 (Johnson et al. 1999). Root pieces were examined under a light microscope for percent AM colonization at 40x magnification using the grid-intersect method. The full intention of the root assessment was to examine a minimum of two-thirds of the root samples collected following harvest (i.e., 2 patches of each of the 3 patch types), but a large portion were misplaced from -80˚C storage during transfer to numerous departmental freezers, a result of on-going building construction and multiple laboratory moves during the analysis.

Statistical Analyses

To determine any interaction effect between the inoculation treatments and patch types on plant performance, a two-way ANOVA was performed on inoculation treatment crossed by patch type (i.e., inoculation treatment x patch type) (JMP-SE 6.0.3©). A chi-squared test was completed for mean mortality rate of each patch type and soil treatment to test for potential differences. Nutrient differences (inorganic N and P) between patch types were examined using one-way ANOVA. Individual relationships between plant performance and soil nutrients were explored using linear correlation analysis.

In the root analysis, ANOVA was used to compare mean AM root colonization differences between the inoculation treatments (pooled by patch type because the sample size was too small to compare between patch types). Linear correlation analysis was performed to establish the relationship between plant performance and root colonization for the total samples (i.e., the combined inoculation treatments), and for the individual inoculation treatments (i.e., sterile or inoculated). To determine the relationship between root colonization and soil P, a linear correlation was performed for both the total samples and each individual inoculation treatment.

For all statistical analyses of the growth assay and root colonization, diagnostic plots were used to check for homogeneity of the variance, and log-transformation of the data was performed to normalize the distribution. Results in tables and graphs were presented as untransformed data. Significance level was set at $\alpha = 0.05$ for all tests.

Results

Growth Assay

There was no significant difference in *A. gerardii* performance between the patch types (Fig. 3.1a and 3.1b; Table 3.1), but there was a significant difference between the inoculation treatments (Fig. 3.2a and 3.2b; Table 3.1). When pooled by patch type, inoculated plants were 3 times larger in mean biomass than plants grown in sterile soil ($p = 0.003$), and the root to shoot ratio was 2.5 times lower for inoculated plants versus those grown in sterile soil ($p = 0.02$) (Fig. 3.1a and 3.1b). Mortality of *A. gerardii* was high (approx. 50%) in this assay (Table 3.2), but occurred early in plant growth (i.e., within the first two weeks following planting) and with no obvious cause of death (i.e., water and temperature levels were held constant). A chi-squared test on the mean plant mortality rate for all patch types and inoculation treatments indicated no significant differences (Table 3.2).

Soil nutrient levels did not significantly differ between the patch types or inoculation treatments according to a one-way ANOVA (Table 3.3). Results for total plant performance fit by nutrient levels reported insignificant linear correlation values for all analyses (Fig. 3.3a, 3.3b and 3.4a), except root: shoot ratio and P, which had an inverse relationship ($r^2 = 0.35$; $p = 0.02$) (Fig. 3.4b).

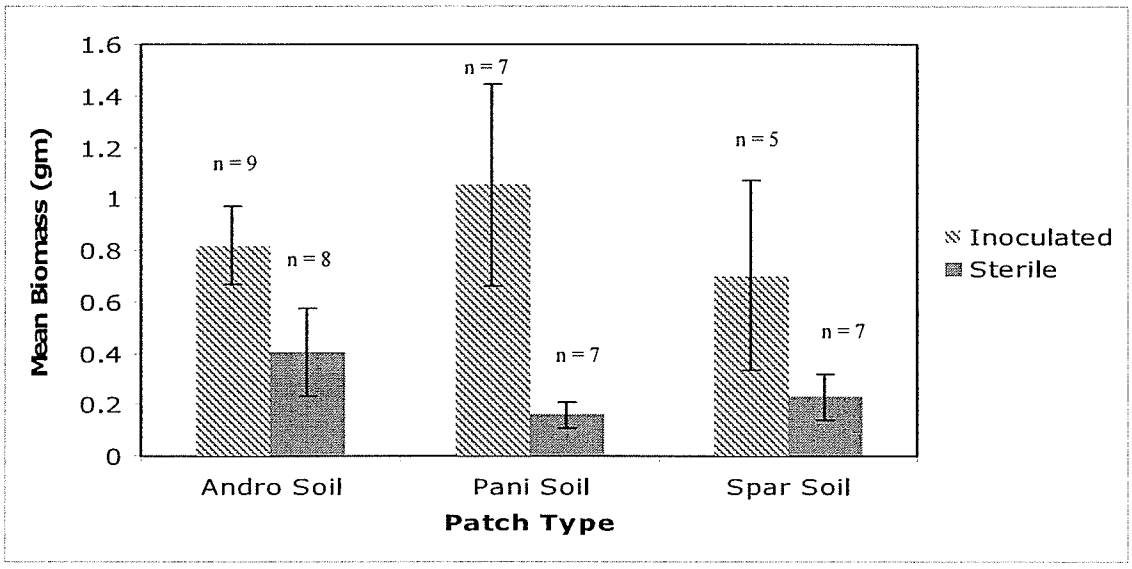


Fig. 3.1a Mean biomass of *A. gerardii* in soil from three species patch types (Andro Soil, Pani Soil, Spar Soil), and under two soil treatments: inoculated and sterile. Standard errors of the means (vertical bars) and sample size are presented for each treatment.

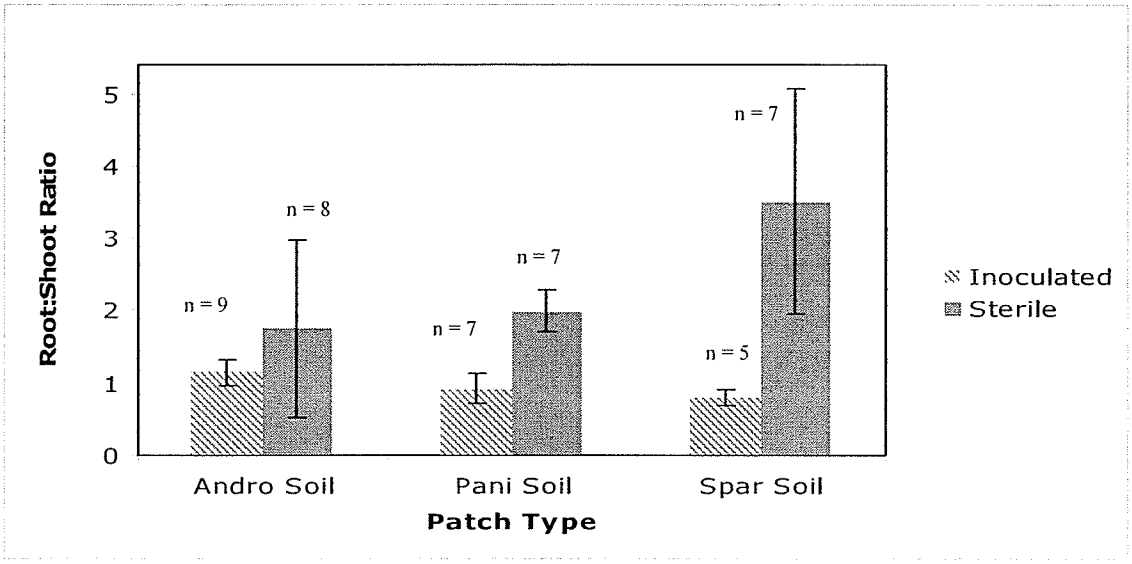


Fig. 3.1b Mean root to shoot ratio of *A. gerardii gerardii* in soil from three species patch types (Andro Soil, Pani Soil, Spar Soil), and under two soil treatments: inoculated and sterile. Standard errors of the means (vertical bars) and sample size are presented for each treatment

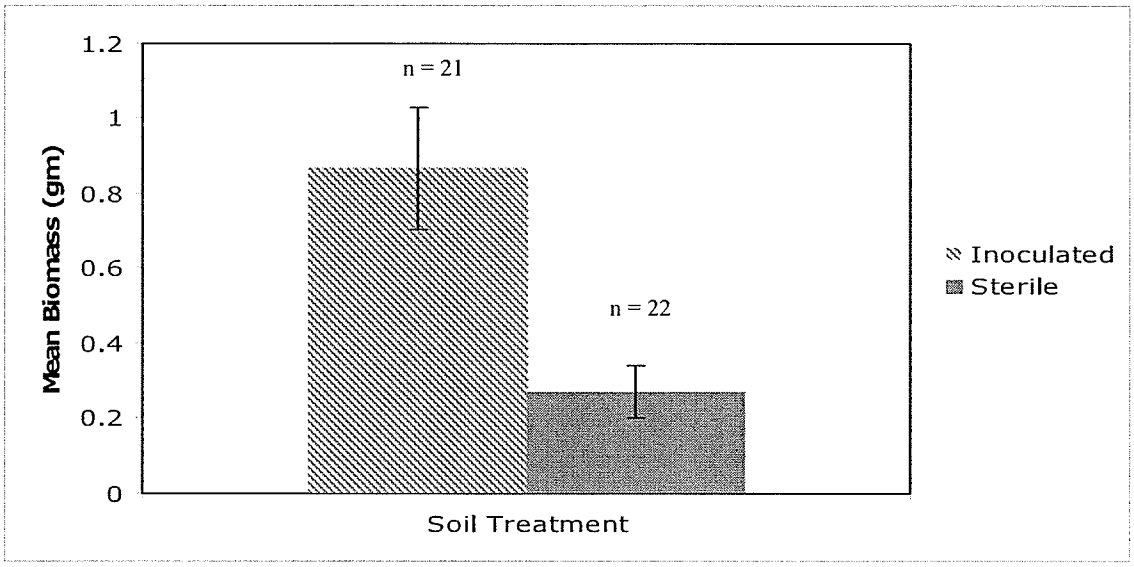


Fig. 3.2a Mean biomass of *A. gerardii* in the inoculated soil treatment compared to the sterile control (pooled by patch type). Standard errors of the means (vertical bars) and sample size are presented for each treatment.

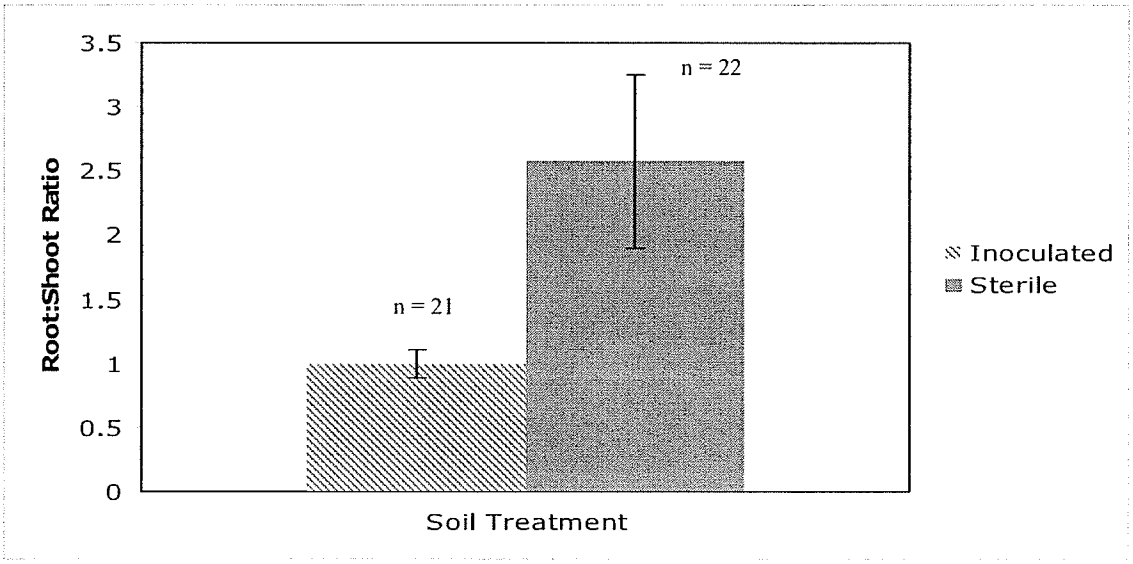


Fig. 3.2b Root to shoot ratio of *A. gerardii* in the inoculated soil treatment compared to the sterile control (pooled by patch type). Standard errors of the means (vertical bars) and sample size are presented for each treatment.

Table 3.1 Two-way ANOVA for the effect of the interaction between inoculation treatment and patch type on performance of *A. gerardii* (mean per plant). Degrees of freedom (df), F-statistic (F), sum of squares (SS), and p values are presented.

	Total Dry Mass				Root to Shoot Ratio			
Source of Variance	SS	df	F	p	SS	df	F	p
Inoculation Treatment	3.65	1	10.5	0.003	13.8	1	6.32	0.02
Patch Type	0.18	2	0.25	ns	3.14	2	0.72	ns
Patch Type x Inoculation Treatment	0.50	2	0.72	ns	5.41	2	1.24	ns
Error	12.8	37			56.8	26		
Total	17.3	42			83.5	31		

ns = not significant ($p > 0.05$)

Table 3.2 Distribution of the plant mortality rate (%) for the inoculation treatments and patch types. A chi-squared test on mean mortality reported no significant differences between inoculation treatments or patch types.

	Andro Patch	Pani Patch	Spar Patch	*Mean Mortality*
Inoculated Treatment	40.0%	53.3%	66.7%	53.3 % ns
Sterile Treatment	46.7%	53.3%	53.3%	51.1% ns
Mean Mortality	43.3% ns	53.3% ns	60% ns	52.2% ns

ns = not significant ($p > 0.05$); Andro Patch = patch type soil of *A. gerardii*; Pani Patch = patch type soil of *P. virgatum*; Spar Patch = patch type soil of *S. pectinata*

Table 3.3 One-way ANOVA for the effect of patch type and inoculation treatment on mean soil nutrient levels (N and P). Degrees of freedom (df), F-statistic (F), sum of squares (SS), and p values are presented.

	Soil N				Soil P			
Source of Variance	SS	df	F	p	SS	df	F	p
Inoculation Treatment	544.3	1	0.40	ns	1.31	1	0.69	ns
Patch Type	6506.1	2	2.40	ns	4.77	2	1.26	ns
Error	23035.8	17			32.3	17		
Total	29904.9	20			38.1	20		

ns = not significant ($p > 0.05$)

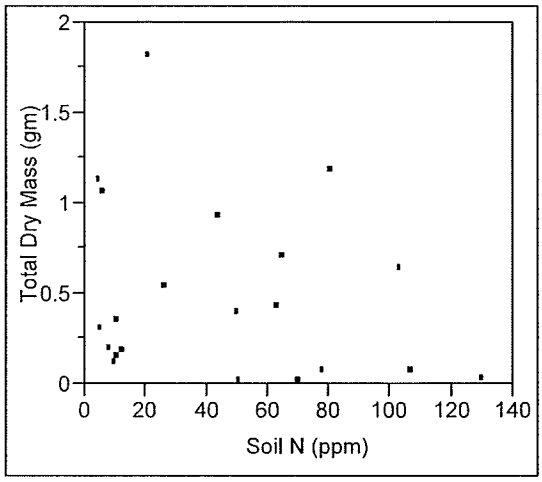


Fig. 3.3a Total dry mass of *A. gerardii* fit by soil N (n = 21) (r^2 = 0.0452; p > 0.05).

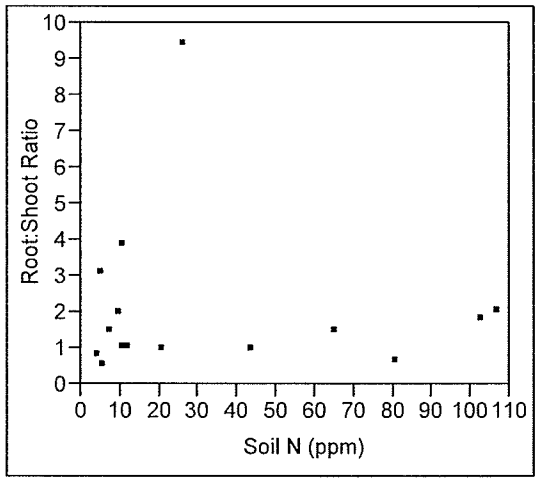


Fig. 3.3b Root to shoot ratio of *A. gerardii* fit by soil N (n = 21) (r^2 = 0.0057; p > 0.05).

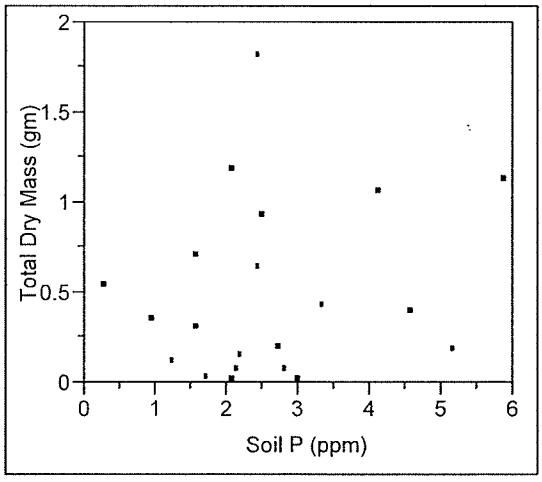


Fig. 3.4a Total dry mass of *A. gerardii* fit by soil P (n = 15) (r^2 = 0.036; p > 0.05).

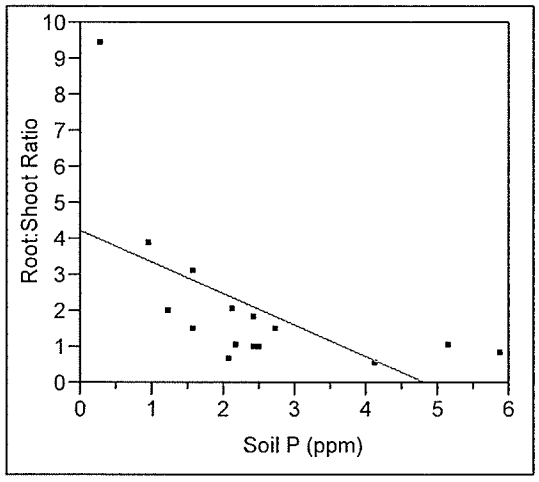


Fig. 3.4b Root to shoot ratio of *A. gerardii* fit by soil P (n = 15) (r^2 = 0.351; p = 0.02; y = - 0.873x + 4.22).

Mycorrhizal Colonization

Mean AM root colonization was significantly higher at 69.5 ± 5.1 % (mean ± S.E.) in the inoculated treatment as compared to 45.0 ± 3.4 % in the control treatment ($p = 0.002$) (Fig. 3.5). The amount of variation within the inoculation treatments was high with individual colonization values between 41-84% for the inoculated treatment roots, and 28%-61% for the sterile treatment roots (data not shown). The relationship between plant performance and root colonization was insignificant for the total samples (Fig. 3.6a and 3.6b), and when separated by each inoculation treatment (data not shown). There was also no relationship between root colonization and soil P for the total samples (Fig. 3.7), and when separated by each inoculation treatment (data not shown).

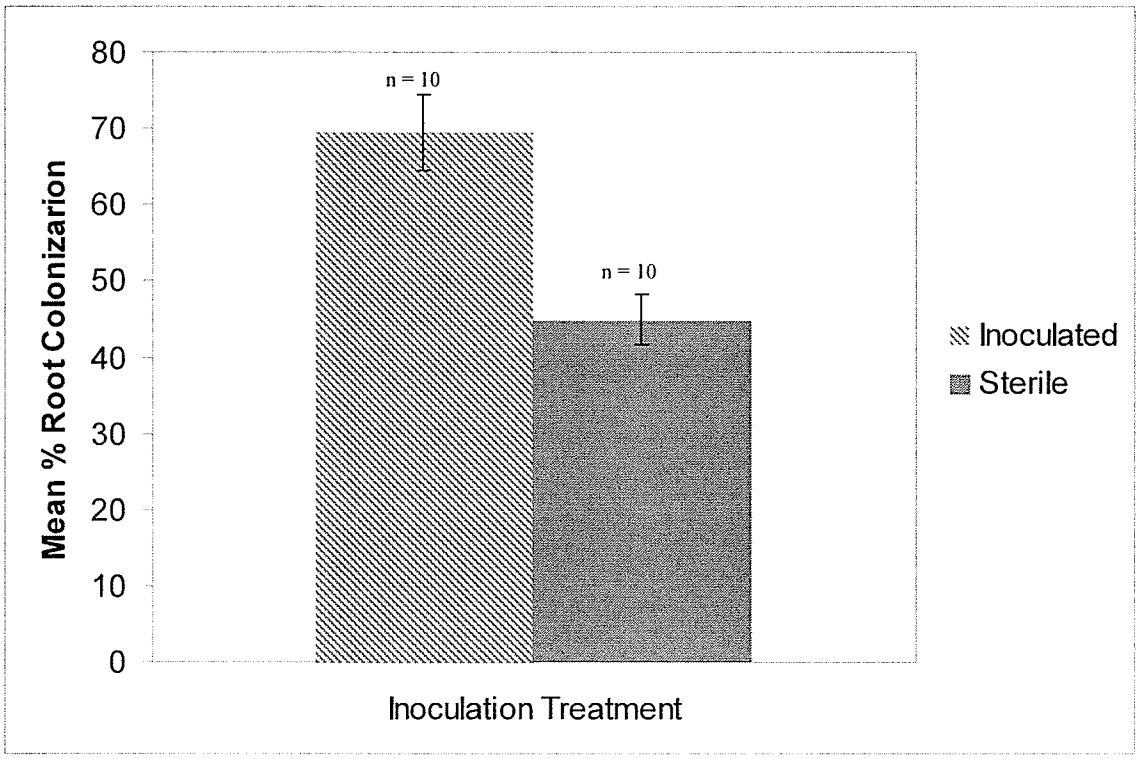


Fig. 3.5 Mean percent AM root colonization for *A. gerardii* in the inoculated treatment compared to the sterile control (pooled by patch type). Standard errors of the means (vertical bars) and sample size are presented for each treatment.

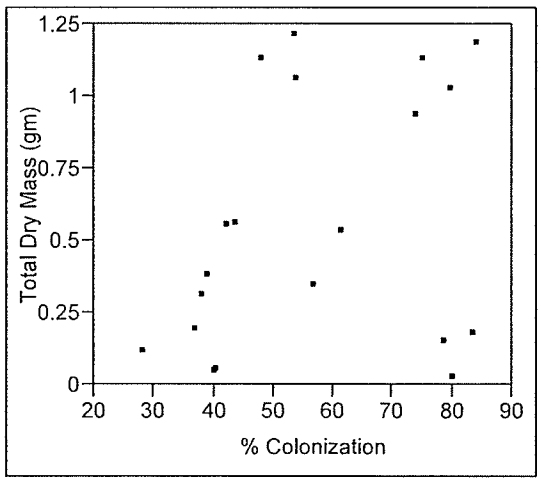


Fig. 3.6a Total dry mass of *A. gerardii* fit by percent AM root colonization (n = 20) ($r^2 = 0.016$; $p > 0.05$).

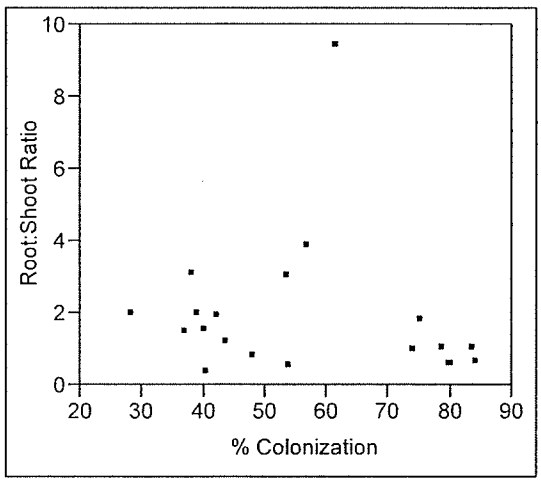


Fig. 3.6b Root to shoot ratio of *A. gerardii* fit by percent AM root colonization (n = 20) ($r^2 = 0.10$; $p > 0.05$).

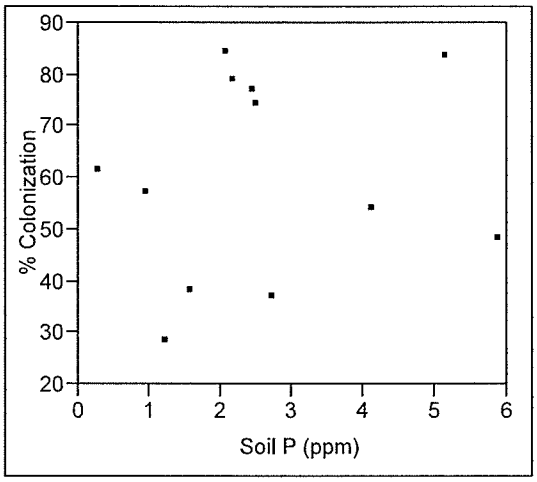


Fig 3.7 AM % root colonization of *A. gerardii* fit by soil P (n = 12) (r^2 = 0.025; $p > 0.05$).

Discussion

Overall, this study demonstrated that there was a strong biotic effect on *A. gerardii*, but there was no patch type effect. The differences in the soil properties (i.e., soil heterogeneity) associated with particular plant patches have been shown to cause variation in the performance and competitive ability of common species within particular grassland communities, but most of this work has focused solely on the abiotic soil effects, while largely ignoring the biotic effects (Fitter 1982; Reynolds et al. 1997). My research is important because it considers the total abiotic and biotic influence of the soil community on growth of a common tallgrass species (*A. gerardii*), which corresponds with recent papers emphasizing that the whole-community interactions within the soil are far too complex to uncouple in assessing effect on the local plant community (Wolfe and Kironomos 2005). This study is also relevant because it focuses on the significance of the biotic soil interactions on plant growth, which has been established in current literature (Chanway et al. 1991; Bever et al. 1997; Bever 2002; Bever 2003, Ehrenfeld et al. 2005, Wolfe and Klironomos 2005, Wardle 2006; Casper and Castelli 2007), while recognizing the importance of arbuscular mycorrhizal fungi (Bever et al. 2001). The role of arbuscular mycorrhizae in individual plant growth and overall community structure has been an increasing area of attention in grassland studies (Wilson and Hartnett 1998; Bever et al. 2001; Wilson et al. 2001; Van der Heijden et al. 2006).

Although high mortality of *A. gerardii* was observed during the assay, death occurred during the initial growth stages and could not be attributed to greenhouse methods such as insufficient water or soil effects such as patch type- or inoculation treatment differences. Thus, all growth results were reported with strong confidence.

Effect of Patch Type on Plants

Soil patch type had no significant effect on the performance of the dominant species (*A. gerardii*). It was expected that there would be variation in the performance of *A. gerardii* due to inherent variation in the abiotic and biotic soil properties of each patch type. The abiotic and biotic variation between patch types is thought to be a result of the changes in the soil community associated with a particular plant species; i.e., due to host-specificity (Ehrenfeld et al. 2005). Nutrients (inorganic N and P), however, did not differ between patch types; a finding confirmed from the permanent plot sampling. Reasons for the lack of patch effect could be related to the distribution of the individual patches sampled in the OHM tallgrass community. It is possible that the patches did not sufficiently vary in spatial location (i.e., only 10-25 m apart along a 500 m transect) or in temporal scale (i.e., collected in a single year) to result in any quantitative effects on plant performance. Grassland communities, including local- temporal and spatial- scale, are considered to be highly variable (Collins 2000). On the other hand, it is also probable that species performance did not differ simply because the 3 study species are similar grasses (i.e., C4 dominants, with widespread distribution across the entire tallgrass region) which may have equivalent soil community effects. In studies where abiotic resources were limiting in the local soil (e.g. high-stress environments), it was concluded that all plants respond in the same basic way; by declining growth rate and acquisition of resource supply (Grime 1977; Chapin 1991). Perhaps a modification of the growth strategy could be inferred in my study, such that when the abiotic resources (i.e., soil N and P) between the 3 species patch types are similar, the plants grow at the same rate. Additionally, although species patch types have been found to alter abiotic soil properties

and affect growth rate in other plant-soil studies (Markham et al. 2008), typically the species are found in dissimilar ecological groups, such as competitive species versus stress-tolerators or grass species versus forb species, rather than from within a single ecological group as in this study (i.e., C4 grass species).

It should also be pointed out that given the vegetation stability patterns determined for the OHM tallgrass community in **Chapter 2,** the lack of patch effect established in this study could not be explained by a short residency time for a species in a particular location. It would be expected based on the vegetation patterns in **Chapter 2** that there would be a patch effect due to the sufficient period of time for abiotic and biotic differences to arise in the soil.

Effect of Inoculation Treatment on Plants

The performance results of the inoculation treatments suggested that there was a strong biotic (plus abiotic) effect of the soil community on plant growth as compared to the abiotic effect alone (i.e., the inoculation treatment had a greater effect on growth than the sterile control treatment), but it did not differ between patch types as predicted by the hypothesis. Overall, plants grown in inoculated soil were 3 times larger in biomass and 2.5 times smaller in root to shoot ratio than plants grown in sterile soil, indicating that inoculated plants put a high effort into increasing shoot growth and total biomass and less effort into producing roots, which was likely as result of advantageous biotic soil components. Equivalent to the patch type results, inorganic N and P did not vary significantly between the inoculation treatments. Given that soil nutrients (i.e., abiotic properties) were comparable, greater plant performance under the inoculation treatment

was attributed to the biotic soil effects. Additionally, because it was also determined that there were no patch type effects on plant performance, a strong effect of soil biota independent of patch type could be concluded.

The biotic soil community, comprised of many diverse microbial species (Bever et al. 1997; Bever 2003), is known to form symbiotic associations with plants in nature that can have direct or indirect effects on growth, ranging from strongly positive (or mutualistic) to strongly negative (or parasitic) (Chanway et al. 1991; Bever 2003). Strong positive effects of the biotic soil community directly on plant performance, as observed in this growth assay, are frequently due to presence of mycorrhizal fungi (Bever 2003) and other free-living bacteria such as rhizobacteria (Chanway et al. 1991). Mycorrhizal fungi, in particular, are known to vastly improve the supply of limiting nutrients (especially available phosphate), to plant roots and subsequently improve plant growth in native prairie soils (Wilson and Hartnett 1998; Wilson et al. 2001; Van der Heijden et al. 2006). However, it should be pointed out that to truly assess whether a positive feedback system between *A. gerardii* and the biotic soil community has occurred in this assay would also require knowing if the plants had a positive effect on the soil microbes.

Effect of Mycorrhizal Colonization on Plants

Many grassland species are obligate mycotrophs, which require association with arbuscular mycorrhizae (AM) to grow to maturity (Wilson and Hartnett 1998; Wilson et al. 2001). In this study, AM colonization of *A. gerardii* was evident through root staining and microscopic observation. It was also found that plants inoculated with the biotic soil

community, which included mycorrhizal fungi, had significantly greater growth than plants grown in sterile soil. Remarkably, however, there was found to be no correlation of mycorrhizal colonization with either plant performance or soil P. This can perhaps be explained by the results of Smith et al. (2004), who concluded that although AM fungi increase plant P supply, there appears to be no relation to plant growth response, P uptake or extent of AM root colonization. Furthermore, the precise mechanism by which plants benefit from mycorrhizal P remains unclear (Smith et al. 2004).

There was a significant difference in mean percent colonization between the inoculation and control soil treatments as expected, with greater colonization in the roots of the inoculated soil due to the incorporation of the biotic soil community containing AM fungi. A study by Smith et al. (1998), found that native species inoculated with AM fungi in a disturbed tallgrass site in Minnesota had a significantly higher percentage of colonized roots as compared to the sterile control. However, the colonization value for the sterile soil treatment in which the biotic component was assumed to be removed (through autoclaving), was relatively high in my study and expected to be negligible. A previous growth assay of *A. gerardii* in a tallgrass soil community from Oak Hammock Marsh (OHM), Manitoba, demonstrated significant root colonization in inoculated soil, but no colonization in sterile soil (Markham, per comm.). On the other hand, the aforementioned study by Smith et al. (1998) found that AM colonization in the selected native species was 44% and 35% in inoculated and sterile soil treatments, respectively, which were values comparable to my study (70% and 45%, respectively). They suggested that high colonization in the sterile control could potentially be attributed to either low levels of mycorrhizae initially present at the site or high influx of mycorrhizal

propagules into the site (Smith et al. 1998). In my study, the growth assay occurred in a greenhouse which contained large vents at both ends and was characterized by cross-flow air movement. Mycorrhizal propagules could have spread from the inoculated to control treatment pots through the action of wind. Additionally, it is possible that mycorrhizae were present in the initial seed stock of *A. gerardii* (collected from the OHM tallgrass community) or in the potting medium (not autoclaved) in which the seedlings were originally germinated, which may have contributed to the higher AM root colonization values in both the sterile and inoculated soil treatments.

Conclusion

No evidence was found in this study to conclude that patch differences in the soil communities of the dominant tallgrass species caused variation in the performance of the dominant species *A. gerardii*. However, it was demonstrated that there was a strong positive effect of the soil biotic components on plant performance. Mycorrhizae present in the soil community were measured in particular (i.e., percent root colonization), and found in high abundance in the inoculated soil (biotic community) as expected, but were surprisingly present in the sterile control soil (abiotic community), where abundance was expected to be nil. In general, it was established that the presence of unidentified microbial species in the biotic soil community of the dominant species patches had an overall positive effect on the dominant species performance. Although only mycorrhizal fungi were examined in this study, it is important to emphasize that the positive effects of the biotic soil are likely due to the diversity of microbial species present in the soil community (Chanway et al. 1991; Bever et al. 1997; Bever 2002; Bever 2003, Ehrenfeld

et al. 2005, Wolfe and Klironomos 2005, Wardle 2006; Casper and Castelli 2007). The soil microbes potentially increased supply of limiting nutrients to the plants, particularly soil P (Smith et al. 2004), but such results were not apparent in this study.

Chapter 4: Biotic and abiotic effects of remnant and restoration soils on the performance of native tallgrass species

Introduction

Plant species can change the abiotic and biotic properties of the soil in terrestrial communities (top-down effects), while the soil can directly affect the performance of plants (bottom-up effects). The bottom-up and top-down effects are linked concurrently in a type of local feedback system (Bever et al. 1997; Bever 2003; De Deyn and Van Der Putten 2005; Ehrenfeld et al. 2005; Wardle 2006; Casper and Castelli 2007). Many of the ecological concepts established on plant and soil relationships in natural plant communities (e.g. mutualisms and nutrient cycling) have been incorporated into the field of restoration ecology (Young et al. 2005). Restoration ecology has rapidly developed to attempt to recover degraded terrestrial communities, which have been previously disturbed through human practices such as farming, industrial mining, and urban development (Bradshaw 1980).

In general, ecologists recognize that the degradation of plant communities alters the abiotic and biotic properties of the soil community and renders an unfertile medium for plant growth, and it is only through restoring the original soil properties that plants will grow successfully (Bradshaw 1980; Perry et al. 1989). Bradshaw (1998) emphasizes that there are three principal issues for consideration: 1) amending the physical habitat (e.g. soil texture, structure, stability and moisture), 2) amending the chemical components, nutrients and toxicity (e.g. macro- and micro-nutrients, pH, heavy metals and salinity), and 3) replacing lost plant species or removing exotics. The importance of

integrating the biotic soil community in plant community re-establishment has also been established, with soil microbes considered to play a central role in overall community health and resilience (Smith et al. 1998; Young et al. 2005). This is illustrated in the restoration of salt marsh ecosystems in the eastern United States, where native grasses have been inoculated with arbuscular mycorrhizal fungi in efforts to increase root colonization, establish species cover, and stabilize salinity levels (McHugh and Dighton 2004). Additionally, restoration of clear-cut forests along the northwestern coast of the United States has been successful through inoculation of conifer seedlings with soil from a local hardwood stand, enhancing mycorrhizal formation in the rhizospheres of the seedling species, and improving seedling survival rates (Perry et al. 1989).

Prairie communities- tallgrass prairie in particular, have also received considerable attention in restoration literature over the past 20 years (Kline and Howell 1987; Smith et al. 1998; Richter and Stutz 2002; Sveinson 2003; Martin et al. 2005; Polley et al. 2005). Interest in the tallgrass region has primarily been generated through the loss of a significant portion (estimated at 99%) of the original North American habitat to agricultural cultivation, and the fact that many remaining fragments of prairie communities (i.e., remnants) are still under impending threat (Samson and Knopf 1994). Furthermore, fear of losses in biological diversity in disturbed prairie areas as native species are being out-competed by many weedy species (Sveinson 2003) has lead to increased research efforts.

Particular prairie restoration studies have found an influence of biotic soil components on establishment of native species. Smith et. al (1998), for example, looked at the inoculation of disturbed sites with arbuscular mycorrhizae (primarily via artificial

potting soil mixed with root fragments, fungal hyphae and spores) during prairie seeding, and presented results showing an increase in mycorrhizal root colonization and percent cover of native grasses, concluding that mycorrhizae play a beneficial role in the restoration of tallgrass communities. However, it appears that few (or possibly no) studies have tested the effects of using fresh soil inoculum (i.e., the abiotic plus biotic components), known as soil transfers in forest-site restorations (Perry et al. 1989), from local tallgrass communities on native species performance. While it is recognized that the biotic changes occurring in the local soil community- in response to a particular aboveground species- can significantly influence plant growth rate (Bever 2002; Bever 2003, Ehrenfeld et al. 2005, Wolfe and Klironomos 2005, Wardle 2006; Casper and Castelli 2007), the complexity of the abiotic and biotic interactions necessitates incorporation of a whole-system approach to accurately evaluate soil effects on the associated plant community (Wolfe and Klironomos 2005).

Andropogon gerardii Vitman (Big bluestem) is a dominant, warm season (C4) grass species widely distributed throughout the northern and southern tallgrass range, including Manitoba tallgrass communities (Van Bruggen 1992; Vance 1999). It commonly reaches 6 feet in height, is red in colour, and is identified by conspicuous "turkey's foot" branching of the seed heads (Van Bruggen 1992). *Veronicastrum virginicu*m (L.) Farw. (Culver's root) is a perennial forb species found in moist habitats within the northern tallgrass range, where it is described as rare in abundance (Van Bruggen 1992). In Manitoba, it is listed as threatened under the Manitoba Endangered Species Act and primarily limited to small tallgrass openings with moist, calcareous sandy-loam soil such as found within the Tall Grass Prairie Preserve at Tolstoi (near the

Manitoba-Minnesota border) (Manitoba Conservation 2001). Stems can reach 6 feet in height, bearing leaves in whorls of 3 to 6, and tubular-shaped flowers are white to pinkish in colour (Van Bruggen 1992).

The goals of this research were to look at the abiotic and biotic effect of soil communities from differing tallgrass sites in Manitoba on the study species *A. gerardii* and *V. virginicum*, and to separate the biotic- from abiotic effects of each soil community on plant performance. I tested the two hypotheses that: a) there will be variation in the performance of *A. gerardii* and *V. virginicum* in soil from different tallgrass restoration and remnant sites, which is expected to be the result of biotic and abiotic differences between the soil communities (Ehrenfeld et al. 2005), and b) the performance of *A. gerardii* will be greater in a abiotic and biotic (inoculated) site soil than in an abiotic (sterile) site soil alone, which is thought to be due to the strong influence of biotic interactions within the soil microbial community (Smith et al. 1998; Young et al. 2005).

Materials and Methods

Study Sites and *Soil Sampling*

Twenty-eight sites in Manitoba were selected for soil core sampling in spring 2006 (Table 4.1; Fig. 4.1). A majority of the sites were chosen in collaboration with the thesis research of P. Mutch, Department of Environment and Geography, University of Manitoba (Mutch 2008). Specifically, his work in urban tallgrass prairies within Winnipeg during this study provided names, locations, and site-history information (e.g. age, area) for 25 study sites (sites 1-25 in Table 4.1; Fig. 4.1). Mutch also provided

Table 4.1 Summary of site number, name, latitude/longitude coordinates, type, age, area, and four soil properties for 28 tallgrass prairie communities within Winnipeg (site 1-25), and near Winnipeg, Manitoba (site 26-28). Under site type, remnant sites refer to largely uncultivated tallgrass areas not previously broken for agriculture (and) or urban development. Restoration sites refer to cultivated tallgrass areas which were broken for agriculture (and) or urban development and planted with native species to re-establish. P = Inorganic phosphate; N = Inorganic nitrogen; N:P = Inorganic nitrogen to phosphate ratio; GWC = gravimetric water content; OM = organic matter.

Site No.	Site Name	Lat/Long coordinates N, W (d:m:s)	Site Type	Age (yrs)	Area (ha)	P (ppm)	N (ppm)	N: P ratio	GWC ($gm \cdot gm^{-1}$)	OM (%)
1*	Big Bluestem Park	49:53:11.934, 97:11:43.950,	Rs	8	0.32	5.88	13.40	2	0.25	14.8
2	Lagimodiere Gaboury Heritage Park	49:53:47.786, 97:06:51.676	Rs	NA	0.25	18.18	20.24	1	0.38	16.6
3*	Plessis Bergen park	49:56:58.044, 97:05:30.297	Rs	NA	1.09	10.39	31.23	3	0.46	14.5
4*	Spence St. park	49:52:53.495, 97:09:16.194	Rs	7	0.07	9.65	17.43	2	0.27	12.2
5	Royalwoods prairie buffer	49:49:32.708, 97:04:24.330	Rs	3	14.6	7.68	13.22	2	0.37	15.5
6*	Harry Collins	49:49:07.682, 97:08:00.605	Rs	10	0.13	15.26	37.08	2	0.42	17.2
7	St Andrews	50:03:38.920, 97:00:10.025	Rs	4	2.70	12.15	26.59	2	0.40	15.0
8	McBeth	49:57:19.845, 97:04:59.093	Rs	10	4.05	6.91	15.81	2	0.22	31.1
9	Living Prairie Museum	49:53:23.963, 97:16:19.801	Rm	NA	13.6	1.19	19.19	16	0.50	18.0
10	Sturgeon Creek	49:52:33.237, 97:16:16.854	Rs	NA	0.27	10.44	27.65	3	0.31	18.1
11	Charleswood Bridge	49:52:15.814, 97:15:51.817	Rs	6	0.17	6.62	29.43	4	0.34	14.8
12	King's Park	49:47:38.372, 97:07:10.164	Rs	11	1.20	10.38	23.02	2	0.34	14.5
13*	University of Manitoba	49:48:24.643, 97:07:59.743	Rs	3	0.09	13.39	14.57	1	0.24	11.4
14	Smith Carter	49:50:01.241, 97:10:37.689	Rs	3	3.93	0.48	9.90	21	0.32	11.7
15	Ferrier prairie	49:57:28.892, 97:07:04.256	Rm	NA	0.32	1.39	21.93	16	0.66	15.5
16	Forks	49:53:11.957, 97:07:46.743	Rs	4	2.21	5.84	24.24	4	0.55	36.5
17	Manitoba Hydro	49:51:16.034, 97:09:35.515	Rs	17	8.09	6.51	17.47	3	0.33	15.6
18	Harbourview	49:56:10.793, 97:01:53.014	Rs	NA	6.96	0.73	17.67	24	0.32	13.3

NA = unknown; * = study sites in assay 2; Rs = Restoration site; Rm = Remnant site

Table 4.1 Cont'd Summary of site number, name, latitude/longitude coordinates, type, age, area, and four soil properties for 28 tallgrass prairie communities within Winnipeg (site 1-25), and near Winnipeg, Manitoba (site 26-28). Under site type, remnant sites refer to largely uncultivated tallgrass areas not previously broken for agriculture (and) or urban development. Restoration sites refer to cultivated tallgrass areas which were broken for agriculture (and) or urban development and planted with native species to re-establish. P = Inorganic phosphate; N = Inorganic nitrogen; N:P = Inorganic nitrogen to phosphate ratio; GWC = gravimetric water content; OM = organic matter.

Site No.	Site Name	Lat/Long coordinates N, W (d:m:s)	Site Type	Age (yrs)	Area (ha)	P (ppm)	N (ppm)	N: P ratio	GWC ($gm \cdot gm^{-1}$)	OM (%)
19	Elmwood High School	49:54:39.211, 97:05:51.814	Rs	15	0.22	6.41	16.98	3	0.29	18.3
20	Warsaw	49:51:44.956, 97:10:20.405	Rs	16	0.13	6.26	24.35	4	0.37	18.9
21	Murray	49:57:20.813, 97:06:10.202	Rs	8	0.05	5.40	21.80	4	0.35	19.1
22	Bay	49:50:55.048, 97:03:54.188	Rs	8	0.16	7.96	21.06	3	0.49	33.3
23*	Wilkes prairie	49:50:32.971, 97:15:01.949	Rm	NA	0.07	1.60	24.93	16	0.28	10.1
24*	Rotary prairie	49:53:51.476, 97:02:02.776	Rm	NA	10.0	2.38	29.17	12	0.45	22.0
25	Plessis prairie	49:52:15.804, 97:01:43.460	Rm	NA	3.66	1.65	15.42	9	0.55	26.7
26*	Oak Hammock Marsh prairie	50:10:40.383, 97:09:36.966	Rm	NA	116	2.85	31.78	11	0.60	24.0
27*	Tall Grass Prairie Preserve (SW & SE 2-1-6E)	49:10:41.614, 96:40:26.244	Rm	NA	3530	1.51	7.61	5	0.37	11.5
28*	St. Charles Rifle Range prairie	49:54:37.208, 97:20:16.804	Rm	NA	85	1.65	38.78	24	0.69	18.5

NA = unknown; * = study sites in assay 2; Rs = Restoration site; Rm = Remnant site

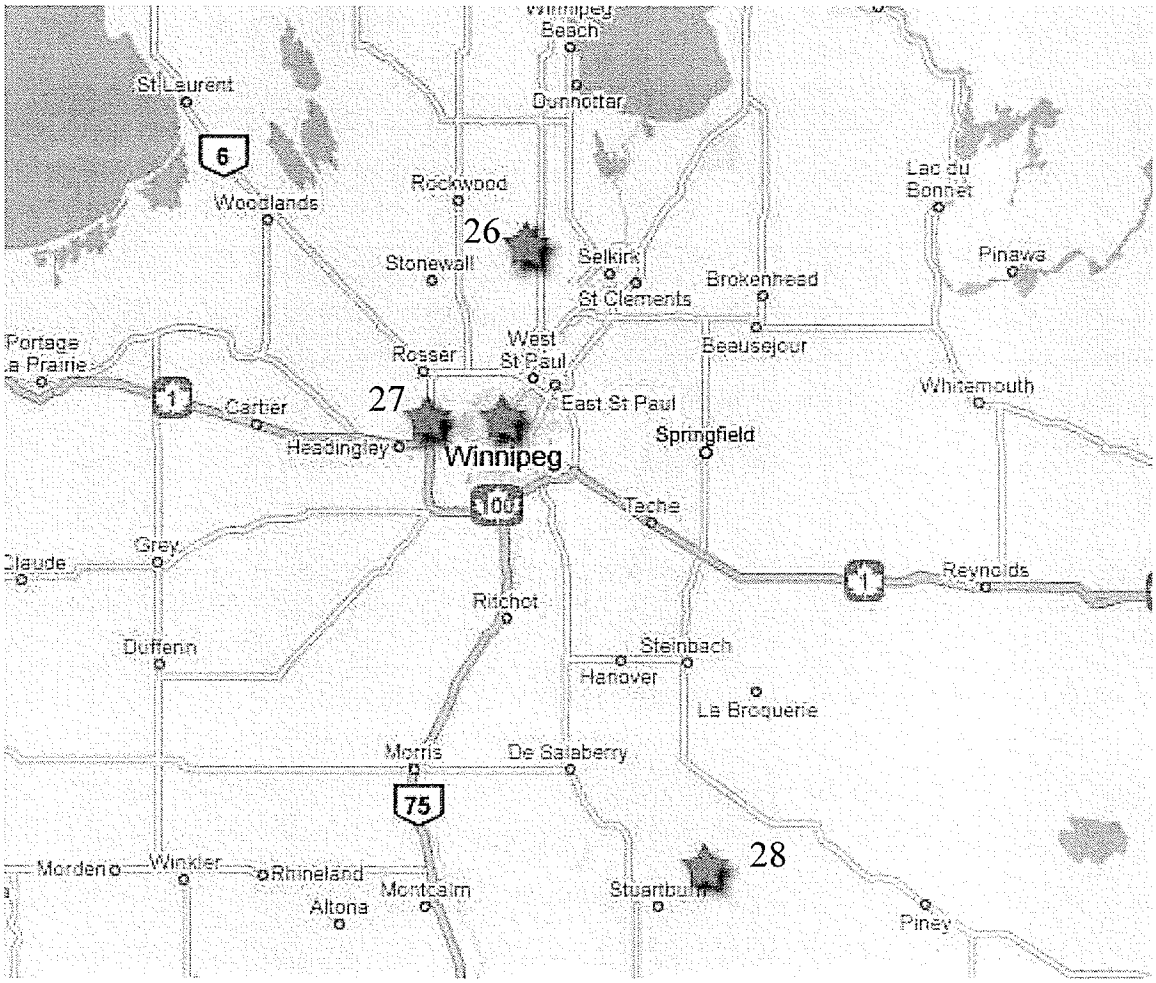


Fig. 4.1 Map of Winnipeg, MB and surrounding areas indicating location of 28 tallgrass sites where soil cores were collected in 2006 (stars). 25 sites were located within the city of Winnipeg (centre star; see Table 4.1), and 3 other sites were located outside the city: Oak Hammock Marsh (26), St. Charles Rifle Range (27), and Tallgrass Prairie Preserve (28) (from Google maps).

information on the past level of site disturbance, with 5 sites identified as (publicly-owned) remnant prairies- i.e., largely uncultivated tallgrass areas, but for which the true classification as original native prairie is unknown. Twenty other sites were described as (private- or publicly-owned) restoration prairies- i.e., cultivated tallgrass areas, which were broken for either agriculture or urban development, and planted with native species (Table 4.1). Three sites, in addition to those of Mutch, were chosen for soil sampling based on classification as the largest and only remnant (i.e., native prairie) areas left in Manitoba outside Winnipeg: 1. St. Charles Rifle Range (SCRR), 2. Oak Hammock Marsh (OHM), and 3. Tallgrass Prairie Preserve (TGPP; SE & SW 2-1-6E) (sites 26-28 in Table 4.1; Fig. 4.1).

A commercial bulb planter was used to collect individual cores (6 cm diameter by 12 cm depth) from the 28 sites, and I was assisted by P. Mutch for a large proportion of the collection. Plots were sampled with the bulb planter using the same methods as in **Chapter 3**, but with 10 plots sampled in each site and 2 cores collected per plot (i.e. 20 cores per site), for a total of 560 soil cores collected.

Experimental Design

Two greenhouse growth assays were independently conducted to test the two hypotheses: assay 1 - compare the performance of *A. gerardii* and *V. virginicum* in soil from 28 local tallgrass remnant and restoration communities (hypothesis a); assay 2 - compare the performance of *A. gerardii* in inoculated and sterile soil from 10 local tallgrass remnant and restoration communities selected from the first assay (hypothesis b). In assay 1, seedlings of *A. gerardii* and *V. virginicum* were planted in fresh soil cores

from 28 sites comprising 8 tallgrass remnants and 20 restorations (Table 4.1). No soil treatments were applied, as the intent of the experiment was to assess the overall effects of the different soil communities on species performance. It was predicted that there would be variation in the performance of the two species across the different soil sites due to differences in the soil communities (i.e., abiotic and biotic conditions) associated with each tallgrass site including size, age, site type (remnant vs. restoration), abiotic properties (nutrients, organic matter, water content), and biotic community composition (mycorrhizal fungi, pathogens, invertebrates, etc., but for which characterization of individual microbial species was not performed in this study).

In assay 2, seedlings of *A. gerardii* were planted in sterile soil from 10 of the 28 sites in assay 1 comprising 5 tallgrass remnants and 5 restorations (Table 4.1). The abiotic and biotic effects of the soil were separated by growing plants in one of two inoculation treatments: 1. inoculation with a small amount of fresh soil (i.e., inoculation treatment), or 2. no soil amendment (i.e., control treatment). The source of the fresh soil used in the inoculation treatment was a small plug of soil collected from each core following harvest of assay 1. The purpose of the sterile soil in which all seedlings were initially planted was to create a background soil of abiotic conditions for each soil site. The inoculation treatment was applied to incorporate biotic conditions with the background soil to assess the abiotic and biotic effects of each soil site. It was predicted that the difference in performance of seedlings under the inoculated treatment (biotic plus abiotic soil conditions) would be significantly greater than the seedlings under the control treatment (abiotic conditions), and this was expected to be due to an additional positive influence of soil biota- present in the fresh soil inocula- on plant performance (i.e., the

effect of the biotic plus abiotic soil conditions would be greater than the abiotic conditions alone).

Assay 1

Cores from the 28 sites were placed in cold-storage (4˚C) for a period of 2-4 weeks between collection and planting assay 1 to maintain the biotic soil community (i.e., to ensure the soil microbes were alive and active). The cores were kept intact (i.e., not broken up or sieved) to preserve the physical soil structure for the growth assay.

Seeds of *Andropogon gerardii*, a local dominant grass, were collected from a tallgrass community at Oak Hammock Marsh, MB (see **Chapter 2** for site description) in 2005, air-dried, and stored at -20˚C prior to seeding. Native seeds of *Veronicastrum virginicum*, a local rare forb, were purchased from a local seed company (Prairie Habitats Inc., Argyle, MB) and cold-stratified (4˚C) in moistened potting mix for 4 weeks prior to seeding. Trays of *A. gerardi*i (assay 1 and 2) and *V. virginicum* (assay 1) were seeded on Turface® (Profile Products, Buffalo Grove, IL) in a greenhouse, with no fertilizer added, and allowed to establish for 1-2 weeks before planting.

Experimental pots (10 cm diameter by 9 cm depth) were set up in mid-July 2006 with one fresh soil core positioned vertically or 'top-up' in each to emulate original orientation in the field. The surrounding space in the pot was packed with perlite to maintain the shape of the core. The plastic polyethylene bag covering each core was then cut back to expose the soil surface, and holes punctured underneath to allow for drainage. One seedling (*A. gerardii* or *V. virginicum*) was transplanted into each soil core according to the species and soil site combination (2 plant species x 28 soil sites x 10

replications), for a total of 560 pots and all were arranged randomly by site on greenhouse tables (but grouped by species). Pots were weeded for the first few weeks as required and watered through a drip line system for 45 seconds every other day to keep the soil moist. The growth experiment was maintained under natural light conditions, average growing-season temperatures (ca. 24˚C daytime/18˚C night-time) and no fertilization for a total of 6 weeks, after which time plants were harvested. In order to minimize changes in the soil conditions in the greenhouse, and prevent plants from outgrowing the small soil cores in which they were planted, the assay was kept short.

Assay 2

A small amount (~ 5 ml) of fresh soil was collected in plastic vials from each soil core immediately following harvest of assay 1 (but prior to breaking up the cores for air-drying), labelled by site, and stored at -80˚C. Vials corresponding to 10 study sites were obtained from the -80˚C freezer and used as the fresh soil in the inoculated treatment in assay 2.

All soil cores were broken-up following assay 1, air-dried, and stored at room temperature for 9 months prior to planting assay 2. Ten sites were chosen based on the results of assay 1- the top 5 and bottom 5 growth-promoting sites of *A. gerardii* were selected, and the soils pooled by site. The 10 pooled soils were autoclaved at 121˚C for 1 hr. The original soil cores were pooled because the intention of the growth assay was to create background abiotic soil properties for each site, as opposed to preserving the physical soil structure.

Autoclaved soil was loosely packed into Ray Leach "Cone-tainers"™ (2.5 cm x 12 cm, Stuewe & Sons Inc., Corvallis, OR), and one seedling of *A. gerardii* was transplanted into each cone-tainer in June 2007 according to the soil treatment and site combination (2 soil treatments x 10 soil sites x 5 replications), for a total of 100 pots. For the inoculated treatment, a small hole was created in the soil around the seedling, filled with approx. 5 ml from a vial of fresh soil (see *Assay 1*), and the soil lightly pushed down around the seedling. Cone-tainers were placed in storage racks (25 per rack), arranged randomly on greenhouse tables, and watered by an overhead-mist system for 2 minutes every other day to keep the soil moist, but not saturated. The growth experiment was maintained under natural light conditions, average growing-season temperatures (ca. 24˚C daytime/18˚C night-time) and no fertilization for a total of 8 weeks, after which time plants were harvested.

Measurements of Growth and Soil Analysis

Immediately following harvest of assay 1 (late August to early September 2006), total plant fresh mass was measured, and dead plants were noted and excluded. Roots were separated from half of the replicates in each site (i.e., root subsample), collected in small plastic vials, and placed in a -80˚C freezer for future mycorrhizal examination. The remaining half of the replicates, which were still intact (i.e., whole plants), were dried at 65˚C and total dry mass was measured. Total dry mass of plants for which a root subsample was collected (i.e., plants sampled for mycorrhizal examination) was determined by using the ratio of total dry mass to total fresh mass of the whole plants (i.e., plants not sampled for mycorrhizal examination), multiplied by the total fresh mass

of the plants sampled for mycorrhizal examination. Following harvest of assay 2 (August 2007), the roots and shoots of each plant were separated and measured equivalent to the methods used in **Chapter 3**, including collection and storage (-80˚C) of (half) root subsamples for future mycorrhizal examination.

In assay 1, soil samples from each site were analyzed for gravimetric water content (GWC) (280 samples = ½ of the total replicates, equally-distributed among sites) immediately following harvest, and for organic matter (OM) (28 samples; all replicates bulked per site) in February 2007 using standard soil methods: GWC - soil was weighed fresh, oven-dried at 105˚C until a constant weight was achieved, and weighed dry (Jarrell et al. 1999); OM – oven-dried (105˚C) soil was pre-weighed in a ceramic crucible, ignited at 500˚C in a muffle furnace for 4-5 hours, and re-weighed (i.e., weight loss on ignition) (Harmon and Lajtha 1999). Random soil samples (dried at 65˚C) from each site (140 samples = ¼ of the total replicates, not equally-distributed among sites) were analyzed for inorganic phosphate (P) in March 2007 and inorganic nitrogen (N) in July 2007 using Olsen's method (Kalra and Maynard 1991) and the Microdiffusion method (Mulvaney 1996), respectively. No soil samples were analyzed in assay 2 because the samples measured in assay 1 included the 10 soil sites.

Mycorrhizal Examination

Roots were examined for percent arbuscular mycorrhizal (AM) colonization in June 2008 using the same clearing, staining, and microscopic assessment (gridline intersect) methods in **Chapter 3** (Johnson et al. 1999). In assay 1, to reduce the number roots of *A. gerardii* and *V. virginicum* to be examined (from approximately 280 samples),

samples were selected from the 28 study sites based on mean inorganic P levels for each site determined during the soil analyses. Soil phosphate is thought to be increased by mycorrhizal fungi and therefore may benefit plant growth (Bever et al. 2001). *A. gerardii* root samples were chosen based on association with sites with high phosphate (P) (sites 3 & 13), medium P (20 & 22) and low P (24, 27 & 28) levels, for a total of 7 sites selected or 31 root samples- 19 samples of which were from restoration sites and 12 were from remnants. *V. virginicum* root samples were also chosen from sites with high P (site 4), medium P (19 & 21) and low P (15, 16, 24 & 26) levels, for a total of 7 sites selected or 19 root samples- 16 samples of which were from restoration sites and 3 were from remnants.

In assay 2, *A. gerardii* root samples were reduced to 6 sites or 60 samples from the original 10 study sites (approximately 100 samples) based on selecting an equal representation of the top and bottom site types (i.e., 3 top-5 and 3 bottom-5 sites) and inoculation treatments (i.e. ½ inoculated and ½ sterile replicates) for examination. Of the 60 root samples selected, 40 samples were from restoration sites and 20 were from remnants.

Statistical Analyses

In assay 1, significant differences between sites (1-28) for *A. gerardii* and *V. virginicum* biomass were examined using a random effects ANOVA model (Model II) (JMP-SE 6.0.3©). A linear correlation was performed on the log-transformed biomass of each site to examine if both species responded in the same manner to the different sites. Individual relationships between plant performance and age (only 16 sites, for which age

was known), area, mean N, mean P, mean GWC, and OM (sample per site) values for the 28 sites were also explored using linear correlations. A stepwise multiple regression was performed to determine which soil variables predict species biomass. Nutrient level (soil N and P) differences between the sites were examined through one-way ANOVA. To avoid pseudoreplication, comparisons between the site types were performed using the mean per site values.

In assay 2, a two-way ANOVA was used to test for an interaction effect between inoculation treatment and site type on plant performance (total biomass, root to shoot ratio). Nutrient level (soil N and P) differences between the site types were evaluated using one-way ANOVA. All ANOVA analyses were performed on the mean per site values. A t-test was performed on the mean (per site) sterile to inoculated (S: I) ratio of total biomass and root to shoot ratio compared with the predicted mean of 1 (i.e., set as no difference between treatments) for the top vs. bottom site types, as well as the restoration vs. remnant site types.

In the root analyses of assay 1 (*A. gerardii* and *V. virginicum*) and assay 2 (*A. gerardii*), linear correlation analysis was used to examine the relationships between root colonization and soil P using the random (and unequally-distributed) soil core P values analyzed for each site, and plant performance and root colonization using the values of each plant. In both assay 1 and 2, differences in root colonization were explored using one-way ANOVA between the remnant vs. restoration site types, and in assay 2, between both the inoculation treatments and the top vs. bottom site types. ANOVA comparisons were performed using the mean per site values.

For all statistical analyses in assay 1 and 2, diagnostic plots of residuals by predicted values were used to check for homogeneity of the variance, and log-transformation of the data was performed to normalize the distribution. Results of all graphs and tables were presented as untransformed data, except where the text indicates the data presented was log-transformed. Significance level was set at $\alpha = 0.05$ for all tests.

Results

Assay 1

The size of the 28 sites ranged from 0.07 ha to 3530 ha, the age of 16 restoration sites varied from 3-17 years, while the age of 4 restoration sites and 8 remnant sites was unknown (Table 4.1). The mean soil properties varied from 0.48 to 18.2 ppm for phosphate (P), 7.61 to 38.8 ppm for inorganic nitrogen (N), 1 to 24 for N:P ratio, 0.22 to 0.69 $gm \cdot gm^{-1}$ for gravimetric water content (GWC), and 10.1 to 36.5% for organic matter (OM) (Table 4.1). Mean values for soil N, P, N:P ratio, GWC and OM were reported solely for the 28 site cores planted with *A. gerardii*, as values for the *V. virginicum* sites were similar. The soil properties between remnant and restoration sites only differed noticeably in soil P, with levels much lower in remnant sites compared to restorations (Table 4.1).

The results of *A. gerardii* and *V. virginicum* performance showed that mean biomass (per plant) varied significantly ($p < 0.0001$) between the 28 sites, with over a 10-fold range in biomass between the remnant and restoration sites (Fig. 4.2a and 4.2b;

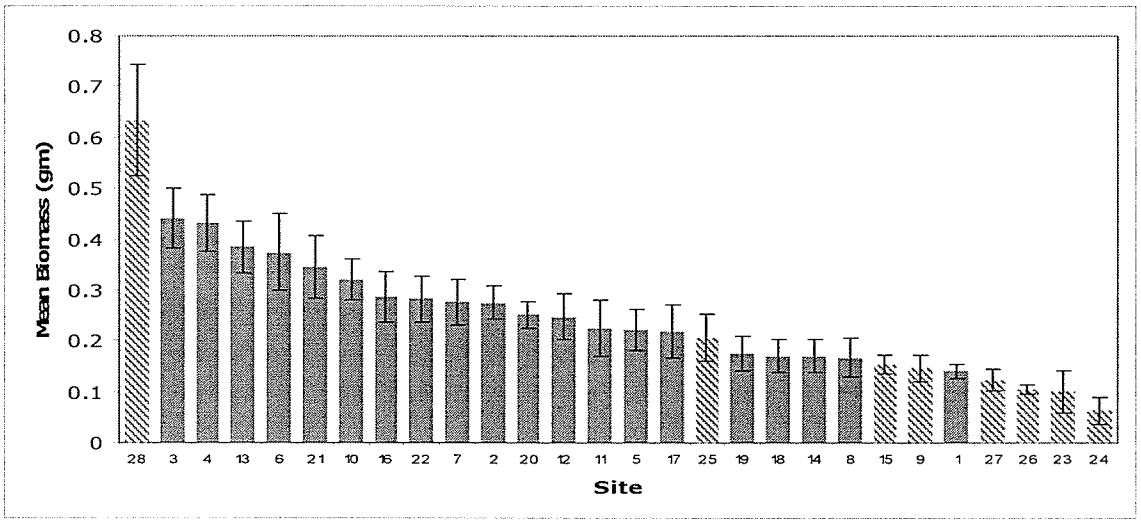


Fig. 4.2a Mean biomass of *Andropogon gerardii* grown in soil from 28 tallgrass sites in Manitoba (see site names in Table 4.1), and separated into two site types: remnant sites (grey stripe) and restoration sites (grey solid). Vertical bars represent standard errors of the means.

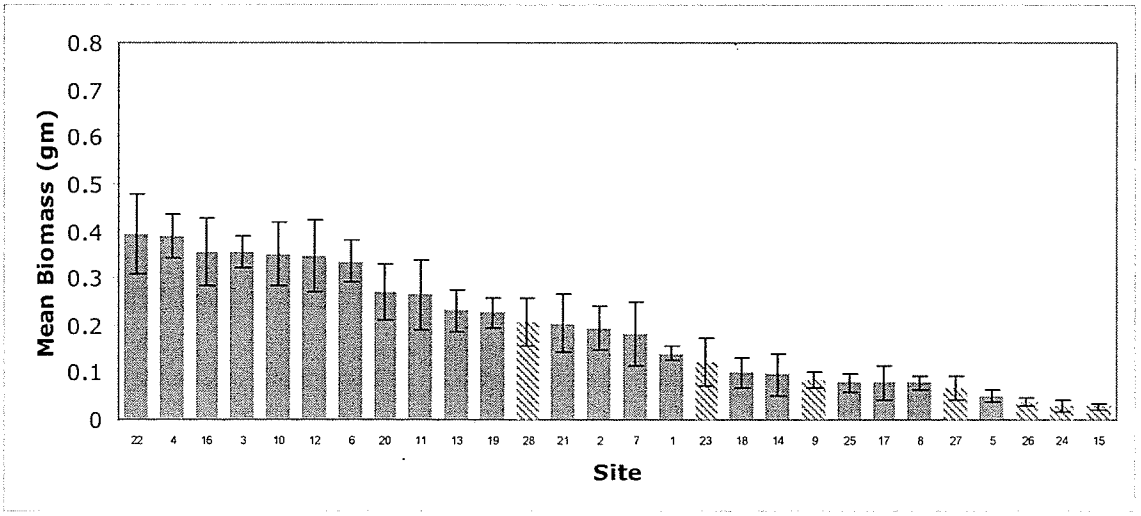


Fig. 4.2b Mean biomass of *Veronicastrum virginicum* grown in soil from 28 tallgrass sites in Manitoba (see site names in Table 4.1), and separated into two site types: remnant sites (grey stripe) and restoration sites (grey solid). Vertical bars represent standard errors of the means.

Table 4.2). Site 28 had a particularly higher biomass for *A. gerardii* (Fig. 4.2a). There was a significant relationship for site biomass between species ($r^2 = 0.55$; $p < 0.0001$), indicating that individual sites had the same effect on plant performance regardless of species present (Fig. 4.3). Mean biomass (per site) of *A. gerardii* was not significantly different ($p = 0.157$) between the remnant and restoration site types (Fig. 4.4a). However, because site 28 was a strong outlier (Fig. 4.4a); it is evident that mean biomass would generally be much higher in the restoration sites compared to the remnant sites (Fig. 4.2a). Performance (mean biomass per site) of *V. virginicum* between site types was significantly different ($p = 0.002$) (Fig. 4.4b; Table 4.2). Plants had a biomass of 0.20 ± 0.02 gm (mean and S.E.) for the restoration sites compared to 0.07 ± 0.04 gm for the remnant sites.

Linear correlations demonstrated positive relationships for the performance of both *A. gerardii* ($p = 0.04$) (Fig. 4.5a) and *V. virginicum* ($p = 0.003$) (Fig. 4.5b) fit by soil P using the mean per site values. There was also a positive relationships for *A. gerardii* performance fit by soil N ($p = 0.03$) (Fig. 4.6a). There were no significant relationships for performance of either species fit by OM (sample per site), GWC (mean per site), site age, and site area (Fig. 4.7a; 4.7b; 4.8a; 4.8b; 4.9a; 4.9b; 4.10a; 4.10b). However, one result of note in the linear correlation of *V. virginicum* performance by (log) area was that high biomass (> 0.2 gm) only occurred in tallgrass sites greater than 3 ha (Fig. 4.10b), which may be particularly interesting because that species is locally rare. A stepwise multiple regression showed that soil P was the most important variable predicting *A. gerardii* biomass, while organic matter was most important for predicting *V. virginicum* biomass (data not shown).

Table 4.2 One-way ANOVA for the effect of site (1-28) and site type (remnant vs. restoration) on total biomass (*A. gerardii* and *V. virginicum*), and on soil nutrient (N and P) levels. Degrees of freedom (df), F-statistic (F), sum of squares (SS), and p values are reported with significant values presented in bold ($p < 0.05$).

	A. gerardii				*V. virginicum*			
Source of Variance	SS	df	F	p	SS	df	F	p
Biomass								
Site (mean per plant)	98.4	27	5.66	**<0.0001**	3.71	27	6.82	**<0.0001**
Site Type (mean per site)	0.03	1	2.12	ns	0.10	1	10.1	**0.002**
Soil N								
Site (mean per core)	3484.7	27	2.49	**0.0011**	--	--	--	NR
Site Type (mean per site)	34.2	1	0.56	ns				NR
Soil P								
Site (mean per core)	3017.9	27	12.5	**<0.0001**	--	--	--	NR
Site Type (mean per site)	247.6	1	18.1	**0.0002**				NR

ns = not significant ($p > 0.05$); NR = not reported because the results are similar to *A. gerardii*

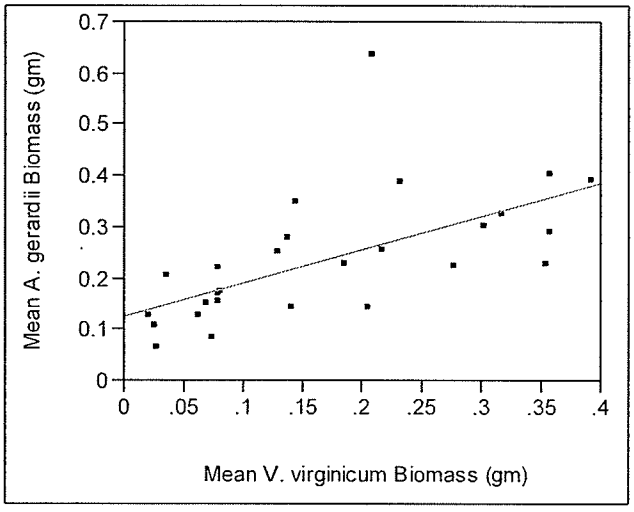


Fig. 4.3 *A. gerardii* mean biomass fit by *V. virginicum* mean biomass for 28 tallgrass sites ($r^2 = 0.55$; $p < 0.0001$; $y = 0.438x + - 0.664$).

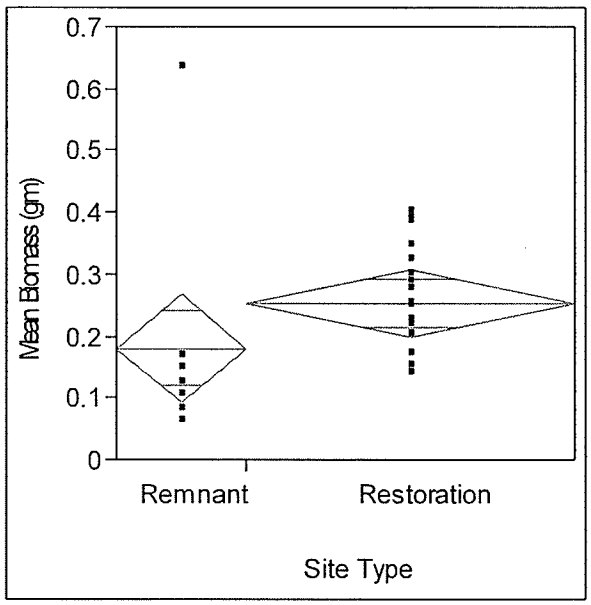


Fig 4.4a *A. gerardii* mean biomass (per site) fit by site type for 28 tallgrass sites. The vertical span of each diamond represents the 95% confidence interval for each group. The horizontal extent along the *x*-axis is proportional to the sample size of each level of the *x* variable. The line across each diamond represents the group mean, while overlap marks are drawn above and below the group mean. For groups with equal sample sizes, overlapping marks indicate that the two group means are not significantly different at the 95% confidence level.

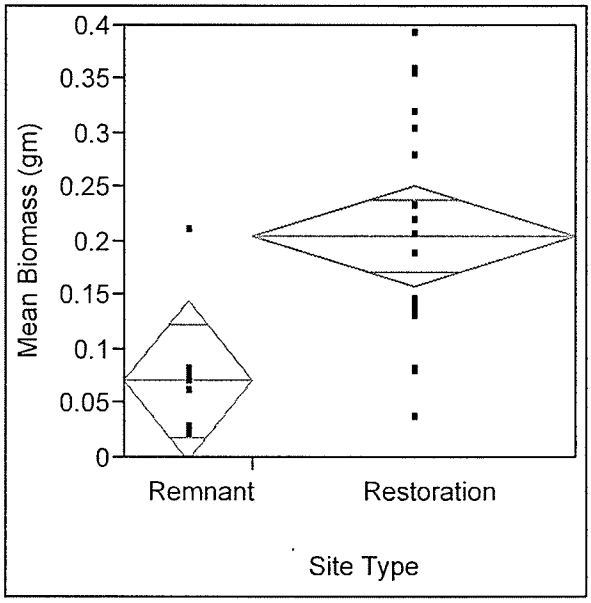


Fig. 4.4b *V. virginicum* mean biomass (per site) fit by site type for 28 tallgrass sites ($p = 0.004$).

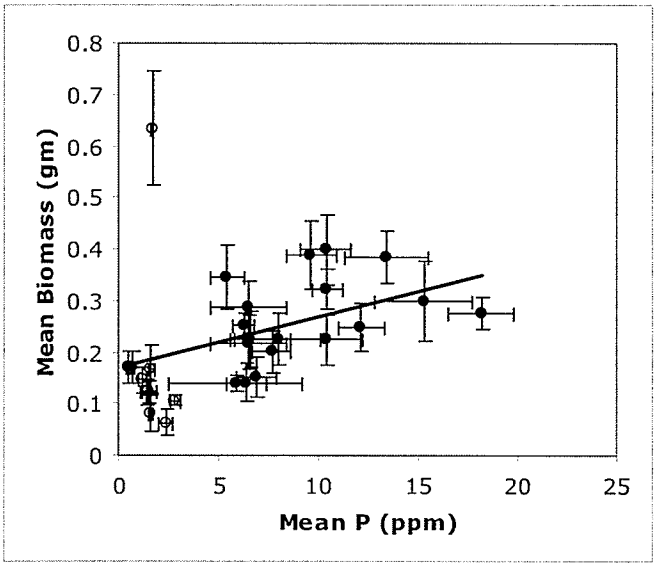


Fig. 4.5a *A. gerardii* mean biomass fit by mean soil P for 28 tallgrass sites (• = restorations, o = remnants). Vertical and horizontal bars indicate standard errors of the means for each site (r^2 = 0.152; p = 0.04; y = 0.010x + 0.168).

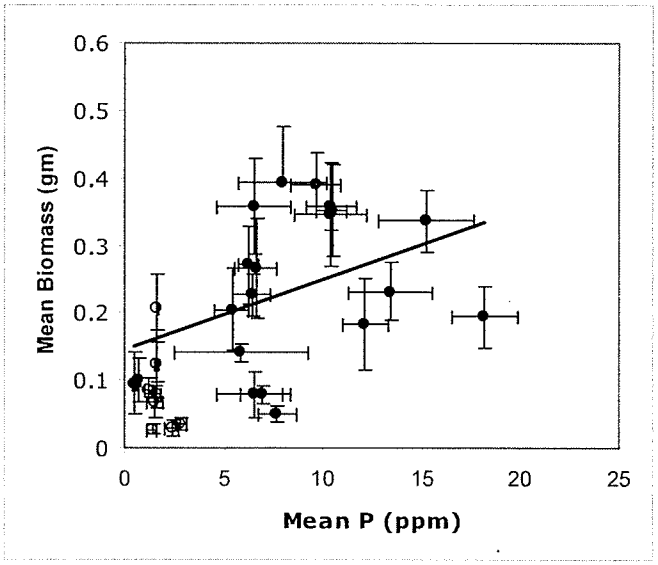


Fig. 4.5b *V. virginicum* mean biomass fit by mean soil P for 28 tallgrass sites (• = restorations, o = remnants). Vertical and horizontal bars indicate standard errors of the means for each site (r^2 = 0.293; p = 0.003; y = 0.013x + 0.080).

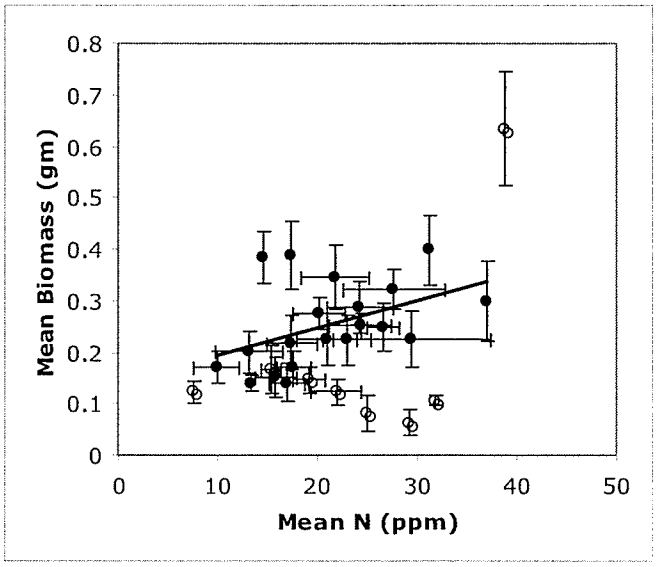


Fig. 4.6a *A. gerardii* mean biomass fit by mean soil N for 28 tallgrass sites (• = restorations, o = remnants). Vertical and horizontal bars indicate standard errors of the means for each site (r^2 = 0.171; p = 0.029; y = 0.007x + 0.090).

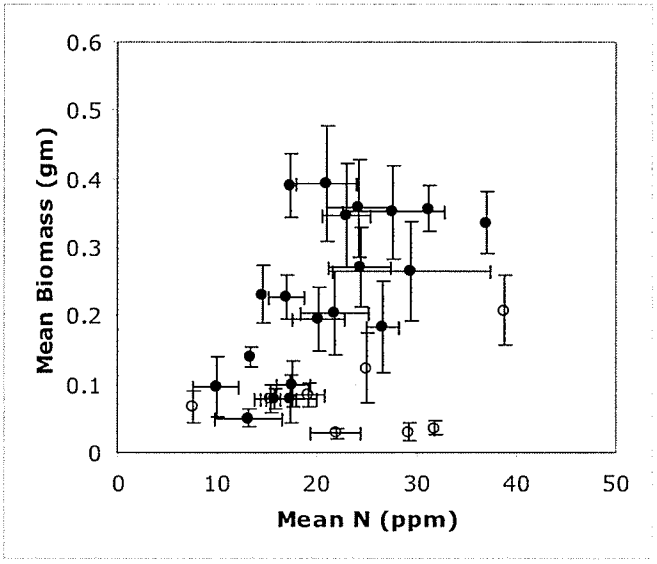


Fig. 4.6b *V. virginicum* mean biomass fit by mean soil N for 28 tallgrass sites (• = restorations, o = remnants). Vertical and horizontal bars indicate standard errors of the means for each site (r^2 = 0.098; p > 0.05).

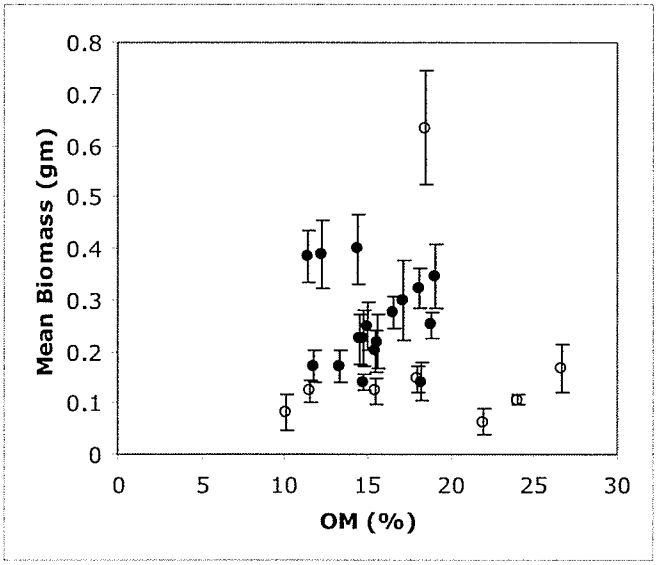


Fig. 4.7a A. *gerardii* mean biomass fit by OM for 28 tallgrass sites (• = restorations, o = remnants). Vertical bars indicate standard errors of the means for each site (r^2 = 0.003; $p > 0.05$).

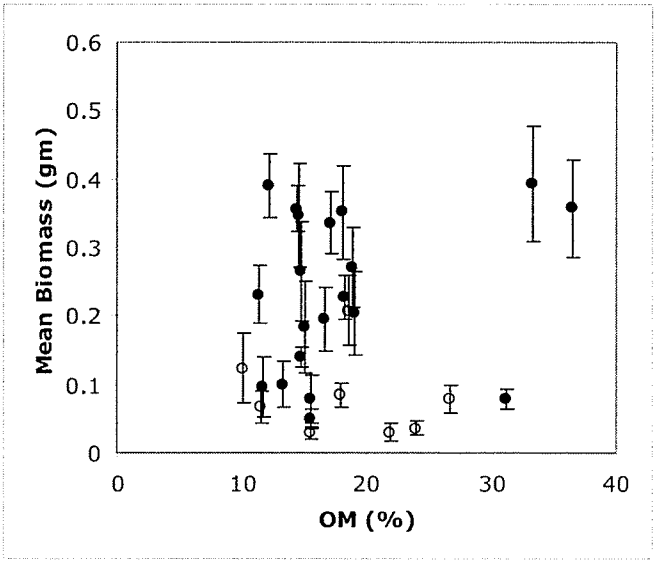


Figure 4.7b *V. virginicum* mean biomass fit by OM for 28 tallgrass sites (• = restorations, o = remnants). Vertical bars indicate standard errors of the means for each site ($r^2 = 0.031$; $p > 0.05$).

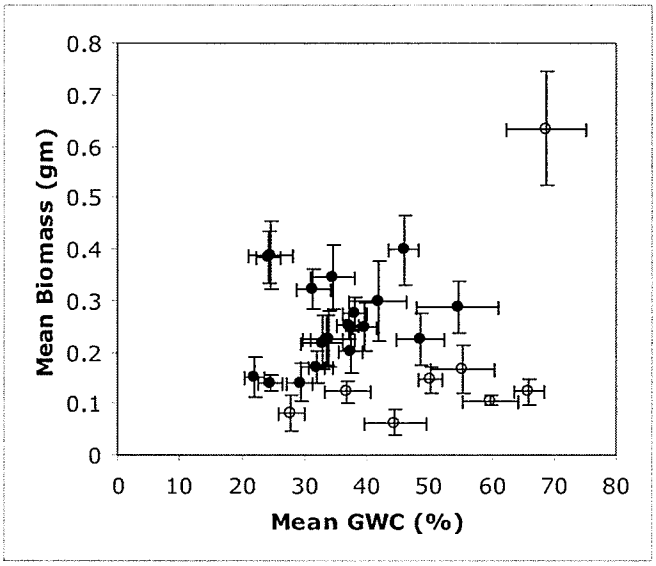


Fig. 4.8a A. *gerardii* mean biomass fit by mean GWC for 28 tallgrass sites (• = restorations, o = remnants). Vertical and horizontal bars indicate standard errors of the means for each site (r^2 = 0.028; $p > 0.05$).

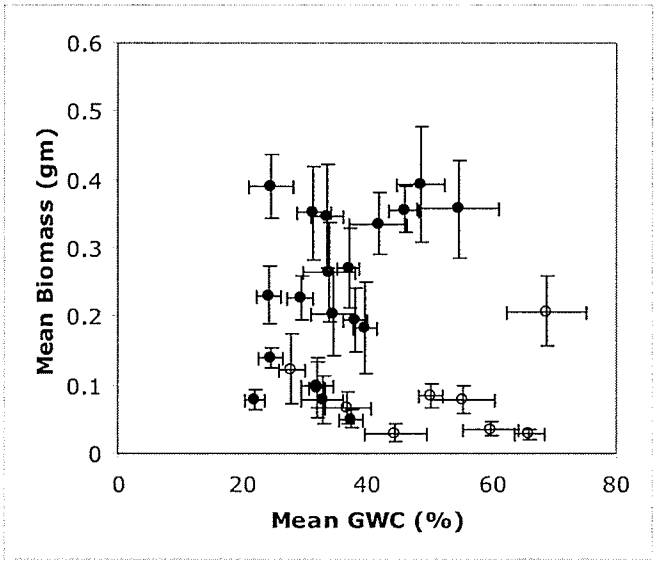


Fig. 4.8b *V. virginicum* mean biomass fit by mean GWC for 28 tallgrass sites (• = restorations, o = remnants). Vertical and horizontal bars indicate standard errors of the means for each site (r^2 = 0.007; $p > 0.05$).

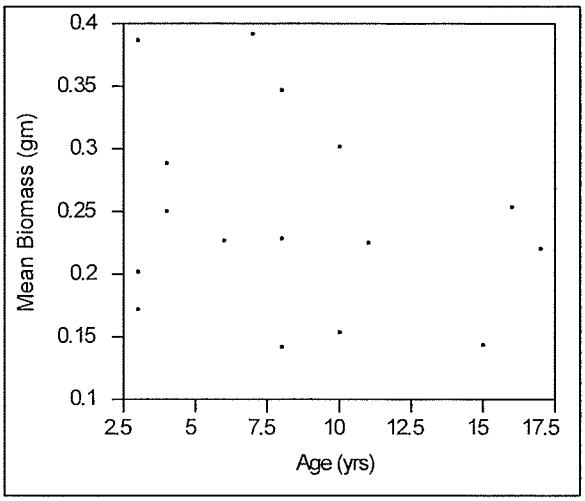


Fig. 4.9a *A. gerardii* mean biomass fit by age for 16 tallgrass restoration sites (12 sites including all remnants were excluded because age was unknown) ($r^2 = 0.068$; $p > 0.05$).

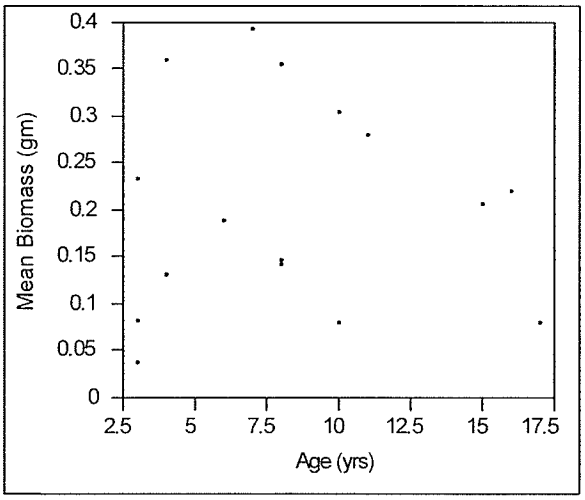


Fig. 4.9b *V. virginicum* mean biomass fit by age for 16 tallgrass restoration sites (12 sites including all remnants were excluded because age was unknown) in assay 1 ($r^2 = 1.09e\text{-}5$; $p > 0.05$).

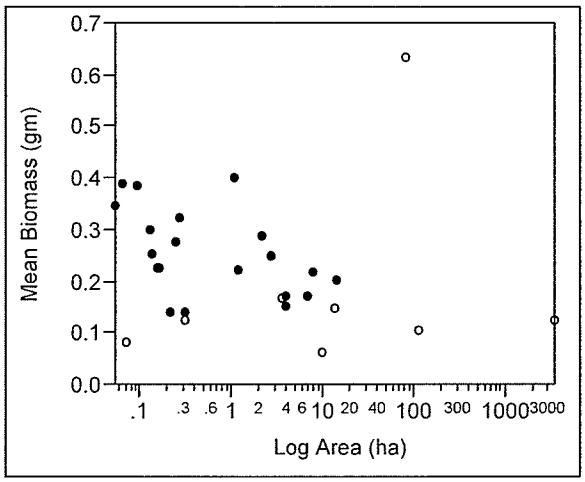


Fig. 4.10a *A. gerardii* mean biomass fit by log area for 28 tallgrass sites (• = restorations, o = remnants) (r^2 = 0.027; $p > 0.05$).

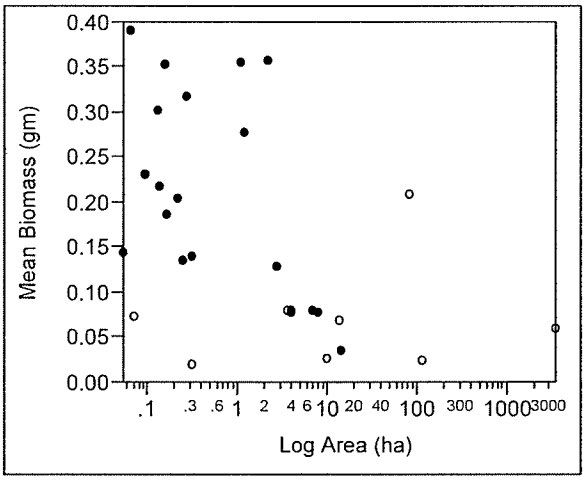


Fig. 4.10b *V. virginicum* mean biomass fit by log area for 28 tallgrass sites (• = restorations, o = remnants) ($r^2 = 0.034$; $p > 0.05$).

Soil N and P levels (mean per core) differed significantly between the 28 sites ($p = 0.011$ for N; $p < 0.0001$ for P) (Table 4.2). Soil P (mean per core), but not N, was significantly different between the sites types ($p = 0.002$) (Table 4.2), with (mean ± standard error) values of 8.36 ± 0.83 ppm for restoration sites and 1.77 ± 1.31 ppm for remnant sites (Fig 4.11).

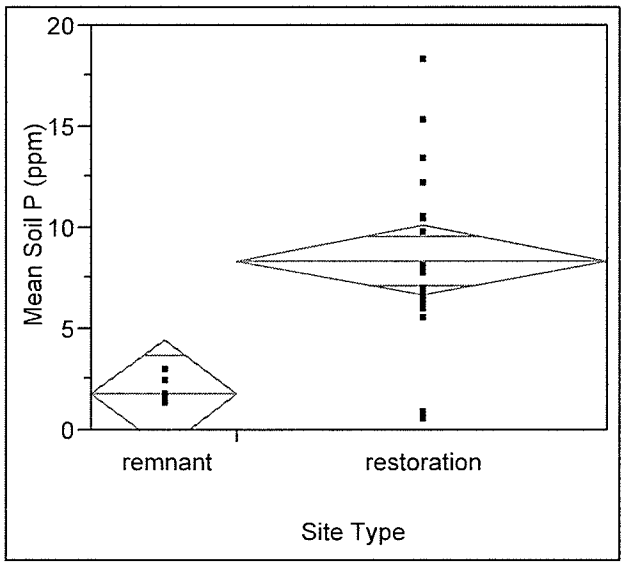


Fig. 4.11 Mean soil P (per site) fit by site type for 28 tallgrass sites (p = 0.0002).

Mycorrhizal Colonization (Assay 1)

The relationship between AM root colonization and plant performance for *A. gerardii* and *V. virginicum* was insignificant for the total values per plant (Fig. 4.12a and 4.12b), or values per plant when separated by the remnant and restoration site types (data not shown). There was no relationship between root colonization and soil P for the total values per soil core (i.e., using the random and unequally-distributed soil P values measured for each site) (Fig. 4.13a and 4.13b), or values per core when separated by the site types (data not shown). ANOVA for colonization differences between the site types using mean per site values also did not significantly differ (data not shown).

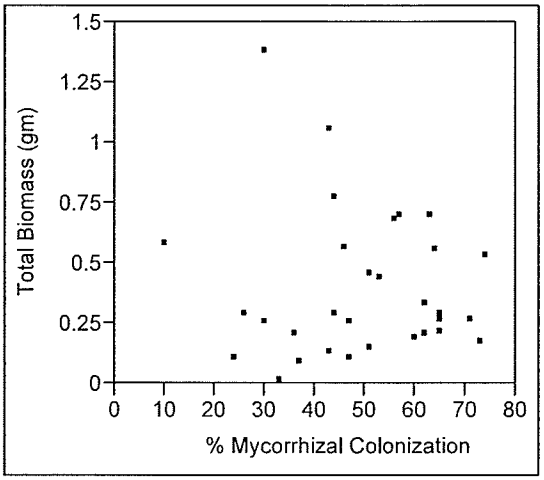


Fig. 4.12a Total biomass (per plant) of *A. gerardii* fit by mycorrhizal colonization (n = 31) ($r^2 = 0.037$; $p > 0.05$).

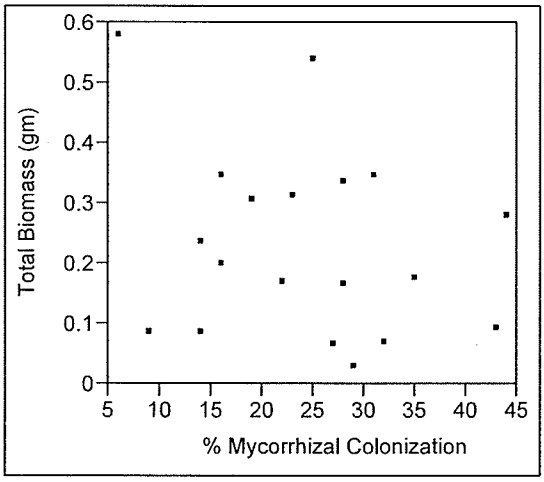


Fig. 4.12b Total biomass (per plant) of *V. virginicum* fit by *m*ycorrhizal colonization (n = 19) ($r^2 = 0.065$; $p > 0.05$).

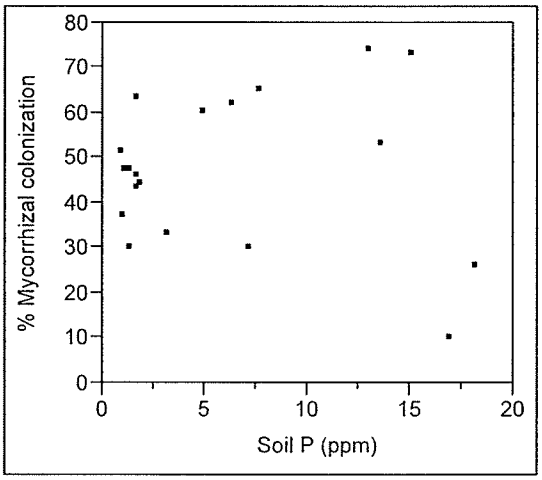


Fig. 4.13a Mycorrhizal colonization of *A. gerardii* fit by soil P (per core) (n = 19) ($r^2 = 0.27$; $p > 0.05$).

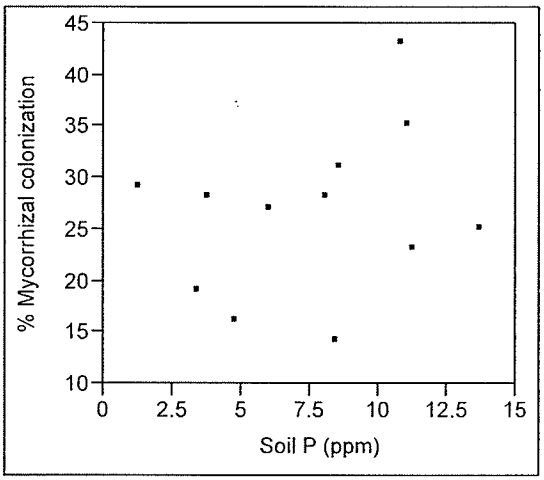


Fig. 4.13b Mycorrhizal colonization of *V. virginicum* fit by soil P (per core) (n = 12) ($r^2 = 0.078$; $p > 0.05$).

Assay 2

The results of a two-way ANOVA showed that *A. gerardii* mean biomass (per site) differed significantly between the inoculation treatments and top vs. bottom site types, with plants grown in inoculated soil 1.6 times larger than those in sterile soil and plants grown in the top sites 3.5 times larger than those in the bottom sites (Fig 4.14a; Table 4.3). Root to shoot ratio only differed significantly between the top vs. bottom site types, with the ratio 1.2 times greater in plants grown in the bottom sites as compared to the top sites (Fig. 4.14b; Table 4.3). The interaction of site type by inoculation treatment on biomass and root: shoot ratio was insignificant (Table 4.3). Soil P (mean per core) differed significantly between the top vs. bottom sites ($p = 0.02$), with values (mean $\pm$ standard error) of 10.10 ± 1.75 ppm in the top sites compared to 2.68 ± 1.75 in the bottom sites (Fig. 4.15). Soil N (mean per core) was not significantly different between the site types, while soil N and P also did not differ between the inoculation treatments (data not shown). The ratio of the mean sterile to inoculated treatment (S:I) for each site calculated for *A. gerardii* biomass was significantly different from 1 for the bottom sites ($p < 0.0001$), but not the top sites, suggesting that inoculation had more of an effect on plant growth for the bottom sites than the top sites. The ratio of S:I for root to shoot ratio was significantly different from 1 for the top sites ($p = 0.022$), but not the bottom sites (Table 4.4). The ratio of the mean sterile to inoculated treatment (S:I) for each site calculated for *A. gerardii* mean biomass was significantly different from 1 for the restoration sites ($p = 0.02$) and remnant sites ($p = 0.002$), while the ratio of S:I for root to shoot ratio was not significant for either of the site types (Table 4.5).

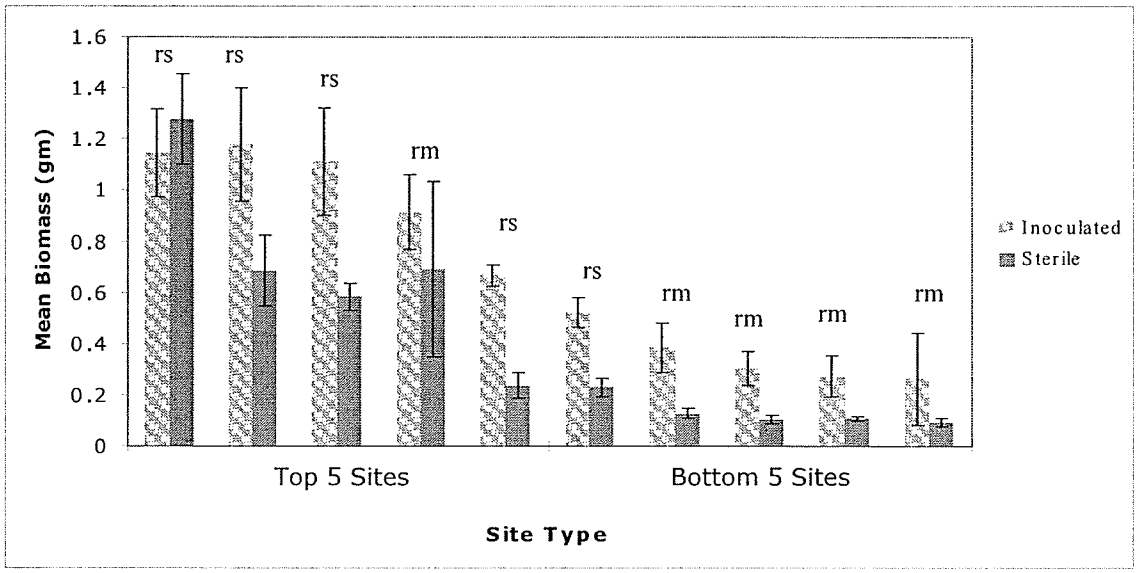


Fig. 4.14a Mean biomass (per site) of *Andropogon gerardii* grown in the top 5 and bottom 5 sites (influencing *A. gerardii* performance in assay 1), and under two inoculation treatments: inoculated and sterile (rm = remnant sites, rs = restoration sites; see Table 4.1).

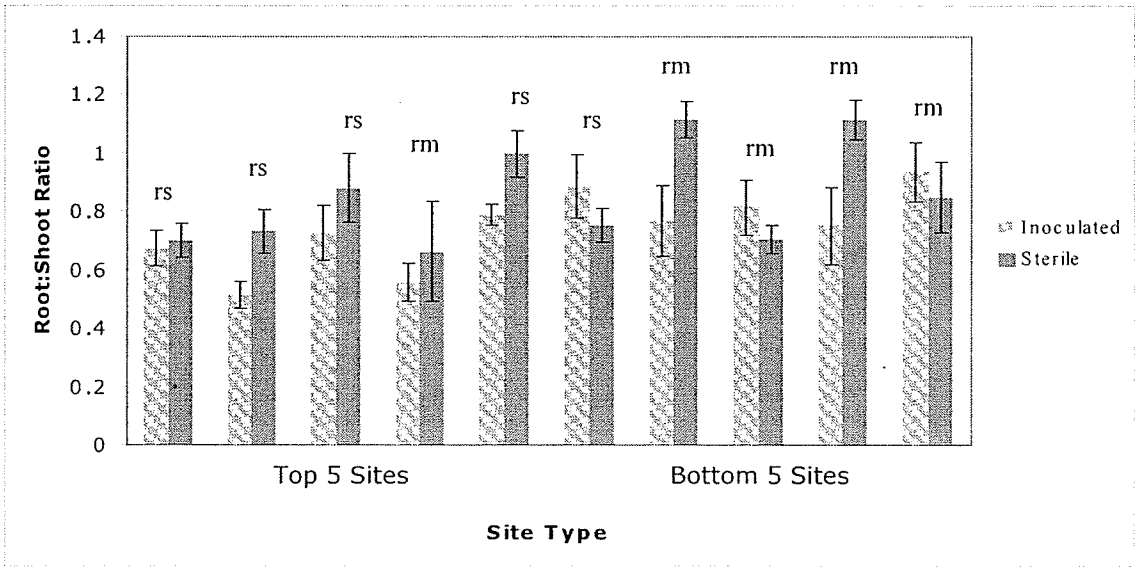


Fig. 4.14b Root to shoot ratio (per site) of *Andropogon gerardii* grown in the top 5 and bottom 5 sites (influencing *A. gerardii* performance in assay 1), and under two inoculation treatments: inoculated and sterile (rm = remnant sites, rs = restoration sites; see Table 4.1). Sites are sorted according to Fig. 4.12a.

Table 4.3 Two-way ANOVA for effect of the interaction between inoculation treatment and top vs. bottom site type on performance of *A. gerardii*. Degrees of freedom (df), F-statistic (F), sum of squares (SS), and p values are reported with significant values presented in bold ($p < 0.05$).

	Total Biomass				Root to Shoot Ratio			
Source of Variance	SS	df	F	p	SS	df	F	p
Inoculation Treatment	0.34	1	6.84	**0.02**	0.06	1	3.10	ns
Top vs. Bottom Site Type	1.85	1	36.8	**<0.0001**	0.11	1	5.51	**0.03**
Inoculation Treatment x Site Type	0.01	1	0.21	ns	0.005	1	0.28	ns
Error	0.80	16	---	---	0.31	16	---	---
Total	3.01	19	---	---	0.48	19	---	---

ns = not significant ($p > 0.05$)

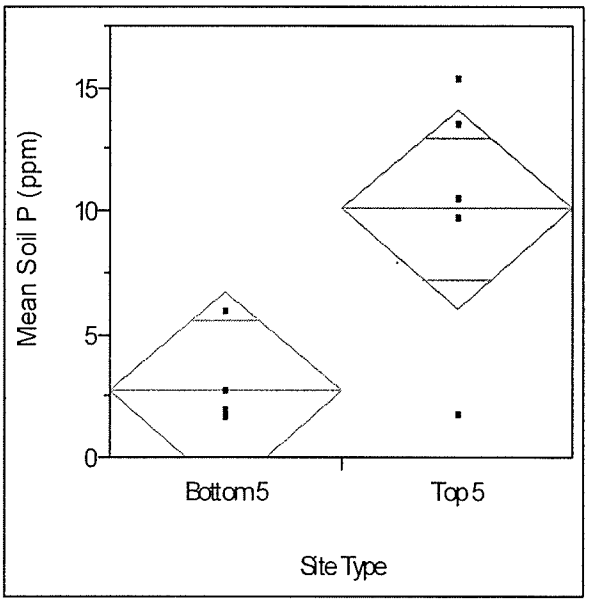


Fig. 4.15 Mean soil P (per site) fit by site type for 10 tallgrass sites ($p = 0.02$).

Table 4.4 *A. gerardii* mean (per site) ratio of sterile to inoculated (S:I) soil inoculation treatments for total biomass and root to shoot ratio when separated by top 5 and bottom 5 site types. A t-test was used to compare the actual mean to no difference (i.e., mean = 1) for the S: I ratio. Mean, degrees of freedom (df), standard deviation (SD), t values, and p values are reported with significance presented in bold ($p < 0.05$).

	Total Biomass S: I Ratio		Root to Shoot S: I Ratio	
	Top 5	Bottom 5	Top 5	Bottom 5
Mean	0.67	0.38	1.23	1.11
SD	0.29	0.04	0.14	0.33
df	4	4	4	4
t	2.57	31.7	3.64	0.77
p	ns	**<0.0001**	**0.02**	ns

ns = not significant ($p > 0.05$)

Table 4.5 *A. gerardii* mean (per site) ratio of sterile to inoculated (S:I) soil inoculation treatments for total biomass and root to shoot ratio when separated by restoration and remnant site types. A t-test was used to compare the actual mean to no difference (i.e., mean = 1) for the S: I ratio. Mean, degrees of freedom (df), standard deviation (SD), t values, and p values are reported with significance presented in bold ($p < 0.05$).

	Total Biomass S: I Ratio		Root to Shoot S: I Ratio	
	Restoration	Remnant	Restoration	Remnant
Mean	0.60	0.44	1.16	1.18
SD	0.30	0.18	0.22	0.29
df	4	4	4	4
t	2.96	6.98	1.60	1.38
p	**0.04**	**0.002**	ns	ns

ns = not significant ($p > 0.05$)

Mycorrhizal Colonization (Assay 2)

AM root colonization (mean per site) was significantly higher at 69.8 ± 2.6% in the inoculated treatment as compared to 36.9 ± 3.2% in the control treatment ($p < 0.0001$) (Fig. 4.16), but the means did not differ significantly between the top vs. bottom sites types and remnant vs. restoration site types (data not shown). The amount of variation within the inoculation treatments was high with individual colonization values between 40 and 85% for the inoculated treatments, and 12% and 60% for the sterile treatments (data not shown). The relationship between plant performance and root colonization was significant (total values per plant), with a positive relationship demonstrated for total biomass ($p = 0.0008$) and a negative relationship for root to shoot ratio ($p = 0.02$) (Fig. 4.17a and 4.17b). When the data was grouped by the inoculation treatments and top vs. bottom sites, only the sterile treatments ($p = 0.02$) (Fig. 4.18a) and top 5 sites ($p = 0.006$) (Fig. 4.18b) were significant, and both were positive relationships. There was also a significant positive relationship between root colonization and soil P ($p = 0.007$) (random values per soil core) (Fig. 4.19), but not when grouped by top vs. bottom sites or sterile vs. inoculated treatments (data not shown).

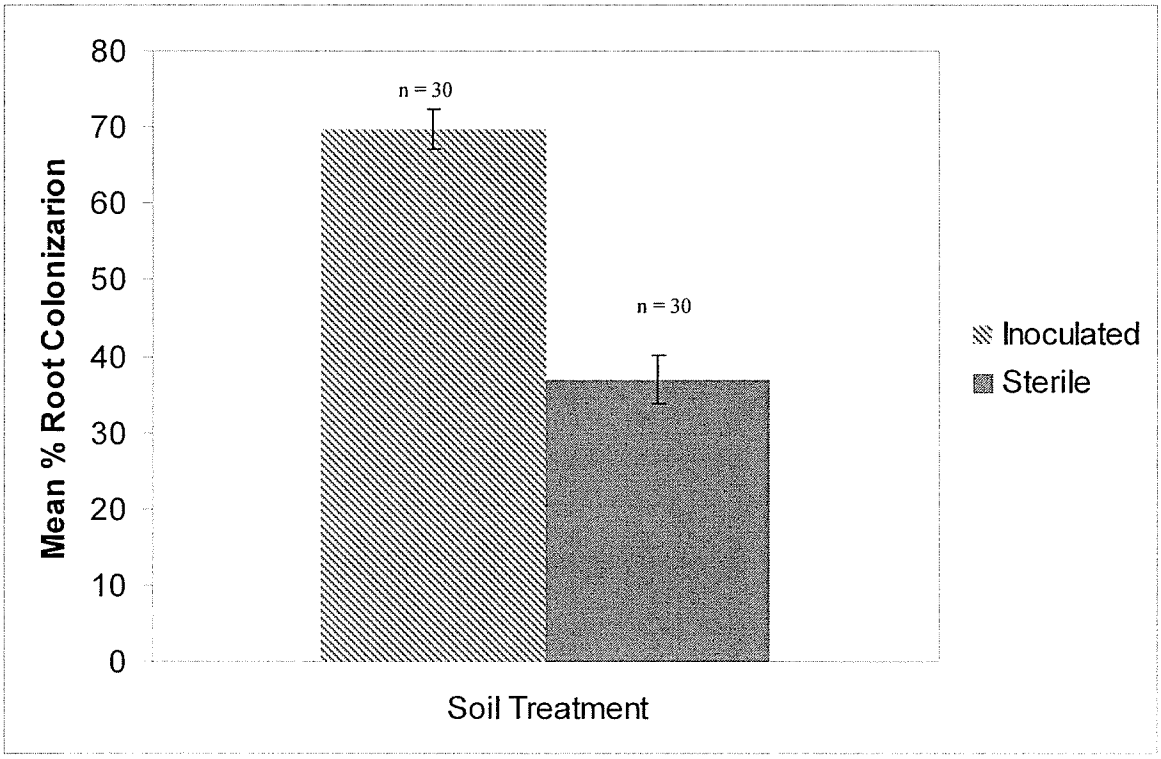


Fig. 4.16 Mean (per site) percent AM root colonization of *A. gerardii* when separated by inoculation treatment (pooled by site). Standard errors of the means (vertical bars) and sample size are presented for each treatment.

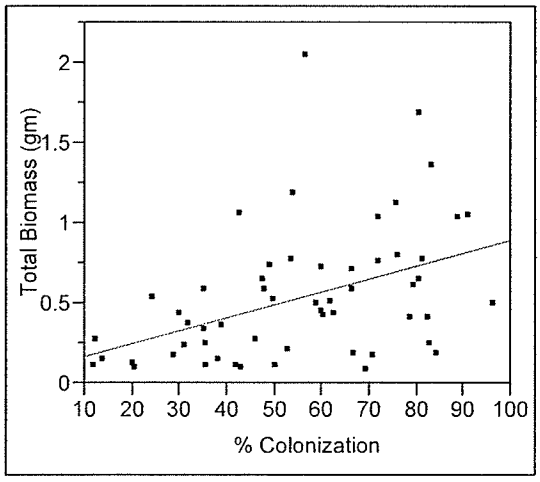


Fig. 4.17a Total biomass (per plant) of *A. gerardii* fit by mycorrhizal colonization when pooled by soil inoculation treatment (n = 56) (r^2 = 0.19; p = 0.0008; y = 0.008x + 0.08).

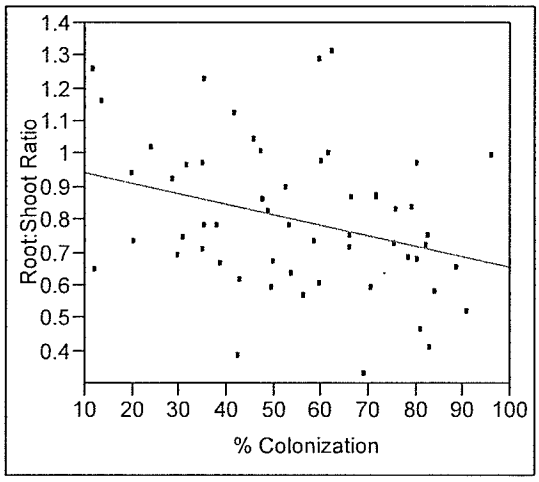


Fig. 4.17b Root to shoot ratio (per plant) of *A. gerardii* fit by mycorrhizal colonization when pooled by soil inoculation treatment (n = 56) (r^2 = 0.10; p = 0.02; y = -0.003x + 0.97).

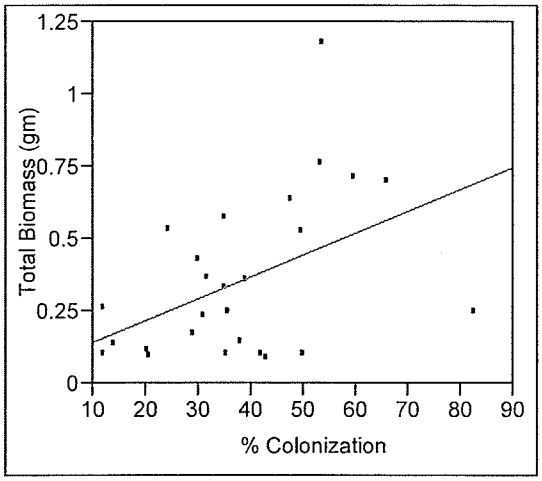


Fig. 4.18a Total biomass (per plant) of *A. gerardii* fit by mycorrhizal colonization for the sterile soil treatment (r^2 = 0.21; p = 0.02; y = 0.008x + 0.06).

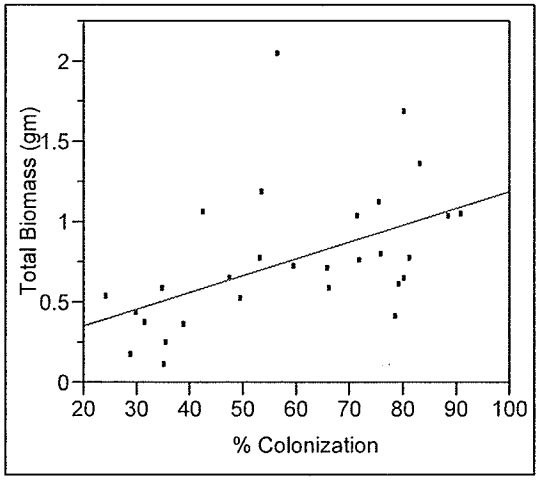


Fig. 4.18b Total biomass (per plant) of *A. gerardii* fit by mycorrhizal colonization for the top 5 sites (r^2 = 0.25; p = 0.006; y = 0.010x + 0.14).

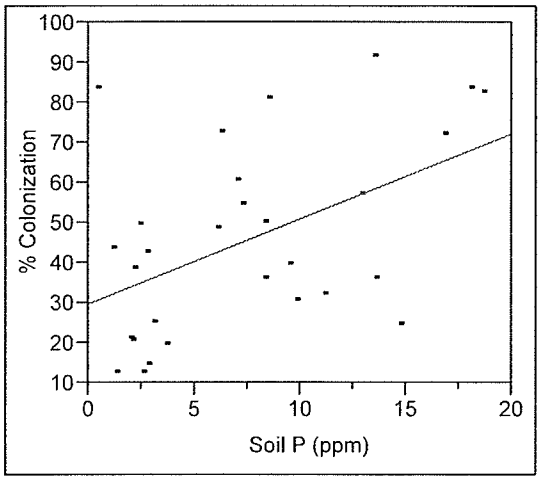


Fig 4.19 Mycorrhizal colonization of *A. gerardii* fit by soil P (per core) (n = 29) (r^2 = 0.24; p = 0.007; y = 2.14x + 29.3).

Discussion

To my knowledge, there are no studies on the effect of local soil site type (i.e., the difference in abiotic and biotic soil conditions between sites) on the performance of native tallgrass species, or on the potential application of such research in tallgrass community restorations. One particular review of European grassland restorations examined the effect of varying plant traits (e.g. life-form and history, environmental associations with soil pH, hydrology and fertility, etc.) between 25 local study sites on native plant performance. However, the influence of variation in the soil communities between the sites was not considered (Pywell et al. 2003). Recognition of differences in the microbial soil communities between natural forests and clear-cut sites in a North American forest restoration is the closest comparison of soil site type effects- particularly the biotic soil effects- on native species performance encountered in the literature (Perry et al. 1989).

My research is important because it considers the total abiotic and biotic influence of the soil community on growth of native tallgrass species. The importance of evaluating the total soil community corresponds with recent papers emphasizing that the whole-community interactions within the soil are far too complex to uncouple in assessing effect on the local plant community (Wolfe and Kironomos 2005). Moreover, assessment of numerous local tallgrasss soil communities on performance of both a common (*A. gerardii*) and uncommon species (*V. virginicum*) (specifically assay 1) has potential for future tallgrass restoration studies in Manitoba using different local soil types to improve establishment of native species. This research (specifically assay 2) is also distinct because it focuses on the significance of the biotic soil interactions on plant

growth (Chanway et al. 1991; Bever et al. 1997; Bever 2002; Bever 2003, Ehrenfeld et al. 2005, Wolfe and Klironomos 2005, Wardle 2006; Casper and Castelli 2007), while recognizing the importance of arbuscular mycorrhizal fungi (Bever et al. 2001). The role of arbuscular mycorrhizae in individual plant growth and overall community structure has been an increasing area of attention in grassland studies (Wilson and Hartnett 1998; Bever et al. 2001; Wilson et al. 2001; Van der Heijden et al. 2006).

Effect of Site Type on Plants

A. gerardii and *V. virginicum* performance differed significantly between the 28 (restoration and remnant) sites in assay 1 as expected. The individual sites had the same effect on species performance as demonstrated by the positive relationship between the ranks of each site. *A. gerardii* plants grown in soil from restoration sites were 2 times larger in biomass than plants grown in remnant sites (but only with site 28 excluded), while *V. virginicum* plants were 3 times larger in restoration sites compared to remnants, which was probably a result of advantageous (abiotic and biotic) soil conditions for the restoration site type. The importance of soil nutrients (i.e., abiotic conditions) was established in this study, with N and P differing significantly between the 28 individual sites. In particular, there was a significant relationship between plant performance for both species and soil P, suggesting that across the sites P limited plant growth. A positive relationship also existed between *A. gerardii* and soil N, but was not significant. Greater species performance in the restoration sites could be explained by a significant difference in soil P, with mean levels 5 times higher in restoration sites as compared to remnants. A higher phosphate level in the restoration sites was likely a result of the past level of

human disturbance characterizing the individual sites. For example, all restoration sites were located in tallgrass communities within Winnipeg and mainly found in personal gardens (pers. obs.), where cultivated soil was amended with organic matter and nutrients in much higher proportions than that found in native tallgrass prairie. In comparison, many of the remnant sites (i.e., relatively native prairies) were located in tallgrass communities outside the city of Winnipeg, where soil was not (or less) cultivated for agriculture and considered poor in nutrients, and are currently managed by local conservation groups (CWHP 1994; DND 2004; Suggett, pers. comm.). Overall, it is likely that some form of natural disturbance is required to cycle P in tallgrass prairie communities.

While prairie remnants typically produced lower biomass than restoration sites, site 28- a particular remnant located at St. Charles Rifle Range (SCRR), Manitoba- produced undoubtedly the largest *A. gerardii* plants. This anomaly may be related to the fact that the SCRR is considered the least (human) disturbed and largest tallgrass prairie remnant left in the Red River Valley of Manitoba. Historical accounts indicate that it was relatively uncultivated for agricultural purposes up until the 1900's when it was designated as Department of National Defense (DND) property (Sveinson 2003; DND 2004). Today DND manages the property exclusively for range training practices, in accordance with the objectives of prairie conservation, and limits access to the public (DND 2004). The SCRR site was recently characterized to be uniquely composed of lake bottom clay soil (as opposed to beach ridge sediments of Glacial Lake Agassiz characterizing the Tall Grass Prairie Preserve area in Manitoba), resulting in slightly different species composition (DND 2004). In general, it appears that this site is

characterized by highly favourable abiotic and biotic soil conditions for growth of local tallgrass species, and the soil is unique in comparison to the other tallgrass sites in this study.

A. gerardii performance varied significantly between the top 5 and bottom 5 site types in assay 2 as expected, with plants grown in the top sites 3.5 times larger in biomass than those grown in the bottom sites, and root to shoot ratio 1 time greater in the bottom sites as compared to the top sites. This indicated that plants grown in the top sites put a higher allocation into increasing shoot growth and total biomass and less allocation into producing roots, which was possibly a result of highly advantageous (abiotic and biotic) soil conditions. Since the top 5 tallgrass sites planted with *A. gerardii* were primarily restoration sites and the bottom 5 sites were remnants, significant performance differences between the top and bottom sites could likely be attributed to respective classification as restoration and remnant sites. Greater plant performance in the top (or restoration) sites compared to bottom (or remnant) sites was explained by a significant difference in soil P, with the mean levels 4 times greater in the top sites. As discussed for assay 1 above, higher phosphate levels in the top sites (or restoration sites) was possibly an outcome of the disturbance histories of the individual sites themselves (i.e., due to the past level of soil cultivation and nutrient amendment).

Effect of Inoculation Treatment on Plants

The results of the inoculation treatments indicated that there was a stronger biotic effect of the soil community on growth of *A. gerardii* (i.e., the inoculation treatment had a greater effect on growth than the sterile control treatment), which was in accordance

with the second hypothesis. Specifically, plants grown in inoculated soil were 1.6 times larger in biomass than plants grown in sterile soil. However, soil N and P did not vary significantly between treatments. Given that soil nutrients (i.e., abiotic conditions) were constant, greater plant performance under the inoculation treatment was attributed to the biotic soil interactions. The ratio of the mean sterile to inoculated soil treatment (S:I) for each site was significant for biomass of the bottom sites but not the top sites, and significant for root to shoot ratio of the top sites but not the bottom sites. This indicated that plants grown in the bottom sites benefited more from the inoculated treatment than those in the top sites. Additionally, the ratio of the mean S:I for each site was significant for biomass of both the restoration sites and remnant sites, while insignificant for root to shoot ratio, indicating that plants equally benefited from the inoculated treatment in both site types.

Effect of Mycorrhizal Colonization on Plants

Many grassland species are obligate mycotrophs, which require association with arbuscular mycorrhizae (AM) to grow to maturity (Wilson and Hartnett 1998; Wilson et al. 2001). AM colonization of *A. gerardii* (and *V. virginicum* in assay 1) was evident through root staining and microscopic observation in both assays. In assay 1, there was no significant difference in AM colonization between the remnant and restoration site types. Remarkably, there was also no correlation for colonization with either plant performance or soil P. This can perhaps be explained by the research of Smith et al. (2004), which concluded that although AM fungi increase plant P supply, there appears to be no relation to plant growth response, P uptake or extent of AM root colonization.

Furthermore, the precise mechanism by which plants benefit from mycorrhizal P remains unclear (Smith et al. 2004).

Conversely, proof that plants benefited from AM colonization was apparent in assay 2. For example, there was a significant relationship between plant performance and root colonization for total biomass (positive relationship) and for root to shoot ratio (negative relationship). A significant relationship between root colonization and soil P was also evident, which suggests that the mycorrhizal fungi may have improved supply of soil P and resulted in positive effects on plant growth (Bever et al. 2001). When the relationship between plant performance and root colonization was separated by the soil inoculation treatments and top vs. bottom site types, only a significant positive relationship was evident for plants grown in the sterile soil treatment and top sites. This suggested that those particular plants highly benefited from the AM colonization. The positive relationship for plants grown in sterile soil was somewhat surprising, as AM colonization was expected to be low or negligible due to removal of the biotic soil community (i.e., through autoclaving), but may be explained by a higher-than-expected mean percent colonization determined for the sterile soil treatment (see below). It is also possible that colonization was significant in the sterile treatments because in those treatments there is a low rate of colonization and the maximum level of benefit to the plants has not yet been reached, resulting in improved growth effects. In comparison, there is a high rate of colonization in the inoculated treatments, and the threshold of benefit may have already been reached, resulting in no further effects on growth.

As expected, there was a significant difference in mean percent colonization between the inoculation and control soil treatments in assay 2. Greater colonization

occurred in the roots of the inoculated soil as a result of incorporation of the biotic soil community containing AM fungi. There was found to be no significant difference in AM colonization between the remnant and restoration site types (or top and bottom site types). One result worthy of note was that the colonization values for the sterile soil treatments (in which the biotic component was assumed to be removed through autoclaving) were relatively high, and expected to be negligible. A previous growth assay of *A. gerardii* in a tallgrass soil community from Oak Hammock Marsh, Manitoba, demonstrated significant root colonization in inoculated soil, but no colonization in sterile soil (Markham, per comm.). This result was particularly interesting because the study was conducted on a rooftop greenhouse, where mycorrhizal contamination rates would be expected to be comparable to my study. A particular study by Smith et al. (1998), however, found that native species inoculated with AM fungi in a disturbed tallgrass site in Minnesota also had significantly higher percentage of colonized roots as compared to the sterile control. Specifically, AM colonization was 44% and 35% for the inoculated and sterile soil treatments, respectively, which were values comparable to my study (70% and 37%, respectively). They suggested that high colonization in the sterile control could potentially be attributed to either low levels of mycorrhizae initially present at the site or high influx of mycorrhizal propagules into the site (Smith et al. 1998). In my study, the two growth assays occurred in a greenhouse containing large vents at both ends and characterized by cross-flow air movement. Mycorrhizal propagules could have spread from the inoculated- to control treatment pots through the action of wind. Additionally, it is possible that mycorrhizae were present in the initial seed stock of *A. gerardii* (collected from the Oak Hammock March tallgrass community) or in the potting medium (not

autoclaved) in which the seedlings were originally germinated, which may have contributed to the higher AM root colonization values in both the sterile and inoculated soil treatments. However, it should be pointed out that comparison of mycorrhizal inoculation effects on plant performance in the greenhouse to studies such as Smith et al. (1998) may not be applicable because there particular research was performed under field conditions. In addition, as noted above, the contamination of sterile controls by inoculated treatments did not occur in a previous performance assay using local tallgrass soil (Markham, pers. comm.), even though the conditions for growth were similar to my study.

Conclusion

There was evidence in this study to conclude that differences in the soil communities of the restoration and remnant tallgrass sites caused variation in the performance of a local dominant grass (*A. gerardii*) and a rare forb (*V. virginicum*). It could also be concluded that differences in the soil communities of the top and bottom sites (corresponding to differences in the restoration and remnant sites, respectively) cause variation in the performance of *A. gerardii*. Restoration sites had an advantageous effect on native species performance compared to remnants, which was believed to be the result of higher P levels characterizing that site type. However, it was also found that the soil community of one particular remnant site (SCRR) had uniquely positive effects on growth of both the dominant and rare species. Further greenhouse studies to examine inoculation effects of this soil community on performance of various local tallgrass species (i.e., dominant and rare species) and results from outplanting such species within

this site would be ideal. In general, it could be concluded that soil inocula from various local tallgrass communities (and perhaps with some soil P amendment of the sites) may be important for improving native species establishment, particularly in future tallgrass restoration projects in Manitoba.

It was also demonstrated in this study that there was a strong positive effect of the soil microbial community on tallgrass species performance. Typically, the poorer the site in soil nutrients- particularly soil P- as found in the remnant sites, the more important the biotic effect of the soil community. Mycorrhizae present in the soil community were specifically measured for percent root colonization and found in significantly high abundance in the inoculated soil (biotic community) as expected, but were surprisingly present in the sterile control soil (abiotic community), where abundance (although much lower than inoculated soil) was expected to be nil. A difference in mycorrhizal abundance between the restoration and remnant sites was not apparent. Although only mycorrhizal fungi were examined in this study, it is important to emphasize that the positive effects of the biotic soil are likely due to the diversity of microbial species present in the soil community (Chanway et al. 1991; Bever et al. 1997; Bever 2002; Bever 2003, Ehrenfeld et al. 2005, Wolfe and Klironomos 2005, Wardle 2006; Casper and Castelli 2007). A specific review paper by Chanway et al. (1991) suggests that certain free-living bacteria (i.e., rhizobacteria) in the soil can enhance plant growth through increasing phosphorus solubilization and nitrogen fixation, suppressing antagonistic bacteria, and producing plant growth substances. In general, the soil microbes may have increased supply of limiting nutrients to the plants, particularly soil P (Smith et al. 2004), but such results were not apparent in this study.

General Conclusion

Research on the plant-soil feedback relationships in Manitoba tallgrass prairie is particularly important given that the factors (i.e., both abiotic and biotic interactions) influencing community structure in the northern tallgrass range are not well known. My research within Manitoba tallgrass communities- particularly a remnant prairie at Oak Hammock Marsh (OHM), is important because it reduces gaps in knowledge of (plant and soil) community dynamics within the northern extent. In reference to the literature, few to no descriptive studies of spatial and temporal changes in local tallgrass community structure (i.e., vegetation and soil) have been published (**Chapter 2**). In addition, while the differences in the soil patch types associated with particular plant species (i.e., the difference in abiotic and biotic soil conditions between patches) have been shown to cause variation in the performance of dominant grass species (e.g. primarily European species), most of this work has focused solely on the abiotic soil effects, while not considering the additional importance of the biotic soil effects (**Chapter 3**). Furthermore, there appears to be no studies on the effect of local soil site type (i.e., the difference in abiotic and biotic soil conditions between sites) and particularly the biotic soil effects on the performance of native tallgrass species, or on the potential application of such research in local tallgrass community restorations (**Chapter 4**).

Overall, through my thesis research 4 specific short-term objectives were accomplished, which included: 1. Determining the spatial dynamics of vegetation patches in tallgrass prairie (**Chapter 2**); 2. Characterizing the abiotic soil conditions of a particular tallgrass prairie community (**Chapter 2**); 3. Determining the effect of soil biota from dominant plant patches on the dominant plant species (**Chapter 3**); and 4.

Determining the extent of variation in the soil communities of other tallgrass prairies (including both remnant and restoration prairies) on plant performance (**Chapter 4**). Results of field sampling within the OHM community in **Chapter 2** demonstrated that spatial structure of the vegetation exists, with patches of a few dominant species found interspersed with many rare species. However, because OHM is characterized as a perennial grassland system and there appears to be slow movement of species patches (i.e., over a 5 year period), the vegetation structure was considered temporally stable. Soil nutrients (inorganic N and P) exhibited trends of temporal and spatial variation which were important in characterizing the abiotic conditions, but were not important in explaining the plant community structure. Additionally, although gravimetric water content, microelevation, and bulk density had low degrees of variation across the site, they were still important environmental variables predicting structure of the vegetation. It was concluded that since nutrient levels fluctuate on a much shorter temporal scale than species dispersal in this system, plant patches are not able to move quickly enough in location to track the yearly change in nutrients. Specifically, it was found that spatial variation occurs within the vegetation structure of the OHM tallgrass community, while temporal fluctuation in soil nutrients (and minimal spatial variation in the important environmental variables) is coupled with temporal stability in vegetation over a 5 year period.

Growth assay results in **Chapter 3** provided no evidence to conclude that patch differences in the soil communities of the 3 dominant tallgrass species (*A. gerardii*, *S. pectinata*, and *P. virgatum*; from within the OHM site) caused variation in the performance of the dominant species *A. gerardii*. Soil nutrients (inorganic N and P) also

did not vary significantly between the patch types or inoculation treatments. However, the results of the inoculation treatments suggested that there was a strong effect of soil biota (i.e., the inoculation treatment had a greater effect on growth than the sterile control treatment), independent of patch type. In general, it was found that the presence of unidentified microbial species in the biotic soil community of the dominant species had an overall positive effect on the dominant species performance.

There was evidence based on the growth assay results in **Chapter 4** to conclude that differences in the soil communities of the restoration and remnant tallgrass sites (located in Manitoba) caused variation in the performance of the local dominant grass *A. gerardii* and rare forb *V. virginicum* (assay 1). Restoration sites had an advantageous effect on native species performance compared to the remnants as a result of higher inorganic P levels characterizing that site type. However, it was also found that the soil community of one particular remnant site (SCRR) had uniquely positive effects on growth of both the dominant and rare species. Additionally, it was concluded through the assays results that differences in the soil communities of the top 5 (or restoration) and bottom 5 (or remnant) sites- i.e., influencing *A. gerardii* and *V. virginicum* growth performance in assay 1- caused variation in the performance of *A. gerardii* (assay 2). Analogous to the results of the restoration sites (assay 1), advantageous effects of the top sites on native species performance was believed to be the result of higher inorganic P levels characterizing that site type. Furthermore, the results of the inoculation treatments suggested that there was a strong biotic effect of the soil community on plant growth (i.e., the inoculation treatment had a greater effect on growth than the sterile control treatment) (assay 2). In general, it was found that the presence of unidentified microbial species in

the biotic soil community of the remnant and restoration sites had an overall positive effect on the native tallgrass species performance, particularly for the nutrient-poor sites (i.e., remnants or bottom sites). It could be concluded that soil inocula from various local tallgrass communities (and perhaps with some soil P amendment of the sites) may be important for improving native species establishment, particularly in future tallgrass restoration projects in Manitoba.

In closing, it should be emphasized that although the overall objective of this research was to examine the plant and soil feedback relationships in Manitoba tallgrass prairie, the scope of the project was more focused on the influence of the abiotic and biotic soil community on performance of local tallgrass species (i.e., a one-way feedback effect). In general, my research concluded that there was a strong positive effect of the biotic soil community on the performance of the study species. In order for the magnitude and direction of a true feedback system to be characterized, the effect of the plant community on the soil community needs to be examined (i.e., a two-way effect). Future studies characterizing the host-specific composition and abundance of soil biota in Manitoba tallgrass prairie would be an ideal complement to my research.

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Appendix 1: Principal Component Analyses

Output files produced by SYN-TAX 2000© with total variance, axis eigenvalues and variable scores reported for the individual variables in three principal component analyses: **A. OHM SPECIES_2002, B. OHM SPECIES_2007, and C. OHM ENVIRONMENT 2006_2007** (data collected from Oak Hammock Marsh [OHM], MB tallgrass prairie community).

A. OHM SPECIES_2002 (50X20)

INPUT AND RUN PARAMETERS

NUMBER OF ROWS =	50
NUMBER OF COLS =	21
TYPE OF ANALYSIS =	PCA FROM COVARIANCES
NO. OF COMPONENTS RETAINED =	2
LABELS FOR OBJECTS =	NOT USED
LABELS FOR VARIABLES =	USED
CORRESP. ANALYSIS =	NOT APPLICABLE
MATRIX =	NOT SAVED
PRINTOUT =	SHORT
VAR. SCORE OPTION =	COVARIANCES OF VAR. WITH COMPONENTS
OBJ. SCORE =	NORMALIZED TO LAMBDA

VARIABLES STATISTICS

POOLED VARIANCE = 5.8851

VARIABLE	MEAN	STD DEV	VARIANCE	VARIANCE AS %
1 ANDger	1.04	.7812	.6102	10.369
2 POApra	.98	.5652	.3195	5.428
3 SPARpec	.67	.7572	.5734	9.743
4 CALneg	.54	.7491	.5611	9.534
5 PANvir	.73	.6774	.4589	7.798
6 HELmax	.70	.5654	.3196	5.431
7 HIEodo	.42	.6566	.4311	7.326
8 GALbor	.52	.5750	.3306	5.618
9 BARE	.53	.4655	.2167	3.682
10 SOLrig	.34	.5347	.2859	4.859
11 ROSark	.43	.4760	.2266	3.850
12 SEDGE	.35	.4818	.2322	3.945
13 SOLcan	.25	.4862	.2364	4.017

14 HELrhom	.17	.4497	.2022	3.436
15 HYPhir	.25	.4322	.1868	3.173
16 GLYlep	.11	.3705	.1373	2.333
17 AGRtrac	.18	.4340	.1884	3.201
18 PRErac	.20	.3520	.1239	2.105
19 ASTeri	.18	.3306	.1093	1.857
20 ZIZapt	.10	.2673	.0715	1.214
21 ASTlae	.08	.2522	.0636	1.081

NUMBER OF POSITIVE EIGENVALUES = 21

SUM OF POSITIVE EIGENVALUES = 0.58851390E+01

EIGENVALUES

0.1377E+01 0.8456E+00 0.6723E+00 0.4773E+00 0.3944E+00
0.3187E+00 0.2890E+00 0.2346E+00 0.2101E+00 0.1733E+00
0.1662E+00 0.1461E+00 0.1100E+00 0.9694E-01 0.8837E-01
0.7756E-01 0.6598E-01 0.4972E-01 0.4296E-01 0.2667E-01
0.2237E-01

EIGENVALUES AS PERCENT

23.40	14.37	11.42	8.11	6.70
5.42	4.91	3.99	3.57	2.94
2.82	2.48	1.87	1.65	1.50
1.32	1.12	.84	.73	.45
.38				

CUMULATIVE PERCENTAGE OF EIGENVALUES

23.40	37.77	49.19	57.30	64.00
69.42	74.33	78.31	81.88	84.83
87.65	90.13	92.00	93.65	95.15
96.47	97.59	98.44	99.17	99.62
100.00				

SQUARE ROOTS OF EIGENVALUES

1.173487	.919583	.819912	.690852	.628003
.564520	.537584	.484383	.458326	.416240
.407717	.382169	.331687	.311351	.297272
.278504	.256866	.222988	.207274	.163320
.149576				

COMPONENT SCORES

1	.652	-1.036
2	.527	-.373
3	.533	-.833

4	-.192	.310
5	.073	-1.403
6	-.056	-.901
7	-.356	-1.647
8	.286	.218
9	-.257	.681
10	1.065	-.643
11	.548	-1.502
12	.531	-.752
13	.631	-1.021
14	.523	-.500
15	1.418	-.066
16	.632	1.313
17	1.579	-.607
18	1.431	-.289
19	1.293	-.115
20	.784	-.806
21	1.498	-.005
22	1.149	-.217
23	.219	1.800
24	1.557	.872
25	1.311	1.464
26	1.183	1.575
27	.754	1.920
28	1.611	1.699
29	.947	1.282
30	1.032	.278
31	.328	-1.193
32	.418	-1.478
33	.953	-.180
34	-.288	-.426
35	-1.561	.051
36	-1.099	.005
37	-1.878	-.102
38	-1.754	-.277
39	-1.754	-.277
40	-1.305	.939
41	-1.356	.775
42	-.491	-.176
43	-1.622	.678
44	-1.599	-.524
45	-1.132	-.862
46	-1.736	.330
47	-1.571	-.311
48	-1.831	1.031
49	-1.811	.596

50 -1.819 .701

SCORES FOR VARIABLES

VARIABLE 1 ANDger
.412 -.590
VARIABLE 2 POApra
-.427 .006
VARIABLE 3 SPARpec
-.056 -.213
VARIABLE 4 CALneg
.495 .426
VARIABLE 5 PANvir
.313 .362
VARIABLE 6 HELmax
-.226 -.106
VARIABLE 7 HIEodo
-.569 .095
VARIABLE 8 GALbor
.393 -.114
VARIABLE 9 BARE
-.114 -.100
VARIABLE 10 SOLrig
-.045 -.179
VARIABLE 11 ROSark
.187 .032
VARIABLE 12 SEDGE
.182 -.057
VARIABLE 13 SOLcan
.039 -.099
VARIABLE 14 HELrhom
-.158 .076
VARIABLE 15 HYPhir
.141 -.176
VARIABLE 16 GLYlep
-.092 -.002
VARIABLE 17 AGRtrac
.086 .070
VARIABLE 18 PRErac
-.062 -.045
VARIABLE 19 ASTeri
.006 .038
VARIABLE 20 ZIZapt
-.014 -.043
VARIABLE 21 ASTlae
-.001 -.056

B. **OHM SPECIES_2007** (50x16)

INPUT AND RUN PARAMETERS

NUMBER OF ROWS =	50
NUMBER OF COLS =	16
TYPE OF ANALYSIS =	PCA FROM COVARIANCES
NO. OF COMPONENTS RETAINED =	2
LABELS FOR OBJECTS =	NOT USED
LABELS FOR VARIABLES =	USED
CORRESP. ANALYSIS =	NOT APPLICABLE
MATRIX =	NOT SAVED
PRINTOUT =	SHORT
VAR. SCORE OPTION =	COVARIANCES OF VAR. WITH COMPONENTS
OBJ. SCORE =	NORMALIZED TO LAMBDA

VARIABLES STATISTICS

POOLED VARIANCE = 4.0265

VARIABLE	MEAN	STD DEV	VARIANCE	VARIANCE AS %
1 ANDger	.91	.8464	.7164	17.792
2 POApra	.36	.6171	.3808	9.458
3 SPARpec	.57	.7742	.5994	14.887
4 CALneg	.45	.7322	.5361	13.315
5 PANvir	.48	.6225	.3875	9.624
6 HELmax	.49	.5923	.3508	8.713
7 GALbor	.13	.3507	.1230	3.055
8 SOLrig	.11	.3287	.1080	2.683
9 SEDGE	.03	.2277	.0518	1.288
10 SOLcan	.12	.4278	.1830	4.544
11 HELrhom	.10	.3441	.1184	2.941
12 GlyLep	.07	.2480	.0615	1.528
13 ASCov	.09	.3183	.1013	2.516
14 ASTeri	.03	.2277	.0518	1.288
15 POA	.04	.2659	.0707	1.756
16 PHA	.11	.4310	.1858	4.614

NUMBER OF POSITIVE EIGENVALUES = 16

SUM OF POSITIVE EIGENVALUES = 0.40264560E+01

EIGENVALUES
0.9925E+00 0.7623E+00 0.6302E+00 0.4020E+00 0.2730E+00
0.2285E+00 0.1685E+00 0.1209E+00 0.9301E-01 0.8469E-01
0.7095E-01 0.6664E-01 0.4675E-01 0.3538E-01 0.3095E-01
0.2016E-01

EIGENVALUES AS PERCENT

24.65	18.93	15.65	9.98	6.78
5.68	4.18	3.00	2.31	2.10
1.76	1.65	1.16	.88	.77
.50				

CUMULATIVE PERCENTAGE OF EIGENVALUES

24.65	43.58	59.23	69.22	76.00
81.67	85.86	88.86	91.17	93.27
95.04	96.69	97.85	98.73	99.50
100.00				

SQUARE ROOTS OF EIGENVALUES

.996231	.873123	.793820	.634032	.522493
.478018	.410493	.347775	.304980	.291011
.266371	.258139	.216227	.188093	.175934
.141996				

COMPONENT SCORES

1	-.350	-1.288
2	.268	-.545
3	-.607	-.582
4	-1.571	.329
5	-1.049	-1.007
6	.328	-.078
7	.261	-1.244
8	.386	-.242
9	.700	-.143
10	.972	-.509
11	-.059	-1.256
12	.379	-1.289
13	-.072	-.789
14	.442	-1.162
15	1.258	-.530
16	.806	.608
17	1.214	.110
18	1.323	-.485
19	1.253	-.214

20	.435	-.530
21	1.489	-.087
22	1.148	-.581
23	.979	1.547
24	.983	1.622
25	1.229	1.653
26	1.022	1.433
27	1.032	1.440
28	1.097	1.488
29	-1.539	.467
30	-1.748	.451
31	-.403	-1.110
32	-.481	-1.133
33	.709	-.309
34	-1.717	.454
35	-1.806	.446
36	-1.717	.454
37	-1.658	.452
38	-1.803	.447
39	-1.526	.509
40	-.311	.602
41	.133	.998
42	-.179	-.699
43	-.364	.496
44	-.055	-.927
45	.422	-1.185
46	-.613	.512
47	.171	-.791
48	-.229	.692
49	-.288	.743
50	-.295	.765

SCORES FOR VARIABLES

VARIABLE 1 ANDger
.355 -.752
VARIABLE 2 POApra
.046 .007
VARIABLE 3 SPARpec
-.653 -.028
VARIABLE 4 CALneg
.499 .314
VARIABLE 5 PANvir
.344 .221
VARIABLE 6 HELmax

-.244 -.015
VARIABLE 7 GALbor
-.075 -.118
VARIABLE 8 SOLrig
-.028 -.005
VARIABLE 9 SEDGE
.011 -.003
VARIABLE 10 SOLcan
.007 -.095
VARIABLE 11 HELrhom
-.004 -.020
VARIABLE 12 GlyLep
.011 .050
VARIABLE 13 ASCov
.060 .114
VARIABLE 14 ASTeri
-.010 .029
VARIABLE 15 POA
.010 -.024
VARIABLE 16 PHA
-.030 .092

C. **OHM ENVIRONMENT 2006_2007** (50x7)

INPUT AND RUN PARAMETERS

NUMBER OF ROWS =	50
NUMBER OF COLS =	7
TYPE OF ANALYSIS =	PCA FROM CORRELATIONS
NO. OF COMPONENTS RETAINED =	2
LABELS FOR OBJECTS =	NOT USED
LABELS FOR VARIABLES =	USED
CORRESP. ANALYSIS =	NOT APPLICABLE
MATRIX =	NOT SAVED
PRINTOUT =	SHORT
VAR. SCORE OPTION =	CORRELATIONS OF VAR. WITH COMPONENTS
OBJ. SCORE =	NORMALIZED TO LAMBDA

VARIABLES STATISTICS

POOLED VARIANCE = .3645

VARIABLE	MEAN	STD DEV	VARIANCE	VARIANCE AS %
1 BD	1.81	.0690	.0048	1.306
2 GWC	1.64	.0666	.0044	1.218
3 OM	1.20	.1833	.0336	9.220
4 pH	7.91	.1536	.0236	6.470
5 N	1.14	.2354	.0554	15.196
6 P	2.89	.3666	.1344	36.864
7 ME	1.31	.3292	.1084	29.726

NUMBER OF POSITIVE EIGENVALUES = 7

SUM OF POSITIVE EIGENVALUES = 0.70000000E+01

EIGENVALUES
0.1731E+01 0.1420E+01 0.1134E+01 0.8841E+00 0.8443E+00
0.5344E+00 0.4512E+00

EIGENVALUES AS PERCENT
24.73 20.29 16.20 12.63 12.06
7.63 6.45

CUMULATIVE PERCENTAGE OF EIGENVALUES
24.73 45.02 61.23 73.86 85.92
93.55 100.00

SQUARE ROOTS OF EIGENVALUES
1.315793 1.191790 1.065026 .940255 .918866
.731048 .671728

COMPONENT SCORES

1	1.739	1.573
2	-.327	-.394
3	-.318	.088
4	-.890	-.945
5	1.249	-.268
6	1.535	-.507
7	-.100	-1.445
8	.257	-.804
9	1.485	.208
10	-1.370	-2.021
11	-.543	.282
12	-1.146	.453
13	-1.358	1.016

14	-.831	.731
15	1.517	1.502
16	.144	2.062
17	-.653	.780
18	.565	.172
19	-.314	1.771
20	.418	2.192
21	-1.341	1.412
22	-1.943	1.434
23	.550	-.588
24	.260	-.947
25	.137	.515
26	.415	.629
27	1.213	.179
28	.671	-.516
29	1.035	1.466
30	.519	1.281
31	-1.105	-1.228
32	-2.636	.016
33	-1.324	-.635
34	-1.150	.318
35	-1.055	.230
36	-3.142	-.315
37	-1.641	-1.867
38	-.918	.437
39	-.706	-.632
40	-.963	-1.264
41	.446	-1.341
42	-1.160	2.261
43	1.569	-1.191
44	1.266	-.477
45	.555	-.699
46	1.804	-2.491
47	.432	-2.025
48	2.141	-1.131
49	1.463	-.894
50	3.549	1.617

SCORES FOR VARIABLES

VARIABLE 1 BD

.809 -.142

VARIABLE 2 GWC

-.429 .561

VARIABLE 3 OM

.266 -.605
VARIABLE 4 pH
.453 .697
VARIABLE 5 N
-.306 .234
VARIABLE 6 P
-.184 -.283
VARIABLE 7 ME
-.699 -.315

Appendix 2: Redundancy Analyses

Output files produced by CANOCO for Windows 4.5© with the correlation matrix, total variance, axis eigenvalues and Monte Carlo Permutation test of the sets of variables in two redundancy analyses: A. **OHM 2002 SPECIES_2006/2007 ENVIRO** and B. **OHM 2007 SPECIES_2006/2007 ENVIRO** (data collected from Oak Hammock Marsh [OHM], MB tallgrass prairie community).

A. OHM 2002 SPECIES_2006/2007 ENVIRO

*** Type of analysis ***
Model Gradient analysis
indirect direct
linear 1=PCA 2= RDA
Type analysis number
Answer = 2

Forward selection of envi. variables = 0
Scaling of ordination scores = 2
Diagnostics = 1
No. of couplets of species number and abundance per line : 0
No samples omitted
Number of samples 50
Number of species 21
Number of occurrences 394
No interaction terms defined
No transformation of species data
No species-weights specified
No sample-weights specified
Centering/standardization by species = 1
Centering/standardization by samples = 0
No. of active samples: 50
No. of passive samples: 0
No. of active species: 21
Total sum of squares in species data = 288.372
Total standard deviation in species data TAU = 0.524061

**** Correlation matrix ****

SPEC AX1	1.0000				
SPEC AX2	0.1428	1.0000			
SPEC AX3	-0.0828	-0.0391	1.0000		
SPEC AX4	0.0316	-0.2353	0.2115	1.0000	
ENVI AX1	0.8282	0.0000	0.0000	0.0000	1.0000
ENVI AX2	0.0000	0.6184	0.0000	0.0000	0.0000
	1.0000				

ENVI AX3	0.0000	0.0000	0.6667	0.0000	0.0000
	0.0000	1.0000			
ENVI AX4	0.0000	0.0000	0.0000	0.4221	0.0000
	0.0000	0.0000	1.0000		
BD	-0.2488	0.3874	0.3063	-0.1353	-0.3004
	0.6264	0.4594	-0.3205		
GWC	0.7953	-0.0661	0.0612	-0.0377	0.9602
	-0.1069	0.0919	-0.0894		
OM	0.0151	0.2708	-0.0807	0.2695	0.0182
	0.4379	-0.1211	0.6386		
pH	0.1842	0.2525	-0.3723	-0.2148	0.2224
	0.4083	-0.5584	-0.5090		
Nitro	0.2220	-0.2900	0.1161	-0.0166	0.2681
	-0.4690	0.1741	-0.0394		
Phos	0.0475	0.0304	-0.0277	-0.1478	0.0573
	0.0492	-0.0415	-0.3501		
MicroE	-0.1178	-0.4632	-0.1118	0.0057	-0.1422
	-0.7490	-0.1676	0.0136		

	SPEC AX1	SPEC AX2	SPEC AX	SPEC AX4	ENVI AX1
	ENVI AX2	ENVI AX3	ENVI AX4		
BD	1.0000				
GWC	-0.3393	1.0000			
OM	0.1521	-0.1449	1.0000		
pH	0.1827	0.1097	-0.2101	1.0000	
Nitro	-0.1521	0.1481	-0.0770	-0.0580	1.0000
Phos	0.0087	0.0859	0.2184	-0.1398	0.0809
	1.0000				
MicroE	-0.3866	-0.0050	-0.1469	-0.3557	-0.0357
	0.0086	1.0000			

	BD	GWC	OM	pH	Nitro
	Phos	MicroE			

N	name	(weighted) mean	stand. dev.	inflation factor
1	SPEC AX1	0.0000	1.2075	
2	SPEC AX2	0.0000	1.6170	
3	SPEC AX3	0.0000	1.4999	
4	SPEC AX4	0.0000	2.3694	
5	ENVI AX1	0.0000	1.0000	
6	ENVI AX2	0.0000	1.0000	
7	ENVI AX3	0.0000	1.0000	
8	ENVI AX4	0.0000	1.0000	
1	BD	1.8116	0.0683	1.4128

2	GWC	1.6418	0.0660	1.2231
3	OM	1.1968	0.1815	1.1956
4	pH	7.9092	0.1520	1.2971
5	Nitro	1.1378	0.2330	1.0646
6	Phos	2.8326	0.3460	1.0891
7	MicroE	1.3074	0.3259	1.3946

**** Summary ****

Axes	1	2	3	4	Total variance
Eigenvalues:	0.154	0.042	0.022	0.017	1.000
Species-environment correlations:	0.828	0.618	0.667	0.422	
Cumulative percentage variance					
of species data:	15.4	19.6	21.9	23.6	
of species-environment relation:	58.0	73.9	82.3	88.7	
Sum of all eigenvalues					1.000
Sum of all canonical eigenvalues					0.266

**** Summary of Monte Carlo test ****

Test of significance of first canonical axis: eigenvalue = 0.154
F-ratio = 7.651
P-value = 0.0020

Test of significance of all canonical axes: Trace = 0.266
F-ratio = 2.170
P-value = 0.0020

B. OHM 2007 Species_2006/2007 Environment

*** Type of analysis ***
Model Gradient analysis
indirect direct
linear 1=PCA 2= RDA
Type analysis number
Answer = 2

Forward selection of envi. variables = 0
Scaling of ordination scores = 2
Diagnostics = 1
No samples omitted
Number of samples 50
Number of species 16
Number of occurrences 155
No transformation of species data
No species-weights specified
No sample-weights specified
Centering/standardization by species = 1
Centering/standardization by samples = 0
No. of active samples: 50
No. of passive samples: 0
No. of active species: 16
Total sum of squares in species data = 197.296
Total standard deviation in species data TAU = 0.496609

**** Correlation matrix ****

SPEC AX1	1.0000				
SPEC AX2	0.1758	1.0000			
SPEC AX3	-0.1118	-0.0682	1.0000		
SPEC AX4	0.0877	0.1936	-0.2764	1.0000	
ENVI AX1	0.7660	0.0000	0.0000	0.0000	1.0000
ENVI AX2	0.0000	0.5141	0.0000	0.0000	0.0000
	1.0000				
ENVI AX3	0.0000	0.0000	0.4825	0.0000	0.0000
	0.0000	1.0000			
ENVI AX4	0.0000	0.0000	0.0000	0.4358	0.0000
	0.0000	0.0000	1.0000		
BD	0.3456	-0.3709	0.1779	-0.0449	0.4511
	-0.7215	0.3687	-0.1031		
GWC	-0.6961	-0.0649	0.0320	0.0211	-0.9087
	-0.1263	0.0663	0.0485		

OM	0.0230	-0.2232	-0.0887	-0.0973	0.0300
	-0.4342	-0.1838	-0.2232		
pH	-0.1579	-0.2175	-0.1429	0.1188	-0.2062
	-0.4231	-0.2962	0.2727		
Nitro	-0.2543	0.1470	0.0776	0.2076	-0.3319
	0.2860	0.1609	0.4765		
Phos	0.0970	-0.1428	-0.2417	0.1762	0.1267
	-0.2778	-0.5009	0.4043		
MicroE	-0.0395	0.2652	-0.2005	-0.2685	-0.0516
	0.5159	-0.4155	-0.6162		

	SPECAX1	SPECAX2	SPECAX3	SPECAX4	ENVIAX1
	ENVIAX2	ENVIAX3	ENVIAX4		

BD	1.0000				
GWC	0.3393	1.0000			
OM	0.1521	-0.1449	1.0000		
pH	0.1827	0.1097	-0.2101	1.0000	
Nitro	-0.1521	0.1481	-0.0770	-0.0580	1.0000
Phos	0.0087	0.0859	0.2184	-0.1398	0.0809
	1.0000				
MicroE	-0.3866	-0.0050	-0.1469	-0.3557	-0.0357
	0.0086	1.0000			

	BD	GWC	OM	pH	Nitro
	Phos	MicroE			

N	name	(weighted) mean	stand. dev.	inflation factor
1	SPEC AX1	0.0000	1.3055	
2	SPEC AX2	0.0000	1.9453	
3	SPEC AX3	0.0000	2.0726	
4	SPEC AX4	0.0000	2.2949	
5	ENVI AX1	0.0000	1.0000	
6	ENVI AX2	0.0000	1.0000	
7	ENVI AX3	0.0000	1.0000	
8	ENVI AX4	0.0000	1.0000	
1	BD	1.8116	0.0683	1.4128
2	GWC	1.6418	0.0660	1.2231
3	OM	1.1968	0.1815	1.1956
4	pH	7.9092	0.1520	1.2971
5	Nitro	1.1378	0.2330	1.0646
6	Phos	2.8326	0.3460	1.0891
7	MicroE	1.3074	0.3259	1.3946

**** Summary ****

Axes	1	2	3	4	Total variance
Eigenvalues:	0.095	0.055	0.024	0.016	1.000
Species-environment correlations:	0.766	0.514	0.482	0.436	
Cumulative percentage variance					
of species data:	9.5	15.1	17.4	19.0	
of species-environment relation:	44.3	70.0	80.9	88.5	
Sum of all eigenvalues					1.000
Sum of all canonical eigenvalues					0.215

**** Summary of Monte Carlo test ****

Test of significance of first canonical axis: eigenvalue = 0.095
F-ratio = 4.425
P-value = 0.0260

Test of significance of all canonical axes: Trace = 0.215
F-ratio = 1.645
P-value = 0.0080