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Increasing atmospheric nitrogen deposition: implications for tallgrass prairie restoration

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A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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INCREASING ATMOSPHERIC NITROGEN DEPOSITION: IMPLICATIONS FOR
TALLGRASS PRAIRIE RESTORATION

Thesis format: Monograph

by

Jennifer M^cPhee

Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

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Abstract

Continued intensification of agriculture and combustion of fossil fuels will increase rates of atmospheric nitrogen (N) deposition over the next century. N is typically a limiting resource for terrestrial plants, and many species are adapted to low-N conditions. Increased N availability can affect both plant biomass and species composition, often favouring N-demanding, adventive species. These effects can be adverse in the context of ecological restoration projects, where the aim is to establish a particular species composition. I used a field experiment in Norfolk County, Ontario, to examine how N addition affects species composition and plant productivity of a tallgrass prairie restoration. I predicted that N addition would increase the abundance of plant species not included in the original seeding. Contrary to my prediction, relative abundance of native, rather than adventive species, increased with N addition, although the latter species were scarce at the site, possibly as a result of dispersal limitation. I conclude that increased N availability can enhance the growth of tallgrass prairie species in the first few years of restoration.

Keywords

Tallgrass Prairie, Restoration, Atmospheric Nitrogen Deposition, Plant Productivity

Co-Authorship Statement

Any manuscript(s) published based on the contents of this thesis will be co-authored by Dr. Hugh Henry, Laura Borden and the late Dr. Jane Bowles. The experimental plots used for this research were designed, maintained and monitored by Dr. Hugh Henry since 2010. Canada Horseweed (*Erigeron canadensis*) data for 2011 were collected by Laura Borden as part of her undergraduate thesis project, and Jane Bowles aided in species identification and data analysis.

In memory of Jane Bowles

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List of Abbreviations

ANOSIM	Analysis of Similarities
ANOVA	Analysis of Variance
C	Carbon
CO ₂	Carbon dioxide
DCA	Detrended Correspondence Analysis
DNA	Deoxyribonucleic acid
g	Grams
H	Hydrogen
H'	Shannon-Wiener diversity index
Km	Kilometre
log	Logarithmic
m	Metre
mm	Millimetres
N	Nitrogen
n	Sample size
N ₂ O	Nitrous oxide
NCC	Nature Conservancy Canada
NH ₃	Ammonia
NO ₃	Nitrate
NO _x	Nitrogen oxides
O	Oxygen
P	Probability
SE	Standard error
Sqrt	Square root
R	Correlation coefficient
Tg	Teragram
y	Year
°C	Degrees Celsius

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Chapter 1 - Introduction

1.1. Atmospheric nitrogen deposition

1.1.1. N in the environment

N is required for the formation of DNA, chlorophyll, and amino acids, and is thus required in high quantities relative to other mineral nutrients for plant growth and reproduction (Graham *et al.* 2006). However, the supply of N available for plant use is limited (Galloway *et al.* 2004). Earth's atmosphere is 78% N, yet, less than one percent of this N is available for use by most plants (Freedman, 2006; Galloway *et al.*, 2004). For plants to be able to use N, it must first be converted from un-reactive N₂ gas to reactive forms of N (Bobbink *et al.* 2010). The triple bond holding the two atoms of N₂ gas together is very strong, therefore a large energy expenditure is needed to separate them (Driscoll 1997). The separation of these two atoms is called N fixation. Natural N fixation can be caused by the high temperatures of lightning or by N fixing microorganisms (Dentener *et al.* 2006). N can also be fixed via anthropogenic activities such as the combustion of fossil fuels, or by the Haber-Bosch process (Galloway *et al.*, 2008). Once two N atoms are separated, H or O atoms can combine with these N atoms to make reactive N compounds such as ammonia (NH₃) or N oxides (NO_x), including nitrous oxide (N₂O), and nitrate (NO₃⁻) (Vitousek *et al.* 1997; Freedman 2006).

Synthetic ammonia produced using the Haber-Bosch process is used in the fabrication of many products (e.g. nylon, plastics, explosives, and fertilizers), and the processes used in the creation of these products give off harmful NO_x and NH₃ gases (Galloway *et al.* 2008). The addition of synthetic fertilizer to agricultural fields has substantially increased food production, but has also increased N pollution in the process (Galloway *et al.* 2008). The combined effects of agricultural intensification and increased release of reactive N, caused by fossil fuel combustion for transportation and industry, has increased emissions of N pollution into the atmosphere over the last century to above that of natural inputs (Galloway *et al.* 2004). Atmospheric N deposition occurs most heavily around industrial and

agricultural areas (Dentener *et al.* 2006), but N pollution can also travel thousands of kilometers once it enters the stratosphere (Bobbink *et al.* 2010). Typically, NH_3 makes up the majority of atmospherically-deposited N near agricultural areas, while NO_3^- is typically deposited near industrial areas (Galloway *et al.* 2004).

Increased emissions of N pollution have increased the rate at which N is being deposited across the landscape in the form of NH_3 and NO_3^- compounds in dust or precipitation (Vitousek *et al.* 1997). Prior to widespread anthropogenic N pollution, natural atmospheric N deposition was not a significant input into ecosystems, biological N fixation being the main contributor (Galloway *et al.* 2008). However, this is no longer the case, and anthropogenic N fixation is now one of the most dominant sources of N for terrestrial ecosystems at 268 Tg N y^{-1} globally, while natural N fixation accounts for about 112 Tg N y^{-1} (Galloway *et al.* 2008). Global atmospheric N deposition rates have more than tripled from 1860 to the early 1990's, from 31.6 to 103 Tg N y^{-1} , and are projected to almost double again by 2050, to 195 Tg N y^{-1} (Galloway 2005). This is more than four times higher than the natural rate of N deposition ($< 50 \text{ Tg N y}^{-1}$ on average) (Galloway *et al.* 2008). In southern Ontario, the current atmospheric N deposition rate is approximately 1 to $2 \text{ g N m}^{-2} \text{ y}^{-1}$, and this rate is expected to increase to between 2 and $5 \text{ g N m}^{-2} \text{ y}^{-1}$ over the next 40 years (Galloway *et al.* 2004; Aherne & Posch 2013).

1.1.2. Plant species composition responses to increased atmospheric N deposition

Due to post-industrial increases in N pollution, atmospheric N deposition is now one of the most critical threats to ecosystem health (Payne *et al.* 2013), especially in areas that have experienced elevated deposition rates for several decades (Phoenix *et al.* 2006). In field experiments examining the combined effects of global change factors, atmospheric N deposition is consistently among the strongest factors altering plant productivity and community composition (Torok *et al.* 2000; Miles & Knops 2009). Understanding how atmospheric N deposition affects plant species composition is especially important in N limited systems, where plants are adapted to low N availability (Tilman 1985). Plant species that are adapted to low N availability are efficient at N sequestration, which intensifies N limitation in the system and limits the establishment of faster growing species that have

higher N demands (Aber & Nadelhoffer 1989; McLendon & Redente 1992). Dentener *et al.* (2006) found that atmospheric N deposition levels have already surpassed the critical N threshold, the exposure level at which significant detrimental effects will begin to occur, in approximately 10% of all naturally occurring vegetation, indicating that ecosystem functions in these systems may already be altered.

When N inputs increase, plants initially responds with increased productivity and biomass (Aerts *et al.* 1999; An *et al.* 2005; Morford *et al.* 2011) and some species are better able to utilize the added N for rapid increases in productivity than others (McLendon & Redente 1992; An *et al.* 2005). Since increased N deposition increases plant productivity in many systems, it can also significantly alter plant species composition (Carson & Barrett 1988; Wedin & Tilman 1990; Torok *et al.* 2000), often in favor of fast-growing adventive species (Miles & Knops 2009). On soils with historically high N availability, which are able to support rapid plant growth, the effects of competition can be intensified (Wilson & Tilman 1991). Once fast-growing species begin to out compete slower growing species for remaining resources, there can be reductions in species diversity and richness (Carson & Barrett 1988; Wedin & Tilman 1990; Torok *et al.* 2000).

1.2. Restoration of plant communities in the context of increased atmospheric N deposition

In the context of global environmental change, the question has arisen as to whether plant communities are best restored to their historical species composition or whether an attempt should be made to develop restoration goals under anticipated future environmental conditions (Temperton *et al.* 2004). The addition of N to an ecosystem often has a greater effect on plant species composition than the addition of phosphorus, potassium, calcium, magnesium, sulfur or trace metals (Tilman 1987). Increases in other factors such as CO₂, drought, temperature and the interactions between these factors can also affect how species react to increased N deposition (Turner & Knapp 1996; Bond 2008). Alteration of plant competition outcomes due to increased atmospheric N deposition could alter the establishment and persistence of species targeted for restoration under traditional strategies.

Despite these general insights into the effects of N addition on plant communities, the specific effects of increased atmospheric N deposition on the outcome of restoration projects have not been widely studied. By recognizing the key factors involved, property managers are able to make more effective decisions about how to restore habitats (Torok *et al.* 2000).

1.3. N deposition and tallgrass prairie restoration

Tallgrass prairies are grassland ecosystems native to central North America that were historically regulated by a fire disturbance regime, as well as by grazing of large mammals (Axelrod 1985; Stephen Packard & Mutel 1997). Prairie grasslands are generally dominated by between one and three grass species that cover more area than all other species combined (Miles & Knops 2009). The dominant species present at a site help determine the species trajectory, at a site by competitively excluding some species and not others (Wedin & Tilman 1992; Nyamai *et al.* 2011). The dominant or matrix species for tallgrass prairies are big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), yellow indiangrass (*Sorghastrum nutans*) and switchgrass (*Panicum virgatum*) (Miles & Knops 2009). These grasses are all native to the central regions of North America (Stephen Packard & Mutel 1997). The majority of grass species in the tallgrass prairies are C₄ photosynthetic bunchgrasses, including *S. scoparium* and *S. nutans*; however, *A. gerardii* can act as either a bunchgrass or a sod-forming grass with very short stolons, depending on the amount of competition it has from other species (McGregor *et al.* 1991; Emery & Gross 2007). Tallgrass prairies also feature an enormous diversity of forbs and woody shrubs. Over 150 plant species are found in tallgrass prairies in southern Ontario, 12 of which are endangered, six are threatened, and two are of special concern for extinction either provincially or nationally (Delaney *et al.* 2000; COSEWIC 2007). Tallgrass prairies farther south have higher species diversity with over 350 species, two thirds of which are forb species (Howe 1994). In southern Ontario, some animal species of concern that are typically found in tallgrass prairies include the Eastern fox snake (*Pantherophis gloydi* Conant), Northern bobwhite (*Colinus virginianus* L.), mottled dusky wing (*Erynnis martialis*

Scudder), American badger (*Taxidea taxus* Schreber) and Henslow's sparrow (*Ammodramus henslowii* Audubon) (COSEWIC 2007).

Due to the extensive habitat loss since the 19th century, tallgrass prairies are now one of the most severely degraded ecosystems in North America (Stephen Packard & Mutel 1997). In 1830, there were about 162 million ha of tallgrass prairie in North America, since then there has been an estimated 82-99% decline in the area cover by this ecosystem (Samson & Knopf 1994). In southern Ontario, the majority of tallgrass prairies have been converted to cropland, and as a result have been reduced from about 1000 km² to only 21 km² after the arrival of European settlers (Bakowsky & Riley 1994; Figure 1.1). This rapid conversion was due to the fact that prairies have deep, productive soils well suited for agricultural use (Curtis 1959; Camill *et al.* 2004). In recent decades, tallgrass prairie restoration efforts have been attempted (Delaney *et al.* 2000), particularly in areas of marginal agricultural potential, such as sandplains.

Tallgrass prairies are useful systems for testing the influence of increased N availability on restoration efforts, in part because “restored” prairie can be established in a relatively short time frame (3-20 years), and also because tallgrass systems have characteristically low soil nutrient availability (Schramm 1990; Seastedt *et al.* 1991). The species found in these prairies are adapted to low nutrient soils, and as a result are likely to be especially susceptible to the effects of increased N deposition (Nyamai *et al.* 2011). On sites with increased N availability of as little as 1-2 g N m⁻² y⁻¹ above natural levels, non-tallgrass species can eliminate native tallgrass species (Tilman 1990; Wedin & Tilman 1992). After a disturbance, such as that of restoration procedures in an area, there may be increased competition intensity associated with increased resource availability, for example light and space (Baer *et al.* 2003). Increased competition intensity in the initial establishment phase of restoration projects may cause this phase to be the most sensitive to changes in soil nutrient content, which would also cause soil conditions to be more important to in determining what species become dominant (Baer *et al.* 2003). Therefore, the first few years after the restoration process has begun are most critical for determining the success of the restoration process because, once tallgrass prairies mature and the species become well established, fast-growing adventive species may no longer able to successfully colonize the area without a disturbance (Gartshore 2011).

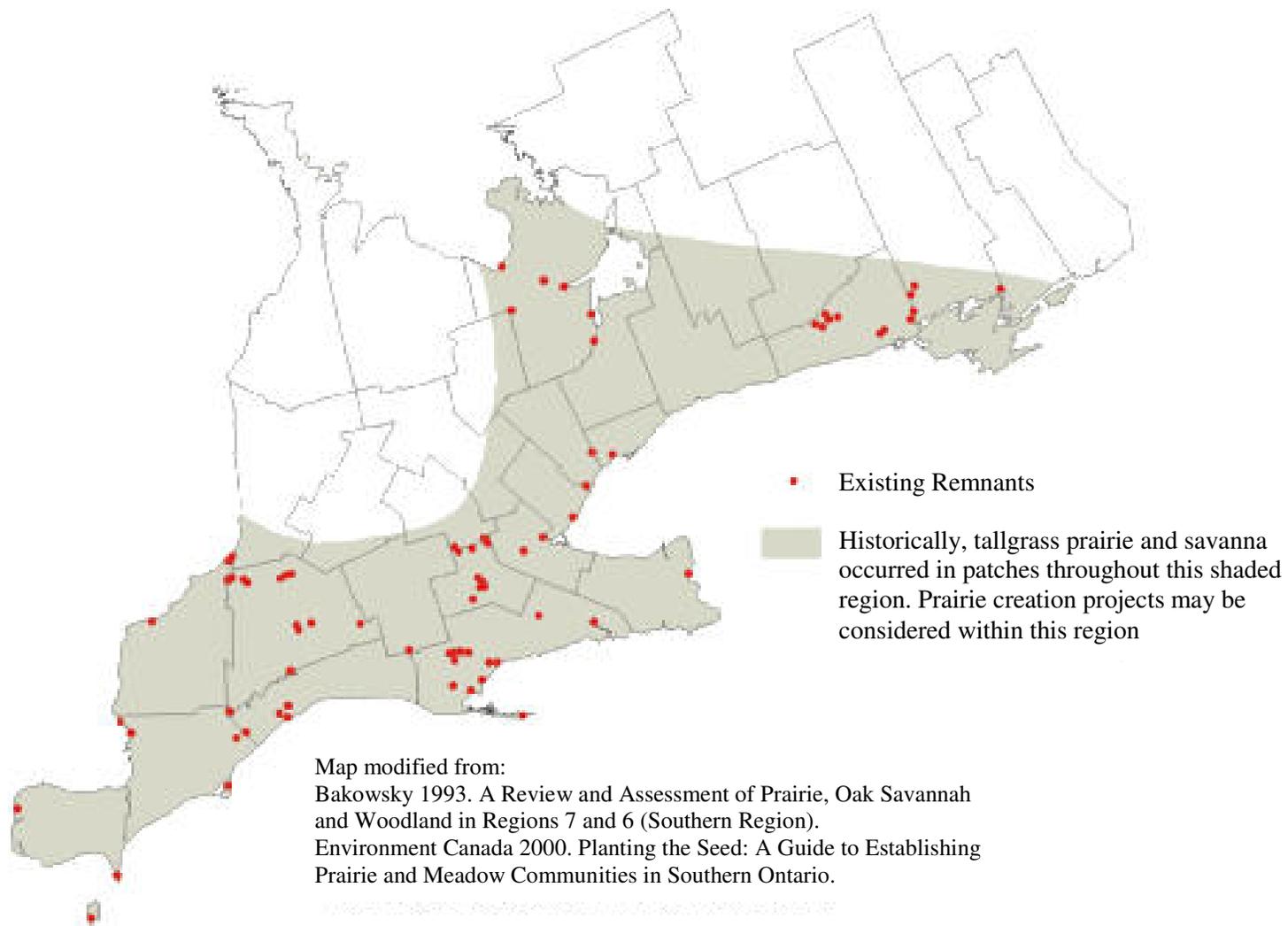


Figure 1.1. Range of historic and current locations of tallgrass prairie and savanna vegetation in southern Ontario, Canada. Re-production permission granted by Tallgrass Ontario (Appendix 1). *Source: Tallgrass Ontario (2013) [map] “Grassland ID: What are tallgrass communities”. Physiographic region: southern Ontario < http://www.tallgrassontario.org/ID_grassland.html>*

1.3.1. Competitive roles of tallgrass prairie species

The majority of tallgrass prairie grasses use the C₄ photosynthetic pathway, yet most of the adventive grasses that threaten to outcompete tallgrass species use the C₃ pathway. There are several key differences between these two plant groups that influence their ability to survive in prairie ecosystems. C₄ photosynthetic grasses in tallgrass prairies can grow as tall as three metres, with roots that can reach four metres deep (Ladd & Oberle 2005), as opposed to C₃ grasses, which do not grow as tall and have mostly horizontal root growth (Barbour *et al.* 1999). The near vertical root growth in tallgrass species allows them to acquire moisture and nutrients deep into soil (Barbour *et al.* 1999; Wedin 2004; Miles & Knops 2009). This high allocation to belowground biomass makes tallgrass C₄ species more resistant to drought, fire, and nutrient limitation than C₃ species (Wilson & Tilman 1991; Ladd & Oberle 2005). C₄ grasses also have high water and N use efficiency, meaning they generally use less N and moisture per unit of biomass compared to C₃ species (Wedin 2004; Miles & Knops 2009). Therefore, in natural, N-limiting conditions, the high N and water use efficiency of C₄ grasses, in conjunction with their deep root system, gives them a competitive advantage over the rapidly growing C₃ species (Wilson & Tilman 1991).

C₄ photosynthetic tallgrass species also differ from C₃, non-tallgrass species in that they have different growth requirements. For example, the photosynthetic temperature optima of C₄ grasses are higher than those of C₃ species (Wedin 2004). In addition, C₃ species have their peak abundance in the cooler part of the growing season, from late spring to early summer (Kemp & Williams 1980; McLendon & Redente 1992), while C₄ species have their peak abundance later in the season, from mid-June to late-August, at a time when the C₃ plants have begun to senesce (Kemp & Williams 1980; Miles & Knops 2009). Because of this temporal separation in peak abundances, the ability of C₃ species to compete with C₄ grasses early in the year is high (Kemp & Williams 1980). Ephemeral species also have an opportunity to establish before the C₄ species outcompete them for light, which increases the biodiversity of these communities (Kemp & Williams 1980). C₄ species have higher light use efficiency (Monteith 1978), but they also have a higher light requirement and lower shade tolerance than C₃ species (Kephart *et al.* 1992). The competitive advantage of C₄ species is reduced if the limiting resource switches from N or moisture to light (Wedin &

Tilman 1990). Therefore, they may lose some of their competitive advantage if they become shaded by a thick litter layer, or by C₃ species earlier in the growing season (Wedin 2004).

Increased atmospheric N deposition on tallgrass prairies could increase the competitive advantage of non-tallgrass species relative to tallgrass species, and ultimately decrease the biodiversity of these systems (Clark & Tilman 2008; Miles & Knops 2009). Under low N availability, the combination of high N use efficiency, poor quality litter and large belowground root biomass for nutrient uptake, will result in a positive feedback loop that internally reinforces the dominance of tallgrass C₄ species by keeping N availability low (Wedin & Tilman 1992; Mack *et al.* 2001; Fargione & Tilman 2005). However, increased N availability may facilitate invasion of these communities by C₃ grasses such as Kentucky bluegrass (*Poa pratensis*), quackgrass (*Elymus repens*), and smooth brome (*Bromus inermis*), which are effective at exploiting high N availability (Bakker & Berendse 1999; Vinton & Goergen 2006; Miles & Knops 2009). Most C₃ grasses, especially *P. pratensis*, can reproduce vegetatively by extending aboveground stolons or belowground roots and rhizomes that interweave to form a dense mat, making it difficult for other species to compete for space (McGregor *et al.* 1991). Also, most of the root growth in non-tallgrass species is horizontal rather than vertical, which makes it difficult for other species to compete for nutrients and moisture (Barbour *et al.* 1999). Turf-forming C₃ grasses that exhibit aggressive vegetative spread inhibit the establishment of other species and reduce biodiversity (Miles & Knops 2009), whereas most C₄ tallgrasses form clumps that allow other species to grow in between these patches (McGregor *et al.* 1991). C₄ grasses facilitate higher biodiversity because the space between bunches allows forb, shrub and tree species to co-exist with the grasses.

Site quality and site history affect species composition in both tallgrass prairie (Stephen Packard & Mutel 1997; Fargione & Tilman 2005; Thorne & Cardina 2007), and non-tallgrass communities (Tilman 1987; Bakker & Berendse 1999; Vinton & Goergen 2006; Payne *et al.* 2013) by influencing the dominant vegetation at the site (Miles & Knops 2009). However, increased atmospheric N deposition can have an influence on species composition by changing the competition dynamics between tallgrass and non-tallgrass species (Wedin & Tilman 1990, 1992, 1993; Tilman & Wedin 1991; Wedin 2004; Clark & Tilman 2008).

Nevertheless, little is known about how the effects of increased atmospheric N deposition on the dominant tallgrass vegetation will alter the trajectory of species composition in the context of tallgrass restoration, especially in the early stages of restoration. It is important to understand how N deposition will effect tallgrass prairie restoration, because tallgrass prairies are so well adapted to low N conditions that any changes in soil N concentrations may give an advantage to the highly competitive, N demanding, non-tallgrass species in early stages of restoration. This could have detrimental effects both ecologically and economically for tallgrass restoration programs.

1.4. Objectives and Hypothesis

I hypothesized that increased atmospheric N deposition will increase the abundance of non-tallgrass herbaceous species in newly restored tallgrass prairies. In order to test this hypothesis, the objective of my research project was to evaluate how N addition at a newly established tallgrass prairie restoration site would alter both plant productivity and species percent composition. I conducted a two-year vegetation survey in a recently established N addition experiment located in a tallgrass prairie restoration site and recorded changes in species composition, abundance, and biomass on a monthly basis. I predicted that N addition would increase plant productivity (i.e. increased rate of biomass production) of species that were not part of the initial tallgrass restoration seeding, and thus shift the species composition away from what was intended.

Chapter 2 - Methods and Materials

2.1. Study Site

My experiment was conducted in Norfolk County, Ontario at a tallgrass prairie restoration site (42.687078, -80.466565) that was established by the Nature Conservancy of Canada. The site is located on a sandplain that would have historically been a mix of tallgrass prairies and deciduous forest (Goodban *et al.* 1997). The restoration site is 20.9 ha in area, and was used as a tobacco farm until the early 2000's. St. William's Nursery was contracted to restore the site in the spring of 2010, at which point seeds were sown from a mix of a tallgrass prairie species, along with several native Carolinian tree species (Appendix 2). The tallgrass prairie seed mix contained only local genotypes, and would represent current/historic species composition that would be expected in local tallgrass prairie communities. The woody species were avoided when plots were selected for the experiment.

In June 2010, N addition plots were established at the site. These plots were arranged in eight experimental blocks at 25 m intervals along a transect. One block consisted of a set of three 2 m × 2 m plots spaced at least 1.5 m apart (Fig. 2.1). N treatments (0, 2, or 6 g N m⁻² y⁻¹) were randomly assigned to the plots within each block, and N was added every year in early May. These rates were chosen to represent current deposition rates, as well as low and high projections for N deposition rates in this region by the year 2050 (Galloway *et al.* 2004). N was added in the form of slow release Osmocote[®] pellets containing ammonium nitrate. A 10 cm buffer zone of N was added around the plots to minimize edge effects.

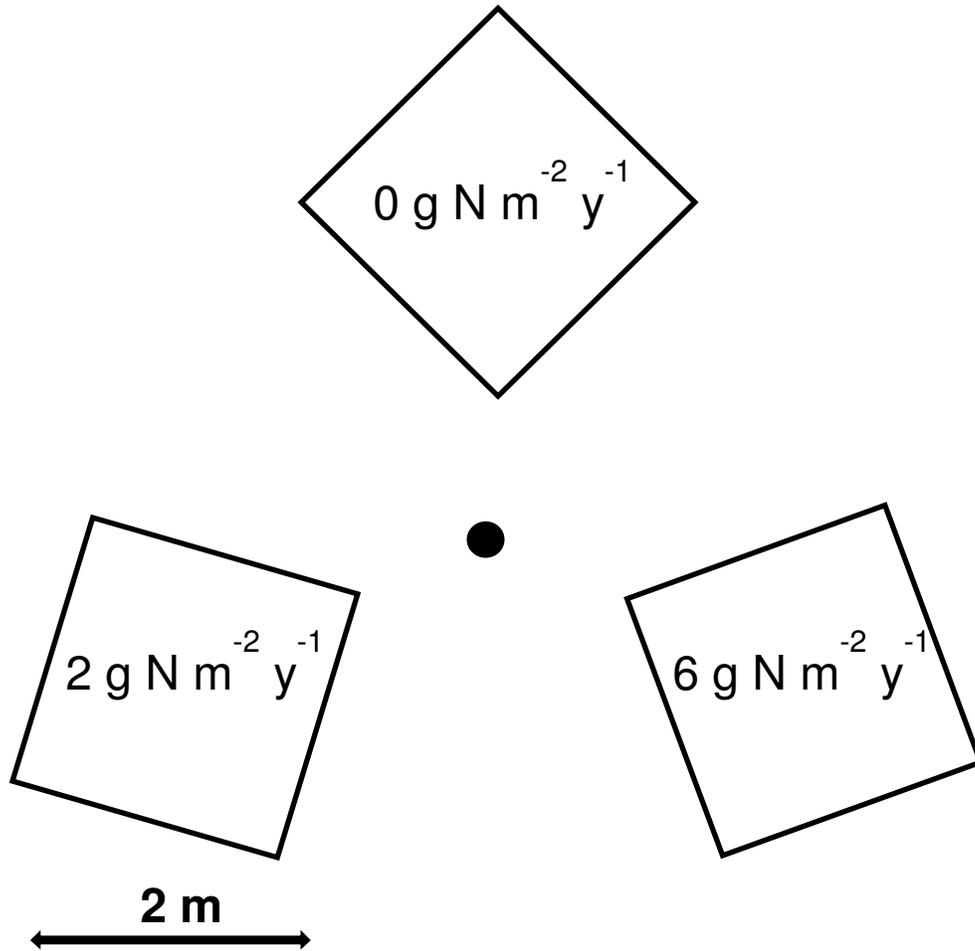


Figure 2.1. Overhead depiction of one of eight experimental blocks spaced 25 m apart along a transect. Each of the three $2 \text{ m} \times 2 \text{ m}$ plots within each block were randomly assigned 0, 2, or $6 \text{ g N m}^{-2} \text{ y}^{-1}$ of added N.

2.2. Data Collection

I conducted vegetation cover estimates at one-month intervals from May through October in both 2012 and 2013 to document changes in species composition and percent cover from within 1 m × 1 m subplots of each plot. I used the Domin-Krajina cover-abundance scale (Mueller-Dombois & Ellenberg 1974) to obtain cover percentage estimates on a per species basis. Due to the nature of the Domin-Krajina scale, using the mid-point of a range of percentages, mean percent cover may be higher than 100%. The total cover percentages could also exceed 100% in plots with more than one leaf layer per unit ground area. I identified species in the field, if possible, and collected representative specimens from outside of the plots. If identification was not possible in the field, I completed the identification of specimens in the herbarium at Western University, Canada.

I collected standing aboveground biomass samples from each plot in 2012, first in the middle of July (peak biomass for many of the species) and again at the beginning of September (peak biomass for tallgrass). I clipped the biomass samples from 25 cm × 25 cm subplots to the soil level, kept them cool, then separated the shoots by species when possible, keeping litter separate. I then dried all of the plant material for at least 48 hours at 65 °C and weighed it.

Belowground biomass samples were collected at the beginning and end of each of the two field seasons. I collected the samples using a 2 cm diameter corer to a depth of 15 cm deep at two uniform locations within each plot for each of the four soil sampling periods. I stored the soil in a refrigerator until further analysis, and then washed the soil from the roots using a series of sieves and reverse osmosis water. I then separated the roots from other organic material and the remaining soil using tweezers, dried the roots at 65°C for at least 48 hours, and weighed them.

2.3. Data analyses

I used the maximum cover percentage (i.e. peak cover from May to October) to represent the abundance of each plant species for each plot during both 2012 and 2013. These values were then used to calculate the total percent cover for various categories, for example functional group, native or adventive (non-native). Data from Rodger (1998) was used to classify species into prairie indicator indicator or non-prairie indicator, while the rest of the floristic quality information was acquired from Total aboveground biomass was also calculated for the same categories. Because there were many species only present in a subset of plots, the plot by species matrix contained many zeros, and in many cases the data did not fit the assumption of normality. In these cases I used Wilcoxon/Kruskal-Wallis tests for Rank Sums, with block as a factor, to analyze treatment effects. For data pooled over species that fit the normality assumption I used an Analysis of Variance (ANOVA) with a randomized block design with N treatment and year as factors. Community diversity was calculated using the Shannon diversity index (H'). The formula to calculate H' is:

$$H' = -\sum(p_i \ln p_i); H' \geq 0$$

where p_i is the relative proportion of total percent cover for the i th species in each plot, Species diversity was calculated for each of the N treatments then averaged across N treatments.

Detrended Correspondence Analysis (DCA) was used to identify clusters of similar plots in ordination space based on how the N addition treatments related to species abundance. The plots that are most similar to each other cluster together in the ordination. I used Analysis of Similarities (ANOSIM), in conjunction with a DCA, to assess treatment effects on relative species abundance at the community level. I conducted all data analysis using JMP 10.0 (SAS Institute Inc., Cary, NC, USA), with the exception of the ANOSIM and DCA, for which I used PAST (Hammer *et al.* 2001).

Chapter 3 - Results

3.1. Interannual weather variability

In the winter of November 2011 through April 2012 the study site experienced relatively dry conditions, with a mean winter temperature 2.7 °C higher than the 1971-2000 normal for the region, and mean monthly winter precipitation 52% less than normal (thus low snow accumulation) (Table 3.1). The mean temperature over the 2012 plant growing season (May to October) was very similar to the climate normal, however there was 37% less precipitation than normal at this time (Table 3.1). The following winter from November 2012 to April 2013, was close to normal with respect to temperature and precipitation, and while the mean temperature for the 2013 growing season (May to October) was 2.2 °C higher than the climate normal, precipitation at this time was only 10% less than normal (Table 3.1).

Table 3.1. Mean monthly temperatures and mean precipitation for winter 2012 (Nov. 2011 to Apr. 2012), growing season 2012 (May 2012 to Oct. 2012), winter 2013 (Nov 2012 to Apr. 2013), and growing season 2013 (May 2013 to Oct. 2013) for Delhi, Ontario, \approx 26 km from the field site, as well as climate normal from 1971 to 2000.

Mean Monthly Temperature ($^{\circ}$ C)	1971-2000	2012	2013
Winter	-0.6	2.2	-1.0
Growing season	16.3	16.1	17.62
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Mean Monthly Precipitation (mm)			
Winter	81.5	39.2	84.9
Growing season	86.8	54.3	78.16

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: Environment Canada, National Climate Data and Information Archive.
<http://climate.weather.gc.ca/data_index_e.html>

3.2. Percent cover estimates

There were no significant effects of N addition on mean total percent cover (i.e. the sum of percent cover values for all species) in 2012, but in 2013 mean total percent cover was significantly higher in the N addition plots than in the ambient plots ($P=0.019$; Fig. 3.1). Averaged among treatments, mean total percent cover was also significantly higher in 2013 than it was in 2012 ($P<0.001$; Fig. 3.1). The percentage of bare ground did not differ significantly among treatments (Fig. 3.2), but overall it was significantly lower in 2013 than it was in 2012 ($P<0.001$; Fig.3.2).

Of the 61 species chosen for the tallgrass prairie restoration seed mix, 100% of the species were native, and 41% of the species were also indicative of tallgrass prairies (Appendix 2). Out of the 61 species originally sown, 22 of these seeded species were recorded in the plots by the third year (Appendix 3). Although only 41% of the species planted as part of the prairie restoration mix were tallgrass species, 65% of the seeded species that were present in the plots were tallgrass prairie species (Appendix 3). Of the 17 species found in the plots that were not originally seeded, 41% were native, and 0% were tallgrass indicators (Appendix 3).

In both years, mean total percent native species cover was higher than that of adventive species ($P<0.001$; Fig. 3.3). The mean total percent cover of native species increased significantly with N addition in both 2012 ($P=0.022$) and 2013 ($P=0.019$), and there were no significant effects of N addition on the mean total percent cover of adventive species in either year (Fig. 3.3). There were no adventive grasses present in any of the sub-plots used for cover sampling. *Elymus trachycaulus* was the only grass species present that was not indicative of tallgrass prairies, and it occurred with the lowest cover values in the $0 \text{ g N m}^{-2} \text{ y}^{-1}$ plots in both years (2012, $P=0.049$; 2013, $P=0.013$; data not shown).

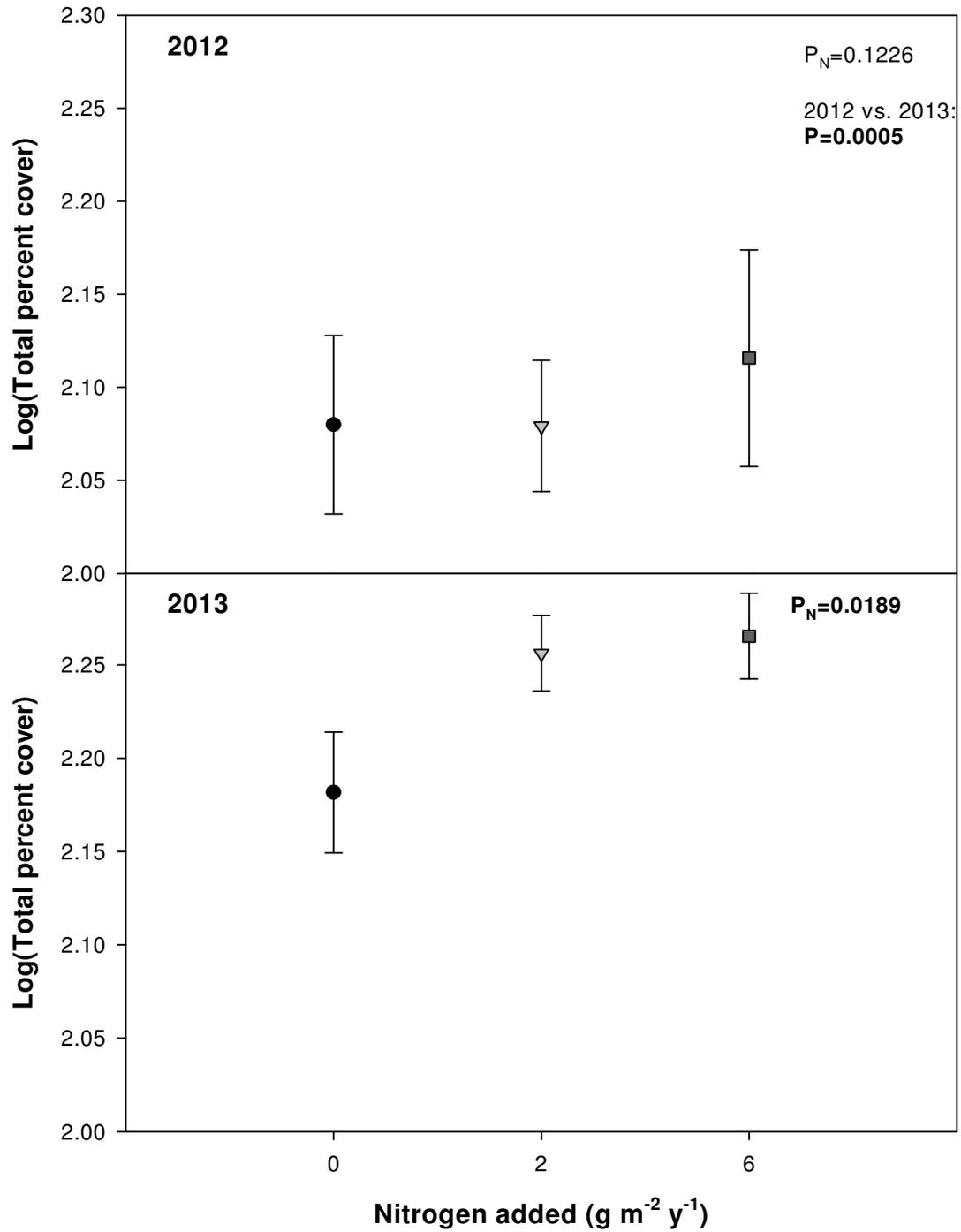


Figure 3.1. Log transformed data for mean total percent cover ± 1 SE by N treatments for the 2012 and 2013 growing seasons ($n=8$). P-values obtained using Wilcoxon/Kruskal-Wallis tests. Statistically significant values are bolded ($P < 0.05$). Data for comparison across years were pooled over N treatments.

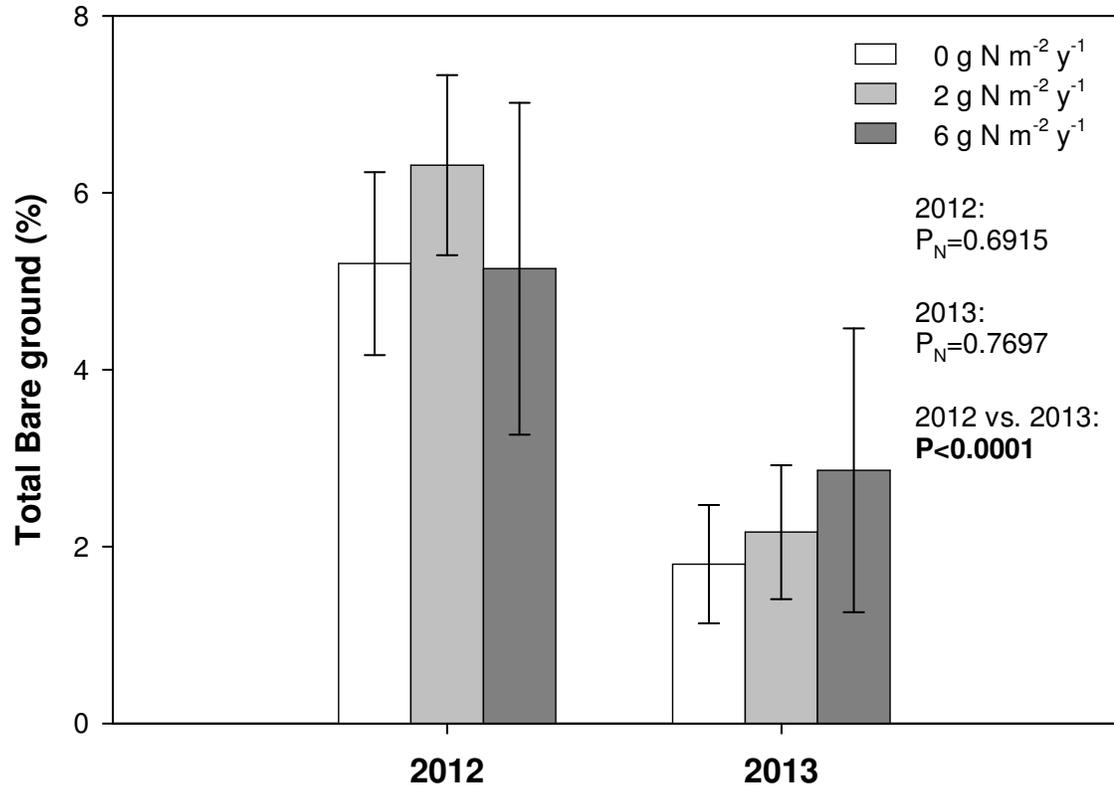


Figure 3.2. Mean total percent of bare ground ± 1 SE by N treatments for the 2012 and 2013 growing seasons (n=8). P-values obtained using a one-way ANOVA. Statistically significant values are bolded (P<0.05). Data for comparison across years were pooled over N treatments.

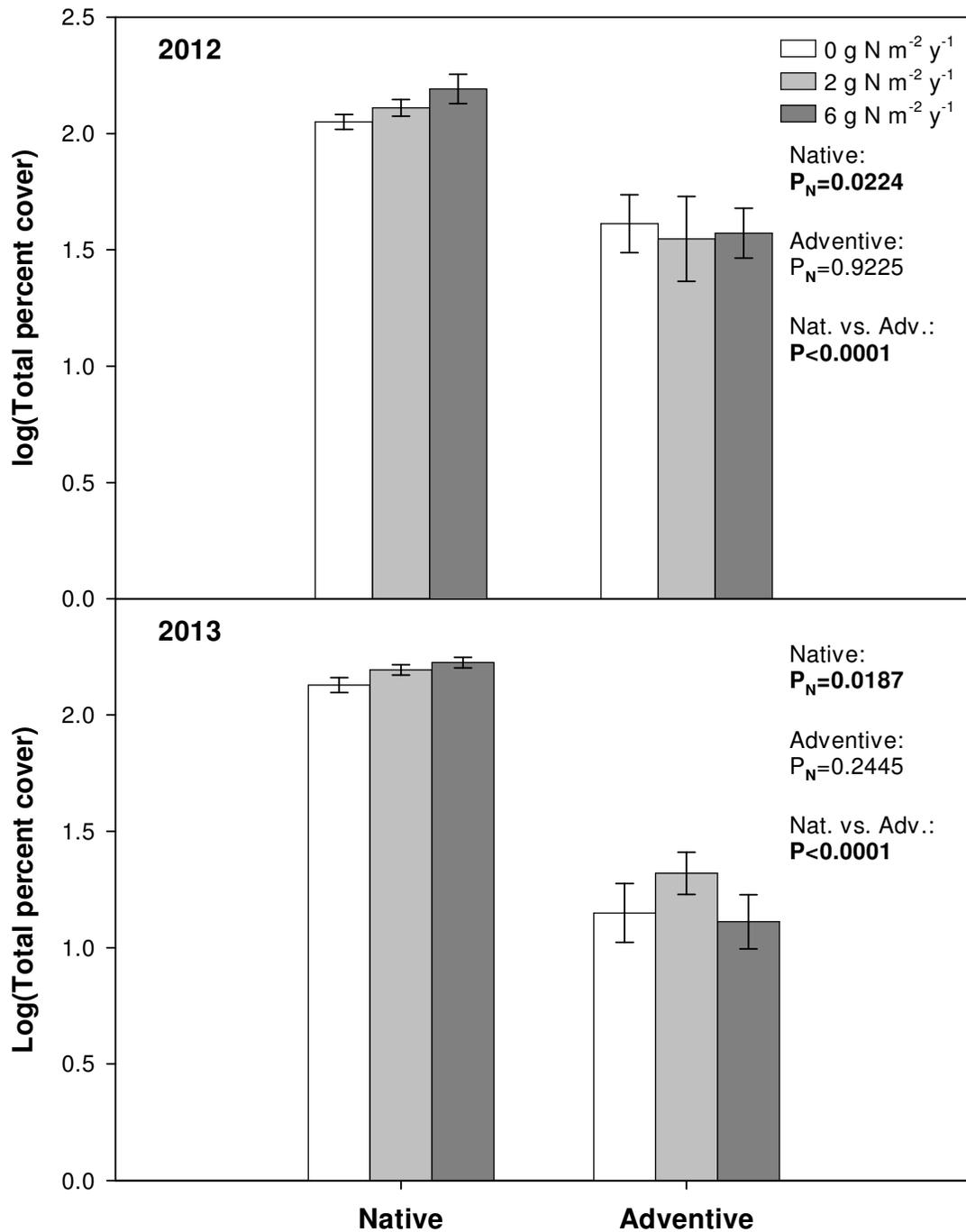


Figure 3.3. Log transformed data for mean total percent cover ± 1 SE separated by N treatments for both native or adventive species for the 2012 and 2013 growing seasons ($n=8$). P-values obtained using Wilcoxon/Kruskal-Wallis tests. Statistically significant values are bolded ($P<0.05$). Data for comparison between native and adventive species were pooled over N treatments.

In 2012, there were no significant differences in mean total percent cover between seeded and non-seeded species, nor were there any significant treatment effects on these categories (Fig. 3.4). However, in 2013, species that were seeded as part of the original restoration seed mix followed a trend similar to that of native flora, in that they had significantly larger mean total percent cover than that of non-seeded species ($P < 0.001$). The mean total percent cover of seeded species also increased significantly with N addition ($P = 0.027$), whereas there was no significant effect of N addition on the mean total percent cover of non-seeded species (Fig. 3.4).

The mean total percent cover of tallgrass prairie indicator species was not significantly different than that of non-prairie indicator species in 2012, whereas prairie species had a higher mean total cover in 2013 ($P < 0.001$; Fig. 3.5). In 2012, the mean total percent cover did not significantly increase with N addition for prairie indicator species (Fig. 3.5). However, in 2013, the mean total percent cover of prairie indicator species increased in response to N addition ($P = 0.035$). The mean total percent cover of non-prairie indicator species was also significantly higher in the $2 \text{ g N m}^{-2} \text{ y}^{-1}$ than the $0 \text{ g N m}^{-2} \text{ y}^{-1}$ treatment in 2012, yet there was no significant increase in response to $6 \text{ g N m}^{-2} \text{ y}^{-1}$ relative to $0 \text{ g N m}^{-2} \text{ y}^{-1}$ or $2 \text{ g N m}^{-2} \text{ y}^{-1}$ ($P = 0.0224$; Fig. 3.5)

Mean total percent cover differed significantly among life history strategies between 2012 and 2013. In 2012, the mean total percent cover of biennials increased in response to N addition ($P = 0.031$), but there was no significant N addition effect in 2013 (Fig. 3.6). Conversely, in 2012 the mean total percent cover of perennials was not significantly affected by N addition, whereas in 2013 it increased significantly in response to N ($P = 0.041$; Fig. 3.6).

In 2012 by functional group, 68% of the mean total percent cover was represented by forbs, followed by 28% by grasses, 4 % by legumes and less than 1% of by horsetail. In 2013, the mean total percent cover of forbs decreased to 58%, while the percent cover of grasses (35%), legumes (5%) and horsetails (3%) increased.

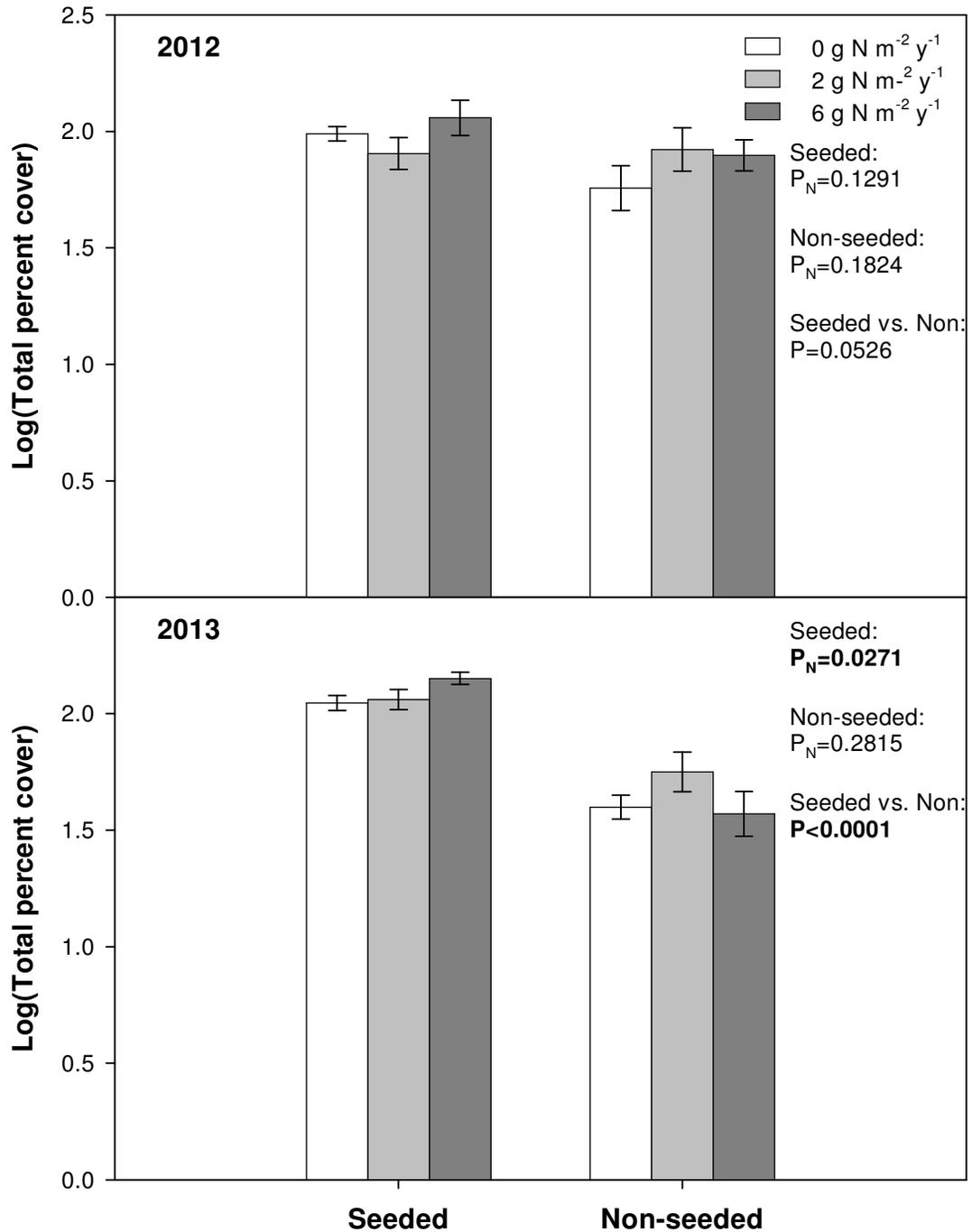


Figure 3.4. Log transformed data for mean total percent cover ± 1 SE separated by N treatments for both seeded and non-seeded for the 2012 and 2013 growing seasons ($n=8$). P-values obtained using Wilcoxon/Kruskal-Wallis tests. Statistically significant values are bolded ($P<0.05$). Data for comparison between seeded and non-seeded species were pooled over N treatments.

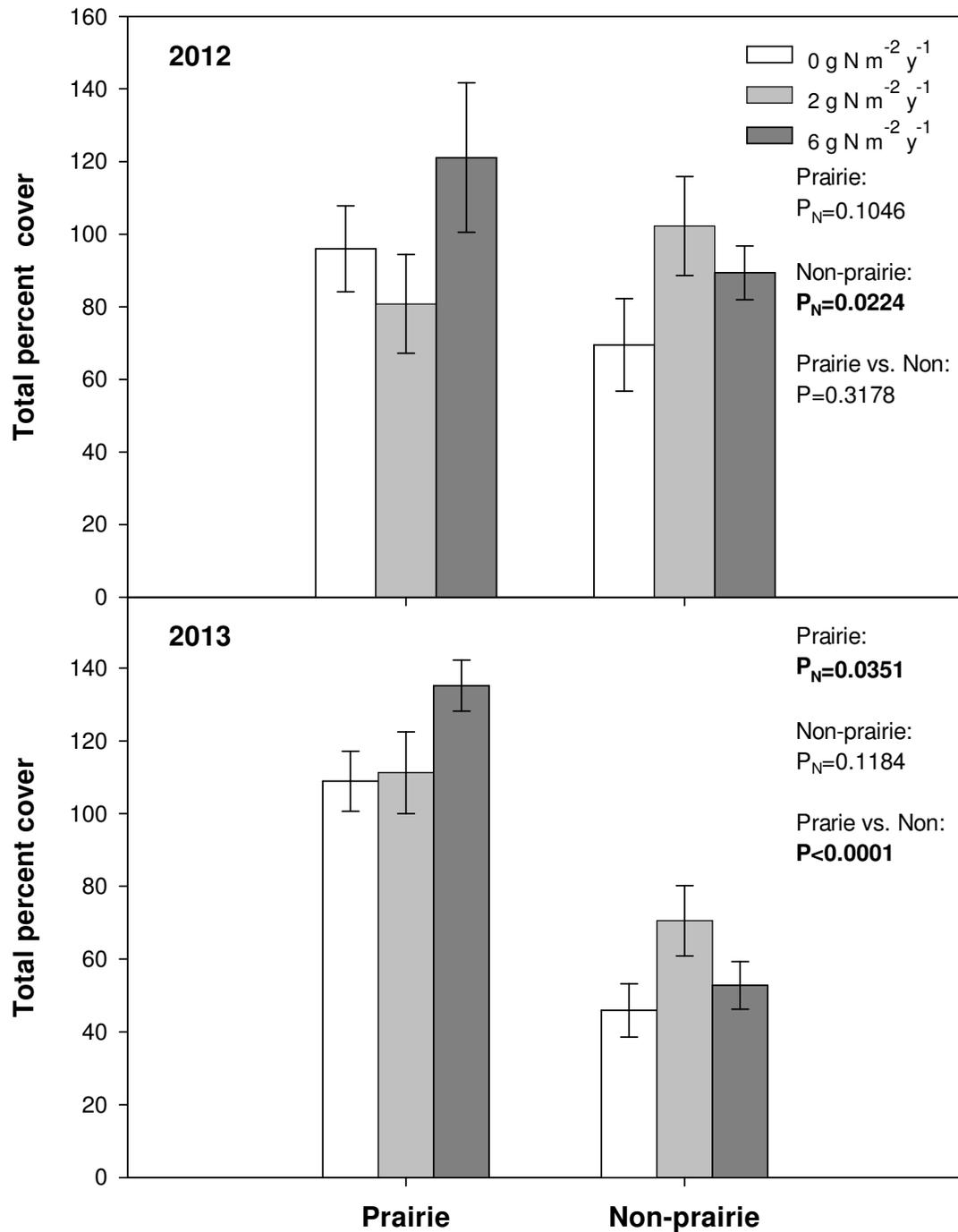


Figure 3.5. Mean total percent cover ± 1 SE separated by N treatments for prairie indicator species and non-prairie indicator species for the 2012 and 2013 growing seasons ($n=8$). P-values obtained using a one-way ANOVA. Statistically significant values are bolded ($P<0.05$). Data for comparison between prairie and non-prairie species were pooled over N treatments.

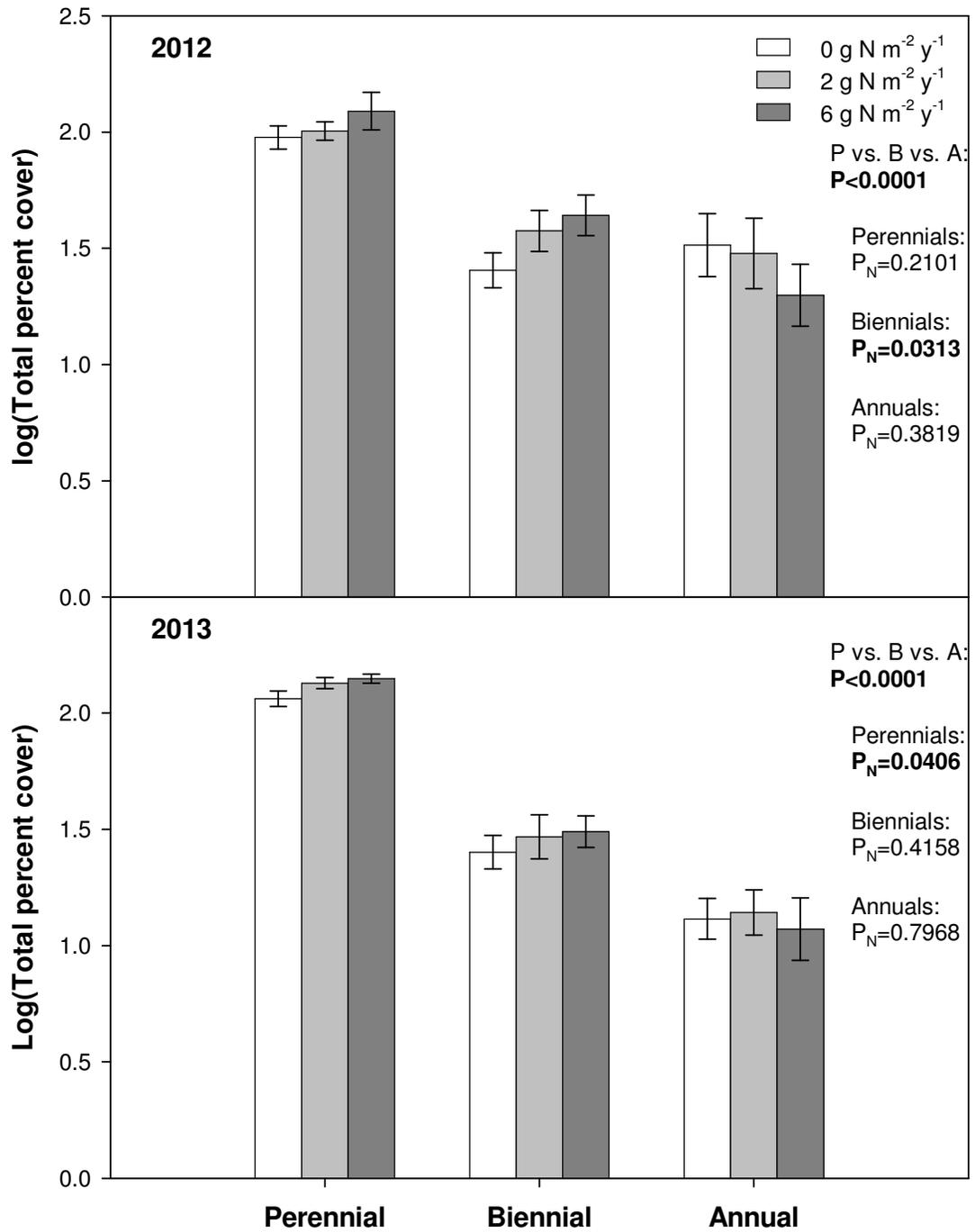


Figure 3.6. Log transformed data for mean total percent cover ± 1 SE separated by N treatments for each growth habit for the 2012 and 2013 growing seasons ($n=8$). P-values obtained using Wilcoxon/Kruskal-Wallis tests. Statistically significant values are bolded ($P < 0.05$). Data for comparison between growth habits were pooled over N treatments.

Among functional groups, the results for both 2012 and 2013 were similar with respect to N response. In both years the mean total percent cover was highest for non-leguminous forbs and grasses, and there was approximately half as much percent cover for legumes and horsetails (Fig. 3.7). The mean total percent cover of forbs was lowest in the 0 g N m⁻² y⁻¹ plots (2012, P=0.020; 2013, P=0.003), while the mean total percent cover of legumes was highest in the ambient N plots for 2013, but not 2012 (2013, P=0.019; Fig. 3.7). Although there was a trend of horsetail decreasing with increasing N in 2013, there were no significant effects of N on the mean total percent cover of grasses or horsetails (Fig. 3.7).

Prior to my research, a preliminary vegetation survey was completed in 2011 during the first year of growth after seeding (Borden 2012). Vegetative sampling was completed in both August and September to catch the peak biomass of both the cool season and warm season species. The main finding from Borden (2012) was that Canadian horseweed (*Erigeron canadensis*) increased in cover with added N in 2011 (P=0.010). However, unlike 2011, there were no effects of N addition in subsequent years (2012, P=0.144; 2013, P=0.191; Fig. 3.8). Although there were no effects of cover in 2012 or 2013 with N addition, there was a significant decrease in each year (P<0.001; Fig 3.8). Total cover of *E. canadensis* was 87% lower in 2012 than it was 2011, and by 2013 cover was 92% lower than in 2011.

When analyzed by native and adventive species categorized by functional group, the effects of N addition on mean total percent cover were driven by native species. When mean total percent forb cover was divided into native and adventive species, mean total percent cover was lowest for native forbs in ambient N plots for both 2012 (P=0.015) and 2013 (P=0.006), but there were no significant effects of N addition on adventive species in either year (Fig. 3.9). Although, native legumes also covered significantly more of the plot area than adventive species (P<0.001), the mean total percent cover of native legumes decreased, rather than increased, with N addition in 2013 (P=0.006; Fig. 3.10). In 2013, native legumes also had much higher mean total percent cover under ambient N than the adventive species (P=0.021; Fig. 3.10). All species in the grass and horsetail categories were native (Appendix 3).

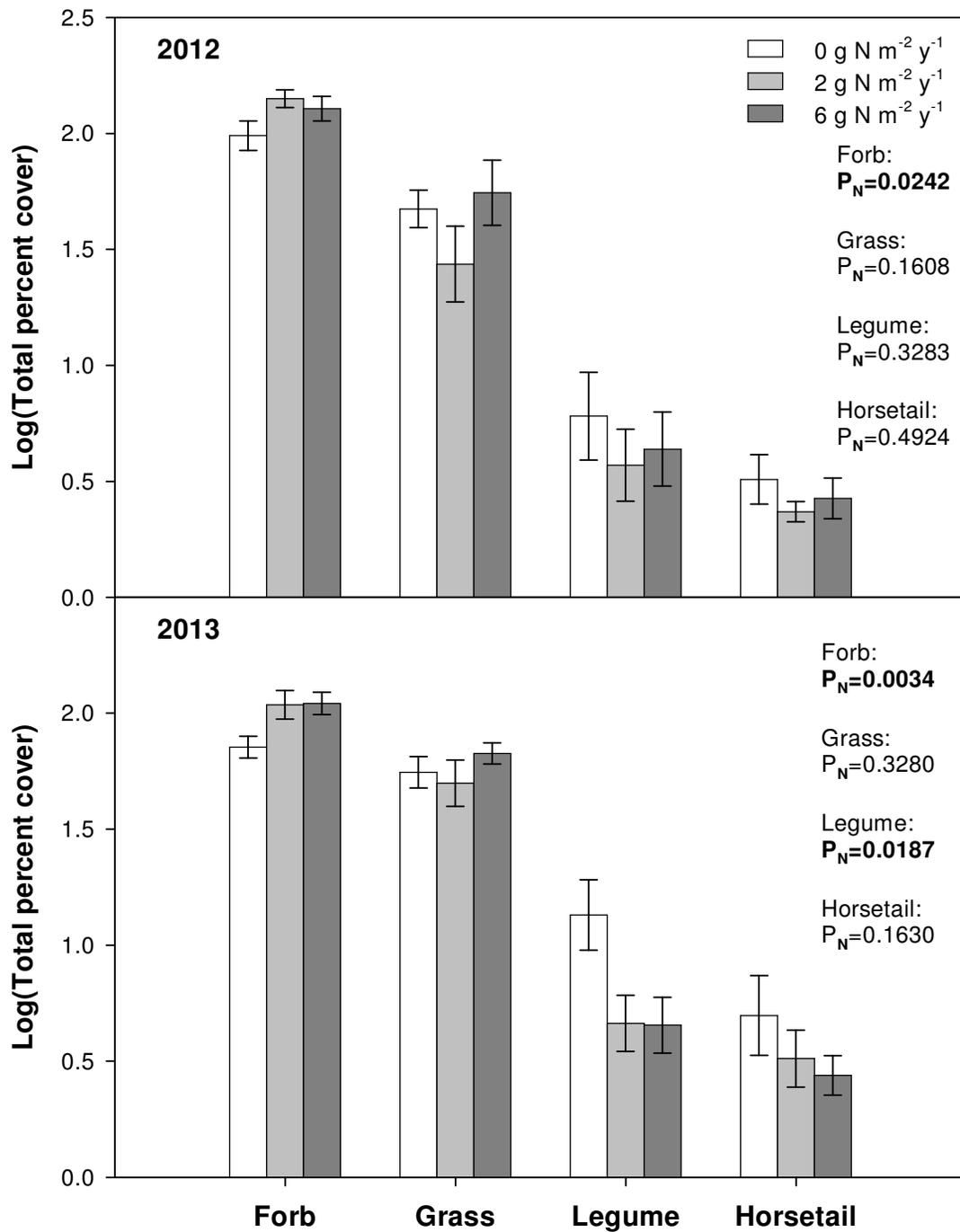


Figure 3.7. Log transformed data for mean total percent cover ± 1 SE separated by functional group for each N treatments for the 2012 and 2013 growing seasons (n=8). P-values obtained using Wilcoxon/Kruskal-Wallis tests. Statistically significant values are bolded ($P < 0.05$). Data for comparison between functional groups were pooled over N treatments.

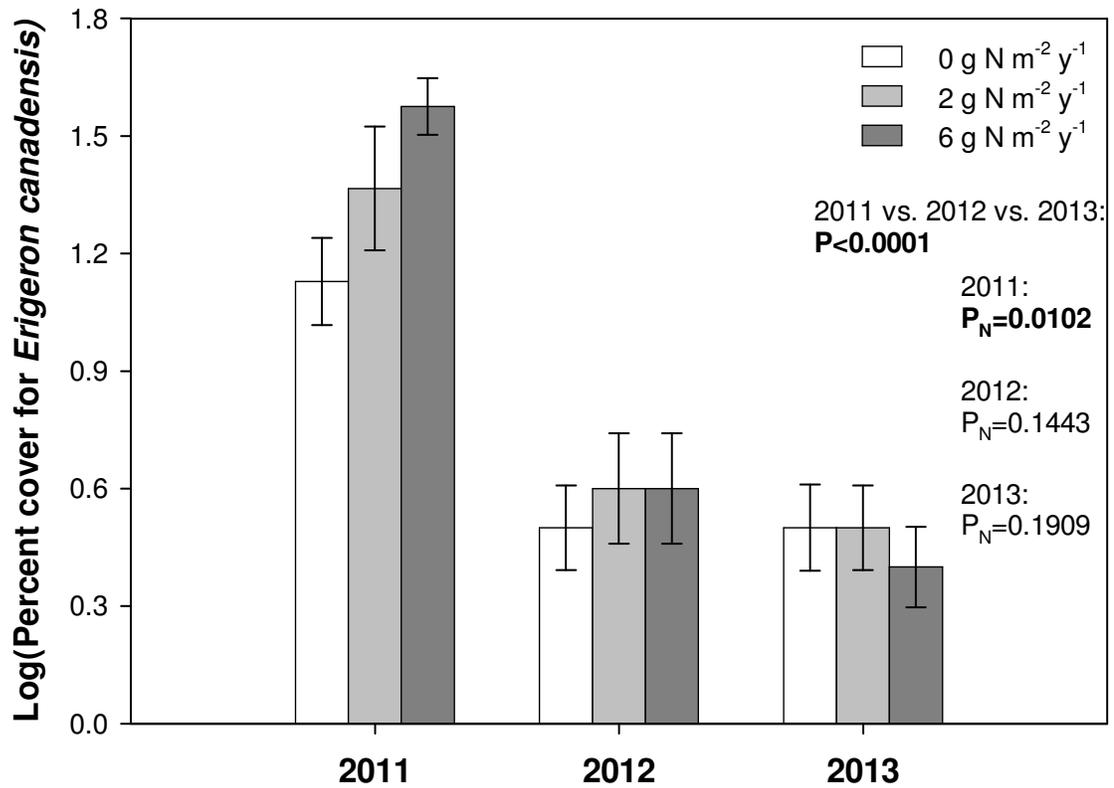


Figure 3.8. Log transformed data for mean *Erigeron canadensis* percent cover ± 1 SE by N treatments for the 2011, 2012, and 2013 growing seasons (n=8). P-values obtained using Wilcoxon/Kruskal-Wallis tests. Data for 2011 was obtained from Borden (2012). Statistically significant values are bolded (P<0.05). Data for comparison across years were pooled over N treatments.

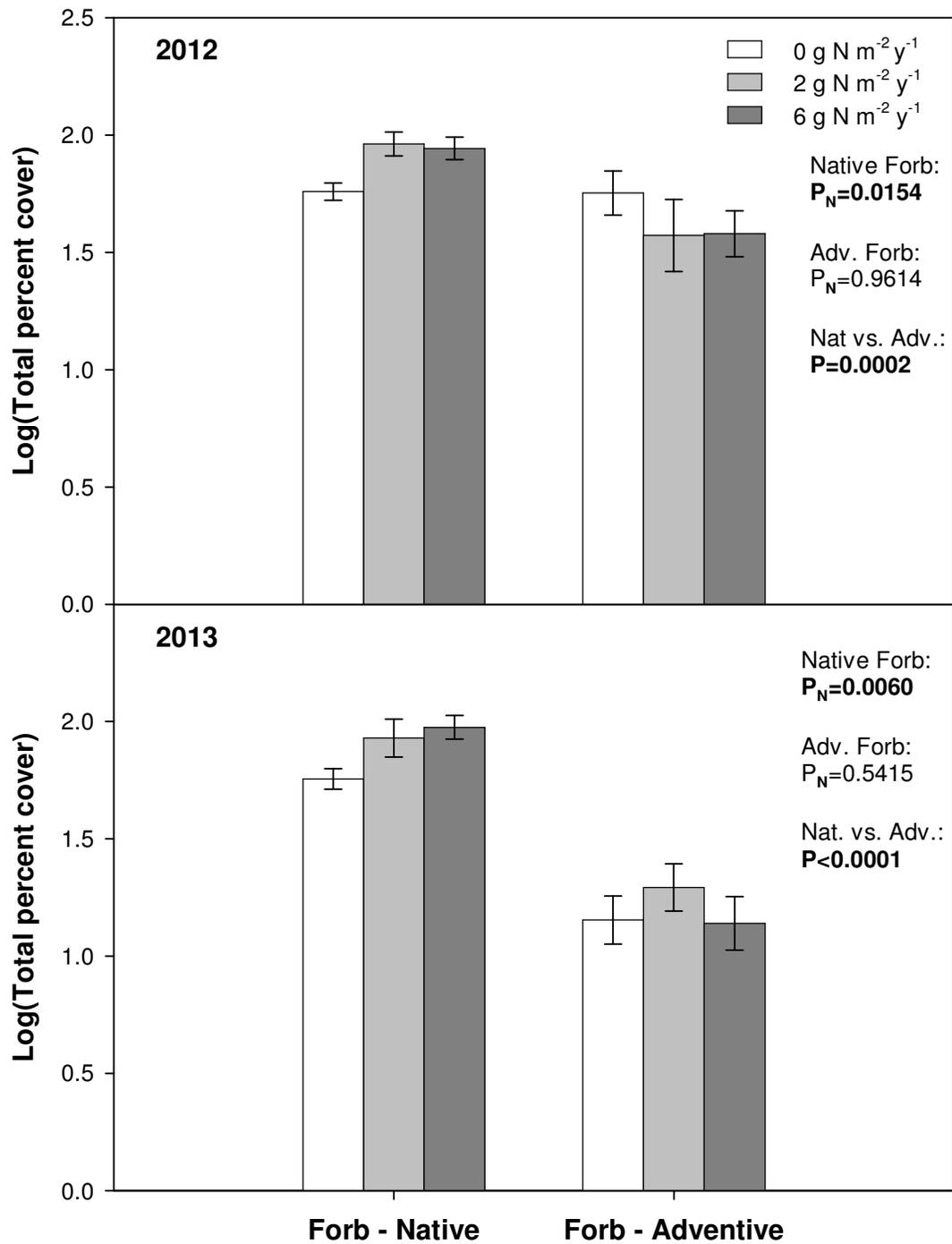


Figure 3.9. Log transformed data for mean total percent cover ± 1 SE separated by N treatments for both native and adventive forbs for the 2012 and 2013 growing seasons (n=8). P-values obtained using Wilcoxon/Kruskal-Wallis tests. Statistically significant values are bolded ($P < 0.05$). Data for comparison between native and adventive forbs were pooled over N treatments.

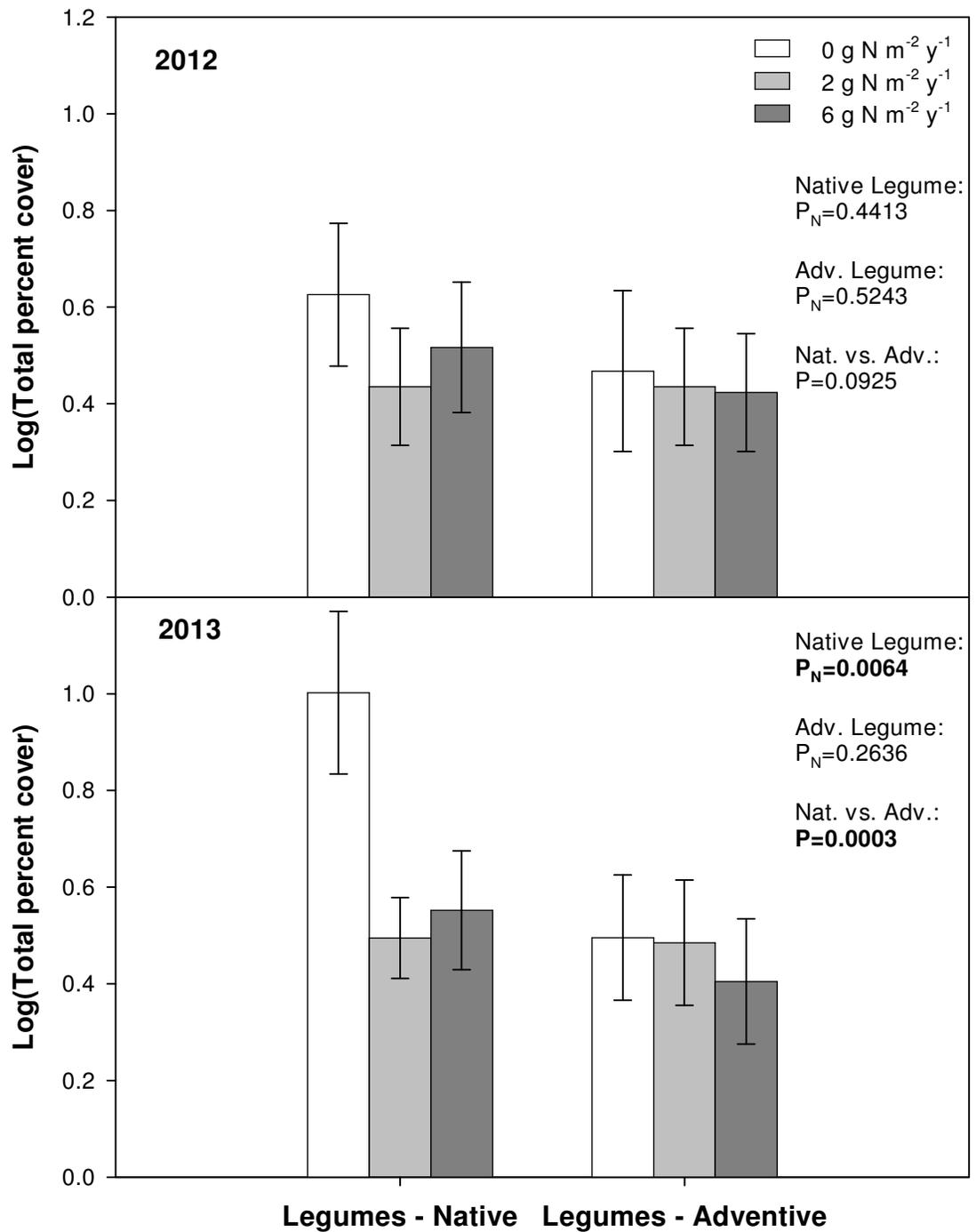


Figure 3.10. Log transformed data for mean total percent cover ± 1 SE separated by N treatments for both native and adventive legumes for the 2012 and 2013 growing seasons (n=8). P-values were obtained using Wilcoxon/Kruskal-Wallis tests. Statistically significant values are bolded ($P < 0.05$). Data for comparison between native and adventive legumes were pooled over N treatments.

3.2.2 Community structure analysis

An ANOSIM test showed that there were significant differences in community structure between plots given $0 \text{ g N m}^{-2} \text{ y}^{-1}$, and $6 \text{ g N m}^{-2} \text{ y}^{-1}$ for both 2012 ($P=0.002$) and 2013 ($P=0.035$), however the R-values denoting the percentages of variance explained were not large in either year (Table 3.2). Although the ANOSIM showed a difference between the $0 \text{ g N m}^{-2} \text{ y}^{-1}$ and the $6 \text{ g N m}^{-2} \text{ y}^{-1}$ treatments for 2013, the difference in clusters with respect to the N addition treatments was not clearly evident on the DCA (Fig. 3.11). For 2012, the first two DCA axes also showed that plots from the $0 \text{ g N m}^{-2} \text{ y}^{-1}$ treatment had an assemblage structure more closely related to the $2 \text{ g N m}^{-2} \text{ y}^{-1}$ treatment than the $6 \text{ g N m}^{-2} \text{ y}^{-1}$ treatment (Fig. 3.11). Block 6 was removed from both an ANOSIM test and a DCA because plot 6.3 had an abundance of jagged chickweed (*Holosteum umbellatum*) and very little of any other species in 2012, and thus it was not representative of the rest of the site.

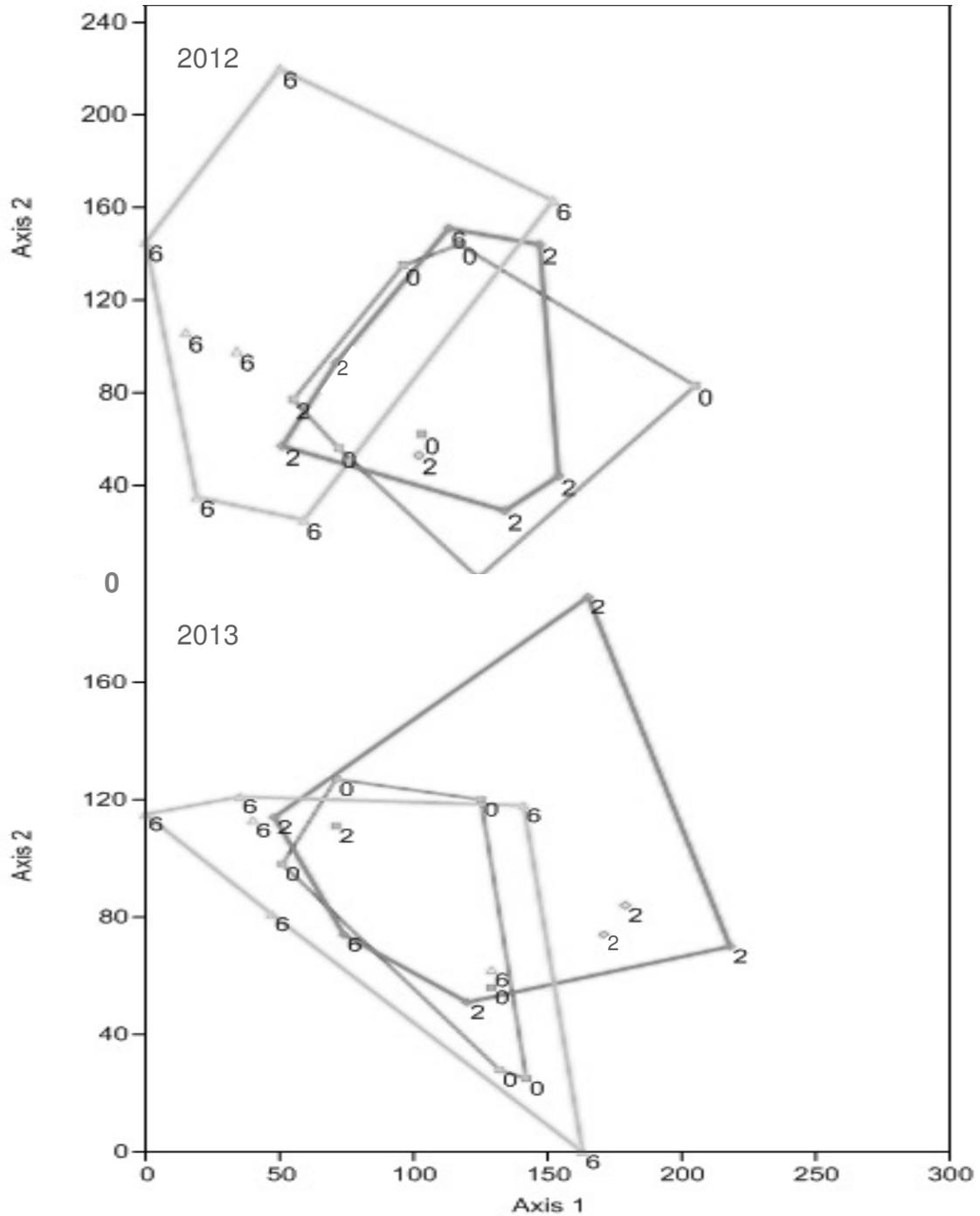


Figure 3.11. Results of the Detrended Correspondence Analysis (DCA) for both 2012 and 2013, showing separation between mean total percent coverage of plant species for N addition treatments (0, 2, and 6 g N m⁻² y⁻¹) along the first two axes (n=7).

Table 3.2. Values derived from Analysis of Similarity (ANOSIM) pairwise comparisons of plant cover percentages using Bray-Curtis values (n=7). The values highlighted in bold are statistically significant ($P < 0.05$). P-values are Bonferroni-corrected.

2012	Structure - Bray-Curtis distance	
	R-values	P-values
0 vs. 2 g N m ⁻² y ₁ ⁻¹	-0.0058	1
0 vs. 6 g N m ⁻² y ₁ ⁻¹	0.4956	0.0021
2 vs. 6 g N m ⁻² y ₁ ⁻¹	0.1429	0.3576
2013	R-values	P-values
0 vs. 2 g N m ⁻² y ₁ ⁻¹	0.1098	0.1361
0 vs. 6 g N m ⁻² y ₁ ⁻¹	0.2551	0.0346
2 vs. 6 g N m ⁻² y ₁ ⁻¹	0.0418	0.2896

3.3 Standing aboveground biomass

There was no significant effect of N addition on total biomass (Fig. 3.12). The biomass of species that were originally seeded as part of the restoration seed mixture were 37% greater than that of non-seeded species ($P < 0.001$; Fig. 3.13). However, N addition did not significantly affect the total biomass of either the seeded or non-seeded species (Fig. 3.13). The biomass of tallgrass indicator species was greater than that of non-indicator species ($P < 0.001$), but there were no significant effects of N addition on the total biomass of either group (Fig. 3.14). Similarly, there was significantly more native than adventive biomass ($P < 0.001$), but there were no significant differences in biomass for either native or adventive species with the addition of N (Fig. 3.15).

When biomass was analyzed by functional group, only horsetails showed a significant response (a decrease) to N addition (Fig. 3.16). However, horsetails biomass was very low in general (Fig. 3.16). Overall, forbs made up the highest percentage of aboveground biomass at 54%, followed by grasses at 40%, then legumes with 5%, and finally horsetails with only 1%.

Litter was removed from the biomass calculations and was treated separately. However, there was no effect of N treatment on litter accumulation (Fig. 3.17).

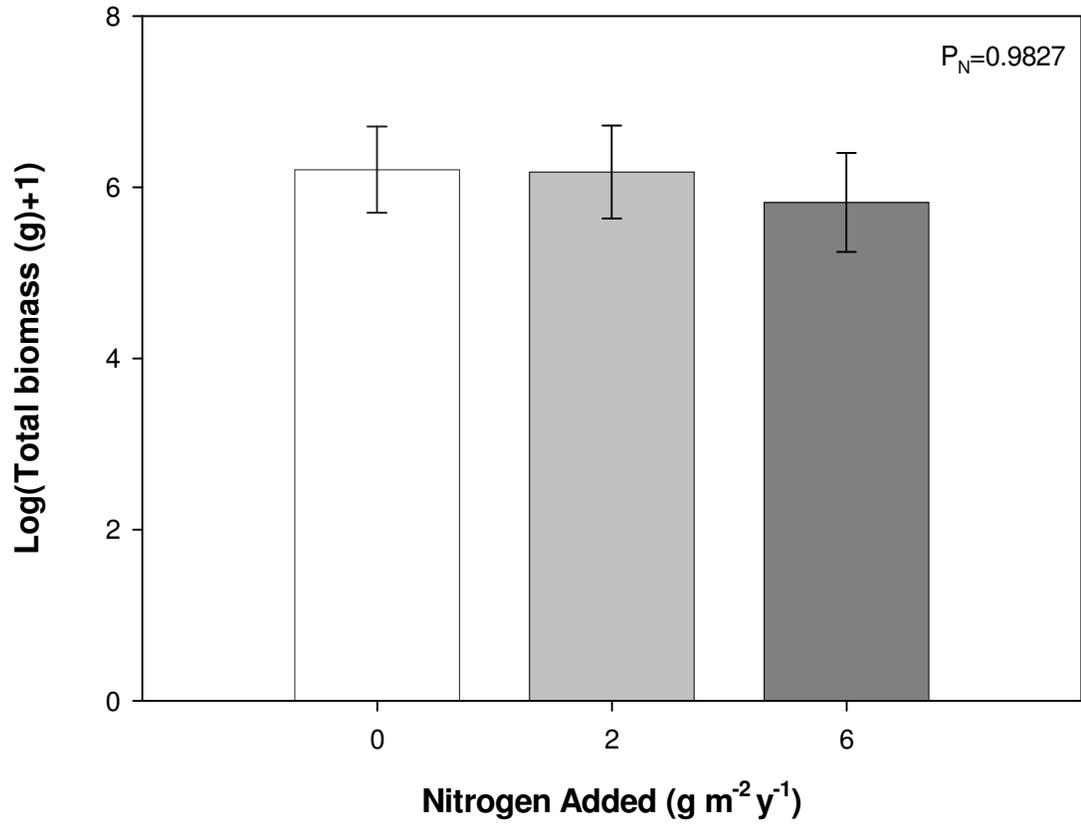


Figure 3.12. Log transformed data for mean total aboveground plant biomass (0.25 m⁻²) with litter removed ± 1 SE by N treatments for the 2012 growing season (n=8). P-values obtained using a one-way ANOVA. Statistically significant values are bolded (P<0.05).

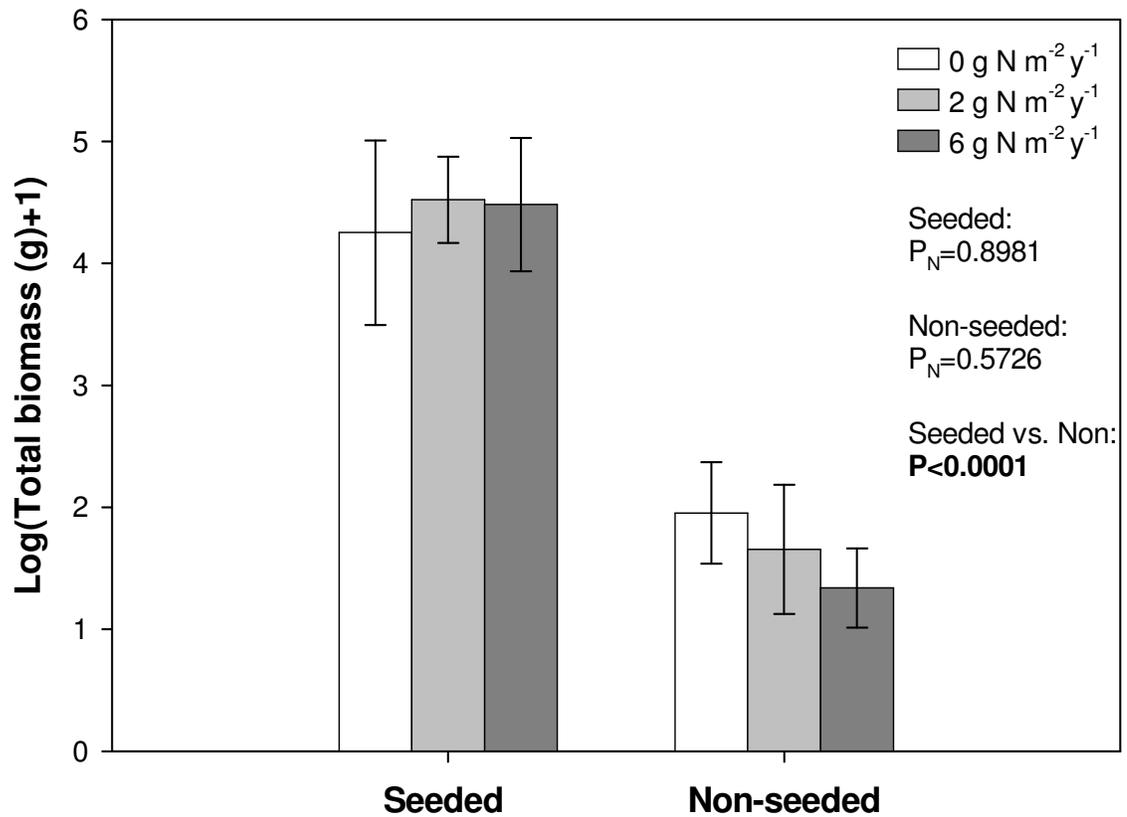


Figure 3.13. Log transformed data for mean total seeded and non-seeded aboveground plant biomass (0.25 m^{-2}) with litter removed ± 1 SE by N treatments for the 2012 growing season ($n=8$). P-values obtained using a one-way ANOVA. Statistically significant values are bolded ($P<0.05$). Data for comparison between seeded and non-seeded species were pooled over N treatments.

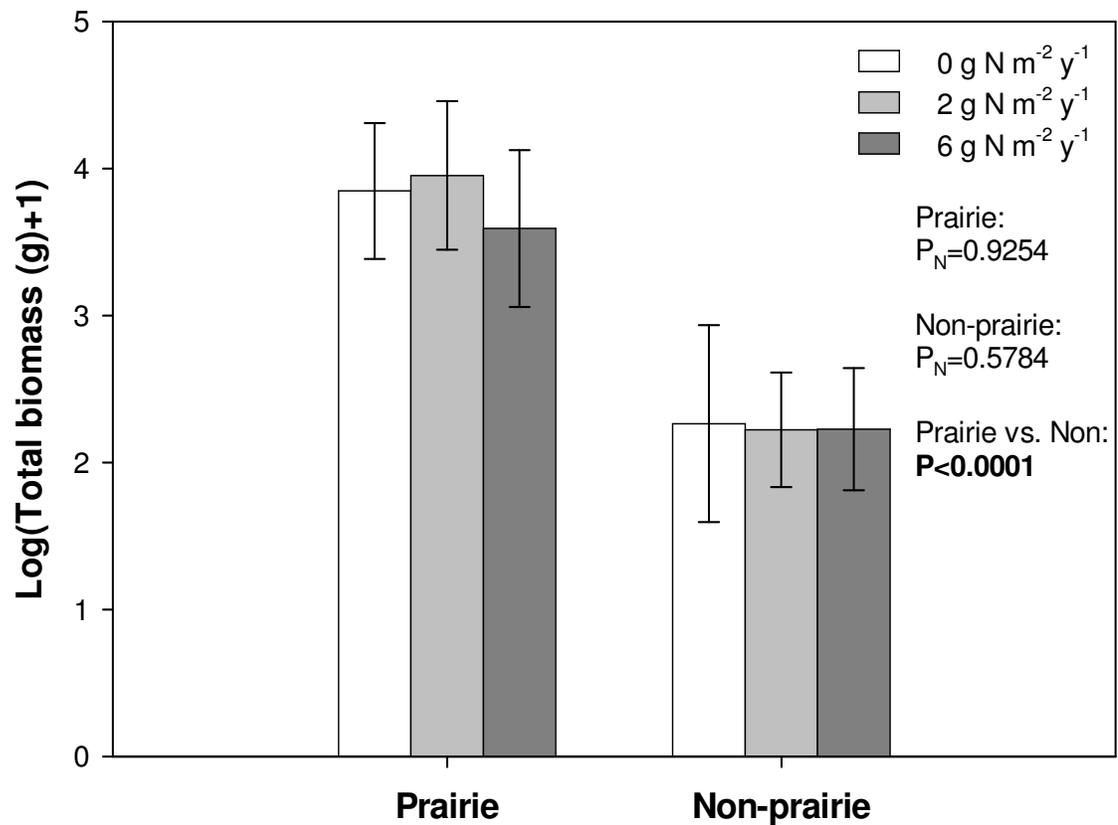


Figure 3.14. Log transformed data for mean total prairie and non-prairie aboveground plant biomass (0.25 m⁻²) with litter removed ± 1 SE by N treatments for the 2012 growing season (n=8). P-values obtained using a one-way ANOVA. Statistically significant values are bolded (P<0.05). Data for comparison between prairie and non-prairie species were pooled over N treatments.

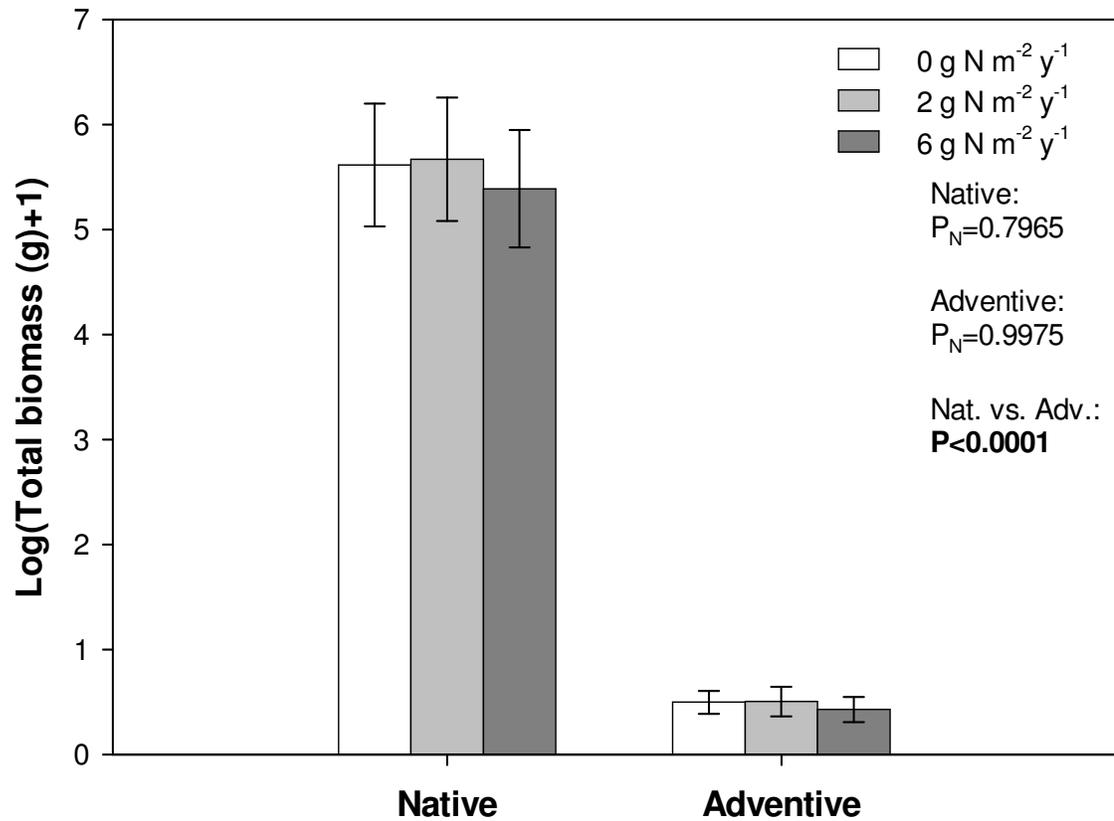


Figure 3.15. Log transformed data for mean total native and adventive aboveground plant biomass (0.25 m²) with litter removed ± 1 SE by N treatments for the 2012 growing season (n=8). P-values obtained using a one-way ANOVA. Statistically significant values are bolded (P<0.05). Data for comparison between native and non-native species were pooled over N treatments.

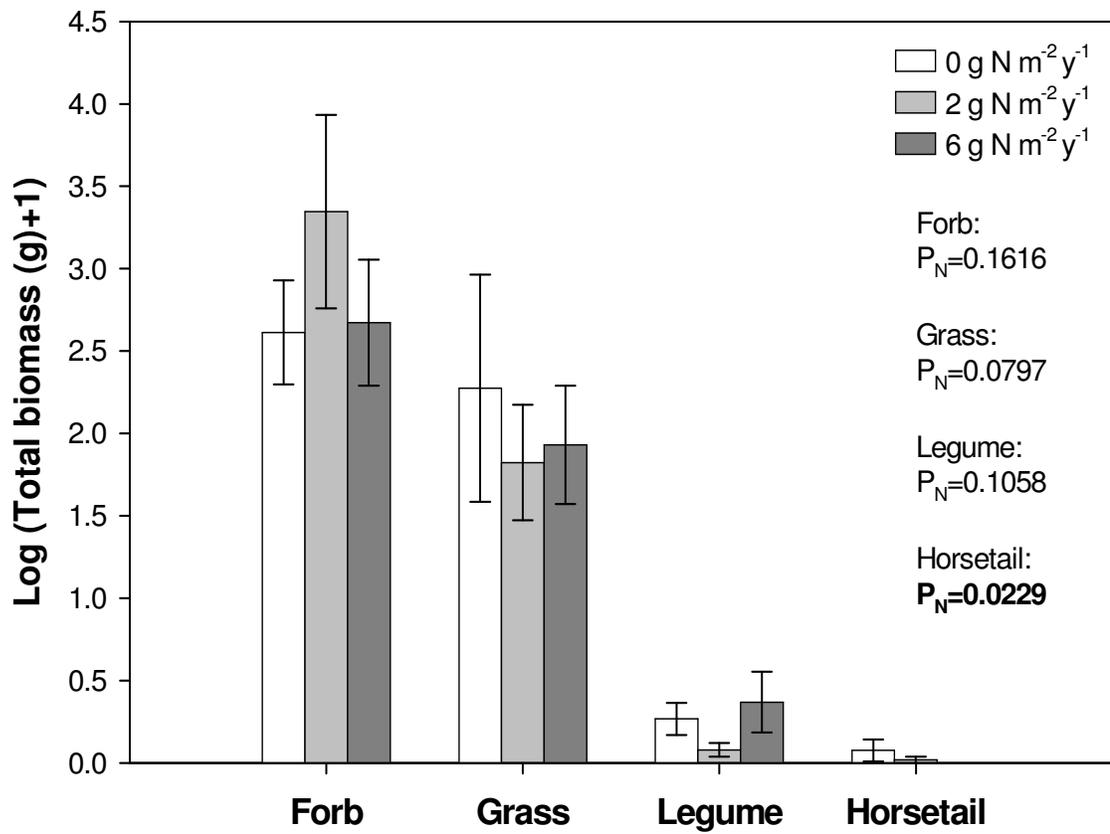


Figure 3.16. Log transformed data for mean total aboveground plant biomass (0.25 m⁻²) by functional group with litter removed ± 1 SE by N treatments for the 2012 growing season (n=8). P-values obtained using Wilcoxon/Kruskal-Wallis tests. Statistically significant values are bolded (P<0.05). Data for comparison between functional groups were pooled over N treatments.

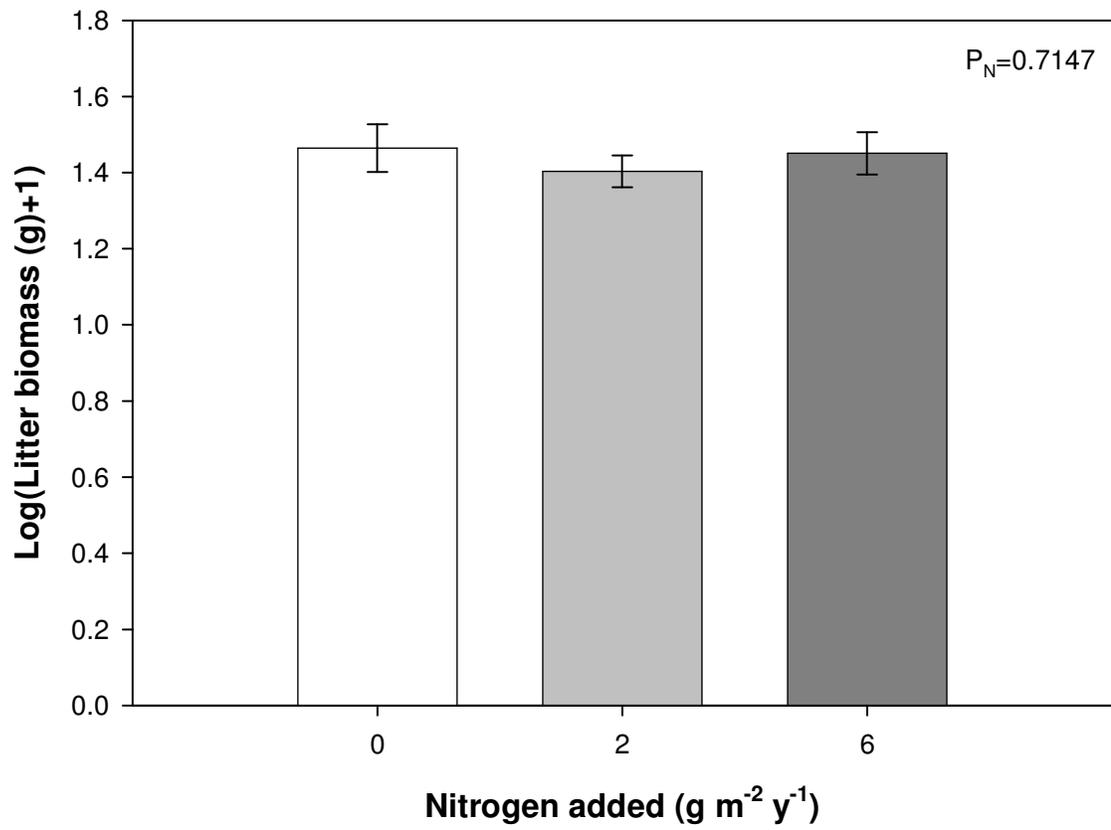


Figure 3.17. Log transformed data for mean total litter biomass (0.25 m⁻²) ±1 SE by N treatments for the 2012 growing season (n=8). P-values obtained using a one-way ANOVA. Statistically significant values are bolded (P<0.05).

3.4 Species Diversity

The Shannon diversity index did not show differences in species diversity between treatments in either year. However, total diversity was higher in 2012 than it was in 2013 ($P=0.032$; Fig. 3.18). Species that were found in 2012, but not 2013, included stinking chamomile (*Anthemis cotula*), common milkweed (*Asclepias syriaca*), smooth hawkweed (*Crepis capillaris*), narrow-leaved hawkweed (*Crepis tectorum*), evening primrose (*Oenothera biennis*), and sundial lupine (*Lupinus perennis*) while *E. canadensis* and *H. umbellatum* were severely reduced. None of these species were prairie indicators with the exception of *L. perennis*. In contrast, Kalm's brome (*Bromus kalmi*), wild bergamot (*Monarda fistulosa*), alsike clover (*Trifolium hybridum*), and one-sided grass (*Elymus trachycaulus*) increased in abundance between 2012 and 2013, and with the exception of *T. hybridum*, all of these species are prairie indicator species.

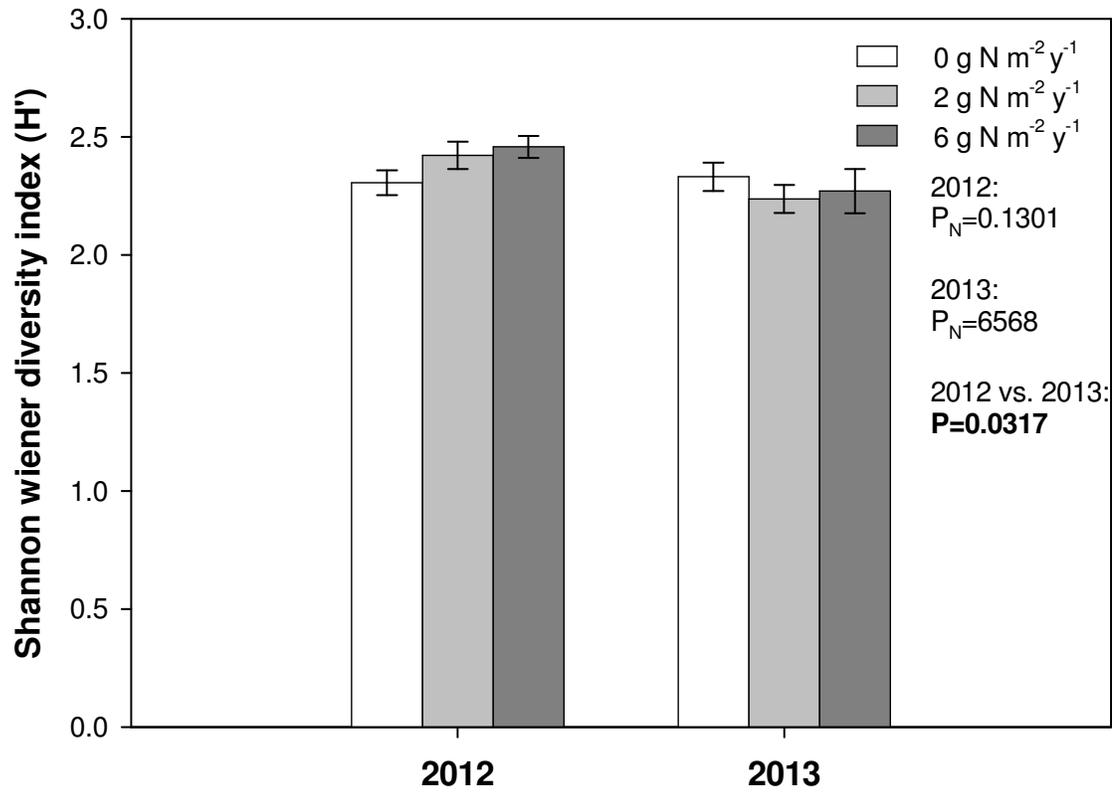


Figure 3.18. Shannon diversity index values ± 1 SE by N treatments for the 2012 and 2013 growing seasons (n=8). P-values obtained using a one-way ANOVA. Statistically significant values are bolded (P<0.05).

3.5 Belowground biomass

Belowground biomass was not significantly affected by N addition in 2012 or 2013 (Fig. 3.19). Root biomass was significantly higher in September than in June for both years ($P < 0.001$; data not shown). However, 2013 did not have significantly different root biomass than 2012 (Fig. 3.19).

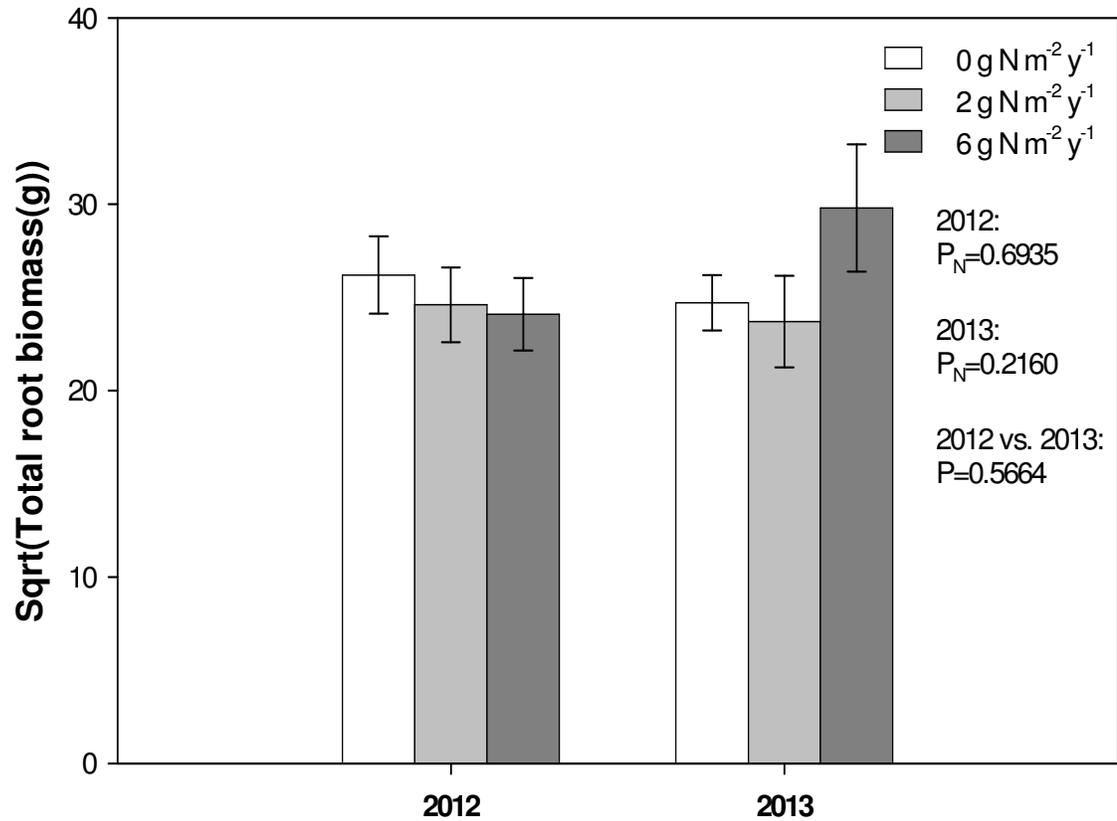


Figure 3.19. Square root transformed data for mean total root biomass ± 1 SE by N treatment for the 2012 and 2013 growing seasons (n=8). P-values obtained using Wilcoxon/Kruskal-Wallis tests. Statistically significant values are bolded ($P < 0.05$).

Chapter 4 - Discussion

I used a N addition experiment in a restored tallgrass prairie, to test the hypothesis that increased atmospheric N deposition will increase the abundance of adventive herbaceous species that are not typically associated with tallgrass prairie. Contrary to my initial prediction, N addition did not significantly increase the relative biomass or abundance of adventive species. Instead, the native species that were part of the restoration seeding showed substantial increases in cover in response to N addition. Non-tallgrass species (some of which were non-adventive, native species, but not part of the restoration seed mix) also increased in response to N in 2012, but this effect was no longer present in 2013.

When cool-season (non-tallgrass) grasses become established at a restoration site, native warm-season grasses often do not respond to N addition (Doll *et al.* 2011), yet they respond positively to the reduced N availability that occurs via C addition (Blumenthal *et al.* 2003; Averett *et al.* 2004). Likewise, there have been studies where neither seed competitive ability or competition between native and adventive perennial prairie species changed with added N (Thomsen *et al.* 2006; Biondini 2007). However, my results were consistent with previous studies where warm-season grass species have responded positively to added N in the absence of competition from other species (Tilman 1987; Baer *et al.* 2003). Therefore, although tallgrass species are effective competitors under N limited conditions, and they may be less effective than some non-tallgrass species at exploiting high N availability, they nevertheless can benefit from added N.

The control of non-seeded species through the combination of seedbank exhaustion (Bakker & Poschlod 1996) and seed dispersal limitation (Bragg & Hulbert 2006) can give an advantage to seeds that are directly sown in restoration projects. Seed limitation can decrease both productivity and species richness by reducing establishment of some species and altering species composition (Tilman 1997; Foster & Tilman 2003; MacDougall & Turkington 2006; Zeiter *et al.* 2006). The dominance of native tallgrass species I observed may have been influenced by the relative availabilities of seeds that were sown versus those

that remained in the seedbank or reached the site through dispersal. In particular, when comparing the list of seeded species (Appendix 2) versus species percent cover(s) (Appendix 3), there were no grasses found within the plots that were not planted as part of the restoration seed mixture. Because the restoration site had been used for agriculture prior to the restoration, and it was tilled and treated with herbicide as part of the restoration process, the presence of both native and adventive species in the seedbank may have been severely reduced. In addition, adventive species were actively controlled outside of the study area at the restoration site with herbicide, which would have limited dispersal into the plots.

4.1 Mean total percent cover

Mean total percent cover (i.e. the sum of species percent cover values) increased with added N in 2013, but there was no effect of treatment on cover in 2012. This difference between 2012 and 2013 was associated with drought conditions in 2012 and normal precipitation in 2013 (Table 3.1). In particular, in grass-dominated systems, drought conditions can become the most influential factor limiting plant growth, diminishing the effects of N addition on plant growth (Hutchison & Henry 2010). In addition, N conservation increases in periods of drought in the C_4 photosynthetic, tallgrass species big bluestem (*Andropogon gerardii*), which may limit the N response of this dominant species in drought years (Hayes 1985).

The rate of N addition also appeared to be important in determining plant cover responses. In 2012, the DCA and ANOSIM results revealed that the relative species abundances in the $0 \text{ g N m}^{-2} \text{ y}^{-1}$ and $2 \text{ g N m}^{-2} \text{ y}^{-1}$ plots were more similar to each other than the abundances in the $6 \text{ g N m}^{-2} \text{ y}^{-1}$ plots. This result suggests that significant responses may only occur at high addition rates, which has been observed elsewhere in N addition experiments (Bobbink et al., 1998), although the chronic effects of lower addition rates may also result in species changes over time (Tilman 1990; Wedin & Tilman 1993; Stevens *et al.* 2010).

4.2 Total plant biomass

Native species accounted for a large proportion of the total aboveground biomass relative to their cover, and likewise, seeded, and prairie indicator species were present at high biomass across all treatments. Although percent cover is typically a good predictor of variation in biomass (MacDonald & Burke 2012), biomass sampling in my study was not effective for discerning differences among N addition treatments. However, biomass clipping typically requires more replicates, larger quadrats, and a greater time commitment than non-destructive methods such as percent cover estimation (Heidelbaugh & Nelson 1996). The size of my biomass quadrats (0.25 m × 0.25m) were consistent with those described elsewhere in the literature for prairie communities (Seastedt *et al.* 1991; Tilman & Wedin 1991; Biondini 2007; Dickson & Busby 2009; Socher *et al.* 2012; Seabloom *et al.* 2013), but it appears that due to the high diversity and spatial heterogeneity in our plots, the biomass quadrats were not large enough to be representative of the overall plot species composition.

To properly represent the plot biomass I may have needed quadrats of at least 1 m × 1 m (Camill *et al.* 2004), but this would have consumed too much of the 2 m × 2 m plot area since the plots are intended to be monitored over the long term (i.e. many years). Similar to the aboveground biomass estimates, the belowground biomass estimates, which relied on 2 cm diameter soils cores, may not have been representative of the high heterogeneity in the plots, which featured high root densities directly beneath the established bunchgrasses.

4.2.1 Litter biomass

High N litter typically breaks down faster than low N litter, which can result in low litter accumulation in high N addition plots (Wedin & Tilman 1990; Lü *et al.* 2013). Likewise, changes in the relative abundances of species in response to N addition can affect overall litter quality, and when a high percentage of non-tallgrass species are present it should result in less litter accumulation than when a high percentage of tallgrass species are present, because of the high N concentration in litter from non-tallgrass species (Pastor *et al.* 1987). However, correlations between litter decomposition and N addition are not ubiquitous (Pastor *et al.* 1987; Tilman 1987), and likewise I did not observe an effect of N addition on

the accumulation of litter biomass. Aside from considering potential litter quality effects, the latter result was not surprising given that total biomass was not significantly affected by N addition.

Litter accumulation has been suggested to reduce species richness, independent of N addition effects (Foster & Gross 1998; Török *et al.* 2010). Reduction of species richness due to a dense litter layer may be of special concern to field sites like the one used for this experiment that are not frequently burned to remove litter accumulation (Knapp & Seastedt 1986). Litter decreases temperature, irradiance, and moisture loss at the soil surface, which may be beneficial to some species, yet detrimental to others (Knapp & Seastedt 1986; Boeken & Orenstein 2001; Török *et al.* 2010). For example, C₄ tallgrass species require high temperatures to germinate and grow, and early successional species are not shade tolerant, therefore, both C₄ tallgrass species and early successional species can exhibit reduced germination, reduced productivity, and increased mortality when a thick litter layer is present (Foster & Gross 1998; Török *et al.* 2010).

4.3 Species specific and functional group responses

4.3.1 Tallgrass prairie and native species

Tallgrass prairie restorations are typically seed limited, and most rare species need to be seeded in order for them to become established (Martin & Wilsey 2006). The majority of species recorded in the plots were native, prairie species that were part of the original restoration seed mix; although only 41% of the original restoration seed mix were tallgrass prairie species, 65% of the seeded species that were recorded as growing in the plot three years later were tallgrass prairie species (Appendix 3). With respect to N addition responses, total cover for tallgrass prairie indicator species followed my prediction in 2012, when the total cover of non-prairie species increased in response to N, and the cover of prairie indicator species was not affected by N addition. However, contrary to my prediction, the abundance of tallgrass indicator species was higher than non-tallgrass indicator species in 2013, and there was no longer a significant effect of N addition on non-

prairie indicator species. This change in composition was accounted for by an increased abundance of native forbs and grass in 2013.

The restoration project in which my experiment was located was unlike other tallgrass prairie restorations because it will not be routinely burned as part of the restoration plan. Instead, the ultimate goal of this particular restoration project was to facilitate forest regeneration in the long term, and burning would be counterproductive because it would discourage the growth of woody species. Three years after the seeding, woody plants contributed very little to percentage of cover in the plots (Appendix 3). However, the lack of burning will likely slow the rate of vegetative reproduction of tallgrass prairie species (Benson & Hartnett 2006). Without frequent burning, N levels may be even more important to tallgrass species, because they will have more competition from non-tallgrass species, and thus more difficulty maintaining dominance (Norris *et al.* 2007).

4.3.2 Pioneer weeds

The first few years after a disturbance are critical for establishing tallgrass species dominance, because of the rapid growth of pioneer species under the high light, low nutrient conditions (Wedin 2004). The first year after seeding, the aboveground biomass of *E. canadensis*, a native species not present in the seeding mix, increased significantly in response to N addition in the experimental plots (Borden 2012). *E. canadensis* was also the main driver of the increased mean percent cover of biennials in response to N addition in 2012. However, when the aboveground biomass of grass and native forb increased in 2013, there was a corresponding decrease in both the aboveground biomass and cover of *E. canadensis*, and there were no longer significant effects of N addition on *E. canadensis* cover. These responses were consistent with the colonization patterns of *E. canadensis*, which is a fast-growing, weedy species that is successful on high N soil, but is not able to compete effectively with tallgrasses for light over time (Tilman 1987; Thébaud *et al.* 1996; Prieur-Richard *et al.* 2000).

In addition to the increased aboveground competition, pioneer weed species such as, *E. canadensis* also would have been influenced by increased belowground competition from tallgrass species. Because tallgrass species allocate such a large proportion of biomass to

belowground growth, they may outcompete weedy species for soil nutrients (Pärtel & Wilson 2002). Tallgrass species may also inhibit the establishment of pioneer weeds, because their deep, near vertical root growth can create a physical barrier to block lateral root growth by other plants, and thus impede colonization (Delaney *et al.* 2000).

4.3.3 Functional groups

Although the total biomass and cover of forbs were higher than those of grasses, the dominant species in each plot was most often a grass species, because the ratio of forb species to grass species was high. In this experiment there were no significant N addition effects on the ratio of grasses to forbs, whereas in other experiments N addition has been shown to decrease this ratio (Stevens *et al.* 2006; Lu *et al.* 2010). Functional group membership was also important for interpreting trends in the establishment of native versus adventive species; for example, only forbs and legumes were represented by species that were adventive, and the increases in total cover with added N in 2013 were due mostly to increases in native forbs. Elsewhere, increased invasion of non-prairie species has been observed in the presence of N fixing legumes, due to increased soil N, whereas there is often decreased invasion when tallgrass species dominate, as a result of decreased soil N (Prieur-Richard *et al.* 2000; Fargione & Tilman 2005). At my site, N fixers such as legumes and field horsetail (*Equisetum arvense*) (Fuji *et al.* 1984) thrived in the low N plots, but an increase in invasion by adventive species was not observed in these plots. Legumes had higher percent cover in low N plots than in high N plots in both 2012 and 2013, and there was a marginally significant trend of increased *E. arvense* cover in low N plots, and *E. arvense* biomass increased significantly in the low N plots. Legumes typically have higher seed invasibility than perennial grasses; therefore, with the reduced vegetative cover in the low N plots, adventive leguminous species had higher establishment rates, as compared to adventive perennial grasses (Tilman 1997).

Both biennial and annual total cover decreased over time, whereas perennial cover increased, which is consistent with the literature (e.g. Camil *et al.* 2004). With respect to the effect of N addition on species composition, biennials increased with added N for 2012, whereas perennials increased with added N for 2013.

4.4 Species diversity

Although species diversity increased between 2012 and 2013, it did not respond significantly to N addition. Since our study revealed increases in mean percent cover of native species that were already found throughout the site with added N, rather than large increases in cover of adventive species, added N did not affect species diversity. This result is not consistent with diversity responses to the manipulation of N availability in other experiments. A N reduction study by Baer *et al.* (2003) suggested that in the early years of a tallgrass prairie restoration project, decreased productivity coupled with low N availability increased light availability, and hence increased species diversity by increasing the abundance of light-demanding pioneer species. The opposite has been observed in other early successional tallgrass prairie N addition experiments, where the diversity of pioneer species decreased (Tilman 1987). Likewise, when sites mature and species become better established over time, species diversity often decreases with N addition (Tilman 1987; Wedin & Tilman 1990; Pyšek & Lepš 1991; Tilman & Pacala 1993; Reich *et al.* 2001; Fargione & Tilman 2005; Clark & Tilman 2008; Bobbink *et al.* 2010). Although the species richness and diversity in a plant community can be an important determinant of invasibility (Tilman 1997), the dominant cover at a site may be a better invasibility indicator (Smith *et al.* 2004).

4.5 Future directions

Future studies could add N at a rates higher than projected rates of atmospheric N deposition for 2050 in order to get a better understanding of what would happen to species composition at restoration sites under extremely high deposition conditions. For example, N treatments of 5 to 56 g N m⁻² y⁻¹ have been used in old-field (Foster & Gross 1998), semiarid grassland (Lü *et al.* 2013) and forest community experiments (Lu *et al.* 2010). At very high N levels there can be a decrease in productivity due to sensitivities to toxicity of soil by mobilization of Al, Fe, and Mn and acidification, which can also change species composition (Bakker & Berendse 1999; Stevens *et al.* 2004, 2011; Horswill *et al.* 2008). The effect of increased N on productivity is compounded by the presence of N fixing

bacteria, because the soil pH is decreased when N fixing bacteria oxidize NH_3 into two protons (Bakker & Berendse 1999). When N accumulates you can also see a greater disparity in biomass between low and high N addition sites (Tilman 1987).

Terrestrial plant communities containing rare species can be especially susceptible to increased rates of N addition (Vandenberg *et al.* 2011; Stevens *et al.* 2011), yet as tallgrass prairies mature, they may be less susceptible to invasive species because the dominant native species have time to establish, and are consequently better able to compete with incoming species (Gartshore 2011). Thus, understanding invasion dynamics in the early stages of tallgrass prairie restoration, as it relates to increasing atmospheric N deposition, is important to help the tallgrass prairie reach maturity and become able to self-regulate. The importance of seed limitation was not monitored in this study, but may have had an effect on the invasion of adventive species. It would have been interesting to monitor how much the seedbank and seed rain attributed to species composition in context of the N addition treatments.

In addition to increased rates of atmospheric N deposition, other global change factors such as CO_2 , warming, or precipitation regime alteration (Alley *et al.* 2013) may interact to affect community composition and invasability in restored tallgrass prairie. Future studies could explore interactions between these factors and N in the context of tallgrass prairie restoration, and it is anticipated that C_4 , tallgrass species may react differently than C_3 non-tallgrass species to these factors (An *et al.* 2005; Lattanzi 2010). Both climate warming and increased concentrations of atmospheric CO_2 may shift the competitive advantage towards C_4 tallgrasses, since their already high N and CO_2 use efficiencies may be further enhanced by increased temperatures, while the corresponding efficiencies of non-tallgrass, C_3 species do not seem to be affected by increased temperatures (Ward *et al.* 1999; An *et al.* 2005). As for anthropogenic alteration of the precipitation cycle, this is expected to result in more extreme droughts and flooding in the future (Alley *et al.* 2013) Compared to non-tallgrass C_3 species, the increased water-use efficiency allowed by decreased stomatal opening, and deep root system make C_4 tallgrass species superior competitors under low soil moisture conditions (Ward *et al.* 1999; Wedin 2004). Therefore, C_4 , tallgrass species may have a competitive advantage over non-tallgrass species in future tallgrass prairie restorations if

interactions with N deposition and other global change factors have additive effects on tallgrass productivity.

In my study, I looked exclusively at the effects of N addition on species composition in tallgrass prairie restoration, however, using C addition to lower N levels as a means of combating atmospheric N deposition has also been suggested (Baer *et al.* 2002, 2003; Blumenthal *et al.* 2003). The addition of C, usually in the form of sawdust or sucrose, may help reduce weedy species in tallgrass sites, but it has had limited success in field trials (Blumenthal *et al.* 2003). There have been decreases in the productivity of tallgrass species associated with C addition, which would leave them vulnerable to shading, and decrease belowground nutrient storage reserves for growth after a fire (Blumenthal *et al.* 2003). C addition can also alter seed germination, which may also influence community composition (Kabouw *et al.* 2010).

4.6 Conclusions

I had predicted that increases in the rate of atmospheric N deposition, consistent with levels projected for southern Ontario over the next 40 years, would jeopardize the establishment and success of tallgrass prairie species by increasing the ability of non-tallgrass prairie species to invade restoration sites. However, after three years of N addition, tallgrass prairie species were able to maintain and even increase in dominance at the restoration site. Nevertheless, because the study site had been previously used for agriculture, and the area surrounding the restoration site was actively controlled for adventive species, the study site may have had a very low residual seedbank, and a low amount of seed rain of adventive species. The dominance of native species may also have resulted from the resilience of the native species to invasion at increased N levels. Therefore, although the site did not show a lasting effect of increased N levels on invasive species, less intensive restorations that have a large residual source of perennial adventive species may experience greater increases in the abundance of adventive species. Based on the increased dominance of both native and tallgrass prairie species with added N in the time scale in the study, it appears that with thorough restoration procedures, which reduce the abundance of adventive seed

establishment, it will be economically and ecologically feasible to plant tallgrass prairies based on past species compositions. However, early successional species composition can be drastically different from that of mature tallgrass prairies (Kindscher & Tieszen 1998; Baer *et al.* 2002). Due to the long-term nature of this study, future monitoring of the vegetation will allow a better determination of whether chronic N addition will influence the species trajectory of the tallgrass prairie restoration.

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Appendices

Appendix 1. Permission to use Figure 1.1.

On Nov 11, 2013, at 1:11 PM, Tallgrass Ontario <info@tallgrassontario.org> wrote:

Hello Jennifer

On behalf of the Tallgrass Ontario Board of Directors, you have permission to use the TgO map of “Current and Historical Tallgrass Vegetation of Southern Ontario” in your master's thesis.

We only request that appropriate credit be given, and if at all possible, TgO receive a digital copy of the completed thesis for our files.

We wish you well with your thesis and hope that you consider becoming [a member of Tallgrass Ontario](#).

Regards

Tom Purdy - Treasurer



Appendix 2. Species list for seeded species at the restoration site, Spring 2010. All species seeded were native.

Tallgrass Prairie Restoration Seed Mix (Spring 2010)

Forbs

Artemisia campestris L. (field woodworm)
Asclepias syriaca L. (common milkweed)
Asclepias tuberosa L. (butterfly milkweed)
Crocanthemum bicknellii (Fernald) Janch. (bicknell's rock rose)
Desmodium canadense (L.) DC (Canada tick-trefoil)
Desmodium paniculatum (L.) DC (panicled tick-trefoil)
Doellingeria umbellata (Mill.) Nees (flat-topped white aster)
Euthamia graminifolia (L.) Nutt. (grass-leaved goldenrod)
Helianthus divaricatus L. (hoary frostweed)
Houstonia longifolia Gaertn. (long-leaved bluets)
Lechea intermedia Britton (large-pod pinweed)
Lechea mucronata Raf. (hairy pinweed)
Lespedeza capitata Michx. (round-headed bush-clover)
Lespedeza hirta (L.) Hornem. (hairy bush-clover)
Lespedeza intermedia (S. Watson) Britton (shrubby bush-clover)
Liatris cylindracea Michx. (slender blazing star)
Lupinus perennis L. (sundial lupine)
Maianthemum stellatum (L.) Link (false Solomon's seal)
Monarda fistulosa L. (wild bergamot)
Oenothera biennis L. (common evening primrose)
Penstemon digitalis Sims (foxglove beardtongue)
Pseudognaphalium macounii (Greene) Kartesz (Macoun's cudweed)
Pseudognaphalium obtusifolium (L.) Hilliard & B.L. Burt (sweet everlasting)
Pycnanthemum virginianum (L.) Fernald & B.L. Rob. (Virginia mountain-mint)
Rudbeckia hirta L. (black-eyed Susan)
Silene antirrhina L. (sleepy catchfly)
Sisyrinchium montanum Greene (blue-eyed grass)
Solidago juncea Aiton (early goldenrod)
Solidago nemoralis Aiton (grey-stemmed goldenrod)
Symphotrichum ericoides (L.) G.L. Nesom (white heath aster)
Symphotrichum laeve (L.) Á. Löve & D. Löve (smooth blue aster)
Symphotrichum oolentangiense (Riddell) G.L. Nesom (sky blue aster)
Symphotrichum pilosum (Willd.) G.L. Nesom (old field aster)
Symphotrichum urophyllum (DC) G.L. Nesom (arrow-leaved aster)
Triodanis perfoliata (L.) Nieuwl. (clasping-leaved Venus' looking-glass)
Viola sagittata Aiton (sand violet)

* = Native (Oldham *et al.* 1995)

Appendix 2 (Continued). Species list for seeded species at the restoration site, Spring 2010.

Tallgrass Prairie Restoration Seed Mix (Spring 2010)

Graminoids

Andropogon gerardii Vitman (big bluestem)
Bromus kalmii A. Gray (kalm's brome)
Elymus trachycaulus (Link) Gould (slender wildrye)
Schizachyrium scoparium (Mich.) Nash (little bluestem)
Scirpus cyperinus (L.) Kunth (common woolly bulrush)
Sorghastrum nutans (L.) Nash (yellow indiagrass)
Sporobolus cryptandrus (Torr.) A. Gray (sand dropseed)

Trees/Shrubs

Carya cordiformis (Wang.) K. Koch (bitternut hickory)
Carya glabra (Mill.) Sweet (pignut hickory)
Carya ovata (Mill.) K. Koch (shagbark hickory)
Ceanothus americanus L. (New Jersey tea)
Cornus amomum Mill. (silky dogwood)
Cornus racemosa Lam. (grey dogwood)
Corylus americana Walter (American hazel)
Crataegus pruinosa (H.L. Wendl.) K. Koch (frosted hawthorn)
Prunus americana Marshall (American plum)
Prunus serotina Ehrh. (black cherry)
Prunus virginiana L. (chokecherry)
Malus coronaria (L.) Mill. (sweet crabapple)
Quercus macrocarpa Michx. (burr oak)
Rhus copallinum L. (winged sumac)
Rhus typhina L. (staghorn sumac)
Rosa blanda Aiton (smooth rose)
Rosa carolina L. (Carolina rose)
Vitis aestivalis Michx. (summer grape)

* = Native (Oldham *et al.* 1995)

Appendix 3. Maximum species cover for the 2012 and 2013 field seasons by N treatment (0, 2, or 6 g N m⁻² y⁻¹).

Forbs	Species	2012			2013		
		0	2	6	0	2	6
Prairie							
*^	<i>Artemisia campestris</i> L. (field woodworm)	3.1	1.8	0.3	1.0	0.8	2.1
*^	<i>Asclepias tuberosa</i> L. (butterfly milkweed)	0.0	0.1	0.1	0.0	0.0	0.3
*^	<i>Monarda fistulosa</i> L. (wild bergamot)	4.2	3.7	9.2	12.0	10.3	35.4
*^	<i>Pycnanthemum virginianum</i> (L.) Fernald & B.L. Rob. (Virginia mountain-mint)	0.0	3.0	2.1	0.1	8.8	3.6
*^	<i>Rudbeckia hirta</i> L. (black-eyed Susan)	15.3	16.8	22.8	14.6	19.7	17.6
*^	<i>Symphyotrichum laeve</i> (L.) Á. Löve & D. Löve (smooth blue aster)	9.6	10.4	8.1	4.9	11.3	6.3
*^	<i>Symphyotrichum oolentangiense</i> (Riddell) G.L. Nesom (sky blue aster)	0.0	0.0	0.0	3.7	3.9	5.5
*^	<i>Symphyotrichum urophyllum</i> (DC) G.L. Nesom (arrow-leaved aster)	6.1	8.6	11.7	1.5	6.0	1.5
Non-prairie							
*	<i>Ambrosia artemisiifolia</i> L. (common ragweed)	0.1	2.2	0.2	0.1	0.0	0.4
	<i>Anthemis cotula</i> L. (stinking chamomile)	0.9	1.9	2.2	0.0	0.0	0.0
*	<i>Arabidopsis lyrata</i> (L.) O'Kane & Al-Shehbaz (lyre-leaved rockcress)	1.3	9.1	14.4	5.7	5.7	6.6
*^	<i>Asclepias syriaca</i> L. (common milkweed)	0.1	0.1	2.1	0.0	0.0	0.0
	<i>Crepis capillaris</i> (L.) Wallr. (smooth hawksbeard)	0.0	0.0	2.1	0.0	0.0	0.0
	<i>Crepis tectorum</i> L. (narrow-leaf hawksbeard)	0.0	0.1	0.1	0.0	0.0	0.0
*	<i>Erigeron annuus</i> (L.) Pers. (annual fleabane)	0.3	0.0	0.3	0.9	0.0	3.6
*	<i>Erigeron canadensis</i> L. (Canada horseweed)	5.3	12.9	7.8	4.5	4.3	4.6
*^	<i>Euthamia graminifolia</i> (L.) Nutt. (grass-leaved goldenrod)	2.2	0.0	0.0	0.7	0.1	0.9
	<i>Holosteum umbellatum</i> L. (jagged chickweed)	32.9	30.4	18.7	4.7	3.3	4.8
*^	<i>Oenothera biennis</i> L. (common evening primrose)	0.9	0.7	0.9	0.0	0.0	0.0
*	<i>Oxalis dillenii</i> Jacq. (slender yellow wood-sorrel)	0.1	1.0	4.8	0.0	2.3	0.9
*	<i>Solidago canadensis</i> L. (Canada goldenrod)	0.0	10.3	0.0	0.3	11.3	0.9
*^	<i>Solidago juncea</i> Aiton (early goldenrod)	5.8	13.0	4.3	6.9	8.7	6.3
	<i>Taraxacum officinale</i> F.H. Wigg. (common dandelion)	4.4	11.1	14.7	3.0	6.6	5.0
	<i>Trifolium hybridum</i> L. (alsike clover)	0.0	0.0	0.0	0.9	0.1	0.1
	<i>Veronica arvensis</i> L. (corn speedwell)	7.8	5.6	3.9	7.7	11.6	5.8

* Native species ^ Seeded

Appendix 3 (continued). Maximum species cover for the 2012 and 2013 field seasons by treatment (0, 2, or 6 g N m⁻² y⁻¹).

Grasses	Species	2012			2013		
		0	2	6	0	2	6
Prairie							
*^	<i>Andropogon gerardii</i> Vitman (big bluestem)	6.1	3.9	19.0	13.9	5.1	10.8
*^	<i>Bromus kalmii</i> A. Gray (kalm's brome)	3.0	5.3	5.9	5.0	11.7	13.1
*^	<i>Schizachyrium scoparium</i> (Mich.) Nash (little bluestem)	21.0	13.4	22.3	21.6	22.5	23.2
*^	<i>Sorghastrum nutans</i> (L.) Nash (yellow indiagrass)	20.4	11.3	16.2	15.8	9.6	10.6
Non-prairie							
*^	<i>Elymus trachycaulus</i> (Link) Gould ex Shinners (slender wildrye)	0.3	1.2	8.7	1.9	8.1	9.9
Trees							
Non-prairie							
*	<i>Betula alleghaniensis</i> Britton (yellow birch)	0.0	0.0	0.0	0.0	3.0	0.0
*^	<i>Carya glabra</i> (Miller) Sweet (pignut hickory)	0.1	0.1	0.0	0.0	0.1	0.0
*^	<i>Quercus macrocarpa</i> Mich. (burr oak)	0.0	0.1	0.1	0.0	0.3	0.1
*	<i>Rhus typhina</i> L. (staghorn sumac)	0.0	0.0	0.0	0.1	0.0	0.9
Legumes							
Prairie							
*^	<i>Desmodium canadense</i> (L.) de Candolle (Canada tick-trefoil)	2.7	0.3	0.3	9.9	0.1	2.2
*^	<i>Lespedeza capitata</i> Mich. (round-headed bush-clover)	4.8	2.2	3.1	4.9	1.6	1.1
Non-prairie							
*^	<i>Lupinus perennis</i> L. (sundial lupine)	0.0	0.1	0.0	0.0	0.0	0.0
	<i>Medicago lupulina</i> L. (black medick)	5.1	2.1	2.1	1.8	2.5	0.9
Fern Ally							
Non-prairie							
*	<i>Equisetum arvense</i> L. (field horsetail)	2.1	0.4	1.2	6.9	2.8	1.3

* Native species

^ Seeded

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- McPhee, JM**, Henry HAL (May, 2013). Increasing atmospheric nitrogen deposition: Implications for tallgrass prairies. Ontario Ethology, Ecology and Evolution (OE³C). Western University, Ontario, Canada.

PUBLISHED REPORTS:

- MacLeod, H, McPhee, JM, & MacDonald, C 2012. Vegetation Enhancement Initiative for Cape Breton Strip Mine Sites 2011: in Mineral Resources Branch, Report of Activities; Nova Scotia Department of Natural Resources.