THESIS

ECOLOGICAL CONSEQUENCES OF INCREASED NITROGEN DEPOSITION IN

THREE NORTHERN GREAT PLAINS GRASSLANDS

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ABSTRACT

ECOLOGICAL CONSEQUENCES OF INCREASED NITROGEN DEPOSITION IN

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Increased nitrogen deposition is an important driver of plant species composition change in terrestrial ecosystems globally. Plant composition change from increased nitrogen inputs can result in substantial species richness declines especially where atmospheric inputs already exceed critical loads. Shifts in community structure can occur through changes in basic ecosystem conditions (such as soil properties) or through alterations in competitive interactions potentially disrupting feedbacks that maintain an ecosystem at a given fertility level. Although there has been a substantial amount of research on the effects of increased N on communities and ecosystems, most studies add large and, relative to natural inputs, unrealistic amounts of N. Thus, responses are often immediate and drastic. This "two-point" approach, comparing control to high-N plots, provides little information about the levels of N inputs at which responses first occur, which is more important for managing and mitigating the effects of increased N deposition in a proactive rather than reactive manner. The overarching goal of this study was to identify response thresholds to N addition in Northern Great Plains grasslands that differed markedly in productivity and soil fertility.

Over two years we assessed responses to increased N inputs (from 2.5 to 100 kg N/ha) in soils, leaf tissue, plant community composition, and aboveground net primary production (ANPP) in three northern mixed prairie grasslands that varied 3-fold in ANPP. The results of the study will enable us to better forecast both ecosystem and community responses to increased fertilization in this understudied region.

After two years of fertilization (with and without water addition) at levels ranging from 0 to 100 kg N/ha, we found significant effects from increased N inputs on ANPP when nitrogen levels exceeded 68 kg N/ha/year and effects on leaf tissue nitrogen, soil nitrogen content and N mineralization rates, particularly when levels exceeded 45 kg/ha/year. Alterations in soil and leaf nitrogen content and ANPP tended to have linear responses and remained consistent across sites. Significant responses occurred even in low ANPP sites (Badlands NP) which experienced a higher relative response.   No significant and consistent effects were found on total species richness, and community metrics such as evenness and diversity indices, plant cover by functional group or canopy cover. Our results suggest that though high fertility and production sites may have a greater absolute biomass response, low fertility and production environments can be quite responsive to nitrogen addition as well. Community change may occur more slowly with these northern grasslands which show little response to two years of increased N inputs.

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CHAPTER ONE: INTRODUCTION

General Nitrogen Fertilization Effects on Ecosystems and Communities

Increased nitrogen deposition is currently an important driver of plant species composition change in terrestrial ecosystems globally (Vitousek et al., 1997; Stevens, 2004; Bobbink, 2010; Pardo, 2011).   As nitrogen deposition increases world-wide even historically low-nitrogen systems may experience increased soil N supply (Holland, 1999; Bobbink and Lamars, 2002; Galloway, 2004). General effects of increased soil nitrogen supply can include increased production, decreased species richness and increased dominance of invasive plants (Huenneke, 1990; Allen, 2006; Harpole et al., 2007; Fenn, 2010; Pardo, 2011a). These effects may vary, depending on how N-limited the ecosystem is.   Most plant communities world-wide are N-limited, so when nitrogen is added, we can expect plant production to increase (Vitousek and Howarth, 1991). Increased production is not always beneficial as it can result in community change and can favor faster-growing species at the expense of rarer or slow-growing ones (Suding, 2005). Changes in community structure can occur through changes in ecosystem conditions or alterations in competitive interactions and may potentially change the feedback loops which maintain an ecosystem at a given fertility level.

Increased plant production due to increased nitrogen supply may be a positive effect of nitrogen deposition. However in some less productive or low nutrient environments, increased production may lead to undesired changes in species composition or richness. Under more benign conditions, as nutrient stress is relieved, stress tolerators are replaced by better competitors (Aerts, 2000; Bai, 2010; Fenn, 2010). Also some less productive environments such as the Badlands Sparse

vegetation complex (where ANPP<100 g/m^2, associated with badlands formations, the main attraction of Badlands National Park); would not benefit from increased plant production and cover. Nitrogen-enhanced variation in productivity matters as well. In nitrogen and water co-limited systems such as the Northern Great Plains, under increased nitrogen deposition, plant production may greatly increase in wet years but because productivity is limited by water as well, show little response in dry years (Black and Wight, 1979). Nitrogen accumulates during drought and can cause even greater production responses in subsequent wet years, opening the window further for possible exotic plant invasion when a wet year does occur (Hooper and Johnson, 1999).

Nitrogen addition studies typically use amounts of nitrogen far exceeding background N deposition levels to ensure immediate and drastic ecosystem responses occur. This study focuses on identifying critical loads for nitrogen deposition, a threshold of N addition response, below which there is no significant response to the given dose. While numerous studies have been done on nitrogen deposition effects on grassland ecology (Huenneke, 1990; Burke and Lauenroth 1998, Bardgett 1999, Foster and Gross, 1999; Burke and Lauenroth 2002; Lowe 2002; Harpole et al., 2007; Clark and Tilman 2008; Pan 2010), few of these studies have been at low levels that mimic current or forecasted N deposition rates (below 10 kg N/ha, Rauzi and Fairbourn, 1983) and few have been done in the unique northern mixed grass prairie system (Jacobsen, 1996). The results of this project will begin to define the dose-response of northern Great Plains grassland vegetation and soils to inorganic N and provide critical information needed to inform policy decisions on air quality emission levels in the region.

We examined three Northern Great Plains sites in South Dakota and assessed aboveground primary plant production and plant community responses to different N fertilization levels. First we determined how nitrogen limited each site is and at what level a nitrogen addition dose increases plant-available nitrogen. Nitrogen addition rates were 0, 2.5, 5, 7.5, 10, 20, 45, 68, and 100 kg N/ha/year with a subset of treatments receiving supplemental water. We then evaluated responses in production, species composition and community structure. Understanding how water limitation and the abiotic properties of the native soil affect responses to nitrogen levels enable us to forecast community responses to increased N deposition and to better understand nutrient cycling in this understudied grassland type.

Nitrogen Fertilization Effects in Northern Great Plains Grasslands

The mixed-grass prairie is the largest remaining prairie in North America with its extent shown in Fig. 1-1. In ecological literature, this vast region is often compared to either the wetter tall-grass prairie ecosystem to the east or the dry short-grass prairie to the west. Plant growth in short-grass prairie is primarily water-limited, while tall-grass prairies are both nitrogen and light-limited (Lauenroth and Dodd, 1978; Burke et al., 1998). Plant growth responses in the mixed prairie are less predictable and to some degree both water and nitrogen limited or co-limited. Year to year variation in precipitation is extremely high and thought to be a key driver in production and community responses (Wight, 1976; Samuel and Hart, 1998). In wet years, the prairie may behave more like a productive tall-grass system while in dry years show little production response to nitrogen and act primarily water-limited. The northern mixed grass prairie is composed of bunch grasses and rhizomatous sod-formers, cool season

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C_3 grasses and warm season C_4 grasses in addition to many forbs which are low in cover and biomass but which make up the bulk of its diversity. Since many functional types and possible competitors are present, the mixed grass prairie is an excellent place to study ecological change. When an ecological change occurs, favoring one type of life form over another, there is an extant species pool that includes many possible functional groups that could benefit from that particular change.

The northern mixed grass prairie includes most of the prairie pothole region, a network of localized wetlands extremely important to migrating bird species (Samson et al., 1998).   Much of the Northern Great Plains region is now under threat from increased nitrogen inputs due to energy development in eastern Wyoming (Baxter, 2008). However the native mixed grass prairie and possible effects from nitrogen deposition are understudied. Range studies done on both native and pasture areas of the Northern Great Plains, inform about plant responses to nitrogen and provide clues as to the extent of nitrogen limitation and nitrogen retention. Fertilization studies have been limited and often involve applying extremely high amounts of nitrogen often in excess of 45 kg N/ha/year for the purpose of range improvement, well above realistic pollution scenarios (10 kg N/ha/year; Lorenz and Rogler, 1972; Wight, 1976; Black and Wight, 1979; Power, 1980). Although nitrogen deposition is projected to increase throughout the region, historically this is an environment well-suited for studying nitrogen effects due to its low background deposition. Our fertilization experiment is located at two sites in Wind Cave NP (Fig.1-2) and one site in Badlands NP (Fig. 1-3).

Based on emissions estimates for the six power plants proposed in the area that would affect the Wind Cave and Badlands airshed, up to 11 kg N/ha/year (a worst-case

scenario) could be deposited across the parks (Baxter, 2008; Krust, 2009). Although

predicting deposition rates in a given location from emissions is difficult because of

wind, topography and other factors, N deposition does tend to closely reflect emissions

(Baron, 2006).   Nitrogen deposition consists of easily measurable wet deposition which

is carried in precipitation and dry windblown deposition which is not routinely measured,

but modeled. Nitrogen deposition is still minimal throughout the study area, though it

has been increasing slightly in recent years (Fig. 1-4). Wet nitrate and ammonium

deposition measurements at Wind Cave National Park (NP) have been measured by

the National Atmospheric Deposition Program (NADP) since 2003. Total inorganic wet

nitrogen deposition in the Wind Cave area over the past 8 years averages 2.65 kg

N/ha/year. The dry deposition component is difficult to measure, though Woodmansee

(1978) estimated dry deposition to be 25 %-50% of the wet deposition. CASTNET

(EPA's Clean Air Status and Trends Network) predicts an additional 1.03 kg N/ha/year

for dry deposition during 2003-2010 (USEPA, 2009). 2011 measurable wet nitrogen

deposition data is not yet available. Similar nitrogen levels have been recorded at the

Cottonwood, SD station, near Badlands National Park where nitrate and ammonium

have been measured since 1983. For the purpose of this study, we assumed 3.68 kg

N/ha/year is the current average amount of background total inorganic nitrogen

deposition based on the average NADP data from 2003-2010 plus an estimate of dry

deposition from CASTNET.

Woodmansee conducted the first nitrogen budget for the Great Plains region in

1978. It has been refined over the decades and later incorporated into the CENTURY

model by Parton and Stewart (1988).   The Cottonwood Site in South Dakota is the only

northern mixed grass prairie site examined in the budget. This site actually loses

nitrogen due to grazing (3 kg/N/year) and is a sink for nitrogen, meaning it loses more

nitrogen than it produces. Due to patchy deposition from animal waste, there is great

heterogeneity in soil nitrogen even within a small area.   Losses of nitrogen from

denitrification, leaching and runoff are minimal. Burke (1997) later showed that North

American grasslands were still suffering from the effects of tillage and estimated that as

much as 30% of available nitrogen has been lost.

Exactly why grasslands are limited by nitrogen is still not completely understood,

but generally available nitrogen is immobilized by microbes, creating low levels of plant

available nitrogen (Wedin, 1996). Native legumes can fix a small amount of available

nitrogen and inputs have been largely confined to animal manure (Woodmansee, 1978).

Although the northern great plains are grass-dominated, disturbances such as grazing

and the low nitrogen levels allow a wide array of forbs to proliferate, making grasslands

quite diverse.

The basic soils characteristic of the Northern Great Plains are expected to buffer

against acidification effects (Fenn et al., 2003) seen in other areas that have suffered

from nitrogen deposition (e.g., Rocky Mountain National Park; Baron, 2006), but the

fertilization effect on even basic soils could produce changes in plant productivity and

composition (Samuel and Hart, 1998). Sensitive vegetation types can respond at a very

low level of increased nitrogen (Fenn, 2003; Pardo, 2011a).

Critical loads for nitrogen are essential for understanding nitrogen-related change

in ecosystems and are a useful tool for policymakers (Porter, 2005.) A critical load for

nitrogen is a threshold of N addition response below which there is no significant

response to the given dose. Though challenging to develop, they have become more widespread in recent years. Critical loads typically involve a study or studies in which nitrogen addition had a particular effect on an ecosystem component. There are no set ecosystem components measured, effect size calculated, or length of time for which a study should be conducted (Porter, 2005). In a country-wide survey of nine major U.S. ecoregions, Pardo et al (2011a-b) compiled relevant studies to project a critical load for nitrogen in each ecosystem type. The Great Plains are estimated to have a critical load of 10-25 kg N/ha/year. These numbers are largely derived from studies at Cedar Creek Minnesota's tall grass prairies. The plains are further divided into tall-grass, mixed-grass and short-grass prairies and have critical loads of 5-15 kg/ha/year, 10-25 kg/ha/year and 10-25 kg/ha/year respectively. Mixed grass prairie critical load estimates are drawn from solely from southern mixed grass studies in Oklahoma. The southern mixed grass prairie is dominated by warm season C_4 grasses as opposed to the northern mixed grass composed largely of C_3 grasses which have different N requirements. While this critical load estimate by ecoregion is valuable and the first of its kind to be compiled, the authors caution that more work needs to be done to refine the critical loads and more studies need to be done at lower nitrogen addition levels without the addition of other supplemental minerals.

Several National Parks have determined critical loads for nitrogen deposition in order to better protect and understand their resources. Williams and Tonnessen (2000) calculated that the critical load for nitrogen acidification of alpine lakes in the Colorado Front Range was a mere 4 kg N/ha of wet deposition. Later Baron (2006) "hindcasted" to suggest the critical load for Rocky Mountain National Park lakes be reduced to 1.5 kg

N/ha/year.   Allen et al. (2006) found that 5 kg N/ha/year addition to already heavily

fertilized Joshua Tree National Park sites had a significant effect on the production of

exotic grasses. Fenn (2011) estimated California grasslands had a critical load of 6

kg/ha/year, above which annual grasses negatively impacted native vegetation.

Approaches to gauging critical loads involve modeling but are ultimately based on

empirical data in the form of fertilization studies to mimic the effects of deposition.

We can expect several effects from nitrogen addition in the mixed-grass prairie from

other long-term range fertilization studies. First, production increases with fertilization

(Power and Alessi, 1971; Lorenz and Rogler, 1972; Power, 1980; Jacobsen, 1996;

Samuel and Hart, 1998). Applications of fertilizer as low as 45 kg N/ha/year can

significantly increase forage production (Lorenz and Rogler, 1973). Also, community

shifts have been observed in the mixed grass prairie over several years of nitrogen

application. *Pascopyrum smithii*, a cool season wheatgrass responds dramatically to

nitrogen addition (Lorenz and Rogler, 1973). *Bouteloua gracilis* (a C_4 grass) cover has

been observed slightly declining under fertilization levels of 45 kg N/ha/year (Lorenz and

Rogler, 1973; Samuel and Hart, 1998). Power (1980) found that native prairie is less

adept at taking up nitrogen than rangelands dominated by introduced species such as

crested wheatgrass and smooth brome.

Nitrogen-cycling in Great Plains Grasslands

Unlike other nutrients which become available for organisms to use following

their liberation from rocks through weathering, nitrogen becomes available through

biological processes. Although the atmosphere is composed mainly of nitrogen, it

remains largely unavailable to organisms save through biological nitrogen fixation.

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Fixation and increasingly deposition are the two main ways nitrogen is introduced into the ecosystem. Nitrogen can leave the ecosystem through a number of pathways, including denitrification, leaching, erosion, harvest and through animal production. Nitrogen cycling is a collection of processes that recycle this biologically mediated nutrient from unavailable forms within plant and microbial biomass into bioavailable forms for other plants and microbes to take up and use for maintenance, growth and reproduction.

Understanding nitrogen limitation in an ecosystem of interest is critical to predicting the impacts of nitrogen addition (Vitousek and Howarth, 1991). How nitrogen limited an ecosystem is will determine its capacity to absorb excess nitrogen, without saturating and exporting nitrogen through leaching and denitrification. A simple way of determining the degree of limitation is by examining the production response of the vegetation to nitrogen. Often in the more arid short grass prairies, nitrogen is co-limited with water (Hooper and Johnson, 1999). Under co-limitation, no additional growth will occur even after fertilization until the plant's water needs are met. The degree of co-limitation with water has a huge effect on patterns of nitrogen uptake. Also responses to nitrogen uptake can differ depending on the precipitation that year. In wet years, more nitrogen is incorporated into more plant biomass while in dry years more of it is stored in higher quality tissue (Black and Wight, 1979). Systems that are not nitrogen-limited can be expected to show little production response to nitrogen.

Soils may exhibit one of two nitrogen cycling strategies (Fig.1-5). Highly productive eastern prairie soils which are less moisture-limited are more conservative in their nitrogen cycling (Burke, 1997; McCulley, 2009). The wetter eastern soils have a

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more closed nitrogen cycle. We would expect them to have higher N retention rates, immobilization, mineralization and nitrogen turnover. Drier less productive semi-arid shortgrass soils are expected to be leakier in their nitrogen use and tend toward a more open system. They have lower nitrogen retention rates, and because of their lower microbial activity, lower immobilization and mineralization rates. These soils are less limited by nitrogen than they are by other factors such as water (Clark et al., 2009). The mixed grass prairie could fluctuate between the two cycles based on nitrogen addition and on the amount of available water. There may be nonlinear thresholds between the two nitrogen cycling strategies where a pulse of nitrogen can send the ecosystem on a trajectory toward a different nitrogen cycling strategy. Identifying thresholds of nitrogen addition rates between different nitrogen cycling strategies is a crucial part of correctly recognizing critical loads.

Understanding regional trends in nitrogen mineralization and retention may help us predict what will happen to nitrogen at our study sites under the particular climatic scenario for our study. The Northern Great Plains have been plagued by drought in recent years (HPRCC, 2011) and have been presumably quite water-limited. We experienced two wet years when water limitation was relieved and so we can expect more nitrogen limitation. There are two major gradients used for ecological studies in the Great Plains. There is a MAT (mean annual temperature) gradient increasing from north to south and a MAP (mean annual precipitation) gradient increasing from west to east paralleled by what Burke and Lauenroth (1997) depict is an increasing average net N mineralization trend in Fig. 1-6. McCulley et al. (2009) actually found that both immobilization and nitrogen pools increase from west to east, but that net nitrogen

mineralization did not increase across the gradient. Though more productive, the eastern soils also had a great demand for nitrogen which contributes to a tight cycle. Just as McCulley examined regional trends in nitrogen cycling, the same trends and responses to wetter soils may be detected on a much smaller spatial scale that includes climatic variability across years.

Plant Uptake and Community Responses to Nitrogen Fertilization

Certain areas in the plains are much less productive than areas with similar climatic regimes due to their substrate, for example the Nebraska Sand Hills and Badlands areas both of which have low nutrient retention among other challenging soil properties. Low soil fertility can contribute to lower plant production. Soil fertility is a complex of soil factors which affect plant growth such as pH, which affects nutrient availability; water holding capacity and soil structure, which determine how much water infiltrates the soil and remains available to plants; salts and minerals, which can have detrimental effects on plant growth; organic matter; and essential nutrients such as nitrogen, phosphorus and potassium. Studying these low production ecosystems in comparison to more productive ecosystems with similar climatic regimes helps tease apart the role soil fertility plays in plant responses. There is no clear consensus in the ecological community but in general, initial productivity of a site is either considered to have no relation to the fertilization effect (Gough, 2000) or nitrogen effects are considered to be relatively greater in more productive communities (Chapin, 1986). Storm (2008) found evidence to the contrary in very low nutrient sand grasslands in Germany which experienced an increase in canopy cover in response to fertilization.

Rangeland studies also provide some guidance as to what to expect from nitrogen effects on soils and roots. Though much of the initial response to fertilization is in the tops of the plant, the roots are extremely N-limited and may be a site for long-term storage of excess nitrogen. Power's (1972) range paper on North Dakota rangeland shows the massive amount of nitrogen that can be immobilized first in a grass's aboveground biomass, then later stored in its roots. He found that up to 200 kg N/ha could be immobilized in roots and soil organic matter in the first year of fertilization. Up to 350 excess kg N/ha could be stored after 3-4 years of fertilization. At doses of less than 180 kg N/ha, the overall soil nitrogen pool did not significantly increase. Background nitrogen in the "check" plots is 20-40 kg N/year, which provides an average of how much nitrogen is in grassland soils. For the first 135 kg N/ha applied, the researchers found no increase in soil N pool. Maximum production (ANPP) occurs at 350 kg N/ha after which nitrogen limitation is overcome and another factor becomes limiting. Even with massive nutrient loading, it takes 3-4 years for this to occur. Power's (1981) thorough long-term experiment looks at the fate of nitrogen during the 6 years of application and subsequent 9 years of recovery. The Northern Great Plains are N-limited and respond with increased biomass to nitrogen fertilization. Even when fertilization stopped, the plants kept taking up nitrogen from the soil. Power's study shows that C_3 grasses respond more readily to the nitrogen and so fertilization may result in an eventual community shift toward C_3 species. Recovery in above-ground biomass may not exceed 35% of the application rate, 30% is stored in the roots and 35% lost through volatilization or immobilized in the soil.

Rauzi's (1983) paper differs from other range papers in that it uses realistic amounts of nitrogen simulating deposition. The authors applied 22, and 34 kg N in a factorial experiment with both spring and fall applications. They found forbs increased under all nitrogen treatments and that western wheatgrass, a C_3 grass, increased in density but that there were no other significant species effects. Crude protein, a measure correlated with leaf nitrogen increased in all treatments. Their conclusion, that low amounts of nitrogen addition results in increased leaf N rather than in a biomass increase, suggests how plants which are also water-limited might store nitrogen in their tissues. Jacobsen's 1996 range paper examines different fertilization rates on dry matter production (ANPP) and forage quality (correlated to leaf N ratio) on forage grasses which cover about half the Northern Great Plains. They investigated the effects of one application of fertilizer on the growth of 10 grass species up to 4 years after fertilization. Responses were still evident after 4 years in increased leaf and root N. Fertilization increased yield even at low amounts of 56kg N/ha. In dry years little above-ground response was seen and results suggest that any extra nitrogen taken up was stored in the roots.

Samuel and Hart (1998) found that blue grama, a C_4 perennial decreased under all levels of nitrogen treatments. Western wheatgrass, a C_3 grass, increased in frequency perhaps because of its early phenology which benefits from nitrogen available early in the season. The ANPP response closely followed precipitation patterns, and increased at all nitrogen levels relative to the control. Increases of 24%, 23% and 39% in the 22 kg N/ha plots over the control in poor, average and good years for ANPP respectively were found, which is highly significant and points to strong N-

limitation. Power (1981) estimated that unfertilized soils contained 20-40 kg N/ha/year that was inorganic and bioavailable.

Grasses are thought to have the advantage over forbs and shrubs under fertilized conditions because of their faster N uptake and use rates (Bobbink, 2010; De Schrivjer, 2011). Indeed many fertilization studies both in the field and in the lab support this supposition. C_3 or cool season grasses often outperform the warm season C_4 grasses and show a disproportionate response when fertilized (Pan, 2010). C_3 grasses in general have lower nutrient use efficiency or NUE (Chapin, 1980; Wedin and Tilman, 1996) meaning they add fewer grams biomass per gram nitrogen absorbed due to the less nitrogen-efficient C_3 photosynthetic pathway. Plant uptake of nitrogen results in either higher litter quantity or quality which has feedback repercussions on the microbial community. Nitrogen unused during dry years may result in a dramatic increase in production when water limitation is finally alleviated (Lorenz and Rogler, 1972; Black and Wight, 1979). Flushes of nitrogen are available in the early spring when considerable wetting/drying and freezing/thawing cycles release nitrogen from microbes making it available to plants. Plants that can take advantage of this early pulse of nitrogen include the bromes and early cool season grasses such as western wheatgrass (Lorenz and Rogler, 1973).

One possible effect of increased soil N supply due to deposition is a shift in community composition. Differences in nutrient uptake can cause community shifts as members of the plant community are outcompeted and replaced by less nutrient efficient species. Nutrient use efficiency is an important concept in community ecology. Nutrient efficient plants are typically slower growing, and retain nitrogen within their

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tissues (Chapin, 1980). They are best at conserving nitrogen and produce low quality litter to enforce a feedback that favors a conservative strategy with slow nutrient cycling (Chapman, 2006). Low NUE plants are more adept at taking up nitrogen and are faster-growing and produce higher quality litter that is cycled quickly. Changes in nutrient acquisition strategies and N cycling at the ecosystem level have serious consequences and can precipitate drastic community change (Wardle 1998; Grime 2002; Fortunel 2004; Chapman, 2006).

Species composition shifts can include favoring faster-growing species over slower-growing species (Liancourt, 2009), grasses over forbs (Huenneke, 1990; Bobbink, 1991; Wedin and Tilman, 1996; Harpole, 2007; Allen 2009), non-legumes over legumes (Huenneke, 1990; Suding, 2005), earlier phenology C_3 grasses over C_4 grasses (Clark and Tilman, 2008; Pan, 2010) and nonnative plants over natives (Huenneke, 1990; Schwinning, 2005; Allen, 2009; Fenn, 2010). While it has been demonstrated that the status of a plant being invasive does not necessarily make it a better competitor for nitrogen (Lowe, 2003), many invasive species do become even better competitors under conditions of increased nitrogen supply. Indeed increased nitrogen supply has consistently lead to greater invasibility across ecosystems.

Fluctuations of soil nitrogen can also increase invasibility (Davis, 2000). One such invasive common to the northern Great Plains is *Bromus tectorum* and its closely related species *Bromus japonicus*. Both species are widespread throughout the mixed grass prairies and found at all my study sites. These annual bromes are fall to winter annuals and among the earliest grasses to green-up in the spring. They successfully avoid drought by completing their life cycle by June or July. If there is an early spring

microbial-based pulse of nitrogen in the spring, they can take advantage of the pulse before later emerging grasses. Bromes favor disturbed soils and have been commonly observed growing preferentially in soils with higher nitrogen content. Any change in the biomass or cover of annual bromes can have ecosystem–wide consequences. Bromes can change fire regimes by increasing fuel loads, change soil moisture patterns, lead to reductions in native diversity through extensive cover and increase erosion due to competition against deeper rooted and more permanent species (Lowe, 2003; Allen, 2009). Power (1981) agreed with Wight (1976) and found that annual bromes were the most responsive grass to nitrogen and native grasses the least. Day and Detling (1990) found that *Poa pratensis*, a naturalized fast-growing C_3 grass increased in biomass on nitrogen-rich bison urine patches. Van Riper (2005) found exponential increases in response to nitrogen addition in a summer annual, *Halegeton glomeratus* in the Badlands Sparse Vegetation complex, where one of my study sites is located.

Lowe and Lauenroth (2003) found that *Bromus tectorum* an invasive grass common to the both the short grass and mixed grass prairie increased in biomass under all levels of fertilization treatments (from 10 kg N/ha to 100 kg N/ha). Because C_3 grasses and especially winter annuals start growth earlier in the season, it is thought they are able to take advantage of nitrogen that has accumulated over the winter and take advantage of available moisture earlier in the year. It must be said that in cold areas such as the Northern Great Plains, nitrification and indeed all microbially-mediated nitrogen transformations are restricted by temperature early in the year when the C_3 grasses begin growth (Lorenz and Rogler, 1973). Less nutrient efficient plants (low NUE) can gain a competitive advantage against high NUE plants when nitrogen is

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added. Some exotic plants such as ruderals are low NUE plants and contribute to quick

nitrogen cycles. They can take advantage of a pulse of nutrients quicker than slower

growing native plants.   In Tilman's 12-year nitrogen addition study in tall grass prairie,

C_3 grasses increased, C_4 grasses decreased and overall diversity declined at all levels

of nitrogen addition (Fig.1-7).

A great number of studies show a decline in local species diversity due to

nitrogen addition, (Huenneke, 1990; Bobbink, 1998; Stevens, 2004; Clark and Tilman,

2008; Bobbink, 2010; Cleland and Harpole, 2010; Fenn, 2010). Suding et al (2005)

summarized abundance-based, function-based and resource-use based mechanisms

for plant species loss due to nitrogen. The authors conclude that the mechanism of loss

is scale dependent and ecosystem-specific.   Greatest species losses occur in sites that

experienced the greatest increase in productivity. Abundance-based losses are

primarily random but explain why rare species are much more likely to be lost than

dominant species. A function-based loss occurs when short-statured species may be

constrained by their height and unable to compete in a more productive environment.

Resource-use based mechanisms says that losses are due to a shift in N cycling in the

ecosystem and low NUE plants can better utilize additions of nutrients than high NUE

plants. High NUE plants such as long-lived perennials, legumes and C_4 plants are often

outcompeted by low NUE plants such as annuals, C_3 plants and grasses. Note that not

all C_3 grasses are low NUE and not all C_4 grasses are high NUE. Controlled

greenhouse conditions may change the NUE, resulting in different NUEs than the same

grasses grown under field conditions (Levang-Brilz and Biondini 2003).   Cleland and

Harpole found that an ecosystem's response to nitrogen, made up of individual species'

responses, depends largely on whether nitrogen is still collectively limiting for that ecosystem or if limitation has shifted to another resource, causing the structure of plant competition to change.   Species adapted to high nitrogen environments are low NUE plants and better nitrogen competitors. Cleland and Harpole found in their meta-analysis that plant communities were more vulnerable to species loss in sites with "low cation exchange capacities, cold regional temperatures and strong production responses to nitrogen."

Objectives and Hypotheses

This study tests the relative nitrogen limitation in a variety of habitats in the Northern Great Plains. We established plots at three sites: an upland site at Wind Cave NP, a lowland site at Wind Cave NP and a low production site of concern at Badlands NP (Maps shown in Figs. 1-2 and 1-3).   The three sites encompass the range of edaphic factors, fertility and initial productivity levels typical of the natural variability of northern Great Plains grasslands. Large herbivore grazing and fire were excluded for the past twenty years for all plots. In 2010 and 2011 we applied fertilization and water treatments at each site to test the relative water and nitrogen limitation. Water treatments were either water addition of 3-6 cm per growing season or ambient rainfall. Nitrogen additions were 0, 2.5, 5, 7.5,10, 20, 45, 68 or 100 kg N/ha/year. Aboveground net primary production by functional group, species richness, evenness, diversity, and percent cover by species were measured each year. This study uses chronic low level levels of nitrogen fertilization to determine the level below which no negative effects from fertilization occur in the ecosystem. Nitrogen mineralization measurements were made early each summer. Tissue chemistry was measured in six species, *Bromus*

tectorum, *Pascopyrum smithii*, *Elymus trachycaulus*, *Andropogon gerardii*, *Poa pratensis* and *Bouteloua curtipendula* throughout 2011. Relative production and changes in species composition or nutrient cycling were recorded for each site and year.

We hope to address the following research questions in the study and address them in this thesis:

1. At what level of N addition do our sites show a significant response at four hierarchical levels of the ecosystem? Abiotic responses include soil inorganic N content and N mineralization rates. Organismal responses include leaf tissue nitrogen in dominant C_3 grasses. Production responses include aboveground annual primary production and C_3 nonnative plant biomass. Community responses include plant species richness and changes in plant cover (e.g. bare ground canopy cover, changes in the C_3: C_4 grass ratio, diversity and evenness).

2. Will the most N-limited site (Badlands) respond more strongly to N addition than less N-limited sites (Wind Cave)?

3. Does initial productivity of a site influence the strength of its response?
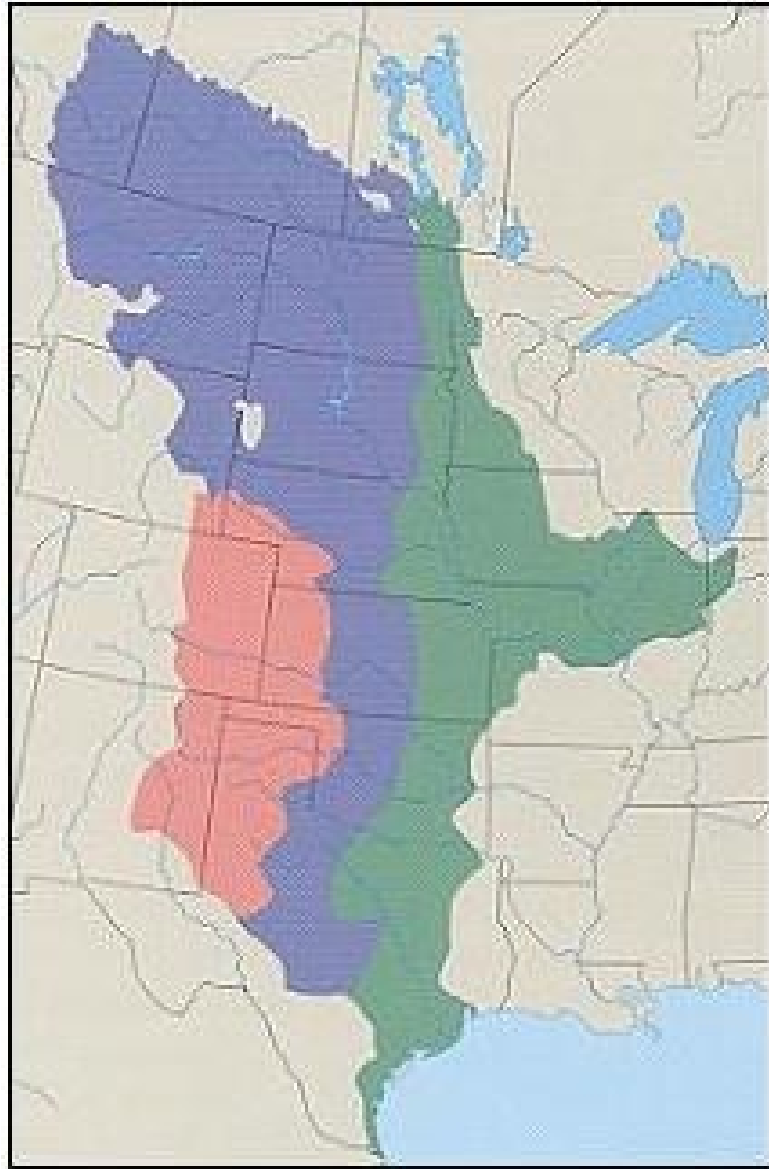
Figure 1-1: Prairie Biomes with shortgrass in pink, mixed-grass in purple and tall-grass in green. (Samson et al., 1998)
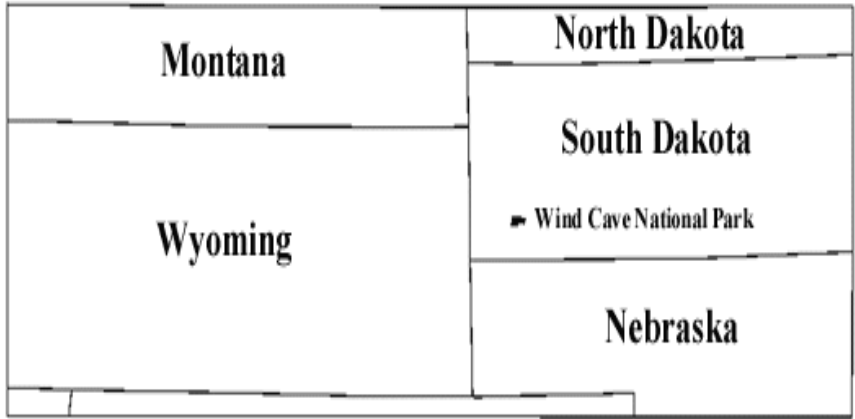
Figure 1-2: Wind Cave National Park location (Cogan, 1999)

Figure 1-3: Badlands National Park Location (Von Loh, 1999)

Wet Nitrogen Deposition
Wind Cave
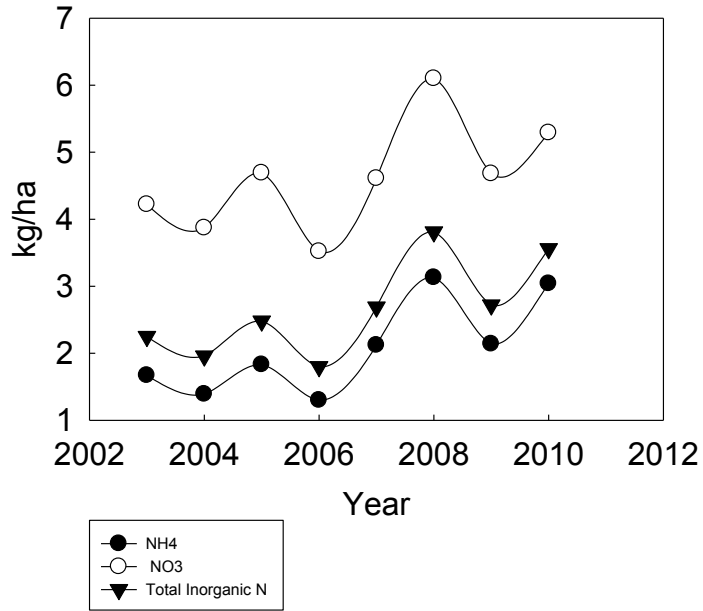
Figure 1-4: Nitrogen deposition at Wind Cave NP 2003-2010 (NADP, 2011).

Wet deposition data collected at the Wind Cave NP NTN (National Trends Network)

SD04 Wind Cave- Elk Mountain site. All analytes shown as kg [analyte]/ ha deposition.

Increasing N limitation

"Open" Nitrogen Cycle
Faster cycling
Less Retention

"Closed" Nitrogen Cycle
Slower cycling
More retention

Lower NUE plants

Higher NUE plants

Less
Immobilization

High
Quality
Litter

More
immobilization

Low
quality
litter

Low C:N ratio

High C: N ratio

N addition

Threshold

Figure 1-5: Slow and Fast pathways of Nitrogen Utilization adapted from Chapman et al, 2006.

Figure 1-6: Mean Annual Precipitation (MAP), Mean Annual Temperature (MAT), Soil
 Carbon, and Average Annual Net Mineralization throughout the Great Plains
 (Burke and Lauenroth, 1997).
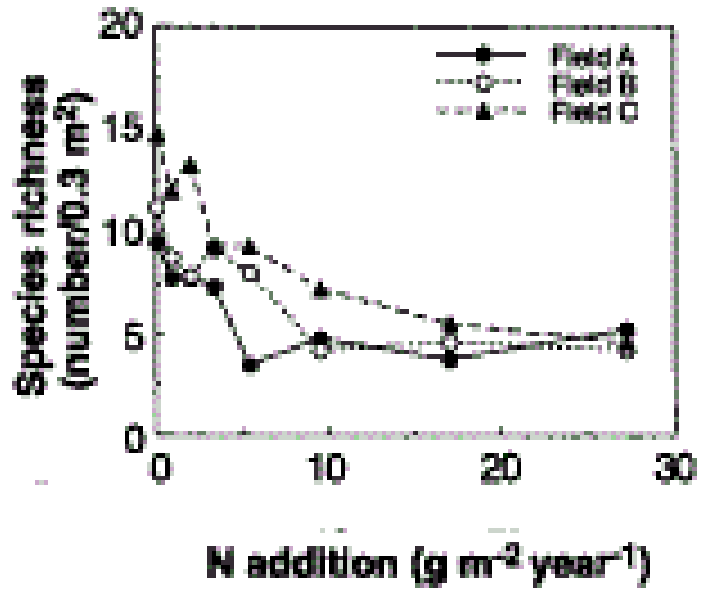
Figure 1-7: Species Richness decline with Nitrogen Addition (Wedin and Tilman, 1996).

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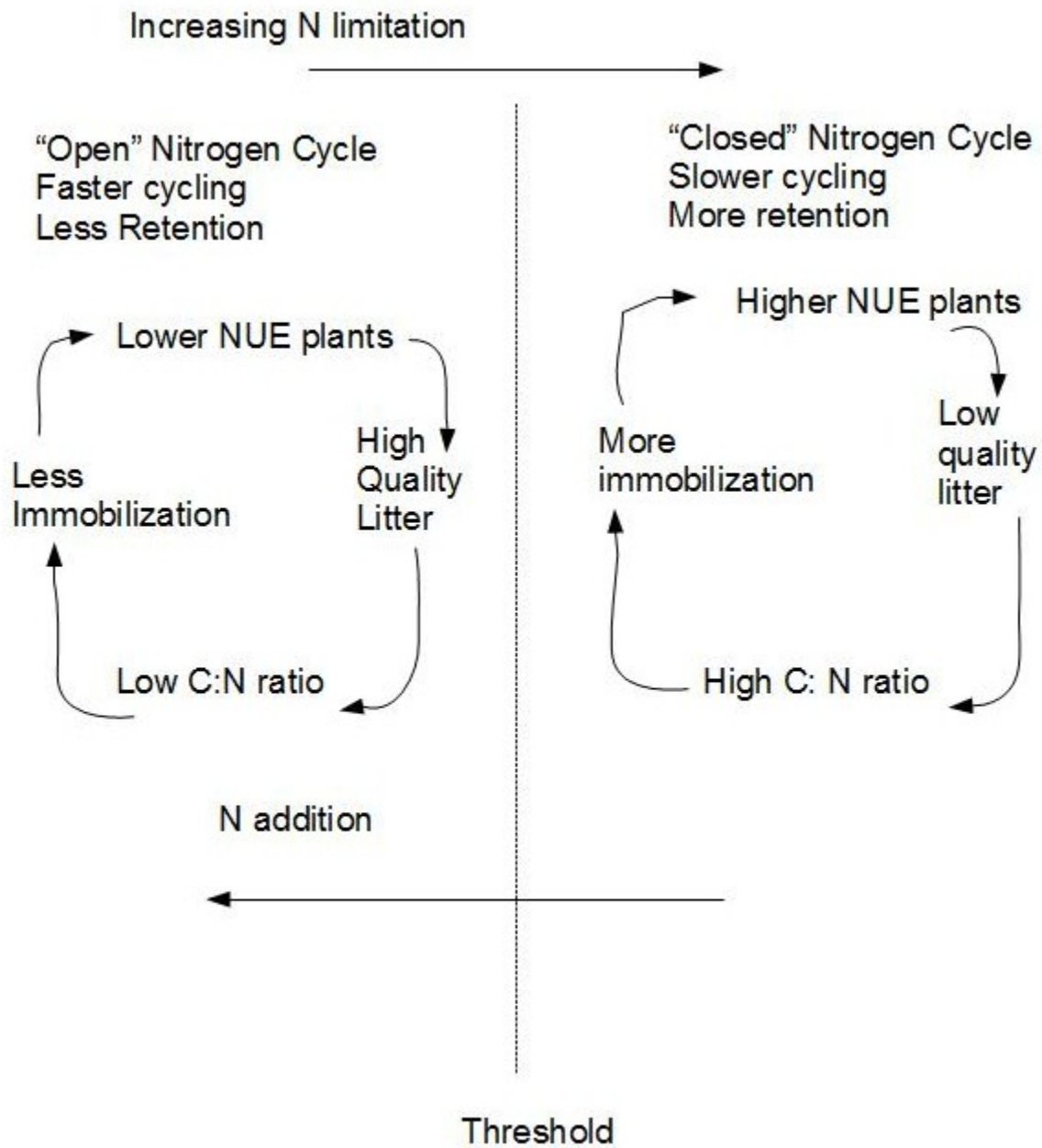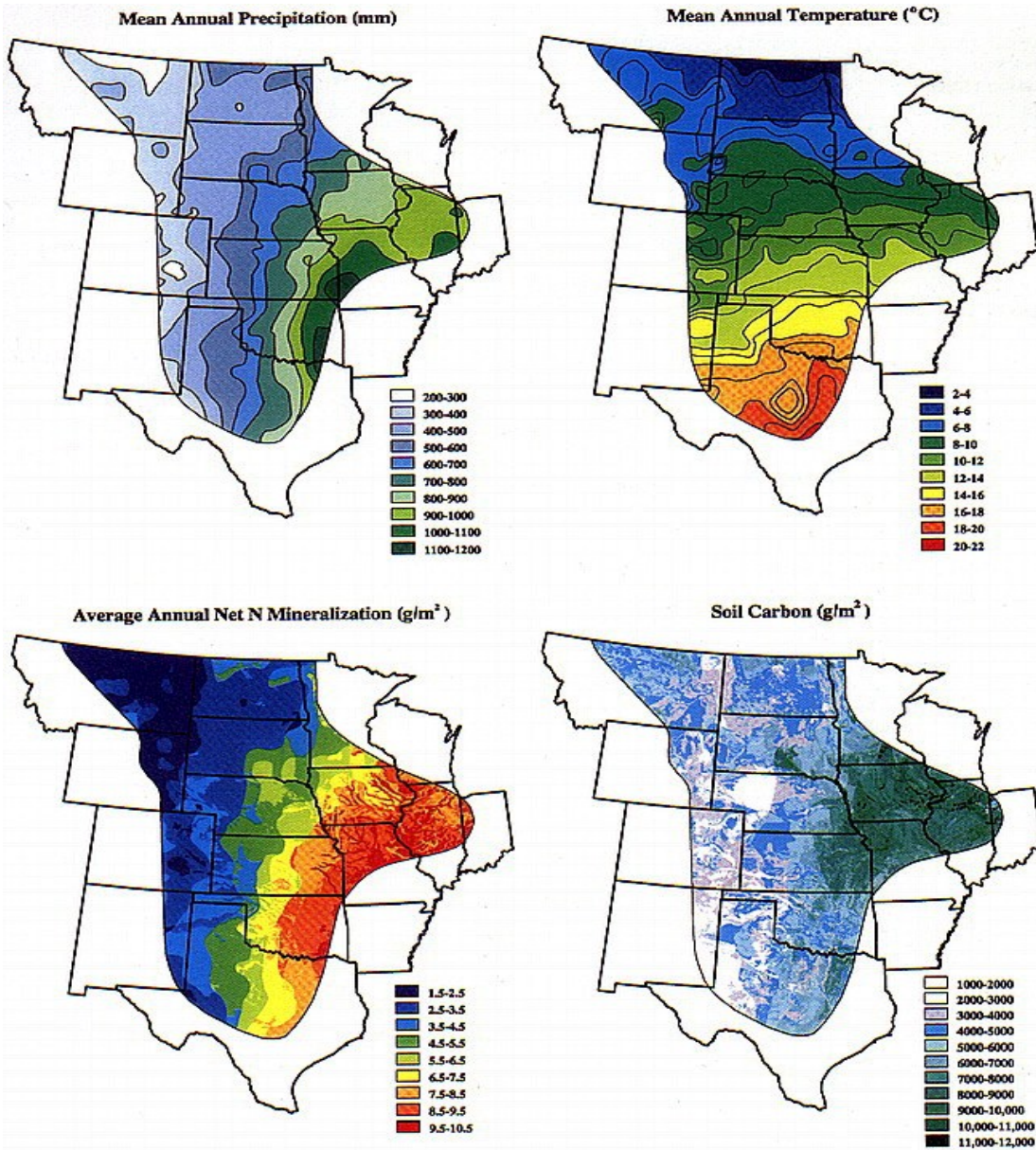Black, A. L. and J. R. Wight (1979). "Range Fertilization- Nitrogen and Phosphorus uptake and recovery over time." Journal of Range Management 32(5): 349-353.

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CHAPTER 2: PLANT COMMUNITY AND PRODUCTION RESPONSES TO

INCREASED NITROGEN INPUTS IN HIGH AND LOW FERTILITY GRASSLANDS

Introduction

Increased nitrogen deposition is one of the primary drivers of plant species

composition change in terrestrial ecosystems globally (Vitousek, 1997; Stevens, 2004;

Bobbink, 2010; Pardo, 2011a-b).   As nitrogen inputs to terrestrial ecosystems increase

world-wide, even historically low-fertility systems may experience increased soil

nitrogen supply (Holland, 1999; Bobbink and Lamars, 2002; Galloway, 2004). General

effects of increases in soil nitrogen include increased plant production,

decreased species richness and increased dominance of invasive plants (Huenneke,

1990; Gough, 2000). These effects may vary depending on the degree of N limitation in

a particular ecosystem, but because most terrestrial plant communities are N-limited,

increased production is almost always expected to result (Vitousek and Howarth,

1991). Increased production is often accompanied by community change. Shifts in

community structure can occur through changes in basic ecosystem conditions (such as

soil properties) or through alterations in competitive interactions, potentially disrupting

feedbacks that maintain an ecosystem at a given fertility level (McNaughton, 1979;

Chapin, 1980; Chapman, 2006).

Although there has been a substantial amount of research on the effects of

increased N on communities and ecosystems, most studies add large and, relative to

natural inputs, unrealistic amounts of nitrogen that exceed ten times the amount of

nitrogen forecast for or currently experienced in the area. Thus, responses are often

immediate and drastic. This "two-point" approach, comparing control to high-nitrogen

plots, provides little information about the levels of nitrogen inputs at which responses first occur (Jorgensen et al, 2005), which is more important for managing and mitigating the effects of increased nitrogen deposition in a proactive rather than reactive manner. The overarching goal of this study was to identify response thresholds to N addition in Northern Great Plains grasslands that differed markedly in soil fertility. At present, N inputs into these ecosystems are relatively low (3.68 kg N/ha/yr) but these are expected to increase up to 10 kg N/ha/year as a worst case scenario with plans for the construction of several fossil fuel based energy production facilities regionally. Over two years we assessed responses to increased N inputs (from 2.5 to 100 kg N/ha) in soils, plant community composition, and aboveground net primary production (ANPP) in grasslands that varied 3-fold in ANPP.

Methods

Study plots were established in the spring of 2010 at three Northern Great Plains grassland sites. One study site was located at Badlands National Park and two sites were established at Wind Cave National Park. Site characteristics are summarized in Table 2-1.

Badlands National Park is located in southwest South Dakota USA. The climate is continental with hot summers and cold winters. Average January high/low is 2.2/ -10.6 °C and average July high/low is 32.8/17.0 °C. Average annual precipitation is 40.6 cm with 75% falling during the growing season (April to September) largely in the form of intense storms. Soils range from being classified as 'no soil', associated with erosional features, to shallow clays and clay loams. In addition to having low nitrogen availability, (control soil N content averaged .57 µg N/g soil), these poor quality soils

have low water holding capacity, (Table 2-1) low organic matter and nutrient (N and P) content, and can be alkaline with a high pH and salt content (NRCS, 2011). Previous experiments have shown little vegetation response to P addition but high response to N and N plus water treatments, suggesting that these grasslands are both nitrogen- and water-limited (Van Riper 2005). We established plots at a site (referred to as the 'BSV' site) representative of the Badlands Sparse Vegetation type (Von Loh et al., 1999). This vegetation occurs at the base of badlands formations and is sparsely vegetated with a mix of C_3 and C_4 grasses, forbs and subshrubs. Subshrubs include *Gutierrezia sarothrae, Eriogonum pauciflorum* and *Yucca glauca*, as well as several *Opuntia* species. Badlands National Park contains many invasive species. Nonnative forbs such as *Salsola tragus*, *Halogeton glomeratus*, and the legume *Melilotus officinalis* are of special concern in Badlands NP. These summer annuals/biennial are highly variable from year to year and represented 13% (2% forb cover and 11% legume cover) of total canopy cover at the BSV site during this study. Nonnative C_3 annual bromes (primarily *Bromus japonicus*, but also *B. tectorum*) were widespread throughout our study area and formed a significant component of the cover (10%)*.* Other common grasses included *Elymus trachycaulus* and *Poa secunda* (native C_3 species) and *Muhlenbergia cuspidata*, *Bouteloua curtipendula*, and *Bouteloua gracilis* (native C_4 species). Vegetated canopy cover was low, with the control plots averaging only 63%.

Wind Cave National Park is also located in southwest South Dakota, on the southern edge of the Black Hills, an isolated extension of the Rocky Mountains in the Northern Great Plains. The climate is slightly milder than that of Badlands (average January high/low3.3/-11.4 °C, average July high/low 31.7/14.0 °C, average annual

precipitation 50 cm), but with similar precipitation seasonality. Two sites that differed in topographic position were selected for study at Wind Cave.

The lowland 'Tree' site was located inside a large bison exclosure in the low-elevation, Red Valley portion of the park. Soils were well-drained sandy loam, neutral (pH 7), relatively high in nutrient and organic matter content (7.8%), and had the highest water holding capacity of the three sites (Table 2-1). Inorganic soil nitrogen averaged .94 µg N/g soil. Plots were located in a typical northern mixed grass prairie with a variety of native C_3 grasses (*Hesperostipa comata*, *Pascopyrum smithii, Nassella viridula, Koeleria macrantha*), C_4 grasses (*Bouteloua gracilis*, *Bouteloua curtipendula*, *Schizachyrium scoparium*), and sedges (*Carex filifolia*, *Carex inops*). The nonnative C_3 grass, *Poa pratensis,* was widespread across the site, as were *Bromus japonicus and Bromus tectorum*. Some plots had high cover of the low shrubs *Symphoricarpus occidentalis* and *Rosa arkansana*. A great diversity of forbs, including *Tradescantia bracteata, Ratibida columnifera* and *Sphaeralcea coccinea*, occurred throughout the site.

The upland 'Hill' site at Wind Cave was also located within a large herbivore exclosure. Plots were laid out along a ridgeline of limestone parent material on a cobbly sandy loam soil with limited water capacity (Table 2-1). Soils were shallow, slightly acidic (pH 5.9) and had 7.7% organic matter. Nitrogen was most available at this site, with 1.57 µg N/g soil. Common C_3 grasses include the native *Hesperostipa comata* and the nonnative *Bromus tectorum* and *Bromus japonicus*. Sedges including *Carex inops* and *Carex filifolia* were frequent. Common C_4 grasses included *Andropogon gerardii*,

Schizachyrium scoparium, *Bouteloua curtipendula*, and *Bouteloua gracilis*. The nitrogen-fixing shrub *Amorpha canescens* was also abundant (26% cover).

Plots (2.5 x 2.5 m) were established at all sites in the spring of 2010 in a randomized complete block design of 5 blocks per site. Nitrogen (NH_4NO_3) addition treatment levels were 0, 2.5, 5, 7.5,10, 20, 45, 68 and 100 kg N/ha added in 4 applications: mid- April, early June, late June and mid-July each year. Four additional treatments crossed nitrogen addition (0, 2.5, 68, or 100 kg N/ha/year) with water addition (20 plots per site). With the goal of adding ten percent of average growing season rainfall, water was added 3 times over the summer of 2010 (early June, mid-July and early August) for a total addition of 3.1 cm added to each water addition plot. Water was applied via backpack sprayer to the plots with the amount applied during application adjusted to maximize infiltration into the soil. In 2011 water addition was increased to 5.5 cm at the Tree site, 6.6 cm at the Hill site (which had available water nearby, hence the greater amount applied) and 5.5 cm at the BSV site, applied over three treatment dates (Fig. A-1).

In 28 plots at each site, we measured in situ nitrogen mineralization over 28 days from mid-June to mid-July (2010 and 2011) using the buried bag method (Eno 1961, Binkley and Hart 1984). Pre- and post determinations of ammonium (NH_4^+) and nitrate (NO_3^-) from four 1.9 cm diameter x 10-cm deep soil cores were based on 2M KCL extractions (24 hour period) with an Alpkem Flow Solution IV auto-analyzer. A portion of the same soil cores were used to determine gravimetric moisture and total C and N content. In addition, inorganic N content was measured pre-growing season in March and post-growing season at the end of September 2011 from four 1.9 cm diameter x 10

cm deep cores per plot. Due to time and financial constraints, soil responses were only measured in half of the N addition treatments (0, 5, 10, 45 and 100 kg N/ha/year).

Aboveground whole plant tissue nitrogen content was measured in 2011 at times appropriate for each species. We selected plants that were fully grown, with at least 4 leaves, but that had not reached the flowering stage yet. *Bromus japonicus* could not be harvested earlier than the flowering stage due to its early phenology. Tissue chemistry samples were collected for *Bromus japonicus* (all three sites), *Poa pratensis* (Tree site), *Pascopyrum smithii* (Tree and Hill sites), and *Elymus trachycaulus* (BSV site) in June 2011, and for *Andropogon gerardii* (Hill site) and *Bouteloua curtipendula* (BSV and Tree sites) in July 2011. Between 7 and 12 tillers per species per plot were harvested and dried. The samples were composited and ground by species, then analyzed with a Leco Tru-Spec Total CN analyzer. In statistical analyses, *P. smithii* and *E. trachycaulus* were analyzed together as native C_3 grasses and *A. gerardii* and *B. curtipendula* together as native C_4 grasses, since a single individual species in each of these functional groups was not sufficiently abundant for sampling at all three sites. Aboveground net primary production (ANPP) was estimated by harvesting all herbaceous aboveground biomass in a 20 cm x 50 cm area in August 2010 and in a different 25 cm x 1 m area in August 2011 in each plot. The larger area was harvested in 2011 to reduce sample variability. Current year's growth of herbaceous species (forbs, graminoids, subshrubs) was considered ANPP. Cactus and succulent (*Yucca glauca*) species were excluded from analysis due to difficulties in distinguishing current from previous years' growth. Biomass was sorted into functional groups and dried for 48 hours at 60 C^o before weighing. Canopy light (PAR) was measured in 2011 using a

linear quantum light meter (LI-250A Li-Cor) 100 cm above ground level and at ground level.

Plant species richness, diversity and cover were measured within the same 1 m x 1 m quadrat both years. Quadrats were surveyed in both June and September for a yearly plant richness value (species by site listed in Table A-2). Canopy cover by species and by substrate (bare ground, litter, etc.) was determined in June by the point-intercept method within the same quadrat. We used 100 points at the BSV site but, due to the higher density of the vegetation, only 50 points were required at the two Wind Cave sites. Diversity was determined with the Shannon-Weiner index (H') and evenness calculated as H'/Hmax, where Hmax= lnS and S is the number of species in the plot. The C_3:C_4 grass cover ratio we hoped to examine was better analyzed as C_4 plant cover as a percent of total grass cover as many plots had either strictly C_3 or C_4 grasses within.

The experimental design was a multi-site randomized complete block design with year as a repeated measure. A mixed linear model was initially used to test first for water and nitrogen addition, as well as their interactions using the REML (Restricted Maximum Likelihood) Method in the MIXED procedure (SAS, 2008). We used a mixed linear model with nitrogen treatments (referred to as 'nrate') as a fixed categorical factor, and site and year as fixed factors. Random factors included blocks nested within site, the interaction of treatment and blocks nested within site, and the interaction of year and block nested within site. The significance of the categorical variable was used to discern significance of the treatment on the response variable. Response variables of interest were soil inorganic N content and N mineralization ('Nmin'), leaf tissue N

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content, ANPP, C_3 nonnative plant biomass, plant species richness, and changes in plant canopy cover including: bare ground cover, $C_3:C_4$ grass ratio, evenness, and diversity. Differences between treatment levels were detected by examining the LSMEANS, using the Fischer-Protected Least Significant Difference to correct for multiple comparisons. Furthermore, the relationship between the transformed response variable and 'nrate' as a continuous variable was explored through regression models where transformed response variable= intercept+ slope*(continuous nitrogen rate). A non-zero slope showed that the response variable did react to the nitrogen addition rate in either a positive or negative manner.

Response variables log-transformed to maintain linearity and homoscedasticity include inorganic N content, N mineralization, soil moisture, ANPP and C_3 nonnative grass biomass. Cover response variables (bareground cover and C_4 percent grass cover) were square root transformed to maintain normality then run in the linear model. The level of significance was $p < 0.05$ for all variables. Degrees of freedom were calculated using the Satterthwaite method except for soil moisture measurements which had missing data so the Kenward-Rogers method was used. All statistics were performed using SAS 9.2 (2008) with the techniques of Littell (2006).

Results

Precipitation was above average during both the 2010 and 2011 growing seasons (HPRCC, 2011). Growing-season rainfall was nearly 60% above average in 2010 at Wind Cave and 61% above average in 2011 at Badlands. Consequently no significant soil moisture differences were seen in the gravimetric soil moisture samples between control and water-treated plots at any of the sites or months measured

(F=0.08, p=0.7732), when soil moisture values were log-transformed to maintain normality. In addition, there were no main (F = 0.27, p = 0.60) or interaction (F=0.97, p= 0.41) effects of water on ANPP. Thus, for all analyses 'nrate' was the only treatment tested and any water addition to plots was ignored.

Soil responses

Only the highest N addition rates ('nrate') of 45 and 100 kg N/ha significantly increased soil inorganic N content, and this was found at all sites (Table 2-2, Fig. 2-1, Fig. A-2). Significant treatment (F=38.38, p<0.0001) and site x treatment (F=4.65, p=0.0001) responses occurred (Table 2-2).   Due to the site x treatment interactions, further testing was required by site (Fig. A-2). N availability in the control plots differed by site with Hill the highest site at 1.52 µg N/g soil compared to BSV's 0.57 µg N/g soil and Tree's 0.94 µg N/g soil. Inorganic nitrogen content increased more strongly at the Hill site, followed closely by the BSV site while there was little response at the Tree site (Fig. A-2). Nitrogen mineralization responses to N addition rate were more variable, with net N mineralization generally increasing in the highest N addition treatment in 2010 but decreasing in 2011, regardless of site (Table 2-2, Fig. A-3). Nitrogen treatment remained effective at altering the nitrogen mineralization rate (Table 2-2, Fig. A-3). Differences in the final soil C:N ratio between the Wind Cave sites (C:N ~12) and the Badlands site (C:N ~33) suggest greater potential N limitation at Badlands (Fig. A-4).

Leaf Tissue N Responses

We tested 6 grass species for whole plant nitrogen content throughout the summer of 2011. All species responded positively to nitrogen addition (Fig. 2-2).

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Native C_3 grasses *Pascopyrum smithii* (dominant at the Tree and Hill sites) and *Elymus*

trachycaulus (dominant at Badlands) were analyzed together with 'nrate' a significant

predictor at p<0.0001. The control differed from the 45 and 100 kg N/ha/year

treatments. *Poa pratensis,* a nonnative C_3 grass, had 'nrate' as a significant predictor

variable, p=0.0003 with the control plots differing significantly from 100 kg N/ha/year

treatment. The other annual C_3 nonnative grass, *Bromus japonicus*, had control leaf

tissue N differ significantly from the 100 kg/ha/year treatment and 'nrate' was significant

at p<0.0001. The C_4 grasses, *Bouteloua curtipendula* and *Andropogon gerardii* were

analyzed together as native C_4 grasses. These grasses differ significantly from controls

at 'nrates' of 45 and 100kg/ha/year. 'Nrate' was a significant predictor variable at F=

4.76, p=0.0076.

Production responses

ANPP increased in both years, at all three sites, with increasing N addition (Table

2-2), but significant increases in ANPP over the control plots were detected only in the

68 and 100 kg N/ha/year levels (Fig.2-3). After log-transforming biomass, the 'nrate'

variable was significant with a p-value of 0.0229. When examining 'nrate' as a

continuous variable using the regression approach, the response of ANPP to N addition

was significant all sites (Table 2-2).

Overall, grasses responded strongly to nitrogen addition regardless of the grass

species or functional type present in the plot. Total grass biomass increased

significantly with 'nrate' (F=2.97, p=0.0052), and the control differed from the 68 and

100 kg N/ha/year treatment levels. However, C_3 nonnative grasses did not increase

significantly with nrate (p= 0.1709) and the control did not differ from any N addition level (Fig.2-4).

The relative response to nitrogen addition was higher at plots with lower initial productivity than those with higher initial productivity (Fig. 2-5). Thus, the Badlands site, which had low baseline productivity showed a proportionally greater N response than sites with higher productivity.

Community Responses

Across all sites, for the species richness response, there was a significant site x treatment interaction (Table 2-2, Fig. 2-6, F=2.28, p= 0.0071). Richness did show a significant response to nitrogen addition only at the BSV site, (F=2.67, p=0.0230) though the control did not significantly differ from any treatment levels there (Table 2-2, Fig. 2-6).

Although species richness did not respond significantly to nitrogen addition levels, the relative response to high nitrogen addition (100 kg N/ha/year) was higher at plots with lower initial productivity than those with higher background productivity (Fig. 2-5). Less productive plots (<100 g/m^2 ANPP$_c$), which tended to be located at Badlands NP, actually experienced a positive species richness increase relative to their control (S_n/S_c>1), while the more productive sites had comparatively lower but still positive relative responses (Fig. 2-5). The species richness relative response (meaning the species richness of fertilized plots compared to the species richness of control plots) tends to be positive (S_n/S_c>1) in plots with lower control plot productivity (ANPP$_c$), while plots with higher control plot productivity have S_n/S_c<1 (Fig. 2-5).

No major shifts in functional groups (native vegetation (F= 0.85, p=0.5620), forbs

(F= 1.70, p=0.1113) or legumes (F=0.56 p= 0.8086)) occurred with nitrogen addition

(Fig. A-5). Neither did it appear that nitrogen addition resulted in lower light levels

(Fig. A-6). Plant community responses had significant site x treatment factors, which

required further testing by site (Table A-4). Although there were some responses in

diversity (F=2.26, p= 0.0285), and evenness (F= 2.35, p=0.0229), responses were

inconsistent with nitrogen addition and generally very small (Table A-3). When testing

the community metric regression lines, the slope was only different from zero for bare

ground cover (t=-4.04, p=0.0001, Table A-3). Likewise when significant treatment-level

differences did exist, there was no pattern in how those levels differed from the control

(Table A-3). The BSV site was the only site in which bare ground cover significantly

decreased with N addition (Fig. 2-7) with differences significant at the 45 and 100 kg

N/ha/year treatments. C_3 nonnative grass cover neither had a significant trend in

relation to N addition levels (F=0.42, p=0.9071) nor did the control differ significantly

from any of the other treatments. The Tree site experienced some slight increases in C_3

nonnative cover (from 72 to 90%), but not until the highest levels of nitrogen addition.

The proportion of grass cover comprised of C_4 natives decreased at the Hill site

(Fig. 2-7), where proportional C_4 grass cover in the control was 47% and was 25% in

the 100 kg N/ha/year treatment but not at the other two sites (Table A-3).

Discussion

The overall goal of this research was to assess the degree of sensitivity of three

relatively pristine Northern Great Plains grassland communities to increased nitrogen

addition, especially at realistic levels expected from atmospheric deposition. We used a

wide range of nitrogen addition levels (0 kg N/ha/year to 100 kg N/ha/year) over two years to identify initial response thresholds. Although significant only in the two highest N treatments in which it was measured, inorganic soil N content tended to increase with increasing N addition throughout the year. This indicates that N treatments were effective in fertilizing the plots and that soil nitrogen remained elevated in treated plots. While it is not surprising that nitrogen would be higher soon after added to the system, it is significant that nitrogen remained elevated year-round, including at the beginning (March) and end (September) of the second growing season (Fig. 2-1). This suggests that excess nitrogen is accumulating in the soils.

Some of this soil nitrogen was taken up by plants and converted both into higher leaf nitrogen content and into biomass. Indeed, leaf nitrogen increased linearly with nitrogen addition, with a 29% increase over the controls in the 100 kg N/ha/year treatment averaged over species. ANPP responded significantly only at higher N addition levels (68 kg N/ha/year and above, with 'Nrate'=100 at 41% more ANPP over the controls). Other studies in the mixed-grass prairie have shown similar increases in leaf N (Rauzi and Fairbourn, 1983; Jacobsen, 1996) and ANPP (Lorenz and Rogler, 1972; Wight 1976; Black and Wight, 1979; Power, 1980; Jacobsen, 1996; Samuel and Hart, 1998), although sometimes at lower N addition levels (Rauzi and Fairbourn, 1983; Samuel and Hart, 1998). Some of these studies (Black and Wight, 1979; Rauzi and Fairbourn, 1983; Jacobsen, 1996) suggest that the primary response of plants in this type of grassland to added N is to accumulate the N in plant tissues, instead of increasing productivity, though both responses are common and likely to occur. In wet years water is less limiting and plants can use the additional nitrogen for biomass

production (Hooper and Johnson, 1999; Harpole, 2007; Pardo 2011). Nitrogen can be stored in leaf and root tissue during dry years to be used by the plant for biomass accumulation when conditions are favorable (Jacobsen, 1996). Our watering treatments were meant to confirm this water x nitrogen limitation, but the high precipitation in our study years essentially swamped our watering treatments. Although the high precipitation may have made our study systems more responsive to N than would occur in an average precipitation or drought year, response to nitrogen at lower addition levels (22 kg N/ha/year) in mixed-grass prairie have been reported in these drier conditions (Samuel and Hart, 1998).

Interestingly, the two dominant nonnative C_3 grasses at our sites tended to show greater increases in leaf tissue N with N addition than did the dominant native C_3 or C_4 grasses (Fig. 2-2). Similarly, Power (1980) found that native prairie is less adept at taking up N than rangelands dominated by *Bromus inermis* and *Agropyron cristatum*, two other nonnative C_3 grasses. The C_3 grasses, native or nonnative, experienced a dramatically greater increase in leaf nitrogen than did our C_4 grasses, presumably due to greater nitrogen demand. This greater demand for nitrogen, evident in the leaf tissue, may indicate that under higher nitrogen conditions, C_3 plants could gain a competitive advantage over C_4 grasses, possibly leading to future composition changes.

Shifts in the relative abundance of individual species and functional groups have been demonstrated in mixed-grass prairie (Lorenz and Rogler, 1972; Samuel and Hart, 1998). For example, *Pascopyrum smithii* density decreased and *Bouteloua gracilis* basal area increased as N fertilization level increased in a North Dakota mixed-grass prairie (Lorenz and Rogler, 1972). Similarly, *P. smithii* frequency increased and *B.*

gracilis and *Carex filifolia* frequency decreased under two levels of N addition in a Wyoming mixed-grass prairie, but biomass responses of these groups was more variable (Samuel and Hart, 1998). We did not see any consistent composition changes across our sites, even for nonnative C_3 grasses, a group that has increased dramatically in other North American grasslands in response to N addition (Lowe and Lauenroth, 2003; Clark and Tilman, 2008; Fig. 2-4, Fig. A-5). Since we sampled biomass at the time of peak herbaceous biomass (late July - early August), the productivity of these early-season grasses may have been underestimated, especially for the annual *Bromus* species that senesce in late June. Our two-year study may not be long enough to capture community changes that often take years to become apparent (Samuel and Hart, 1998; Clark and Tilman, 2008).   Whatever grasses were present in the plot did increase in biomass with nitrogen addition (Fig. 2-4). The particular phenology and anatomy of the grass (warm or cool season) did not matter. Neither did non-legumes experience more biomass change than legumes or nonnatives more than natives (Fig. A-5). The grass response was expected, as grasses are considered to be faster growing than forbs, and more adept at using available nitrogen (Huenneke 1990, Bobbink 1991, Wedin and Tilman 1996; Harpole, 2007; Clark and Tilman, 2008; Allen 2009).

Species composition metrics such as species richness, evenness and diversity varied significantly but not consistently with nitrogen levels (Table 2-2; Fig 2.6; Tables A-3 and A-4). We detected conflicting site x treatment interactions (plant species richness, bareground cover, C_4 cover) that showed the importance of site effects on community composition (Tables A-3 and A-4). For example, species richness tended to

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decrease (though not significantly) with nitrogen addition at Wind Cave, while Badlands plots showed species richness increasing significantly with nitrogen addition (Fig. 2-6). Light levels at ground level did not differ by treatment (Fig. A-6). Reduced canopy light caused directly by fertilization is a common mechanism attributed to N-induced species loss (Suding, 2005).

Bai et al. (2010) experienced similar results, at a mature grassland undergoing a major species richness decline and a decline in productivity. A degraded grassland actually showed positive results in response to nitrogen addition with increased productivity and only a slight decline in community composition.

The greater the nitrogen limitation, the greater the effect nitrogen addition will have in that ecosystem. Plant communities in extremely low fertility soils that are nutrient-limited are limited in ANPP and in the number of species that can survive there by the lack of nutrients (Grime, 2002). Nitrogen addition would relieve extreme conditions and increase production and the number of species that can exist in that harsh environment (Chalcraft, 2008). As moderate low fertility or low nitrogen availability soils shift to higher nitrogen availability, species can be lost later due to more competitive plants' increased dominance and productivity at the expense of less competitive plants (Aerts and Chapin, 2000). Though there is no clear consensus in the ecological community, initial productivity of a site is considered to have no relation to the fertilization effect (Gough, 2000) or nitrogen effects are considered to be relatively greater in more productive communities (Chapin, 1980; Grime, 2002). Storm (2008) found evidence to the contrary in very low nutrient sand grasslands in Germany. However Huenneke (1990) and Clark and Tilman (2008) found that production

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increases in a low productivity grasslands led to species richness reductions.   My results support the idea that though higher production sites may have a greater absolute biomass response, low fertility and low production environments can be quite responsive to nitrogen addition and even the relative response to nitrogen can be greater.

In our study, the low fertility BSV site responded quite strongly to nitrogen addition. The Tree site and BSV site had a similar absolute ANPP response to N addition, an increase of ~100 g/m^2/year between the control and 68 or 100 kg N/ha/year treatments. However, the relative response of the BSV site was stronger: a 120% increase in ANPP vs. 36% increase at the Tree site. The responsiveness of low fertility sites is even more strongly illustrated at the individual plot level. When the N response is measured as the ratio of ANPP in the 100 kg N/m^2/year plot to its within-block control (Fig. 2-5), only two of the 19 plots with a >2-fold ANPP increase were in areas with control productivity >100 g/m^2. Composition and diversity responses to N addition were much more muted, but the lowest fertility site was again the most responsive in that species richness actually increased (Fig. 2-6).

The Hill site at Wind Cave was the least responsive site and probably the least nitrogen-limited. This site's background inorganic soil nitrogen was much higher than the other two sites' (1.52 µg N/g soil compared to BSV's 0.57 µg N/g soil and Tree's 0.94 µg N/g soil), as was the tissue nitrogen content of native cool-season grasses (Fig. 2-2). Higher nitrogen content at the Hill site may have been due to the abundance of the nitrogen-fixing shrub, *Amorpha canescens* (26% canopy cover), which has be shown to increase soil nitrogen and favor C$_3$ grasses (Milby, 2011).

Some important caveats to this study are that that we used sites where grazing is minimal and large ungulates such as elk and bison are excluded. The study sites have not had fire on them for at least twenty years. Literature suggests that both fire and grazing, which are common on the northern mixed grass prairie and maintain ecological features, have important effects on nitrogen cycling. We would expect both of these management practices to mitigate negative effects of nitrogen deposition by recycling it out of grasses and back into the air, or moving it out of the system by denitrification (Vitousek, 1997).

In summary, nitrogen addition at our sites resulted primarily in an increase in leaf and soil nitrogen, followed by significant increases in ANPP at higher levels of nitrogen addition. We found a positive significant increase in soil nitrogen throughout the growing season, even months after application with a significant response at 45 kg N/ha/year. Production increased across sites at levels above 68 kg N/ha/year. Sites with lower initial productivity had proportionally greater production responses to fertilization. Few community-level changes occurred in the time frame of our study and none that were consistent across sites, leading us to suggest that Northern Great Plains grasslands are more resistant to the effects of nitrogen addition than some other grasslands (Harpole, 2007; Clark and Tilman, 2008). To date, significant effects occurred at N addition rates more akin to deliberate fertilization (>45 kg N/ha/year) than those expected from atmospheric deposition caused by fossil-fuel combustion (<10 kg N/ha/year). Further research will determine whether this remains true in the long term.

Table 2-1: Study Site Characteristics

Site characteristic variables derived from NRCS (Natural Resources Conservation Service), 2011. PH values determined from pre-treatment soil cores to 10 cm.

Site	Location	Mean annual temperature (^{o}C)	pH	Mean annual precipitation (mm)	Soil Texture	Slope	Elevation	Soil Available Water capacity
(BSV) Badlands Sparse Vegetation Complex site Badlands, NP	43° 43'31"N 101°58'59"W	8.30	8.6	406	clay loam/ weathered bedrock	3-60%	737 m	.3 cm
(Tree) Lowland Wind Cave NP site	43° 36' 15" N 103° 22' 59" W	6.95	7.0	483	sandy loam	0-3%	1157m	19.8 cm
(Hill) Upland Wind Cave NP Site	43° 44' 38" N 101° 56' 20" W	5.83	5.9	530	cobbly sandy loam	20-40%	1230m	10.2 cm

Table 2-2: Results from ANOVA table for multi-site analysis of nitrogen treatment x site x year and regression results. Treatment is the nitrogen addition rate or 'nrate', and include levels of 0, 2.5, 5, 7.5 10, 20, 45, 68 and 100 kg N/ha/year. A continuous version of 'nrate' is used for a separate regression model which also uses a repeated measures structure as random effects. Inorganic N, N mineralization, ANPP and C_3 biomass values were log-transformed to maintain homoschedasticity for analysis. Bare ground cover values were square-root transformed to maintain normality. Results from site-based analyses for nitrogen treatment x year for response variables in the Tree, Hill and BSV sites are included in Figs 2-1, 2-3, 2-4, and 2-7 and Appendix Figure 2. Regression slopes are calculated for all response variables using the same repeated measures structure as the ANOVA. The slope test tests if the slope of the regression line does not equal zero where transformed response variable= Intercept+slope *continuous nitrogen rate. Significant values are p <.05.

Abiotic responses

Effect	df	Inorganic N content		N Mineralization rate		df	Native C_3 Leaf N content	
		F	p	F	p		F	p
Site	2	48.12	<0.0001	4.74	0.0339	2	22.04	0.0001
Year	1	1.64	0.2151	6.59	0.0116			
Site x Year	2	3.29	0.0585	1.36	0.2598			
Treatment	4	38.38	<0.0001	3.67	0.0114	8	17.6	<0.0001
Site x Treatment	8	4.65	0.0001	0.69	0.6976	16	0.11	0.9988
Year x Treatment	4	0.16	0.9560	1.79	0.1361			
Site x Year x Treatment	8	1.55	0.1463	0.71	0.6798			
Regression slope		0.0044		-0.001			0.0044	
Slope test (if slope=0)		t=9.37, p=.0001		t=-1.89,p=.0661			t=9.16,p=0.0001	

Production responses   **Community responses**

| Effect	df	ANPP		C_3 nonnative grass biomass		df	Plant Species Richness		Bareground Cover	
		F	p	F	p		F	p	F	p
Site	2	41.2	<0.0001	13.94	0.0007	2	13.32	0.0008	60.82	<0.0001
Year	1	4.02	0.0630	5.63	0.0184	1	1.27	0.2603	32.02	<0.0001
Site x Year	2	1.05	0.3747	7.31	0.0008	2	1.17	0.3110	7.58	0.0006
Treatment	8	2.36	0.0229	1.49	0.1709	8	1.01	0.4357	2.69	0.0104
Site x Treatment	16	0.92	0.5473	0.83	0.6541	16	2.28	0.0071	2.15	0.0120
Year x Treatment	8	0.59	0.7874	0.5	0.8531	8	0.1	0.9993	1.87	0.0657
Site x Year x Treatment	16	1.07	0.3853	0.81	0.6733	16	0.33	0.9934	0.65	0.8380
Regression slope		0.00486		0.0065			-0.0023		-0.001	
Slope test (if slope=0)		t=3.98, p=0.0001		t=1.7, p=.0922			t=-.32, p=.7499		t=-4.04,p=0.0001	

Inorganic N content
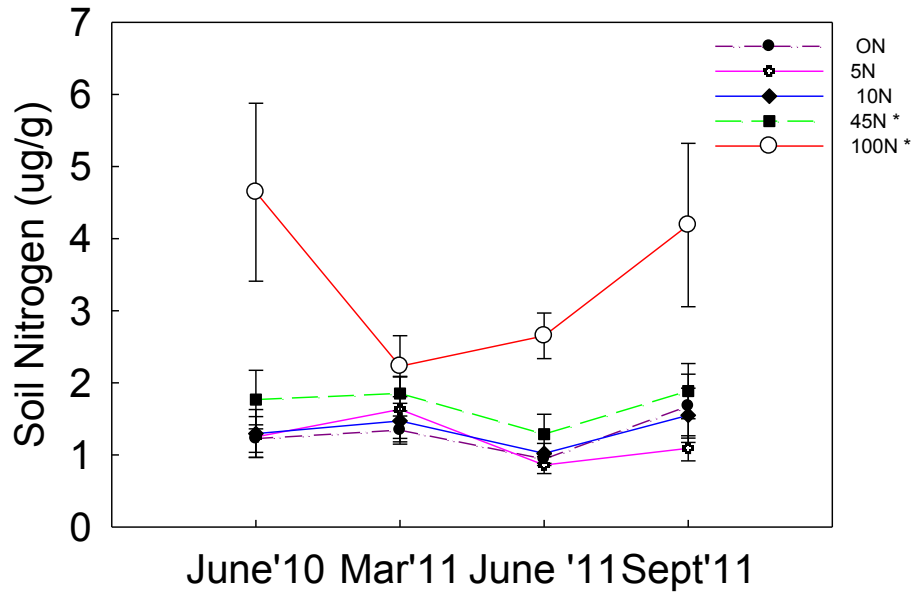
Figure 2-1: Inorganic N content from soils at 10-cm depth 2010 and 2011
Values were log-transformed to maintain homoscedasticity. Error bars represent one standard error of the mean.
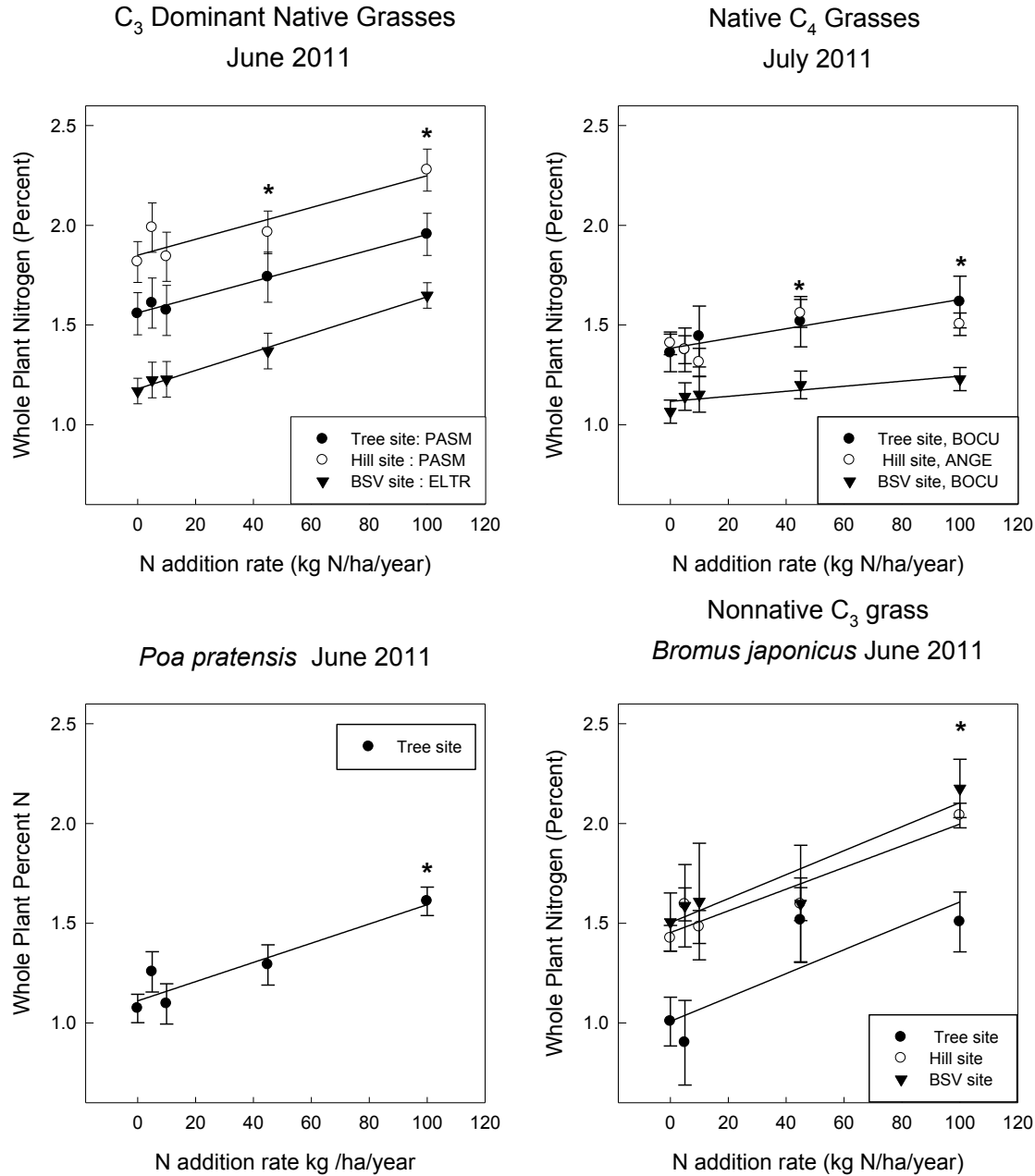
C$_3$ Dominant Native Grasses June 2011

- ● Tree site: PASM
- ○ Hill site : PASM
- ▼ BSV site : ELTR

Native C$_4$ Grasses July 2011

- ● Tree site, BOCU
- ○ Hill site, ANGE
- ▼ BSV site, BOCU

***Poa pratensis* June 2011**

- ● Tree site

Nonnative C$_3$ grass *Bromus japonicus* June 2011

- ● Tree site
- ○ Hill site
- ▼ BSV site

Figure 2-2: Whole plant tissue nitrogen. Asterisks indicate where aboveground tissue N significantly differs from the control ($p<.05$). Native C$_3$ grasses are the dominant grasses at each site: *Pascopyrum smithii* (PASM) at the Wind Cave Hill and Tree Sites and *Elymus trachycaulus* (ELTR) at the Badlands BSV site. C$_4$ grasses include *Bouteloua curtipendula* (BOCU) at the Tree and BSV sites and *Andropogon gerardii* at the Hill site. Error bars represent one standard error of the mean.
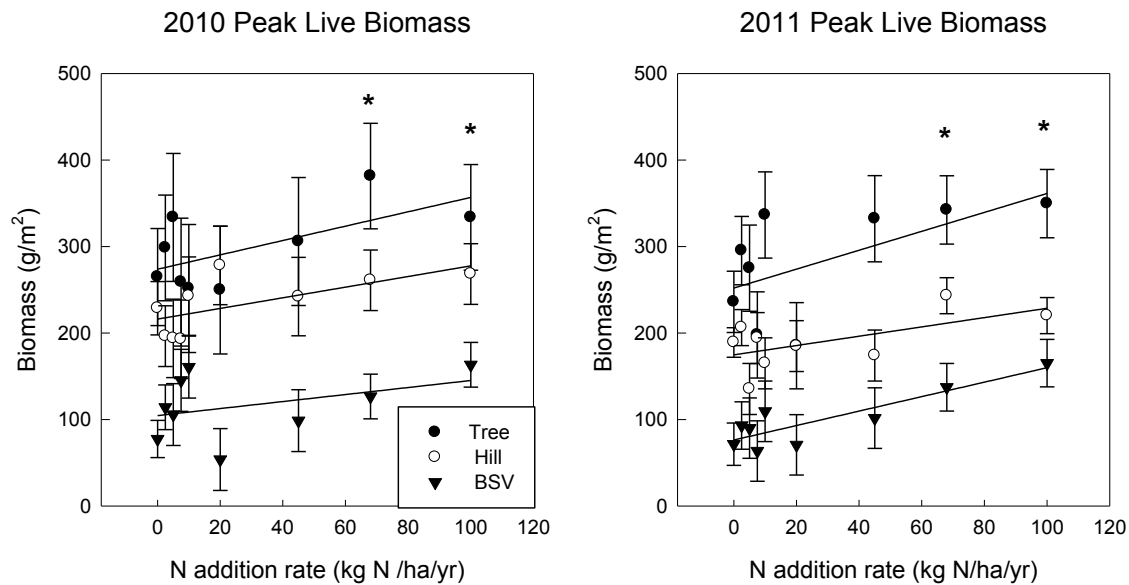
Figure 2-3: Biomass g/m^2 2010 and 2011 by site

Biomass values were log-transformed to maintain homoscedasticity. Using a repeated measures ANOVA over two years, control biomass differs from treatment biomass with an 'nrate'= 68 and 100 kg N/ha/year. 'Nrate' is a significant regression variable with **F=2.36 and p=0.0229**. Error bars represent one standard error of the mean.
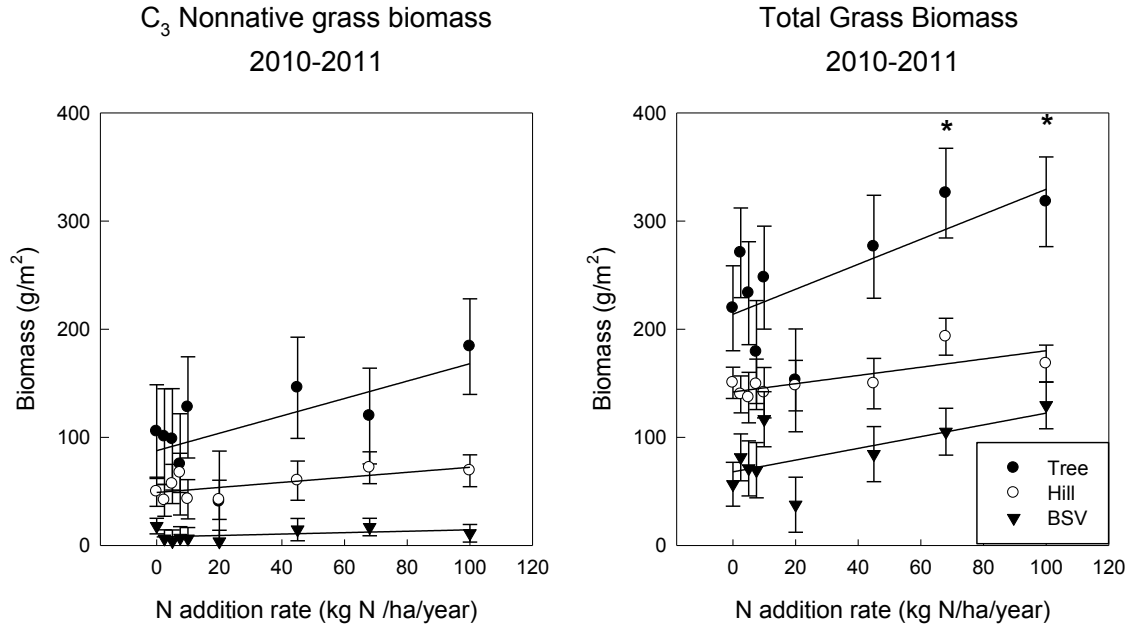
C$_3$ Nonnative grass biomass 2010-2011

Total Grass Biomass 2010-2011

Figure 2-4: C$_3$ nonnative grass biomass 2010 and 2011, by site. Biomass values were log-transformed for analysis to maintain homoscedasticity. Using a repeated measures ANOVA over both seasons, C$_3$ nonnative grass biomass in the control does not differ from any level of treatment biomass (F=1.49 p=.1709). However overall grass biomass did change significantly with treatment **(F=2.97, p=0.0052)** and treatment biomass with an 'nrate'= 68 and 100 kg N/ha/year differed from the control. Error bars represent one standard error of the mean.

Productivity Response based on Initial Productivity at N_{rate}=100kg/ha/year
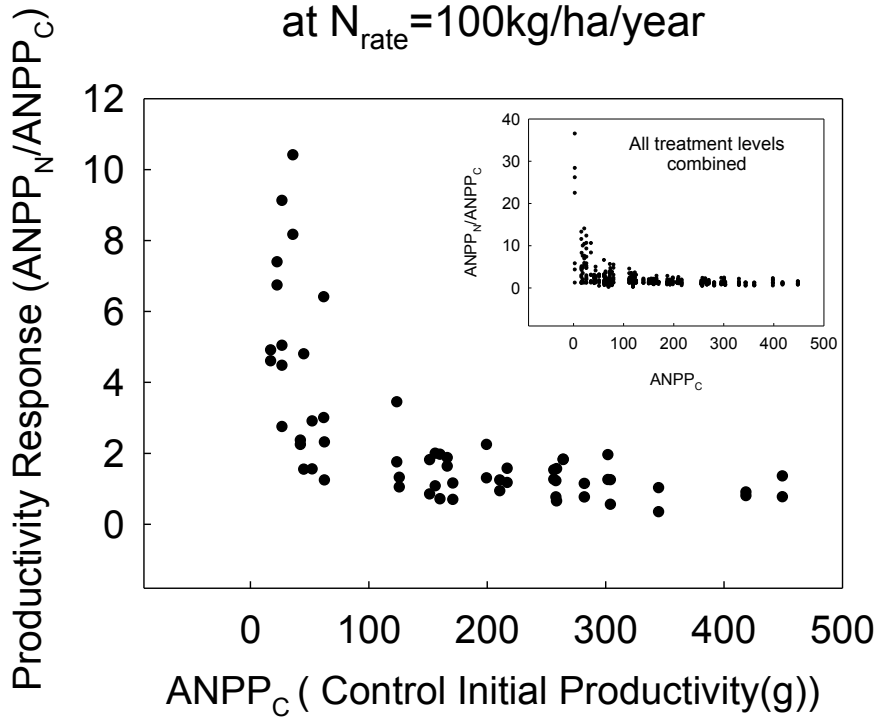
Species Richness Response based on Initial Productivity at N_{rate}= 100kg/ha/year
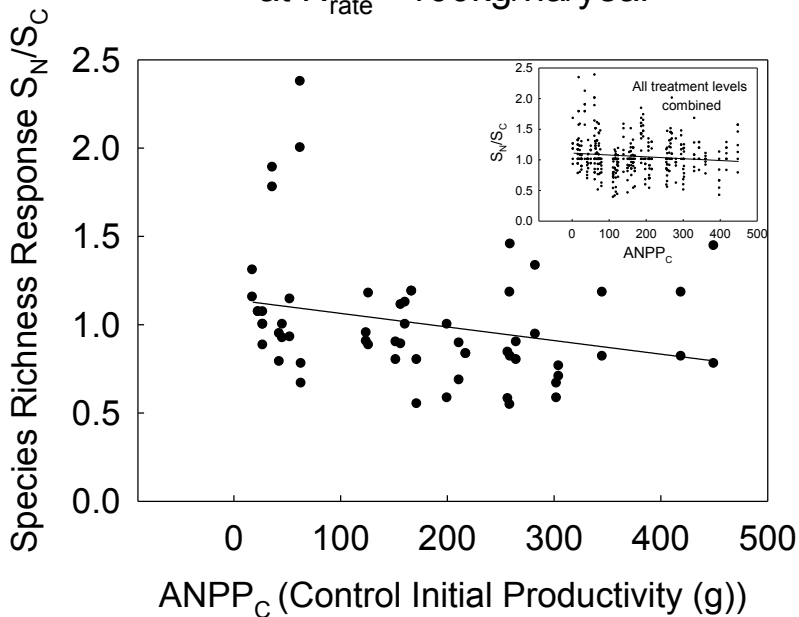
Figure 2-5: Relative production and species richness responses based on initial productivity in the control plot. Production response, which represents the strength of the response for each plot, is a proportion: the ANPP of the treatment plot (in this case

N=100 kg N/ha/year) divided by the ANPP of the control plot (which is a proxy for initial productivity). Many plots with lower control ANPP had a greater production response, and this phenomenon follows an exponential decay line. The inset uses all plots regardless of treatment level and has a similar shape, where only plots with very low productivity responded strongly in response to nitrogen treatment. Likewise a species response (Species richness $_{N\text{-treated plots}}$/ species richness $_{control\ plots}$) shows that plots with very low initial productivity may double their species richness with nitrogen addition, whereas plots with high initial productivity may begin to lose species; though this trend is significant (F=4.5, p=0.0382), slope is minimal (-0.00078 ± 0.00037).
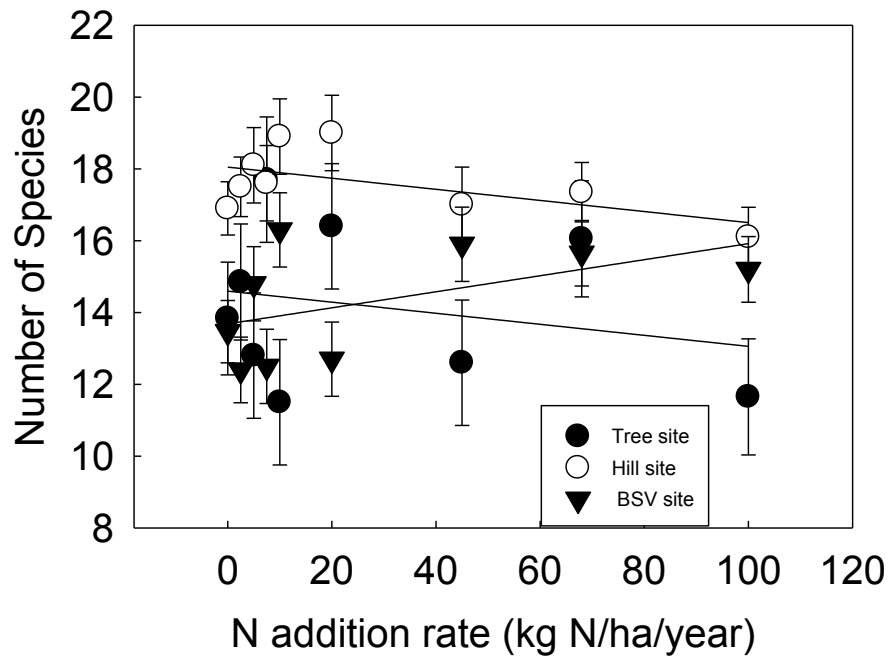
Figure 2-6: Species richness in 2010 and 2011, by site. Species richness is the number of species within a 1 m^2 quadrat and analyzed using a repeated measures ANOVA combining both years. The treatment effect on species richness varied by site (Table 2-2), with species richness increasing positively with the nitrogen addition rate at the BSV site **(F=2.67, p=0.0230)** but not at the other two (p>0.05). Error bars represent one standard error of the mean.

Bare Ground Cover
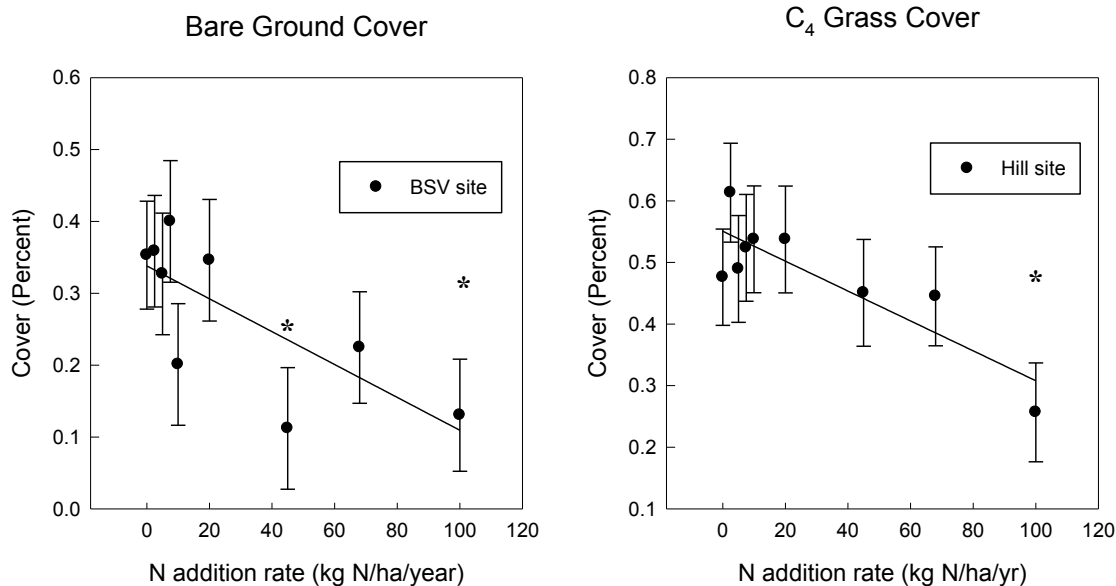
C$_4$ Grass Cover

Figure 2-7: Community change. Both bare ground cover and C$_4$ percent grass cover were square-root transformed to maintain normality. Bare ground cover does respond significantly to nitrogen **(F=2.69, p=0.0104).** The site x treatment interaction was significant (F=2.15 and p=0.0120). At Badlands, the control differed from 45 and 100 kg N/ha/year treatments **(F=2.58, p=0.0271)**, but there were no significant differences at the other two sites (Table A-3). Another community metric, C$_4$ grass as a percent of total grass cover, has marginally significant treatment (F=1.99, p=0.0558) responses driven largely by one site. At the Hill site (F=1.68, p=0.1424), the control differs from 100 significantly. Error bars represent one standard error of the mean.

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CHAPTER 3: CONCLUSIONS

Production responses in our two-year study indicate that all three sites are nitrogen-limited, at least in wet years. We looked for significant differences between our control at each site and the various levels of nitrogen addition to determine the critical load, the level below which no significant change in the response variable occurs. After two consecutive years with higher than normal rainfall (HPRCC, 2011; MesoWest, 2011; Fig.A-1), we cannot show that our sites were water-limited or show a water-nitrogen interaction through our supplemental water treatments. When reverting to more "normal" or dry years in the mixed grass prairies, we may see nitrogen limitation decrease as water becomes more of a limiting factor and the effects from fertilization lessen. Wet years in the mixed grass prairie exacerbate nitrogen limitation as water limitation is relieved (Hooper and Johnson, 1999). Pardo (2011) suggested that critical nitrogen loads for mixed grass prairies are lower in wet years due to increased nitrogen limitation.

Determining critical loads for air pollution is important for developing policy to protect our air, land and water resources (Porter, et al., 2005; Greaver, et al., 2012). Some ecosystems are quite resilient to nitrogen and have the capacity to store a lot of nitrogen, even increasing carbon storage in the soil in the process. Others have been found to be surprisingly sensitive to nitrogen with leaching, plant community and other negative changes in the ecosystem. Despite the fact that most nitrogen studies use large amounts of nitrogen to elicit an immediate and drastic response greater than would be expected under a realistic pollution scenario; recent studies have found that the critical load for nitrogen below which no harmful ecological effects are found, can

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actually be quite low. Pardo (2011) recently synthesized published information to derive critical loads for nitrogen throughout the U.S.   The entire Great Plains region was given an estimated critical load of 10-25 kg N/ha/year, based largely on an experiment in Minnesota (Clark and Tilman, 2008). Pardo divided the Great Plains region into short-, mixed-, and tall-grass prairie and gave an estimate for each. The mixed-grass prairie's critical load of 16.3 kg N/ha/year was based on just one study in Oklahoma (Jorgensen, 2005) with high background deposition of 11 kg N/ha/year. More fertilization studies using chronic low levels of nitrogen should be conducted in both drought and wet years to obtain an accurate critical load for the vast mixed grass prairie region.

Our fertilization study included collecting measurements of various abiotic and biotic indicators to determine their response to a given dose of nitrogen for two years. However determining critical loads is more complicated than looking for a response to a given factor at a given site in a given year (Greaver, et al., 2012). Critical loads can fluctuate depending on the year and weather conditions. Responses may occur in some years but not in others, particular when water-limitation is high (Bai, 2010). Our study, conducted in two wet years in which water apparently was not limiting, probably represents conditions in which a response to nitrogen is more likely. Between- and within-site (macro- and microsite) variations also impact critical load estimates, especially for response variables involving soil nitrogen, which is extremely heterogeneous. Consequently, multiple years and sites are needed to express the full spectrum of possible responses and give a better estimate of the critical load.

We measured a wide variety of response variables at three sites over two years. Our 9 levels of nitrogen addition span the range from realistic for atmospheric

deposition in this region (≤10 kg N/ha/year) to levels more akin to deliberate fertilization (68-100 kg N/ha/yr). With discrete treatments like these, the critical load can only be estimated as somewhere between the level at which a significant response was measured and the next lowest level tested. In addition, due to logistical constraints we were only able to sample a subset of levels for soil N content and leaf tissue N contents (0, 5, 10, 45 and 100 kg N/ha/year). When differences among sites are ignored and all response variables are considered, our results show that the lowest critical load for nitrogen deposition is below 45 kg N/ha/year (Table 3-1). Production changes occurred at a higher level, below 68kg N/ha/year. Species richness, biomass of non-native C_3 grasses, and the relative abundance of C_4 grasses were resistant to change. Other community changes (diversity, evenness) were inconsistent, with significant effects at low nitrogen addition rates (5-20 kg N/ha/yr) but not at higher ones (Table A-3).

Soil properties such as elevated nitrate levels may result in community change in future years (Lowe and Lauenroth, 2003; Jorgensen, 2005, Clark and Tilman, 2008; Pardo, 2011). From this evidence we conclude that at least short term deposition poses no immediate impacts to northern mixed grass prairie ecosystems. Additional monitoring may reveal long-term impacts from chronic nitrogen deposition which often take years to become apparent (Lauenroth and Dodd, 1978; Wedin and Tilman, 1996; Samuel and Hart, 1998; Clark and Tilman, 2008; Bai, 2010).

However, the critical load varied across sites and was determined by different response variables. It should be noted that we are less confident about site-specific critical loads due to the limited number of plots at each site and the resulting decreased statistical power to detect a change. The Badlands sparse vegetation critical load was

between below 45 kg N/ha/year due to elevated soil nitrogen content and canopy cover changes (Table A-5). The lowland Wind Cave site (Tree) had a critical load of 68 kg N/ha/year based on changes in production (Table A-6). The upland Wind Cave site (Hill) had a critical load at 45 kg/ha/year based on soil nitrogen content (Table A-7). Unproductive plots (<100 g/m^2 peak biomass), mainly at Badlands NP, showed the strongest production responses to nitrogen, with up to 10 times more vegetation in the highest nitrogen treatments (100 kg N/ha/year) relative to the control plots (Fig.2-5) .

That leaf nutrient status was the most sensitive parameter is not surprising. Rauzi and Fairbourn (1983) found that crude protein, a measure of tissue N, increased at all levels of treatment (22kg and 34 kg N/ha/year). Likewise in a meta-analysis by Lu et al. (2011), the overall result of N on leaf nitrogen was positive. Soil properties such as elevated nitrate levels may result in community change in future years (Lowe and Lauenroth, 2003; Jorgensen, 2005, Clark and Tilman, 2008; Pardo, 2011). The fact we found no increases in C_3 nonnative plant biomass and no decreases in plant species richness are encouraging, in that at least in the short term, these native grasslands can effectively absorb excess nitrogen with little plant community impact. Other researchers have found that species loss can occur where production responses occur, (Gough, 2000; Clark and Cleland, 2007), though, so additional monitoring may reveal long-term impacts from chronic nitrogen deposition (Lauenroth and Dodd, 1978; Wedin and Tilman, 1996; Samuel and Hart, 1998; Clark and Tilman, 2008; Bai, 2010).

Future research

This study is on-going; after at least two more years of study, we will be able to more confidently ascertain the ecological effects of atmospheric nitrogen on grassland

vegetation in these parks, when growing seasons of different precipitation levels (Samuel and Hart, 1998; Cleland and Harpole, 2007) and longer-term effects (Lauenroth and Dodd, 1978; Clark and Tilman, 2008) may become apparent. Although grasslands have been shown to absorb large amounts of nitrogen by storing it in roots with no production response (Power, 1972; Woodmansee, 1978), nitrogen-induced species loss in other studies and perhaps hinted at in our own (Fig.2-5) show that species richness responses in the mixed grass prairie must be fully investigated to avoid any loss of diversity.

In light of the uncertainty regarding critical loads for park management, I suggest monitoring soil nitrogen levels in order to establish baseline measurements and begin looking for deviations from that baseline as evidence of possible ecological change. Though soil nitrogen can be expensive to measure, it is a reliable and consistent indicator of increased nitrogen levels. In the absence of a fertilization experiment any tissue N or ANPP changes (which also showed significant responses to N addition) may be more difficult to attribute directly to increased nitrogen inputs.

In addition to continuing research at these study sites, a better understanding of how climate, grazing and fire affect ecological responses to nitrogen is essential both for mitigating any effect from nitrogen deposition and understanding ecosystem function under nitrogen deposition conditions. The belowground component of grasslands should also be studied more as it is likely that responses to nitrogen occur there, but they are not often measured due to technical difficulties. Future research should also concentrate on the gaps between N addition levels that we used in order to better pinpoint a critical load for a given year or set of years with more accuracy.

Table: 3-1 Critical loads for Nitrogen, Northern Great Plains 2010-2011			
Response	**Site**	**Critical Dose kg N/ha/year**	**P-value**
Primary Responses			
Aboveground annual net primary production	*All*	*between 45 and 68*	*0.0229*
C$_3$ nonnative biomass	All	n.s.	0.1709
Leaf Tissue N content: Wheatgrass	*All*	*between 10 and 45*	*0.0001*
Secondary Responses			
Inorganic N content	*All*	*between 10 and 45*	*0.0001*
N mineralization rates	*All*	*between 10 and 45*	*0.0114*
Plant species richness	All	n.s.	0.4357
Tissue nitrogen in dominant grass species	*All*	*between 45 and 100*	*0.0001*
Bare ground cover	*Badlands*	*between 20 and 45*	*0.0271*

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APPENDIX

Table A-1: Water addition treatments and precipitation by season

Water Addition (May-August) 2010			
Site	Tree	Hill	BSV
Water added (cm)	3.1	3.1	3.1
Average Rainfall (cm)	25.45	25.45	25.06
Actual Rainfall (cm)	40.03	40.03	26.27
Percent above average	57.29	57.29	4.83
2011			
Site	Tree	Hill	BSV
Water added (cm)	5.5	6.6	5.5
Average Rainfall (cm)	25.45	25.45	25.06
Actual Rainfall (cm)	32.58	32.58	40.43
Percent Above Average	28.02	28.02	61.33

Table A-2: Species list compiled from diversity measurements from 2010 and 2011.

Genus	Species	Functional Group	Tree	Hill	BSV
Achnatherum	*hymenoides*	C_3 native			*
Agropyron	*cristatum*	C_3 nonnative		*	
Allium	*textile*	native forb			*
Alyssum	*alyssoides*	native forb		*	
Ambrosia	*psillostachya*	native forb		*	
Amorpha	*canescens*	shrub		*	
Andropogon	*gerardii*	C_4 native	*	*	
Anenome	*cylindrica*	native forb	*		
Antennaria	*parviflora*	native forb	*	*	
Arabis	*hirsuta*	native forb	*		
Aristida	*purpurea*	C_4 native	*	*	*
Artemisia	*dracunculus*	native forb	*	*	
Artemisia	*frigida*	native forb	*	*	
Artemisia	*ludoviciana*	native forb	*	*	
Asclepias	*pumila*	native forb	*		

Genus	Species	Functional Group	Tree	Hill	BSV
Asclepias	*viridiflora*	native forb	*		
Astragalus	*agrestis*	native legume	*	*	
Astragalus	*crassicarpus*	native legume	*		
Astragalus	*missouriensis*	native legume			*
Astragalus	*racemosus*	native legume			*
Atriplex	*argentea*	native forb			*
Bessia	*wyomingensis*	native forb	*		
Bouteloua	*curtipendula*	C_4 native	*	*	*
Bouteloua	*gracilis*	C_4 native	*	*	*
Bouteloua	*hirsuta*	C_4 native	*	*	
Brassica	*sp.*	native forb	*		
Brickellia	*eupatoroides*	native forb	*	*	
Bromus	*inermis*	C_3 nonnative		*	*
Bromus	*japonicus*	C_3 nonnative	*	*	*
Bromus	*tectorum*	C_3 nonnative	*	*	*
Buchloe	*dactyloides*	C_4 native	*		
Calamovilfa	*longifolia*	C_4 native		*	*
Calochortus	*nuttallii*	native forb			*
Calyophus	*serrulatus*	native forb	*		
Camelina	*microcarpa*	nonnative forb	*	*	
Campanula	*rotundifolia*	native forb	*		
Carex	*duriscula*	sedge		*	
Carex	*filifolia*	sedge	*	*	
Carex	*inops*	sedge	*	*	*
Chamesyce	*sp.*	native forb			*
Chenopodium	*album*	nonnative forb	*	*	*
Chorospora	*tenella*	nonnative forb			*
Cirsium	*arvense*	nonnative forb	*		
Cirsium	*undulata*	native forb	*		*
Collomia	*linearis*	native forb	*	*	
Comandra	*umbellata*	native forb			*
Conyza	*canadensis*	native forb	*	*	*
Cryptanthus	*celesoides*	native forb			*
Dichanthelium	*acuminatum*	C_4 native		*	
Dichanthelium	*oligosanthes*	C_4 native		*	*
Draba	*reptans*	native forb	*		*
Echinacea	*angustifolia*	native forb	*	*	
Echinocereus	*viridiflorus*	cactus		*	

Genus	Species	Functional Group	Tree	Hill	BSV
Elymus	*elmoides*	C$_3$ native	*	*	*
Elymus	*trachycaulus*	C$_3$ native			*
Erigeron	*sp.*	native forb	*		
Erigeron	*glabelis*	native forb	*		
Eriogonum	*pauciflorum*	native forb			*
Escobaria	*missouriensis*	cactus		*	
Euphorbia	*dentata*	native forb		*	
Euphorbia	*spathulata*	native forb	*	*	
Evolvulus	*nuttallianus*	native forb		*	
Galium	*aparine*	native forb	*	*	
Gaura	*coccinea*	native forb	*	*	*
Grindelia	*squarrosa*	native forb		*	*
Gutierrezia	*sarothrae*	shrub			*
Halogeton	*glomeratus*	nonnative forb			*
Hedeoma	*hispida*	native forb	*	*	*
Helianthus	*annuus*	native forb		*	*
Hesperostipa	*comata*	C$_3$ native	*	*	
Heterotheca	*villosa*	native forb			*
Ipomoea	*leptophylla*	native forb		*	
Jupinerus	*scopulorum*	shrub		*	
Koeleria	*macrantha*	native forb	*	*	*
Lactuca	*serriola*	nonnative forb	*	*	*
Lappula	*occidentalis*	native forb	*	*	*
Lepidium	*densiflorum*	nonnative forb		*	
Lepidium	*virginicum*	native forb	*		*
Lesquerella	*arenosa*	native forb	*		
Leucocrinum	*montanum*	native forb	*	*	
Liatrus	*punctata*	native forb	*	*	*
Linum	*rigidum*	native forb	*		*
Lithospermum	*incisum*	native forb	*	*	
Logfia	*arvense*	native forb	*		
Lomatium	*foeniculaceum*	native forb			*
Lygodesmia	*juncea*	native forb	*		
Meliolotus	*officinalis*	nonnative legume	*		*
Mirabilis	*linearis*	native forb			*
Muhlenbergia	*cuspidata*	C$_4$ native	*	*	*
Musineon	*divaricatum*	native forb			*

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Genus	Species	Functional Group	Tree	Hill	BSV
Nassela	*viridula*	C$_3$ native	*	*	*
Nepeta	*cataria*	nonnative forb		*	
Nothocaulus	*cuspidata*	native forb			*
Oligoneuron	*rigidum*	native forb	*		
Onosmodium	*molle*	native forb	*		
Opuntia	*fragilis*	cactus		*	
Opuntia	*macrorhiza*	cactus		*	*
Opuntia	*polyantha*	cactus			*
Oxytropis	*lambertii*	native legume		*	
Oxytropis	*sericea*	native legume			*
Pascopyrum	*smithii*	C$_3$ native	*	*	*
Pediomelum	*argophyllum*	native legume	*		
Pediomelum	*cuspidata*	native legume			*
Pediomelum	*esculenta*	native legume	*	*	*
Penstemon	*albinsis*	native forb			*
Phlox	*andicola*	native forb	*	*	
Phlox	*hoodii*	native legume	*	*	*
Plantago	*patagonica*	native forb		*	*
Poa	*pratensis*	C$_3$ nonnative	*	*	*
Poa	*secunda*	C$_3$ native			*
Polygonum	*convolvulus*	nonnative forb		*	
Potentilla	*pensylvanica*	native forb	*		
Psoralea	*tenuiflora*	native legume	*	*	*
Ratibida	*columnifera*	native forb	*	*	*
Ribes	*aureum*	shrub		*	
Rosa	*arkansana*	shrub	*		
Salsola	*tragus*	nonnative forb			*
Schedonnardus	*paniculatus*	C$_4$ native			*
Schizachryium	*scoparium*	C$_4$ native	*	*	
Senecio	*plattensis*	native forb	*	*	
Senecio	*sp.*	native forb			
Silene	*antirhinnum*	native forb	*	*	
Silene	*latifolia*	nonnative forb	*	*	
Sisrynchium	*montanum*	native forb	*		
Sisymbrium	*altissimum*	nonnative forb		*	*
Sisymbrium	*loeslii*	nonnative forb	*		
Solidago	*missouriensis*	native forb			*
Sonchus	*oleracus*	nonnative forb		*	

Genus	Species	Functional Group	Tree	Hill	BSV
Sphaeralcea	*coccinea*	native forb	*	*	*
Sporobolus	*cryptandrus*	C_4 native	*	*	*
Sporobolus	*heterolepsis*	C_4 native		*	
Symphoricarpus	*occidentalis*	shrub	*		
Symphyotrichum	*ericoides*	native forb	*	*	*
Symphyotrichum	*oblongifolium*	native forb	*	*	
Taraxacum	*officianale*	nonnative forb	*	*	*
Tradescantia	*bracteata*	native forb	*	*	
Tragopogon	*dubious*	nonnative forb	*	*	*
Verbena	*hastata*	native forb	*		
Viola	*canadensis*	native forb	*		
Viola	*nuttallii*	native forb	*	*	
Yucca	*glauca*	cactus	*	*	*
Zigadenus	*venenosus*	native forb	*	*	

Table A-3: Community Responses. Full statistics given across sites and by individual sites for community response metrics diversity (Shannon-Weiner index), evenness, percent bare ground cover(square-root transformed) and C_4 cover as a percent of total grass cover(square-root transformed).   The first line, the F-statistic and p-value are the treatment or 'nrate' effect from the repeated measures ANOVA. The R^2 and slope values are from the plotted LSMEANS of that same ANOVA. The 2nd line gives the nitrogen addition rate or 'nrate' the control differs significantly from. The regression line, derived across sites, uses 'xn' as a continuous variable to test whether the slope of the line is significantly different from zero. A non-zero slope indicates a non-zero linear treatment effect.

Site	Diversity				Evenness			
	F-stat	p-value	R^2	slope	F-stat	p-value	R^2	slope
All Sites	2.26	0.0285	0.037	-3.9E-04	2.35	0.0229	0.1096	-1.6E-04
	Control differs from 5, 20				Control differs from 5, 20			
	Regression: t= -.59, p =.5595				Regression: t= -1.13, p=.2612			
Tree	2.53	0.0295	0.0316	-0.001	2.75	0.0195	0.0103	0.0001
	Control differs from none				Control differs from 20, 68			
Hill	2.02	0.0689	0.1049	-0.0011	0.75	0.6508	0.0968	-0.0002
	Control differs from 5				Control differs from none			
BSV	3.32	0.0063	0.0149	0.0007	3.69	0.003	0.161	-0.0006
	Control differs from 5, 10				Control differs from 10			
Site	Bare ground cover				C_4 as a percent of total grass cover			
	F-stat	p-value	R^2	slope	F-stat	p-value	R^2	slope
All Sites	2.69	0.0104	0.8243	-0.001	1.99	0.0558	0.2228	-9.6E-04
	Control differs from 45, 68, 100				Control differs from none			
	Regression: t=-4.04, p=.0001				Regression: t= -1.94, p= .0551			
Tree	1.41	0.2336	0.2188	-0.000075	1.14	0.3632	0.0519	-0.0007
	Control differs from none				Control differs from none			
Hill	1.81	0.0819	0.1093	-0.000097	1.68	0.1424	0.7398	-0.0024
	Control differs from 68				Control differs from 100			
BSV	2.58	0.0271	0.0659	-0.002	1.23	0.3137	0.0166	0.0003
	Control differs from 45, 100				Control differs from none			

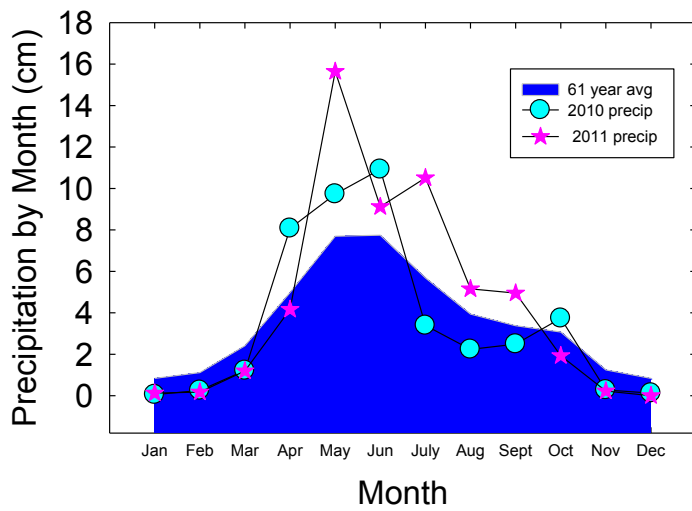| Table A-4: Community response results from ANOVA table for multi-site analysis of nitrogen treatment x site x year					
Effect		Diversity		Evenness	
	df	F	p	F	p
Site	2	5.67	0.0182	6.51	0.0113
Year	1	1.27	0.2601	0.38	0.5359
Site x Year	2	2.2	0.1125	1.1	0.3353
Treatment	8	2.26	0.0285	2.35	0.0229
Site x Treatment	16	2.95	0.0005	2.37	0.0047
Year x Treatment	8	0.21	0.9889	0.45	0.8909
Site x Year x Treatment	16	0.34	0.9926	0.8	0.6815
Effect		Bare ground cover		Percent C4 grass	
	df	F	p	F	p
Site	2	60.82	<0.0001	0.04	0.965
Year	1	32.02	<0.0001	0.3	0.5833
Site x Year	2	7.58	0.0006	2.13	0.1208
Treatment	8	2.69	0.0104	1.99	0.0558
Site x Treatment	16	2.15	0.012	0.99	0.4712
Year x Treatment	8	1.87	0.0657	0.14	0.997
Site x Year x Treatment	16	0.65	0.838	0.18	0.9998

Table A-5: Critical loads for Nitrogen, Badlands NP 2010-2011		
Response	Critical Dose kg N/ha/year	P-value
Primary Responses		
Aboveground annual net primary production	*between 68 and 100*	0.0288
C3 nonnative biomass	n.s.	
Leaf Tissue N content: Elymus trachycaulus	*between 45 and 100*	<.0001
Secondary Responses		
Inorganic N content	*between 10 and 45*	0.0242
N mineralization rates	*between 45 and 100*	0.0281
Plant species richness	n.s.	
Bareground cover	*between 20 and 45*	0.0183

Table A-6: Critical loads for Nitrogen, Lowland Wind Cave, Tree Site 2010-2011		
Response	**Critical Dose kg N/ha/year**	**P-value**
Primary Responses		
Aboveground annual net primary production	*between 45 and 68*	*0.0367*
C$_3$ nonnative biomass	n.s.	
Leaf Tissue N content: Western wheatgrass	*between 45 and 100*	*0.0081*
Secondary Responses		
Inorganic N content	*between 45 and 100*	*0.0003*
N mineralization rates	n.s.	
Plant species richness	n.s.	0.0869

Table A-7: Critical loads for Nitrogen, Upland Wind Cave(Hill Site) 2010-2011		
Response	**Critical Dose kg N/ha/year**	**P-value**
Primary Responses		
Aboveground annual net primary production	n.s.	
C$_3$ nonnative biomass	n.s.	
Leaf Tissue N content: Western wheatgrass	*between 45 and 100*	*0.0003*
Secondary Responses		
Inorganic N content	*between 10 and 45*	*0.0359*
N mineralization rates	n.s.	
Plant species richness	n.s.	0.4612

Figure A-1: Wind Cave and Badlands Precipitation 2010 and 2011, High Plains Regional Climate Center, 2011; MesoWest, 2011.
Long-term average monthly precipitation from HPRCC and monthly 2010 and 2011 precipitation averages from MesoWest stations PINS2 and WCAS2 for Badlands and Wind Cave respectively.

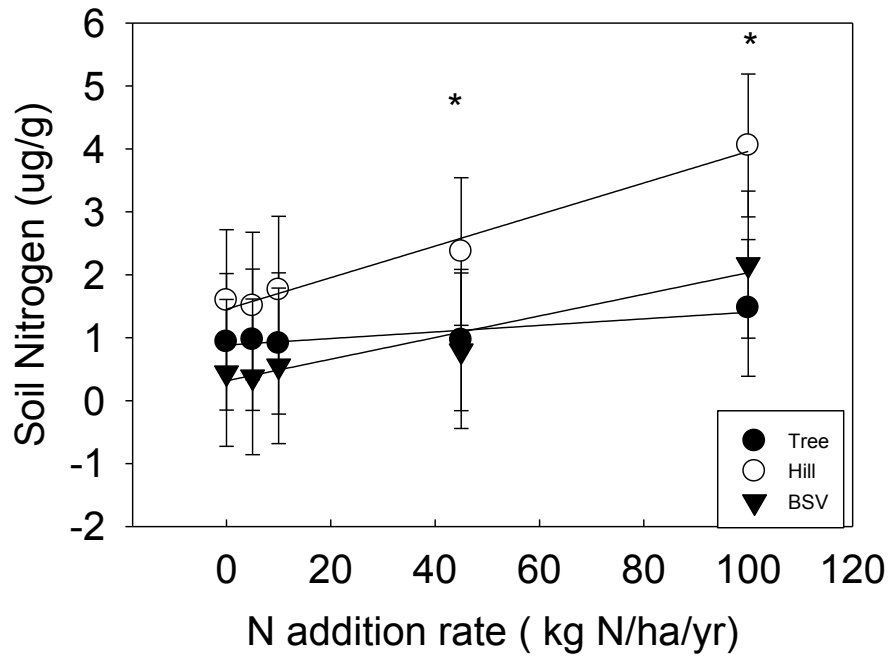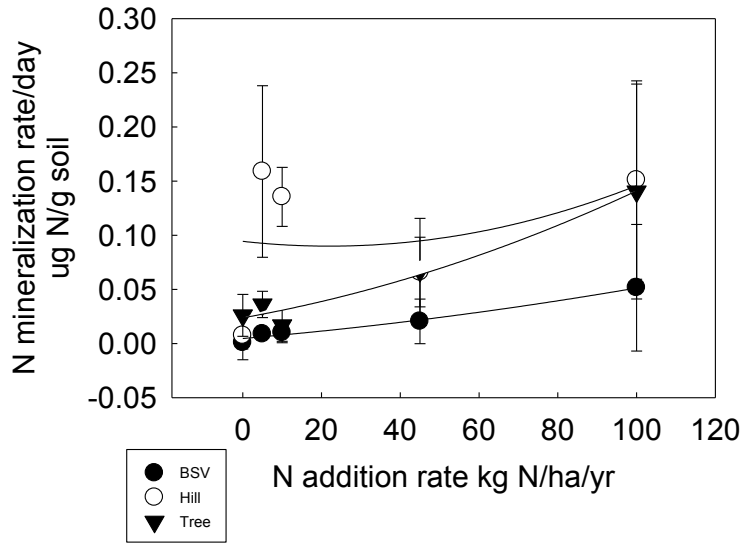# Inorganic soil N content by site 2010-2011

Figure A-2: Inorganic N content by site 2010 and 2011. Values were log-transformed in analyses to maintain homoscedasticity. Asterisks represent nitrogen addition levels across sites which differ from the control rate. Error bars represent one standard error of the mean.

N mineralization June-July 2010
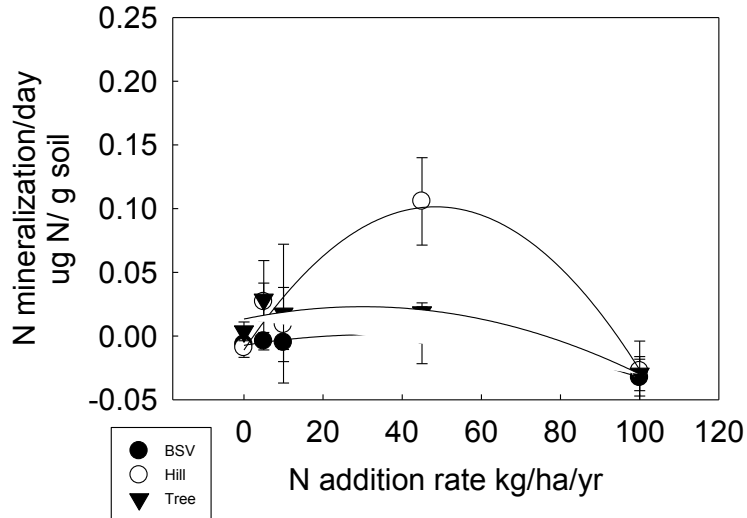

N mineralization June-July 2011

Figure A-3: N mineralization June-July 2010 and 2011, by site.
Net mineralization was calculated as July inorganic N (NH4+NO3) June inorganic N
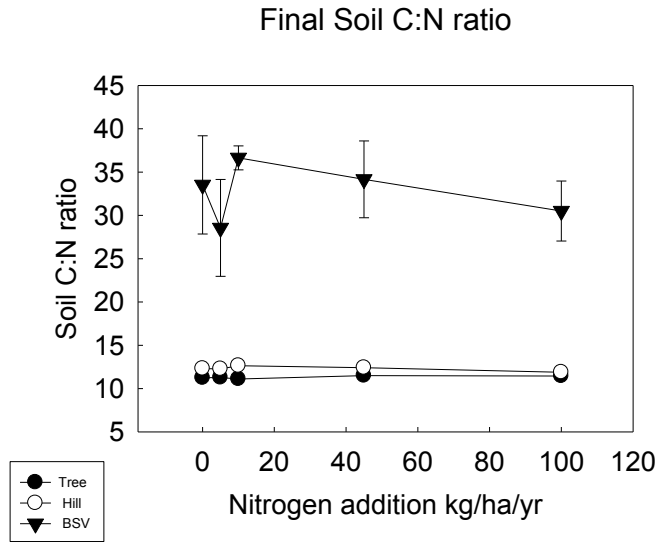during field incubations both summers. Error bars represent one standard error of the
mean.

Figure A-4: Final soil C:N ratio by site, September 2011. Final C:N ratio determined in four bulked 10-cm cores per plot. Error bars represent one standard error of the mean.
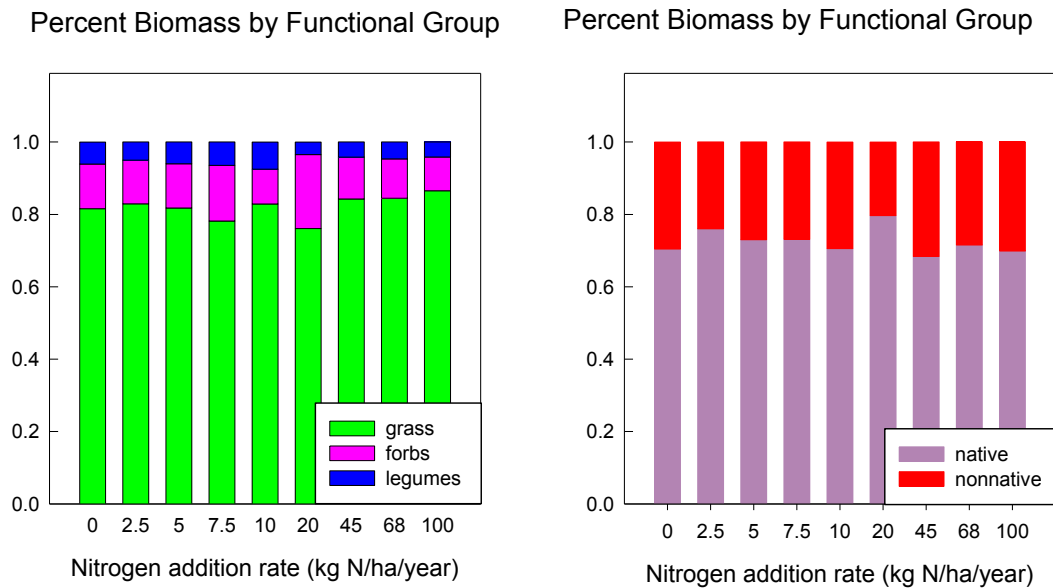

Figure A-5: Percent biomass by functional groups, nativity. Percent biomass measured across site 2010-2011, from biomass taken in late July to early August. No signficant effect of 'nrate' on percent native plants (F= 0.85, p=0.5620), percent forbs (F= 1.70, p=0.1113) or percent legumes (F=0.56 p= 0.8086).

Percent Light Reduction
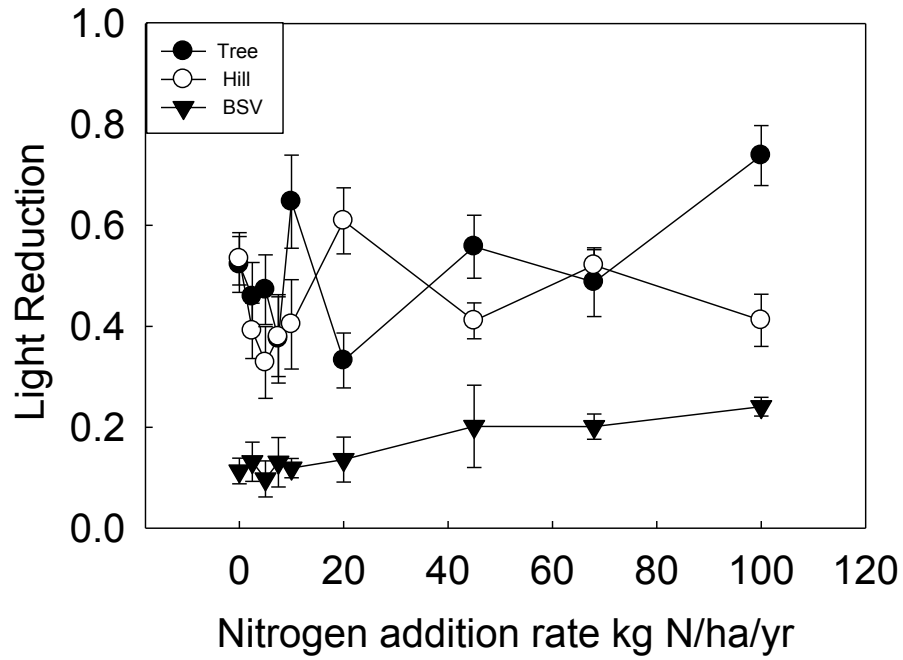## (100cm to Ground Level)

Figure A-6: Canopy light reduction (PAR) 2011, by site. Canopy light reduction measured by light meter in July 2011. Error bars represent one standard error of the mean.