National Park Service
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Natural Resource Stewardship and Science

Potential Nitrogen Critical Loads for Northern Great Plains Grassland Vegetation

Natural Resource Report NPS/NGPN/NRR—2015/989

ON THE COVER
Diverse vegetation in bandlands soil at Badlands National Park
Photograph by Amy Symstad

Potential Nitrogen Critical Loads for Northern Great Plains Grassland Vegetation

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July 2015

U.S. Department of the Interior
National Park Service
Natural Resource Stewardship and Science
Fort Collins, Colorado

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Please cite this publication as:

Symstad, A.J., A.T. Smith, W.E. Newton, and A. K. Knapp. 2015. Potential nitrogen critical loads for northern Great Plains grassland vegetation. Natural Resource Report NPS/NGPN/NRR—2015/989. National Park Service, Fort Collins, Colorado.

NPS 920/129008, July 2015

Contents

Page

Figures....iv

Tables....v

Appendices....vi

Executive Summary....vii

Acknowledgments....viii

1. Introduction....1

2. Methods....5

2.1 Study Area....5

2.2 Experimental Design....8

2.3 Treatment Implementation....9

2.4 Responses Measured and Sampling Methods....10

2.5 Data Analysis....11

3. Results....13

3.1 Supplemental Water Treatments....15

3.2 Variation in Measured Characteristics among the Experimental Sites....16

3.3 Badlands Sparse Vegetation (BSV) Site....17

3.4 Terrace Site....22

3.5 Hilltop Site....26

4. Discussion....29

Nitrogen Critical Loads Derived from This Study....31

Caveats to Estimated Critical Loads....33

Implications of Estimated Critical Loads....34

5. Conclusions....37

Literature Cited....39

Figures

Page

Figure 1. Total (wet + dry) nitrogen (N) deposition (kg/ha/yr), averaged over 2011-2013, in the continental United States (bottom) and northern Great Plains (NGP; top).....5

Figure 2. Photographs illustrating the (a) BSV, (b) Terrace, and (c) Hilltop study sites.....7

Figure 3. Precipitation and nitrogen deposition at Badlands and Wind Cave National Parks during the four years of this study.....8

Figure 4. Soil inorganic nitrogen in **(a)** June of 2010 and 2011 and **(b)** March, June, and September of 2011, and whole-plant tissue carbon to nitrogen ratio (C:N) for three species each in **(c)** 2011 and **(d)** 2013 at the Badlands Sparse Vegetation (BSV) site.....18

Figure 5. 2010 and 2011 least squares means and 95% confidence intervals for plant community response variables sensitive to 2010-2011 experimental treatments at the BSV site. 20

Figure 6. 2012 and 2013 least squares means and 95% confidence intervals for plant community response variables sensitive to experimental treatments at the BSV site. 21

Figure 7. Soil inorganic nitrogen in **(a)** June of 2010 and 2011 and **(b)** March, June, and September of 2011, and whole-plant tissue carbon to nitrogen ratio (C:N) for three species each in **(c)** 2011 and **(d)** 2013 at the Terrace site. 23

Figure 8. Back-transformed least-squares means and 95% confidence intervals for plant community response variables sensitive to experimental treatments at the Terrace site.....25

Figure 9. Distribution of mean (over 2010-2013) *Amorpha canescens* cover in Hilltop site plots. 26

Figure 10. Soil inorganic nitrogen in (a) June of 2010 and 2011 and (b) March, June, and September of 2011, and (c) whole-plant tissue carbon to nitrogen ratio (C:N) for three species in 2011 at the Hilltop site. 27

Figure 11. Back-transformed least squares means and 95% confidence intervals of all non-native (circles) and annual brome grass (squares) proportion of foliar cover adjusted to *Amorpha canescens* cover ("AMCA") = 0 in (a) 2010, (b) 2011, and (c) 2013 at the Terrace site.....29

Figure 12. Box-and-whisker plots of total (wet + dry) annual atmospheric nitrogen deposition at parks in the NGP. 35

Tables

Page

Table 1. Response variables measured in this study, rationale for their inclusion, and the ecosystem characteristics with which they are associated. 3

Table 2. Treatments in first and second halves of the experiment, and block(s) in which each treatment occurred. There were five of each block at each site. 9

Table 3. Results of tests for significant treatment x year or treatment x month effect on response variables. 15

Table 4. Characteristics of the vegetation at the three experimental sites 16

Table 5. Direction (positive, +; negative, -; none, 0) of linear relationships between transformed plant community response variables and experimentally added nitrogen for the BSV site. 19

Table 6. Directions (positive, +; negative, -; none, 0) of linear relationships between transformed plant community response variables and experimentally added nitrogen for the Terrace site. 24

Table 7. Directions (positive, +; negative, -; none, 0) of linear relationships between transformed plant community response variables and experimentally added nitrogen for the Hilltop site. 28

Table 8. Inorganic nitrogen critical loads (kg N/ha/yr) and the response variable from which they are derived for three sites in two northern Great Plains national parks. 32

Appendices

Page

Appendix A: Plant tissue samples 45

Appendix B: Community Composition Results from Nonmetric Multidimensional Scaling Ordination 47

Appendix C: Additional Hilltop Site Plant Community Responses 53

Appendix D: Derivation of Critical Loads 55

Executive Summary

The National Park Service is concerned that increasing atmospheric nitrogen deposition caused by fossil fuel combustion and agricultural activities could adversely affect the northern Great Plains (NGP) ecosystems in its trust. The critical load concept facilitates communication between scientists and policy makers or land managers by translating the complex effects of air pollution on ecosystems into concrete numbers that can be used to inform air quality targets. A critical load is the exposure level below which significant harmful effects on sensitive elements of the environment do not occur. A recent review of the literature suggested that the nitrogen critical load for Great Plains vegetation is 10-25 kg N/ha/yr. For comparison, current atmospheric nitrogen deposition in NGP National Park Service (NPS) units ranges from ~4 kg N/ha/yr in the west to ~13 kg N/ha/yr in the east. The suggested critical load, however, was derived from studies far outside of the NGP, and from experiments investigating nitrogen loads substantially higher than current atmospheric deposition in the region.

Therefore, to better determine the nitrogen critical load for sensitive elements in NGP parks, we conducted a four-year field experiment in three northern Great Plains vegetation types at Badlands and Wind Cave National Parks. The vegetation types were chosen because of their importance in NGP parks, their expected sensitivity to nitrogen addition, and to span a range of natural fertility. In the experiment, we added nitrogen at rates ranging from below current atmospheric deposition (2.5 kg N/ha/yr) to far above those levels but commensurate with earlier experiments (100 kg N/ha/yr). We measured the response of a variety of vegetation and soil characteristics shown to be sensitive to nitrogen addition in other studies, including plant biomass production, plant tissue nitrogen concentration, plant species richness and composition, non-native species abundance, and soil inorganic nitrogen concentration. To determine critical loads for the NGP plant communities in our experiment, we followed the NPS's precautionary principle in assuming that it is better to be cautious than to let harm occur to the environment. Thus, the critical loads we derived are the lowest nitrogen level that any of our data suggest has a measureable effect on any of the response variables measured.

Badlands sparse vegetation, a low-productivity plant community that is an important part of the scenery at Badlands National Park and provides habitat for rare plant species, was the most sensitive of the three vegetation types. More aspects of this vegetation type responded to nitrogen addition, and at lower levels, than at the other two sites. Our data suggest that nitrogen deposition levels of 4-6 kg N/ha/yr may increase biomass production, and consequently the amount of dead plant material on the ground in this plant community. Slightly higher critical loads are suggested for the two more productive vegetation types more characteristic of most NGP grasslands: 6-10 kg N/ha/yr for biomass production, grass tissue nitrogen concentration, or non-native species (especially annual brome grasses) cover. Highly variable results among years, as well as inconsistent responses to an increasing dose of nitrogen within sites, complicated the derivation of critical loads in this experiment, however. A less precautionary approach to deriving critical loads yielded higher values of 10-38 kg N/ha/yr.

Acknowledgments

This work could not have completed without the field help of Chelsea Kincheloe, Mark Visel, Christine Prins, Jessica Milby, Jessica Moran, Emily Meirendorf, Adam Fund, and John Wendt, as well as Jeff, Kassia, and Bengt Symstad. Milt Haar and Beth Burkhart provided logistical support at Badlands and Wind Cave National Parks, respectively. This project was funded by the National Park Service Air Resources Division, with support from the U.S. Geological Survey.

1. Introduction

Congress recognized the importance of clean air in large national parks and wilderness areas when it amended the Clean Air Act in 1997 to mandate that air quality in these areas be protected and enhanced for the enjoyment of future generations (National Park Service Air Resources Division 2002). One reason for preserving and restoring clean air is to protect pollution-sensitive natural resources.

The critical load concept facilitates communication between scientists and policy makers or land managers by translating the complex effects of air pollution on ecosystems into concrete numbers that can be used to inform air quality targets (Porter *et al.* 2005; Burns *et al.* 2008). Specifically, a critical load is "the quantitative exposure to one or more pollutants below which significant harmful effects on sensitive elements of the environment do not occur, according to present knowledge" (Nilsson and Grennfelt 1988). Following the use of the critical load approach to shape air pollution control policy in Europe and Canada, the development and use of critical loads for air pollutants affecting Federal lands in the United States is now gaining momentum (Pardo *et al.* 2011a; Ellis *et al.* 2013; Blett *et al.* 2014).

Critical loads, which are based on specific indicators of ecological change in response to a specific type of pollutant, should be as specific to a location as possible and ideally will protect the most sensitive resources at that location (Porter *et al.* 2005). Reactive nitrogen is an air pollutant of great concern throughout North America because of its potential to acidify or fertilize ecosystems (Fenn *et al.* 2011). Atmospheric nitrogen deposition has increased significantly in the last 150 years due to anthropogenic nitrogen emissions derived from agriculture and fossil fuel combustion, and, despite strides made in reducing some sources, emissions continue to rise in some regions (Galloway *et al.* 2008).

In the northern Great Plains (NGP), rapid development of fossil fuel extraction and increasing agricultural intensity threaten to increase nitrogen emissions and deposition in this area of historically clean air (Hand *et al.* 2012; Ellis *et al.* 2013). The soils in most of the region buffer vegetation resources from the detrimental effects of acid deposition associated with sulfur and nitrogen (Fenn *et al.* 2003). However, because plant growth in NGP grasslands is commonly limited by nitrogen or co-limited by nitrogen and another resource, and because the herbaceous species in these grasslands are capable of rapid response to changing environmental conditions, it is expected that these grasslands will be sensitive to elevated nitrogen (Hooper and Johnson 1999; Clark 2011). Nitrogen fertilization experiments in a variety of North American grasslands have shown significant decreases in species richness from fertilization rates as low as 12 kg N/ha/yr above natural deposition rates (Piper *et al.* 2005; Suding *et al.* 2005; Clark and Tilman 2008). Nitrogen-induced changes in composition include shifts in warm:cool season grass ratios (Samuel and Hart 1998), reductions in legume cover (Piper *et al.* 2005), expansion of forest into grassland (Kochy and Wilson 2001) and sometimes drastic increases in invasive species abundance (Schwinning *et al.* 2005). Experimental nitrogen addition of 45 kg N/ ha/yr led to a 20-fold increase in the biomass of an invasive species in badlands sparse vegetation at Badlands National Park (Van Riper *et al.* 2010). Nitrogen fertilization has also

been shown to affect nitrogen cycling and retention, as well as ecosystem carbon storage, in grasslands (e.g., Wedin and Tilman 1996).

Despite the abundance of nitrogen-addition experiments in North American grasslands, determining a nitrogen critical load for Great Plains grasslands is not straightforward. In a recent review specifically designed to do this, Clark (2011) found only 17 papers that included nitrogen addition treatments of 30 kg N/ha/yr or less – levels commensurate with those expected from atmospheric deposition. Furthermore, 12 of those 17 papers came from just two locations – one of which is on the far eastern edge of, and in a soil type uncommon in, the Great Plains – and the experiments in nine of the 17 papers added nutrients other than nitrogen with the nitrogen. Thus, the critical load of 10-25 kg N/ha/yr set for mixed- and shortgrass prairie of the Great Plains – derived from a study in the southern Great Plains of Oklahoma – is considered only "fairly reliable" (Clark 2011; Pardo *et al.* 2011a), and calls for further experimentation in unstudied areas, including the NGP, have been made.

Here we report the results of an experiment designed to derive a nitrogen critical load for three plant communities in two NGP parks – Badlands National Park (BADL) and Wind Cave National Park (WICA) – in southwestern South Dakota. The three plant communities were chosen based on their importance in NGP parks, their expected sensitivity to nitrogen addition, and to span a range of natural fertility. Badlands sparse vegetation at BADL is a widespread, low-productivity vegetation type within the park that characterizes the interface between badlands formations for which the park was established and the mixed-grass prairie of the NGP; it occurs in other NGP parks (Theodore Roosevelt National Park, Scotts Bluff National Monument); and it was expected to be especially sensitive to nitrogen deposition based on its response to fertilization in previous research (Van Riper *et al.* 2010). Western wheatgrass (*Pascopyrum smithii*) and green needlegrass (*Nassella viridula*) vegetation in an ancient stream terrace at WICA was chosen to represent more fertile soil and productive vegetation characteristic of many NGP native grasslands. Finally, a stony hilltop at WICA was chosen for its expected high species richness and mixture of warm- and cool-season grasses – characteristics expected to make it sensitive to nitrogen addition based on research in other similar areas (Samuel and Hart 1998; Suding *et al.* 2005).

The experiment incorporated a large number of nitrogen addition levels from the very low to the very high compared to expected atmospheric deposition in order to provide dose-response curves useful for more precise pin-pointing of critical load values. Measured response variables concentrated on those of importance to the NPS and that have shown sensitivity to nitrogen addition in other grassland experiments. Table 1 describes these response variables and the rationale for their inclusion.

Table 1. Response variables measured in this study, rationale for their inclusion, and the ecosystem characteristics with which they are associated.

Ecosystem characteristic	Response variable	Rationale for inclusion
Soil nitrogen availability	Soil inorganic nitrogen	Inorganic nitrogen in the soil is an indicator of the nitrogen available for plant growth not being used by the plants, and therefore susceptible to leaching into groundwater.
Plant tissue nitrogen	Native warm-season grass tissue carbon-to-nitrogen ratio (C:N) Native cool-season grass tissue C:N Non-native cool-season grass tissue C:N	The carbon-to-nitrogen ratio (C:N) in aboveground plant tissue is an indicator of soil fertility, whether plants are using additional available nitrogen, and forage quality. *Lower* C:N is associated with higher soil fertility (more available nitrogen), greater utility of added nitrogen, and higher protein content of the plant for grazing animals. Native mixed-grass prairie grasses adapted to stressful, high-competition environments often have higher C:N, and are less responsive to added nitrogen, than invasive species adapted to high-nutrient environments. Also, because of differences in their physiology, cool-season grasses may respond to added nitrogen differently than warm-season grasses. Decreasing tissue C:N with increasing nitrogen addition indicates that the added nitrogen is being utilized by the plants (i.e., experimental treatments were successful), and differences in changes in C:N in response to added nitrogen among species indicate which species are using the added nitrogen.
Aboveground net primary production	Total biomass Total herbaceous biomass Native herbaceous biomass	Increased biomass production in response to added nitrogen indicates that nitrogen is limiting plant growth. Added nitrogen may be taken up by plants (indicated by lower C:N) but not result in greater biomass production if another resource (e.g., water, phosphorus) is also limiting the plant's growth. Total biomass measures the response of the whole plant community; herbaceous biomass the response of the non-woody component of the plant community, which is expected to respond more quickly than the woody component and comprises the vast majority of biomass in grassland communities; and native herbaceous biomass the response of the desired component of the plant community to nitrogen addition.
Foliar cover	Total cover	Plant cover is the measure of species abundance and proxy for community production monitored by the NPS NGP Inventory and Monitoring Network throughout parks in the NGP.
Litter load	Litter biomass	As a mechanical barrier, greater litter biomass may impede water or nitrogen infiltration into the soil, as well as the germination and/or growth of some species. On the other hand, greater litter biomass may help in the retention of moisture and therefore aid the germination of some species (such as annual brome grasses).
	Bare ground cover	High bare ground cover is a characteristic of badlands sparse vegetation but bare ground cover may decrease with added nitrogen.
Non-native species	Non-native biomass Non-native proportion of cover Species richness	Increased contribution of non-native species to the plant community, as well as loss of species, is a widely cited response of grasslands to nitrogen addition (see main text above) and is an undesired outcome from the point of native vegetation maintenance in NPS units.
Community composition and biodiversity	Native species richness Diversity Cool-season graminoid biomass proportion Cool-season graminoid proportion of cover Species cover	Park managers seek to maintain native plant species richness and diversity (distribution of abundance among species). Nitrogen addition in other systems has resulted in a shift of the ratio of warm- to cool-season graminoids (see main text); this shift would impact the seasonality of forage production for grazing animals and other wildlife. Other systems have shown shifts in overall species composition in response to nitrogen addition even when production or cover do not (Pardo *et al.* 2011c).

2. Methods

2.1 Study Area

Current total atmospheric nitrogen deposition in the northern Great Plains (NGP) generally increases from ~3-5 kg/ha/yr in the west to up to 20 kg/ha/yr in the southeast (Figure 1), following increasing precipitation, human population density, and row-crop agriculture from west to east.

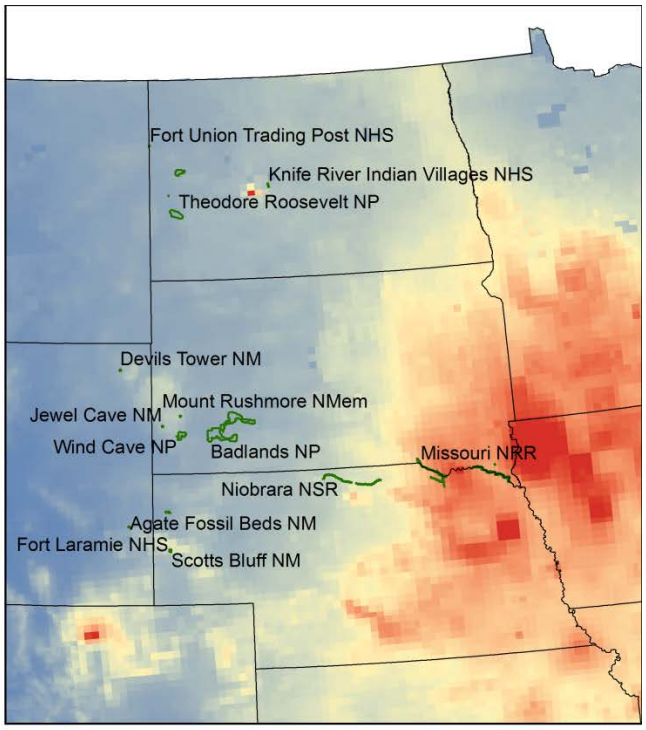
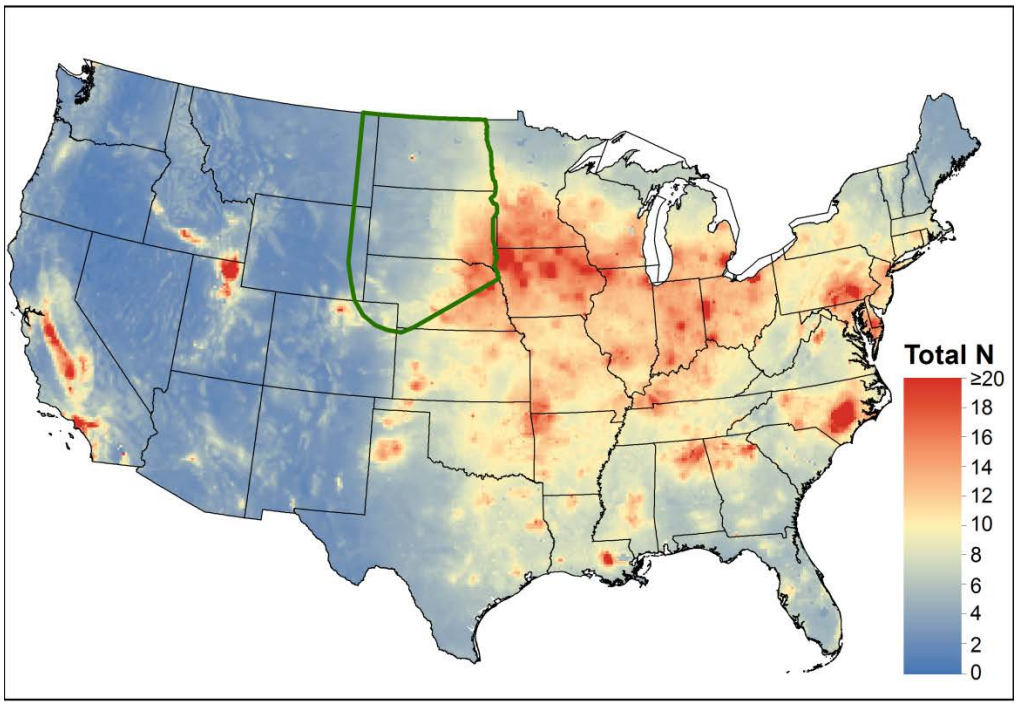


Figure 1. Total (wet + dry) nitrogen (N) deposition (kg/ha/yr), averaged over 2011-2013, in the continental United States (bottom) and northern Great Plains (NGP; top). In the bottom panel, the green outline indicates the region serviced by the NPS Northern Great Plains Inventory & Monitoring Network. In the top panel, parks within this network are shown in green polygons (NHS=National Historic Site, NM = National Monument, NMem=National Memorial, NRR=National Recreational River, NSR=National Scenic River, NP=National Park). Deposition data from the National Atmospheric Deposition Program (2014).



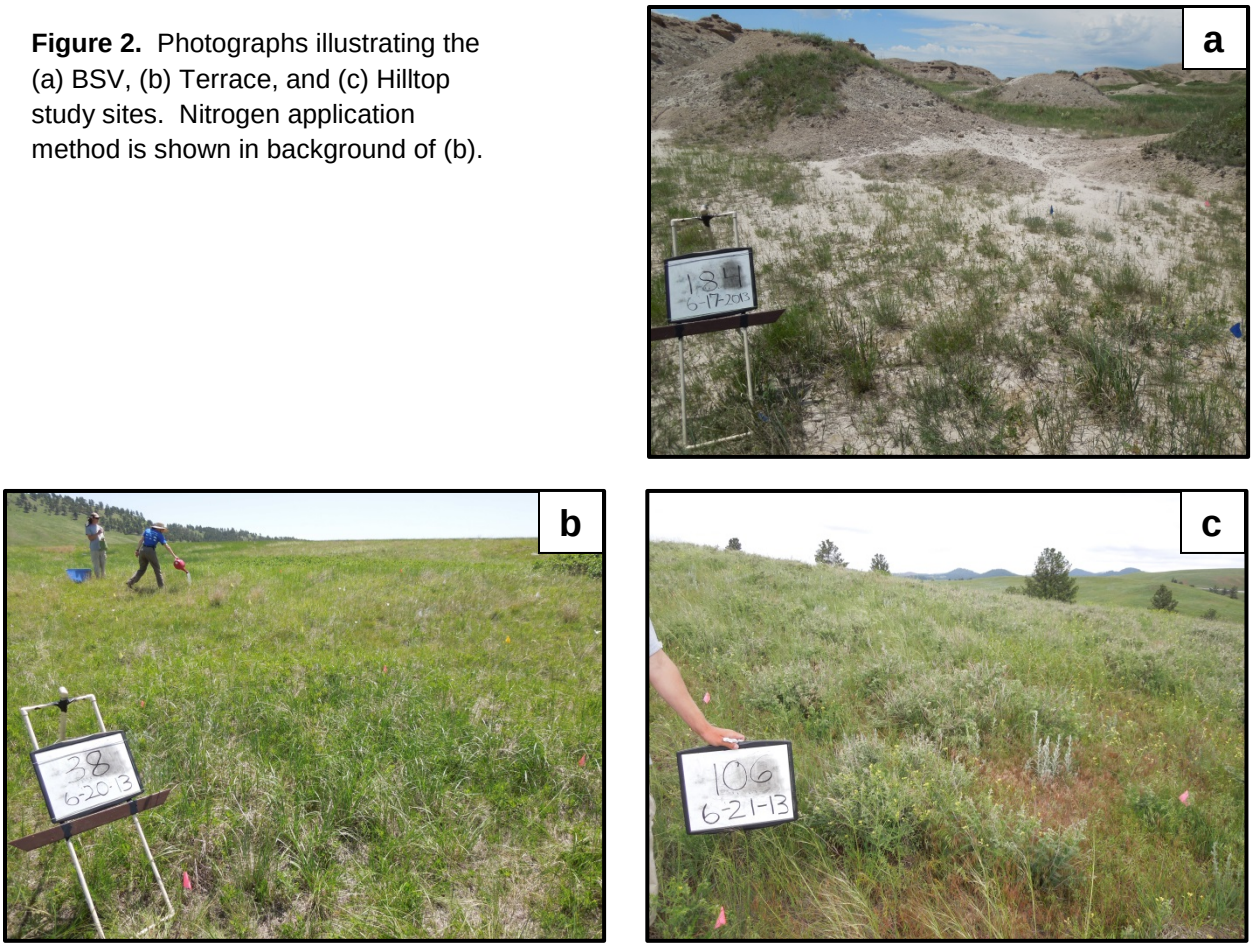
Climate of the region is continental, with hot summers, cold winters, the majority of precipitation falling during the April-September growing season, and high interannual variability in precipitation Vegetation biomass is generally dominated by grasses, with the relative abundance of cool-season (C_3 photosynthetic pathway) and warm-season (C_4 photosynthetic pathway) grass species varying with soil type, slope position, and grazing pressure. Forbs (non-graminoid, generally broadleaf, herbaceous plants) comprise the majority of the plant species in the NGP flora, but the number of grass species is often equal to or sometimes greater than the number of forb species in a small area such as a sample plot. Shrubs and subshrubs are uncommon and trees rare.

For this study, we chose three sites protected from bison to avoid the heterogeneity caused by these large grazers, but they were accessible to other herbivores. The first site ("BSV", Figure 2a) was in the "badlands sparse vegetation complex" vegetation type (Von Loh et al. 1999) near the Badlands National Park (BADL) visitor center (43.756° N, 101.936° W, 737 m above sea level). Experimental plots were established at the base of eroding badlands features, where substrate was silty to clayey alluvial soils low in organic material, nutrients, and water holding capacity and with a neutral to basic pH (NRCS 2010). Dominant plant species were *Elymus trachycaulus* and *Poa secunda* (native cool-season grasses), *Bouteloua gracilis* and *Bouteloua curtipendula* (native warm-season grasses), *Gutierrezia sarothrae* (native subshrub), *Bromus japonicus* (non-native cool-season grass), and *Eriogonum pauciflorum* and *Phlox hoodii* (native forbs). The site was distributed across ten distinct locations (blocks, see "Experimental Design" below) within a ~13-ha area.

The second site ("Terrace", Figure 2b) was on the east side of Wind Cave National Park, in the lower-elevation, Red Valley portion of the park (43.604° N, 103.384° W, 1157 m). Experimental plots were established on ancient stream terraces in well-drained, sandy loam, neutral soil with relatively high nutrient and organic matter content and water-holding capacity (NRCS 2010). Dominant plant species were *Poa pratensis* (non-native cool-season grass), *Pascopyrum smithii*, *Nassella viridula*, and *Hesperostipa comata* (native cool-season grasses), *Bouteloua curtipendula*, *Bouteloua gracilis*, and *Schizachyrium scoparium* (native warm-season grasses), and *Carex filifolia* (native cool-season sedge). Experimental plots were arranged in a ~0.3-ha area within a 5-ha bison exclosure constructed in the mid-1990s.

The third site ("Hilltop", Figure 2c) was near the Wind Cave National Park (WICA) visitor center (43.557° N, 103.473° W, 1230 m). Experimental plots were established on a ridgeline with shallow, slightly acidic, cobbly sandy loam soils with limited water-holding capacity (NRCS 2010). Dominant plant species were *Amorpha canescens* (native nitrogen-fixing shrub), *Bromus japonicus*, *Bromus tectorum*, and *Poa pratensis* (non-native cool-season grasses), *Andropogon gerardii* (native warm-season grass), *Hesperostipa comata* and *Pascopyrum smithii* (native cool-season grasses), and *Carex inops* and *Carex filifolia* (native cool-season sedges). Experimental plots were arranged in a narrow strip (~250 m x 10 m) at the top of the ridge in vegetation chosen for its high apparent species diversity.

Figure 2. Photographs illustrating the (a) BSV, (b) Terrace, and (c) Hilltop study sites. Nitrogen application method is shown in background of (b).



Climate at the two parks is similar, although precipitation is slightly lower at BADL than at WICA (Figure 3). At BADL, annual (September-August) precipitation was near its 30-year average in 2010 and 2013, 23% above average in 2011, and 26% below average in 2012. Growing-season precipitation followed a similar pattern, except in 2013 it was 27% above average. Precipitation variation among years at WICA in a pattern similar to that at BADL, except that at WICA, 2010 was the wetter of the first two years of the study (annual and growing season precipitation 41 and 53% above average; Figure 3). Total atmospheric nitrogen deposition is similar between the two parks, and generally follows a temporal pattern similar to that of precipitation (Figure 3). Average deposition for BADL and WICA over the four years of the experiment was 4.7 and 4.5 kg N/ha/yr, respectively.

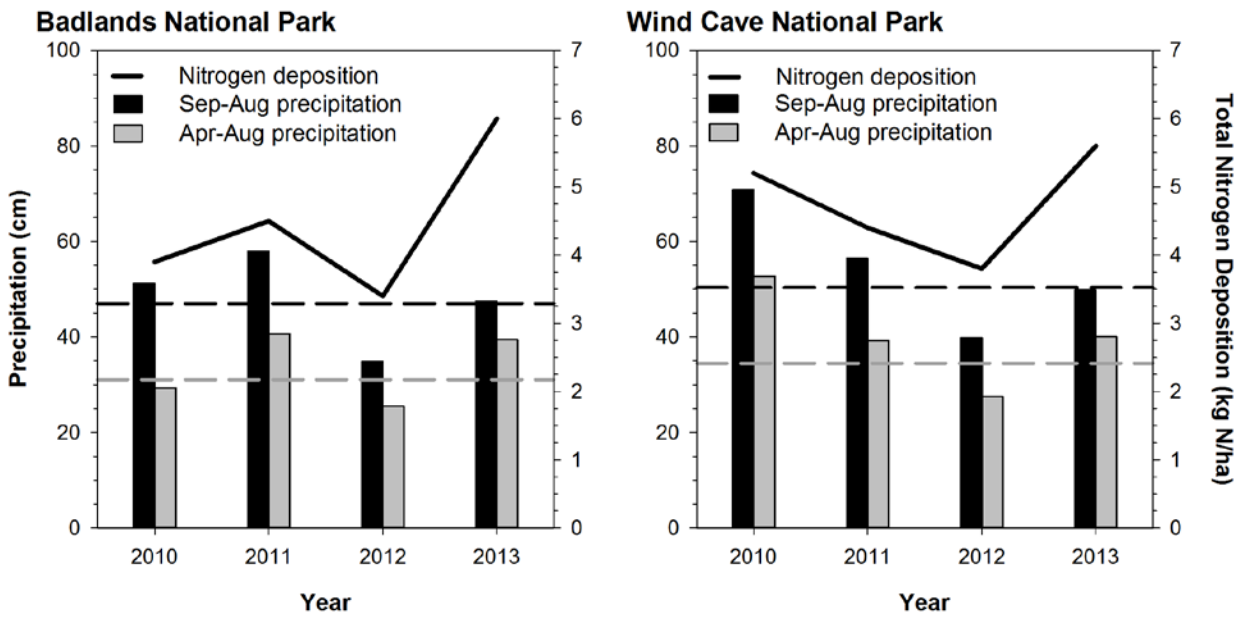


Figure 3. Precipitation and nitrogen deposition at Badlands and Wind Cave National Parks during the four years of this study. Annual (September-August) and growing-season (April-August) precipitation for the four years of this study (2010-2013) are shown as bars, and 1981-2010 precipitation means are shown as dashed lines. Total nitrogen deposition (solid line) is calculated for each park from National Atmospheric Deposition Program data (2014).

2.2 Experimental Design

The experimental design incorporated tradeoffs among the number and distribution of nitrogen addition levels, the number of replicates at each nitrogen level, vegetation heterogeneity, and area and resources available for the study. A high number of nitrogen addition levels evenly spread over a wide range would provide the most robust design for pin-pointing critical loads for various responses, but replication of nitrogen treatments is needed to determine the statistical significance of differences among treatments given the natural heterogeneity of the vegetation within the study sites. Our design in the first two years of the four-year experiment concentrated on nitrogen addition levels in the range expected from atmospheric deposition but also included higher levels. These higher levels were chosen to be comparable to those in previously published experiments from this and other grassland regions (e.g., Lorenz and Rogler 1973; Rauzi and Fairbourn 1983; Tilman 1987; Jacobsen *et al.* 1996) and to serve as surrogates for long-term effects of low-level nitrogen deposition (Clark and Tilman 2008). This design also included treatments that combined nitrogen and water addition treatments to test for the co-limitation of the two resources (Lauenroth *et al.* 1978; Hooper and Johnson 1999).

The resulting design was a partially balanced incomplete block design. A total of thirteen treatments were applied, with nitrogen addition ranging from 0 to 100 kg N/ha/yr and two water addition levels (none and some; see “Treatment Implementation and Sampling Methods”). At each site, seven treatments were assigned randomly to seven, 2.5 m x 2.5 m plots within each of ten blocks. Each block included the control (no nitrogen or water added) and six treatments, with the six treatments

being applied in only half of the blocks (Table 2). Thus, there were five replicates of all treatments except the control, for which there were 10 replicates. The incomplete block design was used because areas of badlands sparse vegetation were too small to accommodate 13 plots and because of the high vegetation heterogeneity at all sites.

In the third year of the study (2012), experimental treatments in some plots were changed (Table 2). Water addition treatments were stopped and replaced by additional nitrogen addition levels. This was done because the first two years of the study showed that significant water addition was infeasible and results from the first two years of the study suggested that additional nitrogen levels above 10 kg N/ha/yr might better determine the critical load for the chosen response variables (Smith 2012).

Table 2. Treatments in first and second halves of the experiment, and block(s) in which each treatment occurred. There were five of each block at each site. Special font indicates treatments in which nitrogen added increased (italics) or decreased (underline) from the first two years of the experiment (2010-2011) to the final two years of the experiment (2012-2013).

Abbreviation: 2010-2011 Treatment	Abbreviation: 2012-2013 Treatment	Block
N0: no nitrogen, no water (control)	N0: no nitrogen, no water (control)	A, B
N0W: no nitrogen, supplemental water	*N15: 15 kg N/ha/yr*	B
N2.5: 2.5 kg N/ha/yr	N2.5: 2.5 kg N/ha/yr	A
N2.5W: 2.5 kg N/ha/yr + supplemental water	*N32: 32 kg N/ha/yr*	A
N5: 5 kg N/ha/yr	N5: 5 kg N/ha/yr	B
N7.5: 7.5 kg N/ha/yr	N7.5: 7.5 kg N/ha/yr	A
N10: 10 kg N/ha/yr	N10: 10 kg N/ha/yr	B
N20: 20 kg N/ha/yr	N20: 20 kg N/ha/yr	A
N45: 45 kg N/ha/yr	N45: 45 kg N/ha/yr	B
N68: kg N/ha/yr	N68: 68 kg N/ha/yr	A
N68W: 68 kg N/ha/yr + supplemental water	N56: 56 kg N/ha/yr	A
N100: 100 kg N/ha/yr	N100: 100 kg N/ha/yr	B
N100W: 100 kg N/ha/yr + supplemental water	N84: 84 kg N/ha/yr	B

2.3 Treatment Implementation

Each year (2010-2013), we applied the assigned amount of nitrogen to each plot with a watering can in four equal allotments in mid-April, early June, late June, and mid-July as ammonium nitrate (NH_4NO_3) salt dissolved in 1 liter of water. Applications were timed to correspond to the average period of greatest rainfall to mimic wet atmospheric nitrogen deposition. Control plots received the same amount of water at the same time as the nitrogen only plots.

We applied supplemental water to applicable plots with a backpack sprayer in three equal amounts in early June, mid-July, and early August of 2010 and 2011. The total amount applied to each plot was 3.1 cm for all sites in 2010, 5.5 cm for the BSV and Terrace sites in 2011, and 6.6 cm for the Hilltop site in 2011. The amounts of water added in 2010 and 2011 were 10% and 18% of the 1981-2010 mean growing-season (April-August) precipitation, respectively, for the BSV site; 9% and 19% of the mean for the Hilltop site; and 9% and 16% of the mean for the Terrace site. However, due to the high growing-season precipitation in 2010 at the Hilltop and Terrace sites (Figure 3), the amount of

water added to these sites in that year was only 6% of actual growing-season precipitation. We tested for lasting effects of water addition on soil moisture by measuring gravimetric moisture content in a 1.9 cm-diameter x 10 cm-deep core taken from each plot in the N0, N0W, N100, and N100W treatments in mid-August 2011.

2.4 Responses Measured and Sampling Methods

In the first two years of the experiment, we used two metrics to measure the effects of the experimental treatments on soil nitrogen availability: total inorganic nitrogen and nitrogen mineralization. We measured total inorganic nitrogen ($NO_3^- + NH_4^+$) in mid-June in 2010 and 2011 in the N0, N5, N10, N45, N100, N0W, and N100W treatments, and in late March, mid-June, and mid-September 2011 in the N0, N5, N10, N45, N100, treatments. Measurements were made on soil combined from four 1.9 cm-diameter x 10 cm-deep cores per plot. Samples were extracted with 2M KCl for 24 hours the extractions analyzed for NO_3^- and NH_4^+ with an Alpkem Flow Solution IV auto-analyzer. We measured nitrogen mineralization from mid-June to mid-July in 2010 and 2011 using the buried bag method (Binkley and Hart 1989) from a subsample of each of the combined samples used for June inorganic nitrogen measurements. In addition, in order to compare soil fertility among sites, we measured total soil carbon and nitrogen in the soil samples taken in September 2011.

Metrics of vegetation response to the experimental treatments were derived from four types of data collected: (1) plant tissue nitrogen and carbon, (2) foliar and ground cover, (3) species presence within a fixed area, and (4) aboveground biomass. Table 1 explains the rationale for the variables calculated from each of these types of data.

(1) We measured whole plant tissue nitrogen and carbon content of each site's dominant native warm-season, native cool-season, and non-native cool-season grass species in a subset of treatments in 2011 and 2013. A tissue sample, collected in mid-June (cool-season species) or mid-July (warm-season species), consisted of 5 to 12 tillers that had at least four leaves but that had not yet flowered for each plot. These were collected outside of the permanent plant cover quadrats and the previous year's biomass harvest quadrat. Tillers from each species were composited by plot, dried, ground, and analyzed for total carbon (C) and nitrogen (N) with a Leco Tru-Spec Total CN analyzer (Colorado State University Soil, Water and Plant Testing Laboratory, Fort Collins, CO). In both years, samples collected and analyzed were limited by plants present in each plot and funds available for analyses. Samples collected in 2011 were from seven treatments (N0, N0W, N5, N10, N45, N100, and N100W) and in 2013 were from all but the N84 and N100 treatments. At the BSV site, species collected were *Bouteloua curtipendula* (2011 only), *Bouteloua gracilis* (2013 only), *Bromus japonicus*, and *Elymus trachycaulus*. Species collected at the Hilltop site were *Andropogon gerardii*, *Bromus japonicus*, *Hesperostipa comata* (2013 only), and *Pascopyrum smithii* (2011 only). At the Terrace site, *Bouteloua curtipendula*, *Pascopyrum smithii*, and *Poa pratensis* were collected. Appendix A shows the number of samples for each species, site, treatment, and year combination.

(2) We measured cover of individual species in mid-June to early July of each year, beginning at the BSV site and ending at the Terrace site. Cover was measured in a fixed, 1 m x 1 m quadrat using the point-intercept method. A pin was inserted vertically through the vegetation canopy at 50 (Hilltop

and Terrace sites) or 100 (BSV site) points arranged on 10-point, parallel, equally spaced lines within each quadrat and all species intercepted by the pin were recorded for each point. Ground cover (plant species, soil, litter, or other) was also recorded for each point. We calculated individual species' percent cover as the sum of points at which the species was intercepted (BSV), or that sum doubled (Hilltop and Terrace). We calculated total plant canopy cover as the sum of all individual species' covers; this value could therefore be greater than 100%. We also calculated Shannon diversity (H'; Magurran 2004), non-native proportion of cover, annual brome grass (*Bromus tectorum* and *Bromus japonicus*) proportion of cover, cool-season graminoid proportion of cover, and bare ground cover.

(3) At the same time and in the same quadrats used for plant cover measurements, we recorded the presence of all vascular plant species rooted within each quadrat. We used these data to calculate total and native plant species richness for each plot.

(4) We estimated aboveground net primary production (ANPP) by harvesting plant biomass at the approximate time of peak biomass each year, beginning in late July at the BSV site and ending in mid-August at the Terrace site. We harvested biomass in quadrats outside of the permanent 1 m^2 quadrats used for cover and richness measurements, and the harvest locations changed each year. Biomass quadrats were 20 cm x 50 cm in 2010, then 25 cm x 100 cm for the remaining three years of the experiment. In 2010 and 2011, all live aboveground vascular plant biomass of plants rooted within the quadrat was harvested and sorted into the following functional groups: warm-season native grass, cool-season native grass, cool-season non-native grass, sedge, shrub, native forb (non-legume), non-native forb (non-legume), native legume, non-native legume, and other. Samples were dried at 60 °C for 48 hours and weighed. Cactus (*Opuntia*, *Escobaria*, and *Echinocereus* spp.) and *Yucca glauca* were not harvested due to difficulties in distinguishing current from previous years' growth. Methods in 2012 and 2013 changed slightly so that only current year's growth of shrubs was harvested to provide a more accurate representation of ANPP. In addition, dead, down litter was harvested from each quadrat, dried, and weighed in 2011-2013. We calculated total, herbaceous, native herbaceous, and non-native biomass, as well as the cool-season graminoid proportion of herbaceous biomass, and litter mass, for each plot.

Hereafter, we refer to response variables derived from data collected by the last three methods as "plant community response variables".

2.5 Data Analysis

We calculated soil and plant tissue carbon-to-nitrogen ratio (C:N) from soil and tissue nitrogen and carbon content. We tested for differences in soil C:N among sites, treatments, and their interaction using a mixed model; treatment means were separated using Fisher's protected Least Significant Difference (LSD). We tested for effect of experimental treatments on plant tissue C:N with different mixed models for each year of tissue sampling (2011 and 2013), each site, and each species, with treatment means separated as for soil C:N. We used repeated-measures analysis of variance (RANOVA) in a partially balanced randomized block design to assess the effects of treatment and year on June inorganic nitrogen, nitrogen mineralization, and the plant community response variables, or treatment and month on inorganic nitrogen measured in 2011 (Littell *et al.* 2006). For

most response variables, we formulated planned contrasts among least-squares means to test for linear and quadratic trends across the nitrogen addition levels and to test for planned pair-wise and group-wise contrasts (Milliken and Johnson 2002). Following the approach of Pardo *et al*. (2011c) for determining the critical load of nitrogen for herbaceous species and shrubs, we emphasized comparing each treatment to the control in these contrasts (Pardo *et al*. 2011b). Because of unequal spacing among nitrogen levels, we used the ORPOL function in SAS PROC IML (SAS Institute Inc. 2012a) to derive orthogonal polynomial coefficients for the contrasts (Kirk 1982). We accommodated the potential presence of autocorrelation across years or months by assuming the first-order auto-regressive model error structure. Except where noted above, analyses were done separately for each response variable and for each study site due to differences in responses among sites indicated in earlier analyses (Smith 2012).

The Hilltop study site, which was selected and established during the dormant season, had unforeseen high abundance of *Amorpha canescens*. Varying abundance of this nitrogen-fixing, native shrub among experimental plots could confound the experimental treatments. Therefore, for this site, we also followed protocols for repeated-measures analysis of covariance (RANCOVA) as prescribed by Milliken and Johnson (2002) to ascertain this species' effect on response variables tested with RANOVA. We first tested if each response variable depended on *Amorpha* abundance (as measured by *Amorpha* cover) for at least one combination of nitrogen level and year. If this was significant at the 0.05 level, we then tested if the relationship was parallel for each nitrogen level by year combination. If sufficient evidence ($P > 0.05$) existed for parallelism in the relationship between each response variable and *Amorpha* cover, we proceeded with a RANCOVA. If there was not sufficient evidence ($P < 0.05$), we proceeded to test for trends across and differences among the nitrogen levels at three levels of AMCA (0, 25, and 50 percent cover).

Response variables departing from normality assumptions (skewness > 1, or |Kurtosis| > 2) were log- or square-root-transformed prior to analyses. Biomass responses were excluded from analyses for two plots in 2010 and one plot in 2013, all from the Hilltop site, because biomass harvest quadrats included very large individuals of *Ipomoea leptophylla*; forb biomass in these plots was 2.8 to 6.8 times that of the next highest forb biomass in the same year and site. June soil inorganic nitrogen and nitrogen mineralization responses were excluded from analyses for two plots in 2010, both from the Hilltop site, because of June inorganic nitrogen values 3 times that of the next highest value. Analyses were conducted using the PROC MIXED procedure in SAS (SAS Institute Inc. 2012b). We considered significance and marginal significance to be at the 0.05 and 0.10 levels, respectively.

As a follow-up to the RANOVAs and RANCOVAs, we used nonmetric multidimensional scaling (NMS) ordination in PC-ORD, version 6.17 (McCune and Mefford 2011) to descriptively assess if separation occurred in plant community composition among experimental treatments (Appendix B). NMS ordination, using the Sorenson distance measure, was done for each study site over all years of the experiment on absolute species cover, so that objects in the ordination were plot x year combinations. Dimensionality for each ordination was determined based on preliminary runs using the "Medium Autopilot" settings and following guidelines in Peck (2010); ordinations presented are

those with the lowest stress value out of 250 runs using all other default settings. Only species occurring in at least 10% of the site's plots in at least one year were included. For each ordination, we visually examined the pair-wise XY graphs for all axes for clusters of plots as related to experimental treatments.

3. Results

There was a significant effect of the experimental treatments on most response variables, but for all of these, the effect of the treatment differed among years of the experiment (significant treatment x year effect, Table 3). Planned contrasts, to test for linear and quadratic trends across nitrogen addition levels and to compare individual treatments to the controls, were completed only for those variables with a significant effect treatment effect. Plots of the NMS ordinations showed no patterns suggesting that plant community composition was affected by experimental treatments at any of the three sites (details in Appendix B).

Table 3. Results of tests for significant treatment x year or treatment x month effect on response variables. June inorganic N (nitrogen) and N mineralization were measured only in 2010 and 2011 in a subset of treatments, and March (Mar), June (Jun) and September (Sep) inorganic N were measured in a smaller subset of treatments in 2011.

	---------- BSV ----------			---------- Terrace ---------			---------- Hilltop ----------		
Response Variable	**df**	***F***	***P***	**df**	***F***	***P***	**df**	***F***	***P***
June inorganic N	13, 21	8.75	<0.001	13, 21	3.79	0.003	13, 19	5.75	<0.001
Mar, Jun, Sep inorganic N	14, 30	4.88	<0.001	14, 30	1.43	0.200	14, 30	2.61	0.013
N mineralization	13, 21	1.82	0.106	13, 21	2.27	0.046	13, 19	2.05	0.075
Total biomass	51, 155	2.49	<0.001	51, 155	3.84	<0.001	51,152	1.15	0.262
Total herbaceous biomass	51, 155	2.54	<0.001	51, 155	2.70	<0.001	51,152	2.13	<0.001
Native herbaceous biomass	51, 155	2.68	<0.001	51, 155	1.94	0.001	51,152	1.22	0.181
Non-native biomass	51, 155	3.41	<0.001	51, 155	1.66	0.009	51,152	3.21	<0.001
Cool-season biomass proportion	51, 153	0.92	0.618	51, 155	1.19	0.207	51, 152	1.31	0.109
Litter biomass	38, 98	2.71	<0.001	38, 98	6.45	<0.001	38, 97	9.63	<0.001
Total species richness	51, 155	8.69	<0.001	51, 155	3.53	<0.001	51, 155	6.05	<0.001
Native species richness	51, 155	6.25	<0.001	51, 155	2.95	<0.001	51, 155	4.10	<0.001
Diversity	51, 155	3.14	<0.001	51, 155	2.30	<0.001	51, 155	2.45	<0.001
Total cover	51, 155	8.58	<0.001	51, 155	8.15	<0.001	51, 155	8.88	<0.001
Non-native cover proportion	51, 154	2.88	<0.001	51, 155	3.43	<0.001	51, 155	6.82	<0.001
Cool-season cover proportion	51, 154	1.95	0.001	51, 155	1.87	0.002	51, 155	3.48	<0.001
Bare ground cover	51, 155	2.68	<0.001	not applicable			not applicable		

3.1 Supplemental Water Treatments

August 2011 soil moisture did not differ among measured treatments (N0, N0W, N100, N100W) at any of the three sites ($P \geq 0.5$), indicating no lasting effect of supplemental water treatments on soil moisture. Furthermore, the mean response of a plant community variable for all watered treatments rarely differed from the mean response of that variable for unwatered treatments with matching nitrogen addition levels (i.e., 0, 2.5, 68, and 100 kg N/ha/yr; $P > 0.1$ for all but four variable-site-year combinations). This indicates there was no overall supplemental water effect on soil and vegetation response variables. However, individual water + nitrogen treatments displayed varying differences from the control at each site. Thus, we address water + nitrogen treatments individually for each site in the following sections, rather than as a whole.

We considered the possibility that treatment effects from the first two years of the experiment might have carried over into the third and fourth years of the experiment, when some treatments changed. To determine whether this happened, we compared the mean change in each plant community

response variable from 2010-11 to 2012-13 among plots in which experimental treatments remained unchanged, plots in which annual nitrogen added increased, and plots in which annual nitrogen added decreased (treatments indicated in Table 2). These changes were similar (means within 1.5 standard error of each other) among the stationary and changed treatments for all plant community response variables and all sites. We therefore concluded that carry-over effects were not substantial.

3.2 Variation in Measured Characteristics among the Experimental Sites

The BSV site was by far the least fertile and least productive of the three sites. Soil C:N was three to four times higher (site effect $P < 0.001$), and much more variable than at the other two sites (Table 4). Aboveground net primary production (ANPP), measured as total biomass, ranged from 0 (in the sampled area) to just 241 g/m^2 in control plots, with an average over all years of 57 g/m^2. Maximum ANPP measured at the site during the experiment was 426 g/m^2. Mean ANPP in control plots at the Hilltop and Terrace sites was four to five times that of the BSV site, and maximum ANPP in the control plots at the Hilltop site was nearly double that of the Terrace site (Table 4).

Table 4. Characteristics of the vegetation at the three experimental sites.

	Soil C:N	ANPP (g/m^2)	Non-native proportion of ANPP	Total species richness (m^{-2})	Non-native proportion of richness	Cool-season proportion of graminoid cover
BSV						
Control mean	38.7	57	0.15	12	0.24	0.32
Control minimum	12.3	0.2	0	3	0	0
Control maximum	61.7	241	0.79	20	0.43	1
Overall maximum[a]	12.3	426	0.85	26	0.54	1
Terrace						
Control mean	10.9	220	0.34	14	0.12	0.68
Control minimum	9.6	82	0	8	0	0
Control maximum	12.0	450	0.56	21	0.30	0.94
Overall maximum[a]	9.7	860	0.95	25	0.67	1
Hilltop						
Control mean	12.4	292	0.15	17	0.24	0.59
Control minimum	11.8	84	0	7	0.10	0.67
Control maximum	13.0	879	0.82	23	0.38	0.88
Overall maximum[a]	11.1	922[b]	0.90	26	0.50	0.91

[a]Minimum for soil C:N, indicating most fertile condition; [b]5,136 when outliers that were not included in statistical analyses are included.

On average, non-native species did not dominate ANPP in control plots at any of the sites. They were more than twice as dominant, in terms of biomass, at the Terrace site than at the other two sites, and non-native species comprised almost all of the ANPP in some treated plots at all sites (Table 4). The Hilltop site was the most, and the BSV site the least, diverse in terms of the total number of species encountered (111 and 90, respectively; 99 at the Terrace site), and mean total species richness in control plots followed the same pattern (Table 4). Non-native species comprised a similar proportion of the flora across sites (18-21%), but they were twice as important to species richness at the 1 m^2 sampling scale at the Hilltop and BSV sites than at the Terrace site (Table 4). Finally, cool-season graminoids were most prevalent at the Terrace site, comprising nearly 70% of the total foliar cover, slightly less prevalent at the Hilltop site, and least prevalent at the BSV site (Table 4).

3.3 Badlands Sparse Vegetation (BSV) Site

Mid-June soil inorganic nitrogen increased significantly ($P \leq 0.001$) with increasing nitrogen addition at the BSV site in both 2010 and 2011, with this trend being driven by the N100 treatment (Figure 4a). This effect of high nitrogen addition on inorganic nitrogen availability was not limited to the growing season, when nitrogen was added, but lasted through the winter and to the end of the growing season, as indicated by significant positive linear trends ($P < 0.001$) and effects of the N45 and N100 treatments on March and September 2011 soil inorganic nitrogen (Figure 4b). In contrast, nitrogen mineralization was unresponsive to experimental treatments (Table 3).

Plant tissue C:N of the three dominant species at the BSV site was influenced little by experimental treatments in the first half of the experiment but was much more responsive in the second half (Figure 4c, d). Among the six treatments and three species in which tissue C:N was measured in 2011, C:N varied with nitrogen addition only for *Elymus trachycaulus*, the dominant native cool-season grass. Compared to the control, tissue C:N was marginally higher in the N0W treatment ($P = 0.063$) and lower in the N100 treatment ($P = 0.006$), with an overall negative trend with increasing nitrogen addition in unwatered treatments (Figure 4c). In 2013, tissue C:N of all three species decreased significantly ($P \leq 0.002$) with increasing nitrogen addition in unwatered treatments, and tissue C:N was generally lower in treatments with at least 45 kg N/ha/yr than in the control. *Bouteloua gracilis* was the most sensitive species, with consistently lower C:N than the control in treatments with at least 20 kg N/ha/yr added and significant but inconsistent responses in treatments with less nitrogen added (Figure 4d). *Bromus japonicus* and *Elymus trachycaulus* also had lower tissue C:N than the control at the N20 level, though not at the N32 level.

Sensitivity of the BSV site to nitrogen addition varied among plant community response variables and sometimes between the first two and final two years of the experiment. Total and herbaceous biomass, which were virtually identical due to the very low abundance of woody species at this site, increased significantly with increasing nitrogen addition in all years of the experiment (Table 5). Although the lowest nitrogen addition level at which total biomass differed from the control in 2010-2011 was 10 kg N/ha/yr, the N20 and N45 treatments were not significantly higher than the control (Figure 5a). Total biomass in the highest nitrogen treatments (68-100 kg N/ha/yr), with or without supplemental water, was consistently higher than the control throughout the experiment at this site, and supplemental water in 2010-2011 or lower nitrogen addition levels (as low as 10 kg N/ha/yr) in 2012-13 yielded marginally significantly higher total biomass (Figures 5a, 6a).

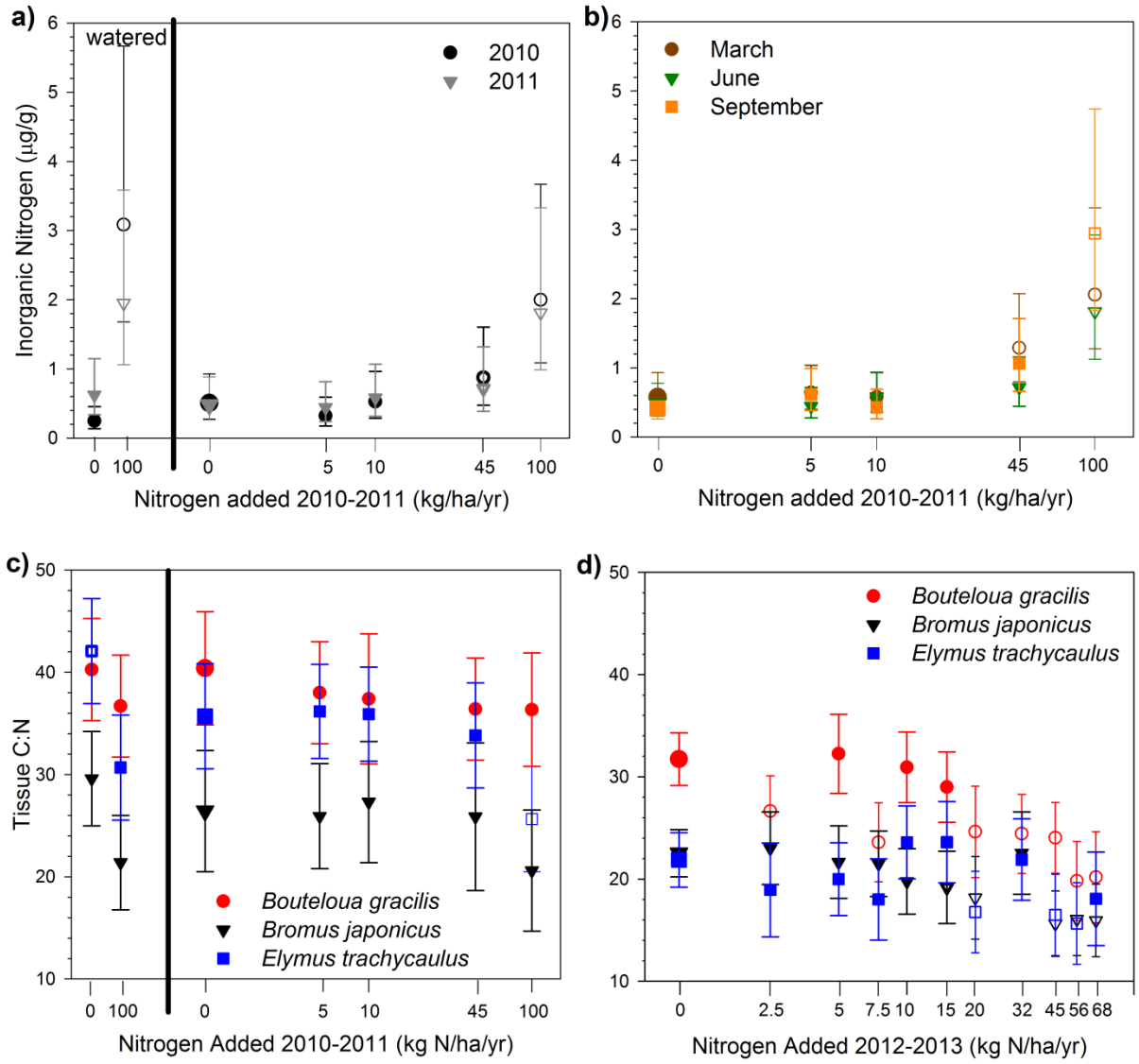


Figure 4. Soil inorganic nitrogen in **(a)** June of 2010 and 2011 and **(b)** March, June, and September of 2011, and whole-plant tissue carbon to nitrogen ratio (C:N) for three species each in **(c)** 2011 and **(d)** 2013 at the Badlands Sparse Vegetation (BSV) site. Supplemental water treatments are to the left of all non-watered treatments. Nitrogen Added (horizontal) axis is on a log scale to improve visibility of all treatments. Controls are indicated by large, filled symbols. Treatments significantly different from that of the control treatment (within species in c and d) are indicated by open, thin-walled symbols; treatments marginally significantly different from the control by open, thick-walled symbols [e.g., N15 *Bromus* in (d)]; and treatments not significantly different from the control by small, filled symbols. Symbols are back-transformed (where necessary) least squares means and 95% confidence intervals.

Table 5. Direction (positive, +; negative, -; none, 0) of linear relationships between transformed plant community response variables and experimentally added nitrogen for the BSV site. Relationships for 2010 and 2011 do not include treatments with supplemental water. Relationships for 2012 and 2013 include all treatments. When necessary, response variables were transformed (T) to meet normality assumptions: S = $\sqrt{(y+0.5)}$; L = ln(y+0.5); N = no transformation. Statistical significance indicated as $^{\dagger}0.05 < P \leq 0.10$; *$0.01 < P \leq 0.05$; **$0.01 < P \leq 0.001$; *** $P < 0.001$.

Response Variable	T	2010	2011	2012	2013
Total biomass	S	+*	+***	+**	+***
Total herbaceous biomass	S	+*	+***	+**	+***
Native herbaceous biomass	S	+†	+***	+***	+***
Non-native biomass	L	0	0	0	0
Litter biomass	L	--	0	+*	+***
Total species richness	N	+*	0	0	+***
Native species richness	N	+†	0	0	0
Diversity	N	0	0	0	0
Total cover	N	+**	+***	0	0
Non-native cover proportion	L	0	0	0	0
C3 cover proportion	N	0	0	0	0

The response of foliar cover was less consistent than that of biomass. In both 2010 and 2011, there was a significant linear increase in cover with increasing nitrogen addition, but no relationship in 2012-13 (Table 5). In 2011, the wettest year of the experiment for this site, cover was higher than the control at and above 10 kg N/ha/yr (Figure 5c). Greater biomass and foliar cover in the first two years of the experiment likely led to litter biomass being greater than the control in many nitrogen treatments in 2012 (N2.5, N10, N45, N68, N80, and N100) and 2013 (N32, N56, N68, N80, N100; Figure 6c). Conversely, bare ground cover did not differ among treatments in any year of the experiment except 2012, when just the N45 treatment had less bare ground than the control (P = 0.034).

Native species comprised, on average, 85% of biomass at this site (standard error 1%). Consequently, native herbaceous biomass response to nitrogen addition was similar to that of total biomass. It increased with increasing nitrogen addition, with the relationship being marginal in 2010 but strong in 2011 to 2013 (Table 5). In the first two years, native herbaceous biomass was higher than in the control in all but the N2.5, N7.5, and N0W treatments (Figure 5b). In the latter two years, it was consistently higher than the control at nitrogen addition levels at or above 32 kg N/ha/yr, though, as with total biomass, the N10 treatment was also significantly higher than the control (Figure 6b). In contrast, non-native species abundance, measured either as biomass or proportion of foliar cover, did not differ among treatments in either 2010-11 or 2012-13 ($P \geq 0.73$). The proportion of foliar cover comprised of C3 species was also unresponsive to treatments ($P \geq 0.71$).

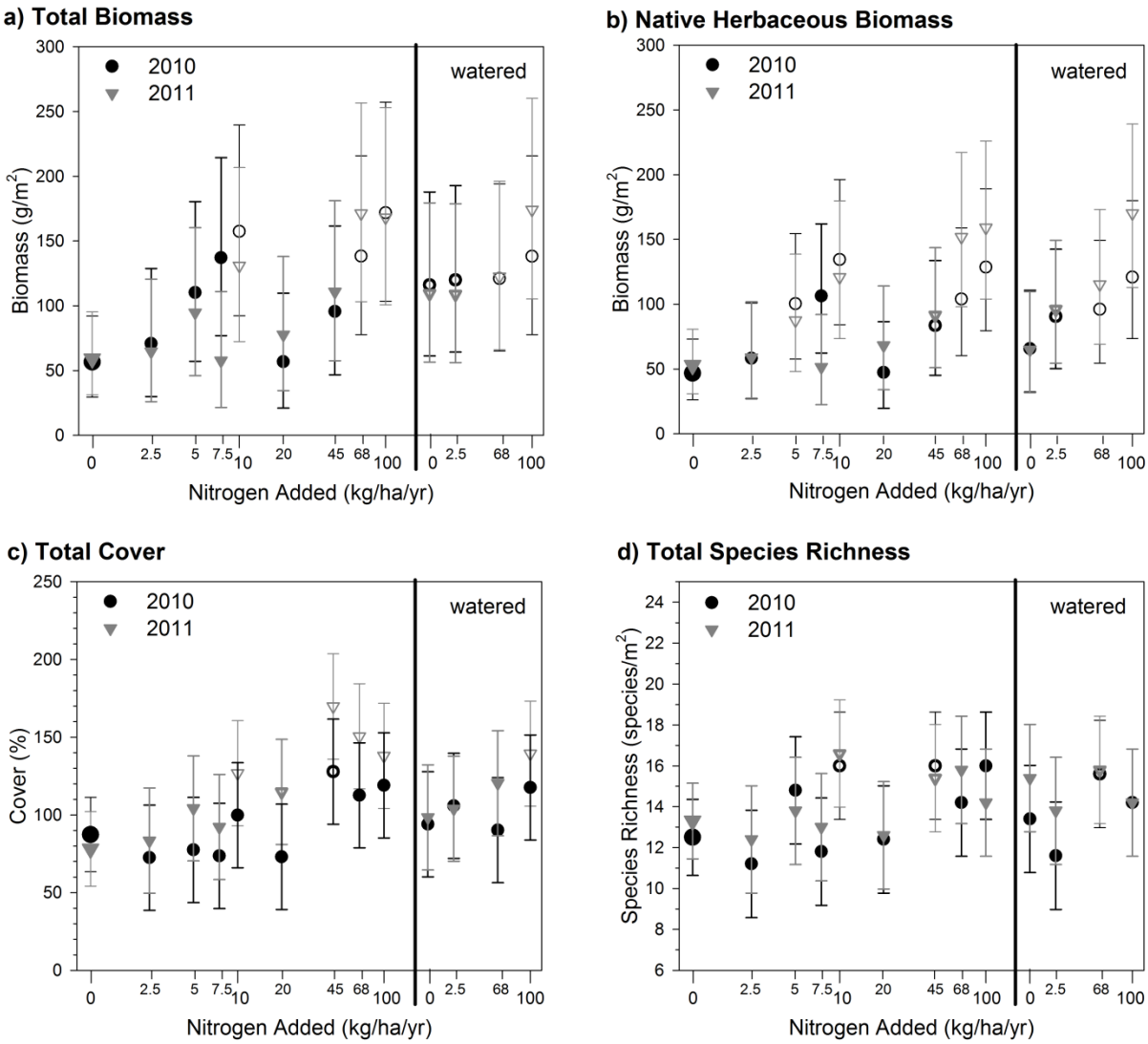


Figure 5. 2010 and 2011 least squares means and 95% confidence intervals for plant community response variables sensitive to 2010-2011 experimental treatments at the BSV site. Treatments are separated between unwatered (left of heavy black line) and watered (right of heavy black line) and aligned along the x-axis by nitrogen added in 2010 and 2011. Nitrogen Added (horizontal) axis is on a log scale to improve visibility of all treatments. Controls are indicated by large, filled symbols. Treatments significantly different from that of the control treatment are indicated by open, thin-walled symbols; treatments marginally significantly different from the control by open, thick-walled symbols; and treatments not significantly different from the control by small, filled symbols. Symbols are back-transformed (where necessary) least squares means and 95% confidence intervals.

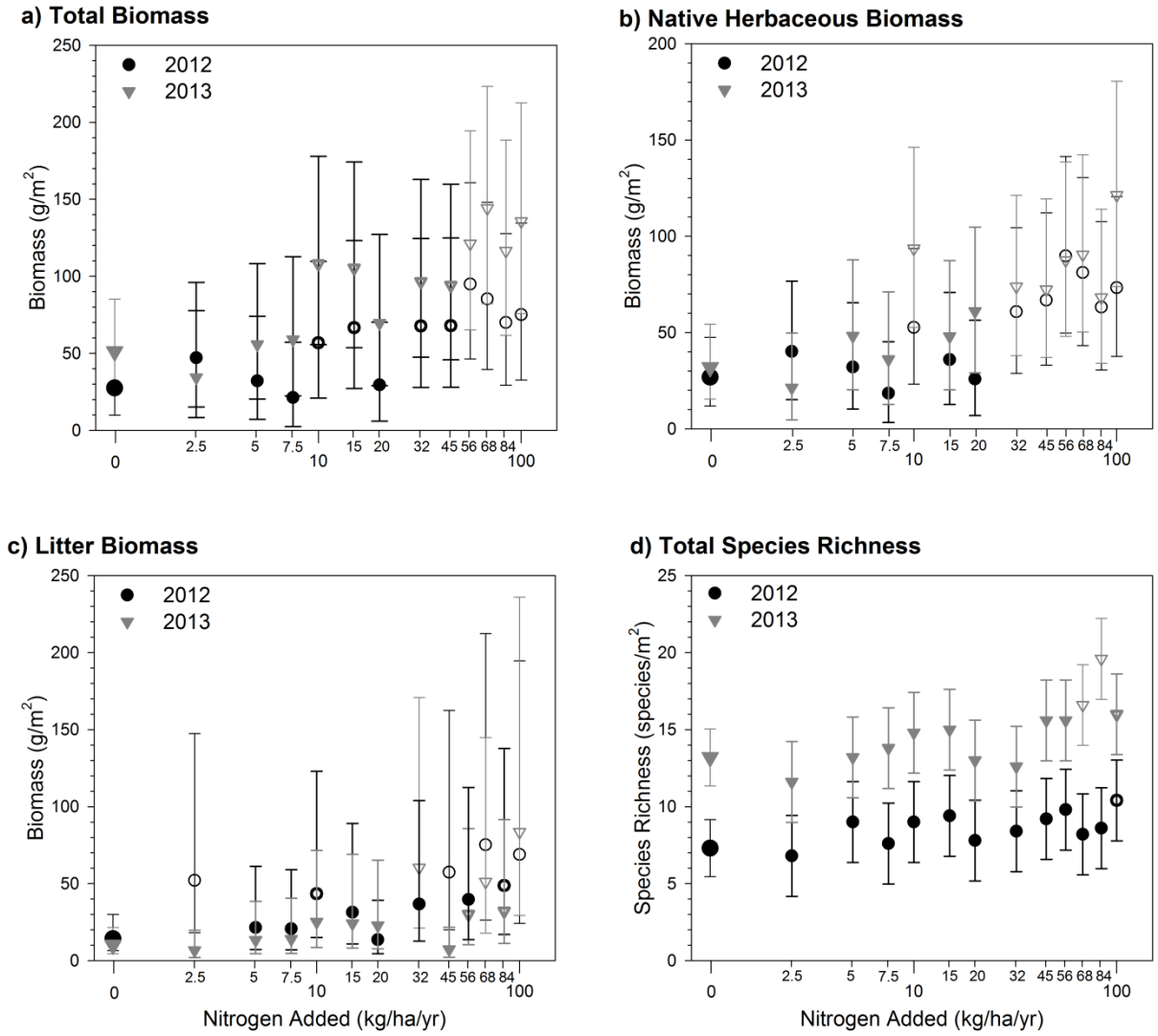


Figure 6. 2012 and 2013 least squares means and 95% confidence intervals for plant community response variables sensitive to experimental treatments at the BSV site. Treatments are aligned along the x-axis by 2012-13 nitrogen addition level. Nitrogen Added (horizontal) axis is on a log scale to improve visibility of all treatments. Controls are indicated by large, filled symbols. Treatments significantly different from that of the control treatment are indicated by open, thin-walled symbols; treatments marginally significantly different from the control by open, thick-walled symbols; and treatments not significantly different from the control by small, filled symbols. Symbols are back-transformed (where necessary) least squares means and 95% confidence intervals.

Total species richness increased with increasing nitrogen addition in 2010 and 2013, but not in the other two years of the experiment (Table 5). Individual treatments differed from the control marginally ($P = 0.066$) in 2010-11, with the N10, N45, and N68W treatments having more species than the control (Figure 5d). The treatment effect in the second half of the experiment was more consistent across nitrogen addition treatments, in that those treatments with at least 68 kg N/ha/yr had more species than the control, but this occurred only in 2013 (Figure 6d). Overall species richness in 2012, a drought year (Figure 3), was only half that of 2013 ($P < 0.001$). In comparison, native species richness increased only marginally ($P = 0.077$) with increasing nitrogen addition in 2010 and

not at all in any of the other years. Furthermore, native species richness did not differ from the control in any treatments in any year, suggesting that increased richness at high nitrogen addition levels was due to an increase in the number of non-native species present in each plot.

3.4 Terrace Site

Soil response to experimental treatments was not as strong at the Terrace site as at the BSV site. In 2010, only with supplemental water did the highest nitrogen addition (100 kg/ha/yr) treatment increase mid-June soil inorganic nitrogen over that of the control. In 2011 mid-June soil nitrogen increased with increasing nitrogen addition ($P = 0.006$), but again only the highest nitrogen addition treatments differed from the control (Figure 7a). Moreover, this effect did not last beyond the time of nitrogen addition. Although there was no statistical difference among months for the marginally significant treatment effect on soil nitrogen in 2011 (treatment effect $P = 0.084$; treatment x month interaction $P = 0.199$), the N100 treatment exhibited a noticeable difference from the control only in June (Figure 7b). A mild ($P = 0.05$) treatment by year interaction for nitrogen mineralization yielded no significant contrasts among treatments.

As with the BSV site, differences in plant tissue C:N among treatments were stronger in 2013 than in 2011. In 2011, the dominant native (*Pascopyrum smithii*) and non-native (*Poa pratensis*) cool-season grasses had a higher C:N ratio than the control in both treatments in which 100 kg N/ha/yr was added, so that, in unwatered treatments, there was a significant ($P \leq 0.017$) linear decrease in tissue C:N as nitrogen added increased (Figure 7c). In 2013, *Poa pratensis* was the most consistent species, with a strong ($P < 0.001$) linear decrease with increasing nitrogen, and treatments with at least 32 kg N/ha/yr having lower C:N than the control (overall treatment effect $P = 0.060$). *Pascopyrum smithii* and *Bouteloua curtipendula*, a common warm-season grass, had patterns similar to that of *Poa*, but they were not significantly different from the control in the N56 treatment (Figure 7d).

More plant community response variables were sensitive to experimental treatments in 2010-11 than in 2012-13, and responses were often idiosyncratic in both periods. Total biomass increased significantly with increasing nitrogen in 2010 and 2011 (Table 6), and it was higher than the control in all but the N2.5, N7.5, and N20 treatments in those years (Figure 8a). Although there was a marginally significant ($P = 0.077$) positive trend in total biomass with increasing nitrogen in 2013 (Table 6), no treatments distinguished themselves from the control in the last two years of the experiment. Total foliar cover had a more idiosyncratic response, being marginally higher than the control in just the N10 and N45 treatments in 2010-2011, and not at all responsive in 2012-13.

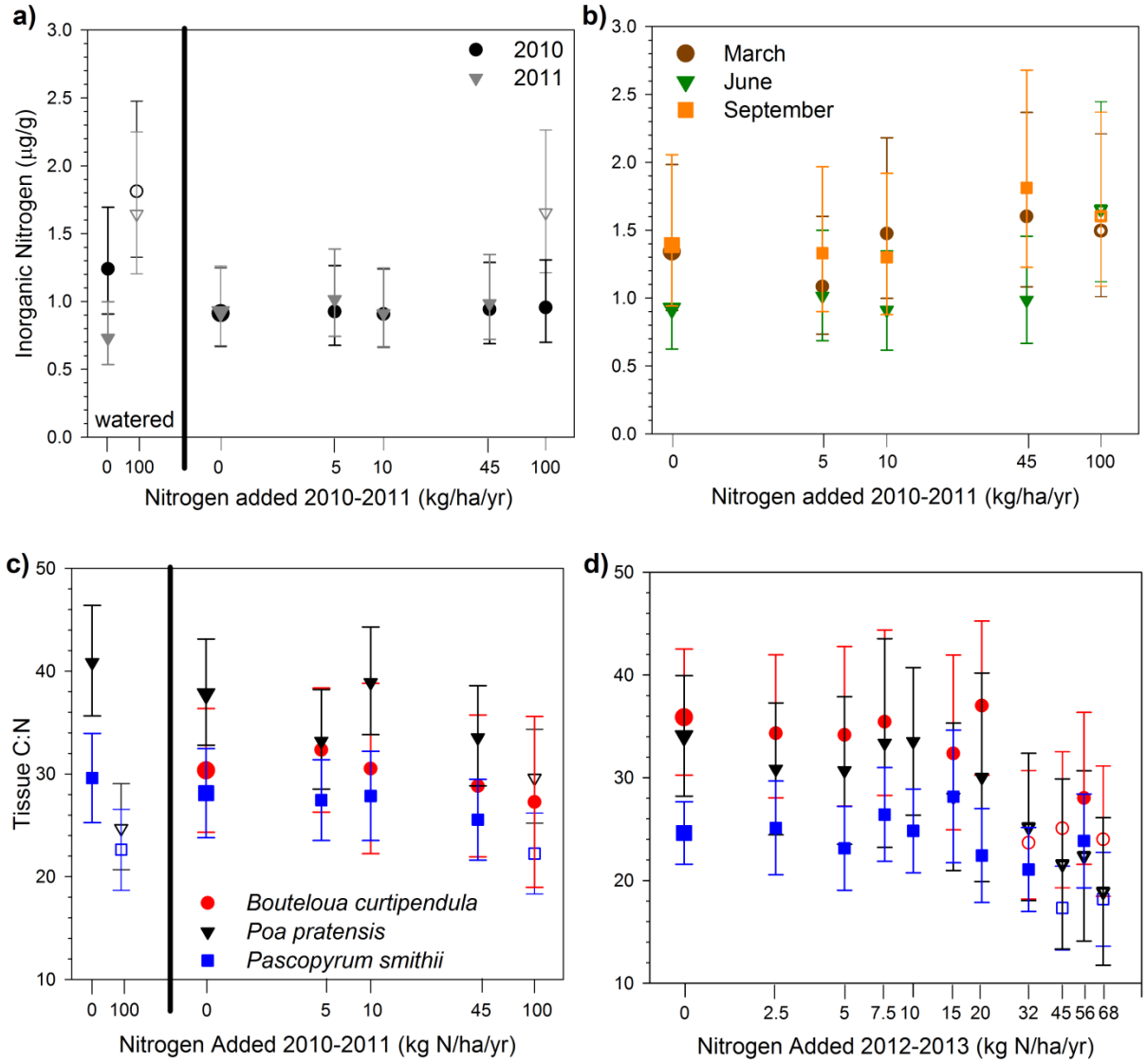


Figure 7. Soil inorganic nitrogen in **(a)** June of 2010 and 2011 and **(b)** March, June, and September of 2011, and whole-plant tissue carbon to nitrogen ratio (C:N) for three species each in **(c)** 2011 and **(d)** 2013 at the Terrace site. Supplemental water treatments are to the left of all non-watered treatments. Nitrogen Added (horizontal) axis is on a log scale to improve visibility of all treatments. Controls are indicated by large, filled symbols. Treatments significantly different from that of the control treatment (within species in c and d) are indicated by open, thin-walled symbols; treatments marginally significantly different from the control by open, thick-walled symbols; and treatments not significantly different from the control by small, filled symbols. Symbols are back-transformed (where necessary) least squares means and 95% confidence intervals.

Table 6. Directions (positive, +; negative, -; none, 0) of linear relationships between transformed plant community response variables and experimentally added nitrogen for the Terrace site. Relationships for 2010 and 2011 do not include treatments with supplemental water. Relationships for 2012 and 2013 include all treatments. Data transformation type (T): S = $\sqrt{(y+0.5)}$; L = ln(y+0.5); N = no transformation. Statistical significance indicated as $^{\dagger}0.05 < P \leq 0.10$; $^{*}0.01 < P \leq 0.05$; $^{**}0.01 < P \leq 0.001$; *** $P < 0.001$.

Response Variable	T	2010	2011	2012	2013
Total biomass	L	+**	+**	0	+†
Total herbaceous biomass	L	0	0	0	0
Native herbaceous biomass	S	0	0	0	0
Non-native biomass	S	0	0	0	0
Litter biomass	N	--	0	0	0
Total species richness	N	0	0	0	-**
Native species richness	N	0	0	-†	-**
Diversity	N	0	0	-*	-†
Total cover	N	0	0	0	0
Non-native cover proportion	N	0	0	0	0
C3 cover proportion	N	0	0	0	0

The only other plant community response variables sensitive to experimental treatments at this site were related to biodiversity, and these responses were generally weak. Total species richness in 2010-11 was marginally lower than the control in the N100W treatment, and it significantly declined with increasing nitrogen in 2013 (Table 6), with the N84 treatment apparently driving this decline (P = 0.058). Native species appear to have driven the total species richness response, since native species richness was significantly lower than the control in the N100W treatment in 2010-11 (Figure 8b) and it declined with increasing nitrogen addition in 2012 (marginally) and in 2013 (Table 6), when both the N84 and N00 treatments were marginally lower than the control (Figure 8c). Finally, diversity, which combines species richness and evenness, responded idiosyncratically in 2010-11, with lower diversity in the N10 and N0W treatments compared to the control (Figure 8c). Diversity also declined with increasing nitrogen addition in 2012 and 2013 (marginally), with the N84 treatment having the strongest difference from the control (Figure 8e).

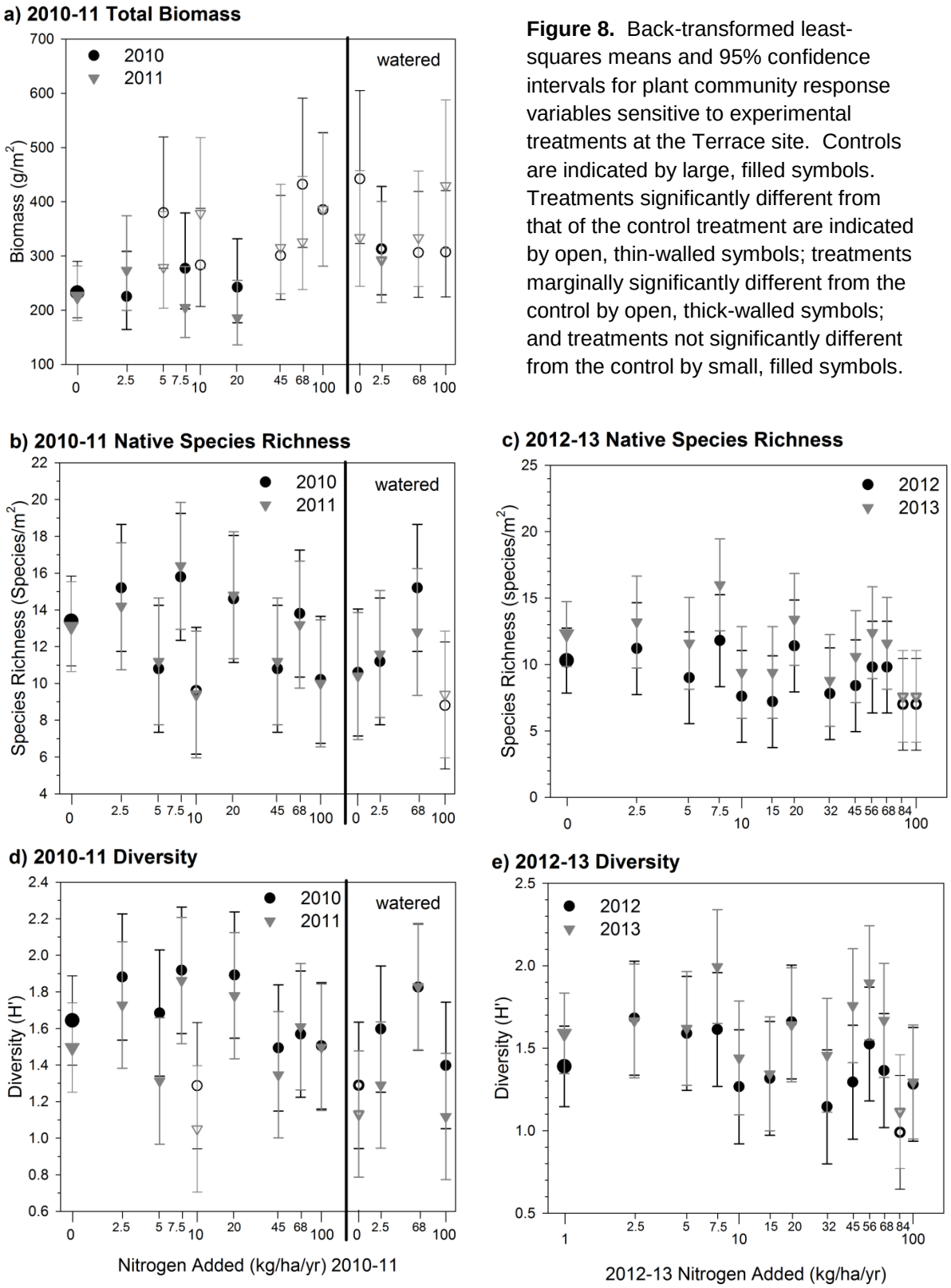
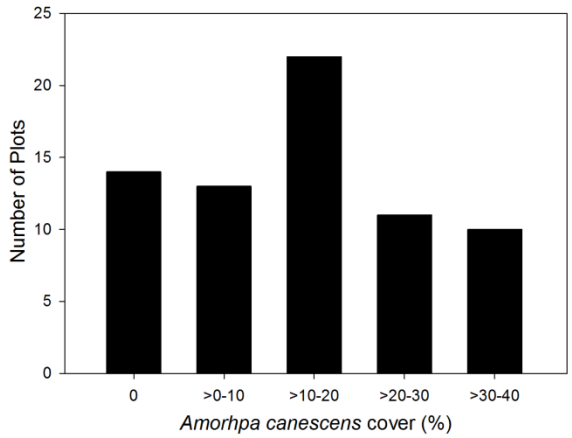


Figure 8. Back-transformed least-squares means and 95% confidence intervals for plant community response variables sensitive to experimental treatments at the Terrace site. Controls are indicated by large, filled symbols. Treatments significantly different from that of the control treatment are indicated by open, thin-walled symbols; treatments marginally significantly different from the control by open, thick-walled symbols; and treatments not significantly different from the control by small, filled symbols.

3.5 Hilltop Site

Amorpha canescens, a native, nitrogen-fixing shrub, contributed between 0 and 39% of the total plant cover in plots at the Hilltop site (Figure 9). Its cover did not vary significantly among years, nitrogen or water treatments, or combinations of these factors ($P \geq 0.11$).

Figure 9. Distribution of mean (over 2010-2013) *Amorpha canescens* cover in Hilltop site plots.



Despite this species' nitrogen-fixing capability, responses of soil inorganic nitrogen and tissue C:N to experimental treatments were not influenced by *Amorpha* cover. In 2010, mid-June soil inorganic nitrogen was marginally higher in the N45 and N100 treatments than in the control, and the N100W treatment was significantly higher than the control and the N100 treatment (Figure 10a). In 2011, this supplemental water enhancement on soil nitrogen did not occur, but mid-June soil nitrogen did increase with increasing nitrogen addition, driven by the significant effect of the N100 treatment in this year (Figure 10a). High nitrogen addition's effect lasted until the end of the 2011 growing season (Figure 10b), but as at other sites, there was no effect of nitrogen addition on mineralization rate ($P = 0.199$).

In the first half of the experiment tissue C:N of both cool-season grasses sampled (*Bromus japonicus* and *Pascopyrum smithii*) decreased significantly with increasing nitrogen addition ($P < 0.002$) and three of the four treatments sampled had lower tissue C:N than the controls in 2011; the N10 treatment did not (Figure 10c). *Andropogon gerardii* tissue C:N also declined with increasing nitrogen addition in 2011 ($P = 0.004$), but only the two higher sampled treatments (N45 and N100) differed at least marginally significantly from the control. In contrast to the other two sites, there were no significant differences between the sampled experimental treatments (N added ≤ 68 kg/ha/yr) and the controls for any of the species sampled at this site in 2013.

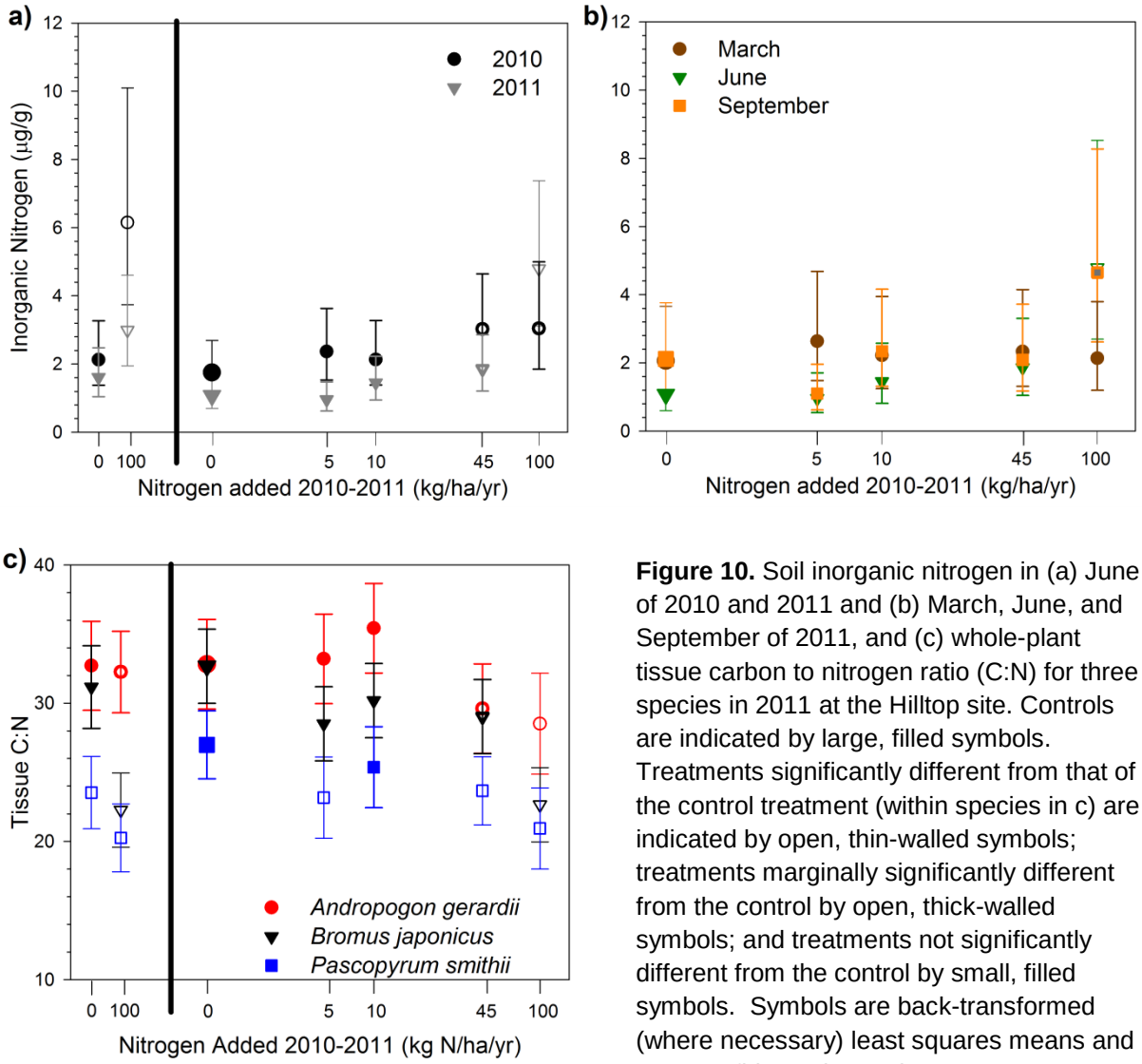


Figure 10. Soil inorganic nitrogen in (a) June of 2010 and 2011 and (b) March, June, and September of 2011, and (c) whole-plant tissue carbon to nitrogen ratio (C:N) for three species in 2011 at the Hilltop site. Controls are indicated by large, filled symbols. Treatments significantly different from that of the control treatment (within species in c) are indicated by open, thin-walled symbols; treatments marginally significantly different from the control by open, thick-walled symbols; and treatments not significantly different from the control by small, filled symbols. Symbols are back-transformed (where necessary) least squares means and 95% confidence intervals.

Experimental treatments, *Amorpha* cover, or their combination did not affect most plant community response variables at this site. Three response variables had significant, but highly idiosyncratic, responses: linear trends and the experimental treatments in which total and native herbaceous biomass differed from the control varied among years and *Amorpha* cover (Table 7, Appendix C), and in 2012-13 diversity was sometimes higher and sometimes lower than in the controls, depending on treatment and year (Appendix C; no effect of *Amorpha* cover). Non-native foliar cover showed a more consistent pattern. In the absence of *Amorpha*, non-native species comprised more of the plant cover in all nitrogen-only treatments with at least 20 kg N/ha/yr added than in the controls in 2010 and 2011, and in many of these treatments in 2013 (Figure 11). Treatments with even less added nitrogen showed significantly higher non-native cover than the control in 2011 and 2013 (Figure 11b,c). Annual brome grasses contributed to this non-native species response, particularly at low to

moderate levels of added nitrogen, but they were not as consistent in their response to added nitrogen as non-native species as a whole (Figure 11).

Table 7. Directions (positive, +; negative, -; none, 0) of linear relationships between transformed plant community response variables and experimentally added nitrogen for the Hilltop site. Relationships for 2010 and 2011 do not include treatments with supplemental water. Relationships are shown for a specific level of *Amorpha canescens* cover (0, 25, or 50, indicated in superscript) when *Amorpha* cover was a significant factor. Data transformation type (T): S = $\sqrt{(y+0.5)}$; L = ln(y+0.5); N = no transformation. Statistical significance indicated as $^{\dagger}0.05 < P \leq 0.10$; $^{*}0.01 < P \leq 0.05$; $^{**}0.01 < P \leq 0.001$; *** $P < 0.001$.

Response Variable	T	2010	2011	2012	2013
Total biomass	L	0	0	0	0
Total herbaceous biomass	L	0	$+^{*,0}$	$+^{\dagger,50}$	0
Native herbaceous biomass	S	0	$-^{*,25}$	0	0
Non-native biomass	S	0	0	0	0
Litter biomass	L	--	0	0	0
Total species richness	N	0	0	0	0
Native species richness	N	0	0	0	0
Diversity	N	0	0	0	$-^{\dagger}$
Total cover	N	0	0	0	0
Non-native cover proportion	N	0	$+^{*,0}$	0	$+^{*,50}$
Annual brome cover prop.	N	0	0	0	$+^{\dagger,50}$
Cool-season cover proportion	N	0	0	0	0

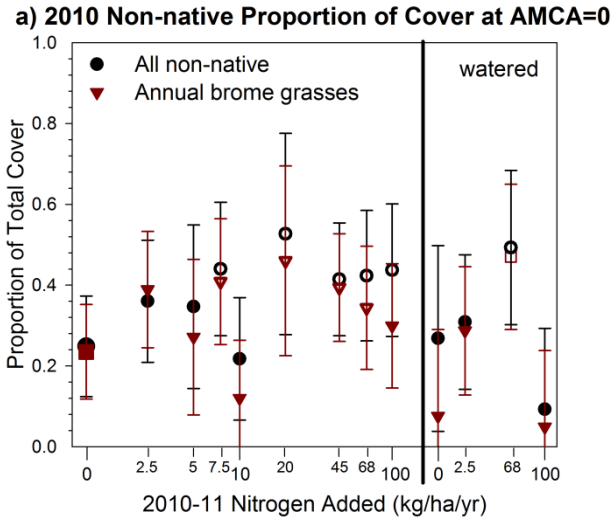
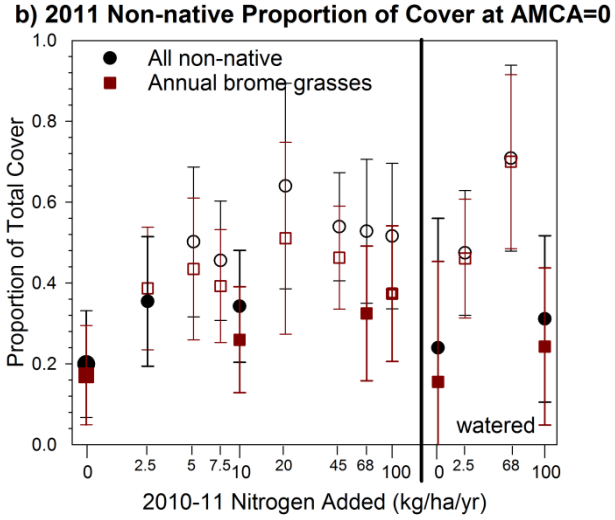
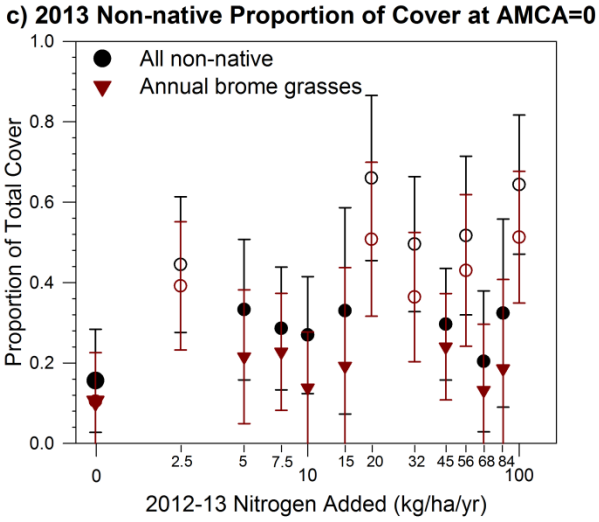


Figure 11. Back-transformed least squares means and 95% confidence intervals of all non-native (circles) and annual brome grass (squares) proportion of foliar cover adjusted to *Amorpha canescens* cover ("AMCA") = 0 in (a) 2010, (b) 2011, and (c) 2013 at the Terrace site. Treatments significantly different from that of the control treatment (within species in c) are indicated by open, thin-walled symbols; treatments marginally significantly different from the control by open, thick-walled symbols; and treatments not significantly different from the control by small, filled symbols. Symbols are back-transformed (where necessary) least squares means and 95% confidence intervals.





4. Discussion

One concern at the outset of this experiment was that, because of the expected co-limitation of water and nitrogen in the semi-arid grasslands of the NGP (Hooper and Johnson 1999; Van Riper *et al.* 2010), adding nitrogen alone would not produce a significant response in the variables we measured, especially under below-average precipitation conditions. Adding water alone did elicit a rare significant response (e.g., total biomass at the BSV and Terrace sites; Figures 5a and 8a), and both biomass and biodiversity were clearly negatively impacted by the 2012 drought (significant year effect in 2012-13, see Figures 6a,d and 8c,e). However, adding nitrogen alone did result in significant responses; supplemental water enhanced a nitrogen addition effect in only one instance (2010 soil nitrogen at the Hilltop site); and variation in responses to added nitrogen among years seem to be more related to the time since the initiation of the experiment than to variability in precipitation among years (Appendix D), which was considerable during the course of the study (Figure 3). Thus, although both water and nitrogen affect various characters of these plant communities, nitrogen deposition alone could significantly impact NGP grassland vegetation.

Nitrogen Critical Loads Derived from This Study

Determining how much nitrogen deposition is needed to elicit a significant change – the critical load – from our study is not as straightforward as hoped, however. In an ideal world, an experiment like ours would yield a monotonically increasing (or decreasing) dose-response curve and a clear threshold above which all doses produce a response significantly different from the untreated control. Few of our response variables behaved in this way. Given that we saw no clear turnover of species or functional groups as nitrogen addition increased (Appendix B), we suggest that the inconsistent responses reflect underlying variability in the environment that our level of experimental replication could not overcome. For example, the consistently low biomass in the N20 treatment at the BSV site throughout the experiment (Figures 5a,b and 6a,b) may be due to the fact that, within each block, the N20 plots tended to have low to moderate biomass at the beginning of the experiment. When resources allow, experiments like ours would be strengthened by multi-year measurements of response variables in each experimental plot *before* treatments are begun to establish a baseline for each plot; responses to the treatments compared to this baseline would provide a clearer picture of the treatment effects.

Despite these complications, our results do provide useful information for deriving nitrogen critical loads for NGP plant communities. We use two different approaches to determine these loads. In both of them, we follow the National Park Service's precautionary principle (National Park System Advisory Board Science Committee 2012) in that we derive critical loads that are likely to be conservative in their potential effects on the environment: we propose the lowest nitrogen level that the data suggest has a measureable effect on any of our response variables as the critical load for a site.

Our first approach is based on minimum significant treatment effects. In it, we recorded two nitrogen addition values for each site x response variable x year combination. A "Method 1m" (where "m" indicates minimum) nitrogen addition value is the minimum nitrogen addition treatment

for which the response variable differed at least marginally significantly ($P < 0.10$) from the control treatment in the given year. A "Method 1c" (where "c" indicates consistent) nitrogen addition value is the lowest nitrogen addition treatment that differed from the control and above which treatments above it also differed from the control in a given year. Then, for each response variable and site, we selected the lowest Method 1m and Method 1c nitrogen addition value from the four years of the experiment. To each of these, we added the total atmospheric nitrogen deposition at the site for the year of that minimum. We designated these sums as the Method 1m and Method 1c critical loads for each response variable and site. Appendix D provides the detailed results of this process. For each site, we selected the lowest Method 1m and Method 1c critical load from across the response variables as the site's Method 1m and Method 1c critical load. These are presented in Table 8.

Table 8. Inorganic nitrogen critical loads (kg N/ha/yr) and the response variable from which they are derived for three sites in two northern Great Plains national parks. For each site, up to three types of critical loads are presented. Method 1m and Method 1c critical loads are derived following a minimum significant treatment effect approach, where the Method 1m critical load is based on the *absolute minimum* experimental nitrogen treatment that produced a response different from the control, and the Method 1c critical load is based on the experimental nitrogen treatment above which higher nitrogen addition treatments *consistently* differed from the control. Method 2 critical loads are derived following a dose-response curve approach, in which the nitrogen dose expected to yield no significant effect is extrapolated from a curve fit to response variables with a consistent response to nitrogen addition.

Critical Load Type	BSV Site	Terrace Site	Hilltop Site
1m	5.9 litter biomass	9.9 total biomass	6.4 (at high *Amorpha* cover) total biomass
1c	21 non-native grass tissue carbon-to-nitrogen ratio	38 native grass tissue carbon-to-nitrogen ratio	9.5 (at low *Amorpha* cover) non-native proportion of cover
2	4.3 total biomass	7.8 native grass tissue carbon-to-nitrogen ratio	(not applicable)

Our second approach ("Method 2") is based on how the magnitude of differences between treatments and the control varies over a nitrogen addition gradient and is adapted from the dose-response curve method of Bowman and colleagues (2006; 2012). In this approach, for each site we selected the response variable x year combination(s) with the most consistent response to increasing nitrogen addition.[1] A fully consistent response is a monotonically increasing or decreasing response to an increasing dose. We calculated the relative magnitude of the effect of each nitrogen addition treatment for this response variable in the selected year, fit an appropriately shaped curve to a plot of the relative effect vs. nitrogen load (nitrogen addition treatment plus atmospheric deposition), then from this curve calculated the nitrogen load at which the relative effect was 0. We consider this nitrogen load the critical load for the site. Appendix D provides the details of this process, and the critical loads calculated from it are in Table 8.

[1] For this approach, we considered not only the response variables in Table 1 and discussed throughout the text, but we also inspected dose response curves of individual species' covers for each site. Only one species-level response was consistent (2010 and 2011 *Elymus trachycaulus* cover at the BSV site), and it yielded a critical load similar to that calculated from a community response variable at this site, so we do not show it here.

Caveats to Estimated Critical Loads

Interpretation of the critical loads presented in Table 8 must be done with a few caveats in mind. First, both of our approaches for deriving critical loads use the control treatments as the baseline. However, the controls, along with all the other treatments, received nitrogen throughout the study in the form of atmospheric deposition, which ranged from 3.4 to 5.9 kg N/ha/yr across the two parks during the course of the study (data from National Atmospheric Deposition Program 2014). In contrast, nitrogen deposition in 1860, before industrial nitrogen fixation began, in our study area has been estimated as 1.0-2.5 kg/ha/yr (Galloway *et al.* 2004). Anthropogenic nitrogen deposition may already have had effects on the grasslands of the NGP, but our study would not be able to detect these. Furthermore, atmospheric deposition is not the only anthropogenic source of nitrogen addition to grasslands in NGP parks. The invasive *Melilotus officinalis* (yellow sweetclover) is a widespread biennial legume that dominates NGP grasslands in some years (e.g., Von Loh *et al.* 1999; Ollila 2014). It has been estimated to fix 40 kg N/ha/yr, and add 12 kg N/ha/yr to Great Plains soils, in non-crop settings (Lesica and DeLuca 2000). Although sweetclover did not dominate in any of our sites during this study, it was present at all sites and was abundant in the year following the study's conclusion, suggesting the potential for it to have been abundant at the sites in years before the study occurred. Nitrogen added to the soil from earlier sweetclover outbreaks may have had lasting effects on the plant communities we measured. In short, we cannot know if the critical loads derived in this study would be applicable in a "pristine" NGP grassland.

Second, Method 1 critical load values are constrained by the intervals between our nitrogen addition treatments. The actual critical load may lie between the critical load we state and the nitrogen load represented by the next lowest nitrogen addition treatment. This caveat is especially relevant to the higher, Method 1c critical loads, which were in the range of nitrogen addition treatments where between-treatment intervals were larger.

Third, the critical load is defined as a level "below which significant harmful effects on sensitive elements of the environment do not occur" (Nilsson and Grennfelt 1988). In our study, significant effects on many response variables often occurred at a low nitrogen addition level, but not at some nitrogen addition levels above that, yielding different Method 1m and Method 1c critical loads for each response variable. Accepting the Method 1m values, rather than the Method 1c values, as the true critical loads assumes that non-significant effects of nitrogen addition treatments between the two values are the idiosyncrasies in a trend rather than the Method 1m nitrogen addition value being the outlier. This may be the preferred approach when adhering to the NPS precautionary principle to "err on the side of protection" (Williams and Tonnessen 2000), but the assumption must be acknowledged when applying the critical loads based on it.

Fourth, critical load is defined in terms of "harmful effects". We measured a wide range of response variables in order to search for the most "sensitive element of the environment", but significant effects on all of these elements may not be considered *harmful*. For example, increased biomass production or concentration of nitrogen in native plant tissues (the basis for many critical loads in Table 8) could be considered beneficial, in that they represent more and higher quality forage for herbivorous wildlife. On the other hand, greater plant biomass production in ecosystems

characterized by their low production, such as in badlands, could alter rates or processes fundamental to those systems, such as high erosion rates and low fire frequency. Moreover, an early biomass response that appears to be beneficial may lead to undesired effects in the longer term, such as greater litter loads hampering germination or growth of desired species (Suttle *et al.* 2007). Thus, the precautionary approach would suggest that any effect driven by nitrogen addition should be considered potentially harmful.

This leads to the final caveat. The length of this study is too short to understand the long-term effects of chronic elevated nitrogen deposition on NGP grasslands. Work in other regions have suggested or demonstrated that chronic low-level deposition can have effects as great as the short-term effects of high-level deposition (Clark and Tilman 2008; Bobbink *et al.* 2010). Some of our results suggest that annual doses of nitrogen produced cumulative effects even within the four years of our study: at the BSV site, significant effects of nitrogen addition on biomass production and plant tissue C:N appeared at lower nitrogen addition levels in the latter half of the study. Thus, we suggest that the unknown magnitude of the cumulative effects of long-term low-level anthropogenic nitrogen deposition is another reason that following the precautionary principle, which yields relatively low critical loads, is warranted.

Implications of Estimated Critical Loads

All of the precautionary Method 1m and Method 2 critical loads we derived from our results, as well as one of the less precautionary Method 1c critical loads, fall below the 10-25 kg N/ha/yr critical load estimated by Clark et al. (2011; 10-25 kg N/ha/yr) for the Great Plains. Instead, these critical loads are similar to critical loads reported for other semi-arid or arid ecoregions of North America. For example, Pardo et al. (2011) reported critical loads of 6 and 3-8.4 kg N/ha/yr for annual grass invasion in California serpentine grassland and vegetation response in North American Desert, respectively.

Moreover, the precautionary critical loads at the BSV site are near total nitrogen deposition values already occurring at that site and at the two other NGP parks with badlands vegetation, Theodore Roosevelt National Park and Scotts Bluff National Monument (Figure 12). Our results suggest that this vegetation, a low-productivity plant community that hosts species rare in more fertile conditions, is more sensitive to nitrogen addition than are the other two types of vegetation in which we worked. This sensitivity is reflected not only in lower critical loads for the similar response variables, but also in more response variables being significantly impacted than in other sites.

Although the Hilltop site is on the opposite end of the productivity spectrum among our study sites (Table 4), the critical loads suggested by our results for this site are not much different from those for the BSV site. One of these critical loads was for the non-native component of the vegetation, a component shown to be sensitive to nitrogen deposition in other portions of the world (Fenn *et al.* 2010; Rao *et al.* 2010; Sharma *et al.* 2010). In our study, the Hilltop site was the only one of the three sites in which non-native species abundance was sensitive to nitrogen addition, even though they comprised a greater proportion of the control vegetation at the Terrace site. This may be because the non-native species component at the Hilltop site was dominated by the annual grasses *Bromus japonicus* (Japanese brome) and *B. tectorum* (downy brome or cheatgrass), at least one of

which is capable of quickly turning available nitrogen into biomass (Evans *et al.* 2001). The response of the non-native component at the Hilltop site was evident only in the absence of *Amorpha*, the nitrogen-fixing native shrub. Although leguminous (often nitrogen-fixing) species are a major portion of the diversity the mixed-grass prairie flora, they are seldom as abundant as at our Hilltop site. Also, *B. tectorum* and *B. japonicus* occur in nearly all NGP parks, and they are abundant in portions of not only WICA and BADL, but also at Scotts Bluff and Devils Tower National Monuments and Fort Laramie National Historic Site (Ashton and Prowatzke 2013a, b, 2014a, b, c, d). Together, these conditions suggest that the critical load relevant to non-native species at the Hilltop site may be applicable to other parks in the region.

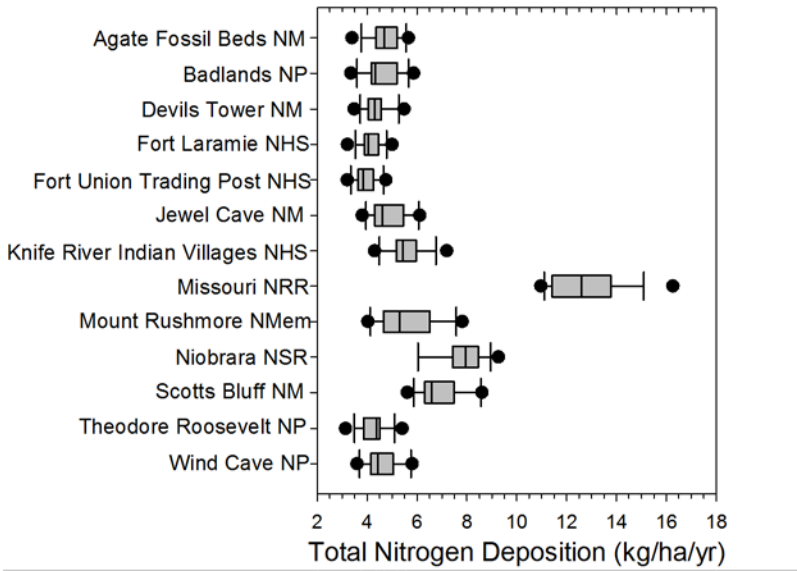


Figure 12. Box-and-whisker plots of total (wet + dry) annual atmospheric nitrogen deposition at parks in the NGP. For each park, annual deposition values from 2000 to 2013 are characterized by their full range (circles), 10th to 90th percentiles (whiskers), 25th to 75th percentiles (box), and median (line within box). Calculated from data from the National Atmospheric Deposition Program (2014).

Although the results from this study suggest that atmospheric nitrogen deposition could already be impacting more sensitive NGP vegetation types, or that only small increases in deposition could have broader impacts, detecting nitrogen-induced changes in NGP vegetation over time or through space will not be easy. This is because the ecosystem characteristics that respond to nitrogen addition also respond to grazing, fire, soil disturbance, and fluctuations in weather (especially precipitation), factors that are generally inescapable in the NGP. The large confidence intervals around our estimates of treatment means, the idiosyncratic responses along a nitrogen gradient, differences in treatment effects among years, and differences in the results between this and another study at BADL (Van Riper and Larson 2009; Van Riper *et al.* 2010) highlight the high natural variability of the response variables that we measured, all of which are characteristics commonly considered for establishing critical loads in herbaceous-dominated ecosystems (Pardo *et al.* 2011c).

5. Conclusions

Critical loads for atmospheric pollutants can be derived with a variety of methods, including steady-state mass balance modeling, dynamic models, and empirical approaches that use gradient studies, field experiments, or observations from long-term studies (Pardo *et al.* 2011a). The empirical approach provides critical loads based on measured ecosystem responses to nitrogen inputs, but experiments designed specifically to investigate the effects of nitrogen inputs at levels commensurate with atmospheric deposition are rare. We provide nitrogen critical loads for three grassland vegetation types important in NGP NPS units that are derived from an experiment using nitrogen inputs ranging from those commensurate with the current, relatively low, ambient deposition rates in the western portion of the NGP to an order of magnitude larger than deposition expected into the middle of the 21st century (Ellis *et al.* 2013).

Our results suggest that the low-productivity, low-cover badlands sparse vegetation at the base of the badlands features that give Badlands and Theodore Roosevelt National Parks their unique scenery could become less sparse, and therefore potentially less hospitable to plant species requiring this type of habitat, under current or only slightly increased atmospheric nitrogen deposition. Our results also suggest that, if deposition rates in the western portion of the NGP were to increase to those already occurring in the easternmost parks of the NGP, annual brome grasses and other opportunistic invaders may be promoted. One disadvantage of the short-term experimental method we used to derive potential critical loads is that it cannot establish the effects of chronic, low-level deposition. Cumulative effects have been seen in a multi-decadal study in a different grassland (Clark and Tilman 2008), and our study showed some evidence of such cumulative effects in the most sensitive of the three vegetation types investigated.

Scientifically based nitrogen critical loads for sensitive ecosystems or ecosystem components aid the NPS and other land management agencies in their consultative role in the air pollution regulatory process (Porter *et al.* 2005). Understanding the impacts that anthropogenic nitrogen inputs have on specific ecosystems may help land managers adjust their management actions to alleviate the impacts of atmospheric nitrogen deposition. Controlling invasive species, both those that contribute nitrogen to ecosystems (i.e., sweetclover) and those that seem to benefit from higher nitrogen (possibly annual brome grasses), is one such action. Further research investigating the effects of fire, grazing, and nitrogen deposition could provide guidance for other ameliorating management actions.

Literature Cited

Ashton, I. W., and M. Prowatzke. 2013a. Plant community composition and structure monitoring for Fort Laramie National Historical Site: 2013 annual report. Natural Resource Technical Report NPS/NGPN/NRDS--2013/585. National Park Service, Fort Collins, Colorado.

Ashton, I. W., and M. Prowatzke. 2013b. Plant community composition and structure monitoring for Scotts Bluff National Monument: 2013 annual report. Natural Resource Technical Report NPS/NGPN/NRDS--2013/570. National Park Service, Fort Collins, Colorado.

Ashton, I. W., and M. Prowatzke. 2014a. Plant community composition and structure monitoring for Agate Fossil Beds National Monument: 2013 annual report. Natural Resource Technical Report NPS/NGPN/NRDS--2014/602. National Park Service, Fort Collins, Colorado.

Ashton, I. W., and M. Prowatzke. 2014b. Plant community composition and structure monitoring for Badlands National Park: 2013 annual report. Natural Resource Technical Report NPS/NGPN/NRDS--2014/601. National Park Service, Fort Collins, Colorado.

Ashton, I. W., and M. Prowatzke. 2014c. Plant community composition and structure monitoring for Devils Tower National Monument: 2013 annual report. Natural Resource Technical Report NPS/NGPN/NRDS--2014/612. National Park Service, Fort Collins, Colorado.

Ashton, I. W., and M. Prowatzke. 2014d. Plant community composition and structure monitoring for Wind Cave National Park: 2013 annual report. Natural Resource Technical Report NPS/NGPN/NRDS--2014/613. National Park Service, Fort Collins, Colorado.

Binkley, D., and S. C. Hart. 1989. The components of nitrogen availability assessments in forest soils. *Advances in Soil Science* 10:57-112.

Blett, T., J. A. Lynch, L. H. Pardo, C. Huber, R. Haeuber, and R. Pouyat. 2014. FOCUS: A pilot study for national-scale critical loads development in the United States. *Environmental Science & Policy* 38:224-236.

Bobbink, R., K. Hicks, J. Galloway, T. Spranger, R. Alkemade, M. Ashmore, M. Bustamante, S. Cinderby, E. Davidson, F. Dentener, *et al.* 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications* 20:30-59.

Bowman, W. D., J. R. Gartner, K. Holland, and M. Wiedermann. 2006. Nitrogen critical loads for alpine vegetation and terrestrial ecosystem response: Are we there yet? *Ecological Applications* 16:1183-1193.

Bowman, W. D., J. Murgel, T. Blett, and E. Porter. 2012. Nitrogen crticial loads for alpine vegetation and soils in Rocky Mountain National Park. *Journal of Environmental Management* 103:165-171.

Burns, D. A., T. Blett, R. Haeuber, and L. Pardo. 2008. Critical loads as a policy tool for protecting ecosystems from the effects of air pollutants. *Frontiers in Ecology and the Environment* 6:156-159.

Clark, C. M. 2011. Great Plains. Pages 117-132 *in* L. H. Pardo, M. J. Robin-Abbott and C. T. Driscoll, editors. Assessment of nitrogen deposition effects and empirical critical loads of nitrogen for ecoregions of the United States. General Technical Report NRS-80. USDA Forest Service, Northern Research Station, Newtown Square, Pennsylvania.

Clark, C. M., and D. Tilman. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* 451:712-715.

Ellis, R. A., D. J. Jacob, M. P. Sulprizio, L. Zhang, C. D. Holmes, B. A. Schichtel, T. Blett, E. Porter, L. H. Pardo, and J. A. Lynch. 2013. Present and future nitrogen deposition to national parks in the United States: critical load exceedances. *Atmospheric Chemistry and Physics* 13:9083-9095.

Evans, R. D., R. Rimer, L. Sperry, and J. Belnap. 2001. Exotic plant invasion alters nitrogen dynamics in an arid grassland. *Ecological Applications* 11:1301-1310.

Fenn, M. E., E. B. Allen, S. B. Weis, S. Jovan, L. H. Geiser, G. S. Tonnesen, R. F. Johnson, L. E. Rao, B. S. Gimeno, F. Yuan, *et al.* 2010. Nitrogen critical loads and management alternatives for N-impacted ecosystems in California. *Journal of Environmental Management* 91:2404-2423.

Fenn, M. E., J. S. Baron, E. B. Allen, H. M. Rueth, K. R. Nydick, L. Geiser, W. D. Bowman, J. O. Sickman, T. Meixner, D. W. Johnson, *et al.* 2003. Ecological effects of nitrogen deposition in the western United States. *Bioscience* 53:404-420.

Fenn, M. E., K. F. Lambert, T. F. Blett, D. A. Burns, L. H. Pardo, G. M. Lovett, R. A. Haeuber, D. C. Evers, C. T. Driscoll, and D. S. Jeffries. 2011. Setting limits: Using air pollution thresholds to protect and restore U.S. ecosystems. Issues in Ecology 14. Ecological Society of America, Washington, DC.

Galloway, J. N., F. J. Dentener, D. G. Capone, E. W. Boyer, R. W. Howarth, S. P. Seitzinger, G. P. Asner, C. C. Cleveland, P. A. Green, E. A. Holland, *et al.* 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry* 70:153-226.

Galloway, J. N., A. R. Townsend, J.-W. Erisman, M. Bekunda, Z. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger, and M. A. Sutton. 2008. Transformation of the nitrogen cycle: recent trends, questions and potential solutions. *Science* 320:889-892.

Hand, J. L., K. A. Gebhart, B. A. Schichtel, and W. C. Malm. 2012. Increasing trends in wintertime particulate sulfate and nitrate ion concentrations in the Great Plains of the United States (2000-2010). *Atmospheric Environment* 55:107-110.

Hooper, D. U., and L. Johnson. 1999. Nitrogen limitation in dryland ecosystems: Responses to geographical and temporal variation in precipitation. *Biogeochemistry* 46:247-293.

Jacobsen, J. S., S. H. Lorbeer, H. A. R. Houlton, and G. R. Carlson. 1996. Nitrogen fertilization of dryland grasses in the Northern Great Plains. *Journal of Range Management* 49:340-345.

Kirk, R. E. 1982. Experimental design: Procedures for the behavioral sciences. 2nd ed. Wadsworth Inc., Belmont, California.

Kochy, M., and S. D. Wilson. 2001. Nitrogen deposition and forest expansion in the northern Great Plains. *Journal of Ecology* 89:807-817.

Lauenroth, W. K., J. L. Dodd, and P. L. Sims. 1978. The effects of water- and nitrogen-induced stresses on plant community structure in a semiarid grassland. *Oecologia* 36:211-222.

Lesica, P. L., and T. H. DeLuca. 2000. Sweetclover: a potential problem for the northern Great Plains. *Journal of Soil and Water Conservation* 55:259-261.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for Mixed Models. 2nd ed. SAS Institute Inc., Cary, NC.

Lorenz, R. J., and G. A. Rogler. 1973. Growth rate of mixed prairie in response to nitrogen and phosphorus fertilization. *Journal of Range Management* 26:365-368.

Magurran, A. E. 2004. Measuring biological diversity. Blackwell Publishing, Oxford.

McCune, B., and M. J. Mefford. 2011. PC-ORD. Multivariate Analysis of Ecological Data, Version 6.0. Gleneden Beach, Oregon: MJM Software Design.

Milliken, G. A., and D. E. Johnson. 2002. Analysis of messy data, volume III: Analysis of covariance. Chapman & Hall/CRC, Boca Raton, Florida.

National Atmospheric Deposition Program (NADP). 2014. Total Deposition Maps, version 2014v02. NADP Total deposition map website. Available at: http://nadp.sws.uiuc.edu/committees/tdep/tdepmaps/ (accessed 15 January 2015).

National Park Service Air Resources Division. 2002. Air Quality in the National Parks, Second Edition. U.S. Department of the Interior, Washington, DC.

National Park System Advisory Board Science Committee. 2012. Revisiting Leopold: Resource stewardship in the national parks. Washington, DC.

Nilsson, J., and P. Grennfelt. 1988. Critical levels for sulphur and nitrogen. Nordic Council of Ministers, Copenhagen, Denmark.

[NRCS] U.S. Department of Agriculture Natural Resources Conservation Service. 2010. NRCS Web Soil Survey website. Available at:

http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx (accessed 30 September 2010).

Ollila, D. 2014. Develop a Grazing Plan for a "Sweet Clover Year". iGrow. Brookings, SD: South Dakota State University Extension. Available at: http://igrow.org/livestock/sheep/develop-a-grazing-plan-for-a-sweet-clover-year/ (accessed 30 June 2014).

Pardo, L. H., M. E. Fenn, C. L. Goodale, L. H. Geiser, C. T. Driscoll, E. B. Allen, J. S. Baron, R. Bobbink, W. D. Bowman, C. M. Clark, *et al.* 2011a. Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. *Ecological Applications* 21:3049-3082.

Pardo, L. H., E. A. Lilleskov, L. H. Geiser, and M. J. Robin-Abbott. 2011b. Methods. Pages 25-35 *in* L. H. Pardo, M. J. Robin-Abbott and C. T. Driscoll, editors. Assessment of nitrogen deposition effects and empirical critical loads of nitrogen for ecoregions of the United States. General Technical Report NRS-80. USDA Forest Service, Northern Research Station, Newtown Square, Pennsylvania.

Pardo, L. H., M. J. Robin-Abbott, and C. T. Driscoll. 2011c. Assessment of nitrogen deposition effects and empirical critical loads of nitrogen deposition effects and empirical critical loads of nitrogen for ecoregions of the United States. General Technical Report NRS-80. USDA Forest Service, Northern Research Station, Newtwon Square, Pennsylvania.

Peck, J. E. 2010. Multivariate Analysis for Community Ecologists: Step-by-Step using *PC-ORD*. MjM Software Design, Gleneden Beach, Oregon.

Piper, J. K., D. N. Billings, and V. J. Leite. 2005. Effects of nitrogen fertilizer on the composition of two prairie plant communities. *Community Ecology* 6:93-100.

Porter, E., T. Blett, D. U. Potter, and C. Huber. 2005. Protecting resources on federal lands: Implications of critical loads for atmospheric deposition of nitrogen and sulfur. *BioScience* 55:603-612.

Rao, L. E., E. B. Allen, and T. Meixner. 2010. Risk-based determination of critical nitrogen deposition loads for fire spread in southern California deserts. *Ecological Applications* 20:1320-1335.

Rauzi, F., and M. L. Fairbourn. 1983. Effects of annual applications of low N fertilizer rates on a mixed grass prairie. *Journal of Range Management* 36:359-362.

Samuel, M. J., and R. H. Hart. 1998. Nitrogen fertilization, botanical composition and biomass production on mixed-grass rangeland. *Journal of Range Management* 51:408-416.

SAS Institute Inc. 2012a. SAS/IML 12.1 User's Guide. SAS Institute Inc., Cary, North Carolina.

SAS Institute Inc. 2012b. SAS/STAT 12.1 User's Guide. SAS Institute Inc., Cary, North Carolina.

Schwinning, S., B. I. Starr, N. J. Wojcik, M. E. Miller, J. E. Ehleringer, and R. L. Sanford. 2005. Effects of nitrogen deposition on an arid grassland in the Colorado Plateau cold desert. *Rangeland Ecology & Management* 58:565-574.

Sharma, G. P., S. A. Muhl, K. J. Esler, and S. J. Milton. 2010. Competitive interactions between the alien invasive annual grass *Avena fatua* and indigenous herbaceous plants in South African Renosterveld: the role of nitrogen enrichment. *Biological Invasions* 12:3371-3378.

Smith, A. 2012. Ecological consequences of increased nitrogen deposition in three northern Great Plains grasslands Master's. Colorado State University, Fort Collins, Colorado.

Suding, K. N., S. L. Collins, L. Gough, C. Clark, E. E. Cleland, K. L. Gross, D. G. Milchunas, and S. Pennings. 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences (USA)* 102:4387-4392.

Suttle, K. B., M. A. Thomsen, and M. E. Power. 2007. Species interactions reverse grassland responses to changing climate. *Science* 315:640-642.

Tilman, D. 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. *Ecological Monographs* 57:189-214.

Van Riper, L. C., and D. L. Larson. 2009. Role of invasive *Melilotus officinalis* in two native plant communities. *Plant Ecology* 200:129-139.

Van Riper, L. C., D. L. Larson, and J. L. Larson. 2010. Nitrogen-limitation and invasive sweetclover impacts vary between two Great Plains plant communities. *Biological Invasions* 12:2735-2749.

Von Loh, J., D. Cogan, D. Faber-Langendoen, D. Crawford, and M. Pucherelli. 1999. USGS-NPS Vegetation Mapping Program, Badlands National Park, South Dakota. Technical Memorandum 8260-00-02. U.S. Bureau of Reclamation Technical Service Center, Denver, Colorado.

Williams, M. W., and K. A. Tonnessen. 2000. Critical loads for inorganic nitrogen deposition in the Colorado Front Range, USA. *Ecological Applications* 10:1648-1665.

Appendix A: Plant tissue samples

Table A1. Number of plant tissue samples used in statistical analysis by species, site, and treatment in 2011.

Site	Treatment	*Andropogon gerardii*	*Bouteloua curtipendula*	*Bromus japonicus*	*Elymus trachycaulus*	*Pascopyrum smithii*	*Poa pratensis*
BSV	N0		4	3	4		
	N0W		5	5	4		
	N5		5	4	5		
	N10		3	3	5		
	N45		5	2	4		
	N100		4	3	4		
	N100W		5	5	4		
Hilltop	N0	4		5		4	
	N0W	4		4		3	
	N5	4		5		2	
	N10	4		5		2	
	N45	4		5		4	
	N100	3		5		2	
	N100W	5		5		4	
Terrace	N0		4			4	4
	N0W		2			4	4
	N5		4			5	4
	N10		2			4	4
	N45		3			5	4
	N100		2			5	4
	N100W		0			5	4

Table A2. Number of plant tissue samples used in statistical analysis by species, site, and treatment in 2013.

Site	Treatment	*Andropogon gerardii*	*Bouteloua curtipendula*	*Bouteloua gracilis*	*Bromus japonicus*	*Elymus trachycaulus*	*Hesperostipa comata*	*Pascopyrum smithii*	*Poa pratensis*
BSV	N0			9	10	9			
	N2.5			5	4	3			
	N5			4	4	5			
	N7.5			4	5	4			
	N10			5	5	5			
	N15			5	4	4			
	N20			3	3	4			
	N32			4	3	4			
	N45			5	5	4			
	N56			4	4	4			
	N68			3	4	3			
Hilltop	N0	9			10		3		
	N2.5	5			5		3		
	N5	4			5		2		
	N7.5	4			5		4		
	N10	4			4		2		
	N15	4			4		2		
	N20	5			5		3		
	N32	5			4		3		
	N45	4			5		2		
	N56	4			4		4		
	N68	4			4		4		
Terrace	N0		7					9	6
	N2.5		5					4	5
	N5		4					5	4
	N7.5		4					4	2
	N10		0					5	4
	N15		3					2	4
	N20		5					4	2
	N32		3					5	4
	N45		3					5	3
	N56		3					4	3
	N68		3					4	4

Appendix B: Community Composition Results from Nonmetric Multidimensional Scaling Ordination

Ordination orders objects that are characterized by their values of multiple variables. In plant ecology, ordination is often used to look for internal (not related to a hypothesized gradient) patterns in community composition. We used the PC-ORD software to conduct a type of ordination called nonmetric multidimensional scaling (NMS) to look for patterns of plant community composition among nitrogen addition treatments and across years of the experiment within each of the three experimental sites. The objects of the ordinations were plots in individual years, and the values by which they were characterized were the canopy cover of individual plant species. Table B1 shows the number of dimensions (axes that convey unique information) selected by the software for the best fit to the data, the final stress of the solution (a measure of how well the solution fits the data), and the probability that a similar final stress could have been obtained by chance for each site.

Table B1. Number of dimensions in the final solution, final stress, and probability (*P*) that a similar final stress could have been obtained by chance (based on Monte Carlo test, 249 runs with randomized data) for each ordination of absolute species cover by site.

Site	Dimensions	Final Stress	*P*
BSV	3	16.54	0.004
Terrace	3	14.71	0.004
Hilltop	4	14.01	0.012

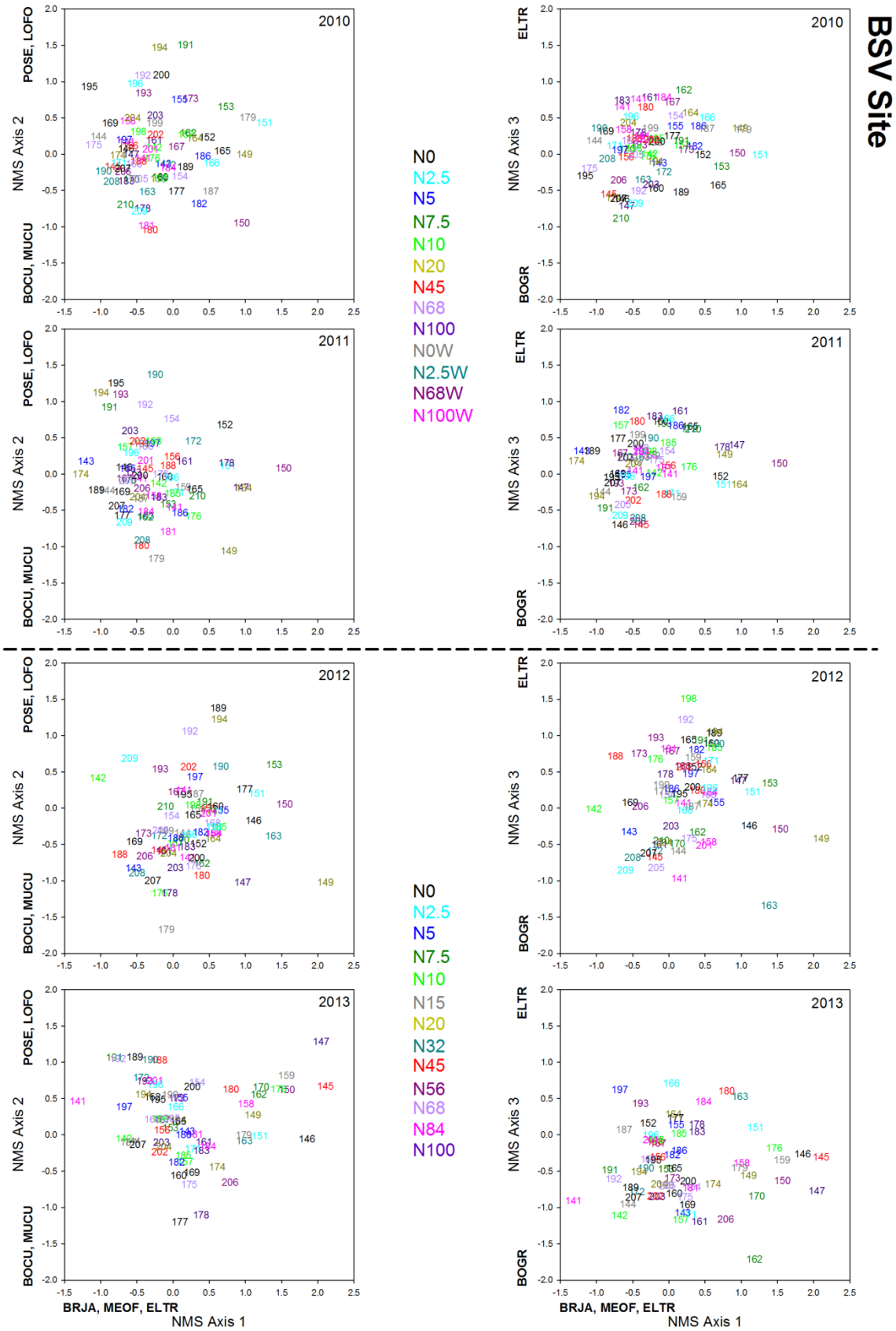
The following pages show the location of each experimental plot in the ordination, by year. Plot symbols are their plot numbers (used for identification in the field) color-coded by treatment as indicated for the first (above dashed line on each page) and second (below dashed line) half of the experiment (Table 2 in main text). Individual plots' colors are consistent across all four years, but the treatment that those colors indicate change between the first and second half of the experiment for plots that received water in 2010-11 (treatments ending in W). Two diagrams are shown for each year because the solution included more than two dimensions (note different axis labels in left and right columns on each page). On each axis, codes for the species most strongly related to each end of the axis are given (species codes are defined in Table B2). Thus, a plot in the bottom left of a diagram has a high amount of the species indicated by the codes on the bottom and left ends of the axes. If experimental treatment had a strong, consistent effect on plant community composition, plot numbers of the same color would cluster together in each diagram for a given site. If a plot's plant community composition were stable throughout the experiment, a plot's symbol would occur in the same location on each of the diagrams within a column on a page.

Examination of the diagrams on the following pages shows that plot numbers of the same color rarely cluster together in any of the diagrams, indicating no strong effect of experimental treatment on composition. Some plots exhibit stability of composition among the four years of the study (e.g., plot 130 at the Hilltop site remains in the lower right quarter of all diagrams for that site), but many do not, indicating substantial variation in community composition from year to year, especially between 2011 and 2012. We assume the dry conditions of 2012 played an important role in

composition shifts from 2011 to 2012, since all treatments, not just the 2010-11 “W” treatments, exhibited these shifts.

Table B2. Four-letter codes and full names of species most strongly related to ordination axes. Nomenclature follows ITIS (http://www.itis.gov/, accessed 6 February 2015).

Code	Scientific Name	Common Name
AMCA	*Amorpha canescens*	leadplant
ANGE	*Andropogon gerardii*	big bluestem
BOCU	*Bouteloua curtipendula*	sideoats grama
BOGR	*Bouteloua gracilis*	blue grama
BRJA	*Bromus japonicus*	Japanese brome
BRTE	*Bromus tectorum*	cheatgrass, downy brome
CAFI	*Carex filifolia*	threadleaf sedge
ECAN	*Echinacea angustifolia*	blacksamson
ELTR	*Elymus trachycaulus*	slender wheatgrass
HECO	*Hesperostipa comata*	needle-and-thread
LOFO	*Lomatium foeniculaceum*	desert biscuitroot
MEOF	*Melilotus officinalis*	yellow sweetclover
MUCU	*Muhlenbergia cuspidata*	plains muhly
NAVI	*Nassella viridula*	green needlegrass
PASM	*Pascopyrum smithii*	western wheatgrass
POPR	*Poa pratensis*	Kentucky bluegrass
POSE	*Poa secunda*	Sandberg bluegrass
SCSC	*Schizachyrium scoparium*	little bluestem
YUGL	*Yucca glauca*	soapweed yucca


BSV Site
2010
2011
2012
2013
POSE, LOFO
BOCU, MUCU
ELTR
BOGR
NMS Axis 2
NMS Axis 3
BRJA, MEOF, ELTR
NMS Axis 1
N0
N2.5
N5
N7.5
N10
N20
N45
N68
N100
N0W
N2.5W
N68W
N100W
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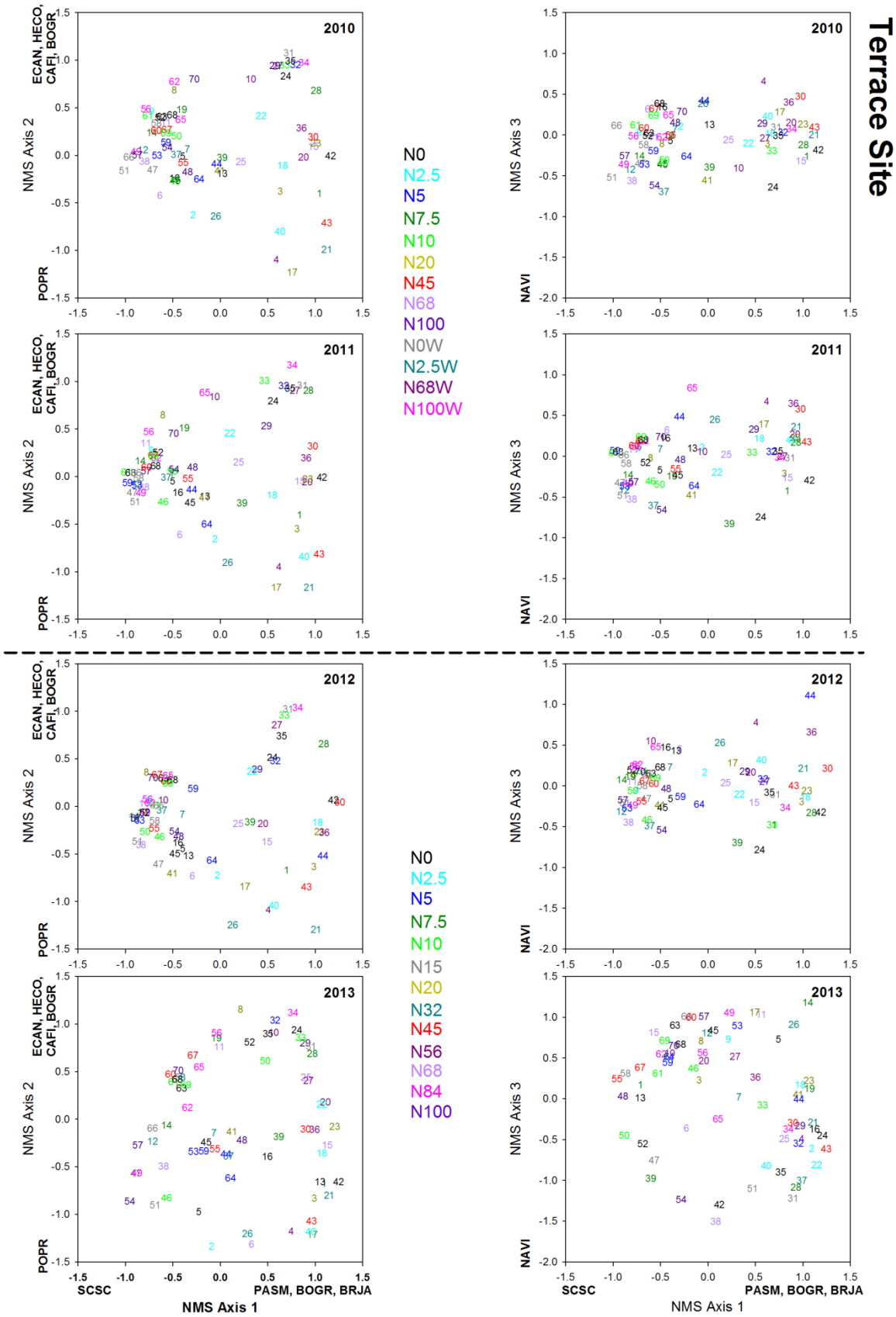
N45
N56
N68
N84
N100


Terrace Site
2010
2011
2012
2013
NMS Axis 2
NMS Axis 3
NMS Axis 1
ECAN, HECO, CAFI, BOGR
POPR
NAVI
SCSC
PASM, BOGR, BRJA
N0
N2.5
N5
N7.5
N10
N20
N45
N68
N100
N0W
N2.5W
N68W
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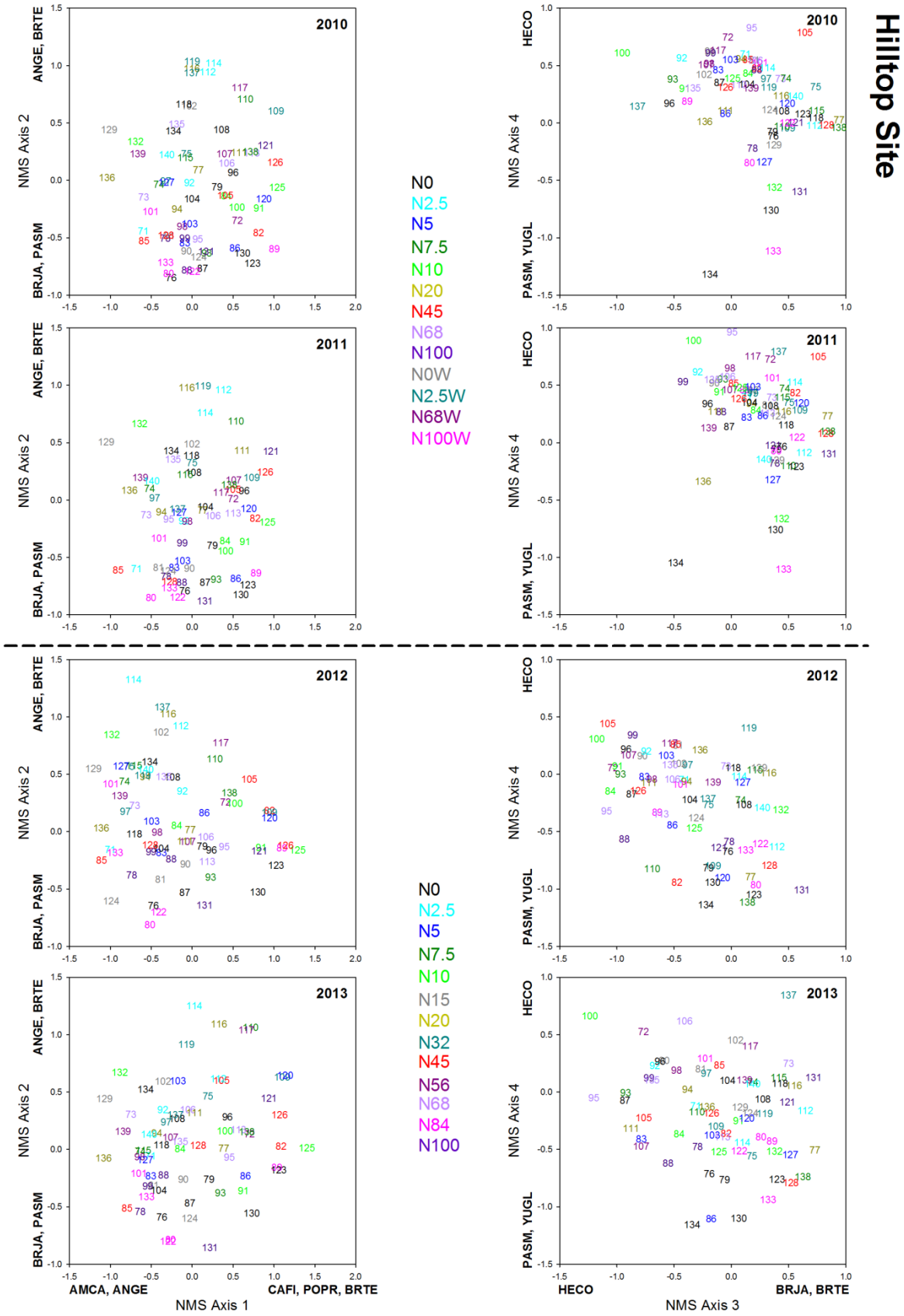
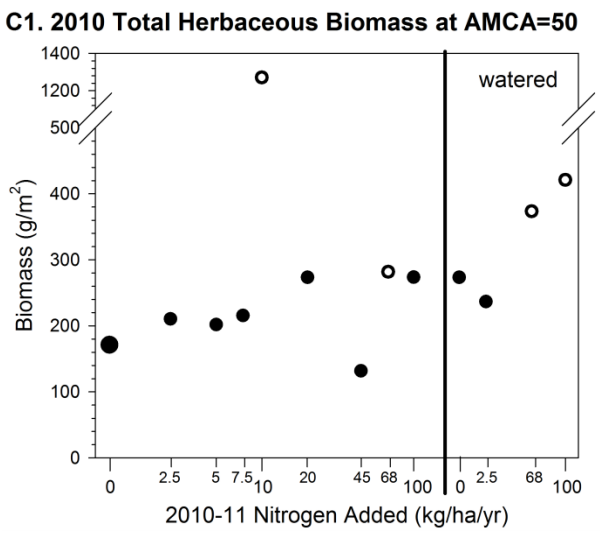
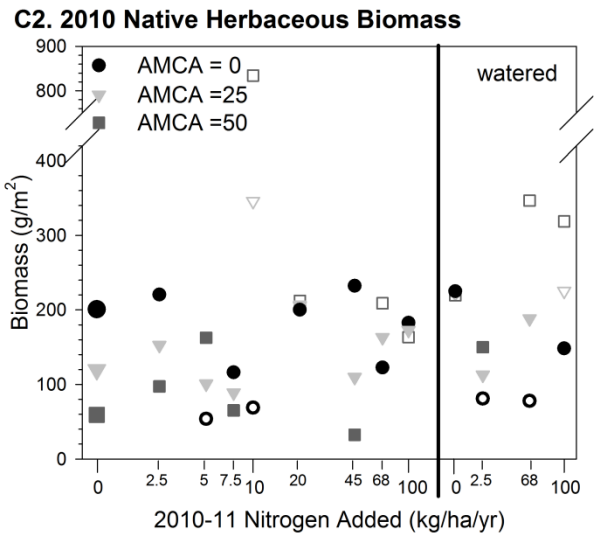
N45
N56
N68
N84
N100


Hilltop Site
2010
2011
2012
2013
NMS Axis 2
NMS Axis 4
NMS Axis 1
NMS Axis 3
ANGE, BRTE
BRJA, PASM
HECO
PASM, YUGL
AMCA, ANGE
CAFI, POPR, BRTE
BRJA, BRTE
N0
N2.5
N5
N7.5
N10
N20
N45
N68
N100
N0W
N2.5W
N68W
N100W
N0
N2.5
N5
N7.5
N10
N15
N20
N32
N45
N56
N68
N84
N100

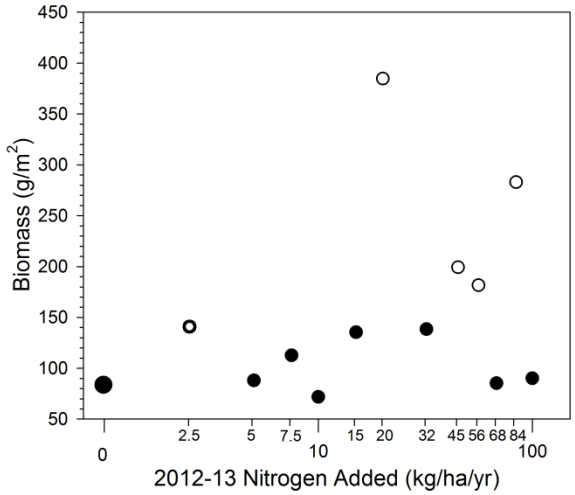
Appendix C: Additional Hilltop Site Plant Community Responses

Responses of total and native herbaceous biomass to nitrogen and water addition treatments at the Hilltop Site were idiosyncratic, in that not only did they vary among years and depend on *Amorpha canescens* cover, but they did not respond consistently to increasing nitrogen addition within year or *Amorpha* cover. Although diversity's significant response to added nitrogen did not depend on *Amorpha* cover, its response was also idiosyncratic among treatments and years. Figures in this Appendix show least squares means of total (C1, C3) and native (C2, C4) herbaceous biomass, as well as diversity (C5), in years and at levels of *Amorpha canescens* cover ("AMCA") in which there was a significant ($P < 0.05$) or marginally significant ($0.05 \leq P < 0.10$) treatment effect. Nitrogen Added (horizontal) axis is on a log scale to improve visibility of all treatments. Controls are indicated by large, filled symbols. Treatments significantly different from that of the control treatment are indicated by open, thin-walled symbols; treatments marginally significantly different from the control by open, thick-walled symbols; and treatments not significantly different from the control by small, filled symbols. Symbols are back-transformed (where necessary) least squares means, and error bars (not available when adjusted for *Amorpha* cover) are 95% confidence intervals.

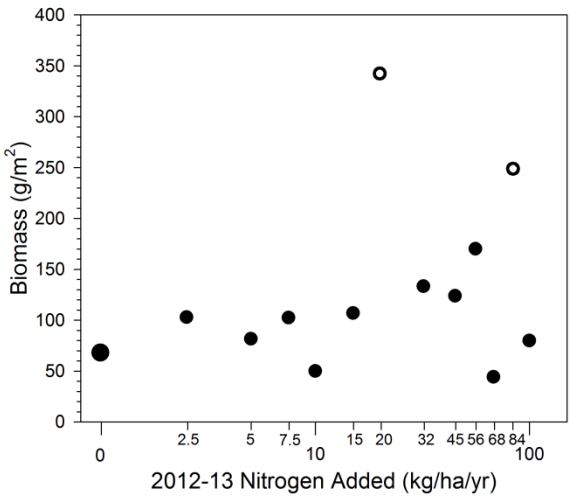




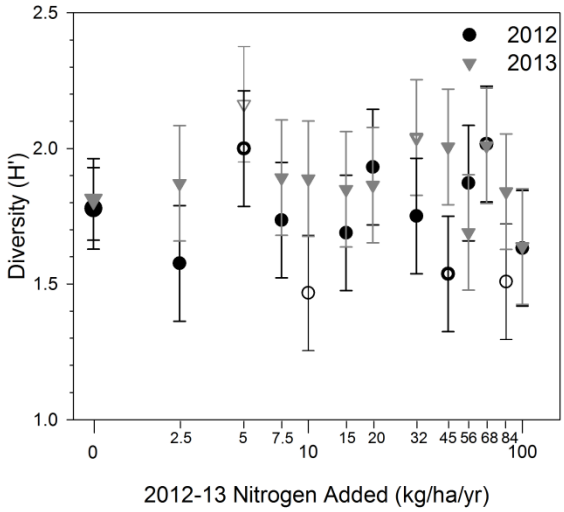
C3. 2012 Total Herbaceous Biomass at AMCA=50



C4. 2012 Native Herbaceous Biomass at AMCA=50



C5. 2012-13 Diversity



Appendix D: Derivation of Critical Loads

We used two approaches to determine nitrogen critical loads for the experimental sites used in this project. The first approach is based on minimum significant treatment effects. The second approach extrapolates dose-response curves to the nitrogen load at which there should be no difference from the control. We did not include water addition treatments in either of these approaches.

Approach 1: Minimum significant treatment effects

In this approach, we started by recording up to two nitrogen addition values for each site x response variable x year combination:

- "Method 1m" nitrogen addition values are in *italics*. They are the lowest nitrogen addition treatment for which the response variable differed significantly (*, $P < 0.05$) or marginally significantly (†, $0.05 \leq P < 0.10$) from the control treatment in the given year.

- "Method 1c" nitrogen addition values are in plain text. They are the lowest nitrogen addition treatment that differed from the control <u>and</u> above which all or nearly all treatments also differed from the control in a given year.

Tables D1, D2, and D3 show the results of this tally for the BSV, Terrace, and Hilltop sites, respectively. Variables not measured in a given year are indicated by "nm"; -- indicates that no treatments differed from the control. Values shared across two years indicate there was no difference in treatment response between those two years. Nitrogen deposition for the site is given for each year at the top of the table (data from National Atmospheric Deposition Program 2014).

Next, we populated the final column in each row with up to two critical loads based on the values in the preceding four columns, with the letter m or c indicating whether the critical load is based on Method 1m or Method 1c nitrogen addition values. Both critical loads are the lowest nitrogen addition value of that type from any year of the experiment plus the estimated total atmospheric nitrogen deposition at the site for the year of that minimum value. Deposition is averaged over the two years when treatment response did not differ between years. Minimum critical loads (one Method 1m and one Method 1c) within each site are in **bold** in the tables.

Table D1. BSV site tally for calculating critical load. All nitrogen addition or critical load values are in kg N/ha/yr.

Ecosystem characteristic	Response variable	Relevant figures	Minimum nitrogen addition values 2010	2011	2012	2013	Method 1m and 1c critical loads
	Nitrogen deposition (kg N/ha) in year →		4.0	4.3	3.4	5.9	
Soil nitrogen availability	Soil inorganic nitrogen	4a,b	45†		nm	nm	c: 49
Plant tissue nitrogen	Native warm-season grass tissue C:N	4c,d	nm	--	nm	*2.5**	m: 8.4
						20*	c: 26
	Native cool-season grass tissue C:N	4c,d	nm		nm	*20**	m: 26
				100*		45*	c: 51
	Non-native cool-season grass tissue C:N	4c,d	nm	--	nm	15†	**c: 21**
Aboveground net primary production	Total biomass	5a, 6a	*10**		*10*†		m: 14
			68*		56*		c: 51
	Total herbaceous biomass	5a, 6a	*10**		*10*†		m: 14
			68*		56*		c: 51
	Native herbaceous biomass	5b, 5b	*5**		*10**		m: 9.1
			45†		32*		c: 38
Foliar cover	Total cover	5c	45†	10*	--	--	m: 14
							c: 49
Litter load	Litter biomass	6c	nm	--	*2.5**		**m: 5.9**
					45*	32*	c: 38
	Bare ground cover		--	--	--	--	
Non-native species	Non-native biomass		--	--	--	--	
	Non-native proportion of cover		--	--	--	--	
	Total species richness[a]	5d, 6d	*10*†				m: 14
					100†	68*	c: 74
Community composition and biodiversity	Native species richness		--	--	--	--	
	Diversity		--	--	--	--	
	C3 biomass proportion		--	--	--	--	
	C3 proportion of cover		--	--	--	--	
	Species cover	Appendix B	--	--	--	--	

[a]Listed with non-native species because native species richness was not responsive to treatments.

Table D2. Terrace site tally for calculating critical load. All nitrogen addition or critical load values are in kg N/ha/yr.

Ecosystem characteristic	Response variable	Relevant figures	---------- Minimum nitrogen addition values ---------- 2010	2011	2012	2013	Method 1m and 1c critical loads
	Nitrogen deposition (kg N/ha) in year →		5.3	4.5	3.9	5.8	
Soil nitrogen availability	Soil inorganic nitrogen	7a,b	--	100*	nm	nm	c: 104
Plant tissue nitrogen	Native warm-season grass tissue C:N	7c,d	nm	--	nm	32*	**c: 38**
	Native cool-season grass tissue C:N	7c,d	nm	100*	nm	32†	**c: 38**
	Non-native cool-season grass tissue C:N	7c,d	nm	100*	nm	45*	c: 51
Aboveground net primary production	Total biomass	8a	*5**				**m: 9.9**
			45*		--	--	c: 50
	Total herbaceous biomass		--	--	--	--	
	Native herbaceous biomass		--	--	--	--	
Foliar cover	Total cover		--	--	--	--	
Litter load	Litter biomass		nm	--	--	--	
	Bare ground cover		--	--	--	--	
Non-native species	Non-native biomass		--	--	--	--	
	Non-native proportion of cover		--	--	--	--	
Community composition and biodiversity	Native species richness	8b,c	*10*†				m: 15
					84†		c: 89
	Diversity	8d,e	*10**		*84*†		m: 15
	Total species richness		--	--	--	--	
	Cool-season biomass proportion		--	--	--	--	
	Cool-season proportion of cover		--	--	--	--	
	Species cover	Appendix B	--	--	--	--	

Table D3. Hilltop site tally for calculating critical load. All nitrogen addition or critical load values are in kg N/ha/yr.

Ecosystem characteristic	Response variable	Relevant figures	---------- Minimum nitrogen addition values ---------- 2010	2011	2012	2013	Method 1m and 1c critical loads
	Nitrogen deposition (kg N/ha) in year →		5.3	4.5	3.9	5.8	
Soil nitrogen availability	Soil inorganic nitrogen	10a,b	45[†]	45[†]	nm	nm	c: 49
Plant tissue nitrogen	Native C4 grass tissue C:N	10c	nm	100*	nm	--	
	Native C3 grass tissue C:N	10c	nm	*5** 45*	nm	--	m: 9.5 c: 49
	Non-native C3 grass tissue C:N	10c	nm	*5** 45[†]	nm	--	m: 9.5 c: 49
Aboveground net primary production	Total biomass		--	--	--	--	
	Total herbaceous biomass	C1,3	*68*[†] [a, b]	--	*2.5*[†] [a]	--	**m: 6.4** [a]
	Native herbaceous biomass	C2,4	20* [a, b]	--	*20*[†] [a]	--	m: 24 [a] c: 25 [a]
Foliar cover	Total cover		--	--	--	--	
Litter load	Litter biomass		nm	--	--	--	
	Bare ground cover		--	--	--	--	
Non-native species	Non-native biomass		--	--	--	--	
	Non-native proportion of cover	11b	*7.5*[†] [c] 20[†] [c]	5* [c]	--	*2.5** [c] 20* [c]	m: 8.3 [c] **c: 9.5** [c]
Community composition and biodiversity	Native species richness		--	--	--	--	
	Diversity	C5	--	--	[d]	*5**	m: 11
	Total species richness		--	--	--	--	
	C3 biomass proportion		--	--	--	--	
	C3 proportion of cover		--	--	--	--	
	Species cover	Appendix B	--	--	--	--	

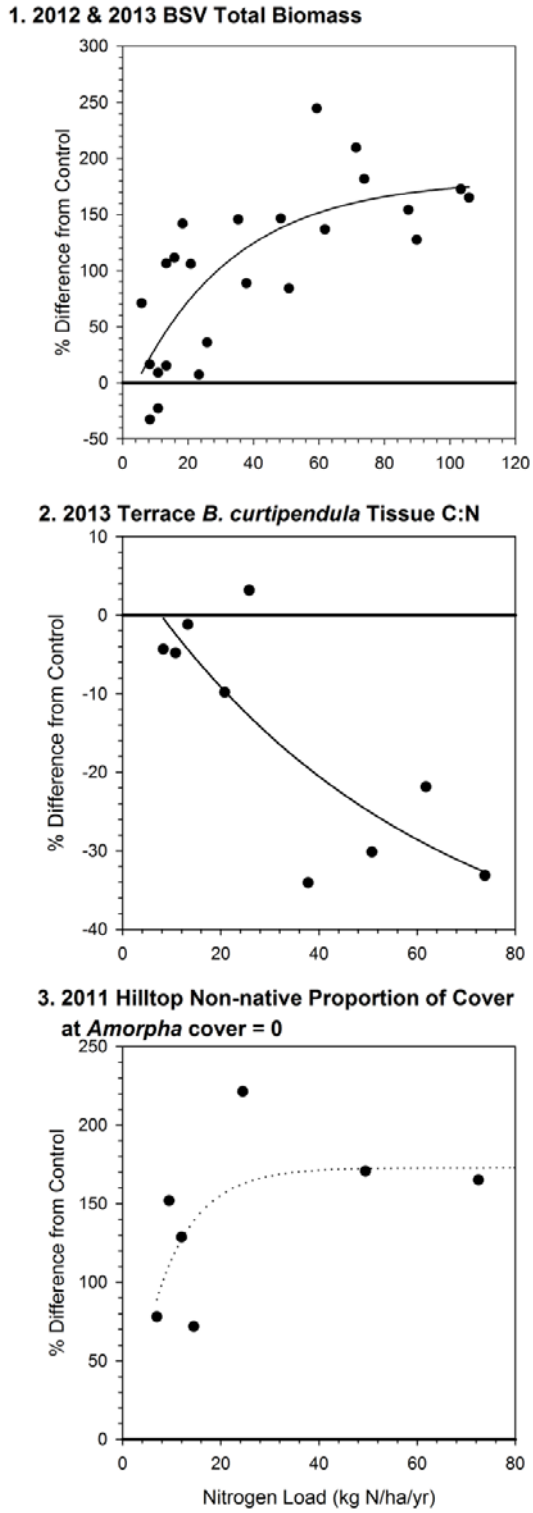
[a]at *Amorpha canescens* cover =50%; [b]unusually high value at 10 kg N/ha/yr, so next highest significant treatment shown; [c]at *Amorpha canescens* cover = 0%; [d]multiple treatments with significant effects, but some greater than and some less than control

Approach 2: Dose-response curve

In this approach, we selected the response variable x year combination with the most consistent response to increasing nitrogen addition from the results for each site presented in Figures 4-8, and 10-11. Only response variable x year combinations with at least marginally significant linear trends, and with at the response measured in at least five nitrogen-addition treatments, were considered for selection. For the BSV site, we selected total biomass in 2012 and 2013 (Figure 6a). For the Terrace site, we selected *Bouteloua curtipendula* (side-oats grama, a native warm-season grass) tissue C:N in 2013 (Figure 7d). For the Hilltop site, we selected 2011 non-native proportion of cover adjusted for no *Amorpha canescens* cover (Figure 11b).

We calculated the relative magnitude of the effect of each nitrogen addition treatment as the percent difference between the treatment's least squares mean and the control's least squares mean for the given response variable and year, then plotted these differences against nitrogen load. Nitrogen load was calculated as the nitrogen addition treatment plus atmospheric deposition for the specific site and year (deposition values in Figure 3). We then fit a curve to each plot and, where appropriate, extrapolated the curve to the point where y = 0 to estimate the nitrogen load at which the effect of nitrogen addition is zero.

An exponential rise to a maximum curve (or decline to minimum) provided a reasonable fit for total biomass in 2012 and 2013 at the BSV site (R^2 = 0.59, P < 0.0001; Figure D1) and for *B. curtipendula* tissue C:N in 2013 at the Terrace site (R^2 = 0.67, P =0.04; Figure D2). These produced estimated critical nitrogen loads of 4.3 and 7.8 kg N/ha/yr, respectively. No curve provided a reasonable fit for 2011 non-native proportion of cover at the Hilltop site (P > 0.2; Figure D3).



Figures D1-3. Dose-response curves for selected response variables.

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NPS 920/129008, July, 2015

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U.S. Department of the Interior

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