

Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands

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Abstract

Nitrogen (N) deposition is widely considered an environmental problem that leads to biodiversity loss and reduced ecosystem resilience; but, N fertilization has also been used as a management tool for enhancing primary production and ground cover, thereby promoting the restoration of degraded lands. However, empirical evaluation of these contrasting impacts is lacking. We tested the dual effects of N enrichment on biodiversity and ecosystem functioning at different organizational levels (i.e., plant species, functional groups, and community) by adding N at 0, 1.75, 5.25, 10.5, 17.5, and 28.0 g N m⁻² yr⁻¹ for four years in two contrasting field sites in Inner Mongolia: an undisturbed mature grassland and a nearby degraded grassland of the same type. N addition had both quantitatively and qualitatively different effects on the two communities. In the mature community, N addition led to a large reduction in species richness, accompanied by increased dominance of early successional annuals and loss of perennial grasses and forbs at all N input rates. In the degraded community, however, N addition increased the productivity and dominance of perennial rhizomatous grasses, with only a slight reduction in species richness and no significant change in annual abundance. The mature grassland was much more sensitive to N-induced changes in community structure, likely as a result of higher soil moisture accentuating limitation by N alone. Our findings suggest that the critical threshold for N-induced species loss to mature Eurasian grasslands is below 1.75 g N m⁻² yr⁻¹, and that changes in aboveground biomass, species richness, and plant functional group composition to both mature and degraded ecosystems saturate at N addition rates of approximately 10.5 g N m⁻² yr⁻¹. This work highlights the tradeoffs that exist in assessing the total impact of N deposition on ecosystem function.

Keywords: aboveground biomass, ecological restoration, ecosystem management, Eurasia steppe, nitrogen enrichment, plant functional group composition, species richness

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Introduction

Although nitrogen (N) is the dominant elemental constituent of Earth's atmosphere, stoichiometric and energetic constraints on its biological fixation to soluble, inorganic forms amenable for plant uptake frequently make it one of the most limiting resources in terrestrial ecosystems (Vitousek & Howarth, 1991). Widespread

nitrogen enrichment through industrial and agricultural processes, however, lessens N limitation and, in so doing, dramatically impacts ecosystem functioning (Galloway *et al.*, 1995; Jordan & Weller, 1996; Vitousek *et al.*, 1997; Phoenix *et al.*, 2003). While its impacts vary considerably among ecosystems (DiTommaso & Aarssen, 1989; Matson *et al.*, 1999; Suding *et al.*, 2005; Clark *et al.*, 2007; LeBauer & Treseder, 2008), N enrichment generally increases aboveground net primary production and reduces plant species richness by favoring a

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small number of opportunistic species, as indicated by grassland studies in Europe (Bobbink *et al.*, 1998; Stevens *et al.*, 2004) and North America (Wedin & Tilman, 1996; Collins *et al.*, 1998; Gough *et al.*, 2000). Such losses in plant richness, in turn, can have marked impacts on ecosystem productivity (Tilman *et al.*, 1996; Hector *et al.*, 1999) and stability (Tilman & Downing, 1994; Pfisterer & Schmid, 2002; Bai *et al.*, 2004; Tilman *et al.*, 2006).

It is unclear, however, to what extent the observed shifts in community composition and functioning are dependent on either the rate of N addition or on properties of the extant community. Ecological theory predicts that N addition to degraded, resource poor communities may alleviate ecosystem N limitation and lead to an increase in diversity. In contrast, N addition to a mature community within the same ecosystem may disrupt mechanisms of community coexistence, leading to a decrease in diversity as a small number of opportunistic species are favored. These changes in community structure are likely influenced by the functional traits that species possess, and recent work suggests that both functional- and neutral- based processes operate simultaneously (Suding *et al.*, 2005). Theory does not predict that these broad processes are rate-specific; however, it has been generally assumed that there exist ecosystem-specific rates of N input for which no deleterious effects occur, an assumption that forms the backbone of a substantial body of environmental legislation (NEGTAP, 2001); However, explicit tests are lacking. Studies that add N at a range of rates generally report qualitative agreement in system responses at low vs. high rates of treatment (Tilman, 1987; Morecroft *et al.*, 1994; Bowman *et al.*, 2006; Britton & Fisher, 2007), suggesting that thresholds, if present, may be lower than previously thought (Stevens *et al.*, 2004; Clark & Tilman, 2008).

From a management perspective, N fertilization has been used to restore the productivity and improve the forage quality of grasslands degraded by overgrazing (Schellberg *et al.*, 1999; Conant *et al.*, 2001). Of the 40% of the Earth's land surface that is grassland, over half has been converted to managed grazing ecosystems with a substantial area already degraded (White *et al.*, 2000). This situation is likely to worsen if grazing pressure continues to increase as projected (Reynolds & Smith, 2002; Asner *et al.*, 2004; Millennium Ecosystem Assessment, 2005). Systems degraded by grazing are often species poor, dominated by less palatable species, and have lowered productivity and vegetation cover, with corresponding increases in wind erosion, nutrient loss, and water runoff (Asner *et al.*, 2004). In such systems N enrichment can increase primary production and ground cover and subsequently reduce wind erosion and soil nutrient loss, slowing the process of anthropo-

genic desertification – a threat confronted by an estimated 41% of the grasslands of the world (Lal, 2001; Malhi *et al.*, 2003; Li *et al.*, 2007).

Reconciliation of these two contrasting perceptions of N enrichment, that it is detrimental for some systems (e.g. unperturbed or natural) but beneficial for others (e.g. degraded), is a result of divergent research goals and insufficient cross-comparisons among fields. Studies in natural ecosystems often focus on biodiversity and ecosystem properties (e.g., production and soil C and/or N pools), while responses in managed ecosystems often focus on ecosystem properties as they refer to ecosystem goods (e.g., forage and livestock production). Clearly these are interrelated topics. However, few studies to date have explicitly compared on a common set of standards both natural and degraded sites within an ecosystem, for which differences in regional species pools, climate, and geomorphology are likely to be minor. The few that have often find that natural systems are less sensitive to N enrichment than currently or recently managed systems (Tilman, 1987, 1993), though this response is far from general (Grime *et al.*, 2000).

Here we report the impacts of a range of N addition rates to two contrasting sites in the Inner Mongolian grassland, which is part of the largest contiguous grassland of the world – the Eurasian steppe. We selected an undisturbed mature grassland and a degraded grassland of the same type to address two primary questions. First, how do several key biotic (i.e. biomass production, species richness, and plant functional group composition) and abiotic (inorganic soil N and soil moisture) ecosystem characteristics respond to various rates of N addition in the Inner Mongolia grassland? And second, do these patterns vary between the degraded and mature sites within this ecosystem?

Materials and methods

Experimental sites

The field experiment was carried out at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS), which is located in the Xilin River Basin, Inner Mongolia Autonomous Region of China (116°42'E, 43°38'N). IMGERS is one of the key members of the Chinese Ecological Research Network (CERN), and is administrated by the Institute of Botany, Chinese Academy of Sciences. Topographic relief exhibits little variation, with elevation ranging from 1250 to 1260 m at our experimental sites. The mean annual temperature in the study area is 0.3 °C with mean monthly temperatures ranging from –21.6 °C in January to 19.0 °C in July. The mean annual precipitation is 346.1 mm with 60–80%

falling during the growing season from May to August. The soil in both sites is classified as dark chestnut (Calcic Chernozem according to ISSS Working Group RB, 1998) or loamy sand in terms of texture with similar physiochemical properties (Table 1).

In 1999, two 120 × 70 m locations with fairly uniform vegetation were established within the permanent research plots of IMGERS as contrasting experimental sites. No fertilizer had been applied in either site before this study. The first site was an undisturbed mature grassland community that had been fenced to exclude large animals since 1979 (Bai *et al.*, 2004). Before beginning the experiment, the mature site was dominated by *Leymus chinensis*, a widely distributed perennial C₃ rhizome grass in the Eurasia steppe region. About 100 meters west of the mature site, the degraded site was an overgrazed area with its dominant species having shifted from the rhizomatous C₃ *L. chinensis* to the non-rhizomatous C₃ bunchgrasses *Stipa grandis* and *Cleistogenes squarrosa* (a C₄ short bunchgrass) due to uncontrolled grazing since the early 1980s (Li, 1988). The degraded site, which had the same plant species composition to the mature site before 1979, was fenced in 1999 six months before the beginning of the experiment. Thus, upon initiation of the experiment, the two sites differed dramatically in structure and function, with the degraded site having fewer species, lower production, and which had experienced a shift in com-

munity composition from dominance by perennial rhizomatous grasses to perennial bunchgrasses, with little change in other functional groups (Table 1).

Experimental design

Identical treatments were applied to both mature and degraded sites. At each site, 162 5 m × 5 m plots were laid out in a randomized block design. Plots were separated by 1-meter walkways. There were 9 replicates for each of the 19 treatments which included a control treatment (i.e. no nutrient addition), and 6 levels of N enrichment at 3 application times. N was added at 0, 1.75, 5.25, 10.5, 17.5, and 28.0 g N m⁻² yr⁻¹ as commercial NH₄NO₃. The three different application times were: (1) the early growing season (May 1–3), (2) the middle of the growing season (July 1–5), and (3) 50% of N applied at each time period (except for the level of 1.75 g N m⁻² yr⁻¹ which was too low to split). To assure that N was the only limiting nutrient (Tilman, 1987), we also added the same amount of P (10 g P₂O₅ m⁻² yr⁻¹), S (0.2 mg m⁻² yr⁻¹), and trace elements (Zn: 190 µg m⁻² yr⁻¹, Mn: 160 µg m⁻² yr⁻¹, B: 31 µg m⁻² yr⁻¹) for all treatments with the exception of control based on the soil census data (Inner Mongolia Soil Census Office & Inner Mongolia Soil and Fertilizer Service, 1994). We did not add K, Mg, Ca and Fe in our experiment because dark chestnut soils were rich in K, Mg, Ca and Fe (Inner Mongolia Soil Census Office & Inner Mongolia Soil and Fertilizer Service, 1994). Since our preliminary analysis found no significant effects of the timing of N addition on the response patterns of biomass, species richness, and functional group composition, we only report the results based on the July data (126 plots in total) in this paper.

Field sampling and measurements

We classified all plants into five functional groups based on life forms, including perennial rhizome grasses (PR), perennial bunchgrasses (PB), perennial forbs (PF), shrubs and semi-shrubs (SS), and annuals (AS) as in Bai *et al.* (2004). Aboveground vegetation was sampled each year between 25 August and 2 September by clipping all plants at the soil surface using a 0.5 × 1 m quadrat randomly placed in the plot with the restriction of no spatial overlap of quadrats among all years. To avoid edge effects, the quadrat was placed at least 50 cm inside each plot. All living vascular plants were sorted to species, and all plant materials, including litter and standing dead, were oven-dried at 65 °C for 48 h and weighed. The dry mass of all living plants per quadrat averaged over the nine replicates for each treatment

Table 1 Soil and plant community properties (means ± SE) of the mature and degraded sites before treatment

	Term	Mature	Degraded
Soil Property (0–20 cm)	SOM (%)	2.76 ± 0.06 ^{NS}	2.87 ± 0.09
	TN (%)	1.73 ± 0.02 ^{NS}	1.77 ± 0.04
	pH	7.52 ± 0.04 ^{NS}	7.54 ± 0.13
	Sand (%)	80.18 ± 0.38 ^{NS}	78.41 ± 1.17
	Silt (%)	17.60 ± 0.50 ^{NS}	19.11 ± 1.04
	Clay (%)	2.22 ± 0.23 ^{NS}	2.48 ± 0.22
Community Property	Richness	51	36
	Biomass (g m ⁻²)	150.32 ± 7.82 ^{**}	116.08 ± 5.52
	PR (%)	41.07 ± 5.52 ^{**}	19.99 ± 2.42
	PB (%)	31.99 ± 4.25 ^{**}	50.65 ± 4.79
	PF (%)	23.42 ± 3.24 ^{NS}	27.80 ± 4.23
	SS (%)	3.17 ± 1.46 ^{NS}	1.44 ± 0.49
	AB (%)	0.35 ± 0.29 ^{NS}	0.12 ± 0.08

Note: Significant differences between sites are reported from one-way ANOVA as NS, $P > 0.05$;

^{**}, $P < 0.01$. The particle size is 0.02–2.0 mm for sand, 0.002–0.02 mm for silt, and <0.002 mm for clay. PR = perennial rhizome grasses, PB = perennial bunchgrasses, PF = perennial forbs, SS = shrubs and semi-shrubs, and AS = annuals.

was used to estimate the aboveground community biomass.

Soil samples were taken from the surface to a depth of 20 cm in early July and early September of 2003. For each plot, three soil cores were collected using a 3-cm diameter soil auger and mixed *in situ* into one composite sample. All soil samples were stored in a refrigerator at 4 °C until being processed and analyzed. Within 10 days of the collection, all soil samples were extracted with 50 mL of 2 M KCl (soil to extractant ratio = 1:10) (Mulvaney, 1996), and ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were analyzed with a FIASTAR 5000 (Dual Channel Soil Nitrogen Analyzer System, FOSS, Höganäs, Sweden). Concentrations of extractable soil NH_4^+ -N and NO_3^- -N were expressed as milligrams per kilogram on the basis of dry soil mass. The concentrations of soil NH_4^+ -N and NO_3^- -N reported here were the averages of the samples from early July and early September for each treatment.

Soil moisture was determined using the gravimetric method. For both sites, three soil cores (3.5 cm in diameter) at each depth of 0–20, 20–40, and 40–110 cm were taken weekly from control plots from May to early September during 2000–2002, and bi-weekly in 2003. The soil samples were weighed before and after being oven-dried at 105 °C for 48 h. We also measured soil moisture in the treatment plots at 0–20 and 20–40 cm at the end of August in 2003. For each treatment, five of the nine replications were selected randomly, and soil moisture was measured using the same method.

Statistical analyses

Statistical analyses were performed using SAS Version 8.0 (SAS Institute, Cary, North Carolina, USA, 2001). Analyses across time were performed using repeated measures ANOVA for both sites, Geisser-Greenhouse adjusted F-statistic to test for multivariate significance, using Year, Site (mature or degraded), N addition rate (categorical), and all interactions as fixed-effects. As anticipated, many high order interactions were significant (i.e. treatment responses differed through time); thus, we ran additional analyses for each field and for each year to determine the pattern and magnitude of response. Regression models with N as a continuous variable were used to determine the general relationship between N addition and various responses (adjustments for non-linearity were made as needed). Regression models with N as an ordinal variable were used to determine the relationship between each rate of N addition and various responses (i.e. to estimate threshold levels). These ANOVAS were followed by a Duncan's multiple-range test to compare the N-addition effects for each rate. To test the dependency of

biomass and species richness (SR) responses to N on soil moisture (SM), we further analyzed the relationships between biomass response ($\text{Biomass}_{\text{treatment}}/\text{Biomass}_{\text{control}}$) and corresponding soil moisture response ($\text{SM}_{\text{treatment}}/\text{SM}_{\text{control}}$) and between species richness response ($\text{SR}_{\text{treatment}}/\text{SR}_{\text{control}}$) and soil moisture response.

Results

Aboveground biomass

Repeated measure ANOVA of aboveground biomass, using site, N-treatment, year, and all interactions as fixed-factors, showed that the effects of site, treatment, and year on aboveground biomass were highly significant, and that all interactions were also highly significant (Table 2). Although neither site responded strongly to treatment in the first year of study (2000), by the fourth year (2003), aboveground biomass increased across the N addition gradient by 98–271% in the mature site, and by 13–62% in the degraded site (Fig. 1). Aboveground biomass tended to increase at all treatment rates in both sites, significantly increasing in the mature site even at the $1.75 \text{ g N m}^{-2} \text{ yr}^{-1}$ rate. Little additional response in either site was found for N addition rates $\geq 10.5 \text{ g N m}^{-2} \text{ yr}^{-1}$. Patterns in the degraded site were less dramatic overall and individual differences between controls and treated plots were only found at higher treatment rates (Fig. 1). Thus, throughout the study period, the undisturbed mature

Table 2 Repeated measures analysis of variance for aboveground biomass and species richness using Site, N-Treatment, Year, and all interactions as fixed-effects

Response	Term	Df	F	P
Aboveground biomass	Site (S)	1	103.74	<0.0001
	N-Treatment (N)	6	36.4	<0.0001
	Year (Y)	3	323.83	<0.0001
	S × N	6	10.3	<0.0001
	S × Y	3	14.99	<0.0001
	N × Y	18	9.49	<0.0001
	S × N × Y	18	4.53	<0.0001
Species richness	Site (S)	1	530.86	<0.0001
	N-Treatment (N)	6	7.35	<0.0001
	Year (Y)	3	106.38	<0.0001
	S × N	6	1.62	0.1590
	S × Y	3	17.5	<0.0001
	N × Y	18	5.32	<0.0001
	S × N × Y	18	2.02	0.0110

Note: There are 168 degrees of freedom for error.

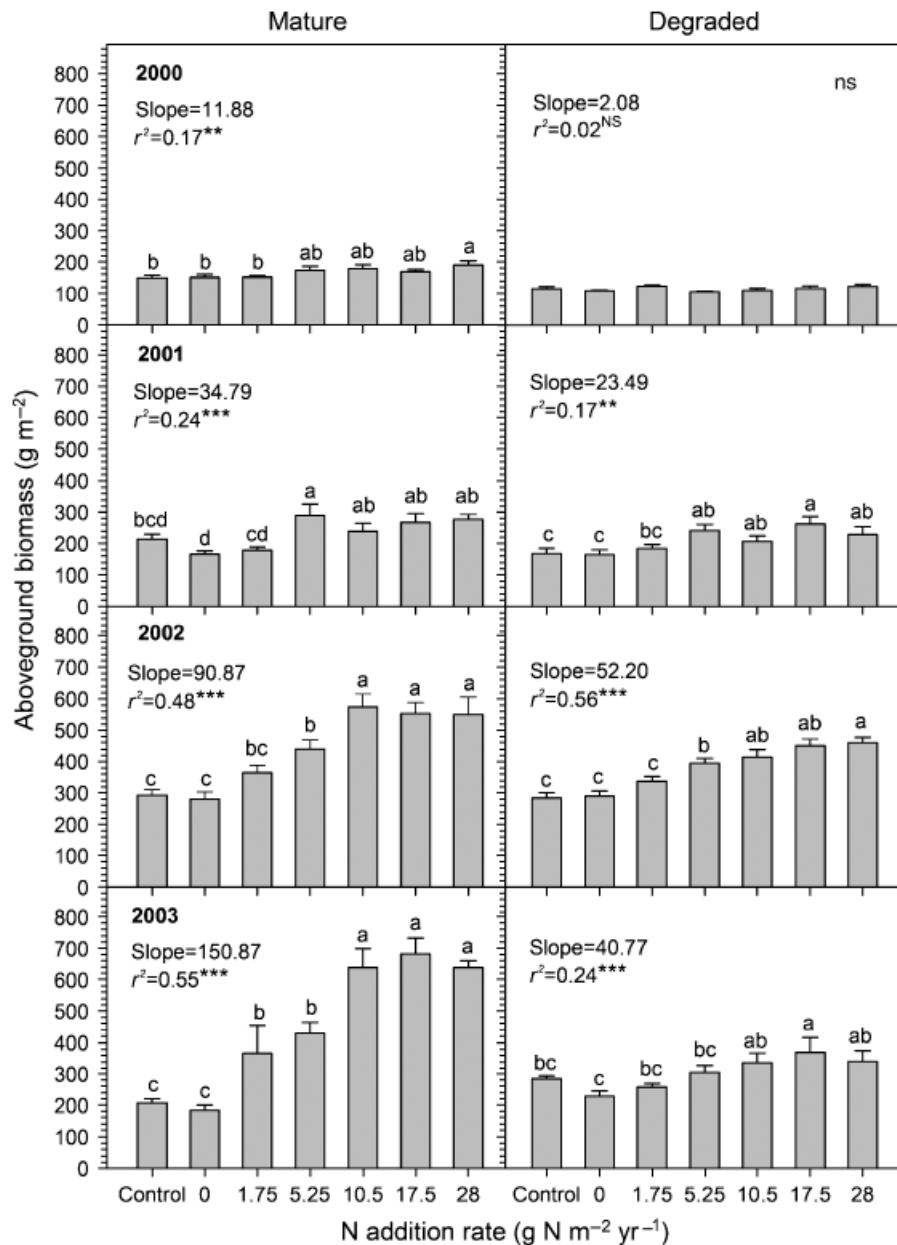


Fig. 1 Effects of N addition on aboveground biomass of the mature and degraded communities. Aboveground biomass for each treatment was the average of nine replicates (error bars denote SEM). Bars with the same letter were not significantly different in Duncan's multiple range tests reported from one-way ANOVA, and NS indicates non-significant ($P > 0.05$). For both sites, regression parameters were estimated each year using log-linear model with N treatment as a continuous predictor, i.e., *Aboveground biomass* = Intercept + *Slope* × $\ln(N)$. Significant differences are reported as NS, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

community showed a stronger overall response to N addition at all treatment rates than the degraded site.

In both the mature and degraded sites, no significant difference in aboveground biomass was found between the control (no nutrients added) and the zero-N treatment (added other nutrients but not N) from year to year or through time (Repeated-measures ANOVA: for the mature site, Time × Treatment, $F_{3,48} = 1.15$,

$P = 0.326$; for the degraded site, Time × Treatment, $F_{3,48} = 1.78$, $P = 0.180$; Fig. 1).

Plant species richness

In general, plant species responded substantially between sites, among treatments, and through time, and there were significant site × year, treatment × year, and

site \times treatment \times year interactions (Table 2). At the mature site, species richness increased with N addition in 2000, demonstrated no response in 2001, and significantly declined in 2002 and 2003 (Fig. 2). By the fourth year of the study, species richness in the mature site had declined by 20–36% across the N addition gradient; and, there was a significant reduction at the lowest treatment

rate when compared with controls in 2002 and with the zero-N added treatment in 2003. Species richness in the degraded site responded more weakly in general, with no change in the first two years and significantly declining with N addition in 2002 and 2003 (Fig. 2). Individual differences with controls, however, were only significant at higher treatment rates. There were

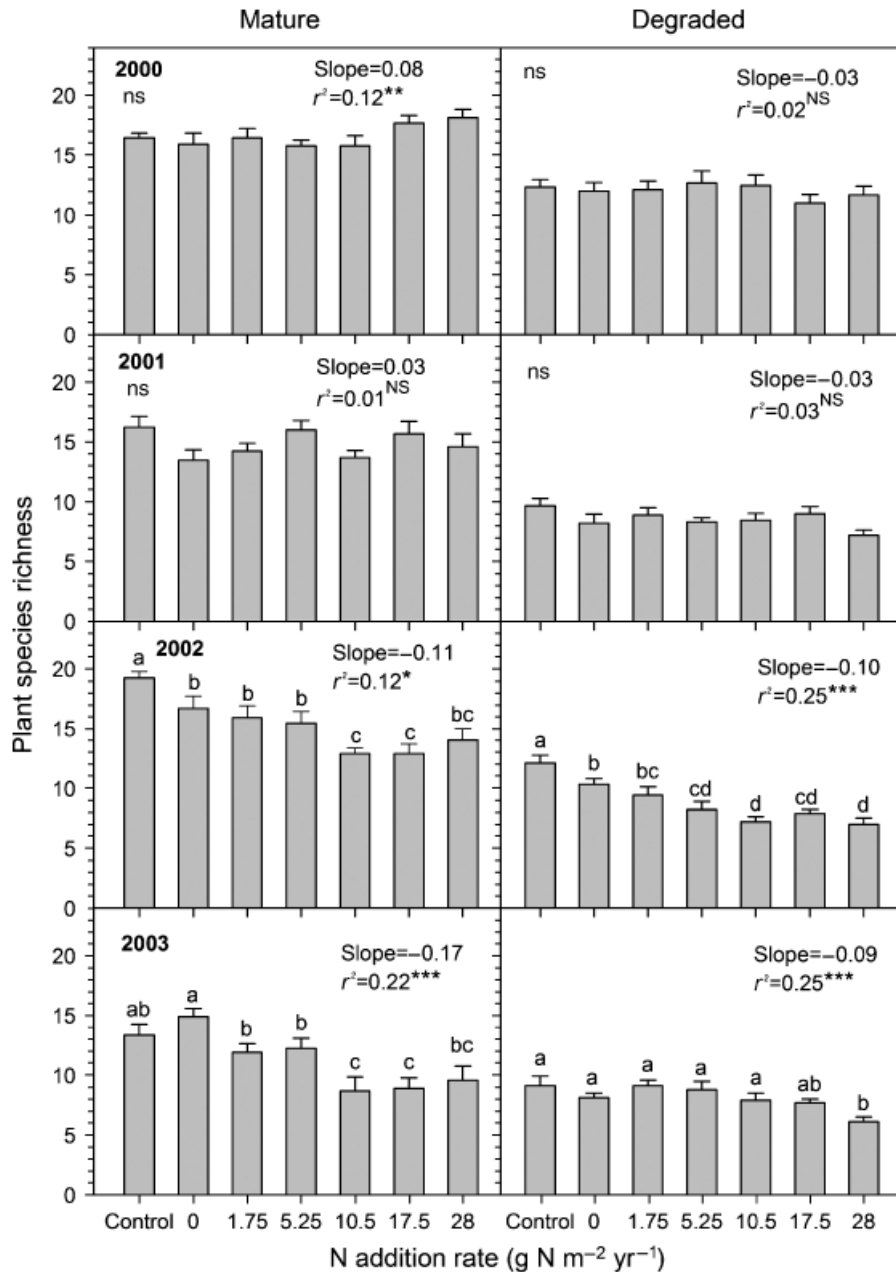


Fig. 2 Effects of nitrogen addition on plant species richness of the mature and degraded communities. Species richness is the average number of plant species in the 9 replicates of each treatment using a $0.5 \times 1.0 \text{ m}^2$ quadrat (error bars denote SEM). For both sites, regression parameters were estimated each year using log-linear model with N treatment as a continuous predictor, i.e., $\text{Species richness} = \text{Intercept} + \text{Slope} \times \text{Ln}(N)$. All symbols are derived as from Fig. 1.

no consistent differences between the control and zero-N treatment in either site (Fig. 2) or through time (analyses not shown). Our results further showed that species richness response was negatively correlated with aboveground biomass response at the mature site ($R^2 = 0.21$, $P = 0.0005$), while no significant relationship between species richness response and aboveground biomass response was found at the degraded site ($P > 0.05$).

Plant functional group composition

Patterns of functional group responses to treatment in the two sites were strikingly different both quantitatively and qualitatively (Table 3). For each of five plant functional groups, however, the effects of site, year, and site \times year were highly significant (Table 3). At the mature site, there were no significant treatment effects for any functional group in the first two years of the study (Fig. 3). However, beginning in 2002, annuals (AS), which had been nearly absent in 2000, increased strongly across the N addition gradient, dominating by 2003 and leading to a sharp decline in perennial rhizome grasses (PR), perennial bunchgrasses (PB), and perennial forbs (PF) functional groups (Fig. 3). No significant response was found for shrubs and subshrubs (SS) over the four years (Fig. 3). Compared to zero-N treatment, the average aboveground biomass of the AS functional group in 2003 increased by approximately 20-fold at the two lowest N addition rates and 50-fold at the three highest, comprising 62% and 91% of the aboveground production respectively in these treatment groups. In contrast, by 2003 the average aboveground biomass of the PR, PB, and PF functional groups declined respectively by 53%, 44%, and 54% at the two

lowest treatment rates and by 95%, 94%, and 92% at the three highest.

There was a markedly different functional response in the degraded site (Fig. 4). Although there were no treatment effects in 2000, beginning in 2001 and continuing to 2002, the aboveground biomass of both PR and PB functional groups increased, with a corresponding decline in PF species (Fig. 4). Between 2002 and 2003, there was a continued increase in PR species and a dramatic decline in PB species. No significant response was found in the aboveground biomass of AS or SS functional groups over the four years (Fig. 4).

These observed shifts in functional composition were consistent at low and high N addition rates in the mature site, but were only significant in the degraded site at higher N addition rates. At both sites, no significant difference in the aboveground biomass of PFGs was found between the control and the zero-N treatment for all functional groups and all years, except perennial forbs at the degraded site in 2002 and 2003 (Figs 3 and 4).

Soil inorganic N

Our results showed that there was a significant treatment effect on soil inorganic N concentrations (NO_3^- -N plus NH_4^+ -N), whereas no significant site and site \times treatment effects were found (ANOVA: Treatment, $F_{6,56} = 13.35$, $P < 0.0001$; Site, $F_{1,56} = 2.36$, $P = 0.1298$; Treatment \times Site, $F_{6,56} = 1.05$, $P = 0.4058$). For both the mature and degraded sites, soil N availability (growing season NO_3^- -N and NH_4^+ -N concentrations) remained relatively unchanged at N addition rates $\leq 10.5 \text{ g N m}^{-2} \text{ yr}^{-1}$ (Fig. 5). At N addition rates $> 10.5 \text{ g N m}^{-2} \text{ yr}^{-1}$, soil NO_3^- -N and NH_4^+ -N increased dramatically (Fig. 5).

Soil moisture

The soil moisture at 0–20 cm depth differed substantially between sites and among treatments at the time of peak aboveground biomass in 2003 (ANOVA: Site, $F_{1,56} = 11.92$, $P = 0.0011$; Treatment, $F_{6,56} = 4.04$, $P = 0.0020$; Site \times Treatment, $F_{6,56} = 0.66$, $P = 0.6831$); it was higher in the mature than the degraded site, and declined with increasing N addition rate at both sites (Fig. 6). For the mature site, a similar pattern of declining soil moisture was found at 20–40 cm depth across the N addition gradient, with no significant differences between the two sites and among treatments for the degraded site (ANOVA: Site, $F_{1,56} = 3.01$, $P = 0.0881$; Treatment, $F_{6,56} = 2.79$, $P = 0.0192$; Site \times Treatment, $F_{6,56} = 0.49$, $P = 0.8100$).

Table 3 *P* values of repeated measures analysis of variance for each plant functional group using Site, N-Treatment, Year, and all interactions as fixed-effects

Term	PR	PB	PF	SS	AS
Site (S)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
N-Treatment (N)	<0.0001	0.106	0.0065	0.3408	<0.0001
Year (Y)	<0.0001	<0.0001	<0.0001	0.0003	<0.0001
S \times N	<0.0001	0.1577	0.3523	0.3839	<0.0001
S \times Y	<0.0001	<0.0001	0.0005	0.0008	<0.0001
N \times Y	0.0013	<0.0001	0.0053	0.5864	<0.0001
S \times N \times Y	<0.0001	0.2059	0.1694	0.8696	<0.0001

Note: There are 168 degrees of freedom for error. PR = perennial rhizome grasses, PB = perennial bunchgrasses, PF = perennial forbs, SS = shrubs and semi-shrubs, and AS = annuals.

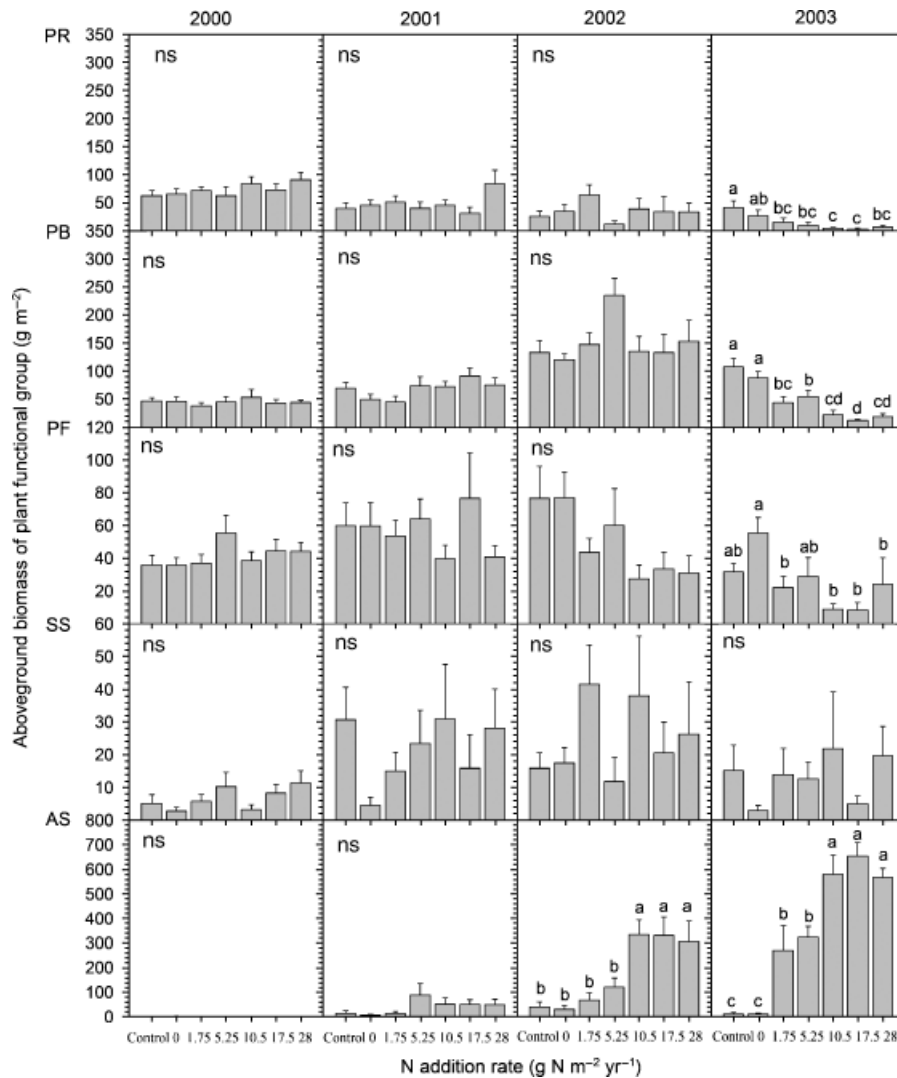


Fig. 3 Responses of the aboveground biomass of PFGs to N addition rates at the mature site (error bars denote SEM). The aboveground biomass of each PFG was the average of the nine replicates of a treatment (note difference in scales). Bars with the same letter were not significantly different in Duncan's multiple range tests reported from one-way ANOVA, and NS indicates non-significant ($P > 0.05$). PR = perennial rhizome grasses, PB = perennial bunchgrasses, PF = perennial forbs, SS = shrubs and semi-shrubs, and AS = annuals.

Although the average soil moisture contents in control plots varied between the two sites, among the different years, and with time within a growing season, it was consistently higher in the mature than the degraded site at three different soil depths (Fig. S1). All interactions between site, year, and sampling date were significant using repeated measures ($P < 0.01$). From May to early August when plants grew most vigorously, soil moisture for the mature site was, on average, about 30% higher than that of the degraded site throughout the soil profile (Fig. S1).

Our further analysis showed that aboveground biomass response was negatively correlated with soil

moisture response at 0–20 cm for both the mature ($R^2 = 0.25$, $P = 0.0054$) and degraded sites ($R^2 = 0.39$, $P = 0.0002$; Fig. 7). On average, soil moisture decreased by 24% for the mature site and by 17% for the degraded site at the two highest N addition rates when compared to the control treatment (Fig. 7). No significant relationship, however, was found between species richness response and soil moisture response for both the mature and degraded site ($P > 0.05$).

Discussion

We found that the aboveground biomass and species richness responses were qualitatively similar between

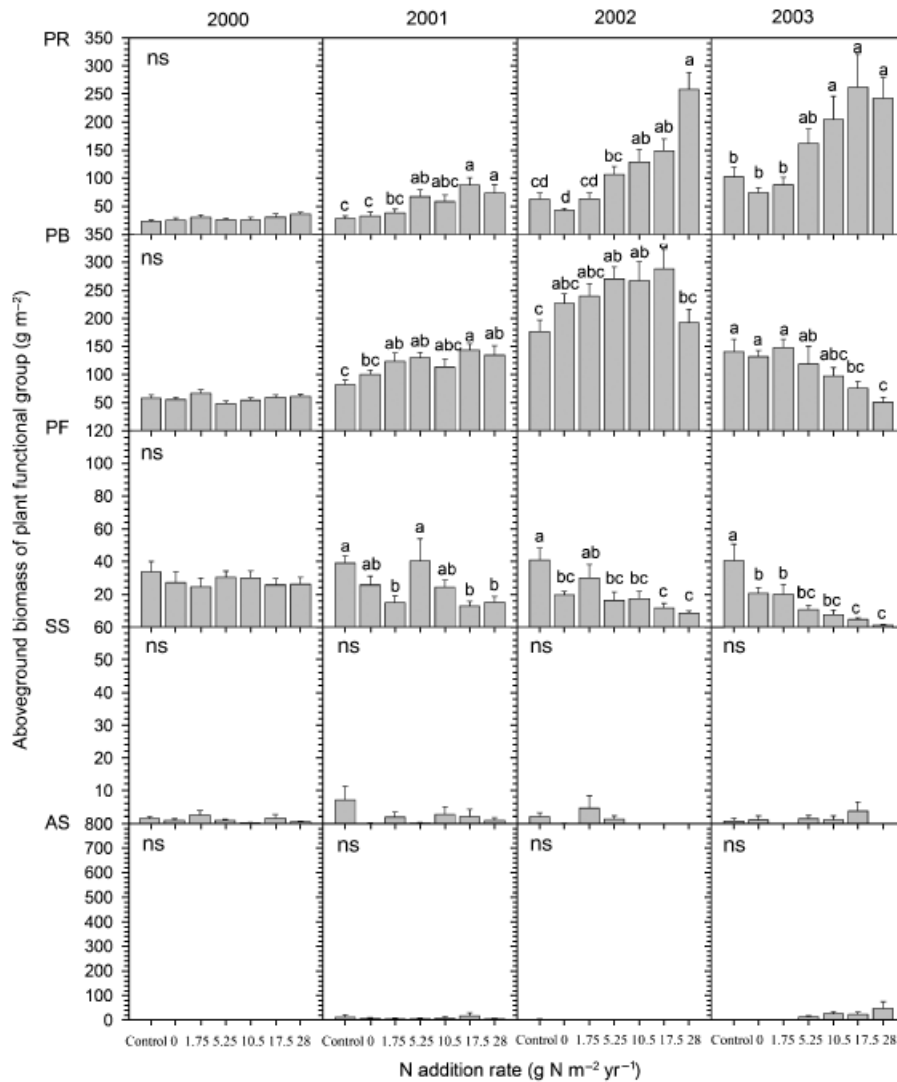


Fig. 4 Responses of the aboveground biomass of PFGs to N addition rates at the degraded site (error bars denote SEM). The aboveground biomass of each PFG was the average of the nine replicates of a treatment. All symbols are derived as from Fig. 3.

the two sites and across the N addition gradient, although large quantitative differences emerged. These quantitative differences appeared to be driven by divergent functional responses in the two sites.

Patterns of aboveground biomass

Our experiment in the Inner Mongolian grassland demonstrated that although the qualitative effects of N addition were similar between sites, their quantitative effects varied substantially. Aboveground biomass in both communities tended to increase relative to controls at all rates of N addition, saturating at rates $\geq 10.5 \text{ g N m}^{-2} \text{ yr}^{-1}$. By 2003, increased aboveground biomass was detected for every treatment rate in the mature community; and, although individual differ-

ences were only significant for higher treatment rates in the degraded community, trends were qualitatively similar. The magnitude of these increases, however, was markedly different, with average aboveground biomass increased by 199% at the mature site and 41% at the degraded site after 4 years. These patterns suggest three conclusions. First, N was limiting at both mature and degraded sites, addition of which at any rate increased aboveground biomass. As such, we did not find strong evidence for a critical threshold within the range tested here, where N addition at rates below this threshold might be unavailable to plants as microbial and abiotic processes likely outcompeted plants for excess N (Johnson *et al.*, 2000; Barrett *et al.*, 2002; Davidson *et al.*, 2003). Second, there appeared to be a saturation rate of N addition above which the production potential of the

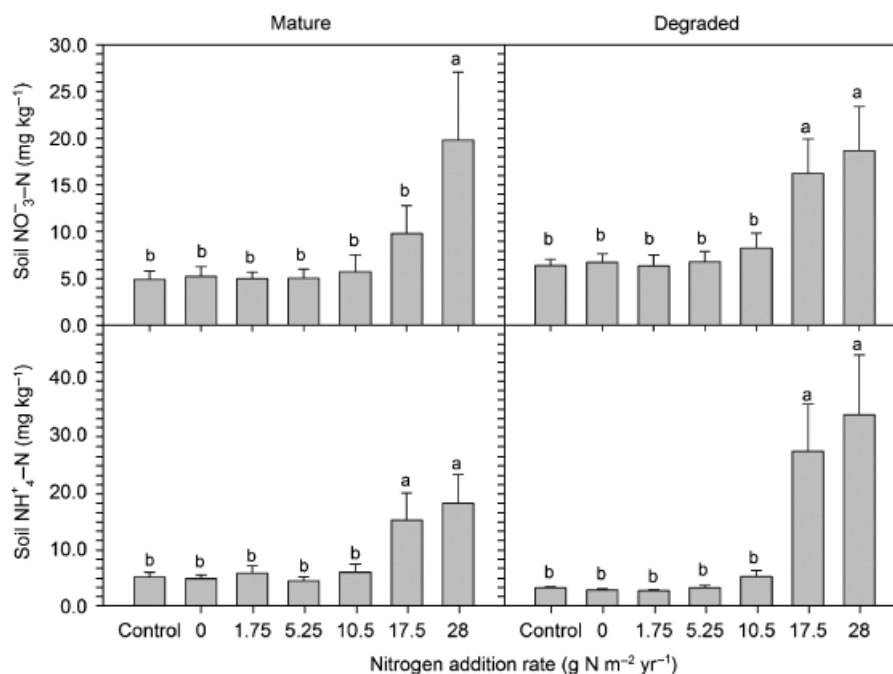


Fig. 5 Seasonal average extractable soil NO₃⁻-N and NH₄⁺-N concentration in the fourth year (error bars denote SEM). Bars with the same letter were not significantly different in Duncan's multiple range tests reported from one-way ANOVA.

existing community was saturated with N. At input rates above this, there was little additional increase in aboveground biomass and N began showing up in the extractable soil pools, as other resources likely became limiting (e.g. light, water, phosphorous). Third, the difference observed in the magnitude of aboveground biomass increase between the two sites suggest that site-specific dynamics such as differences in plant communities, soil properties, or both, modulate the impact of a given input rate of N.

We hypothesize that the differential responses in aboveground biomass at the two sites stem from differences in plant-soil feedbacks influencing water availability. The soils in the degraded and mature sites were initially similar in many physiochemical properties; however, the mature site had consistently higher soil moisture in control plots during the 4 years of study, particularly from May to early August when plants grew most vigorously. High vegetation cover and abundant litter in the mature site likely increased the water-conserving capacity for both rain and snow (Bai *et al.* unpublished data), as compared with the degraded site. This relationship with water was supported by our observations that the greatest absolute responses in aboveground biomass were found in wet years (2002 and 2003, Bai *et al.*, 2008). A negative correlation between aboveground biomass response and soil moisture response in both the mature and degraded sites indicates that the productivity response

to N addition is dependent on soil moisture. Thus, these findings highlight the importance of coupled N and water biogeochemical cycles in grassland and dryland ecosystems (Lauenroth *et al.*, 1978; Hooper & Johnson, 1999; Knapp & Smith, 2001; Liu *et al.*, 2009).

Patterns of species richness

Similar to aboveground biomass, we found qualitative agreement between the two sites in the impacts of N addition on species richness, though quantitative differences were large. Specifically, we found a greater magnitude of species loss in the mature site compared with the degraded site. This was likely a result of differential responses of plant functional groups in the two sites mediated by N and water availability. These findings differ from Tilman (1987, 1993) which found a weaker overall response to N enrichment in a native savannah grassland compared to three abandoned agricultural fields. These varied responses are probably not due to any fundamental difference between legacies left by heavy grazing vs. agriculture, as both often deplete soil nutrients (Ritchie *et al.*, 1998; Brathen *et al.*, 2007). Rather, we suggest that the dynamics in both studies result from interactions with the availability of other resources. The weak response in native savannah of Minnesota as compared with nearby abandoned agricultural fields was explained primarily by less litter buildup in the native savannah

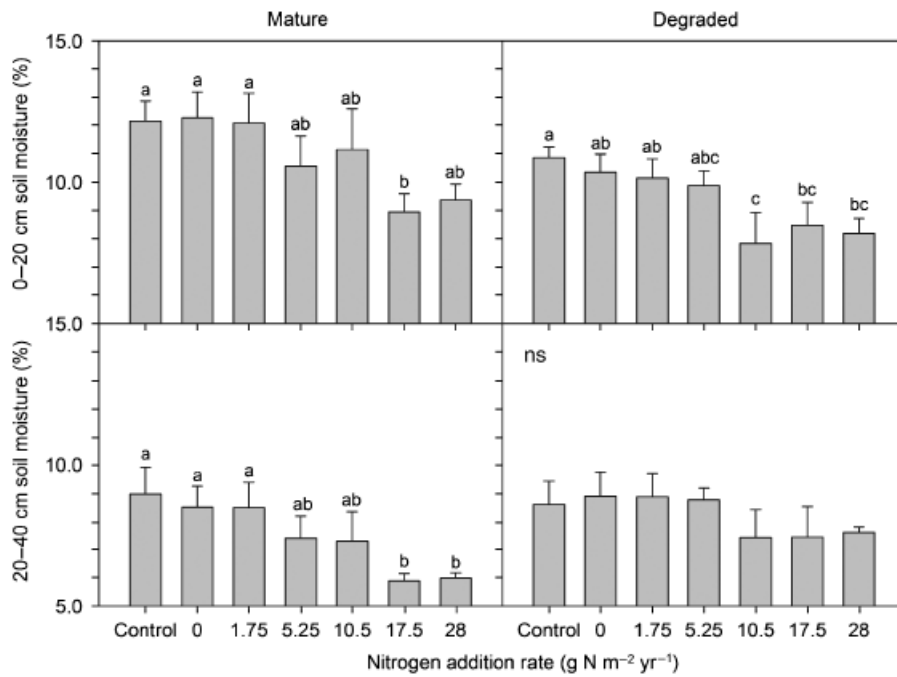


Fig. 6 Responses of soil moisture to N addition rates at the mature and degraded site (error bars denote SEM). The soil moisture was the average of the five replicates of a treatment. Bars with the same letter were not significantly different in Duncan's multiple range tests reported from one-way ANOVA.

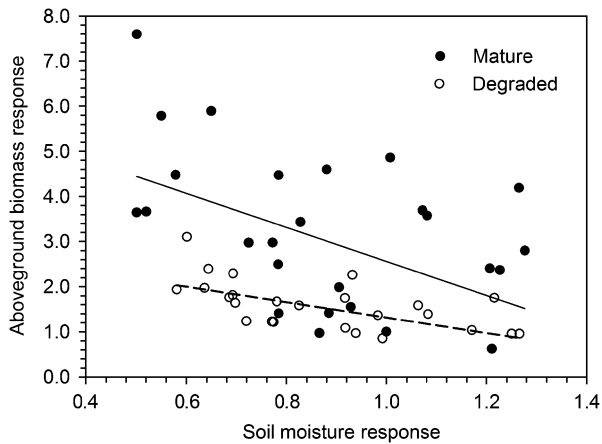


Fig. 7 The relationship between aboveground biomass response and soil moisture response in the mature and degraded sites.

due to periodic burning, resulting in more colonization sites and less potential for light limitation following N addition (Tilman, 1993). Light may have already been limiting in the mature site due to the accumulation of a thick litter layer; and, in the degraded site, heavy grazing likely prevented the accumulation of litter as forage was removed. The strong response in the mature site may have also been influenced by its higher soil moisture, resulting in more severe limitation by N availability alone. We found that species loss increased

linearly with production response, which explained 21% of the variation in richness response in the mature site. Regardless of the exact mechanism, our results demonstrate that natural sites are not generally less sensitive to N-induced reductions in diversity, and instead suggest that the relative responses between ecosystems and between communities within an ecosystem likely depend on interactions with other potentially limiting factors.

Shift in plant functional group composition

The most conspicuous effects of N addition were the shifts in aboveground biomass of different PFGs at the two sites. Surprisingly, fast-growing annuals that are usually abundant only in the early stages of grassland succession (Inouye *et al.*, 1987; Li & Xu, 2002), almost completely replaced the perennials in the mature site. The rapid growth of annuals (e.g. *Axyris amaranthoides*, *Chenopodium glaucum*, and *Cannabis sativa* L. f. *ruderalis*) was facilitated by their species traits, including abundant seed production, rapid growth, and tall stature. In contrast, the decline in abundance of perennials (e.g., *L. chinensis*, *S. grandis*, *Achnatherum sibiricum*, *Agropyron cristatum* and *Carex korshinskyi*) was attributable to their conservative resource-use strategies, with great leaf C:N and root:shoot ratios, high water use efficiency, and high nonstructural carbohydrates reserve in the below-

ground organs (Xu *et al.*, 1994; Chen *et al.*, 2001). These results support the functional-based hypothesis that N enrichment allows species with acquisitive resource-use strategies to exclude those with conservative resource-use strategies (Diaz *et al.*, 2004; Suding *et al.*, 2005). However, these dynamics clearly interacted with site characteristics, as no similar functional pattern was observed in the degraded site. At the degraded site, we found that the perennial rhizome grass (*L. chinensis*) became increasingly dominant as the experiment progressed. Annuals were unable to take advantage of the added N possibly due to insufficient soil moisture affecting germination, whereas drought-tolerant and rhizomatous perennials with high recruitment rates, including *L. chinensis* and *A. cristatum* (a C₃ bunchgrass with short rhizomes), benefited most from N enrichment (Chen *et al.*, 2001). The rapid dominance of annuals following N addition in other previously degraded sites was short-lived (<2 years, Tilman, 1987) likely because the perennial grasses were more dominant in the long run on these dry-sandy soils as opposed to the more moist soils of the mature grassland of Inner Mongolia. However, only long-term sampling of these plots will resolve whether dominance by annuals is transient in this ecosystem, or sustained as a result of less limitation by non-N resources. We suggest that it is the interplay between nitrogen and water that drives the differential responses of PFGs between the two sites, consequently governing the trajectory of ecosystem dynamics.

Implications for biodiversity conservation and ecosystem management

Our results clearly demonstrate that ecosystem sensitivity to low N input rates are likely to vary among ecosystems. In the mature site, production increased and species richness decreased significantly even at the 1.75 g N m⁻² yr⁻¹ treatment rate, an input rate comparable to estimated critical thresholds for several herbaceous communities of Europe (Bobbink *et al.*, 2002). Patterns in the degraded site were much less distinct, possibly resulting from several mechanisms, including a lower number of species initially present, different functional responses, or interactions with other resources.

It seems logical that at sufficiently low rates of N enrichment there is likely to be a net benefit to ecosystems. This apparently inflammatory statement is founded on two generally accepted propositions. First, it is likely that there exist ecosystem-specific thresholds for N enrichment, below which there are no negative ecological impacts as the extant community is capable of sequestering the additional N (Nilsson & Grennfelt, 1988; Bobbink *et al.*, 2002). Although the currently

estimated values of these thresholds have been called into question (Stevens *et al.*, 2004; Clark & Tilman, 2008), their hypothesized existence forms the backbone of a substantial body of environmental legislation on transboundary pollution (NEG-TAP, 2001). Second, Liebig's Law of the Minimum predicts that in N limited ecosystems, common throughout the temperate world, N addition is likely to stimulate plant production. Acceptance of these two propositions leads to the conclusion that there exist rates of N addition for which there are increases in production with no reductions in diversity. We found no strong evidence for this conclusion over the range tested, and indeed many studies find qualitatively similar responses at low vs. high N input rates (Morecroft *et al.*, 1994; Bowman *et al.*, 2006; Clark & Tilman, 2008). These findings suggest that critical thresholds may be lower than previously supposed, and highlight the urgency in assessing the impacts of the low N input rates experienced over much of the globe.

Similar to other studies, we detected evidence for the N addition treatment in the plant community (i.e. changes in production, richness, function) at lower rates than we detected changes in soil (i.e. changes in extractable inorganic N) (Wedin & Tilman, 1996; Bowman *et al.*, 2006). Specifically, we found increases in soil N at rates $\geq 10.5 \text{ g N m}^{-2} \text{ yr}^{-1}$ in both sites, but increases in biomass and decreases in richness at rates as low as 1.75 g N m⁻² yr⁻¹ in the mature site and at rates as low as 5.25 g N m⁻² yr⁻¹ in the degraded site. These findings support the suggestions of Bowman *et al.* (2006) that plant responses rather than soil responses may provide more sensitive measures of the impacts of human-caused N deposition.

Previous work in this system has demonstrated that *L. chinensis*, which had the highest relative biomass and lowest interannual variability of biomass production among all species at the undisturbed mature community, plays a key role in dampening ecosystem variability (Bai *et al.*, 2004). Thus, despite decreased richness reducing some the redundancy aspect of ecosystem resilience (Naeem, 1998), the increased dominance of *L. chinensis* may facilitate restoring the ecosystem resilience of degraded communities to precipitation fluctuations. Our findings were also corroborated by a recent study indicating that the increased production of dominant species compensated the negative effect of nonrandom species loss (Smith & Knapp, 2003). In the mature site, however, the decreased abundance of *L. chinensis* coupled with reductions in richness predicts a decrease in both aspects of ecosystem resilience.

Our results have important implications for restoring and managing the biodiversity and ecosystem functioning of the vast semiarid grasslands in the Eurasian Steppe region which stretches from eastern China in

Asia to Hungary in Europe. In mature and moderately grazed grasslands, N enrichment will generally increase aboveground biomass and decrease plant species richness. These changes will likely reduce ecosystem resilience to variation in precipitation because of declined species diversity and altered PFG compositions (Bai *et al.*, 2004). Though qualitatively similar, N enrichment to degraded or heavily grazed grasslands leads to small increases in aboveground biomass and decreases in richness. However, increased aboveground biomass, especially of rhizomatous perennial grasses, may reduce the nutrient and water losses through wind erosion and runoff (Li *et al.*, 2007), improve forage quality (Schellberg *et al.*, 1999), and increase grassland resilience to precipitation variability (Bai *et al.*, 2004). Clearly, there are a set of contrasting goals for restoration of ecosystem services (Suding *et al.*, 2004), among which the trade-offs may exist between the increases in productivity and forage quality vs. losses of biodiversity.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Soil moisture in control plots with depth for the mature and degraded sites averaged over all four years (error bars indicate SEM). Significant differences between sites are reported from one-way ANOVA as NS, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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