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Cumulative and partially recoverable impacts of nitrogen addition on a temperate steppe

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Abstract. Atmospheric nitrogen (N) deposition has been shown to decrease biodiversity and change nutrient cycles in terrestrial ecosystems. However, our understanding of ecological responses to chronic N addition and ecological recovery of grassland from N enrichment is limited. Here we present evidence from an 11-year grassland experiment with a range of N addition rates (0, 30, 60, 120, 240, and 480 kg N·ha⁻¹·yr⁻¹) in Inner Mongolia, China. Chronic N addition led to a reduction in species richness, Shannon diversity index, and soil pH and an increase in aboveground biomass, foliar N, and soil mineral N. High N addition rates (240 and 480 kg N·ha⁻¹·yr⁻¹) showed significant effects in the first and second years, which stabilized over time. Nitrogen addition at low rates (30 and 60 kg N·ha⁻¹·yr⁻¹) took longer (e.g., three years or more) to achieve significant effects. The negative impacts of high N addition (480 kg N·ha⁻¹·yr⁻¹) were reduced and species richness, Shannon diversity index, and soil pH showed a limited but rapid recovery with the cessation of N addition. Our findings suggest serious and cumulative impacts of N addition on plant and soil communities but the potential for partial system recovery over time if N inputs decline or cease.

Key words: aboveground biomass; ecosystem restoration; grassland; nitrogen deposition; species composition; species richness.

INTRODUCTION

Nitrogen (N) limitation to plant growth is widespread in terrestrial ecosystems (Vitousek and Howarth 1991). Global N enrichment, attributed to anthropogenic inputs from agriculture and industry (Galloway et al. 2008), can ameliorate N deficiency. However, increased N deposition is generally regarded as a key threat to global biodiversity, impacting ecosystem functioning and services through, e.g., soil acidification (Sala et al. 2000, Hooper et al. 2005, Niu et al. 2009). Research on impacts of N deposition at regional and field scales has identified multiple effects through surveys, in situ experiments, and meta-analysis, particularly using simulated N deposition experiments (Morecroft et al. 1994, Stevens et al. 2004, Bobbink et al. 2010, Duprè et al. 2010, Van den Berg et al. 2010, Phoenix et al. 2012). In general, N stimulates plant growth and increases net primary production by enhancing soil N availability (Phoenix et al. 2012). However, plants with different growth habits have differential responses to N addition due to their multiple N-use strategies (Klanderud and Totland 2005, Yang et al. 2011). These varied responses will result in limiting

resource (mineral nutrients, water, light, etc.) redistribution and ecological niche competition for both below- and aboveground processes (Grime 1973). For example, plant species that can quickly utilize available nutrients (e.g., grasses) generally benefit more from extra N than other species (e.g., forbs), leading to greater aboveground biomass and cover of these species and the inhibition of other species (Xia and Wan 2008, Song et al. 2011). In turn, enhanced plant growth drives more intense evapotranspiration and photosynthesis, thereby accelerating the depletion of soil mineral nutrients and water (Harpole and Tilman 2007, Lü and Han 2010). This adds to the negative effects of acidification and eutrophication (Silvertown et al. 2006, Hautier et al. 2014). Impacts have been observed on plant species (Clark and Tilman 2008), plant litter accumulation (Tilman 1993, Foster and Gross 1998), the seed bank (Basto et al. 2015), microbial enzyme activities (Kardol et al. 2010, Zeng et al. 2016), resource competition, and community composition (Klanderud and Totland 2005).

Much research has concentrated on the restoration of degraded ecosystems, with long-term experiments revealing varying degrees of recovery from the over-supply of N, in terms of plant diversity (Shi et al. 2014, Storkey et al. 2015, but compare Isbell et al. 2013), soil N pools (including soil mineral N, microbial biomass N, and total organic N), together with soil pH (O'Sullivan et al. 2011). A recent study suggested that the minimum

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time of recovery for grassland was between 1.8 and 20 years (Stevens 2016).

China is experiencing intense atmospheric N pollution (Richter et al. 2005) with annual bulk N deposition increasing from 13.2 kg N/ha in the 1980s to 21.1 kg N/ha in the 2010s (Liu et al. 2013). However, few studies on the impacts of N pollution and recovery have been made in which pollution has decreased, especially long-term research in grassland ecosystems in China. Considering that N deposition in China could profoundly influence nutrient cycling, biodiversity conservation and ecosystem services of grasslands, a continuous long-term experiment studying the effects of increased and decreasing N inputs is necessary.

Here, we conducted an in situ N addition experiment on a temperate steppe in China between 2005 and 2015 in order to (1) provide a comprehensive record of the response of plants and soil to various rates of chronic N addition and (2) explore the extent of recovery after cessation of N addition.

METHODS

Study site and experimental design

Our study was conducted at a semiarid temperate steppe in Duolun County (116°17' E, 42°02' N, 1324 m above sea level), Inner Mongolia, China, part of the Eurasian steppe. From 2005 to 2015, annual precipitation (rain only; snow was not included over the experimental period) averaged 316 mm with 72% falling during the growing season from May to August. The mean annual temperature was 3.3°C and the temperature during the growing season averaged 16.5°C, ranging from 15.5°C in 2015 to 17.5°C in 2013. The local wet/dry N deposition was approximately 10.0 (58% NH_4^+ , 42% NO_3^-) and 5.1 (64% NH_x , 36% NO_x) kg $\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ during 2010–2014 and 2013–2014, respectively (Xu et al. 2015). The soil consists of 62.8% sand, 20.3% silt, and 16.9% clay. According to the FAO classification, the soil is a Haplic Calcisol. The bulk density of the topsoil (0–10 cm) was 1.31 g/cm^3 and the original soil pH (1:2.5, soil:solution ratio in H_2O) was 7.12. The site is a typical steppe community of Mongolian-Manchurian grassland, belonging to the Eurasian steppe. The common grass-forb-legume classification (Tilman et al. 1997, Hector et al. 1999) was used to assign to species. Further and continuous classifications, based on measured or referenced plant traits (such as plant life span, photosynthesis types, and maximum plant height), were conducted to provide more clarity on species diversity (see also Appendix S1: Table S1). The plant species comprise three functional groups with dominant species: grasses (*Stipa capillata*, *Agropyron cristatum*, *Cleistogenes squarrosa*, and *Leymus chinensis*), non-leguminous forbs (called forbs for short in this study; *Artemisia frigida*, *Carex tibetica*, *Potentilla acaulis*, *Potentilla tanacetifolia*, and *Potentilla bifurca*), and legumes

(*Melissilus ruthenicus*, *Astragalus galactites*, *Astragalus scaberrimus*, and *Lespedeza bicolor*).

Five (2005) or six (2006 and after) N addition treatments with five replicates each were established in 2005. The 30 5 × 5 m plots were randomly arranged with a 1-m buffer strip between adjacent plots. N application rates were 0 (N0 as control), 30 (N30, from 2006), 60 (N60), 120 (N120), 240 (N240), and 480 (N480) kg $\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$. N addition was applied as urea in 2005 and NH_3NO_4 from 2006 onward; N was applied to the N480 plots only during 2005 and 2008. There was no N addition to the N480 plots after 2008 so that any ecological restoration could be observed. N was uniformly applied to the plots in three equal amounts at the beginning of June, July and August. The continuous experimental records covered 2005–2015 except for 2007 and 2012 when no data were collected. In these two years, N was applied to all the plots as usual, but with no sampling or measurements.

Sampling and measurement methods

Precipitation data and samples during the observation period were collected using a nearby ombrometer. Temperature data was collected from a local automatic meteorological station. Vegetation sampling accompanied by species analysis was conducted in late August during peak aboveground biomass in 2005, 2006, 2008–2011, 2013–2015. Aboveground biomass and individual number of each species in each plot were measured in a randomly selected quadrat (1 × 1 m), inserted above the canopy. The aboveground biomass of each plant species in each quadrat was cut just above the soil surface, including living and standing dead plants. Each plant species was sampled separately. After oven-drying at 65°C for 48 h, plant samples were weighed on an electronic balance (accuracy ± 0.01 g) for dry matter. Species richness was calculated as the total number of species types within the quadrat. Based on Tilman (1997), and as explained above, plant species are classified into three functional groups (grasses, forbs, and legumes) to analyze the richness, Shannon diversity index, and aboveground biomass. Plant samples were oven-dried and ground to measure foliar N concentration using Kjeldahl digestion (Horwitz 1965) for grasses and forbs.

Soil samples, which were taken from five sampling points per plot at two depths (0–10 cm and 10–20 cm), were collected with a semicylindrical stainless steel auger of 5 cm inner diameter after vegetation sampling in 2005, 2006, 2008–2011, and 2013–2015. The samples were immediately frozen at –20°C and stored for the analysis of soil mineral N ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) and pH. For soil mineral N, 12 g of fresh soil was shaken with 100 mL 2 mol/L KCl (Guaranteed Reagent, Sinopharm Chemical Reagent Co., Ltd., Beijing, China), filtered and analyzed on an AA3 continuous flow analyzer (Bran + Luebbe GmbH, Norderstedt, Germany). Soil water content (%) was measured by oven-drying at 105°C; soil mineral N (kg N/ha) was calculated from the measured $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$

concentrations, soil water content, and soil bulk density. For soil pH, 10 g air-dried soil was shaken with 25 mL deionized water for 0.5 h, allowed to settle for 0.5 h, and the pH of the supernatant measured.

Data evaluation

Assessment of diversity.—The Shannon diversity index (H') is expressed as

$$\sum_{i=1}^n (-P_i \times \ln(P_i))$$

where P_i is the relative number of each species (number of species i /total number of all species \times 100) (O'Connor et al. 2002).

Beta-diversity was calculated using PCoA (Tuomisto and Ruokolainen 2006) in R (R Core Team 2014).

Assessment of species loss.—Proportional species loss was taken as species number in the control plot minus species number in the N treated plot, divided by the species number of control plots.

Statistical analysis

Repeated-measures analysis of variance (ANOVA) was used to test the effects of N addition on the species richness, Shannon diversity index, aboveground biomass and soil mineral N. When the ANOVA results were significant, the Tukey's Honestly Significant Difference (HSD) test was used to determine the significance of the difference between means with a significance level of $P < 0.05$. Two-factor ANOVA was performed to test the effects of N addition, precipitation, and their interaction on species richness and aboveground biomass of the community and three functional groups (grasses, forbs, and legumes), where F test values and P values are given. Linear regression was performed to identify the strength of N addition on the variation of species loss during two observation periods (2005–2008 and 2013–2015). Partial correlation was used to remove the effect of climate (only precipitation in this study) over the effect of nitrogen. Coefficient of determination was used to test the linear correlations with a significance level of $P < 0.05$. All the statistical analyses were conducted using IBM SPSS Version 23.0 (IBM Corporation, Armonk, New York, USA). Figures were drawn using Microsoft Excel 2010 (Microsoft, Redmond, Washington, USA).

RESULTS

Species richness, alpha and beta diversity

Chronic N addition decreased plant species richness (SR) and Shannon diversity index (H') compared with the control (Fig. 1a, b). Compared to the control plots, significant declines in SR and H' generally occurred

when N addition exceeded 120 kg N·ha⁻¹·yr⁻¹ from 2008 and 2009, respectively.

During the observation period, the low N additions (30 and 60 kg N·ha⁻¹·yr⁻¹) caused non-significant 6.6% and 4.0% species losses, respectively, but high N addition rates (120 and 240 kg N·ha⁻¹·yr⁻¹) significantly reduced species numbers by 22.2% and 40.9%, respectively. Species loss for 2013–2015 showed a stronger and more significant ($P < 0.001$) positive relationship (slope = 0.0027, $R^2 = 0.73$) with N addition than that for 2005–2008 (slope = 0.001, $R^2 = 0.23$; Fig. 2). Different functional groups had different responses to N addition. The SR of grasses always remained constant at approximately 3.5 in all plots. However, with increasing N addition, the SR of forbs and legumes decreased significantly with time (Fig. 3). N addition (N) and precipitation (P) showed significant impacts on the SR of the community and three functional groups (Appendix S1: Table S2). The interaction of N addition and precipitation also significantly reduced the SR of the community, forbs, and legumes ($P < 0.01$). However, after partial correlation to remove the effect of precipitation, the effect of N addition on the SR of grasses weakened with a decline in the P value from <0.001 to 0.013 (Appendix S1: Table S3). There were no changes for the SR of community, forbs, and legumes.

Changes in the species richness of different groups is presented according to the species classification based on plant traits in Appendix S1: Fig. S1. Compared to the control plots, C₃ species and perennial species significantly decreased in the plots with high N addition rates (≥ 120 kg N·ha⁻¹·yr⁻¹) from 2010, while those in the N60 plots showed a non-significant decline in the last two years. The number of low-growing species (0–25, 25–50 cm) significantly decreased in N240 and N480 plots from 2010 and in the N120 plots from 2014. Taller species (>50 cm) remained stable except in 2010 and 2015.

On the N480 plots where N addition caused a significant decline in SR during the first four years (2005–2008), when N addition ceased, a rapid recovery of SR at the community level was observed with no significant differences with the control plots in 2011. The SR then stabilized at a level similar to that of N120 from 2011 to 2015. A similar recovery was observed in H' and SR of specific groups (C₃, perennial, low height). The final H' of N480 was between that of N60 and N120 and similar to that of N120 with regard to the SR of specific groups.

Annual Beta diversity at the different N addition rates, representing the differentiation between habitats, is shown in Fig. 4. We found no differences between treatments, but it did vary between years.

Aboveground biomass (AGB) and foliar N concentration

High N addition (over 120 kg N·ha⁻¹·yr⁻¹) significantly increased aboveground biomass (Fig. 5a). The AGB of grasses (AGB_{grass}) increased with N addition (Fig. 5b). AGB_{grass} significantly increased at high N addition rates (240 and 480 kg N·ha⁻¹·yr⁻¹) in the first

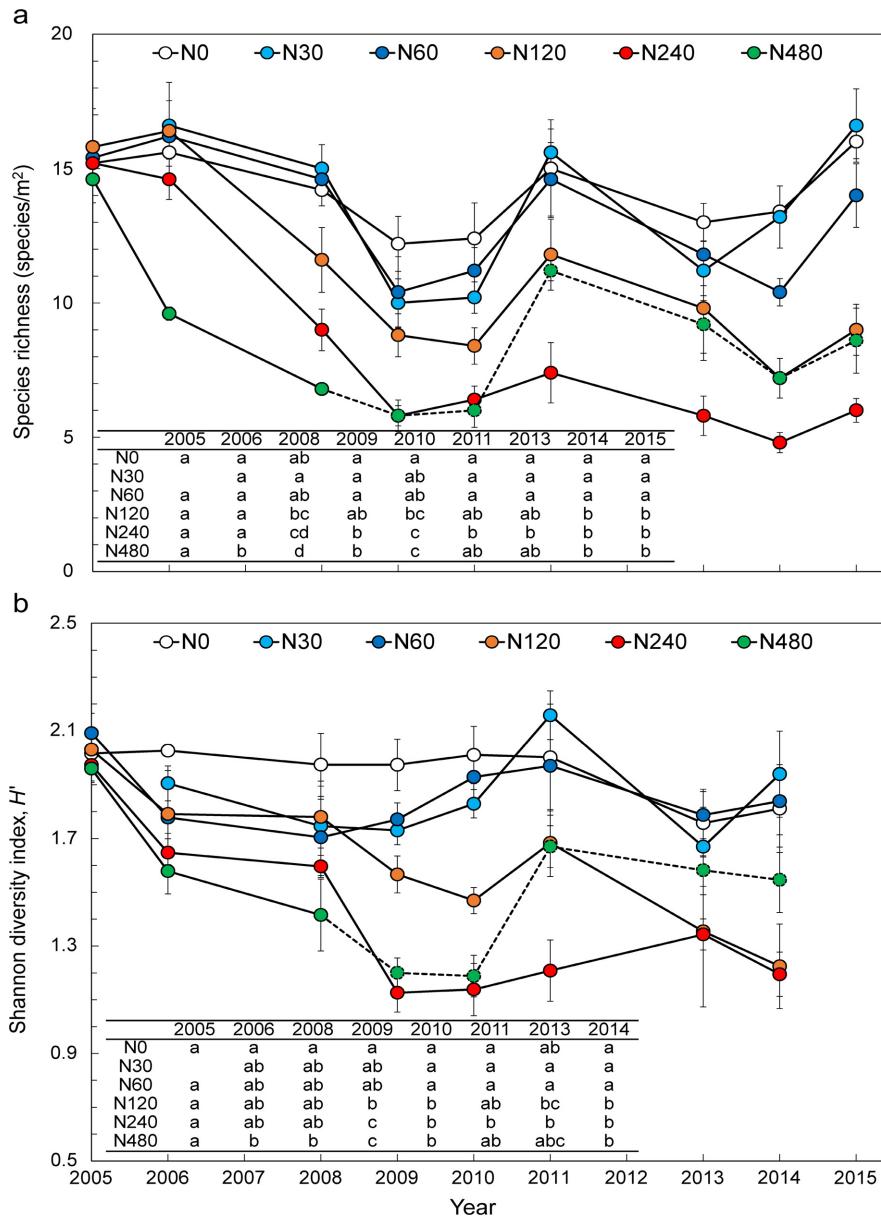


FIG. 1. Temporal trends in plant diversity between 2005 and 2015: changes in (a) species richness and (b) Shannon diversity index for the different N additions through time. Dashed line in N480 (480 kg N·ha⁻¹·yr⁻¹) shows that N application ceased after 2009 (same below). Enclosed tables in the two figures represent the differences between treatments at $P < 0.05$; cells sharing a letter are not significantly different. Note: missing data for H' in 2012 and 2015 resulted from the lack of sampling and analysis. [Color figure can be viewed at wileyonlinelibrary.com]

four years. AGB_{grass} for N120 and N60 was not significantly different from that of N240 in 2010 and 2011. AGB_{grass} had become saturated after seven years of chronic N addition at N60, and showed no further increase even at higher N addition rates. For N480, the highest AGB_{grass} occurred in 2008, after which it decreased until it reached the same level as that of N30 in 2013, due to the cessation of N addition. Compared to grasses, the AGB of forbs (AGB_{forb}) fluctuated with increasing N addition (Fig. 5b). It increased with

increasing N addition in the first year of the experiment, but then showed a negative relationship with N addition until 2009. In the following years, except for 2011, the middle N addition rates (60 and 120 kg N·ha⁻¹·yr⁻¹) always had the lowest AGB_{forb} . AGB_{forb} of N480 showed a rapid decline from 89.7 g/m² in 2005 to 9.7 g/m² in 2006, and remained low until 2009 (the second year after cessation of N addition), after which it quickly returned to the level of the control plot. Compared to grasses and forbs, the AGB of legumes (AGB_{legume}) was

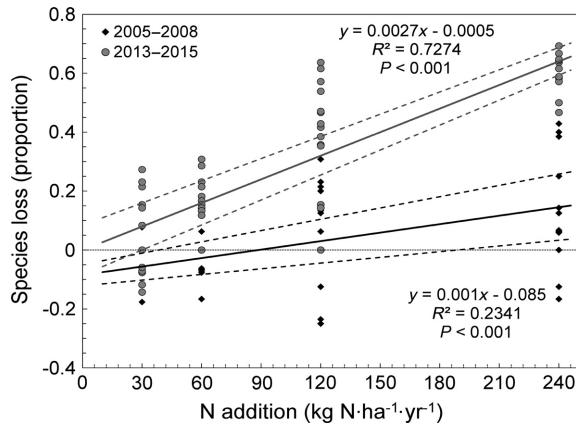


FIG. 2. Proportional species loss by N addition rates for 2005–2008 and 2013–2015. (All plot values during the three-year observation period are fitted to a linear curve with 95% confidence.) [Color figure can be viewed at wileyonlinelibrary.com]

very low and did not show significant change with N addition except in 2015, when there was a significant decline in the AGB_{legume} in N120, N240, and N480.

Foliar N concentrations of both grasses and forbs in all plots (except N480) increased and showed a strong positive response to N from 2008 to 2010 (Appendix S1: Fig. S2), but then remained constant. After 2011, no significant differences in the foliar N concentrations of grasses were found between N treated plots, but those of forbs in N60, N120 and N240 were still significantly higher than those of the N30 and control plots. The highest foliar N concentration occurred in N480 in 2008, followed by N240 in 2009 and finally N120 in 2013, showing that it took longer for foliar N to increase in the lower N application treatments. Foliar N concentration in the N480 plots declined from the peak of 2008 and recovered to the control (N0) level after 2010.

Soil mineral N and pH

Soil mineral N in both soil layers in each year increased with increasing N addition; soil mineral N in the 0–10 cm layer was higher than that in the 10–20 cm layer (Appendix S1: Fig. S3). Soil mineral N in control plots ranged from 9.4 kg N/ha in 2009 to 19.8 kg N/ha in 2013. Soil mineral N in the N120 and N60 plots was

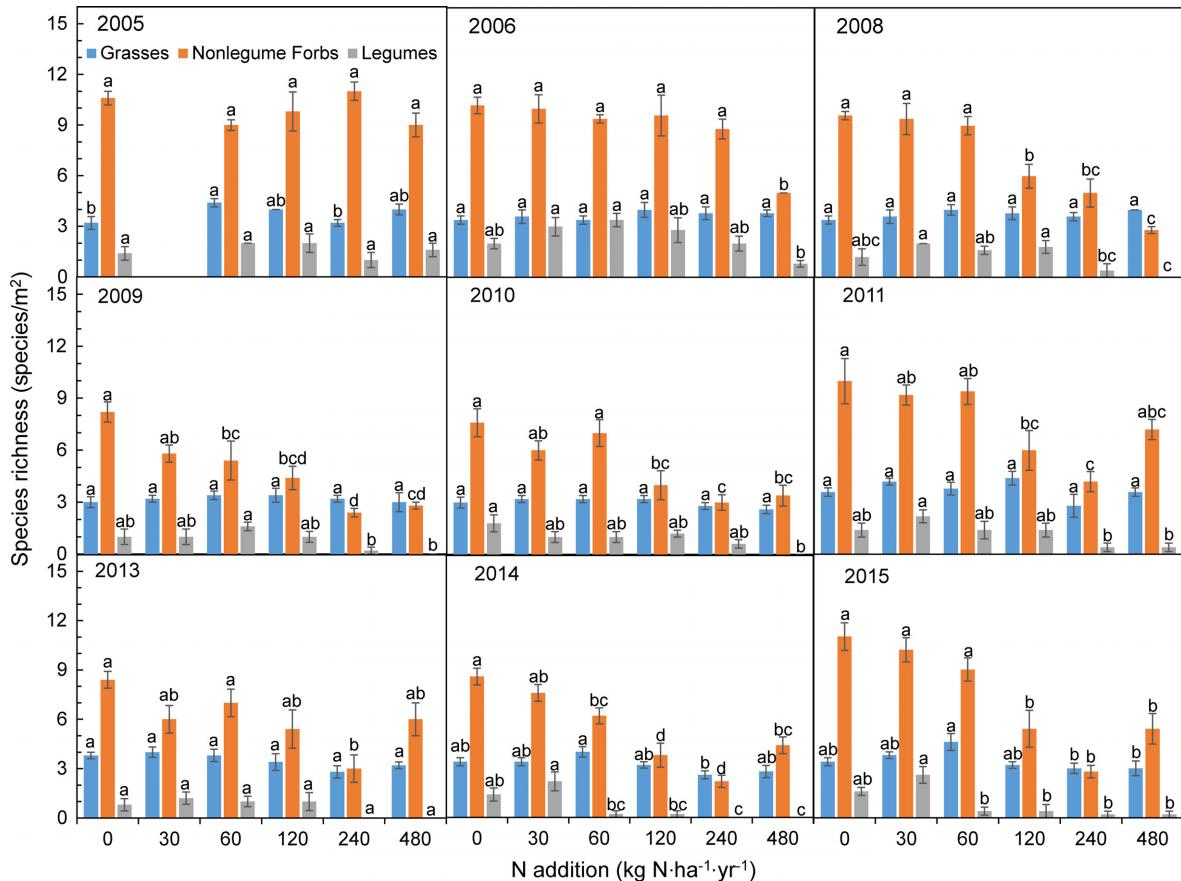


FIG. 3. Effect of N addition on species richness of grasses, forbs and legumes from 2005 to 2015. Values are mean \pm standard error (SE). Lowercase letters on bars indicate the differences between treatment at $P < 0.05$. [Color figure can be viewed at wileyonlinelibrary.com]

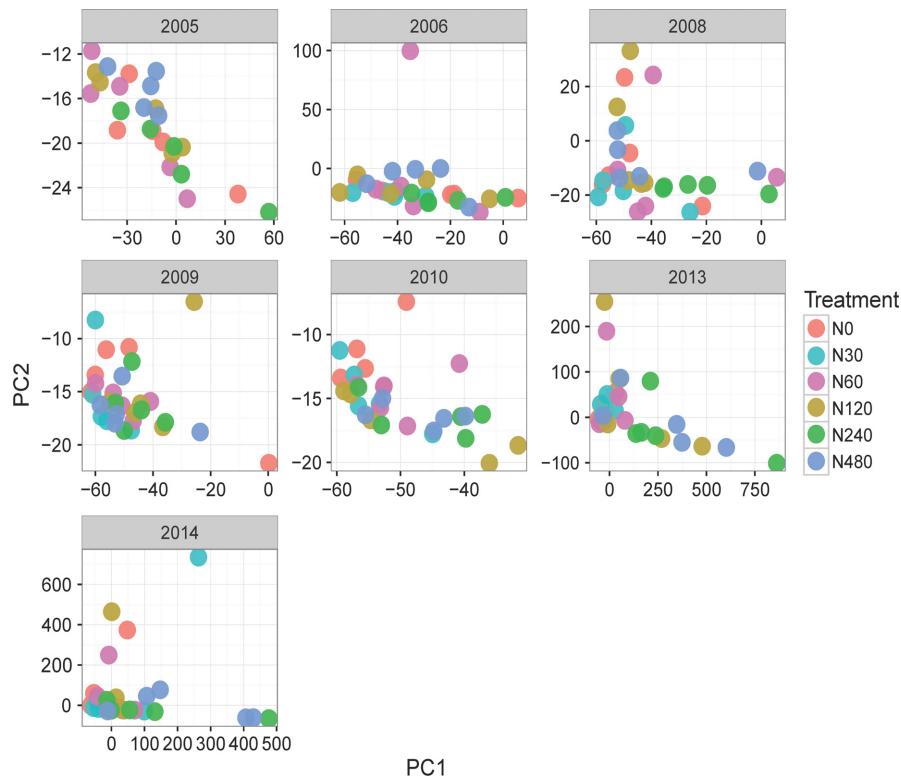


FIG. 4. Principal coordinate analysis (PCoA) shows the changes in beta-diversity through time for the different N addition rates. Note: missing data for 2011 and 2015 resulted from the lack of sampling and analysis. [Color figure can be viewed at wileyonlinelibrary.com]

significantly higher than that in the control plots after 2008 and 2009, respectively. Soil mineral N of the N480 plots in the 10–20 cm layer was significantly lower than N240 plots for the first time in 2009, the first year after the cessation of N addition, but it was still highest in the 0–10 cm layer. In the following five years after N application ceased, mineral N in both soil layers of the N480 plots significantly declined and returned to the control level by 2013. N240 had the highest soil mineral N level after 2010 with most in the top (0–10 cm) layer.

Soil pH in the 0–10 cm layer of the control plots did not change from 2005 to 2015 (Appendix S1: Fig. S4) but decreased with increasing N addition. No significant change in soil pH occurred at 10–20 cm (data not shown).

DISCUSSION

Species richness and diversity

During the observation period, N addition at high rates (N120 and N240) led to an obvious species loss (Fig. 1a), as others have found (Stevens et al. 2004, Clark et al. 2007, De Schrijver et al. 2011, Song et al. 2011, Zhang et al. 2014). However, N addition at low rates (N30 and N60) did not significantly reduce species richness at the community level. This suggests that the critical load of N addition for species loss is between 60 and 120 kg

$\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ at our site over the 11-year study period (effects might have been observed had the experiment continued for longer). This is much higher than the threshold found by Clark and Tilman (2008), where experimental addition of 10 and 34 $\text{kg}\ \text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ led to a significant plant species loss after nine and five years of the experiment, respectively. The difference was possibly due to the differences in grassland properties, functional responses, and resource interactions on the Eurasian grasslands (Bai et al. 2010). Bai et al. (2010) found that the N-induced species loss was lower in a mature healthy grassland (17.5 $\text{kg}\ \text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$) than in a degraded grassland (52.5 $\text{kg}\ \text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$). Soil spatial heterogeneity, such as phosphorus and/or water availability could also limit the effect of N (Li et al. 2015). As in other experiments, we found that species loss with N addition increased over time (Fig. 2). Short-term experiments will almost certainly underestimate the impacts of continuous N addition, and species loss is likely to occur over time as the impact of low N inputs accumulated, as found by Clark and Tilman (2008).

Species with different functional groups or traits had different responses to N addition. In the grass-forb-legume classification, the species richness of grasses is regarded as insensitive to N addition, but we found forbs to be very sensitive, being the primary contributor to species loss (Fig. 3). Nitrogen input will cause competition to shift to limiting factors other than N (Cleland

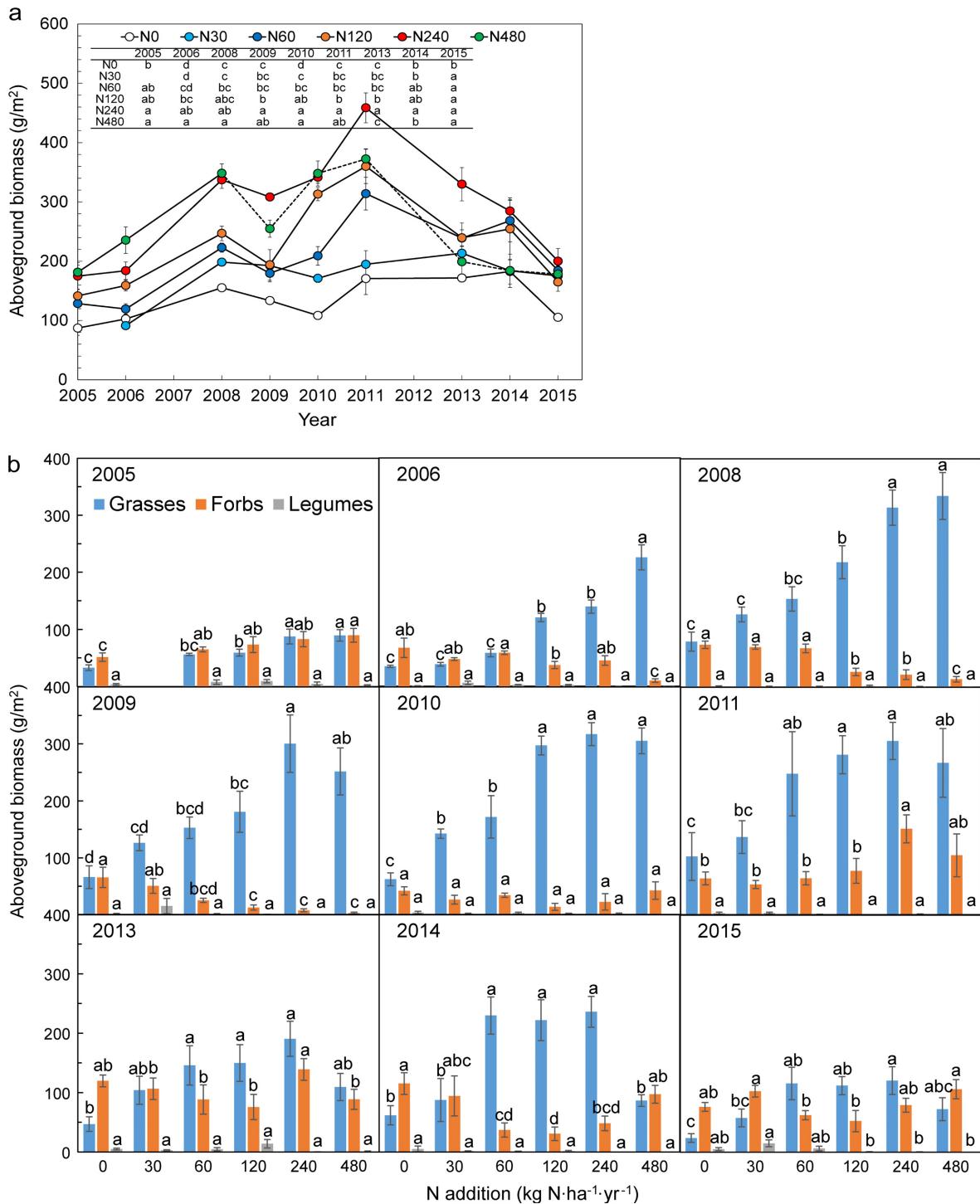


Fig. 5. Effect of N addition on aboveground biomass of (a) community and (b) three functional groups from 2005 to 2015. Values are mean \pm SE. Lowercase letters on bars indicate treatment differences between treatments at $P < 0.05$. [Color figure can be viewed at wileyonlinelibrary.com]

and Harpole 2010). The faster growing and taller canopy of grasses (Suding et al. 2005) stimulated by N can acquire an aboveground advantage in competing for other resources (e.g., light and space), and inhibit or

even exclude other species such as forbs and legumes (Hautier et al. 2009, Borer et al. 2014). Legumes are normally sensitive to N addition (Huenneke et al. 1990, Suding et al. 2005, Clark et al. 2007). In natural

grassland, legumes can improve the soil N status, enhancing the growth of other herbage, especially under N-limiting conditions (Ledgard and Steele 1992). However, legumes were extremely rare at our site, with an SR of approximately 1 during 2005–2015, so the change in SR_{legumes} could not reliably reflect the effect of N on legumes. However, our results support the common finding that forbs are more sensitive to N addition than grasses, resulting from different resource competition caused by different N-use strategies of grasses and forbs. In addition, the accumulated, thick litter layer, resulting from the absence of mowing, also leads to a limitation of light (Bai et al. 2010), which can be corrected by litter removal followed by an increase in species diversity (Bonanomi et al. 2006). Turning to the trait-based classification, the consistent SR of annuals/biennials (AB) benefitted from their ample seed production, rapid growth, and tall stature. In contrast, the decline in SR of perennials was due to their conservative resource-use strategies, e.g., high foliar C:N ratio, high water use efficiency, and so on (Xu et al. 1994, Bai et al. 2010). Generally, N enrichment stimulates species with acquisitive resource use strategies and excludes conservative species (Diaz et al. 2004, Suding et al. 2005). C_4 species at our site mainly comprised annuals with high maximum plant heights and one dominant grass (*C. squarrosa*), which was insensitive to N addition. In contrast, C_3 species with a high proportion of perennials declined with N addition. Short species were more sensitive to N addition than taller species because of light competition. Nitrogen, precipitation and their interaction significantly influenced the species richness of the community and different functional groups, but N alone significantly affected the SR ($P < 0.05$).

Shannon diversity index takes into account both abundance and evenness of the species (Kent and Coker 1992). N addition had a negative effect on H' in this study (Fig 1b), similar to previous studies on grassland (Van Den Berg et al. 2011, Bassin et al. 2013, Humbert et al. 2016). Beta diversity was variably affected by N addition for several reasons. First, Beta diversity may decrease because of reducing micro-environmental heterogeneity in soil available N, favoring species with high adaptability to N (Huston 1979, Tilman 1988, Wisheu and Keddy 1992). Second, the increased AGB may elevate Beta diversity via more alternative community states and/or the effect of temporal stochasticity on species composition (Chase and Leibold 2002, Chase 2003). Third, Beta diversity is productivity dependent under natural conditions, increasing (decreasing) with low (high) productivity (Holt et al. 1994, Van de Koppel et al. 1996, Chalcraft et al. 2008). The differences in Beta diversity between years might be due to the variation in precipitation.

N-induced soil acidification (Appendix S1: Fig. S4) contributed to reduced species diversity (Stevens et al. 2010, Van Den Berg et al. 2011) through (1) increased H^+ and Al^{3+} , which directly damage roots (Kochian

1995, Van Den Berg et al. 2005, Poschenrieder et al. 2008) and soil organisms (e.g., microbial and nematode; Kuperman and Edwards 1997, Raty and Huhta 2003, Rousk et al. 2010), which can in turn affect plant diversity (Van Der Heijden et al. 2008, Bardgett and Wardle 2010); (2) soil nutrient deficiency because of reduced soil exchangeable base cations (e.g., Ca^{2+} , Mg^{2+} , K^+ and Na^+) via leaching (Kochian 1995, Kuperman and Edwards 1997, Kochian et al. 2004).

Aboveground biomass

N input increased AGB via increased plant growth, being more rapid and significant at high N addition rates while the low rates took longer to achieve the same effect for specific functional groups (Fig. 5a, b). AGB had become saturated at N addition $\geq 120 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ from 2008 onward (the fourth year of the experiment), as was found with an N addition of $105 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ in a degraded grassland at high altitude compared to an impact of $17.5 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ at a mature site at lower altitude (Bai et al. 2010). Patterns of AGB in different functional groups varied. AGB_{grass} was positively correlated with increased N addition. After 11 years of the experiment, a significant increase in AGB_{grass} was found for N addition rates $\geq 60 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$. Low N addition rates over time (Humbert et al. 2016) or via increasing addition frequency (Zhang et al. 2015) had similar effects to those of short-term high N addition rates. In contrast, AGB_{forb} showed an inconstant pattern with N, with a negative relationship before 2009 but then an irregular fluctuation after 2010. This might be caused by the combined impact of competition from grass growth and ecological niche redistribution of forbs, rather than N per se. Grasses that can utilize nutrients quickly benefit more from N addition than forbs, as well as being more competitive for ecological niches both below and above ground (Grime 1973, Xia and Wan 2008). Until 2009 when N addition ceased, grasses had the advantage as N addition impeded the growth of forbs and many forbs, generally in the understory, were excluded. After 2010, the “abandoned” ecological niche (James et al. 1984) caused by the loss of forbs was probably reused by other forbs with a similar ecological niche but a higher tolerance to resource competition, explaining the increase in AGB_{forb} in later years. However, the AGB_{legumes} were hardly influenced by N addition due to their extremely low productivity.

Precipitation drove variations in biomass accumulation (Bai et al. 2004, Heisler-White et al. 2008). A significant relationship between AGB and precipitation was found (Appendix S1: Table S2). Although the impact of increased precipitation on AGB would probably be limited by soil N availability (Ren et al. 2017), removing the effect of precipitation weakened the N effect on some functional groups (forbs at our site) (Appendix S1: Table S3). Snow addition, which was not included in our study, can also effect vegetation growth and increase

AGB though increasing precipitation, but its impact is not as effective as that of rainfall (Chimner et al. 2010).

Foliar N, soil mineral N, and pH

N addition increased foliar N concentrations in both grasses and forbs, but with no further increase when N had saturated the plant–soil system, as indicated by a stable AGB and a large N surplus in the soil. The increase of foliar N concentrations in the control plots may result from the background atmospheric N deposition ($15.1 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ in total deposition; Xu et al. 2015) and soil N mineralization ($4.9 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ in the topsoil 0–20 cm of our site; Song et al. 2011), combined with the absence of cutting throughout the experiment, and so N recycling. Hence the total N input via deposition and mineralization ($20 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$, not including N recycling from litter decomposition) was enough to meet the annual plant uptake ($17.3 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$). Thus increased N deposition across China (Liu et al. 2013) can provide most of the N for grazed grasslands, with a little mining of soil N.

It is interesting to note that, in the N30 plots, the foliar N content of grasses tended toward that of the other N treated plots, but that of forbs was always close to that in the control plots (Appendix S1: Fig. S2). This indicated that N was still limiting in N30, there being not enough for forbs after “feeding” the competitive grasses. On the other hand, the foliar N of forbs in N60 increased and reached the level of that in those receiving high rates of N during 2010 and 2013, when the $\text{AGB}_{\text{grasses}}$ had become saturated. Unfortunately, we did not observe the turning point because of missing data in 2011 and 2012.

Increased N addition significantly elevated soil mineral N (Appendix S1: Fig. S3). However, in control plots with limited disturbance, soil mineral N (e.g., NH_4^+ and NO_3^-) showed a large fluctuation, especially for topsoil (0–20 cm). The topsoil N_{min} ranged from 9.4 to 19.8 kg N/ha, possibly due to continuous soil N mineralization, deposition, N recycling, and dust from adjacent N-treated plots. The NH_4^+ surplus would have been retained in the soil and nitrified to NO_3^- or lost via NH_3 volatilization; NO_3^- surplus was generally limited by denitrification and leaching (De Vries and Breeuwsma 1987). Nitrification and NH_3 emissions are important H^+ production processes if NO_3^- is lost via leaching rather than taken up by plants (an H^+ consumption process), causing soil acidification (De Vries and Breeuwsma 1987). Normally, surplus mineral N is retained in the soil because of limited water supply in this arid and semiarid area. However, heavy rain (daily rainfall >20 mm) during the growing season occurred two to four times per year during 2006–2015, causing large NO_3^- leaching losses and further soil acidification. Our results (Appendix S1: Fig. S4) showed that the topsoil (0–10 cm) pH significantly decreased with increasing N addition. Soil pH in the N240 plots declined by two units to from 7.1 at the beginning to about 5.0 in 2010.

The decline in soil pH would reduce plant diversity (Chen et al. 2013) via direct H^+ toxicity and further nutrient deficiencies (Van Miegroet and Cole 1984, Kochian et al. 2004) as soil exchangeable base cations were leached, especially for non-calcareous soils with weak acid neutralization capacities (soil pH < 6.5; Ulrich 1986, De Vries et al. 1989).

Recovery after cessation of N addition

During the observation period, species richness in the unfertilized control plots fluctuated within a range of 80–105% of its original level in 2005. SR in the N480 plots decreased to 45% (48%), 38% (48%), and 40% (48%) of the original (control) level in 2008 (the last year with N addition), 2009, and 2010 (the first two years after N addition ceased), respectively, then increased to about 74% (75%) in 2011 (the third year; Appendix S1: Fig. S5). SR continued to decrease to 50–60% (50–70%) during the most recent three years. At the same time, SR in N240 continued to decrease from 59% (63%) in 2008 to 39% (38%) in 2015. There are two long-term experiments that are relevant to discussions of recovery. One was conducted at a successional grassland at Cedar Creek Ecosystem Science Reserve (Isbell et al. 2013), showing a very limited recovery of plant diversity 20 years after high N addition ($95, 170, 270 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$) ceased. The ratio of the SR of high N addition rates to that of the control plots increased from about 45% in 1991 (the last year of N addition) to about 60% in 2011 (20 years after high N addition ceased). The second long-term experiment is the Park Grass experiment at Rothamsted Research (Storkey et al. 2015), which shows that biodiversity can recover from chronic N inputs. The plant diversity declined to 30% of the original level after 135 years of experimental N addition ($96 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$), then recovered to about 70% of the original level 20 years after N addition was stopped, approximately equal to 88% of the control level in that year (Tilman and Isbell 2015). Our results are more similar to the findings of Isbell et al. (2013). The weak recovery was possibly attributed to the absence of mowing and herbage removal and so further N recycling (Tilman and Isbell 2015). Even if recovery was limited, it quickly achieved a comparable restoration only seven years after N addition ceased as that of the Cedar Creek experiment after two decades. There were also other recovery signs, including (1) both foliar N concentrations (Appendix S1: Fig. S2) and soil mineral N (Appendix S1: Fig. S3) decreased to the control level, (2) plant diversity (SR and H') recovered somewhat, and (3) soil pH stabilized. Our results suggest a limited but fast ecological recovery in the N480 plots as N addition ceased.

CONCLUSIONS

Nitrogen input is a critical factor in ecosystems. Increasing N addition promotes aboveground biomass, foliar N

and mineral N, but a decline in species richness, Shannon diversity index (H'), and soil pH when N input exceeds a threshold. Those influences also increase progressively with time: N addition at low rates takes time to achieve significant effects on species diversity or aboveground biomass compared to those of high rates in shorter times. Our research supports other findings that decreased species diversity can be recovered somewhat, as can declines in aboveground biomass and increases in foliar N and soil mineral N, if N inputs stop or are reduced. We thus conclude that N deposition effects on temperate steppe grasslands are cumulative with time and possibly reversible as N deposition inputs decline. However, further experimental evidence is needed to determine (1) whether plant diversity can recover to the original or control level in the next decade or so and (2) whether the low-diversity state accompanying N addition is stable and will persist.

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SUPPORTING INFORMATION

Additional supporting information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/eap.1647/full>

DATA AVAILABILITY

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.c52kp>