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Nitrogen enrichment enhances the dominance of grasses over forbs in a temperate steppe ecosystem

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Abstract

Chinese grasslands are extensive natural ecosystems that comprise 40 % of the total land area of the country and are sensitive to N deposition. A field experiment with six N rates (0, 30, 60, 120, 240, and 480 $kg\,N\,ha^{-1}\,yr^{-1}$) was conducted at Duolun, Inner Mongolia, during 2005 and 2010 to identify some effects of N addition on a temperature steppe ecosystem. The dominant plant species in the plots were divided into two categories, grasses and forbs, on the basis of species life forms. Enhanced N deposition, even as little as 30 $kg\,N\,ha^{-1}\,yr^{-1}$ above ambient N deposition (16 $kg\,N\,ha^{-1}\,yr^{-1}$), led to a decline in species richness. The cover of grasses increased with N addition rate but their species richness showed a weak change across N treatments. Both species richness and cover of forbs declined strongly with increasing N deposition as shown by linear regression analysis ($p < 0.05$). Increasing N deposition elevated aboveground production of grasses but lowered aboveground biomass of forbs. Plant N concentration, plant $\delta^{15}N$ and soil mineral N increased with N addition, showing positive relationships between plant $\delta^{15}N$ and N concentration, soil mineral N and/or applied N rate. The cessation of N application in the 480 $kg\,N\,ha^{-1}\,yr^{-1}$ treatment in 2009 and 2010 led to a slight recovery of the forb species richness relative to total cover and aboveground biomass, coinciding with reduced plant N concentration and soil mineral N. The results show that N deposition induced changes in soil N transformations and plant N assimilation that are key to changes in species composition and biomass accumulation in this temperate steppe ecosystem.

1 Introduction

Atmospheric nitrogen (N) deposition has become a global concern because of its potential influence on ecosystem productivity, biodiversity and function, especially in many N deposition "hotspots" worldwide (Phoenix et al., 2006). Anthropogenic reactive N emissions from intensive livestock production, chemical fertilizer N application and

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combustion of fossil fuels and biofuels have accelerated global N deposition (Galloway et al., 2008). Many terrestrial ecosystems worldwide are therefore facing an N-enriched environment which may lead to rapid changes in plant production and species richness induced by acidification and eutrophication (Vitousek et al., 1997). A decrease in biodiversity or species richness is the most serious threat to the maintenance of a stable community (Tilman et al., 2006). Nitrogen deposition can stimulate plant growth and enlarge the soil N pool (Storm and Suss, 2008) and long-term continuous N inputs may lead to loss of species diversity and changes in species composition (DeFalco et al., 2003). Compared with NO_3-N addition, Goulding et al. (1998) reported a much larger decrease in species richness of grasses in plots receiving NH_4-N applications in the famous Park grass long-term field experiment, suggesting a larger impact on species richness by acidification than by eutrophication. Stevens et al. (2004) confirmed that grass species richness in acidic soils decreased linearly with increasing N deposition (ranging from 5 to 35 kg N ha^{-1} yr^{-1}) in the UK and every 2.5 kg N ha^{-1} yr^{-1} led to the loss of one grass species per 4 m^2 on average. Clark and Tilman (2008) further found that long-term low level N addition (10 kg N ha^{-1} yr^{-1}) has reduced plant species numbers by 17 % relative to controls receiving ambient N deposition (6 kg N ha^{-1} yr^{-1}), suggesting that chronic but low-level N deposition may have a greater impact on biodiversity than previously thought. In addition, they also demonstrated that the effects of N addition on relative plant species number are reversible in another experiment in which N addition was halted for a decade.

Grasslands are the dominant landscape in China and account for 40 % of the national land area (Sun et al., 2005). They play important roles in supporting the species diversity of plants and animals (Kang et al., 2007). Temperate steppe is a major grassland type in north China and is sensitive to climate change, overgrazing and nutrient supply (Bai et al., 2004; Christensen et al., 2004; Liu et al., 2009). Very limited studies have been conducted on the effects of N deposition (an important nutrient supply) on Chinese grasslands (Liu et al., 2010). In a 4 yr case study conducted in Inner Mongolia, Bai et al. (2010) demonstrated the tradeoffs and thresholds in effects of N addition on grassland biodiversity and ecosystem functioning. Their results suggest that the critical threshold for N-induced species loss to a mature Eurasian grassland may be below 17.5 kg N ha^{-1} yr^{-1}, and changes in aboveground biomass, species richness, and plant functional group composition to both mature and degraded ecosystems saturated at approximately 105 kg N ha^{-1} yr^{-1}. However, the effects of N deposition on communities often depend on other factors such as plants traits, climatic factors, and abiotic characteristics (Maskell et al., 2010). N fertilization is a common practice to increase the productivity and forage quality of degraded or intensively-managed grasslands (Conant et al., 2001). It is therefore very important to take into account the tradeoffs between increased aboveground biomass and plant species biodiversity or ecosystem function when evaluating the effects of N deposition or fertilization. More in-situ N addition field studies at different sites or regions in China are urgently needed to reveal N impacts on temperate grasslands. Here we describe a 6-year field experiment with different N addition rates at Duolun Experimental Station in Inner Mongolia designed to investigate the response of temperate steppe to elevated N deposition. The objectives of this study were to address two major questions. The first was to test how different functional groups in grasslands respond to elevated N deposition and the second to elucidate the role of N enrichment in determining the loss of plant species.

2 Materials and methods

2.1 Study site

The research station is located at Duolun county (42°02′ N, 116°17′ E, 1324 m a.s.l.), a semi-arid area in Inner Mongolia Autonomous Region with a mean temperature of 2.1 °C and maximum and minimum mean monthly temperatures ranging from 18.9 °C in July to −17.5 °C in January. The area is characterized by a continental monsoon climate. Mean annual precipitation is 386 mm, with 91 % distributed from May to October, and mean potential evaporation of 1748 mm. The soil is classified as a chestnut soil (Chinese classification) or Calcic Luvisol according to the FAO classification, with sand, silt, and clay contents of 62.8 ± 0.04 %, 20.3 ± 0.01 %, and 16.9 ± 0.01 %, respectively. Mean soil bulk density and soil pH (in water) are 1.31 g cm^{-3} and 7.12, respectively. Soil (0–10 cm depth) organic C, total N, and total P are 12.3, 1.7, and 0.28 g kg^{-1}. According to Zhang et al. (2008), ambient annual N deposition in this area is about 16 kg N ha^{-1}. The study site has been fenced off since 2001 to preserve the grazing disturbance. The experimental field belongs to a typical steppe community and the dominant plant species in this temperate grassland are *Artemisia frigida* Willd, *Stipa krylovii* Roshev., *Potentilla acaulis* L., *Cleistogenes squarrosa* (Trin.) Keng, *Allium bidentatum* Fisch. ex Prokh., and *Agropyron cristatum* (L.) Gaertn. The main species of grasses and forbs found in the quadrats from 2005 to 2010 are listed in Table 1.

2.2 Experimental design

A randomized block design was used with five (2005) or six (after 2006) treatments and five replicate plots of each treatment. Thirty 5 m × 5 m plots were arranged in a 5 × 6 matrix. The distance between any two adjacent plots was 1 m. N fertilizer was added at rates of 0, 30, 60, 120, 240, and 480 kg N ha^{-1} yr^{-1} as NH_4NO_3 except in the first year (urea was applied in 2005). The 30 kg N ha^{-1} yr^{-1} treatment started in 2006 and N addition in the 480 kg N ha^{-1} yr^{-1} treatment was not applied in 2009 and thereafter to check the residual effect of high N addition on grasslands. The fertilizer for each plot was split into 3 equal foliar applications in early June, July and August. The experiment started in 2005 and continued to 2010 but no measurements were made in 2007.

2.3 Vegetation sampling

All vegetation sampling except for biomass determination was performed non-destructively because the experiment was designed to investigate the long-term responses of the plant community to cumulative N addition. Sampling was conducted in late August (20 to 25 August) in 2005, 2006, 2008, 2009 and 2010 when the sward had

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the highest biomass each year. Visual estimates were made of vegetation cover and species richness to test for changes in the grassland community in response to N addition. One permanent quadrat (1 × 1 m) was established at each subplot in May 2005. During the measurement a frame (1 × 1 m) with 100 equally distributed grids spaced 10 cm apart was placed above the canopy in each quadrat. The percent cover of each species was estimated visually in all grid cells, the sum of which in each quadrat was considered to represent community cover or functional cover. Species richness was defined as the number of different species in one quadrat.

2.4 Plant sampling and analysis

Aboveground vegetation was sampled each year between 25 and 30 August by clipping all plant species at the soil surface. A quadrat (1 × 1 m) was placed within each plot randomly except for avoiding overlap with the permanent quadrat used for vegetation sampling and at least 50 cm away from the edge of the plot to avoid edge effects. All living vascular plants were sorted into species, litter, and standing dead samples which were oven dried for 48 h at 65 °C and weighed. All living plants belonging to forbs and grasses were taken as the aboveground biomass for forbs and grasses. Plant N concentration and N isotope composition (expressed by $\delta^{15}N$) were determined by grinding oven dried samples to < 100 µm followed by micro-Kjeldahl digestion and continuous flow stable isotope ratio mass spectrometry (Delta Plus, Finnigan, Pittsburg, PA).

2.5 Soil sampling and analysis

Soil samples from 0–20 cm depth were collected from all plots with a 5 cm i.d. tube auger and separated into 10 cm depth increments after plant harvest in 2008, 2009 and 2010. One portion of the samples was immediately cooled for transport and stored deep frozen. Within 12 h a 12 g moist subsample was extracted with 100 ml 2 mol L^{-1} KCl solution and shaken for 1 h. The extracts were analyzed for NO_3-N and NH_4-N

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by continuous flow analysis (TRAACS 2000 Analyzer, Bran and Luebbe, Norderstedt, Germany). Another subsample was weighed in a pre-weighed aluminium can for simultaneous determination of soil water content. Ammonium and nitrate values ($mg\,kg^{-1}$) were converted to $kg\,N\,ha^{-1}$ using soil bulk density and soil depth.

2.6 Statistical analysis

Statistical analysis of the data was accomplished by standard analysis of variance and pairs of mean values were compared by least significant difference (LSD) at the 5 % level using the SAS software package (SAS Institute, 1996).

3 Results

3.1 Species richness

Species richness is here expressed as the average number of plant species in the 5 replicates of each treatment determined using a $1.0\,m^2$ quadrat (Fig. 1). There was a negative relationship between N deposition and species richness in general, especially for forbs which decreased significantly with N addition rate and over the years, but there was no obvious influence on grasses whose species richness ranged from 3 to 4 and did not show any change over time. In 2005, 2006 and 2008, species richness of grasses was significantly stimulated by N addition compared to the zero-N control plots. In 2009 and 2010, after five and six years of N addition, no differences in grasses species richness were found across all treatments.

The pattern of response to N addition in forb species richness showed a decline with increasing N addition gradient in contrast to the grasses. There were no responses to N addition in 2005 and 2006, except at 480 $kg\,N\,ha^{-1}\,yr^{-1}$ in which species richness was significantly lower than in the other treatments. In 2008 the species richness decreased significantly at moderate N rates (e.g., 120 and 240 $kg\,N\,ha^{-1}\,yr^{-1}$) compared with the

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control. In 2009 and 2010 there was a further significant decline in species richness of forbs at lower N rates (e.g. 30 and 60 kg N ha^{-1} yr^{-1}), indicating a cumulative effect of N addition on species richness of forbs (Fig. 1). There was no further decline in forb species after cessesion of N addition in the 480 kg N ha^{-1} yr^{-1} treatment after 2009.

3.2 Aboveground biomass

Aboveground biomass of grasses tended to increase with N addition even in the first year at higher N addition rates (e.g. 240 and 480 kg N ha^{-1} yr^{-1}) (Fig. 2). With cumulative N addition, aboveground biomass of grasses increased with time in all treatments, for example from 900 kg ha^{-1} to approximately 3000 kg ha^{-1} in the 480 kg N ha^{-1} yr^{-1} treatment. After 6 years of N addition the grass aboveground biomass saturated at 120 kg N ha^{-1} yr^{-1} treatment and did not show further increase at higher N addition rates. In the 480 kg N ha^{-1} yr^{-1} treatment the grass aboveground biomass showed no significant change in 2009 and 2010 compared with 2008 because N addition had ceased.

The trend in forb aboveground biomass was opposite to that of grasses except for the first year (2005) (Fig. 2). In 2005 forb biomass was significantly higher in all N addition treatments than in the control. By the second year the forb biomass began to exhibit a negative relationship with N addition rate. There was a significant decline at 480 kg N ha^{-1} yr^{-1} from 900 kg ha^{-1} in 2005 to 100 kg ha^{-1} in 2006, with no apparent change in subsequent years. The aboveground biomass of forbs at 120 kg N ha^{-1} yr^{-1} and 240 kg N ha^{-1} yr^{-1} declined significantly in 2008. With continuing N addition over 6 years the forb biomass declined significantly down to 30 kg N ha^{-1} yr^{-1} compared with control plots in 2009. We observed a higher forb aboveground biomass at 480 kg N ha^{-1} yr^{-1} in 2010 than in 2009, showing a recovery of forb biomass two years after cessation of N addition.

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3.3 Relative cover

Figure 3 shows the grass and forb cover as a percentage of the total vegetation cover of the whole plots of each treatment. The cover of grasses and forbs was significantly affected by N addition, the former increasing and the latter decreasing with increasing N rate.

In 2005 there were no statistical differences in grass cover across all N addition rates. Increasing grass cover occurred only at high N addition rates (≥120 kg N ha^{-1} yr^{-1}) in 2006 and 2008. In 2009 and 2010, grass cover was significantly higher in almost all N treatments including the lowest N rate (30 kg N ha^{-1} yr^{-1}) than in the control (Fig. 3). The cessation of N addition in the 480 kg N ha^{-1} yr^{-1} treatment led to a decline in grass cover in 2009 and 2010 (about 80 %) compared with 2008 and 2006 (> 90 %).

Forb cover under different N addition rates followed the opposite trend to grasses. There were no changes in grass cover in 2005 but significant declines were observed in 2006 and 2008 in the plots with N addition rates equal to or higher than 120 kg N ha^{-1} yr^{-1}. Forb cover decreased significantly even at N addition as low as 30 kg N ha^{-1} yr^{-1} in 2009 and 2010. Forb cover was significantly lower in the 480 kg N ha^{-1} yr^{-1} treatment than in 240 kg N ha^{-1} yr^{-1} over the preceding 3 years (2006–2008) but the difference between the two treatments disappeared in 2009 and 2010 because N addition had ceased at the highest N addition rate from 2009.

3.4 Plant N concentration and accumulation

A positive relationship between N addition rate and herbage N concentration is shown in Fig. 4A and B and this effect was pronounced in both grasses and forbs. Addition of 480 kg N ha^{-1} yr^{-1} showed the highest N concentrations in both grasses and forbs in 2008. With cessation of N addition at 480 kg N ha^{-1} yr^{-1}, the highest N concentrations in the two function groups were found in the 240 kg N ha^{-1} yr^{-1} treatment in 2009 and 2010. Significant increases in aboveground N concentration were found at N rates at or above 120 and 60 kg N ha^{-1} yr^{-1} for grasses and forbs, respectively, across three

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years but no significant changes were found at the lower N rates (except grass N concentration at 30 kg N ha^{-1} yr^{-1} in 2009). The aboveground N concentration and the change in N concentration in forbs with N addition were generally higher than those in grasses across all of the treatments. Nitrogen accumulation in grasses increased with N addition, which was enhanced by both increased N concentration and aboveground biomass under elevated N input (Fig. 4C). However, due to the reduction in forb biomass and cover, the N accumulated in forb aboveground parts was significantly lower than that in grasses and followed the opposite trend to N concentration (Fig. 4D). Similar to the pattern of aboveground N concentration, N accumulated in aboveground parts of grasses also showed no differences between the lower N addition rates especially in 2008 and 2009, unless the rate was equal to or above 120 kg N ha^{-1} yr^{-1}. The amounts of N taken up by grasses were significantly higher at 480 kg N ha^{-1} yr^{-1} than in the other treatments in 2008 and N uptake in that treatment decreased with the cessation of N addition in 2009 and 2010. However, the N taken up by forbs declined with N addition in particular when the addition rate was higher than 120 kg N ha^{-1} yr^{-1}, and the higher the N addition rate became, the lower was the N accumulation in forbs. This trend ceased at 480 kg N ha^{-1} yr^{-1} in the last two years because N addition had stopped. This changing pattern of N accumulation indicates that N deposition or fertilization can stimulate plant growth by increasing N nutrient supply while also producing changes in species composition.

3.5 Plant δ^{15}N

Under different addition rates of N fertilizer (δ^{15}N = 6.3 ‰) plant aboveground δ^{15}N varied generally from 0 to 6 ‰ (Fig. 5). Plant δ^{15}N in both grasses and forbs showed positively linear relationships with N addition rate if the 480 kg N ha^{-1} yr^{-1} treatment which ceased in 2009 and 2010 is omitted. Plant δ^{15}N values were close to zero (equal to natural ^{15}N abundance) in the control, consistent with the background soil δ^{15}N value (0.2 ‰) and all these values in added N treatments were higher in 2010 than in 2009. The plant δ^{15}N values for both grasses and forbs in the 480 kg N ha^{-1} yr^{-1} treatment were lower than those in the 240 kg N ha^{-1} yr^{-1} treatment in both years. Compared with grasses, δ^{15}N in forbs increased faster under elevated N addition in both 2009 and 2010. We also found that plant δ^{15}N in both forbs and grasses increased significantly and linearly with increasing plant N concentration (data not shown).

3.6 Soil mineral N

Soil mineral N (NH_4-N and NO_3-N) distributions at the 0–10 and 10–20 cm soil depths are shown in Fig. 6. Most of NH_4-N in this part of the soil profile accumulated in the top 10 cm while NO_3-N mainly appeared at 10–20 cm. Soil mineral N was significantly higher at N rate ≥ 120 kg N ha^{-1} yr^{-1} (2008) or 60 kg N ha^{-1} yr^{-1} (2009 and 2010) compared with the control. In 2008 NH_4-N was significantly higher at 480 kg N ha^{-1} yr^{-1} than in the control or 30 kg N ha^{-1} yr^{-1}, and there were no differences among the other treatments. NO_3-N was lower than NH_4-N across all of the treatments in the 0–10 cm soil layer and increased slightly with increasing N addition rate. Nitrate-N at 480 kg N ha^{-1} yr^{-1} was significantly higher than in the other N treatments. The ratios of NH_4-N to NO_3-N were not affected by N addition treatment in the 0–10 cm soil layer but they delined from 2.2 at the control to 1.1 at the highest N rate in the top 0–20 cm soil (2010). In 2009 and 2010 significant increases in NH_4-N and NO_3-N were found from the N rate of 60 kg N ha^{-1} yr^{-1} in both soil layers. Compared with the 240 kg N ha^{-1} yr^{-1} treatment, a significant decline in mineral N in the 480 kg N ha^{-1} yr^{-1} treatment was found because of the cessation of N addition in the last two years of the study.

4 Discussion

4.1 N addition effects on vegetation, plant N assimilation and δ^{15}N

In the present study we observed a positive and dose-related relationship between N addition and cover or biomass of grasses while the forbs showed an opposite trend.

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Furthermore, the changes in species composition were mainly due to the decline in forb species richness under N enriched conditions. No significant variations were found in grass species richness at elevated N inputs. A significant decline in forb species richness occurred even at the lowest N addition rate of 30 kg N ha^{-1} yr^{-1} in both 2009 and 2010 (Fig. 1). Compared to 2005, the forb species richness in 2010 at an N addition rate of 240 kg N ha^{-1} yr^{-1} was reduced from 12.0 to 3.0, a very large loss of species biodiversity. No further decline in forb species occurred at the highest N addition rate (480 kg N ha^{-1} yr^{-1}) in 2009 or 2010 compared with 2008 and earlier years due to zero N application during the final two years of the study. These are consistent with the findings of Clark and Tilman (2008) who reported that even N additions as low as 10 kg N ha^{-1} yr^{-1} led to a significant loss of sensitive grass species in an acidic grassland in Minnesota and the cessation of N addition for a decade can result in recovery of the main grass species.

Nitrogen deposition has been regarded as the third most important driver of declining species richness after land use change and climate change (Sala et al., 2000). Nitrogen limits plant growth in most terrestrial ecosystems but both N gradient and N addition experiments predict that there is a critical level above which species richness will decline as biomass production increases (Vitousek et al., 2002; Grime, 1973; Gross et al., 2000). The critical level was found to be 15–22 kg N ha^{-1} yr^{-1} for wet and dry heathlands, 14–25 kg N ha^{-1} yr^{-1} for calcareous grassland, and 20–30 kg N ha^{-1} yr^{-1} for intermediate and acid grassland (Bakker and Berendse, 1999). There have been numerous studies on the mechanisms of change in species composition under elevated N deposition. Suding et al. (2005) proposed that two hypotheses, trait-neutral and trait-based, operate simultaneously in relation to the biodiversity-production relationship. On one hand, rare species are at most risk of loss because of their initial low abundance as productivity is enhanced. On the other hand, competition shifts from belowground nutrient resources to aboveground resources (such as light) and, as a result, species that are habituated to unfertilized conditions or constrained in their use of aboveground resources will be more likely to disappear. An additional factor is invasion

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by exotic grasses which have higher N use efficiency than the native species as soil available N increases with elevated N deposition (Rao et al., 2009). Furthermore, all of these factors will be affected by underground process changes as N deposition increases (Dupre et al., 2010). Soil pH is strongly correlated with N deposition and shows a negative association with the proportion of grasses in acidic grassland (Stevens et al., 2004). Species number and diversity are also negatively correlated with increasing N:P and N:K ratios in heathlands and grasslands (Roem and Berendse, 2000; Koerselman and Meuleman, 1996).

In the present study the increase in soil mineral N, especially NO_3-N, under elevated N deposition may be the main explanation for changes in species richness and aboveground biomass of both grasses and forbs. Nitrogen deposition can also influence soil C storage and C:N ratio through biotic and abiotic pathways, which further influence soil gross N mineralization and microbial respiration (West et al., 2006).

Nitrogen deposition has favored grasses at the expense of forbs in our study. This is in accordance with the results of Stevens et al. (2006) who have conducted studies along a geographical deposition gradient in Great Britain and Carroll et al. (2003). Similar responses involving changes in species composition in favor of grasses have also been observed in other studies (Bobbink, 1991; Mountford et al., 1993; Wedin and Tilman, 1996). One possible explanation for this phenomenon is that grasses have higher N use efficiency (NUE) than forbs, resulting in faster growth and suppression of the growth of other plant species (Peppler-Lisbach and Petersen, 2001). Our work provides evidence for this mechanism. We found increased aboveground biomass and N accumulation in grasses but decreased aboveground biomass and N accumulation in forbs with increasing N addition (Figs. 2 and 4). In contrast, plant N concentration and δ^{15}N showed greater responses by forbs than by grasses under N enrichment conditions (Figs. 4 and 5), suggesting that forbs are more "sensitive" to N addition.

Previous studies have predicted that foliar δ^{15}N can be considered to be a good indicator of the source of N for plants (Amundson et al., 2003; Kahmen et al., 2008; Pardo et al., 2006). Assuming that plant δ^{15}N shows a positive relationship with N source,

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this pattern indicates that increasing amounts of mineral N lead to more ^{15}N enrichment in soils with altered N transformation rates and ^{14}N-enriched N losses through NO_3-N, N_2O and N_2 (Kahmen et al., 2008). In the temperate grassland at our study site, denitrification can be regarded as an important pathway of N loss (Tilsner et al., 2003a, b), leading to greater enrichment of NO_3-N than NH_4-N in ^{15}N. With increasing N addition rate the NH_4-N/NO_3-N ratio (according to Fig. 6) declined in soil, causing plants to take up more and more NO_3-N over NH_4-N. Consequently plant δ^{15}N will also increase with declining NH_4-N/NO_3-Nin the soil. Futhermore, because the δ^{15}N of our fertilizer (6.3 ‰) was above natural abundance, more N assimilated by plants would also result in higher δ^{15}N in the aboveground plant parts. The higher δ^{15}N values in forbs at the same N addition rate than grasses suggests that N uptake by forbs could be mostly from transformed added N (with higher ^{15}N enrichment) whereas grass N assimilation was mainly from the original N source. The cessation of fertilizer N application in 2009 and 2010 produced a decline in δ^{15}N in both grasses and forbs in the plots receiving 480 kg N ha^{-1} yr^{-1} compared to 240 kg N ha^{-1} yr^{-1}, clearly showing the effect of N source.

4.2 N addition effects on the soil mineral N pool

As N addition rate increased, more available mineral N accumulated in the soil profile. The amount of residual mineral N (in particular NO_3-N) can affect soil N transformations (e.g., mineralization and nitrification) and also indicates whether or not the soil is N saturated. In the present study NH_4-N accumulated mainly in the topsoil (0–10 cm) and NO_3-N accumulated substantially when the N addition rate was higher than 120 kg N ha^{-1} yr^{-1} either at 0–10 or 10–20 cm soil depth and the ratio of NH_4-N to NO_3-N declined sharply in the highest N addition treatment (Fig. 6). The accumulation of NO_3-N in the soil profile (Fig. 6) can be considered to indicate soil N saturation (Aber et al., 1998; Goulding et al., 1998). This may explain the decline in forb composition under elevated N deposition in our study because under N saturation conditions

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other factors (e.g. P, K, light or water) rather than N become major factors limiting plant growth. Grassland species (e.g., forbs) which are sensitive to N inputs will be at risk of poor survival under elevated N deposition induced soil N saturation.

5 Conclusions

Our results indicate that increased N deposition can lead to loss of forb species richness in temperate steppe ecosystems. Even N inputs as low as 30 kg N ha^{-1} yr^{-1} will lead to changes in species composition, showing an increase in aboveground biomass and cover of grasses and a decrease in forbs together with a decrease in species richness of forbs. A two-year cessation of N addition of 480 kg N ha^{-1} yr^{-1} showed a recovery effect on forb biomass, cover and species richness. As the plants increased their N assimilation the δ^{15}N value increased in both grasses and forbs and there were changes in soil N sources. When the N addition rate was above 60 kg N ha^{-1} yr^{-1}, NO_3-N contents increased sharply in the top 20 cm of the soil profile until at the highest N addition rate the ratio of NH_4-N to NO_3-N declined to 1.1 compared with 2.2 in the zero-N control. Increasing soil NO_3-N and declining NH_4-N/NO_3-N ratio with N addition can lead to changes in soil N transformation rates, soil enrichment with ^{15}N and eventually soil N saturation, all of which may be associated with changes in plant community species richness.

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Table 1. Major species of grasses and forbs within the quadrats (1 × 1 m) from 2005 to 2010.

Functional group	Species	
Grasses	*Achnatherum sibiricum*	
	Agropyron cristatum	
	Cleistogenes squarrosa	
	Leymus chinensis	
	Setaria viridis	
	Stipa krylovii	
Nonlegume Forbs	*Allium neriniflorum*	*Heteropappus altaicus*
	Allium ramosum	*Iris tenuifolia*
	Allium senescens	*Ixeris chinensis*
	Allium tenuissimum	*Lespedeza davurica*
	Androsace umbellata	*Phlomis umbrosa*
	Artemisia capillaris	*Potentilla acaulis*
	Artemisia frigida	*Potentilla anserina*
	Carex korshinskyi	*Potentilla bifurca*
	Chamaerhodos erecta	*Potentilla multifida*
	Chenopodium aristatum	*Potentilla tanacetifolia*
	Chenopodium glaucum	*Salsola collina*
	Cymbaria dahurica	*Saposhnikovia divaricata*
	Dianthus chinensis	*Scorzonera austriaca*
	Dontostemon dentatus	*Sibbaldia adpressa*
	Fagopyrum sagittatum	*Sonchus arvensis*
	Gentiana dahurica	*stellera chamaejasme*
	Gentiana squarrosa	*Thalictrum petaloideum*

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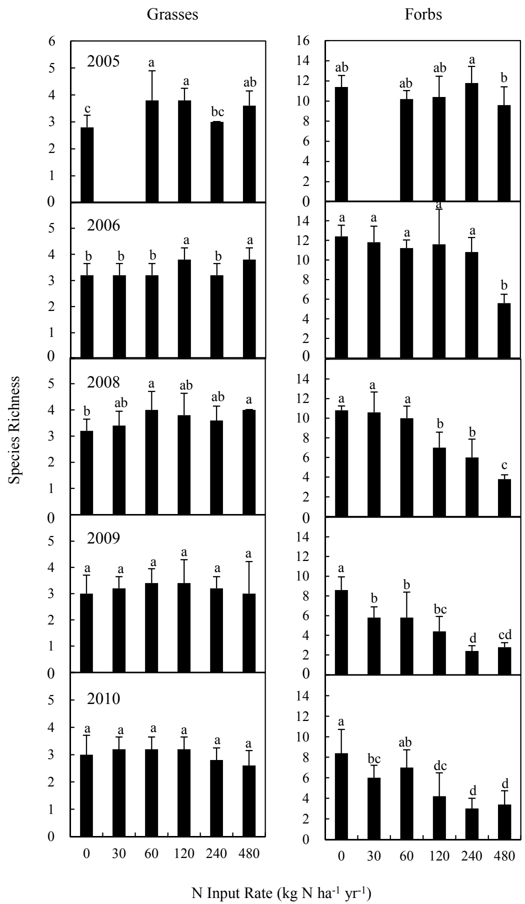

Fig. 1. Relationship between N addition rate and species richness for grasses (left) and forbs (right) from 2005 to 2010. Different letters at bars indicate treatment differences at $P < 0.05$.

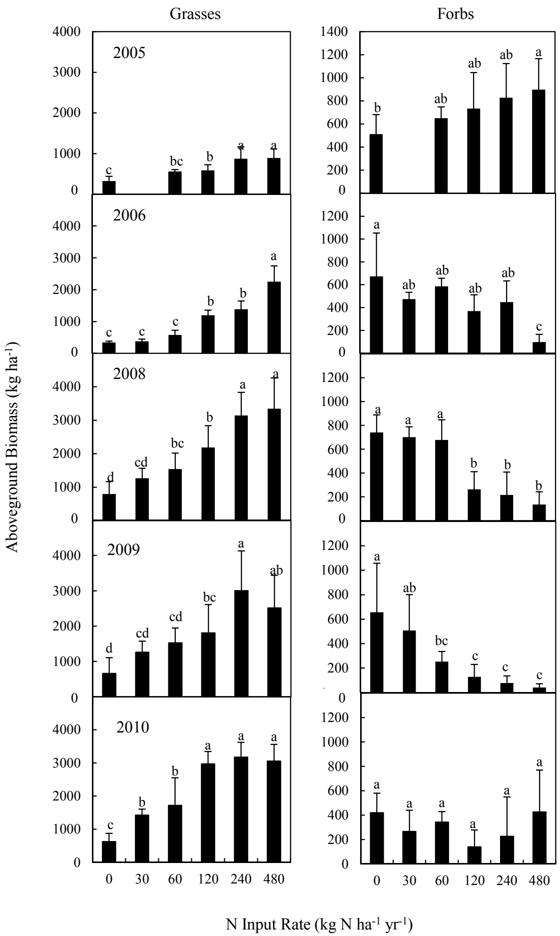

Fig. 2. Response of aboveground biomass of grasses (left) and forbs (right) to N addition from 2005 to 2010. Different letters at bars indicate treatment differences at $P < 0.05$.

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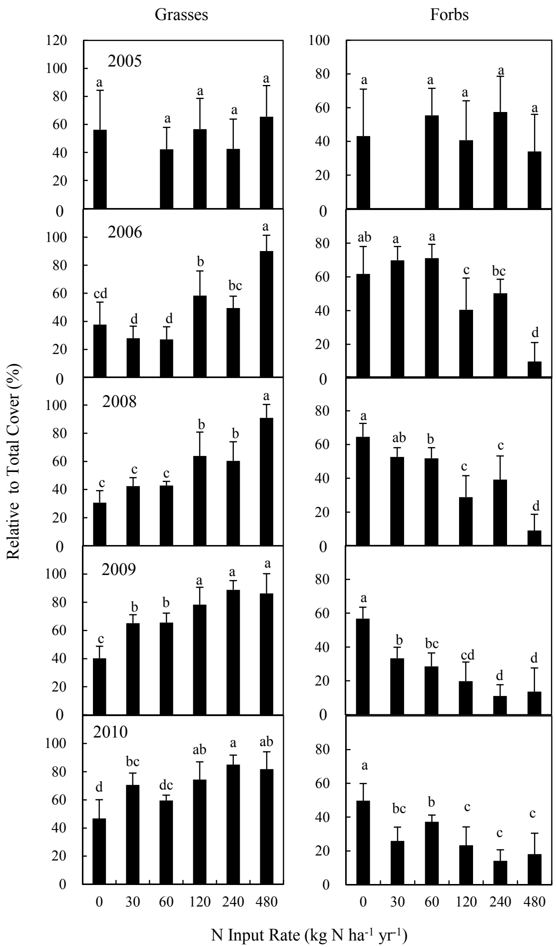

Fig. 3. Relationship between N addition rate and relative cover of grasses (left) and forbs (right) in relation to total cover during 2005 and 2010. Different letters at bars indicate treatment differences at $P < 0.05$.

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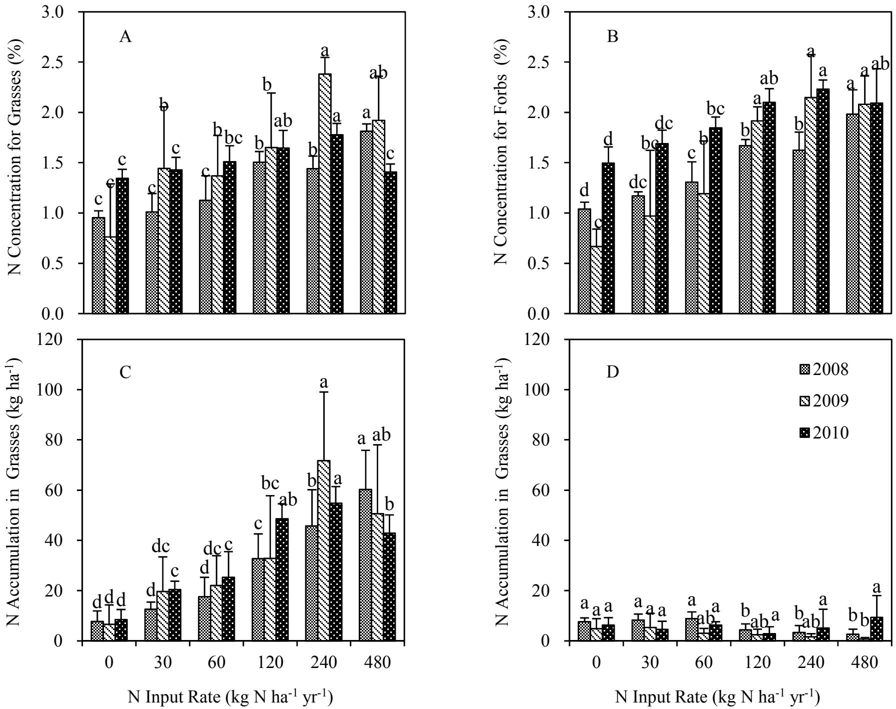

Fig. 4. Responses of aboveground N concentration **(A, B)** and N accumulation **(C, D)** in grasses and forbs to N addition rate from 2008 to 2010. Different letters at bars within the same year indicate treatment differences at $P < 0.05$.

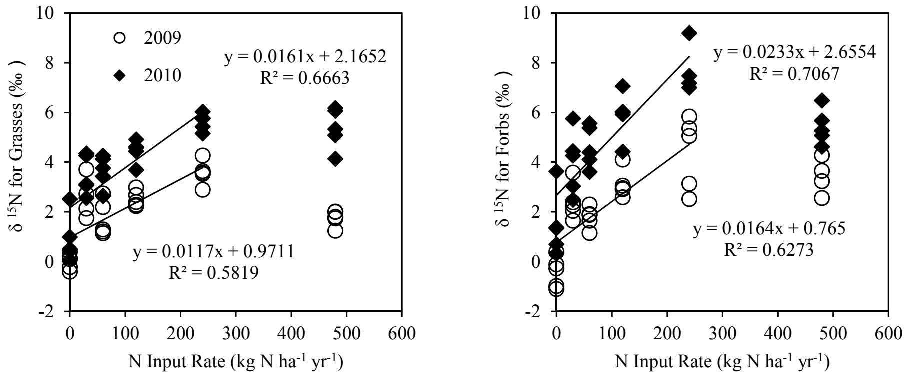

Fig. 5. Relationships between N addition rate and aboveground δ^{15}N value of grasses (left) and forbs (right) during 2009 and 2010.

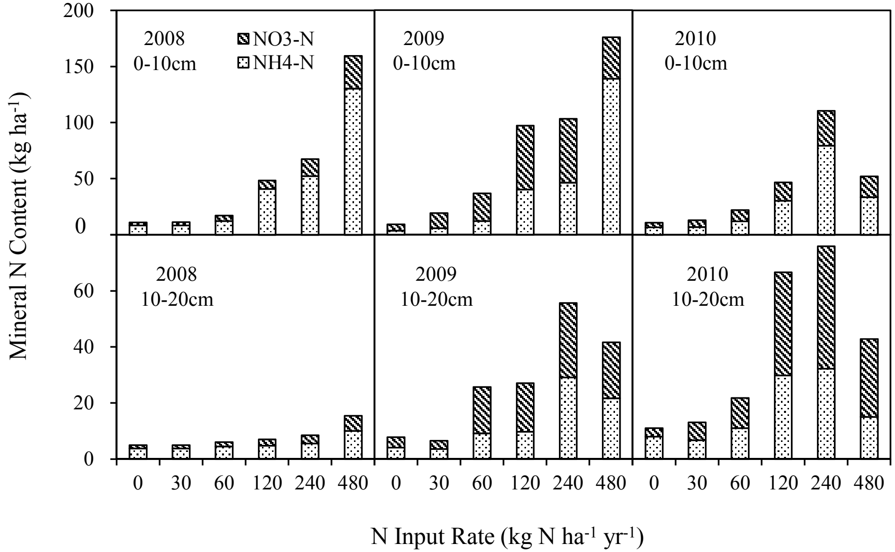

Fig. 6. Soil mineral N content in the top 20 cm of the soil profile from 2008 to 2010.

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