# Mowing mitigates the negative impacts of N addition on plant species diversity

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## Abstract

Increasing availability of reactive nitrogen (N) threatens plant diversity in diverse ecosystems. While there is mounting evidence for the negative impacts of N deposition on one component of diversity, species richness, we know little about its effects on another one, species evenness. It is suspected that ecosystem management practice that removes nitrogen from the ecosystem, such as hay-harvesting by mowing in grasslands, would mitigate the negative impacts of N deposition on plant diversity. However, empirical evidence is scarce.

Here, we reported the main and interactive effects of N deposition and mowing on plant diversity in a temperate meadow steppe with four years data from a field experiment within which multi-level N addition rates and multiple N compounds are considered.

Across all the types of N compounds, species richness and evenness significantly decreased with the increases of N addition rate, which was mainly caused by the growth of a tall rhizomatous grass, *Leymus chinensis*. Such negative impacts of N addition were accumulating with time. Mowing significantly reduced the dominance of *L. chinensis*, and mitigated the negative impacts of N deposition on species evenness.

We present robust evidence that N deposition threatened biodiversity by reducing both species richness and evenness, a process which could be alleviated by mowing. Our results highlight the changes of species evenness in driving the negative impacts of N deposition on plant diversity and the role of mowing in mediating such negative impacts of N deposition.

Keywords: Nitrogen deposition, nitrogen compounds, hay-making, diversity, community evenness, semi-arid grassland, temperate steppe, Erguna

## Introduction

Human activities have substantially altered global N cycling (Vitousek et al. 1997, Galloway et al. 2004), resulting in large amounts of reactive N being deposited into many terrestrial ecosystems (Galloway et al. 2008). For instance, N deposition increased by ~8 kg N $ha^{-1}$ $yr^{-1}$ from 1980 (9.4 kg N $ha^{-1}$ $yr^{-1}$) to 2010 (averaged 21.8 kg N $ha^{-1}$ $yr^{-1}$) in China (Liu et al. 2013), with an average $NO_3^{-}$ deposition of 32.93 kg $ha^{-1}$ $yr^{-1}$ in Chinese terrestrial ecosystems from 2009 to 2014 (Yu et al. 2016). Nitrogen enrichment generally decreases species richness, especially in herbaceous communities (Stevens et al. 2004, Clark and Tilman 2008, Bobbink et al. 2010), and alters community composition (Suding et al. 2005, Dickson et al. 2014), with consequences for ecosystem processes and functioning (Vitousek et al. 1997, Cardinale et al. 2012, Isbell et al. 2015). Many mechanisms have been proposed for the negative impacts of N deposition on species richness, including light limitation (Hautier et al. 2009, Borer et al. 2014), eutrophication (Clark and Tilman 2008), acidification (Stevens et al. 2004), and metal toxicity (Tian et al. 2016).

It is well established that species richness is not a complete surrogate for plant diversity (Wilsey and Potvin 2000). There are many limitations for using species richness as a surrogate for biodiversity, which would not capture the key responses of biodiversity to environmental changes (Hillebrand et al. 2008). However, fewer studies have focused on the impacts of N deposition on other components of plant diversity beyond species richness, for example species evenness, which measures the distribution of abundance or biomass among species in a community (Smith and Wilson 1996). Species evenness plays an important role in driving ecosystem functioning (Hillebrand et al. 2008), in that it has a positive relationship with primary productivity (Wilsey and Potvin 2000, Orwin et al. 2014) and could increase the resistance of plant community to dicot invasion and insect infestation (Wilsey and Polley 2002). However, it should be noted that the impacts of species evenness on ecosystem invasibility are variable depending on phenological traits of target species (Losure et al. 2007). While species evenness could indirectly affect ecosystem processes by affecting species richness (Wilsey and Polley 2004), a recent study reported that species evenness and richness would show synergistic effects on ecosystem functioning (Lembrechts et al. 2018).

Nitrogen deposition generally facilitates the growth of grasses but is unfavorable for that of forbs (Bobbink et al. 2010, Mitchell et al. 2017), and thus changes the relative abundance of species with different functional and morphological traits in grasslands (Suding et al. 2005, Gough et al. 2012). N enrichment is beneficial to competitively dominant species (Stevens et al. 2004, Suding et al. 2005). Whether such changes in community composition and species relative abundance following N deposition would alter species evenness remains unknown. In a tallgrass prairie, Avolio et al. (2014) found that a decade long N addition increased species evenness but the substantial changes in community composition had no effect on species evenness. In a survey of calcareous grasslands in UK, however, van den Berg et al. (2011) reported that species evenness was negatively correlated with N deposition rate.

The divergent responses of plant diversity to N addition have been ascribed to the variation in addition rate, application duration, and climate in different experiments (Phoenix et al. 2012, Humbert et al. 2016). Other factors may also play a role, for instance, the different types of N compounds used for simulating N deposition (Gaudnik et al. 2011, Van Den Berg et al. 2016). Atmospheric deposited N is mainly composed of inorganic N (Jia et al. 2016) with less organic N (Cornell 2011), and is often accompanied by sulfate deposition (Yu et al. 2016). Many types of N compounds have been used in related experiments, including $NH_4NO_3$, $NaNO_3$, $(NH_4)_2SO_4$, $NH_4Cl$, and urea (Phoenix et al. 2012, Stevens and Gowing 2014). In an alpine meadow, Song et al. (2012) showed the idiosyncratic responses of different plant functional groups to different N compounds, with ammonium addition favoring legumes and sedges and nitrate addition favoring forbs. Similarly, species richness in acid grasslands is more sensitive to the reduced N form while that in calcareous grasslands is more sensitive to the oxidized form (van den Berg et al. 2016). Compared with species richness, the effects of different N compounds on species evenness have received less attention.

Ecosystem management practice can also modulate the impacts of N deposition on plant diversity (Bobbink et al. 2010). On one hand, mowing can alter the intrinsic sensitivity of plants to N deposition (Bobbink et al. 1998). On the other hand, it can change the abiotic factors, such as light availability and biotic factors, such as the number of C3 species (Collins et al. 1998). In fertilized plots, mowing is apparently able to mitigate for the negative effects of the dominant species which positively affected by fertilization (Lepš 2014). Mowing for hay-harvesting, as a widely-used ecosystem management practice in grasslands, could preserve plant diversity under the scenarios of N deposition (Collins et al. 1998, Poschlod et al. 2005, Knop et al. 2006). The removal of biomass and N could reduce the accumulation of N in soils (Storkey et al. 2015), which reduces the eutrophication effects. Mowing could increase light availability, therefore promoting low-statured perennial grasses and forbs (Collins et al. 1998, Hautier et al. 2009). While evidence from the European and Chinese grasslands showed that mowing can mitigate the negative effects of N addition on species richness (Socher et al. 2013, Jones et al. 2016, Yang et al. 2012, Zhang et al. 2017), we are not sure how mowing would mediate the N impacts on species evenness.

To investigate the main and interactive effects of N addition (both rates and forms) and mowing on plant diversity, we carried out a four-year field experiment in a temperate meadow steppe in northeastern China. We hypothesized that (1) N addition would decrease species diversity via a biomass promotion of the nitrophilous species, (2) such effects would vary among different types of N compounds, and (3) mowing would mitigate the negative effects of N addition on species diversity by decreasing the dominance of nitrophilous species.

## Materials and Methods

### *Study site*

The experiment was conducted at the Erguna Forest-Steppe Ecotone Research Station (N50°10′46.1′′，E119°22′56.4′′). The field experiment was carried out in a natural steppe which has been fenced since 2013 to prevent livestock grazing, before that the grassland was annually mown for hay-harvesting. The long-term mean annual precipitation of the site is 363 mm and the mean annual temperature is -2.45°C (1957-2016). The soil is classified as chernozem according to the US soil taxonomy classification. The pH of top soil (0-10cm) is 6.8~7.0. The dominant species in this ecosystem are *Leymus chinensis*, *Stipa baicalensis*, *Cleistogenes squarrosa*, *Thermopsis lanceolate*, *Cymbaria dahurica*, and *Carex duriuscula*. Previous study from this region showed that N enrichment increased the aboveground biomass of *L. chinensis* (Zhang et al. 2015), whose maximum photosynthetic rate showed positive responses to N enrichment (Chen et al. 2005). Moreover, *L. chinensis* is a nitrophilous species with higher foliar N concentration than other grasses (Cui et al. 2010).

*Experimental design*

The N addition experiment began in 2014, following a randomized complete block design. There were six rates of N addition (i.e., 0, 2, 5, 10, 20, and 50 g N $m^{-2}$ $yr^{-1}$), five types of N compounds ($NH_4NO_3$, $(NH_4)_2SO_4$, $NH_4HCO_3$, $CO(NH_2)_2$, slow-release $CO(NH_2)_2$, crossed with mowing treatments (non-mown vs. mown). There were 60 treatments with each being replicated by eight times (480 treatment plots in total). The area of each plot was 10 m × 10 m. Nitrogen fertilizers were added annually since 2014, in late May. Fertilizers were mixed with sand (because of the low amount of added fertilizer at low addition rates) and broadcast uniformly by hand. Sand was sieved through less than 2 mm in size, washed in water, and then heated at nearly 250°C for 60 minutes in an iron pan. To avoid potentially confounding effects, all plots received the same amount of sand (0.5 kg per plot). Mowing was conducted annually in late-August at the height of 10 cm above the soil surface to simulate hay harvesting. The harvested biomass was removed from the plots.

### *Field sampling and measurement*

Aboveground biomass was sampled each year between August 10th and 20th by clipping all vascular plants at the soil surface in a 1 m × 1 m quadrat, which was randomly placed in each plot without a spatial overlap of quadrats among different years and at least 50 cm inside the border of each plot to avoid edge effects. All living vascular plants were sorted to species. All plant materials were oven-dried at 65°C for 48h and weighed. Species richness (number of plant species $m^{-2}$) was recorded in the same quadrat in which aboveground biomass was measured. We classified species into the following five plant functional groups based on life forms: perennial rhizomatous grasses (PR), non-leguminous forbs (NF), bunchgrasses (BG), sedges (SE) and legumes(LE).

### *Calculation and statistical analysis*

Species evenness was quantified as $^{1}/_{D}$. D is the Simpson's dominance index and calculated as $D=\sum_{i=1}^{S}(pi)^2$ (vegan package), where $p_i$ is the relative aboveground biomass of species *i* in the community. The relative biomass of *L. chinensis* was calculated as its proportional contribution to the community total aboveground biomass. Repeated-measures ANOVAs (gls function in nlme packages) were conducted to detect the effects of N addition rate, N compounds type, mowing, and their interactions on species evenness, species richness, aboveground biomass of each plant functional group, and relative biomass of *L. chinensis* across 2014 to 2017. The relationship between the relative biomass of *L. chinensis* and species evenness was fitted with quadratic regression in different years, using the lm function, with model selection criterion AIC to choose the best model. All analyses were conducted using R version 3.2.3 (R development core team, 2015)

## Results

### *Effects of N addition and mowing on plant diversity*

Both species evenness and richness significantly decreased with increasing N addition rates ($P < 0.001$, Table 1; Figs. 1 and 2). The effects of N addition on species evenness and richness significantly different among N compounds ($P < 0.05$, Table 1, Figs. 1 and 2), with the $(NH_4)_2SO_4$ treatment showing stronger reduction of evenness and richness averaged across the four years (Fig. 1F, Fig. 2F). Mowing significantly enhanced species evenness and richness ($P < 0.001$, Table1; Figs.1 and 2).

After four years treatments, species evenness declined by 8.5%-55.8% and 13.1%-62.9%, species richness decreased 4%-30% and 2%-45% across the N addition gradient with the presence and absence of mowing, respectively. Species evenness and richness showed significant inter-annual variation ($P < 0.001$, Table1; Figs. 1 and 2), with a decreasing trend as time went on. The effects of N addition rates on species evenness and richness varied among different years as indicated by the significant interaction between both of them (Table 1).

### *Effects of N addition and mowing on aboveground biomass of plant functional groups*

The aboveground biomass of perennial rhizome grasses (PR) significantly increased with increasing the N addition rates, whereas that of bunchgrasses (BG), sedges (SE) and legumes (LE) significantly decreased with increasing N addition (Table S1). Mowing significantly reduced aboveground biomass of PR and increased that of non-leguminous forbs (NF) and SE (Table S1).

### *Effects of N addition and mowing on the relative biomass of L. chinensis*

The relative biomass of *L. chinensis*, a tall perennial rhizomatous grass, significantly increased with the increasing N addition rates ($P < 0.001$, Table1; Fig. 3). The changes of relative biomass of *L. chinensis* varied significantly across different N compounds, with the largest enhancement presenting under the $(NH_4)_2SO_4$ treatment (Fig. S2). Mowing significantly reduced the relative biomass of *L. chinensis* by 19.2% averaged across all the N treatments and years. Mowing and N addition rates did not interact to affect the relative biomass of *L. chinensis* (Table 1), indicating a consistent role of mowing across the N addition gradient.

The relative biomass of *L. chinensis* varied significantly among different years, with higher contribution of *L. chinensis* to the total community biomass at late years than that in early years (Table 1, Fig. 4). From 2014 to 2017, averaged across all the rates and types, N addition increased the relative biomass of *L. chinensis* by 14.5%, 36.9%, 46.2%, and 93.0%, respectively. The effects of both the rates and types of N addition on the relative biomass of *L. chinensis* varied among different years (Table 1).

The relative biomass of *L. chinensis* and species evenness showed significantly quadratic regression in each year. With the increases of the relative biomass of *L. chinensis,* species evenness decreased sharply when the relative biomass of *L. chinensis* was less than 60% and changed slowly higher than 60% (Fig.5).

## Discussion

Species evenness and richness was reduced with the increases of N addition rates, which was consistent with our first hypothesis and findings from previous studies (Stevens et al. 2004, Clark and Tilman 2008, Hillebrand et al. 2007, Bobbink et al. 2010, van den Berg et al. 2011, Niu et al. 2018). Effects of N addition and mowing on perennial rhizomatous grasses (PR) were much stronger than on other plant functional groups, so we deduced that the losses of species evenness and richness in response to N addition resulted from the enhancement of the dominance of the rhizomatous grass, *L. chinensis*. Species evenness decreased with the increases of relative biomass of *L. chinensis,* highlighting the important role of *L. chinensis* in driving the decline of species evenness in response to N enrichment in this ecosystem. Similarly, other studies have reported that N enrichment may reduce species diversity by favoring competitively dominant species (Stevens et al. 2004, Suding et al. 2005). From a plant physiology perspective, *L. chinensis* has higher foliar N concentration than other grasses in this ecosystem (Cui et al. 2010) and its maximum photosynthetic rate shows positive responses to N enrichment (Chen et al. 2005). In natural steppe, *L. chinensis* dominates in sites with high N availability (Chen et al. 2005). *L. chinensis* spreads clonally with runners (in contrast to clumps) following the classification of Cleland et al. (2008), with rhizomes and longer spacers between ramets of the same individual (Wang et al. 2004). The capacity of *L. chinensis* to vegetatively expand by rhizomes would give it competitive advantage under N enrichment. Moreover, *L. chinensis* is a canopy species in this ecosystem. Height would give it benefit for the competition for light, a limiting factor which drives local species extinction following N enrichment thus reduce species richness (Hautier et al. 2009). The above-mentioned physiological and morphological traits would account for the rapid enhancement of dominance of *L. chinensis* in response to N enrichment, with direct consequences on the changes of species evenness and richness.

Although species evenness and richness decreased with increasing N addition rates for all the N compounds examined in this study, the magnitude of such responses varied significantly among different compounds. Across all the five types of N compounds, $(NH_4)_2SO_4$ addition resulted in the lowest evenness due to the strongest increases of the dominance of *L. chinensis*. Soil pH, an important driver for the local extinction of plant species in temperate steppe (Zhang et al. 2014), was lowest in the $(NH_4)_2SO_4$ treatment (Fig. S3). There would be, therefore, more niche space for *L. chinensis* in the $(NH_4)_2SO_4$ treatment due to the losses of other acid-sensitive species. In contrast, *L. chinensis* is tolerant of acid conditions. Lan (2014) found that *L. chinensis* was the sole species showing positive relationship between primary productivity and the degree of acidification in an acid addition experiment in a temperate steppe. While the variation of the effects of reduced and oxidized N on biodiversity is well-recognized (Stevens et al. 2011), our results implied that the combined N and S deposition, which is occurring globally (Dentener et al. 2006), would be a bigger threaten to biodiversity (species evenness and richness) than N deposition alone.

The negative effects of N addition rates on species evenness increased over the duration of our experiment, which is in line with previous results about the changes of species richness in other studies (Isbell et al. 2013, Zhang et al. 2016). For example, species richness has been found to show large decreases during the later years of a three-decade

N addition experiment in tall prairie in US (Isbell et al. 2013) and of a five-year experiment in temperate steppe in China (Zhang et al. 2016), and global mountain grasslands (Humbert et al. 2016). Such temporal changes for the responses of species richness could be ascribed to the increasing of N availability, decreasing of soil pH, and litter accumulation (Clark and Tilman 2010, Dupre et al. 2010). The cumulative negative effects of N deposition on species richness and evenness have important implications for measures of ecosystem functioning, such as primary productivity (Wilsey and Potvin 2000, Cardinale et al. 2007, Orwin et al. 2014).

Our results supported the second hypothesis, in that mowing mitigated the negative effects of N addition on species evenness and richness, mainly due to it reducing the growth of *L. chinensis*. In line with our results, Smith et al. (2017) showed that the effects of mowing on evenness was generally positive for temperate grassland in Australia. Furthermore, mowing also preserved species richness under N enrichment partly reducing the competitive ability of dominant species (Lepš 2014). On the one hand, mowing (and also herbivory) could remove aboveground plant biomass and increase light availability (Borer et al. 2014, Kotas et al. 2017), which would help reduce the dominance of tall herbs and perennial grasses and increase that of small herbs (Hewett 1985, Plassmann et al. 2009). On the other hand, mowing had considerable potential to remove N (Jones et al. 2017). For example, Barker et al. (2004) found that high intensity mowing removed 23% of total system N while regular mowing had been able to maintain the N balance despite at high N deposition levels in heathland (Verhoeven et al. 1996). The lower biodiversity losses in response to N deposition under mown communities not only help maintain ecosystem services under the N enriched conditions (Yang et al. 2012), but also facilitate the biodiversity recovery after the cessation of N deposition (Tilman and Isbell 2015). Consequently, annual mowing with the removal of biomass would be an appropriate management strategy to conserve biodiversity in temperate grasslands.

## Conclusions

Our results demonstrated that N deposition could simultaneously reduce species richness and evenness. The negative impacts of N deposition on biodiversity would be stronger than previous findings based solely on species richness as it substantially reduced species evenness. But, it remains unknown whether and how such shifts in species evenness would contribute to the impacts of N deposition on ecosystem functioning. The N-induced losses of species evenness and richness in mown plots were much lower than that in unmown plots, highlighting the important role of ecosystem management practice in mediating the impacts of N deposition on plant diversity. The higher species evenness and richness in mown plots compared with that in unmown plots may help explain why the biodiversity in mown ecosystems shows a more rapid recovery as N declines (Tilman and Isbell 2015).

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**Table1.** Results (*F* values) of the repeated measures analysis of variance for the effects of mowing(M), N addition rate (R), Year (Y), N compounds type (T) and their interactions on species evenness, species richness and relative biomass of *Leymus chinensis*.

| | *df* | Species evenness | Species richness | Relative biomass of *L. chinensis* |
|---|---|---|---|---|
| R | 5 | 78.63*** | 63.89*** | 27.825*** |
| T | 4 | 7.07*** | 5.41*** | 2.867* |
| M | 1 | 284.80*** | 111.22*** | 195.053*** |
| R×T | 20 | 1.18$^{\text{ns}}$ | 1.01$^{\text{ns}}$ | 2.373** |
| R×M | 5 | 2.51* | 1.35$^{\text{ns}}$ | 0.832$^{\text{ns}}$ |
| T×M | 4 | 0.17$^{\text{ns}}$ | 1.78$^{\text{ns}}$ | 3.774** |
| R×T×M | 20 | 1.05$^{\text{ns}}$ | 0.99$^{\text{ns}}$ | 2.284** |
| Y | 3 | 18.14*** | 106.43*** | 17.195*** |
| Y×R | 15 | 2.15** | 2.21** | 2.561*** |
| Y×T | 12 | 1.43$^{\text{ns}}$ | 1.38$^{\text{ns}}$ | 0.494$^{\text{ns}}$ |
| Y×M | 3 | 1.08$^{\text{ns}}$ | 0.29$^{\text{ns}}$ | 1.409$^{\text{ns}}$ |
| Y×R×T | 60 | 0.96$^{\text{ns}}$ | 0.77$^{\text{ns}}$ | 0.770$^{\text{ns}}$ |
| Y×R×M | 15 | 1.47$^{\text{ns}}$ | 2.11** | 0.517$^{\text{ns}}$ |
| Y×T×M | 12 | 0.87$^{\text{ns}}$ | 1.01$^{\text{ns}}$ | 0.770$^{\text{ns}}$ |
| Y×R×T×M | 60 | 0.68$^{\text{ns}}$ | 0.63$^{\text{ns}}$ | 0.653$^{\text{ns}}$ |

Asterisks denote significant levels: ns, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; and ***, $P \leq 0.001$, respectively.