Different nitrogen saturation thresholds for above-, below-, and total net primary productivity in a temperate steppe

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Abstract

Identifying the thresholds for the positive responses of total net primary productivity (NPP) to nitrogen (N) enrichment is an essential prerequisite for predicting the benefits of N deposition on ecosystem carbon sequestration. However, the responses of below-ground NPP (BNPP) to N enrichment are unknown in many ecosystems, which limits our ability to understand the carbon cycling under the scenario of increasing N availability. We examined the changes in above-ground NPP (ANPP), BNPP, and NPP of a temperate meadow steppe across a wide-ranging N addition gradient (0, 2, 5, 10, 20, and 50 g N m^{-2} yr^{-1}) during five years. Both ANPP and NPP increased nonlinearly with N addition rates. The N saturation threshold for ANPP (T_A) and NPP (T_N) was at the rate of 13.11 and 6.70 g N m^{-2} yr^{-1}, respectively. BNPP decreased with increasing N addition when N addition rates > 5 g N m^{-2} yr^{-1}, resulting in much lower T_N than T_A. Soil N enrichment played a key role in driving the negative impacts of high N addition rates on BNPP, and consequently on the earlier occurrence of N saturation threshold for NPP. Our results highlight the negative effects of soil N enrichment on NPP in natural grasslands super-saturated with N. Furthermore, by considering ANPP and BNPP simultaneously, our results indicate that previous findings from aboveground might have over-estimated the positive effects of N deposition on primary productivity.

Keywords: nitrogen deposition, semi-arid grassland, N saturation threshold,

production, ammonium toxicity, soil N concentration

Introduction

Nitrogen (N) is the primary limiting nutrient for primary productivity in most terrestrial ecosystems (Elser et al., 2007; Vitousek and Howarth, 1991). Accelerating human activities have led to increasing atmospheric N deposition worldwide (Galloway et al., 2008), and the N deposition rate in China during 2011-2015 is estimated as 20.4 ± 2.6 kg N $ha^{-1}yr^{-1}$ (Yu et al., 2019). Critical thresholds occur when the responses of ecological processes to increasing N inputs are non-linear (Groffman et al., 2006; Toms & Lesperance, 2003), as have been found for soil N mineralization, soil respiration, aboveground production, and ecosystem C sequestration (Aber et al., 1998; Aber et al., 1989; Wang et al., 2020). A better understanding of the N saturation threshold for fundamental ecosystem functions is crucial for accurately predicting the ecological consequences of N enrichment, and setting conservation goals and policies (Rockström et al., 2009).

Net primary productivity (NPP) is the primary driver of global C cycling, including photosynthetic and structural pools above- and below-ground. A better understanding of NPP is crucial for detecting biosphere-atmosphere interactions and predicting the responses of ecosystem function to global changes. Nitrogen enrichment generally increases above-ground NPP (ANPP) in many ecosystems (LeBauer & Treseder, 2008; Xia & Wan, 2008). Nevertheless, there is always a threshold for the positive effects of

N (Bai et al. 2010; Bowman et al. 2006), due to the limitation of the maximum photosynthetic rate or the presence of other limiting factors (Elser et al., 2007; Hautier et al., 2009; Peng et al., 2020). Across global grasslands, the mean N saturation threshold of ANPP is estimated as 15 g N m^{-2} $year^{-1}$ (Peng et al., 2020). However, our understanding of the responses of below-ground NPP (BNPP) to N enrichment is more limited. BNPP plays a key role in numerous ecosystem processes. For instance, roots account for more than 60% of plant-origin organic C input into the soil (Peek, 2007; Wang et al., 2019). To date, positive (Yuan & Chen, 2012; Zhang et al., 2015), negative (Bai et al., 2015; Wang et al., 2019), and neutral responses (Gao et al., 2011) of BNPP to N enrichment have been reported. The variation in results is due to the differences in N addition rates, soil nutrient availability (Li et al., 2011; Wang et al., 2019), and plant species composition (Bai et al., 2015; Gao et al., 2011). It remains unknown whether the threshold of BNPP responses to N enrichment parallels that of ANPP and whether divergent or convergent responses between BNPP and ANPP have consequence on the changes of NPP. Although the responses of NPP to N enrichment have been reported in previous studies (Wang et al., 2019; Zhu et al., 2016), understanding about the N saturation threshold for BNPP and NPP is scarce.

Here we propose and test a conceptual framework for understanding the responses of semi-arid grassland NPP and its above- and below-ground components to increasing N addition rates (Fig. 1). Our framework depicts the hierarchical mechanisms underlying the responses of grassland ANPP, BNPP, and NPP (Fig. 1a), based on evidence from temperate semi-arid grasslands. Grassland productivity is generally supposed to be limited by N availability (Vitousek & Howarth, 1991), and thus increasing N addition with lower rates would stimulate both ANPP and BNPP (stage I) (Xia & Wan, 2008; Zhang et al., 2015). At stage II, further increasing N addition rates would lead to plant growth being limited by aboveground factors (light availability) instead of belowground factors (N availability) and plants would consequently allocate more biomass above-ground parts (Bloom et al., 1985). Moreover, increases in soil aluminum and soil NH_4^+-N following acidification related to N addition could depress root growth (Horswill et al., 2008; Li et al., 2014; Tian et al., 2016b), leading to a reduction in BNPP (Aber et al., 1998). The divergent responses of ANPP and BNPP to N addition at this stage may lead to the earlier occurrence of an N saturation threshold for NPP than ANPP (Fig. 1b). At stage Ш, ANPP becomes saturated with N due to the limitations from other factors beyond N, including light availability (Ma et al., 2020; Niu et al., 2010), water availability (Cleland & Harpole, 2010), and soil acidification (Stevens et al., 2015). The magnitude of ecosystem response to increasing N availability would be site- and time-specific (Swindon et al., 2019), as constrained by other resources. For example, a recent study spanning nine NutNet sites showed that the responses of root biomass to N addition depend on background N conditions, with root biomass being increased at sites with low N deposition but decreased at sites with high N deposition (Keller et al., 2023). Uncovering the mechanisms underlying the non-linear responses of NPP and its above- and below-ground components to increasing N addition, which would help improve our mechanistic and quantitative understanding such context-dependent responses of productivity to N enrichment.

Using data from a 5-year field experiment in a temperate meadow steppe in Northeast China, we examined the changes in ANPP, BNPP, and NPP across six N addition rates (0, 2, 5, 10, 20, 50 g N m^{-2} yr^{-1}) and identified their N saturation thresholds. We tested the hypothesis that the N saturation threshold of NPP would be lower than that of ANPP in the temperate steppe.

Materials and methods

Study site

The study site is located in a temperate meadow steppe, near the Erguna Forest-Steppe Ecotone Research Station (50°10′46.1″N, 119°22′56.4″E). The field has been fenced since 2013 to exclude livestock grazing. The long-term mean annual temperature is -2.45°C (1957-2016). Mean annual precipitation is 363 mm, with ~75% occurring from May to August. Our experiment was carried out during 2016-2020, and there were precipitation variability during May to mid-August in each growing season of those five years (Fig. S1). The soil is classified as Loamic Haplic Chernozems by the FAO and as Chernozem in Chinese Soil Classification System. The plant community was dominated by two perennial grasses, *Leymus chinensis* and *Stipa baicalensis*, which together accounted for >60% of the total aboveground biomass.

Experimental design

The N addition experiment was set up in 2014, following a randomized block design. There were six rates of N addition (0, 2, 5, 10, 20, and 50 g N m^{-2} yr^{-1}), crossed with two types of N compounds (NH_4NO_3 and $CO(NH_2)_2$) that are widely used in global N addition experiments. There were a total of 12 treatments, which were replicated eight times in eight blocks. The area of each plot was 10 m × 10 m. Nitrogen fertilizers were mixed with sand (because of the low amount of fertilizer at low addition rates) and broadcast to each plot uniformly by hand in late May since 2014. Sand was sieved through a sieve with 2 mm mesh, washed in water, and then oven-dried at 250°C for 1 hour. To avoid potentially confounding effects, all plots received the same amount of sand (0.5 kg per plot).

Field sampling and measurement

Peak aboveground green biomass at mid-August of each year was used to represent ANPP (Bai et al., 2004). Aboveground biomass was sampled annually from 2016 to 2020 using a 1 × 1 m quadrat, which was randomly placed in each plot without spatial overlap of quadrats among years and at least 50 cm inside the border of each plot to avoid edge effects. All living vascular plants were clipped and sorted to species. Litter in each quadrat was collected after the sampling of aboveground biomass. Plant samples were oven-dried at 65 °C for 48 h and then weighed.

The root ingrowth-core method was used to estimate BNPP (Steingrobe et al., 2001). Three soil cores (7 cm in diameter and 50 cm in depth) were vertically drilled into the soil in each plot in June of 2016, 1 m distance between cores. The collected soil was sieved (mesh size 2 mm) to remove roots. Then, polyester mesh bags (mesh size 1 mm) with root-free sieved soil were inserted into the holes. In mid-September of each year from 2016 to 2020, the mesh bags were carefully pulled out of the holes and the soil was sieved (mesh size 2 mm) to collect roots. After being washed, all root samples were dried at 75°C for 48 h and then weighed. BNPP was calculated based on the average root biomass of three ingrowth cores in each plot. NPP was calculated as the sum of ANPP and BNPP in each plot.

After aboveground biomass and litter sampling at mid-August of each year, three soil cores (0-5 cm depth and 50 cm apart) in each quadrat were collected using a 7 cm diameter soil auger and mixed into one composite sample. Soil samples were sieved through a 2-mm sieve to remove visible roots, plant residues, and stones, and taken to the laboratory for analysis of soil water content (%), soil ammonium (NH_4^+-N; mg kg^{-1}) and nitrate (NO_3^--N; mg kg^{-1}) concentrations. Soil water content was determined using the gravimetric method, with soil samples being weighed before and after being oven-dried at 105°C for 48 h. To measure soil NH_4^+-N and NO_3^--N concentration, 10 g soil sample was extracted with 50 mL of 2 M KCl solution and then analyzed with a FLAstar 5000 Analyzer (Foss Tecator, Hillerød, Denmark). The soil NH_4^+-N and NO_3^-

-N concentration were expressed as mg kg^{-1} dry soil. Soil inorganic N concentration is the sum of soil NH_4^+-N and NO_3^--N concentration. Subsamples of soils were air-dried and used to analyze soil pH, which was measured in a water suspension (soil: water = 1:5) using a pH meter (Thermo Fisher Scientific, America).

Statistical analysis

A mixed model ANOVA was performed to examine the effects of N addition rate, N addition type and their interaction on ANPP, BNPP, NPP, species richness, litter biomass, soil pH, and soil inorganic N concentration. Nitrogen addition rate and type were set as the fixed factors, and block as a random factor (Table S1). As there was no interaction between N addition rate and type in affecting productivity and diversity (all $P > 0.05$; Table S1), data of the two N compound types were combined for further analyses. We further examined the direct and indirect pathways through which N addition and precipitation during May to mid-August in each year from 2017 to 2020 affected ANPP, BNPP, and NPP by structural equation modeling (SEM). We constructed hypothetical causal models including all possible pathways (Fig. S5) and then sequentially removed the non-significant pathways to obtain the best model. The SEM was fitted with linear mixed-effects models (with block as the random effect) using the R package *piecewiseSEM* and *nlme* (Lefcheck, 2016). We used the Fisher's C-test (when $0.05 < P < 1.00$) to confirm the goodness of the modelling results. Species richness, soil pH and soil inorganic N concentration were log-transformed for SEM to meet the assumption of normal distribution.

Quadratic-plus-plateau model was used to identify the threshold for the responses of ANPP, BNPP, and total NPP to increasing N addition rates (Peng et al., 2020). The quadratic-plus-plateau model is defined as:

$$y = ax^2 + bx + c, \text{ if } x \leqslant C \qquad (1)$$

$$y = M, \text{ if } x > C \qquad (2)$$

where y is the ANPP (BNPP or NPP) (g m^{-2} yr^{-1}) and x is the rate of N addition (g N m^{-2} yr^{-1}); a, b and c are the coefficients of the quadratic model (Equation 1), and M is a constant fitting the plateaued phase (Equation 2). The constant C represents the N saturation threshold and the constant M represents the maximum value of ANPP (BNPP or NPP). The model fitting was performed using “easynls” package (Arnhold, 2017). All analyses were conducted using R version 4.1.2 (R Development Core Team, 2021).

Results

Responses of soil and plant community characteristics

Averaged across the four years from 2017 to 2020, there was no difference for inorganic N concentrations and soil pH across the three treatments with lower N addition rates (0 ~ 5 g N m^{-2} yr^{-1}; Fig. 2a,b). Under the treatments with higher N addition rates (10 ~ 50 g N m^{-2} yr^{-1}), soil inorganic N concentrations increased and soil pH decreased with increasing addition rates (Fig. 2a,b). Averaged across the five years from 2016 to 2020, plant species richness decreased with increasing N addition rates, with a reduction of

16.5%, 33.1%, 44.0%, and 56.6% under N5, N10, N20, and N50, respectively (Fig. 2c). Litter biomass increased with increasing N addition rates, with a 50% enhancement under N5 and >150% enhancement under treatments with higher N addition rates (Fig. 2d). The responses of soil inorganic N concentrations and plant community characteristics to N enrichment varied significantly among different years (all $P < 0.001$; Fig. S2).

Nitrogen saturation thresholds for ANPP, BNPP, and NPP

Although NPP varied significantly among different years, it generally had lower saturation threshold than ANPP across the N addition gradient in each year (Fig. S3) and under each of the two N compounds (Fig. S4). Averaged across the five years, NPP and its above- and below-ground components all showed non-linear responses to increasing N addition rates (Fig. 3). With the increases of N addition rates, ANPP increased quickly, with a saturation threshold (T_A) at 13.11 g N m^{-2} yr^{-1}. BNPP tended to increase with N addition, peaked at the rate of 5 g N m^{-2} yr^{-1}, and then decreased under higher N addition rates (Fig. 3). NPP increased with increasing N addition rates, and remained stable when the rates were > 6.70 g N m^{-2} yr^{-1}, with a lower saturation threshold than ANPP (Fig. 3). Specifically, ANPP and BNPP were weakly and positively correlated across plots with N addition rates $\leq$ 5 g N m^{-2} yr^{-1} (Fig. 4a), but were not correlated in plots with > 5 g N m^{-2} yr^{-1} (Fig. 4b).

Different factors driving the responses of productivity under low and high N addition rates

Given that the N saturation threshold for BNPP was 5 g N m^{-2} yr^{-1} and that the responses of soil and plant community characteristics to N addition differed between low and high addition rates (Fig. 2 and Fig. 3), we constructed SEM for the low (N0-N5) and high (N10-N50) N addition rates, separately. Growing season precipitation had direct and positive effects on both ANPP and BNPP, with stronger impacts under high N addition rates (Fig. 5a, b). Under low N addition rates ranging from 0 to 5 g N m^{-2} yr^{-1}, there was direct and positive effect of N enrichment on ANPP, but a significantly negative effect on both ANPP and BNPP (Fig. 5a), probably due to the higher inorganic N accumulation but lower productivity in the years with lower growing season precipitation (Fig. S1, S2, and S3). Under high N addition rates from 10 to 50 g N m^{-2} yr^{-1} (Fig. 5b), the direct and positive effect of N enrichment on ANPP was significant, which would be cancelled out by its indirect and negative effect through soil inorganic N accumulation and biodiversity loss. There was a negative effect of ANPP on BNPP under high N addition rates (Fig. 5b).

ANPP played a more important role than BNPP in driving the variations of NPP at low N addition rates (standardized path coefficient: 0.68 vs 0.64; 0 ~ 5 g N m^{-2} yr^{-1}) and high N addition rates (0.79 vs 0.66; 10 ~ 50 g N m^{-2} yr^{-1}), with the difference being much larger under high N addition rates (Fig. 5).

Discussion

Consistent with results from other grasslands (Bowman et al., 2012; Tian et al., 2016a) as well as from a global meta-analysis (Peng et al., 2020), the responses of ANPP to N addition changed from a linear response to a saturation response with increasing N addition rates in this temperate meadow steppe. Averaged across the five years, the N saturation threshold of ANPP was 13.11 g N m^{-2} yr^{-1} in our ecosystem, which is higher than that in an alpine meadow (4 g N m^{-2} yr^{-1}) (Ma et al., 2020), but quite similar with that in a typical steppe (10.5 g N m^{-2} yr^{-1}) of Inner Mongolia (Bai et al., 2010). Globally, the N saturation threshold for ANPP would vary across different grasslands due to the variations of climate and soil C:N ratio (Peng et al., 2020).

ANPP increased with increasing N inputs when the rates were below the saturation threshold, indicating that N is the limiting nutrient for ANPP in this steppe. The continuous increase of ANPP may attribute to that N enrichment stimulates photosynthesis and increases leaf area (Niu et al., 2010; Ren et al., 2011). However, the SEM results showed negative effect of N addition on ANPP under N0-N5 by increasing soil inorganic N concentration across the five years when precipitation was included in the model, which probably because the higher inorganic N accumulation but lower productivity in the years with lower precipitation (Fig. S1, S2, and S3). Furthermore, we measured soil inorganic N concentrations only once each year, which is an instantaneous varaible closely related to soil moisture and temperature. In N super-saturated grasslands (those receiving more N than the saturation threshold) the positive impacts of increasing N addition rates on ANPP are cancelled out by the negative roles of N-induced changes in other factors, such as soil inorganic N accumulation and the decline of species richness as shown by the SEM results (Fig. 5b). Many biodiversity experiments reported negative impacts of species richness losses on productivity (see Tilman et al., 2014). Stronger light competition (Borer et al., 2014; Hautier et al., 2009), soil acidification, and ammonium toxicity (Stevens et al., 2004; Zhang et al., 2014) are accounting for the N-induced losses of plant diversity. Isbell et al. (2013) found that the positive impacts of N addition on ANPP diminished over time due to the losses of initially dominant plant species. However, a recent work based on 47 grasslands demonstrated the positive impacts of N on aboveground biomass strengthen over time despite increasing species diversity losses (Seabloom et al., 2021). Thus, there is no consensus on how changes in biodiversity will influence N effects productivity, and these seemingly conflicting results might be reconciled by considering how diversity and productivity covary across time and space (Isbell et al., 2013).

In addition, the SEM results showed that N addition had a positive direct but a negative indirect effect on ANPP under N10-N50, in that the increases of soil inorganic N concentration significantly reduced ANPP (Fig. 5). Soil NH_4^+-N accumulation could be toxic to sensitive plant species (Zhang et al., 2014), cause leaf and roots damage (Li et al., 2014), reducing primary productivity (Li et al., 2011; Roosta et al., 2009). For example, De Graaf (1998) found that ammonium concentrations above 100 μM could increase plant mortality and reduce biomass. Together, those results indicate that species richness loss and soil inorganic N accumulation are the driver for the diminishing positive impacts of N addition on ANPP in N saturated temperate steppe.

This study extends our knowledge on the responses of BNPP to N enrichment and inter-annual variations of precipitation, which is less understood than ANPP. First, the responses of BNPP to N addition are highly dependent on the addition rate. Across the wide-ranging N addition gradient and across the five years, BNPP peaked at the addition rate of 5 g N m^{-2} yr^{-1} and then decreased with increasing N rates. Second, we identified the key role of soil inorganic N accumulation in regulating BNPP response to N addition under both low and high N addition rates (Fig. 5). N addition would indirectly reduce BNPP through soil ammonium toxicity in N-saturated and -supersaturated plots. While field evidence for the negative impacts of ammonium toxicity on plant community BNPP are rather scarce, the molecular and genetic mechanisms underlying its toxicity on model plant are well established (Li et al., 2014).

Third, growing season precipitation had direct and positive effect on BNPP in the temperate steppe, providing new evidence for the water-limitation of primary productivity in temperate steppe from belowground perspective. While previous studies have highlighted the importance of precipitation in driving the spatial and temporal variations of ANPP in temperate steppe (Bai et al., 2008; Lü et al., 2018), our results highlight its role in stimulating BNPP.

BNPP played an important role in driving the earlier occurrence of N saturation threshold for NPP than ANPP (6.70 vs. 13.11 g N m^{-2} yr^{-1}). The responses of ANPP, BNPP, and NPP to increasing N addition as found here are generally consistent with the framework proposed in this study (Fig. 1). At the initial stage of N addition, ANPP increased quickly due to the alleviation of N limitation, and thus more roots were required to capture nutrients and water from the soil (Chapin III, 1980). However, the relationship between ANPP and BNPP was rather weak (R^2=0.06, Fig. 4a). The concomitant increases of ANPP and BNPP led to positive responses of NPP to increasing N addition rates at this stage. With continuous N addition, NPP leveled off at the specific N addition rate (e.g. $\geq$ 6.70 g N m^{-2} yr^{-1}), largely due to the trade-off between increases of ANPP and decreases of BNPP. According to the optimal partitioning theory, plants would allocate more biomass to aboveground in response to N supply in order to efficiently capture light (Olff, 1992) and consequently relatively less biomass to belowground. Evidence from other grasslands demonstrated the reduction of BNPP with increasing N addition rates (Gao et al., 2011; Poorter & Nagel, 2000; Wang et al., 2019). These responses indicated that the grassland changed from N-limited to N-saturated, the relationship of ANPP and BNPP is decoupling. At the N supersaturated communities, where N addition rate is higher than the N saturation threshold of ANPP, the enrichment of soil NH_4^+-N depressed the activity and biomass of roots (Li et al., 2014), which caused a decline in BNPP. Overall, ANPP and BNPP were weakly and positively correlated under low N addition rates but not correlated in N-saturated communities. Similarly, Keller et al. (2023) reported no correlation for the responses of aboveground and root production to N addition with a rate of 10 g N m^{-2} yr^{-1} across nine North American grasslands. A recent study in Chihuahuan Desert shrubland also found that the relationship between ANPP and BNPP was weak and that BNPP was unaffected by chronic N enrichment (Brown & Collins, 2023), indicating that BNPP would be controlled by a different set of drivers than ANPP. Globally, ANPP instead of BNPP is sensitive to climate variations (Sun et al. 2021), resulting in different response patterns between ANPP and BNPP along climatic gradients. Our results present further evidence for the differences of their responses along a gradient of soil nutrient availability. The decoupling of ANPP and BNPP has great implications for biogeochemical modeling works, because it would be more difficult to accurately project the changes and contribution of BNPP based on the easily available ANPP data. In addition, our results highlight the importance of viewing ecosystem productivity from a whole-system perspective, and we can not simply predict the responses of total

NPP to N deposition based on above-ground results, which are more often examined in previous studies.

Precipitation is one of key driving factors for plant growth in semi-arid ecosystems (Hooper and Johnson, 1999; Wilcox et al., 2017), as it can stimulate plant growth directly by providing water and indirectly by changing soil nutrient availability (Huxman et al., 2004). In this study, we found that growing season precipitation had direct and positive impacts on ANPP and BNPP (Fig. 5), especially at high N addition rates. Moreover, higher growing season precipitation could release water limitation directly, and had indirect and positive effect on ANPP by alleviating the negative effect of soil inorganic N accumulation in N-enriched grasslands. Given that the increased ANPP in wet years would reduce temporal stability of ANPP in N-enriched grasslands (Wang et al., 2017), our results also support the conclusion that precipitation fluctuations drive ANPP and its temporal stability in N-enriched ecosystems. Both the direct and indirect impacts of precipitation on primary productivity are deserved more attention under the scenarios of increasing N deposition and altering precipitation regime.

Conclusion

This study provides a whole-system perspective on the N saturation threshold of primary productivity in a temperate steppe. We found that ecosystem total primary productivity and its above- and below-ground components showed non-linear responses to increasing N addition rates. The N saturation threshold of NPP occurs earlier than that of ANPP, due to the decline of BNPP in N-saturated communities. As atmospheric N deposition is projected to continue increasing in developing countries, our results suggest that models may overestimate ecosystem total primary productivity based only on ANPP. Our results also shed light on the controllers of NPP responses under different N conditions by uncovering the role of soil inorganic N accumulation in non-N-saturated communities and the role of soil inorganic N accumulation and biodiversity loss in N-saturated communities. Given the importance of primary productivity in driving soil carbon storage in global ecosystems (Plaza et al., 2022), our findings provide empirical footing for improving the biogeochemical models forecasting ecosystem carbon sequestration ability under N deposition.

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Data availability statement

The data that support the findings of this study will be openly available in a public repository after the acceptance of this manuscript.

Authors' contributions

XTL and XGH designed the experiment. XTL conceived the research idea. GJY and ZJZ collected the data. GJY analyzed the data and wrote the first draft, XTL and CS revised the manuscript. All authors contributed to the development of the manuscript.

References

Aber, J. D., Nadelhoffer, K. J., Steudler, P., Melillo, J. M., Bioscience, S., & Jun, N. (1989). Nitrogen saturation in northern forest ecosystems. *Bioscience,39*(6), 378–386.

Aber, J., Mcdowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., Mcnulty, S., Currie, W., Rustad, L., Fernandez, I., Aber, J., Mcdowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Mcnulty, S., Currie, W., Rustad, L., & Fernandez, I. (1998). Nitrogen saturation in temperate forest ecosystems. *Bioscience*, *48*(11), 921–934.

Arnhold, E. (2017). Fit and plot some nonlinear models. Retrieved from https://cran.r-proje ct.org/web/packa ges/easyn ls/index.html.

Bai, W., Guo, D., Tian, Q., Liu, N., Cheng, W., Li, L., & Zhang, W. H. (2015). Differential responses of grasses and forbs led to marked reduction in below-ground productivity in temperate steppe following chronic N deposition. *Journal of Ecology*, *103*(6), 1570–1579. https://doi.org/10.1111/1365-2745.12468

Bai, Y., Han, X., Wu, J., Chen, Z., & Li, L. (2004). Ecosystem stability and compensatory effects in the Inner Mongolia grassland. Nature, 431, 181– 184. https://doi.org/10.1038/natur e02850

Bai, Y., Wu, J., Xing, Q., Pan, Q., Huang, J., Yang, D., & Han, X. (2008). Primary production and rain use efficiency across a precipitation gradient on the Mongolia plateau. *Ecology, 89*(8), 2140–2153.

Bai, Y., Wu, J., Clark, C. M., Pan, Q., Huang, J., Zhang, L., & Han, X. (2010). Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning : evidence from inner Mongolia Grasslands. *Global Change Biology*, *16*, 358–372. https://doi.org/10.1111/j.1365-2486.2009.01950.x

Bloom, A. J., F. Stuart Chapin, I., & Mooney, H. A. (1985). Resource limitation in plants: an economic analogy. *Annual Review of Ecology Evolution and Systematics*, *16*, 363–392.

Borer, E. T., Seabloom, E. W., Gruner, D. S., Harpole, W. S., Hillebrand, H., Lind, E. M., Adler, P. B., Alberti, J., Anderson, T. M., Bakker, J. D., Biederman, L., Blumenthal, D., Brown, C. S., Brudvig, L. A., Buckley, Y. M., Cadotte, M., Chu, C., Cleland, E. E., Crawley, M. J., … Yang, L. H. (2014). Herbivores and nutrients control grassland plant diversity via light limitation. *Nature, 508*(7497), 517–520. https://doi.org/10.1038/nature13144

Bowman, W. D., Murgel, J., Blett, T., & Porter, E. (2012). Nitrogen critical loads for alpine vegetation and soils in Rocky Mountain National Park. *Journal of Environmental Management*, *103*, 165–171. https://doi.org/10.1016/j.jenvman.2012.03.002

Brown, R. F., & Collins, S. L. (2023). As above, not so below : Long-term dynamics of net primary production across a dryland transition zone. *Global Change Biology*, March, 1–13. https://doi.org/10.1111/gcb.16744

Chapin III, F. S. (1980). The mineral nutrition of wild plants. *Annual Review of Ecology Evolution and Systematics*, *11*(1), 233–260. https://doi.org/10.1146/annurev.es.11.110180.001313

Cleland, E. E., & Harpole, W. S. (2010). Nitrogen enrichment and plant communities. In *Annals of the New York Academy of Sciences.1195:* 46-51. https://doi.org/10.1111/j.1749-6632.2010.05458.x

De Graaf, M. C. C., Bobbink, R., Roelofs, J. G. M., & Verbeek, P. J. M. (1998). Differential effects of ammonium and nitrate on three heathland species. *Plant Ecology*, *135*(2), 185–196. https://doi.org/10.1023/A:1009717613380

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., & Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, *10*(12), 1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., … Sutton, M. A. (2008). Transformation of the nitrogen cycle :Recent trends, questions, and potential solutions. *Science*, *320*(5878), 889–892

Gao, Y. Z., Chen, Q., Lin, S., Marcus Giese, & Brueck, H. (2011). Resource manipulation effects on net primary production , biomass allocation and rain-use efficiency of two semiarid grassland sites in Inner Mongolia , China. *Oecologia*, *165*, 855–864. https://doi.org/10.1007/s00442-010-1890-z

Groffman, P. M., Baron, J. S., Blett, T., Gold, A. J., Goodman, I., Gunderson, L. H., Levinson, B. M., Palmer, M. A., Paerl, H. W., Peterson, G. D., Poff, N. L. R., Rejeski, D. W., Reynolds, J. F., Turner, M. G., Weathers, K. C., & Wiens, J. (2006). Ecological thresholds: The key to successful environmental management or an important concept with no practical application? *Ecosystems*, *9*(1), 1–13. https://doi.org/10.1007/s10021-003-0142-z

Hautier, Y., Niklaus, P. A., & Hector, A. (2009). Competition for light causes plant biodiversity loss after eutrophication. *Science*, *324*(5927), 636–638. https://doi.org/10.1126/science.1169640

Hooper, D. U., Adair, E. C., Cardinale, B. J., Byrnes, J. E. K., Hungate, B. A., Matulich, K. L., Gonzalez, A., Duffy, J. E., Gamfeldt, L., & Connor, M. I. (2012). A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature*, *486*(7401), 105–108. https://doi.org/10.1038/nature11118

Horswill, P., O'Sullivan, O., Phoenix, G. K., Lee, J. A., & Leake, J. R. (2008). Base cation depletion, eutrophication and acidification of species-rich grasslands in response to long-term simulated nitrogen deposition. *Environmental Pollution*, *155*(2), 336–349. https://doi.org/10.1016/j.envpol.2007.11.006

Huxman, T.E., Smith, M.D., Fay, P.A., Knapp, A.K., Shaw, M.R., Lolk, M.E., … Williams, D.G., 2004. Convergence across biomes to a common rain-use efficiency. *Nature*, *429*, 651–654. https://doi.org/10.1038/nature02561

Isbell, F., Reich, P. B., Tilman, D., Hobbie, S. E., Polasky, S., & Binder, S. (2013). Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. *Proceedings of the National Academy of Sciences*, 110(25), 11911–11916. https://doi.org/10.1073/pnas.1310880110

Keller, A. B., Walter, C. A., Blumenthal, D. M., Borer, E. T., Collins, S. L., DeLancey, L. C., Fay, P. A., Hofmockel, K. S., Knops, J. M. H., Leakey, A. D. B., Mayes, M. A., Seabloom, E. W., & Hobbie, S. E. (2023). Stronger fertilization effects on aboveground versus belowground plant properties across nine U.S. grasslands. *Ecology, 104*(2), e3891. https://doi.org/10.1002/ecy.3891

LeBauer, D. S., & Treseder, K. K. (2008). Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology, 89*(2), 371–379. https://doi.org/10.1890/06-2057.1

Li, B., Li, G., Kronzucker, H. J., Baluška, F., & Shi, W. (2014). Ammonium stress in Arabidopsis: Signaling, genetic loci, and physiological targets. *Trends in Plant Science*, *19*(2), 107–114. https://doi.org/10.1016/j.tplants.2013.09.004

Li, B., Shi, W., & Su, Y. (2011). The differing responses of two Arabidopsis ecotypes to ammonium are modulated by the photoperiod regime. *Acta Physiologiae Plantarum*, *33*(2), 325–334. https://doi.org/10.1007/s11738-010-0551-5

Lü, X. T., Liu, Z. Y., Hu, Y. Y., & Zhang, H. Y. (2018). Testing nitrogen and water co-limitation of primary productivity in a temperate steppe. *Plant and Soil*, *432*(1–2), 119–127. https://doi.org/10.1007/s11104-018-3791-6

Ma, F., Song, B., Quan, Q., Zhang, F., Wang, J., Zhou, Q., & Niu, S. (2020). Light competition and biodiversity loss cause saturation response of aboveground net primary productivity to nitrogen enrichment. *Journal of Geophysical Research: Biogeosciences*, *125*(3), 1–12. https://doi.org/10.1029/2019JG005556

Niu, S., Wu, M., Yi, H., Jianyang, X., Zhang, Z., Haijun, Y., & Wan, S. (2010). Nitrogen effects on net ecosystem carbon exchange in a temperate steppe. *Global Change Biology*, *16*, 144–155. https://doi.org/10.1111/j.1365-2486.2009.01894.x

Olff, H. (1992). Effects of light and nutrient availability on dry matter and N allocation in six successional grassland species - Testing for resource ratio effects. *Oecologia*, *89*(3), 412–421. https://doi.org/10.1007/BF00317420

Peek, M. S. (2007). Explaining Variation in Fine Root Life Span. In K. Esser (Ed.), *Progress in Botany* (pp. 382– 398). Springer

Peng, Y., Chen, H. Y. H., & Yang, Y. (2020). Global pattern and drivers of nitrogen saturation threshold of grassland productivity. *Functional Ecology*, *34*(9), 1979–1990. https://doi.org/10.1111/1365-2435.13622

Plaza, C., García-Palacios, P., Berhe, A. A., Barquero, J., Bastida, F., Png, G. K., Rey, A., Bardgett, R. D., & Delgado-Baquerizo, M. (2022). Ecosystem productivity has a stronger influence than soil age on surface soil carbon storage across global biomes. *Communications Earth and Environment*, *3*(1), 233. https://doi.org/10.1038/s43247-022-00567-7

Poorter, H., & Nagel, O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: A quantitative review. *Australian Journal of Plant Physiology*, *27*(6), 595–607. https://doi.org/10.1071/pp99173

R Development Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Ren, H., Xu, Z., Huang, J., Clark, C., Chen, S., & Han, X. (2011). Nitrogen and water addition reduce leaf longevity of steppe species. *Annals of Botany, 107*(1), 145–155. https://doi.org/10.1093/aob/mcq219

Rockström1, J., Steffen, W., Noone, K., Persson, Å., F. Stuart Chapin, I., & F., L. E. (2009). A safe operating space for humanity. *Nature*, *461*, 472–475.

Roosta, H. R., Sajjadinia, A., Rahimi, A., & Schjoerring, J. K. (2009). Responses of cucumber plant to NH_4^+ and NO_3^- nutrition: The relative addition rate technique vs. cultivation at constant nitrogen concentration. *Scientia Horticulturae*, *121*(4), 397–403. https://doi.org/10.1016/j.scienta.2009.03.004

Seabloom, E. W., Adler, P. B., Alberti, J., Biederman, L., Buckley, Y. M., Cadotte, M. W., Collins, S. L., Dee, L., Fay, P. A., Firn, J., Hagenah, N., Harpole, W. S., Hautier, Y., Hector, A., Hobbie, S. E., Isbell, F., Knops, J. M. H., Komatsu, K. J., Laungani, R., … Borer, E. T. (2021). Increasing effects of chronic nutrient enrichment on plant diversity loss and ecosystem productivity over time. *Ecology, 102*(2), e03218. https://doi.org/10.1002/ecy.3218

Steingrobe, B., Schmid, H., & Claassen, N. (2001). The use of the in- growth core method for measuring root production of arable crops - Influence of soil and root disturbance during installation of the bags on root ingrowth into the cores. *European Journal of Agronomy, 15*, 143– 151. https://doi.org/10.1016/S1161 - 0301(01)00100 - 9

Stevens, C. J., Dise, N. B., Mountford, J. O., & Gowing, D. J. (2004). Impact of Nitrogen Deposition on the Species Richness of Grasslands. *Science, 303*(5665), 1876–1879. https://doi.org/10.1126/science.1094678

Stevens, C. J., Lind, E. M., Hautier, Y., Harpole, W. S., Borer, E. T., Hobbie, S., Seabloom, E. W., Ladwig, L., Bakker, J. D., Chu, C., Collins, S., Davies, K. F., Firn, J., Hillebrand, H., La Pierre, K. J., MacDougall, A., Melbourne, B., McCulley, R. L., Morgan, J., … Wragg, P. D. (2015). Anthropogenic nitrogen deposition predicts local grassland primary production worldwide. *Ecology*, *96*(6), 1459–1465. https://doi.org/10.1890/14-1902.1

Sun, Y., Yang, Y., Zhao, X., Tang, Z., Wang, S., & Fang, J. (2021). Global patterns and climatic drivers of above- and belowground net primary productivity in grasslands. *Science China Life Sciences, 64*(5), 739–751. https://doi.org/10.1007/s11427-020-1837-9

Swindon, J. G., Burke, I. C., & Lauenroth, W. K. (2019). Seasonal Patterns of Root Production with Water and Nitrogen Additions Across Three Dryland Ecosystems. *Ecosystems, 22*(7), 1664–1675. https://doi.org/10.1007/s10021-019-00364-y

Tian, D., Wang, H., Sun, J., & Niu, S. (2016a). Global evidence on nitrogen saturation of terrestrial ecosystem net primary productivity. *Environmental Research Letters*, *11*(2). https://doi.org/10.1088/1748-9326/11/2/024012

Tian, Q., Liu, N., Bai, W., Li, L., Chen, J., Reich, P. B., Yu, Q., Guo, D., Smith, M. D., Knapp, A. K., Cheng, W., Lu, P., Gao, Y., Yang, A. N., Wang, T., Li, X., Wang, Z., Ma, Y., Han, X., & Zhang, W. H. (2016b). A novel soil manganese mechanism drives plant species loss with increased nitrogen deposition in a temperate steppe. *Ecology, 97*(1), 65–74.

Tilman, D., Isbell, F., & Cowles, J. M. (2014). Biodiversity and ecosystem functioning. *Annual Review of Ecology, Evolution, and Systematics, 45*, 471–493. https://doi.org/10.1146/annurev-ecolsys-120213-091917

Toms, J. D., & Lesperance, M. L. (2003). Piecewise regression: A tool for identifying ecological thresholds. *Ecology*, *84*(8), 2034–2041.

Vitousek, P. M., & Howarth, R. W. (1991). Nitrogen limitation on land and in the sea : How can it occur ? *Biogeochemistry*, *13*(2), 87–115.

Wang, C., Ren, F., Zhou, X., Ma, W., Liang, C., Wang, J., Cheng, J., Zhou, H., & He, J. S. (2020). Variations in the nitrogen saturation threshold of soil respiration in grassland ecosystems. *Biogeochemistry*, *148*(3), 311–324. https://doi.org/10.1007/s10533-020-00661-y

Wang, J., Knops, J. M. H., Brassil, C. E., & Mu, C. (2017). Increased productivity in wet years drives a decline in ecosystem stability with nitrogen additions in arid grasslands. *Ecology*, *98*(7), 1779–1786. https://doi.org/10.1002/ecy.1878

Wang, J., Gao, Y., Zhang, Y., Yang, J., Smith, M. D., Knapp, A. K., Eissenstat, D. M., & Han, X. (2019). Asymmetry in above- and belowground productivity responses to N addition in a semi-arid temperate steppe. *Global Change Biology*, *25*(9), 2958–2969. https://doi.org/10.1111/gcb.14719

Wilcox, K.R., Shi, Z., Gherardi, L.A., Lemoine, N.P., Koerner, S.E., Hoover, D.L., … Luo, Y., 2017. Asymmetric responses of primary productivity to precipitation extremes: a synthesis of grassland precipitation manipulation experiments. *Global Change Biology, 23*, 4376–4385. https://doi.org/10.1111/gcb.13706

Xia, J., & Wan, S. (2008). Global response patterns of terrestrial plant species to nitrogen addition. *New Phytologist*, *179*(2), 428–439. https://doi.org/10.1111/j.1469-8137.2008.02488.x

Yu, G., Jia, Y., He, N., Zhu, J., Chen, Z., Wang, Q., Piao, S., Liu, X., He, H., Guo, X., Wen, Z., Li, P., & Ding, G. (2019). Stabilization of atmospheric nitrogen deposition in China over the past decade. *Nature Geoscience, 12*(6), 424–429. https://doi.org/10.1038/s41561-019-0352-4

Yuan, Z. Y., & Chen, H. Y. H. (2012). A global analysis of fine root production as affected by soil nitrogen and phosphorus. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1743), 3796–3802. https://doi.org/10.1098/rspb.2012.0955

Zhang, T., Guo, R., Gao, S., Guo, J., & Sun, W. (2015). Responses of plant community composition and biomass production to warming and nitrogen deposition in a temperate meadow ecosystem. *PLoS ONE*, *10*(4). https://doi.org/10.1371/journal.pone.0123160

Zhang, Y., Lü, X., Isbell, F., Stevens, C., Han, X., He, N., Zhang, G., Yu, Q., Huang, J.,

& Han, X. (2014). Rapid plant species loss at high rates and at low frequency of N addition in temperate steppe. *Global Change Biology, 20*(11), 3520–3529. https://doi.org/10.1111/gcb.12611

Zhu, K., Chiariello, N. R., Tobeck, T., Fukami, T., & Field, C. B. (2016). Nonlinear, interacting responses to climate limit grassland production under global change. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(38), 10589–10594. https://doi.org/10.1073/pnas.1606734113

Figure 1 (a) Potential driving factors for the responses of above-ground (ANPP), below-ground (BNPP), and total net primary productivity (NPP) to increasing N addition rates under different stages (I, II, and III); (b) Schematic illustration of the occurrence of N saturation threshold of ANPP (T_A), BNPP (T_B), and NPP (T_N).

Figure 2 Effects of different N addition rates on the concentration of soil inorganic (a), soil pH (b), species richness (c), and litter biomass (d) in a temperate meadow steppe. The soil data shown are averaged across four years (2017-2020) and plant data shown are averaged across five years (2016-2020) as 16 replications ± SE. Different letters indicate significant difference at $P < 0.05$.

Figure 3 Changes of aboveground net primary productivity (ANPP), belowground net primary productivity (BNPP), and net primary productivity (NPP) across a gradient of N addition rate in a temperate meadow steppe. The data shown are the five year (2016-2020) means with 16 replications ± SE.

Figure 4 Relationships between above-ground and below-ground net primary productivity under N0 to N5 treatment (a) and N10 to N50 treatment (b) from 2016 to 2020 in a temperate meadow steppe.

Figure 5 Results of structural equation modelling (SEM) showing the direct and indirect effects of N enrichment and growing season precipitation (May to mid-August) on aboveground net primary productivity (ANPP), belowground net primary productivity (BNPP), and net primary productivity (NPP) from 2017 to 2020 under N0 to N5 treatment (a) and N10 to N50 treatment (b). Solid blue and red arrows represent significant positive and negative correlation, respectively (asterisks denote significant levels: *, $P \leq 0.05$; **, $P \leq 0.01$; and ***, $P \leq 0.001$, respectively), dashed arrow represents marginal significant correlation ($0.05 < P < 0.1$). Arrow width is proportional to the strength of the correlation. The numbers represent standardized path coefficients. Percentages next to endogenous variables indicate the variance explained by the model (R^2).