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Soil water regulates plant diversity response to gradual and step nitrogen addition

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Abstract

Atmospheric nitrogen (N) deposition has a gradual and slow impact on N inputs in
ecosystems. However, our current understanding of the effects of N deposition largely
relies on step N addition experiments. In this study, we investigated the contrasting
impacts of step versus gradual N addition on plant community biomass and species

richness in a typical temperate grassland. Additionally, we measured soil pH, inorganic N concentration, and moisture to explore the potential mechanisms underlying species loss with N addition. Our findings revealed that both gradual and step N addition significantly increased plant aboveground biomass, with increases of 150% and 221% respectively. However, step N addition resulted in a significant reduction in plant species richness by 18%, while gradual N addition did not significantly alter species richness. The positive correlation between species richness and soil water content indicated that soil moisture played a crucial role in determining the extent of species loss under step versus gradual N additions. Collectively, our study suggests that step N additions may overestimate the impact of nitrogen addition on plant biomass and biodiversity in comparison to gradual N addition.

KEYWORDS

Plant biomass, grassland, gradual N, step N, soil moisture, species richness

1 INTRODUCTION

Global nitrogen (N) deposition, predominantly attributed to anthropogenic sources such as artificial fertilizer spread and fossil fuel combustion, is recognized as a pivotal factor in global climate change (Galloway et al. 2008). Generally, in real ecosystems, N deposition occurs gradually and increases at a slow pace (Galloway et al. 2008). It is worth noting that existing studies introduced N fertilization abrupt and fixedly, often at specific levels such as 5 or 10 g N m⁻² yr⁻¹ (Xiao et al. 2021, Keller et al. 2023, Wheeler et al. 2023, Yu et al. 2023). However, it is important to acknowledge that the instantaneous addition of N in fixed amounts fails to accurately reflect the gradual process of N deposition observed in real-world ecosystems (Luo et al. 2009, De Boeck et al. 2015). This discrepancy probably leads to an over or down-response of ecosystem processes, potentially deviating from the actual dynamics and responses found in natural environments. Therefore, what we should genuinely concern about is the impact of both gradual and step changes in the global change factors. In particular, we seek to

investigate whether ecosystems exhibit disparate responses when confronted with gradual versus step increases in N deposition. Does a sudden increase in N lead to a transient overshoot, and if so, what are the underlying mechanisms driving this response? Conducting manipulative experiments that accurately replicate the gradual changes of global environmental factors encountered in the real world presents significant challenges. Some scholars have noted that there is not a perfect match between the experimental studies involving step increases and the complex dynamics of real-world global environmental changes (Luo et al. 2009, De Boeck et al. 2015). Their finding suggests that abrupt changes in environmental factors can elicit an exaggerated response in ecosystem functioning over the short term (Luo et al. 2009, De Boeck et al. 2015). Several experiments and models have been conducted to investigate the contrasting effects of gradual and step warming and increasing CO₂ levels on ecosystem processes (Luo and Reynolds 1999, Dafeng Hui 2002, Shen et al. 2009). However, to data, there is a notable scarce of relevant research specifically focused on the response of ecosystem processes to gradual N addition.

Elevated atmospheric N deposition exerts various effects on terrestrial ecosystems, including plant photosynthesis and growth, ecosystem productivity, and plant biodiversity (Zhang et al. 2016a, Soons et al. 2017, Dai et al. 2019, Lama et al. 2020, Seabloom et al. 2021, Zhang et al. 2021, Jia et al. 2022b, Meng et al. 2022, Keller et al. 2023). Numerous studies have demonstrated that N deposition lead to increases in plant aboveground biomass while contributing to declines in plant diversity and ecosystem stability (Clark and Tilman 2008, Fang et al. 2012, Seabloom et al. 2021, Ke et al. 2023, Keller et al. 2023). The mechanisms underlying the effects of nitrogen addition on plant community productivity are relatively clear as there were many studies in field experiments (Ke et al. 2023, Keller et al. 2023). The impacts of atmospheric nitrogen deposition on biodiversity loss in grassland ecosystems is a significant and concerning global issue (Galloway et al. 2008, Bobbink et al. 2010, Payne et al. 2013, Clark et al. 2019). Biodiversity plays a crucial role in sustaining the functionality of ecosystems and the provision of vital ecosystem services (Hooper et al. 2005, Cardinale et al. 2012). Therefore, understanding the mechanisms underlying the loss of species diversity is

crucial for species conservation efforts.

The potential mechanisms underlying the species loss after N addition are still uncertain (Band et al. 2022). Three main explanations were proposed: (1) the “nitrogen detriment hypothesis” – N addition alters the abiotic or biotic conditions, including litter accumulation (Fang et al. 2012), ammonium toxicity (Britto and Kronzucker 2002), soil acidification (Fang et al. 2012, Tian et al. 2022, Ke et al. 2023), soil microbiome (Farrer and Suding 2016), and thus increases plant susceptibility to various stress agents and results in species loss; (2) the “niche dimension hypothesis” – high levels of soil resources (nutrients) reduce the potential for resource-based niche partitioning, and species loss in fertilized plots is strongly and positively related to the number of added resources (Harpole and Tilman 2007); (3) the “biomass-driven competition hypothesis” – high levels of soil resources increase plant aboveground biomass, thereby intensifying competitive interactions (Grime 1973, Newman 1973). Many researchers suggest that light competition drives species loss under high soil fertility because the canopy and/or fast-growing species have preferential access to light resources (Grime 1973, DeMalach et al. 2017). Besides, others suspected that species loss were the result of a combination of light and belowground competition (Grime 1973). It is important to note that these explanations are not mutually exclusive, and the relative importance of each mechanism may vary depending on specific environmental conditions and ecological contexts (Band et al. 2022). In water-scarce regions, N deposition has been observed to stimulate aboveground biomass, thereby potentially increasing water uptake by plants (Yang et al. 2011, Liu et al. 2017) and thus increasing competition for water among species and plant capacity to resist drought (Pivovarovoff et al., 2016; Chen et al., 2022). Consequently, soil water may regulate plant species in arid and semiarid ecosystems (Su et al. 2019).

In this study, we conducted a three-year field experiment in the semi-arid grassland of the Loess Plateau in China to examine the impacts of gradual versus step N addition on plant community biomass and species richness. The study aimed to address two key questions: (1) whether gradient and step N addition elicit different effects on plant biomass and species richness, and (2) which mechanism primarily drives the response

of plant species to gradual and step N addition.

2 MATERIALS AND METHODS

2.1 Experimental site

We conducted a N addition field experiment in a semiarid grassland located at Gansu Provincial Dryland Agroecology Observation and Research Station, Lanzhou University, China (36°02'N, 104°25'E; 2400 m asl). The grassland is characterized by a moderate temperate semi-arid climate (Zheng et al. 2023), the mean annual air temperature was about 5.6 °C during the study period of 2017-2019, and the mean annual precipitation was 408 mm. Soils are classified as *Dark loessial* and *Brunisolic* soils (CSTCRG 1995) and are composed of 9.5% clay, 11.2% sand, and 79.3% silt in the 0-20 cm soil profile. Grazing by large mammals was prevented by fencing. The plant communities are dominated by a mix of grasses, including graminoids (*Leymus secalium*, *Poa annua* L, *Stipa breviflora*), and forbs (*Artemisia capillaris*, *Heteropappus altaicus*, *Artemisia annua*, *Leontopodium nanum*, *Pedicularis kansuensis*, *Potentilla bifurca*).

2.2 Experimental design

We set up twelve 2×2 m plots in a randomized block design in 2016. In each block, we included an ambient, a gradual N addition (Gradual N), and a step nitrogen addition (Step N). Each treatment had four replicates. Plots were separated by 1m walkways. The gradual N treatment mimicked a gradual increase in N deposition over time, where we added 4 g N m⁻² yr⁻¹ in 2017, 10 g N m⁻² yr⁻¹ in 2017, and 16 g N m⁻² yr⁻¹ in 2019. In each year, we applied N in four times during the growing season (April to July), with an increase of 0.385 g N m⁻² each time/month. The step N treatment is a commonly used manipulation in the field experiment, where we added 16 g N m⁻² yr⁻¹ in all 3 years and applied 4 g N m⁻² each month during the growing season (April to July). Nitrogen was applied in the form of ammonium nitrate (NH₄NO₃). The gradual and step N treatments resulted in the same N loading (16 g N m⁻² yr⁻¹) in the last year of

experiment (i.e., 2019), while they differed in the total amount of N added (30 versus 48 g N m⁻² in 3-year, respectively). In each application, we dissolved NH₄NO₃ in 400 mL of distilled water and evenly sprayed the solution into the N treatment plot. The control/ambient plots were also received 400 mL of distilled water. The total amount of water added to each plot corresponded to 0.5 mm of extra precipitation annually.

2.3 Field Sampling and Measurements

We measured the plant aboveground biomass in mid-August by clipping all plants above the soil surface from three 0.5×0.5m quadrats randomly placed in each plot. One time every year after growth season from 2017 to 2019. We sampled 12 quadrates for each treatment. Plant material was oven-dried at 80°C to constant weight in the laboratory. We classified the plants into two functional groups based on life form, that is, grasses and forbs, and recorded the species number of each plot. After removing aboveground biomass, we collected roots from each sampling quadrat using the soil core (9 cm diameter, 60 cm depth) method. The soil was washed in a 0.15 mm mesh bag and dried at 65°C to constant weight. We then collected soil samples (soil core with 2.5 cm diameter and 20 cm depth) from each sampling quadrat. Fresh soils were passed through a 2-mm sieve, roots and rocks were removed. Later, we homogenized soil samples. The composite soil for each plot was divided into two parts: one part was stored at 4°C immediately after collection for later measurement of soil inorganic nitrogen, soil pH, and soil moisture content; the other part was air dried and stored at room temperature. Soil inorganic nitrogen (IN) concentration (mg kg⁻¹), including nitrate (NO₃⁻-N) and ammonium (NH₄⁺-N), was measured by potassium chloride solution spectrophotometry. Soil pH was measured with a pH meter on a 1:2.5 soil:water suspension. Gravimetric moisture content was measured by drying soil subsamples at 105°C for 48 h. We measured aboveground biomass, species richness and soil properties every year from 2017 to 2019, and measured belowground biomass only in 2019 to minimize destruction of the plots.

2.4 | Statistical analyses

We calculated the temporal mean of plant community aboveground biomass, species richness, soil inorganic nitrogen, pH, and water content across the three sampling years. Then, we used Random Block ANOVAs with treatment as fixed independent factor and block as random independent factor, followed by LSD multiple-range tests to examine the effects of N addition on these variables. We used Linear mixed effects model to analyse the relationship between nitrogen (N) loading and soil pH, soil water content, NO_3^- -N, and NH_4^+ -N under gradual and step N addition. Later, we used Pearson correlation analysis to examine the correlations among soil inorganic nitrogen, biomass, soil pH, soil water content, and species richness. Finally, we used a structural equation model to examine the possible mechanisms underlying the responses of species richness to N addition. N addition was introduced in the model as a continuous, the value of each case was used the three-year mean of N loading. We employed chi-square (χ^2), p-value (p), the goodness of fit index (GFI) and comparative fit index (CFI), and root-mean-square error of approximation (RMSEA) to evaluate the goodness of model fitting (Grace et al. 2015), where $p > 0.05$, $\text{GFI} > 0.90$, $\text{CFI} > 0.95$, $\text{RMSEA} \leq 0.08$ suggested a reasonable fitting. We performed all the above analyses with R 4.2.3 (R Foundation for Statistical Computing). The graphs made use of origin 2023b and R package (ggplot2).

3 RESULTS

3.1 Responses of plant biomass and species richness

Compared with the ambient level, gradual and step N addition significantly increased 3-year average plant community aboveground biomass by 150% and 221%, respectively (Figure 1a). The step N addition increased plant community aboveground biomass by 28% relative to the gradual N addition (marginally significant, $p=0.05$) (Figure 1a). The gradual and step N addition significantly increased plant community belowground biomass by 35% and 54% in 2019, respectively (Figure 1d). However,

there was no significant difference between the step and gradual N addition. The N addition significantly increased grasses and forbs aboveground biomass by 272% and 154%, respectively; while the gradual N addition significantly increased grasses and forbs aboveground biomass by 195% and 92%, respectively (Figure 1b-c).

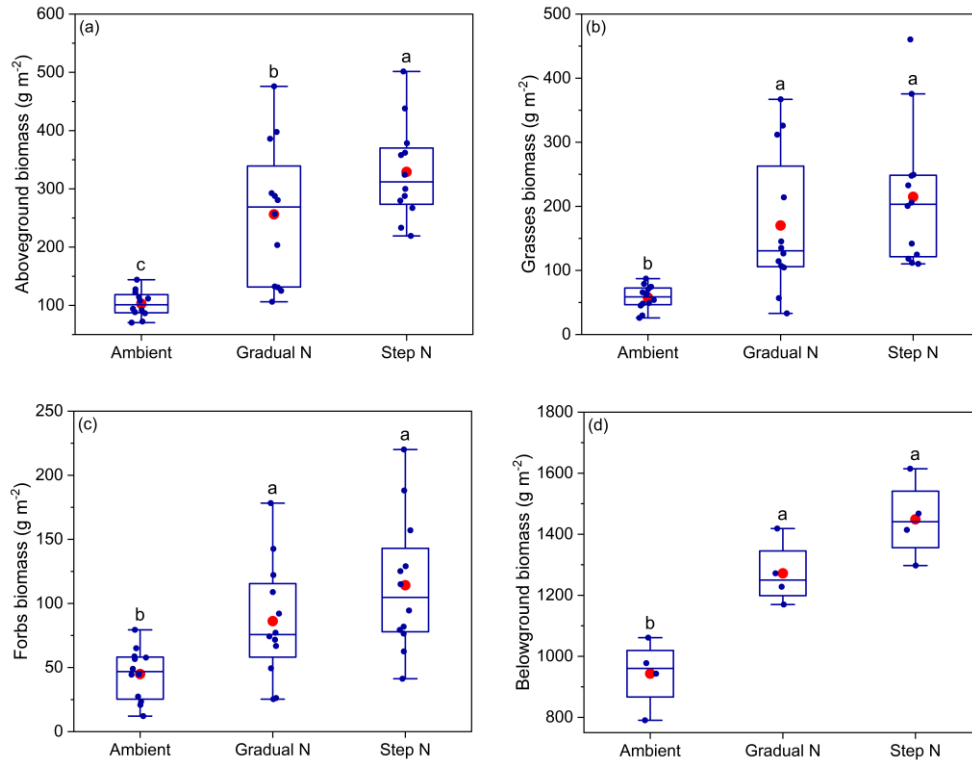


Figure 1 Effects of gradual and step nitrogen (N) addition on plant community biomass. (a) Aboveground biomass, (b) Grasses aboveground biomass, (c) Forbs aboveground biomass, (d) Belowground biomass. Aboveground biomass showed the all values of 2017– 2019, and belowground biomass was measured in 2019 only. Different letters indicate significant differences among treatments. Boxes show the median, 25th and 75th percentiles. Red dots are the mean values.

Averaged over the 3-year, step N addition significantly decreased species richness by 18% ($p = 0.034$) relative to ambient level, while gradual N showed no significant effects on species richness (Figure 2a). This result was consistent if we only used species richness measured in the last of of experiment (Figure 2b). In summary, step N tended to decrease species richness more intensely than gradual N addition did (Figure 2).

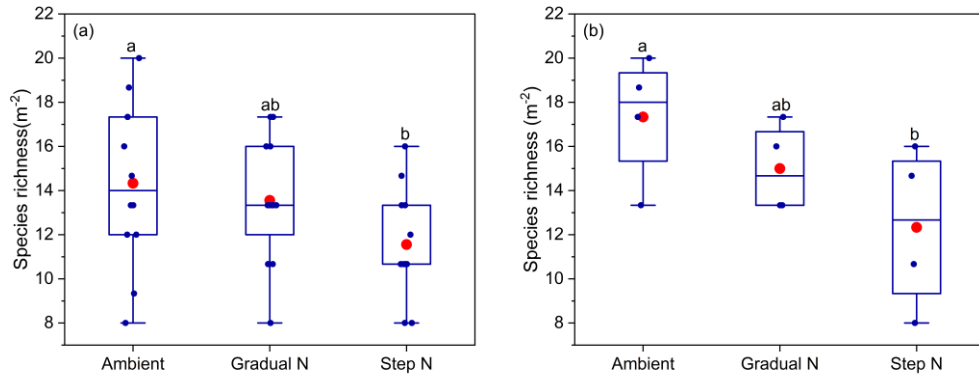


Figure 2 Effects of gradual and step nitrogen (N) addition on plant species richness. **(a)** Average species richness during 2017–2019, and **(b)** species richness in 2019. Different letters indicate significant differences among treatments. Boxes show the median, 25th and 75th percentiles. Red dots are the mean values.

3.2 Changes in soil pH, inorganic nitrogen, and soil water content

Soil pH did not show significant trend with N loading (Figure 3a). Soil NO₃⁻-N increased significantly with N loading (Figure 3b), while soil water content decreased significantly with N loading under gradual and step N addition (Figure 3c). Soil NH₄⁺-N concentration showed no significant trend with N loading (Figure 3d).

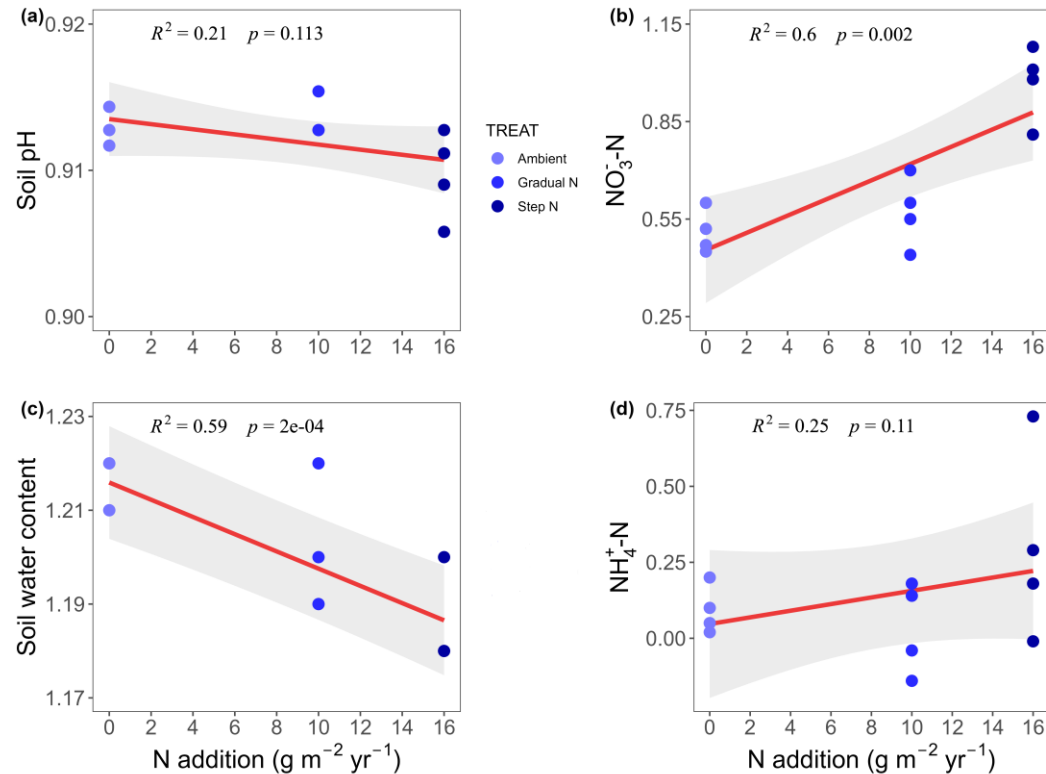


Figure 3 Linear mixed effects model analysis between nitrogen (N) loading and (a) soil pH, (b) soil water content, (c) NO_3^- -N, and (d) NH_4^+ -N under gradual and step N addition, and the circles of different shades of colour represent logarithm of the average of 2017-2019 of different treatments. The gradual and step N treatments had 10 and 16 $\text{g N m}^{-2} \text{ yr}^{-1}$ in 3-year, respectively.

3.3 Mechanism of changes in plant species richness

The correlation analysis showed significant positive relationships between plant aboveground biomass and soil NO_3^- -N, inorganic N concentration, and N addition (Figure 4, $p < 0.05$). Plant belowground biomass and soil NO_3^- -N concentration and N addition were positively (Figure 4, $p < 0.05$). Plant aboveground and belowground biomass and soil water content were also positively correlated (Figure 4, $p < 0.001$). Plant species richness was positively related to soil water content (Figure 4, $p < 0.05$), suggesting soil water importantly regulated species.

The SEM model adequately fitted the data describing interaction pathways among plant and soil in response to N addition ($\chi^2 = 0.381$, $p = 0.996$, RMSEA = 0.000, $df = 5$; Figure 5). The model revealed that N addition significantly increased plant aboveground biomass and soil inorganic N, while plant aboveground biomass decreased soil water content and reduced species richness. Soil water was negatively linked to aboveground biomass, which was consistent with our expectation that higher aboveground biomass under N addition would cause greater water demand and consumption.

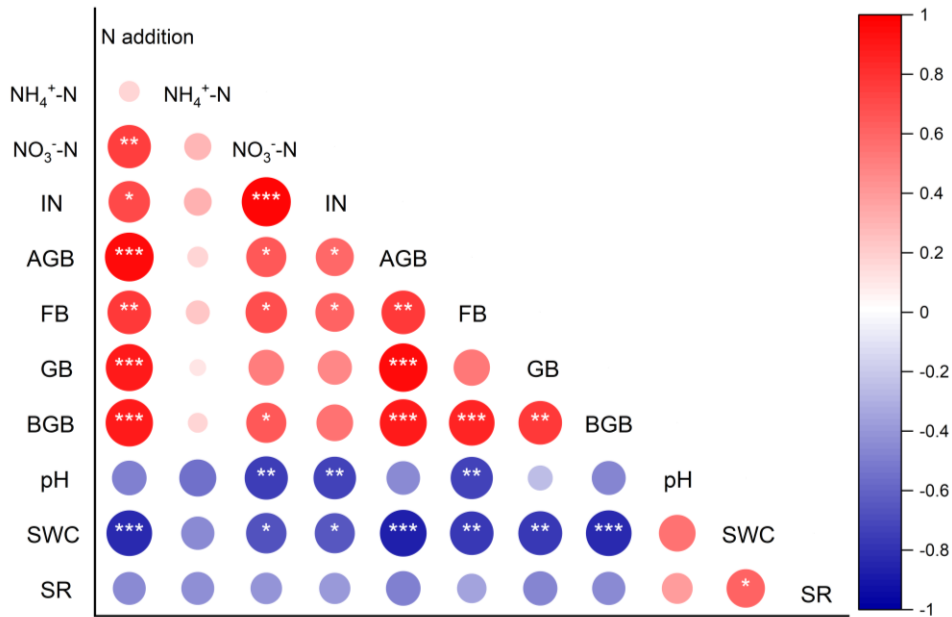


Figure 4 Correlations of soil inorganic nitrogen (IN), ammonium nitrogen ($\text{NH}_4^+\text{-N}$), nitrate nitrogen ($\text{NO}_3^-\text{-N}$), aboveground biomass (AGB), grasses aboveground biomass (GB), forbs aboveground biomass (FB), and belowground biomass (BGB), pH, soil water content (SWC) and species richness (SR). *p < 0.05, **p < 0.01 and ***p < 0.001.

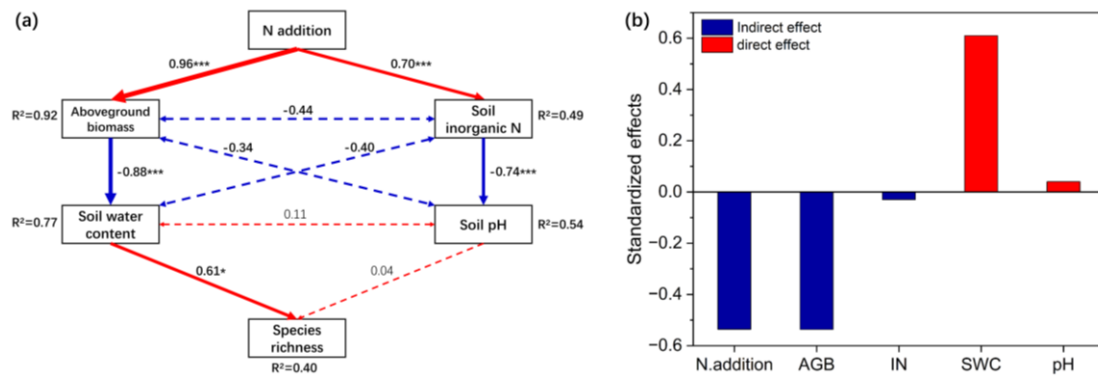


Figure 5 Structural equation model showing pathways through which nitrogen addition influences species richness. **(a)** The final model fits the data adequately based on the chi-square and RMSEA tests ($\chi^2 = 0.218$, $p = 0.999$, RMSEA = 0.000, $df = 5$). Red and blue solid arrows represent significant positive and negative pathways, respectively. Red and blue dashed arrows represent no significant positive and negative pathways, respectively. Numbers adjacent to the arrows are standardized path coefficients with different significance levels: *p < 0.05, **p < 0.01, and ***p < 0.001. The R² of each dependent variable is the proportion of variance explained by the model. **(b)** Direction

of total, direct, and indirect effects of biotic and abiotic factors on plant species richness.

4 DISCUSSION

4.1 The differences in plant community responses under gradual and step nitrogen addition

The study highlighted that the differences in the effects of gradual and step N addition on plant biomass and species richness. Nitrogen addition increased plant biomass, which was consistent with previous studies (Clark and Tilman 2008, Jia et al. 2022a, Ke et al. 2023). Particularly, step N addition exhibited a significantly positive effect on plant aboveground biomass compared to gradual N addition, suggesting that the conventional practice of step N addition tended to overestimate the effect on plant aboveground biomass, which was consistent with our initial hypothesis. This may be due to that step N addition significantly increased soil inorganic N concentration (Figure 3 b), which had a positive effect on plant aboveground biomass (Figure 4). Furthermore, step N addition increased soil nitrate nitrogen, which subsequently led to an increase in belowground biomass (Figure 3b, Figure 4). It a possible complementary explanation is that in gradual N deposition microbes have more change to take up N than when N is added at great dosis and plants have more capacity to take up it.

In reality, N deposition increases gradually and slowly. Up to now, there is no relevant research on gradual N addition, only a few experiments and modeling assessed the differences in the response of ecosystem processes to gradual and step warming and CO₂ elevation (Luo and Reynolds 1999, Dafeng Hui 2002, Shen et al. 2009). Their results showed that step perturbations generally resulted in larger and had longer differentiated impacts than did gradual perturbations. Zhang et al. (Zhang et al. 2014, Zhang et al. 2015a, Zhang et al. 2015b, Zhang et al. 2018) divided the same amount of N evely into 12- versus 2-addition per year, and found significant impacts on plant community structure and species competition only under the 2-addition treatment (Zhang et al. 2015). Our study used a gradual N increasing approach, and suggested

that conventional step N addition might overestimate the effects of N on plant biomass and species richness.

4.2 Potential drivers of species to gradual and step nitrogen addition

We found that only step N addition significantly reduced species richness, indicating that step N overestimates the effect on species loss (Figure 2). To explore the underlying mechanisms of the negative effects of N addition on species richness, we further assessed the responses of soil pH, soil $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, soil water content, and plant biomass to N addition. It has long been proposed that N addition would decrease grassland biodiversity through competition for light and soil resources, e.g. the “biomass-driven competition hypothesis” (Newman 1973, DeMalach et al. 2017, Band et al. 2022). In this study, correlation analysis and structural equation modeling showed no direct and significant correlation between biomass and species richness. There were significant negative links between plant biomass and soil water content, and positive link between soil water content and species richness (Figure 5). Increased plant aboveground biomass under N addition would consume more soil water by transpiration (A et al. 2019), and increased rainfall interception and canopy evaporation and decreased rainwater infiltration to soil (Zhang et al. 2016b). Once soil water content declines, the shallow-rooted and weaker competitive species may be excluded (Yang et al. 2018). Therefore, the reduced species richness in gradual and step N treatments could be attributed to lower soil water content in this treatment than ambient (Figure 2, Figure 3c), pointing to the importance of water as a limiting resource in the semiarid grasslands (Zhu et al. 2020). This is conceptually similar to low light availability, associated with increased plant biomass, limiting species diversity in a number of other resource enrichment studies (Hautier et al. 2009, Borer et al. 2014, Zhu et al. 2020).

The niche dimension hypothesis posits that increasing the number of added resources in resource addition experiments should decrease species richness (Ren et al. 2010, Harpole and Suding 2011). Only one resource was added to our field experiment,

and the addition of one resource also reduced species richness compared to the ambient. The “nitrogen detriment hypothesis” is also used to explain the species loss under N addition. In general, N addition can induce soil acidification, and then drive plant species loss directly or indirectly (Tian and Niu 2015, Ke et al. 2023). Soil acidification is directly harmful to plant growth (Bobbink et al. 2010) and the tolerance of plant species to soil acidification is different (Yadav et al. 2020, Tian et al. 2022), resulting in the loss of some vulnerable species. In contrast, although N addition significantly increased soil inorganic N and caused a significant decrease in soil pH, there was no significant correlation between soil pH and species richness (Figure 4 and Figure 5). In addition, ammonia toxicity can also affect species richness (Tian et al. 2016), nevertheless, N addition did not significantly affect NH_4^+ -N concentrations in our experiments, probably soils in the grassland were alkline ($\text{pH} > 8.0$). In summary, our findings suggested soil water strongly regulate species loss in this semiarid grassland under N addition.

CONCLUSION

Through nitrogen addition field experiments in a semiarid temperate grassland, we investigated the differential effects of gradient and step nitrogen additions on plant community biomass and species richness. Our findings revealed significantly stronger impacts of step N addition compared to gradual N addition on plant community aboveground biomass and species richness. This suggests that conventional gradual N additions may lead to an overestimation of the effects on grassland communities. Furthermore, we observed a greater decline in plant species richness under step N addition compared to gradual N addition, indicating that the effects of N deposition on species richness may be exaggerated when step N additions are utilized.

The observed reduction in species richness following N addition was associated with decreased soil water content. Therefore, soil moisture plays a significant role in regulating the response of plant species richness to N addition in semiarid environments.

In fact, step N addition resulted in a higher increase in both aboveground and belowground biomass compared to gradient N addition. This led to a greater decrease in soil water content and subsequently intensified competition for water, thereby contributing to a higher magnitude of species loss compared to gradient N addition.

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