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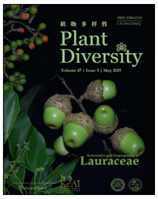

Research paper

# Plant use of water across soil depths regulates species dominance under nitrogen addition

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ABSTRACT

The primary mechanism driving plant species loss after nitrogen (N) addition has been often hypothesized to be asymmetric competition for light, resulting from increased aboveground biomass. However, it is largely unknown whether plants' access to soil water at different depths would affect their responses, fate, and community composition under nitrogen addition. In a semiarid grassland exposed to 8-years of N addition, we measured plant aboveground biomass and diversity under four nitrogen addition rates (0, 4, 10, and 16 g $m^{-2}$ $year^{-1}$), and evaluated plant use of water across the soil profile using oxygen isotope. Aboveground biomass increased significantly, but diversity and shallow soil-water content decreased, with increasing rate of nitrogen addition. The water isotopic signature for both plant and soil water at the high N rate indicated that *Leymus secalinus* (a perennial grass) absorbed 7% more water from the subsurface soil layer (20–100 cm) compared to *Elymus dahuricus* (a perennial grass) and *Artemisia annua* (an annual forb). *L. secalinus* thus had a significantly larger biomass and was more abundant than the other two species at the high N rate but did not differ significantly from the other two species under ambient and the low N rate. Species that could use water from deeper soil layers became dominant when water in the shallow layers was insufficient to meet the demands of increased aboveground plant biomass. Our study highlights the importance of water across soil depths as key driver of plant growth and dominance in grasslands under N addition.

## 1. Introduction

The deposition of atmospheric nitrogen (N) has increased in terrestrial ecosystems around the world over the last six decades, mainly due to the combustion of fossil fuels and the widespread use of agricultural fertilizers (Galloway et al., 2003, 2008; Peñuelas et al., 2020). Increased N deposition and fertilization can have detrimental effects on terrestrial ecosystem structure and function (Liu et al., 2011; Borer and Stevens, 2022). Nitrogen is a key element limiting plant growth (Elser et al., 2007), and thus, a modest increase in N input can alleviate soil N limitation, accelerate plant growth, increase aboveground production, and in some cases, enhance soil carbon storage (Li et al., 2015; Liang et al., 2020; Shi et al., 2024). Excessive inputs of N, however, can have negative effects, such as soil acidification (Lu et al., 2014), water eutrophication (Zhan et al., 2017), and biodiversity loss (Midolo et al., 2019), all of which can strongly affect the functions, services, and stability of terrestrial and aquatic ecosystems (Bai et al., 2010; Hautier et al., 2014, 2020).

Nitrogen addition generally increases plant aboveground biomass and cover often leading to a reduction in species diversity

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(Sala et al., 2000; Stevens et al., 2004; Lu et al., 2021), but the underlying mechanism driving the loss of diversity remains uncertain (Dickson et al., 2014; Xiao et al., 2021; Eskelinen et al., 2022). Many studies have suggested that increased aboveground biomass and cover after N addition would not only intensify asymmetric interspecific competition for light and exclude shade-intolerant species (Hautier et al., 2009; Borer et al., 2014; Eskelinen et al., 2022), but also increase competition for other resources, such as soil water (Li et al., 2024, 2025; Pei et al., 2024) and phosphorus (Sardans and Peñuelas, 2012; Luo et al., 2022; Yu et al., 2023). Indeed, not all communities that experience a decline in species richness with added N have closed canopies. Therefore, mechanisms other than light competition must drive diversity loss in some plant communities. Greater production and leaf area under N addition likely increases plant demand for soil water, especially in regions with low mean annual rainfall (Porporato et al., 2004; Chen et al., 2008; Huo et al., 2018). While this has raised the possibility that soil water may drive species loss, so far there is a lack of empirical evidence that links plant water usage (e.g., by $^{18}O$ isotope measurements) with the fate of species after N addition.

Competition for soil water among coexisting plants will greatly affect their growth (Chen et al., 2023). The use of water in a healthy and sustainable plant–soil system would not usually exceed the supply of soil water, leading to water deficit (Chen et al., 2015). N addition can increase the water demand of plants by increasing leaf area and transpiration (A et al., 2019), which may cause greater demands for soil water (Zhu et al., 2020) and thus potentially intensify competition belowground. Increased aboveground biomass after N addition decreases plant diversity (Bai et al., 2010; Zhang et al., 2022), potentially due to a greater overall demand for soil water, thereby affecting species coexistence (Bai et al., 2010). On the other hand, greater N supply can also improve water-use-efficiency in the short term (Lu et al., 2019) but reduce it over the long term when linked to other limitations such as phosphorus availability (Huang et al., 2016). Less attention, however, has been paid to the response of soil water across depth (i.e., shallow vs deep soils) and its interaction with dominant species and changes in species abundances after N addition.

Based on mass balance theory, ecosystem function is mainly determined by the traits of dominant plants in a community rather than by rare and uncommon species (Grime, 1998). Hence, the stability of the dominant species is critical to ecosystem stability (Zelikova et al., 2014; Ma et al., 2017). That is, the dynamics of dominant species in a community can be more important than the dynamics of rare species under environmental change (Smith and Knapp, 2003). The dynamics of the dominant species may also affect the generality of the diversity-stability relationship (Polley et al., 2007). Species gain was more important than species loss in determining plant diversity after a decade of experimental N addition (Ladouceur et al., 2022; Zhao et al., 2022). A subsequent study also suggested that the dominant species determined the effects of N addition on ecosystem stability (Jia et al., 2022). Variation in the abundance of the dominant species will influence species interactions and plant diversity, with uncertain consequences on community dynamics and stability (Klanderud and Totland, 2005; Hillebrand et al., 2008). Conserving dominant species is important for stable functioning in ecosystems undergoing environmental change (Hou et al., 2023). Therefore, it is crucial to understand how changes in the dominance of plant species in a community after N addition, as well as the underlying mechanisms, affect function and stability.

To determine whether soil water regulated composition and dominance in a plant community after N addition, we measured aboveground biomass and diversity, and estimated water use across soil depths using oxygen isotope measurements in a semi-arid grassland exposed to four rates of N addition for eight years. We hypothesized that the strategies of water use by different plant species would strongly regulate their dominance after N addition. We aimed to explore (1) changes in plant species richness and biomass under different N addition rates, and (2) the differential responses of plant species with different water use strategies to N additions.

## 2. Materials and methods

### 2.1. Experimental site

The study was conducted at the Dryland Agroecology Research Station (36°02′N, 104°25′E, 2400 m a.s.l.) of Lanzhou University in Gansu Province, China. The site has a temperate semi-arid climate (Huang et al., 2021), with an annual mean air temperature of 4.0 °C, mean maximum temperature of 15.7 °C in July, and mean minimum temperature of −12.6 °C in January. Mean annual precipitation during 2014–2023 was 338 mm, with about 80% falling during the growing season from April to September (Zheng et al., 2023). The site has a dark loessial soil, with a field water-holding capacity of about 20% and a permanent wilting coefficient of 4.5% (Shi et al., 2003). The study site is a natural grassland and was fenced in 2003 to prevent grazing and human disturbance. The plant communities are dominated by a mix of grasses, including graminoids (*Leymus secalium* (Georgi) Tzvel., *Poa annua* L., *Stipa breviflora* L., *Elymus dahuricus* Turcz.), and forbs (*Artemisia capillaris* Thunb., *Aster altaicus* Willd., *Artemisia annua* L., *Convolvulus arvensis* L., *Pedicularis kansuensis* L., *Potentilla bifurca* L.). Legume species are rare and generally have relatively low abundance and biomass in the study area.

### 2.2. Experimental design

We conducted an N addition experiment with four rates (0, 4, 10, and 16 g N $m^{-2}$ $y^{-1}$, representing ambient, low, intermediate, and high rates of N addition, respectively), starting from 2016 with a completely randomized block design (Fig. S1). Each treatment had four replicates, resulting in sixteen 2 × 2 m plots in total, with a 1 m buffer between plots. We dissolved $NH_4NO_3$ in 400 mL of distilled water, and evenly sprayed the solution in the treatment plots during April–July each year since 2016. The ambient (0 g N $m^{-2}$ $y^{-1}$) plots 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. There is no significant difference in the baseline of aboveground biomass and soil water content among blocks (Fig. S2), therefore, the grassland is generally uniform in composition and production.

### 2.3. Field sampling and measurements

We classified all plant species into four functional groups based on their life forms: perennial and annual grasses, and perennial and annual forbs. Aboveground biomass was sampled in mid-August 2023. All aboveground parts of the plants were cut at ground level in a 1 × 1 m quadrat placed in the center of each 2 × 2 m plot, and the number of individual plants of each species in each plot was recorded. Biomass was separated by species and oven-dried at 70 °C to constant weight in the laboratory. We calculated the Shannon–Weiner diversity index and Simpson's dominance index as:

$$\text{Simpson's index} = \sum P_i^2 \qquad (1)$$

$$\text{Shannon–Weiner index} = -\sum P_i \ln P_i \tag{2}$$

where $P_i$ is the ratio of the number of individuals of species $i$ to the total number of individuals in the community.

Previous studies suggested that plant biomass rather than plant identity is the primary determinant for soil moisture, therefore, we selected three abundant species: two perennial grasses (*Leymus secalinus* and *Elymus dahuricus*) and one annual forb (*Artemisia annua*) that accounted for 73% of community biomass under the high N addition rate. Also, these three species occurred in all plots. The three representative species all had sufficient biomass for measuring $\delta^{18}O$ in plant tissue water. Other species were only observed in a portion of the experimental plots or did not have sufficient biomass for extracting plant water, thus were not selected for the measurement of $\delta^{18}O$. We collected the non-green rhizome-binding fraction of each plant for measuring water isotopes (Chang et al., 2019). The samples of plant water were rapidly loaded into 50-ml sampling vials sealed with parafilm to avoid isotopic fractionation and stored at −20 °C before analysis.

Soil samples were collected from 1 m depth profile using a hand auger and no roots were found below 1 m. We collected the samples at regular intervals of 10 cm in the top 20 cm and at intervals of 20 cm in the 20–100 cm soil depth. We divided each sample into two subsamples. One subsample was used to measure gravimetric soil water content (SWC) by drying at 105 °C to a constant weight. The other subsample was transported to the laboratory and stored at −20 °C until water was extracted for isotopic analysis.

### 2.4. Analysis of the composition of oxygen isotope

We used a vacuum distillation system (LI–2000, LICA, China) to extract soil and plant-stem water. The extraction efficiency was up to 98% (Wang et al., 2019a; Jia et al., 2024). We measured $\delta^{18}O$ from extracted soil water using an isotope ratio infrared spectroscopy (IRIS) analyzer—L2130i liquid water isotope analyzer (Picarro, USA), with an analytical precision of ±0.025‰. Extracting water from plant tissues using cryogenic vacuum distillation can co-distill organic materials (e.g., methanol and ethanol) that may affect the spectroscopy and cause erroneous stable isotope values when analyzing with the IRIS method (West et al., 2006). However, the Isotope Ratio Mass Spectrometer (IRMS) method can overcome this shortcoming (West et al., 2010; Wang et al., 2019a). Therefore, in order to eliminate the effects of the trace amount of soluble organic matter (Huang et al., 2023), $\delta^{18}O$ for xylem water were determined by IRMS interfaced with an elemental analyzer (Delta V + TC/EA, Thermo Finnigan Scientific Inc., USA), with an analytical precision of ±0.4‰. Moreover, no pronounced discrepancy was observed in soil water isotopic compositions analyzed using the two instruments (Schultz et al., 2011; Pei et al., 2023). The measurements are expressed as parts per thousand (‰) deviations from the Vienna Standard Mean Ocean Water (V-SMOW):

$$\delta^{18}O = (R_s/R_{std} - 1) \tag{3}$$

where $R_s$ and $R_{std}$ are the stable isotopic ratios of heavy to light isotopes ($^{18}O/^{16}O$) in the water samples and the V-SMOW sample.

### 2.5. Determination and quantification of water sources

The water table at this site is > 60 m below the soil surface, which is unavailable for plants (Huang et al., 2021). No precipitation fell in the week before soil sampling, so the water in the soil layers was regarded as the only source utilized at the time by the plants. Isotopes were not fractionated during uptake by plants, thus we could determine the water source taken up by the plants by comparing the stable isotope composition of the xylem and soil water. We detected the intersection of the xylem water isotopic vertical line and the soil water at different depths, which indicated the main depth from which plants took up soil water (Ehleringer and Dawson, 1992). We estimated the proportion of plant water taken up across the soil profile using the Bayesian isotope mixing model MixSIAR (Stock and Semmens, 2013; Wang et al., 2019b; Gai et al., 2023). To facilitate analysis and comparison, the water sources from different soil depths were combined into three layers (0–20, 20–40, and 40–100 cm) based on the distributions of the isotopic values and SWC.

### 2.6. Measurements of stomatal conductance

We determined plant water uptake by measuring leaf stomatal conductance for the dominant plant species (*Leymus secalinus*) in the community. We measured stomatal conductance in three fully expanded green leaves in each plot using a LI-6400 Portable Photosynthesis System (LI-COR, Lincoln, USA). The minimum equilibration time for each set of measurements was 3 min. The measurements were performed between 10:00 and 12:00 h.

### 2.7. Statistical analyses

We used Random Block ANOVAs to examine the effects of N addition on biotic and abiotic factors among treatments, including plant biomass and diversity, leaf stomatal conductance, plant water uptake, and soil-water content, with N addition treatment as the independent fixed factor and block as the independent random factor. We performed Random Block ANOVAs in R packages '*car*' and '*agricolae*'. We then used Pearson correlation analysis to examine the correlations among these variables. In addition, we used a structural equation model (SEM) to examine the direct and indirect effects of N addition on soil-water content, plant water use across the soil profile and species dominance. We employed $p$-value, the goodness of fit index (GFI), root-mean-square error of approximation (RMSEA) and standardized root mean square residual (SRMR) to evaluate the goodness of model fitting (Grace et al., 2015; Yao et al., 2025), where $p > 0.05$, GFI > 0.90, RMSEA ≤ 0.08, SRMR ≤ 0.08 suggested a reasonable fitting. We performed SEM using the 'sem' function in the '*lavaan*' package. All the above analyses were conducted with R 4.2.3 (R Foundation for Statistical Computing).

## 3. Results

### 3.1. Responses of plant aboveground biomass and diversity to nitrogen addition

Plant community aboveground biomass increased significantly, while diversity decreased, as the rate of N addition increased (Fig. 1). Community aboveground biomass was 94% and 124% larger, but species richness was 12% and 41% lower, at the low (4 g N $m^{-2}$ $y^{-1}$) and high (16 g N $m^{-2}$ $y^{-1}$) rates, respectively (Fig. 1a). Simpson's dominance index was 54% larger (Fig. 1c), while the Shannon–Weiner diversity index was 25% lower (Fig. 1d) at the high rate of N addition compared to ambient level. N addition did not significantly affect the average height of the plants in the community (Fig. S3).

Compared to ambient level, aboveground biomass of perennial grasses was 162%, 329%, and 485% larger at the low, intermediate, and high rates of N addition, respectively (Fig. 2a). Perennial grasses dominated the plant communities at the intermediate and high rates, representing 44% and 56% of total community aboveground

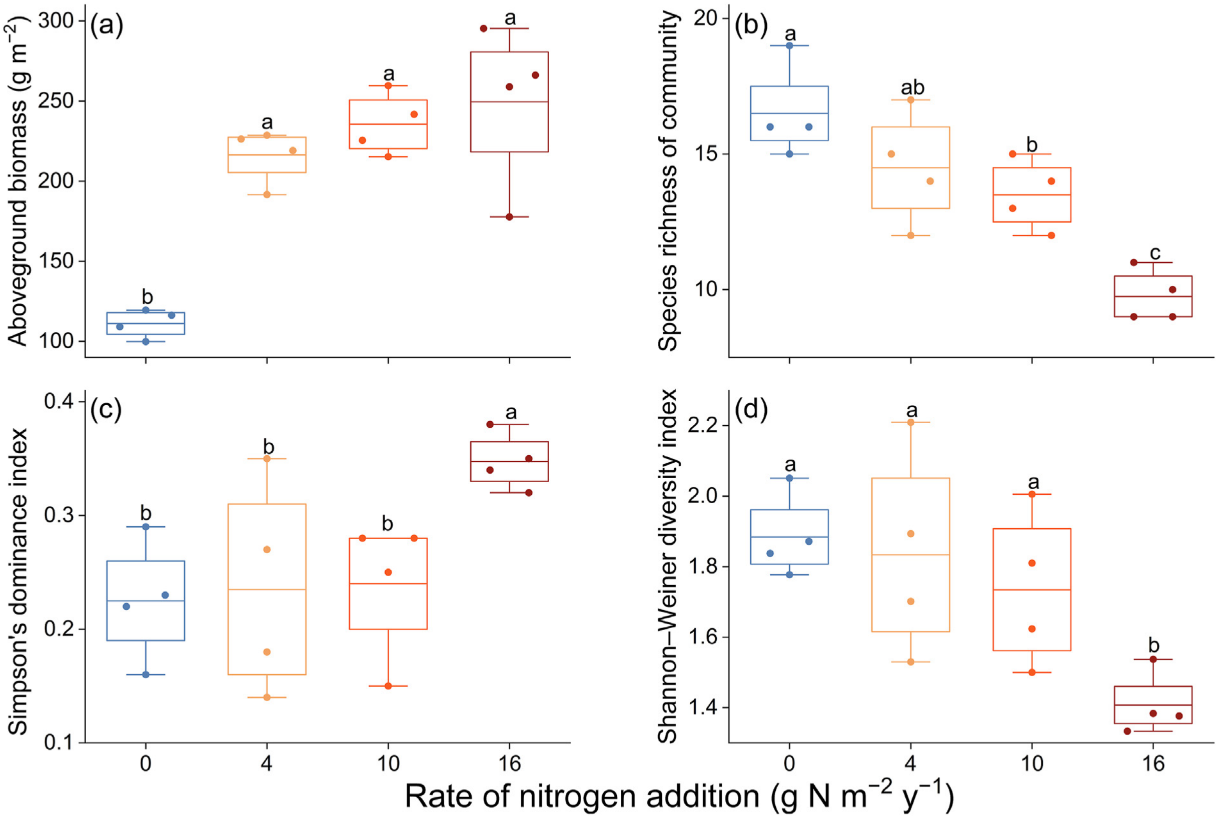

**Fig. 1.** Effects of nitrogen (N) addition on (a) aboveground biomass, (b) species richness, (c) Simpson's dominance index, and (d) the Shannon–Weiner diversity index. Different letters indicate significant differences among treatments.

biomass, respectively. N addition did not significantly affect aboveground biomass of annual grasses, nor perennial and annual forbs (Fig. 2b–d). N addition did not significantly affect species richness of the perennial and annual grasses (Fig. 2e and f). Species richness of perennial and annual forbs, however, decreased as the rate of N addition increased (Fig. 2g and h). Compared to ambient, species richness of perennial forbs was 21% and 45% lower at the intermediate and high N addition rates, respectively. Species richness of annual forbs was 33% and 66% lower at the intermediate and high rates relative to ambient, respectively.

### 3.2. Responses of stomatal conductance and soil water

Stomatal conductance of *Leymus secalinus*, a dominant perennial grass species, was 143%, 101%, and 66% significantly greater at the low, intermediate, and high rates of N addition, respectively, relative to ambient (Fig. 3a). SWC was significantly lower in the 0–20 cm layer at the high N addition rate than at the intermediate N addition rate (Fig. 3b). SWC was 19%–27% lower in the 20–40 cm layer and 12%–16% lower in the 40–100 cm layer at the low to high N addition rates. SWC was significantly greater in the 20–100 cm layers than the surface layer. SWC in the ambient, low, and intermediate N treatments was 43%, 17%, and 21% greater in the 20–40 cm layer than the 0–20 cm layer, respectively. SWC at the high N addition rate was greatest in the 40–100 cm layer, which was 21% greater than in the 0–20 cm layer.

We identified significant negative correlations between aboveground biomass and SWC in the 20–40 cm layer (Fig. S4, $p < 0.05$) and the 40–100 cm layer ($p < 0.01$). SWC in the 40–100 cm layer was also negatively correlated with stomatal conductance ($p < 0.05$). Species richness was correlated negatively with aboveground biomass ($p < 0.05$) but positively with SWC in the 20–40 cm layer ($p < 0.05$). These findings indicated that the response of biomass and diversity to N addition was closely linked to soil water availability at different depths.

### 3.3. Aboveground biomass and abundance of three representative plant species under nitrogen addition

Aboveground biomass was significantly larger for *Elymus dahuricus* than the other two species under ambient N and did not differ significantly in the low-N treatment (Fig. 4a). Aboveground biomass was significantly larger for *Leymus secalinus* than *E. dahuricus* and *Artemisia annua* in the intermediate-N and high-N treatments. Aboveground biomass in the high-N treatment was 1224% and 318% larger for *L. secalinus* than *E. dahuricus* and *A. annua*, respectively. The abundances of the three species did not differ significantly in the ambient, low-N, and intermediate-N treatments (Fig. 4b). Abundance in the high-N treatment was 1335% and 138% greater for *L. secalinus* than *E. dahuricus* and *A. annua*, respectively. These findings indicated that plant community reordering occurred as *L. secalinus* became more dominant after N addition.

### 3.4. Plant water use across the soil profile

The $\delta^{18}O$ of xylem water was significantly lower for *Leymus secalinus* than *Elymus dahuricus* and *Artemisia annua* (Fig. S5). The $\delta^{18}O$ intersection points between the soil water and xylem water in *L. secalinus* were mainly below 20 cm, and those for the other two species were mainly above 20 cm. The results of the Bayesian isotope mixing model supported these findings; *L. secalinus*

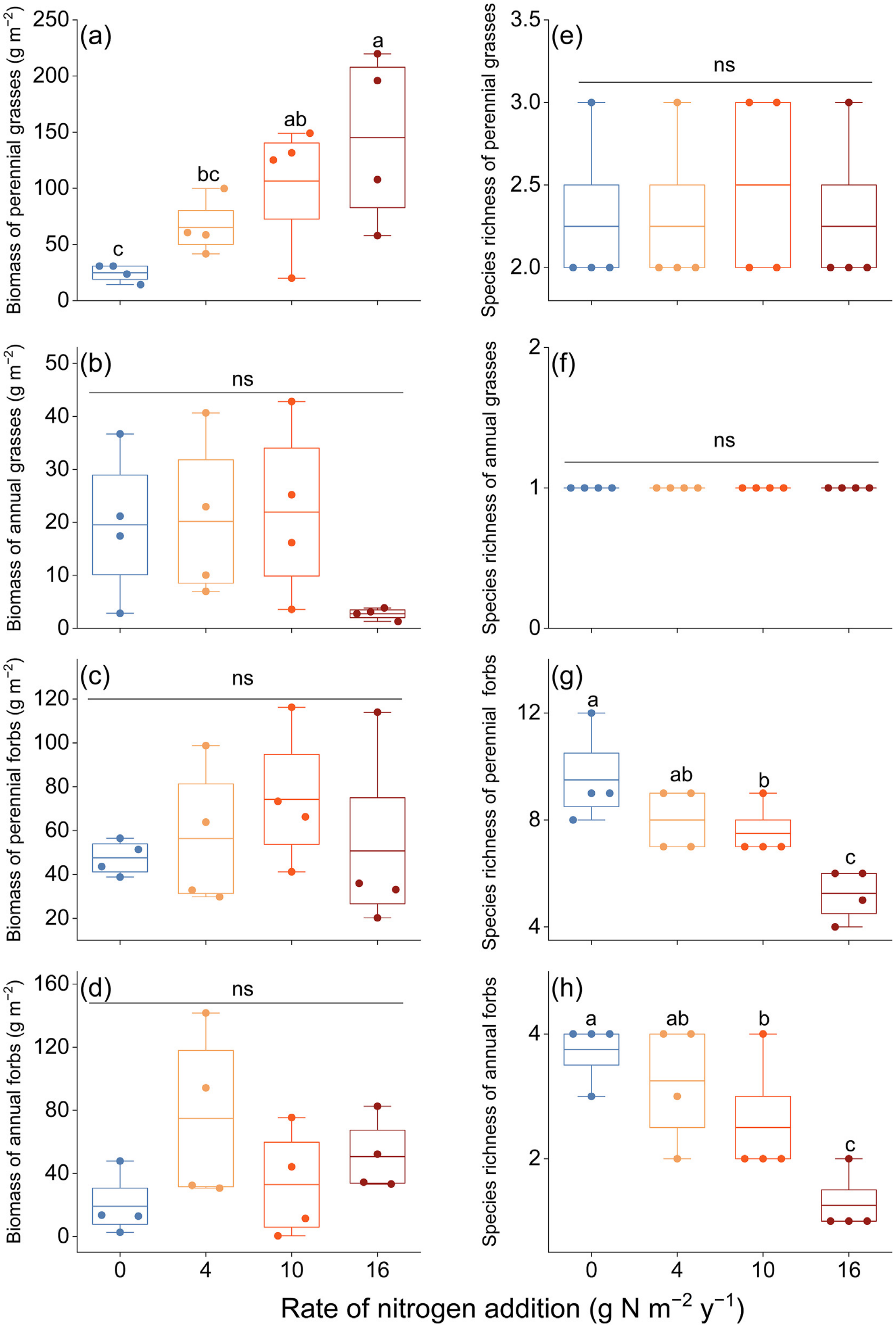

**Fig. 2.** Effects of nitrogen (N) addition on aboveground biomass and species richness for different plant functional groups. Different letters indicate significant differences among the treatments. ns means no significant difference among the treatments.

obtained the largest proportion of water from the 20–100 cm soil depth, and this proportion increased with the rate of N addition (Fig. 5). *L. secalinus* obtained 7% more water compared with the other two species from the 20–100 cm soil depth at the intermediate and high rates of N addition.

Based on the correlations among aboveground biomass, soil water, species richness, and plant water uptake across soil depths (Figs. S4 and S6), we developed structural equation models (SEMs). The SEMs adequately fitted the data describing interaction pathways among soil water content and N addition (Fig. 6a), proportion

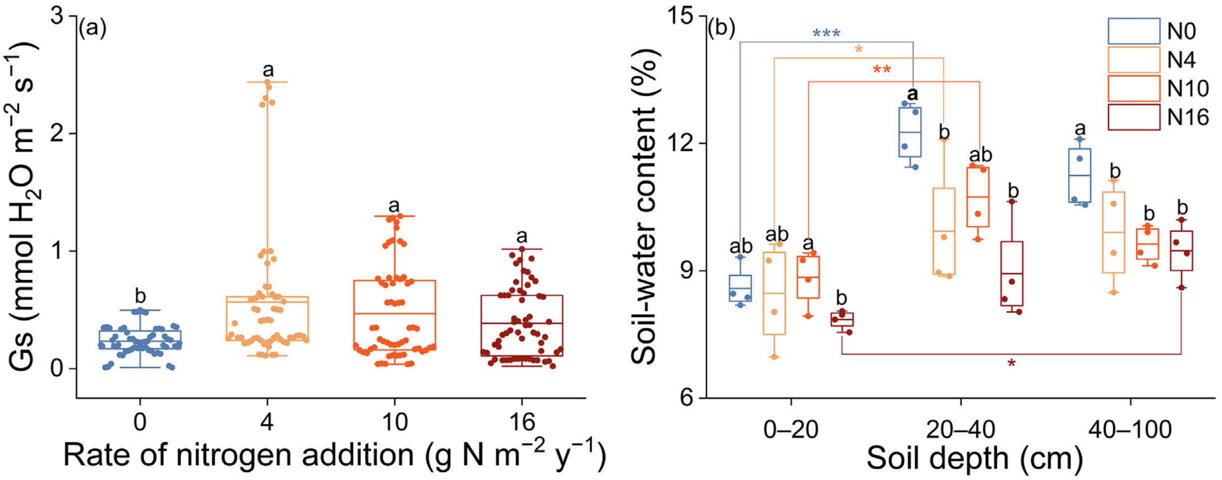

**Fig. 3.** Effects of nitrogen (N) addition on (a) stomatal conductance and (b) soil-water content. Different letters indicate significant differences among the treatments. Significant differences among the soil layers: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

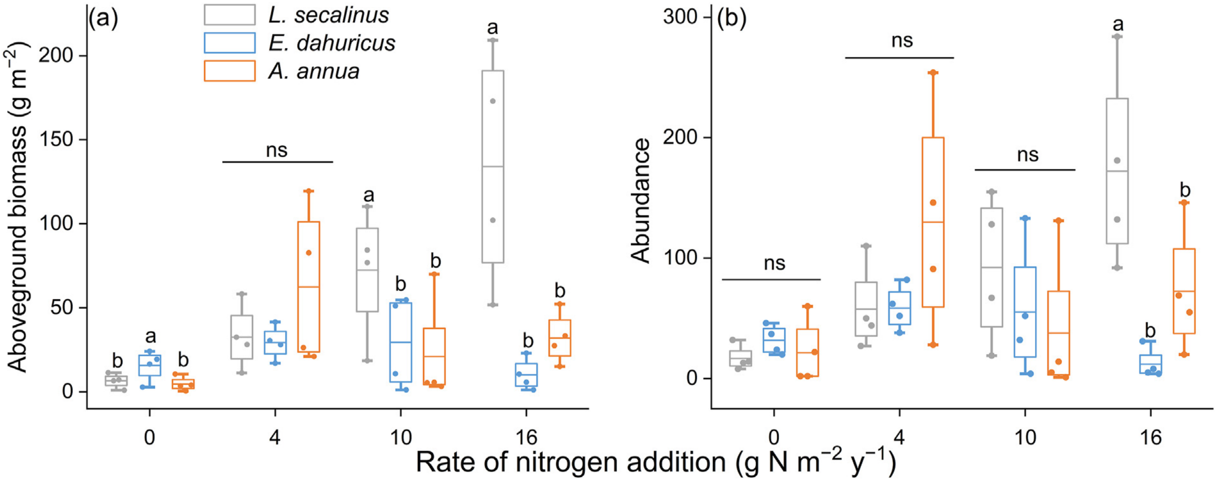

**Fig. 4.** Aboveground biomasses and abundances of *Leymus secalinus*, *Elymus dahuricus*, and *Artemisia annua* under nitrogen (N) addition. Different letters indicate significant differences among the three plant species under the same N addition rate. ns, no significant difference among the species.

of deep water use by species, and their dominance (Fig. 6b). N addition significantly increased plant aboveground biomass and decreased soil water content of deep soil layer. Therefore, with the consumption of soil water, the abundance and biomass of species largely depended on their use of water from the deep soil layer at the intermediate and high rates of N addition. The correlation analysis affirmed these findings. Aboveground biomass is significantly and positively correlated with the proportion of water taken up from the deep soil layer under the intermediate and high rate of N addition (Fig. S6, $p < 0.05$). These findings suggested that the capacity of the plants to take up water from deep soil layers is linked to their dominance after N addition.

## 4. Discussion

### 4.1. Changes in plant species richness and biomass under nitrogen addition and the underlying mechanism

We attributed the increase in aboveground biomass of the grassland community after N addition mainly to the increase in the biomass of a few perennial grasses (Fig. 2a) and attributed the decrease in species richness due to the loss of forb species (Fig. 2g and h). N addition generally increases plant productivity but decreases plant diversity in many N-limited terrestrial ecosystems (Suding et al., 2005; Clark and Tilman, 2008; Reich, 2009). Different functional groups, however, may respond distinctively to N addition (Tian et al., 2016, 2020; Clark et al., 2019). The proportion of grass biomass in our study increased from 40% under ambient to 57% at the high-N rate, thus suggesting that grasses may have advantages over forbs after N addition in this system.

We also found that N addition did not affect grass species richness (Fig. 2e and f), while the intermediate and high rates of N addition significantly decreased the diversity of the forb species (Fig. 2g and h). In addition, the root biomass of perennial grasses became more and more dominant as the rate of N addition increased (Fig. S7). This was consistent with the observation that N addition promoted the growth of grasses and suppressed the growth of forbs. Most grass species are nitrophilous, and recent research has found that belowground traits of grasses can regulate the responses of species to N addition (Tian et al., 2020). Typically, grass roots exploit a larger belowground space by increasing the size of their root sheath under N addition, which enhances their belowground competitive capacity for nutrients and water. By comparison, forb roots rarely form rhizosheaths and thus have

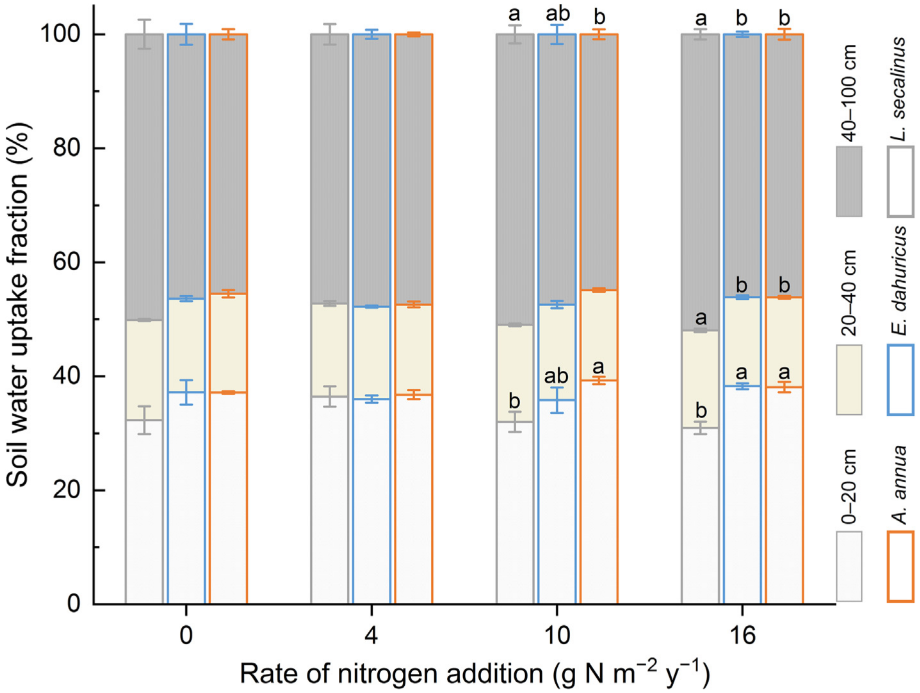

**Fig. 5.** The proportion of water taken up from different soil layers. Column heights represent the mean value of the relative contribution and error bars are the standard errors of the means, which were calculated using stable isotope $\delta^{18}O$ in plant and soil water coupled with a Bayesian mixing model MixSIAR. Different letters indicate significant differences in the proportions of water taken up from the same soil layer among different species.

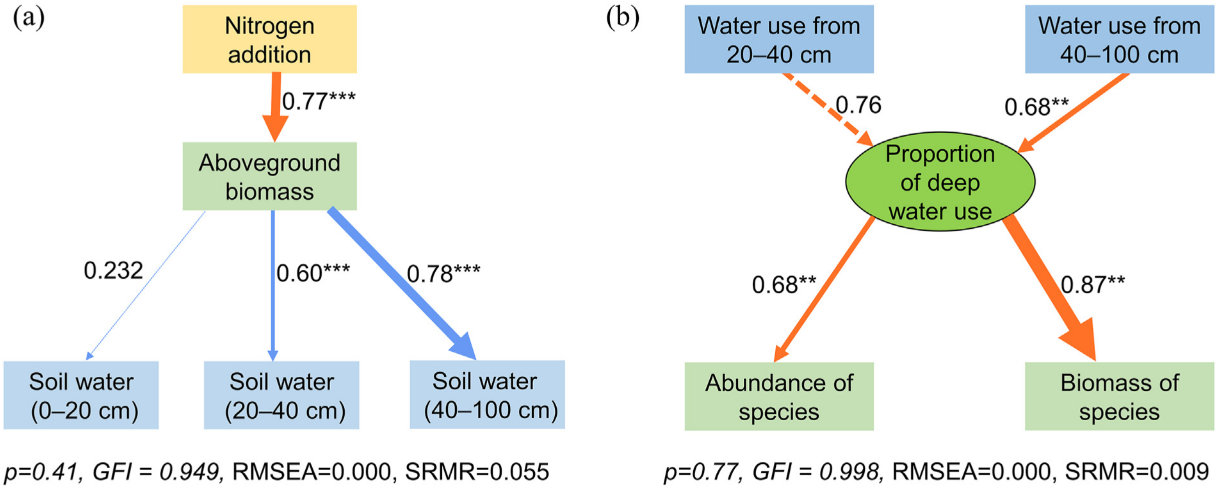

**Fig. 6.** Structural equation model showing pathways through which (a) nitrogen addition (0, 4, 10, and 16 g N m$^{-2}$ y$^{-1}$) increases community aboveground biomass and decreases soil water content, (b) Species water use of the deep soil layer increases their dominance (measured as abundance and biomass) under the intermediate and high N addition rates. There is no significant link to the surface soil layer. The ellipse and squares denote latent and observable variables, respectively. Proportion of deep water use is the latent variable and is estimated by analyzing the covariance matrix of water uptake from 20 to 40 cm and 40–100 cm by the three representative plant species (*Leymus secalinus*, *Elymus dahuricus* and *Artemisia annua*) under intermediate and high rates of N addition. Orange and blue solid arrows represent significant positive and negative pathways, respectively. Orange and blue dashed arrows represent non-significant positive and negative pathways, respectively. Numbers adjacent to the arrows are standardized path coefficients with different significance levels: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

weaker ability to compete for belowground resources (Tian et al., 2022). The dominance of grasses in our study therefore increased with N addition. This increase also accounted for the significantly greater Simpson's dominance index and the significantly lower Shannon–Weiner diversity index at the high rate of N addition (Fig. 1c and d).

In summary, distinctive responses of functional groups to high N addition affected the composition of the plant community. Our study shows that a more acquisitive strategy regarding resource uptake provided an advantage as soil resource availability (N and water) changes. Strategies that allow for greater spatial occupation and increased capacity to uptake resources confer a competitive advantage.

### 4.2. *Differential responses of plant species with different water use strategies to nitrogen additions*

Soil water content decreased as the rate of N addition increased, especially in the 40–100 cm layer, which was closely linked to greater water uptake due to increased stomatal conductance and aboveground biomass (Figs. 3a and S4). The decrease in diversity after N addition was also linked to the decrease in soil water (Fig. S4). In addition, it is likely that the greater biomass of dominant species rather than rare species reduced soil water content, because the biomass of the rare species did not change significantly with N addition (Fig. S8). Water is one of the most important factors limiting plant survival and growth in arid and semi-arid regions (Porporato et al., 2004; Huo et al., 2018; Li et al., 2023). Previous research found that insufficient soil water decreased the germination rate of some annual species (Bai et al., 2010). Wetter grasslands had both greater plant biomass and diversity than drier grasslands (Zheng et al., 2023), and wetter grasslands were less likely to lose plant diversity than drier grasslands under inorganic and organic fertilization (Flores-Moreno et al., 2016; Shi et al., 2024). The availability of soil water is thus a key factor maintaining the structure and function of grassland ecosystems under N deposition, especially in semi-arid areas.

*Leymus secalinus* is a perennial grass that can access deeper soil water compared to other species in our experimental grassland, thus allowing it to meet its water needs and increase its dominance at intermediate and high rates of N addition (Fig. 5). The proportion of *L. secalinus* biomass in the community significantly increased from 6% under ambient deposition to 51% at the high rate of N addition. The capacity to adjust water use strategy in conjunction with variation in SWC is critical for plant survival in water-limited ecosystems (McCole and Stern, 2007; Wu et al., 2016). Increased aboveground biomass after N addition may also increase the demand for soil water due to greater leaf area (Li et al., 2023), so water in the surface soil layer that is usually limited and unpredictable would be rapidly consumed by plants during the growing season (McCole and Stern, 2007; Prechsl et al., 2015; Priyadarshini et al., 2016). Therefore, root biomass in the 20–60 cm soil layer significantly increased with N addition, while there was no significant difference in root biomass among different treatments in 0–20 cm soil depth (Fig. S9). When water in the surface soil layer is insufficient to meet demand from increased aboveground biomass, species that can use water in deeper layers would become dominant in the community. In contrast, less competitive plant species that can only obtain water from shallow soil layers would not be able to increase their abundance and biomass (Fig. S10).

Patterns of plant water use are affected by soil water availability (Gow et al., 2018) and distribution of fine roots (Wang et al., 2021). Many plants can adjust the soil depth of water uptake through dynamic changes in root distribution (Lanning et al., 2020; Pei et al., 2023). This capacity to shift water use along with soil water availability represents a stress-tolerant strategy and ecological plasticity of plants (Grime, 1977; Valladares et al., 2007), and is crucial for plant survival under water stress that may occur under climate change. Species with greater stress tolerance and ecological plasticity can maximize efficiency with scarce resources. Such species generally have a greater capacity to adapt to stressful environments. In addition, plant species that can shift water sources can maximize their soil water use and reduce interspecific competition with those that can only utilize shallow water sources (Wang et al., 2019a). Therefore, plant water use characteristics provide important information for screening species for vegetation restoration and grassland management. The combination of plant species with different water use characteristics may form a more resilient community, which not only improves the utilization of soil water resources by plants, but also promotes the sustainable development of grassland ecosystems.

Overall, our research uncovered the causal link between dominance and species water use strategies after N addition. Dominant species with greater biomass and abundance relative to other species in the community were key to understanding the impacts of global change on ecological functions (Hillebrand et al., 2008; Sasaki and Lauenroth, 2011; Avolio et al., 2019). Dominant species may alter biotic conditions and interact with other species, thereby strongly influencing community dynamics and ecosystem functioning (Grime, 1998). Several studies have shown that dominant species were not only key to maintaining community stability (Jia et al., 2022; Hou et al., 2023), but also shaped the recovery dynamics of grasslands following extreme drought (Bazzichetto et al., 2024). The response of dominant species to environmental change is a crucial determinant of whether or not the ecosystem will continue to provide needed levels of functions and services into the future (Gitlin et al., 2006). Therefore, it is important to reveal the mechanisms that underlie the capacity of species to dominate. We argue for a greater focus on the dynamics of dominant species in the context of changes in community structure, particularly the mechanisms, traits and conditions that enable species to dominate. This will contribute to predictions of community assembly and impacts of environmental drivers on ecosystems, which can lead to the creation of more effective grassland management strategies under climate change (Avolio et al., 2019).

## 5. Conclusion

Nitrogen addition increased the aboveground biomass of perennial grasses, decreased the diversity of forbs, and ultimately affected community structure and function. We found that greater aboveground biomass and stomatal conductance increased the consumption of and the competition for soil water. These dynamics favored species with greater capacity for water acquisition in deeper soil layers resulting in decreased plant community diversity. Species that could use water from deeper soil layers (mostly grasses) became dominant in the community when shallow soil water was insufficient to meet the demands of increased aboveground biomass. Our study integrated plant community structure with plant water use across the soil profile, and provided a belowground mechanism for explaining changes in species dominance under N enrichment.

## CRediT authorship contribution statement

**Fu-Qiang Huang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Josep Peñuelas:** Writing – review & editing, Funding acquisition. **Jordi Sardans:** Writing – review & editing, Funding acquisition. **Scott L. Collins:** Writing – review & editing. **Kai-Liang Yu:** Writing – review & editing. **Man-Qiong Liu:** Investigation, Formal analysis, Data curation. **Jiu-Ying Pei:** Investigation, Formal analysis, Data curation. **Wen-Bin Ke:** Investigation, Formal analysis, Data curation. **Jian-Sheng Ye:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

## Data availability statement

The data that support the findings of this study are openly available in figshare at doi: https://doi.org/10.6084/m9.figshare.25887580.v1.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pld.2025.02.006.

## References

A, Y.L., Wang, G.Q., Liu, T.X., et al., 2019. Vertical variations of soil water and its controlling factors based on the structural equation model in a semi-arid grassland. Sci. Total Environ. 691, 1016–1026. https://doi.org/10.1016/j.scitotenv.2019.07.181.

Avolio, M.L., Forrestel, E.J., Chang, C.C., et al., 2019. Demystifying dominant species. New Phytol. 223, 1106–1126. https://doi.org/10.1111/nph.15789.

Bai, Y.F., Wu, J.G., Clark, C.M., et al., 2010. Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. Glob. Change Biol. 16, 358–372. https://doi.org/10.1111/j.1365-2486.2009.01950.x.

Bazzichetto, M., Sperandii, M.G., Penone, C., et al., 2024. Biodiversity promotes resistance but dominant species shape recovery of grasslands under extreme drought. J. Ecol. 112, 1087–1100. https://doi.org/10.1111/1365-2745.14288.

Borer, E.T., Seabloom, E.W., Gruner, D.S., et al., 2014. Herbivores and nutrients control grassland plant diversity via light limitation. Nature 508, 517–520. https://doi.org/10.1038/nature13144.

Borer, E.T., Stevens, C.J., 2022. Nitrogen deposition and climate: an integrated synthesis. Trends Ecol. Evol. 37, 541–552. https://doi.org/10.1016/j.tree.2022.02.013.

Chang, E.H., Li, P., Li, Z.B., et al., 2019. Using water isotopes to analyze water uptake during vegetation succession on abandoned cropland on the Loess Plateau, China. Catena 181, 104095. https://doi.org/10.1016/j.catena.2019.104095.

Chen, H.S., Shao, M.A., Li, Y.Y., 2008. Soil desiccation in the loess plateau of China. Geoderma 143, 91–100. https://doi.org/10.1016/j.geoderma.2007.10.013.

Chen, Y.P., Wang, K.B., Lin, Y.S., et al., 2015. Balancing green and grain trade. Nat. Geosci. 8, 739–741. https://doi.org/10.1038/ngeo2544.

Chen, Z.X., Wang, G.H., Yang, X.L., et al., 2023. Agricultural water management. Agric. Water Manag. 279, 108206. https://doi.org/10.1016/j.agwat.2023.108206.

Clark, C.M., Simkin, S.M., Allen, E.B., et al., 2019. Potential vulnerability of 348 herbaceous species to atmospheric deposition of nitrogen and sulfur in the United States. Nat. Plants 5, 697–705. https://doi.org/10.1038/s41477-019-0442-8.

Clark, C.M., Tilman, D., 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451, 712–715. https://doi.org/10.1038/nature06503.

Dickson, T.L., Mittelbach, G.G., Reynolds, H.L., et al., 2014. Height and clonality traits determine plant community responses to fertilization. Ecology 95, 2443–2452. https://doi.org/10.1890/13-1875.1.

Ehleringer, J.R., Dawson, T.E., 1992. Water uptake by plants: perspectives from stable isotope composition. Plant Cell Environ. 15, 1073–1082. https://doi.org/10.1111/j.1365-3040.1992.tb01657.x.

Elser, J.J., Bracken, M.E., Cleland, E.E., et al., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x.

Eskelinen, A., Harpole, W.S., Jessen, M.T., et al., 2022. Light competition drives herbivore and nutrient effects on plant diversity. Nature 611, 301–305. https://doi.org/10.1038/s41586-022-05383-9.

Flores-Moreno, H., Reich, P.B., Lind, E.M., et al., 2016. Climate modifies response of non-native and native species richness to nutrient enrichment. Philos. Trans. R. Soc. B-Biol. Sci. 371, 20150273. https://doi.org/10.1098/rstb.2015.0273.

Gai, H.Q., Shi, P.J., Li, Z., 2023. Untangling the uncertainties in plant water source partitioning with isotopes. Water Resour. Res. 59, e2022WR033849. https://doi.org/10.1029/2022WR033849.

Galloway, J.N., Aber, J.D., Erisman, J.W., et al., 2003. The nitrogen cascade. BioScience 53, 341–356. https://doi.org/10.1641/0006-3568(2003)053[0341:TNC]2.0.CO;2.

Galloway, J.N., Townsend, A.R., Erisman, J.W., et al., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320, 889–892. https://doi.org/10.1126/science.1136674.

Gitlin, A.R., Sthultz, C.M., Bowker, M.A., et al., 2006. Mortality gradients within and among dominant plant populations as barometers of ecosystem change during extreme drought. Conserv. Biol. 20, 1477–1486. https://doi.org/10.1111/j.1523-1739.2006.00424.x.

Gow, L.J., Barrett, D.J., O'grady, A.P., et al., 2018. Subsurface water-use strategies and physiological responses of subtropical eucalypt woodland vegetation under changing water-availability conditions. Agric. For. Meteorol. 248, 348–360. https://doi.org/10.1016/j.agrformet.2017.10.005.

Grace, J.B., Scheiner, S.M., Schoolmaster, J., et al., 2015. Structural equation modeling: building and evaluating causal models. In: Fox, G.A., et al. (Eds.), Ecological Statistics: Contemporary Theory and Application. Oxford University Press, UK, pp. 168–199. https://doi.org/10.1093/acprof:oso/9780199672547.003.0009.

Grime, J.P., 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. 111, 1169–1194. https://doi.org/10.1086/283244.

Grime, J.P., 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. J. Ecol. 86, 902–910. https://doi.org/10.1046/j.1365-2745.1998.00306.x.

Hautier, Y., Niklaus, P.A., Hector, A., 2009. Competition for light causes plant biodiversity loss after eutrophication. Science 324, 636–638. https://doi.org/10.1126/science.11696.

Hautier, Y., Seabloom, E.W., Borer, E.T., et al., 2014. Eutrophication weakens stabilizing effects of diversity in natural grasslands. Nature 508, 521–525. https://doi.org/10.1038/nature13014.

Hautier, Y., Zhang, P.F., Loreau, M., et al., 2020. General destabilizing effects of eutrophication on grassland productivity at multiple spatial scales. Nat. Commun. 11, 5375. https://doi.org/10.1038/s41467-020-19252-4.

Hillebrand, H., Bennett, D.M., Cadotte, M.W., 2008. Consequences of dominance: a review of evenness effects on local and regional ecosystem processes. Ecology 89, 1510–1520. https://doi.org/10.1890/07-1053.1.

Hou, G., Shi, P.L., Zhou, T.C., et al., 2023. Dominant species play a leading role in shaping community stability in the northern Tibetan grasslands. J. Plant Ecol. 16, rtac110. https://doi.org/10.1093/jpe/rtac110.

Huang, F.Q., Wei, J.Z., Song, X., et al., 2021. $\delta^2$H and $\delta^{18}$O in precipitation and water vapor disentangle seasonal wind directions on the Loess Plateau. Sustainability 13, 6938. https://doi.org/10.3390/su13126938.

Huang, L.M., Pei, Y.W., Jia, X.X., et al., 2023. Multi-species plantation intensifies soil water competition and groundwater depletion in a water-limited desert region. For. Ecol. Manag. 537, 120953. https://doi.org/10.1016/j.foreco.2023.120953.

Huang, Z.Q., Liu, B., Davis, M., et al., 2016. Long-term nitrogen deposition linked to reduced water use efficiency in forests with low phosphorus availability. New Phytol. 210, 431–442. https://doi.org/10.1111/nph.13785.

Huo, G.P., Zhao, X.N., Gao, X.D., et al., 2018. Seasonal water use patterns of rainfed jujube trees in stands of different ages under semiarid plantations in China. Agric. Ecosyst. Environ. 265, 392–401. https://doi.org/10.1016/j.agee.2018.06.028.

Jia, X.T., Tao, D.X., Ke, Y.G., et al., 2022. Dominant species control effects of nitrogen addition on ecosystem stability. Sci. Total Environ. 838, 156060. https://doi.org/10.1016/j.scitotenv.2022.156060.

Jia, X.X., Bai, X., Liu, C.G., et al., 2024. Differences in plant water use between check-dam land and slope land on the Loess Plateau: significance for vegetation restoration. Agric. Ecosyst. Environ. 362, 108849. https://doi.org/10.1016/j.agee.2023.108849.

Klanderud, K., Totland, O., 2005. Simulated climate change altered dominance hierarchies and diversity of an alpine biodiversity hotspot. Ecology 86, 2047–2054. https://doi.org/10.1890/04-1563.

Ladouceur, E., Blowes, S.A., Chase, J.M., et al., 2022. Linking changes in species composition and biomass in a globally distributed grassland experiment. Ecol. Lett. 25, 2699–2712. https://doi.org/10.1111/ele.14126.

Lanning, M., Wang, L.X., Benson, M., et al., 2020. Canopy isotopic investigation reveals different water uptake dynamics of maples and oaks. Phytochemistry 175, 112389. https://doi.org/10.1016/j.phytochem.2020.112389.

Li, H.L., Chen, J., Peñuelas, J., et al., 2024. Water limitation drives species loss in grassland communities after nitrogen addition and warming. Proc. R. Soc. B-Biol. Sci. 291, 20240642. https://doi.org/10.1098/rspb.2024.0642.

Li, H.L., Peñuelas, J., Collins, S.L., et al., 2025. Water limitation as a driver of species richness decline in global grasslands under nutrient addition. Plant Soil 1–10. https://doi.org/10.1007/s11104-025-07253-5.

Li, H.L., Terrer, C., Berdugo, M., et al., 2023. Nitrogen addition delays the emergence of an aridity-induced threshold for plant biomass. Natl. Sci. Rev. 10, nwad242. https://doi.org/10.1093/nsr/nwad242.

Li, W.B., Jin, C.J., Guan, D.X., et al., 2015. The effects of simulated nitrogen deposition on plant root traits: a meta-analysis. Soil Biol. Biochem. 82, 112–118. https://doi.org/10.1016/j.soilbio.2015.01.001.

Liang, X.Y., Zhang, T., Lu, X.K., et al., 2020. Global response patterns of plant photosynthesis to nitrogen addition: a meta-analysis. Glob. Change Biol. 26, 3585–3600. https://doi.org/10.1111/gcb.15071.

Liu, X.J., Duan, L., Mo, J.m., et al., 2011. Nitrogen deposition and its ecological impact in China: an overview. Environ. Pollut. 159, 2251–2264. https://doi.org/10.1016/j.envpol.2010.08.002.

Lu, P., Hao, T.x., Li, X., et al., 2021. Ambient nitrogen deposition drives plant-diversity decline by nitrogen accumulation in a closed grassland ecosystem. J. Appl. Ecol. 58, 1888–1898. https://doi.org/10.1111/1365-2664.13858.

Lu, X.H., Ju, W.M., Jiang, H., et al., 2019. Effects of nitrogen deposition on water use efficiency of global terrestrial ecosystems simulated using the IBIS model. Ecol. Indic. 101, 954–962. https://doi.org/10.1016/j.ecolind.2019.02.014.

Lu, X.K., Mao, Q.G., Gilliam, F.S., et al., 2014. Nitrogen deposition contributes to soil acidification in tropical ecosystems. Glob. Change Biol. 20, 3790–3801. https://doi.org/10.1111/gcb.12665.

Luo, M., Moorhead, D.L., Ochoa-Hueso, R., et al., 2022. Nitrogen loading enhances phosphorus limitation in terrestrial ecosystems with implications for soil carbon cycling. Funct. Ecol. 36, 2845–2858. https://doi.org/10.1111/1365-2435.14178.

Ma, Z.Y., Liu, H.Y., Mi, Z.R., et al., 2017. Climate warming reduces the temporal stability of plant community biomass production. Nat. Commun. 8, 15378. https://doi.org/10.1016/j.agrformet.2021.108526.

McCole, A.A., Stern, L.A., 2007. Seasonal water use patterns of Juniperus ashei on the Edwards Plateau, Texas, based on stable isotopes in water. J. Hydrol. 342, 238–248. https://doi.org/10.1016/j.jhydrol.2007.05.024.

Midolo, G., Alkemade, R., Schipper, A.M., et al., 2019. Impacts of nitrogen addition on plant species richness and abundance: a global meta-analysis. Global Ecol. Biogeogr. 28, 398–413. https://doi.org/10.1111/geb.12856.

Pei, J.Y., Zheng, Y., Yu, Y., et al., 2024. Soil water regulates plant diversity response to gradual and step nitrogen addition. Plant Soil 1–14. https://doi.org/10.1007/s11104-024-06938-7.

Pei, Y.W., Huang, L.M., Zhang, Y.L., et al., 2023. Water use pattern and transpiration of Mongolian pine plantations in relation to stand age on northern Loess Plateau of China. Agric. For. Meteorol. 330, 109320. https://doi.org/10.1016/j.agrformet.2023.109320.

Peñuelas, J., Janssens, I.A., Ciais, P., et al., 2020. Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. Glob. Change Biol. 26, 1962–1985. https://doi.org/10.1111/gcb.14981.

Polley, H.W., Wilsey, B.J., Derner, J.D., 2007. Dominant species constrain effects of species diversity on temporal variability in biomass production of tallgrass prairie. Oikos 116, 2044–2052. https://doi.org/10.1111/j.2007.0030-1299.16080.x.

Porporato, A., Daly, E., Rodriguez-Iturbe, I., 2004. Ecology. Am. Nat. 164, 625–632. https://doi.org/10.1086/424970.

Prechsl, U.E., Burri, S., Gilgen, A.K., et al., 2015. No shift to a deeper water uptake depth in response to summer drought of two lowland and sub-alpine $C_3$-grasslands in Switzerland. Oecologia 177, 97–111. https://doi.org/10.1007/s00442-014-3092-6.

Priyadarshini, K., Prins, H.H., de Bie, S., et al., 2016. Seasonality of hydraulic redistribution by trees to grasses and changes in their water-source use that change tree–grass interactions. Ecohydrology 9, 218–228. https://doi.org/10.1002/eco.1624.

Reich, P.B., 2009. Elevated $CO_2$ reduces losses of plant diversity caused by nitrogen deposition. Science 326, 1399–1402. https://doi.org/10.1126/science.1178820.

Sala, O.E., Stuart Chapin, F., Armesto, J.J., et al., 2000. Global biodiversity scenarios for the year 2100. Sci. Total Environ. 287, 1770–1774. https://doi.org/10.1126/science.287.5459.1770.

Sardans, J., Peñuelas, J., 2012. The role of plants in the effects of global change on nutrient availability and stoichiometry in the plant-soil system. Plant Physiol. 160, 1741–1761. https://doi.org/10.1104/pp.112.208785.

Sasaki, T., Lauenroth, W.K., 2011. Dominant species, rather than diversity, regulates temporal stability of plant communities. Oecologia 166, 761–768. https://doi.org/10.1007/s11104-023-05907-w.

Schultz, N.M., Griffis, T.J., Lee, X., et al., 2011. Identification and correction of spectral contamination in $^2H/^1H$ and $^{18}O/^{16}O$ measured in leaf, stem, and soil water. Rapid Commun. Mass Spectrom. 25, 3360–3368. https://doi.org/10.1002/rcm.5236.

Shi, T.S., Collins, S.L., Yu, K., et al., 2024. A global meta-analysis on the effects of organic and inorganic fertilization on grasslands and croplands. Nat. Commun. 15, 3411. https://doi.org/10.1038/s41467-024-47829-w.

Shi, Z.Y., Liu, W.Z., Guo, S.L., et al., 2003. Moisture properties in soil profiles and their relation to landform at Zhonglianchuan small watershed. Agric. Res. Arid Areas 21, 101–104.

Smith, M.D., Knapp, A.K., 2003. Dominant species maintain ecosystem function with non-random species loss. Ecol. Lett. 6, 509–517. https://doi.org/10.1046/j.1461-0248.2003.00454.x.

Stevens, C.J., Dise, N.B., Mountford, J.O., et al., 2004. Impact of nitrogen deposition on the species richness of grasslands. Science 303, 1876–1879. https://doi.org/10.1126/science.1094678.

Stock, B., Semmens, B., 2013. MixSIAR GUI User Manual V3, vol. 1. https://conser.ver.iugocafe.org/user/brice.semmens/MixSIAR.

Suding, K.N., Collins, S.L., Gough, L., et al., 2005. Functional-and abundance-based mechanisms explain diversity loss due to N fertilization. Proc. Natl. Acad. Sci. U.S.A. 102, 4387–4392. https://doi.org/10.1073/pnas.0408648102.

Tian, Q.Y., Liu, N.N., Bai, W.M., et al., 2016. A novel soil manganese mechanism drives plant species loss with increased nitrogen deposition in a temperate steppe. Ecology 97, 65–74. https://doi.org/10.1890/15-0917.1.

Tian, Q.Y., Lu, P., Zhai, X.F., et al., 2022. An integrated belowground trait-based understanding of nitrogen-driven plant diversity loss. Glob. Change Biol. 28, 3651–3664. https://doi.org/10.1111/gcb.16147.

Tian, Q.Y., Yang, L.Y., Ma, P.F., et al., 2020. Below-ground-mediated and phase-dependent processes drive nitrogen-evoked community changes in grasslands. J. Ecol. 108, 1874–1887. https://doi.org/10.1111/1365-2745.13415.

Valladares, F., Gianoli, E., Gómez, J.M., 2007. Ecological limits to plant phenotypic plasticity. New Phytol. 176, 749–763. https://doi.org/10.1111/j.1469-8137.2007.02275.x.

Wang, J., Fu, B.J., Jiao, L., et al., 2021. Age-related water use characteristics of Robinia pseudoacacia on the Loess Plateau. Agric. For. Meteorol. 301, 108344. https://doi.org/10.1016/j.agrformet.2021.108344.

Wang, J., Fu, B.J., Lu, N., et al., 2019a. Water use characteristics of native and exotic shrub species in the semi-arid Loess Plateau using an isotope technique. Agric. Ecosyst. Environ. 276, 55–63. https://doi.org/10.1016/j.agee.2019.02.015.

Wang, J., Lu, N., Fu, B.J., 2019b. Inter-comparison of stable isotope mixing models for determining plant water source partitioning. Sci. Total Environ. 666, 685–693. https://doi.org/10.1016/j.scitotenv.2019.02.262.

West, A.G., Goldsmith, G.R., Brooks, P.D., et al., 2010. Discrepancies between isotope ratio infrared spectroscopy and isotope ratio mass spectrometry for the stable isotope analysis of plant and soil waters. Rapid Commun. Mass Spectrom. 24, 1948–1954. https://doi.org/10.1002/rcm.4597.

West, A.G., Patrickson, S.J., Ehleringer, J.R., 2006. Water extraction times for plant and soil materials used in stable isotope analysis. Rapid Commun. Mass Spectrom. 20, 1317–1321. https://doi.org/10.1002/rcm.2456.

Wu, H.W., Li, X.Y., Jiang, Z.Y., et al., 2016. Contrasting water use pattern of introduced and native plants in an alpine desert ecosystem, Northeast Qinghai–Tibet Plateau. China. Sci. Total Environ. 542, 182–191. https://doi.org/10.1016/j.scitotenv.2015.10.121.

Xiao, Y., Liu, X., Zhang, L., et al., 2021. The allometry of plant height explains species loss under nitrogen addition. Ecol. Lett. 24, 553–562. https://doi.org/10.1111/1365-2745.13928.

Yao, S.R., Hu, W.G., Ji, M.F., et al., 2025. Distribution, species richness, and relative importance of different plant life forms across drylands in China. Plant Divers. 47, 273–281. https://doi.org/10.1016/j.pld.2024.09.007.

Yu, G.C., Chen, J., Yu, M.X., et al., 2023. Eighteen-year nitrogen addition does not increase plant phosphorus demand in a nitrogen-saturated tropical forest. J. Ecol. 111, 1545–1560. https://doi.org/10.1111/1365-2745.14118.

Zelikova, T.J., Blumenthal, D.M., Williams, D.G., et al., 2014. Long-term exposure to elevated $CO_2$ enhances plant community stability by suppressing dominant plant species in a mixed-grass prairie. Proc. Natl. Acad. Sci. U.S.A. 111, 15456–15461. https://doi.org/10.1073/pnas.1414659111.

Zhan, X.Y., Bo, Y., Zhou, F., et al., 2017. Evidence for the importance of atmospheric nitrogen deposition to eutrophic Lake Dianchi, China. Environ. Sci. Technol. 51, 6699–6708. https://doi.org/10.1021/acs.est.6b06135.

Zhang, R., Shen, H., Dong, S.K., et al., 2022. Effects of 5-year nitrogen addition on species composition and diversity of an alpine steppe plant community on Qinghai-Tibetan plateau. Plants 11, 966. https://doi.org/10.3390/plants11070966.

Zhao, M., Zhang, H.X., Baskin, C.C., et al., 2022. Intra-annual species gain overrides species loss in determining species richness in a typical steppe ecosystem after a decade of nitrogen enrichment. J. Ecol. 110, 1942–1956. https://doi.org/10.1111/1365-2745.13928.

Zheng, Y., Pei, J.Y., Fang, C., et al., 2023. Determining the harvest frequency to maintain grassland productivity and minimum nutrient removal from soil. Plant Soil 487, 79–91. https://doi.org/10.1007/s11104-023-05907-w.

Zhu, J.T., Zhang, Y.J., Yang, X., et al., 2020. Synergistic effects of nitrogen and $CO_2$ enrichment on alpine grassland biomass and community structure. New Phytol. 228, 1283–1294. https://doi.org/10.1111/nph.16767.