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Nitrogen addition amplified water effects on species composition shift and productivity increase

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Abstract

Aims Water and nitrogen (N) are two key resources in dryland ecosystems, but they may have complex interactive effects on the community structure and ecosystem functions. How future precipitation (rainfall vs snowfall) change will impact aboveground net primary production (ANPP) is far from clear, especially when combined with increasing N availability.

Methods In this study, we investigated changes in community productivity, abundance and aboveground biomass of two dominant plant functional groups (PFGs), i.e. perennial rhizome grasses (PR) and perennial bunchgrasses (PB) under the impacts of increased precipitation (rainfall vs snowfall) combined with N addition in a semiarid temperate steppe.

Important Findings Summer rainfall augmentation marginally increased community ANPP, whereas it significantly increased the abundance and aboveground biomass of PR, but not those of PB. Summer rainfall addition increased the fraction of PR biomass (f_{PR}) while decreased that of PB (f_{PB}). Spring snow addition had no effect on aboveground biomass of either compositional PFG although it marginally increased community ANPP. Nitrogen addition significantly increased community ANPP with greater increase in PR under summer rainfall addition, indicating strong interactive effects on community ANPP largely by enhancing PR biomass. We also found a nonlinear increase in the positive effect of nitrogen addition on productivity with the increased precipitation amount. These findings indicate an amplified impact of precipitation increase on grassland productivity under the accelerated atmospheric N deposition in the future.

Keywords aboveground net primary production, community structure, temperate steppe, nitrogen addition, snow and water addition

增加氮素放大了水分对物种组成变化和生产力提高的影响

摘要：水分和氮素是旱地生态系统中的重要资源，二者对群落结构和生态系统功能的影响存在着复杂的交互作用。未来降水(降雨与降雪)变化将如何影响地上净初级生产力(ANPP)尚未研究清楚，尤其是当降

水变化与氮沉降引起的氮有效性增加相互结合时。在本研究中，我们探讨了半干旱温带典型草原对增加降水(降雨与降雪)和氮素的响应，包括群落生产力、两种主要植物功能群、多年生根茎禾草(PR)和多年生丛生禾草(PB)的多度和地上生物量的变化。研究结果发现，夏季增雨略微增加了群落的ANPP，显著增加了PR的多度和地上生物量，而对PB的多度和地上生物量没有影响。夏季增雨提高了PR生物量在ANPP中的占比，降低了PB生物量的占比。春季增雪对上述两个植物功能群地上生物量均无显著影响，尽管它对群落ANPP有一定的提高。在夏季增雨条件下，氮素添加显著增加了群落ANPP，主要是由于增加了PR的生物量，说明氮和水分添加对群落ANPP存在较强的交互作用，并在很大程度上通过提高PR生物量来实现。我们发现随着降水量的增加，施氮对生产力的正效应呈非线性增加。上述结果表明，在未来大气氮沉降增加的情景下，降水增加对草地生产力的影响将得到进一步放大。

关键词：地上净初级生产力，群落结构，温带草原，氮素添加，雨雪添加

INTRODUCTION

Precipitation is one of the most important factors limiting ecosystem productivity in arid and semiarid regions (Bai *et al.* 2008; Knapp and Smith 2001; Wang *et al.* 2012). Precipitation is predicted to increase by about 30% at the end of this century specifically in the mid-latitudes of Northern Hemisphere (Cholaw *et al.* 2003; IPCC 2013; Zhang *et al.* 2010). The predicted change in precipitation may have substantial effects on the changes in ecosystem functioning (Fay *et al.* 2011). However, how increases in precipitation in both snowfall and rainfall will impact plant species composition and primary productivity remains largely elusive (Gherardi and Sala 2019). Increasing precipitation in different seasons (e.g. spring vs summer) may have significantly different impacts on plant growth in temperate grasslands (Li *et al.* 2019). Previous studies have focused more on the effects of rainfall change rather than snowfall. Most studies have showed that summer rainfall increase can significantly enhance primary productivity in semiarid grasslands (Gong *et al.* 2015; Heisler-White *et al.* 2008; Lü *et al.* 2014; Xu *et al.* 2013; Zhang *et al.* 2019) although no significant response was also found in some specific type of grassland such as sandy grassland (Yu *et al.* 2009). In addition, different types or even genotypes of compositional species might respond differently to summer rainfall change (Aspinwall *et al.* 2017; Heisler-White *et al.* 2008). Therefore, how the semiarid steppe may respond to the summer rainfall change is still not clear yet (Song *et al.* 2019).

Snowfall is also an important form of precipitation in the middle latitudes, and is expected to increase with climate changes (Ma *et al.* 2012), although it is not clear how much will be increased. Snowmelt could increase soil moisture especially in the early growing seasons (Li *et al.* 2020; Rammig *et al.* 2010) and might have a lasting effect on plant growth during the entire growing season (Bombonato and Gerdol 2012; Chimner *et al.* 2010; Fan *et al.* 2013). In a recent study, aboveground biomass was found increasing significantly with the depth of snowpack in a temperate steppe with a stronger effect in the relatively drier areas, indicating the importance of snowfall for primary productivity in the study area (Liu *et al.* 2018a). Therefore, it is critically important to understand how future snowfall increase will affect ecosystem dynamics under future climate change scenarios.

Apart from precipitation, nitrogen (N) is another limiting factor for vegetation productivity in arid and semiarid ecosystems (Hooper and Johnson 1999; LeBauer and Treseder 2008; Wang *et al.* 2010). Studies have reported an increasing effect of N addition on primary productivity in semiarid grasslands (Gong *et al.* 2015; Zhang *et al.* 2015a). Since N deposition will continue to increase at least in the near future (Schlesinger 2009), it is important to evaluate the response of species, plant functional groups (PFGs), communities and ecosystems to N addition, including its interactive effects with projected change in precipitation. In addition, how primary productivity may respond to changes of more than one limiting resource is still under debate. Previous studies have demonstrated that community primary productivity is generally colimited by multiresources (Eskelinen and Harrison 2015; Harpole *et al.* 2007; Hooper and Johnson 1999; Lü *et al.* 2018). However, other studies have also shown a shift may exist from one limiting resource to another, e.g. from soil water to N (Brauer *et al.* 2012; Niu *et al.* 2010; Ren *et al.* 2017), or vice versa (Harpole *et al.* 2017). Thus, it is important to know whether the colimitation of multiresources or a shift of limiting resources is a prevailing resource use pattern in those arid and semiarid grassland ecosystems.

There is a remarkable difference in resource use among different plant species and corresponding PFGs in a community, and thus they may respond differently to change in the availability of resources such as water and N (Heisler-White *et al.* 2008). For example, N addition in a tallgrass prairie shifted the plant composition by decreasing abundance and productivity of C_4 grasses while increasing those of perennials and forbs (Avolio *et al.* 2014). In alpine grasslands in the Tibetan Plateau, a shift of plant species composition was also found from sedges and forbs to grasses caused by declining soil water availability (Liu *et al.* 2018b). Nitrogen addition could also lead to a shift in the canopy dominance from lower to upper species in a temperate steppe (Niu *et al.* 2010). Consequently, changes in species competition in a community will have important implications on how community responds to alterations in resources (Harpole *et al.* 2016; Vila and Sardans 1999).

In this study, we investigated responses of primary productivity (and relevant ecosystem properties) and the underlying mechanisms to water (spring snow and summer rainfall) and N addition in a semiarid steppe in Inner Mongolia, China. We aimed to test the following hypotheses: (i) spring snow addition will have a relatively weaker impacts on the composition of aboveground production than summer rainfall addition as snowfall accounts for a small proportion of annual precipitation; (ii) N addition will have weaker effects on the composition of aboveground production under spring snow than summer rainfall increase for efficient use of additional N by increased precipitation in growing seasons; (iii) carbon (C) assimilation capacity of the different plant species and the aboveground productivity of different PFGs will respond differently to water and N addition for their different requirements of resource use; and (iv) there is a shift in the limiting factor (from water to N or vice versa) in determining the community productivity.

MATERIALS AND METHODS

Site description

The study was conducted in the Inner Mongolia Grassland Ecosystem Research Station, Institute of Botany, the Chinese Academy of Sciences. The site is classified as semiarid temperate steppe and dominated by C_3 grasses (e.g. *Leymus chinensis*, *Lc*; *Stipa grandis*, *Sg*). According to the climate data from the meteorological station of the Inner Mongolia Grassland Ecosystem Research Station (IMGERS), the long-term mean annual temperature is 0.4 °C (1982–2013), and mean annual precipitation is 333.3 mm, with 91.9% (306.3 mm) falling in the form of rainfall and the rest as snowfall. Plant growth generally reaches peak in August. During this study, amount of precipitation by snowfall was 40.3, 31.9, 37.9 and 77.6 mm, and rainfall amounts were 169.6, 163.6, 306.7 and 191.0 mm from November to August 2010, 2011, 2012 and 2013, respectively (Supplementary Fig. S1).

Experimental design and treatments

A randomized block design was used in this study to incorporate three types of water treatments (no water addition, spring snow addition and summer rainfall addition) and two levels of N addition (with and without N addition), resulting in a total of six treatments randomly assigned to six plots in one block. There were five blocks as replicates, resulting in a total of 30 plots. Each plot occupied an area of 25 m^2 (5 m × 5 m) and the distance between two adjacent plots was 1 m. Treatments were labeled as following: control (N0W0), spring snow addition (N0W1), summer rainfall addition (N0W2), nitrogen addition only (N1W0), spring snow addition plus nitrogen addition (N1W1) and summer rainfall addition plus nitrogen addition (N1W2). In this study, we focused on the impacts of increasing amount of snow water, thus we implemented snow addition in March, just before the snow meltdown. At the beginning of March from 2010 through 2013, 25 mm water equivalent of snow was added every year by taking snow from nearby open place and distributed them evenly onto spring snow addition plots right before snowmelt according to the local air temperature. Considering the relatively small quantity of snowfall in this area, we added more snow (~100% of mean annual value) than the predicted values (~30% increase). Similarly, a 10 mm water from a nearby well was added manually from a container weekly starting from mid-June 2010 until it reached a total of 100 mm each year (~30% increase for the mean annual rainfall) in the summer water addition plots. For the N addition treatment, urea fertilizer was applied because of its availability from market and added at a level of 10 g N m^{-2} each year since July

2009. A more detailed description about the design of the experiment can be found in Zhang *et al.* (2015b).

Aboveground production

All plants were collected by species in a harvesting quadrat (1 m × 1 m) within each plot by strip-clipping (1 m × 0.2 m) in mid-August when plants reached their peak growth. A different strip was used each year. Plant samples were taken back to the laboratory and the living aboveground parts were separated from the dead ones. Samples were oven-dried at 65 °C for about 48 h until reaching constant weight. The dry biomass was determined by species and the sum of each compositional species was used as the current year aboveground net primary production (ANPP) at the community level. The ANPP was also considered as the aboveground productivity in this study. Based on the dominant life forms in our study site, species were categorized into four PFGs according to a previous study (Bai *et al.* 2004): perennial rhizome grasses (PR), perennial bunchgrasses (PB), perennial forbs (PF) and annuals or biennials (AB). The fraction of aboveground biomass of each PFG was calculated by dividing ANPP by each PFG biomass. In addition, PR and PB were the major PFGs as their combined relative aboveground biomass could exceed 90% in most plots. All the plant species appeared in the study site are listed in Supplementary Table S1.

The abundance of plant species was determined in a survey quadrat (1 m × 1 m) within each plot in July of each experimental year. The abundance of each dominant PFG was simply calculated by summing up the number of individuals (PF and AB) or culms (PR and PB) of each species involved.

Soil water content

Soil volumetric water content (SWC) was measured on clear days at 10 cm deep during CO_2 gas exchange measurement with a TDR-200 probe (Spectrum Technologies Inc., Plainfield, IL, USA) in each plot. The measurement was conducted once every 10 days in 2010, while once every week in 2011–2013 from May till the end of growing seasons.

Data analyses

Linear mixed model was performed to analyze the main and interactive effects of spring snow addition or summer rainfall addition and N addition on SWC, ANPP, aboveground biomass and the fraction of the four PFGs as well as the abundance of the dominant functional groups (i.e. PR and PB).

We used the structural equation modeling (SEM) to establish pathways that could explain the effects of water and N addition on changes in aboveground biomass of PR and PB and their fractions in the community across the years from 2011 to 2013. In the SEM analysis, the final model was selected based on Goodness of Fit Index (GFI), Comparative Fit Index (CFI) and the Akaike Information Criteria (AIC). The final model was fitted with a nonsignificant χ^2 test ($P > 0.05$), higher GFI and CFI ($P > 0.9$) and lower AIC. All above mentioned analyses were performed using SPSS 23.0 for windows (SPSS Inc., Chicago, IL, USA) except for the SEM, which was calculated by the Amos 20.0 (Amos Development Corporation, Meadville, PA, USA).

RESULTS

Community and PFG aboveground production

Community aboveground biomass (ANPP surrogate) in the control plots, i.e. with neither water addition nor N enhancement, was 193.7, 332.6, 478.8 and 293.9 g m^{-2} from 2010 to 2013, respectively, with a mean value of 324.8 g m^{-2} (Fig. 1a). The aboveground biomass of functional group PR was 49.3, 57.4, 106.7 and 146.7 g m^{-2} from 2010 to 2013, respectively, with a mean value of 90.0 g m^{-2} (Fig. 1b). Correspondingly, the aboveground biomass of PB was 138.5, 266.0, 369.7 and 144.9 g m^{-2} in the same 4 years with a mean value of 229.8 g m^{-2} (Fig.1c). Functional groups PF and AB accounted much less of community ANPP with the mean value of 4.8 g m^{-2} for PF (Fig. 1d) and 0.2 g m^{-2} for AB (Fig. 1e).

Overall across the four study years, water addition only marginally enhanced community ANPP with spring snow addition increasing ANPP by 4.0% and summer rainfall addition increasing ANPP by 4.4% (Table 1; Fig. 1). At the PFG level, spring snow addition had no effects on aboveground biomass of either PFG while summer rainfall addition significantly increased that of PR by 99.4% (Table 1; Fig. 1).

The effects of N addition on aboveground biomass also varied with PFGs and the form of additional water. For example, it increased community ANPP by 16.6%, and increased PB and AB biomass by 25.5% and 968.3%, but decreased PR and PF biomass by 2.2% and 58.4%, respectively, under spring snow addition (Table 1; Fig. 1). When combined with summer rainfall addition, N addition increased community ANPP by 24.7%, increased PR biomass by 27.9%, and PB biomass by 19.3%, but had no influence on PF

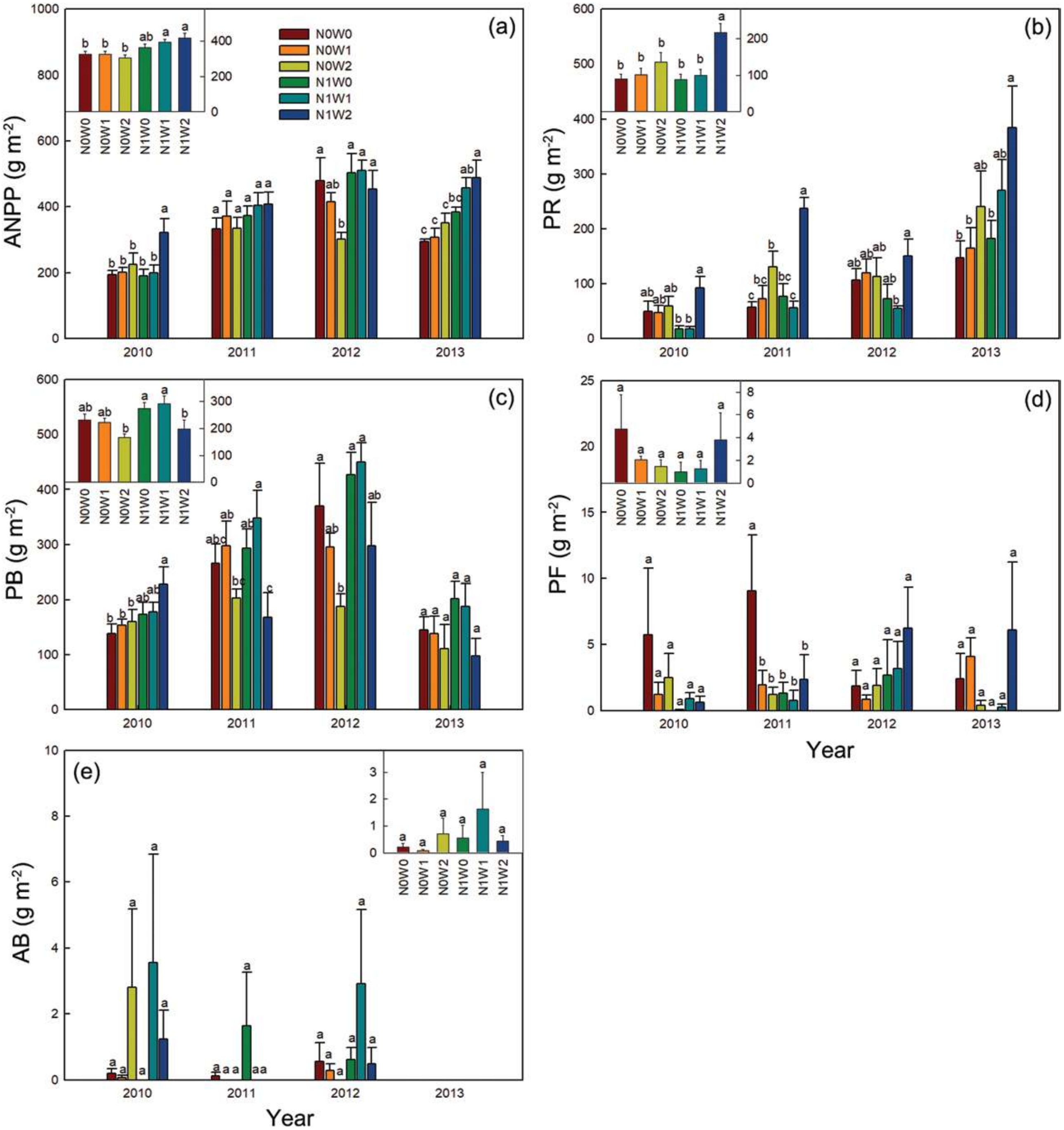

Figure 1: Mean values (mean ± SE) of aboveground biomass of (**a**) community (ANPP, g m^{-2}), (**b**) PR (g m^{-2}), (**c**) PB (g m^{-2}), (**d**) PF (g m^{-2}) and (**e**) AB (g m^{-2}) with inset figures showing the 4-year mean. N0W0, control; N0W1, spring snow addition; N0W2, summer rainfall addition; N1W0, nitrogen addition; N1W1, spring snow + nitrogen addition; N1W2, summer rainfall + nitrogen addition. Different letters on the bar indicate significant differences among treatments ($P < 0.05$).

and AB (Table 1; Fig. 1). In addition, the interactive effects were generally found insignificant between N addition and spring snow addition on community ANPP and the biomass of any compositional PFG. However, there were some interactive effects of increased precipitation and N addition. Increased precipitation enhanced PR biomass in the ambient N plots, but enhanced PR biomass and decreased PF biomass in the enriched N plots. Nitrogen addition decreased PR biomass in the ambient precipitation plots, but enhanced PR biomass and decreased PF biomass in the increased precipitation plots (Table 1).

Table 1: Results (P values) of linear mixed model on the effects of water addition (W) including spring snow and summer water addition, N addition (N) and their interactions on ANPP, aboveground biomass of PR, PB, PF and AB, on the fraction and abundance of PR (f_{PR}, PR_a) and PB (f_{PB}, PB_a) and SWC over 2010 to 2013

Water addition		ANPP	PR	PB	PF	AB	f_{PR}	f_{PB}	PR_a	PB_a	SWC
Spring snow	W	**0.06** (4.0%)	0.95	0.72	0.72	0.87	0.83	0.69	0.39	0.91	0.30
	N	**<0.001** (16.6%)	**0.07** (−2.2%)	**0.02** (25.5%)	**0.01** (−58.4%)	**0.10** (968.3%)	**0.001** (−23.6%)	**0.001** (11.3%)	0.52	0.22	0.75
	W × N	0.26	0.43	0.85	0.50	0.68	0.54	0.79	**0.08** (−13.4%)	0.77	0.80
Summer rainfall	W	**0.08** (4.4%)	**<0.001** (99.4%)	0.17	0.88	0.42	**<0.001** (81.7%)	**<0.001** (−26.3%)	**<0.001** (116.9%)	0.32	**<0.001** (28.5%)
	N	**<0.001** (24.7%)	**0.07** (27.9%)	**0.09** (19.3%)	0.65	0.42	0.80	0.94	0.62	0.46	0.26
	W × N	0.21	**0.02** (140.3%)	0.74	**0.02** (−19.7%)	0.91	**0.04** (73.5%)	**0.02** (−29.9%)	**0.08** (117.1%)	**0.10** (−22.1%)	0.19

Boldfaced numbers indicate significant treatment effects ($P < 0.1$) with change percentage being given in parentheses.

The fraction of aboveground biomass of PR (f_{PR}) in the control plots was 24.9%, 18.0%, 25.0% and 48.8% from 2010 to 2013, respectively, and the mean was 29.2% (Fig. 2a), while the fraction of PB (f_{PB}) was 72.0%, 79.2%, 74.3% and 50.4% from 2010 to 2013, respectively, and the mean was 69.0% (Fig. 2b). Effects of water addition on aboveground biomass fraction of different PFGs varied with the type of water addition. Summer rainfall addition increased f_{PR} by 81.7% but decreased f_{PB} by 26.3% while spring snow addition had largely no effect on either f_{PR} or f_{PB} (Table 1; Fig. 2a and b). The N addition displayed remarkably different effects on the biomass fraction of PFGs over the four experimental years (Table 1; Fig. 2). It decreased f_{PR} by 23.6% while increased f_{PB} by 11.3% under spring snow addition (Table 1; Fig. 2a and b), but had no significant effects on f_{PR} and f_{PB} under summer rainfall addition. In addition, the f_{PR} was significantly increased by 73.5% while f_{PB} was significantly decreased by 29.9% in plots with combined addition of summer rainfall and N (Table 1; Fig. 2a and b).

Based on the average response ratio over the four experimental years, responses to spring snow and summer rainfall addition were remarkably different in aboveground biomass of the two dominant PFGs and community ANPP. The response to spring snow addition was largely weak (<12%) and with large variation among replicates, causing no significant N addition effect (Fig. 3a). The response of aboveground biomass of PR was positive to summer rainfall addition alone, and it was significantly enhanced by the combined addition of N while the response of aboveground biomass of PB was negative to summer water addition regardless of N addition (Fig. 3b). The response ratio of ANPP was marginally negative to summer rainfall addition alone, but turned significantly positive when combined with N addition (Fig. 3b).

To examine the potential shift in water and N limitations on ANPP, we constructed a precipitation gradient by pooling all data from different water addition treatments to investigate how community ANPP changed with precipitation with or without N addition. We found that growing season precipitation (sum of both natural and experimental) could explain much higher variation of ANPP and the ANPP generally increased with precipitation from less than 100 mm till around 300 mm when the ANPP reached a saturation level (Fig. 4). Correspondingly, the N effects changed from being insignificant to significantly positive with increasing amount of the growing season precipitation.

PFG abundance

Over the four experimental years, the average abundance of the two dominant PFGs, PR_a and PB_a, was 201.6 and 355.1 individuals m^{-2} in the control plots. Summer rainfall addition increased PR_a by 116.9%, but showed no effect on PB_a, while spring snow addition alone had no impact on the abundance of either PFG (Table 1; Fig. 5). The N addition alone showed neutral effect on the PR_a and PB_a but marginally decreased PR_a by 13.4% when combined with spring snow addition and increased PR_a by 117.1% and decreased PB_a by 22.1% when combined with summer rainfall addition (Table 1).

Pathways of water and N addition on aboveground biomass of PR, PB and their biomass fractions

Results from the SEM analysis showed that water addition caused a positive but N addition caused a

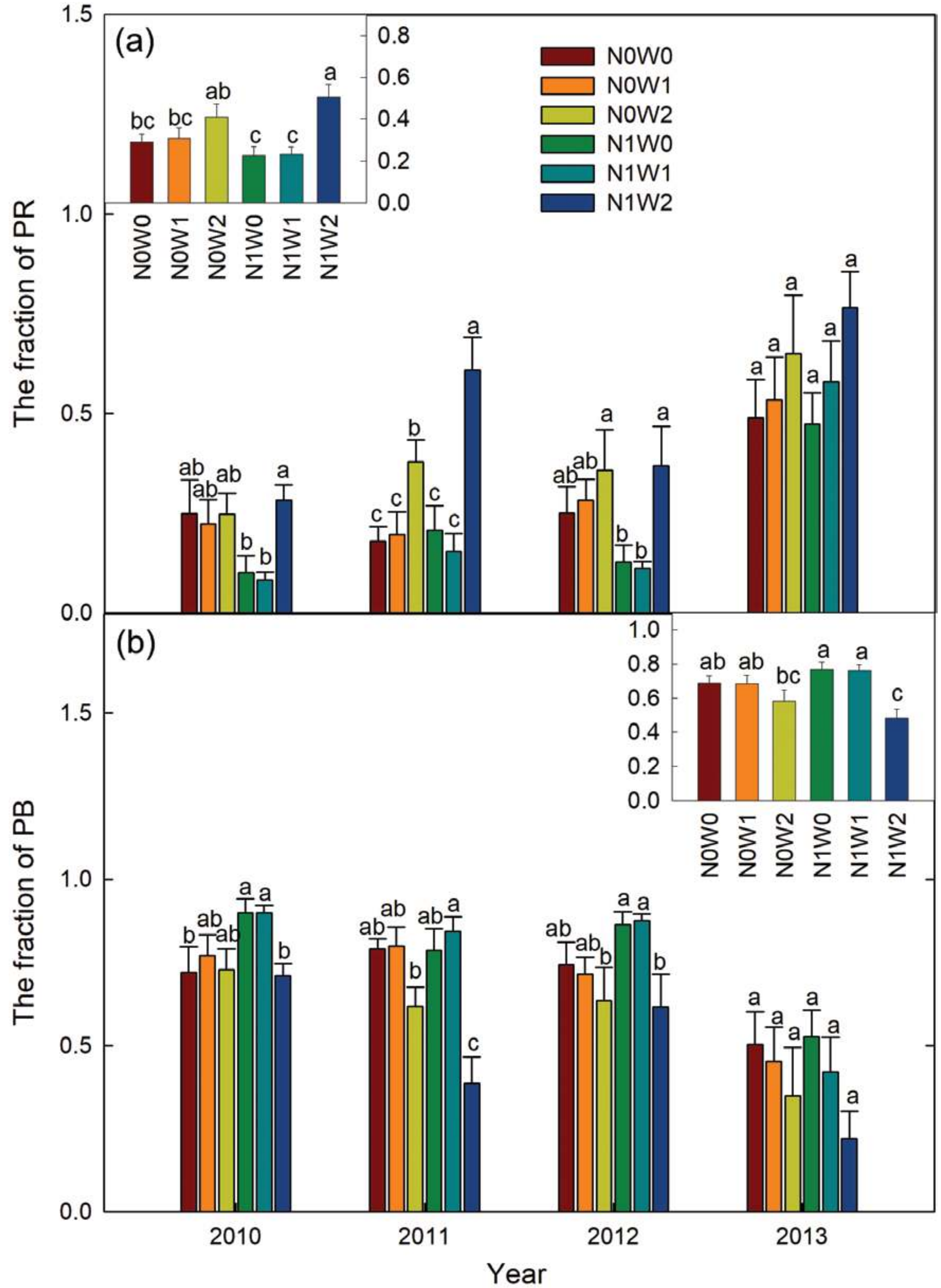

Figure 2: The fraction of aboveground biomass of (**a**) PR and (**b**) PB with inset figures showing the 4-year mean (2010–13). Values are shown as mean ± SE. Different letters on the bar indicate significant differences among treatments ($P < 0.05$). See Fig. 1 for treatment explanation.

marginally negative impact on soil moisture, which further increased abundance of PR but decreased that of PB (Fig. 6). In addition, water addition exerted direct and positive impacts on net photosynthesis (Pn) of PR and abundance of PB while N addition caused direct and positive influences on Pn of PB and abundance of PR. Greater Pn and abundance of PR, and lower abundance of PB promoted aboveground biomass of PR, which contribute to greater f_{PR} but lower f_{PB} (Fig. 6). Correspondingly, greater Pn and abundance of PB, and lower abundance of PR stimulated aboveground biomass of PB, which contributed to greater f_{PB} but lower f_{PR} (Fig. 6).

DISCUSSION

Spring snow and summer rainfall addition impacts PFGs differently

Water is one of the most important driving factors for the Inner Mongolia steppe. We found that the community ANPP could be well predicted by accumulative precipitation of June to August (sum of both the natural and experimental). The ANPP increased rapidly with higher precipitation until ANPP reached a plateau (Fig. 4). This indicates the importance of growing season precipitation to community ANPP in the studied system regardless of N status.

Precipitation impacts on the abundance and aboveground biomass of different PFGs are different. Summer water addition caused marginal increase in the community ANPP due to the significant increase in PR while no significant responses for other PFGs. Summer water addition increased PR biomass by enhancing the C assimilation of *L. chinensis* (the dominant species of PR) especially in combination with N addition, but largely no significant impacts on that of *S. grandis* (the dominant species of PB) (Supplementary Fig. S2a and b). Our SEM analysis also supported this by showing that water addition directly regulated Pn of *L. chinensis*, but not for that of *S. grandis* (Fig. 6). Different rooting system and tillering strategy of the two PFGs may cause this difference in responses to precipitation increase. We speculated that the deeper rooting system of PR (*L. chinensis*) allowed them to take up more belowground resources such as water and nutrients from the deeper soil layers. Our results also showed that aboveground biomass of the two dominant PFGs (i.e. PB and PR) responded to summer water addition in the different directions, resulting in augmented PR biomass while no change of PB biomass. In contrast, the increase in snowfall only showed limited and inconsistent effects, as it showed neutral impact on any of the four compositional PFGs, although there were marginal effects on ANPP at the community level (Table 1).

The trivial effects of snowfall addition on ANPP in the study area could be due to the following reasons. First, our experiment applied snow addition right before snowmelt, and thus only considered the amount of snow water added while ignored impacts in other soil physicochemical processes caused by the coverage of snowpack during the winter time. A previous study showed that deepened snow cover during winter time could increase soil moisture especially in early growing seasons and inorganic

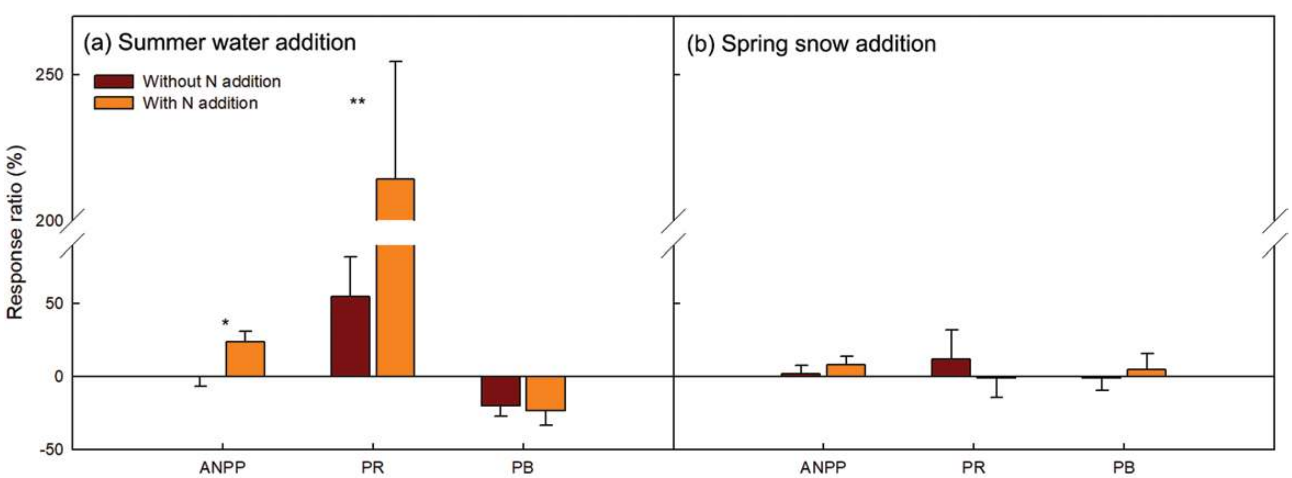

Figure 3: Response ratio of ANPP, aboveground biomass of PR and PB to (**a**) summer rainfall and (**b**) spring snow addition under different N addition treatment. * or ** on each group of bars represent significant differences in *t*-test, at levels of $P < 0.05$ and $P < 0.01$, respectively.

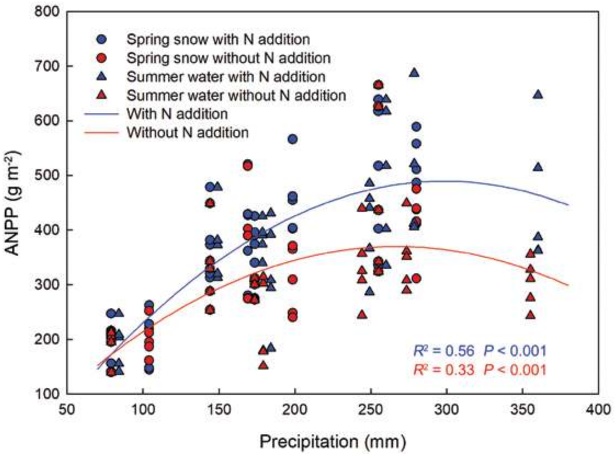

Figure 4: The relationship of the ANPP with total water amount (natural rainfall + water addition) of June–August under spring snow and summer rainfall addition with or without N addition during the four study years.

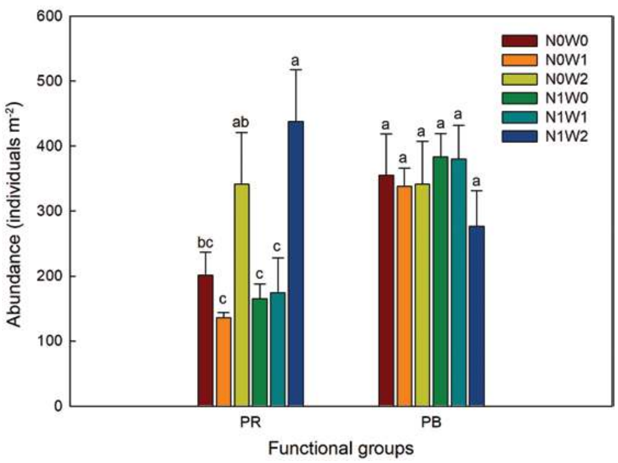

Figure 5: Mean abundance of PR (individuals m^{-2}) and PB (individuals m^{-2}) from 2010 to 2013. Values are shown as mean ± SE. Different letters on the bar indicate significant differences among treatments (P < 0.05). See Fig. 1 for treatment explanation.

N availability under snow cover, thus enhance net ecosystem exchange of CO_2 and belowground biomass although there was no significant effect on aboveground biomass (Li *et al.* 2020). In relatively dry areas, ANPP could also be increased by deepened snow coverage especially with annual precipitation being less than 300 mm (Liu *et al.* 2018a). Second, water from snowfall contributed only a small proportion of the total annual precipitation. The snowfall (including snow addition) accounted for 22.7% (2011), 13.1% (2012) and 29.7% (2013) of the annual precipitation (spring snowfall in November–April + summer rainfall in May–October) during the study period. Third, snowfall generally happens during the nongrowing season, and consequently it does not synchronize with the plant water demand. Considering that the projected increase of snowfall is only by 30% (as opposed to 100% increase in our experiment), we therefore expect that the effects of additional snowfall will be limited in this semiarid steppe because of its small amount and the timing of occurrence.

Nitrogen addition amplified the precipitation effect on ANPP

Both water and N availability are the limiting factors to productivity in terrestrial ecosystems, but their relative importance on ANPP is perhaps difficult to quantify (Burke *et al.* 1997). Our results showed that the N addition significantly enhanced community ANPP although inconsistent effects were found on different PFGs in the spring snow treatment and the effects were only significant on the two dominant PFGs (i.e. PR and PB) in the summer rainfall treatment, indicating that N also significantly limits ANPP in this temperate steppe (Table 1; Niu *et al.* 2010). Moreover, increasing N availability can also enhance ANPP response to precipitation increase (Fig. 4). This largely corroborates results on ecosystem C exchange of this system (Zhang *et al.* 2015b).

Our results indicate that the increase of N availability stimulates water effect on ANPP, which corroborates the meta-analysis study results at a global scale (Xia and Wan 2008). Moreover, N limitation can be aggravated by precipitation increase according to results from a previous study in this semiarid area if the increasing rate of N deposition is lower than the demand of plants (Ren *et al.* 2017). Our results also showed that increase in water supply caused decrease in green leaf nutrient (N and P) concentrations and N addition could lead to the imbalance between N and P uptake (Supplementary Table S2 and Fig. S2c and d). Such results indicate multiple resource limitation of the ecosystem productivity in temperate grasslands, which was also found in other ecosystem types (Schleuss *et al.* 2020; Yuan and Chen 2015). Based on abovementioned points, we can infer that summer precipitation is more important than N availability when precipitation is relatively low, but N availability became more limiting when precipitation reaches a threshold (Fig. 4), indicating that an emergent shift in the resource limitation from water supply to nutrient availability with increasing precipitation. Our results show that three stages can be distinguishable in the response of ANPP to N addition along with the

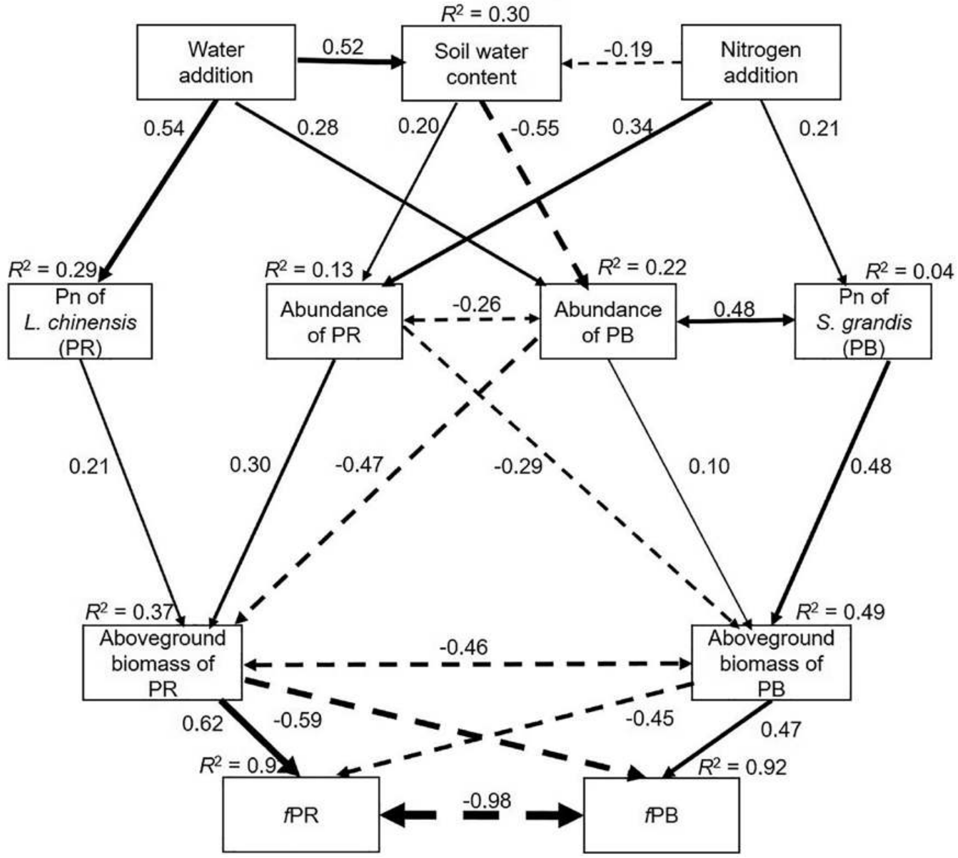

Figure 6: SEM analysis to examine the main controlling effects of water and N addition on the fraction of PR biomass (f_{PR}) and PB biomass (f_{PB}) by affecting their aboveground biomass, abundance and the net photosynthetic rate (Pn) of the dominant species (*Leymus chinensis* and *Stipa grandis*). The model was fitted as χ^2 = 40.04, P = 0.10, GFI = 0.90, CFI = 0.98, AIC = 112.04, df = 30, n = 54. Square boxes in the model indicate the measurable variables with solid and dashed arrows showing significant positive and negative effects between two variables (P < 0.05), respectively. The R^2 showed the proportion of the variance for the target variable in the square box explained by the SEM models.

precipitation increase in this semiarid grassland system (Fig. 4): (i) only precipitation is limiting when precipitation is low, (ii) colimitation of both N and precipitation when precipitation is at the medium level and (iii) only N was limiting when precipitation is high. Although we only considered one level of N fertilization in this study, community ANPP may also reach plateau when N addition reaches a high level according to a study on worldwide grasslands (Peng *et al.* 2020). We thus predict that the limitation of community ANPP may shift to other resources such as light, when both water and N reach their optimal availability (Niu *et al.* 2009). Our results also suggest that limitation will generally transfer from one resource to another when the availability of one resource increases to a sufficient level while colimitation of the two resources will occur before the shift.

Water addition combined with elevated N availability caused differential responses among species and corresponding PFGs

Our results showed that different plant species or PFGs, displayed significantly divergent and even contrasting responses to change in resource availability. Our results from the SEM analysis not only showed how precipitation and N addition affected the aboveground biomass of the two dominant PFGs and their biomass fractions in the community, but also demonstrated the competitive compensatory interactions among the two dominant plant species and their representing PFGs.

As *L. chinensis* was the only species of PR functional group in our study site, and *S. grandis* contributed more than 60% biomass of PB functional group, the response of PR and PB to water and N treatments can largely be reflected by the two dominant species.

Our results showed that the summer rainfall addition significantly enhanced the Pn of *L. chinensis* (PR) but showed largely insignificant effects on that of *S. grandis* (PB) (Supplementary Table S2). This can explain the increase in PR abundance but not for that of PB abundance under summer rainfall addition treatment (Table 1). As a result, the biomass fraction of PR increased while biomass fraction of PB decreased especially in our fourth experimental year (i.e. 2013). This also indicates that *L. chinensis* (PR) benefits more from the predicted environmental change scenarios than *S. grandis* (PB) (Table 1; Supplementary Table S2). Consequently, the growth of PR was stimulated while growth of PB was suppressed especially when ANPP reached the highest level under high precipitation combined with N addition. This is also supported by the strongly negative correlations between the two dominant PFGs in the abundance, aboveground biomass production and their biomass fraction in the community (Fig. 6). Our results suggest that a shift in the dominance of plant species and PFGs can be resulted from combined precipitation and N changes.

The different responses to precipitation and N supply increase between the two dominant species and the two PFGs can be explained as follows. First, they have different resource use strategy. According to a previous study, *L. chinensis* (PR) was more adapted to a relatively fertile soil environment with high water and nutrient supply while *S. grandis* (PB) could survive poor soil environment (Chen *et al.* 2005). Our results showed that N addition increased N uptake of the two dominant species, *L. chinensis* and *S. grandis*. However, it only increased the photosynthesis of *L. chinensis* but not that of *S. grandis* especially under summer rainfall addition (Supplementary Table S2). Second, the two species have different rooting system and tillering strategy. According to a field survey, PB plants generally have fibrous rooting system with either dense or sparse bunch of tillers, while PR plants have strong rhizomatous rooting system via clonal growth with marginally deeper root distribution in the soil profile (Chen *et al.* 2001). Results from a recent study show that N addition can increase the rhizome/shoot ratios of PR while decrease root/shoot ratios of PB (Tian *et al.* 2020), indicating root growth was enhanced for PR species while weakened for PB species after N addition. Our results showed that the abundance of PR was greatly enhanced while the abundance of PB was suppressed by the combined addition of N and summer rainfall partially because of the efficient clonal reproduction of *L. chinensis*. As a result, aboveground biomass of PR was greatly stimulated by addition of water and N either alone or interactively.

CONCLUSIONS

Our results illustrate that water addition, especially when combined with N addition, can enhance the dominance of some species and PFGs (e.g. PR) via suppressing growth of other species and PFGs (e.g. PB), leading to a shift in plant species composition. Both the shift in resource limitation from water to N and their colimitation were found to take effect in controlling change of community ANPP. Our results suggest that the Inner Mongolian steppe may experience a gradual transition in community structure as different plant species, thus PFGs, respond at different paces and directions to the increase in precipitation and atmospheric N deposition.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Table S1: All plant species appeared in the study community categorized into four PFGs: i.e. perennial rhizome grass (PR), perennial bunchgrasses (PB), perennial forbs (PF) and annuals or biennials (AB).

Table S2: Linear mixed model analysis results (*P* values) on effects of water addition (W) as either spring snow or summer rainfall addition, nitrogen (N) addition, and their interactions on the net photosynthetic rate of *Leymus chinensis* (Pn_{LC}) and *Stipa grandis* (Pn_{SG}) over 2011–13, on the foliar N and phosphorus (P) concentrations of *Leymus chinensis* (N_{LC} and P_{LC}) and *Stipa grandis* (N_{SG} and P_{SG}) over 2010–13.

Figure S1: Precipitation (mm) from November to the following August in 2010, 2011, 2012 and 2013.

Figure S2: Characteristics of the two dominant species, *Leymus chinensis* and *Stipa grandis*, (**a**) net photosynthetic rate (Pn, μmol CO_2 m^{-2} s^{-1}) (2011–13), (**b**) foliar N:P ratio, (**c**) foliar N concentration (%) and (**d**) foliar P concentration (%) (2010–13).

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Conflict of interest statement. The authors declare that they have no conflict of interest.

REFERENCES

Aspinwall MJ, Fay PA, Hawkes CV, *et al*. (2017) Intraspecific variation in precipitation responses of a widespread C-4 grass depends on site water limitation. *J Plant Ecol* **10**:310–321.

Avolio ML, Koerner SE, La Pierre KJ, *et al*. (2014) Changes in plant community composition, not diversity, during a decade of nitrogen and phosphorus additions drive above-ground productivity in a tallgrass prairie. *J Ecol* **102**:1649–1660.

Bai YF, Han XG, Wu JG, *et al*. (2004) Ecosystem stability and compensatory effects in the Inner Mongolia grassland. *Nature* **431**:181–184.

Bai Y, Wu J, Xing Q, *et al*. (2008) Primary production and rain use efficiency across a precipitation gradient on the Mongolia plateau. *Ecology* **89**:2140–2153.

Bombonato L, Gerdol R (2012) Manipulating snow cover in an alpine bog: effects on ecosystem respiration and nutrient content in soil and microbes. *Clim Change* **114**:261–272.

Brauer VS, Stomp M, Huisman J (2012) The nutrient-load hypothesis: patterns of resource limitation and community structure driven by competition for nutrients and light. *Am Nat* **179**:721–740.

Burke IC, Lauenroth WK, Parton WJ (1997) Regional and temporal variation in net primary production and nitrogen mineralization in grasslands. *Ecology* **78**:1330–1340.

Chen SP, Bai YF, Zhang LX, *et al*. (2005) Comparing physiological responses of two dominant grass species to nitrogen addition in Xilin River Basin of China. *Environ Exp Bot* **53**:65–75.

Chen SH, Zhang H, Wang LQ, *et al*. (2001) *Root Systems of Grassland Plants in Northern China*. Changchun, China: Jilin University Press.

Chimner RA, Welker JM, Morgan J, *et al*. (2010) Experimental manipulations of winter snow and summer rain influence ecosystem carbon cycling in a mixed-grass prairie, Wyoming, USA. *Ecohydrology* **3**:284–293.

Cholaw B, Cubasch U, Lin YH, *et al*. (2003) The change of North China climate in transient simulations using the IPCC SRES A2 and B2 scenarios with a coupled atmosphere-ocean general circulation model. *Adv Atmos Sci* **20**:755–766.

Eskelinen A, Harrison SP (2015) Resource colimitation governs plant community responses to altered precipitation. *Proc Natl Acad Sci U S A* **112**:13009–13014.

Fan L, Li Y, Tang L, *et al*. (2013) Combined effects of snow depth and nitrogen addition on ephemeral growth at the southern edge of the Gurbantunggut Desert, China. *J Arid Land* **5**:500–510.

Fay PA, Blair JM, Smith MD, *et al*. (2011) Relative effects of precipitation variability and warming on tallgrass prairie ecosystem function. *Biogeosciences* **8**:3053–3068.

Gherardi LA, Sala OE (2019) Effect of interannual precipitation variability on dryland productivity: a global synthesis. *Glob Change Biol* **25**:269–276.

Gong XY, Fanselow N, Dittert K, *et al*. (2015) Response of primary production and biomass allocation to nitrogen and water supplementation along a grazing intensity gradient in semiarid grassland. *Eur J Agron* **63**:27–35.

Harpole WS, Potts DL, Suding KN (2007) Ecosystem responses to water and nitrogen amendment in a California grassland. *Glob Change Biol* **13**:2341–2348.

Harpole WS, Sullivan LL, Lind EM, *et al*. (2016) Addition of multiple limiting resources reduces grassland diversity. *Nature* **537**:93–96.

Harpole WS, Sullivan LL, Lind EM, *et al*. (2017) Out of the shadows: multiple nutrient limitations drive relationships among biomass, light and plant diversity. *Funct Ecol* **31**:1839–1846.

Heisler-White JL, Knapp AK, Kelly EF (2008) Increasing precipitation event size increases aboveground net primary productivity in a semi-arid grassland. *Oecologia* **158**:129–140.

Hooper DU, Johnson L (1999) Nitrogen limitation in dryland ecosystems: responses to geographical and temporal variation in precipitation. *Biogeochemistry* **46**:247–293.

IPCC (2013) *Climate Change 2013: The Physical Science Basis: Summary for Policymakers: IPCC WGI Fifth Assessment Report*. New York, USA: Cambridge University Press.

Knapp AK, Smith MD (2001) Variation among biomes in temporal dynamics of aboveground primary production. *Science* **291**:481–484.

LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**:371–379.

Li Y, Hou L, Song B, *et al*. (2019) Seasonal distribution of increased precipitation in maternal environments influences offspring performance of *Potentilla tanacetifolia* in a temperate steppe ecosystem. *J Plant Ecol* **12**:742–750.

Li P, Sayer EJ, Jia Z, *et al*. (2020) Deepened winter snow cover enhances net ecosystem exchange and stabilizes plant community composition and productivity in a temperate grassland. *Glob Change Biol* **26**:3015–3027.

Liu W, Allison SD, Li P, *et al*. (2018a) The effects of increased snow depth on plant and microbial biomass and community composition along a precipitation gradient in temperate steppes. *Soil Biol Biochem* **124**:134–141.

Liu H, Mi Z, Lin L, *et al*. (2018b) Shifting plant species composition in response to climate change stabilizes grassland primary production. *Proc Natl Acad Sci U S A* **115**:4051–4056.

Lü X-T, Dijkstra FA, Kong D-L, *et al*. (2014) Plant nitrogen uptake drives responses of productivity to nitrogen and water addition in a grassland. *Sci Rep* **4**:4817.

Lü X-T, Liu Z-Y, Hu Y-Y, *et al*. (2018) Testing nitrogen and water co-limitation of primary productivity in a temperate steppe. *Plant Soil* **432**:119–127.

Ma J, Wang H, Zhang Y (2012) Will boreal winter precipitation over China increase in the future? An AGCM simulation under summer "ice-free Arctic" conditions. *Chin Sci Bull* **57**:921–926.

Niu S, Wu M, Han Y, *et al*. (2010) Nitrogen effects on net ecosystem carbon exchange in a temperate steppe. *Glob Change Biol* **16**:144–155.

Niu S, Yang H, Zhang Z, *et al*. (2009) Non-additive effects of water and nitrogen addition on ecosystem carbon exchange in a temperate steppe. *Ecosystems* **12**:915–926.

Peng Y, Chen HYH, Yang Y (2020) Global pattern and drivers of nitrogen saturation threshold of grassland productivity. *Funct Ecol* **34**:1979–1990.

Rammig A, Jonas T, Zimmermann NE, *et al*. (2010) Changes in alpine plant growth under future climate conditions. *Biogeosciences* **7**:2013–2024.

Ren H, Xu Z, Isbell F, *et al*. (2017) Exacerbated nitrogen limitation ends transient stimulation of grassland productivity by increased precipitation. *Ecol Monogr* **87**:457–469.

Schlesinger WH (2009) On the fate of anthropogenic nitrogen. *Proc Natl Acad Sci U S A* **106**:203–208.

Schleuss PM, Widdig M, Heintz-Buschart A, *et al*. (2020) Interactions of nitrogen and phosphorus cycling promote P acquisition and explain synergistic plant-growth responses. *Ecology* **101**:e03003.

Song J, Wan S, Piao S, *et al*. (2019) A meta-analysis of 1,119 manipulative experiments on terrestrial carbon-cycling responses to global change. *Nat Ecol Evol* **3**:1309–1320.

Tian Q, Yang L, Ma P, *et al*. (2020) Below-ground-mediated and phase-dependent processes drive nitrogen-evoked community changes in grasslands. *J Ecol* **108**:1874–1887.

Vila M, Sardans J (1999) Plant competition in Mediterranean-type vegetation. *J Veg Sci* **10**:281–294.

Wang L, D'Odorico P, Evans JP, *et al*. (2012) Dryland ecohydrology and climate change: critical issues and technical advances. *Hydrol Earth Syst Sci* **16**:2585–2603.

Wang L, D'Odorico P, O'Halloran LR, *et al*. (2010) Combined effects of soil moisture and nitrogen availability variations on grass productivity in African savannas. *Plant Soil* **328**:95–108.

Xia J, Wan S (2008) Global response patterns of terrestrial plant species to nitrogen addition. *New Phytol* **179**:428–439.

Xu X, Sherry RA, Niu S, *et al*. (2013) Net primary productivity and rain-use efficiency as affected by warming, altered precipitation, and clipping in a mixed-grass prairie. *Glob Change Biol* **19**:2753–2764.

Yu Z-Y, Zeng D-H, Jiang F-Q, *et al*. (2009) Responses of biomass to the addition of water, nitrogen and phosphorus in Keerqin sandy grassland, Inner Mongolia, China. *J For Res* **20**:23–26.

Yuan ZY, Chen HYH (2015) Negative effects of fertilization on plant nutrient resorption. *Ecology* **96**:373–380.

Zhang B, Cadotte MW, Chen S, *et al*. (2019) Plants alter their vertical root distribution rather than biomass allocation in response to changing precipitation. *Ecology* **100**:e02828.

Zhang Y, Feng J, Isbell F, *et al*. (2015a) Productivity depends more on the rate than the frequency of N addition in a temperate grassland. *Sci Rep* **5**:12558.

Zhang YG, Nearing MA, Zhang XC, *et al*. (2010) Projected rainfall erosivity changes under climate change from multimodel and multiscenario projections in Northeast China. *J Hydrol* **384**:97–106.

Zhang X, Tan Y, Li A, *et al*. (2015b) Water and nitrogen availability co-control ecosystem CO_2 exchange in a semiarid temperate steppe. *Sci Rep* **5**:15549.