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Correspondence and requests for materials should be addressed to X.T.L. (lvxiaotao@iae.ac.cn)

Plant nitrogen uptake drives responses of productivity to nitrogen and water addition in a grassland

Xiao-Tao Lü[1], Feike A. Dijkstra[2], De-Liang Kong[3], Zheng-Wen Wang[1] & Xing-Guo Han[1]

[1]State Key Laboratory of Forest and Soil Ecology, Chinese Academy of Sciences, Shenyang 110164, China, [2]Department of Environmental Sciences, The University of Sydney, Camden, NSW, 2570, Australia, [3]School of Life Sciences, Henan University, Henan 475004, China.

Increased atmospheric nitrogen (N) deposition and altered precipitation regimes have profound impacts on ecosystem functioning in semiarid grasslands. The interactions between those two factors remain largely unknown. A field experiment with N and water additions was conducted in a semiarid grassland in northern China. We examined the responses of aboveground net primary production (ANPP) and plant N use during two contrasting hydrological growing seasons. Nitrogen addition had no impact on ANPP, which may be accounted for by the offset between enhanced plant N uptake and decreased plant nitrogen use efficiency (NUE). Water addition significantly enhanced ANPP, which was largely due to enhanced plant aboveground N uptake. Nitrogen and water additions significantly interacted to affect ANPP, plant N uptake and N concentrations at the community level. Our observations highlight the important role of plant N uptake and use in mediating the effects of N and water addition on ANPP.

Increased atmospheric nitrogen (N) deposition and altered precipitation regimes can have profound impacts on terrestrial carbon and nutrient cycling[1,2], with potentially strong feeds back to climate change[3]. Grasslands cover vast areas of the Earth's surface and provide a range of ecosystem services, from forage production to the preservation of biodiversity. Such ecosystem services provided by grasslands are challenged by global climate change[4]. In semi-arid grassland ecosystems, which are generally assumed to be limited by N and water availability, increased N inputs and annual precipitation often stimulate primary productivity[1,5,6]. Moreover, those two factors can interact in complex ways that may not be simply predicted by the additive effects of individual drivers.

Nitrogen limitation of primary productivity is common in most ecosystems all over the world[7]. Changes in plant N use strategies play an important role in how ecosystems respond to global change factors. There are several indices that have been widely used to quantify plant adaptation to changes of resource availability resulting from global change factors. One index is N use efficiency (NUE), which measures the amount of biomass produced per unit of N taken up by plants from the soil[8]. Plant N concentration has large implications for its NUE, with decreasing NUE at higher plant N concentrations[9,10]. Increased N availability often leads to an enhancement of plant N concentrations[11] and thus a decline in plant NUE[12,13] due to a lower dry matter production per unit of N taken up by plants[14] or/and a shorter mean residence time of N in the plant[15]. Increased N uptake would play an important role in sustaining higher rates of primary production in ecosystems with enhanced N inputs[7,14]. Consequently, the responses of plant growth would be dependent on the balance between the alteration of plant N uptake and NUE after N addition.

The role of N in regulating ecosystem structure and function are influenced by other factors[16] such as water availability in semi-arid ecosystems[1]. Herbaceous dominated systems, such as grasslands, exhibit greater inter-annual variability in ANPP than woody dominated systems, and may be more sensitive to future changes in precipitation[17]. It is predicted that precipitation regimes will change in the future, with increasing precipitation at the mid-latitude regions[18]. At a regional level, ANPP of the temperate steppe increased with increasing mean annual precipitation[5]. At a local level, water addition significantly enhanced both above- and below-ground NPP in the temperate steppe[19]. As water availability increases, plant growth is expected to be more limited by N[20]. There are at least two mechanisms underlying the increase in productivity under enhanced water conditions: increased N uptake from the soil and more efficient use of the N assimilated by plants[10]. However, the relative importance of those two processes in supporting higher rates of primary production under increased precipitation remains unknown.

Nitrogen deposition and increased precipitation may have interdependent effects on grasslands[8]. More importantly, those two global change factors occur at the same time. The interactive effects of N deposition and increased precipitation remain largely unknown. Even within the few related studies that have been published, there is no consensus on the effects of N and water amendment on primary production in grasslands, where both additive[8,21] and non-additive effects[6,19] have been reported. One of the factors accounting for the different results among these studies could be the variation of ambient precipitation, as it has been found that the effects of N and water addition largely depend on ambient precipitation in different years[21,22]. Another factor could be the species-specific N use strategy of plants and their responses to resource amendment. For example, N addition decreased the NUE of C3 perennial grasses but had no effects on NUE of C4 grasses and C3 perennial forbs in a temperate steppe[21]. While considerable data exist on species-level NUE responses to the enhancement of N and water availability, the main and interactive effects of N and water addition on plant community NUE are seldom examined. Species level NUE may not be representative of plant responses at a community level, as communities are generally composed of plant species with different N use strategies[9,14] and community composition usually changes in response to variation of N and water availability[1,6].

As a typical semiarid grassland, the temperate steppe in northern China is generally limited by water and N availability[5] and is sensitive to global change factors[23], such as N deposition and alteration of precipitation regime[6,24]. To examine the role of N use strategies in regulating the response of primary production to increased water and N availability, we carried out a field experiment in a semiarid temperate steppe in northern China. We tested the hypothesis that both increased N and water availability would increase ANPP due to increased plant N uptake in this semi-arid ecosystem.

Results

Soil inorganic N content was higher in 2007 than 2008 ($F = 28.9$, $P < 0.001$; Fig. 1a). Both N ($F = 202.2$, $P < 0.001$) and water addition ($F = 59.0$, $P < 0.001$) significantly increased soil inorganic N content (Fig. 1a). Across all treatments, ANPP was higher in 2007 than 2008 ($F = 9.9$, $P < 0.01$; Fig. 1b). Across the two years, water addition significantly enhanced ANPP ($F = 80.6$, $P < 0.001$; Fig. 1b) and N addition had no impacts on ANPP ($F = 1.9$, $P = 0.19$; Fig. 1b). Nitrogen and water additions interacted to affect ANPP ($F = 4.4$, $P = 0.05$; Fig. 1b), to the effect that the increases in ANPP under water addition were significantly lower in the N fertilized than in unfertilized plots during the two years ($F = 7.4$, $P = 0.015$; Fig. 2).

Nitrogen uptake by aboveground parts showed substantial interannual variation, with higher N uptake in 2007 than 2008 ($F = 21.6$, $P < 0.001$; Fig. 3a). Both N and water additions significantly stimulated plant N uptake (N: $F = 24.5$, $P < 0.001$; Water: $F = 61.3$, $P < 0.001$; Fig. 3a). The interactive effect of N and water addition on plant N uptake was significant ($F = 8.0$, $P = 0.013$), with higher enhancement of N addition without water addition (ambient precipitation) than with water addition (increased precipitation). There was interannual variation of plant N concentrations across the two years ($F = 12.1$, $P < 0.01$; Fig. 3b). Nitrogen addition significantly increased plant N concentrations at the community level, whereas the effect of water addition was not significant ($F = 159.7$, $P < 0.001$; Fig. 3b). There was a significant interaction between N and water addition on plant N concentrations at the community level ($F = 33.9$, $P < 0.001$; Fig. 3b). For the eight dominant species that together contributed more than 85% of ANPP, N addition significantly increased plant N concentrations in most cases (all $P < 0.05$; Fig. 4). In contrast, the effects of water addition on N concentrations varied greatly among species, showing positive (A.c. and C.s.), negative (C.k.), and neutral effects (Fig. 4).

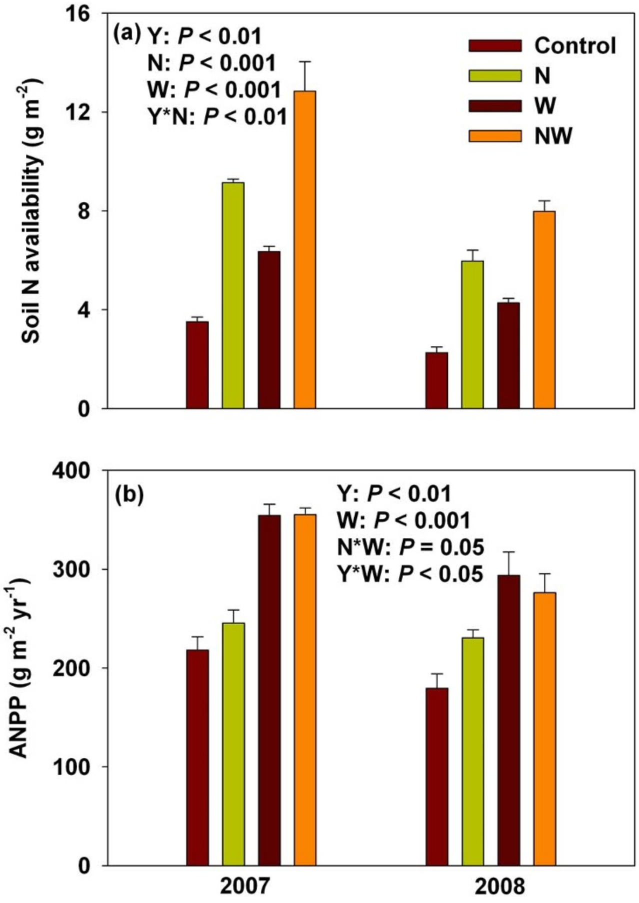

Figure 1 | Soil inorganic N content (a) and ANPP (b) in response to nitrogen and water addition in a semi-arid grassland of northern China. Error bars represent 1 SE. Repeated measurement ANOVA *P* values are reported when $P \leq 0.05$.

Discussion

Nitrogen limitation of primary production is widely distributed in various ecosystems[7]. An increase in net primary production in response to the addition of a limiting nutrient is considered as the classic test of nutrient limitation in a particular ecosystem[25]. Across the two years in this study, N addition showed no significant effect on ANPP, indicating that N would not be the primary limiting factor in this ecosystem. We suspect that the lack of response in ANPP after N addition resulted from an offset between enhanced plant N uptake and decreased plant NUE.

Consistent with our hypothesis, we found that N addition enhanced both plant N concentrations and uptake. Positive effects of N addition on plant N concentrations at both community and species levels as found in this study suggest that N addition causes negative effects on plant NUE. Negative relationships between NUE and soil N availability were observed in a variety of ecosystems, including grasslands[26], forests[27], and peatlands[13]. However, others found no relationship between NUE and soil N availability due to the inherent trade-offs between the two components of NUE, N productivity and mean residence time of N[14,28]. Decreased NUE at the community level in response to N addition may result from lower photosynthesis under enriched N conditions[29]. Nitrogen addition may stimulate the growth of annual forbs at the expense of grasses as found in a similar ecosystem[30], because grasses generally have higher NUE than forbs as reflected by the lower N status in grasses

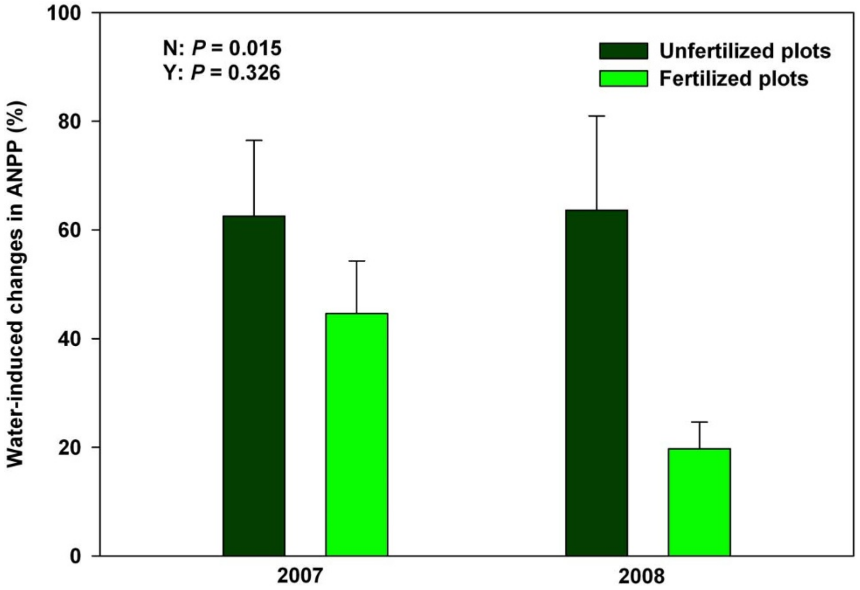

Figure 2 | Water-induced changes in ANPP in the unfertilized and fertilized plots in 2007 and 2008. P-values in the figures represent the difference of water effects between the fertilized and unfertilized treatments and these differences between years.

(Fig. 4). Further, NUE could decrease when plant growth becomes limited by other factors. For example, increased P limitation has been triggered by nitrate addition in an annual grassland in Central California[31].

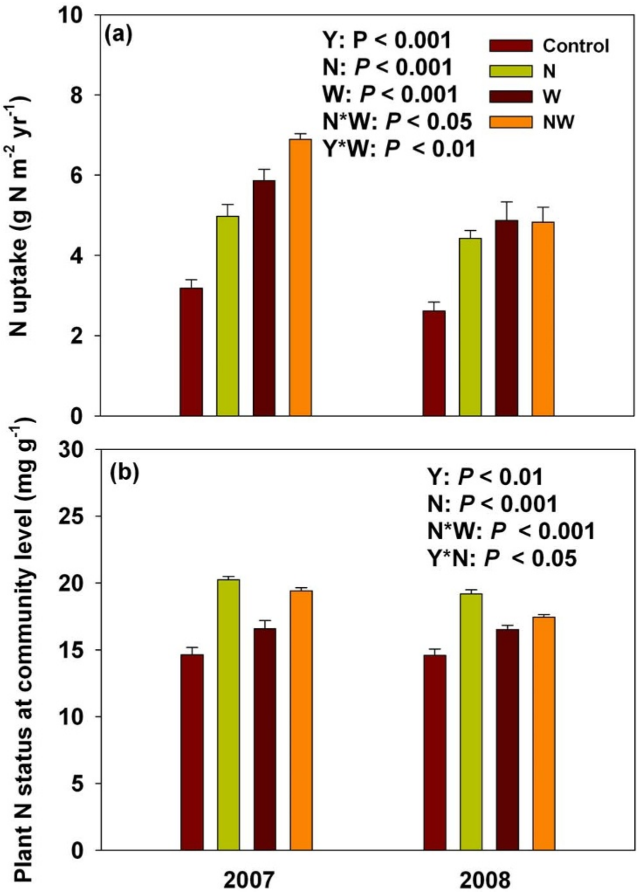

Figure 3 | Soil inorganic N content (a) and plant N status at the community level (b) in response to nitrogen and water addition in a semi-arid grassland of northern China. Error bars represent 1 SE. Repeated measurement ANOVA P values are reported when $P \leq 0.05$.

We attribute the increased plant N uptake to higher N availability in soil under fertilized conditions. Average soil inorganic N content was 2.5 times higher in the fertilized plots than that in the control in both 2007 and 2008. Nitrogen addition greatly enhanced the plant N pool, largely due to higher N concentrations in plant tissues in the N enriched plots (Fig. 4). In a previous study[32], we have shown that N addition significantly increased N concentrations in green leaves of four dominant species in the same ecosystem, which gives further evidence that N addition significantly enhanced plant N status in this ecosystem. As community biomass showed no response to N addition, the enhancement of plant N uptake was mainly driven by changes in plant N concentrations.

Water addition significantly enhanced ANPP in this semiarid grassland, irrespective of the ambient precipitation in the two hydrologically different years. Similar observations were reported in other semiarid grasslands receiving various rates of water addition[19,33]. Moreover, Niu et al. (2009) reported that precipitation plays an important role in regulating ecosystem C fluxes in the temperate steppe, such as net ecosystem exchange, ecosystem respiration, and gross ecosystem production[6]. Water availability also has important implications for the responses of ecosystem C and water fluxes to climatic change in the temperate steppe[34]. At the regional level, ANPP increased significantly with increasing mean annual precipitation in the Inner Mongolia grassland of northern China[5]. Together, results from this study and others indicate that water availability is an important driver for primary production and ecosystem C sequestration in the temperate steppe of northern China. The potential changes in precipitation regimes as predicted by climatic models may exert significant impacts on ecosystem functioning of the temperate steppe.

A variety of mechanisms have been proposed to explain the positive effects of increased precipitation on ANPP in the arid and semiarid grassland. The most common explanation is that plants use limiting nutrients, such as N, more efficiently under increased precipitation[33]. However, our results show that water addition did not alter plant N concentrations and consequently plant NUE at the community level in this semiarid grassland, which is also in contrast with our initial hypothesis. Water addition significantly enhanced N concentrations in both green and senesced leaves of four dominant species in this ecosystem[32]. Higher foliar N concentrations could lead to higher photosynthetic rates under increased precipitation, which

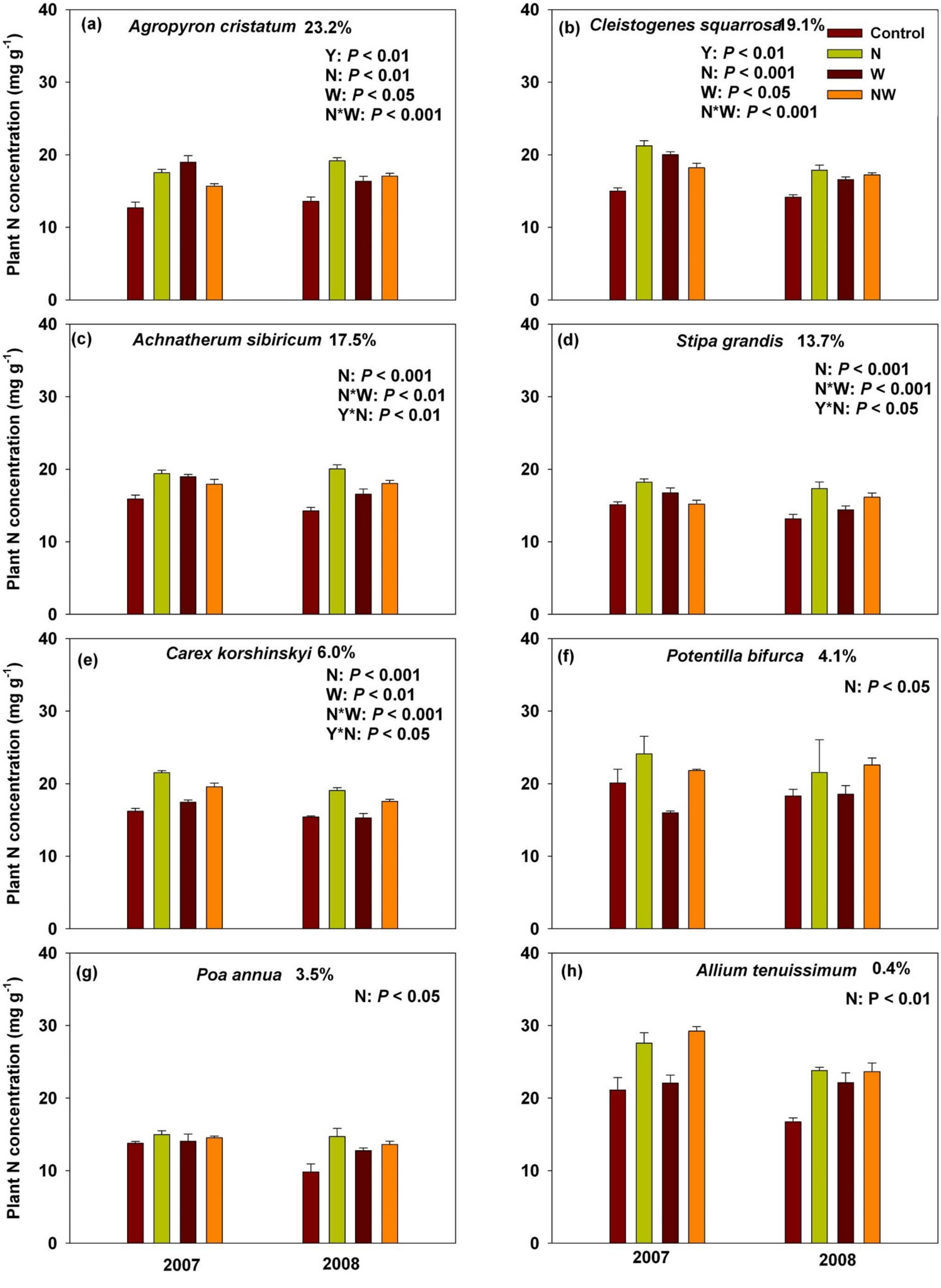

Figure 4 | Plant N status at the species level for eight dominant species in response to nitrogen and water addition in a semi-arid grassland of northern China. C3 grasses: A.c., A.s., S.g., and P.a.; C3 sedge: C.k.; C4 grass: C.s.; C3 forb: P.b. and A.t.. Error bars represent 1 SE. Repeated measurement ANOVA *P* values are reported when $P \leq 0.05$.

has been reported by Niu et al. in other water addition experiments carried out in the temperate steppe of northern China[34]. In the present study, however, we found that water addition seldom altered shoot-level and community-level N status, implying a hierarchical response of plant N to water addition from organ to community level. The neutral response of shoot N concentrations and thus NUE to water addition would be a result of similar relative increases in photosynthesis and plant N uptake rates with increasing precipitation. Yuan and Li[35] reported no effect of soil water status on NUE of six species growing in two habitat types with contrasting soil water supply due to the trade-off between N productivity and its mean residence time (MRT), in that species from habitats with high soil water status had higher N productivity but lower MRT than those from habitat with low soil water status.

We attribute the higher ANPP in water addition plots to the increases in the plant N uptake. Several mechanisms may contribute to the greater uptake of N by plants under increased precipitation. First, the increase in soil water availability may accelerate soil N cycling. Water addition increased soil N availability, as observed in this study, and may also increase the rate of soil N mineralization in this temperate steppe[36]. Consequently, more N would be supplied for plant uptake under increased precipitation. Secondly, water addition has been found to increase fine root production in nearby ecosystems[19,33], allowing plants to explore more of the soil volume for available N. Third, growth of mycorrhizal fungi may benefit from increased C allocation to roots and also from pulsed water availability[37], such as the water addition in this study. It is well-known that mycorrhizal fungi contribute to the uptake and transport of organic and inorganic N[38]. Increases in plant N uptake and ANPP in response to increased precipitation would result in higher litter production both in quality and quantity, indicating that increased precipitation would lead to a positive plant-soil feedback with respect to ecosystem N cycling in this semiarid grassland.

There is no consensus about the relative importance of N and water in regulating ecosystem function of grasslands. We found that water addition stimulated ANPP while N addition did not affect ANPP in this semiarid grassland, suggesting that water availability was more important than N availability in regulating primary production in this ecosystem. The effect of water addition on ANPP was much stronger than that of N addition. In the dry year 2007, ANPP in the +N and +W plots was 12.6% and 62.5% higher than that in the control plots, respectively. In the wet year, these numbers were 28.6% and 63.6%. These results are consistent with Niu et al. who reported that ecosystem carbon and water fluxes and their responses to climate change largely depend on water availability in the temperate steppe of northern China[34]. It is notable that water and N availability interacted to affect plant growth and plant N uptake and use, as indicated by the significant interactive effects of N and water addition on those variables. For example, the enhanced ANPP with water addition was much lower in the fertilized plots than that in the ambient N plots. Those results suggest that the stimulation of plant growth with increased precipitation would be weakened with increased N availability resulting from both atmospheric deposition and global warming in this temperate steppe[22]. The interactive effects between increasing water and N availability on plant N use and primary productivity deserve more attention in modeling responses of terrestrial ecosystems under scenarios of increasing N deposition and altered precipitation regime.

It seems self-contradictory to our assertion that this ecosystem was more water-limited than N-limited when in fact ANPP was lower in the wet year (2008) than the dry year (2007). We propose that the low ANPP in 2008 was related to the precipitation patterns but not to the precipitation amount in the growing season, especially in July and August. The January-July precipitation is considered the dominant driver for ANPP in this temperate steppe[39]. The total precipitation from January to July was 151.5 mm in 2007 and 238.8 mm in 2008. There were seven precipitation events larger than 10 mm in 2008 (averaged 21 mm per event), accounting for more than 60% of the total amount of precipitation from January to July. In contrast, there were four precipitation events larger than 10 mm in 2007 (averaged 14 mm per event), contributing 38% of the total precipitation from January to July. It is easy to see that the amount of precipitation was largely dominated by large precipitation events in 2008. Pulsed water supply could lead to increased N loss in semiarid grasslands[40,41], which may explain our observation that soil inorganic N content was lower in the wet year than in the dry year. In fact, soil inorganic N content in the top 10 cm of the soil in all treatments sharply decreased after an extreme rainfall (63.3 mm) at the end of July in 2008[42]. Decreased soil N availability is the main reason for lower plant N uptake in lower ANPP in 2008 than in 2007. While an increase in precipitation event size increased ANPP in a semi-arid grassland in Colorado due to the alteration in soil moisture[43], our results highlight the importance of N availability in mediating the response of ANPP to altered precipitation regime.

In conclusion, we found that water addition rather than N addition enhanced ANPP in a temperate steppe of northern China. Given the predicted increases of precipitation in mid-latitude regions, the temperate steppe would play an important role in C sequestration due to the increased ANPP as illustrated in this study and the increased net ecosystem exchange found in nearby ecosystems[6]. However, it should be noted that the positive effects of increasing precipitation would be lower under N enriched conditions. Nitrogen enrichment had negative, and increased precipitation had neutral, effects on plant NUE at the community level. However, both factors showed positive effects on plant N uptake, although these effects may occur through different mechanisms. The neutral effect of N addition on ANPP possibly resulted from the offset between enhanced plant N uptake and decreased plant NUE. On the other hand, increases in plant N uptake accounted for the enhanced ANPP after water addition. The variation of ANPP in the two hydrologically different years illustrates that precipitation patterns may exert a strong effect on primary production, largely through its effect on soil N availability. Our findings highlight the importance of plant N uptake in mediating the responses of ecosystem functioning to N enrichment and increased precipitation in the semiarid temperate steppe in northern China.

Materials and methods

Study site. Our experimental site was located in the Xilin River Basin, near the Inner Mongolia Grassland Ecosystem Research Station of the Chinese Academy of Sciences (116°42′E, 43°38′N and 1250 m a.s.l.). The mean annual temperature in this area is 0.3°C, with the lowest monthly temperature (−21.6°C) in January and the highest (19.0°C) in July. The mean annual precipitation is 346 mm, with about 80% occurring in the growing season from May to September. The field experiment was carried out on a natural steppe community, which has been fenced to prevent grazing by large animals since 1999. The soil is a dark chestnut according to the Chinese classification, or Calcic-orthic Aridisol according to US Soil Taxonomy, with 48.6% sand, 26.1% silt, and 25.3% clay in the top 10 cm soil. Mean soil bulk density of the top 10 cm soil-depth is 1.23 g cm^{-3}, and the soil pH is 7.5. The plant community was dominated by *Stipa grandis* P. Smirn., *Achnatherum sibiricum* (Linn.) Keng, and *Agropyron cristatum* (L.) Gaertn, which are widely distributed perennial C3 grasses in the Eurasia steppe.

To examine the effects of increased N and water availability on community composition and ecosystem functioning of the temperate steppe we established five replicate blocks of four 4 m × 4 m plots, separated by 1 m aisles in 2007. Each treatment was replicated five times. We added N in May and July each year, as urea, in dry form in two applications totaling 17.5 g N m^{-2} yr^{-1}. This rate of N addition would alleviate N limitation in the grassland of this region, and therefore the amount of N added was much greater than the local atmospheric N deposition. For water addition, 10 mm of tap water was manually applied with a sprayer each week from May to September each year. Water was always applied after 16:00 h to prevent rapid loss by evaporation. In total, water was added 18 times in each year, amounting to an additional 180 mm precipitation, which is about 50% of the long-term mean annual precipitation in this region. The observations in the present study were from two hydrologically contrasting growing seasons, with a total precipitation of 240 mm in 2007 (dry year, with 192 mm in the growing season) and 362 mm in 2008 (wet year, with 295 mm in the growing season) (see Supplementary Fig. S1 online).

Consequently, we took this opportunity to test the inter-annual variation of responses of ANPP and N use strategies to N and water addition.

Field sampling and measurement. Aboveground net primary production (ANPP) was determined as the peak aboveground biomass during the growing season of each year. As the standing crop of the temperate steppe reaches its annual peak at the middle to end of August, the estimated community biomass would be an approximate of ANPP in this ecosystem. Aboveground biomass was sampled in each plot by clipping all vascular plants at the soil surface within a 1 m × 1 m quadrat in mid-August of each year. All plants were sorted by species, and plant samples for each species from each plot were separated into leaves, stems, flowers, fruits, and current year senesced parts if there were any. All the samples were oven-dried at 65°C for 48 h and weighed. After being ground with a ball mill (Retsch MM 400; Retsch, Haan, Germany), N concentrations of plant samples were analyzed with a PE-2400 CHN analyzer (Perkin-Elmer, Foster City, USA).

Plant N concentrations were quantified at both species and community levels. Plant N concentration for the dominant species was calculated as the biomass-weighted average N concentration among different organs (leaf, stem, fruit, flower, senesced parts). Plant N concentration at the community level was calculated as the biomass weighted average N concentration among different species. Nitrogen uptake was defined as the N content in the peak biomass of the community.

Soil samples were taken from 0–10 cm, 10–20 cm, and 20–30 cm depth each month from June to October in each year. For each plot, five soil cores from each of the three soil depths were collected using a 3-cm-diameter soil auger and mixed into one composite sample. Fresh soil samples (10 g) were extracted with 50 ml of 2 M KCl. The filtered soil extraction was used to determine ammonium (using the salicylate method) and nitrate concentration (cadmium reduction method) with a continuous flow spectrophotometer (FIAstar 5000; Foss Tecator, Denmark). Total inorganic N concentration was the sum of ammonium and nitrate concentrations. The mean value of inorganic N concentration in each plot across the growing season in each year was used in this study. Gravimetric water moisture was determined by drying soil at 105°C for 48 h.

Statistical analysis. Data were tested for normality using the Kolmogorov-Smirnov test and for equality of error variance using Levene's test. For plant N concentrations in *Allium tenuissimum*, we log-transformed our data to meet model assumptions, whereas for all other analyses we used untransformed data. Repeated measurement ANOVAs were used to examine the effects of N and water addition on soil inorganic N concentrations, ANPP, community N uptake and plant N concentrations at species and community levels, with block as a random factor. Between-subject effects were evaluated as N or water addition treatments and within-subject effects were year. Water effects on ANPP were calculated as [100 × (W - control)/control] in the unfertilized plots and [100 × (NW - N)/N] in the fertilized plots. Here, N, W, and NW refer to N addition, water addition and addition of both N and water, respectively, in the above calculations. Two-way ANOVA was used to examine the effects of N addition and year on the water-induced changes in ANPP. All analyses were conducted using SPSS 13.0 (SPSS Inc., Chicago, USA). The significance level was set at 0.05.

1. Harpole, W. S., Potts, D. L. & Suding, K. N. Ecosystem responses to water and nitrogen amendment in a California grassland. *Glob Change Biol* **13**, 2341–2348 (2007).
2. Gruber, N. & Galloway, J. N. An Earth-system perspective of the global nitrogen cycle. *Nature* **451**, 293–298 (2008).
3. Heimann, M. & Reichstein, M. Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* **451**, 289–292 (2008).
4. Maestre, F. T., Salguero-Gomez, R. & Quero, J. L. It is getting hotter in here: determining and projecting the impacts of global environmental change on drylands Introduction. *Phil Trans R Soc B-Bio Sci* **367**, 3062–3075 (2012).
5. Bai, Y. F. *et al.* Primary production and rain use efficiency across a precipitation gradient on the Mongolia plateau. *Ecology* **89**, 2140–2153 (2008).
6. Niu, S. L. *et al.* Non-Additive Effects of Water and Nitrogen Addition on Ecosystem Carbon Exchange in a Temperate Steppe. *Ecosystems* **12**, 915–926 (2009).
7. LeBauer, D. S. & Treseder, K. K. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**, 371–379 (2008).
8. Hirose, T. Nitrogen use efficiency revisited. *Oecologia* **166**, 863–867 (2011).
9. Aerts, R. & Chapin, F. S. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Adv Ecol Res* **30**, 1–67 (2000).
10. McCulley, R. L., Burke, I. C. & Lauenroth, W. K. Conservation of nitrogen increases with precipitation across a major grassland gradient in the Central Great Plains of North America. *Oecologia* **159**, 571–581 (2009).
11. Xia, J. Y. & Wan, S. Q. Global response patterns of terrestrial plant species to nitrogen addition. *New Phytol* **179**, 428–439 (2008).
12. Aerts, R. & de Caluwe, H. Nitrogen Use Efficiency of Carex Species in Relation to Nitrogen Supply. *Ecology* **75**, 2362–2372 (1994).
13. Bridgham, S. D., Pastor, J., Mcclaugherty, C. A. & Richardson, C. J. Nutrient-use efficiency - a litterfall Index, a model, and a test along a nutrient-availability gradient in North-Carolina peatlands. *Am Nat* **145**, 1–21 (1995).
14. Iversen, C. M., Bridgham, S. D. & Kellogg, L. E. Scaling plant nitrogen use and uptake efficiencies in response to nutrient addition in peatlands. *Ecology* **91**, 693–707 (2010).
15. Yuan, Z. Y., Li, L. H., Huang, J. H., Han, X. G. & Wan, S. Q. Effect of nitrogen supply on the nitrogen use efficiency of an annual herb, *Helianthus annuus* L. *J Integr Plant Biol* **47**, 539–548 (2005).
16. Agren, G. I., Wetterstedt, J. A. M. & Billberger, M. F. K. Nutrient limitation on terrestrial plant growth - modeling the interaction between nitrogen and phosphorus. *New Phytol* **194**, 953–960 (2012).
17. Knapp, A. K. & Smith, M. D. Variation among biomes in temporal dynamics of aboveground primary production. *Science* **291**, 481–484 (2001).
18. Solomon, S. *et al.* (eds.). *Climate Change 2007: The Physical Science Basis - Summary For Policymakers.* Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 996 pp. (2007).
19. Gao, Y. Z., Chen, Q., Lin, S., Giese, M. & Brueck, H. Resource manipulation effects on net primary production, biomass allocation and rain-use efficiency of two semiarid grassland sites in Inner Mongolia, China. *Oecologia* **165**, 855–864 (2011).
20. Hooper, D. U. & Johnson, L. Nitrogen limitation in dryland ecosystems: Responses to geographical and temporal variation in precipitation. *Biogeochemistry* **46**, 247–293 (1999).
21. Gong, X. Y. *et al.* Tradeoffs between nitrogen- and water-use efficiency in dominant species of the semiarid steppe of Inner Mongolia. *Plant Soil* **340**, 227–238 (2011).
22. Xia, J. Y., Niu, S. L. & Wan, S. Q. Response of ecosystem carbon exchange to warming and nitrogen addition during two hydrologically contrasting growing seasons in a temperate steppe. *Glob Change Biol* **15**, 1544–1556 (2009).
23. Christensen, L., Coughenour, M. B., Ellis, J. E. & Chen, Z. Z. Vulnerability of the Asian typical steppe to grazing and climate change. *Clim Chang* **63**, 351–368 (2004).
24. Niu, S. L. *et al.* Nitrogen effects on net ecosystem carbon exchange in a temperate steppe. *Glob Change Biol* **16**, 144–155 (2010).
25. Chapin, F. S., Vitousek, P. M. & Vancleve, K. The Nature of Nutrient Limitation in Plant-Communities. *Am Nat* **127**, 48–58 (1986).
26. Yuan, Z. Y. *et al.* Nitrogen response efficiency increased monotonically with decreasing soil resource availability: a case study from a semiarid grassland in northern China. *Oecologia* **148**, 564–572 (2006).
27. Tateno, R. & Takeda, H. Nitrogen uptake and nitrogen use efficiency above and below ground along a topographic gradient of soil nitrogen availability. *Oecologia* **163**, 793–804 (2010).
28. Eckstein, R. L. & Karlsson, P. S. Above-ground growth and nutrient use by plants in a subarctic environment: Effects of habitat, life-form and species. *Oikos* **79**, 311–324 (1997).
29. Talhelm, A. F., Pregitzer, K. S. & Burton, A. J. No evidence that chronic nitrogen additions increase photosynthesis in mature sugar maple forests. *Ecol Appl* **21**, 2413–2424 (2011).
30. Bai, Y. F. *et al.* 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 (2009).
31. Menge, D. N. L. & Field, C. B. Simulated global changes alter phosphorus demand in annual grassland. *Glob Change Biol* **13**, 2582–2591 (2007).
32. Lü, X. T. & Han, X. G. Nutrient resorption responses to water and nitrogen amendment in semi-arid grassland of Inner Mongolia, China. *Plant Soil* **327**, 481–491 (2010).
33. Li, J. Z., Lin, S., Taube, F., Pan, Q. M. & Dittert, K. Above and belowground net primary productivity of grassland influenced by supplemental water and nitrogen in Inner Mongolia. *Plant Soil* **340**, 253–264 (2011).
34. Niu, S. L. *et al.* Water-mediated responses of ecosystem carbon fluxes to climatic change in a temperate steppe. *New Phytol* **177**, 209–219 (2008).
35. Yuan, Z. Y. & Li, L. H. Soil water status influences plant nitrogen use: a case study. *Plant Soil* **301**, 303–313 (2007).
36. Wang, C. H., Wan, S. Q., Xing, X. R., Zhang, L. & Han, X. G. Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China. *Soil Biol Biochem* **38**, 1101–1110 (2006).
37. Birhane, E., Sterck, F. J., Fetene, M., Bongers, F. & Kuyper, T. W. Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. *Oecologia* **169**, 895–904 (2012).
38. Hawkins, H. J., Johansen, A. & George, E. Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant Soil* **226**, 275–285 (2000).
39. Bai, Y. F., Han, X. G., Wu, J. G., Chen, Z. Z. & Li, L. H. Ecosystem stability and compensatory effects in the Inner Mongolia grassland. *Nature* **431**, 181–184 (2004).
40. Austin, A. T. *et al.* Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia* **141**, 221–235 (2004).
41. Dijkstra, F. A., Augustine, D. J., Brewer, P. & von Fischer, J. C. Nitrogen cycling and water pulses in semiarid grasslands: are microbial and plant processes temporally asynchronous? *Oecologia* **170**, 799–808 (2012).
42. Lü, X. T., Kong, D. L., Pan, Q. M., Simmons, M. E. & Han, X. G. Nitrogen and water availability interact to affect leaf stoichiometry in a semi-arid grassland. *Oecologia* **168**, 301–310 (2012).

43. Heisler-White, J. L., Knapp, A. K. & Kelly, E. F. Increasing precipitation event size increases aboveground net primary productivity in a semi-arid grassland. *Oecologia* **158**, 129–140 (2008).

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Author contributions

X.T.L. and X.G.H. conceived and designed the experiment. X.T.L. and D.L.K. carried out the field experiment. X.T.L., D.L.K., Z.W.W. and F.A.D. analyzed the data. All the authors contributed to the writing of the manuscript.

Additional information

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