

# Nitrogen and water availability interact to affect leaf stoichiometry in a semi-arid grassland

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**Abstract** The effects of global change factors on the stoichiometric composition of green and senesced plant tissues are critical determinants of ecosystem feedbacks to anthropogenic-driven global change. So far, little is known about species stoichiometric responses to these changes. We conducted a manipulative field experiment with nitrogen (N;  $17.5 \text{ g m}^{-2} \text{ year}^{-1}$ ) and water addition (180 mm per growing season) in a temperate steppe of northern China that is potentially highly vulnerable to global change. A unique and important outcome of our study is that water availability modulated plant nutritional and stoichiometric responses to increased N availability. N addition significantly reduced C:N ratios and increased N:P ratios but only under ambient water conditions. Under increased water supply, N addition had no effect on C:N ratios in green and senesced leaves and N:P ratios in senesced leaves, and significantly decreased C:P ratios in both green and senesced leaves and N:P ratios in green leaves. Stoichiometric ratios varied greatly among species. Our results suggest that N and water addition and species identity can affect stoichi-

ometric ratios of both green and senesced tissues through direct and interactive means. Our findings highlight the importance of water availability in modulating stoichiometric responses of plants to potentially increased N availability in semi-arid grasslands.

**Keywords** C:N:P · Precipitation regime · Senesced leaves · Steppe · Stoichiometric ratios

## Introduction

Carbon and nutrient concentrations and their relative ratios in green and senesced leaves can significantly influence ecosystem processes (Aerts and Chapin 2000; Sterner and Elser 2002). They can also change in response to global change factors, such as elevated  $\text{CO}_2$  concentration (Billings et al. 2003), increased N deposition (Esmeijer-Liu et al. 2009), decreased biodiversity (Knops et al. 2001) and interactions among these factors (Henry et al. 2005; Knops et al. 2007; Novotny et al. 2007). Concurrently, potentially altered precipitation regimes resulting from global climate change may exert strong controls on community composition and ecosystem function of arid and semi-arid areas (Huxman et al. 2004; Suttle et al. 2007; Bai et al. 2008; Gerten et al. 2008). Furthermore, variation in precipitation regimes interact with other global change factors to affect ecosystem processes (Weltzin et al. 2003; Henry et al. 2006; Harpole et al. 2007). Yet, we still know little about plant stoichiometric responses to synchronous changes in precipitation regimes and N availability, which may potentially limit our ability to predict related positive and negative feedbacks in ecosystem processes. Moreover, better understanding of plant stoichiometric responses to concurrent alterations in N and water availability is critical for

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projection of nutrient cycling dynamics under future global change scenarios.

Human activities have directly and indirectly increased ecosystem N availability (Vitousek et al. 1997; Gruber and Galloway 2008). N deposition and addition often result in higher availability of soil inorganic N (Galloway and Cowling 2002) and thus in increased N concentrations and decreased C:N ratios in green and senesced plant leaf tissue (Henry et al. 2005; Novotny et al. 2007; Xia and Wan 2008). Increased N availability would be expected to increase N:P ratios and plant growth until growth becomes P-limited (Perring et al. 2008). However, empirical studies have shown varied results. While some studies have reported positive effects of N addition on leaf N:P ratios (Henry et al. 2006; Menge and Field 2007), others found no effects (Phoenix et al. 2003; Kozovits et al. 2007; Novotny et al. 2007). These inconsistencies may be attributed to species-specific response patterns of plants (Novotny et al. 2007), variation in the amount of N added (Güsewell 2005), variability in environmental conditions of micro-sites (Pugnaire 2001) and variation in the duration of each experiment (Güsewell et al. 2002). Given the mixed results of these studies and the location bias of the existing experiments (mainly from Europe and North America), additional evidence from various ecosystem types and from different continents are critically needed to identify general patterns in plant tissue stoichiometric responses to N addition or deposition. Moreover, a meta-analysis of existing data should be conducted to resolve existing contradictions, and a more general model is needed to assess mechanisms behind site specific differences.

An increasing body of evidence indicates that changes in the amount, frequency and seasonality of precipitation have important consequences for ecosystem structure and functioning (Knapp et al. 2002; Huxman et al. 2004; Heisler-White et al. 2008), and that these effects are more evident in arid and semi-arid ecosystems (Gerten et al. 2008). Increased precipitation can directly enhance soil water availability (Zavaleta et al. 2003; Harpole et al. 2007), one of the limiting factors of ecosystem processes in arid and semi-arid areas (Hooper and Johnson 1999; Bai et al. 2008). Increased precipitation shows notably positive effects on net primary production in the temperate steppe (Bai et al. 2008). Soil water availability can influence soil N availability through several microbial-mediated pathways, such as litter decomposition (Liu et al. 2006) and N mineralization (Wang et al. 2006). In addition, water availability influences leaf phenology (Penuelas et al. 2009) and rate of photosynthesis (Patrick et al. 2007), which in turn influence plant growth and primary productivity. Thus, it is reasonable to expect a change in plant nutritional status corresponding to variation in precipitation regimes. However, research on the influence of altered precipitation regimes on

plant leaf nutritional status is rare. In an annual grassland in California, Henry et al. (2006) found that increased precipitation did not affect the N:P ratio of aboveground tissue, but it changed C and N concentrations in freshly senesced litter of graminoids (Henry et al. 2005).

To examine potential impacts of increased soil N and water availability and their interaction on the stoichiometry of green and senesced leaves, we conducted a field experiment with N and water addition in a semi-arid temperate steppe in northern China that is predicted to be highly vulnerable to climate change (Christensen et al. 2004). We applied N and water, alone and in combination, and sampled five dominant plant species. We hypothesized that (1) plants exposed to enriched N would have lower foliar C:N ratios, higher N:P ratios in green leaves (N:Pg) and senesced leaves (N:Ps) and higher C:P ratios in senesced leaves (C:Ps), due to an expected increase in P resorption in response to N addition, and (2) water addition would lead to lower C:N ratios and higher N:P ratios considering the positive effects of soil water availability on soil inorganic N availability (Wang et al. 2006) and higher C:P ratios in both green (C:Pg) and senesced leaves (C:Ps), due to dilution of P in response to higher growth rates from increased available water (Bai et al. 2008). Based on these two hypotheses, effects of interactions between N and water addition on leaf stoichiometric ratios were expected to be limited.

## Materials and methods

### Study site

This study was conducted in a fenced grassland (exclusion of large mammal grazers since 1999) located near the Inner Mongolia Grassland Ecosystem Research Station (IMGERS, 43°38'N, 116°42'E, 1,250 m a.s.l.) in northern China. Long-term (1980–2006) mean annual precipitation is 345 mm with 70% occurring in June, July and August. Mean annual temperature is 1.1°C with monthly mean temperature ranging from −20.8°C in January to 16.4°C in July. The soil is Calcic-orthic Aridisol in the US soil taxonomy classification system. Mean bulk density is 1.29 g cm<sup>-3</sup>, and soil pH is 7.2. The dominant plant species at this site are *Stipa grandis* P. Smirn., *Agropyron cristatum* (L.) Gaertn., *Achnatherum sibiricum* (Linn.) Keng. and *Cleistogenes squarrosa* (Trin.) Keng, comprising more than 75% of the total aboveground biomass.

### Experimental design

We used a complete randomized block design with four treatments (Control, C; N addition, +N; water addition, +W; and both N and water addition, +NW) replicated five times,

resulting in 20 plots of 4 m × 4 m. Plots were separated by 1-m buffers. On 10 May 2005, we compared aboveground litter biomass, root biomass and soil total N and inorganic N in the plots and found no significant difference ( $P > 0.05$ ). We added N (urea in dry form) during the rainy days of mid-May and early July 2007 and 2008, totaling 17.5 g N m<sup>-2</sup> year<sup>-1</sup>. This amount was selected because it is in the range found to have a significant effect on primary productivity in this area (Bai et al. 2008). For water addition treatments, 10 mm of tap water was manually applied with a sprayer every 7 days throughout the growing season (May–September) in 2007 and 2008. The aim of the water-addition treatment was to increase long-term mean annual precipitation by approximately 50%. Water was applied after 16:00 to prevent rapid loss by evaporation. In total, water was added 18 times each year, amounting to 180 mm year<sup>-1</sup>. The ambient annual precipitation was 240 mm (192 mm for May–September, 42 rain days) in 2007 and 362 mm (295 mm for May–September, 47 rain days) in 2008.

#### Field sampling and measurement

Aboveground biomass was sampled in each plot using a 1 m × 1 m quadrat from 12 to 14 August 2008 by clipping all plots at the soil surface. All living vascular plants were sorted into species and then oven-dried at 65°C for 48 h and weighed. The dry mass for all living plants in each quadrat averaged over the five replicates for each treatment was used to estimate the aboveground community biomass. Plant aboveground net primary production (ANPP) was determined as the peak aboveground biomass during the growing season (Bai et al. 2008).

Soil samples were taken from the surface to a depth of 15 cm on 15 August 2008. For each plot, five soil cores were collected using a 3-cm-diameter soil auger and mixed into one composite sample. Gravimetric water moisture was determined by drying at 105°C for 48 h. Soil samples (10 g) were extracted with 50 ml of 2 M KCl. The filtered soil extraction was used to determine ammonium and nitrate concentration with a continuous flow spectrophotometer (FIAstar 5000; Foss Tecator, Denmark). Available soil P was measured using the Olsen method (Olsen et al. 1954).

We collected leaf samples from five dominant plant species: three C3 grasses (*A. cristatum*, *S. grandis* and *A. sibiricum*), one C4 grass (*C. squarrosa*) and a sedge (*Carex korshinskyi*). Fresh foliar samples for all five species were collected on 16 August 2008. Except for *C. squarrosa*, senesced leaves of all species were collected on 20 October 2008. Because senesced leaves of *C. squarrosa* were easily blown away by strong wind after senescence, these leaves were collected on 1 October 2008 when leaves had turned brown. All senesced leaves were directly collected from

plants rather than from leaf litter. Leaves with obvious evidence of mechanical damage or biotic alteration (e.g. herbivory, bird droppings, disease) were not sampled. Similar leaves in terms of size, shape, and color for each species were sampled. Samples were dried at 65°C for 48 h, then ground with a ball mill (Retsch MM 400; Retsch, Haan, Germany) and analyzed for C, N and P content. Carbon and N concentrations were analyzed with a PE-2400 CHN analyzer (Perkin-Elmer, Foster City, USA). Phosphorus concentration was determined by persulfate oxidation followed by colorimetric analysis (Schade et al. 2003). Mass ratios of C:N, C:P and N:P are used here to facilitate comparisons with previous studies (Güsewell 2004; He et al. 2008).

#### Statistical analysis

Levene's test was used to test for normality of all data before statistical analysis. Two-way ANOVAs were used to test the effects of N and water addition on soil water moisture, plant available N and P in soils, and peak aboveground biomass. A three-way ANOVA was used to test the effects of species identity, N addition, water addition and their possible interactions for each stoichiometric ratio. Significant differences among treatment means were analyzed using Tukey's multiple comparison post hoc test. When analyses revealed species-treatment interactions for certain variables, two-way ANOVAs were conducted for each species. All statistical analyses were conducted with SPSS version 13.0 (SPSS, Chicago, IL, USA).

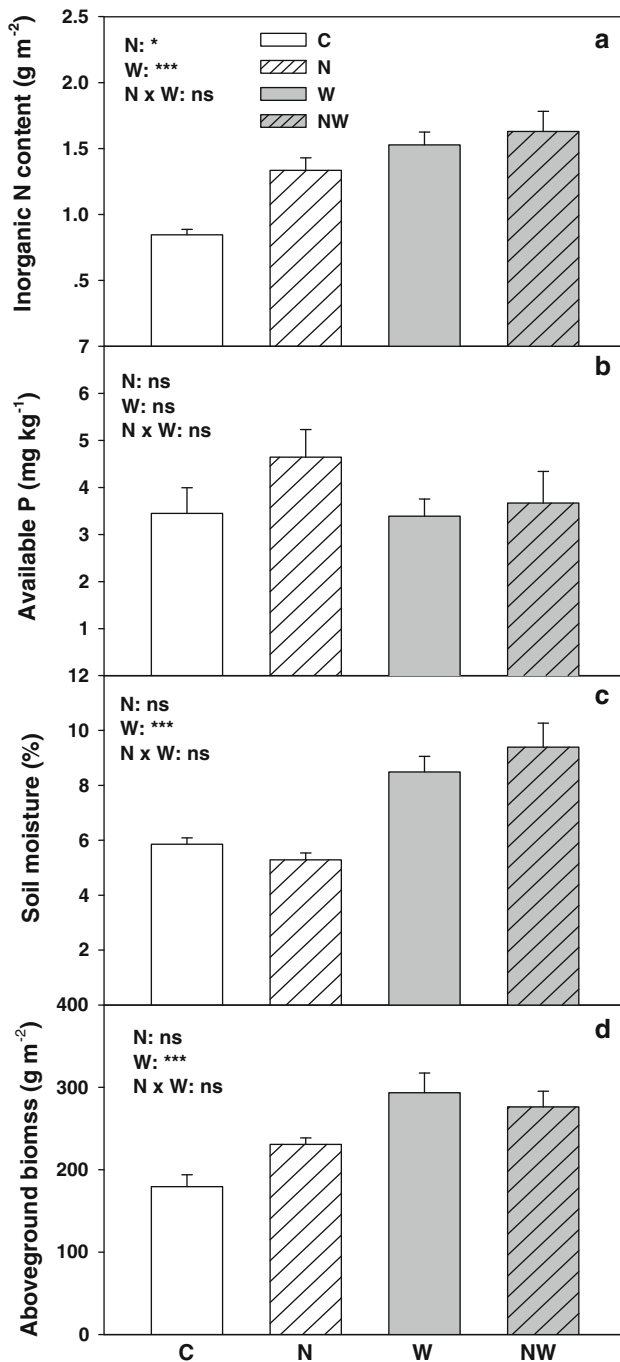
## Results

#### Responses of key resources and ANPP

Amounts of soil inorganic N were significantly enhanced by both N ( $P < 0.05$ ) and water addition ( $P < 0.001$ ; Fig. 1a). However, neither N addition nor water addition had a significant influence on plant-available P concentrations in soil (Fig. 1b). There was no significant interaction between N and water addition in their effects on plant-available P concentrations in soil. Water addition significantly enhanced soil moisture and ANPP in this community (all  $P < 0.001$ ), whereas N addition showed no significant effect (Fig. 1c, d). The combination of N and water addition did not lead to a higher ANPP than did the +N or +W treatments alone.

#### Responses of leaf nutrient concentrations

Mature and senesced leaf N (Ng and Ns) and P (Pg and Ps) concentrations varied significantly among species (Table 1; Fig. 2). N addition on average led to a greater increase in



**Fig. 1** Responses of soil inorganic N content (a), available P concentration (b), soil moisture (c), and aboveground biomass (d) to N and water addition. Error bars +1SE

Ng and Ns than did the addition of water (Fig. 2a, b). However, in the combination treatment, the effects of N and water addition were not additive, which is reflected by a significant interaction (Table 1). Water addition significantly decreased Pg and Ps (Table 1; Fig. 2c, d). Nitrogen and water addition significantly interacted in their effects on

Pg and Ps, in that water addition generally reduced P concentrations in ambient N conditions only (Fig. 2c, d).

#### Stoichiometric responses

Stoichiometric ratios differed among the five species (Table 1; Fig. 3). In the controls, the highest C:Ng and C:Pg were observed in *S. grandis* and *C. korshinskyi*, and the lowest values occurred in *A. cristatum*. Patterns of stoichiometric ratios differed among species and between green and senesced leaves (Fig. 3). Species with the highest stoichiometric ratios in green leaves did not necessarily have the highest ratio in senesced leaves.

Across all species, there was a significant interaction between N and water addition in their effects on C:N ratios in green and senesced leaves (C:Ng, C:Ns) (Table 1). Within the same water treatment level, N addition significantly decreased C:Ng and C:Ns under ambient water conditions only (Fig. 3a, b). In most cases, water addition under the ambient N treatment significantly reduced C:Ns and C:Ng, but water addition under the N-enriched treatment had no effect (Fig. 3a, b). Species responded differently to water addition as shown by significant species identity  $\times$  water addition interactions for both C:Ng and C:Ns. Compared with the control, water addition alone (+W treatment) significantly reduced C:Ng in only three species (*S. grandis*, *A. sibiricum*, and *C. squarrosa*) (Fig. 3a). Water addition significantly decreased C:Ns in four species but not in *C. korshinskyi* (Table S1; Fig. 3b).

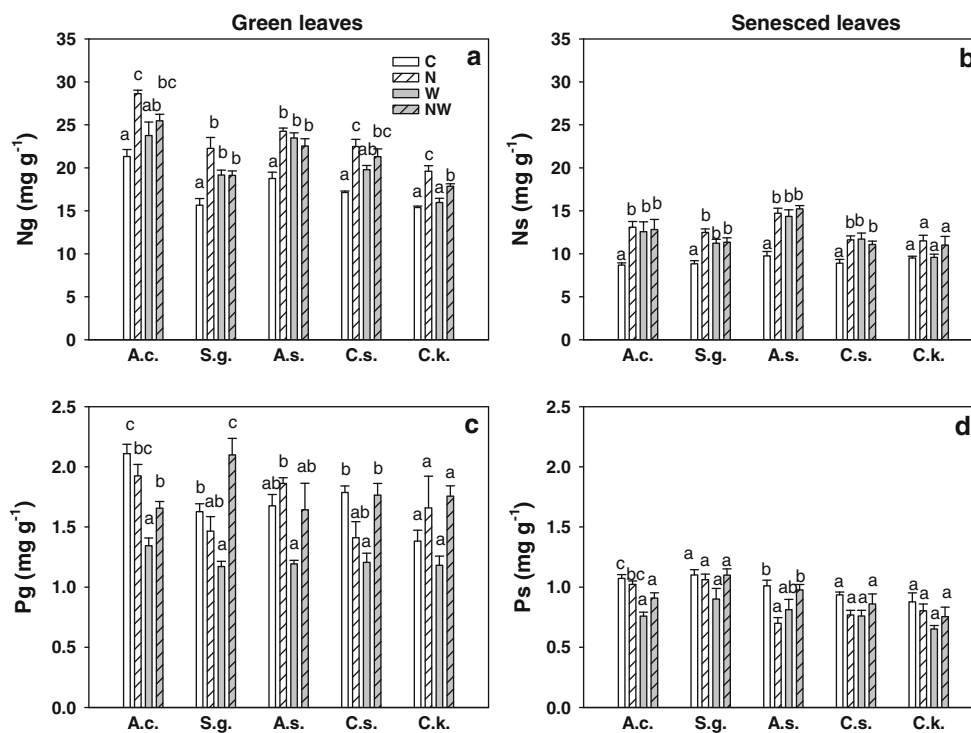
There was significant interaction between N and water addition in their effects on C:Pg (Table 1). Nitrogen addition reduced C:Pg only under increased water supply. Similarly, water addition led to a higher C:Pg only under ambient N conditions (Fig. 3c). In most species, N addition showed no significant effect on C:Ps (Fig. 3d). Water addition had a significant effect on C:Ps, while the combination of N and water addition had no effect, which was reflected by a significant N  $\times$  W interaction (Table 1; Fig. 3d). There was a significant interaction between species identity and water addition that affected C:Ps; all species responded to water addition in the same way, but the response was only statistically significant for *A. cristatum* and *C. korshinskyi* (Table S1).

When comparing the effect of +N, +W and +NW on N:Pg, the addition of water on top of N addition tended to cancel the positive effect of +N on N:Pg (Fig. 3e, f). Species responded differently to water addition as indicated by the significant species identity  $\times$  water interaction (Table 1). The N:Pg in four out of the five species was significantly higher in the +W treatment than in the control (Fig. 3e). There was a significant interaction between N and water addition in their effects on N:Ps (Table 1).

**Table 1** Results (*F* values) of three-way ANOVAs on the effects of species identity (*S*), nitrogen addition (*N*), water addition (*W*) and their interactions on N concentration (*[N]*), P concentration (*[P]*) and C:N, C:P and N:P ratios in green and senesced leaves

Source	Green leaf					Senesced leaf				
	[N]	[P]	C:N	C:P	N:P	[N]	[P]	C:N	C:P	N:P
S	63.1***	3.2*	50.2***	2.9*	4.0**	15.3***	14.4***	10.0***	11.6***	11.7***
N	100.5***	26.4***	128.5***	34.3***	0.1	49.0***	0.1	70.7***	0.6	14.7***
W	0.8	14.3***	2.5	18.8***	17.1***	17.4***	12.6***	32.0***	8.3**	24.1***
S × N	1.2	2.27	1.2	2.1	1.5	1.2	1.3	0.4	1.4	1.7
S × W	1.3	4.7**	3.0*	2.5	4.2**	2.8*	3.1*	2.9*	2.8*	1.8
N × W	56.6***	38.3***	91.4***	32.8***	78.3***	30.9***	30.9***	37.8***	34.6***	63.8***
S × N × W	1.6	2.8*	1.4	2.4	2.1	1.4	1.2	2.4	1.1	4.0**

\* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001



**Fig. 2** Responses of N (a, b) and P (c, d) concentrations in green (Ng, Pg) and senesced leaves (Ns, Ps) of five plant species to N and water addition. Error bars +1SE. Bars within species with dissimilar letters

denote significant difference (*P* < 0.05). *A.c.* *Agropyron cristatum*, *S.g.* *Stipa grandis*, *A.s.* *Achnatherum sibiricum*, *C.s.* *Cleistogenes squarrosa*, *C.k.* *Carex korshinskyi*

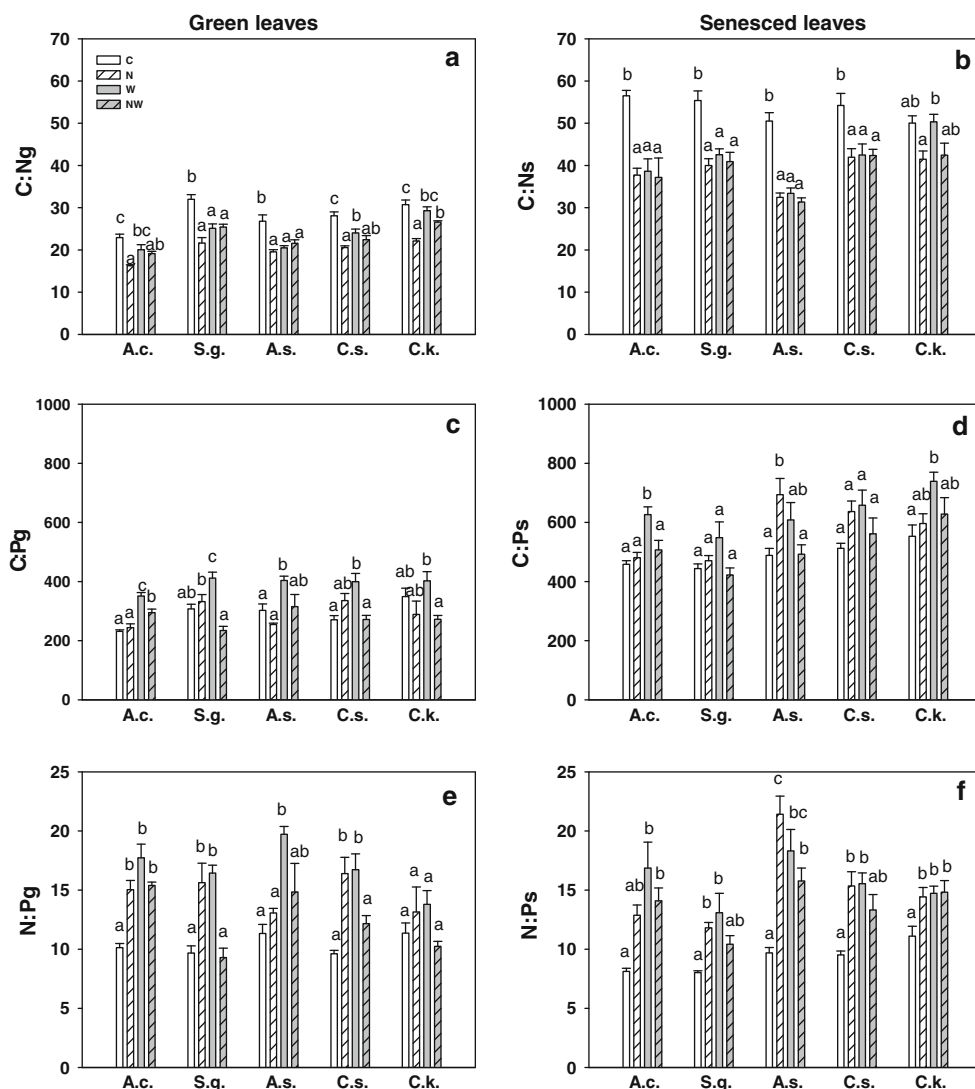
For each individual species, N addition increased N:P<sub>s</sub> under ambient water supply (except *A. cristatum*), but showed slightly negative and neutral effects when comparing the +W and the +NW treatments (Fig. 3f). Four out of five species responded to N addition with an increased N:P<sub>s</sub> under the ambient water level. Four species had higher N:P<sub>s</sub> (though not statistically significant) in the +W treatment than in the +NW treatment, while the sedge *C. korshinskyi* had equal N:P<sub>s</sub> in those two treatments (Fig. 3f).

### Discussion

#### Resource limitation of plant species and ANPP

Species examined in the present study showed significant differences in nutritional status and stoichiometric ratios of both green and senesced leaves. Differences in green leaf stoichiometric ratios among species are consistent with previous studies of grasslands in China (He et al. 2006, 2008). The nutrient quality (Hobbie 2005; Manzoni et al. 2008)

**Fig. 3** Responses of green and senesced leaf C:N ratios (**a, b**), C:P ratios (**c, d**) and N:P ratios (**e, f**) in five plant species to N and water addition. Error bars +1SE. Bars within species with dissimilar letters denote significant difference ( $P < 0.05$ ). A.c. *Agropyron cristatum*, S.g. *Stipa grandis*, A.s. *Achnatherum sibiricum*, C.s. *Cleistogenes squarrosa*, C.k. *Carex korshinskyi*



and stoichiometric ratio of litter (Güsewell and Gessner 2009) play an important role during litter decomposition and ecosystem nutrient cycling. Thus, differences in senesced leaf nutrient concentrations and stoichiometric ratios among species in this study imply that potential changes in plant community composition and structure in response to increased N availability (Clark and Tilman 2008; Bai et al. 2010), altered precipitation regimes (Zavaleta et al. 2003; Knapp et al. 2006; Harpole et al. 2007) and their interaction (Dukes et al. 2005; Henry et al. 2006) will have strong effects on plant-mediated nutrient dynamics in semi-arid grasslands.

Water addition significantly enhanced ANPP of this grassland ecosystem, indicating that this ecosystem was water-limited. In the control plots, N:Pg of all five species was below 14, and N:Pg in three out of the five species was around 10, indicating N-limitation for plant growth in this semi-arid grassland (at least for the five dominant species examined in the present study) (Koerselman and Meuleman

1996; Güsewell 2004). However, we did not find a statistically significant effect of N addition on ANPP, which may be due to the short duration of the present study. Although N addition can significantly stimulate plant growth and primary production in this area (Bai et al. 2008, 2010), it has been shown to take more than 3 years to see the positive effect of increased N availability (Bai et al. 2010). It is well known that fertilization experiments yield ambiguous results with respect to nutrient limitation due to various experimental methods used (Güsewell 2004). These results also suggest that N:P ratios may not always be good predictors for the types of nutrient limitations, especially under water-limited conditions. In plots receiving additional water, N:Pg was higher than 16 in four out of the five species examined in the present study, suggesting that plant growth would be P-limited rather than N-limited after alleviating water limitation. This was partly supported by the result that N addition did not enhance ANPP even when water-limitation was alleviated (+W vs. +NW; Fig. 1d).

It seems that water availability plays an important role in determining the status of nutrient limitations of dominant grasses in this semi-arid grassland.

#### Effects of N addition on stoichiometric ratios

In the present study, the amount of N added was much higher than estimates for future N deposition for this region ( $2\text{--}5\text{ g N m}^{-2}\text{ year}^{-1}$ ) (Galloway et al. 2004). This level of N addition ( $17.5\text{ g N m}^{-2}\text{ year}^{-1}$ ) was selected to increase productivity (Bai et al. 2008) rather than to simulate future N deposition for the region. Consistent with our hypothesis, N addition significantly increased  $N_g$  and  $N_s$  and decreased C: $N_g$  and C: $N_s$ . Both an increased  $N_g$  and a resultant decline in C: $N_g$  following N addition are well documented (Henry et al. 2005; Xia and Wan 2008). Increased  $N_g$  leads to decreased N resorption efficiency (Kobe et al. 2005). Thus, more N will remain in senesced leaves, resulting in lower C: $N_s$ . An important result in this study is that N addition led to a higher  $P_g$  and consequently a lower C: $P_g$  under elevated water conditions (+NW vs. +W). We suspect that the increased  $P_g$  resulted from enhanced P uptake. Enhanced root surface phosphatase activity following N addition has been found to increase plant P uptake, though this process may be species-specific (Fujita et al. 2010). Although N addition reduced C: $P_g$  under elevated water conditions (+W vs. +NW; Fig. 3c), it did not significantly affect C: $P_s$ . We attribute this to the elevated P resorption in the N-enriched environment, especially when water was not limiting (Lü and Han 2010).

Our hypothesis that N: $P_g$  and N: $P_s$  would be enhanced after N addition was partly supported by changes in N: $P_s$  but not by N: $P_g$ , though the positive effect of N addition on N: $P_s$  was only observed under ambient water conditions (Fig. 3f). We attribute the unchanged N: $P_g$  to the simultaneous increases of  $N_g$  and  $P_g$  following N addition in this study. Similarly, in a study conducted in the same area, Zhang et al. (2004) found that N:P ratios of two dominant species (*L. chinensis* and *C. korshinskyi*) remained constant after 2 years of N fertilization. In contrast, Menge and Field (2007) reported that the N:P ratio of grasses significantly increased after N addition ( $7\text{ g m}^{-2}\text{ year}^{-1}$ ) in an annual grassland in North America. These inconsistencies indicate a need to conduct a meta-analysis of existing data to assess the underlying mechanisms accounting for those site-specific differences. The positive effect of N addition under ambient water conditions on N: $P_s$  was due to higher  $N_s$  and unchanged  $P_s$  in response to N addition. We postulate that responses of nutrient resorption to N addition account for the changes in nutrient concentrations in senesced leaves. This is in agreement with results from a previous study (Lü and Han 2010), in which we found that N addition would lead to lower N resorption and higher P resorption in the

same community. Additionally, in terms of certain stoichiometric ratios, green and senesced leaves showed different response patterns to N addition (Fig. 3e, f), which agrees with another study that focused on the responses of green tissue and leaf litter to elevated  $\text{CO}_2$  (Billings et al. 2003). Results from this study and from Billings et al. (2003) suggest that nutrient resorption plays an important role in mediating the responses of senesced leaves and consequently the potential responses of leaf litter to global change factors. Moreover, these results also illustrate the importance of examining both green and senesced leaves when the aim of a study is to determine how N availability may ultimately affect plant-mediated nutrient dynamics.

#### Effects of water addition on stoichiometric ratios

No effects of water addition on C: $N_g$  were observed across all five species. However, when these species were analyzed individually, we found different response patterns (Fig. 2a): C: $N_g$  decreased in *A. sibiricum*, marginally increased in *C. korshinskyi* and remained unchanged in *A. cristatum*, *S. grandis* and *C. squarrosa* (Table S1). These results indicate that *A. sibiricum* was much more sensitive to water addition in terms of leaf N concentration than were the other species. As *A. sibiricum* is the canopy species in this community, enhanced leaf N concentration in response to water addition may favor a higher rate of photosynthesis. Species-specific responses of leaf nutritional status to global change factors have also been reported in other studies (Billings et al. 2003; Aerts et al. 2009). In this study, water addition significantly reduced C: $N_s$ . We attribute this to lower N resorption following water addition (Lü and Han 2010). Given that litter decomposition usually depends on both litter quality and environmental conditions, our results indicate that increased precipitation would stimulate litter decomposition not only because it can improve soil physical conditions but also because it can improve litter quality.

In accordance with our hypothesis, C: $P_g$  and C: $P_s$  were lower in ambient water than in elevated water conditions. This can be explained by the unchanged plant available P in soils and the increased ANPP in response to water addition. Plant available P concentrations in the soil were not affected by either N addition or by water addition in this study. Phosphorus is derived from weathering rock, and the decomposition of P is a chemical process instead of a biological process (Aerts and Chapin 2000). Although N addition may stimulate the activity of root surface phosphatase, as reported by Fujita et al. (2010), and subsequently enhance leaf P concentration, our data suggest that the direct effect of water addition on phosphatase activity may be limited. Increased precipitation, especially January–July precipitation, can significantly stimulate plant growth and

primary productivity in semi-arid grasslands of northern China (Bai et al. 2004, 2008). Here, we found water addition significantly increased peak aboveground biomass by 60%. Thus, it is reasonable to expect a lower Pg and a higher C:Pg in response to water addition due to a growth dilution effect. A higher C:Ps derived from green leaves could be achieved with a constant P resorption rate or with increased P resorption in response to decreasing Pg, which is a general pattern suggested from a global-scale dataset (Kobe et al. 2005).

In the present study, water addition increased N:Pg and N:Ps due to unchanged (in green leaves) or enhanced (in senesced leaves) N concentrations and simultaneously reduced P concentrations in response to water supply. Positive responses of soil N mineralization (Wang et al. 2006) and inorganic N concentration to increased water availability in this system may contribute to this result (Fig. 1a). Foliar N:P ratios play important roles in influencing ungulate grazing (Augustine et al. 2003) and insect herbivory (Elser et al. 2000). Similarly, litter N:P ratios determine the relative importance of decomposers during decomposition and ultimately nutrient dynamics in an ecosystem (Güsewell and Gessner 2009). Thus, our results suggest that altered precipitation regimes that are predicted for northern China (Ding et al. 2007) will have important implications for plant-mediated nutrient cycling in this semi-arid grassland. It is notable that results of this study were based on the simulation of increased precipitation by regular addition of water (=10 mm precipitation per week). As precipitation will be characterized by greater extremes and more erratic fluctuations in the future, it will be interesting to understand the stoichiometric responses of dominant plants to stochastic changes of additional precipitation.

#### Interactive effects of N and water addition

Our data demonstrate significant interactions between N and water addition to affect plant nutrient concentrations and all stoichiometric ratios in both green and senesced leaves. Under ambient water conditions, decreased C:Ng and C:Ns in response to N addition were in line with results from other studies (Esmeijer-Liu et al. 2009; Novotny et al. 2007). Under elevated water conditions, however, N addition showed no effect on C:Ng and C:Ns. This inconsistency may be explained by the fact that this system is water-limited, as indicated by the large increase of ANPP in response to water addition (Fig. 1d). As a result, the increased leaf N concentration resulting from N addition was diluted by increased plant growth in response to water addition. Similar to the findings of Novotny et al. (2007), N addition did not change C:Pg under ambient water conditions. Under elevated water conditions, however, N addition produced a lower C:Pg resulting from higher Pg. We

found higher C:Ps despite C:Pg being unaffected in response to N addition under ambient water levels. This can be attributed to increased P resorption in N-enriched environments (Lü and Han 2010). In contrast, when both N and water were added, higher N availability resulted in a lower C:Ps, indicating that more P remained in leaf litter, compared to water addition only. Nitrogen addition significantly increased both N:Pg and N:Ps in ambient water plots. Similar patterns have been observed in other ecosystems (Menge and Field 2007). When water availability was enhanced, higher foliar P concentration and unaltered N concentration in response to N addition resulted in a lower foliar N:P ratio. However, N:Ps was not affected by N addition under higher water availability, suggesting that simultaneously alleviating N and water limitation would have limited influence on N:Ps in this ecosystem. All these results suggest that water availability will modulate the effects of increasing N availability on plant nutritional status and stoichiometric ratios. Considering the increasing atmospheric N deposition associated with changing precipitation regimes, the interactive effects of changing N and water availability should be fully considered in the future to address their impacts on ecosystem nutrient cycling.

#### Conclusions

In this study, we investigated plant leaves rather than whole plants, but the nutritional status of leaves is closely related to that of other plant tissues (Kerkhoff et al. 2006). Similar to previous studies, we found decreased C:N ratios and increased N:P ratios with increasing N availability but only under ambient water conditions. The different response patterns of stoichiometric ratios to N addition between ambient and elevated water availability suggest that water availability plays an important role in mediating plant stoichiometry responses to increased N availability in this semi-arid grassland. Thus, our results provide further support for the assumption that grassland ecosystem responses to N addition will be strongly dependent on precipitation regimes (Weltzin et al. 2003; Harpole et al. 2007). Given the varied responses of green and senesced leaves to changes in N and water availability, we suggest that both green and senesced tissues should be included when investigating plant-mediated nutrient dynamics in ecosystems. Our results show that stoichiometric ratios vary greatly among different species. This implies that changes in community structure and composition in response to altered N availability and precipitation regimes that have been observed in various ecosystems would have as much of an effect as global change factors on plant stoichiometry and related nutrient dynamics. These findings will improve our understanding of responses of plant-mediated nutrient



cycling to simultaneous changes in precipitation and N availability in arid and semi-arid areas.

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