REGULAR ARTICLE

Nutrient resorption responses to water and nitrogen amendment in semi-arid grassland of Inner Mongolia, China

Xiao-Tao Lü · Xing-Guo Han

Abstract Plant growth and ecosystem processes in semi-arid and arid areas are typically limited by nutrient and water availability. Alleviation of these resource stresses by future increases in reactive nitrogen and precipitation may affect internal plant nutrient cycling. The effect of increased nitrogen and water supply on foliar N and P concentrations, resorption efficiency and proficiency in four dominant species was studied in semi-arid grassland in northern China. Four treatments (Control, N addition, water addition and N plus water additions) were replicated in five 4×4 m plots per treatment. We found significant interspecific differences in leaf nutrient and resorption variables. N addition and water addition increased N concentration but had no effect on P concentration in green leaves. Both N addition and water addition reduced plant N resorption proficiency. N addition increased and water addition had no effect on P resorption proficiency. N addition and water addition decreased N resorption efficiency while increasing P resorption efficiency, but responses to N and water addition differed among species. N addition significantly interacted with water addition to affect N resorption efficiency and proficiency but did not affect that of P. These results suggest that species identity, N availability and water availability interact to affect plant nutrient resorption processes. Our analyses provide a better understanding of plant internal nutrient retranslocation in response to potential changes in nitrogen and water availability.

Keywords N:P ratio · Nitrogen addition · Nutrient concentration · Soil water availability · Temperate steppe · Typical steppe

X.-T. Lü · X.-G. Han (✉)
State Key Laboratory of Vegetation and Environmental Change, Institute of Botany,
the Chinese Academy of Sciences,
Beijing 100093, China
e-mail: xghan@ibcas.ac.cn

X.-T. Lü
Graduate University of the Chinese Academy of Sciences,
Yuquan Road,
Beijing 100049, China

Introduction

Most of the Earth's ecosystems are experiencing increased input of nitrogen due to the more than doubled anthropogenic production of reactive nitrogen (Galloway et al. 2004). Moreover, as an indirect effect of climate warming, soil nitrogen availability will likely increase due to faster nitrogen mineralization (Rustad et al. 2001). Altered nitrogen availability has been shown to impact ecosystem structure and function, such as species composition, productivity and mineral biogeochemical cycling (Aerts et al. 2003; Clark and Tilman 2008; Stevens et al. 2004; Vitousek et al. 1997). Meanwhile, annual precipitation in northern Asia and East Asia,

especially summer precipitation, is predicted to increase by more than 10% this century (Ding et al. 2007). The amount and pattern of precipitation have important influences on community structure and ecosystem functioning (Harpole et al. 2007; Knapp et al. 2002; Suttle et al. 2007; Weltzin et al. 2003).

Semi-arid ecosystems such as temperate steppe of Inner Mongolia may be particularly vulnerable to changes in nutrient and water availability (Christensen et al. 2004). Several ecosystem processes in temperate steppe are limited by and sensitive to both nitrogen and water availability (Niu et al. 2008; Bai et al. 2008; Piao et al. 2003; Yuan et al. 2006; Zhang and Han 2008). In addition, the effects of nitrogen and water availability on ecosystem processes are interdependent (Bai et al. 2008; Harpole et al. 2007). Soil microbial activity is dependent on soil water availability in semiarid grassland (Liu et al. 2009). Increased soil water moisture usually results in a higher N mineralization rate and thus soil inorganic N availability (Wang et al. 2006), which is crucial for plant growth. Moreover, soil mineral N is dissolved in the soil solution, and presence of water affects soil mineral N mobility and loss. In turn, N addition can affect soil water availability by increasing plant transpiration, thereby more rapidly depleting soil water, which is related to higher photosynthetic rate. However, limited field experiments have examined ecosystem responses to these interacting global change factors (Dukes et al. 2005; Harpole et al. 2007; Henry et al. 2005; Zhang and Han 2008).

Understanding the changes in leaf nutrient concentration and resorption responses of grassland plants in a changing climate scenario is critical to our understanding of the functioning of grassland ecosystems. It is well known that leaf nutrient concentration plays an important role in photosynthesis, primary productivity, herbivory and several other carbon and nutrient cycling processes (Aerts 1997; Chen et al. 2005; Schade et al. 2003). Nutrient resorption from senescing organs has important consequences for perennial plants, especially those that live in nutrient limited environments. Through retranslocation, such nutrients can be stored or directly used by plants, thus making plants less dependent on current environmental nutrient supply (Aerts and Chapin 2000). In addition, nutrient resorption has important consequences for nutrient cycling in ecosystems (Aerts 1997).

Nutrient resorption can be quantified by two indices, namely nutrient resorption efficiency (defined as the proportion of the mature leaf nutrient pool that is resorbed) and nutrient resorption proficiency (defined as the terminal nutrient concentration in senesced leaves) (Aerts 1996; Killingbeck 1996). Nutrient resorption responses to changing nutrient availability have been extensively studied. Nutrient resorption efficiency is less responsive to altered soil fertility than nutrient resorption proficiency (Eckstein et al. 1999; Killingbeck 1996; Kozovits et al. 2007; van Heerwaarden et al. 2003), and recent studies indicate that nitrogen resorption may remain unchanged or decrease with increasing supply of fertilizer in some nutrient limited ecosystems (Güsewell 2005; Kozovits et al. 2007; Soudzilovskaia et al. 2007; van Heerwaarden et al. 2003). Moreover, most studies have not demonstrated a clear relationship between P resorption and fertilizer addition (Kozovits et al. 2007; van Heerwaarden et al. 2003). Few studies have investigated nutrient resorption responses to increased water availability (Soudzilovskaia et al. 2007), and studies on the effects of simultaneous and interacting fertility and water changes on nutrient resorption processes are almost completely lacking.

In arid and semi-arid grassland ecosystems, where plant growth is usually co-limited by nutrient and water availability, plant internal nutrient resorption processes may have particular importance for the fitness of plant populations (Aerts and Chapin 2000; Aerts et al. 2007; Yuan et al. 2005a; Yuan et al. 2005b). To test the hypothesis that potential increases of N and water availability will result in significant changes in plant nutrient concentration and nutrient resorption, we conducted a fertilization and precipitation addition experiment in natural grassland of Inner Mongolia. We investigated the effects of N addition and water addition on 1) nutrient concentrations and 2) nutrient resorption in both green and senesced leaves of three graminoid species, *Agropyron cristatum*, *Achnatherum sibiricum* and *Stipa grandis*, and one forb species, *Potentilla bifurca*.

Materials and methods

Study site

The study was conducted at a long-term experiment site (43°33′ N, 116°40′ E, 1268 m asl) fenced since 1999, which is managed by the Inner Mongolia Grassland Ecosystem Research Station (IMGERS,

43°38′ N, 116°42′ E) of the Chinese Ecological Research Network (CERN). The site represents typical steppe which is widely distributed in Inner Mongolia and dominated by *Leymus chinensis* and *Stipa grandis*. At this site, average annual precipitation is 350 mm, with 80% occurring from June to September. Annual precipitation in 2007 was 240 mm, or 70% of the average. Mean annual temperature is 1.1°C, and mean growing season length is about 150 days (Wang et al. 2006). Soil is characterized as chestnut (Chinese classification), which corresponds to Calcis-orthic Aridisol in the US Soil Taxonomy, with 48.6% sand, 26.1% silt and 25.3% clay (top 10 cm). Mean soil bulk density is 1.29 g cm^{-3}, and pH is approximately 6.78.

Experimental design

In May 2007, 20 plots measuring 4×4 m were established with four treatments (Control, C; N addition, + N; water addition, + W; and both N and water addition, + NW) replicated five times separated by 1-m buffers. Treatments were randomly assigned to plots within each block. We added N in May and July 2007, as urea, in dry form in two applications totaling 17.5 g N m^{-2}. The amount of fertilizer was based on recommendations for alleviating nitrogen limitation in typical steppe of this region (Bai et al. 2008; Pan et al. 2005). For water addition treatments, 10 mm of tap water was manually applied with a sprayer each week from 27 May to 23 September 2007. Water was always applied after 16:00 h to prevent rapid loss by evaporation. In total, water was added 18 times during this experiment, amounting to 180 mm. This resulted in a doubling of precipitation compared with plots that received no additional water (192 mm of ambient rainfall from May to September 2007).

Sampling and chemical analysis

Leaf collection was conducted in a 2×2 m subplot located in the center of each plot. In each subplot, we chose and numbered 60 shoots for each of the graminoid species and 15 individuals of the forb. We selected two fully expanded leaves of almost the same size from each graminoid individual and six leaves from each individual forb. One of the numbered mature graminoid leaves and three leaves of each forb were sampled in all plots during the first

week of August 2007 (the peak of the growing season). In all, there were 60 leaves for each graminoid and 45 leaves for the forb species in each plot. The other numbered graminoid and forb leaves were marked with ink or with a red thread. These leaves were monitored weekly starting at the beginning of September, and the recently senesced forb leaves, which were dry and yellow, were collected. In the second week of October, all senesced but still attached leaves were collected from each of the marked individuals. The leaves were transported to the laboratory, oven dried at 70°C for 48 h, and then weighted. Leaves of each species were combined per plot and ground to pass through a 40-mesh sieve using a mechanical mill. Total N concentrations were determined colorimetrically by the Kjeldahl acid-digestion method with an Alpkem auto-analyzer (Kjektec System 1026 distilling unit, Sweden) after extraction with sulfuric acid. Total P concentration was measured by persulfate oxidation followed by colorimetric analysis (Schade et al. 2003). N and P concentrations were expressed on a mass basis.

The top 10 cm of soil from each plot were sampled with a soil corer (5 cm in diameter) and analyzed for gravimetric water moisture and inorganic N (NH_4^+–N and NO_3^-–N) every month from June to October 2007. Gravimetric water moisture was determined by drying at 105°C for 24 h. To analyze inorganic N, 50 ml of 2 M KCl solution was added to a 10 g soil sample. The mixture of soil and extractant was shaken for 1 h on a reciprocal shaker, and then the soil suspension was filtered (Whatman No. 1 filter paper). Soil solutions were kept frozen prior to analysis for NH_4^+–N and NO_3^-–N on a FIAstar 5000 Analyzer (Foss Tecator, Denmark).

Calculation and statistical analysis

For each species in each plot, the nutrient pool of leaves (60 graminoid and 45 forb leaves) was calculated from the total leaf mass of each species and the leaf nutrient concentration. Nutrient resorption efficiency (RE), defined as the proportion of mature leaf nutrient pool that is resorbed, was calculated as:

$$RE = \left(1 - Nutrient_{senesced}/Nutrient_{green}\right) \times 100\%$$

in which $Nutrient_{senesced}$ and $Nutrient_{green}$ is the N or P pool of the senescent and the green leaves, respectively.

Nutrient resorption proficiency is defined as the level to which nutrient concentration is reduced during the resorption process. Higher resorption proficiency means to lower nutrient concentration in senesced leaves (Killingbeck 1996).

Data were tested for normality using the Kolmogorov–Smirnov test, and when non-normal, data were log-transformed. Repeated measures ANOVA was used to examine N addition and water addition effects on soil moisture and inorganic N concentration over the growing season in 2007. A three–way ANOVA with species, nitrogen and water addition as the main factors was performed to examine all possible interactions. Because of significant species × N–addition interaction and species × water–addition interaction affecting nutrient concentrations and resorption efficiencies, we further analyzed the effects of N addition and water addition for each species individually. The effects of treatments on nutrient concentration and nutrient resorption were analyzed by one-way ANOVAs for each species followed by a Tukey's post-hoc test. All analyses were conducted with SPSS version 11.5.

Results

Soil moisture and nutrient availability

Nitrogen addition significantly decreased soil moisture at a depth of 0–10 cm by 6.4% averaged across the entire experimental period ($P=0.043$, Table 1; Fig. 1a). Water addition significantly increased soil moisture ($P<0.001$), and there was no interaction

between N and water addition affecting soil moisture ($P=0.19$). Water addition significantly interacted with sampling date to affect soil moisture ($P<0.001$). Soil moisture did not differ among treatments in June (Fig. 1a). Nitrogen addition significantly increased soil NH_4^+-N ($P<0.001$, Fig. 1b), NO_3^--N ($P<0.001$, Fig. 1c) and inorganic nitrogen concentration ($P<0.001$, Fig. 1d). Sampling date significantly interacted with N addition to impact mineral nitrogen concentrations (Table 1).Water addition significantly increased NO_3^--N concentration ($P=0.049$, Table 1), while not affecting NH_4^+-N ($P=0.304$) and inorganic nitrogen concentration ($P=0.212$).

Interspecific differences

There were highly significant differences in leaf nutrient variables and resorption parameters among the four species (Table 2). In the controls, N and P concentrations in both green and senesced leaves were higher in the forb *P. bifurca* than the three graminoids (Fig. 2a–d). Nitrogen resorption efficiency in the controls varied from 55% to 70%, with the lowest efficiency in the forb *P. bifurca* ($56.97\pm0.66\%$) and the highest in *A. sibiricum* ($67.26\pm1.08\%$) (Fig. 2e). Differences in phosphorus resorption efficiency among species were significant but small (Fig. 2f).

Nutrient concentration in green leaves and nutrient resorption proficiency

Nitrogen addition ($P<0.001$, Table 2) and water addition ($P<0.001$) significantly increased green leaf N concentrations (Fig. 2a). Species significantly interacted with N addition to affect green leaf N concentration ($P<0.05$, Table 2). When we further analyzed the effects of nitrogen addition for each species individually, however, N addition significantly increased green leaf N concentration for all four species (Table 3). There was a significant interaction between nitrogen addition and water addition affecting green leaf N concentration ($P<0.05$, Table 2). Neither N addition nor water addition, or their interaction, affected P concentrations in green leaves (Table 2). When analyzed for each species individually, N addition, water addition and their interaction had significant effects on green leaf P concentration in *A. sibiricum* (Table 3). N addition had a significant positive effect on green leaf P concentration in

Table 1 Results (*P*-values) of repeated measures ANOVA on the effects of nitrogen addition (*N*), water addition (*W*), sampling date (*D*) and their interactions on soil moisture (*SM*), ammonium (*Amm*), nitrate (*Nit*) and inorganic nitrogen (*In-N*) concentrations

	SM	Amm	Nit	In-N
N	0.043	<0.001	<0.001	<0.001
W	<0.001	0.304	0.049	0.212
N × W	0.190	0.160	0.196	0.076
D	<0.001	0.012	0.180	0.002
D × N	0.885	0.004	0.012	0.020
D × W	<0.001	0.542	0.651	0.713
D × N × W	0.795	0.639	0.076	0.333

Fig. 1 Soil water content (**a**), ammonium (**b**), nitrate (**c**) and inorganic nitrogen (**d**) concentrations in typical steppe soils (0–10 cm) from June until October 2007. *Error bars* are SE (*n*=5). Different letters indicate significant differences (*P*≤0.05) among treatments within the same month
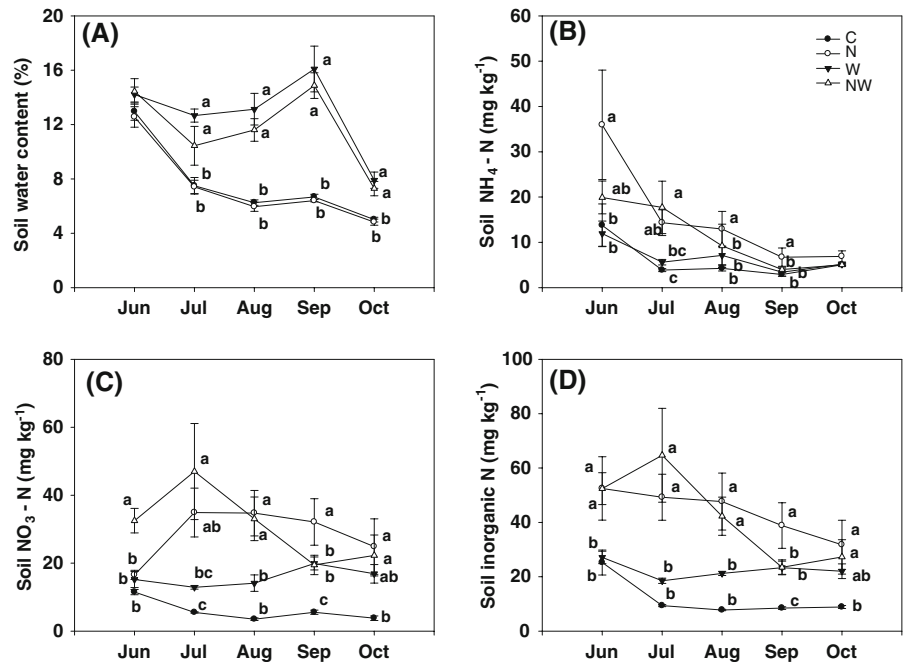
A. sibiricum under ambient water levels, but it did not affect P concentration in watered plots (Fig. 2b). In the controls, green leaf N/P ratios were lowest in the forb *P. bifurca* (20.14±1.18, mean ± SE) and the highest in *A. sibiricum* (33.57±2.15).

N concentrations in senesced leaves were significantly increased by N addition (*P*<0.001, Table 2) and water addition (*P*<0.001), i.e. N addition and water addition significantly decreased N resorption proficiency. The effect of N addition interacted with plant species identity (*P*<0.05, Table 2), as senesced leaf N concentrations increased in three out of four species when N was added (Table 3, Fig. 2c). N addition and water addition significantly interacted to affect senesced leaf N concentration in *A. cristatum* (*P*=0.009, Table 3), as N addition only significantly increased senesced leaf N concentration under ambient water condition (Fig. 2c).

N addition significantly reduced senesced leaf P concentration (*P*=0.008, Table 2), whereas neither water addition (*P*=0.959) nor its interaction with N addition (*P*=0.543) affected P concentration. Species identity affected the response of senesced leaf P concentration to water addition (*P*=0.041, Table 2; Fig. 2d). While the effect of water addition on senesced leaf P concentration in *A. sibiricum* (*P*=0.098, Table 3) and *P. bifurca* (*P*=0.098) were marginally significant, water addition had no effects on *A. cristatum* (*P*=0.548) and *S. grandis* (*P*=0.187).

Effect of N and water amendment on nutrient resorption efficiency

Both N addition (*P*<0.001, Table 2) and water addition (*P*<0.001, Table 2) produced a significant

Table 2 Results (*P*-values) of three-way ANOVAs on the effects of species, N addition (*N*), water addition (*W*) and their interactions on N and P concentrations in green leaves (*Ng* and *Pg*) and senesced leaves (*Ns* and *Ps*), N resorption efficiency (*NRE*) and P resorption efficiency (*PRE*)

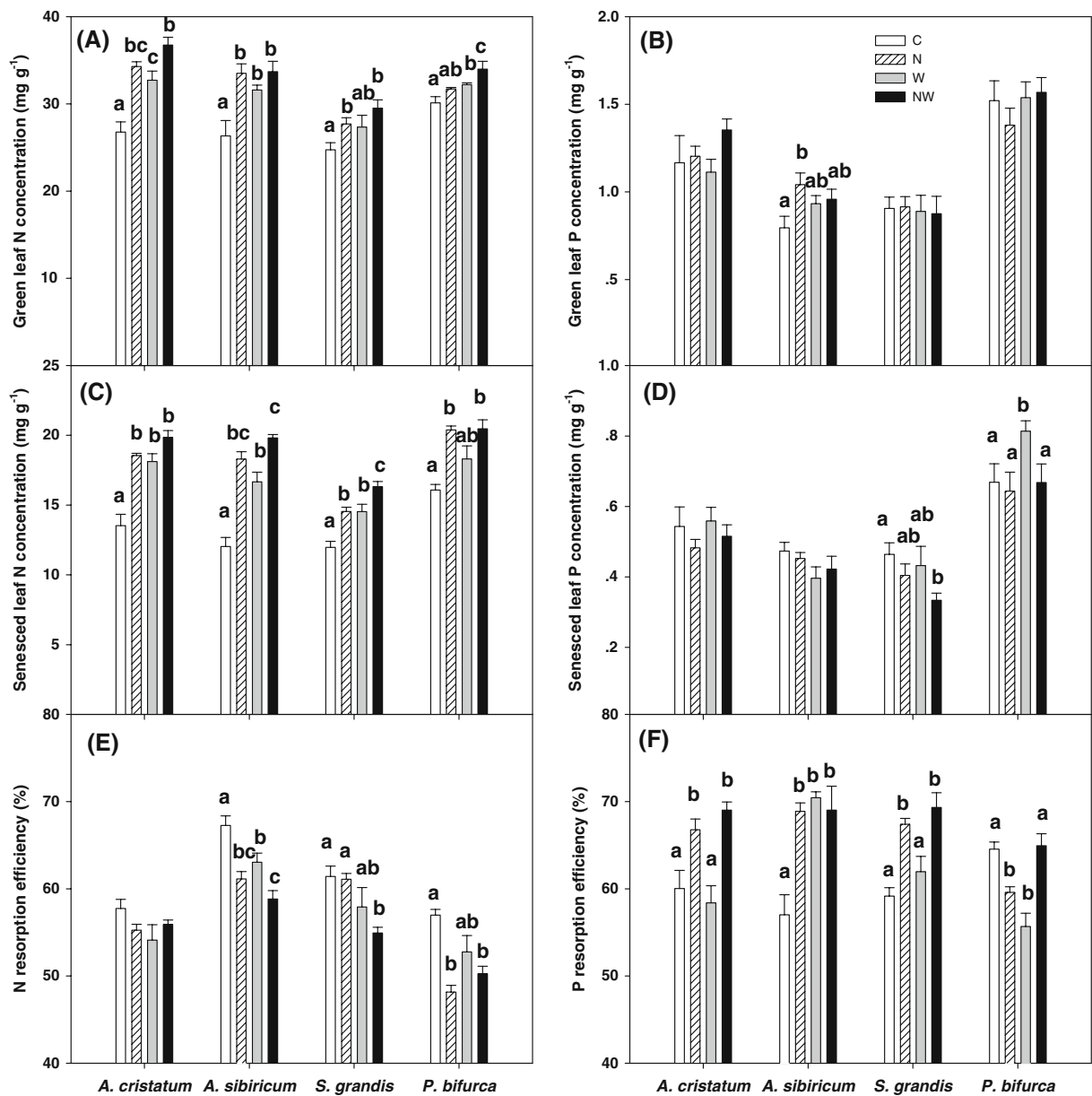|	Ng	Pg	Ns	Ps	NRE	PRE
Species	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
N	<0.001	0.328	<0.001	0.008	<0.001	<0.001
W	<0.001	0.243	<0.001	0.958	<0.001	0.014
Species × N	0.015	0.129	0.017	0.371	0.003	0.016
Species × W	0.409	0.500	0.058	0.041	0.093	0.002
N × W	0.020	0.499	<0.001	0.543	0.037	0.518
Species × N × W	0.207	0.165	0.344	0.454	0.049	<0.001

Fig. 2 Nitrogen (**a**, **c**) and phosphorus (**b**, **d**) concentrations in green and senesced leaves and nitrogen (**e**) and phosphorus resorption efficiency (**f**) of the four dominant species from the typical steppe of Inner Mongolia in the control (*C*), nitrogen addition (*N*), water addition (*W*) and both nitrogen and water addition (*NW*) plots. *Error bars* are SE (*n*=5). Different letters indicate significant differences (*P*≤0.05) among treatments for each species

decline in N resorption efficiency (Fig. 2e). Response of N resorption efficiency to N addition differed among species (significant species-nitrogen interaction; *P*< 0.01, Table 2). While N resorption efficiencies of *A. cristatum* (*P*=0.754, Table 3) and *S. grandis* (*P*= 0.238) showed no response to N addition, *A. sibiricum* (*P*<0.001) and *P. bifurca* (*P*<0.001) had lower efficiencies (Fig. 2e). Species identity appeared to

modulate the response of plant N resorption efficiency to N and water addition, as indicated by a significant three-way interaction (*P*<0.05; Table 2). While N addition led to lower resorption efficiency in both ambient and added water conditions for *A. sibiricum* and *P. bifurca*, *A. cristatum* and *S. grandis* showed no response to N addition under both water conditions (Fig. 1e).

Table 3 Results (*P* values) of two-way ANOVAs on the effects of nitrogen addition (*N*), water addition (*W*) and their interaction on nitrogen and phosphorus concentration in green leaves (*Ng* and *Pg*) and senesced leaves (*Ns* and *Ps*), nitrogen resorption efficiency (*NRE*) and phosphorus resorption efficiency (*PRE*) for each species

	Ng	Pg	Ns	Ps	NRE	PRE
A. cristatum						
N	<0.001	0.165	<0.001	0.204	0.754	<0.001
W	<0.001	0.620	<0.001	0.548	0.201	0.851
N × W	0.084	0.303	0.009	0.834	0.070	0.248
A. sibiricum						
N	0.002	<0.001	0.926	0.095	<0.001	0.014
W	0.040	<0.001	0.079	0.098	0.005	0.002
N × W	0.055	0.013	0.427	0.230	0.347	0.003
S. grandis						
N	0.019	0.976	<0.001	0.049	0.238	<0.001
W	0.040	0.730	<0.001	0.187	0.003	0.102
N × W	0.686	0.888	0.859	0.613	0.339	0.754
P. bifurca						
N	0.011	0.293	<0.001	0.095	<0.001	0.080
W	0.002	0.136	0.078	0.098	0.369	0.145
N × W	0.881	0.183	0.101	0.230	0.015	<0.001

P resorption efficiency was significantly reduced by N addition ($P < 0.001$, Table 2) and water addition ($P < 0.05$). There was a significant interaction between species identity and N addition, which was driven by a lack of change in P resorption efficiency for *P. bifurca* ($P = 0.08$, Table 3). The interaction between species and water addition was also significant as only *A. sibiricum* significantly increased its resorption efficiency when water was added ($P = 0.002$, Table 3), while there was no change in the other species (Table 3). The significant three-way interaction between species, N addition and water addition ($P < 0.001$, Table 2), indicates that plant species responded idiosyncratically to N and water addition. N addition resulted in increased P resorption efficiency in most cases, except in *A. sibiricum* under water addition and *P. bifurca* under ambient water (Fig. 2f).

Discussion

Nutrient concentration of green leaves

In this study we included four dominant plant species that comprised more than 80% of aboveground primary productivity in plots. These species showed highly significant differences in green and senesced leaf nutrient concentrations. Plant foliar N concentrations ranged from 24.73 mg g^{-1} in *S. grandis* to 30.12 mg g^{-1} in the forb *P. bifurca* in the control plots. These results were higher than results of other studies conducted in the same region (15 – 20 mg g^{-1} for graminoids, Chen et al. 2005, and an average of 18.8 mg g^{-1} for graminoids and 28.8 mg g^{-1} for forbs, Yuan et al. 2005b) but were similar to results of a large scale study of leaf N in grassland biomes of China (with an average of 26.8 mg g^{-1} for Inner Mongolia, with 24.0 mg g^{-1} for grasses and 30.6 mg g^{-1} for forbs, He et al. 2006). In contrast, the foliar P concentrations (0.79 – 1.52 mg g^{-1}) were lower than the mean value of 87 species from typical steppe of Inner Mongolia (1.8 mg g^{-1}) (He et al. 2008). Together, higher N concentration and lower P concentration led to high N:P ratios, ranging from 17 to 35. This was consistent with results from previous studies (Han et al. 2005; He et al. 2008) which showed high N:P ratios in China's flora.

N and P limitation of net primary production in terrestrial ecosystems occurs globally (Elser et al. 2007; LeBauer and Treseder 2008), and biomass N:P ratios would be appropriate indicators for nutrient limitation of plants (Güsewell et al. 2003). Productivity is limited by P when the N:P ratio is higher than 16, it is limited by N when the ratio is lower than 14, and it is co-limited by N and P when the ratio is between 14 and 16 (Koerselman and Meuleman 1996). The high plant N:P ratio in this study indicates that the growth of these plants may be limited by P, contrary to the commonly held view that this system is limited by N (Pan et al. 2005). In fact, in this experiment N addition did not significantly increase the peak season biomass, while water addition led to a >60% increase in biomass regardless of N addition (X.T. Lü, unpublished data). Several factors may contribute to this pattern. First, this study was conducted in an area that had been fenced from grazing for more than 8 years. The nutrient cycling of fenced sites generally differs from that of unfenced sites in that gross nitrogen mineralization rate and thus inorganic N availability are higher in the soil of fenced plots (Holst et al. 2007; Wang et al. 2006). Higher inorganic N availability in the soil has a positive effect on N concentration in plant green tissue (Richardson et al. 2005; Yuan et al. 2005a).

Also, N cycling is a biological process, while P cycling is a physical and chemical process that is less influenced by biotic and abiotic conditions (Aerts and Chapin 2000). At the same time, Han et al. (2005) proposed that low soil P availability at the national scale accounts for a low leaf P and high N:P ratio in China's flora. Finally, this study was conducted in a small area and focused on four dominant species. It is well known that the N:P ratios can vary greatly among different species (Han et al. 2005; He et al. 2008) and environments (Reich and Oleksyn 2004). Therefore, analyzing responses of more species or species in several environments may give clearer patterns.

Effects of N and water amendment on leaf nutrient status

In this study, both N addition and water addition significantly increased the N concentration of green leaves (Table 2). The positive response of foliar N concentration may have resulted from the increased inorganic nitrogen availability following N and water addition (Table 1). In spite of simultaneously allevi-ating N and soil moisture stress in the + NW treatment, foliar N concentrations were not signifi-cantly different from the + N and + W treatments for all species except the forb, P. bifurca. These results suggest that the effect of N and water addition on foliar N concentration may be non-additive.

All treatments had limited effects on green leaf P concentrations of the four species except for the forb P. bifurca under the + N treatment. This contradicts the findings of van Heerwaarden et al. (2003), who reported a generally decreasing P concentration in green leaves in six sub-arctic bog species after 4 years of N fertilization. The difference may be understand-able considering the fact that our results are based on the first-year effects of fertilization, whereas van Heerwaarden et al. (2003) examined a longer-term effect. Short-term responses are mainly driven by the nutrient conditions experienced by plants before fertilizer addition, while long-term patterns reflect the effect of changed competitive interaction after fertilization (Fynn and O'Connor 2005; Güsewell et al. 2003). Several previous studies have shown that short-term responses of the ecosystem to altered nutrient availability can be different from that of long-term responses (Chapin and Shaver 1985; Clark

and Tilman 2008; Inouye and Tilman 1995). In all, our findings suggest that, in the short-term, plants experience an increase in foliar N concentration and an unpredictable change in P concentration in the typical steppe with increasing N and water availability.

Leaf nutrient resorption efficiency and proficiency in typical steppe species

The species in this study showed high N and P resorption efficiency. All the species retranslocated more than 57% of N and P during leaf senescence (Fig 2e, f). The graminoid A. sibiricum withdrew the most amount of leaf N (67%), while the forb P. bifurca showed the highest value of P resorption efficiency (64%). Aerts (1996) reported a typical resorption efficiency value of 50% for N and 52% for P for deciduous and evergreen shrubs and trees. Given the fact that nutrient resorption efficiency of graminoids is usually lower than that of other plant life-forms in this region (Yuan et al. 2005b), our data suggest that plant nutrient resorption efficiency in typical steppe of Inner Mongolia is higher than the average values from many types of ecosystems throughout the world.

In the control plots, the average N concentration of senesced leaves ranged from 12 to 16 mg g^{-1}, and mean P concentration in the leaf litter varied between 0.46 and 0.67 mg g^{-1}. Killingbeck (1996) suggested limits of <0.7% and >1% for complete resorption of N and <0.05% and >0.08% for incomplete resorption of P in senesced leaves of deciduous species. Following these criteria, N resorption was incomplete for all four species in this study. In contrast, P resorption could be classified as intermediate or complete. In a P-limited savanna ecosystem of South Africa, Kozovits et al. (2007) found complete P resorption and intermediate or incomplete N resorption. The resorption patterns of N and P are consistent with the finding that N concentration was higher and P concentration was lower in the plants of this study. All results indicate that the plants in this fenced grassland ecosystem might be more limited by P than by N.

Effects of N and water amendment on nutrient resorption

In general, N addition and water addition reduced leaf N resorption efficiency (Table 2). Such a decrease is

most likely due to the increase of green leaf nitrogen concentration under these treatments. The relationship between nutrient resorption efficiency and green leaf nutrient status, however, is elusive. In an un-weighted meta-analysis focused on woody species, Aerts (1996) found no or a weak relationship between nutrient status and N and P resorption from mature leaves. In contrast, in a meta-analysis of 297 perennial species of different life-forms, Kobe et al. (2005) concluded that nutrient resorption efficiency decreased with increasing green leaf nutrient concentration. However, species responses to N addition in this study were idiosyncratic. N addition reduced N resorption efficiency in two out of four species but had no effects on the other two. Similarly, Huang et al. (2008) found reduced N resorption efficiency for two out of six species in response to three years of N addition at a nearby grassland. The fact that N addition and water addition interacted to affect N resorption efficiency indicates that the response of internal plant N resorption processes to changes of N availability may be mediated by soil water availability. This provides further evidence that community and ecosystem responses to increased N availability are mediated by changes in precipitation (Harpole et al. 2007; Weltzin et al. 2003).

P resorption efficiency was also affected by the treatments in this study. N addition significantly increased P resorption efficiency of the three graminoids but did not affect that of the forb species. This is partly in agreement with results of van Heerwaarden et al. (2003) who found that P resorption efficiency did not change in four, increased in one and decreased in one out of six species after four years of N fertilization. Regarding changes in P resorption efficiency, species responded idiosyncratically to water addition, with the only significant increase occurring in *A. sibiricum* (Table 3). It is interesting to note that water addition had no effects on the patterns of P resorption efficiency response to N addition in the + NW treatment (Fig. 2f) given the reduced P resorption efficiency when N and water were added separately (Table 2). These results indicate that N addition alone would lead to higher P resorption efficiency and that soil water availability would not alter this pattern.

N addition led to a decrease in N resorption proficiency for all species except *A. sibiricum*, which is consistent with previous results based on fertilization experiments (Huang et al. 2008; van Heerwaarden et al. 2003). This is caused by increased green leaf N concentration and reduced or unchanged N resorption efficiency as reported by Soudzilovskaia et al. (2007). It is noticeable that the effects of N addition and water addition on N resorption efficiency were species-specific as indicated by the significant species-N interaction and marginally significant species-water interaction. P resorption proficiency increased in *S. grandis*, while showing no response to N addition in other species (Table 3). Neither water addition nor its interaction with N addition affected P resorption efficiency (Table 2). These results could be explained by the fact that the species in this study had complete or intermediate resorption of P in the control plots. Perhaps because of plant physiological nutrient concentration needs, those species that already exhibited complete resorption in the control plots would be unable to extract additional P from senescing leaves (van Heerwaarden et al. 2003).

Increased N concentration in senesced leaves under all treatments would lead to higher quality litter. Litter decomposition is also stimulated after N addition for graminoids and forbs in grasslands of Inner Mongolia (Liu et al. 2006). Given the lower senesced leaf P concentration in this study, however, we suspect that a stronger P limitation on litter decomposition would occur as a result of potentially increased N and water availability. On the other hand, given the species-specific responses of senesced leaf N concentration to simultaneous changes in N and water supplies, changing community composition as a result of global climate change should be considered. These results also support the suggestion that changes in vegetation composition and trophic structure should be considered when estimating plant-mediated effects on nutrient cycling in response to both nutrient supply (Clark and Tilman 2008; Soudzilovskaia et al. 2007; van Heerwaarden et al. 2003) and precipitation (Suttle et al. 2007).

Conclusions

Our study has shown that both nitrogen addition and water addition can affect plant nutrient status and resorption processes. Furthermore, nitrogen availability and water availability interacted to affect N resorption efficiency and proficiency. Importantly,

the effects of N and water availability amendment on leaf nutrient and resorption parameters differed among species. This implies that changes in community structure and composition would have large influences on plant-mediated nutrient cycling pathways. Given the potential increase in nutrient and precipitation availability in the semi-arid grassland of northern China as a result of global climate change, this study provides critical insight into possible changes in plant internal nutrient retranslocation, litter quality and consequently the amount of nutrients returned to the ecosystem. These findings improve our understanding of nutrient resorption response to potential climate change in arid and semi-arid grassland ecosystems. Although the amount of nutrient and water addition used in this study represents an extreme case of global climate change, it is notable that the potential responses at the individual species and community levels are substantial.

References

Aerts R (1996) Nutrient resorption from senescing leaves of perennials: are there general patterns? J Ecol 84:597–608

Aerts R (1997) Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos 79:439–449

Aerts R, Chapin FS (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv Ecol Res 30:1–67

Aerts R, Cornelissen JHC, van Logtestijn RSP, Callaghan TV (2007) Climate change has only a minor impact on nutrient resorption parameters in a high-latitude peatland. Oecologia 151:132–139

Aerts R, de Caluwe H, Beltman B (2003) Is the relation between nutrient supply and biodiversity co-determined by the type of nutrient limitation? Oikos 101:489–498

Bai YF, Wu JG, Xing Q, Pan QM, Huang JH, Yang DL, Han XG (2008) Primary production and rain use efficiency across a precipitation gradient on the Mongolia plateau. Ecology 89:2140–2153

Chapin FS, Shaver GR (1985) Individualistic growth response of tundra plant species to environmental manipulations in the field. Ecology 66:564–576

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

Christensen L, Coughenour MB, Ellis JE, Chen ZZ (2004) Vulnerability of the Asian typical steppe to grazing and climate change. Clim Change 63:351–368

Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451:712–715

Ding YH, Ren GY, Zhao ZC, Xu Y, Luo Y, Li QP, Zhang J (2007) Detection, causes and projection of climate change over China: an overview of recent progress. Adv Atmos Sci 24:954–971

Dukes JS, Chiariello NR, Cleland EE, Moore LA, Shaw MR, Thayer S, Tobeck T, Mooney HA, Field CB (2005) Responses of grassland production to single and multiple global environmental changes. Plos Biology 3:1829–1837

Eckstein RL, Karlsson PS, Weih M (1999) Leaf life span and nutrient resorption as determinants of plant nutrient conservation in temperate-arctic regions. New Phytol 143:177–189

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–1142

Fynn RWS, O'Connor TG (2005) Determinants of community organization of a South African mesic grassland. J Veg Sci 16:93–102

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vorosmarty CJ (2004) Nitrogen cycles: past, present, and future. Biogeochemistry 70:153–226

Güsewell S (2005) Nutrient resorption of wetland graminoids is related to the type of nutrient limitation. Funct Ecol 19:344–354

Güsewell S, Koerselman W, Verhoeven JTA (2003) Biomass N : P ratios as indicators of nutrient limitation for plant populations in wetlands. Ecol Appl 13:372–384

Han WX, Fang JY, Guo DL, Zhang Y (2005) Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. New Phytol 168:377–385

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

He JS, Fang JY, Wang ZH, Guo DL, Flynn DFB, Geng Z (2006) Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grassland biomes of China. Oecologia 149:115–122

He JS, Wang L, Flynn DFB, Wang XP, Ma WH, Fang JY (2008) Leaf nitrogen : phosphorus stoichiometry across Chinese grassland biomes. Oecologia 155:301–310

Henry HAL, Cleland EE, Field CB, Vitousek PM (2005) Interactive effects of elevated CO_2, N deposition and climate change on plant litter quality in a California annual grassland. Oecologia 142:465–473

Holst J, Liu CY, Bruggemann N, Butterbach-Bahl K, Zheng XH, Wang YS, Han SH, Yao ZS, Yue J, Han XG (2007) Microbial N turnover and N-oxide ($N_2O/NO/NO_2$) fluxes in semi-arid grassland of Inner Mongolia. Ecosystems 10:623–634

Huang JY, Zhu XG, Yuan ZY, Song SH, Li X, Li LH (2008) Changes in nitrogen resorption traits of six temperate grassland species along a multi-level N addition gradient. Plant Soil 306:149–158

Inouye RS, Tilman D (1995) Convergence and divergence of old-field vegetation after 11 yr of nitrogen addition. Ecology 76:1872–1887

Killingbeck KT (1996) Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. Ecology 77:1716–1727

Knapp AK, Fay PA, Blair JM, Collins SL, Smith MD, Carlisle JD, Harper CW, Danner BT, Lett MS, McCarron JK (2002) Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. Science 298:2202–2205

Kobe RK, Lepczyk CA, Iyer M (2005) Resorption efficiency decreases with increasing green leaf nutrients in a global data set. Ecology 86:2780–2792

Koerselman W, Meuleman AFM (1996) The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. J Appl Ecol 33:1441–1450

Kozovits AR, Bustamante MMC, Garofalo CR, Bucci S, Franco AC, Goldstein G, Meinzer FC (2007) Nutrient resorption and patterns of litter production and decomposition in a Neotropical Savanna. Funct Ecol 21:1034–1043

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

Liu P, Huang JH, Han XG, Sun OJ, Zhou Z (2006) Differential responses of litter decomposition to increased soil nutrients and water between two contrasting grassland plant species of Inner Mongolia, China. Appl Soil Ecol 34:266–275

Liu WX, Zhang Z, Wan SQ (2009) Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. Glob Change Biol 15:184–195

Niu SL, Wu MY, Han Y, Xia JY, Li LH, Wan SQ (2008) Water-mediated responses of ecosystem carbon fluxes to climatic change in a temperate steppe. New Phytol 177:209–219

Pan QM, Bai YF, Han XG, Yang J (2005) Effects of nitrogen additions on a *Leymus chinensis* population in typical steppe of Inner Mongolia. Acta Phytoecologica Sinica 29:311–317

Piao SL, Fang JY, Chen AP (2003) Seasonal dynamics of terrestrial net primary production in response to climate changes in China. Acta Bot Sin 45:269–275

Reich PB, Oleksyn J (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. Proc Natl Acad Sci USA 101:11001–11006

Richardson SJ, Peltzer DA, Allen RB, McGlone MS (2005) Resorption proficiency along a chronosequence: responses among communities and within species. Ecology 86:20–25

Rustad LE, Campbell JL, Marion GM, Norby RJ, Mitchell MJ, Hartley AE, Cornelissen JHC, Gurevitch J (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126:543–562

Schade JD, Kyle M, Hobbie SE, Fagan WF, Elser JJ (2003) Stoichiometric tracking of soil nutrients by a desert insect herbivore. Ecol Lett 6:96–101

Soudzilovskaia NA, Onipchenko VG, Cornelissen JHC, Aerts R (2007) Effects of fertilisation and irrigation on 'foliar afterlife' in alpine tundra. J Veg Sci 18:755–766

Stevens CJ, Dise NB, Mountford JO, Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. Science 303:1876–1879

Suttle KB, Thomsen MA, Power ME (2007) Species inter-actions reverse grassland responses to changing climate. Science 315:640–642

van Heerwaarden LM, Toet S, Aerts R (2003) Nitrogen and phosphorus resorption efficiency and proficiency in six sub-arctic bog species after 4 years of nitrogen fertilization. J Ecol 91:1060–1070

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7:737–750

Wang CH, Wan SQ, Xing XR, Zhang L, Han XG (2006) Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China. Soil Biol Biochem 38:1101–1110

Weltzin JF, Loik ME, Schwinning S, Williams DG, Fay PA, Haddad BM, Harte J, Huxman TE, Knapp AK, Lin GH, Pockman WT, Shaw MR, Small EE, Smith MD, Smith SD, Tissue DT, Zak JC (2003) Assessing the response of terrestrial ecosystems to potential changes in precipitation. Bioscience 53:941–952

Yuan ZY, Li LH, Han XG, Huang JH, Jiang GM, Wan SQ (2005a) Soil characteristics and nitrogen resorption in Stipa krylovii native to northern China. Plant Soil 273:257–268

Yuan ZY, Li LH, Han XG, Huang JH, Jiang GM, Wan SQ, Zhang WH, Chen QS (2005b) Nitrogen resorption from senescing leaves in 28 plant species in a semi-arid region of northern China. J Arid Environ 63:191–202

Yuan ZY, Li LH, Han XG, Chen SP, Wang ZW, Chen QS, Bai WM (2006) Nitrogen response efficiency increased mono-tonically with decreasing soil resource availability: a case study from a semiarid grassland in northern China. Oecologia 148:564–572

Zhang JF, Han XG (2008) N_2O emission from the semi-arid ecosystem under mineral fertilizer (urea and superphosphate) and increased precipitation in northern China. Atmos Environ 42:291–302