

RESEARCH PAPER

Combined effects of nitrogen addition and litter manipulation on nutrient resorption of *Leymus chinensis* in a semi-arid grassland of northern China

X. Li*, J. Liu*, J. Fan, Y. Ma, S. Ding, Z. Zhong & D. Wang

Institute of Grassland Science, Key Laboratory of Vegetation Ecology, Northeast Normal University, Ministry of Education, Changchun, China

Keywords

Grassland; litter manipulation; nitrogen fertilisation; nutrient concentration; resorption efficiency; resorption proficiency.

CorrespondenceD. Wang, Institute of Grassland Science, Key Laboratory of Vegetation Ecology, Northeast Normal University, Ministry of Education, Changchun 130024, China.
E-mail: wangd@nenu.edu.cn

*These authors contributed equally to this work.

Editor

H. Rennenberg

Received: 19 November 2013; Accepted: 28 January 2014

doi:10.1111/plb.12172

ABSTRACT

Plant growth in semi-arid ecosystems is usually severely limited by soil nutrient availability. Alleviation of these resource stresses by fertiliser application and aboveground litter input may affect plant internal nutrient cycling in such regions. We conducted a 4-year field experiment to investigate the effects of nitrogen (N) addition ($10 \text{ g N} \cdot \text{m}^{-2} \cdot \text{year}^{-1}$) and plant litter manipulation on nutrient resorption of *Leymus chinensis*, the dominant native grass in a semi-arid grassland in northern China. Although N addition had no clear effects on N and phosphorus (P) resorption efficiencies in leaves and culms, N fertilisation generally decreased leaf N resorption proficiency by 54%, culm N resorption proficiency by 65%. Moreover, N fertilisation increased leaf P resorption proficiency by 13%, culm P resorption proficiency by 20%. Under ambient or enriched N conditions, litter addition reduced N and P resorption proficiencies in both leaves and culms. The response of P resorption proficiency to litter manipulation was more sensitive than N resorption proficiency: P resorption proficiency in leaves and culms decreased strongly with increasing litter amount under both ambient and enriched N conditions. In contrast, N resorption proficiency was not significantly affected by litter addition, except for leaf N resorption proficiency under ambient N conditions. Furthermore, although litter addition caused a general decrease of leaf and culm nutrient resorption efficiencies under both ambient and enriched N conditions, litter addition effects on nutrient resorption efficiency were much weaker than the effects of litter addition on nutrient resorption proficiency. Taken together, our results show that leaf and non-leaf organs of *L. chinensis* respond consistently to altered soil N availability. Our study confirms the strong effects of N addition on plant nutrient resorption processes and the potential role of aboveground litter, the most important natural fertiliser in terrestrial ecosystems, in influencing plant internal nutrient cycling.

INTRODUCTION

Nutrient resorption from senescing tissues is a critical physiological process for plant nutrient conservation (Aerts 1996), which minimises the dependence of terrestrial plants on soil nutrient availability and thus has important consequences for plant growth, reproduction and competitive ability (Killingbeck 1996; Aerts & Chapin 2000). As one of the key determinants of litter chemistry and litter decomposition, plant nutrient resorption also significantly influences soil nutrient cycling in terrestrial ecosystems (Kozovits *et al.* 2007). Plant nutrient resorption can be quantified as nutrient resorption efficiency (percentage of a nutrient withdrawn from green leaves before leaf abscission; van Heerwaarden *et al.* 2003a) and nutrient resorption proficiency (level to which a nutrient is reduced during senescence; Killingbeck 1996). Up to now, patterns and determinants of plant nutrient resorption have mostly been investigated in forest ecosystems, and much less is known for grass species in temperate grasslands.

As atmospheric deposition of biologically active N has increased dramatically over the past few decades (Galloway

et al. 2004), a number of studies have examined the potential effects of N deposition on internal plant nutrient cycling (Aerts *et al.* 2007; Lü *et al.* 2011; Wang *et al.* 2013). There is plenty of evidence that increased inorganic N supply can have a significant impact on both N resorption proficiency and resorption efficiency in plants (Soudzilovskaia *et al.* 2007; Huang *et al.* 2008). However, while plant N resorption proficiency often shows a clear decreased tendency (Cordell *et al.* 2001; van Heerwaarden *et al.* 2003b; Kobe *et al.* 2005) as soil N availability increases both between (Wright & Westoby 2003) and within species (Richardson *et al.* 2005; Norris & Reich 2009), the response of plant N resorption efficiency to altered soil fertility still seems to be inconsistent: N resorption may remain unchanged (Chapin & Moilanen 1991; Vitousek 1998; Yuan & Chen 2009), reduced (Vergutz *et al.* 2012) or be enhanced (Birk & Vitousek 1986; Yuan *et al.* 2005) with increasing supply of fertiliser in different habitats. On the other hand, it was gradually recognised that increased N input could cause a shift from N-limited towards P-limited ecosystems (Meng & Field 2007), which may potentially affect the process of P resorption in plants. Indeed, there are currently many theories that predict

N fertilisation should lead to more conservative use of P (e.g. higher P resorption) in terrestrial ecosystems (Lü *et al.* 2013). However, compared to the intensive research on the response of N resorption to soil N availability, empirical evidence of the effects of N addition on plant P resorption is still rare (but see Lü *et al.* 2013; Mao *et al.* 2013).

Aboveground litter represents a major process for transferring nutrients from vegetation to soils, and has long been recognised as the crucial factor in controlling soil nutrient availability in terrestrial ecosystems (Vitousek 1982; Vitousek & Sanford 1986; Clark *et al.* 2001). Research conducted in the Netherlands showed that only 3 years of litter harvesting can decrease the available soil N by 8%, and cause a reduction in ammonium and nitrate concentrations of 56% and 31%, respectively (Baar & ter Braak 1996). Furthermore, Park & Matzner (2003) reported that 2 years of litter addition led to a 38% increase in dissolved organic N in a temperate deciduous forest in Bavaria, Germany. Similarly, long-term litter removal in southern China resulted in a decrease in total soil N and P of up to 40% and 37%, respectively (Peng *et al.* 2003). Given these significant effects of plant litter on soil nutrient availability, it seems reasonable to expect that plant nutrient resorption, which is assumed to be highly sensitive to soil nutrient status (Eckstein *et al.* 1999), might be affected by plant litter manipulation.

Over the past several decades, leaf nutrient resorption has been thoroughly studied from the molecular to the whole-plant level. In contrast, whether and to what extent nutrients are resorbed from non-leaf organs such as stems and roots are much less studied (Freschet *et al.* 2010). Despite a lack of systematic studies, there are several lines of evidence suggesting that non-leaf organs such as grass culms and roots might be an important source of nutrients for plant resorption. For example, N and P resorption efficiencies in plant stems range from 45% to 80% and 55% to 91%, respectively (Aerts & de Caluwe 1989; Freschet *et al.* 2010; Lü *et al.* 2012). Since the non-leaf organs often account for a large proportion of the total plant biomass and play an important role in plant nutrient economy in many terrestrial ecosystems (Ren *et al.* 2009; Craine *et al.* 2010), knowledge of the response of nutrient resorption from non-leaf organs to soil nutrient availability is of primary importance.

To examine the organ-specific responses of nutrient concentrations and nutrient resorption patterns to N addition and litter manipulation, we examined leaf and culm nutrient concentrations of the dominant grass *Leymus chinensis* in an N-limited grassland of northern China. We hypothesised that (i) N fertilisation would not affect leaf and culm resorption efficiencies for either N or P, but would lead to lower leaf and culm resorption proficiencies for N and higher values for P; and (ii) under either ambient or enriched N conditions, aboveground litter addition would decrease both N and P resorption (proficiencies and efficiencies) in leaves and culms, because plant litter can provide key elements (especially N and P) that plants need for growth (Kaspari *et al.* 2008; Sayer & Tanner 2010).

MATERIAL AND METHODS

Study site

The experiment was carried out at the Grassland Ecological Research Station of Northeast Normal University, Jilin Province, China (44°45' N, 123°45' E). This site is character-

ised by a semi-arid continental monsoon, with cold dry winters and warm rainy summers. Annual mean temperature ranges from 4.6 to 6.4 °C and annual precipitation is 280–400 mm, with 70% falling in June–August. Annual potential evapotranspiration is approximately three times as much as annual precipitation. The soil is a mixed salt-alkali meadow steppe (Salid Aridisol, US Soil Taxonomy) with 28.9% sand, 40.1% silt and 31.0% clay (top 10 cm; Zhu 2004). Mean soil bulk density is 1.2 g·cm⁻³, and soil pH range from 8.5 to 10.0. In the surface soil horizon (30 cm), soil organic matter content is low (ca. 12 mg·g⁻¹), soil total N content ranges from 2.2 to 2.5 mg·g⁻¹, and soil total P content ranges from 0.23 to 0.27 mg·g⁻¹ (Ba *et al.* 2012). This study site lies in the eastern region of the Eurasian steppe zone. The perennial rhizomatous grass *Leymus chinensis* is the dominant plant species, accounting for 60–85% of the total aboveground biomass (Wang & Ba 2008). Other species include grasses such as *Phragmites australis*, *Calamagrostis epigejos* and *Chloris virgata*; legumes such as *Lespedeza davurica* and *Medicago ruthenica*; and forbs such as *Artemisia scoparia*, *A. mongolica* and *Carex duriuscula*. See Zhu *et al.* (2012) for details of the site characteristics.

Experimental design

In early May 2009, we established six blocks at the study site, each consists of six 3 × 4 m plots. Experimental treatments representing two levels of N (ambient N, enriched N) and three levels of litter (litter removed – 0L, litter left intact – 1L, litter added – 2L) were randomly assigned to the six treatment plots within each block. Blocks were separated by a 2-m walkway and plots within each block were separated with 1-m buffers.

Nitrogen was applied to the N addition plots as commercial ammonium nitrate fertiliser (NH₄NO₃) at a rate of 10 g N·m⁻²·year⁻¹. From 2009 to 2012, the fertiliser was applied in the rainy days of early May in each year. The N addition rate was relatively high compared to the sum of agricultural inputs and atmospheric deposition in this region. We chose this rate of N addition for our experiment to ensure a large response of plant nutrient resorption, and to mimic the potential response of this ecosystem to N addition that may occur in the future. Litter treatments were performed in early May 2009 and in late October from 2009 to 2012. Plant litter was removed from the appropriate plots (litter removal plots), pooled and homogenised, then an average amount added to the litter addition plots. We performed litter treatments separately among the treatments with or without N fertilisation.

Field sampling and measurements

In early August (peak of growing season) 2012, 20 shoots of *L. chinensis* with similar stature were randomly selected within each plot. We harvested ten of these and then collected two fully expanded leaves (only blades were included) and one corresponding culm (both sheaths and stems were included) per shoot to measure biomass and nutrient concentrations of these organs. Another ten shoots were marked with plastic labels, and then two fully expanded leaves per shoot were tagged with small pieces of red plastic foil. All tagged organs were harvested after complete senescence in mid-October, in the same way as the green organs.

To calculate plant nutrient resorption, the tagged senesced leaves and culms were used, along with their green counterparts.

All plant samples (both green and senesced leaves and culms) were oven-dried at 65 °C for 48 h, weighed separately, and then the average mass per leaf and culm calculated. Plant samples were ground and stored to measure N and P concentrations. For each plant sample, 0.1 g subsample was digested with H₂SO₄-H₂O₂, then N concentration (mg·g⁻¹) was measured using modified Kjeldahl wet digestion with a 2300 Kjektec Analyzer Unit (FOSS, Höganäs, Sweden); P concentration (mg·g⁻¹) was measured using the molybdenum blue colorimetric method (Murphy & Riley 1962). Total N or P pool of an individual organ (for both green and senesced material) was calculated from the individual organ weight (g) and its N or P concentration. Given the mass loss during plant organ senescence, N or P resorption efficiency (NRE or PRE) was calculated as the ratio of the difference in N or P pool between green and senesced organ to green organ N or P pool (Lü *et al.* 2012). N or P resorption proficiency (NRP or PRP) was quantified as the senesced organ (both leaves and culms) nutrient concentration, with lower organ nutrient concentration indicating higher nutrient resorption proficiency (Killingbeck 1996).

Statistical analysis

All data were tested for normality with the Shapiro–Wilk W statistic (Shapiro & Wilk 1965); non-normally distributed parameters were transformed prior to analysis of variance. We first tested for block effects for all response variables, and found that there were no significant main effects of blocks and no significant interactions between blocks and treatments. Therefore, the effects of blocks were not considered in this study. Two-way ANOVA was performed to evaluate the effects of N addition, litter manipulation and their interaction on nutrient (N and P) concentrations of green and senesced organs (leaves and culms), N:P ratio of green leaves and nutrient resorption efficiencies of leaves and culms. We conducted t-tests to evaluate whether N fertilisation influenced these response variables within each litter treatment. Under either ambient or enriched N condition, we further conducted Tukey tests to examine the mean difference of each response variable across the three litter treatments. All statistical analyses were performed using SPSS (SPSS 13.0 for Windows; SPSS Inc., Chicago, IL, USA).

RESULTS

Effects of N addition and litter manipulation on green organ nutrient concentrations

Nitrogen concentrations in green leaves and culms were significantly affected by N addition and litter manipulation (Table 1). Moreover, litter manipulation and the interaction between N addition and litter manipulation significantly affected green leaf P concentration, while N addition and litter manipulation significantly influenced green culm P concentration (Table 1).

Within each litter treatment, N fertilisation strongly increased N concentration in both green leaves and culms (Fig. 1A), whereas N fertilisation significantly decreased P concentration in these two organs, except for green leaf P concentration in the plots with double litter (Fig. 1B). Generally, N and P concentrations in both green leaves and culms increased along the litter gradient (from plots without litter to plots with double litter; Fig. 1A and B). Under ambient N con-

Table 1. Results of two-way ANOVA for the effects of nitrogen (N), litter (L) and their interaction (N × L) on nutrient variables and nutrient resorption of leaves and culms for *Leymus chinensis*.

	leaf		culm	
	F	P	F	P
[N]g				
N	71.003	<0.001	86.643	<0.001
L	8.356	<0.001	4.974	0.014
N × L	1.336	0.271	2.710	0.083
[P]g				
N	2.773	1.106	30.932	<0.001
L	8.263	<0.001	11.670	<0.001
N × L	4.859	0.015	0.318	0.730
[N]s				
N	66.412	<0.001	104.840	<0.001
L	6.418	0.005	1.534	0.232
N × L	0.974	0.389	0.280	0.758
[P]s				
N	6.254	0.018	20.000	<0.001
L	33.621	<0.001	10.194	<0.001
N × L	4.240	0.024	0.669	0.520
[N]g:[P]g				
N	48.406	<0.001	–	–
L	2.739	0.081	–	–
N × L	2.936	0.068	–	–
NRE				
N	17.435	<0.001	16.345	<0.001
L	1.589	0.221	5.227	0.011
N × L	0.241	0.787	4.142	0.026
PRE				
N	1.863	0.182	0.302	0.587
L	9.330	<0.001	0.945	0.400
N × L	0.363	0.698	0.841	0.441

The *F*-ratios and their level of significance are presented. [N]g and [P]g represent N and P concentrations in green organs; [N]s and [P]s represent N and P concentration in senesced organs; [N]g:[P]g represents N:P in green leaves; NRE and PRE represent N and P resorption efficiency, respectively.

ditions, green leaf N concentration was lowest in the plots without litter and highest in the plots with litter left intact (Fig. 1A); P concentration in green culms was also lowest in plots without litter, but was highest in plots with double litter (Fig. 1B). Under enriched N conditions, the plots with double litter had significantly higher nutrient (N and P) concentrations in both green leaves and culms than the plots without litter, except for N concentration in green culms (Fig. 1A and B).

The ratio of N:P in green leaves was significantly affected by N addition (Table 1). Under ambient N conditions, green leaf N:P ranged from 14 to 15, whereas this value increased to 17–22 under enriched N conditions (Fig. 2). Under ambient N conditions, green leaf N:P did not differ significantly across the three litter treatments; under enriched N conditions, green leaf N:P decreased significantly with increasing litter amount (Fig. 2).

Effects of N addition and litter manipulation on senesced organ nutrient concentrations

Nitrogen concentrations in senesced leaves and culms were both significantly affected by N addition, while senesced leaf N concentration was also affected by litter manipulation

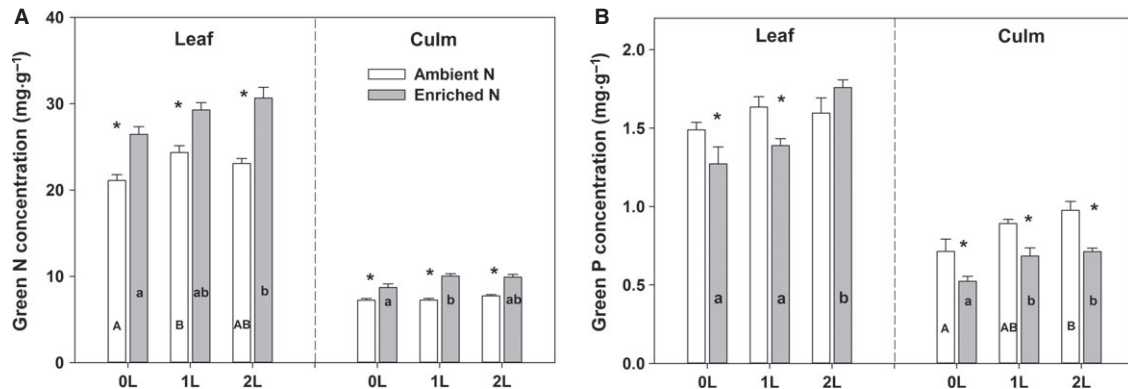


Fig. 1. Effects of N (ambient N and enriched N) and litter (litter removal–0L; litter intact–1L and litter addition–2L) on N (A) and P (B) concentrations in green leaves and culms of *Leymus chinensis*. Uppercase represent differences within the three litter treatments under ambient N condition, lowercase letters represent differences within the three litter treatments under enriched N condition, and * indicates significant N effect within each litter treatment ($P < 0.05$). Error bars indicate ± 1 SE.

(Table 1). On the other hand, N addition and litter manipulation significantly influenced P concentration in both senesced leaves and culms, while the interaction between litter manipulation and N addition also significantly affected senesced leaf P concentration (Table 1).

Within each litter treatment, N fertilisation strongly increased N concentration in both senesced leaves and culms (Fig. 3A), whereas N addition generally decreased P concentrations in these two organs (Fig. 3B). Similar to nutrient concentrations in green organs, N and P concentrations in senesced leaves and culms also increased along the litter gradient (Fig. 3A and B). Under ambient N conditions, senesced organ N and P concentrations in the plots with double litter were significantly higher than in the plots without litter, except for N concentration in senesced culms (Fig. 3A and B). Under enriched N conditions, N concentration in both senesced organs did not differ significantly from each other among the three litter treatments (Fig. 3A). However, P concentrations in senesced leaves and culms were significantly higher in the plots with double litter than that in the plots without litter (Fig. 3B).

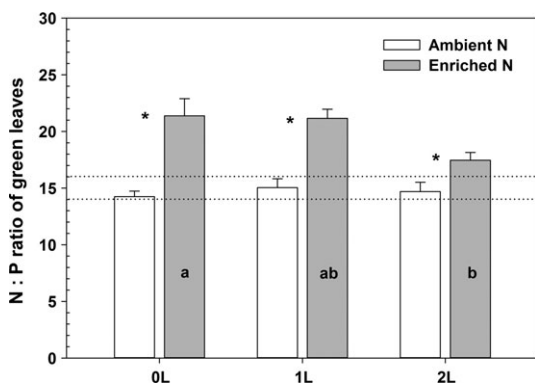


Fig. 2. Effects of N (ambient N and enriched N) and litter (litter removal–0L; litter intact–1L and litter addition–2L) on green leaf N:P of *Leymus chinensis*. Lowercase letters represent differences within the three litter treatments under enriched N condition, and * indicates significant N effect within each litter treatment ($P < 0.05$). Error bars indicate ± 1 SE.

Effects of N addition and litter manipulation on leaf and culm nutrient resorption efficiency

Nitrogen resorption efficiency in leaves was significantly affected by N addition, while N resorption efficiency in culms was simultaneously affected by N addition, litter manipulation and their interaction (Table 1). P resorption efficiency in leaves was only affected by litter manipulation (Table 1).

Within each litter treatment, N fertilisation generally decreased N resorption efficiencies in leaves and culms (Fig. 4A). However, there were no clear effects of N fertilisation on leaf and culm P resorption efficiencies (Fig. 4B). Under ambient N conditions, leaf N resorption efficiency was highest in the plots without litter, and lowest in the plots in which litter was left intact (Fig. 4A). Under enriched N conditions, the plots without litter had significantly higher culm N resorption efficiency and leaf P resorption efficiency than the plots with double litter (Fig. 4A and B). Both N and P resorption efficiencies in leaves and culms generally decreased along the litter gradient, except for culm P resorption efficiency under ambient N conditions (Fig. 4A and B).

DISCUSSION

Effects of N addition and litter manipulation on nutrient concentrations of green organs

In our study, N addition significantly increased N concentrations in green leaves and culms of *L. chinensis* (Fig. 1A). This result supports the previous suggestion that fertilisation usually induces positive responses on N concentrations in plant tissues (Lü *et al.* 2011; Mao *et al.* 2013). In contrast, we found that N fertilisation generally decreased P concentrations in both leaves and culms (Fig. 1B). In northern Sweden, van Heerwaarden *et al.* (2003b) also reported that N addition (at $10 \text{ g N}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$) could induce lower green leaf P concentrations in two woody species (*Vaccinium uliginosum* and *Betula nana*) after 4 years of treatment. However, our results are surprisingly inconsistent with the findings of other studies conducted in nearby regions. For example, in a freshwater wetland of northeast China, Mao *et al.* (2013) found that leaf and stem P concentrations for *Deyeuxia angustifolia* were not affected by N fertilisation, while N addition (at $12 \text{ g N}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$) only

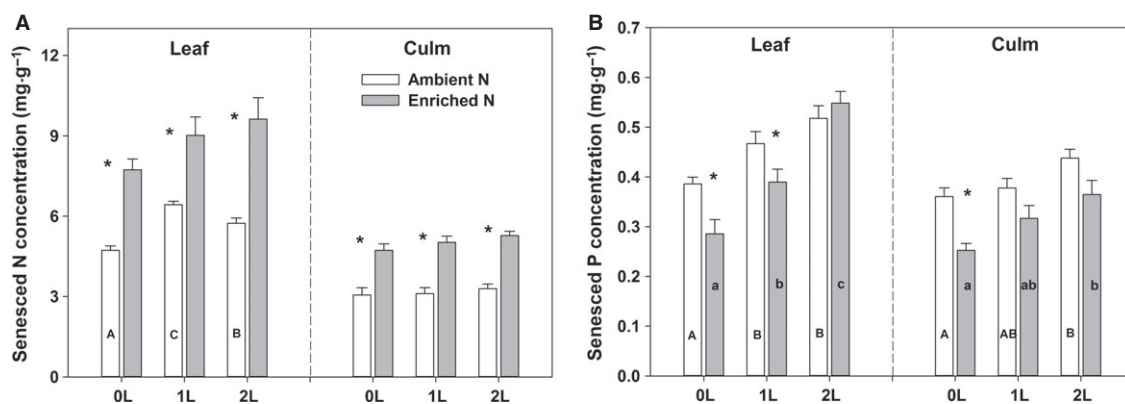


Fig. 3. Effects of N (ambient N and enriched N) and litter (litter removal–0L; litter intact–1L and litter addition–2L) on N (A) and P (B) concentrations in senesced leaves and culms of *Leymus chinensis*. Uppercase represent differences within the three litter treatments under ambient N condition, lowercase letters represent differences within the three litter treatments under enriched N condition, and * indicates significant N effect within each litter treatment ($P < 0.05$). Error bars indicate ± 1 SE.

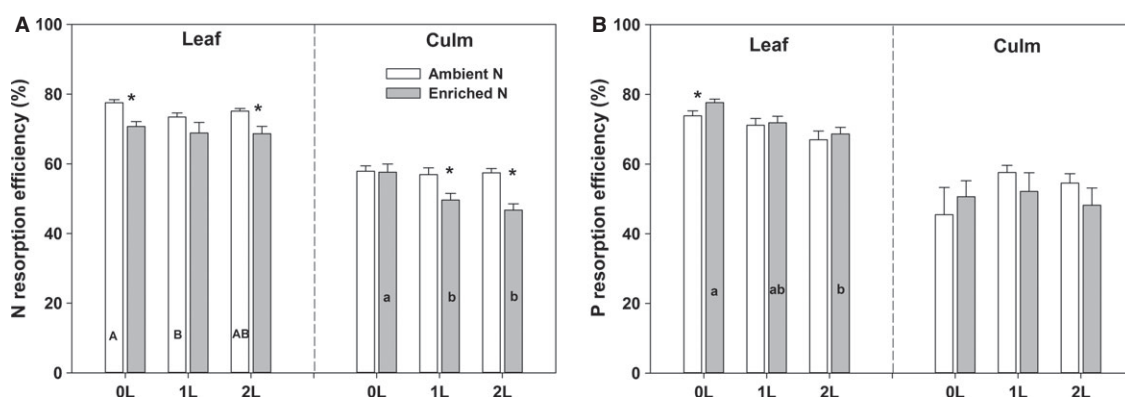


Fig. 4. Effects of N (ambient N and enriched N) and litter (litter removal–0L; litter intact–1L and litter addition–2L) on N (A) and P (B) resorption efficiencies in leaves and culms of *Leymus chinensis*. Uppercase represent differences within the three litter treatments under ambient N condition, lowercase letters represent differences within the three litter treatments under enriched N condition, and * indicates significant N effect within each litter treatment ($P < 0.05$). Error bars indicate ± 1 SE.

caused a significant decline in P concentration in *Glyceria spiculosa* stems. In a semi-arid grassland of Inner Mongolia, China, Lü *et al.* (2012) showed that N fertilisation (at $17.5 \text{ g N} \cdot \text{m}^{-2} \cdot \text{year}^{-1}$) can even significantly increase green leaf P concentration in *Achnatherum sibiricum*. The exact cause of the inconsistency between our results and these other studies is still unclear, but as noted in several previous studies, the responses of plant tissue P concentrations to N fertilisation could be related to species identity (van Heerwaarden *et al.* 2003b), the initial levels of nutrients in the soil (Aerts & Chapin 2000; Lü *et al.* 2012), N fertilisation rates (Lü *et al.* 2013) and even habitat type (Kobe *et al.* 2005).

Under both ambient and enriched N conditions, litter addition generally exerted a positive effect on green organ nutrient concentrations (Fig. 1A and B). In particular, litter addition led to a significantly higher P concentration in both green leaves and culms (Fig. 1B). To date, there is limited empirical work reporting the influence of litter manipulation on plant nutrient status, and almost no data available to compare to the results obtained in our studies. We infer that the positive responses of green organ nutrient concentrations may result from the increased soil nutrient availability following litter addition.

Foliar N:P ratios have been suggested as good indicators of nutrient limitation for plants in terrestrial ecosystems (Koerselman & Meuleman 1996; Güsewell 2004). Koerselman & Meuleman (1996) suggested that plant growth is limited by N when the N:P ratio is below 14, is limited by P when the ratio is above 16, and is co-limited by N and P when the ratio is between 14 and 16. In our study, green leaf N:P ratios were between 14 and 16 under ambient N conditions, which suggests that N and P co-limited plant growth (Fig. 2). Under N enriched conditions, green leaf N:P ratios increased significantly and exceeded the critical value of 16, suggesting that plant growth might be limited by P. More importantly, we found that litter addition can lead to significantly lower green leaf N:P ratios under N enriched conditions, which indicates that aboveground litter could potentially alleviate P limitation under scenarios of enhanced N deposition.

Effects of N addition and litter manipulation on leaf and culm nutrient resorption proficiency

In accordance with our hypothesis, N fertilisation causes significantly lower N resorption proficiency in both leaves and culms

for the dominant grass in temperate grassland (Fig. 3A), which is consistent with earlier studies in a sub-arctic peat bog (van Heerwaarden *et al.* 2003b), oak savanna (Norris & Reich 2009), freshwater wetland (Mao *et al.* 2013) and temperate steppe (Lü & Han 2010). P proficiency in leaves and culms generally increased under enriched N conditions, except for leaf P proficiency in the plots with double litter (Fig. 3B). As N addition can stimulate primary production (and thus P uptake), it is generally suggested that increasing N addition would lead to increased P limitation to plant growth, and finally result in a more conservative use of P in plants (Meng & Field 2007). On the other hand, plants may reabsorb more P to balance the increased organ N concentrations to maintain stoichiometric homeostasis under enriched N conditions (Yu *et al.* 2010). N addition had no effects on leaf and culm P proficiencies in the plots with double litter (Fig. 3B), indicating that the above-ground litter input may indirectly mediate the response of P proficiency to the changes in soil N availability.

The potential role of aboveground litter input in influencing the processes of plant nutrient resorption has been reported in tropical forests (Sayer 2006; Sayer & Tanner 2010). Here, our results showed that plant litter can also significantly affect plant nutrient resorption in grassland ecosystems: under ambient and enriched N conditions, litter addition generally decreased N and P proficiencies in both leaves and culms of *L. chinensis* (Fig. 3A and B). More interestingly, we note that the response of P resorption proficiency to litter manipulation appeared to be more sensitive than N resorption proficiency. Under both ambient and enriched N conditions, leaf and culm P resorption proficiencies decreased strongly with increasing litter amount. However, litter manipulation only significantly affected N resorption proficiency in leaves under ambient N conditions (Fig. 3A and B). Given plant litter usually acts as a complete fertiliser that provides all the elements (especially N and P) plants need for growth (Sayer *et al.* 2012), our results imply that plant growth in this semi-arid grassland may be more limited by P rather than by N.

Indeed, there is plenty of evidence that plant nutrient resorption proficiency often increases in parallel to soil nutrient availability (Wright & Westoby 2003; Norris & Reich 2009). Yet, as most ecosystems often exhibit some degree of patchiness in their soil fertility conditions (Ettema & Wardle 2002), the effects of N and litter manipulations on plant nutrient resorption in our study may also be influenced by the spatial heterogeneity of soil properties. This assumption is partially supported by a recent study in northern China. Lü *et al.* (2012) reported that micro-scale variation in resource availability (such as soil inorganic N and soil moisture) can strongly impact both leaf and culm N resorption proficiencies and absolute leaf N resorption of *L. chinensis*.

The reduced N and P resorption proficiencies following litter addition have implications for nutrient cycling dynamics, as higher nutrient concentrations in litter generally lead to a faster decomposition rate. The manipulation of aboveground litter can thus affect decomposition rate and change nutrient cycling in this grassland ecosystem. Additionally, we should note that except for aboveground organs, a large proportion of plant lit-

ter input is derived from belowground organs (Yuan & Chen 2010). In grasslands and forests, belowground litter can represent approximately 33% and 48% of annual litter input, respectively (Freschet *et al.* 2013). Given this fact, it is reasonable to expect that the belowground litter may also play an important role in influencing ecosystem nutrient dynamics. Nevertheless, the number of relevant studies is currently still quite limited.

Effects of N addition and litter manipulation on leaf and culm nutrient resorption efficiency

We hypothesised that N resorption efficiency in leaves and culms would not be affected by N fertilisation, but we found that N fertilisation generally decreased N resorption efficiency in leaves and culms (Fig. 4A). The decrease in N resorption efficiency is probably related to the increased N concentrations in green organs after N addition, as a higher green organ nutrient concentration generally leads to lower nutrient resorption efficiency at a global scale (Kobe *et al.* 2005; Vergutz *et al.* 2012). Generally, there were no clear effects of N addition on P resorption efficiency in either leaves or culms (Fig. 4B). We were not surprised by this result, as previous studies showed that P resorption efficiency might increase (Lü & Han 2010), decrease (van Heerwaarden *et al.* 2003b) or remain unchanged (Kozovits *et al.* 2007) after N fertilisation. Compared with the strong effects of litter manipulation on nutrient proficiency in leaves and culms (Fig. 3A and B), the effects of aboveground litter input on nutrient resorption efficiency seems to be less significant (Fig. 4A and B). Nevertheless, we noted that leaf N nutrient resorption efficiency under ambient N conditions, leaf P resorption efficiency and culm N resorption efficiency under enriched N conditions all decreased with increasing litter amount, suggesting aboveground litter has the potential to influence plant resorption efficiency in this grassland ecosystem.

CONCLUSIONS

Our study is the first, to our knowledge, to simultaneously examine the effects of N addition and litter manipulation on plant nutrient resorption. Our results showed that N fertilisation strongly influences nutrient resorption in both leaves and culms of *L. chinensis*, the keystone and dominant species in the eastern Eurasian steppes. More importantly, as the most important natural fertiliser in terrestrial ecosystems, this study confirms the potential role of aboveground litter in influencing plant internal nutrient cycling, especially in nutrient-limited habitats.

ACKNOWLEDGEMENTS

We thank E.Q. Li, Q.H. Ma, X.C. Fan and R. Liu for assistance in the field and lab. This work was supported by the State Agricultural Commonwealth Project (201003019), the National Natural Science Foundation of China (No. 31230012, 31302012, 31370453) and the Fundamental Research Funds for the Central Universities (12QNJ019).

REFERENCES

- Aerts R. (1996) Nutrient resorption from senescing leaves of perennials: are there general patterns? *Journal of Ecology*, **84**, 597–608.
- Aerts R., Chapin F.S. (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research*, **30**, 1–67.
- Aerts R., de Caluwe H. (1989) Aboveground productivity and nutrient turnover of *Molinia caerulea* along an experimental gradient of nutrient availability. *Oikos*, **54**, 320–324.

- Aerts R., Cornelissen J.H.C., van Logtestijn R.S.P., Callaghan T.V. (2007) Climatic change has only a minor impact on nutrient resorption parameters in a high-latitude peatland. *Oecologia*, **151**, 132–139.
- Ba L., Ning J., Wang D., Facelli E., Facelli J.M., Yang Y., Zhang L. (2012) The relationship between the diversity of arbuscular mycorrhizal fungi and grazing in a meadow steppe. *Plant and Soil*, **352**, 143–156.
- Baar J., ter Braak C.J.F. (1996) Ectomycorrhizal sporocarp occurrence as affected by manipulation of litter and humus layers in Scots pine stands of different age. *Applied Soil Ecology*, **4**, 61–73.
- Birk E.M., Vitousek P.M. (1986) Nitrogen availability and nitrogen use efficiency in loblolly pine stands. *Ecology*, **67**, 69–79.
- Chapin F.S., Moilanen L. (1991) Nutritional controls over nitrogen and phosphorus resorption from Alaskan birch leaves. *Ecology*, **72**, 709–715.
- Clark D.A., Brown S., Kicklighter D.W., Chambers J.Q., Thomlinson J.R., Ni J., Holland E.A. (2001) Net primary production in tropical forests: an evaluation and synthesis of existing field data. *Ecological Applications*, **11**, 371–384.
- Cordell S., Goldstein G., Meinzer F.C., Vitousek P.M. (2001) Regulation of leaf life-span and nutrient-use efficiency of *Metrosideros polymorpha* trees at two extremes of a long chronosequence in Hawaii. *Oecologia*, **127**, 198–206.
- Craine J.M., Towne E.G., Nippert J.B. (2010) Climate controls on grass culm production over a quarter century in a tallgrass prairie. *Ecology*, **91**, 2132–2140.
- Eckstein R.L., Karlsson P.S., Weih M. (1999) Leaf life span and nutrient resorption as determinants of plant nutrient conservation in temperate-arctic regions. *New Phytologist*, **143**, 177–189.
- Ettema C.H., Wardle D.A. (2002) Spatial soil ecology. *Trends in Ecology & Evolution*, **17**, 177–183.
- Freschet G.T., Cornelissen J.H.C., van Logtestijn R., Aerts R. (2010) Substantial nutrient resorption from leaves, stems and roots in a subarctic flora: what is the link with other resource economics traits? *New Phytologist*, **186**, 879–889.
- Freschet G.T., Cornwell W.K., Wardle D.A., Elumeeva T.G., Liu W., Jackson B.G., Onipchenko V.G., Soudzilovskaia N.A., Tao J., Cornelissen J.H.C. (2013) Linking litter decomposition of above- and below-ground organs to plant–soil feedbacks worldwide. *Journal of Ecology*, **101**, 943–952.
- Galloway J.N., Dentener F.J., Capone D.G., Boyer E.W., Howarth R.W., Seitzinger S.P., Asner G.P., Cleveland C.C., Green P.A., Holland E.A., Karl D.M., Michaels A.F., Porter J.H., Townsend A.R., Vörösmarty C.J. (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry*, **70**, 153–226.
- Güsewell S. (2004) N: P ratios in terrestrial plants: variation and functional significance. *New Phytologist*, **164**, 243–266.
- van Heerwaarden L.M., Toet S., Aerts R. (2003a) Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions. *Oikos*, **101**, 664–669.
- van Heerwaarden L.M., Toet S., Aerts R. (2003b) Nitrogen and phosphorus resorption efficiency and proficiency in six sub-arctic bog species after 4 years of nitrogen fertilization. *Journal of Ecology*, **91**, 1060–1070.
- Huang J.Y., Zhu X.G., Yuan Z.Y., Song S.H., Li X., Li L.H. (2008) Change in nitrogen resorption traits of six temperate grassland species along a multi-level N addition gradient. *Plant and Soil*, **306**, 149–158.
- Kaspari M., Garcia M.N., Harms K.E., Santana M., Wright S.J., Yavitt J.B. (2008) Multiple nutrients limit litterfall and decomposition in a tropical forest. *Ecology Letters*, **11**, 35–43.
- Killingbeck K.T. (1996) Nutrients in senesced leaves: key to the search for potential resorption and resorption proficiency. *Ecology*, **77**, 1716–1727.
- Kobe R.K., Lepczyk C.A., Iyer M. (2005) Resorption efficiency decreases with increasing green leaf nutrient in a global data set. *Ecology*, **86**, 2780–2792.
- Koerselman W., Meuleman A.F.M. (1996) The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology*, **33**, 1441–1450.
- Kozovits A.R., Bustamante M.M.C., Garofalo C.R., Bucci S., Franco A.C., Goldstein G., Meinzer F.C. (2007) Nutrient resorption and patterns of litter production and decomposition in a Neotropical Savanna. *Functional Ecology*, **21**, 1034–1043.
- Lü X.T., Han X.G. (2010) Nutrient resorption responses to water and nitrogen amendment in semi-arid grassland of Inner Mongolia, China. *Plant and Soil*, **327**, 481–491.
- Lü X.T., Cui Q., Wang Q.B., Han X.G. (2011) Nutrient resorption response to fire and nitrogen addition in a semi-arid grassland. *Ecological Engineering*, **37**, 534–538.
- Lü X.T., Freschet G.T., Flynn D.F.B., Han X.G. (2012) Plasticity in leaf and stem nutrient resorption proficiency potentially reinforces plant–soil feedbacks and microscale heterogeneity in a semi-arid grassland. *Journal of Ecology*, **100**, 144–150.
- Lü X.T., Reed S., Yu Q., He N.P., Wang Z.W., Han X.G. (2013) Convergent responses of nitrogen and phosphorus resorption to nitrogen inputs in a semi-arid grassland. *Global Change Biology*, **19**, 2275–2784.
- Mao R., Song C.C., Zhang X.H., Wang X.W., Zhang Z.H. (2013) Response of leaf, sheath and stem nutrient resorption to 7 years of N addition in freshwater wetland of Northeast China. *Plant and Soil*, **364**, 385–394.
- Meng D.N.L., Field C.B. (2007) Simulated global changes alter phosphorus demand in annual grassland. *Global Change Biology*, **13**, 2582–2591.
- Murphy J., Riley J.P. (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, **27**, 31–36.
- Norris M.D., Reich P.B. (2009) Modest enhancement of nitrogen conservation via retranslocation in response to gradients in N supply and leaf N status. *Plant and Soil*, **316**, 193–240.
- Park J.H., Matzner E. (2003) Controls on the release of dissolved organic carbon and nitrogen from a deciduous forest floor investigated by manipulations of aboveground litter inputs and water flux. *Biogeochemistry*, **66**, 265–286.
- Peng S., Ren H., Wu J., Lu H. (2003) Effects of litter removal on plant species diversity: a case study in tropical *Eucalyptus* forest ecosystems in South China. *Journal of Environmental Sciences*, **15**, 367–371.
- Ren H.Y., Zheng S.X., Bai Y.F. (2009) Effects of grazing on foliage biomass allocation of grassland communities in Xilin river basin, Inner Mongolia. *Chinese Journal of Plant Ecology*, **33**, 1065–1074.
- Richardson S.J., Peltzer D.A., Allen R.B., McGlone M.S. (2005) Resorption proficiency along a chronosequence: responses among communities and within species. *Ecology*, **86**, 20–25.
- Sayer E.J. (2006) Using experimental manipulation to assess the roles of litter in the functioning of forest ecosystems. *Biological Reviews*, **81**, 1–31.
- Sayer E.J., Tanner E.V. (2010) Experimental investigation of the importance of litterfall in lowland semi-evergreen tropical forest nutrient cycling. *Journal of Ecology*, **98**, 1052–1062.
- Sayer E.J., Wright S.J., Tanner E.V.J., Yavitt J.B., Harms K.E., Powers J.S., Kaspari M., Garcia M.N., Turner B.L. (2012) Variable responses of lowland tropical forest nutrient status to fertilization and litter manipulation. *Ecosystems*, **15**, 387–400.
- Shapiro S.S., Wilk M.B. (1965) An analysis of variance test for normality (complete samples). *Biometrika*, **52**, 591–611.
- Soudzilovskaia N.A., Onipchenko V.G., Cornelissen J.H.C., Aerts R. (2007) Effects of fertilisation and irrigation on ‘foliar afterlife’ in alpine tundra. *Journal of Vegetation Science*, **18**, 755–766.
- Vergutz L., Manzoni S., Porporato A., Novais R.F., Jackson R.B. (2012) Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. *Ecological Monographs*, **82**, 205–220.
- Vitousek P.M. (1982) Nutrient cycling and nutrient use efficiency. *American Naturalist*, **119**, 553–572.
- Vitousek P.M. (1998) Foliar and litter nutrients, nutrient resorption, and decomposition in Hawaiian *Metrosideros polymorpha*. *Ecosystems*, **1**, 401–407.
- Vitousek P.M., Sanford R.L. Jr (1986) Nutrient cycling in moist tropical forest. *Annual Review of Ecology and Systematics*, **17**, 137–167.
- Wang D.L., Ba L. (2008) Ecology of meadow steppe in northeast China. *The Rangeland Journal*, **30**, 247–254.
- Wang M., Murphy M.T., Moore T.R. (2013) Nutrient resorption of two evergreen shrubs in response to long-term fertilization in a bog. *Oecologia*, **174**, 365–377.
- Wright I.J., Westoby M. (2003) Nutrient concentration, resorption and lifespan: leaf traits of Australian sclerophyll species. *Functional Ecology*, **17**, 10–19.
- Yu Q., Chen Q.S., Elser J.J., Cease A., He N.P., Wu H.H., Zhang G.M., Wu J.G., Bai Y.F., Han X.G. (2010) Linking stoichiometric homeostasis with ecosystem structure, functioning and stability. *Ecology Letters*, **13**, 1390–1399.
- Yuan Z.Y., Chen H.Y.H. (2009) Global-scale patterns of nutrient resorption associated with latitude, temperature and precipitation. *Global Ecology and Biogeography*, **18**, 11–18.
- Yuan Z.Y., Chen H.Y.H. (2010) Fine root biomass, production, turnover rates, and nutrient contents in boreal forest ecosystems in relation to species, climate, fertility, and stand age: literature review and meta-analyses. *Critical Reviews in Plant Sciences*, **29**, 204–221.
- Yuan Z.Y., Li L.H., Han X.G., Huang J.H., Jiang G.M., Wan S.Q., Zhang W.H., Chen Q.S. (2005) Nitrogen resorption from senescing leaves in 28 plant species in a semi-arid regional of northern China. *Journal of Arid Environments*, **63**, 191–202.
- Zhu T.C. (2004) *Biological ecology of Leymus chinensis*. Jilin Science and Technology Press, Changchun, China (In Chinese).
- Zhu H., Wang D.L., Wang L., Bai Y.G., Fang J., Liu J.S. (2012) The effects of large herbivore grazing on meadow steppe plant and insect diversity. *Journal of Applied Ecology*, **49**, 1075–1083.