

Nitrogen deposition promotes phosphorus uptake of plants in a semi-arid temperate grassland

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Abstract

Background and aims Nitrogen (N) deposition greatly influences ecosystem processes through the alteration of plant nutrition; however, there is limited understanding about the effects of phosphorus (P) inputs, especially within the backdrop of N deposition.

Methods Here we investigated the plant stoichiometric responses to P addition under both ambient conditions and with N addition in a temperate grassland in Inner Mongolia over a two-year period.

Results The effects of P addition on foliar nutrition and stoichiometric ratios were highly dependent on the presence of N supply, in that P addition showed no significant impacts on foliar N or P concentrations (or pools in community biomass) or N:P under ambient N conditions, but enhanced foliar N in dominant species and foliar P in almost all species (and pools in community biomass) with N addition. These results may be explained by P addition significantly changing the soil properties (soil pH and available P) when applied in combination with N additions. Moreover, there was no biomass response to nutrient additions.

Conclusions Our results suggest that N deposition may enhance the absorption of additional P by plants in semi-arid grasslands, and that P limitation with increased N deposition might be less important for limiting plant growth than previously anticipated.

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Introduction

Increases in reactive nitrogen (N) availability resulting from human activities have substantially altered the structure and function of many terrestrial ecosystems (Galloway et al. 2004), with important consequences for biogeochemical cycles (Lü et al. 2013; Niu et al. 2010; Stevens et al. 2011; Zhang et al. 2014). For example, increased N inputs generally lead to higher foliar N concentrations (Lü et al. 2012; Sardans and

Peñuelas 2012), lower plant carbon (C) to N ratios (C:N), and higher N:P ratios (Bobbink et al. 1998; Bragazza et al. 2004; Pardo et al. 2007; Yesmin et al. 1996). In contrast to the well-known effects of N enrichment on plant nutrient status, large uncertainties remain about the influences of P additions on nutrient cycling, especially in grassland ecosystems.

Given that human activities are also substantially altering the global P cycle (Elser and Bennett 2011), it is particularly important to explore the effects of P availability on nutrient cycling. A global budget approach estimates that there has been at least a 75 % increase in P accumulation in soils since preindustrial times (Bennett et al. 2001). Increases in P inputs to ecosystems may affect nutrient dynamics by affecting nutrient utilization and storage. For example, in temperate grasslands, P enrichment increased total soil C storage due to significant increases in C inputs from litter and roots (He et al. 2013). In addition, P concentrations in senesced leaves from four dominant species in a semi-arid grassland increased following P addition (Li et al. 2012), suggesting that the remobilization of nutrients in plants might change with P addition. Furthermore, a global study showed that both leaf N and P concentrations were positively related to soil P availability (Ordoñez et al. 2009), but most of these data were collected from forests and shrublands. Hitherto, there has been little understanding about the effects of anthropogenic P inputs on the nutrient uptake of plants in grasslands. Given the ecological and economic importance of grasslands (covering 13.37 % of the total land area of the world including Antarctica and Greenland, Gong et al. 2013) and their sensitivity to global change factors such as changes in nutrient availability (Ceulemans et al. 2013; Lü et al. 2012), studies in grassland ecosystems are urgently needed.

Previous studies have shown that P effects on plant nutrition may be regulated by N addition due to the synergistic interaction between N and P in plant metabolism (Güsewell 2004; Niklas et al. 2005). Nitrogen may enhance plant acquisition of P in a number of ways. First, in calcareous grasslands, N deposition may reduce the loss of available P due to precipitation with base cations by decreasing the concentrations of those ions such as Ca^{2+} in soils (Horswill et al. 2008). Second, N additions in the form of ammonium compounds, may increase soil P availability in grasslands by decreasing soil pH

(Phoenix et al. 2012; Zhálnina et al. 2015). Third, N enrichment from deposition may stimulate the activity of root phosphomonoesterase, an enzyme catalyzing the mineralization of organic P in soil (Johnson et al. 1999; Olde Venterink 2011). Fourth, plants may assimilate more P to maintain N:P homeostasis when N concentrations in plant tissue are increased by N addition (Yu et al. 2011). For example, it has been reported that P uptake in *Calluna vulgaris* was positively correlated with N deposition rates (Rowe et al. 2008). Hence, a number of mechanisms may lead to N deposition enhancing the uptake of P by plants.

In this study, we investigated the effects of N and P addition on plant N and P concentrations and N:P ratios at both community- and species-levels in a temperate grassland of Inner Mongolia. We hypothesized that (1) P addition alone would not increase plant P because Inner Mongolia grasslands are mainly limited by N (Yu et al. 2010), and (2) the effect of P input would be enhanced by N addition because of the positive effect of N addition on plant P uptake.

Materials and methods

Study site

This experiment was carried out in a temperate semi-arid steppe near the Inner Mongolia Grassland Ecosystem Research Station (43°38'N, 116°42'E, 1250 m a.s.l.) in 2006 and 2007. Long-term (1980–2008) mean annual temperature at the site is approximately 0 °C; mean monthly temperature ranges from –21.6 °C in January to 19.0 °C in July. Annual precipitation was 304 mm in 2006 and 240 mm in 2007, lower than mean annual precipitation (346.1 mm), about 80 % of which falls during the growing season from May to September. The study area had been fenced since 1999 to prevent grazing by large animals. The soil is classified as a dark chestnut or Calcic-Orthic Aridisol (Calcic Chernozem according to ISSS Working Group RB 1998). Mean bulk density of the top soil (0–10 cm) is 1.3 g cm⁻³ and soil pH is 7.5. Soil organic carbon, total N and total P concentrations measured for the top 10 cm are 18.9, 1.9 and 0.4 g kg⁻¹, respectively. The dominant plant species are *Leymus chinensis*, *Stipa grandis*, *Koeleria cristata* and *Agropyron cristatum*, accounting for nearly 80 % of the total biomass.

Experimental design and sampling

Four treatments, with six replicates each, were established for this experiment. The four nutrient addition treatments included: 1) control (no N or P added), 2) 1.55 g P m⁻² (as KH₂PO₄), 3) 2.8 g N m⁻² (as urea), and 4) 2.8 g N + 1.55 g P m⁻² (N + P treatment hereafter). The N addition rate was made in reference to the level of atmospheric N deposition in this region (Liu et al. 2013), while the P addition rate was based on the available N:P and specific calcium conditions in the soil of this study area (Office and Service 1994), as well as the level of N deposition. Each plot was 6 × 8 m in size and separated by a 1 m buffer. All fertilizers, mixed with sand, were applied to plots in May 2006 and 2007. Thirteen species were selected for foliar nutrient sampling in this study: *Axyris amarantoides* (AA), *Agropyron cristatum* (AC), *Allium ramosum* (AR), *Achnatherum sibiricum* (AS), *Allium tenuissimum* (AT), *Chenopodium glaucum* (CG), *Carex korshinskyi* (CK), *Cleistogenes squarrosa* (CS), *Koeleria cristata* (KC), *Kochia prostrate* (KP), *Leymus chinensis* (LC), *Poa sphondylodes* (PS) and *Stipa grandis* (SG). The biomass of these species accounted for 95 % of the total aboveground biomass.

All plants were clipped at ground level within a 1 m × 1 m quadrat in July 2006 and 2007 and oven-dried at 60 °C and weighed. Fifty fully expanded, healthy leaves of the 13 species were sampled from each plot, ground and mixed thoroughly for N and P analysis. Leaf N concentrations were analyzed using the micro-Kjeldahl method (Sparks 1996), while leaf P concentrations were measured by the ammonium molybdate method after persulfate oxidation (Sparks 1996). Meanwhile, soil samples (three per plot) were collected at a depth of 0–10 cm (which contains about 60 % of total belowground biomass, Fan et al. 2009) and composited to determine available P and soil pH, two soil parameters most likely to affect the P uptake. Samples for available P measurement were extracted by NaHCO₃. The concentrations of P in all solutes were determined by the ammonium molybdate method. Gravimetric soil water content was determined after oven-drying subsamples at 105 °C for 24 h. Soil pH was measured with a glass electrode (Jenway 3320, UK, soil:water ratio = 1:2.5).

Data analysis and statistics

Community nutrient pools (N and P) were calculated by summing the products of each species' foliar nutrient

concentration and its biomass across all 13 species in each plot. Tukey HSD post hoc tests were used to quantify significant differences of plant nutrition, soil properties (soil pH and available P) and community aboveground biomass among treatments ($P < 0.05$). To estimate the possible effects of soil properties on plant nutrients, Pearson's product-moment correlations were performed between soil parameters and plant nutrients at both community- and species-levels. All statistical analyses were performed using software R, version 3.1.2 (R Core Team 2014).

Results

At the community-level, when N or P were added alone, neither significantly affected N or P pools or N:P (although an increasing trend was observed in foliar N and P after N addition), while the combined nutrient addition (N + P treatment) significantly increased N and P pools, and decreased N:P (Fig. 1). One exception to these significant effects was that no change in the N pool was observed in 2006.

At the species-level, N or P additions alone did not change foliar N concentrations in almost all species in either 2006 or 2007. However, the N + P treatment increased foliar N concentrations by 9.09 %. More specifically, the N + P treatment enhanced the foliar N concentrations of the dominant species, *L. chinensis*, *S. grandis*, *A. cristatum* and *C. squarrosa* (which together accounted for 67.99 % of the total biomass), with the exceptions of *C. squarrosa* in 2006 and of *L. chinensis* in 2007 (Fig. 2). Foliar N of *P. sphondylodes*, *A. ramosum* and *C. glaucum* in 2006 and *K. cristata* in 2007 also increased with the N + P treatment (Fig. 2).

Similarly, N or P addition alone failed to exert significant influence on foliar P concentrations regardless of species identity and year, with the exceptions of *K. cristata* and *A. tenuissimum* in 2007 (Fig. 3). However, the N + P treatment significantly increased foliar P concentration by 27.16 %. Consistent responses were seen in all species in 2006 (except *C. korshinskyi*) and in nine of the thirteen species in 2007.

In both years, foliar N:P of all species, except *C. squarrosa* and *A. tenuissimum* in 2007, showed no response to N addition alone (Fig. 4). Similarly, foliar N:P in all species did not change with P addition alone (Fig. 4). However, some species in the N + P treatment

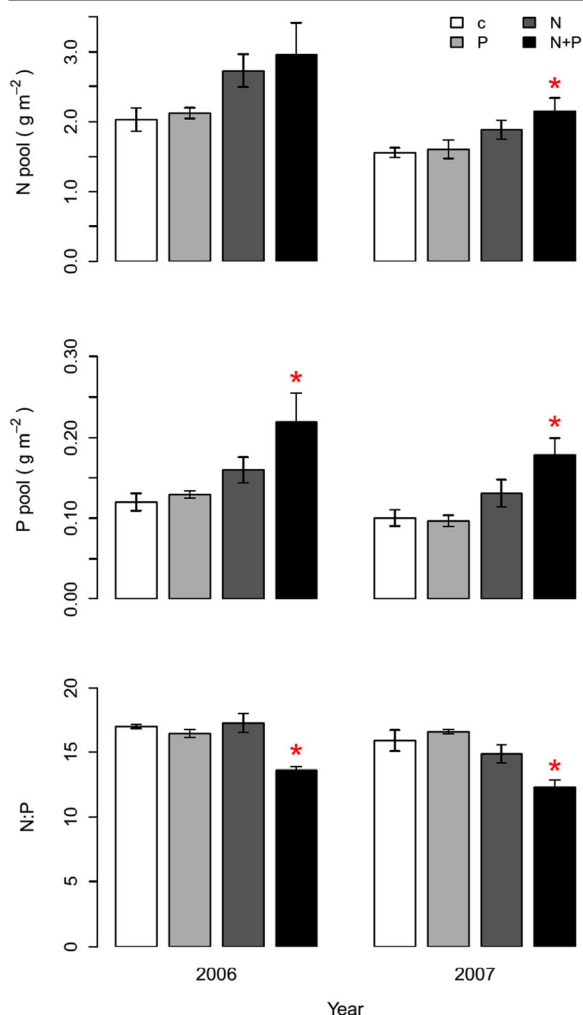


Fig. 1 Effects of N and P additions (c: control, N: N addition, P: P addition, N + P: both N and P addition) on community nutrient pools and N:P in the Inner Mongolia grassland. Data are shown as means \pm SE. Bars with asterisks are significantly different from corresponding control treatments ($P < 0.05$)

had significantly lower foliar N:P than in the control treatment (Fig. 4).

The N addition alone and N + P treatments decreased soil pH significantly in both years (Fig. 5). Soil pH also decreased significantly with P addition in 2007, but less than the response observed with N addition (Fig. 5). In 2006, P addition significantly increased available P by 152.48 % when added in combination with N and by only 21.47 % without N additions (Fig. 5), and similar enhancements were observed in 2007, with 143.79 % and 19.61 % increases, respectively. The community aboveground biomass did not respond to nutrient treatments significantly both in 2006 and 2007 (Fig. 6),

however there were increasing trends with N addition (16.7 %) and with N + P addition (28.3 %).

Plant stoichiometry was sensitive to soil pH and available P at both the community- and species-level. More specifically, N in community biomass was significantly positively related to available P (Table 1). The P pool was negatively related to soil pH and positively related to available P (Table 1). Community N:P showed a positive correlation with soil pH and a negative correlation with available soil P (Table 1). At the species level, most of the negative correlations between foliar N and soil pH were not significant, while significantly positive correlations between foliar N in most species and available P were observed (Table 2). Foliar P in most species was negatively related to soil pH and positively related to available P (Table 2). In particular, foliar P concentrations in all 13 species were significantly positively correlated with available P (Table 2). Foliar N:P ratios were positively and negatively correlated with soil pH and available P in almost all species, respectively (Table 2).

Discussion

In the temperate grassland of Inner Mongolia studied here, N + P additions generally increased plant nutrient concentrations (or pools) and decreased N:P ratios at both community- and species-levels, while neither N nor P addition alone changed plant stoichiometry. The uptake of added P by plants was promoted by N availability at both levels of biological organization, in that P uptake was increased by P addition combined with N addition, while no response was observed in the absence of N. Furthermore, this pattern seems to be robust across years, as similar responses were observed for many species in both 2006 and 2007.

We found no significant effects of N addition alone on plant nutrients or stoichiometry at the community- or species-levels in either year of the study. This may, in part, be due to the relatively low N addition rate ($2.8 \text{ g N m}^{-2} \text{ yr}^{-1}$) imposed when compared to other studies adding N at rates of $10 \text{ g N m}^{-2} \text{ yr}^{-1}$ or higher (Han et al. 2014; Lü et al. 2012). In addition, responses of foliar chemistry to atmospheric N deposition are generally less apparent in grassland ecosystems than that in other ecosystems (Phoenix et al. 2012). The lack of change in plant nutrients and N:P with the addition of N alone suggests that low rates of N deposition alone

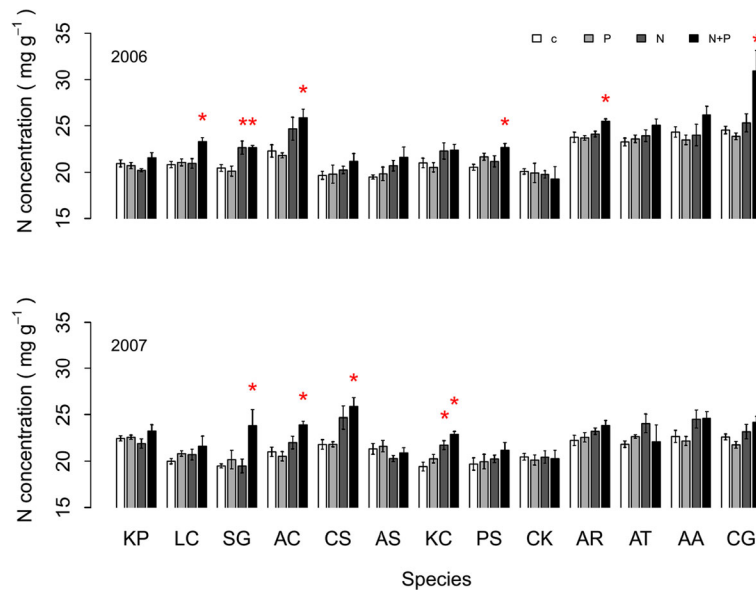


Fig. 2 Effects of N and P additions (c: control, N: N addition, P: P addition, N + P: both N and P addition) on foliar N concentrations of 13 plant species in the Inner Mongolia grassland. Data are shown as means ± SE. Bars with asterisks are significantly different from corresponding control treatments ($P < 0.05$). KP: *Kochia prostrata*, LC: *Leymus chinensis*, SG: *Stipa grandis*, AC:

Agropyron cristatum, CS: *Cleistogenes squarrosa*, AS: *Achnatherum sibiricum*, KC: *Koeleria cristata*, PS: *Poa sphondyloides*, CK: *Carex korshinskyi*, AR: *Allium ramosum*, AT: *Allium tenuissimum*, AA: *Axyris amarantoides*, CG: *Chenopodium glaucum*

would not result in N-P imbalance in temperate grasslands, at least within a short time scale. While there is increasing evidence that long-term low-level N deposition could result in changes in diversity, productivity and community composition (Clark and Tilman 2008; Phoenix et al. 2012), it is still an open question whether

the responses of foliar nutrient concentrations or pools in community would be changed by low rates of N loading in the long-term.

No pronounced effects of P addition alone on plant N or P or N:P was found for almost all species or for the community. Although many studies have reported that P

Fig. 3 Effects of N and P additions (c: control, N: N addition, P: P addition, N + P: both N and P addition) on foliar P concentrations of 13 plant species in the Inner Mongolia grassland. Data are shown as means ± SE. Bars with asterisks are significantly different from corresponding control treatments ($P < 0.05$). See Fig. 2 for species full names

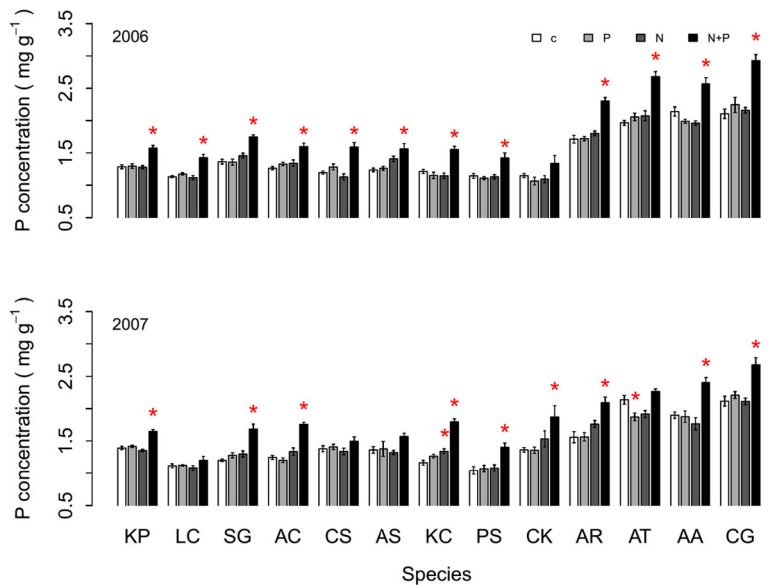
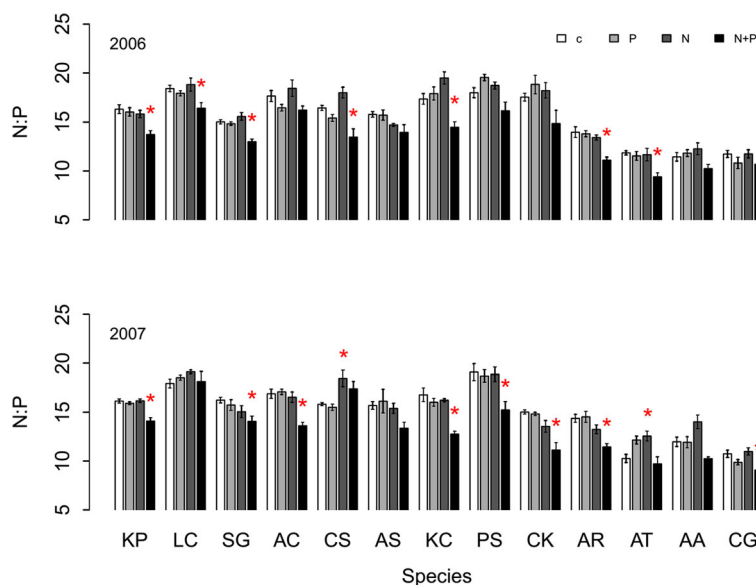


Fig. 4 Effects of N and P additions (c: control, N: N addition, P: P addition, N + P: both N and P addition) on foliar N:P of 13 plant species in the Inner Mongolia grassland. Data are shown as means \pm SE. Bars with asterisks are significantly different from corresponding control treatments ($P < 0.05$). See Fig. 2 for species full names



fertilization generally increases plant P nutrition in forest ecosystems (Prietz et al. 2008; Prietz and Stetter 2010), our results suggested that the mild increases in

available P after low-level P addition ($1.55 \text{ g P m}^{-2} \text{ yr}^{-1}$) observed in the semi-arid grassland studied here failed to affect plant P uptake at both community- and species-levels. This result further bolsters support for the findings of a transect investigation showing that leaf P concentrations across Inner Mongolia grasslands in China were not correlated with soil P availability

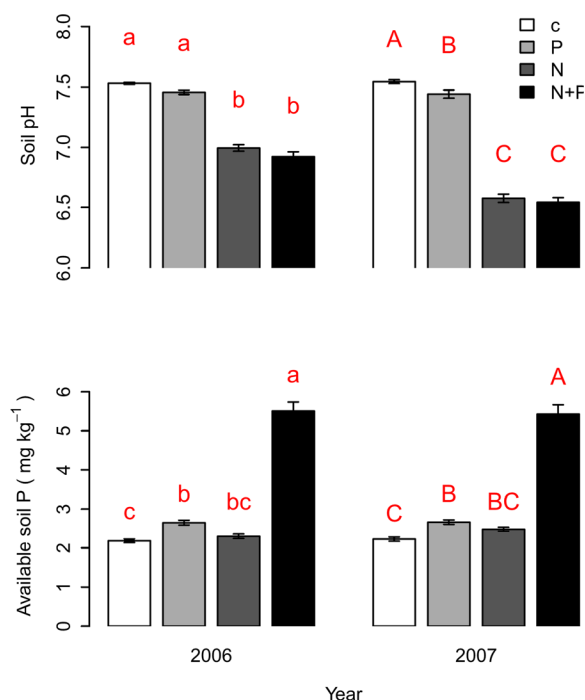


Fig. 5 Effects of N and P additions (c: control, N: N addition, P: P addition, N + P: both N and P addition) on soil pH and available soil P concentrations in the Inner Mongolia grassland. Data are shown as means \pm SE. Different letters above bars denote significant differences among treatments ($P < 0.05$)

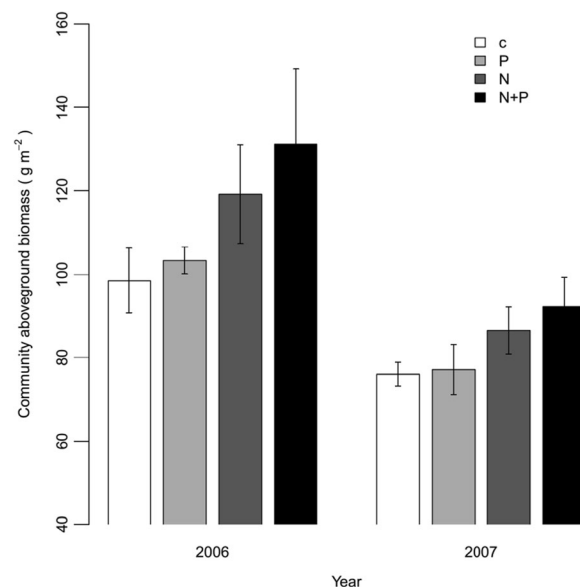


Fig. 6 Effects of N and P additions (c: control, N: N addition, P: P addition, N + P: both N and P addition) on community aboveground biomass in the Inner Mongolia grassland. Data are shown as means \pm SE

Table 1 Pearson's product-moment correlation (*r*-values) between nutrient pools (N pools, P pools and N:P) in community biomass and soil chemistry (soil pH and available P)

Soil parameter	N pool	P pool	N:P
Soil pH	-0.222	-0.385 **	0.555 ***
Available P	0.385 **	0.605 ***	-0.708 ***

P* < 0.01; *P* < 0.001

based on the data of 57 species at 32 sites (Geng et al. 2011). Another experiment conducted in our study area showed that the organ biomass and total biomass of *L. chinensis*, the dominant species in the community, also did not respond to P addition (Zhang 2003). Collectively, these results, together with the responses of aboveground biomass in this study, suggest that plant growth in our focal grassland are not limited by P availability. This may be because in the area we studied, the concentration of exchangeable Ca²⁺ in surface soils (0–18 cm) is as high as about 0.15 % (Office and Service 1994). Thus, the added P could be easily bound to these abundant Ca²⁺ and become unavailable to plants (Vitousek et al. 2010). Given that the responses of plant P to fertilization could change with time (Li et al. 2016), the P concentrations in plant tissues and P pools in community biomass in response to P additions may be

altered if continued over the long-term. Yet, such long-term studies are currently lacking in Inner Mongolia grassland systems.

Although responses to N and P additions alone were weak, the effects of P addition on plant nutrient concentrations and nutrient pools were amplified greatly by N addition in both years, confirming our expectation. However, the significant changes in plant stoichiometry after N + P treatment were not accompanied by biomass responses in both 2006 and 2007, but there was a tendency for biomass to increase with N + P addition (28.3 % compared to the control treatment), with this effect significant after 4 years of treatment (unpublished data). This delayed response is consistent with the results from European wet meadows (Olde Venterink et al. 2001). These results suggest that the community is co-limited by N and P, as observed in many natural ecosystems (Fay et al. 2015; Lannes et al. 2016). It could also be classified as 'serial limitation' according to Harpole et al. (2011), because community biomass increased significantly with a higher level of N addition (Bai et al. 2010) and with N + P addition after 4 years of treatment (unpublished data). Previous studies in this area also suggested that ecosystem productivity may be N and P co-limited and mainly limited by N availability (Bai et al. 2010; Lü et al. 2013; Yu et al. 2010).

Table 2 Pearson's product-moment correlation (*r*-values) between foliar nutrients (N concentrations, P concentrations and N:P) in 13 species and soil chemistry (soil pH and available P)

Species	Foliar N		Foliar P		Foliar N:P	
	Soil pH	Available P	Soil pH	Available P	Soil pH	Available P
<i>Kochia prostrata</i>	-0.178	0.321 *	-0.459 **	0.838 ***	0.433 **	-0.769 ***
<i>Leymus chinensis</i>	-0.278	0.470 **	-0.159	0.584 ***	-0.084	-0.322 *
<i>Stipa grandis</i>	-0.315*	0.443 **	-0.500 ***	0.766 ***	0.410 **	-0.629 ***
<i>Agropyron cristatum</i>	-0.388 **	0.486 ***	-0.609 ***	0.837 ***	0.383 **	-0.588 ***
<i>Cleistogenes squarrosa</i>	-0.571 ***	0.358 *	-0.240	0.634 ***	-0.350 *	-0.270
<i>Achnatherum sibiricum</i>	-0.085	0.130	-0.368 *	0.554 ***	0.365 *	-0.496 ***
<i>Koeleria cristata</i>	-0.555 ***	0.459 **	-0.648 ***	0.862 ***	0.424 **	-0.714 ***
<i>Poa sphondylodes</i>	-0.162	0.390 **	-0.390 **	0.762 ***	0.364 *	-0.652 ***
<i>Carex korshinskyi</i>	-0.043	-0.165	-0.535 ***	0.458 **	0.516 ***	-0.517 ***
<i>Allium ramosum</i>	-0.274	0.413 **	-0.531 ***	0.774 ***	0.555 ***	-0.736 ***
<i>Allium tenuissimum</i>	-0.093	0.073	-0.263	0.693 ***	0.162	-0.552 ***
<i>Axyris amaranthoides</i>	-0.323 *	0.341 *	-0.245	0.725 ***	-0.012	-0.522 ***
<i>Chenopodium glaucum</i>	-0.175	0.541 ***	-0.375 **	0.822 ***	0.213	-0.333 *
All species	-0.221***	0.276***	-0.218***	0.375***	0.159***	-0.320***

P* < 0.05; *P* < 0.01; ****P* < 0.001

However, under the case of simultaneous co-limitation (sensu Harpole et al. 2011), addition of either N or P alone normally causes increased N or P uptake without a response in biomass production (Olde Venterink et al. 2001).

The correlations between plant nutrition and soil chemistry indicated that increases in P uptake, the key outcome of this study, may be accounted for by the significant changes in soil properties under the combined N and P addition, though changes in soil pH were also observed with N addition alone. Phosphorus availability in the soil increased dramatically in the N + P treatment, which may directly lead to the enhancement in P uptake at both the species- and community-levels in this treatment. The unique response of P availability in the N + P treatment suggested that the increased N supply may prevent the precipitation of additional P with soil Ca^{2+} . The significant decrease in soil pH could result in increases in P availability because increases in soil acidity may increase the mobility of P. A similar increase in P availability after N addition has also been supported by other studies: experiments conducted in Inner Mongolia and British grasslands, as well as a glasshouse research showed that the activity of phosphomonoesterase and phosphodiesterase were generally positively correlated with N addition rates (Johnson et al. 1999; Olde Venterink 2011; Zhang et al. 2013). These enzymes would contribute to plant P uptake and assimilation by mobilizing P from ester-bonded forms and producing phosphate ions that were available for plant uptake (Sinsabaugh et al. 2008). Besides the increase in available soil P, the increased plant N concentration after N additions may have contributed to an enhancement in P uptake because of the physiological mechanism of N:P homeostasis in plant species and in the community (Yu et al. 2010). This enhanced uptake ultimately leads to higher foliar P concentrations and P pools after N and P are added in combination.

Previous studies have showed that P limitation is widespread in terrestrial ecosystems and it is predicted that more than 50 % of the mineable P-reserve will be depleted by mid-late twenty-first century (Elser et al. 2007; Peñuelas et al. 2013; Vitousek et al. 2010). This highlights the ecological significance of P uptake efficiency in plants. Our results suggest that N deposition might provide an advantage for plants (particularly for the faster growing species but less for the slower growing species, Yu et al. 2015) by enhancing P uptake in P fertilized semi-arid grasslands, although it may also

substantially influence ecosystem structure and function through changes in stature and nutrient content of plants and ecosystem-level processes such as carbon sequestration. In this study, we only investigated nutrients in leaf tissue, without data on roots, stems and reproductive organs. Future research comparing the differences in the stoichiometric response to nutrient addition among organs would provide a more accurate estimation of the responses at the community level. We note that our results might have limited implications on the effects of N deposition, because urea was used in this study and deposition occurs mainly in other forms of N. However, previous studies suggest that N fertilizer form may have limited effects on plant responses to N addition (Tian et al. 2016), although they had a significant impact on other ecosystem properties, such as soil carbon process (Du et al. 2014). As long-term N deposition also changes soil properties that are related to P availability, such as soil acidity and base cation status (Horswill et al. 2008; Phoenix et al. 2012), the effect of N deposition on P fertilization might be consistent across different temporal scales, and P limitation as a consequence of increased N deposition might be less important for limiting plant growth than previously anticipated.

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