Phosphorus does not alleviate the negative effect of nitrogen enrichment on legume performance in an alpine grassland

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Abstract

Aims

Nitrogen (N)-fixing legumes, despite being highly phosphorus (P)-demanding, constitute an important plant functional group and play key roles in N-poor ecosystems such as alpine grasslands. However, legume performance, including biomass, abundance and species richness, is expected to change, because anthropogenic activities have drastically increased soil N and P availability worldwide. We conducted a field experiment to assess the effects of N and P addition, alone and in combination, on legume performance in an alpine grassland, and identified and clarified the mechanisms underlying these changes.

Methods

A three year field experiment of N addition ($10\,g\,N\,m^{-2}\,year^{-1}$), P addition ($5\,g\,P\,m^{-2}\,year^{-1}$), and N + P combined addition (both N and P, same amounts as solo treatments) was conducted in an alpine grassland on the Tibetan Plateau in China from 2011 to 2013. Effects of nutrient addition were assessed at the community level (aboveground net primary production (ANPP), height and light intensity), functional group level (biomass, species richness, relative height, relative coverage and relative density of legumes) and species level (foliar N, P concentration of two legumes).

Important findings

Overall, adding N alone significantly increased ANPP by 20.82%, but adding P alone did not; whereas, addition of N and P together resulted in a large increase in ANPP (+37.03%) than addition of either alone, indicating potential co-limitation of alpine grasslands. In contrast, adding P alone significantly promoted legume performance as measured by 65.22% increase in biomass and 58.45% increase in relative abundance, while adding N alone reduced legume performance as measured by 39.54% decrease in biomass and 50.36% in relative abundance. Combining P and N addition did not mitigate the negative effect of N addition on legume performance and, surprisingly, suppressed legume biomass by 53.14% and relative abundance by 63.51%. N and P addition altered the balance of light competition between grasses and legumes as indicated by the changes in light levels, plant heights and litter accumulation. However, there were no obvious changes in legume species richness in response to N and P within our experimental timeframe. This study provides further evidence of the importance of P as a co-limiting nutrient in alpine grasslands, contrary to the traditional view that N limitation predominates in such regions. The contrasting effects of N and P addition on legume performance provide important insights into potential changes in legume performance in nutrient-limited grasslands following N and P enrichment under climate change, with implications for nutrient management in alpine grasslands.

INTRODUCTION

Co-limitation by nutrients, especially by nitrogen (N) and phosphorus (P), is increasingly recognized in terrestrial eco-systems worldwide (Elser *et al.* 2007; Harpole *et al.* 2011). Many nutrient manipulation studies have been conducted globally to test the hypothesis that nutrients are co-limiting (e.g. Nutrient Network, see Fay *et al.* 2015). However, com-parable evidences for alpine grasslands is relatively limited. Although the responses of community performance (e.g. biomass, richness and abundance) as a whole to nutrient additions have been well documented in past decades, how plant functional groups respond to nutrient addition remains poorly understood. Plant functional groups may differ in their responses to nutrient addition (Avolio *et al.* 2014; Isbell *et al.* 2013; Pennings *et al.* 2005; Suding *et al.* 2005). Therefore, it is necessary to understand the effects of nutrient addition on the performance of plant functional groups, especially leg-umes, which strongly affect N and P availability, but have not been well studied in alpine grasslands.

Legumes can fix N by means of symbionts (Bordeleau and Prévost 1994), and transfer a portion of the fixed N to non-leguminous neighbours (Temperton *et al.*, 2007), thus playing an important role in terrestrial ecosystem functions (Spehn *et al.* 2002; Vitousek *et al.* 2002), especially in typi-cally N-limited regions such as alpine grasslands (Jin *et al.* 2013; Soudzilovskaia *et al.* 2012; Spehn *et al.* 2002; Yang *et al.* 2011a).

Phosphorus availability could greatly affect the productivity and abundance of legumes (Vitousek and Field 1999). On the one hand, legumes require P for growth, root development, and formation of nodules with symbionts (Batterman *et al.* 2013; Novotny *et al.* 2007); on the other hand, the symbionts also demand P-rich molecules because of the high energy cost of the nitrogenase reaction (Augusto *et al.* 2013; Divito and Sadras 2014). However, soil-available P is scarce in most eco-systems (Han *et al.* 2005), limiting legume performance and abundance.

Increased anthropogenic activities have enhanced avail-ability of N and/or P even in typically nutrient-poor ecosys-tems such as alpine grasslands (Galloway *et al.* 2008; Peñuelas *et al.* 2012; Xu *et al.* 2014). Nevertheless, studies have still not explicitly test how changes in soil N and P availability would affect legume performance (e.g. production, species richness and abundance).

Previous studies have indicated that N addition suppresses legume performance as measured by lowered production, abundance and richness (Avolio *et al.* 2014; Isbell *et al.* 2013) via competition from faster growing grasses for resources, such as light (Bobbink 1991; Hautier *et al.* 2009; Suding *et al.* 2005) and water (Bobbink 1991). Furthermore, N addition also suppresses legumes by N-induced soil acidification (Lu *et al.* 2011). More importantly, because of the higher rate of N deposition than that of P deposition (Peñuelas *et al.* 2012; Vitousek *et al.* 2010), increased N availability in soils would shift the intrinsic N-vs.-P-limitation balance (Elser *et al.* 2009; Peñuelas *et al.* 2012), resulting in intense competition between plants, and a depletion of soil P, which is critical for legumes because of their high P-demand (Augusto *et al.* 2013; Silvertown *et al.* 2006; Spehn *et al.* 2002).

Despite the limiting nature of P on legume function, few studies have examined how legumes would respond to P enrichment in P-poor regions. Furthermore, we know even less about the interactive effects of N and P enrichment on legume performance. Specifically, it is unclear whether P add-ition could alleviate the negative effects of N enrichment on legumes when both N and P are added to such potentially N and P co-limited areas. Results from the few existing stud-ies have been equivocal, reporting positive (Grünzweig and Korner 2003), neutral (Avolio *et al.* 2014) and negative (Sun *et al.* 2016) interactive effects of N and P additions on legume biomass, and none have directly addressed legume perform-ance in the context of combined N and P enrichment, espe-cially for alpine grasslands on the Tibetan Plateau (but see Sun *et al.* 2016).

The Tibetan Plateau is an ideal location for examining the effects of N and P on legume performance in alpine grass-lands. These grasslands are nutrient-poor due to lower decomposition and mineralization rates, although researchers historically assumed that they are mainly N- but not P-limited because the soil is relative young (Reich and Oleksyn 2004). In this study, we took advantage of these suitable conditions to assess legume performance in response to N and P enrich-ment. We hypothesized that: (i) N addition suppresses legume performance, while (ii) P addition promotes legume perfor-mance, (iii) combining P addition with N addition alleviates the negative effects of N on legumes and (iv) nutrient addi-tions change the species richness of legumes as predicted by the literature.

METHODS

Study site

The study site was an alpine meadow near the Haibei National Field Research Station of Alpine Grassland Ecosystem, Northwest Institute of Plateau Biology, Chinese Academy of Sciences (37°37′N, 101°12′E; altitude: 3250 m), in Qinghai Province. This site has been overgrazed in the past but has been fenced and only grazed during non-growing seasons since 2001. The mean annual temperature is −1.1°C (1981–2010). Mean annual precipitation is 490 mm, of which more than 80% occurs during the growing season from May to September (Wang *et al.* 2014; Zhao and Zhou 1999). The soil is classified as Gelic Cambisols (FAO classification); soil characteristics are presented in Supplementary Table S1. The vegetation type is a typical *Kobresia humilis* (C.A.Mey. ex Trautv.) Serg. meadow. The dominant grasses species are *Stipa aliena* Keng, *Elymus nutans* Griseb. and *Festuca ovina* L. The leg-umes found in the experimental site include *Medicago archi-ducis-nicolai* Sirj., *Tibetia himalaica* (Baker) H.P.Tsui, *Oxytropis*

kansuensis Bunge, *O. qinghaiensis* Y.H. Wu, *Astragalus chilienshanensis* Y.C.Ho and *Thermopsis lanceolata* R.Br.

Experimental design

In May 2009, we fenced an experimental area of 1 ha to exclude grazing disturbance, and in May 2011, we started a fertilization experiment. A completely randomized design was used with four treatments comprising four replicates each (6×6 m per plot, 16 plots in total). The four treatments were as follows: Control (with no nutrient addition); N addition (urea, 10 g N m^{-2} year^{-1}); P addition (superphosphate, 5 g P m^{-2} year^{-1}) and NP addition (both N and P, same amounts as solo treatments). Plots received N and/or P amendments were applied three times each year. The granular fertilizer was divided into three equal parts and was applied by hand broadcasting on 1 June, 1 July and 1 August in 2011 and 2012, and 1 June, 31 June, and 2 August in 2013.

Plant sampling and analysis

In mid-August of each year, a 50×50 cm quadrat was randomly selected within each plot for biomass harvest. Aboveground parts of plants were clipped at the ground surface and then sorted by species before dry-weight measurement (drying at 65°C for >48 h), and above-ground net primary production (ANPP) of the community and plant functional groups (grasses, sedges, legumes and forbs) were determined. Standing dead matter and litter in quadrats were also collected and measured as litter stock.

In 2012 and 2013, we estimated pre-harvest plant heights by species in quadrats in each plot, then calculated community height, and the relative height of legume functional group. Individual plant heights were measured using steel tape measures. Community height was the average of species' heights. Relative height of legumes was calculated as the average height of legumes divided by the community height. Similar data for other functional groups were also collected but are not reported in detail in this paper.

In the 2012 growing season, light transmission through the canopy was measured above and below the canopy (approximately at the soil surface) using a Quantum PAR meter (Apogee MQ-200 PAR Meter, USA) on three sunny days. Light intensity (percentage) under the canopy was calculated as the ratio of light intensity below to that above the canopy.

We also determined foliar N and P concentrations of two main legume species (*T. himalaica* and *M. archiducis-nicolai*) in 2012. Total N content was assayed using an elemental analyzer (2400 II CHNS/O Elemental Analyzer; Perkin-Elmer, Boston, MA, USA). Total P content was determined by the molybdate colorimetric method after ascorbic acid reduction (Murphy and Riley 1962).

Statistical analysis

We tested the effects of N, P, year, and their interactions on measured variables using a linear-mixed effects model, with 'block' as a random factor. In general, only significant treatment effects and their interactions are reported. When the analysis indicated that there were significant N × year, P × year or N × P × year interactions, we ran separate analyses of variance for each year, taking N and P as main factors, and block as a random factor. When *F*-tests were significant, means were compared using Tukey's HSD test. Significance was set at *P* < 0.05. Analyses were performed in R software version 3.0.1 (R Development Core Team 2013), using the 'nlme' (Pinheiro *et al.* 2013) and 'multcomp' (Hothorn *et al.* 2008) package. In addition, relationships between legumes biomass and the standing litter biomass were also determined by linear regression analysis using R. Graphs were created using SigmaPlot (version 12.5, Systat Software, Inc., Point Richmond, CA).

RESULTS

Response at community level

There were significant effects of nutrients, year and their interactions on ANPP (Table 1), and these effects were mostly similar in the three years, although the trends were generally stronger and more significant in 2011 and 2012 (Fig.1a). Overall, N addition alone significantly increased ANPP by 20.82%, but P alone did not; N and P combined resulted in larger increases of ANPP (+37.03%) than either N or P alone (Fig. 1a).

Nutrient additions led to an asymmetry changes in plant heights among species and thus community height as ANPP changed. Average across 2012 and 2013, combined NP addition, but not addition of either nutrient alone, significantly increased community height, as indicated by a significant N × P interaction (*P* = 0.019) (Table 2; Fig. 1b).

Legume performance

Legume biomass was significantly affected by nutrients, year and their interactions (Table 2; Fig. 2a). Legume biomass

Table 1: Effects of nitrogen (N, 0 and 10 g m^{-2}) and phosphorus (P, 0 and 5 g m^{-2}) additions over time (year) on ANPP of community and community height from 2011 to 2013

Source	df	ANPP		df	Community height	
		F	*P*		*F*	*P*
N	1.33	57.53	*<0.001*	1.21	33.20	*<0.001*
P	1.33	8.09	*0.007*	1.21	13.49	*0.001*
N × P	1.33	2.959	0.095	1.21	6.50	*0.019*
Year	2.33	111.37	*<0.001*	1.21	46.74	*<0.001*
N × year	2.33	12.38	*<0.001*	1.21	35.66	*<0.001*
P × year	2.33	0.93	0.406	1.21	0.43	0.522
N × P × year	2.33	8.372	*0.001*	1.21	4.30	**0.038**

Results are from mixed-effects model for repeated-measures ANOVA. Significant differences (*P* < 0.05) are bolded and italicized. Abbreviations: ANOVA = analysis of variance; df = degree of freedom.
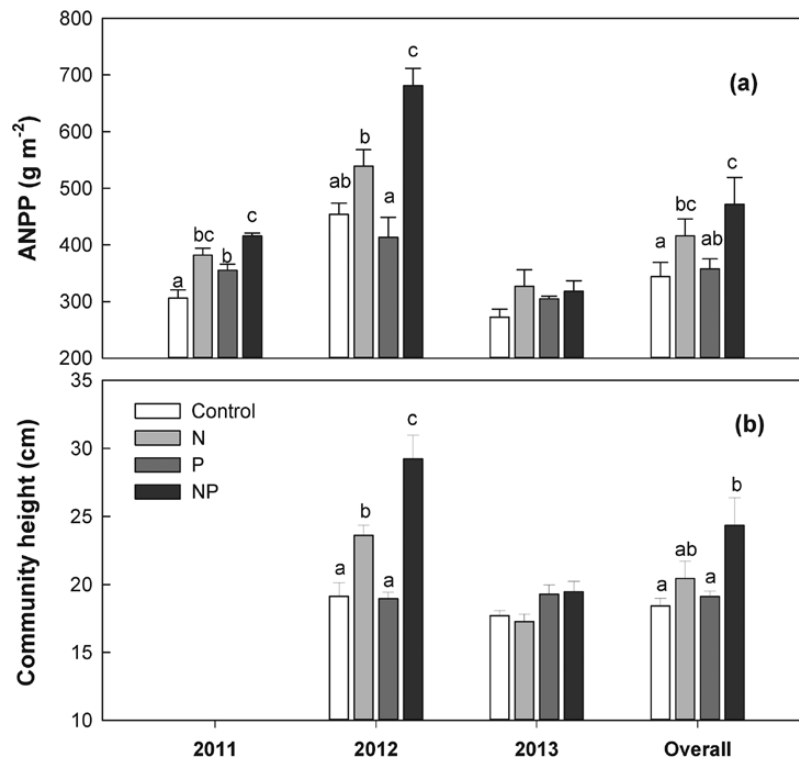
Figure 1: Effects of N and P additions on ANPP of community (**a**), community height (**b**) from 2011 to 2013 (mean + SE). Letters denote significant differences at *P* < 0.05. Abbreviation: SE, standard error.

Table 2: Effects of nitrogen (N, 0 and 10 g m^{-2}) and phosphorus (P, 0 and 5 g m^{-2}) additions over time (year) on biomass, richness and relative height of legumes from 2011 to 2013

Source	df	Biomass		df	Relative height		df	Richness	
		F	*P*		*F*	*P*		*F*	*P*
N	1,33	126.43	*<0.001*	1.21	21.75	*<0.001*	1,33	1.401	0.245
P	1,33	13.51	*<0.001*	1.21	2.20	0.153	1,33	0.623	0.436
N × P	133	8.21	*<0.001*	1.21	0.51	0.485	1,33	0.156	0.696
Year	2,33	31.51	*<0.001*	1.21	13.61	*0.001*	2,33	3.541	*0.040*
N × year	2,33	7.53	*0.002*	1.21	4.62	*0.040*	2,33	0.817	0.450
P × year	2,33	2.13	0.135	1.21	0.01	0.913	2,33	0.272	0.763
N × P × year	2,33	3.11	0.058	1.21	5.44	**0.030**	2,33	0.506	0.608

Results are from mixed-effects model for repeated-measures ANOVA. Significant differences (P < 0.05) are bolded and italicized. Abbreviations: ANOVA = analysis of variance; df = degree of freedom.

ranged from 4.93 g m^{-2} to 83.56 g m^{-2}, contributing to 1.4–28.70% of ANPP. Across the three years, the average legume biomass decreased by 39.54% with N addition alone, but increased by 65.22% with P addition alone. Surprisingly, legume biomass decreased by 53.14% with NP addition, more than the decrease with N addition alone. Accordingly, the legume biomass percentage increased by 69.25% with P addition, but decreased by 50.24% with N addition alone, and by 63.51% with NP addition (Supplementary Table S2 and Fig. S1a).

Nutrient addition did not significantly influence legume height. However, nutrient addition altered the relative height of legumes compared with the mean community height (relative height, hereafter) (Table 2; Fig. 2c). Across 2012 and 2013, N addition significantly decreased the relative height by 21.36%, but P and NP addition did not (Fig. 2c).

Additionally, relative coverage and relative density of legumes followed similar trends as legume biomass and biomass percentage. Across 2012 and 2013, N addition significantly decreased the relative coverage of legumes by 38.73%, P addition significantly increased it by 74.72%, while NP addition significantly decreased it by 51.10% (Supplementary Table S2 and Fig. S1b). Similarly, across 2012 and 2013, N addition significantly decreased relative density of legumes by 52.45%,
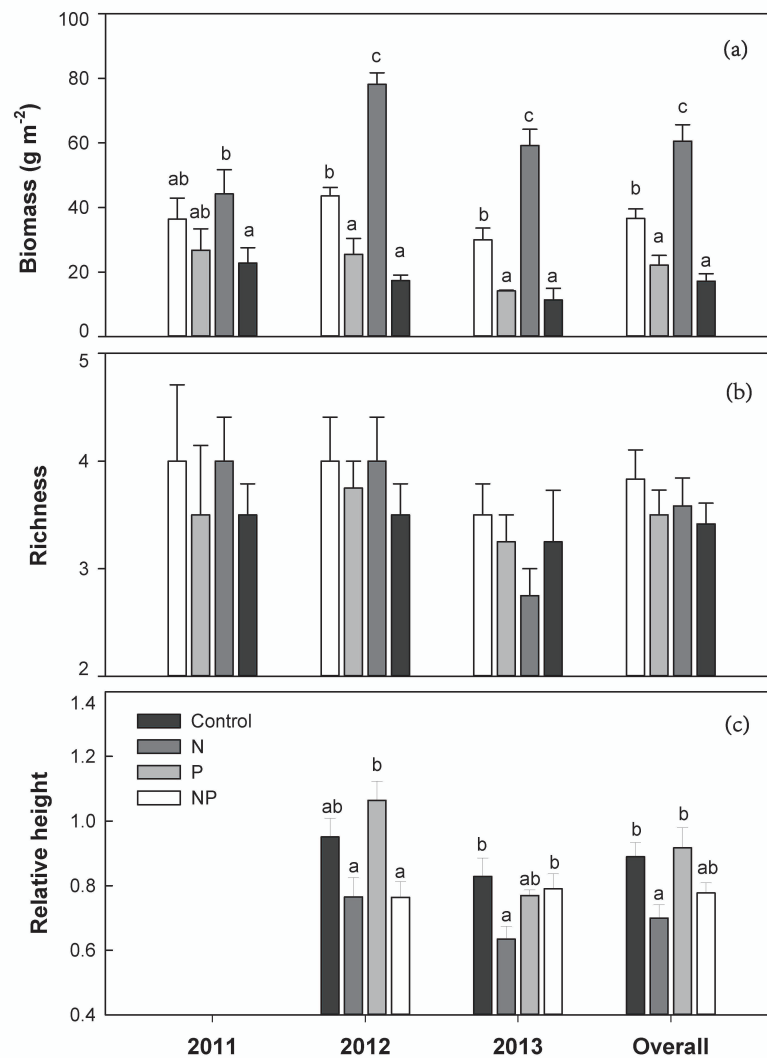
Figure 2: Effects of N and P additions on above-ground biomass of legumes (legume biomass, **a**), richness (**b**) and relative height (**c**) of legumes from 2011 to 2013 (mean + SE). Letters denote significant differences at $P < 0.05$. Abbreviation: SE, standard error.

P addition significantly increased it by 49.36%, while NP addition significantly decreased it by 61.51% (Supplementary Table S2 and Fig. S1b and c).

Contrary to the significant effects of treatments on legume biomass and height, the average species richness of legumes was 3.6, and no significant species loss was found across all treatment (Table 2; Fig. 2b). Moreover, although legume richness was different across the three years ($F_{2,23} = 3.541$, $P = 0.040$), no significant interactive effect of nutrient vs. year on richness was found. In addition, N and P additions had neither main effects nor interaction effects.

Nutrient-induced changes in productivity and plant height, altered light penetration in the canopy considerably. In 2012, addition of both nutrients significantly decreased the light intensity in the lower canopy (Fig. 3).

With increase in ANPP after nutrient addition, more standing litter accumulated and created more shade. There was a negative correlation between legume biomass and litter stock (Fig. 4, $R^2 = 0.22$, $P < 0.001$).

Two common legumes species in all plots, *T. himalaica* and *M. archiducis-nicolai* together constitute approximately 75% of total legume biomass. N addition alone significantly increased foliar N concentration [N] of *T. himalaica*, but had no effect on [N] of *M. archiducis-nicolai* (Fig. 5a and b), indicating that legumes' [N] response are species-specific. Moreover, the interaction between N and P was significant, indicating that the response of foliar [N] to N addition depended on whether or not the P was co-added. However, N addition alone did not significantly affect the foliar P concentration [P] of either species (Fig. 5c and d). In contrast, changes of [P] in plots with P addition were more
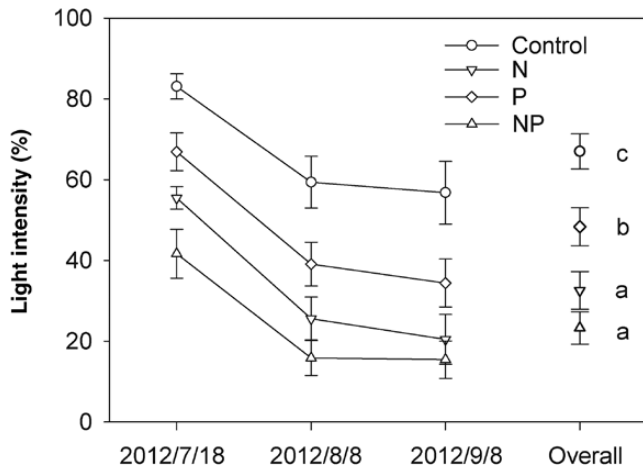
Figure 3: Effects of N and P additions on light intensity under the canopy in 2012.
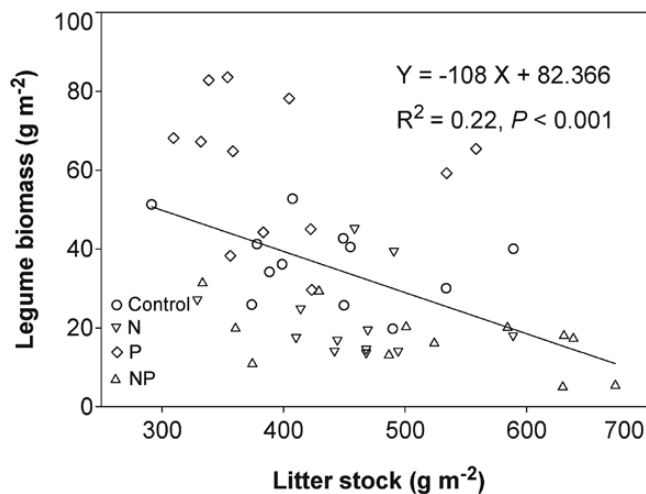

Figure 4: Relationship between litter stock and legume biomass across all years. The solid line represents the linear regression used to estimate legume biomass from standing litter biomass.

distinct: for both species, addition of either P alone or NP significantly increased [P], but had no effect on [N].

DISCUSSION

After adding N and P to an alpine grassland on the Tibetan plateau for three years, we observed that N and P, both alone and in combination, drastically changed the performance of legumes. Our results were consistent with some, but not all, of our hypotheses.

Negative effects of N addition on legumes

In support of our first hypothesis, N addition significantly suppressed legume biomass. This result is consistent with the observations in Park Grass Experiments (Bobbink 1991), a temperate grassland (Yang *et al.* 2011b) and an alpine

grassland in the same region (Song *et al.* 2012; Sun *et al.* 2016; Xu *et al.* 2014). Several factors may have caused this effect. First, as in previous studies, N increased ANPP (Fig. 1a) and community height (Fig. 1b), but decreased light transmission (Fig. 3) and thereby shifted the competitive advantage for light (Bobbink 1991; Hautier *et al.* 2009; Silvertown *et al.* 2006; Vitousek and Field 1999) among different functional groups, especially grasses and legumes (Suding *et al.* 2005; Xu *et al.* 2014), and thus decreased legume biomass. Moreover, the consistent responses of relative coverage and relative density to N addition (Supplementary Fig. S1b and c), could also partially explain such 'top–down' control of legume performance by light.

Second, we speculated that N addition aggravated P limitation of legumes' growth and N-fixation, because grasses would need more P for N-induced faster growth. However, when supplied with sufficient N, grasses have the upper hand against legumes in competition for P (Bobbink 1991; Hacker *et al.* 2015; Silvertown *et al.* 2006).

Third, previous studies indicated that N-induced soil acidification (Lu *et al.* 2011) also suppressed legume performance. However, after four years nutrients addition, we did not observe significant soil acidification in this study (Supplementary Table S1 and Fig. S2), suggesting that soil acidification was unlikely to have influenced legume performance, at least during the study period.

Positive effects of P addition on legumes

In support of our second hypothesis, we found that P addition significantly increased legume biomass and other performance metrics. It is clear that the enhancement of soil P availability is the most likely factor driving these changes (see foliar [P] of two main legume species in Fig. 5c and d). These results are consistent with previous reports (Bobbink 1991; Grünzweig and Korner 2003; Novotny *et al.* 2007), and provide strong evidence that legumes growing in alpine grasslands are severely P limited.

In addition, we speculated that the inherent N limitation of other non-leguminous plants, which must rely solely on soil nitrogen, presented a favourable condition for legumes to fix nitrogen and get a competitive edge in light exposure to increase productivity (Suding *et al.* 2005; Temperton *et al.* 2007). Moreover, N addition increased the foliar [N] in *T. himalaica* (Fig. 5a) in 2012, suggesting that legumes in alpine grassland may co-limited by soil P and N content, but P's essential role in N-fixation probably makes it the ultimate limiting nutrient for legume production. This speculation was consistent with our results that P addition only increased legume biomass (Fig. 2a), but did not increase ANPP (Fig. 1a). These results are inconsistent with results from previous studies that legumes promoted ANPP and N availability (Marquard *et al.* 2009; Mulder *et al.* 2002; Oelmann *et al.* 2011). Our results on legume biomass and ANPP have three implications. First, at severe P limitation, legumes growing in N-poor alpine grasslands rely strongly, but not completely, on N-fixation
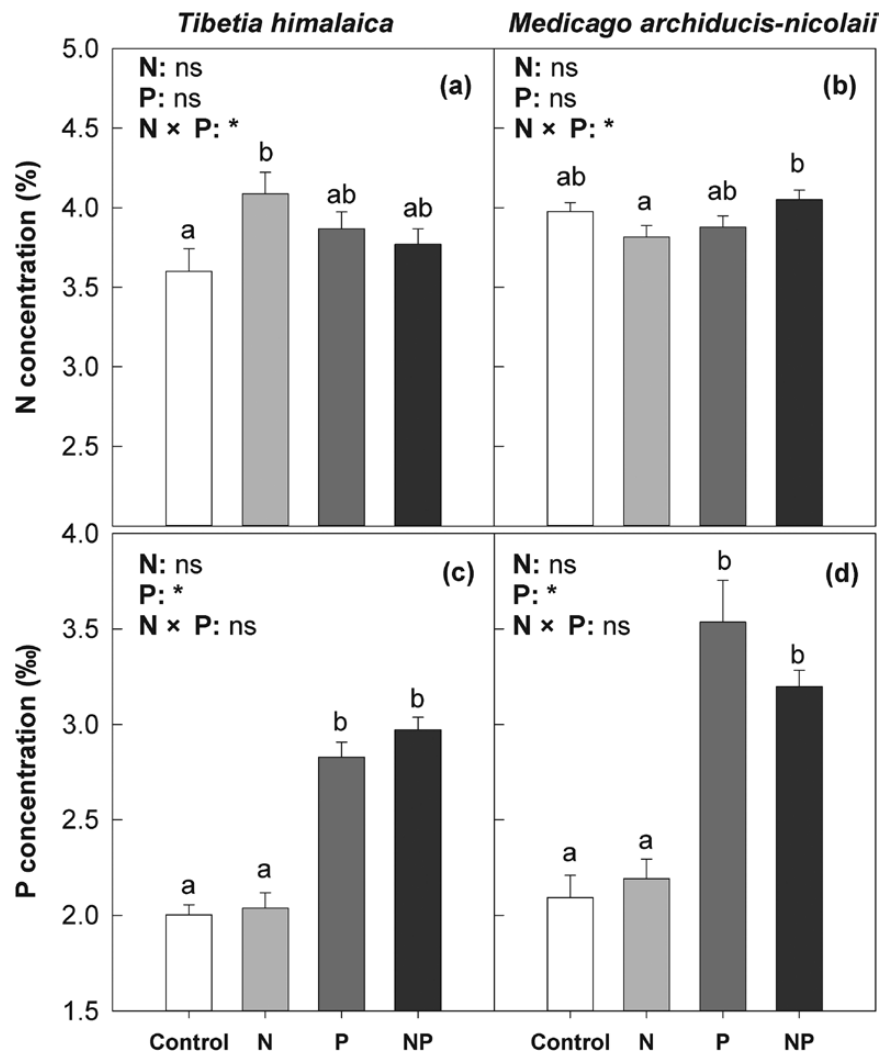
Figure 5: Effects of N and P additions on foliar N (**a**, **b**), P concentrations (**c**, **d**) of two legumes, *Tibetia himalaica* (left) and *Medicago archiducis-nicolai* (right) (mean + SE) in 2012. ANOVA *P*-values are reported (ns: no significant, *P < 0.05). Letters denote significant differences at *P* < 0.05. Abbreviation: ANOVA, analysis of variance; SE, standard error.

(Liu *et al.* 2013; Xu *et al.* 2014). Second, despite facilitation of legume growth by P addition, the low soil temperatures in alpine grasslands, which are far below the optimum of 20–35°C for nitrogenase activity (Dart and Day 1971), may reduce the magnitude of facilitation (Yang *et al.* 2011a). Third, in alpine grassland, the facilitation of legumes to other plants in the community may take some time as the decomposition of those leguminous plant litter may take long time than the study timeframe. This could be another explanation for the observed positive effect of P addition on legume biomass, but negligible effect on ANPP, in our study.

P did not alleviate the negative effect of N on legume performance

Compared to the individual effects of N and P addition, the combined effect of N and P on legumes performance has received less attention in grassland ecosystems. Although

there are several studies addressing how combined N and P addition influences legumes, their results remain controversial (Avolio *et al.* 2014; Grünzweig and Korner 2003; Sun *et al.* 2016).

Contrary to our third hypothesis, NP addition suppressed legume performance more than N addition alone, indicating that P did not alleviate the negative effects of N on legume performance (Fig. 2). This result is consistent with a recent study conducted in an alpine grassland (Sun *et al.* 2016), which found that NP addition decreased legume biomass, irrespective of amount of which the P added with N. Although the addition of N and P together precluded nutrient competition between legumes and grasses, the more rapid increase in community height resulting from grasses growth (Fig. 1b) increased light competition to a higher degree than what was seen following N addition (Fig. 3). Moreover, the negative correlation between legume biomass and litter stock found in our

study (Fig. 4) suggests that the litter accumulated after nutrient enrichment can aggravate light competition. Together, these data suggest that light competition is the major mechanism driving P-induced changes in legume performance, with legumes and dominant grasses having the advantage in low-N (P-addition) and high-N (N- and NP-addition) scenarios, respectively. These findings highlight the importance of height-mediated light competition in determining legume performance under N addition.

Neutral effect of N and P on legumes richness

In general, increased N availability enhances ANPP and reduces species richness (Song *et al.* 2011; Suding *et al.* 2005; Sun *et al.* 2016). Moreover, legumes have a greater probability of being lost from communities with N additions than non-leguminous species (Isbell *et al.* 2013; Suding *et al.* 2005). In contrast, P additions may favour legumes by releasing them from P limitation without releasing other plant groups from N limitation. However, contrary to our hypothesis that nutrient additions would affect legume species richness, we found no evidence that N enrichment decreased, or P addition increased, legume species richness significantly. Therefore, the alterations in biomass in our study were not from species loss or immigration, but from large reductions or increases in the abundance of legumes. It is not clear why nutrient addition did not affect legume species richness, but perhaps the fertilization effect was not completely apparent within just three years because the legumes in our study are perennials. Therefore, a longer experiment with more detailed analysis is required to determine whether these nutrient additions could affect the species richness of legumes.

CONCLUSIONS

In summary, this study provides further evidence of the importance of P as a co-limiting nutrient in alpine grasslands, in contrast to the traditional view that N limitation predominates in such regions. These results improve our understanding of the potential importance of legumes in alpine meadows (in which their contribution to community biomass depends on nutrient availability). Our findings suggest that the effects of N and P on legumes are mediated by competition with grasses for light, and that legumes are likely to respond to N and P co-limitation or enrichment differently than other functional groups because of their ability to fix N. The finding that legume abundance depends on nutrient availability may partially explain why legumes contribute little biomass to both alpine and temperate grasslands. It also provides important insights into potential changes in the performance of legumes in nutrient-limited grasslands following N and P enrichment with anthropogenic changes in nutrient inputs. Moreover, the contrasting and interactive effects of N and P addition on legume performance indicate that P alone does not alleviate the

negative effect of N on legume performance, even though N and P both limit community biomass in alpine grasslands. The rapid change in functional group performance under nutrient amendment might lower community stability and thus, future ecosystem function. Therefore, special cautions should be taken when adding nutrients to improve community productivity in alpine grasslands on the Tibetan plateau.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology online*.

ACKNOWLEDGEMENTS

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