Effect of nitrogen and phosphorus addition on leaf nutrient concentrations and nutrient resorption efficiency of two dominant alpine grass species

LIU Yalan[1,2,3], LI Lei[1,2*], LI Xiangyi[1,2], YUE Zewei[1,2,3], LIU Bo[4]

[1] State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China;
[2] Cele National Station of Observation and Research for Desert Grassland Ecosystems, Cele 848300, China;
[3] University of Chinese Academy of Sciences, Beijing 100049, China;
[4] Shandong Provincial Key Laboratory of Soil Conservation and Environmental Protection, College of Resources and Environment, Linyi University, Linyi 276000, China

Abstract: Nitrogen (N) and phosphorus (P) are two essential nutrients that determine plant growth and many nutrient cycling processes. Increasing N and P deposition is an important driver of ecosystem changes. However, in contrast to numerous studies about the impacts of nutrient addition on forests and temperate grasslands, how plant foliar stoichiometry and nutrient resorption respond to N and P addition in alpine grasslands is poorly understood. Therefore, we conducted an N and P addition experiment (involving control, N addition, P addition, and N+P addition) in an alpine grassland on Kunlun Mountains (Xinjiang Uygur Autonomous Region, China) in 2016 and 2017 to investigate the changes in leaf nutrient concentrations (i.e., leaf N, Leaf P, and leaf N:P ratio) and nutrient resorption efficiency of *Seriphidium rhodanthum* and *Stipa capillata*, which are dominant species in this grassland. Results showed that N addition has significant effects on soil inorganic N (NO_3^--N and NH_4^+-N) and leaf N of both species in the study periods. Compared with green leaves, leaf nutrient concentrations and nutrient resorption efficiency in senesced leaves of *S. rhodanthum* was more sensitive to N addition, whereas N addition influenced leaf N and leaf N:P ratio in green and senesced leaves of *S. capillata*. N addition did not influence N resorption efficiency of the two species. P addition and N+P addition significantly improved leaf P and had a negative effect on P resorption efficiency of the two species in the study period. These influences on plants can be explained by increasing P availability. The present results illustrated that the two species are more sensitive to P addition than N addition, which implies that P is the major limiting factor in the studied alpine grassland ecosystem. In addition, an interactive effect of N+P addition was only discernable with respect to soil availability, but did not affect plants. Therefore, exploring how nutrient characteristics and resorption response to N and P addition in the alpine grassland is important to understand nutrient use strategy of plants in terrestrial ecosystems.

Keywords: leaf nutrient concentration; nutrient resorption efficiency; leaf N:P ratio; N addition; P addition; *Seriphidium rhodanthum*; *Stipa capillata*

1 Introduction

Nitrogen (N) and phosphorus (P) are two important nutrients for plant growth (Zhang et al., 2017). The imbalanced N and P deposition induced by human activities, such as fertilization and fossil fuel combustion (Delonge et al., 2008), affects the primary production of plants and profoundly influences the structure and function of ecosystems (Sardans et al., 2012; Chen et al., 2015; Monuca et al., 2021). To explore how nutrient cycling between plants and soil in different ecosystems responds to nutrient deposition, researchers conducted many experimental studies (Chen et al., 2015; Li et al., 2016). For example, Tian and Niu (2015) reported that excessive N addition influences nutrient cycling between plants and soil and intensifies soil acidification in a global meta-analysis. Yang (2018) established an experiment of external N and P addition in a subtropical forest and concluded that P addition improves leaf P concentration but has no effect on P resorption efficiency (PRE). These studies help explain the impacts of nutrient addition on plant growth and nutrient cycling processes in ecosystems (Chen et al., 2015; Tian and Niu, 2015).

Leaf nutrient concentrations are the most vital indicators of plant growth (Yang, 2018; Xu et al., 2021) and are generally positively correlated with soil nutrient availability when the ecosystem is limited by nutrient resources (Garnier, 1998). They are also correlated with internal nutrient resorption, which is a critical nutrient conservation strategy for plants (Killingbeck, 1996; Vergutz et al., 2012). Nutrient resorption efficiency (NuRE) is used to quantify nutrient resorption in plants (Wright and Westoby, 2003; Chen et al., 2015). Generally, P concentration less than 0.5 mg/g in senesced leaves denotes a high PRE, and N concentration higher than 7.0 mg/g in senesced leaves signifies a low N resorption efficiency (NRE) (Killingbeck, 1996). Furthermore, negative relationships between NuRE and leaf nutrient concentrations have been widely reported (Chen et al., 2012; Lü et al., 2013; Zheng et al., 2020). Lü et al. (2013) demonstrated that NRE of six sub-arctic bog species decreases following four years of experimental N addition. However, positive and neutral relationships between NuRE and leaf nutrient concentrations have also been revealed (Aerts, 1996; Chen et al., 2015). For example, a meta-analysis conducted by Yuan and Chen et al. (2015) showed that NuRE does not change with nutrient addition, possibly because nutrient concentrations in plants depend more on external nutrient supply rather than internal resorption in some ecosystems. This finding differs from that of Yang (2018), who found that approximately 50% of green leaf nutrients are resorbed from senesced leaves. These contradictory results suggest that further studies on NuRE response to nutrient addition are needed to understand nutrient cycling in terrestrial ecosystems.

Ecological stoichiometric characteristics are also important indices of plant growth. From freshwater to terrestrial ecosystems, N:P ratio is widely employed to elucidate nutrient limitations (Güsewell, 2004; Elser et al., 2007; Jin et al., 2020). Koerselman and Meuleman (1996) reported that plants in a wetland ecosystem are limited by P when N:P ratio is higher than 16 and limited by N when the ratio is lower than 14. Zhang et al. (2004) revealed that an N:P ratio lower than 21 signifies N limitation while a ratio higher than 23 suggests P limitation in a steppe ecosystem in Inner Mongolia Autonomous Region, China. These results indicate that N:P ratio is determined by ecosystem types and plant species (Verterink et al., 2001; Menge and Field, 2007). However, recent studies about N:P ratio have tended to focus on green leaves while neglecting senesced leaves (Liu et al., 2013; Zhang et al., 2017). Persson et al. (2010) concluded that N:P ratios in green leaves are more stable than those in senesced leaves due to nutrient resorption (Chen et al., 2015). Therefore, understanding how increasing N and P addition influences N:P ratio in senesced leaves is also important.

Alpine grassland is a typical terrestrial ecosystem, and numerous studies have reported that this ecosystem is limited by N or co-limited by N and P (Elser et al., 2007; Xu et al., 2014; Zhang et al., 2021). Many researchers have illustrated that long-term nutrient deposition accelerates nutrient cycling between plants and soil with concomitant impact on nutrient limitation. For example, Tian and Niu (2015) showed that N addition decreases soil pH but increases P desorption. Peñuelas et al. (2013) suggested that long-term N inputs shift ecosystem limitation from N to P. However, our understanding of nutrient cycling responses to external nutrient inputs in alpine grasslands is

limited (Sardans et al., 2012). In addition, N and P are believed to be linked tightly in nutrient cycle processes, and the interactive effects of these nutrients appear to differ from the effect of N or P addition alone (Peng et al., 2019). Moreover, the interactive influence of the addition of these major nutrients in alpine grasslands is less well understood. Hence, in order to determine how increasing N and P deposition influences terrestrial ecosystems, it is necessary to explore the effects of N and P addition, as well as the combination of N and P, on leaf nutrient concentrations and nutrient resorption efficiency in alpine grasslands.

Here, we conducted an experiment (involving control, N addition, P addition, and N+P addition) and sampled two dominant alpine grass species (i.e., *Seriphidium rhodanthum* and *Stipa capillata*) in an alpine grassland on Kunlun Mountains, Xinjiang Uygur Autonomous Region of China in 2016 and 2017. This study aims to: (1) explore the responses of leaf N, leaf P, N:P ratio, and NuRE to external nutrient addition in this alpine grassland; (2) assess the interactive effect of these nutrients on this ecosystem; and (3) evaluate whether N and P addition alleviates or enhances limitation in this alpine ecosystem.

2   Materials and methods

2.1   Study area and experimental design

This study was conducted in an alpine grassland (36°08′03″N, 81°06′33″E; 3100 m a.s.l.) on Kunlun Mountains, Xinjiang Uygur Autonomous Region of China in 2016 and 2017. The topographic conditions in this region were uniform. The annual mean air temperature was approximately 5.2°C in 2016 and 3.8°C in 2017 (Fig. 1). The monthly mean temperature ranged from –5.5°C in February to 15.5°C in July in 2016 and from –8.0°C in January to 12.6°C in July in 2017. The total precipitation was 482 mm in 2016 and 561 mm in 2017, with most occurring from May to October. *S. rhodanthum* and *S. capillata* are the two dominant grass species in this region. *S. capillata* covers more than 70% of the total aboveground biomass, and *S. rhodanthum* accounts for more than 20%.
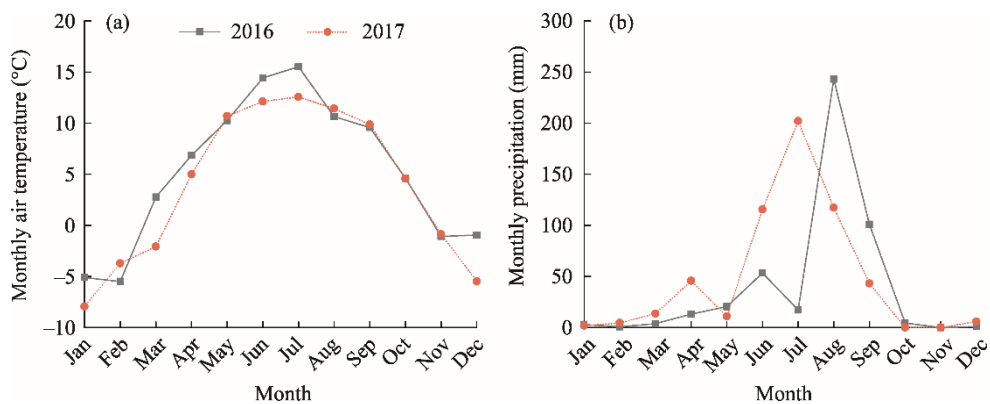

Fig. 1   Monthly air temperature (a) and monthly precipitation (b) in 2016 and 2017

This study simulated N and P addition via four treatments: control (no fertilization), N addition (16 g N/(m^2·a)), P addition (3 g P/(m^2·a)), and N+P addition (16 g N/(m^2·a) and 3 g P/(m^2·a)). The nutrient addition protocol was based on an extant study on the Bayanbulak alpine grasslands of Xinjiang, China (Yue et al., 2016). Sixteen 2 m×3 m plots were established (four replicates for each treatment) for each species. All plots were separated by at least 1 m, and each plot was randomly arranged. In late April, monobasic potassium phosphate and urea were mixed with soil from the test plots and spread evenly on the surface of the soil.

2.2   Sampling and measurements

Plant and soil collection was carried out following the methods of Lü et al. (2013) with minor modifications. One hundred leaves (fully expanded) of *S. capillata* and *S. rhodanthum* with similar

size, respectively, were chosen from the center of each plot. Half of the samples were collected as the green group in August of both years, and the rest samples were marked and collected as the senesced group in October of both years. All samples were dried at 75°C for 48 h, milled, and sieved (1 mm mesh) for chemical property determination. Soil samples were collected in mid-August of 2016 and 2017. Five points were selected in each plot, and soil samples were collected using a soil drilling sampler with a diameter of 5 cm. All soil samples were completely mixed and passed through a 2-mm mesh to eliminate roots and other debris.

Leaf N concentration was analyzed using an auto elemental analyzer (Vario EL III, Elementar Analysensysteme GmbH, Hanau, Germany). Leaf P concentration was measured by Mo-Sb colorimetric method after $H_2SO_4+HClO_4$ digestion. Soil inorganic N (NO_3^--N and NH_4^+-N) was determined by the Kjeldahl method using an Alpkem autoanalyzer (FIAstar 5000, Foss Tecator, Denmark). Soil available P was determined by Mo-Sb colorimetric method (Chen et al., 2015).

2.3   Calculation of nutrient resorption efficiency (NuRE)

In this study, we calculated NuRE (i.e., PRE and NRE) based on nutrient concentrations in leaves (Lü et al., 2013).

$$\text{NuRE (\%)}=((\text{Nutrient}_{\text{green}}-\text{Nutrient}_{\text{senesced}})/\text{Nutrient}_{\text{green}})\times100\%, \tag{1}$$

where $\text{Nutrient}_{\text{green}}$ is the nutrient concentration in green leaves (mg/g) and $\text{Nutrient}_{\text{senesced}}$ is the nutrient concentration in senesced leaves (mg/g).

2.4   Statistical analysis

Linear mixed effect models were used to evaluate the effects of year, N addition, P addition, and N+P addition on soil inorganic N, soil available P, leaf N, leaf P, leaf N:P ratio, NRE, PRE, and NRE:PRE ratio. We set year as a random factor to evaluate the effects of temperature, precipitation, and other meteorological factors on N and P concentrations in soil and plants. N addition, P addition, and N+P addition were set as fixed factors to evaluate the effects of N addition, P addition, and the interactive effect of N addition and P addition on nutrient stoichiometric characteristics of soil and plants. One-way ANOVA was performed to test the influences of N addition, P addition, and N+P addition on soil inorganic N, soil available P, leaf N, leaf P, leaf N:P ratio, NRE, PRE, and NRE:PRE ratio. An ANOVA post-hoc test, specifically the least significant difference method, was performed to determine differences between individual treatments. Pearson correlation analysis was conducted to determine the relationship between leaf nutrient concentrations and soil nutrient concentrations. R 4.0.4 software was used to perform all analyses, and $P=0.05$ was used to detect statistical significance.

3   Results

3.1   Soil inorganic N and available P concentrations

The random factor year showed a significant effect on soil inorganic N and available P concentrations (Table 1). N addition significantly affected soil inorganic N but did not affect soil available P; P addition significantly affected soil available P but did not influence soil inorganic N. Moreover, N+P addition was only discernable with respect to soil available P. The effects of N addition and N+P addition on soil inorganic N were positive in both years (Fig. 2). A significant increase in soil available P was observed in both years in P addition and N+P addition treatments, and the highest values were observed following P addition alone (Fig. 2).

Table 1   *P*-values from linear mixed models for soil inorganic nitrogen (N) and soil available phosphorus (P)

Parameter	Year	N addition	P addition	N+P addition
Soil inorganic N	<0.001***	<0.001***	0.211	0.240
Soil available P	<0.001***	0.842	<0.001***	0.008**

Note: **, *P*<0.01; ***, *P*<0.001.

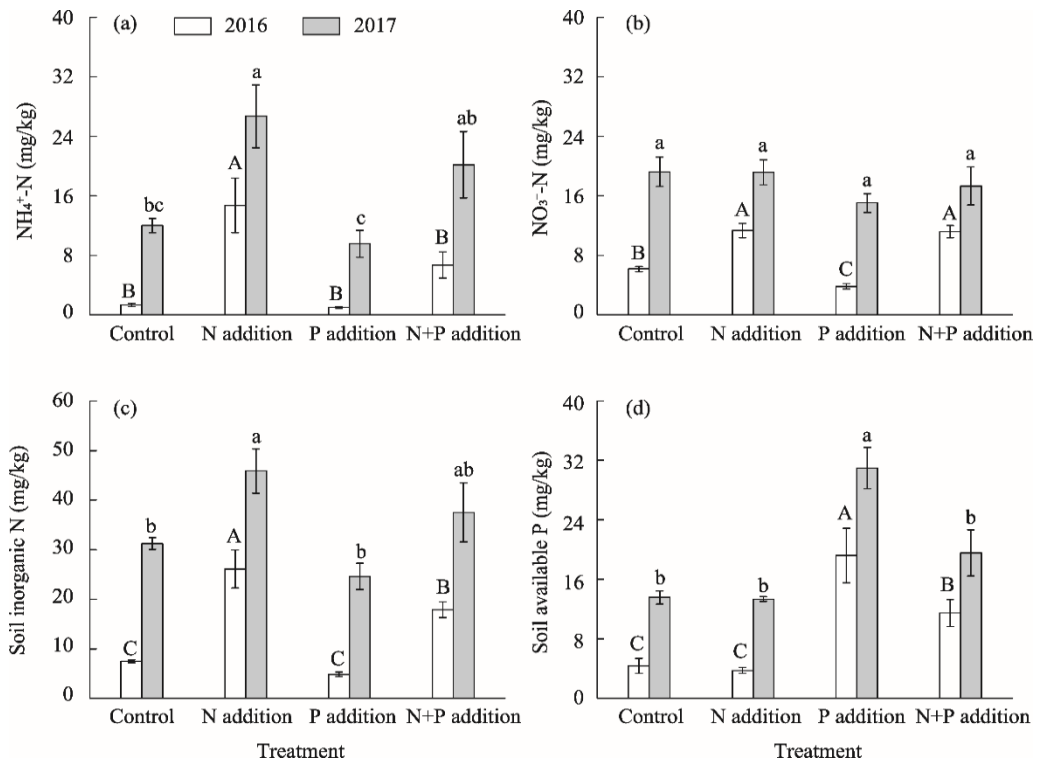**Fig. 2**   Responses of NH$_4^+$-N (a), NO$_3^-$-N (b), soil inorganic N (c), and soil available P (d) to the four treatments (control, N addition, P addition, and N+P addition). Different uppercase letters indicate the significant differences among the four treatments in 2016, and different lowercase letters indicate the significant differences among the four treatments in 2017 (P<0.05). Bars mean standard errors.

3.2   Leaf nutrient concentrations and nutrient resorption efficiency (NuRE)

The random factor year had significant effects on leaf N and leaf N:P ratio in green leaves and leaf P in senesced leaves of *S. rhodanthum* (Table 2). It significantly influenced leaf N and leaf P in green and senesced leaves and leaf N:P ratio in senesced leaves of *S. capillata*. N addition affected leaf N and leaf N:P ratio in green and senesced leaves of *S. capillata* and senesced leaves of *S. rhodanthum*. P addition also influenced leaf P and leaf N:P ratio in the two species, except green leaves of *S. rhodanthum*. N addition and N+P addition increased leaf N in *S. capillata* in both years (Fig. 3). P addition and N+P addition increased leaf P in the two species. However, leaf P did not change in either of the two species following N addition, and leaf N also did not change in response to P addition. An interactive effect of N+P addition was only observed in senesced leaves of *S. capillata* (Table 3). N addition exerted positive effects on leaf N:P ratio in green and senesced leaves of *S. capillata* (Fig. 4). N:P ratios in green and senesced leaves of the two species in P addition and N+P addition treatments were lower than that in control treatment. Moreover, leaf N:P ratio in *S. capillata* was higher than that in *S. rhodanthum*.

Table 2   *P*-values from linear mixed models for leaf nutrient characteristics of *Seriphidium rhodanthum*

| Parameter | S. rhodanthum | | | | | |
| | Green leaves | | | Senesced leaves | | |
	Leaf N	Leaf P	Leaf N:P ratio	Leaf N	Leaf P	Leaf N:P ratio
Year	<0.001***	0.051	<0.001***	0.112	0.022*	0.480
N addition	0.005**	0.670	0.540	0.023*	0.660	0.007**
P addition	0.221	0.001**	0.573	0.651	0.001**	0.001**
N+P addition	0.243	0.771	0.772	0.354	0.842	0.078

Note: *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001.

Table 3 *P*-values from linear mixed models for leaf nutrient characteristics of *Stipa capillata*

Parameter	S. capillata					
	Green leaves			Senesced leaves		
	Leaf N	Leaf P	Leaf N:P ratio	Leaf N	Leaf P	Leaf N:P ratio
Year	<0.001***	<0.001***	0.711	<0.001***	<0.001***	<0.001***
N addition	0.002***	0.922	<0.001***	<0.001***	0.552	<0.001***
P addition	0.442	<0.001***	<0.001***	0.362	<0.001***	<0.001***
N+P addition	0.431	0.792	0.063	0.523	0.451	0.008**

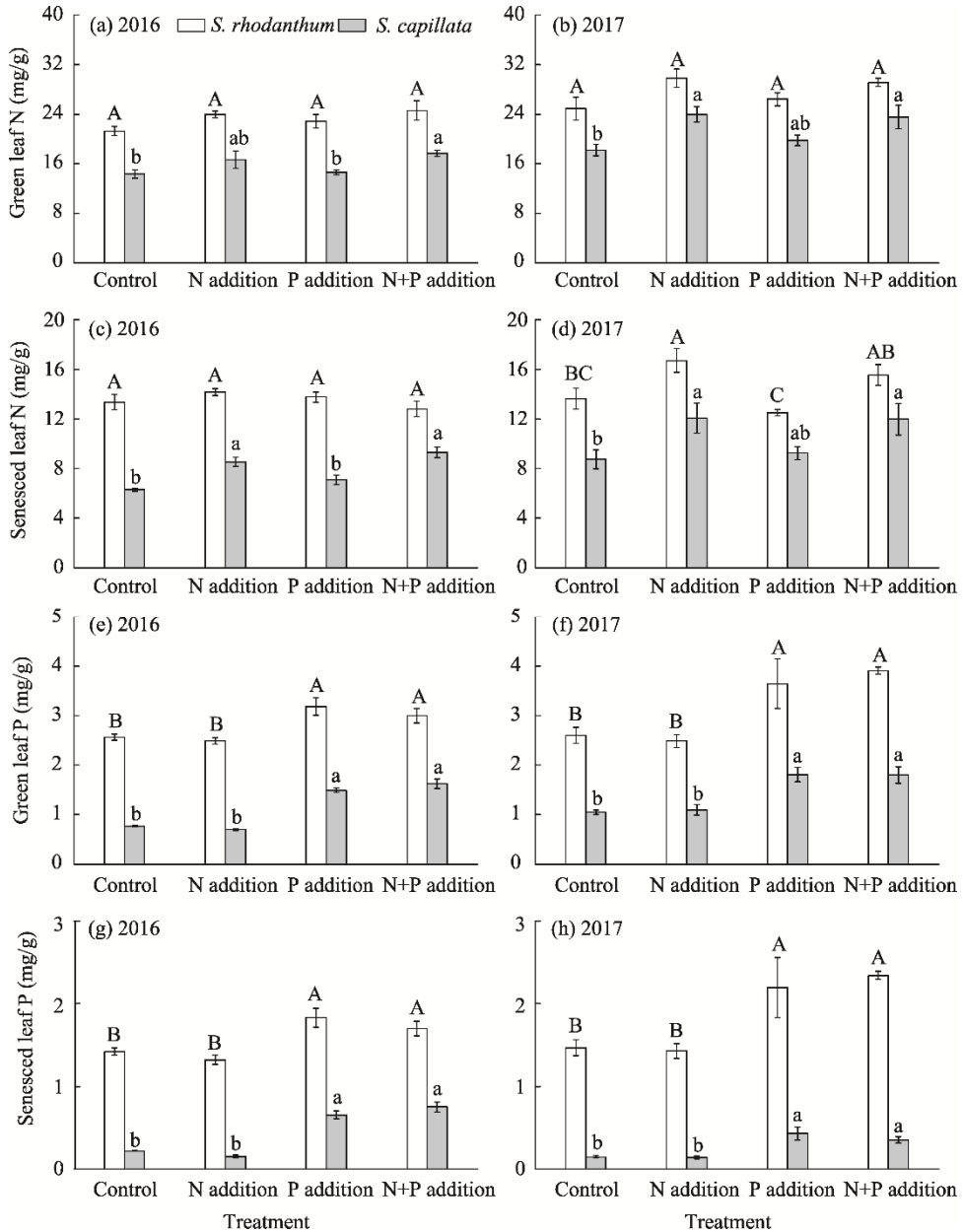Note: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Fig. 3 N and P concentrations in green and senesced leaves of *Seriphidium rhodanthum* and *Stipa capillata* in response to the four treatments in 2016 (a, c, e, g) and 2017 (b, d, f, h). Different uppercase letters indicate the significant differences among the four treatments in *S. rhodanthum*, and different lowercase letters indicate the significant differences among the four treatments in *S. capillata* ($P<0.05$). Bars mean standard errors.

Fig. 4 N:P ratio in green and senesced leaves of *S. rhodanthum* and *S. capillata* in response to the four treatments in 2016 (a, c) and 2017 (b, d). Different uppercase letters indicate the significant differences among the four treatments in *S. rhodanthum*, and different lowercase letters indicate the significant differences among the four treatments in *S. capillata* (*P*<0.05). Bars mean standard errors.
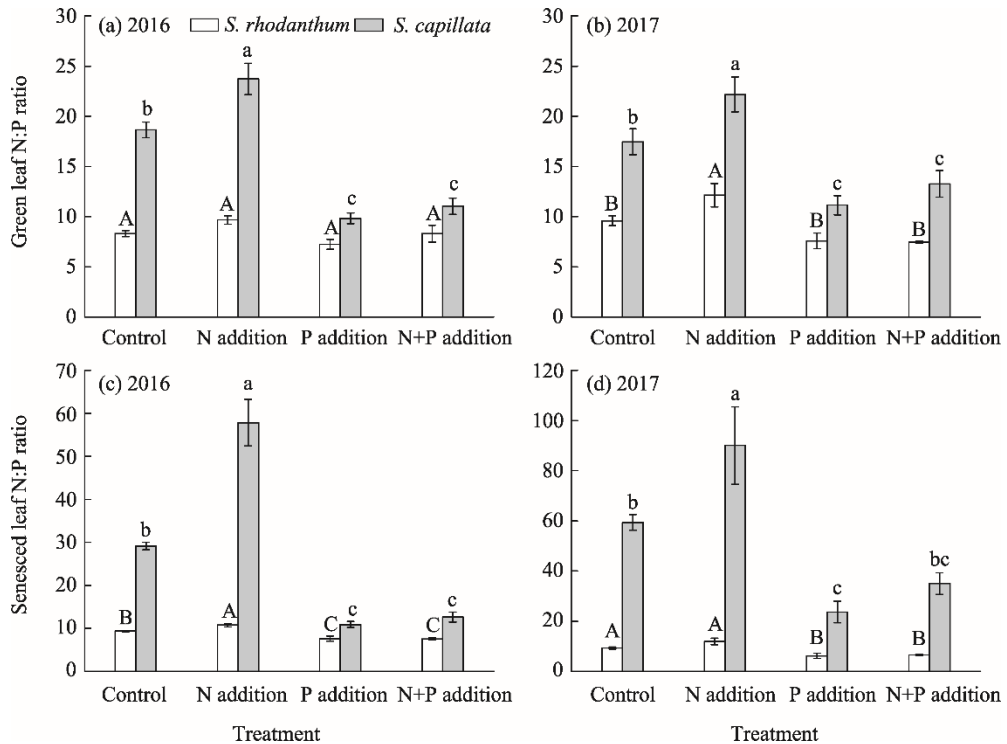
The random factor year exerted significant effects on PRE and NRE:PRE ratio of the two species and NRE of *S. rhodanthum* (Table 4). P addition significantly influenced PRE and NRE:PRE ratio of *S. rhodanthum* and *S. capillata*, while N addition influenced NRE:PRE ratio of *S. capillata* only. Furthermore, no interactive effects of N+P addition on NRE, PRE, and NRE:PRE ratio were observed. PRE of *S. capillata* was lower following P addition compared with control treatment in both years, but PRE of *S. rhodanthum* showed no significant change following P addition (Fig. 5).

Table 4 *P*-values from linear mixed models for nutrient resorption efficiency of *S. rhodanthum* and *S. capillata*

Parameter	*S. rhodanthum*			*S. capillata*		
	NRE	PRE	NRE:PRE ratio	NRE	PRE	NRE:PRE ratio
Year	0.006**	0.003**	<0.001***	0.962	<0.001***	<0.001***
N addition	0.683	0.531	0.811	0.073	0.291	0.054*
P addition	0.122	0.021*	0.019*	0.522	<0.001***	0.011*
N+P addition	0.791	0.906	0.713	0.790	0.359	0.443

Note: *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001; NRE, nutrient resorption efficiency; PRE, phosphorus resorption efficiency.

3.3 Relationship between leaf nutrient concentrations and soil nutrient concentrations

Green leaf N had a positive relationship with soil inorganic N for *S. capillata* but exhibited no relationship for *S. rhodanthum* (Table 5). Leaf N generally showed no relationship with soil available P. In both species, green leaf P had a positive relationship with soil available P and showed no relationship with soil inorganic N. Green leaf N:P ratio was negatively correlated with soil available P. In terms of senesced leaf N:P ratio in *S. rhodanthum* and *S. capillata*, it had a positive relationship with soil inorganic N and a negative relationship with soil available P in the two years. Moreover, compared with *S. rhodanthum*, *S. capillata* was more sensitive to external nutrient addition, and both species were more sensitive to P addition than N addition.
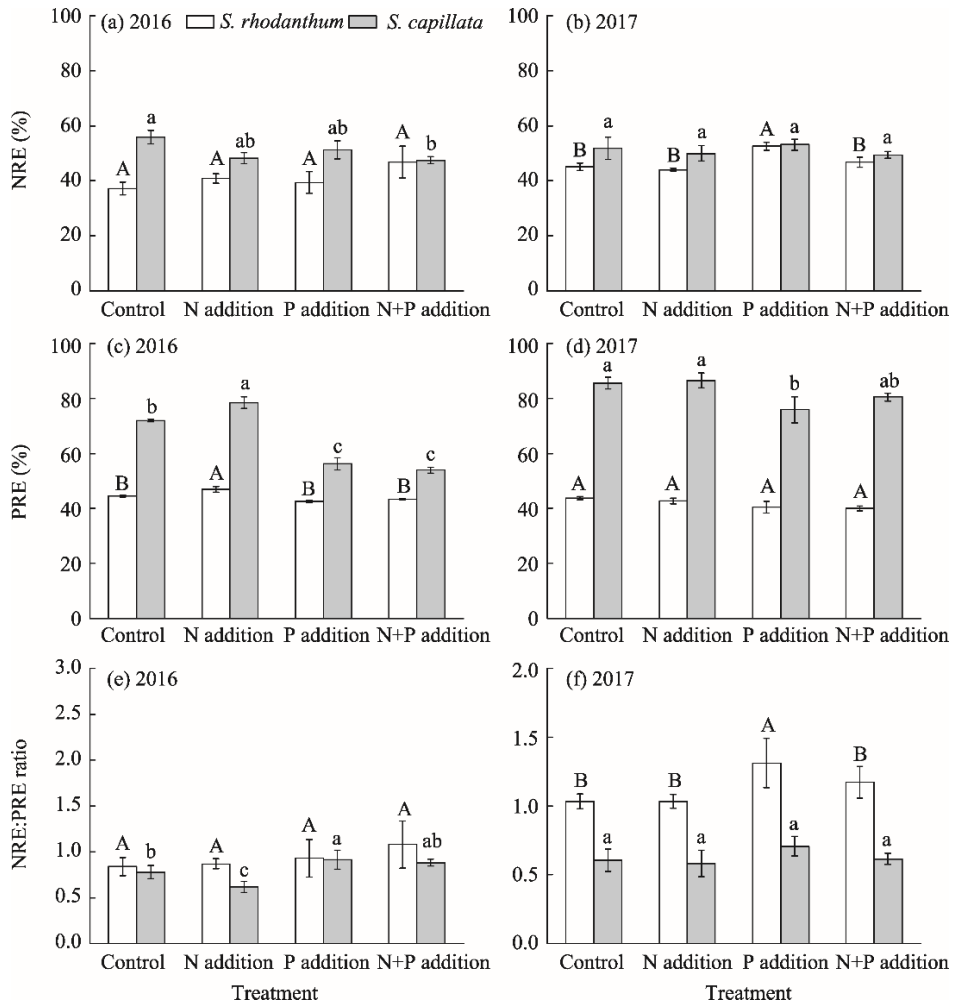
Fig. 5 NRE, PRE, and NRE:PRE ratio of *S. rhodanthum* and *S. capillata* in response to the four treatments in 2016 (a, c, e) and 2017 (b, d, f). NRE, nutrient resorption efficiency; PRE, phosphorus resorption efficiency. Different uppercase letters indicate the significant differences among the four treatments in *S. rhodanthum*, and different lowercase letters indicate the significant differences among the four treatments in *S. capillata* ($P<0.05$). Bars mean standard errors.

4   Discussion

4.1   Leaf nutrient concentrations

N addition had a positive effect on soil inorganic N and leaf N, while P addition had a positive effect on soil available P and leaf P although leaf and soil nutrient concentrations showed significant differences between 2016 and 2017. These results are similar to what has been found in previous studies (e.g., N addition increases soil inorganic N and P addition increases soil available P and leaf P) (Xu and Timmer, 1999; Yuan and Chen, 2015; Deng et al., 2016). Many studies have also reported positive relationships between leaf nutrient concentrations and soil nutrient concentrations (Chen et al., 2015; Huang et al., 2016; Li et al., 2016). Hence, the obvious differences of leaf nutrient concentrations in this study might be due to the different soil nutrient concentrations in the two years. We speculated that the reason for the differences of soil nutrient concentrations in both years might be caused by different precipitation amounts. Soil nutrient concentrations would increase with increasing precipitation (Yuan and Chen, 2009; Zhao et al., 2017). For example, Zhao et al. (2017) reported that soil C and N concentrations increase with increasing precipitation gradient on the Tibetan Changtang Plateau, China. Brant and Chen (2015)

Table 5 Correlation coefficients between leaf nutrient concentrations and soil nutrient concentrations for *S. rhodanthum* and *S. capillata* in 2016 and 2017

Species	Year	Leaf nutrient concentration	Soil nutrient concentration	
			Soil inorganic N	Soil available P
S. rhodanthum	2016	Green leaf N	0.30	0.27
		Green leaf P	−0.39	0.76**
		Green leaf N:P ratio	−0.09	−0.12
		Senesced leaf N	−0.09	−0.45
		Senesced leaf P	−0.24	−0.84**
		Senesced leaf N:P ratio	0.56*	−0.74*
	2017	Green leaf N	0.23	−0.08
		Green leaf P	−0.17	0.53**
		Green leaf N:P ratio	0.26	−0.64*
		Senesced leaf N	−0.39	−0.46
		Senesced leaf P	−0.49	−0.82**
		Senesced leaf N:P ratio	0.62*	−0.63*
S. capillata	2016	Green leaf N	0.65**	−0.85
		Green leaf P	−0.24	0.68**
		Green leaf N:P ratio	0.54*	−0.61*
		Senesced leaf N	0.69**	−0.12
		Senesced leaf P	−0.45	0.68**
		Senesced leaf N:P ratio	0.43	−0.67**
	2017	Green leaf N	0.50*	0.26
		Green leaf P	−0.45	0.71**
		Green leaf N:P ratio	0.58	−0.74**
		Senesced leaf N	0.52*	−0.33
		Senesced leaf P	−0.14	0.73**
		Senesced leaf N:P ratio	0.27	−0.70**

Note: *, $P<0.05$; **, $P<0.01$.

revealed that plant PRE decreases with increasing precipitation because soil P availability increases. However, following N addition and N+P addition, leaf N of *S. rhodanthum* showed no changes except for senesced leaves in 2017. This may reflect delayed responses of the two species to nutrient addition (Bowman et al., 2006; Bai et al., 2010), or plants are insensitive to short-term N addition but could adjust physiological demand to utilize high available N during long-term nutrient addition (Chen et al., 2015). Our results also showed that senesced leaves of *S. rhodanthum* are more sensitive to N addition than green leaves, which resonates with the findings of Persson et al. (2010), who noted that green leaves of some plants are more stable than senesced leaves due to homeostatic regulation and nutrient resorption (Chen et al., 2015). In our study, P concentrations in green and senesced leaves varied with P addition in both years. This result is similar to previous studies showing that green leaf N does not change with N addition but leaf P is significantly influenced by P addition in N-saturated ecosystems, implying P limitation (Yang, 2018; Xu et al., 2021).

For *S. capillata*, N addition and P addition had significant effects on green leaf N:P ratio and senesced leaf N:P ratio, which implied that this species is sensitive to external nutrient addition and the nutrient use strategy of *S. capillata* is different from that of *S. rhodanthum*. These results indicate that green leaf nutrient concentrations of *S. capillata* depend more on direct absorption rather than resorption compared with those of *S. rhodanthum*. Killingbeck (1996) showed that plants have a high P use efficiency when P concentration in senesced leaves is lower than 0.5 mg/g and a low N use efficiency when N concentration in senesced leaves is higher than 7.0 mg/g. Our data revealed that P concentrations in green and senesced leaves of both species are <0.5 mg/g, which suggests that both species have a high P use efficiency. Furthermore, our data showed that the two species are more sensitive to P addition than N addition and the degree of variations under P addition was greater than that under N addition. This finding is similar to previous studies. For

instance, Bobbink (1991) reported that in a chalk grassland in Holland, leaf P concentrations increase to a greater extent than leaf N concentrations. This pattern has also been reported in alpine tundra (Bowman et al., 1993) and other P-limited ecosystems (Sword et al., 2004; Güsewell, 2005; Ostertag, 2010). These results also suggest that nutrient addition would change leaf nutrient concentrations, which consequently facilitates litter decomposition and accelerates nutrient cycling between plants and soil (Zhang et al., 2008). In addition, these results support the finding that the influence of nutrient addition depends on the experimental duration and species (Mayor et al., 2014). Chen et al. (2015) reported some differences between short-term and long-term results in terms of grass responses to nutrient addition. Tian and Niu (2015) suggested that long-term N addition intensifies soil acidification and increases P availability through P desorption (Wang et al., 2011). Therefore, more long-term studies are necessary to investigate how external nutrient addition influences nutrient cycling between plants and soil.

4.2   Nutrient resorption efficiency (NuRE)

N addition did not change NuRE of the two species in either year, which is similar to previous studies on ecosystems that are not limited by N (Huang et al., 2016; Long et al., 2016; Yang, 2018). This result suggests that leaf N of the two species in this alpine grassland ecosystem is more regulated by external N addition than internal resorption (Yang, 2018). Moreover, NRE:PRE ratio of the two species in N addition treatment was lower than that in control treatment (except *S. rhodanthum* in 2016), which implies that the two species have a high efficiency of P utilization. These results also support the hypothesis that N is not the major limiting element in the alpine grassland system. P addition decreases PRE of *S. capillata*, similar to a previous study showing that PRE has a negative relationship with leaf P (Lü et al., 2013). Our data indicated that P addition in the two years not only changes P absorption directly but also influences the internal resorption of *S. capillata*. P inputs exert no influence on PRE of *S. rhodanthum*, which is consistent with the findings of Yuan and Chen (2015), who found that P addition increases P concentrations but has no effect on PRE of some plants in P-limited ecosystems. The possible reason might be that these plants cannot adjust nutrient characteristics quickly in response to environmental change. The two species (*S. capillata* and *S. rhodanthum*) demonstrated different strategic responses to P addition. These results confirmed that species is the dominant factor for nutrient dynamics (He et al., 2008). Hence, more further studies on plant responses to external nutrient addition are needed to predict community succession in alpine grasslands.

Many studies have explored how leaf N, N:P ratio, and NRE respond to N addition and how P cycling between plants and soil responds to P addition (Lawrence, 2001; Huang et al., 2016; Long et al., 2016). However, researches concerning the interactive effects of N and P are limited. Previous experiments have shown that increasing N deposition could increase, decrease, or have no influence on leaf P and soil P concentrations and PRE via affecting phosphatase enzymes (Rodríguez and Fraga, 1999; Marklein and Houlton, 2012; Tian and Niu, 2015). For example, in an old-growth boreal forest, Xu et al. (2021) reported that leaf P and other nutrients do not change with soil N concentrations though the long-term N addition increases leaf P in some extent compared to control treatment. However, Yang (2018) concluded that N addition does not change leaf P in P-limited tropical forests. In this study, we found that leaf P concentrations are not very responsive to N addition. The results of the linear mixed effect model showed that no interactive effect of N and P can be observed. These findings also support the conclusion that the effects of N addition on plant P concentrations are minimal in a non-N limitation ecosystem (Chen et al., 2015; Yang, 2018), and these results showed that plants could adjust leaf nutrient concentrations to adopt the change of ecosystem limitation (Jin et al., 2020).

4.3   Ecosystem limitation

Previous studies have explained that a high N:P ratio indicates that plants are limited by P, while a low N:P ratio signifies plants are limited by N (Chen et al., 2015; Zhang et al., 2017). Koerselman and Meuleman (1996) showed that the threshold values for N and P limitation are 14 and 16, respectively. N:P ratio in green or senesced leaves generally increases with N addition, and P

becomes the major limiting factor subsequently; however, the ratio decreases with P addition, and N will become the major limiting factor (Tilman, 1982). In our experiment, N addition improved green leaf N:P ratio of *S. capillata* from more than 16 in the control group to more than 20 and increased senesced leaf N:P ratio to more than 50. P addition and N+P addition decreased the ratio to below 14 in both green and senesced leaves. The results also support that *S. capillata* is limited by P rather than N. The responses of N:P ratio of *S. rhodanthum* to nutrient addition differed from those of *S. capillata*. Although the ratio in green and senesced leaves of *S. rhodanthum* had different responses to external nutrient addition, the values were lower than 14 in the four treatments. This might be because *S. rhodanthum* had higher leaf P concentrations and was insensitive to N addition. These results also showed that the critical N:P ratio for nutrient limitation is determined by species, and N:P ratio could not be the only indicator to show the limitation for plants.

　　As *S. capillata* represents more than 70% and *S. rhodanthum* occupies more than 20% of the aboveground biomass in the study area, the limitation of the two species can be regarded as the limitation of this alpine grassland. According to the responses of leaf N, leaf P, leaf N:P ratio, NRE, PRE, and NRE:PRE to N addition and P addition for the two species, the ecosystem is limited by P rather than N. This result contradicts earlier research, which found that alpine grasslands are limited by N or N and P, which may be explained by the alleviation of N limitation of this ecosystem under long-term N deposition (Long et al., 2016). More studies are needed to strengthen our understanding of nutrient limitation in the context of long-term global nutrient deposition.

5　Conclusions

Our results showed that the alpine grassland on the Kunlun Mountains is not a N-limited ecosystem and the interactive effect of N and P is not evident. The responses of the two species (*S. rhodanthum* and *S. capillata*) to nutrient addition are species-specific. *S. capillata* is more sensitive to P addition and has a higher P use efficiency than *S. rhodanthum*. Given that *S. rhodanthum* is insensitive to N addition, increasing N addition would support the growth of *S. capillata*. However, although N addition supports the growth of *S. capillata*, this alpine grassland is P-limited and *S. capillata* is mainly limited by P. Future studies should explore the responses of the two species to nutrient addition over a longer period.

Acknowledgements

References

Aerts R. 1996. Nutrient resorption from senescing leaves of perennials: are there general patterns? Journal of Ecology, 84(4): 597–608.

Bai Y F, Wu J G, Clark C M, et al. 2010. Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. Global Change Biology, 16(1): 358–372.

Bobbink R. 1991. Effects of nutrient enrichment in Dutchchalk grassland. Journal of Applied Ecology, 28(1): 28–41.

Bowman W D, Theodose T A, Schardt J C, et al. 1993. Constraints of nutrient availability on primary production in two alpine tundra communities. Ecology, 74(7): 2085–2097.

Bowman W D, Gartner J R, Holland K, et al. 2006. Nitrogen critical loads for alpine vegetation and terrestrial ecosystem response: are we there yet? Ecological Applications, 16(3): 1183–1193.

Brant A N, Chen H Y H. 2015. Patterns and mechanisms of nutrient resorption in plants. Critical reviews in plant sciences, 34(5): 471–486.

Chen F S, Niklas K J, Chen G S, et al. 2012. Leaf traits and relationships differ with season as well as among species groupings in a managed Southeastern China forest landscape. Plant Ecology, 213(9): 1489–1502.

Chen F S, Niklas K J, Liu Y, et al. 2015. Nitrogen and phosphorus additions alter nutrient dynamics but not resorption efficiencies of Chinese fir leaves and twigs differing in age. Tree Physiology, 35(10): 1106–1117.

Delonge M, D'Odorico P, Lawrence D. 2008. Feedbacks between phosphorus deposition and canopy cover: the emergence of multiple stable states in tropical dry forests. Global Change Biology, 14(1): 154–160.

Deng M F, Liu L L, Sun Z Z, et al. 2016. Increased phosphate uptake but not resorption alleviates phosphorus deficiency induced by nitrogen deposition in temperate *Larix principis-rupprechtii* plantations. New Phytologist, 212(4): 1019–1029.

Lü X T, Reed S, Yu Q, et al. 2013. Convergent responses of nitrogen and phosphorus resorption to nitrogen inputs in a semiarid grassland. Global Change Biology, 19(9): 2775–2784.

Elser J J, Bracken M E S, Cleland E E, et al. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters, 10(12): 1135–1142.

Elser J J, Anderson T, Bergström A K, et al. 2009. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science, 326(5954): 835–837.

Feller I C, McKee K L, Whigham D F, et al. 2003. Nitrogen vs. phosphorus limitation across an ecotonal gradient in a mangrove forest. Biogeochemistry, 62(2): 145–175.

Fornara D A, Banin L, Crawley M J. 2013. Multi-nutrient vs nitrogen-only effects on carbon sequestration in grassland soils. Global Change Biology, 19(12): 3848–3857.

Garnier E. 1998. Interspecific variation in plasticity of grasses in response to nitrogen supply. In: Population Biology of Grasses. Cambridge: Cambridge University Press, 155–181.

Güsewell S. 2004. N:P ratios in terrestrial plants: variation and function significance. New phytologist, 168(2): 377–385.

Güsewell S. 2005. High nitrogen: phosphorus ratios reduce nutrient retention and second-year growth of wetland sedges. New Phytologist, 166(2): 537–550.

He J S, Wang L, Flynn D F B, et al. 2008. Leaf nitrogen: phosphorus stoichiometry across Chinese grassland biomes. Oecologia, 155: 301–310.

Huang J, Yu H, Lin H, et al. 2016. Phosphorus amendment mitigates nitrogen addition-induced phosphorus limitation in two plant species in a desert steppe, China. Plant and Soil, 399(1–2): 221–232.

Jin X M, Yang L X, Yang X G, et al. 2020. Effects of N and P fertilization on the biomass and ecological stoichiometric characteristics of *Agropyron michnoi* in sandy grasslands. Chemistry and Ecology, 36(10): 938–952.

Killingbeck K T. 1996. Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. Ecology, 77(6): 1716–1727.

Koerselman W, Meuleman A F. 1996. The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. Journal of Applied Ecology, 33(6): 1441–1450.

Lawrence D. 2001. Nitrogen and phosphorus enhance growth and luxury consumption of four secondary forest tree species in Borneo. Journal of Tropical Ecology, 17(6): 859–869.

Li L, Gao X P, Li X Y, et al. 2016. Nitrogen (N) and phosphorus (P) resorption of two dominant alpine perennial grass species in response to contrasting N and P availability. Environmental and Experimental Botany, 127: 37–44.

Li Y, Niu S, Yu G. 2016. Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: a meta-analysis. Global Change Biology, 22(2): 934–943.

Liu J X, Huang W J, Zhou G Y, et al. 2013. Nitrogen to phosphorus ratios of tree species in response to elevated carbon dioxide and nitrogen addition in substropical forest. Global Change Biology, 19(1): 208–216.

Long M, Wu H H, Smith M D, et al. 2016. Nitrogen deposition promotes phosphorus uptake of plants in a semi-arid temperate grassland. Plant and Soil, 408(1–2): 475–484.

Marklein A R, Houlton B Z. 2012. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. New Phytologist, 193(3): 696–704.

Mayor J R, Wright S J, Turner B L. 2014. Species-specific responses of foliar nutrients to long term nitrogen and phosphorus additions in a lowland tropical forest. Journal of Ecology, 102(1): 36–44.

Menge D N L, Field C B. 2007. Simulated global changes alter phosphorus demand in annual grassland. Global Change Biology. 13(12): 2582–2591.

Monuca W, Kathryn B, Eric W, et al. 2021. Response of fungal endophyte communities within *Andropogon gerardii* (Big bluestem) to nutrient addition and herbivore exclusion. Fungal Ecology, 51: 10143, doi: 10.1016/j.funeco.2021.101043.

Ostertag, R. 2010. Foliar nitrogen and phosphorus accumulation responses after fertilization: an example from nutrient-limited Hawaiian forests. Plant and Soil, 334(1–2): 85–98.

Peng Y F, Peng Z P, Zeng X T, et al. 2019. Effects of nitrogen-phosphorus imbalance on plant biomass production: a global perspective. Plant and Soil, 436(1–2): 245–252.

Peñuelas J, Poulter B, Sardans J, et al. 2013. Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. Nature Communications, 4: 2934, doi: 10.1038/ncomms3934.

Persson J, Fink P, Goto A, et al. 2010. To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. Oikos, 119(5): 741–751.

Rodríguez H, Fraga R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances, 17(4): 319–339.

Sardans J, Rivas-Ubac A, Peñuelas J. 2012. The C:N:P stoichiometry of organisms and ecosystems in a changing world: A review and perspectives. Perspectives in Plant Ecology, Evolution and Systematics, 14(1): 33–47.

Sword S M A, Goelz J C G, Chamber J L, et al. 2004. Long-term trends in loblolly pine productivity and stand characteristics in response to thinning and fertilization in the West Gulf region. Forest Ecology and Management, 192(1): 71–96.

Tian D S, Niu S L. 2015. A global analysis of soil acidification caused by nitrogen addition. Environmental Research Letters, 10(2): 19–24.

Tilman D. 1982. Resource Competition and Community Structure. Princeton: Princeton University Press.

Venterink H O, Vliet Revd, Wassen M J. 2001. Nutrient limitation along a productivity gradient in wet meadows. Plant and Soil, 234(2): 171–179.

Vergutz L, Manzoni S, Porporato A, et al. 2012. Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecological Monographs, 82(2): 205–220.

Wang X, Guppy C N, Watson L, et al. 2011. Availability of sparingly soluble phosphorus sources to cotton (*Gossipium hirsutum* L.), wheat (*Triticum aestivum* L.) and white lupin (*Lupinus albus* L.) with different forms of nitrogen as evaluated by a ^{32}P isotopic dilution technique. Plant and Soil, 348(1–2): 85–98.

Wright I J, Westoby M. 2003. Nutrient concentration, resorption and lifespan: leaf traits of Australian sclerophyll species. Function Ecology, 17(1): 10–19.

Xu L, Xing A, Du E, et al. 2021. Effects of nitrogen addition on leaf nutrient stoichiometry in an old-growth boreal forest. Ecosphere, 12(1): ecs2.3335, doi: 10.1002/ecs2.3335.

Xu X, Timmer V R. 1999. Growth and nitrogen nutrition of Chinese fir seedlings exposed to nutrient loading and fertilization. Plant and Soil, 216(1–2): 83–91.

Xu X, Wanek W, Zhou C, et al. 2014. Nutrient limitation of alpine plants: Implications from leaf N:P stoichiometry and leaf δ15N. Journal of Plant Nutrient and Soil Science, 177(3): 378–387.

Yang H. 2018. Effects of nitrogen and phosphorus addition on leaf nutrient characteristics in a subtropical forest. Trees, 32(2): 383–391.

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(1): 11–18.

Yuan Z Y, Chen H Y H. 2015. Negative effects of fertilization on plant nutrient resorption. Ecology, 96(2): 373–380.

Yue P, Li K, Gong Y, et al. 2016. A five-years study of the impact of nitrogen addition on methane uptake in alpine grassland. Scientific Reports, 6: 32064, doi: org/10.1038/srep32064.

Zhang D, Hui D, Luo Y, et al. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. Journal of Plant Ecology, 1(2): 85–93.

Zhang L X, Bai Y F, Han X G. 2004. Differential response of N:P stoichiometry of *Leymus chinensis* and *Carex Korshinskyi* to N additions in a steppe ecosystem in Nei Mongol. Acta Botanica Sinica, 46(3): 259–270.

Zhang Q F, Xie J S, Lyu M, et al. 2017. Short-term effects of soil warming and nitrogen addition on the N:P stoichiometry of *Cunninghamia lanceolata* in subtropical regions. Plant and Soil, 411(1–2): 395–407.

Zhang X Y, Jia J, Chen L T, et al. 2021. Aridity and NPP constrain contribution of microbial necromass to soil organic carbon in the Qinghai-Tibet alpine grasslands. Soil Biology and Biochemistry, 156: 108213, doi: 10.1016/j.soilbio.2021.108213.

Zheng L L, Zhao Q, Sun Q Y, et al. 2020. Nitrogen addition elevated autumn phosphorus retranslocation of living needles but not resorption in a nutrient-poor *Pinus sylvestris* var. *Mongolica* plantation. Forest Ecology and Management, 468: 118174, doi: 10.1016/j.foreco.2020.118174.

Zhao G S, Shi P L, Wu J S, et al. 2017. Foliar nutrient resorption patterns of four functional plants along a precipitation gradient on the Tibetan Changtang Plateau. Ecology and Evolution, 7(18): 7201–7212.