

# Nitrogen and phosphorus resorption of *Artemisia scoparia*, *Chenopodium acuminatum*, *Cannabis sativa*, and *Phragmites communis* under nitrogen and phosphorus additions in a semiarid grassland, China

L.J. Li<sup>1,2</sup>, D.H. Zeng<sup>2,3</sup>, R. Mao<sup>2</sup>, Z.Y. Yu<sup>2,3</sup>

<sup>1</sup>Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin, P.R. China

<sup>2</sup>State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, P.R. China

<sup>3</sup>Daqinggou Ecological Station, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, P.R. China

## ABSTRACT

A factorial nitrogen (N) × phosphorus (P) addition experiment was conducted to evaluate responses of leaf nutrient resorption to increased soil N and P availability in a semiarid grassland in Keerqin Sandy Lands, China. Four plant species were selected, among which *Artemisia scoparia* and *Chenopodium acuminatum* were dominant species in the control and P-added plots, and *Cannabis sativa* and *Phragmites communis* were dominant in the N- and N + P-treated plots. Results showed that N and P resorption varied substantially among species ( $P < 0.01$ ). A general trend of decrease in N resorption efficiency (NRE) and N resorption proficiency (NRP) was observed in response to increased soil N availability for all species, except *P. communis* only for NRE. Similarly, P resorption proficiency (PRP) decreased in response to P addition for all species, whereas P resorption efficiency (PRE) was not affected by P addition. Species responded differently in terms of PRE and PRP to N addition, whereas no changes in NRE and NRP occurred in response to P addition except *P. communis* for NRE. Our results suggest that increased soil nutrient availability can influence plant-mediated nutrient cycling directly by changing leaf nutrient resorption and indirectly by altering species composition in the sandy grassland.

**Keywords:** litter decomposition; nitrogen limitation; nutrient availability; sandy grassland; species composition

Nutrient resorption is considered to be an important indicator of plant nutrient conservation strategies (Aerts and Chapin 2000). Nutrient resorption is an important process during leaf senescence, which can contribute significantly to whole plant nutrient retention (Carrera et al. 2003). Through nutrient resorption, nutrients are stored by plants and directly available for further use, minimizing nutrient losses and making plant species less dependent on current nutrient supply (Aerts and Chapin 2000).

Increased soil nitrogen (N) availability was observed in many terrestrial ecosystems during the

past decades (Bobbink et al. 2010). Responses of nutrient resorption to changing N availability have been extensively studied (Huang et al. 2012). The results are less conclusive because some studies have shown a decrease in nutrient resorption with increases in soil N availability (Huang et al. 2012), but others have shown a neutral effect (Soudzilovskaia et al. 2007). Inter-species variation and soil nutrient status might be two important factors for the inconsistent responses (Kobe et al. 2005). Furthermore, few studies investigated nutrient resorption responses to increased soil phosphorus (P) availability and to its interactions

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with increased soil N availability (Kozovits et al. 2007), especially in grasslands (Lü and Han 2010).

Many ecosystems in China experience increases in nutrient availability due to fertilizer applications (Lü et al. 2012) and atmospheric N deposition in recent decades (Lü and Tian 2007). Semi-arid ecosystems such as the Keerqin sandy grasslands, characterized by nutrient deficiency, may be particularly sensitive to changes in nutrient availability. Thus, understanding changes in leaf nutrient resorption under increased soil nutrient availability is crucial for exploring plant nutrient conservation strategies and nutrient cycling in the grasslands. The aim of this study was to investigate responses of nutrient resorption of four dominant herbaceous species to N and/or P additions. We hypothesized that nutrient additions might decrease leaf nutrient resorption efficiency and proficiency.

## MATERIAL AND METHODS

**Experimental site.** The study was conducted at the Daqinggou Ecological Station (42°58'N, 122°21'E), located in the southeast of Keerqin Sandy Lands, Northeast China. The site is a sandy flat grassland. Annual average precipitation is 450 mm, with more than 60% falling in June to August. Annual average temperature is about 6.4°C, with the lowest and highest mean monthly temperatures occurring in January (−12.5°C) and in July (23.8°C), respectively. Soils are characterized by coarse texture and loose structure.

**Experimental design.** In 2004, we randomly assigned 24 plots of 4 m × 4 m with visually similar vegetation to four treatments (control, N addition, P addition, and N + P addition). Each of the 24 plots was surrounded by a 2-m buffer strip. Nitrogen was added as urea/NH<sub>4</sub>NO<sub>3</sub> at a rate of 20 g N/m<sup>2</sup>/year. Phosphorus was added as NaH<sub>2</sub>PO<sub>4</sub> at a rate of 4.4 g P/m<sup>2</sup>/year. The fertilizers were dissolved in 16 L water and applied in late April–early May (30%) and mid-June (70%) each year. Control plots only received 16 L water without fertilizer application each time.

**Sampling and analyses.** Our previous studies (Li et al. 2010, Zeng et al. 2010) showed that increased N (but not P) availability led to a dramatic shift in species composition and dominance, thus we selected four dominant plant species from the four treatments in the present study, among which *Artemisia scoparia* (biennial forb) and *Chenopodium acuminatum* (annual forb) were dominant in the control and P-added plots, and *Cannabis sativa* (annual forb) and *Phragmites*

*communis* (perennial grass) were dominant in the N- and N + P-added plots. On August 2, 2009, twenty mature and fully expanded green leaves of each selected species were collected at random from each plot and mixed to produce a sample. On August 23, we collected the same number of recently senesced but still attached leaves in the same plots. The leaves were transported to the laboratory, oven-dried at 60°C for 48 h, and then ground for determination of total N (TN) and total P (TP) concentrations.

Soils (0–15 cm) were sampled with a soil corer (2.5 cm in diameter) in August 2009. Eight soil cores were randomly collected in each plot and were mixed to form a composite sample. After removing visible litters, soil samples were sieved (2 mm) and divided into two subsamples. One subsample was used to determine soil inorganic N (IN, NO<sub>3</sub><sup>−</sup>-N and NH<sub>4</sub><sup>+</sup>-N) concentrations. Another subsample was air-dried for determination of available P (AP) concentration and soil pH, and a portion of the air-dried samples was ground and sieved (0.25 mm) for determination of soil organic C (SOC), TN and TP concentrations.

Air-dried soil samples (sieved through a 2-mm sieve) were used to determine soil pH (soil:water ratio 1:2.5). Organic C in soil samples was determined by using the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>-H<sub>2</sub>SO<sub>4</sub> wet oxidation method of Walkley and Black (Nelson and Sommers 1996). Total N and TP concentrations of soil samples and leaf materials were determined with a continuous flow autoanalyzer (AutoAnalyzer III, Bran + Luebbe GmbH, Norderstedt, Germany). The concentrations of NO<sub>3</sub><sup>−</sup>-N and NH<sub>4</sub><sup>+</sup>-N were also determined on the continuous flow autoanalyzer. Soil AP was measured by the continuous flow autoanalyzer in Mehlich 1 extraction as described by Sims (2000).

**Calculations and statistical analysis.** Nutrient resorption was quantified by nutrient resorption efficiency and proficiency (Killingbeck 1996). It was assumed that there were no nutrient losses by leaching from senesced leaves during nutrient translocation from leaves to roots because there is very little precipitation in this season in our study site. Nutrient resorption efficiency was calculated as:

$$RE = (\text{nutrient}_g - \text{nutrient}_s) / \text{nutrient}_g \times 100\%$$

Where: nutrient<sub>s</sub> and nutrient<sub>g</sub> – the N or P pool of the senesced and green leaves, respectively. Nutrient concentration in senesced leaves was used as an indicator of the nutrient resorption proficiency, defined as the level to which nutrient is reduced during leaf senescence.

Table 1. Soil pH, organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), inorganic nitrogen (IN,  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N), and available phosphorus (AP) under different treatments

Treatment	pH ( $\text{H}_2\text{O}$ )	SOC (mg/g)	TN (mg/g)	TP (mg/g)	IN ( $\mu\text{g/g}$ )	AP ( $\mu\text{g/g}$ )
Control	6.65 (0.02) <sup>a</sup>	4.47 (0.25) <sup>a</sup>	0.33 (0.02) <sup>a</sup>	0.08 (0) <sup>c</sup>	3.84 (0.09) <sup>b</sup>	17.40 (0.83) <sup>c</sup>
N	5.35 (0.04) <sup>b</sup>	5.16 (0.15) <sup>a</sup>	0.40 (0.01) <sup>a</sup>	0.08 (0) <sup>c</sup>	8.26 (1.77) <sup>a</sup>	16.22 (2.16) <sup>c</sup>
P	6.63 (0.08) <sup>a</sup>	5.18 (0.49) <sup>a</sup>	0.37 (0.03) <sup>a</sup>	0.12 (0) <sup>a</sup>	4.78 (0.43) <sup>ab</sup>	56.17 (0.43) <sup>a</sup>
N + P	5.43 (0.05) <sup>b</sup>	5.24 (0.17) <sup>a</sup>	0.34 (0.04) <sup>a</sup>	0.09 (0.01) <sup>b</sup>	6.20 (1.04) <sup>a</sup>	36.66 (0.53) <sup>b</sup>

Values are means with standard error in parentheses ( $n = 6$ ). Different letters within a column indicate a significant difference at  $P < 0.05$

All analyses were performed by the SPSS V13.0 (SPSS Inc., Chicago, Illinois, USA). Treatment effects on soil properties were analyzed by one-way ANOVAs with *LSD*. A three-way ANOVA with species, N and P additions as the main factors was performed to examine all possible interactions on N and P concentrations in senesced leaves ( $\text{N}_s$  and  $\text{P}_s$  for short, respectively), nitrogen resorption efficiency (NRE) and phosphorus resorption efficiency (PRE). The effects of treatment on leaf nutrient concentrations and resorption efficiencies were examined for each species individually based on one-way ANOVAs with *LSD*.

## RESULTS

**Soil properties.** Nitrogen addition decreased ( $P < 0.05$ ) soil pH and increased ( $P < 0.05$ ) soil IN concentration, either alone or combined with P (Table 1). In addition, soil TP and AP concentrations were higher (both  $P < 0.05$ ) in the P-added plots (P and N + P) than the control, respectively. However, SOC and TN concentrations were not significantly impacted by N and P additions ( $P > 0.05$ , Table 1).

**N and P resorption proficiency.** Species ( $P < 0.001$ ), N addition ( $P < 0.001$ ), and their interactions ( $P = 0.007$ ) significantly affected  $\text{N}_s$  (Table 2). When analyzed for each species individually, N addition increased  $\text{N}_s$  in all species (all  $P < 0.05$ ), i.e. N addition decreased N resorption proficiency (NRP); whereas P addition had no significant effects on  $\text{N}_s$  for all species (all  $P > 0.05$ , Figure 1A). The responses of  $\text{N}_s$  to N + P addition were generally similar to those to N addition.

The addition of P increased  $\text{P}_s$  for all the four species (all  $P < 0.05$ ), i.e. P addition decreased P resorption proficiency (PRP); whereas  $\text{P}_s$  was decreased by N addition for *C. sativa* and *P. communis* (both  $P < 0.05$ ). There were no changes of  $\text{P}_s$  in all species with N + P addition ( $P > 0.05$ ).

**N and P resorption efficiency.** The NRE was significantly affected by species, N addition and their interactions ( $P < 0.001$ ;  $P < 0.001$ ;  $P = 0.002$ , respectively; Table 2). When analyzed for each species individually, N and N + P additions led to lower NRE of all species ( $P < 0.05$ ) except *P. communis* ( $P > 0.05$ ); whereas P and N + P additions increased NRE of *P. communis* ( $P < 0.05$ , Figure 2A).

Nitrogen addition decreased PRE of *C. sativa* and increased that of *P. communis* (both  $P < 0.05$ ), whereas it had negligible effects on that of *A. scoparia* and *C. acuminatum* (both  $P > 0.05$ , Figure 2B). Phosphorus and N + P additions did not affect PRE for all species (Figure 2B).

## DISCUSSION

Consistent with our hypothesis, we found a general trend of decreased N resorption (NRE and NRP) of all species investigated in response to increased soil N availability, except for *P. communis* (only for NRE). Such a decrease in N resorption probably resulted from the increase of

Table 2. Results ( $P$  values) of three-way ANOVAs on the effects of species (S), N addition (N), P addition (P), and their interactions on nitrogen ( $\text{N}_s$ ) and phosphorus ( $\text{P}_s$ ) concentrations in senesced leaves, and nitrogen (NRE) and phosphorus (PRE) resorption efficiency

Sources	$\text{N}_s$	$\text{P}_s$	NRE	PRE
S	< 0.001	0.002	< 0.001	< 0.001
N	< 0.001	< 0.001	< 0.001	0.005
P	0.730	< 0.001	0.880	0.047
S × N	0.007	0.041	0.002	0.093
S × P	0.114	0.019	0.054	0.158
N × P	0.811	0.004	0.954	0.430
S × N × P	0.972	0.164	0.928	0.345

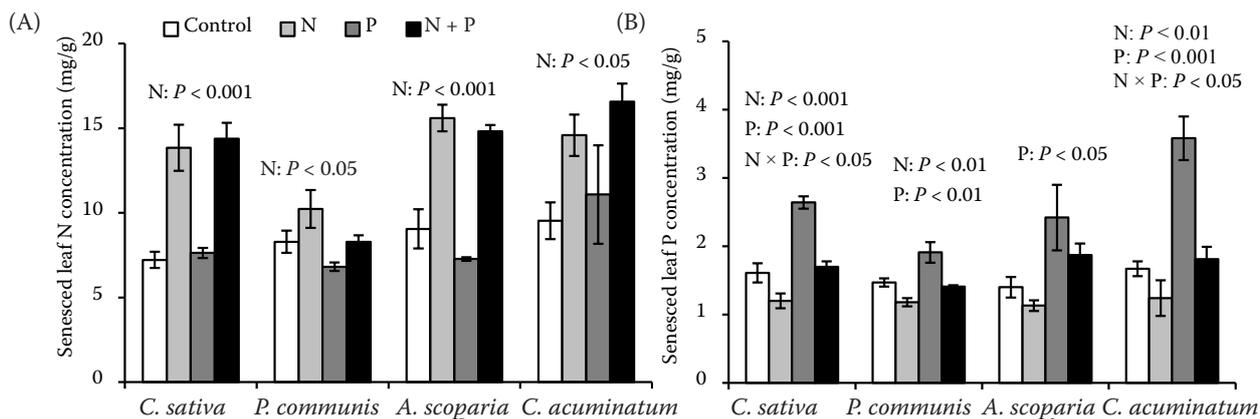


Figure 1. Responses of nitrogen (A) and phosphorus (B) concentrations in senesced leaves of four herbaceous species to N and P additions. Bars are mean values with standard error ( $n = 6$ ). Different letters indicate a significant difference ( $P < 0.05$ ) among treatments for each species individually (based on one-way ANOVA with *LSD*)

N concentration in green leaves with increased N supply (Kobe et al. 2005), implying that plants will absorb more N from soils and become less dependent on N resorbed from senescing leaves. The similar results were also found by other studies (e.g., Huang et al. 2012). Inconsistent with our results, Soudzilovskaia et al. (2007) found that N addition did not alter NRE for most of the analyzed species in an N-limited alpine tundra ecosystem. Soil moisture status and its interaction with soil N availability (Lü and Han 2010) might lead to the different responses of NRE to N addition. Similarly, PRP decreased in response to P addition for all investigated species, whereas PRE was not affected by P addition. The different patterns may be a result of nutrient resorption proficiency being more sensitive to nutrient availability than resorption efficiency (Killingbeck 1996).

Inconsistent with our hypothesis, no marked changes were observed for N resorption (NRE and NRP) to increased soil P availability, except an increase in NRE only for *P. communis* under P addition. The increase in NRE of *P. communis* may be related to a shift from P limitation to N limitation for *P. communis* with P addition. In addition, the different response of NRE of *P. communis* with other species to P addition might be partly explained by the fact that grass species are more efficient in N resorption than other life-forms (Yuan and Chen 2009). Species-specific responses of PRP and PRE to N addition were observed, which was consistent with the study performed in the similar semi-arid grassland (Huang et al. 2012). In our study, N addition decreased  $P_s$  of *C. sativa* and *P. communis*, whereas it had no effects on *A. scoparia* and *C. acuminatum*. The different patterns among spe-

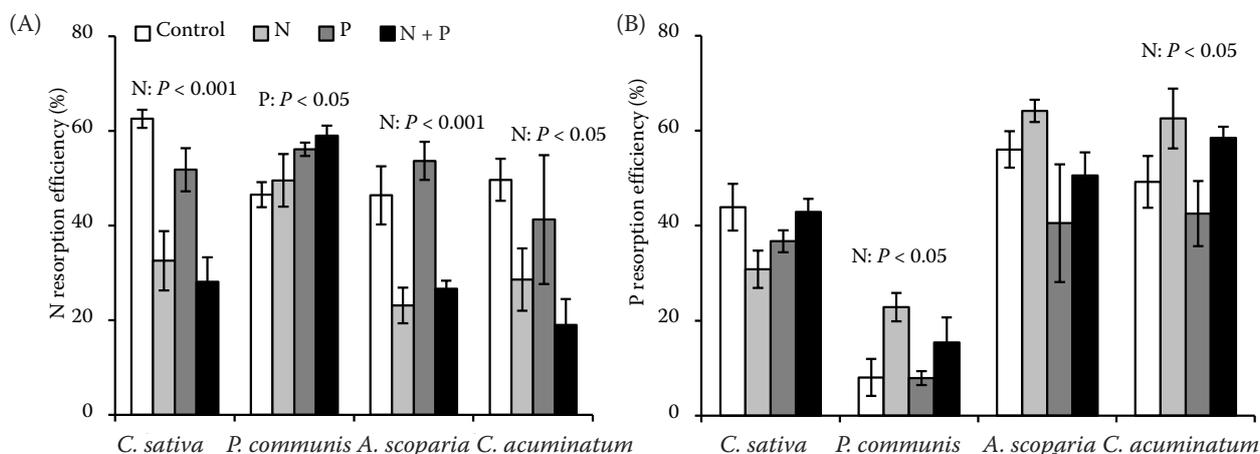


Figure 2. Responses of nitrogen (A) and phosphorus (B) resorption efficiency of four herbaceous species to N and P additions. Bars are mean values with standard error ( $n = 6$ ). Different letters indicate a significant difference ( $P < 0.05$ ) among treatments for each species individually (based on one-way ANOVA with *LSD*)

cies might be related to the different types of nutrient limitation. The increased PRP for *C. sativa* and *P. communis*, combined with increased N/P ratio of senesced leaf in response to N addition ( $P < 0.001$ , data not shown), suggests that N addition possibly results in a shift from N limitation to P limitation and thus strong P control over the leaf decomposition, as observed in other studies (e.g., van Heerwaarden et al. 2003). In contrast, P might not become a main limiting factor for *A. scoparia* and *C. acuminatum* under N addition, as reflected by no marked changes in PRP and N/P ratio under N addition.

Nutrient concentration in senesced leaves, together with litter quantity, determines the amount of nutrients returned to soils (van Heerwaarden et al. 2003). The increased  $N_s$  with N addition, together with increased litter quantity (Li et al. 2011a), would add to the amount of N returned to soils by leaf litter production. Moreover, leaf litter decomposition is mainly controlled by litter N concentration (Li et al. 2011b), and thus litters with high N concentration following N addition would decompose faster. Therefore, given the expected increases in soil nutrient availability due to human activities (Gruber and Galloway 2008), our fertilization study may contribute to predicting the potential effects of soil nutrient enrichment on nutrient cycling, including the nutrient partitioning between the resorption and decomposition pathways.

Huang et al. (2012) reported that both leaf mass- and leaf area-based approaches produced comparable resorption efficiency values in a similar semi-arid area. In our study, it is needed to obtain a mass loss calibration factor by comprising nutrient resorption efficiency expressed on the basis of leaf mass and leaf area (Vergutz et al. 2012).

In conclusion, N addition generally decreased N resorption (NRE and NRP) and P addition decreased PRP for all investigated species. Species responded differently in terms of PRE and PRP to N addition. No changes in PRE, NRE, and NRP were observed in response to P addition. Given N-induced shifts of species composition (Zeng et al. 2010), our study implies that species-specific responses in leaf nutrient resorption to N addition can largely affect plant-mediated nutrient cycling in the semiarid grassland.

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*Corresponding author:*

Prof. De-Hui Zeng, Chinese Academy of Sciences, Institute of Applied Ecology, 72 Wenhua Rd, Shenyang 110016, P.R. China  
e-mail: zengdh@iae.ac.cn

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