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Author for correspondence:

Jin-Sheng He

e-mail: jshe@pku.edu.cn

[†]These authors contributed equally to this work.

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Leaf P increase outpaces leaf N in an Inner Mongolia grassland over 27 years

Zhaorong Mi^{1,4,†}, Yuanyuan Huang^{2,†}, Huijie Gan², Wenjia Zhou², Dan F. B. Flynn^{1,3} and Jin-Sheng He^{1,2}

¹Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, 23 Xinning Road, Xining 810008, People's Republic of China

²Department of Ecology, College of Urban and Environmental Sciences, and Key Laboratory for Earth Surface Processes of the Ministry of Education, Peking University, 5 Yiheyuan Road, Beijing 100871, People's Republic of China

³The Arnold Arboretum of Harvard University, 1300 Centre St., Boston, MA 02131, USA

⁴University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing 100049, People's Republic of China

The dynamics of leaf nitrogen (N) and phosphorus (P) have been intensively explored in short-term experiments, but rarely at longer timescales. Here, we investigated leaf N : P stoichiometry over a 27-year interval in an Inner Mongolia grassland by comparing leaf N : P concentration of 2006 with that of 1979. Across 80 species, both leaf N and P increased, but the increase in leaf N lagged behind that of leaf P, leading to a significant decrease in the N : P ratio. These changes in leaf N : P stoichiometry varied among functional groups. For leaf N, grasses increased, woody species tended to increase, whereas forbs showed no change. Unlike leaf N, leaf P of grasses and forbs increased, whereas woody species showed no change. Such changes may reflect N deposition and P release induced by soil acidification over the past decades. The interannual effect of precipitation may somewhat have reduced the soil available N, leading to the more modest increase of leaf N than of leaf P. Thus, leaf N : P stoichiometry significantly responded to long-term environmental changes in this temperate steppe, but different functional groups responded differently. Our results indicate that conclusions of plant stoichiometry under short-term N fertilization should be treated with caution when extrapolating to longer timescales.

1. Introduction

The status and dynamics of leaf nitrogen (N) and phosphorus (P) are closely linked to many aspects of biological processes, from cellular physiology to ecosystem productivity [1]. Therefore, leaf N : P stoichiometry has been studied intensively to understand the relationships between plants and the external environment [2]. Many studies have shown that both leaf N and P often respond to short-term environmental changes. However, there are few data on how leaf N and P respond to long-term environmental changes [3]. It is important to fill this knowledge gap to better understand ecosystem functions and how plants respond to environmental changes.

The semi-arid Inner Mongolia grassland, which is an important component of the Eurasian grassland biome, is predicted to be sensitive to environmental changes [4], such as increased temperature, decreased precipitation, accelerated N deposition and soil acidification [5,6]. Given the long-term continuous scientific survey of this region, this grassland biome is ideal for investigating the stoichiometric responses of leaf N and P to these long-term environmental changes. Here, we compared recently measured leaf N and P concentrations with published data from 1979 [7] to investigate plant stoichiometric responses to long-term environmental changes and their underlying mechanisms.

Table 1. Results of linear mixed-effects models on the effects of year (Y), functional group (FG) and their interaction on leaf N, P and N : P ratio. Italicized values indicate significance at $p < 0.05$.

		numDF	denDF	leaf N		leaf P		N : P ratio	
				F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
overall ($n = 80$)	Y	1	77	2.85	0.096	15.78	<0.001	14.89	<0.001
	FG	2	68	7.42	0.001	16.59	<0.001	14.02	<0.001
	Y \times FG	2	77	6.29	0.003	1.31	0.276	1.26	0.289
grass ($n = 13$)	Y	1	12	28.15	<0.001	29.60	<0.001	3.73	0.078
forb ($n = 48$)	Y	1	47	0.19	0.664	5.63	0.022	10.22	0.003
woody ($n = 19$)	Y	1	18	3.52	0.077	2.40	0.139	1.04	0.321
matched species ($n = 31$)	Y	1	28	4.27	0.048	15.61	<0.001	14.8	<0.001
	FG	2	19	3.33	0.058	9.08	0.002	8.35	0.003
	Y \times FG	2	28	1.67	0.206	0.88	0.427	2.7	0.084
grass ($n = 6$)	Y	1	5	21.70	0.006	5.06	0.074	2.12	0.205
forb ($n = 15$)	Y	1	14	0.02	0.896	9.44	0.008	19.10	<0.001
woody ($n = 10$)	Y	1	9	8.54	0.017	2.42	0.154	0.13	0.723

2. Material and methods

(a) Study area and field sampling

This study was conducted in the Xilin River Basin (43.43–44.48° N, 115.53–117.20° E), Inner Mongolia of northern China. This area has a semi-arid continental temperate steppe climate. The mean annual temperature is 2.4°C and mean annual precipitation is 278 mm (1953–2011). The dominant soil types are Chernozem and Kastanozems [7].

Samples were collected during the peak growing season (late July) in 2006. The sampling sites were chosen based on the survey in 1979 and were subjected to minimal grazing and anthropogenic disturbances. Eighty species from 28 families including three functional groups were measured in 1979 [7] and resampled in this study. Thirty-one species, referred to as ‘matched species’ hereafter, were collected from the same sites as in 1979. The remaining species were sampled from similar plant communities nearby, because they were not found at the same places as in 1979 (electronic supplementary material, table S1). For each species, leaves from five to 10 individuals were collected randomly following a protocol detailed previously [8]. Soil property measurements were conducted during a soil investigation campaign in 2007 (electronic supplementary material, table S2).

(b) Leaf chemical analysis

Leaf N concentrations of 2006 were determined by combustion method using an elemental analyser (2400 II CHNS/O Elemental Analyzer, Perkin-Elmer, Boston, MA, USA). Since leaf N concentrations in 1979 were determined by the Kjeldahl N determination method, leaf N concentrations of 11 randomly selected species were measured simultaneously by both the Kjeldahl and combustion methods (electronic supplementary material, figure S1). Regression between leaf N measured by the two methods showed a ratio of 1.115, which is similar to many previous studies [9]. Thus, leaf N concentrations of 1979 were transformed using this ratio to eliminate methodological bias. Leaf P concentrations were measured in both years by the molybdate/stannous chloride method after $H_2SO_4-H_2O_2-HF$ digestion [8].

(c) Statistical analysis

We performed linear mixed-effects model analysis using year and functional group as fixed factors and using site and species as random factors. We performed this analysis both for the 80 species overall and 31 matched species. For each functional group, we performed linear mixed-effects model analysis using year as the fixed factor and using site and species as random factors to examine the difference of leaf N : P stoichiometry between 1979 and 2006. All statistical analyses were performed in R v. 3.1.1.

3. Results

Compared with 1979, leaf N of 2006 was 5.0% (relative percentage change, hereafter) and 8.9% higher for overall and matched species, respectively (tables 1 and 2, and figure 1a). Compared with 1979, grass leaf N of 2006 was 38.3% and 26.7% higher, and woody species leaf N was 8.4% and 15.3% higher for overall and matched species, respectively, whereas forbs showed no significant change (figure 2a).

Leaf P of 2006 was 16.0% and 25.2% higher than that of 1979 for overall and matched species, respectively (tables 1 and 2, and figure 1b). Compared with 1979, grass leaf P of 2006 was 53.6% and 39.8% higher, and forbs leaf P of 2006 was 13.4% and 33.6% higher for overall and matched species, respectively, whereas woody species showed no significant change (figure 2b).

Compared with 1979, leaf N : P ratios were 13.7% and 17.5% lower for overall and matched species, respectively (tables 1 and 2, and figure 1c). Among the three functional groups, only forbs showed a significant decrease in leaf N : P ratio (figure 2c).

4. Discussion

This study provides the first evidence of the stoichiometric changes of leaf N and P over a 27-year interval in

Table 2. Leaf N, P and N : P ratios for the overall species, matched species and different functional groups in 1979 and 2006.

	leaf N (mg g ⁻¹)		leaf P (mg g ⁻¹)		N : P ratio	
	1979	2006	1979	2006	1979	2006
overall (<i>n</i> = 80)						
mean	26.44	27.77	1.50	1.74	19.28	16.63
median	24.91	26.48	1.42	1.72	18.06	16.72
CV	0.35	0.26	0.41	0.26	0.37	0.27
grass (<i>n</i> = 13)						
mean	18.94	26.20	0.84	1.29	25.00	20.56
median	18.97	25.81	0.78	1.31	22.14	21.72
CV	0.13	0.17	0.34	0.19	0.36	0.16
forb (<i>n</i> = 48)						
mean	29.66	29.14	1.57	1.78	19.69	16.87
median	27.81	27.65	1.48	1.76	19.44	17.14
CV	0.34	0.28	0.34	0.26	0.31	0.25
woody (<i>n</i> = 19)						
mean	23.41	25.37	1.77	1.94	14.35	13.36
median	23.75	25.50	1.63	1.94	14.38	13.52
CV	0.21	0.23	0.36	0.19	0.31	0.26
matched species						
mean	25.15	27.39	1.39	1.74	19.83	16.36
median	24.16	26.18	1.34	1.79	19.83	15.66
CV	0.35	0.28	0.43	0.26	0.35	0.28
grass (<i>n</i> = 6)						
mean	18.97	24.03	0.83	1.16	25.65	21.09
median	18.61	23.67	0.76	1.09	24.30	22.00
CV	0.16	0.11	0.43	0.20	0.37	0.14
forb (<i>n</i> = 15)						
mean	29.04	29.31	1.40	1.87	21.07	16.03
median	27.68	27.58	1.30	1.90	20.76	15.19
CV	0.36	0.33	0.40	0.24	0.21	0.29
woody (<i>n</i> = 10)						
mean	23.01	26.52	1.71	1.90	14.46	14.02
median	24.35	26.77	1.71	1.85	14.91	14.95
CV	0.25	0.24	0.32	0.14	0.35	0.24

the Inner Mongolia grassland, which could be representative for semi-arid temperate grasslands.

It is likely that the changes in leaf stoichiometry over the 27-year interval were mainly induced by the alteration in soil nutrient availability. There are two possible processes influencing soil N availability. On one hand, accelerated N deposition at a rate of 10–15 kg ha⁻¹ a⁻¹ in the Inner Mongolia Steppe tends to increase N availability [5]. On the other hand, the below-average precipitation of 2006 may decrease soil N availability compared with 1979, in which the above-average precipitation may increase soil N availability in the study area (electronic supplementary material, figure S2) [10]. The increased N deposition may overwhelm the reduction due to the lower precipitation and result in an

increase in soil N availability, leading to a higher leaf N in 2006 than in 1979.

Soil P availability is mainly controlled by the chemical balance between insoluble and soluble forms of phosphate, which is largely influenced by soil pH [11]. During the past decades, China's grasslands have suffered severe acidification with soil pH decreasing by 0.67 units in the typical steppe, driven largely by the significant atmospheric N and sulfur deposition [6]. Therefore, the acidification process would release phosphate radicals, increasing soil P availability, thus enhancing the plant phosphate uptake and leaf P concentration [12]. The below-average precipitation of 2006 may have increased soil P availability relative to 1979, in which the above-average precipitation may not influence

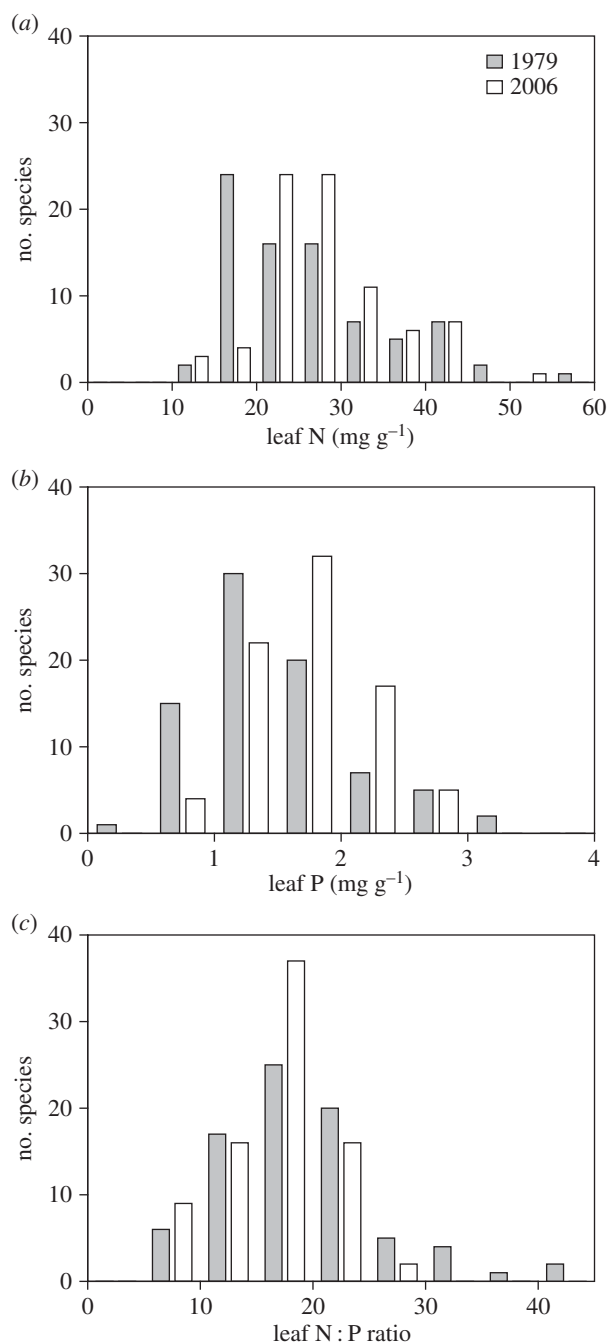


Figure 1. Frequency histograms of (a) leaf N, (b) leaf P and (c) leaf N:P ratios for overall 80 species of 1979 (grey bars) and 2006 (white bars).

soil P availability [13]. The relatively lower increase of leaf N than of P resulted in a lower leaf N:P ratio of 2006 than of 1979. The lower N:P ratio of 2006 may reflect the alleviation of P limitation of this region [14].

The changes in leaf stoichiometry varied among functional groups, reflecting the varying abilities of species to take advantage of the changes in available nutrients in particular traits related to the ability of plant nutrient uptake and resorption. For instance, herbaceous plants (grasses and forbs) have greater specific root length [11] and higher nutrient resorption [15] than woody plants. Previous studies found that N addition benefits grasses at the expense of forbs [16], indicating a competitive advantage of N for grasses to some extent. This competitive advantage and the greater mobility of soil inorganic available N than of P [11] may explain the significant increase of leaf N in grasses but relatively stability in forbs,

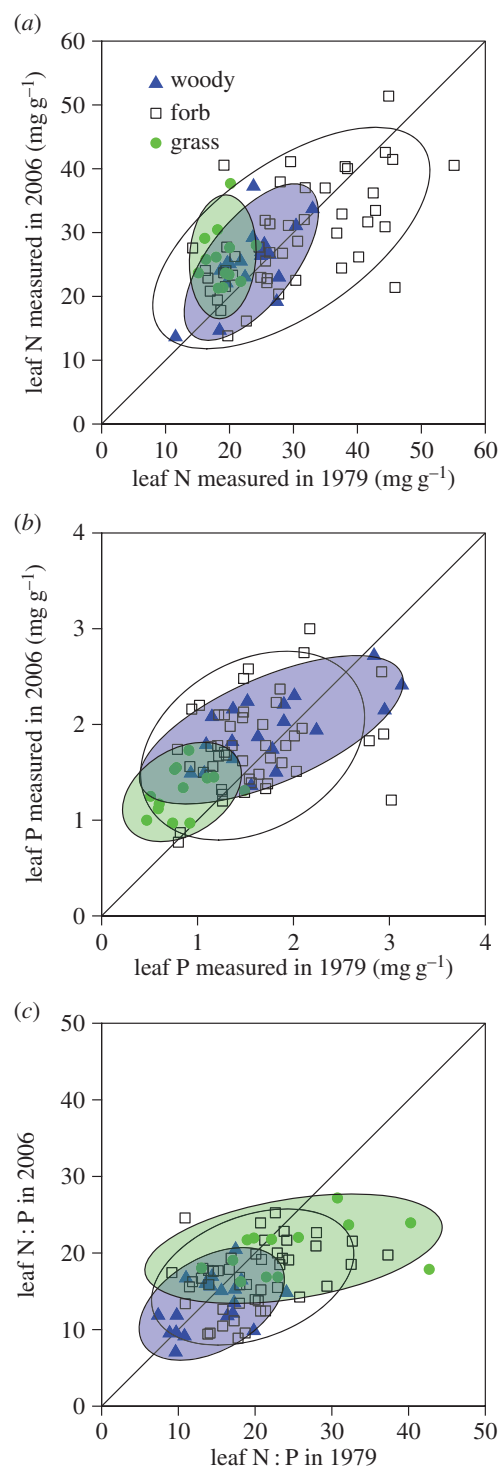


Figure 2. Relationships between leaf N and P stoichiometry of the 80 species of 1979 and 2006. Each circle includes 90% of the data for each functional group. The 1:1 line is shown. (Online version in colour.)

while both grasses and forbs showed a significant increase in leaf P. Therefore, only forbs showed a significant decrease in leaf N:P ratio. Our results diverge somewhat from those of short-term manipulative experiments [16,17]. The results of short-term experiments may be due to the same or larger total amount of N added manually once or several times per year which may generate pulse effects, compared with N deposition in long-term natural conditions. Therefore, different functional groups respond in divergent ways to these changes, which may be attributed to the various inherent nutrient acquisition abilities of individual species [18–20].

Our study suggests that environmental changes will result in significant changes in plant stoichiometry. Different functional groups showed differential responses to long-term environmental changes, reflecting their different nutrient acquisition strategies [21]. The different plant N:P stoichiometry between our study and artificial N addition experiments indicates that conclusions drawn from studies of short-term N fertilization should be treated with caution when extrapolating to longer timescales.

Data accessibility. All stoichiometric data used for analysis are available in the electronic supplementary material.

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