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Scaling of nitrogen and phosphorus across plant organs in shrubland biomes across Northern China

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Allocation of limiting resources, such as nutrients, is an important adaptation strategy for plants. Plants may allocate different nutrients within a specific organ or the same nutrient among different organs. In this study, we investigated the allocation strategies of nitrogen (N) and phosphorus (P) in leaves, stems and roots of 126 shrub species from 172 shrubland communities in Northern China using scaling analyses. Results showed that N and P have different scaling relationships among plant organs. The scaling relationships of N concentration across different plant organs tended to be allometric between leaves and non-leaf organs, and isometric between non-leaf organs. Whilst the scaling relationships of P concentration tended to be allometric between roots and non-root organs, and isometric between non-root organs. In arid environments, plant tend to have higher nutrient concentration in leaves at given root or stem nutrient concentration. Evolutionary history affected the scaling relationships of N concentration slightly, but not affected those of P concentration. Despite fairly consistent nutrients allocation strategies existed in independently evolving lineages, evolutionary history and environments still led to variations on these strategies.

Allocation of limiting resources is an important strategy for plants to adapt to changes in their environment¹, which has been widely observed in the allocation of biomass^{2–4}, functional traits⁵ and morphological plasticity^{6,7}. Nutrients availability plays an important role in ecosystem function and development, since some essential nutrient elements, such as nitrogen (N) and phosphorus (P), are considered limiting resources in nature^{8,9}. Allocation of nutrients involves in two main ways: strategic allocation of different nutrients within a specific organ and allocation of the same nutrient among organs. It has been widely observed that how different nutrients are coupled within a specific organ, such as leaves^{10–12}, stems¹³, fine roots^{14,15} and twigs¹⁴. However, relatively little is known about how the same nutrient is allocated among organs. Plants need to allocate nutrients they absorb into different organs to meet the requirements of multiple functions such as growth, reproduction, nutrient storage and defense¹. Nutrient allocation also reflects a plant's relative investments of essential molecules to different organs, such as N-rich proteins and P-rich RNAs¹⁶.

Previous studies have demonstrated that evolutionary history, environmental stresses and plant functional groups are possible factors influencing the relationship of nutrients among different organs^{16–19}. From an evolutionary perspective, closely related species may share a similar evolutionary history and contain life history strategy^{5,20,21}. Exploring how plants with different evolutionary history allocate N and P among organs can improve our understanding of correlated evolution¹⁶. In a meta-analysis, Kerkhoff et al.¹⁶ found that the scaling relationships of N and P concentrations between plant organs changed between metabolic and structural organs and proposed a general relationship describing N and P allocation among organs across diverse plant lineages. From a life-history perspective, exploring how plants allocate N and P among organs can improve our understanding of nutrient utilization strategies under climate change scenarios²². Plants need to balance the allocation of limiting resource to maximize their growth and may change allocation strategies as a response to changes in their environments such as climate and soil nutrient availability^{1,17,18,22,24}. For example, Sardans & Peñuelas²²



Table 1 | Summary of reduced major axis (RMA) regression results

	Individual level				Species level				PICs				P value for likelihood ratio test				
	b _{ind.}	95% CI	r ²	P	N	b _{sp}	95% CI	r ²	P	N _{sp}	b _{PIC}	95% CI	r ²	P	N _{hip}	b _{ind} vs. b _{sp}	b _{sp} vs. b _{PIC}
Roots vs. leaves:																	
N storage	1.20	1.16–1.25	0.88	<0.001	335	1.30	1.21–1.41	0.85	<0.001	98	1.20	1.10–1.30	0.82	<0.001	98	0.078	0.167
P storage	1.16	1.11–1.2	0.87	<0.001	334	1.26	1.15–1.37	0.82	<0.001	98	1.20	1.09–1.32	0.79	<0.001	98	0.083	0.470
N concentration	1.79	1.68–1.91	0.37	<0.001	613	2.07	1.79–2.38	0.51	<0.001	107	2.04	1.72–2.43	0.20	<0.001	107	0.073	0.721
P concentration	1.52	1.42–1.64	0.22	<0.001	608	1.56	1.34–1.83	0.33	<0.001	107	1.61	1.38–1.89	0.32	<0.001	107	0.770	0.365
Stems vs. leaves:																	
N storage	1.06	1.03–1.09	0.91	<0.001	357	1.10	1.02–1.18	0.87	<0.001	104	1.02	0.97–1.08	0.92	<0.001	104	0.355	0.117
P storage	1.02	0.99–1.05	0.91	<0.001	358	1.07	0.99–1.15	0.86	<0.001	105	1.01	0.95–1.07	0.92	<0.001	105	0.242	0.204
N concentration	1.55	1.45–1.66	0.23	<0.001	655	1.72	1.49–1.99	0.37	<0.001	115	1.74	1.45–2.09	0.04	0.025	115	0.201	0.962
P concentration	1.15	1.07–1.24	0.12	<0.001	650	1.07	0.91–1.26	0.23	<0.001	116	1.15	0.96–1.37	0.08	<0.001	116	0.411	0.571
Roots vs. stems:																	
N storage	1.12	1.09–1.16	0.91	<0.001	343	1.14	1.07–1.22	0.89	<0.001	103	1.16	1.07–1.25	0.84	<0.001	103	0.617	0.778
P storage	1.12	1.08–1.16	0.89	<0.001	344	1.15	1.06–1.23	0.85	<0.001	104	1.18	1.07–1.26	0.8	<0.001	104	0.599	0.612
N concentration	1.14	1.09–1.19	0.67	<0.001	652	1.22	1.11–1.33	0.75	<0.001	113	0.94	0.80–1.11	0.34	<0.001	113	0.185	0.008
P concentration	1.34	1.26–1.43	0.29	<0.001	646	1.48	1.26–1.74	0.22	<0.001	113	1.50	1.25–1.81	0.03	0.050	113	0.286	0.894

Abbreviations: PIC, phylogenetically independent contrast; CI, confidence interval. Regression slopes (b_{ind.}, b_{sp} and b_{PIC}) estimates in bold are significantly different from 1, indicate that the scaling relationship of the two traits are anisometric.

found a higher allocation of nutrients to leaves than to woody biomass in more humid climatic conditions. Plants may contain higher N concentrations in leaves to better adapt to arid environments^{25–29} through exploiting greater light availability²⁵ while reducing stomatal conductance and increasing water use efficiency^{28,29}. Plant functional groups have also been shown to influence allocation of nutrients through different photosynthetic pathways, fraction of woody tissue and N-fixation ability^{16–19}. Further studies are needed for a better understanding of the combined impacts of climate, soil and evolutionary history on the allocation of nutrient among plant organs.

Nutrients such as N and P frequently limit plant growth and play important roles in plant functioning^{14,30,31}. As a major element in proteins, N in plant tissues is related to various functions, such as photosynthesis in leaves, tissue respiration, conversion and storage of photosynthates and chemical defense in all plant organs^{32,33}. As a major element of RNAs, P is mainly involved in the production of proteins^{32,34}. Since different plant organs perform different functions in plant growth, the requirements of N and P will differ. For example, leaf N and P concentrations were found to have different scaling relationships with leaf photosynthetic capacity²³. These differences may lead to differences in the scaling relationships of N and P concentrations among plant organs. Additionally, N and P concentrations in plants are widely reported to be more similar among closely related species³⁵. Finally, plants are able to coordinate various organs with respect to absorption and allocation of limiting resources and adaptation to environmental constraints¹⁸; for example, plants tend to increase leaf N concentration to maintain growth under dry conditions^{25–29}. Given the aforementioned ability of plants to adapt to different allocation strategies, we made the three following hypotheses. First, N and P have different scaling relationships across organs because of their different physiological functions. Second, the scaling relationships of N and P across organs depend on the phylogenetic relatedness of the plants; more similar nutrient allocation strategies exist in more closely related species. Third, we hypothesize that the scaling relationship of nutrients among plant organs changes with environmental factors, such as soil nutrients and precipitation; particularly, we expect higher leaf N concentration at given root or stem N concentration in more arid regions.

We tested the aforementioned hypotheses using an extensive investigation of N and P storage in different organs of shrubs across northern China. Compared to trees, shrubs are generally smaller and relatively more uniform in size among species because they do not have large trunks or roots, which weaken the “dilution effects” of N and P allocation to structural components of woody plants¹⁶. Shrubland represent a major vegetation type in Northern China with community types varying from east to west China along an aridity gradient³⁶. In this study, we explored the relationships of N and P among leaves, stems and roots of shrubs in Northern China using scaling approach, which has been applied in exploring generalities of patterns and trade-offs in many plant traits^{10,16}. Nutrient allocation among plant organs involves two facets: concentration and biomass storage¹⁶. We compared the scaling relationship for both concentrations and storages of N or P among leaves, stems and roots. The comparison has been conducted at following scales: 1) individual vs. species vs. phylogenetic levels; 2) species from the family Fabaceae vs. those from other families; 3) samples from different soil nutrient concentration levels and 4) samples from different climatic regions.

Results

Scaling of N and P among organs. Both concentrations and storages of N and P showed significant correlation among all organs at the individual, species, and phylogeny levels (Table 1).

At the individual level, the slopes of N concentration in all organ pairs were significantly different from 1; the slopes for root vs. leaf and stem vs. leaf were significantly larger than that of root vs. stem. At the species level, the slopes were all significantly different from 1;

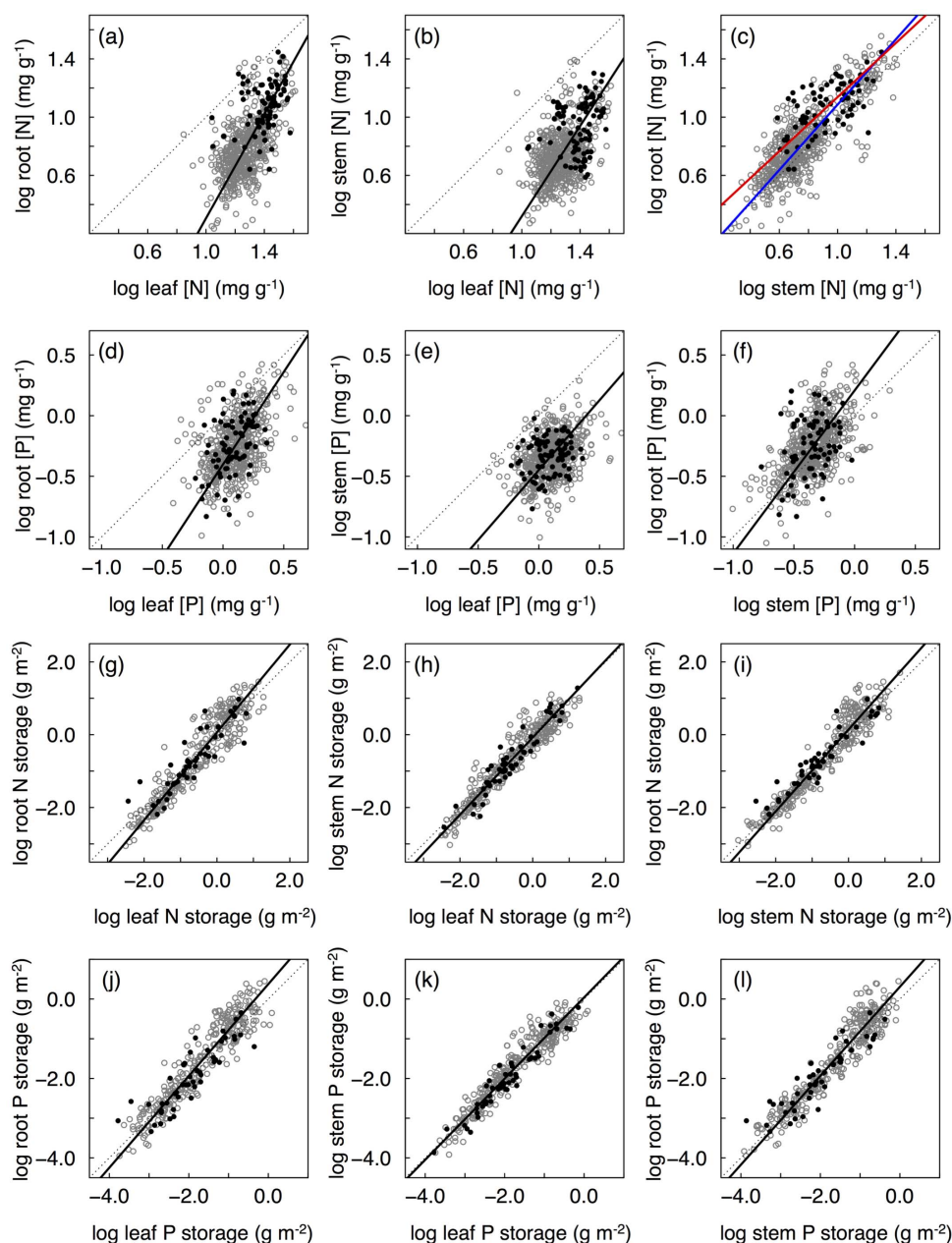


Figure 1 | Scatterplots showing the RMA regressions of N concentration (a–c), P concentration (d–f), N storage (g–i) and P storage (j–l) among organs for legume (black solid dots) and non-legume (gray open cycles) shrubs. Separate lines indicate the slopes of non-legumes (blue) and legumes (red) are significantly different (likelihood ratio tests, $P < 0.05$), whereas a single black line indicates otherwise. Lines with the slopes equal to 1 are shown with dotted lines.

the slope was significantly larger for root vs. leaf and stem vs. leaf than for root vs. stem. At the phylogeny level, the PIC analyses also revealed a similar pattern to individual and species level (Table 1). The slope of the root vs. stem was not significantly different from 1 when PIC is applied. There were no significant differences between the slopes at individual level and species level ($P > 0.05$). Slopes at the species level were not significantly different ($P > 0.05$) from the phylogeny level except the root vs. stem ($P = 0.012$).

Scaling relationship of P concentration among organs was different from that of N (Table 1). At the individual level, the slopes of P concentration in all organ pairs were significantly different from 1; the slope for root vs. leaf or root vs. stem was more deviated than for stem vs. leaf. At the species level, the slope was significantly larger for root vs. leaf and root vs. stem than for stem vs. leaf (Table 1). The PIC analysis depicted that, at the phylogeny level, the slope was significantly larger for root vs. leaf and root vs. stem than for stem vs. leaf.

There were no significant differences between the individual, species and phylogeny level ($P > 0.05$).

The RMA slopes for N and P storages of the same organ pairs were similar at all three levels. For N storage at individual and species levels, the slopes of root vs. leaf, stem vs. leaf and root vs. stem were significantly larger than 1. The PIC analysis depicted that, at the phylogeny level, the slopes of root vs. leaf and root vs. stem were significantly larger than 1, while that of stem vs. leaf was equivalent to 1. For P storage at all levels, the slopes of root vs. leaf and root vs. stem were significantly larger than 1, while the slopes of stem vs. leaf were equivalent to 1.

Influence of functional type on scaling of N and P among organs. All RMA regression slopes were not significantly different between legumes and non-legumes ($P > 0.05$), except the root N concentration vs. stem N concentration ($P = 0.04$) (Figure 1).



All RMA regression slopes of N and P storages among different plant organs were not significantly different between legumes and non-legumes ($P > 0.05$).

Influence of soil nutrient on scaling of N and P among organs. The slopes of N concentration among organs were not significantly different across different soil total N concentration (STN) levels, except that at the highest STN level. The slope of root vs. stem was significantly different than the slopes at the other two STN levels (high STN vs. low STN: $P = 0.009$ and high STN vs. medium STN: $P = 0.005$) (Figure 2a). The slopes of N storage among organs were not significantly different across different STN levels ($P > 0.05$) (Figure 2d).

At different soil total P concentration (STP) levels, the slopes of P concentration among organs were not significantly different across different STP levels, except a slightly larger slope of root vs. stem at the highest STP (high STP vs. medium STP: $P = 0.017$) (Figure 2b). The slopes of P storage among organs were not significantly different across different STP levels ($P > 0.05$) (Figure 2e).

Influence of aridity on scaling of N and P among organs. The slopes of N concentration in root vs. leaf were significantly higher in semi-arid/semi-humid (Semi) and humid (Humid) regions than in arid regions (Arid) (Arid vs. Semi: $P = 0.020$ and Arid vs. Humid: $P = 0.014$). Similarly, the slopes of stem vs. leaf N concentration were significantly higher in Semi and Humid than in Arid (Arid vs. Semi: $P = 0.031$ and Arid vs. Humid: $P = 0.049$). The slope of root vs. stem was not significantly different among three climatic regions (Figure 2c).

The slope of P concentration in root vs. stem was not significantly different between Arid and Semi, and significantly higher in Humid than in Semi (Arid vs. Humid: $P = 0.048$ and Semi vs. Humid: $P < 0.001$). The slopes of stem vs. leaf and root vs. stem were not significantly different among three climatic regions (Figure 2c).

The slopes of N and P storages among different organs were quite similar at different climatic regions (Figure 2f). The slopes of N and P storage in root vs. leaf were not significantly different between Arid and Semi, but were significantly lower in Humid than in Semi ($P = 0.001$ and 0.006 for N and P, respectively). The slopes of P storage in stem vs. leaf were significantly higher in Arid than in Semi ($P = 0.028$), and were not significantly different between Semi and Humid. Slopes of N storage were not significantly different among three climatic regions. The slopes in root vs. stem were significantly higher in Semi than in Arid and Humid (Arid vs. Semi: $P = 0.001$ for both N and P; Semi vs. Humid: $P < 0.001$ for both N and P).

Discussion

Using concentrations and storages of N and P in leaves, stems and roots of 126 shrub species from 172 shrubland sites, we studied the allocation strategies of N and P among organs through a scaling approach. Both concentrations and storages of N and P between all pairs of plant organs are significantly correlated at individual, species and phylogeny levels (Table 1). The coordinate variations of nutrients across different organs demonstrate that plant organs are not independent. The allocation of nutrients among organs might be another important part of life history strategy for plants. For example, plants with high leaf nutrient concentration would also have high nutrient concentration in stem and root to increase nutrient uptaking and phloem loading to meet the requirement of higher photosynthesis rate and photosynthate export. At the community level, this coordination would also help to predict changes in nutrient storage in plants under the climate change scenarios, since increased dominance by species with higher nutrient concentration and storage in one organ will lead to predictable increases in nutrient concentration and storage in other organs¹⁶.

Concentrations of nutrients in lower organs increase with those in the neighboring upper organs in a power law form (e.g., roots vs. stems, stems vs. leaves, see Figures 1 and 2, Table 1). This result is consistent with Brouwer's hypothesis that plant organs are competing for nutrients during growth, and that the organ nearest to the nutrient source will be most successful^{37–39}, as nutrients are transported to distant organs only after the needs by adjacent organs are met³⁹.

As hypothesized, N and P concentrations show different scaling relationships among organs. Consistent with Kerkhoff et al.¹⁶, we find that scaling relationships of N concentration in photosynthetic organ (leaf) vs. N concentration in non-photosynthetic organs (stem and root) tend to be allometric, while that of N concentration between stem vs. root is very close to (although not) isometric (Table 1). Particularly, there is a faster increase of N concentration in non-leaf organs as leaf N concentration increases ($b > 1.0$), partly because non-leaf organs are closer to the nutrient source (soil)^{37–39}. A second possible reason might be related to the activity of vascular tissues. Phloem tissues require more N investments and higher rates of N cycling for the high rate of photosynthate transportation and photosynthetic activity^{16,40}. Among the non-photosynthetic organs, we also observe that the scaling relationships are not strictly isometric, partly because of the difference in distance to the nutrient sources^{37–39}. Contrary to N concentration, the scaling slopes of P concentration between non-root organs are close to 1, but higher in root vs. non-root organs, partly because that root is the closest organ to P source. This difference between the scaling relationships of N and P concentrations among organs might be because of different requirements of N and P among plant organs to perform various functions. However, it is difficult to fully understand these differences without measuring important physiological processes of plant organs, such as photosynthesis, respiration, nutrient absorption and transportation, or plant functional traits which can represent these physiological processes. Future studies including plant physiological processes will help to further explain the mechanisms behind the scaling relationships of N and P across plant organs.

In contrast to concentrations, the scaling slopes of N and P storages among plant organs are quite similar, which indicates that the allocations of N and P in plants largely depends on the allocation of biomass. This result is reasonable because both N and P are only small portions of total plant tissue, and the pools of N and P in plant tissues mainly reflect the tissue size rather than composition.

Most scaling relationships remain unchanged after phylogeny was controlled, indicating that the scaling relationships of nutrient concentrations across plant organs are independent of phylogenetic relationships between species. We also observed the same scaling relationships for most organ pairs between legume and non-legume shrubs. Although plant N and P concentrations are considered phylogenetically conservative traits^{16,35,41}, that was not the case for the allocation of N and P among organs in this study. The scaling relationships of nutrient concentration across most organs are not simply a result of shared evolutionary history of these species¹⁶.

However, we still found significant difference between species and phylogeny level relationships for root N concentration vs. stem N concentration. Similarly, we found significantly different scaling relationships in root N concentration vs. stem N concentration between legumes and non-legumes. These results indicate that the N fixing pathways used by legumes impact the allocation strategy of N in shrubs. The difference in the scaling of N between legumes and non-legumes supports the hypothesis that plant functional group affects plant traits as well as their scaling relationships^{16,42}. Therefore, it is important to take plant functional group into consideration when exploring plant nutrient allocation strategies.

We did not observe a significant influence of STN and STP on the allocation of nutrients among organs, suggesting that plants do not change their nutrients allocation strategies even under soil nutrients

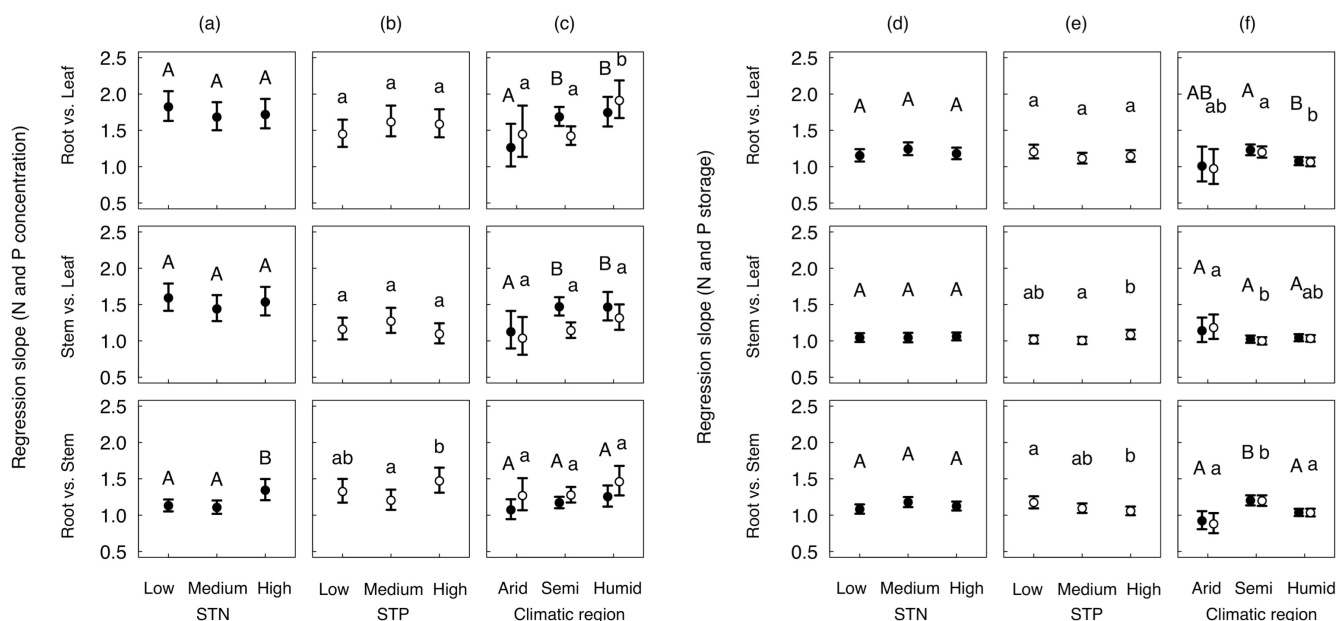


Figure 2 | Comparisons of the RMA regression slopes of N concentration and storage (solid dots), P concentration and storage (open cycles) among different STN levels (a), (d), STP levels (b), (e) and climatic regions (c), (f). Line bars show 95% confidence intervals (CI). Letters above the line bars show the results of likelihood ratio tests (uppercase for N concentration and storage, lowercase for P concentration and storage). Slopes with the same letters are not significantly different ($P \geq 0.05$), while those with different letters are significantly different ($P < 0.05$).

deficiency. Available soil N and P can be absorbed directly by plants, and thus might influence N and P concentrations in plants^{43,44}. However, recent studies on leaf traits of shrubs and grasses conducted across Inner Mongolia suggests that total and available soil nutrient concentrations explain similar and a very small amount of variance in leaf N and P concentrations of shrub species^{45,46}. These results indicate that in the region studied, total soil nutrient concentrations do not differ from available soil nutrient concentrations in explaining or predicting plant nutrient concentrations, which is consistent with our results. Other soil properties such as soil age⁴⁷, soil freezing and disturbance¹⁷ may impact nutrient allocation in plants; however, these factors are not included in the present study. Further studies are needed to examine the effect of soil properties on the allocation of nutrient in plants.

Plants in arid conditions tend to have higher leaf N concentration in order to exploit light²⁵ and increase the water use efficiency at the expense of lowering N-use efficiency^{28,29}. As expected, increases of N concentration in non-photosynthetic organs are even faster in humid than in arid regions (Figure 2c). Our result supplements these theories by considering the relationship of nutrient concentrations among plant organs. Although the increase of N concentration in non-leaf organs is always faster than the increase in leaf N concentration in all the three climate regions, leaf N concentration tends to be higher at given root or stem N concentration under arid region than humid region. The scaling relationships of P concentration between non-leaf organs and leaves showed similar variation as N concentration among three climate regions. Another possible reason might be that water is the limiting factor for vegetation growth in shrublands of Northern China and increases in water availability promotes plant growth⁴⁸. During growth, more photosynthate and nutrients will be transported to non-photosynthetic organs¹³. This result indicates that shrub species in Northern China display a strategy that favors higher nutrient concentration in leaves than in non-photosynthetic organs in dry environments. The variation tendencies of RMA slopes of N and P storages are almost the same under different aridity conditions, which indicates that aridity can influence plant nutrient allocation through variation in plant biomass

allocation. In semi-arid/semi-humid regions, plants tend to allocate more biomass to their root systems than to aboveground organs.

In summary, we investigated the scaling relationship of N and P in leaves, stems and roots based on samples of 126 shrub species from 172 shrubland communities in Northern China. We found that plants have different strategies of allocation for N and P. The scaling relationships of N concentration across different plant organs tended to be allometric between leaves and non-leaf organs, and isometric between non-leaf organs. Whilst the scaling relationships of P concentration tend to be allometric for root vs. stem and for root vs. leaf, it tends to be isometric for stem vs. leaf. The scaling relationships of both concentrations and storages of N and P among organs are affected by aridity, but not by soil nutrient concentration. In arid environments, plant tend to have higher nutrient concentration in leaves at given root or stem nutrient concentration. Most scaling relationships of concentrations and storages of N and P across plant organs don't exhibit phylogenetic signal, except that of N concentration, which could be attribute to the difference of legumes and non-legumes in N utilization. We therefore conclude that consistent nutrient scaling relationships among plant organs exist in independently evolving lineages, but factors such as climate and N-fixation ability lead to variations in these relationships.

Methods

Study site and investigation. This study was carried out at 172 shrubland sites extending 46.1 degrees in longitude (86.7–132.8°E) and 18.7 degrees in latitude (33.7–52.4°N) in Northern China between July and September (mostly July and August) 2011 (Figure 3). Three 5 m × 5 m plots were selected at each site to represent the natural shrubland communities. We identified all individuals to the species level. In each plot, leaf, stem and root biomass were harvested, and all biomass were harvests separately for each species. For each shrub species encountered in the community, we selected three to five individuals and collected fully expanded leaves, stems and roots (mostly coarse roots in the top 30 cm of soil) at each site. Plant samples were oven-dried and ground after being transported to the laboratory. In total, we sampled 702 individuals of 126 shrub species from 71 genera and 33 families. All of the shrub species sampled were deciduous except the *Pinus tabulaeformis* in one study site.

We collected soil samples using three one-meter-deep pits along the diagonal of each plot. For each profile, soil at the depths of 0–10, 10–20, 20–30, 30–50, 50–70 and 70–100 cm was sampled and soil samples from the same depth were well mixed. Soil

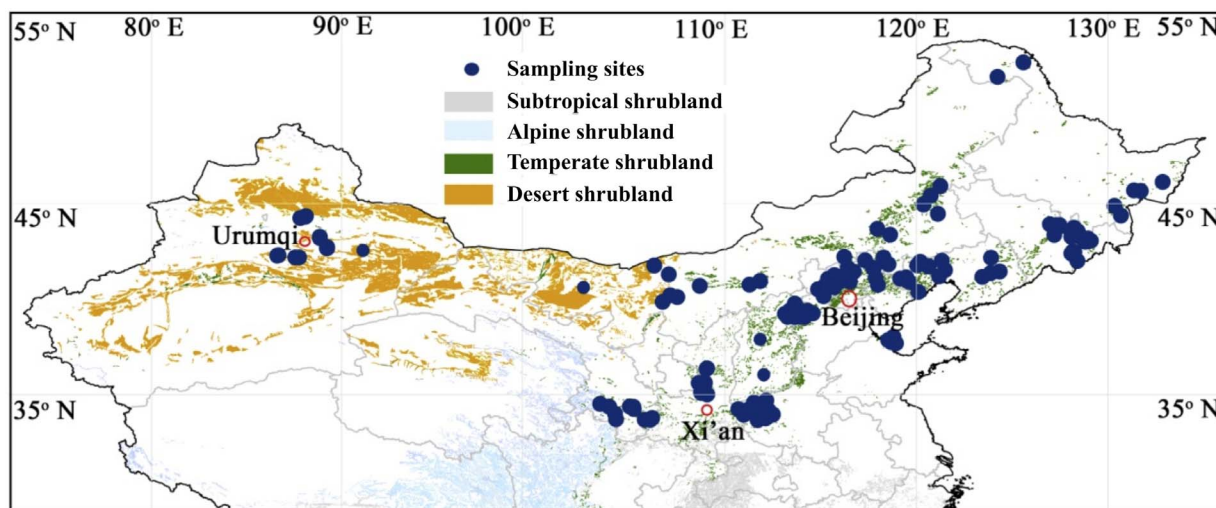


Figure 3 | Locations of the sampling sites. The background shows the distribution of shrubland biomes in the northern part of China based on the “Vegetation map of the People’s Republic of China (1 : 1000000)”³⁶. The map was generated using ArcGIS 10.3 (<http://www.esri.com/>).

samples were air-dried, had roots removed and were ground to pass through a 100-mesh sieve.

Monthly precipitation and mean monthly temperature was obtained from the WorldClim website⁴⁹ (available at www.worldclim.org; resolution of 1 km).

Measurements. The plant and soil samples were analyzed at the Measurement Center of the Institute of Botany, Chinese Academy of Sciences. Total nitrogen concentrations of soil (STN) and plant samples (leaf, stem and root N concentrations) were analyzed using an elemental analyzer (2400 II CHNS; Perkin-Elmer, Boston, MA, USA) under 950°C for combustion then reduced to 640°C. Total phosphorus concentrations of soil (STP) and plant samples (leaf, stem and root P concentrations) were analyzed using the molybdate/ascorbic acid method after $H_2SO_4-H_2O_2$ digestion⁵⁰. We only used STN and STP at 0–10 cm depth interval for the analyses because STN and STP at this depth interval were highly correlated with those at the other five depth intervals.

Scaling of N and P among different organs. A scaling approach, $Y = aX^b$, was used to examine the covariation in N and P concentrations because both concentration and storage of N and P tend to be log normally distributed^{16,51}. The power function can be expressed in the form of a linear regression equation after log-transforming, where the exponent b is the regression slope and a is the regression intercept. The N and P storages of organs were calculated by multiplying the concentrations of N and P of the organs by the corresponding biomasses.

We first applied reduced major axis (RMA) to examine the correlation of nutrients among organs. An important advantage of RMA regression compared with ordinary least square regression is that RMA minimizes sums of squares in X and Y simultaneously. We assigned the scaling relationship between Y and X as isometric when the 95% confidence interval (CI) of b contains 1; the scaling relationship is otherwise allometric. A b above 1 indicates that, on average, Y increases faster than linearly with X; whereas a b below 1 indicates Y increases slower than linearly with X^{10,16}. In the RMA analyses, we set the organs closer to the top of the plant as the X variable.

To examine the effects of soil nutrient on the relationship of nutrient concentration and storage across organs, we divided all individuals into three equal subgroups based on soil nutrient (STN and STP) and compared the exponential slopes among different soil and climate levels (STN < 0.9, $0.9 \leq STN \leq 2.3$ and $STN > 2.3$ for low, medium and high STN, respectively; STP < 0.47, $0.47 \leq STP \leq 0.63$ and $STP > 0.63$ for low, medium and high STP, respectively).

We also identified three climatic areas to examine the effects of aridity on the scaling relationships of nutrient concentrations and storages across plant organs based on the aridity index in growing season (GAI) defined as the ratio of total precipitation to potential evapotranspiration from May to October⁵². Potential evapotranspiration was calculated using the Thornthwaite equation⁵³. GAI increases from arid to humid climate. We divided the range of GAI of the studied region into three groups: arid regions, with $GAI < 0.59$ (the least one third of the GAI, Arid); semi-arid/semi-humid regions, with GAI between 0.59 and 1.17 (the median one third of the GAI, Semi); and humid regions, with $GAI > 1.17$ (the highest one third of the GAI, Humid).

To test the influence of plant functional groups on the scaling relationship of nutrient concentrations and storage across plant organs, we further grouped all samples into legumes (samples from the family Fabaceae) and non-legumes (samples from other families), as the legume shrubs fix nitrogen whereas the non-legume shrubs do not.

A likelihood ratio test⁵⁴ was used to test the heterogeneity between RMA regression slopes of different groups, i.e., different soil nutrient level, climatic regions, and functional groups.

Phylogenetic tree and phylogenetic analyses. We also looked for the phylogenetic signals of all RMA regression slopes. To do this, we first constructed a phylogenetic tree for the 126 species using Phylomatic⁵⁵ based on APG III topology⁵⁶. The branch lengths were determined using BLADJ algorithm within the Phylocom software (<http://www.phylodiversity.net/phylocom/>) and the node ages were fossil-estimated⁵⁷. We then applied a phylogenetically independent contrast (PIC) analysis⁵⁸. PIC analysis allows us to calculate N-1 standardized contrasts of a trait using a series of trait data for N species. We then conducted RMA regression at both species-level data and PICs, we tested the effects of evolutionary history on covariation of N and P concentrations and storages across organs by comparing the RMA regression slopes at species-level and the PIC analysis using the likelihood ratio test.

All analyses were performed using RStudio with the basic, smatr and picante packages (<http://www.R-project.org/>).

- Bazzaz, A. F. & Grace, J. *Plant resource allocation* (Academic Press, San Diego, 1997).
- Niklas, K. J. & Enquist, B. J. Invariant scaling relationships for interspecific plant biomass production rates and body size. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 2922–2927 (2001).
- Niklas, K. J. & Enquist, B. J. On the vegetative biomass partitioning of seed plant leaves, stems, and roots. *Am. Nat.* **159**, 482–497 (2002).
- Enquist, B. J. & Niklas, K. J. Global allocation rules for patterns of biomass partitioning in seed plants. *Science* **295**, 1517–1520 (2002).
- Reich, P. B. *et al.* The evolution of plant functional variation: traits, spectra, and strategies. *Int. J. Plant Sci.* **164**, S143–S164 (2003).
- Fransen, B., de Kroon, H. & Berendse, F. Root morphological plasticity and nutrient acquisition of perennial grass species from habitats of different nutrient availability. *Oecologia* **115**, 351–358 (1998).
- Grime, J. P. & Mackey, J. M. L. The role of plasticity in resource capture by plants. *Ecol. Evol.* **16**, 299–307 (2002).
- Koerselman, W. & Meuleman, A. F. M. The vegetation N : P ratio: a new tool to detect the nature of nutrient limitation. *J. Appl. Ecol.* **33**, 1441–1450 (2007).
- LeBauer, D. S. & Treseder, K. K. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**, 371–379 (2008).
- Wright, I. J. *et al.* The worldwide leaf economics spectrum. *Nature* **428**, 821–827 (2004).
- Han, W., Fang, J., Guo, D. & Zhang, Y. Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. *New Phytol.* **168**, 377–385 (2005).
- He, J. *et al.* Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grassland biomes of China. *Oecologia* **149**, 115–122 (2006).
- Helmissari, H.-S. & Siltala, T. Variation in nutrient concentrations of *Pinus sylvestris* stems. *Scand. J. For. Res.* **4**, 443–451 (1989).
- Guo, D. L., Mitchell, R. J. & Hendricks, J. J. Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest. *Oecologia* **140**, 450–457 (2004).



15. Gordon, W. S. & Jackson, R. B. Nutrient concentrations in fine roots. *Ecology* **81**, 275–280 (2000).
16. Kerkhoff, A. J., Fagan, W. F., Elser, J. J. & Enquist, B. J. Phylogenetic and growth form variation in the scaling of nitrogen and phosphorus in the seed plants. *Am. Nat.* **168**, E103–E122 (2006).
17. Craine, J. M., Lee, W. G., Bond, W. J., Williams, R. J. & Johnson, L. C. Environmental constraints on a global relationship among leaf and root traits of grasses. *Ecology* **86**, 12–19 (2005).
18. Liu, G. *et al.* Coordinated variation in leaf and root traits across multiple spatial scales in Chinese semi-arid and arid ecosystems. *New Phytol.* **188**, 543–553 (2010).
19. Li, A., Guo, D., Wang, Z. & Liu, H. Nitrogen and phosphorus allocation in leaves, twigs, and fine roots across 49 temperate, subtropical and tropical tree species: a hierarchical pattern. *Funct. Ecol.* **24**, 224–232 (2010).
20. Swenson, N. G. & Enquist, B. J. Ecological and evolutionary determinants of a key plant functional trait: wood density and its community-wide variation across latitude and elevation. *Am. J. Bot.* **94**, 451–459 (2007).
21. Fortunel, C., Fine, P. V. A. & Baraloto, C. Leaf, stem and root tissue strategies across 758 Neotropical tree species. *Funct. Ecol.* **26**, 1153–1161 (2012).
22. Sardans, J. & Peñuelas, J. Tree growth changes with climate and forest type are associated with relative allocation of nutrients, especially phosphorus, to leaves and wood. *Glob. Ecol. Biogeogr.* **22**, 494–507 (2013).
23. Reich, P. B. *et al.* Evidence of a general 2/3-power law of scaling leaf nitrogen to phosphorus among major plant groups and biomes. *Proc. R. Soc. B Biol. Sci.* **277**, 877–883 (2010).
24. Han, W. X., Fang, J. Y., Reich, P. B., Ian Woodward, F. & Wang, Z. H. Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. *Ecol. Lett.* **14**, 788–796 (2011).
25. Cunningham, S. A., Summerhayes, B. & Westoby, M. Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecol. Monogr.* **69**, 569–588 (1999).
26. Wright, I. J., Reich, P. B. & Westoby, M. Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Funct. Ecol.* **15**, 423–434 (2001).
27. Farquhar, G. D., Buckley, T. N. & Miller, J. M. Optimal stomatal control in relation to leaf area and nitrogen content. *Silva Fenn.* **36**, 625–627 (2002).
28. Wright, I. J., Reich, P. B. & Westoby, M. Least-cost input mixtures of water and nitrogen for photosynthesis. *Am. Nat.* **161**, 98–111 (2003).
29. Palmroth, S. *et al.* On the complementary relationship between marginal nitrogen and water-use efficiencies among *Pinus taeda* leaves grown under ambient and CO₂-enriched environments. *Ann. Bot.* **111**, 467–77 (2013).
30. Güsewell, S. N:P ratios in terrestrial plants: variation and functional significance. *New Phytol.* **164**, 243–266 (2004).
31. Vitousek, P. M. *Nutrient cycling and limitation: hawai'i as a model system* (Princeton University Press, Princeton, 2004).
32. Sterner, R. W. & Elser, J. J. *Ecological stoichiometry: the biology of elements from molecules to the biosphere* (Princeton University Press, Princeton, 2002).
33. Reich, P. B. *et al.* Scaling of respiration to nitrogen in leaves, stems and roots of higher land plants. *Ecol. Lett.* **11**, 793–801 (2008).
34. Vrede, T., Dobberfuhl, D. R., Kooijman, S. A. L. M. & Elser, J. J. Fundamental connections among organism C:N:P stoichiometry, macromolecular composition, and growth. *Ecology* **85**, 1217–1229 (2004).
35. Stock, W. D. & Verboom, G. A. Phylogenetic ecology of foliar N and P concentrations and N:P ratios across Mediterranean-type ecosystems. *Glob. Ecol. Biogeogr.* **21**, 1147–1156 (2012).
36. Editorial committee of Vegetation map of China. *Vegetation map of the People's Republic of China (1 : 1000,000)* (Geological Publishing House, Beijing, 2007).
37. Brouwer, R. Nutritive influences on the distribution of dry matter in the plant. *Neth. J. Agric. Sci.* **10**, 361–376 (1962).
38. Brouwer, R. Functional equilibrium: sense or nonsense? *Neth. J. Agric. Sci.* **31**, 335–348 (1983).
39. Yang, Z. & Midmore, D. J. Modelling plant resource allocation and growth partitioning in response to environmental heterogeneity. *Ecol. Modell.* **181**, 59–77 (2005).
40. Marschner, H., Kirkby, E. A. & Engels, C. Importance of cycling and recycling of mineral nutrients within plants for growth and development. *Bot. Acta.* **110**, 265–273 (1997).
41. Broadley, M. R. *et al.* Phylogenetic variation in the shoot mineral concentration of angiosperms. *J. Exp. Bot.* **55**, 321–336 (2004).
42. Reich, P. B. *et al.* Variation in growth rate and ecophysiology among 34 grassland and savanna species under contrasting N supply: a test of functional group differences. *New Phytol.* **157**, 617–631 (2003).
43. McNeill, A. & Unkovich, M. [The nitrogen cycle in terrestrial ecosystems] *Nutrient cycling in terrestrial ecosystems* [Marschner, P. & Rengel, Z. (eds.)] [37–64] (Springer, Heidelberg, 2007).
44. Bünemann, E. K. & Condron, L. M. [Phosphorus and sulphur cycling in terrestrial ecosystems] *Nutrient cycling in terrestrial ecosystems* [Marschner, P. & Rengel, Z. (eds.)] [65–92] (Springer, Heidelberg, 2007).
45. Liu, C. *et al.* Relative effects of phylogeny, biological characters and environments on leaf traits in shrub biomes across central Inner Mongolia, China. *J. Plant Ecol.* **6**, 220–231 (2013).
46. Geng, Y., Wu, Y. & He, J.-S. Relationship between leaf phosphorus concentration and soil phosphorus availability across Inner Mongolia grassland. *Chinese J. Plant Ecol.* **35**, 1–8 (2011).
47. Lambers, H., Raven, J. A., Shaver, G. R. & Smith, S. E. Plant nutrient-acquisition strategies change with soil age. *Trends Ecol. Evol.* **23**, 95–103 (2008).
48. Bai, Y. *et al.* Primary production and rain use efficiency across a precipitation gradient on the Mongolia plateau. *Ecology* **89**, 2140–2153 (2008).
49. Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. & Jarvis, A. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* **25**, 1965–1978 (2005).
50. Jones, J. B. *Laboratory guide for conducting soil tests and plant analysis*. (CRC Press, New York, 2001).
51. Warton, D. I., Wright, I. J., Falster, D. S. & Westoby, M. Bivariate line-fitting methods for allometry. *Biol. Rev. Camb. Philos. Soc.* **81**, 259–291 (2006).
52. UNEP. *World atlas of desertification* (Arnold Publisher, London, 1992).
53. Thornthwaite, C. W. & Hare, F. K. Climatic classification in forest. *Unasylva* **9**, 51–59 (1955).
54. Warton, D. I. & Weber, N. C. Common slope tests for bivariate errors-in-variables models. *Biometrical J.* **44**, 161–174 (2002).
55. Webb, C. O. & Donoghue, M. J. Phylomatic: tree assembly for applied phylogenetics. *Mol. Ecol. Notes* **5**, 181–183 (2005).
56. Bremer, B. *et al.* An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Bot. J. Linn. Soc.* **161**, 105–121 (2009).
57. Wikström, N., Savolainen, V. & Chase, M. W. Evolution of the angiosperms: calibrating the family tree. *Proc. Biol. Sci.* **268**, 2211–2220 (2001).
58. Felsenstein, J. Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15 (1985).

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Author contributions

X.Y. and Z.T. conceived and designed the study, conducted analyses and wrote the paper. X.Y., Z.T., C.J., H.L., W.M., A.M., Z.S., W.S., T.W., X.P.W., X.W., S.Y., M.Y. and C.Z. contributed data, discussed the draft manuscript and interpreted the results.

Additional information

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