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

Xian Yang1, Zhiyao Tang1, Chengjun Ji1, Hongyan Liu1, Wenhong Ma2, Anwar Mohhamot3, Zhaoyong Shi4, Wei Sun5, Tao Wang1, Xiangping Wang6, Xian Wu5, Shunli Yu6, Ming Yue7 & Chengyang Zheng1

1Department of Ecology, College of Urban and Environmental Sciences and Key Laboratory for Earth Surface Processes, Peking University, Beijing, China, 2College of Life Science, Inner Mongolia University, Hohhot, China, 3Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, China, 4College of Agriculture, Henan University of Science and Technology, Luoyang, China, 5College of Forestry, Beijing Forestry University, Beijing, China, 6Institute of Botany, Chinese Academy of Science, Beijing, China, 7College of Life Science, Northwest University, Xi’an, China.

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.
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 through exploiting greater light availability while reducing stomatal conductance and increasing water use efficiency. Plant functional groups have also been shown to influence allocation of nutrients through different photosynthetic pathways, fraction of woody tissue and N-fixation ability. 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. 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. As a major element of RNAs, P is mainly involved in the production of proteins. 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 capacity. 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 species. Finally, plants are able to coordinate various organs with respect to absorption and allocation of limiting resources and adaptation to environmental constraints; for example, plants tend to increase leaf N concentration to maintain growth under dry conditions. 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 plants. Shrubland represent a major vegetation type in Northern China with community types varying from east to west China along an aridity gradient. 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. Nutrient allocation among plant organs involves two facets: concentration and biomass storage. 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 conditions 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;
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 ($P > 0.05$) from the phylogeny level except the root vs. stem ($P = 0.012$).

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).

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.

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

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, as nutrients are transported to distant organs only after the needs by adjacent organs are met.

As hypothesized, N and P concentrations show different scaling relationships among organs. Consistent with Kerkhoff et al., 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). 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 photosynthesize transportation and photosynthetic activity. 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. 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 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, 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 species.

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. 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.
deficiency. Available soil N and P can be absorbed directly by plants, and thus might influence N and P concentrations in plants. Recent studies on leaf traits of shrubs and grasses conducted across Inner Mongolia suggest 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. 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 age, soil freezing and disturbance 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 light and increase the water use efficiency at the expense of lowering N-use efficiency. As expected, increases of N concentration in non-photosynthetic organs are even faster in humid than in arid regions. 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 growth. During growth, more photosynthate and nutrients will be transported to non-photosynthetic organs. 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 was harvested 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
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 website (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 molydate/ascorbic acid method after H₂SO₄-H₂O₂
digestion. 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. 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 simulta-
neously. 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 \( X^{ab} \). 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 and STP) and compared the exponential slopes among different
organs by the corresponding biomasses.

A likelihood ratio test 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 Phylomatic based on APG III topology (Academic Press, San Diego, 1997). The branch
lengths were determined using BLADJ algorithm within the Phylcom software
(http://www.phylodiversity.net/phylcom/) and the node ages were fossil-
estimated. We then applied a phylogenetically independent contrast (PIC)
analysis (Academic Press, San Diego, 1997). 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/).

1. Bazzaz, A. F. & Grace, J. Plant resource allocation (Academic Press, San Diego,
1997).
2. 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).
3. Niklas, K. J. & Enquist, B. J. On the vegetative biomass partitioning of seed plant
leaves, stems, and roots. *Am. Nat.*, 159, 482–497 (2002).
4. Enquist, B. J. & Niklas, K. J. Global allocation rules for patterns of biomass
partitioning in seed plants. *Science*, 295, 1517–1520 (2002).
5. Reich, P. B. *et al.* The evolution of plant functional variation: traits, spectra, and
strategies. *Int. J. Plant Sci.*, 164, S143–S164 (2003).
6. 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).
7. Grime, J. P. & Mackey, J. M. L. The role of plasticity in resource capture by plants.
*Ecol. Ecol.*, 16, 299–307 (2002).
8. Koerselman, W. & Medler, 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).
9. LeBauer, D. S. & Treseder, K. K. Nitrogen limitation of net primary productivity in
terrestrial ecosystems is globally distributed. *Ecology*, 89, 371–379 (2008).
10. Wright, I. J. *et al.* The worldwide leaf economics spectrum. *Nature*, 428, 821–827
(2004).
11. 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).
12. 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).
13. Heldmaier, H.-S. & Silitza, T. Variation in nutrient concentrations of *Pinus
sylvestris* stems. *Scand. J. For. Res.*, 4, 443–451 (1989).
14. 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).

![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)." The map was generated using ArcGIS 10.3.](http://www.esri.com/)
