Variations of leaf N, P concentrations in shrubland biomes across Northern China: phylogeny, climate and soil

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Abstract

Concentrations of leaf nitrogen (N) and phosphorus (P) are two key traits of plants for ecosystem functioning and dynamics. Foliar stoichiometry varies remarkably among life forms. However, previous studies have focused on the stoichiometric patterns of trees and grasses, leaving a significant knowledge gap for shrubs. In this study, we explored the intraspecific and interspecific variations of leaf N and P concentrations in response to the changes in climate, soil property, and evolutionary history. We analysed 1486 samples composed of 163 shrub species from 361 shrubland sites in Northern China encompassing 46.1 degrees (86.7°E -132.8 °E) in longitude and 19.8 degrees (32.6°N -52.4 °N) in latitude. Leaf N concentrations decreased with precipitation, while leaf P concentrations decreased with temperature and increased with precipitation and soil total P concentrations. Both leaf N and P concentrations were phylogenetically conserved, but leaf P concentrations were less conserved than leaf N concentrations. At the community level, climate explained more interspecific variation of leaf nutrient concentrations, while soil nutrients explained most of the intraspecific variation. These results suggested that leaf N and P concentrations responded to climate, soil, and phylogeny in different ways. Climate influenced the community chemical traits through the shift in species composition, whereas soil directly influenced the community chemical traits. These findings are not all consistent with findings from previous studies based on data from other regions and vegetation types, and therefore complemented our knowledge of broad biogeographic patterns of leaf chemical traits.

1 Introduction

Understanding how and why plant stoichiometry varies among species and sites is, in general, an important step towards understanding terrestrial ecosystem properties, including biogeochemical cycles, plant trait evolution, plant community structure and their functional characteristics in a changing climate (Westoby and Wright, 2006). Concentrations of leaf nitrogen (N) and phosphorus (P) play a crucial role in ecosystem functioning and dynamics

(Sterner and Elser, 2002; Wright et al., 2004; Kerkhoff et al., 2006; Ordoñez et al., 2009; Vitousek et al., 2010). Leaf N concentrations are critical for photosynthesis, plant production and litter decomposition (LeBauer and Treseder, 2008), while P is a limiting nutrient responsible for energy storage, cell structure, and the composition of DNA and RNA. Despite their shared key functional purpose of photosynthetic carbon assimilation and transpiration (Elser et al., 2003; Reich and Oleksyn, 2004; Wright et al., 2004; Kerkhoff et al., 2006; Chen et al., 2013), plant leaves vary dramatically in N and P concentrations, partly because of the differences in climate, soil, vegetation types, and developing history among sites (Westoby and Wright, 2006). For example, leaf N and P concentrations are higher in herbs than in woody plants, and higher in deciduous than in evergreen species (Kerkhoff et al., 2006), and increase with latitude at large scales (McGroddy et al., 2004; Reich and Oleksyn, 2004; Han et al., 2005, 2011; Kerkhoff et al., 2006). Studying the patterns of leaf N and P concentrations is important for understanding the macroecological patterns in plant stoichiometry and related driving factors (Han et al., 2005).

Several hypotheses have been proposed to explain the patterns of plant stoichiometry (Elser et al., 2003; Reich and Oleksyn, 2004). Among these hypotheses, the plant physiology hypothesis (Woods et al., 2003; Reich and Oleksyn, 2004), the biogeochemical hypothesis (McGroddy et al., 2004; Reich and Oleksyn, 2004) and the species composition hypothesis (Reich and Oleksyn, 2004; He et al., 2006) have most often been reported. The plant physiology hypothesis proposes that the concentrations of N and P in plant tissues increase to offset the decreases in plant metabolic rate as the ambient temperature decreases (Woods et al., 2003; Reich and Oleksyn, 2004). Studies in arid regions also proposed that plants tend to have higher leaf N concentrations to better adapt to arid environments (Cunningham et al., 1999; Wright et al., 2003) through exploiting greater light availability (Cunningham et al., 1999) while reducing stomatal conductance and transpiration rates (Wright et al., 2003; Luo et al., 2015). The biogeochemical hypothesis suggests that the concentrations of N and P in plant tissues are controlled by the availability of soil N and P, and thus, the concentrations of N and P in plant tissues are highly correlated with those in the soil (McGroddy et al., 2004;

Reich and Oleksyn, 2004). The species composition hypothesis suggests that species composition was the primary determinant of stoichiometry, with climatic variables having little effect, which is supported by recent studies conducted from tropical forest to alpine grassland biomes (Townsend et al., 2007; He et al., 2008). In addition, the differences in stoichiometry among species may be highly correlated with the phylogenetic relatedness of the species involved, as the related traits may be phylogenetically conserved (Kerkhoff et al., 2006; Stock and Verboom, 2012).

All hypotheses have received supports from empirical studies by using meta data (McGroddy et al., 2004; Reich and Oleksyn, 2004; Wright et al., 2004; Han et al., 2005; Ordoñez et al., 2009; Stock and Verboom, 2012) or standardized large-scale samplings (He et al., 2006, 2008; Fyllas et al., 2009; Liu et al., 2013; Chen et al., 2013). These hypotheses may function simultaneously, and none of them has been proved to be particularly superior to the others. Particularly, most of these studies have been biased for trees in forests (McGroddy et al., 2004; Townsend et al., 2007; Chen et al., 2013) and herbaceous plants in grasslands (Craine et al., 2005; He et al., 2006, 2008). Reports on measurements of leaf chemistry from shrubland communities are rare (but see Liu et al., 2013). There is an urgent need for a closer evaluation of plant nutrient use strategies under the greater ecological context. As foliar stoichiometry may vary remarkably among life forms (Wright et al., 2004; Han et al., 2005; Kerkhoff et al., 2006), it is therefore necessary to test these hypotheses based on the stoichiometry of shrubs before any consensus can be reached.

Shrubland covers more than 1.23 million km^2 (or 12.5% of the total) in China. The community types vary gradually from temperate shrubland in the northeast to desert shrubland in the northwest China (Editorial Committee of Vegetation Map of China, 2007). Shrubland is the climax vegetation adapted to the arid climate of Northern China. A survey on the shrubs in northern China and their relationship to the climate, soil properties and species composition can considerably improve our understanding of the patterns of foliar stoichiometry for shrubs and the patterns in shrubland communities. In this study, we explored the patterns of leaf N and P concentrations of shrubs and their relationships to the climate, soil and evolutionary history in Northern China. We examined the following hypotheses.

1. First, we hypothesized that both leaf N and P concentrations may decrease with mean annual temperature based on the plant physiology hypothesis; and leaf N concentrations may decrease with increasing precipitation, as plants in arid regions may contain higher N concentrations to better adapt to arid environments.

2. Second, we hypothesized that P concentrations in leaf are more strongly correlated with its availability in soil than N concentrations. This is because that in contrast to soil N, P is particularly low in soils in China (Han et al., 2005), and plants may absorb P from soil when it is available.

3. Finally, we hypothesized that leaf N concentrations are more phylogenetically conserved than leaf P concentrations, because traits that define species competition on limited resources are less likely to be phylogenetically conserved as they are under strong selection and more adapted to the environment. According to Fyllas et al. (2009), leaf N concentrations tend to be more genetically constrained, while leaf P concentrations tend to be more environmentally constrained and have a higher level of plasticity.

2 Materials and methods

2.1 Sampling collection and measurements

This study was conducted based on an investigation of 361 shrubland sites, including 289 temperate, 69 desert and 3 subalpine sites, encompassing 19.8 degrees in latitude (32.6 - 52.4 °N) and 46.1 degrees in longitude (86.7 -132.8 °E) in Northern China (Fig. 1). The sampling was conducted in the summer (July to September) of 2011, 2012 and 2013. At each site, three plots of 5×5 m^2, with distances of 5-50 m between edges of nearby plots, were selected to present the natural shrubland communities. We identified all shrub species in each plot, and harvested leaf, stem and root biomass separately for each species. The dominant life form in all sites was shrub, which accounts for 87.3% aboveground biomass on average.

Fully expanded sun leaves of at least five individuals of each species were collected and assembled in fabric bags then dried in the sun. Leaf samples were then transported to the laboratory and oven-dried at 65°C for 72 hours. In total, we collected 1486 samples composed of 163 species from 38 families and 86 genera, with 91 species sampled from more than one site.

At each plot, we removed the litter layer and excavated three pits to a depth of 1m to collect soil samples at the diagonal of the plot. For each profile, soil samples were taken at depths of 0-10, 10-20, 20-30, 30-50, 50-70 and 70-100 cm. The soil samples from the same depth were mixed; visible roots were removed at the laboratory during mixing.

An elemental analyzer (2400 II CHNS; Perkin-Elmer, Boston, MA, USA) was employed to measure the total N concentrations of the soils (STN) and leaves with a combustion temperature of 950°C and a reaction temperature of 640 °C. The molybdate/ascorbic acid method was applied to measure total P concentrations in the soils (STP) and leaves after H_2SO_4-H_2O_2 digestion (Jones, 2001). Soil pH was measured using a pH meter (S20P-K; Mettler-Toledo, Greifensee, Switzerland) in 1: 2.5 soil-water suspension. As STN and STP from the 0-10 cm depth interval were highly correlated with those from other depth intervals (Table S1), we only used STN and STP from the 0-10 cm depth interval.

We also extracted mean annual temperature (MAT) and precipitation (AP) from the WorldClim spatial climate data (resolution at ca 1km, available at www.worldclim.org/). The MAT in the study sites ranged from -4.1 to 16.0 °C, and the AP ranged from 15 to 974 mm. Please refer to Yang et al. (2014) for more detailed information on data collections.

2.2 Tests of the effects of climate and soil on leaf N and P concentrations

To test the plant physiology and biogeochemical hypotheses (the first and second hypotheses), we examined effects of climate, soil properties and evolutionary history on the leaf N and P concentrations and leaf N: P ratios by plotting the concentrations against environmental factors using all data (treating all observations as equal). Leaf N and P concentrations and leaf N:P ratios were log base 10 transformed to normalize their distributions before analysis.

We followed Lepš et al. (2011) to assess the relative contributions of intraspecific and interspecific variability effects on biomass weighted site-average leaf N, P concentrations and N:P ratios along the climatic and soil nutrient gradients. For each site, we calculated "specific" site-average leaf N, P concentrations and N: P ratios and "fixed" site-average leaf N, P concentrations and N:P ratios with the formulas below:

$$\text{Specific average} = \sum_{i=1}^{S} p_i x_j \qquad (1)$$

$$\text{Fixed average} = \sum_{i=1}^{S} p_i x_i \qquad (2)$$

where S is the number of species in a study site, p_i is the proportion of the ith species based on aboveground biomass (leaf and stem biomass) in the site, x_i is the fixed mean leaf N, P concentrations or N:P ratios of the ith species for all study sites where the species exists, and x_j is the specific mean leaf N, P concentrations or N:P ratios of the ith species for the given site. The variation of specific average values may be attributed to both intraspecific and interspecific leaf chemical trait variations, while the variation of fixed average values is solely affected by interspecific leaf chemical trait variation. Therefore, the effect of intraspecific variability can be estimated as:

$$\text{Intraspecific variability} = \text{Specific average} - \text{Fixed average} \qquad (3)$$

We then used each of the three parameters as a single response variable in general linear model (GLM) regressions with climatic and soil nutrient factors as explanatory variables. The decomposition of sum of squares (SS) can be used across the three GLM models:

$$SS_{\text{Specific}} = SS_{\text{Fixed}} + SS_{\text{Intraspecific}} + SS_{\text{covariance}} \qquad (4)$$

We could then extract the SS for each of the three GLM models explained by each of the environmental factors. In this way, we decomposed the total variation of leaf N, P concentrations or N:P ratios into parts explained by intraspecific variation, interspecific variation and their covariance; we also quantified how much variability in each part can be explained by each environmental factor. We analysed both main-effect GLM models and the

GLM models with interaction terms. Since the results for the main effects of environmental variables were same, and the variation explained by interaction terms were relatively small compared to the main effects, we only presented the main-effect models for simplicity, and showed the models with interaction terms in the supplementary material (Table S2).

Ecological data on large scales often display spatial autocorrelation, and the presence of such pattern in the residuals of a statistical model may result in significant type I error (Dormann, 2007). We tested for spatial independence of the residuals of the models using Moran's I index (Moran, 1950), and found that the Moran's I of the residuals of all the models were not significant (Fig. S1), indicating that the environments included in the models removed the spatial autocorrelation in the leaf nutrient concentrations (Diniz-Filho et al., 2003).

Statistical and phylogenetic analyses were performed using R 3.1.1 (R Development Core Team, 2014) with the ape (Paradis et al., 2004) and picante packages (Kembel et al., 2010). Spatial analyses were conducted using SAM 4.0 (Rangel et al., 2010).

2.3 Phylogenetic signal test

To examine the phylogenetic signal of leaf N and P concentrations and test our third hypothesis, we constructed a phylogenetic tree for the 163 species by using Phylomatic (Webb and Donoghue, 2005) based on APG III topology (Bremer et al., 2009). We then adjusted the branch length using BLADJ algorithm within the Phylocom software (http://www.phylodiversity.net/phylocom/; Wikström et al., 2001). We then calculated the K statistic (Blomberg et al., 2003) to quantify the magnitude of phylogenetic signal of leaf N, P concentrations and N:P ratios. For each species, we first calculated the mean leaf N and P concentrations. To test if the phylogenetic conservatism of leaf N concentrations is caused by legumes (species from Fabaceae) or succulent species, we also calculated the K statistic of leaf N after dropping the clade of Fabaceae or succulent plants. The significance (*p*-values) was evaluated by comparing the variance of independent contrasts for each trait to the expected values obtained by shuffling leaf trait data across the tips of the phylogenetic tree for 999 times. The P-value can be used to test whether the phylogenetic signal in each trait is larger than the null expectation, while the K statistic can be used to estimate the strength of phylogenetic signal. A significant P-value indicates that the phylogenetic signal of the trait was non-random, compared to the prediction of the random-tip-shuffling model.

To quantify the strength of phylogenetic signals of species' environmental traits, we calculated K statistics for mean climate (MAT and AP) and soil chemistry (STN and STP) of all sites each species occurring.

3 Results

3.1 Effects of climate and soil on leaf N and P concentrations

At the individual level, leaf N concentrations ranged from 4.26 to 46.80 mg g^{-1} (mean =21.91, std =6.84) (Table 1; Fig. S2). They decreased with increasing AP (R^2 =0.1, p <0.001) and STN (R^2 =0.13, p <0.001), increased with increasing soil pH (R^2 =0.02, p <0.001), while showed no significant correlation with MAT (R^2 <0.01, p =0.227) (Fig. 2). Leaf P concentrations ranged from 0.16 to 4.80 mg g^{-1} (mean =1.30, std =0.53) (Table 1; Fig. S2). They increased with increasing AP (R^2 =0.03, p <0.001) and STP (R^2 =0.02, p <0.001), while decreased with increasing MAT (R^2 =0.03, p <0.001) and soil pH (R^2 =0.03, p <0.001) (Fig. 2). Leaf N:P ratios changed from 4.07 to 145.76 (mean =18.69, std =8.40) (Table 1; Fig. S2). Leaf N:P ratios increased with increasing MAT (R^2 =0.04, p <0.001) and soil pH (R^2 =0.07, p <0.001), while decreased with increasing AP (R^2 =0.18, p <0.001), STN (R^2 =0.07, p <0.001) and STP (R^2 =0.06, p <0.001) (Fig. 3).

At the community level, climatic variables explained 3.4% of the total variation in leaf N concentrations, and 8.2% of the total variation in leaf P concentrations. Only AP significantly influenced leaf N concentrations, while all environmental factors except STN and soil pH significantly influenced leaf P concentrations. AP explained the most variation in leaf N:P ratios (20.6%), while the effects of other factors were not significant (Table 2).

When the total variation of leaf N and P concentrations were decomposed into intraspecific and interspecific variations, GLM analyses showed that AP and STN explained 5.5% and 2.5%

(p <0.001) of the interspecific variation of leaf N concentrations, respectively. None of MAT, AP, STN, STP and soil pH significantly influenced intraspecific variation of leaf N concentrations (p >0.05 for all). For leaf P concentrations, MAT and AP accounted for 1.2% (p <0.01) and 3.5% (p <0.001) interspecific variation; STN and STP explained 1.1% (p <0.01), and 3.5% (p <0.001) of intraspecific variation, respectively (Table 2; Fig. 4). For leaf N:P ratios, AP accounted for 20.0% (p <0.001) of the interspecific variation; STP explained 1.1% (p <0.01) of the intraspecific variation, respectively (Table 2; Fig. 4). As temperate and desert shrublands distributed in different climates, we further conducted GLM analyses for the two major shrubland types, separately. Temperate shrublands showed similar results to that with all data pooled. For desert shrublands, however, none of the environmental factors significantly influenced leaf N concentrations, and precipitation was the major factor influencing leaf P concentrations and N:P ratios through shifts in species composition (Fig. S3; Table S3).

3.2 Phylogenetic signals of leaf N and P concentrations

Leaf N concentrations exhibited a significantly non-random phylogenetic signal (K =0.31, p <0.001), while leaf P concentrations showed a significant but weaker phylogenetic signal (K =0.24, p <0.01) among all species (Table 1). The phylogenetic signal for leaf N concentrations remained significant when legumes (K =0.30, p <0.001) or succulent plants were excluded (K =0.30, p <0.001) (Table 1).

4 Discussion

Using foliar stoichiometry of 163 shrub species from 361 shrubland sites, we investigated patterns of leaf N and P concentrations in shrublands of Northern China. We focused our discussion on leaf N and P concentrations instead of their ratio because leaf N:P was strongly driven by both leaf N and P concentrations and was predictable based on leaf N and P concentrations. Given that leaf C concentrations are relatively stable, leaf N and P concentrations can be good indicators of C:N and C:P ratios (Reich, 2005). We found that mean leaf N (21.91 mg g^{-1}) and P (1.30 mg g^{-1}) concentrations of shrubs in Northern China shrubland were similar to those in shrubs across China (mostly distributed in forests as understory species, Han et al., 2005), but lower than those in grasses (Han et al., 2005; He et al., 2006, 2008) and higher than those in trees in China (Han et al., 2005) (Fig. S4). The "leaf economics spectrum", proposed by Wright et al. (2004), runs from life strategies characterized by low rates of metabolism, low N and P concentrations, and extended leaf longevity, to life strategies characterized by high rates of metabolism, high N and P concentrations, and short leaf longevity (Wright et al., 2004). Our result indicated differences in life strategies between shrubs and trees or herbaceous plants. Our results also suggested that the inclusion of shrubs is necessary to explore the patterns of leaf stoichiometry in relation to climate and soil property.

There are some novel findings concerning the patterns of leaf stoichiometry, which we discuss below.

4.1 Influence of climate on leaf N and P concentrations

Leaf N and P concentrations responded to climate in different ways. Consistent with our hypothesis, leaf N concentrations decreased with precipitation. This is partly due to the higher leaf N concentrations of plants in desert shrublands. Higher leaf N concentrations have been suggested as a general property of arid-zone plants (Wright et al., 2003). It is widely reported that plants tend to increase leaf N to exploit greater light availability while reducing stomatal conductance and transpiration rates (Cunningham et al., 1999; Wright et al., 2003; Luo et al., 2015). Succulence is such an adaption for plants to drought and salinity by accumulating nitrogen-containing compounds in their leaves to maintain water balance and therefore resulting in succulent plants having higher leaf N concentrations than other plants (Mansour, 2000) (Fig. S4). In contrast, leaf P concentrations increased with precipitation. P is derived primarily from the weathering of soil inorganic components and the degradation of organic matters (Aerts and Chapin, 1999). Increases in precipitation may amplify P availability in soil by facilitating the decomposition of litter in arid regions. 301 of the study sites have an aridity index (the ratio of total precipitation to potential evapotranspiration) of <1, indicating that precipitation is generally lower than evapotranspiration in this region. The positive correlation between soil total phosphorous concentrations and precipitation (R^2 =0.21, p <0.001) and the lower soil total phosphorous concentrations in desert shrublands is in line with such a hypothesis.

Leaf P decreased with mean annual temperature, which was consistent with the plant physiology hypothesis that plant P may increase to offset the decreases in plant metabolic rates as ambient temperature decreases (Reich and Oleksyn, 2004). However, in contrast to other studies (Reich and Oleksyn, 2004; Han et al., 2005; Chen et al., 2013), we did not observe a decrease in leaf N concentrations with temperature. Many previous studies were conducted in regions where temperature and precipitation were highly positively correlated (Ordoñez et al., 2009; Chen et al., 2013). The weak correlation between mean annual temperature and annual precipitation in our study region (Pearson’s correlation R =-0.01) allowed us to test the major influencing climatic factor of leaf N concentrations without problems of collinearity. We found that precipitation, not temperature, significantly influenced leaf N concentrations in the study region.

4.2 Influence of soil N and P concentrations on leaf N and P concentrations

We observed a significantly positive correlation between leaf P concentrations and soil total phosphorous concentrations, but not between leaf N concentrations and soil total nitrogen concentrations. We acknowledge that the available soil N and P, though in a small quantity, can be readily absorbed and utilized by plants (Bünemann and Condron, 2007; McNeill and Unkovich, 2007), and may be better indicators of soil fertility than total element concentrations (Ordoñez et al., 2009). Unfortunately, we did not include these two measures in our study. Nevertheless, we note that organic materials, which constitute the majority mass of soil total N and P, can be directly utilized by many plants that couple with mycorrhizal fungi (Aerts and Chapin, 1999). This makes the total element concentrations, including total N and P, the most effective indicators for soil nutrient level.

The positive correlation between leaf and soil P concentrations might be due to low soil P concentrations. Although leaf P concentrations are higher in shrublands of Northern China than in forests in China (Zhang et al., 2005), it is significantly lower than those in the rest of the world (Han et al., 2005). It is widely reported that a leaf N and P ratios (N:P) <14 indicates N limitation, whereas a N:P >16 indicates P limitation, in the ecosystem (Aerts and Chapin, 1999; Koerselman and Meuleman, 1996). In this study, mean leaf N:P is 18.69, which is significantly greater than 16 (One sample t-test: p <0.001). This means that shrublands of Northern China are P limited. In the P limited ecosystems, plants may absorb P and deposit P in an inorganic form when P in soil is abundant (Sterner and Elser, 2002), resulting a positive correlation between leaf and soil P concentrations. However, leaf N concentrations did not increase with soil N concentrations, which is likely due to N is not limited in soil. Several recent studies found similar results that leaf N concentrations did not increase with soil N concentrations (Ordoñez et al., 2009; Liu et al., 2013; Maire et al., 2015). In addition, soil pH is an integrated index of soil nutrient availability, and is correlated with various processes such as soil enzymatic and microbial activities (Sinsabaugh and Follstad Shah, 2012). Higher soil pH generally indicates higher availability of nutrients held in soil organic matter and lower costs of plant N acquisition when maintaining photosynthesis rate (Maire et al., 2015). This is consistent with our observation that leaf N concentrations increased with soil pH. However, the effect of soil pH became insignificant in the multiple regressions (Table 2), which might due to the strong negative correlation between precipitation and soil pH in this region (R^2 =0.40, p <0.001).

4.3 Influence of environmental factors at the intraspecific and interspecific variation of leaf N and P concentrations

Environmental factors explained more variance in leaf P concentrations than N concentrations at the community level (Table 2). However, the explanatory powers of climate and soil for leaf N and P concentrations are comparatively low. We speculate that other factors, such as soil age, may also have effects on the leaf stoichiometry, (Vitousek et al., 2010; Hayes et al., 2014), but these were not included in our model. Interspecific variation of leaf N and P concentrations is caused by changes in species composition, and intraspecific variation of leaf N and P concentrations is driven by environmental variations. Leaf P was jointly influenced by climate and soil nutrient. Climate influenced the community leaf P concentrations through shift in species composition, whereas soil influences the community P concentrations directly. Compared with other environmental factors, leaf N concentrations were to a larger extent affected by precipitation through species turnover.

The phylogenetic signal analysis also indicated that the temperature and precipitation niches of species exhibited phylogenetic signal, while the soil niche did not (except for soil pH, which also exhibited a phylogenetic signal). This result was consistent with the previous conclusion that climate explained more interspecific variation of leaf chemical traits and influenced species composition. Both results indicated that climate influences the community chemical traits mainly through the shift in species composition (He et al., 2008), whereas soil directly influences the community chemical traits. Changes in leaf chemical traits along temperature and precipitation gradients are likely due to differences in species composition along the gradient. Particularly, annual precipitation showed the strongest phylogenetic signal, largely due to the large gradient in precipitation across the study region and the dramatic variation in species composition adapted to aridity gradient.

4.4 Influence of phylogeny on leaf N and P concentrations

Leaf N concentrations exhibited strong, while leaf P concentrations exhibited weak, phylogenetic conservatism. Legumes and succulent species had high leaf N concentrations (Fig. S4), and may significantly increase the K value of leaf N concentrations. However, the K-value remained almost unchanged after excluding theses species. Therefore, the phylogenetic conservatism of leaf N concentrations did not result from the inclusion of some clades that have higher leaf N concentrations.

Plants disperse and evolve in response to environmental conditions that vary over both time and space (Kerkhoff et al., 2006). In this process, adaptive traits that are shaped by the environment conditions tend to show weaker phylogenetic signal (Losos, 2008). In this study, leaf N concentrations were not influenced by soil nutrients, and we surmise that the influence of climate on leaf N concentrations mainly works through species turnover. Leaf N concentrations therefore exhibited significant phylogenetic signal. In contrast, leaf P concentrations were significantly influenced by soil nutrients, and their conservation was therefore weakened.

5 Conclusions

We investigated the leaf N and P concentrations of 163 shrub species sampled at 361 sites in Northern China, and related the N and P concentrations to the climate, soil conditions, and species phylogenetic information. We found that leaf N and P concentrations responded to climate, soil, and evolutionary history differently. Leaf P concentrations were jointly driven by soil P concentrations and climate, whereas leaf N concentrations were mainly driven by precipitation. Both leaf N and P concentrations were phylogenetically conserved, but leaf P concentrations were less conserved than leaf N concentrations, which could be attributed to the mechanism that plants acquire P. Changes in leaf chemical traits along the climatic gradient were mainly due to differences in species composition along the gradient, whereas soil influenced the community chemical traits directly. Future studies of the biogeochemical implications and the evolutionary basis of plant nutrient concentrations in various regions, plant forms and other plant organs are important to understand the macroecological patterns and mechanisms of plant nutrient concentrations.

Author contribution

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

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Table 1. Arithmetic mean, range and phylogenetic signal (K-value) of leaf N and P concentrations and environmental variables for shrubs in Northern China.

	Mean (95% CI)	Range	K
Leaf N (mg g^{-1})	21.91 (21.56-22.26)	4.26-46.80	0.31^{a}
Leaf N (mg g^{-1}) non-legumes	20.95 (20.62-21.28)	4.26-45.81	0.30^{a}
Leaf N (mg g^{-1}) non-succulent	21.86 (20.48-21.14)	4.26-46.80	0.33^{a}
Leaf P (mg g^{-1})	1.30 (1.27-1.33)	0.16- 4.80	0.24^{b}
Leaf N:P	18.69 (18.26-19.12)	4.07-145.76	0.24^{a}
MAT (°C)	7.18 (7.01-7.36)	-20.03	0.26^{a}
AP (mm)	478.79 (467.82-489.76)	15-974	0.53^{a}
Soil pH	8.35 (8.29-8.41)	5.48-10.29	0.37 ^{a}
STN (mg g^{-1})	1.84 (1.76-1.93)	0.05-18.03	0.23 ^{NS}
STP (mg g^{-1})	0.56 (0.54-0.58)	0.12-3.20	0.17 ^{NS}

Abbreviations:

CI, confidence interval.

MAT, mean annual temperature.

AP, annual precipitation.

STN, soil total nitrogen.

STP, soil total phosphorus.

^{a} $p<0.001$.

^{b} $p< 0.01$.

^{NS} not significant.

Table 2. Summary of main-effect general linear models for leaf N and P concentrations and N:P ratios of shrubs in Northern China.

		Interspecific variation		Intraspecific variation		Total variation		
		F	SS	F	SS	F	SS	SS%
Leaf N	MAT	1.5	43.2^{NS}	0.4	6.3^{NS}	0.4	17.0^{NS}	0.1
	AP	26.4	737.2^{a}	2.7	38.7^{NS}	10.1	450.0^{b}	3.3
	STN	12.1	337.8^{a}	1.1	16.8^{NS}	4.7	208.7^{c}	1.6
	STP	0.2	5.6^{NS}	2.2	31.4^{NS}	0.3	11.5^{NS}	0.1
	Soil pH	0.2	6.7^{NS}	0.1	1.6^{NS}	0.3	14.3^{NS}	0.1
	Residual		7969.6		3969.6		12748.8	
	Total		9100.1		4064.4		13450.3	5.2
Leaf P	MAT	7.5	1.1^{b}	2.1	0.3^{NS}	8.5	2.5^{b}	2.6
	AP	22.2	3.3^{a}	1.8	0.2^{NS}	18.0	5.3^{a}	5.6
	STN	6.2	0.9^{c}	7.5	1.0^{b}	<0.1	$<0.1^{NS}$	<0.1
	STP	0.5	0.1^{NS}	24.6	3.3^{a}	8.1	2.4^{b}	2.5
	Soil pH	3.3	0.5^{NS}	<0.1	$<0.1^{NS}$	1.9	0.5^{NS}	0.6
	Residual		42.8		36.6		84	
	Total		48.8		41.4		94.7	11.3
Leaf N:P	MAT	0.1	2.0^{NS}	0.6	11.6^{NS}	0.5	22.5^{NS}	0.1
	AP	131.2	3055.8^{a}	0.1	1.9^{NS}	75.4	3203.7^{a}	20.6
	STN	1.1	25.5^{NS}	0.1	1.5^{NS}	0.4	16^{NS}	0.1
	STP	0.3	6.2^{NS}	9.0	167.1^{b}	2.5	106.8^{NS}	0.7
	Soil pH	1.6	37.2^{NS}	0.1	1.6^{NS}	1.3	54.8^{NS}	0.4
	Residual		6640.5		5064.1		12111.5	
	Total		9767.2		5247.8		15515.3	21.9

Abbreviations: MAT, mean annual temperature.

AP, annual precipitation.

STN, soil total nitrogen.

STP, soil total phosphorus.

[a] $p<0.001$.

[b] $p< 0.01$.

[c] $p< 0.05$.

[NS] not significant.

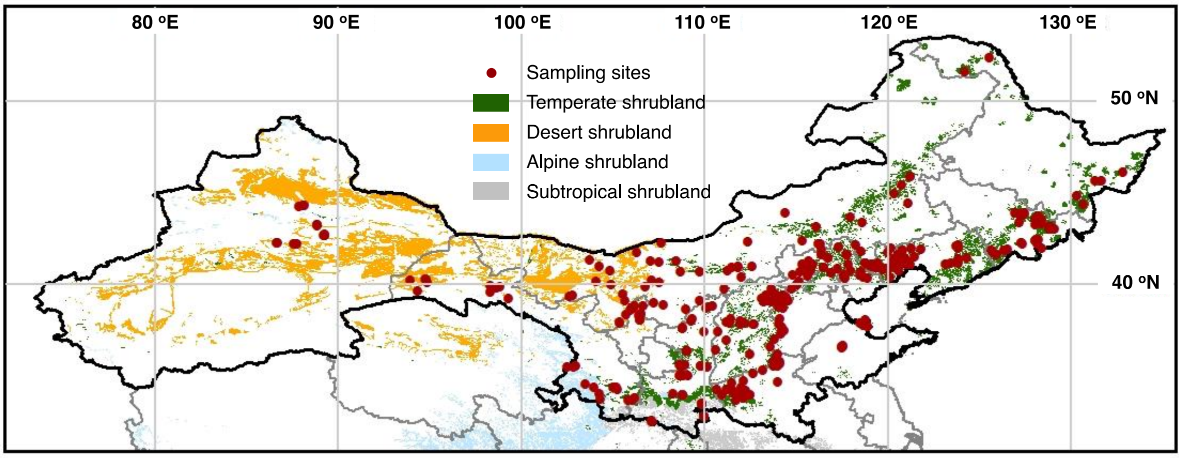

Figure 1. Locations of the sampling sites based on shrublands in Northern China.

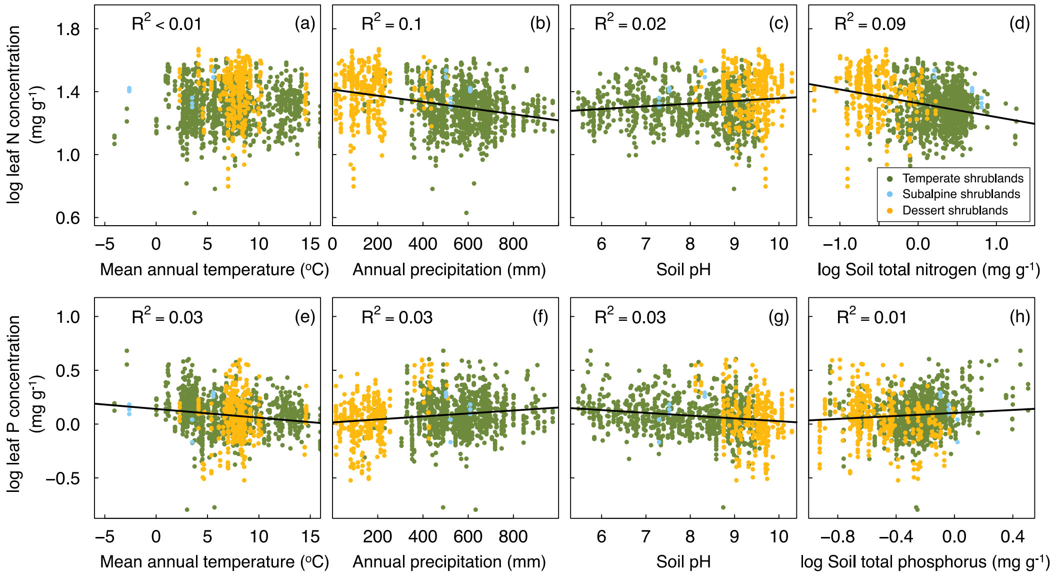

Figure 2. Changes of logtransformed leaf nitrogen and phosphorus concentrations in relation to climate (mean annual temperature and annual precipitation) and soil nutrient (soil pH and soil total nitrogen and phosphorus concentrations) for shrubs in Northern China. Green, yellow and blue dots represent samples from temperate, subalpine, and desert shrublands, respectively. Solid lines represent regressions significant at P<0.001.

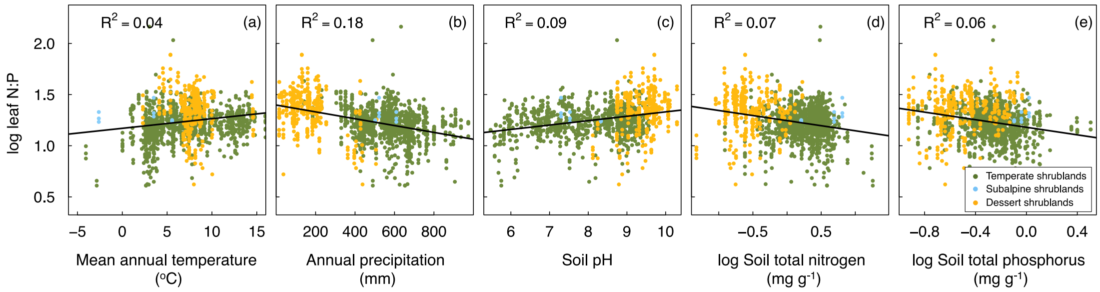

Figure 3. Changes of logtransformed leaf N:P ratios in relation to climate (mean annual temperature and annual precipitation) and soil nutrition (soil pH and soil total nitrogen and phosphorus concentrations) for shrubs in Northern China. Green, yellow and blue dots represent samples from temperate, subalpine, and desert shrublands, respectively. Solid lines represent regressions significant at $P<0.001$.

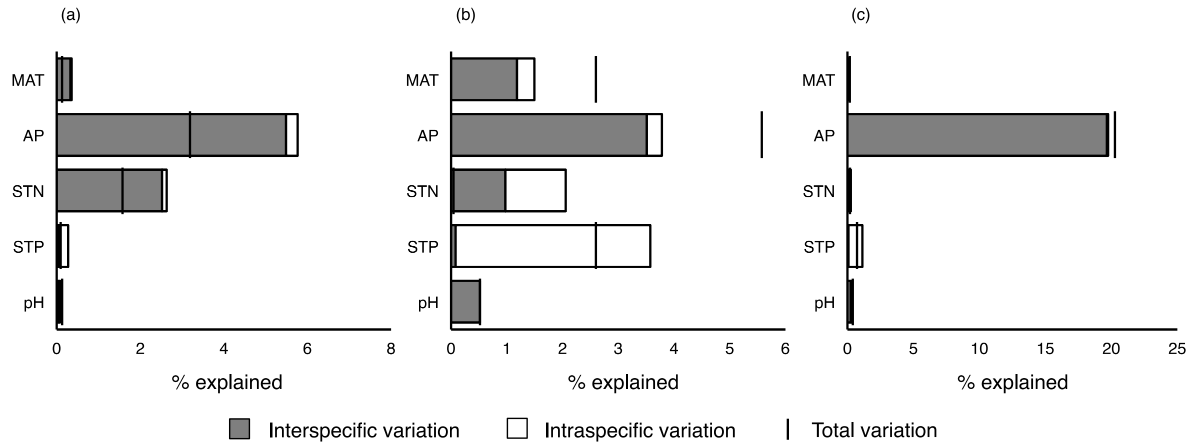

Figure 4. Decomposition of total variation in leaf N (a), P (b) and N:P (c) of shrubs in shrubland of Northern China. Gray shading corresponds to interspecific variation, and open part corresponds to intraspecific variation. Black bar denotes total variation. The space between the top of the column and the black bar corresponds to the covariance between interspecific and intraspecific variations.

Supplementary material

Fig. S1. Variation of Moran's I along distance bands for observed (black open) and residuals in general linear models (black dot), for inter-specific (left column, a, d, g), intra-specific (central column, b, e, h), and total (right column c, f, i) variations in the leaf nitrogen (upper row, a-c), phosphorus (middle row, d-f) concentrations and leaf N:P (lower row, g-i).

Fig. S2. Histograms showing the distributions of leaf N (a) and P concentrations (mg g−1) (b) and N:P ratios (c) for all observations.

Fig. S3. Decomposition of total variation in leaf N (a, d) and P (b, e) concentrations and N:P ratios (c, f) of shrubs in temperate shrubland (a-c) and desert shrubland (d-f). Gray shading corresponds to interspecific variation, and open part corresponds to intraspecific variation. Black bar denotes total variation. The space between the top of the column and the black bar corresponds to the covariance between interspecific and intraspecific variations.

Fig. S4. Different leaf N (black) and P (grey) concentrations among life forms (a) and different leaf N concentration among functional groups (b) in China. In (a), data for "tree" and "shrub" were from Han et al. (2005); data for "herb" were from Han et al. (2005) and He et al. (2006); data for "shrub*" were from this study. Letters above the error bars show the results of multiple comparisons tests. Life forms and functional groups with same letters are not significantly different, while different letters are significantly different ($p< 0.05$).

Table S1. Pearson correlation coefficients (R) of soil total nitrogen (STN) and phosphorus (STP) concentrations between different depth intervals.

Table S2. Summary of general linear models for leaf N and P concentrations and N:P ratios of shrubs in Northern China with interaction terms.

Table S3. Summary of main-effect general linear models for leaf N and P concentrations and N:P ratios of shrubs in temperate shrubland (TS) and desert shrubland (DS).