

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

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17

1 **Abstract**

2 Concentrations of leaf nitrogen (N) and phosphorus (P) are two key traits of plants for
3 ecosystem functioning and dynamics. Foliar stoichiometry varies remarkably among life
4 forms. However, previous studies have focused on the stoichiometric patterns of trees and
5 grasses, leaving a significant knowledge gap for shrubs. In this study, we explored the
6 intraspecific and interspecific variations of leaf N and P concentrations in response to the
7 changes in climate, soil property, and evolutionary history. We analysed 1486 samples
8 composed of 163 shrub species from 361 shrubland sites in Northern China encompassing
9 46.1 degrees (86.7°E -132.8 °E) in longitude and 19.8 degrees (32.6°N -52.4 °N) in latitude.
10 Leaf N concentrations decreased with precipitation, while leaf P concentrations decreased
11 with temperature and increased with precipitation and soil total P concentrations. Both leaf N
12 and P concentrations were phylogenetically conserved, but leaf P concentrations were less
13 conserved than leaf N concentrations. At the community level, climate explained more
14 interspecific variation of leaf nutrient concentrations, while soil nutrients explained most of
15 the intraspecific variation. These results suggested that leaf N and P concentrations responded
16 to climate, soil, and phylogeny in different ways. Climate influenced the community
17 chemical traits through the shift in species composition, whereas soil directly influenced the
18 community chemical traits. New patterns were discovered using our observations on specific
19 regions and vegetation types, which improved our knowledge of broad biogeographic
20 patterns of leaf chemical traits.

21

22 **1 Introduction**

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

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

15 Several hypotheses have been proposed to explain the patterns of plant stoichiometry (Elser
16 et al., 2003; Reich and Oleksyn, 2004). Among these hypotheses, the plant physiology
17 hypothesis (Woods et al., 2003; Reich and Oleksyn, 2004), the biogeochemical hypothesis
18 (McGroddy et al., 2004; Reich and Oleksyn, 2004) and the species composition hypothesis
19 (Reich and Oleksyn, 2004; He et al., 2006) have most often been reported. The plant
20 physiology hypothesis proposes that the concentrations of N and P in plant tissues increase to
21 offset the decreases in plant metabolic rate as the ambient temperature decreases (Woods et
22 al., 2003; Reich and Oleksyn, 2004). Studies in arid regions also proposed that plants tend to
23 have higher leaf N concentrations to better adapt to arid environments (Cunningham et al.,
24 1999; Wright et al., 2003) through exploiting greater light availability (Cunningham et al.,
25 1999) while reducing stomatal conductance and transpiration rates (Wright et al., 2003; Luo
26 et al., 2015). The biogeochemical hypothesis suggests that the concentrations of N and P in
27 plant tissues are controlled by the availability of soil N and P, and thus, the concentrations of
28 N and P in plant tissues are highly correlated with those in the soil (McGroddy et al., 2004;

1 Reich and Oleksyn, 2004). The species composition hypothesis suggests that species
2 composition was the primary determinant of stoichiometry, with climatic variables having
3 little effect, which is supported by recent studies conducted from tropical forest to alpine
4 grassland biomes (Townsend et al., 2007; He et al., 2008). In addition, the differences in
5 stoichiometry among species may be highly correlated with the phylogenetic relatedness of
6 the species involved, as the related traits may be phylogenetically conserved (Kerkhoff et al.,
7 2006; Stock and Verboom, 2012).

8 All hypotheses have received supports from empirical studies by using meta data (McGroddy
9 et al., 2004; Reich and Oleksyn, 2004; Wright et al., 2004; Han et al., 2005; Ordoñez et al.,
10 2009; Stock and Verboom, 2012) or standardized large-scale samplings (He et al., 2006, 2008;
11 Fyllas et al., 2009; Liu et al., 2013; Chen et al., 2013). These hypotheses may function
12 simultaneously, and none of them has been proved to be particularly superior to the others.
13 Particularly, most of these studies have been biased for trees in forests (McGroddy et al.,
14 2004; Townsend et al., 2007; Chen et al., 2013) and herbaceous plants in grasslands (Craine
15 et al., 2005; He et al., 2006, 2008). Reports on measurements of leaf chemistry from
16 shrubland communities are rare (but see Liu et al., 2013). There is an urgent need for a closer
17 evaluation of plant nutrient use strategies under the greater ecological context. As foliar
18 stoichiometry may vary remarkably among life forms (Wright et al., 2004; Han et al., 2005;
19 Kerkhoff et al., 2006), it is therefore necessary to test these hypotheses based on the
20 stoichiometry of shrubs before any consensus can be reached.

21 Shrubland covers more than 1.23 million km² (or 12.5% of the total) in China. The
22 community types vary gradually from temperate shrubland in the northeast to desert
23 shrubland in the northwest China (Editorial Committee of Vegetation Map of China, 2007).
24 Shrubland is the climax vegetation adapted to the arid climate of Northern China. A survey
25 on the shrubs in northern China and their relationship to the climate, soil properties and
26 species composition can considerably improve our understanding of the patterns of foliar
27 stoichiometry for shrubs and the patterns in shrubland communities. In this study, we
28 explored the patterns of leaf N and P concentrations of shrubs and their relationships to the

1 climate, soil and evolutionary history in Northern China. We examined the following
2 hypotheses.

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

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

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

17

18 **2 Materials and methods**

19 **2.1 Sample collection and measurements**

20 This study was conducted based on an investigation of 361 shrubland sites, including 289
21 temperate, 69 desert and 3 subalpine sites, encompassing 19.8 degrees in latitude (32.6 -
22 52.4 °N) and 46.1 degrees in longitude (86.7 -132.8 °E) in Northern China (Fig. 1). The
23 sampling was conducted in the summer (July to September) of 2011, 2012 and 2013. At each
24 site, three plots of 5×5 m², with distances of 5-50 m between edges of nearby plots, were
25 selected to present the natural shrubland communities. We identified all shrub species in each
26 plot, and harvested leaf, stem and root biomass separately for each species. The dominant life
27 form in all sites was shrub, which accounts for 87.3% aboveground biomass on average.

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

6 At each plot, we removed the litter layer and excavated three pits to a depth of 1m to collect
7 soil samples at the diagonal of the plot. For each profile, soil samples were taken at depths of
8 0-10, 10-20, 20-30, 30-50, 50-70 and 70-100 cm. The soil samples from the same depth were
9 mixed; visible roots were removed at the laboratory during mixing.

10 An elemental analyzer (2400 II CHNS; Perkin-Elmer, Boston, MA, USA) was employed to
11 measure the total N concentrations of the soils (STN) and leaves with a combustion
12 temperature of 950°C and a reaction temperature of 640 °C. The molybdate/ascorbic acid
13 method was applied to measure total P concentrations in the soils (STP) and leaves after
14 H₂SO₄-H₂O₂ digestion (Jones, 2001). Soil pH was measured using a pH meter (S20P-K;
15 Mettler-Toledo, Greifensee, Switzerland) in 1: 2.5 soil-water suspension. As STN and STP
16 from the 0-10 cm depth interval were highly correlated with those from other depth intervals
17 (Table S1), we only used STN and STP from the 0-10 cm depth interval.

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

22 **2.2 Tests of the effects of climate and soil on leaf N and P concentrations**

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

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

$$6 \text{ Specific average} = \sum_{i=1}^S p_i x_j \quad (1)$$

$$7 \text{ Fixed average} = \sum_{i=1}^S p_i x_i \quad (2)$$

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

$$16 \text{ Intraspecific variability} = \text{Specific average} - \text{Fixed average} \quad (3)$$

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

$$20 SS_{\text{Specific}} = SS_{\text{Fixed}} + SS_{\text{Intraspecific}} + SS_{\text{Covariance}} \quad (4)$$

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

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

5 Ecological data on large scales often display spatial autocorrelation, and the presence of such
6 pattern in the residuals of a statistical model may result in significant type I error (Dormann,
7 2007). We tested for spatial independence of the residuals of the models using Moran's I
8 index (Moran, 1950), and found that the Moran's I of the residuals of all the models were not
9 significant (Fig. S1), indicating that the environments included in the models removed the
10 spatial autocorrelation in the leaf nutrient concentrations (Diniz-Filho et al., 2003).

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

14 **2.3 Phylogenetic signal test**

15 To examine the phylogenetic signal of leaf N and P concentrations and test our third
16 hypothesis, we constructed a phylogenetic tree for the 163 species by using Phylomatic
17 (Webb and Donoghue, 2005) based on APG III topology (Bremer et al., 2009). We then
18 adjusted the branch length using BLADJ algorithm within the Phylocom software
19 (<http://www.phylodiversity.net/phylocom/>; Wikström et al., 2001). We then calculated the K
20 statistic (Blomberg et al., 2003) to quantify the magnitude of phylogenetic signal of leaf N, P
21 concentrations and N:P ratios. For each species, we first calculated the mean leaf N and P
22 concentrations. To test if the phylogenetic conservatism of leaf N concentrations is caused by
23 legumes (species from Fabaceae) or succulent species, we also calculated the K statistic of
24 leaf N after dropping the clade of Fabaceae or succulent plants. The significance (*p*-values)
25 was evaluated by comparing the variance of independent contrasts for each trait to the
26 expected values obtained by shuffling leaf trait data across the tips of the phylogenetic tree
27 for 999 times. The P-value can be used to test whether the phylogenetic signal in each trait is

1 larger than the null expectation, while the K statistic can be used to estimate the strength of
2 phylogenetic signal. A significant P-value indicates that the phylogenetic signal of the trait
3 was non-random, compared to the prediction of the random-tip-shuffling model.

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

7

8 **3 Results**

9 **3.1 Effects of climate and soil on leaf N and P concentrations**

10 At the individual level, leaf N concentrations ranged from 4.26 to 46.80 mg g⁻¹ (mean =21.91,
11 std =6.84) (Table 1; Fig. S2). They decreased with increasing AP ($R^2 =0.1$, $p <0.001$) and
12 STN ($R^2 =0.13$, $p <0.001$), increased with increasing soil pH ($R^2 =0.02$, $p <0.001$), while
13 showed no significant correlation with MAT ($R^2 <0.01$, $p =0.227$) (Fig. 2). Leaf P
14 concentrations ranged from 0.16 to 4.80 mg g⁻¹ (mean =1.30, std =0.53) (Table 1; Fig. S2).
15 They increased with increasing AP ($R^2 =0.03$, $p <0.001$) and STP ($R^2 =0.02$, $p <0.001$), while
16 decreased with increasing MAT ($R^2 =0.03$, $p <0.001$) and soil pH ($R^2 =0.03$, $p <0.001$) (Fig.
17 2). Leaf N:P ratios changed from 4.07 to 145.76 (mean =18.69, std =8.40) (Table 1; Fig. S2).
18 Leaf N:P ratios increased with increasing MAT ($R^2 =0.04$, $p <0.001$) and soil pH ($R^2 =0.07$, p
19 <0.001), while decreased with increasing AP ($R^2 =0.18$, $p <0.001$), STN ($R^2 =0.07$, $p <0.001$)
20 and STP ($R^2 =0.06$, $p <0.001$) (Fig. 3).

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

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

1 ($p < 0.001$) of the interspecific variation of leaf N concentrations, respectively. None of MAT,
2 AP, STN, STP and soil pH significantly influenced intraspecific variation of leaf N
3 concentrations ($p > 0.05$ for all). For leaf P concentrations, MAT and AP accounted for 1.2%
4 ($p < 0.01$) and 3.5% ($p < 0.001$) interspecific variation; STN and STP explained 1.1% (p
5 < 0.01), and 3.5% ($p < 0.001$) of intraspecific variation, respectively (Table 2; Fig. 4). For leaf
6 N:P ratios, AP accounted for 20.0% ($p < 0.001$) of the interspecific variation; STP explained
7 1.1% ($p < 0.01$) of the intraspecific variation, respectively (Table 2; Fig. 4). As temperate and
8 desert shrublands distributed in different climates, we further conducted GLM analyses for
9 the two major shrubland types, separately. Temperate shrublands showed similar results to
10 that with all data pooled. For desert shrublands, however, none of the environmental factors
11 significantly influenced leaf N concentrations, and precipitation was the major factor
12 influencing leaf P concentrations and N:P ratios through shifts in species composition (Fig.
13 S3; Table S3).

14 **3.2 Phylogenetic signals of leaf N and P concentrations**

15 Leaf N concentrations exhibited a significantly non-random phylogenetic signal ($K = 0.31$, p
16 < 0.001), while leaf P concentrations showed a significant but weaker phylogenetic signal (K
17 $= 0.24$, $p < 0.01$) among all species (Table 1). The phylogenetic signal for leaf N
18 concentrations remained significant when legumes ($K = 0.30$, $p < 0.001$) or succulent plants
19 were excluded ($K = 0.30$, $p < 0.001$) (Table 1).

20

21 **4 Discussion**

22 Using foliar stoichiometry of 163 shrub species from 361 shrubland sites, we investigated
23 patterns of leaf N and P concentrations in shrublands of Northern China. We focused our
24 discussion on leaf N and P concentrations instead of their ratio because leaf N:P was strongly
25 driven by both leaf N and P concentrations and was predictable based on leaf N and P
26 concentrations. Given that leaf C concentrations are relatively stable, leaf N and P
27 concentrations can be good indicators of C:N and C:P ratios (Reich, 2005). We found that

1 mean leaf N (21.91 mg g⁻¹) and P (1.30 mg g⁻¹) concentrations of shrubs in Northern China
2 shrubland were similar to those in shrubs across China (mostly distributed in forests as
3 understory species, Han et al., 2005), but lower than those in grasses (Han et al., 2005; He et
4 al., 2006, 2008) and higher than those in trees in China (Han et al., 2005) (Fig. S4). The “leaf
5 economics spectrum”, proposed by Wright et al. (2004), runs from life strategies
6 characterized by low rates of metabolism, low N and P concentrations, and extended leaf
7 longevity, to life strategies characterized by high rates of metabolism, high N and P
8 concentrations, and short leaf longevity (Wright et al., 2004). Our result indicated differences
9 in life strategies between shrubs and trees or herbaceous plants. Our results also suggested
10 that the inclusion of shrubs is necessary to explore the patterns of leaf stoichiometry in
11 relation to climate and soil property.

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

14 **4.1 Influence of climate on leaf N and P concentrations**

15 Leaf N and P concentrations responded to climate in different ways. Consistent with our
16 hypothesis, leaf N concentrations decreased with precipitation. This is partly due to the
17 higher leaf N concentrations of plants in desert shrublands. Higher leaf N concentrations have
18 been suggested as a general property of arid-zone plants (Wright et al., 2003). It is widely
19 reported that plants tend to increase leaf N to exploit greater light availability while reducing
20 stomatal conductance and transpiration rates (Cunningham et al., 1999; Wright et al., 2003;
21 Luo et al., 2015). Succulence is such an adaption for plants to drought and salinity by
22 accumulating nitrogen-containing compounds in their leaves to maintain water balance and
23 therefore resulting in succulent plants having higher leaf N concentrations than other plants
24 (Mansour, 2000) (Fig. S4). In contrast, leaf P concentrations increased with precipitation. P is
25 derived primarily from the weathering of soil inorganic components and the degradation of
26 organic matters (Aerts and Chapin, 1999). Increases in precipitation may amplify P
27 availability in soil by facilitating the decomposition of litter in arid regions. 301 of the study

1 sites have an aridity index (the ratio of total precipitation to potential evapotranspiration) of
2 <1, indicating that precipitation is generally lower than evapotranspiration in this region. The
3 positive correlation between soil total phosphorous concentrations and precipitation (R^2
4 =0.21, $p < 0.001$) and the lower soil total phosphorous concentrations in desert shrublands is
5 in line with such a hypothesis.

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

17 **4.2 Influence of soil N and P concentrations on leaf N and P concentrations**

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

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

22 **4.3 Influence of environmental factors at the intraspecific and interspecific** 23 **variation of leaf N and P concentrations**

24 Environmental factors explained more variance in leaf P concentrations than N
25 concentrations at the community level (Table 2). However, the explanatory powers of climate
26 and soil for leaf N and P concentrations are comparatively low. We speculate that other
27 factors, such as soil age, may also have effects on the leaf stoichiometry, (Vitousek et al.,

1 2010; Hayes et al., 2014), but these were not included in our model. Interspecific variation of
2 leaf N and P concentrations is caused by changes in species composition, and intraspecific
3 variation of leaf N and P concentrations is driven by environmental variations. Leaf P was
4 jointly influenced by climate and soil nutrient. Climate influenced the community leaf P
5 concentrations through shift in species composition, whereas soil influences the community P
6 concentrations directly. Compared with other environmental factors, leaf N concentrations
7 were to a larger extent affected by precipitation through species turnover.

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

19 **4.4 Influence of phylogeny on leaf N and P concentrations**

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

26 Plants disperse and evolve in response to environmental conditions that vary over both time
27 and space (Kerkhoff et al., 2006). In this process, adaptive traits that are shaped by the

1 environment conditions tend to show weaker phylogenetic signal (Losos, 2008). In this study,
2 leaf N concentrations were not influenced by soil nutrients, and we surmise that the influence
3 of climate on leaf N concentrations mainly works through species turnover. Leaf N
4 concentrations therefore exhibited significant phylogenetic signal. In contrast, leaf P
5 concentrations were significantly influenced by soil nutrients, and their conservation was
6 therefore weakened.

7

8 **5 Conclusions**

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

22

23 **Author contribution**

24 X. Y. and Z. T. conceived and designed the study, X. Y., Z. T. and X. C., conducted analyses
25 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.
26 T. contributed data, discussed the draft manuscript and interpreted the results.

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10

1 Table 1. Arithmetic mean, range and phylogenetic signal (K-value) of leaf N and P
 2 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.

1 Table 2. Summary of main-effect general linear models for leaf N and P concentrations and
 2 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.

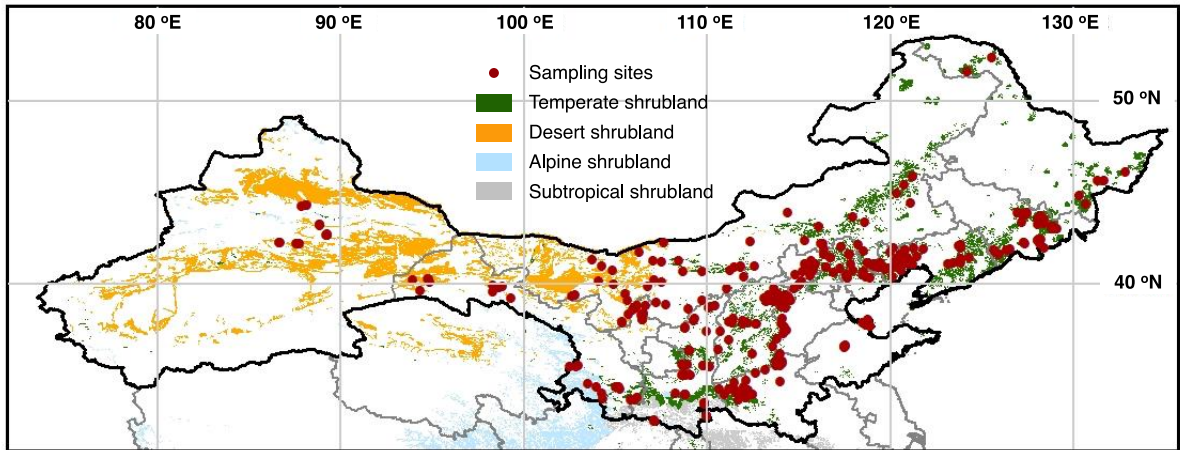
STP, soil total phosphorus.

^a $p < 0.001$.

^b $p < 0.01$.

^c $p < 0.05$.

^{NS} not significant.



1

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

3

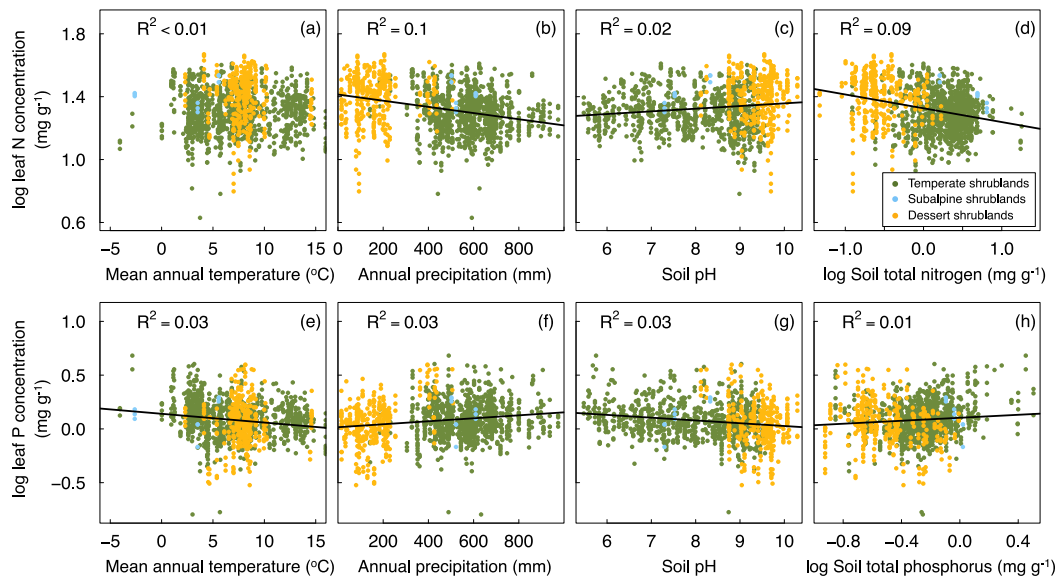
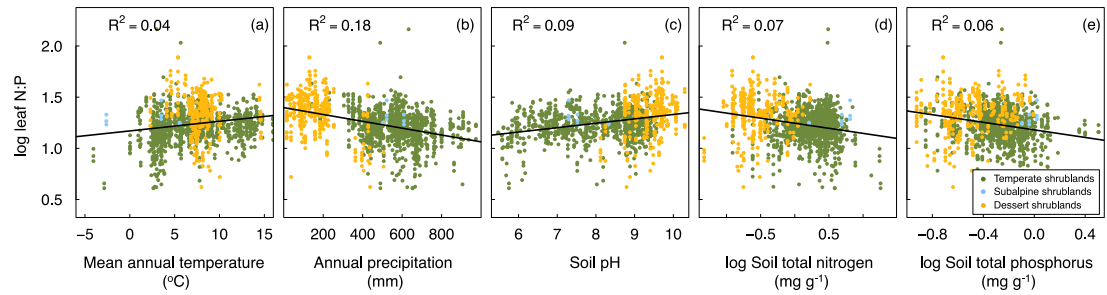
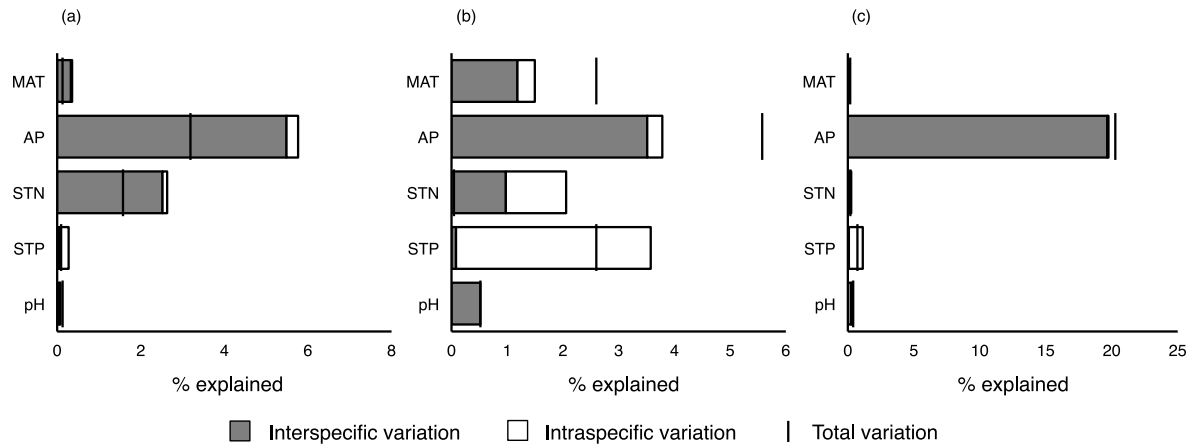


Figure 2. Changes of logtransformed leaf nitrogen and phosphorus concentrations in relation to climate (mean annual temperature and annual precipitation) and soil nutrient (soil pH and soil total nitrogen and phosphorus concentrations) for shrubs in Northern China. Green, yellow and blue dots represent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



1

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



1

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

1 **Supplementary material**

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

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

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

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

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

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

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

25