

# C:N:P stoichiometry of Ericaceae species in shrubland biomes across Southern China: influences of climate, soil and species identity

Qiang Zhang<sup>1,2</sup>, Qing Liu<sup>3</sup>, Huajun Yin<sup>3</sup>, Chunzhang Zhao<sup>3</sup>,  
Lin Zhang<sup>4</sup>, Guoying Zhou<sup>5</sup>, Chunying Yin<sup>3</sup>, Zhijun Lu<sup>6</sup>,  
Gaoming Xiong<sup>1</sup>, Yuelin Li<sup>7</sup>, Jiaxiang Li<sup>8</sup>, Wenting Xu<sup>1</sup>,  
Zhiyao Tang<sup>9</sup> \* and Zongqiang Xie<sup>1,2</sup> \* \* \* \* \*<sup>o</sup>

<sup>1</sup> State Key Laboratory of Vegetation and Environment Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

<sup>2</sup> University of Chinese Academy of Sciences, Beijing 100049, China

<sup>3</sup> Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration and Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China

<sup>4</sup> Key Laboratory of Alpine Ecology and Biodiversity of Chinese Academy of Sciences, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100101, China

<sup>5</sup> Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008

<sup>6</sup> Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, China

<sup>7</sup> South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

<sup>8</sup> College of Forest, Central South University of Forestry & Technology, Changsha 410004, China

<sup>9</sup> Department of Ecology, College of Urban and Environmental Sciences and Key Laboratory for Earth Surface Processes, Peking University, Beijing 100871, China

\*Correspondence address. Department of Ecology, College of Urban and Environmental Sciences and Key Laboratory for Earth Surface Processes, Peking University, No. 5 Yiheyuan Road, Haidian District, Beijing 100871, China. Tel/Fax: +86-10-6275-4039; E-mail: [zytang@urban.pku.edu.cn](mailto:zytang@urban.pku.edu.cn)

\*\*Correspondence address. State Key Laboratory of Vegetation and Environment Change, Institute of Botany, Chinese Academy of Science, No.20 Nanxincun, Xiangshan, Beijing 100093, China. Tel/Fax: +86-10-6283-6284; E-mail: [xie@ibcas.ac.cn](mailto:xie@ibcas.ac.cn)

## Abstract

### Aims

Carbon (C), nitrogen (N) and phosphorus (P) stoichiometry strongly affect functions and nutrient cycling within ecosystems. However, the related researches in shrubs were very limited. In this study, we aimed to investigate leaf stoichiometry and its driving factors in shrubs, and whether stoichiometry significantly differs among closely related species.

### Methods

We analyzed leaf C, N and P concentrations and their ratios in 32 species of Ericaceae from 161 sites across southern China. We examined the relationships of leaf stoichiometry with environmental variables using linear regressions, and quantified the interactive and independent effects of climate, soil and species on foliar stoichiometry using general linear models (GLM).

### Important Findings

The foliar C, N and P contents of Ericaceae were 484.66, 14.44 and 1.06 mg g<sup>-1</sup>, respectively. Leaf C, N and P concentrations and their

ratios in Ericaceae were significantly related with latitude and altitude, except the N:P insignificantly correlated with latitude. Climate (mean annual temperature and precipitation) and soil properties (soil C, N and P and bulk density) were significantly influenced element stoichiometry. The GLM analysis showed that soil exerted a greater direct effect on leaf stoichiometry than climate did, and climate affected leaf traits mainly via indirect ways. Further, soil properties had stronger influences on leaf P than on leaf C and N. Among all independent factors examined, we found species accounted for the largest proportion of the variation in foliar stoichiometry. These results suggest that species can largely influence foliar stoichiometry, even at a lower taxonomic level.

**Keywords:** biogeographic pattern, phylogenetic effect, closely related species, Ericoid Mycorrhiza, shrub

Received: 31 August 2017, Revised: 23 April 2018, Accepted: 29 August 2018

## INTRODUCTION

Plants require ca. 30 elements to complete their life cycle (Ågren 2008; Mengel and Kirkby 2001). Leaf element properties strongly affect plant physiological processes, nutrient cycling, and the responses of ecosystems to global climate change (Ågren 2008; Amatangelo and Vitousek 2008; Mueller *et al.* 2010). The most important and essential elements to plants are carbon (C), nitrogen (N) and phosphorus (P), which provide structural basis and drive metabolic processes in plants. C, which makes up roughly 50% of plant dry mass, is a major structural component of living materials (Hessen *et al.* 2004). N is a fundamental component of all plant enzymes, and P is a vital component of genetic material, energy storage, and cell structure. These three elements are strongly coupled in their biochemical processes. P-rich ribosomes manufacture N-rich proteins, which constitute the C and energy-harvesting organs (Ågren 2008; Hessen *et al.* 2004).

In recent decades, ecological stoichiometry and the factors controlling stoichiometry have drawn great attention at regional and global scales (McGroddy *et al.* 2004; Thompson *et al.* 1997; Yang *et al.* 2015b). Previous studies have reported diverse biogeographic patterns in plant stoichiometry, which may result from different factors, such as climate conditions, soil characteristics, species compositions, and plant growth forms (Chen *et al.* 2013; Han *et al.* 2005, 2011). Several hypotheses have been proposed to explain the patterns in plant stoichiometry, including the temperature-plant physiological hypothesis (Reich and Oleksyn 2004; Woods *et al.* 2003), the biogeochemical hypothesis (McGroddy *et al.* 2004; Reich and Oleksyn 2004), the soil substrate age hypothesis (Reich and Oleksyn 2004), the species composition hypothesis (Reich and Oleksyn 2004), and the growth rate hypothesis (Elser *et al.* 2003; Sterner and Elser 2002). These hypotheses try to give reasonable explanations for these diverse patterns from various perspectives. Understanding the responses of plant stoichiometry to climate and soil conditions would help us elucidate the characteristics of nutrient fluxes across ecological gradients and predict the influences of global climate change.

Although there are long history about the research on plant stoichiometry, the factors driving variation in plant stoichiometry are not well understood (Ågren 2008). First, most ecological plant stoichiometry studies have focused on trees (McGroddy *et al.* 2004; Townsend *et al.* 2007) and herbs (Hao *et al.* 2015; He *et al.* 2006, 2010), but the related researches in shrubs were very limited. As one of the main ecosystem types, shrub lands cover more than 0.69 million km<sup>2</sup> in China (Ministry of Environmental Protection of the People's Republic of China and Chinese Academy of Sciences 2015). It has been reported that leaf stoichiometry can vary remarkably among growth forms (Han *et al.* 2005; Wright *et al.* 2004). Therefore, it is necessary to elucidate the stoichiometric traits of shrubs in order to improve our understanding about ecological processes and nutrient cycling.

Second, the leaf stoichiometry among species showed different patterns with environment changing (Han *et al.* 2005; Reich and Oleksyn 2004). A large proportion of the inter-species variation in leaf stoichiometry can be attributed to phylogenetic effects (Thompson *et al.* 1997; Zhang *et al.* 2012). According to Watanabe *et al.*'s (2007) reports, the majority of genetic variation in elements occurred between species within the same family. However, most previous studies investigating the phylogenetic effects on plant stoichiometry have been conducted on broad phylogenetic scales (family level or above) (Broadley *et al.* 2003, 2004; Yang *et al.* 2015a). Researches that have investigated closely related species include Yang *et al.* (2015b) and Hao *et al.* (2015) which focused on herbs, and Kang *et al.* (2011) about forests. According to our knowledge, this is the first time to compare foliar stoichiometry of phylogenetically proximal shrub species. Third, although global syntheses incorporate extraordinarily rich datasets encompassing multiple geographic locations, species, and growth forms (Han *et al.* 2005; Ordoñez *et al.* 2009; Reich and Oleksyn 2004), it is difficult to ensure consistent data collection approaches are implemented in extremely large-scale studies (He *et al.* 2006; Yang *et al.* 2015b). Standardization of large-scale sampling protocols is essential to minimize the effects of sampling error on variation, particularly when relatively weak relationships, such as between foliar N and climate variables (He *et al.* 2006), are being examined.

In order to address the aforementioned knowledge gaps, we explored the biogeographic patterns of leaf C:N:P stoichiometry in Ericaceous plant along environmental gradient across China. Ericaceae is one of the most widely distributed families in China, with a distribution from the tropical to cool temperate zone and encompassing plains, plateaus, and mountains (Feng 1988). Especially in the alpine and subalpine zones of west and southwest of China, the dominant plant communities above the tree-line are almost all Ericaceae shrublands (Yang *et al.* 1999). Across this immense geographical distribution range of Ericaceae shrubs, there is a broad gradient of mean annual temperature (MAT), mean annual precipitation (MAP), and soil properties, providing an ideal study system to examine the stoichiometry patterns of closely related species at a national scale. In addition, a special kind of endophytic mycorrhiza is widely distributed in Ericaceous plants, which be called Ericoid Mycorrhiza (ERM; Leake and Read 1991). The ERM help Ericaceous plants absorb nutrients and survive in environment with poor nutrients (Cairney *et al.* 2003), which make the Ericaceous species special in the ways of nutrient acquisition.

In this study, we intend to address the following questions: what is special for the foliar C:N:P stoichiometry of Ericaceous species compared with other woody species? How do the climate and soil factors affect the leaf C:N:P stoichiometry of Ericaceous species? Are there any differences in the responses of leaf C, N, P and their ratios to the potential drivers? In order to further understand the mechanisms driving variation

in leaf stoichiometry, we then distinguished shared and pure effects of the different components shaping the patterns in leaf stoichiometry.

## MATERIALS AND METHODS

### Sample collection and element measurements

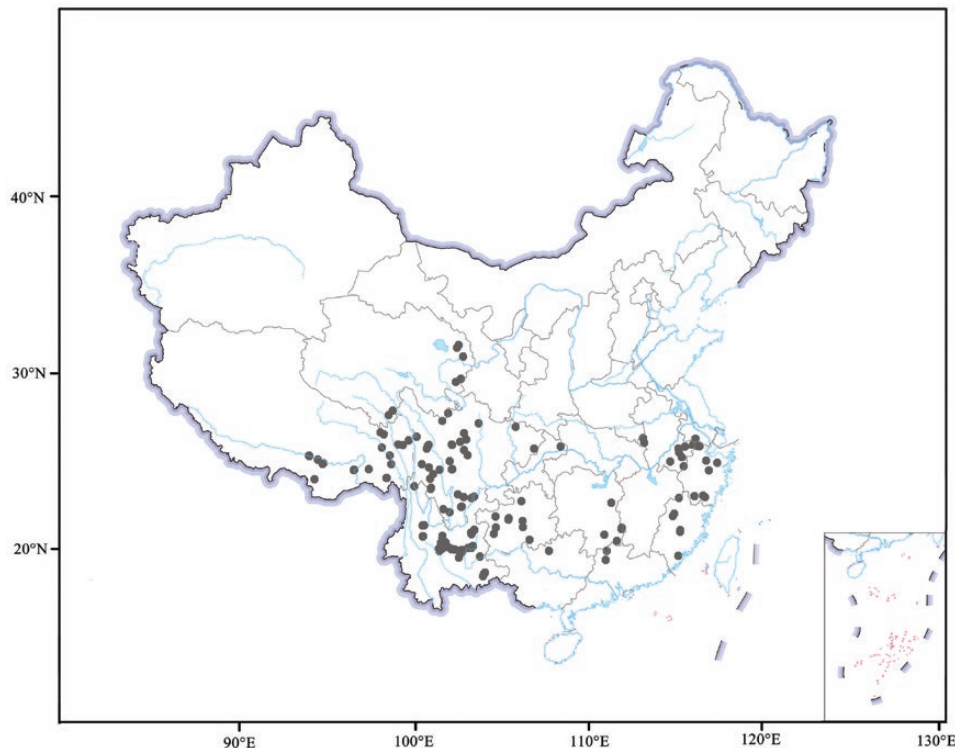
This study was conducted at 161 shrubland sites in China, spanning a range of 13.74 degrees in latitude from 23.66°N to 37.40°N and 29.86 degrees in longitude from 91.25°E to 121.11°E, and varying in altitude from 27 to 4906 m (Fig. 1). Sampling occurred from July to September. At each sampling site, we measured latitude, longitude and altitude, and selected three 5 × 5 m<sup>2</sup> plots, which were separated by 5–50 m, to represent the natural shrubland. For each Ericaceae species encountered in a plot, three to five samples of fully expanded sun-exposed leaves were collected and pooled as one individual. In total, we collected 210 individuals of 32 shrub species in Ericaceae (including 29 evergreen species and 3 deciduous species). Leaf samples were transported to the laboratory and oven-dried at 65°C for 72 h and then ground.

Within each plot, we excavated three 1-m-deep pits along the diagonal of plot to collect soil samples. For each profile, soil samples were collected at the depths of 0–10, 10–20, 20–30, 30–50, 50–70 and 70–100 cm, or deep to the base rock. The three soil samples from the same depth were mixed well and air-dried; then roots and gravels were removed and samples were ground to pass through a 100-mesh sieve before elemental analysis.

Plant and soil C, N and P concentrations were measured at the Measurement Center of the Institute of Botany, Chinese Academy of Sciences. Total carbon and nitrogen concentrations of soil (STC and STN) and plant leaf samples were analyzed using an elemental analyzer (2400 II CHNS; Perkin-Elmer, Boston, MA, USA). Samples were combusted at 950°C, after which temperature was reduced to 640°C. Total phosphorus concentration of soil (STP) and plant leaf samples were measured using the molybdate/ascorbic acid method after H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> digestion (Jones 2001). Soil bulk density (BD) was determined at depths of 0–10, 10–20, 20–30, 30–50, 50–70 and 70–100 cm using the cutting ring core method. Soil samples in the rings were dried in the laboratory at 105°C to constant weight and then removed gravels in it for calculation of BD. As analyses showed STC, STN, STP and BD were highly correlated among different depths, we only used measurements performed on 0–30 cm depth samples for the following analyses. The BD and STC of soil at depths of 0–30 cm were calculated by the following formula ('V' represented the volume of soil):

$$BD_{0-30\text{cm}} = (BD_{0-10\text{cm}} \times V_{0-10\text{cm}} + BD_{10-20\text{cm}} \times V_{10-20\text{cm}} + BD_{20-30\text{cm}} \times V_{20-30\text{cm}}) / (V_{0-10\text{cm}} + V_{10-20\text{cm}} + V_{20-30\text{cm}})$$

$$STC_{0-30\text{cm}} = (STC_{0-10\text{cm}} \times BD_{0-10\text{cm}} \times V_{0-10\text{cm}} + STC_{10-20\text{cm}} \times BD_{10-20\text{cm}} \times V_{10-20\text{cm}} + STC_{20-30\text{cm}} \times BD_{20-30\text{cm}} \times V_{20-30\text{cm}}) / (BD_{0-10\text{cm}} \times V_{0-10\text{cm}} + BD_{10-20\text{cm}} \times V_{10-20\text{cm}} + BD_{20-30\text{cm}} \times V_{20-30\text{cm}})$$



**Figure 1:** the geographic locations of sampling sites.

The calculation methods of STN and STP of soil at depths of 0–30 cm were the same as that of STC.

We calculated MAT and MAP at each site based on National Ecosystem Research Network of China records from 1951 to 2000, with a resolution of  $1 \times 1$  km.

### Data analysis

Leaf element concentrations were averaged at the species level for each sampling site. This dataset was statistically summarized to show the mean, sample size and other statistical characteristics. Element concentrations and stoichiometric ratios were  $\log_{10}$ -transformed before analyses to improve the data normality. We calculated the means of leaf nutrient concentrations and ratios of all species within each sites and then examined the relationships of leaf stoichiometry traits with latitude, altitude, and environmental variables using linear regressions. Environmental variables examined included climate data (MAT and MAP) and soil data (soil C, N and P concentrations and BD). In order to quantify the relative effects of climate, soil and species on leaf element concentrations and ratios, general linear models (GLM) were applied. We separated the variation explained by the different factors into independent effects of individual factors and interactive effects of combined factors (Borcard *et al.* 1992; Heikkinen *et al.* 2005). All statistical analyses were conducted using R version 3.1.1 (R Development Core Team 2014).

## RESULTS

### Patterns of leaf C, N, P and their ratios in Ericaceae

Mean concentrations ( $\pm$  SE) of C, N and P were  $484.66 \pm 1.66$ ,  $14.44 \pm 0.29$  and  $1.06 \pm 0.03$  mg g<sup>-1</sup>, respectively, and mean stoichiometric ratios ( $\pm$  SE) for C:N, C:P and N:P were  $36.40 \pm 0.74$ ,  $527.67 \pm 14.37$  and  $14.78 \pm 0.36$ , respectively (Table 1). The relative variability of these six leaf chemical traits was characterized using coefficients of variation (CV). Among the six leaf chemical traits, leaf P, C:P and N:P were most variable, with CVs of 0.38, 0.39 and 0.35, respectively. The next most variable leaf traits were N (CV = 0.29) and C:N (CV = 0.29). The coefficient of variation for leaf C concentration (CV = 0.05) was distinctly smaller than other leaf traits (Table 1), which suggests that carbon concentration is relatively stable among locations and species.

### Variations of leaf C, N and P stoichiometry in Ericaceae with environment variables

Generally, leaf C, N and P concentrations and their ratios were significantly related to latitude and altitude, except N:P was not significantly correlated with latitude (Fig. 2). Leaf C, N and P concentrations increased but C:N and C:P ratios decreased with increasing latitude ( $P < 0.01$ ,  $r^2 = 0.06, 0.36, 0.14, 0.34$  and  $0.13$ , respectively). As altitude increased, leaf C, N and P concentrations increased ( $P < 0.01$ ,  $r^2 = 0.56, 0.17$  and  $0.47$ , respectively), whereas C:N, C:P and N:P ratios decreased ( $P < 0.01$ ,  $r^2 = 0.08, 0.38$  and  $0.26$ , respectively).

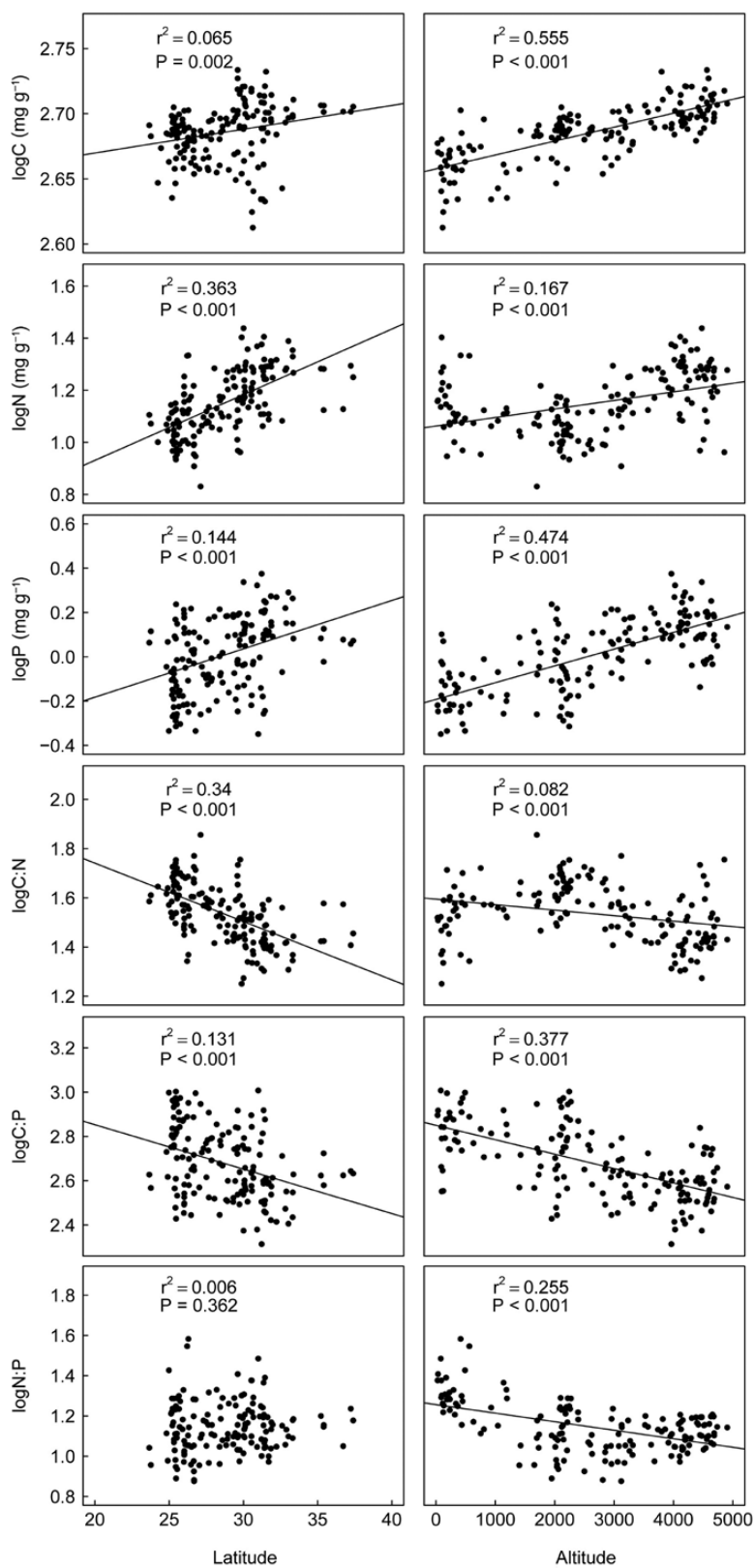
Leaf chemical traits were also significantly related to climate (MAT and MAP) (Fig. 3) and soil conditions (soil C, N and P concentrations and BD) (Fig. 4). In general, leaf C, N and P concentrations responded to environmental variables in the same direction: negatively with MAT, MAP and BD, and positively with soil C, N and P concentrations. The responses of leaf stoichiometry (C:N, C:P and N:P ratios) to environmental conditions were exactly opposite to those of leaf C, N and P concentrations. Leaf C:N, C:P and N:P ratios correlated positively with MAT, MAP and BD, while negatively with soil C, N and P concentrations. Additionally, leaf P correlated more strongly than leaf C or N with soil C, N and P concentrations.

### Effects of climate, soil and species on leaf C, N, P and their ratios in Ericaceae

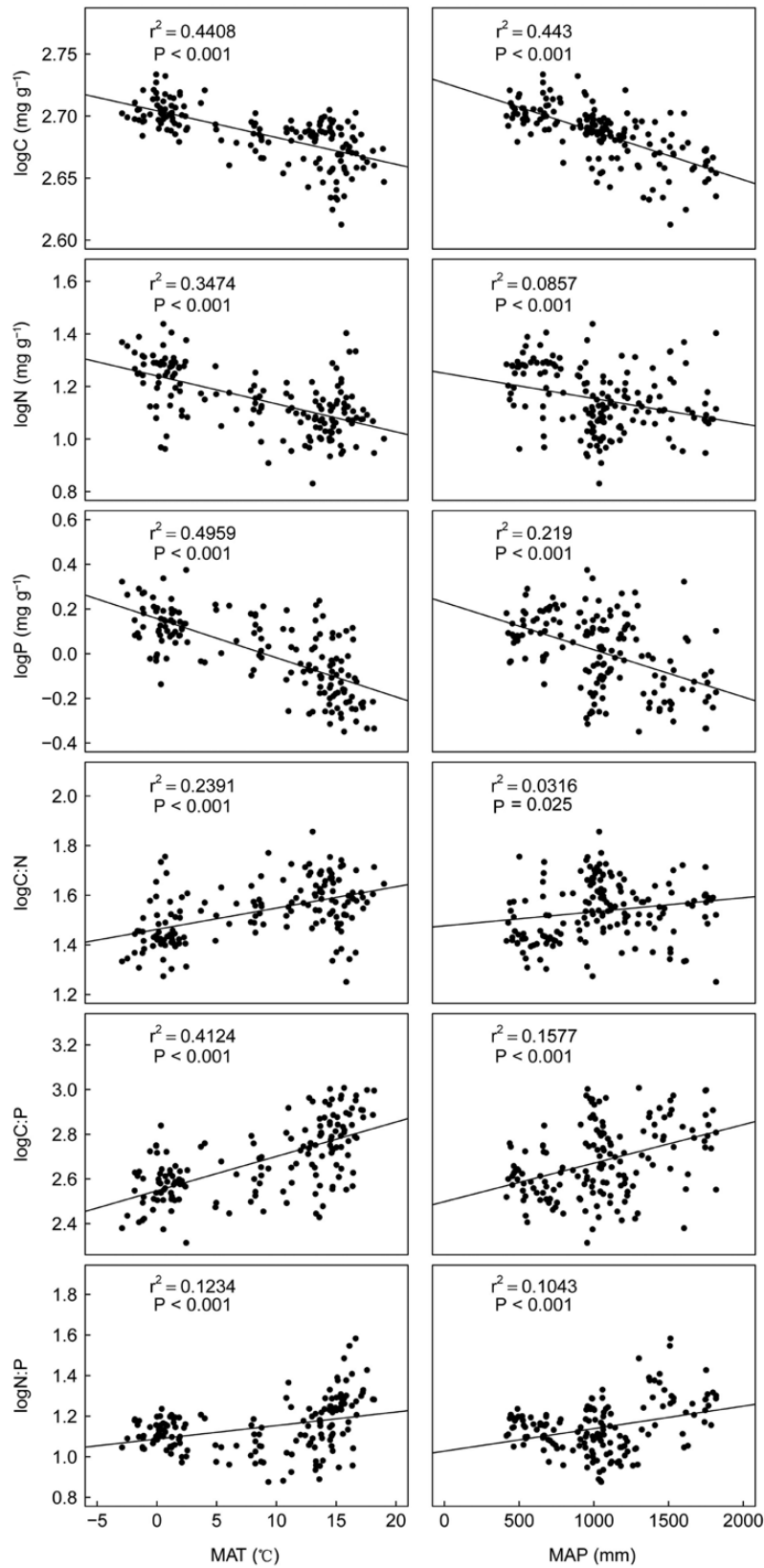
The GLM analysis showed that climate, soil and species together explained an average of 63.1% of the variation in leaf C, N, P and their ratios (Table 2). The major contributors to the variation differed among leaf traits. In general, the interactive effects of climate, soil and species explained the highest proportion of the total variation in leaf chemical traits, 23.3% (from 9.0% to 35.67%). The interactive effects of climate and species explained the largest proportion (25.8%) of the variation in leaf C. Examining the independent factors effects, we found that species, which explained 15.7% (from 12.8% to 19.0%) of the variation, was the most influential independent factor; soil explained 7.0% (from 1.8% to 12.8%) of the variation; and climate explained the smallest fraction of the total variance, 1.1% (from 1.0% to 1.2%).

**Table 1:** statistics of leaf C, N and P concentrations and their ratios in Ericaceous plants

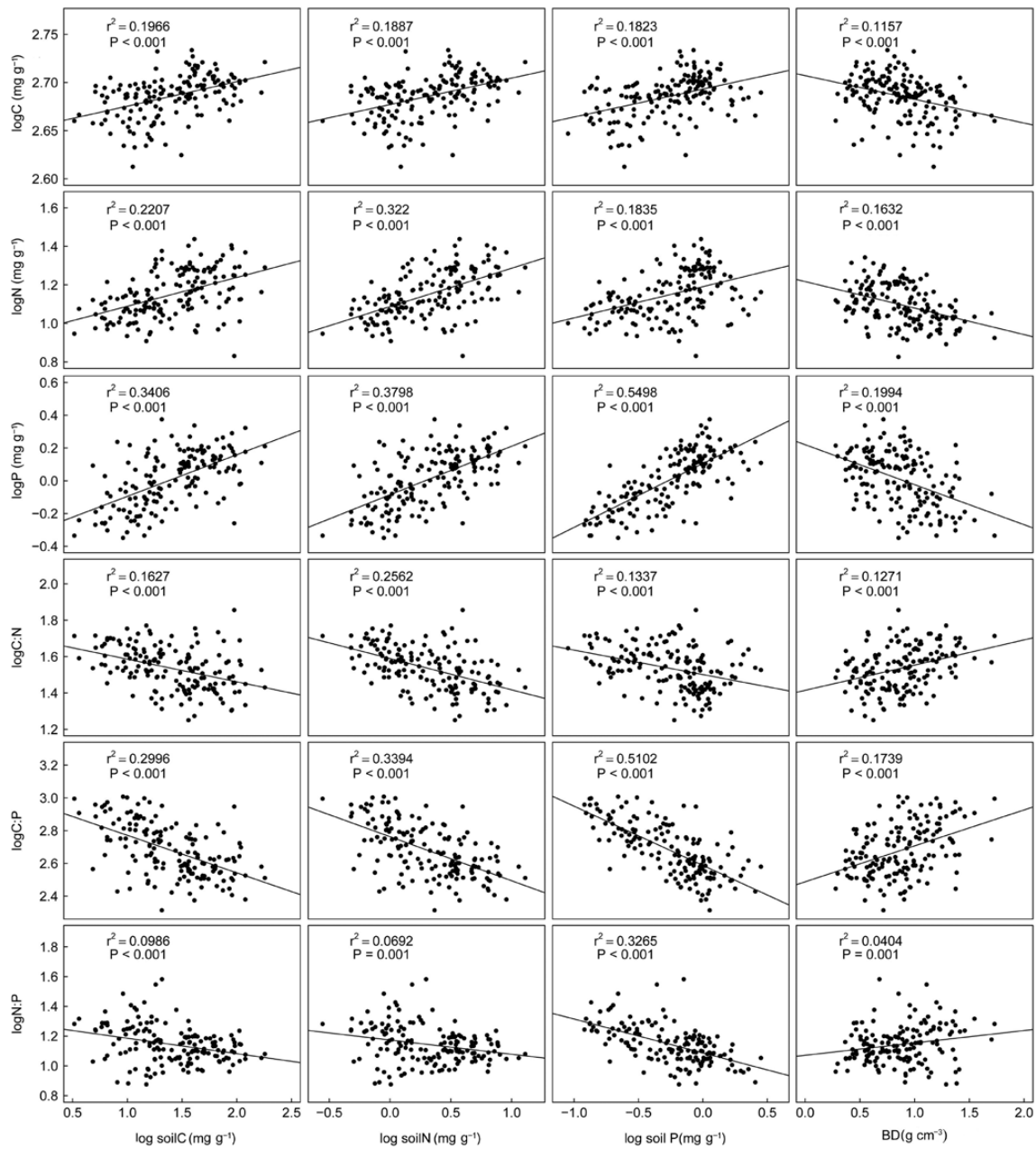
	<i>n</i>	Mean	Min	Max	SE	CV	Skewness	Kurtosis
C (mg g <sup>-1</sup> )	209	484.66	409.75	541.43	1.66	0.05	-0.53	0.21
N (mg g <sup>-1</sup> )	208	14.44	6.66	27.43	0.29	0.29	0.57	-0.29
P (mg g <sup>-1</sup> )	207	1.06	0.41	2.37	0.03	0.38	0.51	-0.35
C:N ratio	207	36.40	17.81	74.27	0.74	0.29	0.82	0.58
C:P ratio	206	527.67	205.94	1153.08	14.37	0.39	0.83	0.05
N:P ratio	206	14.78	6.85	40.70	0.36	0.35	1.76	5.06



**Figure 2:** relationships of leaf C, N and P concentrations and C:N, C:P and N:P ratios with latitude and altitude. Regression lines are shown only for relationships that were significant at  $P \leq 0.05$ .



**Figure 3:** relationships of leaf C, N and P concentrations and C:N, C:P and N:P ratios with MAT and MAP. Regression lines are shown only for relationships that were significant at  $P \leq 0.05$ .



**Figure 4:** relationships of leaf C, N and P concentrations and C:N, C:P and N:P ratios with soil properties. Regression lines are shown only for relationships that were significant at  $P \leq 0.05$ .

## DISCUSSION

### Patterns of leaf C, N, P and their ratios in Ericaceae

The analysis of this study indicated that the mean leaf C concentration of Ericaceous plants was similar to the values reported for global terrestrial plant species ( $46.4 \pm 3.21\%$ , Elser *et al.* 2000) and a global scale estimate of shrub leaf carbon content using the derived data from public publications ( $475.2 \text{ mg g}^{-1}$ , from  $355.1$  to  $594.2 \text{ mg g}^{-1}$ , Zheng *et al.* 2007). The mean leaf N and P concentrations in Ericaceous plants

(most of which were evergreen plants in this study) were lower than those in global terrestrial plants ( $18.3$  and  $1.42 \text{ mg g}^{-1}$ , respectively; Reich and Oleksyn 2004) and shrubs across China ( $19.1$  and  $1.11 \text{ mg g}^{-1}$ , respectively; Han *et al.* 2005). This is because leaf N and P levels tend to be higher in herbs than in woody plants, and in deciduous than in evergreen species (Elser *et al.* 2003; Han *et al.* 2005; Sterner and Elser 2002). When compared with evergreen woody plants in eastern China ( $18.5 \text{ mg g}^{-1}$  for N and  $1.01 \text{ mg g}^{-1}$  for P, Chen *et al.* 2013), the Ericaceous plants still had a lower N concentration.

**Table 2:** summary of the GLM for the effects of climate, soil and species on leaf element concentrations and their ratios

Element	Total effects ( $r^2$ , %)				Independent and interactive effects ( $r^2$ , %)							
	Full	Climate	Soil	Species	a.			ab	ac	bc	abc	
					Climate	Soil	Species					
C	66.6	46.7	20.6	63.5	1.2	1.8	19.0	0.1	25.8	-0.9	19.6	
N	59.6	33.3	37.3	52.3	1.0	4.9	16.0	1.5	5.3	5.4	25.6	
P	72.7	46.6	49.8	62.6	1.1	8.2	12.7	0.8	9.0	5.2	35.7	
C:N	55.3	26.1	32.7	47.0	1.3	5.6	17.6	1.5	3.7	6.0	19.6	
C:P	68.3	38.8	45.5	57.5	1.0	9.0	15.0	0.7	6.7	5.4	30.4	
N:P	56.3	13.4	39.5	41.2	1.1	12.7	13.6	1.2	2.1	16.5	9.0	

In the partial GLM, leaf stoichiometric traits variations were partitioned into different components: a, b and c denote the independent effects of climate, soil, and species, respectively; ab, ac and bc represent the interactive effects between climate and soil, climate and species, soil and species, minus abc; and abc represent the shared effects of climate, soil and species together.

The low nutrient contents in Ericaceae may be due to most of their habits are nutrient poor (Cairney and Meharg 2003); thus, the availability of soil nutrients was lower.

Leaf N:P ratio of Ericaceous plants was larger than ratio reported for global vegetation (11.8; Reich and Oleksyn 2004), but consistent with the ratio of Chinese vegetation (14.4; Han *et al.* 2005). This implies that Chinese plants are more P-limited than global plants, as identified previously (Han *et al.* 2005; Zhang *et al.* 2012). Compared with Ericaceae, the species in the same sites but belongs to other families had greater leaf N:P ratio (19.19, data were unpublished). In addition, the evergreen woody plants in eastern China also had a higher leaf N:P ratio than Ericaceae (Chen *et al.* 2013). This result maybe indicated that the ERM promoted the absorption of P by Ericaceae, which alleviated the P-limited. The phenomenon that Ericaceous plants are so widely distributed in the world and most of their habits are nutrient poor maybe is closely related to the ERM. In addition, we found that carbon is the most stable element, followed by nitrogen and phosphorus. This is consistent with the stability of limiting elements hypothesis reported by Han *et al.* (2011), which suggested elements that are required in higher would be more stable and less sensitive to environmental gradients.

### Biogeographic patterns

We expected to detect distinct biogeographic patterns in element concentrations and stoichiometry of Ericaceae species, given the geographic patterns in climate and soil conditions. Our results showed that leaf C, N, P concentrations and their ratios of Ericaceous plants were significantly related to altitude. Consistent with our results, several previous studies have obtained the similar patterns on foliar stoichiometry (e.g. Kang *et al.* 2011; Yang *et al.* 2015b). It has been recognized that lower temperature and shorter growing season at high altitudes are correlated with higher leaf N and P contents in terrestrial plants (Körner 1989; Reich and Oleksyn 2004). However, in contrast with Yang *et al.*'s (2015b) reports, our study showed that C concentration increased with increasing altitude in Ericaceous plants. This inconsistency may be due

to the predominance of evergreen plants in our study sites. Evergreen plants tend to contain higher leaf lignin when altitude increasing, because lignin can enhance the mechanical support of leaves (leaf strength or elasticity) and resist cold-induced damage at high altitude (Lütz 2010; Zhang *et al.* 2012).

In this study, we also observed a significant latitudinal gradient for most leaf element concentrations and stoichiometry parameters, except the N:P ratio (Fig. 2). The significant latitudinal trends of leaf stoichiometry in Ericaceous plants are in agreement with findings reported by both global scale (Kerkhoff *et al.* 2005; Reich and Oleksyn 2004) and Chinese scale studies (Han *et al.* 2005). The insignificant correlation between N:P ratio and latitude is not alone in our study. For example, Han *et al.* (2005) reported that foliar N:P ratio of terrestrial species in China was weakly correlated with latitude. There are two possible explanations for the trends observed in this study: First, the latitudinal span of sites (23.66 to 37.40°N) is small and mostly limited to subtropical regions. Second, the altitudinal gradient in China is so dominant that it overrides the possible underlying latitudinal effect (Yang *et al.* 2015b). For instance, the Tibetan Plateau, which is located at relatively low latitude but has lower temperatures than some higher latitude locations, has changed the latitudinal gradient of climate.

### Climate effects on leaf stoichiometry of Ericaceae

Macroclimate variables, such as MAT and MAP, are likely direct drivers of the variation in leaf stoichiometry along geographic gradients. Our results showed that MAT and MAP had significant effects on leaf element concentrations and stoichiometry in Ericaceae (Fig. 3). Leaf N and P concentrations were negatively related with MAT. This result supports the Temperature-Plant Physiological Hypotheses (Woods *et al.* 2003), which argued that plants at lower temperatures tend to contain greater leaf N and P in order to offset repressed biochemical reaction rates (Körner 1989; Woods *et al.* 2003). However, the negative relationships between MAP and leaf N and P concentrations may be due to different reasons. A higher leaf N concentration has been described as an



adaptation to low rainfall (Wright *et al.* 2005b). High leaf N concentration in plants from arid zones is linked to greater internal CO<sub>2</sub> drawdown during photosynthesis, allowing plants under low-rainfall to achieve higher photosynthetic rates at a given stomatal conductance (Wright *et al.* 2002). This would lead to more efficient use of water in carbon fixation processes (Wright *et al.* 2001, 2002). Moreover, plants can maintain water balance and resist environmental stress by accumulating nitrogen-containing compounds (e.g. amino acids, imino acids, and polyamines; Mansour 2000). As soil P is mainly derived from rock weathering and easily influenced by shifts in climate (Chen *et al.* 2013; Lambers *et al.* 2008), the negative relationship between leaf P concentration and MAP is partly due to the leaching of soil P under high precipitation (Chen *et al.* 2013; Ordoñez *et al.* 2009). In this study, soil P concentration was lower in higher-rainfall habitats (online supplementary Fig. S1) is strong evidence in support of this conclusion.

In contrast with several previous studies (Fyllas *et al.* 2009; Yang *et al.* 2015b), we found leaf C content of Ericaceous plants was negatively correlated with MAT and MAP (Fig. 3). Global and regional studies have shown that evergreen plants tend to extend leaf lifespan in colder environments (Wright *et al.* 2005a; Zhang *et al.* 2010). The extension of leaf lifespan, according to the cost-benefit theory, requires more carbon investment in leaves (Kikuzawa 1991; Mediavilla *et al.* 2008), and therefore is correlated with higher leaf C content (van Ommen Kloeke *et al.* 2012; Zhang *et al.* 2012). Plant species inhabiting arid and semiarid regions tend to possess high leaf mass per area (LMA; Maximov 1929; Wright *et al.* 2005b). Leaf traits associated with high LMA (e.g. thick leaf blades; small and thick-walled cells) have been interpreted as adaptations to dry conditions (Wright *et al.* 2005b). Increases in leaf C content are consistent with these arid habitat-adaptive leaf traits, pointing to an increased investment of carbon in constitutive compounds, for example the lignin and phenolics (Bertiller *et al.* 2006).

The numerous researches have examined the changes in leaf stoichiometry across climatic gradients, and diverse patterns have been observed. For example, Yang *et al.* (2015b) detected only weak effects of climate on leaf N and P stoichiometry across northern China, which is in contrast with the more typically observed patterns that foliar N and P were negatively correlated with MAT (Han *et al.* 2005; Kerkhoff *et al.* 2006; Reich and Oleksyn 2004). Across 702 wild plant species in China, however, it was shown that the leaf N and P increased with increasing MAT (Zhang *et al.* 2012). These inconsistent patterns suggest that the influences of climate factors on foliar element stoichiometry can vary greatly among climatic regions and may be offset by plant growth forms, species compositions, or soil nutrients. Furthermore, although we did detect significant relationships between foliar elements and climate in this study, we found that the direct effects of climate on broad-scale patterns of variation in leaf traits was quite modest (Table 2), which is consistent with

some previous reports (He *et al.* 2010; Ordoñez *et al.* 2009; Townsend *et al.* 2007). Therefore, we suggest that climate affects foliar traits mainly through indirect ways.

### Soil effects on leaf stoichiometry of Ericaceae

Considering many previous studies obtained soil information from the soil database rather than the field measurement (e.g. Yang *et al.* 2015b), we predicted that the soil properties measured in the field in our research would more strongly related to plant nutrients. Just as we predicted, the links between foliar stoichiometry and soil properties were very strong for Ericaceous species. Terrestrial plants take up most of their mineral elements from soil, therefore nutrient levels within soil should be major determinants of element concentrations in plants, and increased nutrient availability in soil will promote plant growth and affect the leaf elemental patterns (Chapin *et al.* 2011; Foulds 1993; Lynch and Clair 2004). Further, different from plants of other families mainly absorb inorganic nutrients in soil, Ericaceous species are largely dependent on ERM to utilize organic nutrients in soil (Bending and Read 1996, 1997; Cairney and Meharg 2003). Therefore, the links between foliar and soil total C, N, P were more strong for Ericaceous species.

The effects of soil N on leaf P and soil P on leaf N seem determined by the tight coupling of leaf N and P in metabolic processes (Güsewell 2004; Niklas *et al.* 2005). A large part of the soil C comes from the decomposition of litters from plants (Janzen 2004), thus it closely related to foliar nutrients. In addition, higher N and P contents can promote synthesis of enzymes, which play critical roles in carbon assimilation and primary production (Ågren 2008; Hessen *et al.* 2004; LeBauer and Treseder 2008). Other than the chemical properties, plant element concentrations are also largely affected by the physical characteristics of soil (Castle and Neff 2009). Soil bulk density (BD) is an indicator of soil porosity versus soil compaction (Brady and Weil 2001), which reflects the soil capacity for structural support, water and solute movement and aeration. Therefore, soil BD has direct effects on plant root growth and nutrient absorption. Our results showed that leaf C, N and P concentrations were negatively correlated with soil BD (Fig. 4). Through further analysis we found a significantly negative relationship between soil BD and soil nutrient contents (online supplementary Fig. S2). Generally, soil with relatively low BD is rich in organic matter and has high soil porosity, which is conducive to root growth and nutrient and water uptake. Plants growing in these soils tend to have high leaf element concentrations.

Through GLM analysis, we found that soil properties had stronger influences on leaf P than on leaf N (Table 2). This result may be due to the sources of soil N and P are different: soil N primarily comes from the atmosphere through multiple N-fixation mechanisms, whereas soil P is largely acquired from rock weathering and its diffusivity in soil is lower than N (Aerts and Chapin 1999; Lambers *et al.* 2008). Thus, soil P is easily influenced by environmental shifts, and consequently

influences leaf P concentration. In addition, the GLM analysis showed that independent soil effects were greater than pure climate effects for the variation in leaf traits, suggested that soil has greater direct effects on leaf stoichiometry than climate does in Ericaceae. However, it should be noted that climate factors can indirectly affect leaf elements by altering soil biogeographical processes, which will influence soil nutrient availability in a great extent (Ordoñez *et al.* 2009; Reich 2005).

### Variation in leaf stoichiometry across Ericaceous species

Phylogenetic signals of plant foliar traits have been detected in both geographically broad surveys and field experiments (e.g. Castle and Neff 2009; Kerkhoff *et al.* 2006; McGroddy *et al.* 2004). There are several possible reasons for differences of leaf traits among species (when environmental effects are controlled): (i) phylogenetic differences (He *et al.* 2010); (ii) effects of growth form, e.g. leaf N and P levels significantly differ between herbs and woody plants (Han *et al.* 2005; Reich and Oleksyn 2004); and (iii) architecture-related effects, e.g. differences in plant size and structure (Elser *et al.* 2010; Kerkhoff *et al.* 2006; Schmid and Bazzaz 1994). Furthermore, the degree of the phylogenetic signal within a clade is related to the phylogenetic scale (Cavender-Bares *et al.* 2006; Hao *et al.* 2015). However, most studies have been conducted across large phylogenetic distances, which cannot eliminate the effects of growth form and plant size. By comparing closely related species, of the same growth form and similar size, we were able to test if the phylogenetic signal existed at the tips of the phylogeny. Moreover, we minimized the bias due to sampling technique by using the uniform sampling protocol for the entire study. Finally, we collected plants at the same phenological stage in order to control for seasonal variation in foliar nutrient attributes (Milla *et al.* 2007).

Our results confirmed the importance of species, even at a low taxonomic level, in determining the variation in leaf traits of shrub. Since our analysis excluded the architecture- and growth form-related effects on leaf characteristics, the species influences detected in this study were likely linked to different strategies among species to adapt to diverse environmental conditions. For example, species, such as *Rhododendron nivale*, *Rhododendron telmateium*, *Rhododendron capitatum* and *Rhododendron thymifolium*, which are adapted to alpine environments generally have high leaf C, N and P concentrations. Furthermore, element acquisition pathways can differ among species. Plants have evolved diverse physiological traits, such as mycorrhizae, cluster roots and snow roots, to enhance nutrient absorption (Lambers *et al.* 2008; Onipchenko *et al.* 2009). Symbiotic relationships with mycorrhizae are common in Ericaceous plants, where the symbionts are called ERM (Leake and Read 1991). ERM can absorb complex organic nitrogen and phosphorus and transfer them to their host plants (Bending and Read 1996, 1997; Burke and Cairney 1997). Variation in symbiotic mycorrhizal fungi and habitat can significantly influence the ability of Ericaceous plants to

absorb nutrients and the nutrient types they take up (Cairney *et al.* 2000; Chen *et al.* 1999; Leake and Miles 1996). Currently, compared with the large number of Ericaceae species, few of them have been examined for symbiotic relationships with mycorrhizae. Further research on the obligate parasitism of ERM is necessary in order to clarify the influences of ERM on nutrient absorption in Ericaceous plants.

### Independent and interactive effects on leaf stoichiometry

Climate, soil traits and species all influence plant element compositions in complex ways (Hao *et al.* 2015; Hou 1982). GLM showed that climate, soil and species together accounted for a substantial fraction of the biogeographic variation in leaf elements. However, significant collinearity between these factors could potentially obscure their true influences. For a better understanding of these drivers, we partitioned the variance explained by multiple factors into independent effects of individual factors and their interactive effects. The results showed independent effects of species on leaf stoichiometry were stronger than those of climate and soil, suggesting that the geography of leaf elements was largely controlled by plant species, and supporting the species composition hypothesis (Reich and Oleksyn 2004).

The interactive effects of climate and species accounted for 8.8% of the average variation in leaf stoichiometry, and those of climate, soil and species accounted for another 23.3%. However, the independent effect of climate accounted for only 1.1% of the variation (Table 2). These results indicate that, rather than directly changes leaf traits, climate affects plants mainly through changing biogeographical processes and plant species compositions (Ordoñez *et al.* 2009; Reich 2005; Wright *et al.* 2001, 2005b). For example, climate factors can affect soil temperature and water status, which will influence N uptake and utilization by plants (Dong *et al.* 2001). Meanwhile, MAT and MAP can influence species distributions (Ordoñez *et al.* 2009), which in turn affect leaf element concentrations since root nutrient uptake capacities differ among species (Broadley *et al.* 2001). Therefore, global climate change will strongly influence foliar stoichiometry, and subsequently ecosystem nutrient cycles. The GLM analysis performed in this study also showed a considerable proportion of unexplained variation (an average of 36.9%) in leaf stoichiometry, which may be stem from various sources, such as disturbance levels, competition intensity, or unquantified micro-environmental conditions. This unexplained variance provides interesting starting points for future research. The synthesis of all these factors influencing leaf elements will help us further understand the broad-scale patterns in plant traits and predict the changes of nutrient cycling in ecosystem under global climate change.

### SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

## FUNDING

This work was supported by the ‘Strategic Priority Research Program - Climate Change: Carbon Budget and Related Issues’ of the Chinese Academy of Sciences (#XDA05050300).

## ACKNOWLEDGEMENTS

We are grateful to Yahan Chen, Xuejun Yang, Jieli Ge and Yang Wang for helpful suggestions on this manuscript. We would also like to thank Christine Verhille at the University of British Columbia for her assistance with English language and grammatical editing of the manuscript

*Conflict of interest statement.* None declared.

## REFERENCES

- Aerts R, Chapin FS III (1999) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Adv Ecol Res* **30**:1–67.
- Ågren GI (2008) Stoichiometry and nutrition of plant growth in natural communities. *Annu Rev Ecol Evol S* **39**:153–70.
- Amatangelo KL, Vitousek PM (2008) Stoichiometry of ferns in Hawaii: implications for nutrient cycling. *Oecologia* **157**:619–27.
- Bending GD, Read DJ (1996) Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi. *Soil Biol Biochem* **28**:1603–12.
- Bending GD, Read DJ (1997) Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. *Mycol Res* **101**:1348–54.
- Bertiller MB, Mazzarino MJ, Carrera AL, et al. (2006) Leaf strategies and soil N across a regional humidity gradient in Patagonia. *Oecologia* **148**:612–24.
- Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological variation. *Ecology* **73**:1045–55.
- Brady NC, Weil RR (2001) *The Nature and Properties of Soil*, 13th edn. Upper Saddle River, NJ: Prentice Hall.
- Broadley MR, Bowen HC, Cotterill HL, et al. (2003) Variation in the shoot calcium content of angiosperms. *J Exp Bot* **54**:1431–46.
- Broadley MR, Bowen HC, Cotterill HL, et al. (2004) Phylogenetic variation in the shoot mineral concentration of angiosperms. *J Exp Bot* **55**:321–36.
- Broadley MR, Willey NJ, Wilkins JC, et al. (2001) Phylogenetic variation in heavy metal accumulation in angiosperms. *New Phytol* **152**:9–27.
- Burke R, Cairney J (1997) Carbohydrase production by the ericoid mycorrhizal fungus *Hymenoscyphus ericae* under solid-state fermentation conditions. *Mycol Res* **101**:1135–9.
- Cairney J, Meharg A (2003) Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. *Eur J Soil Sci* **54**:735–40.
- Cairney J, Sawyer N, Sharples J, et al. (2000) Intraspecific variation in nitrogen source utilisation by isolates of the ericoid mycorrhizal fungus *Hymenoscyphus ericae* (Read) Korf and Kernan. *Soil Biol Biochem* **32**:1319–22.
- Castle S, Neff J (2009) Plant response to nutrient availability across variable bedrock geologies. *Ecosystems* **12**:101–13.
- Cavender-Bares J, Keen A, Miles B (2006) Phylogenetic structure of Floridian plant communities depends on taxonomic and spatial scale. *Ecology* **87**:109–22.
- Chapin FS III, Matson PA, Vitousek P (2011) *Principles of Terrestrial Ecosystem Ecology*. New York, NY: Springer.
- Chen A, Chambers S, Cairney J (1999) Utilisation of organic nitrogen and phosphorus sources by mycorrhizal endophytes of *Woollsia pungens* (Cav.) F. Muell. (Epacridaceae). *Mycorrhiza* **8**:181–7.
- Chen Y, Han W, Tang L, et al. (2013) Leaf nitrogen and phosphorus concentrations of woody plants differ in responses to climate, soil and plant growth form. *Ecography* **36**:178–84.
- Dong S, Scagel CF, Cheng L, et al. (2001) Soil temperature and plant growth stage influence nitrogen uptake and amino acid concentration of apple during early spring growth. *Tree Physiol* **21**:541–7.
- Elser J, Acharya K, Kyle M, et al. (2003) Growth rate–stoichiometry couplings in diverse biota. *Ecol Lett* **6**:936–43.
- Elser JJ, Fagan WF, Denno RF, et al. (2000) Nutritional constraints in terrestrial and freshwater food webs. *Nature* **408**:578–80.
- Elser J, Fagan W, Kerkhoff A, et al. (2010) Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytol* **186**:593–608.
- Feng GM (1988) *Rhododendron of China*, Vol. I. Beijing: Science Press, 2–9.
- Foulds W (1993) Nutrient concentrations of foliage and soil in South-western Australia. *New Phytol* **125**:529–46.
- Fyllas NM, Patino S, Baker T, et al. (2009) Basin-wide variations in foliar properties of Amazonian forest: phylogeny, soils and climate. *Biogeosciences* **6**:2677–708.
- Güsewell S (2004) N:P ratios in terrestrial plants: variation and functional significance. *New Phytol* **164**:243–66.
- Han W, Fang J, Guo D, et al. (2005) Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. *New Phytol* **168**:377–85.
- Han W, Fang J, Reich PB, et al. (2011) Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. *Ecol Lett* **14**:788–96.
- Hao Z, Kuang Y, Kang M (2015) Untangling the influence of phylogeny, soil and climate on leaf element concentrations in a biodiversity hotspot. *Funct Ecol* **29**:165–76.
- He JS, Fang J, Wang Z, et al. (2006) Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grassland biomes of China. *Oecologia* **149**:115–22.
- He JS, Wang X, Schmid B, et al. (2010) Taxonomic identity, phylogeny, climate and soil fertility as drivers of leaf traits across Chinese grassland biomes. *J Plant Res* **123**:551–61.
- Heikkinen RK, Luoto M, Kuussaari M, et al. (2005) New insights into butterfly–environment relationships using partitioning methods. *P Roy Soc B-Bio Sci* **272**:2203–10.
- Hessen DO, Ågren GI, Anderson TR, et al. (2004) Carbon sequestration in ecosystems: the role of stoichiometry. *Ecology* **85**:1179–92.
- Hou H (1982) *The Vegetation Geography of China with Reference to the Chemical Composition of Dominant Plants*. Beijing: Science Press.
- Janzen HH (2004) Carbon cycling in earth systems—a soil science perspective. *Agr Ecosyst Environ* **104**:399–417.
- Jones JB (2001) *Laboratory Guide for Conducting Soil Tests and Plant Analysis*. New York: CRC Press.
- Kang H, Zhuang H, Wu L, et al. (2011) Variation in leaf nitrogen and phosphorus stoichiometry in *Picea abies* across Europe: an analysis based on local observations. *Forest Ecol Manag* **261**:195–202.

- Kerkhoff AJ, Enquist BJ, Elser JJ, *et al.* (2005) Plant allometry, stoichiometry and the temperature-dependence of primary productivity. *Global Ecol Biogeogr* **14**:585–98.
- Kerkhoff AJ, Fagan WF, Elser JJ, *et al.* (2006) Phylogenetic and growth form variation in the scaling of nitrogen and phosphorus in the seed plants. *Am Nat* **168**:E103–22.
- Kikuzawa K (1991) A cost-benefit analysis of leaf habit and leaf longevity of trees and their geographical pattern. *Am Nat* **138**:1250–63.
- Körner C (1989) The nutritional status of plants from high altitudes. *Oecologia* **81**:379–91.
- Lambers H, Chapin FS III, Pons T (2008) *Plant Physiological Ecology*, 2nd edn. New York: Springer-Verlag.
- Leake J, Miles W (1996) Phosphodiesterases as phosphorus sources for mycorrhizal fungi and their host plants. 1. Phosphodiesterase production and the utilization of DNA as a phosphorus source by the ericoid mycorrhizal fungus *Hymenoscyphus Ericae* (Read) Korf & Kerman. *New Phytol* **132**:435–44.
- Leake J, Read D (1991) 20 experiments with ericoid mycorrhiza. *Method Microbiol* **23**:435–59.
- LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**:371–9.
- Lütz C (2010) Cell physiology of plants growing in cold environments. *Protoplasma* **244**:53–73.
- Lynch JP, Clair SBS (2004) Mineral stress: the missing link in understanding how global climate change will affect plants in real world soils. *Field Crop Res* **90**:101–15.
- Mansour M (2000) Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol Plantarum* **43**:491–500.
- Maximov NA, Yapp RH (1929) *The Plant in Relation to Water: A Study of the Physiological Basis of Drought Resistance*. Australia: Allen & Unwin.
- McGroddy ME, Daufresne T, Hedin LO (2004) Scaling of C:N:P stoichiometry in forests worldwide: implications of terrestrial redfield-type ratios. *Ecology* **85**:2390–401.
- Mediavilla S, Garcia-Ciudad A, Garcia-Criado B, *et al.* (2008) Testing the correlations between leaf life span and leaf structural reinforcement in 13 species of European Mediterranean woody plants. *Funct Ecol* **22**:787–93.
- Mengel K, Kirkby E (2001) *Principles of Plant Nutrition*, 5th edn. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Milla R, Palacio S, Maestro-Martinez M, *et al.* (2007) Leaf exchange in a Mediterranean shrub: water, nutrient, non-structural carbohydrate and osmolyte dynamics. *Tree Physiol* **27**:951–60.
- Ministry of Environmental Protection of the People's Republic of China, Chinese Academy of Sciences (2015). *Regionalization of the National Ecological Function in China* (Revised Edition). [http://www.zhb.gov.cn/gkml/hbb/bgg/201511/t20151126\\_317777.htm](http://www.zhb.gov.cn/gkml/hbb/bgg/201511/t20151126_317777.htm)
- Mueller KE, Diefendorf AF, Freeman KH, *et al.* (2010) Appraising the roles of nutrient availability, global change, and functional traits during the angiosperm rise to dominance. *Ecol Lett* **13**:E1–E6.
- Niklas KJ, Owens T, Reich PB, *et al.* (2005) Nitrogen/phosphorus leaf stoichiometry and the scaling of plant growth. *Ecol Lett* **8**:636–42.
- van Ommen Kloeke A, Douma J, Ordonez J, *et al.* (2012) Global quantification of contrasting leaf life span strategies for deciduous and evergreen species in response to environmental conditions. *Global Ecol Biogeogr* **21**:224–35.
- Onipchenko VG, Makarov MI, van Logtestijn RS, *et al.* (2009) New nitrogen uptake strategy: specialized snow roots. *Ecol Lett* **12**:758–64.
- Ordoñez JC, van Bodegom PM, Witte JPM, *et al.* (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecol Biogeogr* **18**:137–49.
- R Development Core Team (2014) *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. <http://www.R-project.org>
- Reich PB (2005) Global biogeography of plant chemistry: filling in the blanks. *New Phytol* **168**:263–6.
- Reich PB, Oleksyn J (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. *Proc Natl Acad Sci USA* **101**:11001–6.
- Schmid B, Bazzaz F (1994) Crown construction, leaf dynamics, and carbon gain in two perennials with contrasting architecture. *Ecol Monogr* **64**:177–203.
- Sterner RW, Elser JJ (2002) *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton: Princeton University Press.
- Thompson K, Parkinson JA, Band SR, *et al.* (1997) A comparative study of leaf nutrient concentrations in a regional herbaceous flora. *New Phytol* **136**:679–89.
- Townsend AR, Cleveland CC, Asner GP, *et al.* (2007) Controls over foliar N:P ratios in tropical rain forests. *Ecology* **88**:107–18.
- Watanabe T, Broadley MR, Jansen S, *et al.* (2007) Evolutionary control of leaf element composition in plants. *New Phytol* **174**:516–23.
- Woods H, Makino W, Cotner J, *et al.* (2003) Temperature and the chemical composition of poikilothermic organisms. *Funct Ecol* **17**:237–45.
- Wright IJ, Reich PB, Cornelissen JH, *et al.* (2005a) Assessing the generality of global leaf trait relationships. *New Phytol* **166**:485–96.
- Wright IJ, Reich PB, Cornelissen JH, *et al.* (2005b) Modulation of leaf economic traits and trait relationships by climate. *Global Ecol Biogeogr* **14**:411–21.
- Wright IJ, Reich P, Westoby M (2001) Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Funct Ecol* **15**:423–34.
- Wright IJ, Reich PB, Westoby M (2002) Least-cost input mixtures of water and nitrogen for photosynthesis. *Am Nat* **161**:98–111.
- Wright IJ, Reich PB, Westoby M, *et al.* (2004) The worldwide leaf economics spectrum. *Nature* **428**:821–7.
- Yang X, Chi X, Ji C, *et al.* (2015a) Variations of leaf N, P concentrations in shrubland biomes across northern China: phylogeny, climate and soil. *Biogeosciences* **12**:18973–98.
- Yang HB, Fang RZ, Jin CL (1999) *Flora of China*, Vol. **57**, 1. Beijing: Science Press, 13–213.
- Yang X, Huang Z, Zhang K, *et al.* (2015b) C:N:P stoichiometry of *Artemisia* species and close relatives across northern China: unravelling effects of climate, soil and taxonomy. *J Ecol* **103**:1020–31.
- Zhang L, Luo T, Zhu H, *et al.* (2010) Leaf life span as a simple predictor of evergreen forest zonation in China. *J biogeogr* **37**:27–36.
- Zhang SB, Zhang JL, Slik J, *et al.* (2012) Leaf element concentrations of terrestrial plants across China are influenced by taxonomy and the environment. *Global Ecol Biogeogr* **21**:809–18.
- Zheng S, Shangguan Z (2007) Spatial patterns of leaf nutrient traits of the plants in the Loess Plateau of China. *Trees* **21**:357–70.