Changes in the stoichiometry of *Castanopsis fargesii* along an elevation gradient in a Chinese subtropical forest

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ABSTRACT

Elevation is important for determining the nutrient biogeochemical cycle in forest ecosystems. Changes in the ecological stoichiometry of nutrients along an elevation gradient can be used to predict how an element cycle responds in the midst of global climate change. We investigated changes in concentrations of and relationships between nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) in the leaves and roots of the dominant tree species, *Castanopsis fargesii,* along an elevation gradient (from 500 to 1,000 m above mean sea level) in a subtropical natural forest in China. We analyzed correlations between *C. fargesii*'s above-ground biomass and stoichiometry with environmental factors. We also analyzed the soil and plant stoichiometry of this *C. fargesii* population. Our results showed that leaf N decreased while leaf K and Ca increased at higher elevations. Meanwhile, leaf P showed no relationship with elevation. The leaf N:P indicated that *C. fargesii* was limited by N. Elevation gradients contributed 46.40% of the total variance of ecological stoichiometry when assessing environmental factors. Our research may provide a theoretical basis for the biogeochemical cycle along with better forest management and fertilization for this *C. fargesii* population.

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INTRODUCTION

The global forest ecosystem is affected by increasing global temperatures caused by excess greenhouse gases from anthropogenic activities (*Wang et al., 2016*). Global warming has affected nutrient element cycling in forest ecosystems (*Penuelas & Matamala, 1990*; *Penuelas & Matamala, 1993*; *Penuelas et al., 2020*; *Sardans et al., 2015*) and its impact on the biogeochemical cycle is difficult to ignore. Global warming may alter the distribution of nutrient elements by impacting plant metabolism, thus affecting nutrient transfer in plant organs (*Gavito et al., 2005*; *Jónsdóttir, Khitun & Stenström, 2005*; *Yan, Zhu & Yang,*

2017). Two the large-scale effects of global warming on element cycling have been studied to date: latitudinal effects over large areas (*He et al., 2006*; *Han et al., 2011*; *Ordoñez et al., 2009*; *Zhang et al., 2012*; *Fang et al., 2019*) and altitude effects. These are crucial factors for determining how variations in temperature and relative climatic changes drive ecological processes (*Körner, 2007*; *Normand et al., 2009*).

Recently, studies on plant stoichiometry changes along elevation gradients have not shown uniform patterns of change. Changes in plant N has shown an increase (*Richardson, Berlyn & Gregoire, 2001*; *Shi, Körner & Hoch, 2006*), decrease (*Li & Sun, 2016*; *Soethe, Lehmann & Engels, 2008*; *Van De Weg et al., 2009*), or no linear correlation (*Macek et al., 2012*) with increasing elevation. Most studies have shown that plant P decreased with increasing elevation (*Soethe, Lehmann & Engels, 2008*; *Tanner, Vitousek & Cuevas, 1998*; *Vitousek et al., 1992*; *Wang et al., 2018*). Elevational gradients may lead to significant variations in regional microclimate and soil properties (*Zeng et al., 2018*; *Chang et al., 2016*) , which further affects the nutrient cycling of a plant-soil system in forest ecosystems. This suggests that there is closely-coupled element cycling between C, N, and P in the plant-soil system (*Elser et al., 2000*). *Deng et al. (2008)* demonstrated that plant P had a significant positive correlation with soil P in the subtropical forest karst area. Plant stoichiometry was also affected by environmental factors. Studying plant stoichiometry and its correlation with environmental factors may provide a theoretical understanding of plants' nutritional requirements and their mutual feedback with the environment (*Li et al., 2019*). *Reich & Oleksyn (2004)* showed that leaf N and P increases from the tropics to mid-latitudes due to temperature-related plant physiological stoichiometry and soil substrate age; these elements decreased at higher latitudes because cold temperatures affect biogeochemistry. *Ma et al. (2015)* found that root N and P were negatively correlated with annual average temperature and annual precipitation. Many studies of plant stoichiometry have focused on C, N, and P but few have considered the stoichiometry of other crucial elements, including K, Ca, and Mg.

Fagaceae is a vital subtropical plant in China that is important for maintaining and promoting the element cycling of subtropical forest ecosystems. *Zheng et al. (2017)* showed that there were significant differences between leaf and root C, N, and P in seedlings and young trees of *Castanopsis fissa* and other plant organs. *Chang et al. (2013)* showed that P was the most important element for limiting plant productivity in the subtropical forest ecosystem. *Castanopsis fargesii* is one of the main species of the subtropical forests of China; however, there is little information on its stoichiometry, which affects the regional forest ecosystem and element cycle characteristics. Previous studies have focused on the ecological characteristics of *C. fargesii*, including its community structure, photosynthetic characteristics, community biomass, and soil organic carbon (*Song et al., 2003*; *Zhao et al., 2005*; *Qian et al., 2004*; *Gong et al., 2015*; *Dai et al., 2018*). However, changes *C. fargesii* 's stoichiometry along elevation gradients have not been well-studied (*Liu et al., 2019*; *Dai et al., 2018*).

Temperatures are known to change with increasing elevation, even over short distances (*Tan & Wang, 2016*; *Paudel et al., 2019*). The elevational changes in temperature and soil nutrients may have an effect on plant stoichiometry (*Normand et al., 2009*; *Sundqvist,*
Sanders & Wardle, 2013; *Yu et al., 2013*). Thus, we conducted experiments with five elevation gradients as a proxy for the effects of global warming in a subtropical natural forest on Guoyan Mountain in southern China. We sought to investigate the plant (specifically in its leaves and roots) and soil stoichiometry (N, P, K, Ca, and Mg) in *C. fargesii* populations along elevation gradients and determine the relative importance of elevation to the total variation of ecological stoichiometry.

MATERIAL AND METHODS

Study area description
The study was conducted in the Guoyan Mountain Natural Reserve on Wuyi Mountain (17°29′ ~118°14′E, 26°38′ ~27°12′N) in the northwestern Fujian Province. Its peak elevation was 1,383.7 m (Fig. 1). The study area had a moderate-subtropical monsoon climate with an average annual temperature of 19 °C and an average annual rainfall of 2,051 mm. *C. fargesii* was one of the dominant species along the elevation gradients in the study area, which ranged from 500 to 1,000 m.

Sampling
Elevation gradients were established from 500 to 1,000 m and covered *C. fargesii*'s natural habitat. Forests were intentionally planted below this range according to our field observations. Five 30 m × 30 m plots were established at 100 m intervals (i.e., 500–600 m, 600–700 m, 700–800 m, 800–900 m, and 900–1,000 m, respectively, which were denoted as 500 m, 600 m, 700 m, 800 m, and 900 m). Three plots were established at 500 m where fewer *C. fargesii* were found and are close to bamboo plantations. Five *C. fargesii* specimens were selected randomly in each plot and leaf samples were collected from each tree that exhibited good growth conditions. Root samples were collected from the same five trees. Soil samples were collected from around each tree at three depths (0–20 cm, 20–40 cm, and 40–60 cm) using a soil sampling auger with a diameter of five cm. All samples from each plot were mixed thoroughly and stored at 4 °C before being transported to a laboratory. The specimens' biodiversity index, height, and diameter at breast height (DBH) were also recorded (Table 1).

Laboratory analyses
Roots were deposited into 0.15 mm net bags and washed under running water. Fine roots (d < 2 mm) were separated. The leaves and roots were cleaned with distilled water and dried at 50 °C to a constant weight. All plant parts were ground prior to analysis. We removed stones and visible litter from the soil samples and then air-dried and sieved the soil through a two mm nylon mesh. We used 0.10 g of leaf and root material and 0.20 g of soil material to determine the N content using a Vario Max CN analyzer (Elementar, Germany). The leaf and soil P, K, Ca, and Mg concentrations were determined using Inductively Coupled Plasma- Mass Spectrometry (ICP-MS) (PE Optima 8000) following H_2SO_4/$HClO_4$ and HF/$HClO_4$ digestion.
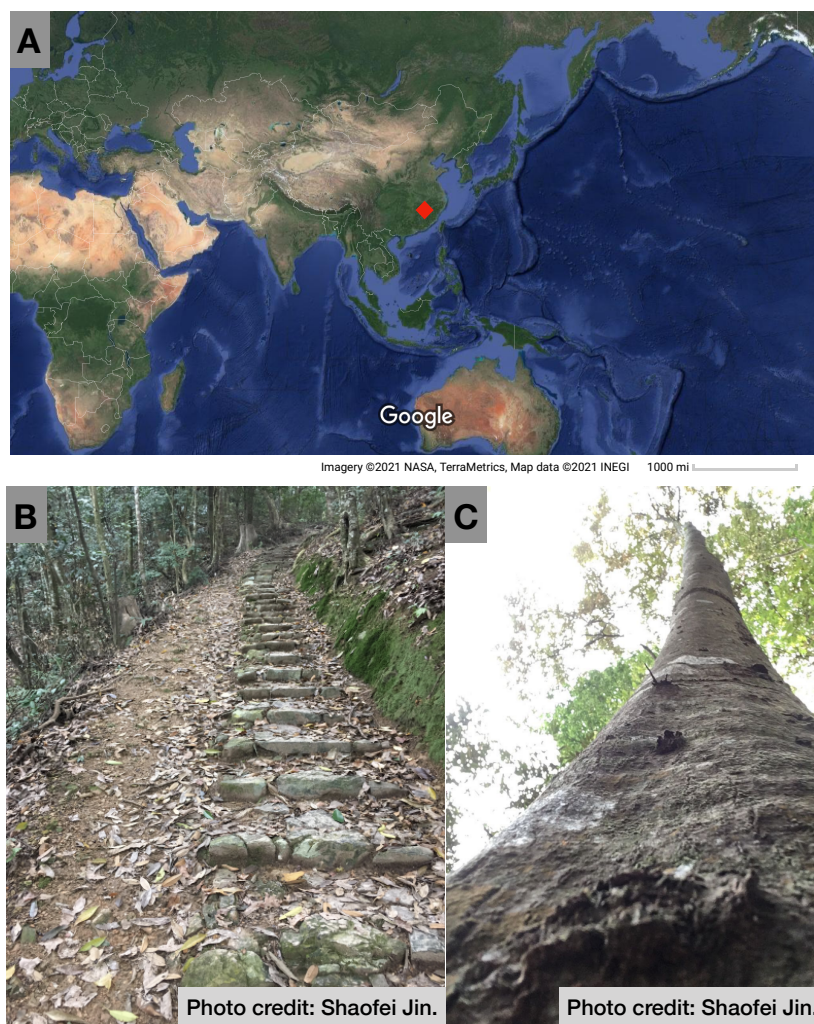
Figure 1 **(A) Location of this study, (B) Ancient road in the Guoyan Mountain Natural Reserve, and (C) Photo of *Castanopsis fargesii.*** Photo credit: Shaofei Jin. Map data ©2021 Google.

Table 1 **Importance value index, DBH, and tree height of *C. fargesii* at different elevations ($n = 15$).**

Elevation (m)	Importance value index	DBH (cm)	Tree height (m)
400	0	NA	NA
500	38.70	15.50 ± 1.15	12.33 ± 0.76
600	43.46	17.06 ± 2.19	14.40 ± 0.80
700	26.09	14.22 ± 2.36	12.30 ± 3.99
800	40.53	13.00 ± 1.99	9.80 ± 2.22
900	71.12	10.58 ± 1.04	9.80 ± 0.94
1,000	0	NA	NA

Notes.
DBH, diameter at breast height; NA, not available..

Estimating above-ground biomass of *C. fargesii*

The above-ground biomass (AGB) of *C. fargesii* in each plot was estimated according to the *2006 IPCC Guidelines for National Greenhouse Gas Inventory* (*Eggleston et al., 2006*). AGB was obtained as follows: Eqs. (1) and (2)

$$AGB = V \times n \times S \div s \times BCEFs \qquad (1)$$
$$V = g_{1.3} \times (H + 3) \times f_3 \qquad (2)$$

where V is the volume of an individual *C. fargesii*; $g_{1.3}$ is the basal area of breast-height; H is the tree height; f_3 is the experimental form factor; the value of the broad-leaved trees is 0.40 (*Meng, 2006*); S is the 1 hm^2 of the stand area; s is the stand area of each plot; *BCEFs* is the biomass conversion and expansion factor of growing-stock. Its value is 0.66 in subtropical regions (*Eggleston et al., 2006*).

Statistical analysis

Leaf, root, and soil stoichiometry variations at different elevations were compared using one-way ANOVA. Multiple comparisons were performed using Tukey-HSD post hoc tests. The correlations between the environmental factors, plant tissues, and soil stoichiometry of the *C. fargesii* community were determined using redundancy analysis (RDA). All data were checked to assess whether they met the assumptions of homogeneity and normality. All analyses and figures were determined at a significance level of $p < 0.05$ using R software. All raw data are shown in Supplemental File 1.

RESULTS

Variations in the plant stoichiometry of *C. fargesii* along the elevation gradients

The mean N, P, K, Ca, and Mg concentrations in the leaves were 14.76 ± 1.60 mg/g, 2.00 ± 0.16 mg/kg, 41.59 ± 8.71 mg/kg, 20.68 ± 7.14 mg/kg, and 8.83 ± 1.51 mg/kg, respectively. The mean N, P, K, Ca, and Mg concentrations in the roots were 12.53 ± 1.91 mg/g, 1.96 ± 0.43 mg/kg, 18.56 ± 4.29 mg/kg, 10.89 ± 4.29 mg/kg, and 5.30 ± 1.56 mg/kg, respectively. Significant differences ($P < 0.05$) in the leaf (Fig. 2) and root (Fig. 3) ecological stoichiometry were found among different elevation gradients. The linear regressions between the ecological stoichiometry and the elevation gradients are shown in Fig. S1 and Fig. S2, respectively. The greatest leaf N concentration was found at the lowest elevation. The mean leaf N:P along the elevation was 7.50 ± 0.63. A significantly positive correlation was found between leaf K and elevation. The leaf and root Ca increased significantly at higher elevations.

Correlations between AGB and plant stoichiometry

Variations in *C. fargesii*'s AGB at different elevations are shown in Fig. 4. The greatest AGB was found at 600 m. The AGB showed a significantly negative correlation with leaf Ca and no correlation with other leaf stoichiometry. The AGB also showed a significantly positive correlation with root K and root P. There was a significantly negative correlation between the AGB and root Ca (Fig. 5).
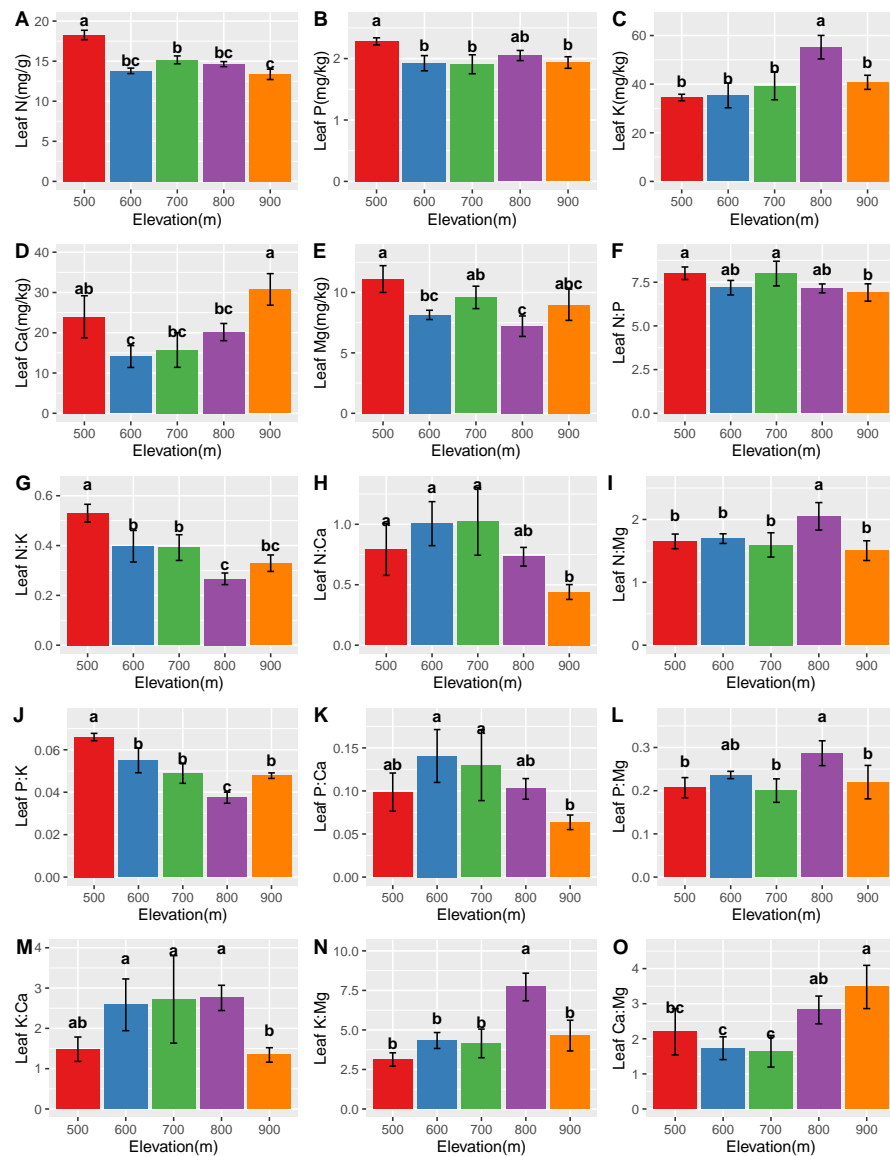
Figure 2 **Variations in the leaf N, P, K, Ca, and Mg concentrations (A–E) and the leaf N:P, N:K, N:Ca, N:Mg, P:K, P:Ca, P:Mg, K:Ca, K:Mg, and Ca:Mg ratios (F–O) at different elevations.** The error bars represent standard deviation. Different letters indicate that there are significant differences among different elevations ($p < 0.05$).

Relationships between soil stoichiometry and plant stoichiometry in the *C. fargesii* population

There were significantly positive correlations between soil N, leaf Ca, and root Ca. There were significantly negative correlations between soil N, leaf N, and leaf Mg. However, there was no correlation between soil P and leaf and root nutrients. Soil K was found to be positively correlated with root P and negatively correlated with leaf Ca, leaf Mg, and root Ca. Soil Ca was found positively correlated with leaf N and leaf P. There was a significantly
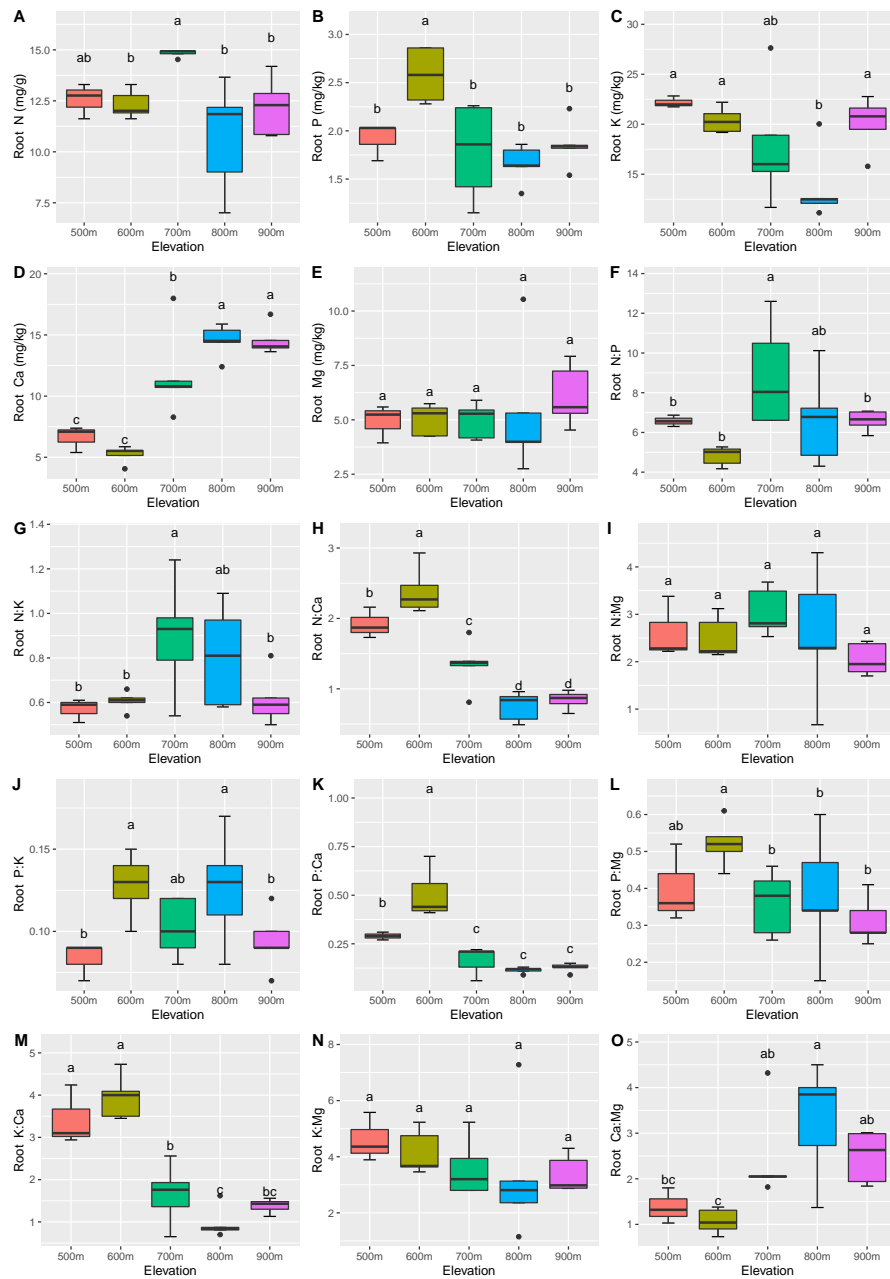
Figure 3 **Variations in the root N, P, K, Ca, and Mg concentrations (A–E) and the root N:P, N:K, N:Ca, N:Mg, P:K, P:Ca, P:Mg, K:Ca, K:Mg, and Ca:Mg ratios (F–O) at different elevations.** The error bars represent standard deviation. Different letters indicate that there are significant differences among different elevations ($p < 0.05$).

positive correlation between soil Mg and leaf K, and a negative correlation between soil and leaf Mg, root N, and root K (Table 2). The changes in the soil stoichiometry of the *C. fargesii* community are shown in Fig. 6.
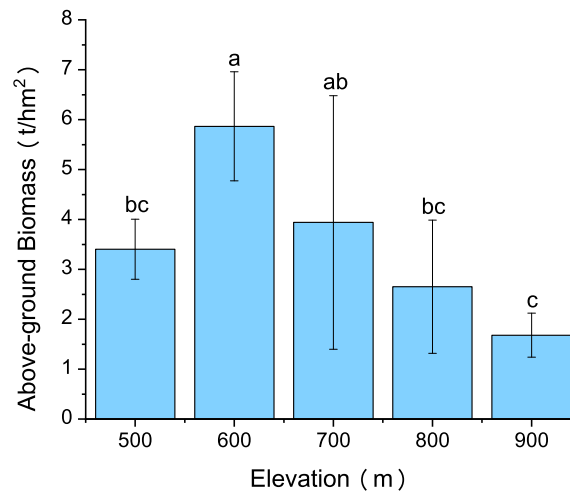
Figure 4 **Changes in the above-ground biomass of *C. fargesii* along the elevation gradients.** The error bars represent the standard deviation. Different letters indicate that there are significant differences among different elevations ($p < 0.05$).
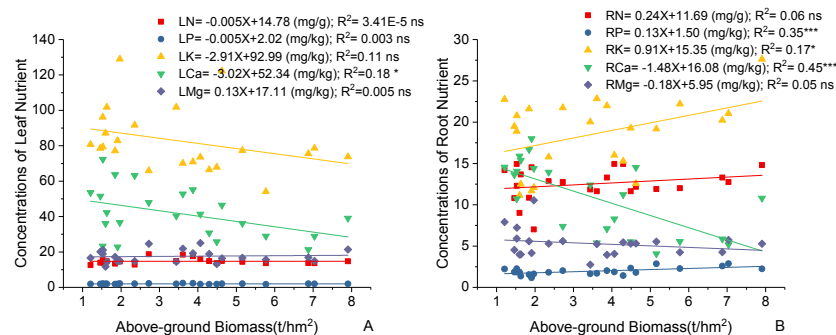
Figure 5 **Linear regression between (A) leaf nutrients, (B) root nutrients (N, P, K, Ca, Mg) and above-ground biomass of the *C. fargesii*.** LN, leaf N concentrations; LP, leaf P concentrations; LK, leaf K concentrations; LCa, leaf Ca concentrations; LMg, leaf Mg concentrations; RN, root N concentrations; RP, root P concentrations; RK, root K concentrations; RCa, root Ca concentrations; RMg, root Mg concentrations. ns, not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Relationships between environmental factors and leaf and root nutrients in the *C. fargesii* population

RDA analysis identified two main axes of environmental variation that together accounted for 58.73% of the total variance of ecological stoichiometry (Fig. 7, Tables 3 and 4). The first RDA axis contributed 38.99% of total variance which was explained by soil pH and elevation. The second RDA axis contributed 19.74% of total variance and was mainly explained by soil moisture and soil temperature. We show the ranking of environmental variables in the order of contribution in Table 3. Elevation, soil moisture, soil temperature, and soil pH contributed 46.4%, 33.8%, 16%, and 3.9%, respectively.

Table 2 Correlation analysis between soil stoichiometry and plant stoichiometry of *C. fargesii*.

Soil stoichiometry	Leaf stoichiometry					Root stoichiometry				
	Leaf N	Leaf P	Leaf K	Leaf Ca	Leaf Mg	Root N	Root P	Root K	Root Ca	Root Mg
Soil N	−0.46***	−0.16	0.17	0.44***	−0.21*	−0.14	−0.056	0.028	0.4***	0.012
Soil P	0.059	0.11	−0.048	0.2	0.026	−0.085	0.14	0.081	0.064	−0.0035
Soil K	−0.29*	−0.051	−0.011	−0.34**	−0.35**	−0.22*	0.66***	0.059	−0.48***	−0.00094
Soil Ca	0.36**	0.37**	−0.036	0.065	0.03	−0.25*	0.15	−0.024	−0.24*	−0.12
Soil Mg	−0.068	0.2	0.62***	−0.037	−0.52***	−0.35**	−0.0079	−0.49***	0.21*	−0.091

Notes.

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$

No asterisk denotes no significant correlation coefficient was found.

DISCUSSION

Plant stoichiometry responses to elevation

We determined that the greatest leaf N concentration of *C. fargesii* was found at the lowest elevation (Fig. 2). This result was consistent with previous studies (*Li & Sun, 2016*; *Soethe, Lehmann & Engels, 2008*; *Van De Weg et al., 2009*). Lower temperatures were found at higher elevations, which decreased the decomposition and mineralization of organic matter (*Hobbie et al., 2000*; *Hobbie, Nadelhoffer & Högberg, 2002*; *He et al., 2016*) and thus decreased the availability of leaf N (*Reich & Oleksyn, 2004*). Leaf N is typically determined by plant growth characteristics, whereas leaf P is determined by plant growth and environment. Therefore, leaf N is more stable and consistent than leaf P (*Chen et al., 2013*). According to *Güsewell (2004)*, leaf N:P <10 represents the N limitation, and N:P >20 represents the P limitation. *C. fargesii* was limited by N but not P, since N was lower than that in lower-elevation plants in China (*Han et al., 2011*; *Zhang et al., 2012*). There were significant differences in root N and P across elevations. Root N and P were greater than those of lower-elevation plants in China (*Ma et al., 2015*). There was a significantly positive correlation between leaf K and elevation, which was supported by the findings of *Du et al. (2017)*. This indicates that the ecology at higher elevations did not restrict K uptake by plants. Leaf Ca increased with elevation except at 500 m. Root Ca increased at higher elevations. The lower temperatures at higher elevations promoted Ca uptake by plants, which aided in their defense against the cold (*Plieth et al., 1999*). *C. fargesii* required more Ca at higher elevations and we predict that its demands at the same elevation may decrease in the future with global warming and altered forest lines.

Correlations between AGB and *C. fargesii* plant stoichiometry

C. fargesii's highest AGB (Fig. 4) and importance value (Table 1) were found at 600 m, indicating that this environment is more suitable for growth than those at other elevations. However, the AGB decreased as elevation increased and species' tendency to migrate to a higher elevation was not noticeable despite being affected by warming temperatures and the tree line moving up. Leaf and root Ca were significantly negatively correlated with
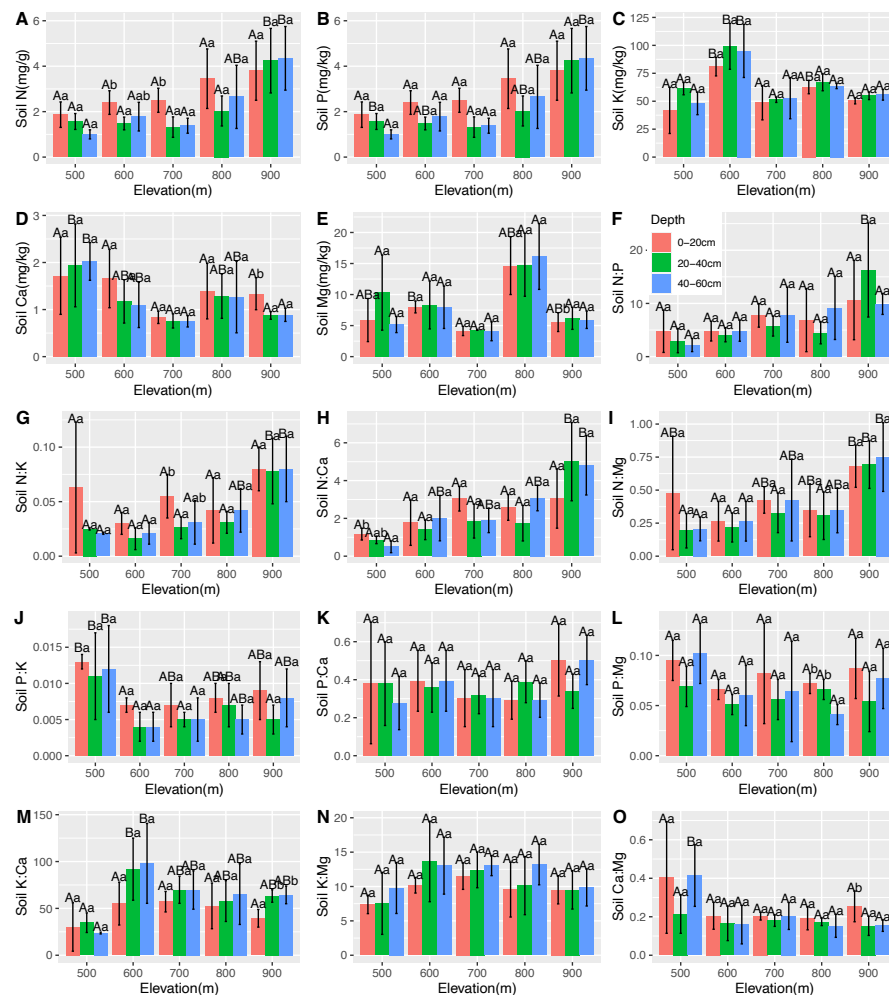
Figure 6 **Variations in the soil N, P, K, Ca, and Mg contents (A–E) and the soil N:P, N:K, N:Ca, N:Mg, P:K, P:Ca, P:Mg, K:Ca, K:Mg, and Ca:Mg ratios (F–O) at different elevations and soil depths.** The error bars represent standard deviation. Different uppercase letters within the panels indicate that there are significant differences between different elevations ($p < 0.05$), and different lowercase letters within the panels indicate that there are significant differences between different soil depths ($p < 0.05$).

C. fargesii AGB indicating that *C. fargesii* with a larger above-ground biomass had less demand for Ca. *C. fargesii* at a higher elevation may require more Ca than those at lower elevations in our research area. Root P and K were significantly positively correlated with AGB indicating that *C. fargesii* with a larger AGB had more demand for P and K. In general, P-limiting was found in the acidic soil of tropical and subtropical regions. However, we found that P-limiting did not exist in our research area and P was absorbed by plant roots as an essential nutrient in P-rich soils. Estimating the AGB of *C. fargesii* at different elevations and analyzing the relationship between AGB and the concentration of plant stoichiometry may provide a basis for *C. fargesii* management and fertilization in the future.
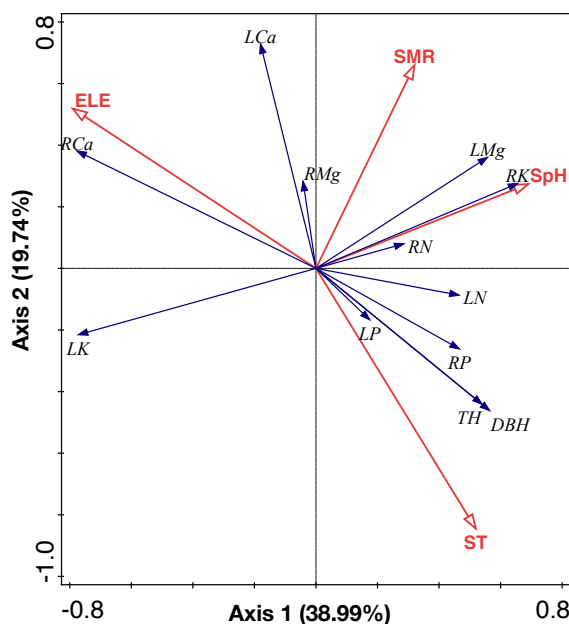
Figure 7 RDA analysis of environmental factors, leaf and root nutrient of the *C. fargesii* community in the Guoyan Mountain Natural Reserve. ST, soil temperature; ELE, elevation; SM, soil moisture; SpH, soil pH; LN, leaf N concentrations; LP, leaf P concentrations; LK, leaf K concentrations; LCa, leaf Ca concentrations; LMg, leaf Mg concentrations; RN, root N concentrations; RP, root P concentrations; RK, root K concentrations; RCa, root Ca concentrations; RMg, root Mg concentrations.

Table 3 Summary of environmental factor at different elevation gradients.

Elevation (m)	Soil temperature (° C)	Soil moisture (%)	Soil pH
500	17.13 ± 7.13	14.12 ± 4.12	5.34 ± .342
600	17.93 ± 7.93	15.98 ± 5.98	4.73 ± .738
700	16.40 ± 0.36	14.02 ± 4.02	4.67 ± .672
800	16.28 ± 6.28	13.04 ± 3.04	4.42 ± .424
900	14.70 ± 4.70	18.82 ± 8.82	4.70 ± .702

Table 4 Relative contribution of environmental variables to the variance of ecological stoichiometry of the *C. fargesii* community.

Order	Variable	Contribution (%)	F	p
1	Elevation	46.4	8.2	0.002
2	Soil moisture	33.8	8.0	0.002
3	Soil temperature	16.0	4.4	0.006
4	Soil pH	3.9	1.1	0.394

Relationships between the soil stoichiometry, environmental factors, and plant stoichiometry in the *C. fargesii* population

Soil influences plant growth, productivity, and distribution (*Condit et al., 2013*) which are relevant to the biogeochemical cycling of nutrients in terrestrial ecosystems (*Izquierdo, Houlton & van Huysen, 2013*; *Tian et al., 2010*). Previous studies have demonstrated that soil P was strongly related to leaf N and P (*Han et al., 2005*; *Hedin, 2004*). However, we found that soil P showed no relationship with leaf stoichiometry, indicating that soil P did not govern nutrient accumulation in leaves from the study area. Leaf Ca and Mg were found to be negatively correlated with soil K. Previous studies have shown that a high concentration of one cation could cause an imbalance in the concentrations of other cations in the soil (*Hailu et al., 2015*). Therefore, Ca and Mg absorption by plants was negatively affected by the excess K in the soil (*Hailu et al., 2015*; *Xue et al., 2019*). There was a negative relationship between the soil and leaf N, but a previous study showed that soil N was not correlated with leaf N across Chinese grasslands (*He et al., 2010*). We explored the relative contribution of elevation gradients and soil microenvironment (soil temperature, soil pH, and soil moisture rate) to ecological stoichiometry variation. Our results show that elevation was the most important factor impacting the stoichiometry of *C. fargesii*. However, elevation had the least impact on stoichiometry variation for *Pinus taiwanensis* within our study area, which indicates that this evergreen broad-leaf species is more sensitive than coniferous trees. These results imply that the ecological stoichiometry of *C. fargesii* will alter during global warming.

CONCLUSIONS

Leaf N, K, and Ca levels within *C. fargesii* were significantly related to elevation, while leaf P and Mg showed no relationship with elevation. The leaf N:P indicated that *C. fargesii* was limited by N in this subtropic forest ecosystem in China. Soil P showed no relationship with leaf stoichiometry. The elevation explained nearly half of the variation of ecological stoichiometry in *C. fargesii.* Our results may improve our understanding of the biogeochemical cycle for nutrients in the subtropical forests of China.

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Danping Liu and Dexiang Zheng conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Yaoyao Xu and Yifei Chen conceived and designed the experiments, performed the experiments, prepared figures and/or tables, and approved the final draft.
- Hesong Wang and Ku Wang conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Xiaoli Liao performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Changxiong Chen conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Jiangjiang Xia conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Shaofei Jin conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability

The raw data are available in the Supplementary File.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.11553#supplemental-information.

REFERENCES

Chang E, Chen T, Tian G, Chiu C. 2016. The effect of altitudinal gradient on soil microbial community activity and structure in moso bamboo plantations. *Applied Soil Ecology : A Section of Agriculture, Ecosystems & Environment* **98**:213–220 DOI 10.1016/j.apsoil.2015.10.018.

Chang Y, Zhong Q, Cheng D, Xu C, Hu B, Zhang Z. 2013. Stoichiometric characteristics of C, N, P and their distribution pattern in plants of *Castanopsis carlesii* natural forest in Youxi. *Journal of Plant Resources and Environment* **22**:1–10 (In Chinese with English abstract).

Chen Y, Han W, Tang L, Tang Z, Fang J. 2013. Leaf nitrogen and phosphorus concentrations of woody plants differ in responses to climate, soil and plant growth form. *Ecography* **36**:178–184 DOI 10.1111/j.1600-0587.2011.06833.x.

Condit R, Engelbrecht BMJ, Pino D, Perez R, Turner BL. 2013. Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. *Proceedings of the National Academy of Sciences of the United States of America* **110**:5064–5068 DOI 10.1073/pnas.1218042110.

Dai A, Liu X, Wang B, Dai W. 2018. Annual spatial and temporal variations of soil organic carbon and its components in *Castanopsis fargesii* forest. *Journal of Zhejiang A & F University* **35**:405–411 (In Chinese with English abstract).

Deng Y, Jiang Z, Lan F, Luo W, Qin X. 2008. Biogeochimical characteristics of elements in soil-plant system in tropical and subtropical typical karst area in Guangxi. *Ecology and Environment* **17**:1140–1145 (In Chinese with English abstract).

Du B, Ji H, Peng C, Liu X, Liu C. 2017. Altitudinal patterns of leaf stoichiometry and nutrient resorption in Quercus variabilis in the Baotianman Mountains, China. *Plant and Soil* **413**:193–202 DOI 10.1007/s11104-016-3093-9.

Eggleston S, Buendia L, Miwa K, Ngara T, Tanabe K. 2006. *2006 IPCC guidelines for national greenhouse gas inventory*. Hayama: Institute for Global Environmental Strategies (IGES).

Elser J, Sterner R, Gorokhova E, Fagan W, Markow T, Cotner J, Harrison J, Hobbie S, Odell G, Weider L. 2000. Biological stoichiometry from genes to ecosystems. *Ecology Letters* **3**:540–550 DOI 10.1111/j.1461-0248.2000.00185.x.

Fang Z, Li DD, Jiao F, Yao J, Du HT. 2019. The latitudinal patterns of leaf and soil C:N:P stoichiometry in the loess plateau of China. *Frontiers in Plant Science* **10**:85 DOI 10.3389/fpls.2019.00085.

Gavito ME, Olsson PA, Rouhier H, Medina-Penafiel A, Jakobsen I, Bago A, Azcon-Aguilar C. 2005. Temperature constraints on the growth and functioning of root organ cultures with arbuscular mycorrhizal fungi. *New Phytologist* **168**:179–188 DOI 10.1111/j.1469-8137.2005.01481.x.

Gong S, Wang D, Dai W, An X, Liu H. 2015. Content and mineralization characteristics of soil organic carbon under *Castanopsis Fargesii* forests in different growth periods. *Bulletin of Soil and Water Conservation* **35**:59–63 (In Chinese with English abstract).

Güsewell S. 2004. N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist* **164**:243–266 DOI 10.1111/j.1469-8137.2004.01192.x.

Hailu H, Mamo T, Keskinen R, Karltun E, Gebrekidan H, Bekele T. 2015. Soil fertility status and wheat nutrient content in Vertisol cropping systems of central highlands of Ethiopia. *Agriculture & Food Security* **4**:19 DOI 10.1186/s40066-015-0038-0.

Han W, Fang J, Guo D, Zhang Y. 2005. Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. *New Phytologist* **168**:377–385 DOI 10.1111/j.1469-8137.2005.01530.x.

Han WX, Fang JY, Reich PB, Ian Woodward F, Wang ZH. 2011. Biogeography and variability of eleven mineral elements in plant leaves across gradients of
climate, soil and plant functional type in China. *Ecology Letters* **14**:788–796 DOI 10.1111/j.1461-0248.2011.01641.x.

He J, Fang J, Wang Z, Guo D, Flynn DFB, Geng Z. 2006. Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grassland biomes of China. *Oecologia* **149**:115–122 DOI 10.1007/s00442-006-0425-0.

He J, Wang X, Schmid B, Flynn DFB, Li X, Reich PB, Fang J. 2010. Taxonomic identity, phylogeny, climate and soil fertility as drivers of leaf traits across Chinese grassland biomes. *Journal of Plant Research* **123**:551–561 DOI 10.1007/s10265-009-0294-9.

He X, Hou E, Liu Y, Wen D. 2016. Altitudinal patterns and controls of plant and soil nutrient concentrations and stoichiometry in subtropical China. *Scientific Reports* **6**: DOI 10.1038/srep24261.

Hedin LO. 2004. Global organization of terrestrial plant-nutrient interactions. *Proceedings of the National Academy of Sciences of the United States of America* **101**:10849–10850 DOI 10.1073/pnas.0404222101.

Hobbie SE, Nadelhoffer KJ, Högberg P. 2002. A synthesis: the role of nutrients as constraints on carbon balances in boreal and arctic regions. *Plant and Soil* **242**:163–170 DOI 10.1023/A:1019670731128.

Hobbie SE, Schimel JP, Trumbore SE, Randerson JR. 2000. Controls over carbon storage and turnover in high-latitude soils. *Global Change Biology* **6**:196–210 DOI 10.1046/j.1365-2486.2000.06021.x.

Izquierdo JE, Houlton BZ, van Huysen TL. 2013. Evidence for progressive phosphorus limitation over long-term ecosystem development: examination of a biogeochemical paradigm. *Plant and Soil* **367**:135–147 DOI 10.1007/s11104-013-1683-3.

Jónsdóttir IS, Khitun O, Stenström A. 2005. Biomass and nutrient responses of a clonal tundra sedge to climate warming. *Canadian Journal of Botany* **83**:1608–1621 DOI 10.1139/b05-129.

Körner C. 2007. The use of 'altitude' in ecological research. *Trends in Ecology & Evolution* **11**:569–574 DOI 10.1016/j.tree.2007.09.006.

Li S, Gou W, Wang H, Wu G, Su P. 2019. Characteristics of C,N,P, and their response to soil water and salt in leaves of Lycium ruthenicum in the lower reaches of the Heihe River. *Acta Pedologica Sinica* **39**:7189–7196 (In Chinese with English abstract).

Li X, Sun K. 2016. The effect of climatic factors on leaf traits of a non-leguminous nitrogen fixing species *Hippophae tibetana* (Schlecht.) along the altitudinal gradient in the eastern Tibetan plateau, China. *Nature Environment and Pollution Technology* **15**:189–194.

Liu J, Chen J, Chen G, Guo J, Li Y. 2019. Enzyme stoichiometry indicates the variation of microbial nutrient requirements at different soil depths in subtropical forests. *PLOS ONE* **15**:e220599 DOI 10.1101/711069.

Ma Y, Zhong Q, Jin B, Lu H, Guo B, Zheng Y, Li M, Cheng D. 2015. Spatial changes and influencing factors of fine root carbon, nitrogen and phosphorus stoichiometry of plants in China. *Chinese Journal of Plant Ecology* **39**:159–166 (In Chinese with English abstract) DOI 10.17521/cjpe.2015.0015.

Macek P, Klimeš L, Adamec L, Doležal J, Chlumská Z, Bello FD, Dvorský M, Řeháková K. 2012. Plant nutrient content does not simply increase with elevation under the extreme environmental conditions of Ladakh, NW Himalaya. *Arctic, Antarctic, and Alpine Research* **44**:62–66 DOI 10.1657/1938-4246-44.1.62.

Meng X. 2006. *Forest measurement.* Beijing: China Forestry Publishing House.

Normand S, Treier UA, Randin C, Vittoz P, Guisan A, Svenning J. 2009. Importance of abiotic stress as a range-limit determinant for European plants: insights from species responses to climatic gradients. *Global Ecology and Biogeography* **18**:437–449 DOI 10.1111/j.1466-8238.2009.00451.x.

Ordoñez JC, Van Bodegom PM, Witte JM, Wright IJ, Reich PB, Aerts R. 2009. A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography* **18**:137–149 DOI 10.1111/j.1466-8238.2008.00441.x.

Paudel BR, Dyer AG, Garcia JE, Shrestha M. 2019. The effect of elevational gradient on alpine gingers (*Roscoea alpina* and *R. purpurea*) in the Himalayas. *Peer J* **7**:e7503 DOI 10.7717/peerj.7503.

Penuelas J, Fernández-Martínez M, Vallicrosa H, Maspons J, Zuccarini P, Carnicer J, Sanders TGM, Krüger I, Obersteiner M, Janssens IA, Ciais P, Sardans J. 2020. Increasing atmospheric CO_2 concentrations correlate with declining nutritional status of European forests. *Communications Biology* **3**:125 DOI 10.1038/s42003-020-0839-y.

Penuelas J, Matamala R. 1990. Changes in N and S leaf content, stomatal density and specific leaf area of 14 plant species during the last three centuries of CO_2 increase. *Journal of Experimental Botany* **41**:1119–1124 DOI 10.2307/23695111.

Penuelas J, Matamala R. 1993. Variations in the mineral composition of herbarium plant species collected during the last three centuries. *Journal of Experimental Botany* **44**:1523–1525 DOI 10.1093/jxb/44.9.1523.

Plieth C, Hansen UP, Knight H, Knight MR. 1999. Temperature sensing by plants: the primary characteristics of signal perception and calcium response. *The Plant Journal : for Cell and Molecular Biology* **18**:491–497 DOI 10.1046/j.1365-313X.1999.00471.x.

Qian L, Wu C, Hong W, Song P, Fan H. 2004. Species-individuals relationships of *Castanopsis fargesii* community in the middle subtropics. *Chinese Journal of Eco-Agriculture* **12**:170–173 (In Chinese with English abstract).

Reich PB, Oleksyn J. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America* **101**:11001–11006 DOI 10.1073/pnas.0403588101.

Richardson AD, Berlyn GP, Gregoire TG. 2001. Spectral reflectance of *Picea rubens* (Pinaceae) and *Abies balsamea* (Pinaceae) needles along an elevational gradient, Mt. Moosilauke, New Hampshire, USA. *American Journal of Botany* **88**:667–676 DOI 10.2307/2657067.

Sardans J, Janssens IA, Alonso R, Veresoglou SD, Rillig MC, Sanders TG, Carnicer J, Filella I, Farré-Armengol G, Peñuelas J. 2015. Foliar elemental composition of

European forest tree species associated with evolutionary traits and present environmental and competitive conditions. *Global Ecology and Biogeography* **24**:240–255 DOI 10.1111/geb.12253.

Shi P, Körner C, Hoch G. 2006. End of season carbon supply status of woody species near the treeline in western China. *Basic and Applied Ecology* **7**:370–377 DOI 10.1016/j.baae.2005.06.005.

Soethe N, Lehmann J, Engels C. 2008. Nutrient availability at different altitudes in a tropical montane forest in Ecuador. *Journal of Tropical Ecology* **24**:397–406 DOI 10.1017/S026646740800504X.

Song P, Hong W, Wu C, Fan H, Chen R, Qian L, Zhu H, Tu Y. 2003. Highness class structure of *Castanopsis fargesii* secondary forest in the middle subtropic. *Chinese Journal of Applied & Environmental Biology* **9**:460–464 (In Chinese with English abstract).

Sundqvist MK, Sanders NJ, Wardle DA. 2013. Community and ecosystem responses to elevational gradients: processes, mechanisms, and insights for global change. *Annual Review of Ecology, Evolution, and Systematics* **44**:261–280 DOI 10.1146/annurev-ecolsys-110512-135750.

Tan Q, Wang G. 2016. Decoupling of nutrient element cycles in soil and plants across an altitude gradient. *Scientific Reports* **6**: DOI 10.1038/srep34875.

Tanner EVJ, Vitousek PM, Cuevas E. 1998. Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. *Ecology* **79**:10–22 DOI 10.2307/176860.

Tian H, Chen G, Zhang C, Melillo JM, Hall CAS. 2010. Pattern and variation of C:N:P ratios in China's soils: a synthesis of observational data. *Biogeochemistry* **98**:139–151 DOI 10.1007/s10533-009-9382-0.

Van De Weg MJ, Meir P, Grace J, Atkin OK. 2009. Altitudinal variation in leaf mass per unit area, leaf tissue density and foliar nitrogen and phosphorus content along an Amazon-Andes gradient in Peru. *Plant Ecology & Diversity* **2**:243–254 DOI 10.1080/17550870903518045.

Vitousek PM, Aplet G, Turner D, Lockwood JJ. 1992. The Mauna Loa environmental matrix: foliar and soil nutrients. *Oecologia* **89**:272–382.

Wang A, Wang X, Tognetti R, Lei J, Pan H, Liu X, Jiang Y, Wang X, He P, Yu F, Li M. 2018. Elevation alters carbon and nutrient concentrations and stoichiometry in *Quercus aquifolioides* in southwestern China. *Science of the Total Environment* **622-623**:1463–1475 DOI 10.1016/j.scitotenv.2017.12.070.

Wang G, Wang T, Kang H, Mang S, Riehl B, Seely B, Liu S, Guo F, Li Q, Innes JL. 2016. Adaptation of Asia-Pacific forests to climate change. *Journal of Forestry Research* **27**:469–488 DOI 10.1007/s11676-016-0218-1.

Xue X, Wu X, Wang W, Luo X, Wang D, Zhang Y, Zou B. 2019. Progress of potassium, magnesium and their interaction in plant-soil system. *Soils* **51**:1–10 (In Chinese with English abstract).

Yan T, Zhu J, Yang K. 2017. Leaf nitrogen and phosphorus resorption of woody species in response to climatic conditions and soil nutrients: a meta-analysis. *Journal of Forestry Research* **29**:1–9 DOI 10.1007/s11676-017-0519-z.

Yu F, Wang D, Shi X, Yi X, Huang Q, Hu Y. 2013. Effects of environmental factors on tree seedling regeneration in a pine-oak mixed forest in the Qinling Mountains, China. *Journal of Mountain Science* **10**:845–853 DOI 10.1007/s11629-013-2548-1.

Zeng X, Fan Y, Lin K, Zhao P, Yuan P, Zhou J, Zhang Q, Cheng L, Xu J, Chen Y. 2018. Characteristics of soil phosphorus fractions and microbial communities in *Pinus taiwanensis* Hayata forests at different altitudes in a subtropical region of China. *Acta Ecologica Sinica* **38(18)**:6570–6579 (In Chinese with English abstract) DOI 10.5846/stxb201805071014.

Zhang S, Zhang J, Slik JWF, Cao K. 2012. Leaf element concentrations of terrestrial plants across China are influenced by taxonomy and the environment. *Global Ecology and Biogeography* **21**:809–818 DOI 10.1111/j.1466-8238.2011.00729.x.

Zhao G, Wang B, Li S, Bai X, Deng Z. 2005. Photosynthetic characteristics of dominant species *Castanopsis fargesii* and *Castanopsis sclerophylla* in different leaf ages in Dagangshan Mountain, Jiangxi Province. *Acta Agriculturae Universitatis Jiangxiensis* **27**:161–165 (In Chinese with English abstract).

Zheng D, Cai Y, Yang Y, Zhong Z, Miu S, Wu W. 2017. Stoichiometric characteristics of C, N and P in the dominant species sapling organs of *Castanopsis fissa* natural forest in Northern Fujian. *Forest Research* **30**:154–159 (In Chinese with English abstract).