


## Article

# Elevation Alone Alters Leaf N and Leaf C to N Ratio of *Picea crassifolia* Kom. in China's Qilian Mountains

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**Abstract:** Leaf stoichiometry of plants can respond to variation in environments such as elevation ranging from low to high and success in establishing itself in a given montane ecosystem. An evaluation of the leaf stoichiometry of Qinghai Spruce (*Picea crassifolia* Kom.) growing at different elevations (2400 m, 2600 m, 2800 m, 3000 m, and 3200 m) in eastern China's Qilian Mountains, showed that leaf carbon (LC) and leaf phosphorus (LP) were similar among elevations, with ranges of 502.76–518.02 g·kg<sup>−1</sup>, and 1.00–1.43 g·kg<sup>−1</sup>, respectively. Leaf nitrogen (LN) varied with changes of elevation, with a maxima of 12.82 g·kg<sup>−1</sup> at 2600 m and a minima of 10.74 g·kg<sup>−1</sup> at 2800 m. The LC:LN under 2400 m and 2600 m was lower than that under other elevations, while LC:LP and LN:LP were not different among these elevations. Except for LN and LC:LN, *P. crassifolia*'s other leaf stoichiometries remained relatively stable across elevations, partly supporting the homeostasis hypothesis. Variations in leaf stoichiometry across elevations were mainly linked to mean annual precipitation, mean annual temperature, soil pH, and the soil organic C to soil total N ratio. *P. crassifolia* growth within the study area was more susceptible to P limitation.

**Keywords:** topographic factor; environmental changes; plant adaptation; leaf elements; nutrient limitation

## 1. Introduction

Over the past several decades, leaf stoichiometry—leaf carbon (C), nitrogen (N), and phosphorus (P) (LC, LN, LP, respectively) and their ratios—has been shown to be a key tool in the study of element cycling, nutrient limitations for plant growth, and response of plants to environmental change [1]. In terrestrial ecosystems, C, N, and P are usually coupled to the processes of primary production, respiration, and decomposition [2,3]. P is important for the production of N-rich proteins by ribosomes, which, in turn, help plants perform photosynthesis and further enhance C accumulation [4]. Patterns in the variation of plant element levels and their relationships with environmental factors have been intensively studied at local [5–7], regional [8–11] and global scales [12,13]. In temperate forest ecosystems situated along elevation gradients in northeastern China's Changbai Mountains, increasing elevation led to a rise in LC and declines in LN and LP [6]. In this

setting, plant life form (e.g., herb, shrub, or tree) was the dominant factor influencing leaf stoichiometry in 175 plant species. Similarly, plant ecotypes and taxonomic groups proved to be the main determinants of leaf stoichiometry of 348 tree species in China's forests [11]. It was further noted that LN and LP increased with latitude, from tropical to cooler and drier zones, then decreased slightly at colder temperatures. Based on a total of 12,055 plant species distributed amongst 486 sites worldwide, Tian et al. [13] found greater LN and LP at high (vs. low) latitudes, and that different life-forms, plant growth rates and soil nutrient availabilities were the main factors influencing the scaling exponent of LN and LP.

Situated at the intersection of the Loess, Qinghai-Tibet and Mengxin plateaus, the Qilian Mountains represent an important ecological barrier from northwest China, and feature a fragile environment, easily eroded, and disturbed by humans [4]. The unique characteristics of the climate and the complex topographic features lead to significant differences in temperature and precipitation along elevational gradients, thereby providing an ideal natural laboratory to study the response of plants to environmental changes [14,15]. Ranging vertically from 2600 m to 3300 m, Qinghai Spruce (*Picea crassifolia* Kom.) is an indigenous evergreen conifer tree species, occupying a large proportion of the coldest part of the evergreen coniferous forest belt on the northern Qilian Mountains [16]. The species provides significant ecological functions, including the conservation of water resources and biodiversity, and the maintenance of ecological safety [17]. Recently, studies of *P. crassifolia* have focused on its growth response and adaption to global warming from a physiological perspective [18,19]. Yet knowledge remains limited regarding the response of *P. crassifolia*'s leaf stoichiometry to environmental changes along elevational gradients. An analysis of *P. crassifolia* leaf stoichiometry at different elevations could provide support for a mechanistic hypothesis explaining the plant communities' responses to a changing environment, thereby offering a potential means of predicting C, N, and P stoichiometry in other species [20,21]. Such knowledge could also help in selecting species for ecological protection [22].

In the present study, our aims were to: (i) determine how *P. crassifolia* leaf stoichiometry changed with elevation; (ii) screen the main driving factors affecting leaf stoichiometry changes; and (iii) explore the relationship between the leaf stoichiometry of *P. crassifolia* and dominant driving factors. Our hypothesis was that, given the existence of large hydrothermal differences across elevations, temperature and precipitation determine leaf stoichiometry of *P. crassifolia*.

## 2. Materials and Methods

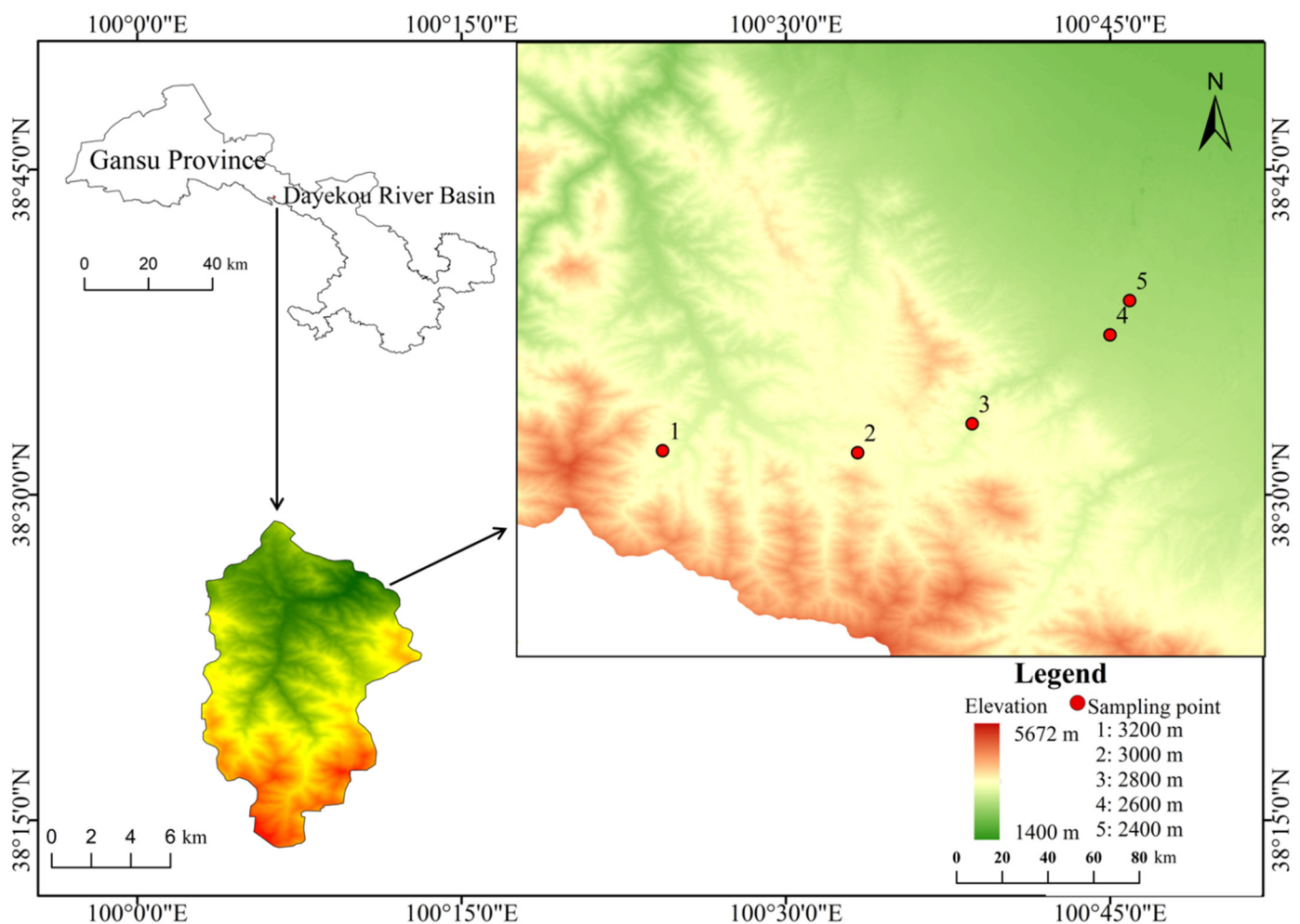
### 2.1. Study Area

Situated in the central Qilian Mountains, the study area is subject to a mean annual temperature of 5.4 °C, and a mean annual rainfall of 433.6 mm. The area's wide range in elevation (2300–4600 m) makes for an equally wide range of combined hydrological and thermal conditions, resulting in different types of vegetation at different elevations. From low to high elevations, the vegetation zones and their corresponding soil types are: dry shrub steppe and mountain chestnut-calcium soil (2100–2400 m), mountainous forest steppe and forest gray-brown soil (2400–3200 m), alpine shrub steppe and alpine scrub-meadow soil (3200–3900 m), and alpine cold desert meadow and alpine cold desert soil (3900–4600 m) [23]. The dominant species, *P. crassifolia*, is distributed in patches or strips on the north-facing slopes at elevations ranging from 2400 m to 3300 m.

### 2.2. Sampling and Management

In August 2018, we collected soil samples and new leaves of *P. crassifolia* at elevations of 2400 m, 2600 m, 2800 m, 3000 m and 3200 m along a north-facing gradient (Figure 1). Three randomly situated 10 m × 10 m plots were established at each elevation for leaf sampling, and 10 g of leaves per plot were collected. Three quadrats (1 m × 1 m) were identified along the diagonal line of each plot. In each quadrat, soils were sampled at

0–0.10, 0.10–0.20 and 0.20–0.40 m depths, respectively, as soil C and nutrients are mainly in the 0–0.40 m soil depth [23,24], using a 35-mm diameter soil drill.



**Figure 1.** The five sample sites at 2400 m, 2600 m, 2800 m, 3000 m and 3200 m in the study area.

Leaf samples were dried to a constant weight at 65 °C for 48 h. Samples were then ground and stored in tin foil for later determination of LC, LN and LP. Soil samples were air-dried and ground through a 2 mm mesh sieve prior to laboratory analysis. LC and soil organic carbon (SOC) were determined using a volumetric potassium dichromate method [25]. Both LN and soil total nitrogen (STN) were measured following selenium-catalyzed  $\text{H}_2\text{SO}_4\text{-K}_2\text{SO}_4\text{-CuSO}_4$  digestion of the soil samples [26]. Soil ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) and nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ) were determined by a SmartChem 200 element analyzer (AMS, Rome, Italy). LP and soil total phosphorus (STP) were extracted using  $\text{H}_2\text{O-H}_2\text{SO}_4$  and determined using the Olsen molybdenum-antimony colorimetric method [27]. Using a Sartorius PB-10 pH meter (Sartorius, Beijing, China), soil pH was determined (water:soil = 2.5:1).

### 2.3. Data Analysis

Soil nutrient values at 0–0.4 m were the average of the measured values in each layer. All the nutrient ratios were mass based. The mean annual temperature (MAT) and mean annual precipitation (MAP) were calculated following Zhao et al. [17,28] as:

$$\text{MAT} = 20.957 - 0.00549H - 0.166Y + 0.0089X, R^2 = 0.98 \quad (1)$$

$$\text{MAP} = 1690.6235 + 0.119H - 75.264Y + 12.405X, R^2 = 0.92 \quad (2)$$

where H is elevation, Y is latitude, X is longitude, and  $R^2$  is the regression coefficient.

The Fisher test was used to determine the effect of elevation on each indicator. A *t*-test was used to examine the significant differences in leaf stoichiometric indicators and environmental factors among different elevations ( $p \leq 0.05$ ). The correlations between leaf stoichiometric indicators and environment factors were determined using the Pearson Correlation Coefficient. Redundancy analysis (RDA) was used to identify the dominant environmental factors of leaf stoichiometry [29]. The R ‘vegan’ package was used to perform RDA [30], while other data analysis was conducted using SPSS 22.0 software (SPSS Inc. Chicago, IL, USA).

### 3. Results

#### 3.1. Variation in Soil Properties and *P. crassifolia* Leaf Stoichiometry across Elevation

In the present study, elevation had a significant influence on all of the measured soil properties but, for *P. crassifolia*, elevation had a significant effect only on LN and LC: LN (Table 1). Overall, SOC ( $86.83 \text{ g}\cdot\text{kg}^{-1}$ ) and STN ( $5.90 \text{ g}\cdot\text{kg}^{-1}$ ) at 3200 m were significantly greater than that at other elevations (Table 2).  $\text{NH}_4^+\text{-N}$  first decreased then increased with elevation, and its minimum value ( $6.17 \text{ mg}\cdot\text{kg}^{-1}$ ) at 2800 m was significantly lower than that at other elevations.  $\text{NO}_3^-\text{-N}$  ( $4.94 \text{ mg}\cdot\text{kg}^{-1}$ ) at 3200 m was significantly greater than that at other elevations except 2600 m. STP fluctuated across elevations, and its values at 2600 m ( $0.63 \text{ g}\cdot\text{kg}^{-1}$ ) and 3000 m ( $0.62 \text{ g}\cdot\text{kg}^{-1}$ ) were significantly greater than that at other elevations. The SOC:STN at 2600 m (12.35) was significantly lower than at other elevations, but its value among these elevations was similar. The SOC:STP and STN:STP changed with elevation, both reaching the maximum values at 3200 m (166.82, 11.30, respectively), and were significantly greater than at other elevations. Soil pH decreased with increasing elevation and reached a minimum (7.44) at 3200 m, significantly lower than at other elevations (Table 2).

**Table 1.** Effects of elevation on soil properties and leaf stoichiometric characteristics of *P. crassifolia*.

Measured Parameters	Effect of Elevation	
	<i>p</i>	df
SOC ( $\text{g}\cdot\text{kg}^{-1}$ )	<b>&lt;0.001</b>	4
STN ( $\text{g}\cdot\text{kg}^{-1}$ )	<b>&lt;0.001</b>	4
STP ( $\text{g}\cdot\text{kg}^{-1}$ )	<b>&lt;0.001</b>	4
SOC:STN	<b>&lt;0.001</b>	4
SOC:STP	<b>&lt;0.001</b>	4
STN:STP	<b>&lt;0.001</b>	4
$\text{NH}_4^+\text{-N}$ ( $\text{mg}\cdot\text{kg}^{-1}$ )	<b>&lt;0.001</b>	4
$\text{NO}_3^-\text{-N}$ ( $\text{mg}\cdot\text{kg}^{-1}$ )	<b>&lt;0.001</b>	4
pH	<b>&lt;0.001</b>	4
LC ( $\text{g}\cdot\text{kg}^{-1}$ )	0.158	4
LN ( $\text{g}\cdot\text{kg}^{-1}$ )	<b>&lt;0.001</b>	4
LP ( $\text{g}\cdot\text{kg}^{-1}$ )	0.203	4
LC:LN	<b>&lt;0.001</b>	4
LC:LP	0.388	4
LN:LP	0.428	4

SOC = soil organic carbon, STN = soil total nitrogen, STP = soil total phosphorus,  $\text{NH}_4^+\text{-N}$  = soil ammonium nitrogen,  $\text{NO}_3^-\text{-N}$  = soil nitrate nitrogen; LC = leaf carbon concentration, LN = leaf nitrogen concentration, LP = leaf phosphorus concentration. *p*, probability value; df, degree of freedom. Significant values are in bold.

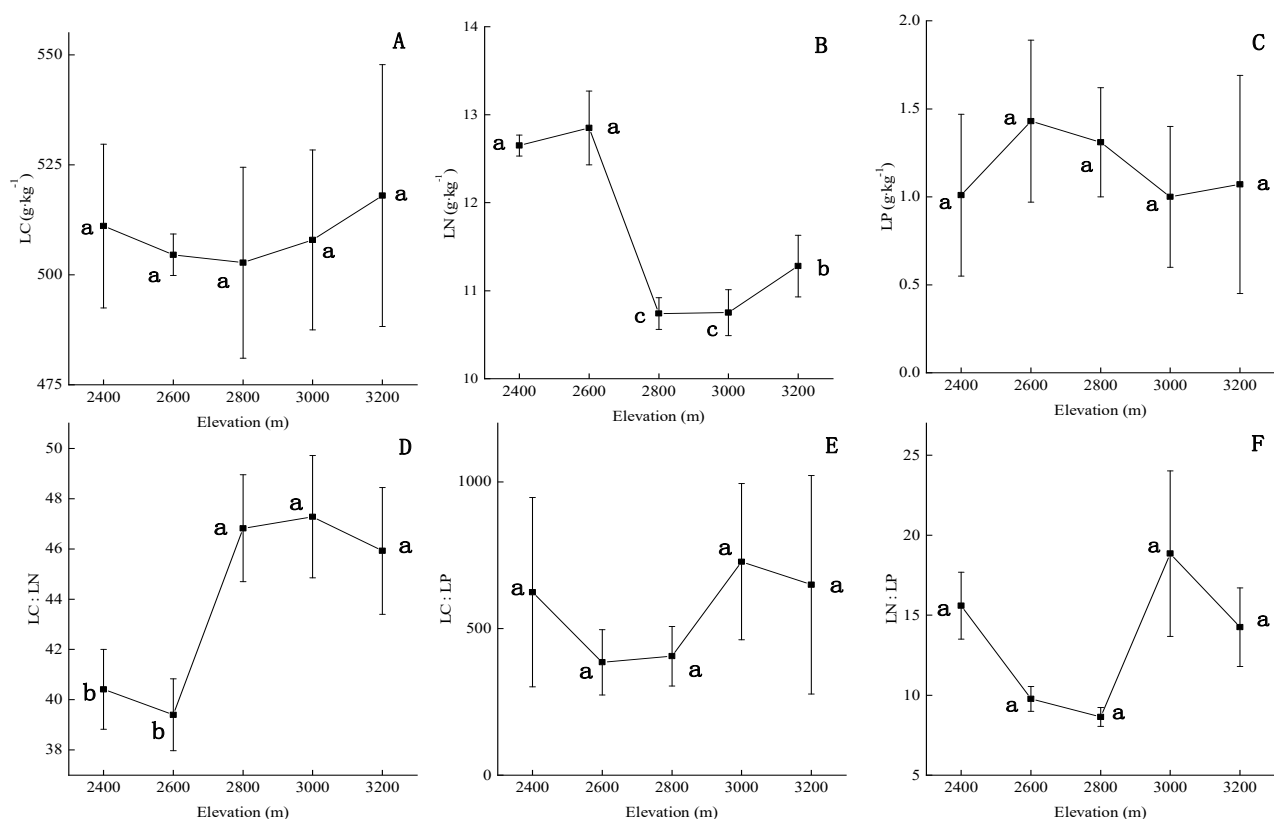
Across elevations, LC ranged from  $502.76$  to  $518.02 \text{ g}\cdot\text{kg}^{-1}$ , and LP from  $1.0$  to  $1.43 \text{ g}\cdot\text{kg}^{-1}$ , and there was no significant difference among the elevations (Figure 2A,C). The maximum LN value ( $12.82 \text{ g}\cdot\text{kg}^{-1}$ ) was reached at 2600 m and was significantly greater at 2800 m, 3000 m, and 3200 m. The minimum LN value ( $10.74 \text{ g}\cdot\text{kg}^{-1}$ ) at 2800 m was significantly lower than the value at 2400 m or 3200 m, but did not differ significantly from that at 3000 m (Figure 2B). The trends in leaf stoichiometric ratios (LC:LP and LN:LP) at

different elevations were similar, but LC:LN at 2400 m and 2600 m was significantly lower than at other elevations (Figure 2D–F).

**Table 2.** Soil nutrient contents, mean annual temperature and mean annual precipitation across the elevational gradient within the study area.

Parameter	Elevation (m)				
	2400	2600	2800	3000	3200
SOC ( $\text{g}\cdot\text{kg}^{-1}$ )	50.03 $\pm$ 9.88 BC	46.00 $\pm$ 5.01 C	49.06 $\pm$ 11.50 BC	56.40 $\pm$ 7.68 B	86.83 $\pm$ 7.92 A
STN ( $\text{g}\cdot\text{kg}^{-1}$ )	3.58 $\pm$ 0.73 BC	3.72 $\pm$ 0.38 BC	3.28 $\pm$ 0.70 C	3.98 $\pm$ 0.75 B	5.90 $\pm$ 0.43 A
STP ( $\text{g}\cdot\text{kg}^{-1}$ )	0.54 $\pm$ 0.08 B	0.63 $\pm$ 0.09 A	0.48 $\pm$ 0.08 B	0.62 $\pm$ 0.05 A	0.52 $\pm$ 0.05 B
SOC:STN	13.99 $\pm$ 0.83 A	12.35 $\pm$ 0.48 B	14.90 $\pm$ 0.78 A	14.33 $\pm$ 1.62 A	14.74 $\pm$ 1.37 A
SOC:STP	92.41 $\pm$ 13.79 BC	74.12 $\pm$ 5.72 C	107.60 $\pm$ 42.23 B	90.40 $\pm$ 10.09 B	166.82 $\pm$ 22.45 A
STN:STP	6.59 $\pm$ 0.82 B	6.01 $\pm$ 0.60 B	7.15 $\pm$ 2.47 B	6.40 $\pm$ 1.14 B	11.30 $\pm$ 0.92 A
NH <sub>4</sub> <sup>+</sup> -N ( $\text{mg}\cdot\text{kg}^{-1}$ )	8.07 $\pm$ 0.73 A	7.42 $\pm$ 0.55 A	6.17 $\pm$ 2.47 B	8.11 $\pm$ 0.54 A	8.14 $\pm$ 1.55 A
NO <sub>3</sub> <sup>-</sup> -N ( $\text{mg}\cdot\text{kg}^{-1}$ )	1.65 $\pm$ 0.41 C	4.34 $\pm$ 2.88 AB	3.43 $\pm$ 0.56 B	3.37 $\pm$ 0.42 B	4.94 $\pm$ 1.52 A
pH	7.86 $\pm$ 0.10 A	7.86 $\pm$ 0.09 A	7.83 $\pm$ 0.27 A	7.75 $\pm$ 0.18 A	7.44 $\pm$ 0.24 B
Longitude	100°21'55" E	100°17'13" E	100°14'28" E	100°13'19" E	100°22'35" E
Latitude	38°37'5" N	38°34'4" N	38°33'9" N	38°33'5" N	38°38'15" N
MAT (°C)	2.26	1.17	0.08	−1.02	−2.68
MAP (mm)	304.32	330.93	353.70	378.96	410.09

MAP = mean annual precipitation, MAT = mean annual temperature, NH<sub>4</sub><sup>+</sup>-N = soil ammonium nitrogen, NO<sub>3</sub><sup>-</sup>-N = soil nitrate nitrogen, SOC = soil organic carbon, STN = soil total nitrogen, STP = soil total phosphorus. Row-wise non-matching upper case letters (e.g., A, B, BC, ...) indicate a significant difference in soil properties amongst elevations ( $p \leq 0.05$ ). Soil parameters were taken from 0–0.40 m depth. E = Eastings and N = Northings.

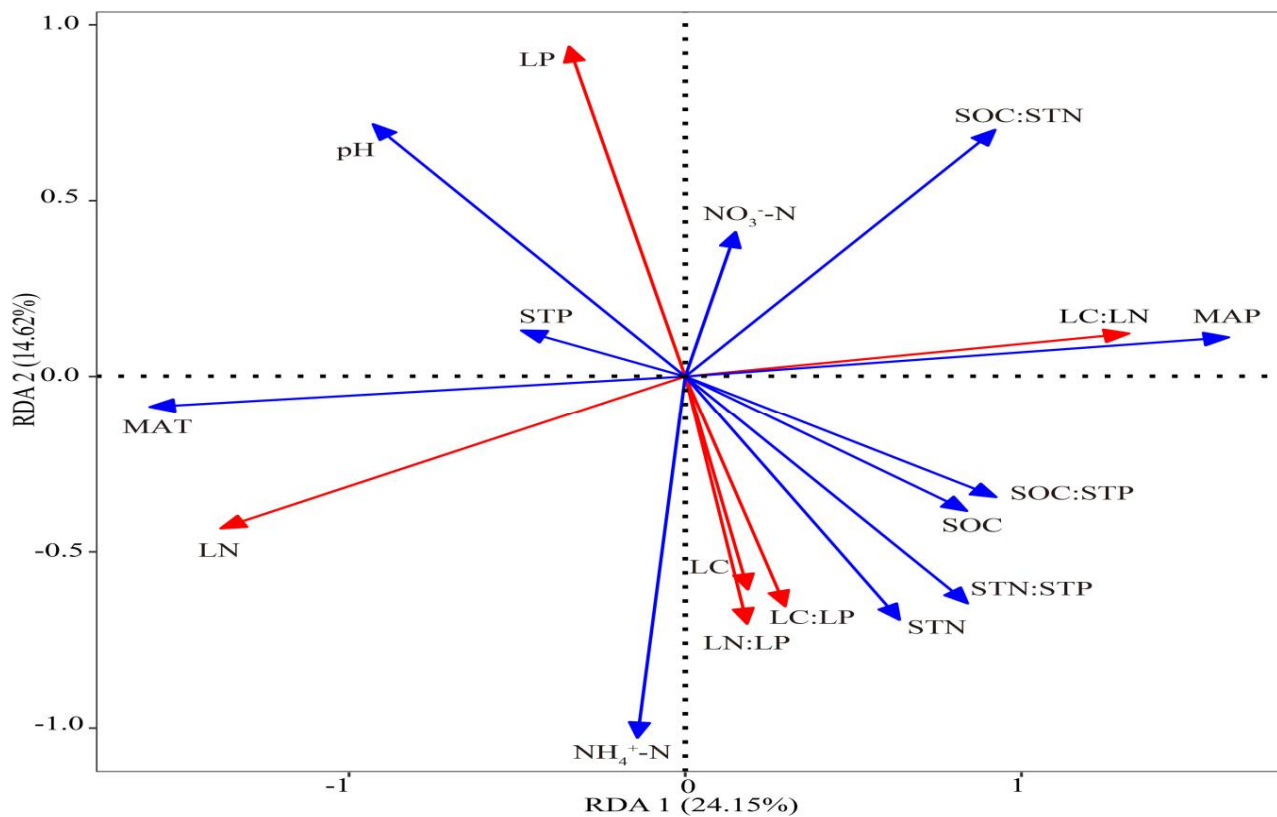


**Figure 2.** *P. crassifolia* LC (A), LN (B), and LP (C) and LC:LN (D), LC:LP (E), and LN:LP (F) ratios at different elevations. LC = leaf C concentration; LN = leaf N concentration, LP = leaf P concentration. For individual plots, non-matching lowercase letters (e.g., a, b, ...) indicate a significant difference in leaf stoichiometry between elevations.



### 3.2. Dominant Factors Affecting the Leaf Stoichiometry of *P. crassifolia*

From the RDA, the eigenvalue of the two axes was 38.77%, the first axis accounting for 24.15% and the second for 14.62% (Figure 3). MAP, MAT, pH, and SOC:STN significantly influenced the variation of leaf stoichiometry, and explained the total variation by 15.9%, 15.4%, 7.4%, and 7.1%, respectively (Table 3).



**Figure 3.** Redundancy analysis (RDA) for the leaf stoichiometric indices of *P. crassifolia* and environmental indices, including ratios of soil nutrients, for elevation. LC = leaf C concentration, LC:LN = LC:LN, LC:LP = LC:LP, LN:LP = LN:LP, MAP = mean annual precipitation, MAT = mean annual temperature, LN = leaf N concentration,  $\text{NH}_4^+ - \text{N}$  = soil ammonium nitrogen,  $\text{NO}_3^- - \text{N}$  = soil nitrate nitrogen, LP = leaf P concentration, SOC = soil organic carbon, STN = soil total nitrogen, STP = soil total phosphorus.

**Table 3.** The dominant factors influencing leaf stoichiometry of *P. crassifolia* across the elevational gradient based on RDA.

Environmental Factors	RDA1	RDA2	$R^2$	$p^*$	Explains (%)	Pseudo-F	$p^{**}$
SOC ( $\text{g} \cdot \text{kg}^{-1}$ )	0.99426	−0.10702	0.1222	0.078	1.7	2.2	0.076
STN ( $\text{g} \cdot \text{kg}^{-1}$ )	0.87152	−0.49036	0.0965	0.126	1.5	2	0.134
STP ( $\text{g} \cdot \text{kg}^{-1}$ )	−0.99995	−0.01024	0.0406	0.41	0.3	0.7	0.604
pH	−0.95203	0.30602	0.1687	<b>0.024</b>	4.7	3.5	<b>0.012</b>
SOC:STN	0.88126	0.47263	0.2418	<b>0.003</b>	3.9	3.3	<b>0.016</b>
SOC:STP	0.99858	−0.05328	0.1469	<b>0.043</b>	2.2	2.6	0.060
STN:STP	0.95268	−0.30397	0.1378	<b>0.042</b>	2.5	2.7	0.050
$\text{NH}_4^+ - \text{N}$ ( $\text{mg} \cdot \text{kg}^{-1}$ )	−0.45172	−0.89216	0.1216	0.076	0.3	0.7	0.484
$\text{NO}_3^- - \text{N}$ ( $\text{mg} \cdot \text{kg}^{-1}$ )	0.63894	0.76926	0.0251	0.552	0.7	1.8	0.156
MAT ( $^{\circ}\text{C}$ )	−0.98159	−0.19099	0.4647	<b>0.001</b>	13.7	7.9	<b>0.002</b>
MAP (mm)	0.98013	0.19838	0.4814	<b>0.001</b>	15.9	8.1	<b>0.002</b>

$\text{NH}_4^+ - \text{N}$  = soil ammonium nitrogen,  $\text{NO}_3^- - \text{N}$  = soil nitrate nitrogen, SOC = soil organic carbon, STN = soil total nitrogen, STP = soil total phosphorus. \* indicates that the environmental factor was significantly ( $p < 0.05$ ) related to the two RDA axes, while \*\* indicates that the variance of each environmental factor contributed ( $p < 0.05$ ) to the total variance. Significant values are in bold.

Based on the results of RDA, we further investigated the correlation between the four dominant factors and leaf stoichiometry of *P. crassifolia*. The LC was negatively related to soil pH, while LN was positively related to MAT and negatively correlated with SOC:STN and MAP. Of the stoichiometric ratios, LC:LN was positively correlated with SOC:STN and MAP, but negatively correlated with pH and MAT. The LP, LC:LP and LN:LP were not correlated with any of the dominant factors (Table 4).

**Table 4.** The Pearson correlations between the dominant factors from the RDA and leaf stoichiometry of *P. crassifolia*.

	pH	SOC:STN	MAT (°C)	MAP (mm)
LC (g·kg <sup>−1</sup> )	−0.301 *	−0.064	−0.139	0.13
LN (g·kg <sup>−1</sup> )	0.276	−0.483 **	0.670 **	−0.684 **
LP (g·kg <sup>−1</sup> )	0.219	0.044	0.099	−0.093
LC:LN	−0.370 *	0.392 **	−0.654 **	0.664 **
LC:LP	−0.229	−0.114	−0.125	0.127
LN:LP	−0.206	−0.134	−0.069	0.069

LC = leaf carbon concentration, LN = leaf nitrogen concentration, LP = leaf phosphorus concentration; SOC = soil organic carbon, STN = soil total nitrogen, MAT = mean annual temperature, MAP = mean annual precipitation. \*  $p < 0.05$ , \*\*  $p < 0.01$ .

## 4. Discussion

### 4.1. Effect of Elevation on Soil Properties

Elevation had a significant influence on soil properties (Table 1), due to temperature and precipitation, plant composition, geological substrates, light resources, and even the disturbance regime varying along the elevation gradient [1,3,14]. In the study area, although the sampled sites were all located on north-facing slopes and the dominant species was *P. crassifolia*, we found that the composition of understory vegetation gradually changed as elevation increased, from herbaceous plants to shrubby plants. This, in combination with variations in other environmental factors as mentioned above, led to changes in soil properties. However, Tewksbury et al. [31] and Godgift et al. [32] found that soil properties did not change with changing environments along an elevation gradient due to the confounding effect of stand structure, growth patterns, litter quality, plant production and soil parent material over the long term. These inconsistent results suggest that elevation has a complicated effect on soil properties, or that the relationship between soil properties and elevation is site dependent.

### 4.2. Overall Patterns of Leaf Stoichiometry of *P. crassifolia* across the Elevation Gradient

In the study area, as biotic and abiotic factors changed with elevation, plant stoichiometry along the elevation gradient was also affected [33,34]. LC, LP, LC:LP, and LN:LP were relatively stable over the elevation range of 2400 m to 3200 m (Figure 1, Table 2), inconsistent with the biogeochemical hypothesis or soil substrate age hypothesis [35]. This indicated the maintenance of a high stability of internal chemical components through regulation of nutrient concentrations and their ratios and suggested the plants were able to adapt to external environmental changes having a potential impact on their growth [36]. This was mainly attributable to the genetic evolution of *P. crassifolia* and its long-term adaptation to the habitat in the study area with its unique climatic conditions [33], through a form of conservative nutrient utilization strategy [34,37].

LN and LC:LN varied significantly with elevation (Figure 1, Table 2). The relatively lower LN at high (vs. low) elevations was likely because lower temperatures slowed soil nutrient turnover and reduced availability, which resulted in little N accessible for plant uptake at high elevations [38]. However, some studies found LN constant across elevations [7], showing a species- and habitat-dependent behavior of LN [7,38]. In our study, LN increased from 3000 m to 3200 m, mainly related to the change of micro-habitats. With a further reduction in temperature, *P. crassifolia* needed more nutrients to maintain a normal

metabolic rate and support the evolution of functional tissues under low temperature stress [39], which is consistent with the ‘Temperature-plant physiological’ hypotheses [35]. Therefore, 3000 m may be the critical point of the adaption of *P. crassifolia* to temperature. As LN varied and LC changed little with elevation, variation in LC:LN was similar to that of LN. This also indicated that in the study area, LC:LN of *P. crassifolia* was mainly influenced by LN.

Generally, high values of LC:LN and LC:LP indicated a low growth rate and high C assimilation [40]. In the present study area, the LC:LN (43.97) and LC:LP (558.27) of *P. crassifolia* were much greater than those of the Loess Plateau (28.6 and 404, respectively) [41] and global flora (22.5 and 232, respectively) [42], indicating a lower growth rate and high C assimilation of *P. crassifolia* in the Qilian mountains, which has been confirmed in other studies [10,43].

#### 4.3. Effects of MAP and MAT on Leaf Stoichiometry of *P. crassifolia*

From the RDA results, about 40% of the total variation in leaf stoichiometry of *P. crassifolia* could be explained by the measured parameters (Figure 2). Of these environmental factors, MAP and MAT were the main explanatory variables, accounting for 30% of the total variation (Table 3), thereby supporting our hypothesis.

In the present study, MAP was negatively correlated with LN, positively correlated with LC:LN, but not correlated with other indicators (Table 4). The LN of *P. crassifolia* decreased with an increase in MAP, as reported in other studies [20]. This may be because increased precipitation alleviates drought stress and protects the plant’s photosynthetic apparatus [44]. Accordingly, as the photosynthetic metabolism rises, N, which is the main component of the enzyme, is consumed in greater quantities [44]. However, other studies found a positive correlation [39] or no correlation [45] between LN and MAP. The reason for this disparity may be related to different plant responses to water availability and different adaptation strategies for survival [6,7]. With a constant LC and a negative relationship between MAP and LN, the LC:LN showed a significantly positive relationship with MAP, suggesting that the greater the precipitation, the slower the growth rate of *P. crassifolia* [40]. *P. crassifolia* growth was sensitive to high MAP; plants may consume greater amounts of N under high MAP than under lower MAP, as mentioned above.

In contrast to MAP, MAT was positively correlated with LN and negatively correlated with LC:LN, and had no correlation with other indicators (Table 4). This was mainly related to the limitation of low temperatures on the rate of nutrient turnover [35]. Generally, low temperatures can decrease soil microbial activity and in turn, reduce the decomposition rate of organic materials and the release of soil nutrients that could subsequently be absorbed by plants [46]. However, some studies found that MAT was negatively correlated with LN [37,38], because plants growing at low temperatures require more nutrients to maintain normal eco-physiological processes [35,47]. In addition, MAT may also affect LN by regulating SOC:STN; that is, the decreased temperature inhibits the microbial activity and reduces the rate of N mineralization, resulting in a high SOC:STN [46,48] (details of SOC:STN influencing LN in Section 4.4). The LC:LN was significantly and negatively related to MAT, indicating that *P. crassifolia* grew faster at low elevations than at high elevations due to the moderate temperature and soil nutrients such as STP at 2600 m (Table 1).

#### 4.4. Effects of Soil pH and SOC:STN on Leaf Stoichiometry of *P. crassifolia*

Soil pH was negatively related to LC and LC:LN, but showed no correlation with other indices (Table 4). The relationship between LC and soil pH was consistent with other studies [35,49]. Under drought stress and the associated shortage of water in stomata, a high soil pH (7.75), such as that found in the study area, limited photosynthesis, which further inhibited leaf C accumulation [36]. Moreover, the negative relationship between LC:LN and soil pH further confirmed that, in the study area, *P. crassifolia* grew faster at low elevations than at high elevations. In contrast to other studies [44], in the present study LN and LP



showed no correlation with soil pH. This was mainly because *P. crassifolia* developed a good nutrient storage mechanism and improved its efficiency in resource utilization, thereby enhancing its capacity to face the study area's harsh growing environment [44,50,51]. Low nutrient requirements have been suggested to be among the most important plant strategies to conserve nutrients [37,51]. However, studies have found both positive and negative relationships between soil pH and LN and LP [44,52,53], indicating that plants independently choose their resource utilization strategies according to differences in the growth environment [51].

SOC:STN was negatively related to LN and positively related to LC:LN, but was not correlated with other indicators (Table 4). SOC:STN was significantly negatively related to LN, concurring with other studies [54]. The higher the SOC:STN, the weaker the microbial activity and thus the lower the soil availability of N [55–57]. With a decline in soil available N, LN will decrease. Notably, nutrient concentrations are not only relevant to microbial communities, but are also related to bulk density when they are presented as nutrient pools (concentration times bulk density) [58–60]. In this case, soil bulk density might change the relationship between soil nutrients and leaf stoichiometry, because it varies widely with elevation in the study area [61,62], but this needs further exploration. LC:LN increased with SOC:STN, suggesting high SOC:STN can limit growth of *P. crassifolia* as available soil N is mainly used by soil microbes [63].

#### 4.5. Effects of Other Factors on Leaf Stoichiometry of *P. crassifolia*

Although MAP, MAT, soil pH and SOC:STN were found to be the main factors that influenced the leaf stoichiometry of *P. crassifolia* across elevations, they only explained 38.77% of the total variation, indicating that other important but undocumented and non-measured factors may be involved. These other factors could include properties of the bedrock [64], disturbance level [54,65], genetic inheritance of species [64], intra- and inter-species competition [7,23,66], phylogenetics [10], and limitation of other nutrient elements. Both Ca [67] and K [68] have been shown to be important factors influencing leaf stoichiometry.

As *P. crassifolia* is one of the most important species in the unique alpine ecosystems of the Qilian Mountains, which harbor a wide spectrum of plant growth forms [7], further studies of *P. crassifolia* leaf stoichiometry, considering multiple additional factors, should be undertaken.

#### 4.6. Nutrient Limitations of *P. crassifolia* in the Study Area

Previous studies have shown that the LN:LP might be an important indicator of nutrient limitation [33]. Different researchers have judged these elements limiting plant growth on the basis of different LN:LP thresholds. For example, a study by Koerselman et al. [69] on plant communities in wetland systems showed that plant growth was limited by N when LN:LP was less than 14, by P when it was greater than 16, and by both N and P when it was between 14 and 16. However, Gusewell [70] found that plants were limited by N when the LN:LP was less than 10, by P when it was greater than 20, and that fertilization had no significant effect on the LN:LP when it was between 10 and 20. Therefore, nutrient limitations based on LN:LP are more speculative, and actual soil stoichiometry may be more useful [71]. Previous studies found that an SOC:STN below 25 indicated sufficient N for plant growth [62]. Since the SOC:STN in the study area was 14.06, it was inferred that the growth of *P. crassifolia* may not be limited by N. Coupled with global warming and N deposition, the rate of net N mineralization would be accelerated [71], and N available for *P. crassifolia* growth will further increase. In the study area, SOC:STP was 106.27, significantly higher than 99.0 in severely P deficient soils [66]. In addition, STP ( $0.56 \text{ g} \cdot \text{kg}^{-1}$ ) was lower than the global average ( $2.8 \text{ g} \cdot \text{kg}^{-1}$ ) [72]. Therefore, we speculated that *P. crassifolia* growth may be susceptible to P limitation in the present study area.

Several studies have shown that high P concentrations are essential for fast growth to compete for a spot in the canopy and access to light (e.g., [65]). However, in high elevation

systems, plants take up P not for rapid growth, but for resistance to harsh conditions such as low temperatures and drought [1,34,39]. Given low P concentration in the topsoil, *P. crassifolia* may have to absorb P in deep soil through roots [73,74], or by resorption from the litter to ensure its growth requirements [14].

## 5. Conclusions

Our study provided a detailed picture of the spatial patterns of the leaf stoichiometry of *P. crassifolia* at varying elevations in the Qilian Mountains. It identified the environmental factors causing differences in some leaf nutrient levels of *P. crassifolia* (especially for LN and LC:LN) at different elevations. However, other leaf stoichiometries exhibited a strong homeostasis to different habitats, thereby offering a great potential to adapt to future climate change. Accordingly, the protection of *P. crassifolia*, should be strengthened to ensure the safe and sustainable development of forest ecosystems in the Qilian Mountains, thus ensuring the long-term development of the region.

Due to MAP and MAT contributing more to the total variance of leaf stoichiometry of *P. crassifolia* than soil pH and SOC:STN across elevations in the study area, *P. crassifolia* was apparently more sensitive to MAP and MAT than soil properties. However, these factors only explained 38.77% of the variation. Other, presently unidentified factors influencing the patterns of leaf stoichiometry at different elevations should be considered in future research.

N versus P limitation based on leaf N:P is speculative. After examining soil nutrient levels, we found that *P. crassifolia* in the Qilian Mountains was susceptible to P limitation, but this needs to be further explored.

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