Leaf stoichiometry of Leontopodium lentopodioides at high altitudes on the northeastern Qinghai-Tibetan Plateau, China postprint

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Abstract

Altitude affects leaf stoichiometry by regulating temperature and precipitation, and influencing soil properties in mountain ecosystems. We investigated leaf carbon concentration (C), leaf nitrogen concentration (N), leaf phosphorus concentration (P), and their stoichiometric ratios in Leontopodium lentopodioides (Willd.) Beauv., a widespread species in degraded grasslands, to explore its response and adaptation strategy to environmental changes along four altitude gradients (2500, 3000, 3500, and 3800 m a.s.l.) on the northeastern Qinghai-Tibetan Plateau (QTP), China. Leaf C varied significantly but without any clear trend with increasing altitude. Leaf N showed an increasing trend, while leaf P changed little with altitude, exhibiting a lower value at 3500 m than at other altitudes. Similarly, leaf C:P and N:P ratios changed little with altitude, but both had greater values at 3500 m than at other altitudes. However, leaf C:N exhibited a decreasing trend with increasing altitude. Soil NH_4^+-N, soil pH, soil total phosphorus (STP), mean annual temperature (MAT), and mean annual precipitation (MAP) were identified as the main factors driving variations in leaf stoichiometry of L. lentopodioides across all altitudes, with NH_4^+-N alone accounting for 50.8% of the total variation. Specifically, leaf C and N were mainly controlled by MAT, soil pH, and NH_4^+-N, while leaf P was controlled by MAP and STP. In the study area, the growth of L. lentopodioides appears to be primarily limited by STP. These results strengthen our understanding of plant growth plasticity in response to environmental changes and provide new information for global grassland management and restoration.

Full Text

Preamble

Leaf Stoichiometry of *Leontopodium lentopodioides* at High Altitudes on the Northeastern Qinghai-Tibetan Plateau, China

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Abstract: Altitude affects leaf stoichiometry by regulating temperature and precipitation, and influencing soil properties in mountain ecosystems. We investigated leaf carbon concentration (C), leaf nitrogen concentration (N), leaf phosphorus concentration (P), and their stoichiometric ratios in *Leontopodium lentopodioides* (Willd.) Beauv., a widespread species in degraded grasslands, to explore its response and adaptation strategy to environmental changes along four altitude gradients (2500, 3000, 3500, and 3800 m a.s.l.) on the northeastern Qinghai-Tibetan Plateau (QTP), China. Leaf C varied significantly but without any clear trend with increasing altitude. Leaf N showed an increasing trend, while leaf P changed little with altitude, exhibiting a lower value at 3500 m than at other altitudes. Similarly, leaf C:P and N:P ratios changed little with altitude, but both had greater values at 3500 m than at other altitudes. However, leaf C:N exhibited a decreasing trend with increasing altitude. Soil NH_4^+-N, soil pH, soil total phosphorus (STP), mean annual temperature (MAT), and mean annual precipitation (MAP) were identified as the main factors driving variations in leaf stoichiometry of *L. lentopodioides* across all altitudes, with NH_4^+-N alone accounting for 50.8% of the total variation. Specifically, leaf C and N were mainly controlled by MAT, soil pH, and NH_4^+-N, while leaf P was controlled by MAP and STP. In the study area, the growth of *L. lentopodioides* appears to be primarily limited by STP. These results strengthen our understanding of plant growth plasticity in response to environmental changes and provide new information for global grassland management and restoration.

Keywords: alpine area; environmental changes; leaf elements; nutrient limitation; Qilian Mountains

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1 Introduction

A variety of elements closely control the growth of biological organisms and maintain ecosystem balance (Moe et al., 2005; Ågren and Weih, 2012). These elements include carbon (C), nitrogen (N), and phosphorus (P). Carbon constitutes about 50% of a plant's dry mass (Ågren, 2008). Nitrogen, a crucial component of proteins and enzymes, plays a vital role in plant photosynthesis, respiration, and litter decomposition (Thomson et al., 2012; Yang et al., 2018). Phosphorus, an essential element in ribosome production, affects the synthesis of N-rich proteins (Ågren, 2008; Reich et al., 2009). Therefore, C, N, and P are interactive, and their composition in organisms determines key ecosystem processes (Vitousek et al., 2010). In the context of global biodiversity loss and global change, ecological stoichiometry focusing on the balances (relative proportions) of these elements and their interactions in ecosystems has emerged as an important field (Elser et al., 1996, 2000; Sterner and Elser, 2002; Moe et al., 2005). It not only provides a new method to integrate evolutionary biology and ecosystem science but also plays a critical role in understanding plant functional traits (He et al., 2009), plant productivity (Tang et al., 2018), ecosystem carbon storage (Reich et al., 2005; Hu et al., 2021), and ecosystem function (Tian et al., 2019; Lin et al., 2022).

Leaves, the main organs of plant photosynthesis, are the primary sites for material and energy exchange between plants and the environment (Sun et al., 2017). Leaf stoichiometry is essential for understanding plant nutrient limitation, nutrient utilization efficiency, and adaptation strategies to environmental change, as well as for formulating rational management policies (Xia et al., 2014; Su et al., 2022). Leaf stoichiometry has been studied widely at both the plant community level (Bai et al., 2012; Xu et al., 2014; Sardans et al., 2016; Gong et al., 2020) and the individual species level (Ai et al., 2017; Wang et al., 2019; Cao et al., 2020; Liu et al., 2020; Su et al., 2021; Tao et al., 2021) under various disturbances and environmental changes such as altitude variation.

Generally, altitude can control plant growth metabolic rate and nutrient uptake efficiency by influencing various environmental gradients such as climate and soil properties (Qin et al., 2016; Feng et al., 2021). At the regional scale, altitude is a vital and fundamental driver of environmental change (Jiang et al., 2019). The high altitude, low CO_2 concentration, strong radiation, and low temperature in the Qinghai-Tibetan Plateau (QTP), China make its plants extremely sensitive to environmental changes and have led to the development of unique adaptation mechanisms through long-term natural selection under environmental stress (Xu et al., 2005). In this region, leaf stoichiometry of some plants has been studied previously. For instance, Wang et al. (2019) found that leaf N and P concentrations of *Juniperus przewalskii* Komarov in the northeastern QTP varied significantly with temperature. Cao et al. (2020) reported that leaf C, N, and P of *Oxytropis ochrocephala* Bunge in the Qilian Mountains (northeastern QTP) were significantly higher at high altitudes than at low altitudes. However, Guo et al. (2021) observed that leaf C and N of *Stellera chamaejasme*

L. on the QTP remained relatively stable, in accordance with homeostasis theory that suggests the stability of leaf nutrient composition when plants face environmental changes (Sterner and Elser, 2002). These results indicate that the response of leaf stoichiometry to environmental changes is species-specific, which benefits metabolic and phenological processes and nutrient allocation for increasing plant growth rate through self-regulation (Kang et al., 2011; Li et al., 2015).

Leontopodium lentopodioides (Willd.) Beauv. is a common perennial herbaceous plant in degraded grasslands on the QTP. Zhang et al. (2020) observed that the coverage and abundance of *L. lentopodioides* populations gradually decreased from 2900 to 3800 m, and the spatial distribution shifted from aggregated to random. Sun et al. (2016) found that the structural morphology of its leaves changed with increasing altitude, such as increasing stomatal density in the upper and lower epidermis to capture more CO_2 and strengthening palisade and spongy tissues to resist strong radiation and low temperature. However, leaf stoichiometry of *L. lentopodioides* along altitudinal gradients in this region has not been documented.

In the present study, we aimed to: (1) explore changes in leaf stoichiometry of *L. lentopodioides* from 2500 to 3800 m and identify the dominant factors influencing these changes; (2) identify direct and indirect factors affecting leaf nutrients of *L. lentopodioides* along altitude; and (3) identify the main factor limiting *L. lentopodioides* growth on the northeastern QTP. Based on current theories and existing studies, we hypothesize that altitude affects leaf stoichiometry of *L. lentopodioides* through its effects on climate and soil properties.

2.1 Study Area

The Qilian Mountains are located in the northeastern part of the QTP, Northwest China (36°30′–39°42′N, 93°30′–103°01′E). These mountains have a high western and low eastern topography, with altitudes between 2000 and 5500 m a.s.l. Mountains above 4600 m are covered with snow year-round, and glaciers are widely distributed, making them the birthplace of many rivers. With a plateau and continental climate, these mountains have a mean annual temperature (MAT) of 6.21°C and mean annual precipitation (MAP) of 233.95 mm (Liu et al., 2020). Grassland is the most important vegetation type, accounting for about 53% of the total area (Yang et al., 2022). The vegetation mainly includes *Potentilla chinensis* Ser., *Elymus dahuricus* Turcz., *Agropyron cristatum* (L.) Gaertn., *Carex tristachya* Thunb., *Stipa capillata* Linn., etc. The soil types are mainly mountain gray cinnamon soil, chestnut soil, and alpine meadow soil (Zhu et al., 2016).

2.2 Sampling and Analyses

In August 2018, based on the distribution altitude (2200–3800 m) of *L. lentopodioides* in the Qilian Mountains, we conducted a field survey. To control for confounding variables, we selected hillsides with sunny aspects and similar slopes at four altitudes: 2500, 3000, 3500, and 3800 m [Figure 1: see original paper]. The basic characteristics of each site are presented in Table 1 . At each altitude gradient, three 10 m × 10 m sample plots with 10-m intervals were randomly placed, for a total of 12 plots (4 altitudes × 3 plots). From each plot, 12–15 healthy individuals of approximately the same size were selected, and 4–5 leaves from the middle of each plant were collected and stored in envelopes. Additionally, three 1 m × 1 m quadrats were established within each plot to eliminate the effect of small-scale spatial heterogeneity on soil properties, resulting in a total of 36 quadrats (12 plots × 3 quadrats). In each quadrat, after manually removing surface litter, soil samples at 0–10, 10–20, and 20–40 cm depths were collected from the center and corners using a 35-mm diameter soil auger. Soil samples from the same depth within each quadrat were mixed in self-sealing bags, carefully labeled, and transported to the laboratory.

In the laboratory, leaves were oven-dried for 48 h at 65°C to constant weight, ground by ball milling, packed in tin foil, and labeled. After air-drying at room temperature and removing stones, roots, and other debris, soil samples were split into two portions. One portion was passed through a 10-mesh sieve for determination of soil available nitrogen (AN) and pH, while the other portion was passed through a 100-mesh sieve for determination of soil organic carbon (SOC), soil total nitrogen (STN), and soil total phosphorus (STP). SOC and leaf C were determined by the potassium dichromate oxidation-external heating method. STN and leaf N were analyzed using the Kjeldahl method (Nelson and Sommers, 1982) following digestion with H_2SO_4 and K_2SO_4-$CuSO_4$-Se accelerator, distillation by Kjeldahl's nitrogen analyzer, and titration with dilute sulfuric acid. STP and leaf P were obtained by H_2SO_4-$HClO_4$ digestion and estimated by molybdenum antimony colorimetry (Olsen et al., 1954). Soil pH was measured with a Sartorius PB-10 meter at a soil-to-water ratio of 1.0:2.5. Soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were determined using the SmartChem Discrete Auto Analyzer 200 (AMS/Westco, Italy). Briefly, 5 g of soil was mixed with 50 mL of 2 mol/L KCl, shaken for 1 h, and the extracted filtrate was analyzed for NH_4^+-N and NO_3^--N.

2.3 Data Analysis

Studies have shown that MAT and MAP can be calculated using the following formulas (Zhao et al., 2005, 2006):

$$\mathrm{MAT} = 20.96 - 5.49 \times 10^{-3} \times \mathrm{ALT} - 8.91 \times 10^{-5} \times \mathrm{LONG} + 0.17 \times \mathrm{LAT}, \quad R^2 = 0.98$$

$$\mathrm{MAP} = 1.68 \times 10^{2} + 0.12 \times \mathrm{ALT} - 75.26 \times \mathrm{LONG} + 12.41 \times \mathrm{LAT}, \quad R^2 = 0.92$$

where MAT is mean annual temperature (°C), MAP is mean annual precipitation (mm), ALT is altitude (m), LONG is longitude, LAT is latitude, and R^2 is the regression coefficient.

To verify data independence, we first tested a linear mixed model (LMM) for the fixed effect of altitude and the random effect of plots using R v.4.1.1 software (Liu et al., 2018). After normalizing all data, SPSS v.22.0 software (SPSS Inc., Chicago, IL) was used for t-tests to determine differences in parameters between altitudes. Redundancy analysis (RDA) in R software with ggrepel, vegan, and ggplot2 packages (Yang et al., 2018) was used to identify dominant environmental factors affecting leaf stoichiometry of *L. lentopodioides*. Structural equation modeling (SEM) in Amos v.24.0 software (Smallwaters Corporation, Chicago, IL, USA) was applied to estimate direct and indirect effects of dominant factors on leaf C, N, and P. Model fit was demonstrated by chi-square test (^{2}) ($P > 0.05$), normed chi-square (NC) in the range of 0–2, goodness-of-fit index (GFI) (> 0.9), and root mean square error of approximation (RMSEA) (< 0.05) (Schermelleh-Engel et al., 2003). Data in figures are presented as mean ± standard error at a significance level of $P < 0.05$.

3.1 Effects of Altitude and Plot on Soil Properties and Leaf Stoichiometry of *L. lentopodioides*

LMM results showed that, except for STP, altitude had a fixed effect on all leaf stoichiometry parameters of *L. lentopodioides* and soil properties, while plot had a random effect on only a few parameters such as STN, NH_4^+-N, SOC:STN, pH, leaf N, and leaf C:N (Table 2), indicating that altitude, rather than plot, was the primary factor affecting leaf stoichiometry of *L. lentopodioides*.

3.2 Variations in Soil Properties at Different Altitudes

Variations in SOC, STN, and NH_4^+-N with increasing altitude were similar, with higher values at 3500 and 3800 m than at 3000 m and below, suggesting that the combination of low temperature and high precipitation at high altitudes contributed to SOC and soil nutrient accumulation. SOC:STP and STN:STP showed sharp increases at 3500 m, while SOC:STN showed a relatively slow increase with altitude, indicating that the capacities of soil N and P mineralization, represented by soil stoichiometric ratios, differed among altitudes. Soil was alkaline at 2500–3000 m and weakly acidic at 3500 m and above (Table 3) due to high SOC content.

3.3 Variations in Leaf Stoichiometry of *L. lentopodioides* at Different Altitudes

With increasing altitude, leaf C of *L. lentopodioides* varied from 343.74 to 397.54 g/kg with significant differences among altitudes (Fig. 2a [Figure 2: see original paper]), implying that *L. lentopodioides* at high altitudes had potential for carbon dioxide uptake and climate change mitigation. Leaf N and P of *L. lentopodioides* showed relative stability at 2500–3000 m but changed sharply at 3500 m, with N increasing by 32.33% and P decreasing by 66.16%. Both elements showed an increasing trend from 3500 to 3800 m (Fig. 2b and c), indicating that high leaf nutrient concentrations may help plants adapt to harsh environments such as low temperature.

Leaf C:N of *L. lentopodioides* significantly declined with increasing altitude, ranging from 10.81 to 14.32 (Fig. 2d [Figure 2: see original paper]), indicating that *L. lentopodioides* improved N utilization efficiency in response to low temperature stress at high altitudes. The trend of leaf C:P was highly similar to that of N:P, with both ratios reaching their highest values at 3500 m—2–3 times higher than at other altitudes (Fig. 2e and f [Figure 2: see original paper])—suggesting that *L. lentopodioides* grew slowly due to low soil TP at this altitude.

3.4 Effects of Environmental Factors on Leaf Stoichiometry of *L. lentopodioides*

RDA results showed that soil properties and climatic factors explained 77.87% of the total variation (Fig. 3 [Figure 3: see original paper]). NH_4^+-N, pH, STP, MAT, and MAP were the dominant factors contributing to variation in leaf stoichiometry of *L. lentopodioides* at different altitudes, with contributions in the following order: NH_4^+-N > pH > STP > MAT > MAP (Table 4), indicating that leaf stoichiometry of *L. lentopodioides* was primarily determined by edaphic and climatic conditions rather than by plant community composition or interspecific competition. Soil properties had larger effects than climate.

SEM results further revealed that environmental factors explained 71%, 98%, and 34% of the variations in leaf C, N, and P, respectively (Fig. 4 [Figure 4: see original paper]). MAT directly affected leaf C, while soil pH affected leaf C indirectly through two paths: soil pH–NH_4^+-N and soil pH–STN:STP (Fig. 4a). MAT exerted a direct negative influence on leaf N. Soil pH affected leaf N mainly through three paths: soil pH, soil pH–NH_4^+-N, and soil pH–NO_3^--N–NH_4^+-N (Fig. 4b). MAP not only directly affected leaf P but also indirectly affected leaf P by influencing NO_3^--N and STN:STP, while STP indirectly affected leaf P (Fig. 4c). SEM results imply that each environmental factor played a different role in shaping leaf stoichiometry of *L. lentopodioides*.

4 Discussion

Although element concentrations and their ratios in *L. lentopodioides* showed no clear trends with increasing altitude (Fig. 2), LMM results showed that the fixed effect of altitude on leaf stoichiometry was significant, as reported in other studies (Du et al., 2017; Su et al., 2022), indicating that plants can actively adjust their nutritional requirements to maintain better growth and development in response to altitude-driven environmental changes (Cernusak et al., 2010).

Generally, altitude substantially impacts leaf stoichiometry by controlling MAT and MAP and affecting soil properties (Zhang et al., 2019). For example, variation in leaf stoichiometry of *O. ochrocephala* at different altitudes in the Qilian Mountains was mainly determined by SOC:STP, SOC, and MAT (Cao et al., 2020), while *S. chamaejasme* was mainly determined by MAT, MAP, and SOC:STN (Su et al., 2021). In the present study, altitude's impact on leaf stoichiometry of *L. lentopodioides* was mainly driven by NH_4^+-N, soil pH, STP, MAT, and MAP, supporting our hypothesis (Table 4; Fig. 3). Among these, NH_4^+-N was the most dominant environmental factor. NH_4^+-N can influence various growth metabolic processes such as photosynthesis and respiration by affecting the synthesis of chlorophyll, proteins, and enzymes in plants (Pandey et al., 2015; Fu et al., 2021). We found that the crucial factors influencing leaf stoichiometry differ among plant species even along similar altitudinal gradients in the Qilian Mountains. Because *L. lentopodioides*, *O. ochrocephala* (Cao et al., 2020), and *S. chamaejasme* (Su et al., 2021) belong to the Compositae, Leguminosae, and Thynchophyceae families, respectively, their mechanisms for nutrient capture and storage may differ (Aerts and Chapin, 1999). Furthermore, differences in community composition and soil nutrient status at sampling sites (Tables 1 and 3) (Cao et al., 2020; Su et al., 2021) may also be partly responsible (Treseder and Vitousek, 2001; Wang et al., 2014). Therefore, studies at the single-species level are required to understand each plant species' adaptation strategies to environmental changes.

4.1 Dominant Factors Influencing Leaf C, N, and P of *L. lentopodioides*

Climatic variables (MAT and MAP) were dominant factors driving changes in leaf C, N, and P of *L. lentopodioides* at different altitudes. Leaf C and N were particularly sensitive to MAT, while leaf P was sensitive to MAP (Fig. 4). MAT directly and positively affected leaf C of *L. lentopodioides* (Fig. 4a) because MAT can influence enzyme activity and plant photosynthesis and C sequestration capacity (Wu et al., 2011). Conversely, MAT directly and negatively affected leaf N of *L. lentopodioides* (Fig. 4b). Leaf N was generally lower at low versus high altitude in this study (Fig. 2b), consistent with the hypothesis that low temperatures reduce plant enzyme efficiency and ribonucleic acid (RNA) synthesis efficiency, and that plants might increase leaf N to compensate for reduced physiological efficiency (Reich and Oleksyn, 2004). MAP directly and positively affected leaf P of *L. lentopodioides* (Fig. 4c), with similar results reported by Su et al. (2022). In high-altitude areas with high precipitation and low temperature (Table 1), *L. lentopodioides* may absorb more P into leaves to promote synthesis of soluble proteins and proline, preventing cell freezing and dehydration and improving cold resistance (Puhakainen et al., 2004; Reich and Oleksyn, 2004; Patton et al., 2007). However, Ding et al. (2012) reported contrary results because low MAP (230.12–360.18 mm) in their study area meant plants did not need to store large amounts of P in leaf non-photosynthetic tissues to resist drought stress as precipitation increased.

Soil pH, an important chemical property, significantly affected leaf C and N. For example, soil pH directly and negatively affected leaf N (Fig. 4b), consistent with Gong et al. (2017). Generally, in alkaline soils, salt content shows a colinear relationship with soil pH (Zhao et al., 2018). In our study area, soil at 2500–3000 m was alkaline (pH = 8.42–8.52) (Table 3), and high soil pH represented high soil salinity (Sun et al., 2017), which would directly impair N uptake by *L. lentopodioides* roots and result in reduced leaf N (Cramer et al., 1986; Rong et al., 2015). Additionally, both soil pH and MAP indirectly affected leaf nutrient concentrations of *L. lentopodioides* by influencing soil properties (Fig. 4). Soil pH affected leaf C by influencing NH_4^+-N and STN:STP, and leaf N by NH_4^+-N and NO_3^--N, while MAP affected leaf P by influencing NO_3^--N and STN:STP (Fig. 4). Soil pH and MAP are often related to the activities of many microorganisms (Chen et al., 2021), which can affect STN replenishment by influencing litter decomposition and affect soil available nitrogen (SAN) by regulating the rate of soil organic matter decomposition and N mineralization (Fierer et al., 2009; Hou et al., 2018; Huang et al., 2018; Liu et al., 2019; Li et al., 2020). Moreover, increasing MAP can increase litter yield and promote STN accumulation by facilitating plant growth and biomass accumulation (Bai et al., 2008; Nakagawa et al., 2019). In contrast, STP is mainly derived from parent rock weathering, influenced by soil parent material, and much less sensitive to the environment than STN (Aerts and Chapin, 1999), as shown in our results (Table 1). Therefore, variation in STN with changes in soil pH and MAP was responsible for STN:STP variation.

Soil nutrients and stoichiometry also significantly affected leaf nutrient concentrations. NH_4^+-N was a critical factor influencing leaf C and N of *L. lentopodioides* (Fig. 4a and b) because N significantly affects plant chlorophyll synthesis as the main component of chlorophyll, directly influencing photosynthesis (Fu et al., 2021). Although soil total nitrogen (TN) is typically used to measure basic soil N fertility, soil available nitrogen (SAN) (NH_4^+-N and NO_3^--N) is more closely related to plant growth (Fois et al., 2009; Pandey et al., 2015). Compared with NO_3^--N, NH_4^+-N may be more easily absorbed and utilized by plants because plants can use less ATP and energy to absorb NH_4^+-N and assimilate it into amino acids (Ruan and Giordano, 2017). Therefore, *L. lentopodioides* growing in alpine environments may preferentially absorb NH_4^+-N, thus saving growth costs to favor plant growth (Miller and Bowman, 2002), though this requires further research. The conversion of mobile NO_3^--N to non-fluid NH_4^+-N (Fig. 4b) is a protective mechanism for soil N to prevent N loss, which would provide more NH_4^+-N for *L. lentopodioides* growth and thus promote its growth (Cheng et al., 2022). NO_3^--N also affected plant nutrient uptake (Fig. 4c) because NO_3^--N, as an anion, can promote plant uptake of cations such as K, Ca, and Mg and inhibit uptake of P and other anions (Ruan et al., 2000). Moreover, studies have shown that NO_3^--N acts as a signaling molecule to regulate plant growth (Crawford and Glass, 1998; Medici et al., 2019), which may also account for the effect of NO_3^--N on leaf P (Fig. 4c). STN:STP, as one of the soil nutrient limitation indicators, not only affects plant N and P supply and soil microbial activities (Fanelli et al., 2008; Tang et al., 2018; Zhao et al., 2018) but also affects plant photosynthesis because plant C fixation cannot be achieved without protease involvement (i.e., N), and protease assembly also demands large amounts of nucleic acid replication (i.e., P) (An et al., 2011). Therefore, STN:STP significantly affected leaf C and P (Fig. 4a and c).

4.2 Variations in Leaf Stoichiometric Ratios and Nutrient Limitation of *L. lentopodioides*

Leaf C:N of *L. lentopodioides* decreased, while leaf C:P increased and then decreased with increasing altitude. Both ratios reached their minimum values at 3800 m (Fig. 2d and e). Since leaf C:N and C:P reflect plant N and P utilization efficiency and growth rate (Elser et al., 2003; Sun et al., 2017), the N and P nutrient utilization efficiency and growth rate of *L. lentopodioides* may be relatively high at 3800 m.

Compared with single nutrient concentrations, nutrient ratios can more accurately reflect the nutrient supply status of the environment for plant growth (Yan et al., 2016). Koerselman and Meuleman (1996) proposed that plants are P-limited when leaf N:P > 16, N-limited when leaf N:P < 14, and co-limited by N and P when $14 \leq$ N:P ≤ 16. In this study, leaf N:P ratios of *L. lentopodioides* at all altitudes were greater than 16. However, leaf N:P not only reflects soil nutrient limitation but also relates to plant genetic characteristics (Reich and Oleksyn, 2004). Accordingly, there is considerable uncertainty in determining nutrient limitation of *L. lentopodioides* growth by leaf N:P alone (Du et al., 2020; Hou et al., 2021). Therefore, it is essential to assess growth limitation in conjunction with soil nutrient status. In this study, the mean SOC:STN was 9.76, lower than 25.00, indicating high soil N mineralization capacity and abundant SAN supply for plant growth in this area (Jiang et al., 2019). The mean STP (0.39 g/kg) was lower than the Chinese average (0.65 g/kg), while the mean SOC:STP (130.45) and STN:STP (12.23) were higher than the national averages (52.70 and 5.10, respectively) (Han et al., 2005; Tian et al., 2010). Considering that plants need more P at high altitudes than at low altitudes to resist low temperature (Cao et al., 2020; Niu et al., 2021), and in combination with the positive indirect effect of STP on leaf P (Fig. 4c), the growth of *L. lentopodioides* in the study area may be mainly limited by STP. In recent years, studies have shown that N deposition on the QTP is continuously increasing (Liu et al., 2013). In this case, N-induced plant demand for P will likely further exacerbate P limitation of *L. lentopodioides* growth (Li et al., 2016).

5 Conclusions

This study investigated changes in leaf stoichiometry of *L. lentopodioides* at 2500, 3000, 3500, and 3800 m on the northeastern QTP. Variations in leaf C and N were substantial, but leaf P changed little with increasing altitude. Except for leaf C:N, which showed a decreasing trend with altitude, altitude had little effect on leaf C:P and N:P. These results demonstrate that *L. lentopodioides* can adjust its leaf C and N in response to altitude changes to ensure normal growth, and based on leaf N:P, *L. lentopodioides* growth in this area was mainly limited by STP.

In the study area, the effects of soil properties on leaf stoichiometry of *L. lentopodioides* were larger than those of climatic conditions. Specifically, leaf C and N were mainly determined by NH_4^+-N, followed by soil pH and MAT, while leaf P was mainly determined by STP and MAP. The C sequestration capacity of *L. lentopodioides* leaves may be elevated under future warming and increased N deposition in the study area.

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