Variations in leaf traits of *Juniperus przewalskii* from an extremely arid and cold environment

Abstract

How leaf traits vary with environmental and climatic variables in cold and arid environments is an essential issue in environmental ecology. Here, we analyzed the variations in leaf nitrogen (N) and phosphorus (P) stoichiometry and leaf dry matter content (LDMC) in Qilian juniper (*Juniperus przewalskii* Kom.) growing in 14 environmentally different plots on the northeastern Tibetan Plateau. The results showed that the N and P concentrations, N:P ratio and LDMC of Qilian juniper were 10.89 mg.g⁻¹, 1.04 mg.g⁻¹, 10.80 and 483.06 mg.g⁻¹, respectively. The spatial coefficients of the variations in leaf N and P stoichiometry were significantly higher than the seasonal ones, and the correlations of leaf N and P concentrations with spatial variables were stronger than their correlations with the season. During the growing season, only the leaf N concentration and N:P ratio significantly increased. Soil nutrients were highly positively significantly correlated with leaf P concentrations but negatively correlated with the N:P ratio and LDMC. However, leaf N concentrations showed no significant correlations with soil nutrients. We suggest that the effects of temperature on the N concentration and LDMC were stronger than the effects of drought, while those on the P concentration and N:P ratio were weaker. Drought reduced leaf N and P concentrations and increased the N:P ratio and LDMC. In the arid region, with an increasing mean annual temperature (MAT), leaf N concentration significantly decreased, and LDMC significantly increased. In the semi-arid region, as MAT increased, leaf N and P concentrations significantly increased and LDMC and the N:P ratio significantly decreased. These opposite results supported the growth rate hypothesis that plant N and P concentrations increase while the N:P ratio and LDMC decrease as the growth rate increases.

Keywords nitrogen and phosphorus stoichiometry, leaf dry matter content, Qilian juniper, spatial and seasonal, climate and soil, the growth rate hypothesis

1. Introduction

Leaf dry matter content (LDMC, i.e., the ratio of the leaf dry mass relative to the saturated fresh mass) is an important trait in comparative plant ecology because it plays an important role in many critical aspects of plant growth, plant survival and the use of resources ([Osnas et al. 2013](#); [Vendramini et al. 2002](#); [Wilson et al. 1999](#); [Wright et al. 2004](#)). Plants tend to invest more in LDMC in a hostile environment ([Shipley et al. 2005](#); [Wright and Westoby 2000](#)), and plant productivity may decrease, while resistance to cold or drought may increase with an increasing LDMC ([Vendramini et al. 2002](#); [Wilson et al. 1999](#)). Additionally, nitrogen (N) and phosphorus (P) are generally considered to be the most important limiting nutrient elements for plant growth ([Agren 2008](#); [Agren et al. 2012](#); [Elser et al. 2000a](#); [Penuelas et al. 2013](#); [Sterner and Elser 2002](#); [Wieder et al. 2015](#)). N is a vital element in plant proteins and chlorophyll, thus influencing the photosynthetic rate ([Elser et al. 2000b](#); [Evans 1989](#); [Norby et al. 2010](#); [Sterner and Elser 2002](#)), while P is an indispensable element in plant nucleic acids, which rapidly increase with rapid plant growth ([Agren 2008](#); [Elser et al. 2003](#); [Elser et al. 2000b](#)). Furthermore, leaf N and P stoichiometry affect LDMC in a series of ways, such as influencing plant photosynthesis ([Elser et al. 2003](#); [Evans 1989](#); [Tang et al. 2018](#)), metabolism ([Elser et al. 2000b](#); [Reich and Oleksyn 2004](#); [Sterner and Elser 2002](#)), and cell expansion ([Singh et al. 2006](#)).

These leaf traits are closely related to soil and climate ([Chen et al. 2013](#); [Ordonez et al. 2009](#); [Yang et al. 2015](#); [Zhang et al. 2018d](#); [Zhang et al. 2016b](#)). Plants principally acquire nutrients from the soil ([Chapin et al. 2002](#)), with soil nutrient content representing an important factor regulating plant N:P stoichiometry ([Gusewell 2004](#)). Climate could impact leaf traits by affecting plant metabolism and growth rates ([Elser et al. 2003](#); [Elser et al. 2000b](#); [Reich and Oleksyn 2004](#)). The growth rate hypothesis suggests that with an increasing growth rate, LDMC, N-rich proteins and P-rich mRNAs will increase rapidly in the plant, and the rate of the increase in mRNA is greater than that of protein ([Agren 2008](#); [Elser et al. 2003](#); [Elser et al. 2000b](#); [Vendramini et al. 2002](#); [Wilson et al. 1999](#)). Simultaneously, soil nutrients and climate generally

show geographical regularity ([Kang et al. 2011](#); [Wu et al. 2012](#); [Yang et al. 2015](#); [Zhang et al. 2018a](#); [Zhang et al. 2018d](#)).

Thus, the study of variations in leaf N and P stoichiometry and LDMC across different geographical, climatic and soil variables generally reveals significant environmental patterns ([Kang et al. 2011](#); [Luo et al. 2006](#); [Migita et al. 2007](#); [Reich and Oleksyn 2004](#); [Tang et al. 2018](#); [Wright et al. 2004](#); [Yang et al. 2015](#); [Zhang et al. 2018a](#); [Zhang et al. 2018d](#); [Zhang et al. 2016b](#)), which not only promote the understandings of plant functional characteristics and survival strategies ([Elser et al. 2003](#); [Reich and Oleksyn 2004](#); [Tang et al. 2018](#); [Zhang et al. 2016b](#)), but also provide important insights for the modeling of future changes in biogeochemical cycles ([Elser et al. 2000b](#); [Liu et al. 2013](#); [Ordonez et al. 2009](#); [Zhang et al. 2018d](#)) and vegetation cover under climate change ([Penuelas et al. 2013](#); [Zhang et al. 2016b](#)).

However, to our knowledge, most of these studies have only focused on variations in a single geographical variable such as latitude (LAT), longitude (LON), altitude (ALT) or season (SEA) ([Kang et al. 2011](#); [Luo et al. 2006](#); [Migita et al. 2007](#); [Reich and Oleksyn 2004](#); [Tang et al. 2018](#); [Zhang et al. 2018a](#); [Zhang et al. 2018d](#)). Additionally, they have been concentrated in warm-humid, warm-arid or cold-humid environments ([Kang et al. 2011](#); [Liu et al. 2013](#); [Wu et al. 2012](#); [Yasumura and Ishida 2011](#); [Zhang et al. 2018a](#); [Zhang et al. 2018d](#); [Zhang et al. 2015](#)) and based on an unverified assumption that the overall trend of leaf traits with temperature is uninfluenced by other environmental factors such as drought ([Kang et al. 2011](#); [Wu et al. 2012](#); [Zhang et al. 2018a](#)).

Here, we report the results of an ecological stoichiometric study in Qilian juniper (*Juniperus przewalskii* Kom.), an endemic and dominant species that is widely distributed on the cold and arid northeastern Tibetan Plateau (NETP). This region, as part of the earth's third pole, not only encompasses the highest altitude and most complex terrain in the world but also exhibits a wide range of climatic conditions, including different levels of drought ([Yao et al. 2012](#)).

In this environment, Qilian juniper plays a key role in maintaining soil stability, conserving water, and mitigating regional droughts and floods ([Gao et al. 2013](#); [Zhang et al. 2018b](#)). Previous dendrochronology, cambial phenology, and model simulation studies have indicated that this species is highly sensitive to temperature and drought ([Gou et al. 2015a](#);

[Gou et al. 2015b](#); [Zhang et al. 2013](#); [Zhang et al. 2018b](#); [Zhang et al. 2018c](#); [Zhang et al. 2016a](#); [Zhang et al. 2014](#)) and, thus, represents an ideal study subject.

Specifically, our objectives were (1) to identify the leaf traits of Qilian juniper and their paired correlations in the cold and arid environment, (2) to compare the spatial and seasonal variations in the leaf traits with LAT, LON, ALT, and SEA, (3) to explore the effects of climatic and soil variables on the leaf traits, and (4) to examine the growth rate hypothesis that plant N and P concentrations increase, while the N:P ratio and LDMC decrease as the growth rate increases.

2. Materials and Methods

2.1. Study area and plot selection

We selected 14 plots in which Qilian juniper was present to cover a broad environmental gradient ([Fig. 1](#), [Table1](#)) on the NETP in China. All plots were located on south-facing slopes and were selected to nearly encompass the distribution boundaries of Qilian juniper in the NETP from the southernmost boundary (TLG) to the northernmost boundary (LCG₁₋₂), from the easternmost boundary (TLG) to the westernmost boundary (DLH₁₋₅), and from the lowest altitude boundary (LCG₂) to the highest altitude boundary (DLH₁). Two elevational transects consisting of 5 plots each were established at QYG₁₋₅ and DLH₁₋₅. The transect at QYG₁₋₅ extended from the valley bottom at 3170 m a.s.l. up to the upper forest limit at 3470 m a.s.l. with one plot per every ~75 m gain in elevation. DLH₁₋₅ stretched from the lower forest boundary at 3580 m a.s.l. up to the upper forest limit at 3980 m a.s.l. with one plot per every 100 m elevation increase.

According to the 11 meteorological stations in this study area, mean annual temperature (MAT) ranged from -4 °C to 6 °C and significantly decreased with increasing LAT and ALT. Mean annual precipitations (MAP) ranged from less than 100 mm to more than 500 mm, significantly increasing toward the east ([Fig. 1](#)). Because the Qilian Juniper forests are open (canopy coverage is <10%), tree-to-tree interactions between adult individuals are expected to be minimal, and environmental and climatic factors are expected to directly impact variations in leaf traits. According to MAP, the 14

plots could be divided into arid (MAP <200 mm) and semiarid (MAP >300 mm) regions ([Huang et al. 2012](#)). QYG1-5, TLG, and YHLG were located in the semiarid region, and DLH1-5 and LCG1-2 were located in the arid region ([Fig. 2](#)).

[.....Fig. 1 near here.....](#)

[.....Table 1 near here.....](#)

[.....Fig. 2 near here.....](#)

2.2. Sampling and processing

Leaves were collected 7 times (May., Early Jun., Late Jun., Early Jul., Late Jul., Aug., and Sep.) at TLG and 6 times (May., Early Jun., Late Jun., Jul., Aug., and Sep.) at the other plots during the growing season in 2013 from representative Qilian junipers individuals at each plot (4 junipers at TLG, DLH₂, LCG₂, and 5 at the other sampled plots).

At each sampling date, leaves from 8 branches from different canopy directions were collected from each selected tree with a tree pruner. The leaves collected from each tree were evenly mixed, sealed in plastic bags and brought back to the laboratory. A total of 398 samples were collected in the 14 sampling plots. In each plot, soil samples were randomly collected from 30 points in the 0-10 cm and 10-30 cm layers using a soil sampler (diameter 5 cm).

The processing and measurement of the collected samples were completed in the MOE Key Laboratory of Western China's Environmental Systems. First, the leaves were microwaved to stop metabolic activities and then dried at 65 °C until a constant weight was reached. After drying, the leaves were crushed and sieved through a 0.2 mm grid. The fresh soil samples were sieved through a 0.25 mm mesh, and roots and visible organic debris were removed by hand. Approximately 100 g of each soil sample was air-dried in a ventilated room to analyze the soil organic carbon (SOC), soil total nitrogen (STN), and soil total phosphorus (STP). The remaining portion of each soil sample was stored in a refrigerator for soil available nitrogen (SAN) and soil available phosphorus (SAP) analysis. To measure leaf N and P concentrations, the leaf samples were digested in a 380 °C H₂O₂-H₂SO₄ environment to decompose their organic matter ([Jones Jr 2001](#)). SOC analysis was conducted using the traditional wet digestion method. STN was extracted from the

soil using the semimicro Kjeldahl method ([Bremner et al. 1996](#)). Part of each soil sample was acidified with $\text{HClO}_4\text{-H}_2\text{SO}_4$ to measure STP ([Parkinson and Allen 1975](#)). SAN was extracted with a KCl (2 mol L^{-1}) solution ([Bremner et al. 1996](#)), and a portion of each fresh soil sample was processed with NaHCO_3 (0.5 mol L^{-1}) for SAP determination ([Jones Jr 2001](#)). Finally, the concentrations of N and P in all the solutions were detected using a Smart Chem 200 automatic chemical analyzer (AMS company Italy). For each soil variable, the value used here was the average from the two depths in each plot. In addition, for the 98 leaf samples collected in September, the leaf fresh weight and leaf dry weight were measured, and the ratio of leaf dry weight to leaf fresh weight was calculated as LDMC ([Perez-Harguindeguy et al. 2013](#)).

2.3. Data analysis

Leaf traits in different regions were compared by single factor analysis of variance (one-way ANOVA) after checking for normality and homogeneity of variance, followed by Tukey's multiple comparison test. The leaf N and P stoichiometry on different dates were compared by one-way repeated measures ANOVA and the Bonferroni post hoc test after checking for normality and homogeneity of variance. The data were confirmed with the Kolmogorov-Smirnov test for normality and with the Levene's test for homogeneity of variance. All data were $\log_{10}$ -transformed before variance analysis to improve the data normality.

Spatial (among plots, $n=14$) and seasonal (among sampling dates, $n=6$, at TLG the arithmetic mean of leaf N and P stoichiometry in Early Jul. and Late Jul. were calculated as the leaf N and P stoichiometry in Jul.) coefficients of variation in leaf N and P stoichiometry were calculated to assess spatial and temporal variability. The coefficients of variation of each plot among sampling dates were calculated as seasonal coefficients of variation, and we obtained 14 seasonal coefficients of variation (because of 14 plots). Similarly, the coefficients of variation for each date among the plots were calculated as spatial coefficients of variation, and we obtained 6 spatial coefficients of variation (because 6 dates were included). Student's t-test was used to identify significant differences in the coefficients of variation among

plots and dates after checking for normality.

To explore the presence of environmental associations in leaf N and P concentrations, N:P ratio and LDMC, Pearson's correlation and regression analysis was performed between the different leaf traits and between the leaf traits and the environmental variables (i.e., LAT, LON, ALT, SEA, MAT, SOC, STN, STP, SAN and SAP). The regressions were linear unless a nonlinear fit improved homoscedasticity or fit.

To identify the relationship between the soil property variables and leaf traits, we performed a redundancy analysis (RDA). The resulting ordination diagram shows the soil variables and leaf traits as vectors whose magnitude and angle indicate the statistical significance and magnitude of the correlations with an ordination axis or another vector.

To demonstrate the effects of MAT and drought on the leaf traits, we used leaf N and P concentrations, leaf N:P ratios and LDMC as a single response variable in general linear model (GLM) regressions with MAT and drought as explanatory variables. Then, a partial general linear model (partial GLM) separated the variance explained by each individual factor and their interactions. Two drought gradients were chosen: the arid region with MAP below 200mm and the semiarid region with MAP above 300 mm. In the arid region, three MAT gradients were chosen: LCG₁ (-3.01 °C), DLH₃ (-1.23 °C), and DLH₅(0.07 °C). Similarly, in the semiarid region with MAP above 300 mm, we selected QYG₁ (-2.94 °C), QYG₄ (-1.64 °C), and TLG (-0.20 °C) as temperature gradients.

As meteorological data, we used the data from the closest meteorological stations considering a lapse rate of 0.65 °C/100 m. For statistical analysis, we used IBM SPSS Statistics 22.0 (SPSS Inc. USA) and CANOCO 5.0 (Micro computer Power, Inc.) and ORIGIN 8.6 (OriginLab Corporation USA) and R 3.2.1 for data plotting.

151

152 **3. Results**

153 **3.1. Leaf traits in the cold and arid environment**

154 Considering all plots and sampling dates, the overall mean leaf N and P concentrations, N:P ratio and LDMC were 10.89

155 $\text{mg}\cdot\text{g}^{-1}$ (range 7.50-16.30), $1.04 \text{ mg}\cdot\text{g}^{-1}$ (0.57-2.13), 10.80 (4.68-17.81) and $483.06 \text{ mg}\cdot\text{g}^{-1}$ (375.90-537.55), respectively
156 ([Table 2](#)).

157 Correlation analysis between the leaf traits indicated significant positive correlations between the N and P concentrations
158 ($p<0.001$), between the N concentration and N:P ratio ($p<0.01$), and between the N:P ratio and LDMC ($p<0.001$) ([Table](#)
159 [3](#)). Significant negative correlations were found between the P concentration and N:P ratio ($p<0.001$), the N
160 concentration and LDMC ($p<0.01$), and the P concentration and LDMC ($p<0.001$).

161 [.....Table 2 near here.....](#)

162 [.....Table 3 near here.....](#)

163 **3.2. Spatial and seasonal variations in leaf traits**

164 The spatial coefficients of variation (maximum 22%) in leaf N and P stoichiometry were significantly higher than the
165 seasonal coefficients of variation (usually below 10%) ($p<0.001$), and the ranking of the spatial coefficients of the
166 variation in leaf traits from high to low was as follows: leaf P concentration>leaf N:P ratio>leaf N concentration ($p<0.05$,
167 by the t-test) ([Table 2](#)).

168 Variations in leaf traits were also observed in association with both the spatial variables and SEA, but the correlations of
169 the leaf traits with the spatial variables were generally stronger than those with SEA ([Fig. 3](#)). The correlations of the N
170 concentration with both LAT ($p<0.01$) and LON ($p<0.001$) were positive but that with ALT was negative ($p<0.001$). The
171 P concentration significantly increased with LON ($p<0.001$) and decreased with LAT ($p<0.01$) and ALT ($p<0.001$). The
172 N:P ratio was significantly positively correlated with LAT ($p<0.001$), and both the N:P ratio ($p<0.001$) and LDMC
173 ($p<0.01$) were significantly negatively correlated with LON. Only the N concentration ($p<0.001$) and N:P ratio ($p<0.05$)
174 were found to significantly change with SEA.

175 [.....Fig. 3 near here.....](#)

176 **3.3. Effects of soil nutrients on leaf traits**

177 Constrained redundancy analysis (RDA) showed that the soil nutrients (SOC, STN, STP, SAN, SAP) explained 51.7% of
178 the total variation in the leaf traits, with axes 1 and 2 explaining 49.3% and 2.1% of the total variation, respectively ([Fig.](#)
179 [4](#)). The order of their effect on leaf traits was as follows: STP>SOC>STN>SAP>SAN. In detail, all the soil variables
180 were highly positively correlated with leaf P concentrations ($p<0.001$), but negatively correlated with the N:P ratio
181 ($p<0.001$) and LDMC ($p<0.001$, except for SAN) ([Fig. 3, 4](#)). However, leaf N concentrations showed no significant
182 correlations with any soil variables (except SOC).

183 [.....Fig. 4 near here.....](#)

184 **3.4. Effects of climatic variables on leaf traits**

185 Similar to the spatial variables, the climatic variables were associated with the observed variations in the leaf traits. MAT
186 was not always a significant factor when the whole plots were considered, except for the N:P ratio ($p<0.001$) ([Fig. 3](#)). In
187 contrast, in the semiarid region (MAP>300 mm), leaf N ($p<0.001$) and P ($p<0.001$) concentrations were significantly
188 positively correlated with MAT, and the N:P ratio ($p<0.01$) and LDMC ($p<0.001$) were significantly negatively correlated
189 with MAT. In the arid region (MAP<200 mm), leaf N concentration ($p<0.001$) was significantly negatively correlated
190 with MAT, and LDMC ($p<0.01$) was significantly positively correlated with MAT. Hence, there were opposite variations
191 in leaf N concentrations and LDMC with MAT in the arid region and semiarid region. One-way ANOVA showed that leaf
192 N and P concentrations were generally higher, while the N:P ratio and LDMC were generally lower in the plots in the
193 semiarid region than in those in the arid region ([Table 2, Fig. 3](#)). Therefore, drought may reduce leaf N and P
194 concentrations and increase the N:P ratio and LDMC.

195 The results of variation partitioning indicated that both MAT (a) and drought (b) significantly affected the leaf traits
196 ($p<0.001$) ([Fig. 5](#)), while the interaction effect (ab) was quite important for leaf N:P ratio and almost nil for the N
197 concentration. Additionally, the independent effects of MAT (a) on the N concentration and LDMC were stronger than
198 those of drought, while those on the P concentration and N:P were weaker (b). According to all variances explained by

MAT, drought and their interactive effect (a+b+ab), the ranking of the sensitivity of leaf traits to the environment in descending order was as follows: LDMC>leaf P concentration>leaf N:P ratio>leaf N concentration.

[.....Fig. 5 near here.....](#)

4. Discussion

4.1. Benchmarking Qilian juniper leaf traits

This study clearly shows that leaf N and P stoichiometry of Qilian juniper in extremely arid and cold environments is inferior to both the mean global and Chinese N and P stoichiometry of terrestrial vegetation ([Han et al. 2005](#); [Reich and Oleksyn 2004](#)) ([Table 4](#)), probably because the global and China's terrestrial vegetation include all types of life forms, such as herbs, grasses, and angiosperm trees, which display greater N and P stoichiometry than conifers ([Reich and Oleksyn 2004](#)).

While the P concentration of Qilian juniper exhibited similar values to those for conifers across China, the N concentration and N:P ratio presented relatively low values compared with the means for conifer species across China ([Han et al. 2005](#)) ([Table 4](#)), possibly because the low temperature and intense drought conditions in our study area compared with other regions across China decrease the decomposition and dissolution rates of soil minerals, leading to reduced availability of soil N and P ([Hobbie et al. 2002](#); [Hobbie et al. 2000](#)). However, the arid environment also reduces the leaching rate of soil P ([Reich and Oleksyn 2004](#); [Vitousek 1982](#); [Vitousek and Sanford 1986](#)), which could explain why the P concentration was still comparable.

Leaf N and P concentrations of Qinghai spruce (*Picea crassifolia* Kom.) ([Hu et al. 2007](#)) and Chinese pine (*Pinus tabulaeformis* Carr.) ([Cao et al. 2018](#)) in western China were similar to those of Qilian juniper, possibly because Qinghai spruce, Chinese pine and Qilian juniper occur in a similar environment (i.e., cold and arid regions). ([Cao et al. 2018](#); [Gou et al. 2015a](#); [Gou et al. 2015b](#); [Hu et al. 2007](#); [Zhang et al. 2018b](#); [Zhang et al. 2016a](#)).

221 Leaf N and P concentrations of Norway spruce (*Picea abies* L.) in Europe and *Metasequoia glyptostroboides* Hu & W.C.
222 Cheng along the eastern coastline of China were distinctly higher than those of Qilian juniper ([Kang et al. 2011](#); [Zhang et](#)
223 [al. 2018a](#)) ([Table 4](#)), probably because the growth rates of Norway spruce and *M. glyptostroboides* are generally higher
224 than that of Qilian juniper ([Esper et al. 2012](#); [Gou et al. 2015b](#); [Helama et al. 2018](#); [Ponocna et al. 2018](#); [Tumajer et al.](#)
225 [2017](#); [Zhang et al. 2013](#); [Zhang et al. 2018b](#); [Zhang et al. 2018c](#)) and N and P concentrations increase with increasing
226 growth rates ([Agren 2008](#); [Elser et al. 2003](#); [Elser et al. 2000b](#)).

227 [.....Table 4 near here.....](#)

228 **4.2. Environmental effects on leaf traits**

229 All spatial and seasonal coefficients of the variation in leaf traits were less than 25% ([Table 2](#)), indicating that leaf traits
230 of Qilian juniper were relatively stable and independent of the external environment ([Sternier and Elser 2002](#)). The
231 significant increase in leaf N concentration during the growing season ([Fig. 3](#)) might be related to the important role of N
232 in chlorophyll and in photosynthetic organs ([Elser et al. 2000b](#); [Evans 1989](#); [Norby et al. 2010](#); [Sternier and Elser 2002](#)),
233 which are more mature at the end of the growing season, as observed in Burma oak ([Yasumura and Ishida 2011](#)). The
234 seasonal variability and correlations in leaf traits were weaker than the spatial variability and correlations, most likely
235 because Qilian juniper is an evergreen species and seasonal variation has less of an effect on its leaf functional
236 characteristics. However, the spatial variations are expected to induce long-term, stable variations in leaf traits for
237 adaptation to different climate and soil properties ([Reich and Oleksyn 2004](#); [Tang et al. 2018](#); [Yang et al. 2015](#); [Zhang et](#)
238 [al. 2018d](#)), confirming our observations. Even at the genetic level, spatially different environments will select Qilian
239 juniper with different leaf traits ([Elser et al. 2000b](#); [Sternier and Elser 2002](#)).

240 The significant correlation of leaf P concentration and N:P ratio with soil nutrients ([Fig. 3, 4](#)) may be due to the fact that
241 plants mainly acquire P from soil nutrients ([Chapin et al. 2002](#); [Gusewell 2004](#)). LDMC was significantly negatively
242 correlated with soil nutrients, probably because higher soil nutrients indicate a better habitat and plants tend to invest less

in LDMC in a better environment ([Shipley et al. 2005](#); [Wright and Westoby 2000](#)). The correlations of leaf N concentrations with MAT and drought reached the level of significance, but those with soil nutrients did not, possibly for the following reasons. First, plant N cycling is a complex and lengthy process, and plants might obtain N from several other sources (e.g., biological N fixation or significantly increasing atmospheric N deposition) ([Jia et al. 2014](#); [Zhu et al. 2015](#); [Zhu et al. 2016](#)). Second, MAT and drought may directly impact leaf N concentration via metabolism and growth rate, rather than via soil nutrients ([Agren 2008](#); [Elser et al. 2003](#); [Elser et al. 2000b](#); [Reich and Oleksyn 2004](#)).

Our observations showed that the effect of temperature on leaf N concentration was stronger than that of drought while the effect of temperature on leaf P concentration was weaker than that of drought ([Fig. 5](#)), which may be related to the close relationships between temperature and N-rich chloroplasts and chlorophyll ([Elser et al. 2000b](#); [Evans 1989](#); [Norby et al. 2010](#); [Sterner and Elser 2002](#)). In contrast, the growth rate, which is mainly limited by drought in this study area ([Gou et al. 2007](#); [Gou et al. 2015a](#); [Gou et al. 2012](#); [Tian et al. 2007](#); [Zhang et al. 2013](#); [Zhang et al. 2018b](#); [Zhang et al. 2016a](#); [Zhang et al. 2009](#); [Zhang et al. 2014](#)), had a much greater impact on the P concentration than the N concentration ([Agren 2008](#); [Elser et al. 2003](#); [Elser et al. 2000b](#)). The total effects of temperature, drought and their interaction (a+b+ab), the correlations of soil variables ([Fig. 3, 4](#)), and the spatial coefficient of variation ([Table 2](#)) were significantly greater for leaf P concentration than leaf N concentration, probably because plant elements with lower concentrations (e.g., leaf P) might be more sensitive to environmental gradients than plant elements with higher concentrations (e.g., leaf N) ([Kang et al. 2011](#); [Singh et al. 2006](#); [Tang et al. 2018](#)).

4.3. The growth rate hypothesis

In general, with an increasing growth rate LDMC is expected to decrease ([Vendramini et al. 2002](#); [Wilson et al. 1999](#)). Additionally, with an increasing growth rate, N-rich proteins and P-rich mRNAs will increase rapidly in the plant, and the increasing rate of mRNA is greater than that of protein ([Elser et al. 2003](#); [Elser et al. 2000b](#)). Thus, both plant N and P concentrations will increase and the N:P ratio will decrease with an increasing of growth rate ([Agren 2008](#); [Elser et al.](#)

265 [2003](#); [Elser et al. 2000b](#)), which could explain why both leaf N and P concentrations of Qilian juniper were significantly
 266 negatively correlated while the N:P ratio was significantly positively correlated with LDMC ([Table 3](#)).

267 We observed opposite variations in leaf N concentration and LDMC with MAT in the semiarid region and the arid region
 268 ([Fig. 3, 6a, c, e, and g](#)), while higher leaf N and P concentrations and a lower N:P and LDMC were observed in the semiarid
 269 region than in the arid region ([Table 2, Fig. 3](#)), which may be due to the growth rate hypothesis that plant N and P
 270 concentrations increase, while the N:P ratio and LDMC decrease as the growth rate increases ([Agren 2008](#); [Elser et al.](#)
 271 [2003](#); [Elser et al. 2000b](#); [Vendramini et al. 2002](#); [Wilson et al. 1999](#)).

272 In the semiarid region (MAP>300 mm), a large number of tree-ring analyses have clearly shown that temperature is
 273 positively correlated with the tree radial growth of Qilian juniper in this environment ([Fig. 6i](#)) ([Gou et al. 2007](#); [Gou et al.](#)
 274 [2012](#); [Zhang et al. 2013](#); [Zhang et al. 2009](#); [Zhang et al. 2014](#)), which may be due to the close positive relationship
 275 between temperature and photosynthesis ([Elser et al. 2000b](#); [Evans 1989](#); [Norby et al. 2010](#); [Sterner and Elser 2002](#)). The
 276 growth rate of Qilian juniper would therefore be accelerated with increasing MAT. Accordingly, our observations that
 277 leaf N and P concentrations were significantly positively correlated and the N:P ratio and LDMC were significantly
 278 negatively correlated with MAT in the semiarid region ([Fig. 3, 6a and e](#)) agreed with the growth rate hypothesis, which
 279 could be confirmed by the significant decreases in leaf N and P concentrations and increase in LDMC with the
 280 elevational gradients at QYG₁₋₅ in the semiarid region ([Fig. 6b and f](#)).

281 In contrast, in the arid region (MAP<200 mm), the growth of Qilian juniper was severely limited by drought ([Fig. 6k](#)),
 282 even leading to a shrubby appearance in some cases ([Gou et al. 2007](#); [Gou et al. 2015a](#); [Gou et al. 2012](#); [Tian et al. 2007](#);
 283 [Zhang et al. 2013](#); [Zhang et al. 2018b](#); [Zhang et al. 2016a](#); [Zhang et al. 2009](#); [Zhang et al. 2014](#)). A large amount of
 284 tree-ring data has also shown that temperature is negatively correlated with the tree radial growth of Qilian juniper in this
 285 environment ([Fig. 6j](#)) ([Gou et al. 2015b](#); [Tian et al. 2007](#); [Zhang et al. 2016a](#)). Therefore, increasing MAT could lead to
 286 aggravation of drought and inhibit the growth rate of Qilian juniper in the arid region. Leaf N concentration was

negatively correlated with MAT, and LDMC was positively correlated with MAT in the arid region ([Fig. 3, 6c and g](#)), which also supported the growth rate hypothesis. The initial increase and later decrease in leaf N concentration ([Fig. 6d](#)) from low to high altitudes at DLH₁₋₅ in the arid region were consistent with a transition from drought restriction to low-temperature restriction of growth rate, and LDMC significantly decreased with the elevational gradient at DLH₁₋₅ ([Fig. 6f](#)), which also confirmed our speculation.

Drought strongly inhibited the growth rate of Qilian juniper on the NETP, and the growth rate of Qilian juniper was significantly higher in the semiarid region than in the arid region ([Fig. 6k](#)) ([Gou et al. 2007](#); [Gou et al. 2015a](#); [Gou et al. 2012](#); [Tian et al. 2007](#); [Zhang et al. 2013](#); [Zhang et al. 2018b](#); [Zhang et al. 2016a](#); [Zhang et al. 2009](#); [Zhang et al. 2014](#)). Hence, leaf N and P concentrations were significantly higher in the semiarid region than in the arid region, while the N:P ratio and LDMC were significantly lower in the semiarid region than in the arid region ([Table 2, Fig. 3](#)), which is consistent with the growth rate hypothesis.

The fifth assessment report of the Intergovernmental Panel on Climate Change showed that temperature will increase significantly in the future, especially in cold regions at high latitudes ([IPCC 2013](#)). Hence, in environments with greater MAP in which the increase in MAT might lead to an increase in the growth rate of conifers, leaf N and P concentrations might increase and leaf N:P and LDMC might decrease in the future. However, in arid environments with less MAP in which an increase in MAT may lead to the intensification of drought, leaf N and P concentrations might decrease and leaf N:P and LDMC might increase in the future.

.....[Fig. 6 near here](#).....

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465

466 **Table 1** Location and tree characteristics (mean $\pm$ standard deviation) of the 14 selected plots.

Plots	LAT (N)	LON (E)	ALT	MAT	SOC	STN	STP	SAN	SAP	Slope (°)	Height (m)	DBH (cm)
A.R. DLH1	37°29'23"	97°25'14"	3980	-2.53	68.6	3.23	0.56	16.5	3.26	30	9.3 $\pm$ 1.9	39.4 $\pm$ 8.5
A.R. DLH2	37°29'19"	97°24'24"	3880	-1.88	55.3	4.43	0.59	18.9	4.82	25	10.5 $\pm$ 1.8	35.6 $\pm$ 5.3
A.R. DLH3	37°29'17"	97°23'28"	3780	-1.23	43.1	2.89	0.43	17.0	2.28	25	10.0 $\pm$ 2.5	38.2 $\pm$ 10.8
A.R. DLH4	37°29'22"	97°22'01"	3680	-0.58	64.2	3.39	0.45	18.3	3.28	30	11.2 $\pm$ 2.7	34.1 $\pm$ 6.5
A.R. DLH5	37°28'37"	97°20'26"	3580	0.07	80.1	7.17	0.76	25.3	8.57	35	10.9 $\pm$ 2.4	29.4 $\pm$ 2.4
A.R. LCG1	39°36'50"	97°53'01"	3100	-3.01	21.3	2.13	0.53	5.97	2.40	30	7.4 $\pm$ 1.0	25.8 $\pm$ 5.1
A.R. LCG2	39°36'46"	97°53'10"	3000	-2.36	23.6	1.94	0.41	6.52	1.75	25	7.2 $\pm$ 0.9	24.6 $\pm$ 3.3
S. A.R. QYG1	38°10'46"	100°25'35"	3420	-2.94	59.5	3.37	0.43	17.9	4.62	30	11.1 $\pm$ 1.5	37.0 $\pm$ 4.3
S. A.R. QYG2	38°10'50"	100°25'37"	3470	-3.27	57.6	3.22	0.42	19.1	3.42	35	6.7 $\pm$ 0.5	17.1 $\pm$ 2.2
S. A.R. QYG3	38°10'41"	100°25'27"	3320	-2.29	53.6	3.46	0.52	19.9	6.04	25	11.2 $\pm$ 1.3	39.0 $\pm$ 6.1
S. A.R. QYG4	38°10'15"	100°26'06"	3220	-1.64	89.0	5.08	0.61	16.3	3.95	25	11.2 $\pm$ 1.1	34.7 $\pm$ 5.5
S. A.R. QYG5	38°09'41"	100°25'55"	3170	-1.32	96.0	6.57	0.71	29.7	6.62	15	10.1 $\pm$ 1.1	30.6 $\pm$ 4.5
S. A.R. TLG	36°43'20"	102°37'60"	3100	-0.20	100.5	5.91	0.86	17.5	4.89	25	6.3 $\pm$ 0.5	24.2 $\pm$ 3.0
S. A.R. YHLG	38°14'43"	99°46'33"	3250	-2.39	105.9	8.79	1.59	21.6	10.9	35	10.7 $\pm$ 2.2	33.5 $\pm$ 8.4

467 A.R.= arid region. S.A.R.= semiarid region. LAT = latitude. LON = longitude. ALT = altitude (m a.s.l.). MAT = mean annual temperature
468 (°C). SOC = soil organic carbon (mg $\cdot$ g⁻¹). STN=soil total nitrogen (mg $\cdot$ g⁻¹). STP= soil total phosphorus (mg $\cdot$ g⁻¹). SAN = soil
469 available nitrogen (mg $\cdot$ kg⁻¹). SAP = soil available phosphorus (mg $\cdot$ kg⁻¹). DBH = diameter at breast height.

470

471 **Table 2** Leaf nitrogen (N, mg·g⁻¹) and phosphorus (P, mg·g⁻¹) concentrations, N:P ratio, and leaf dry matter content (LDMC, mg·g⁻¹)

472 of Qilian juniper (*Juniperus przewalskii*) and their spatial and seasonal coefficients of variation grouped by plots and sampling dates.

Category	N				P				N : P ratio				LDMC
	Mean	SD	Spatial CV	Seasonal CV	Mean	SD	Spatial CV	Seasona l CV	Mean	SD	Spatial CV	Seasonal CV	Mean
A.R. DLH1	10.09ab	1.12	-	11.06%	0.95abc	0.10	-	10.90%	10.74cde	0.91	-	8.46%	460.66bc
A.R. DLH2	10.22abc	0.73	-	7.11%	1.03bcd	0.07	-	6.68%	10.09cd	0.78	-	7.72%	476.32cde
A.R. DLH3	9.99ab	0.49	-	4.91%	0.88a	0.05	-	5.28%	11.47e	0.69	-	5.99%	495.66def
A.R. DLH4	10.93bcd	0.90	-	8.28%	0.93ab	0.06	-	6.36%	11.86ef	0.74	-	6.28%	505.82ef
A.R. DLH5	9.23a	0.38	-	4.16%	0.92ab	0.06	-	6.42%	10.12cd	0.77	-	7.57%	519.36f
A.R. LCG1	11.30cd	0.46	-	4.04%	0.89a	0.05	-	5.16%	12.95f	0.67	-	5.15%	499.92def
A.R. LCG2	11.10bcd	0.55	-	4.94%	0.89a	0.04	-	4.52%	12.61ef	0.66	-	5.27%	502.60def
S. A.R. QYG1	10.47bc	0.26	-	2.50%	0.91ab	0.08	-	9.28%	11.78ef	1.05	-	8.87%	506.18ef
S. A.R. QYG2	11.60d	0.57	-	4.88%	1.06cd	0.09	-	8.64%	11.08de	0.73	-	6.62%	513.00ef
S. A.R. QYG3	11.01bcd	0.56	-	5.12%	1.02bcd	0.08	-	7.45%	10.85de	0.58	-	5.30%	489.14cdef
S. A.R. QYG4	11.00bcd	0.58	-	5.26%	1.12d	0.04	-	4.02%	9.89c	0.48	-	4.82%	477.92cde
S. A.R. QYG5	13.26d	0.92	-	6.95%	1.15d	0.09	-	8.20%	11.69ef	0.92	-	7.91%	465.12cd
S. A.R.TLG	11.79d	0.55	-	4.69%	1.33e	0.08	-	6.28%	8.95b	0.37	-	4.10%	425.73ab
S. A.R.	10.77bcd	0.65	-	6.01%	1.51e	0.11	-	7.35%	7.39a	0.59	-	8.04%	416.62a
YHLG													
May.	10.71B	1.07	9.97%	-	1.06	0.23	21.64%	-	10.58A	1.70	16.11%	-	
Early Jun.	10.31A	0.96	9.28%	-	1.03	0.20	19.89%	-	10.38A	1.70	16.35%	-	
Late Jun.	10.71AB	1.30	12.17%	-	1.02	0.16	15.32%	-	10.80AB	1.41	13.02%	-	
Jul.	11.00BC	0.80	7.25%	-	1.03	0.17	16.21%	-	11.00AB	1.63	14.86%	-	
Aug.	11.58C	1.08	9.30%	-	1.06	0.18	17.21%	-	11.19B	1.46	13.05%	-	
Sep.	11.09BC	1.19	10.76%	-	1.05	0.24	22.74%	-	10.95AB	1.71	15.61%	-	
Mean	10.89	1.52	9.79%	5.71%	1.04	0.25	18.84%	6.90%	10.80	1.93	14.83%	6.58%	483.06
p		<0.001					<0.001					<0.001	

473 Different lowercase letters in the same column represent significant differences between the plots (p<0.05, Tukey post hoc test) and

474 different capital letters in the same column represent significant differences between the seasons ($p < 0.05$, Bonferroni post hoc test).

475 A.R= arid region. S.A.R.= semiarid region. SD= standard deviation. CV= coefficients of variation. The means are the arithmetic

476 average among all samples. P indicates the confidence levels of the differences between the spatial CV and the seasonal CV, tested

477 with the t-test.

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Table 3 Summary of regression analyses among leaf nitrogen (N, $\text{mg}\cdot\text{g}^{-1}$) and phosphorus (P, $\text{mg}\cdot\text{g}^{-1}$) concentrations, the N:P ratio, and leaf dry matter content (LDMC, $\text{mg}\cdot\text{g}^{-1}$).

	N vs. P	N vs. N:P ratio	P vs. N:P ratio	LDMC vs. P	LDMC vs. N	LDMC vs. N:P ratio
r	+0.52	+0.13	-0.78	-0.76	-0.35	+0.70
r²	0.28	0.02	0.6	0.58	0.12	0.50
slope	0.83	0.17	-0.67	-2.52	-0.70	1.82
intercept	-0.85	0.85	1.03	-0.79	0.82	1.61
p	<0.001	<0.01	<0.001	<0.001	<0.01	<0.001
n	398	398	398	67	67	67

483 r: Pearson's correlation coefficient, r^2 : the explained variance.

507 **Table 4** Comparison of leaf nitrogen (N, mg·g⁻¹) and phosphorus (P, mg·g⁻¹) concentrations and the N:P ratio obtained in Qilian
 508 juniper (*Juniperus przewalskii*) in the present study with those in previous studies (the global flora, Chinese flora, Chinese conifers,
 509 *Picea crassifolia* and *Pinus tabuliformis* in western China, *Metasequoia glyptostroboides* along the eastern coastline of China, and
 510 *Picea abies* across Europe).

Data source	N (mg·g ⁻¹)		P (mg·g ⁻¹)		N:P		Reference
	Range	Mean	Range	Mean	Range	Mean	
Juniperus przewalskii	7.50-16.30	10.89	0.57-2.13	1.04	4.68-17.81	10.80	Present study
Global flora	4.1–59.9	18.3	0.1–6.99	1.42	2.6–111.8	11.8	(Reich and Oleksyn 2004)
Chinese flora	6.25-52.61	18.6	0.05-10.27	1.21	3.28-78.89	14.4	(Han et al. 2005)
Global conifers	8-30	-	0.5-4	-	5-20	-	(Reich and Oleksyn 2004)
Chinese conifers	-	11.7	-	1.06	-	13	(Han et al. 2005)
Picea crassifolia	6.73-13.51	9.75	0.72-1.71	0.97	-	10.05	(Hu et al. 2007)
Pinus tabuliformis	-	11.3	-	0.9	-	-	(Cao et al. 2018)
Metasequoia glyptostroboides	19.9-31.5	25.04	1.53-2.02	1.75	12.74-15.57	14.34	(Zhang et al. 2018a)
Picea abies	9.45-19.15	13.37	0.4-3.5	1.41	4.99-19.29	9.76	(Kang et al. 2011)

512 **Fig. 1** Map of the northeastern Tibetan Plateau with the locations of the 14 plots (red triangles) and closest meteorological stations
 513 (black dots). The graph below indicates the variations of mean annual temperature (MAT) with altitude (ALT) and latitude (LAT) and
 514 the variation of mean annual precipitation (MAP) with the longitude (LON) of the meteorological stations.

517 **Fig. 2** Spatial distribution of mean annual precipitation (MAP) on the northeastern Tibetan Plateau as interpolated from meteorological
518 station data within the study area.

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Fig. 3 Correlations matrix between the examined leaf traits and variables.

The leaf traits are as follows: N= leaf nitrogen concentrations (n=398, 195 in the arid region, and 203 in the semi-arid region); P=leaf phosphorus concentrations (n=398, 195 in the arid region, and 203 in the semi-arid region); N:P=leaf N:P ratio (n=398, n=195 in the arid region, and n=203 in the semi-arid region); and LDMC=leaf dry matter content (n=67, n=33 in the arid region, and n=34 in the semi-arid region). The variables are as follows: LAT=latitude; LON=longitude; ALT=altitude; SEA=season; DRO=drought; MAT=mean annual temperature; MAT_A=MAT in the arid region (mean annual precipitation<200 mm); MAT_S=MAT in the semiarid region (mean annual precipitation>300 mm); SOC=soil organic carbon; STN=soil total nitrogen; STP=soil total phosphorus; SAN=soil available nitrogen; and SAP=soil available phosphorus. Colors indicate the Pearson correlation coefficients between the leaf traits and the variables except for DRO. +, significantly positive correlation. -, significantly negative correlation. For DRO, red and + represent promotion of DRO, and blue and - represent inhibition of DRO, tested by one-way ANOVA.

536 **Fig. 4** Two-dimensional RDA scatter diagram for identifying the relationships between leaf traits (blue arrows) and soil
 537 variables (red arrows).

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539 The leaf traits are as follows: Leaf N=leaf nitrogen concentrations; Leaf P=leaf phosphorus concentrations; Leaf

540 N:P=leaf N:P ratio; and LDMC=leaf dry matter content. The soil variables are as follows: SOC=soil organic carbon;

541 STN=soil total nitrogen; STP=soil total phosphorus; SAN=soil available nitrogen; and SAP=soil available phosphorus.

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551 **Fig. 5** Variation partitioning of mean annual temperature (MAT) and drought for leaf nitrogen (N, $\text{mg}\cdot\text{g}^{-1}$) (A) and phosphorus (P,
 552 $\text{mg}\cdot\text{g}^{-1}$) (B) concentrations, the N:P ratio (C), and leaf dry matter content (LDMC, $\text{mg}\cdot\text{g}^{-1}$) (D).

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 554 a and b indicate the independent effects of MAT and drought, respectively; ab denotes the interactive effect. Bold numbers indicate a
 555 significant effect ($p<0.05$).

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566 **Fig. 6** Variations in leaf nitrogen (N, $\text{mg}\cdot\text{g}^{-1}$) concentrations and leaf dry matter content (LDMC, $\text{mg}\cdot\text{g}^{-1}$) in Qilian juniper (*Juniperus*
 567 *przewalskii*) with increasing mean annual temperature (MAT) and altitude (ALT) in the semiarid region (QYG₁₋₅) and the arid (DLH₁₋₅)
 568 region. The graph below shows the correlation coefficients of the tree-ring width of Qilian juniper (*Juniperus przewalskii*) with
 569 monthly climatic factors during the growing season.

570 Linear fit of (a) MAT and N in the semiarid region ($n=203$ $r=0.31$ $p<0.001$); (b) ALT and N at QYG₁₋₅ ($n=145$ $r=-0.39$ $p<0.001$); (c)
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573 MAT and N in the arid region ($n=195$ $r=-0.28$ $p<0.001$); (e) MAT and LDMC in the semiarid region ($n=34$ $r=-0.58$ $p<0.001$); (f) ALT
574 and LDMC at QYG₁₋₅ ($n=25$ $r=0.87$ $p<0.001$); (g) MAT and LDMC in the arid region ($n=33$ $r=0.48$ $p<0.01$); and (h) ALT and LDMC
575 at DLH₁₋₅ ($n=24$ $r=-0.83$ $p<0.001$); and quadratic fit of (d) ALT and N at DLH₁₋₅ ($n=142$ $r=-0.23$ $p<0.05$). Correlation coefficients of
576 (i) tree-ring width in the semiarid region with temperature (T) ([Gou et al. 2012](#)); (j) tree-ring width in the arid region with T ([Tian et al.](#)
577 [2007](#)); and (k) tree-ring width in the study region with the Palmer drought severity index (PDSI) ([Zhang et al. 2009](#)). The bars filled
578 with color indicate that the significance level is above 0.05.

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