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PHYSIOLOGICAL AND ECOLOGICAL RESPONSES OF AN ALPINE PLANT *PICEA LIKIANGENSIS* AT DIFFERENT ALTITUDINAL GRADIENTS

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Abstract

Picea likiangensis (Franch.) Pritz., an evergreen alpine conifer woody plant species is endemic to Yulong snow mountains, Northwest China. It plays a crucial role in maintaining the succession and stability of an ecosystem. In this study, a multitude of physiological indices, such as chlorophyll, malondialdehyde, soluble sugar, proline, soluble protein, antioxidant enzymes (ascorbate peroxidase, catalase, peroxidase and superoxide dismutase), nutrient content (carbon, nitrogen and phosphorus), C/N ratio, N/P ratio and $\delta^{13}C$, were measured in the needles of *P. likiangensis* current-year sunny branches grown at four different altitudes (ranging from 2900 to 3350 m). Our results revealed significant changes in the leaf physiological and ecological traits of *P. likiangensis* along altitudinal gradients. The contents of chlorophyll (chlorophyll a, chlorophyll b and total chlorophyll), total phosphorus and total nitrogen of *P. likiangensis* reached the maximum values at 3200 m altitude, indicating that *P. likiangensis* has the highest photosynthetic capacity at this zone. In addition, the concentrations of soluble sugar, proline and soluble protein were increased along altitudinal gradients, suggesting that *P. likiangensis* exhibits high tolerance to abiotic stress via osmotic adjustment. Furthermore, the activities of ascorbate peroxidase, catalase and peroxidase were remarkably increased from 3200 to 3350 m altitude, implying that *P. likiangensis* had suffered from heavy abiotic stress in high-altitude area. Altogether, our findings revealed that 3200 m altitude might be the optimum zone for the growth of *P. likiangensis* in Yulong Snow Mountain, Northwest China. Nevertheless, the expansion of *P. likiangensis* population was limited by harsh environment at altitudes above 3350 m.

Key words: *Picea likiangensis*; Chlorophyll; Antioxidant enzyme activity; Nutrient content; Altitudinal gradient.

Introduction

Altitudinal gradient is one of the major environmental factors that affect the morphological and physiological characteristics of various plant species, as well as their distribution patterns, especially at high-altitude mountains (Yang *et al*., 2016; Cui *et al*., 2018). With the increase in altitude, the alpine plants are exposed to higher light intensity, larger diurnal temperature fluctuations, and lower atmospheric pressure and partial CO_2 pressure (Li *et al.*, 2004; Oh *et al.*, 2013). Therefore, altitudinal gradients provide ideal natural conditions to assess the physiological and ecological responses of alpine plants to dynamic environmental changes (Siles & Margesin, 2017; Cui *et al*., 2018). Compared to lowland plants, alpine plants exhibit different types of adaptations in responses to biotic and abiotic environmental factors (Shi *et al*., 2006; Wang *et al*., 2014; Ma *et al*., 2015). Previous reports showed that high-altitude plants tended to have lower growth rate and leaf area, while higher photosynthetic capacity and carbon assimilation efficiency to protect them from abiotic stresses (Shi *et al*., 2006; Oh *et al*., 2013). However, it remains unclear how these high-altitude plants maintain their adaptation and survival strategies under alpine environments. Therefore, unraveling the morphological and physiological features of alpine trees at different altitudes is essential to understand their regulatory mechanism in response to extreme altitudes.

Previous studies have suggested that a harsh environment at high altitude is the main reason that limits the distribution and physiological function of various alpine plants (Berli *et al*., 2013; Dogra *et al*., 2013; Guo *et al*., 2016). Cui *et al*., (2018) demonstrated that the distribution, growth rates and reproduction of *Leymus secalinus* were negatively correlated with an increase in altitude on the Qinghai-Tibetan Plateau. In addition, an optimum growth altitude and suitable zones for various alpine species are existed, which can be determined by multiple environmental factors (Cai *et al*., 2012; Guo *et al*., 2016). Guo *et al.*, (2016) reported that 4100 m altitude was a suitable zone for *Abies georgei* var. *smithii* in Sygera Montain, as reflected by the high chlorophyll concentration of leaves, photosynthetic rate and physiological plasticity indicator. Besides, the leaf physiological function traits showed an increasing or decreasing trend after deviating from the optimum growth altitude (Cai *et al*., 2012; Guo *et al*., 2016). Moreover, as the main organ of photosynthesis, plant leaves are extremely sensitive to various stresses. Thus, a detailed quantitative characterization of leaf functional traits along the altitudinal gradients is needed to understand the specific adaptive strategies of plants.

The contents of mineral nutrients are very important for plant metabolism and growth. Nitrogen (N) is closely related to the proteins of photosynthetic machinery, especially Rubisco and chlorophyll biosynthesis (Grassi and Minotta, 2000). Phosphorus (P) plays an important role in constructing lipids, membranes, nucleic acids and bioenergetic molecules (e.g., ATP). In addition, leaf carbon isotope composition ($\delta^{13}C$) is positively correlated with the water-use efficiency (WUE) and diffusive CO_2 uptake by plants (Sparks & Ehleringer, 1997; Weih, 2001; Hamerlynck *et al*., 2004). Bresson *et al.*, (2011) found that alpine plants exhibited a higher proportion of biomass to roots, lower growth rate and C/N ratio, but higher leaf N content and $\delta^{13}C$ value at high-altitude environments. In the studies of *Quercus aquifolioides*, Li *et al*., (2006, 2009) observed that the N content and $\delta^{13}C$ values of leaves were increased with increasing altitude, especially when the altitude was above 2800 m, but reduced at the altitude below 2800 m; while the ratio of C/N showed an opposite pattern. This may be attributed to their xerophytic characteristics and the optimum distribution zone (Li *et al*., 2006). However, there is a lack of physiological evidence in these studies. Therefore, it is important for us to further understanding the morphological and physiological variations of plant species along altitudinal gradients.

Under ongoing altitude, most alpine plant populations can gradually acclimate and withstand the rapid changes in environmental conditions (Lindner *et al*., 2010). Several studies have revealed that the climate change adaptation of alpine plants is not only reflected by their morphological structure but also their physiological traits, including antioxidant defenses, hormone levels, cell membrane lipids and osmotic adjustment (Lindner *et al*., 2010; Schleuss *et al*., 2015). It has been widely accepted that when the plants are exposed to adverse environmental conditions (e.g., low temperature and drought stress), a high amount of reactive oxygen species (ROS) can be generated, thus resulting in the peroxidation of lipid in the membrane and damage of plant cellular components. More importantly, most alpine plants can activate both enzymatic and non-enzymatic antioxidants, such as ascorbate oxidase (APX), catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD), each of which plays important roles in scavenging ROS and preventing oxidative damage.

P. likiangensis is endemic and a dominant tree species in Yulong Snow Mountain, Northwest China. It is an evergreen conifer, distributed within an altitude range of 2500-3800 m, and plays a crucial role in maintaining biodiversity conservation in maintaining the ecosystem stability and succession, *P. likiangensis* has been widely used to assess the relationship between radial growth and climate variables (Zhang *et al*., 2018), as well as evaluate soil microbial community succession along altitudinal gradients (Luo *et al*., 2018). However, the physiological and ecological characteristics of *P. likiangensis* in response to altitudinal variation remain largely unknown. In this study, we measured the physiological indices, foliage N, C, P content and carbon isotope composition ($\delta^{13}C$), nutrient content and antioxidant enzyme activities of *P. likiangensis* at different altitudes. Our main objectives were: (i) to elucidate the eco-physiological adaption processes of alpine plant; (ii) to evaluate the relationship between the geographical distributions and eco-physiological characteristics of *P. likiangensis*; and (iii) to reveal the population expansion potential of *P. likiangensis* at high altitude.

Materials and Methods

Plant growth and experimental sites: The experiment was carried out at different altitudes of 2900, 3050, 3200 and 3350 m, on the eastern slope (approximately 40°) of Yulong Snow Mountain (26°59′ to 27°17′ N, 100°04′ to 100°15′ E, approximately 3400 m a.s.l) (Fig. 1; Guo *et al*., 2009). The average yearly temperature of this location is 6.3°C, and the highest and lowest average monthly temperatures are 13.2°C (July) and -3.5 °C (January), respectively. The dry season (from November to May) is characterized by higher temperature, stronger solar radiation, lower precipitation and drier air compared to the rainy season (from June to October). The mean annual precipitation is 967.5 mm, which mainly occurs between June and August. The soil types of the study sites are mainly composed of red soil, humus soil and mountain dark brown forest soil, with pH of 6.47 - 8.26. At 2900 m altitude, the plantation area is predominated by *P. likiangensis* and *Pinus yunnanensis* man-made forest. At 3050 m altitude, the plantation area is covered by *P. likiangensis*, *Pinus densata* and *Quercus aquifolioides* Fir-spruce mixed forest. At 3200 m altitude, the plantation area is dominated mainly by *P. likiangensis*. At 3350 altitude, the plantation area is predominated by *P. likiangensis* and *Abies fabri* Fir-spruce mixed forest.

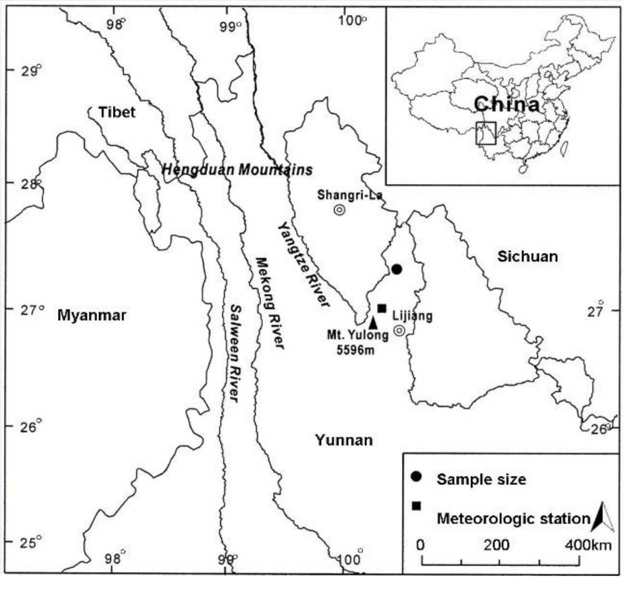

Fig. 1. Map of the experiment area in Yulong Snow Mountain Natural Reserve (Lijiang, northwestern Yunnan). Black circle indicates the sample size, while black square indicates the meteorological station (modified from Guo *et al*., 2009).

Experimental design and sampling: At each altitude, *P. likiangensis* samples were harvested on the eastern slopes (about 40°) of Yulong Snow Mountain at a 150 m vertical interval, starting from May 12, 2013 to May 20, 2013. Under the same altitudinal gradient, a total of three 50 × 50 m temporary plots, 20 m away from each other, were randomly selected. In each sample plot, one composite current-year branchlet sample was collected from ten well-developed *P. likiangensis* at each altitude. The leaves were immediately harvested from branchlets and placed in liquid nitrogen for subsequent measurement of physiological parameters as well as the contents of various nutrients and elements.

Leaf chlorophyll concentration measurements: Total chlorophyll was extracted using ethanol according to Wellburn & Lichtenthaler (1984). Fresh leaves (0.2 g) were ground using a mortar, and then extracted with 20 ml of 95% ethanol on ice (0ºC) for 12 hours in the dark, until the green chlorophyll disappeared from the leaves. The absorbances of leaf samples were measured on a spectrophotometer at 470, 649 and 665 nm. The contents of chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (TC) were measured at 649 and 665 nm; while the content of carotenoid (Car) was measured at 470 nm.

Determination of soluble sugar, soluble protein, and proline content: The concentrations of soluble sugar were detected according to the method reported by Wang (2006). Fresh leaves (0.2 g) were cut into pieces and extracted with 15 ml of distilled water in a heated bath for 20 minutes. Then, 1 ml of the extract and 5 ml of anthrone reagent were placed into a test tube and continuously heated in the boiling water bath for 10 minutes. After that, the reaction was immediately stopped by placing the test tube in ice-cold water, and the absorbance of the samples was measured at 520 nm. Meanwhile, the concentrations of soluble protein and proline were measured using the methods of Bradford (1976) and Bates *et al*., (1973), respectively. In brief, 0.5 g of leaf sample was extracted with 8 ml of 3% aqueous sulfosalicylic acid in a heated bath for 10 minutes. Then, 2 ml of supernatant was removed, added into a clean tube, mixed with acidic ninhydrin reagent and glacial acetic acid (2 ml each), and kept in the boiling water bath for 30 minutes. Finally, the reaction was stopped by adding 4 ml of ice-cold toluene, and the absorbance of the samples was measured at 520 nm.

Determination of antioxidant enzymes and MDA levels: At first, fresh leaf sample (0.2 g) was extracted with 8 ml of 0.1M phosphate-buffered saline (PBS; pH 7.8) containing 1% polyvinylpyrrolidone. Liquid nitrogen-cold pestle and mortar were used throughout the extraction process. After centrifugation at 12,000× g for 20 minutes at 4°C, the resulting supernatant was subjected to enzyme activity assays. The activities of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) were determined according to the methods reported by Duan *et al.*, (2005), Luck (1965), Tuna *et al.*, (2008) and Zaharieva *et al.*, (1999), respectively. Meanwhile, the concentration of MDA was measured using the method proposed by Draper *et al.*, (1993).

Leaf C, N and P and carbon isotopic composition analyses: The fresh leaves were cleaned thoroughly with deionized water, dried at 60°C for 72 hours, ground to a fine powder, and then stored in plastic vials for subsequent elemental concentration analyses. The contents of N and C were detected using an element analyzer (Vario-MAX-CN; Elementar Analysensysteme, Germany); while the content of P was analyzed with an inductively coupled plasma optical emission spectroscopy (7000DV; Perkin Elmer, USA). Stable carbon ($\delta^{13}C$) isotopic composition in leaf samples was detected using an Advantage Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, USA).

Statistical analysis

The statistical analyses were carried out using SPSS version 19.0 (SPSS, Chicago, IL, USA). Homogeneity of variances and normality of distributions were tested for all variables before statistical testing. Leaf physiological and ecological traits among different altitudinal gradients were compared by the Duncan's multiple range test after one-way ANOVA. Figure plotting was performed using Origin 8.5 (Microcal Inc., Northampton, MA, USA). The significance level was set at $p<0.05$.

Results

Varying contents of leaf chlorophyll along altitudinal gradients: As shown in Table 1, the chlorophyll (including Chl a, Chl b and TC) contents of *P. likiangensis* leaf samples were increased gradually at higher altitudes and reached a peak value at 3200 m altitude. Notably, the levels of Chl a, Chl b, TC and Car were 706.85, 365.61, 1072.19 and 279.67 μg g^{-1}, respectively (Table 1). Although the contents of chlorophylls were decreased when the altitude was increased from 3200 to 3350 m, the total chlorophyll content of *P. likiangensis* leaves was highly accumulated at an altitude of 3050-3350 m, ranging from 971 to 1072 μg g^{-1}, when compared to its value (750μg g^{-1}) at lower altitude (2900 m). In addition, the ratio of Chl a to Chl b was increased significantly at higher altitudes (Table 1).

Variations in leaf lipid peroxidation and osmotic adjustment along altitudinal gradients: Leaf malondialdehyde (MDA) was used as a marker of lipid peroxidation to estimate the oxidative damage of *P. likiangensis* leaves at different altitudes. As shown in Fig. 2a, the MDA content demonstrated an increasing trend from 2900 m (about 6.62) to 3200 m (about 7.32) with slight difference, and significantly increased (about 8.95) at 3350 m altitude. Notably, the content of MDA at 3350 m altitude was 1.36 times higher than that at 2900 m altitude (Fig. 2a). It was also found that the concentration of proline exhibited a similar trend with that of MDA along altitudinal gradients (Fig. 2b). Besides, the contents of soluble sugar and soluble protein in *P. likiangensis* leaves had similar increasing trends, and both showed a significant increase along altitudinal gradients (Fig. 2c, d).

Changes in the activities of antioxidant enzymes along altitudinal gradients: To investigate whether the activities of APX, CAT, POD and SOD could be altered in plant response to oxidative stress along altitudinal gradients, their activities were measured in *P. likiangensis*. As shown in Table 2, the enzymatic activities of APX, CAT and POD were significantly increased with increasing altitude, reaching their maximum values at 3350 m altitude. Interestingly, under high altitude (3350 m) conditions, the activities of APX (about 2.58-fold), CAT (about 5.91-fold) and POD (about 10.48-fold) were obviously increased compared to the values at low altitude (2900 m). We also noticed that the activity of SOD was not significantly improved during the elevation of altitude from 2900 to 3350 m (Table 2).

Table 1. Chlorophyll content of *P. likiangensis* leaves along altitudinal gradients.

Altitude (m)	Chl a (μg g^{-1} FW)	Chl b (μg g^{-1} FW)	TC (μg g^{-1} FW)	Chl a/b	Car (μg g^{-1} FW)
2900	483.52 ± 10.27 c	267.39 ± 6.54 c	750.91 ± 16.73 c	1.81 ± 0.003 b	189.45 ± 0.025 c
3050	692.29 ± 59.07 a	360.20 ± 19.19 a	1052.50 ± 74.09 ab	1.92 ± 0.032 ab	214.49 ± 0.048 b
3200	706.58 ± 55.77a	365.61 ± 21.10 a	1072.19 ± 74.72 a	1.93 ± 0.009 ab	279.67 ± 0.063 a
3350	653.05 ± 61.85 b	318.77 ± 27.59 b	971.82 ± 89.32 b	2.05 ± 0.021 a	193.51 ± 0.037 bc

Notes: Values are mean ± SE (n = 5). Different letters indicate significant differences between samples ($p < 0.05$)

Table 2. Antioxidant enzyme activities of *P. likiangensis* leaves along altitudinal gradients.

Altitude (m)	SOD (Unit g^{-1} FW)	CAT (μmol H_2O_2 min^{-1} g^{-1} FW)	APX (μmol H_2O_2 min^{-1} g^{-1} FW)	POD (μmol guaiacol min^{-1} g^{-1} FW)
2900	892.42 ± 12.26 b	1.90 ± 0.13 d	13.26 ± 1.88 c	0.338 ± 0.043 d
3050	903.57 ± 8.01 ab	2.06 ± 0.35 c	17.54 ± 3.71 c	0.806 ± 0.142 c
3200	904.40 ± 9.19 ab	9.01 ± 1.26 b	29.40 ± 2.29 b	1.744 ± 0.302 b
3350	908.03 ± 9.70 a	11.24 ± 1.86 a	34.29 ± 3.69 a	3.544 ± 0.166 a

Notes: Values are mean ± SE (n = 5). Different letters indicate significant differences between samples ($p<0.05$)

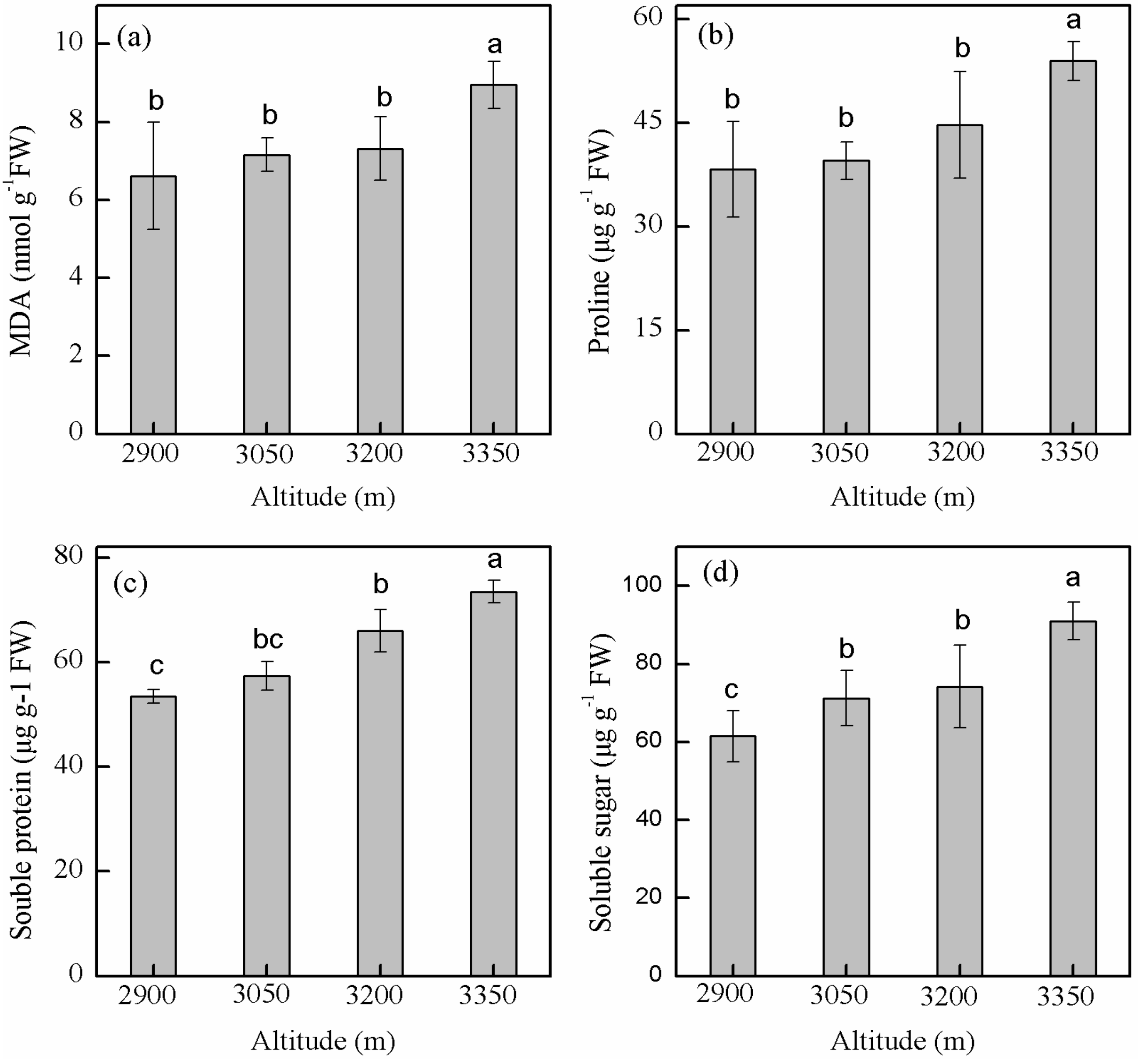

Fig. 2. Concentrations of malondialdehyde (MDA), proline, soluble sugar and soluble protein at different altitudes. Data are presented as the mean ± SE of five replicates.

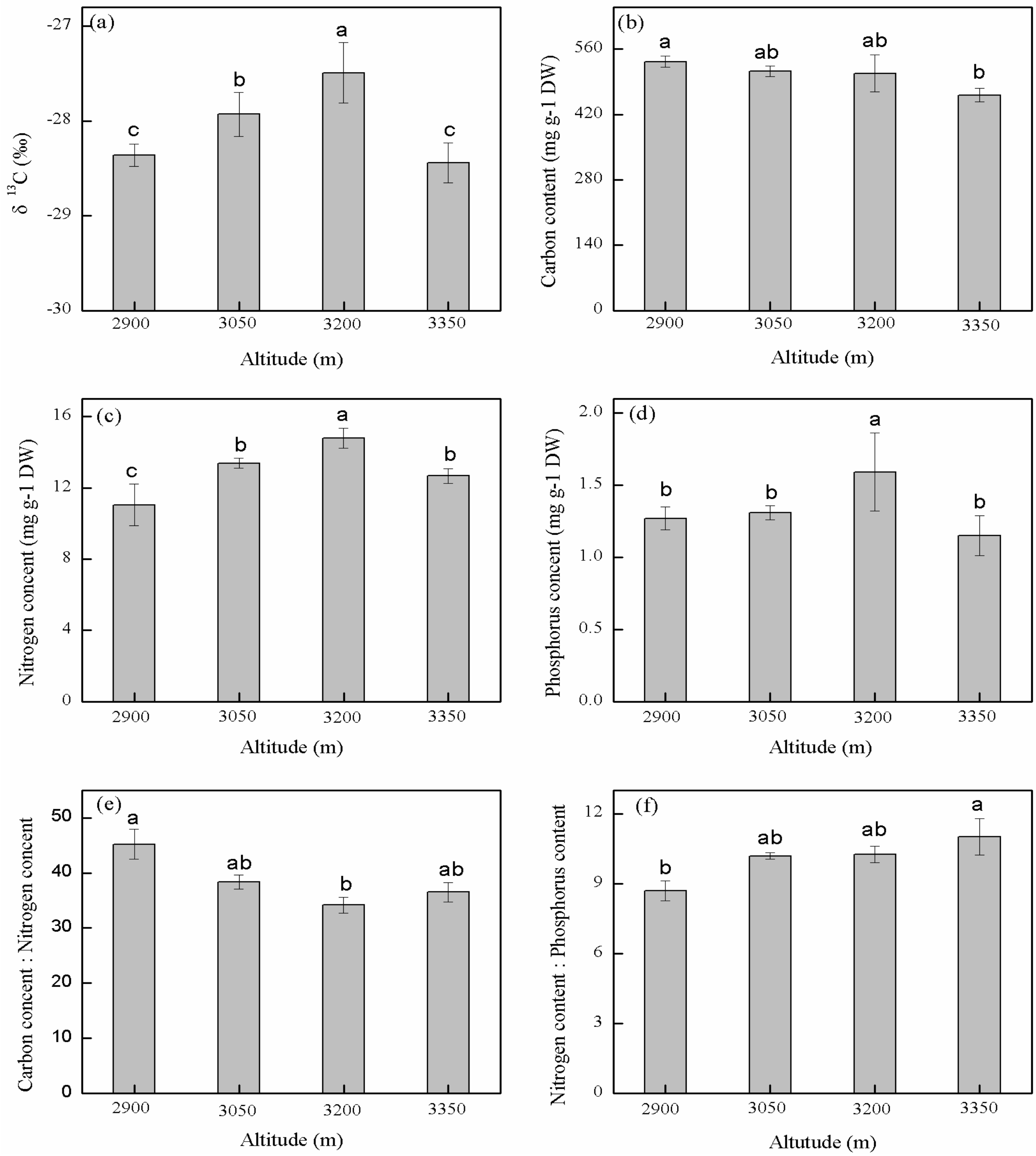

Fig. 3. The leaf δ13C, carbon (C), nitrogen (N) and phosphorus (P) content, C/N ratio and N/P ratio of *Picea Likiangensis* at different altitudes. Data are presented as the mean ± SE of five replicates.

Variations in the leaf δ^{13}C and nutrient contents along altitudinal gradients: Changes in the leaf nutrient concentration (e.g., C, N and P content), nutrient ratio (e.g., C/N and N/P) and δ^{13}C (a representative parameter of water use efficiency) of *P. likiangensis*are at different altitudes showed that there was a significant elevation effect (Fig. 3). The contents of N, P and δ^{13}C were increased from 2900 to 3200 m, achieved the peak values at 3200 m altitude, and obviously decreased at 3350 m (Fig. 3a, c, d), indicating that the most critical altitude for the alterations of N, P and δ^{13}C is 3200 m altitude. Moreover, the foliage C content showed a slight decrease tendency with increasing altitude (Fig. 3b). In contrast, the leaf N/P ratio exhibited a significant increasing trend (Fig. 3f). Besides, when the altitude was below 3200 m, the levels of leaf δ^{13}C, N and P increased significantly with increasing altitude (Fig. 3a, c, d); while the value of foliage C/N ratio exhibited an opposite pattern (Fig. 3e). It was found that the foliage N, P and δ^{13}C increased by 33.91%, 64.6% and 3.1%, respectively, from 2900 to 3200 m altitude.

Discussion

Variation in leaf chlorophyll content along altitudinal gradients: Leaf chlorophyll, as the major pigments perceiving blue and red light, is crucial for photosynthesis. The biosynthesis and degradation of chlorophyll are largely affected by various environmental signals (Li *et al*., 2013; Guo *et al*., 2016; Cui *et al*., 2018). Previous studies reported that the content of leaf chlorophyll was reduced with increasing altitude (Li *et al*., 2013; Cui *et al*., 2018), which might be attributed to the higher UV-B irradiation and lower temperature at higher altitude (Ashraf and Harris, 2013; Hazrati *et al*., 2016). In this study, the highest contents of Chl a, Chl b, TC and CAR in *P. likiangensis* were observed at 3200 m altitude, and then decreased when the altitude was above 3200 m (Table 1), suggesting that *P. likiangensis* may exhibit the highest photosynthetic capacity at 3200 m altitude.In addition, our previous work has revealed that 3200 m altitude is the core area for *P. likiangensis* survival in Yulong Snow Mountain (Cheng *et al*., 2014). This is likely due to the high variations in the physiological plasticity and regional adaptation of *P. likiangensis* at different habitats (Cui *et al*., 2018). First, the increased light intensity along altitudinal gradients might largely promote the biosynthesis of chlorophyll in *P. likiangensis*, while the decreased chlorophyll content at high altitude (3350 m) might be attributed to the inhibition of chlorophyll biosynthesis under low temperature conditions. Second, the reduced content of chlorophyll could help *P. likiangensis* to defend against oxidative damage and photoinhibition by decreasing the light energy absorption (Osone and Tateno, 2005). Third, we observed that the ratio of Chl a/b was significantly increased with increasing altitude (Table 1), which might enhance the performance of *P. likiangensis* under low-intensity light or shade conditions. In addition, compared to other alpine plant species, the higher contents of CAR could protect the chlorophyll molecules of *P. likiangensis* by absorbing a portion of light at high altitude (Cui *et al*., 2018).

Variations in leaf MDA concentration, osmotic adjustment substance content and antioxidative enzymes activities along altitudinal gradients: As the altitude increases, alpine plants may suffer from more severe abiotic stresses, which in turn leads to the accumulation of ROS, and subsequently induce cellular damage and lipid peroxidation in the membrane (Demiral & Türkan, 2005). MDA, a stable end product of lipid peroxidation, is an important index that reflects plant membrane injury caused by free radicals under various environmental stresses (Xu *et al*., 2008). In the present study, we observed that the concentration of MDA was remarkably increased when the altitude was above 3200 m, which could be resulted from a harsh growth environment (e.g., high altitude, low temperature and high solar radiation). Under unfavorable growth conditions, the plant osmoregulation and self-defense system can be stimulated to cope with oxidative stress and increase their tolerance to high-altitude environment.

Previous research indicated that osmotic regulation substances (soluble sugar, proline and soluble protein) were positively correlated with plant adaptation (Ashraf & Harris, 2013). Meanwhile, our results demonstrated that the contents of soluble sugar, proline and soluble protein were significantly increased in *P. likiangensis* along altitudinal gradients (Fig. 2). Similar results were also reported in other alpine plant species, including *Abies faxoniana* (Ran *et al*., 2013) and *Picea crassfolia* (Zhao *et al*., 2008). The increased contents of soluble sugar, proline and soluble protein in plants could increase cellular fluid, decrease osmotic potential, stabilize cell membranes and enhance their radical scavenging ability (Ma *et al*., 2015).

On the other hand, with increasing altitude, the harsher environment can lead to a higher level of ROS and a more serious oxidative damage in plant cells (Cui *et al*., 2018). Under this condition, plant antioxidant enzymes (e.g., CAT, APX and POD), which serve as the important components of antioxidant defense system interacting with each other to remove ROS and increase tolerance to abiotic stresses, can be activated. In our study, *P. likiangensis* population grown at 3200 and 3500 m altitude located near the Jinxiu valley White Water River and closer to the snowcapped mountains, thus their temperature was much lower than that of other two experiment sites. Additionally, we found that the activities of APX, CAT and POD were significantly increased in the leaves of *P. likiangensis* population grown at 3200 and 3500 m altitude, compared to those grown at 2900 and 3050 m altitude (Table 2). All these findings indicated that the harsh environment at high altitude (very low temperature and high light intensity) could stimulate the self-protective mechanism of *P. likiangensis* and was the crucial limiting factor for its expansion.

Variations in leaf $\delta^{13}C$ and **nutrient content along altitudinal gradients:** The *P. likiangensis* population grown at 3200 and 3500 m altitude is considered as pure spruce near the Jinxiu valley white water river. Therefore, the high soil moisture and extremely low air temperature may influence the contents of nutrient and numerous cellular processes in *P. likiangensis*. As shown in Fig. 3, the leaf $\delta^{13}C$, C/N, N/P ratio, and C, N and P concentrations of *P. likiangensis* leaves were not linearly increased with altitude. The concentrations of N and P reached their maximum values at 3200 m altitude and decreased when the altitude was above 3200 m (Fig. 3c, d), but the C/N ratio exhibited an opposite pattern (Fig. 3e). This pattern was consistent with a previous study (Wang *et al*., 2017) showing that the high leaf N content and C/N ratio of plants at high altitude might be ascribed to the low efficiency of physiological processes at lower temperatures. It is worth noting that the ratio of C/N can be used to estimate the long-term nutrient use efficiency (NUE) (Chen *et al*., 2005), which also indicates the amount of carbon fixed per unit nitrogen. In our study, the *P. likiangensis* populations grown at 3200 and 3500 m altitudes were relatively near to the snowcapped mountains and easily subjected to the wind blew from the snowcapped mountains. Therefore, low air temperature and low CO_2 concentration may reduce the efficiency of photosynthesis and other numerous physiological processes (e.g., nutrient uptake) in *P. likiangensis* at high altitude.

It has been observed that the value of $\delta^{13}C$ has a close relationship with long-term WUE and stomatal conductance (Marshall & Zhang, 1994; Hultine & Marshall, 2000). In this study, we observed that the leaf $\delta^{13}C$ value was increased with increasing altitude (below 3200 m), and decreased above 3200 m, suggesting that *P. likiangensis* can achieve higher WUE and lower NUE (indicated by C/N) at 3200 m altitude. Consistently, Li *et al*., (2009) have reported that high altitude plants achieved higher WUE at expense of decreasing NUE. Therefore, there is a possible trade-off between NUE and WUE at 3200 m altitude, which reflects the ability of *P. likiangensis* to maximize the efficiency of resource usage. In addtion, there was an interactive effect between elevation and environmental factors on the phenology of trees, and it was found the growth of Norway spruce (*Picea abies*) at lower altitudes initiated earlier and ceased later than at the highest altitude (Miller *et al*., 2020). Previous studies thought the precipitation and temperature were the limiting factors at lower and higher altitudes respectively (Yu *et al*., 2011). In our study, the change of $\delta^{13}C$ value along the altitude indicated that the precipitation was more limited at 3200 m site.

Conclusion

In this study, the physiological properties and nutrients traits of *P. likiangensis,* a typical alpine plant, were quantified at different altitudinal gradients. We found that the chlorophyll contents of *P. likiangensis* leaves were increased gradually and reached a peak value at 3200 m altitude, and significantly decreased at 3500 m altitude, indicating that *P. likiangensis* suffered from the harsh abiotic stress at 3350 m altitude (Table 1). On the other hand, the altitude below 3200 m had no obvious advantages to the growth of *P. likiangensis*, mainly due to the resource competition with other tree species because of the warm and humid climate, the good soil conditions and the plenty of plants in the lower altitude. Moreover, the levels of MDA (Fig. 2a), antioxidant enzymes (APX, CAT, and POD; Table 2), and osmotic adjustment substances (soluble sugar, proline and soluble protein; Fig. 2) were significantly increased at higher altitudes. Furthermore, the concentrations of $\delta^{13}C$ (an indicator of WUE), N and P achieved their maximum values at 3200 m altitude (Fig. 3). Overall, we concluded that 3200 m altitude was the optimum zone for the growth of *P. likiangensis* in Yulong Snow Mountain.

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