

Original Research

Elevation Effects on Growth Pattern and Resource Allocation of *Picea crassifolia* in Qilian Mountains, Northwest China

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Abstract

Environmental variances in temperature, moisture, and soil fertility caused by elevation gradients affect tree growth. However, the variability in regional climate and differences in tree physiology give rise to much uncertainty regarding the effects of elevation on tree growth patterns and resource allocation. In order to clarify the growth-limiting factors and physiological mechanisms of trees at different elevations, we selected *Picea crassifolia* (Qinghai spruce) at high (3300 m), middle (2850 m) and low (2585 m) elevations to analyze the interannual trends of tree radial growth and the resource allocation trade-offs involving nonstructural carbohydrates (NSC). The results were as follows: 1) the limiting factor for the growth of Qinghai spruce changed from growth limitation to carbon limitation with an increase in elevation; this was supported by the higher concentrations of NSC and its components in the whole plant and all tissues at middle and low elevations compared to high elevation, while the basal area increment (BAI) showed a decreasing trend at middle and low elevations and an increasing trend at high elevation. 2) The greatest concentrations of NSC were found in the leaves and thick roots at all three elevations, but soluble sugar (SS) and starch (ST) mainly accumulated in overground tissues (leaves and trunks) and underground tissues (fine and thick roots), respectively. 3) The ratios of soluble sugar to starch (SS/ST) at high elevations were significantly greater than those at middle and low elevations, indicating that more carbon was used for growth at high elevations, while more carbon was used for long-term energy storage at middle and low elevations. The results elucidate the growth constraints of Qinghai spruce at different elevations and the resource allocation trade-off mechanism, and offer reference points for ecological adaptation theory and conservation measures for trees at different elevations.

Keywords: nonstructural carbohydrates, growth limitation, carbon limitation, elevation effects, *Picea crassifolia*

Introduction

Global climate change has a significant impact on forests and, particularly, on the growth regimes of trees at different elevations. As the ecological transition zone between closed forest and alpine vegetation, the alpine forest line is very sensitive to climate change and is considered an ideal monitor of global climate change. Although low temperatures are thought to be the main determinant of alpine tree line formation at increasing elevations, the mechanism by which low temperatures affect tree line growth is unclear. Various hypotheses have been proposed in the debate around the causes of alpine stand formation, but it is generally agreed that either the carbon limitation hypothesis or the growth limitation hypothesis most accurately explains the physiological mechanism of alpine stand formations [1, 2]. The carbon limitation hypothesis suggests that low temperatures, drought, short growing seasons, and other environmental stress factors dysregulate the relationship between carbon absorption and carbon consumption in plants, resulting in insufficient carbon supply and thus causing the formation of alpine forest lines [3]. The growth limitation hypothesis suggests that low temperatures prevent sufficient carbon from being used by tree growth, thus limiting tree growth and leading to the formation of an alpine forest line [4].

NSC is an important energy supply and temporary solute reservoir during plant growth and metabolism, and it is closely related to changes in environmental factors, making it an important mechanism by which plants adapt to environmental changes through physiological regulation [5-7]. In particular, the concentrations of NSC in plant tissues (mainly soluble sugar and starch) are thought to result from the balance between plant carbon assimilation and carbon consumption (i.e., supply and demand), and indicate whether alpine forest line plants are carbon-limited or growth-limited. *Betula ermanii* on Changbai Mountain in Northeast China, a timberline tree species in Lapland, eastern Finland, showed lower NSC concentrations at high elevations than at low elevations, and all suffered from carbon limitation [8, 9]. *Pinus sylvestris* var. *mongolica* in Northeast Asia, *Myricaria elegans* in the arid Himalayas, and *Abies georgei* var. *smithii*, *Juniperus saltuaria*, *Betula platyphylla* and *Larix potaninii* var. *macrocarpa* in the eastern Himalayas were higher at higher elevations than at lower elevations [10-12], indicating growth limitation. Some studies have found that carbon storage is prioritized over tree growth under harsh environmental conditions (including cold, drought, wind, etc.). Tree survival depends more on metabolic carbon demand than on carbon demand for growth [13], this prioritization being a conservative strategy that often occurs regardless of environmental stress [14-16]. However, in most cases, high-elevation trees have much greater NSC concentrations than low-elevation trees [17], suggesting that growth limitation does not necessarily occur at the tree line. Low-

elevation tree growth is usually affected by drought stress. NSC concentrations increase under mild water stress conditions because growth demand drops faster than rates of photosynthesis. However, when subjected to extreme drought, the concentrations of NSC will drop due to the inhibition of photosynthesis and increases in carbon storage resulting from respiration, metabolism and defense [18, 19]. As such, the limiting factors of tree growth at different elevations are uncertain, and require further study.

The allocation pattern of NSC in plant tissues is influenced by environmental factors and reflects the adaptation strategy of plants [20]. The NSC compositions and concentrations of different tissues can indicate the balance between supply and demand of photosynthetic products under certain environmental conditions, as well as the availability of carbohydrate reserves. Leaves are the most metabolically active tissues of the plant, and are the source of NSC. Additionally, the higher concentrations of NSC leaves are related to its role as a tissue of photosynthetic carbon assimilation, which somewhat influences the metabolic rate of the leaves [21]. Additionally, roots are important sinks and mineral nutrient uptake tissues of NSC, and the growth and functional maintenance of roots requires large amounts of carbon storage in order to provide energy and facilitate carbon skeleton. Therefore, the roots also tend to contain higher concentrations of NSC. In addition, SS, active carbon storage substances in the plant's body, are preferentially directed towards actively growing tissues, such as leaves. ST is an inactive carbon storage material that is associated with plant resilience. Additionally, it is preferentially directed towards the roots, as part of the plant growth strategy of the long-term storage of energetic material to deal with adverse circumstances [22, 23]. That is, higher SS concentrations help plants to successfully survive cold and long winters. Meanwhile, the interconversion of SS and ST is considered to be an effective plant stress-tolerance mechanism; the higher the elevation, the greater the SS/ST [3]. In conclusion, the distribution pattern of NSC and its components between different elevations and tissues could reflect the resource allocation trade-off occurring in plants.

Tree rings can record the growth and vigor of trees, with the advantages of climate sensitivity, precise dating, continuity, high resolution, and wide geographical distribution [24]. They have thus become important tools for studying global climate change characteristics and plant response mechanisms [3]. The basal area increment (BAI) of trees, calculated via tree width chronology, can be used to quantify the patterns of tree radial growth, because it is a more biologically meaningful variable that overcomes the bias associated with data transformation and the decline in ring-width with age. By comparing the patterns in BAI changes at different elevations, we can better understand the growth trends of trees and thus analyze the limiting factors of tree growth at each elevation. For example, *Picea abies*, *Picea crassifolia* and *Juniperus przewalskii*

all showed declined growth patterns in the context of global warming, based on the BAI trends [25-28]. In conclusion, the BAI patterns between elevations could reflect plant growth patterns.

The Qilian Mountains are located in the northeastern part of the Qinghai-Tibet Plateau, a transition zone between the East Asian monsoon and westerly wind belts, and an important ecological security barrier in the northwest of China. In addition, Qinghai spruce, as a major component species of the forest ecosystem in the central Qilian Mountains, plays an extremely important ecosystem service role in maintaining biodiversity, water conservation, net primary productivity, and carbon sequestration in the Qilian Mountains [29]. As a typical elevation-dependent tree, the growth conditions of Qinghai spruce vary significantly with elevation. Therefore, studying the growth pattern and resource allocation of Qinghai spruce at different elevations is beneficial to better understanding the influence of elevation factors on tree growth, and supports the growth restriction theory of dominant trees in the study area. In recent years, some scholars have analyzed the NSC concentration in various tissues and the resource allocation characteristics of Qinghai spruce [30],

and also explored the causes of growth cessation in Qinghai spruce under the climate change [31]. However, no combined BAI was available to study the factors limiting the growth of Qinghai spruce at different elevations [32, 33]. In this paper, we have calculated the BAI of Qinghai spruce using the original tree ring width chronology method and compared the patterns of change in NSC and its components' concentrations between the elevations and tissues available in the Qilian Mountains. The aims were 1) to clarify the interannual variation trend of BAI at different elevations and describe the growth pattern of Qinghai spruce, 2) to compare NSC concentrations at different elevations and explore the growth-limiting mechanisms of Qinghai spruce, and 3) to analyze the inter-tissue NSC distribution and clarify the resource allocation trade-off of Qinghai spruce.

Materials and Methods

Study Area

The Qilian Mountains (36°30'-39°30'N, 93°31'-103°00'E) are located in the northeastern part of the

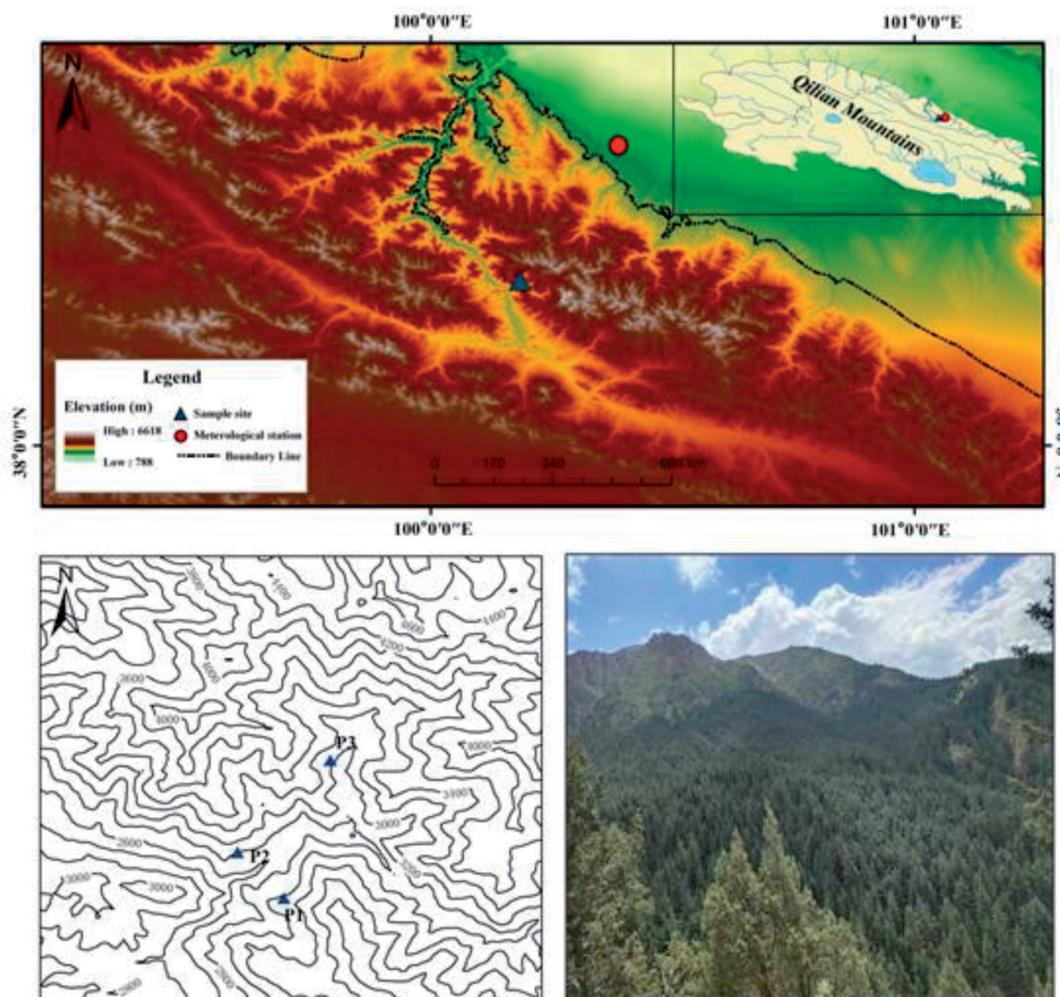


Fig. 1. Locations of the sampling sites.

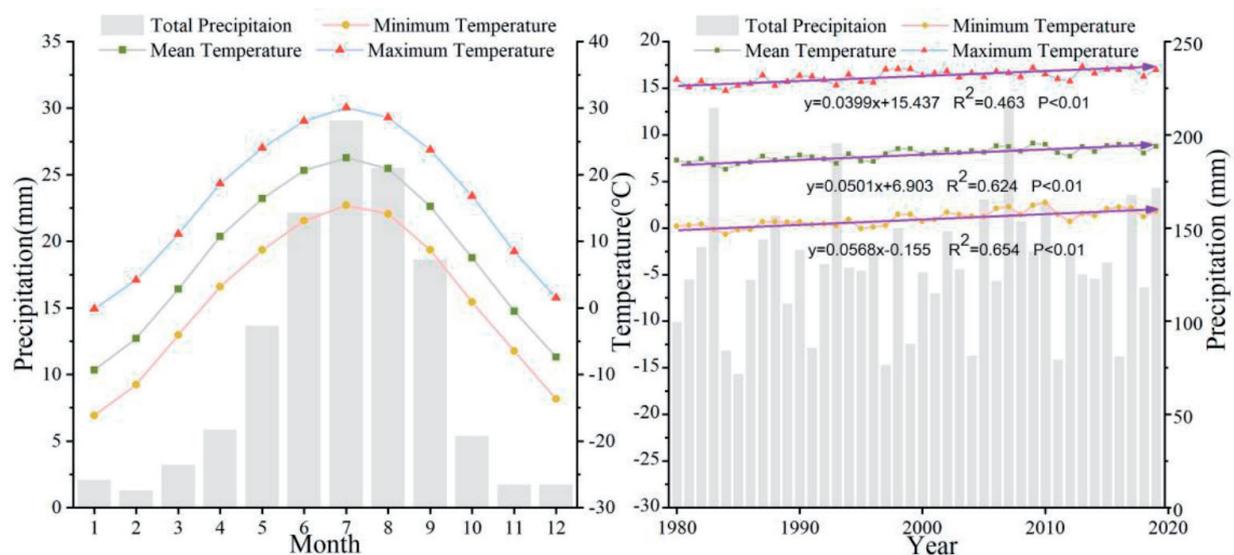


Fig. 2. Monthly and annual mean temperature and total precipitation in the Qilian Mountains during 1980-2019.

Qinghai-Tibet Plateau, which is an important mountain range in northwestern China and is the boundary between arid and non-arid areas of China. The sampling site was in the Xishui Forestry in the Qilian Mountain Reserve (Fig. 1). This region has an alpine mountain forest grassland climate with significant vertical gradients in temperature and precipitation. The average annual temperature is 7.9°C, and the annual total precipitation is 130.3 mm, with more than 70% of rainfall concentrated between June and September (Fig. 2). The elevation ranges from 2400 m to 3800 m. The soil types in the watershed mainly include mountain chestnut calcium soil, mountain gray-brown soil, subalpine scrub meadow soil, and alpine cold desert soil. The Qinghai spruce is the dominant species of Qilian Mountain's forests, and the main constituent of the water-conserving forestry, with an elevation range of 2500-3300 m on shady slopes and semi-shady slopes. The dominant shrub species are *Potentilla fruticosa*, *Caragana jubata* and *Salix gilashanica*, and the main herbs are *Polygonum viviparum*, *Carex atrata* and *Stipa*.

Sampling and Pre-Treatment

Based on the distribution characteristics of Qinghai spruce in this area, we established three sampling sites at high (P3: 3300 m), middle (P2: 2850 m) and

low elevations (P1: 2585 m) in Table 1. To minimize the effects of differences in light and needle surface temperature on carbohydrates, each sampling was conducted at noon [34]. At each sample site, five Qinghai spruce were selected that were upright, healthy, undamaged, non-isolated, and of the same size (age, height, and diameter at breast height), and samples of leaves, stems, thick roots (>5 mm in diameter) and fine roots (<2 mm in diameter) were collected. Leaf samples were taken from unshaded mature branches two years of age on the uphill face. Fine root samples were obtained via excavation at a depth of 5-30 cm into the soil layer. Three root cores were drilled with a growth cone at the base of each sample tree, i.e., in the root with the highest branching grade, for deriving thick root samples. In addition, cores of 25 healthy Qinghai spruce trees were drilled at 1.3 m (around breast height) using growth cones, with each core taken along parallel and vertical planes in each tree, as per the international standard for tree annual ring measurement. Among these, five tree cores were used as stem samples.

Tree Growth Measurement

The tree ring cores were brought back to the laboratory and processed according to the basic principles of tree chronology and standard procedures.

Table 1. Basic characteristics of sample sites.

Sample site	Elevation (m)	Slope direction	Slope (°)	Average tree height (m)	DBH (cm)	Average crown width (m)
P1	2585	North	19	16.5	31.1	4.2
P2	2850	Northwest	17	14.5	30.8	4.0
P3	3300	North	30	20.3	41.0	4.1

DBH diameter at breast height

First, the sample core was air-dried and fixed in a wood groove with white latex. Next, the sample cores were ground with 120, 400 and 600 mesh sandpapers until the growth ring boundaries were clear. Then, the initial age was determined under a microscope according to the width of the tree ring; missing and false rings were judged, and the tree ring width was measured using a Lintab 6 (RINNTECH e.K. Heidelberg, Germany) with a 0.001 mm resolution. After this, the results of the measured sample sequences were quality-checked using COFECHA software to eliminate errors in dating and width measurements, thus ensuring that each chronicle was accurately dated [35]. Finally, the original tree ring widths were measured with the individual series by calculating the bi-weight robust means chronology using the ARSTAN program.

NSC and Its Component Concentrations Measurement

The soluble sugar and starch were measured separately using UV spectrophotometer (Cary 60), and the NSC content sum of the two contents of soluble sugar and starch. Both the SS and ST concentrations were determined via the anthrone colorimetric method [36], by which 0.1 g of each tissue sample was weighed into a 10.0 ml centrifuge tube, and 5.0 ml of 80% ethanol solution was added to a boiling water bath for 15 min. The solution was cooled to room temperature and centrifuged at 4000 rpm for 10 min. The supernatant was transferred into a 50.0 ml volumetric flask 3 times, and the volume was made up to 50.0 ml with ultra-pure water for the determination of SS.

Then, 2.0 ml distilled water was added to the precipitates taken from the supernatant, which were gelatinized in a boiling water bath for 15 min. After cooling, 6.0 ml 9.2 mol l⁻¹ HClO₄ was added, which then stood overnight in a water bath at 80°C. The supernatant was cooled to room temperature and centrifuged at 4000 rpm for 10 min. The supernatant was placed into a 50.0 ml volumetric flask for the determination of ST.

The SS and ST concentrations were read from the glucose standard curve. The concentrations of SS, ST and NSC were expressed as percentage dry weight (%DM).

Data Analysis

The growth pattern and trends in tree radial growth in response to climate change were determined by calculating the basal area increment (BAI) of Qinghai spruces from the original measured ring width series. The BAI will remain consistent for decades in trees that are not senescent and not subject to environmental stress [37]. However, negative BAI growth indicates that tree radial growth has been inhibited by environmental stress. The formula for calculating BAI is as follows:

$$BAI_t = BA_t - BA_{t-1} = \pi((R_{t-1} + TRW_t)^2 - (R_{t-1})^2) \quad (1)$$

where BA is the basal area of the continuous cross section, R is the length of the tree ring from the core to year $t-1$, and TRW is the original annual ring width measured in year t .

The soluble sugar, starch, and NSC concentrations were calculated using the formula:

$$SS(\%) = (C \cdot VT_1) / (V_1 \cdot W) \quad (2)$$

$$ST(\%) = (C \cdot VT_2) / (V_1 \cdot W) \quad (3)$$

$$NSC = SS + ST \quad (4)$$

where SS represents soluble sugars, ST represents starch, NSC represents nonstructural carbohydrates, C is the value displayed by the photometer (μg), VT_1 is the volume of the extraction solution (ethanol extraction) (ml), VT_2 is the volume of the extraction solution (perchloric acid extraction) (ml), V_1 is the volume of the aspirated sample liquid (ml), and W is the weight of the selected tissue sample (g).

The concentrations of soluble sugar, starch and NSC in a whole Qinghai spruce were calculated via the concentrations of soluble sugar, starch and NSC in each tissue in relation to the biomass, via the following formula:

$$C_{\text{whole}} = \frac{\sum C_{\text{tissue}} \times B_{\text{tissue}}}{\sum B_{\text{tissue}}} \quad (5)$$

where C_{whole} is the concentration of whole plant NSC and its components (%DM), C_{tissue} is the concentration of NSC and its components in each tissue NSC, and B_{tissue} is the biomass of each tissue (g).

Statistical analysis was performed using the SPSS 12.0 statistical software. One-way ANOVA and the least significant difference method (LSD) were used to compare the statistical differences between different data groups. Two-way ANOVA was performed to analyze the effects of elevation, tissue, and the interaction between elevation and tissue on the concentrations of NSC and each of its component, as well as SS/ST, and the software Origin 2018 was used for graphing.

Results and Discussion

BAI Trends of Qinghai Spruce at Different Elevations

The trends in the BAI of Qinghai spruce were different at each of the three elevations (Fig. 3), with significant reductions at low elevations

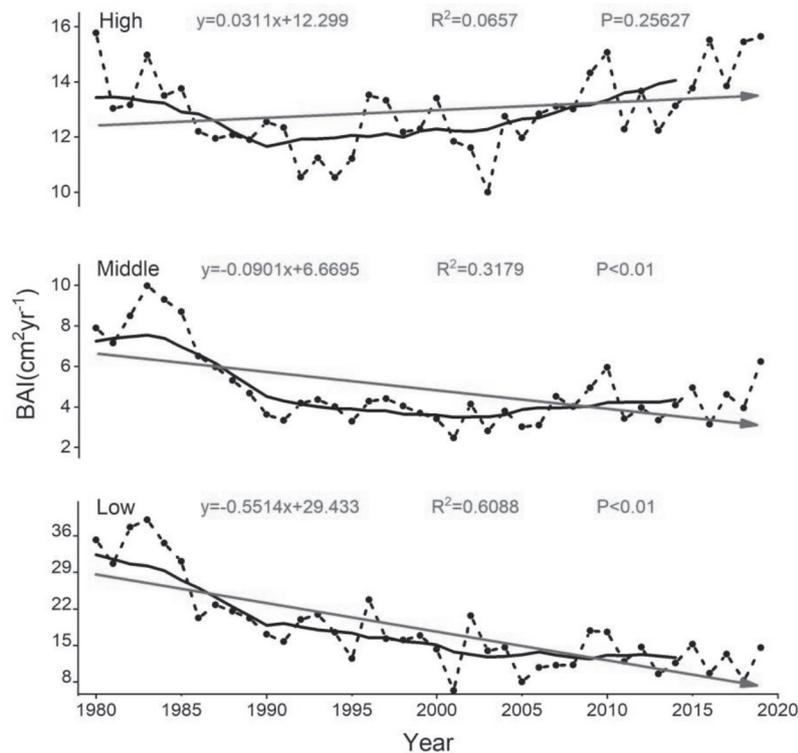


Fig. 3. BAI trends of Qinghai spruce at different elevations.

($-0.5514 \text{ cm}^2\text{y}^{-1}/10\text{a}$, $P<0.01$) and middle elevations ($-0.0901 \text{ cm}^2\text{y}^{-1}/10\text{a}$, $P<0.01$). There was a steady but non-significant upward trend in the BAI of Qinghai spruce at high elevations ($0.0311 \text{ cm}^2\text{yr}^{-1}/10\text{a}$, $P>0.05$).

Concentrations of NSC and its Component in the Whole Plant of Qinghai Spruce at Different Elevations

The SS, ST and NSC concentrations of Qinghai spruce all showed increasing then decreasing trends with increasing elevation ($P<0.01$). Specifically, SS concentrations showed the following order: middle elevation ($2.179\pm 0.029\%$)>low elevation ($1.803\pm 0.019\%$)>high elevation ($1.603\pm 0.021\%$). The ST concentrations were as follows: middle elevation ($8.600\pm 0.126\%$)>low elevation ($7.009\pm 0.114\%$)>high elevation ($3.381\pm 0.071\%$). The NSC concentrations were as follows: middle elevation ($10.780\pm 0.136\%$)>low elevation ($8.812\pm 0.122\%$)>high elevation ($4.984\pm 0.884\%$). In contrast, SS/ST showed the opposite trend ($P<0.01$), as follows: high elevation ($0.475\pm 0.006\%$)>low elevation ($0.258\pm 0.004\%$)>middle elevation ($0.254\pm 0.004\%$)(Fig. 4).

Comparison of the Concentration of NSC and Its Components Among Qinghai Spruce Tissues at Different Elevations

The trends in SS, ST and NSC concentrations in tissues at different elevations were very similar, with

all of them increasing first and then decreasing with increasing elevation ($P<0.01$) (Fig. 5).

As regards the SS concentrations in tissues, the leaf values at low, middle and high elevations were $3.524\pm 0.082\%$, $3.954\pm 0.052\%$ and $3.030\pm 0.101\%$, respectively. The stem values at low, middle and high elevations were $0.952\pm 0.006\%$, $1.163\pm 0.034\%$ and $0.867\pm 0.039\%$, respectively. The thick root values were ranked as follows: middle elevation ($1.464\pm 0.021\%$)>low elevation ($1.090\pm 0.067\%$)>high elevation ($0.906\pm 0.052\%$). The order of fine root values was as follows: middle elevation ($2.136\pm 0.104\%$)>low elevation ($1.647\pm 0.020\%$)>high elevation ($1.608\pm 0.069\%$).

As regards the ST concentrations in the tissues, the leaf values at low, middle and high elevations were $5.955\pm 0.228\%$, $7.768\pm 0.166\%$ and $4.157\pm 0.186\%$, respectively. The stem values at low, middle and high elevations were $7.493\pm 0.327\%$, $8.688\pm 0.248\%$ and $2.560\pm 0.178\%$, respectively. The thick root values were ranked as follows: middle elevation ($9.389\pm 0.354\%$)>low elevation ($8.913\pm 0.338\%$)>high elevation (4.125 ± 0.226). The fine root values were as follows: middle elevation ($8.555\pm 0.085\%$)>low elevation ($5.676\pm 0.194\%$)>high elevation ($2.683\pm 0.202\%$).

As regards the NSC concentrations in tissues, the leaf values at low, middle and high elevations were $9.479\pm 0.300\%$, $11.723\pm 0.181\%$ and $7.187\pm 0.176\%$, respectively. The stem values at low, middle and high elevations were $8.445\pm 0.324\%$, $9.851\pm 0.255\%$ and $3.427\pm 0.175\%$, respectively. The thick root values

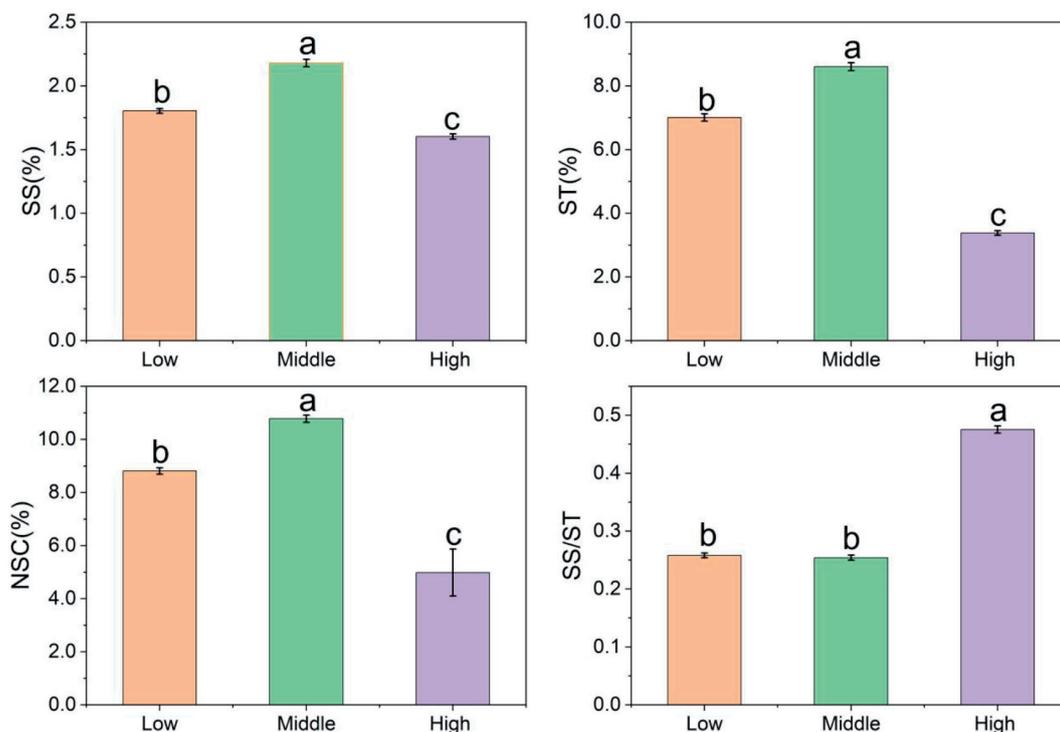


Fig. 4. Variations in NSC, SS, and ST concentrations and SS/ST in whole plants for Qinghai spruce at different elevations (Error bars represent standard deviation; different lowercase letters indicate significant differences among different elevation).

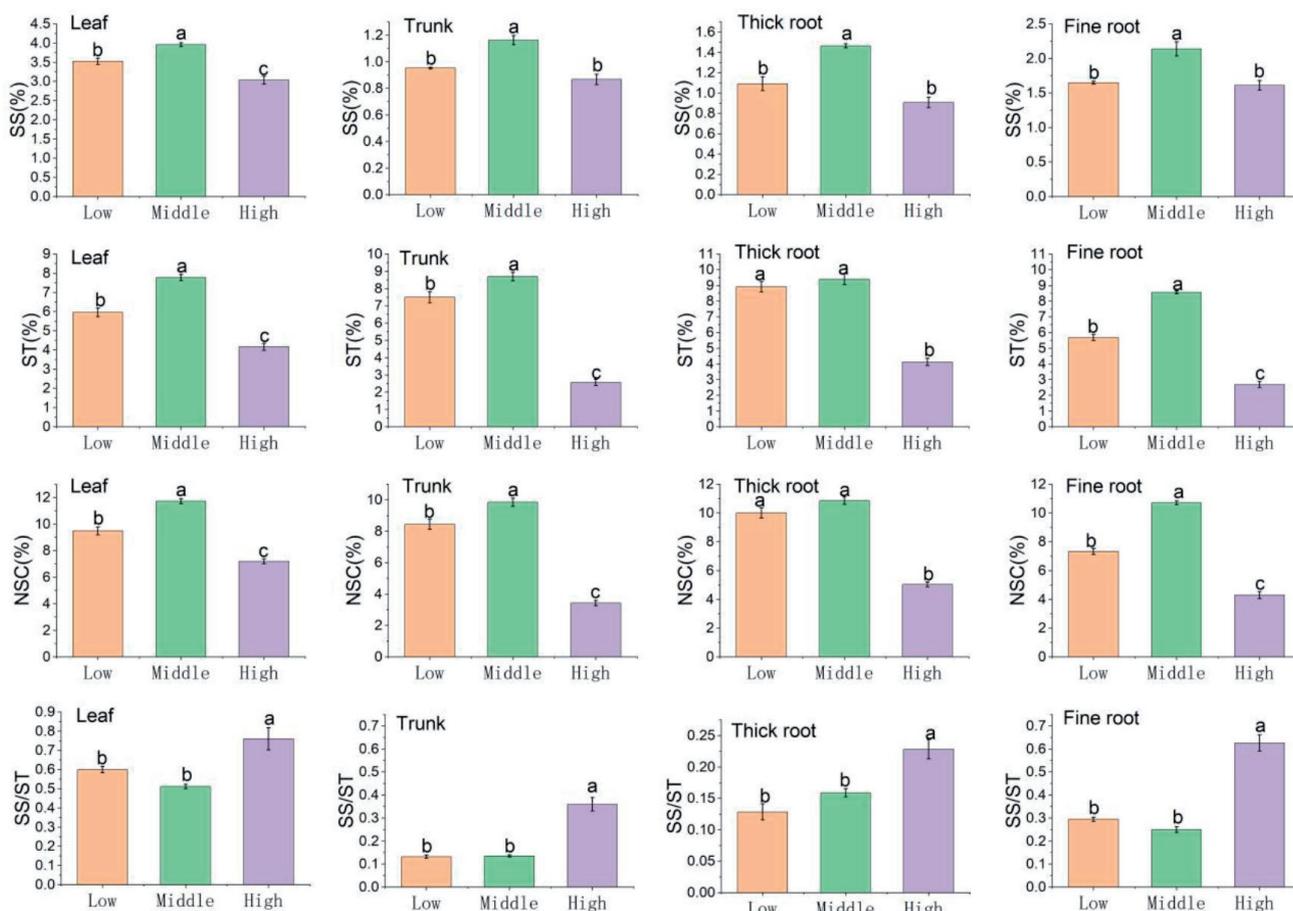


Fig. 5. Variations in NSC, SS, and ST concentrations and SS/ST in different tissues of Qinghai spruce at different elevations (Error bars represent standard deviation; different lowercase letters indicate significant differences among different elevation).

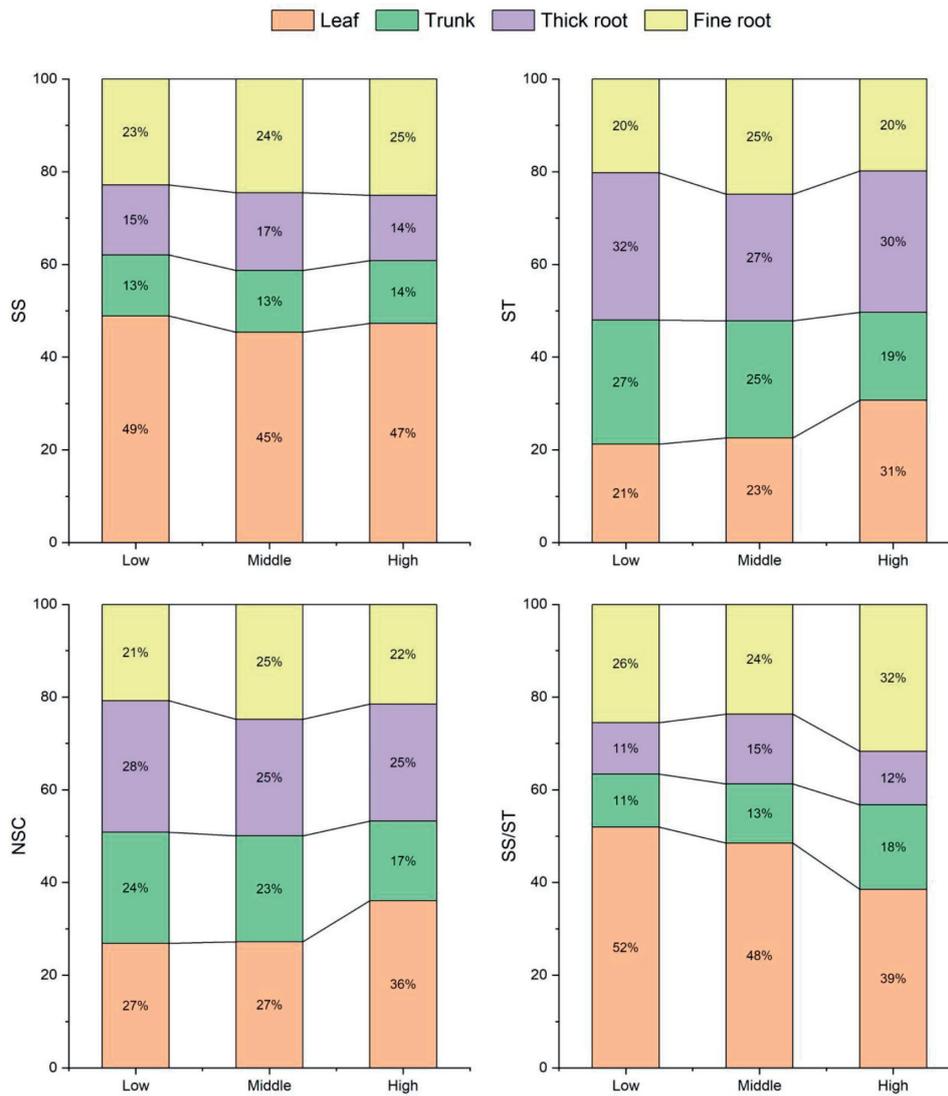


Fig. 6. Distribution pattern of NSC, SS, and ST concentrations and SS/ST in different tissues of Qinghai spruce at different elevations.

were as follows: middle elevation ($10.854 \pm 0.255\%$) > low elevation ($10.003 \pm 0.365\%$) > high elevation ($5.031 \pm 0.175\%$). The order of the fine root values was as follows: middle elevation ($10.691 \pm 0.135\%$) > low elevation ($7.322 \pm 0.211\%$) > high elevation ($4.291 \pm 0.242\%$).

The SS/ST, and the concentrations of total NSC and its components, were different at different elevations for each tissue, and the ratios were significantly higher at high elevations than at middle and low elevations ($P < 0.01$). The leaf values at low, middle and high elevations were $0.599 \pm 0.016\%$, $0.512 \pm 0.011\%$ and $0.759 \pm 0.058\%$, respectively. The stem values at low, middle and high elevations were $0.131 \pm 0.007\%$, $0.135 \pm 0.005\%$ and $0.359 \pm 0.029\%$, respectively. The order of thick root values was as follows: high elevation ($0.228 \pm 0.015\%$) > middle elevation ($0.159 \pm 0.007\%$) > low elevation ($0.228 \pm 0.015\%$). The order of fine root values was: high elevation ($0.625 \pm 0.036\%$) > low elevation ($0.294 \pm 0.009\%$) > middle elevation ($0.250 \pm 0.0130\%$).

Allocation Patterns of the Concentration of NSC and its Components in Qinghai Spruce at Different Elevations

SS and ST showed opposite distribution patterns in different tissues of Qinghai spruce at the three elevations (Fig. 6), and SS mainly accumulated in the overground tissues at 62%, 58%, and 61% at the three elevations, respectively. The accumulation levels in leaves were higher, with values of 48.9%, 45.3%, and 47.2%; those in stems were 13.2%, 13.3%, and 13.5%; in thick roots they were 15.1%, 16.8%, and 14.1%; and those in fine roots were 22.8%, 24.5%, and 25.1% at the three elevations. However, the ST mainly accumulated in underground tissues at 54%, 52%, and 50% at the three elevations, respectively. The proportions in the thick roots were higher, at 31.8%, 27.3%, and 30.5%; those in leaves were 21.2%, 22.6%, and 30.7%; those in stems were 26.7%, 25.3%, and 18.9%; and those in fine roots were 20.2%, 24.9%, and 19.8% at the three elevations, respectively.

Table 2. Effects of elevation, tissue, and their interactions on NSC, SS and ST concentrations and SS/ST.

Source of variation	Parameter	SS	MS	F	P
Elevation	SS	26.711	6.678	135.228	<0.01
	ST	1807.929	451.982	537.595	<0.01
	NSC	2255.129	563.782	611.881	<0.01
	SS/ST	6.517	1.629	63.777	<0.01
Tissue	SS	183.626	61.209	1239.508	<0.01
	ST	53.552	17.851	21.232	<0.01
	NSC	109.297	36.432	39.541	<0.01
	SS/ST	11.748	3.916	153.287	<0.01
Elevation×Tissue	SS	26.380	2.198	44.517	<0.01
	ST	131.358	10.947	13.020	<0.01
	NSC	123.908	10.326	11.207	<0.01
	SS/ST	1.932	0.161	6.304	<0.01

The distribution pattern of SS/ST was similar to that of total NSC, with higher values in leaves and thick roots than in stem and fine roots. The proportion of total NSC was higher in leaves, at 26.9%, 27.2%, and 36.1%; the proportion in thick roots followed, with values of 28.4%, 25.2%, and 25.2%; the fine roots showed values of 20.8%, 24.8%, and 21.5%; and the stems exhibited 24.0%, 22.8%, and 17.2% at the three elevations, respectively. The proportions of SS/ST were higher in leaves, with values of 52.0%, 48.5%, and 38.5%; the proportions in thick roots followed, with values of 11.1%, 15.1%, and 11.6%; the fine roots had values of 25.5%, 23.7%, and 31.7%; and the stems displayed values of 11.4%, 12.8%, and 18.2% at the three elevations, respectively.

Variation of Sources of NSC and Its Component Concentrations for Qinghai Spruce

Elevations, tissues, and the interaction of the two significantly affected the concentrations of SS, ST, and NSC, as well the SS/ST (Table 2). The effects on SS concentration and SS/ST of the different factors were ranked as follows: tissue (SS: 1239.508 and SS/ST: 153.287) > elevation (SS: 135.228 and SS/ST: 63.777) > interaction of elevation and tissue (SS: 44.517 and SS/ST: 6.304). The effects on ST concentration and NSC could be ranked as follows: elevation (ST: 537.595 and NSC: 611.881) > tissue (ST: 21.232 and NSC: 39.541) > interaction of elevation and tissue (ST: 13.020 and NSC: 11.207).

Growth-Limiting Factors of Qinghai Spruce at Different Elevations

Elevation gradients relate to dramatic changes in many environmental factors, such as temperature,

humidity, light, and soil, making them ideal for studying plant responses to heterogeneous environments [1]. Additionally, environmental heterogeneity in elevation gradients will lead to certain changes in the morphology and physiology of trees. Qinghai spruce is distributed across a range of elevations, and there are obvious altitudinal differences in growth conditions. By studying the relation of elevation with its growth-limiting factors, we can better understand the influence of elevation over the growth of Qinghai spruce. In this study, the BAI of Qinghai spruce showed decreasing trends at low and middle elevations, while the opposite was the case at high elevations (Fig. 3). Moreover, the concentrations of soluble sugar, starch and NSC in the whole plant and tissues were the highest at middle elevations and the lowest at high elevations (Figs 4, 5). This indicates that Qinghai spruce was growth-limited at low and middle elevations, and carbon-limited at high elevations.

In this study, NSC concentrations increased and then decreased significantly with increasing elevations, confirming that the carbon was a limiting factor of tree growth. Consequently, our results support the tree carbon limitation hypothesis at high elevations. Empirical evidence of carbon limitation was also given in NSC concentrations in *Nothofagus pumilio*, and in the NSC concentrations in fir trunks and roots measured between closed forests and the alpine line [38, 39, 40]. These findings are similar to our findings, and further confirm our conclusion. Lower temperatures are related to limited carbohydrate formation in trees due to the association between higher elevations and lower temperatures. The photosynthetic rate decreases, or the photosynthetic season is shortened, along with decreasing temperature as the elevation rises from 2850 m to 3300 m, resulting in an insufficient supply of photosynthetic products, leading to a decrease in

the NSC concentrations of trees [3, 2, 10]. It has also been shown that NSC concentration increases with increasing latitude, so there is no carbon limitation at high elevations. However, sufficient carbon for tree growth was not available due to the low temperature, resulting in the limitation of tree growth. This result of tree growth limitation was also observed in the mixed deciduous evergreen forests of the southern Andes in Chile, as well as in Norway spruce and European larch, and larch in the eastern Himalayan treeline region, at different elevations [41-43]. The conclusions of these scholars' studies were contrary to ours, which may have resulted from differences in the region and tree species. There was no general consistency in the trends of NSC concentration with elevation in plants from different regions, species and seasons. We also found that the BAI increased at high elevations, contrastingly from the middle and low elevations, indicating the good growth trend of the trees (Fig. 3). The annual average radial growth of Qinghai spruce gradually increased, the sensitivity of trees to environmental changes decreased, and the limiting effect of climatic factors on tree growth gradually weakened with increasing elevation [44].

The BAI at middle and low elevations showed a downward trend, and the NSC concentration was higher (Figs 3, 4). Thus, the growth of trees at middle and low elevations was restricted. The trees at low and middle elevations are not carbon-constrained, with access to abundant carbohydrates, and so they might be affected by other environmental factors that limit growth. For example, the limiting factor for tree growth under current atmospheric CO₂ is not carbon resources but environmental factors (such as low temperature and drought) or carbon utilization activity restrictions caused by development restrictions [1]. The debate between carbon and growth limitation also extends to the effects of water stress on tree growth [15]. The growth of trees, especially height and radial increase, is often accompanied by an increase in hydraulic limitation, meaning reduced growth may not be due to reduced carbon uptake. Some studies suggest that water deficit prevents photosynthesis from facilitating growth, resulting in growth limitation [45]. In mountainous areas, drought stress with less precipitation in the growing season is a major climatic factor limiting tree growth in low-elevation areas, with a positive correlation with tree radial growth at low elevations [46-48]. When the supply of soil water decreases and the evaporation demand of trees increases with the occurrence of water drought stress, xylem embolism occurs in plants, thus impeding the transport of water and drying out tree tissues [49]. This impedes the division of tree cells and even kills them, affecting the growth of trees. Meanwhile, the closing of stomata prevent any further loss of water also forces photosynthesis to decline, and the plant has to slow down its metabolic rate in order to survive [50]. Modern molecular methods have extensively demonstrated that plant respiration and growth are downregulated, and

energy storage is upregulated, after the photosynthetic rate is reduced by various processes taking place in the early stages of water stress. This was consistent with our findings. Trees in the Tatra Mountains of Poland, Kansas in America, and the Andes in South America were all found to be subject to growth restriction at middle and low elevations [51-53].

This study shows that the growth limitation and carbon limitation hypotheses can not only explain the formation mechanism of the alpine forest line, but can also explain the factors limiting tree growth at different elevations. The limiting factor of Qinghai spruce growth changed from carbon limitation to growth limitation between high elevations and middle and low elevations, which indicates that the mechanism of forest formation is significantly affected by elevation.

Trade-Offs in Resource Allocation of Qinghai Spruce at Different Elevations

The growth-limiting factors affecting Qinghai spruce are ultimately a matter of resource allocation, and nonstructural carbohydrates are an important resource for tree growth. Therefore, the effects of elevation on the growth of Qinghai spruce can be better revealed by exploring the resource allocation trade-offs taking place in nonstructural carbohydrates of Qinghai spruce at different elevations. Moreover, the different distributions of NSC in the tissues also reflect their survival strategies in heterogeneous environments.

Due to the differences in the physiological metabolic activities and physiological functions of plant tissues, their NSC concentrations vary greatly [13, 54]. In this study, the NSC concentration of Qinghai spruce was higher in leaves and thick roots. However, SS mainly accumulated in aboveground tissues, with the highest proportion in leaves, and ST mainly accumulated in the underground tissues, with a high percentage in thick roots at different elevations (Fig. 6). The results show that the leaves were the main carbohydrate-producing tissue and the sources of NSC, and the roots were the main carbohydrate storage tissue. Similar results have been found in other studies; the SS, as an active carbon storage substance in tree tissues, is preferentially directed towards the flourishing tissues. At the same time, SS, as the main form in which carbohydrate is transported and metabolized, is directed towards the stem to repair conduit embolization [55]. In addition, the leaves are the main carbon assimilation tissues in trees, acting as a "carbon source" that provides carbohydrates for plant growth [56, 57]. Therefore, the SS concentration in the leaves was higher than in the stem and root. On the contrary, the concentrations of ST in the leaves were lower than in the roots, and the concentration of ST in thick roots was higher than that in fine roots. This may be because the thick roots can act as storage tissues, whereas the fine roots function as water-absorbers [58]. In particular, the NSC concentration in the thick roots of evergreen

broad-leaved trees was mainly in the form of ST [59]. The highest ST concentrations being found in the roots is consistent with studies on *Linum suffruticosum* and *Lepidium subulatum* in northeastern Spain, as well as on 13 populations of *Erica australis* in central Spain, and on *Pinus strobus* and *Quercus rubra* in North America [60-62].

The SS/ST levels in the whole plant and the tissues of Qinghai spruce at high elevations were significantly higher than those at middle and low elevations (Figs 4, 5), indicating that the prioritization of carbon at high elevations would change, and more carbon would be used for growth under conditions of low-temperature stress [2]. At the same time, Qinghai spruce at high elevations convert more ST into SS in the trade-off of resource allocation, in order to be resistant to low-temperature stress. In contrast, Qinghai spruces at middle and low elevations converted more SS into ST, which was used for long-term energy storage. At middle and low elevations, Qinghai spruces can better adapt to the drought-stressed environment by regulating their resource allocation, with higher concentrations of SS, ST, and NSC. In addition, Qinghai spruce growth in low-elevation regions is restricted by drought stress, which causes a decrease in the amount of carbon consumed in the body, making the trees appear to accumulate passively after growth limitation [57]. Meanwhile, we found that the level of NSC in the thick roots of Qinghai spruce was higher than that in the leaves in the low-elevation environment. This is because the roots of Qinghai spruce, as a shade-tolerant species, are usually allocated more NSC to adapt to the shady environment and thus improve its survival rate [59]. Additionally, the NSC storage level of Qinghai spruce was increased in the mid-altitude region, which might be due to the active accumulation of carbon that helps them to adapt to the higher intensity of intraspecific and interspecific competition [63, 64].

In summary, this research on the factors limiting growth in Qinghai spruce at different elevations and the concentration variation patterns of NSC and its components in various tissues, as well as on the strategies for adaptation to differently elevated habitats, will expand our understanding of the physiological and ecological adaptation mechanisms of Qinghai spruce to regional environments, and provide basic data for further analyses of the changes in carbon sequestration taking place in Qinghai spruce at different elevations.

Conclusion

The effects of elevation on the growth patterns and resource allocation of plants could serve as a reference for the conservation and utilization of plant resources in alpine environments. In our study, the patterns of BAI interannual variation in Qinghai spruce in the Qilian Mountains of northwestern China were the opposite of those of NSC concentration distribution between

tissues with changes in elevation. This result proves that the trees at middle and low elevations were subjected to growth limitations and that those at high elevations were subjected to carbon limitation. Meanwhile, Qinghai spruce at high elevations used more carbon for growth, with lower NSC concentrations and higher SS/ST in the whole plant and tissues, while those at low and middle elevations used more carbon for long-term energy storage, with higher NSC concentrations and lower SS/ST in the whole plant and tissues. Therefore, we should pay attention to the growth dynamics of Qinghai spruce in the Qilian Mountains, focusing on the carbon absorption and deployment taking place in high-elevation trees and the strengthened water regulation taking place in low-elevation trees in order to protect the subalpine forest ecosystems in Northwest China.

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Conflict of Interest

The authors declare no conflict of interest.

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