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SHORT COMMUNICATION

Shoot growth and seasonal changes of nonstructural carbohydrate concentrations at the upper and lower distribution limits of three conifers

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Abstract Nonstructural carbohydrate (NSC) concentration in plant organs is an indicator of a balance between carbon sources (i.e., photosynthesis) and sinks (i.e., growth). Understanding how NSC concentrations change with altitude would help determine altitudinal changes in plant growth. This study compared shoot growth and seasonal changes in NSC concentrations of current-year and 1-year-old needles and branch woods between the upper and lower distribution limits of subalpine conifers *Abies veitchii* (1600–2000 m a.s.l.), *A. mariesii* (2000–2400 m a.s.l.), and *Pinus pumila* (2400–2800 m a.s.l.) in Japan. The lengths of 1-year-old shoots were shorter at the upper distribution limits for the three species, and concentrations and branch woods were all high in spring but decreased toward summer, increasing from summer to autumn. No clear difference was found for either parameter between upper and lower distribution limits for each species. Therefore, this study suggests that growth reduction at the upper distribution limits is due to reduction of both sink and source activities, with similar degrees for each species. However, further studies of sink and source activities, such as temperature-dependent photosynthesis and growth traits, are necessary to reveal clearly the cause of this growth reduction in high altitudes.

Keywords Distribution limit · Nonstructural carbohydrate · Seasonal change · Sink · Source · Tree line

Introduction

Altitude greatly affects various aspects of plants (e.g., ecology and physiology) by regulating environmental conditions, such as temperature and precipitation. The length of the growing season decreases with increasing altitude, except for the tropical zone, which have no temperature seasons. Growing-degree days must exceed a certain threshold for each plant species to restart growth from the dormant period (Fosaa et al. 2004). Photosynthetic rates decrease in cool conditions because of temperature dependency of photosynthesis (DeLucia and Smith 1987). Therefore, the annual carbon gain of plants decreases at high altitudes, which in turn reduces plant growth.

Nonstructural carbohydrate (NSC) is stored in plant organs, such as root, wood xylem, and leaves (Hansen and Beck 1994; Schaberg et al. 2000; Kabeya and Sakai 2003; Takahashi and Goto 2012). Starch and soluble sugar are different forms of NSC. Starch, the primary storage polysaccharide in plants, consists of chains of glucose molecules, while soluble sugar (e.g., sucrose) is transported from leaves to other parts of the plant body (Raven et al. 1991). NSC concentrations change seasonally. In deciduous trees in particular, NSC stored in leaves is transported to wood xylem before leaf fall and is used for growth the following year (Wong et al. 2003). NSC also relates to cold tolerance, i.e., concentrations increase to raise cold tolerance (Chatterton et al. 1989). Concentration of soluble sugar increases in winter compared with that of starch (Kibe and Masuzawa 1992; Wong et al. 2003), and NSC increases by carbon gain due to photosynthesis (i.e., source), while plants grow using NSC (i.e., sink). Therefore, NSC concentration is determined by the balance between sink and source activities. It also relates to

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phenology because the balance between sink and source activities differs seasonally. Concentrations are high in spring, before the start of growth, decreases after the start of growth, and then are constant or increase after summer (Gansert and Sprick 1998).

Dominant species change along altitudinal gradients. For example, dominant subalpine evergreen conifer species change with altitude on Mt. Norikura (3026 m above sea level) in central Japan: *Abies veitchii* Lindl. 1600–2000 m a.s.l., *A. mariesii* Mast. 2000–2500 m a.s.l., and *Pinus pumila* Regel 2500–2800 m a.s.l. (Miyajima et al. 2007). Previous studies have examined effects of climate on interannual variations of growth of these three species and showed that annual growth rates are greater in years that have a warmer summer (Takahashi 2003; Takahashi et al. 2003, 2011). This tendency is more conspicuous at higher altitudes, indicating that tree growth is more limited by lower temperature at higher altitudes along an altitudinal gradient (1600–2800 m a.s.l.) on Mt. Norikura. Tree growth is also often regulated by low summer temperatures at high altitudes in other regions (Adams and Kolb 2005; Wang et al. 2005; Massaccesi et al. 2008; Peng et al. 2008). Both sink and source activities decrease at high altitudes because of lower temperature and shorter growing season at higher altitudes, which in turn possibly affect NSC concentrations of plant organs. However, no study has compared seasonal variations of NSC concentrations between upper and lower distribution limits for the three species (*A. veitchii*, *A. mariesii* and *P. pumila*) dominating at different altitudes.

The purpose of this study was to examine altitudinal variations of shoot length and seasonal changes in NSC concentrations in needles and branch woods from spring to autumn in these three species at their upper and lower distribution limits on Mt. Norikura. In particular, the study answers the following questions: (1) How do NSC (starch and soluble sugar) concentrations change seasonally? (2) Do NSC concentrations of each organ differ between the upper and lower distribution limits for each species?

Materials and methods

Study site

This study was done at the upper and lower distribution limits of subalpine conifers *A. veitchii*, *A. mariesii*, and *P. pumila* on the east slope of Mt. Norikura (36°06′N, 137°33′E, 3026 m a.s.l.) in central Japan. The altitudes of the lower and upper distribution limits of the study sites were 1600 and 2000 m a.s.l. for *A. veitchii*, 2000 and 2400 m a.s.l. for *A. mariesii*, and 2400 and 2800 m a.s.l. for *P. pumila*. Although *A. veitchii* and *A. mariesii* grow below and above 2000 m a.s.l., respectively, they segregate physiognomically at this altitude (Miyajima et al. 2007). Altitudes of 1600 and 2800 m a.s.l. are the lower and upper distribution limits, respectively, of the subalpine zone.

Although *Tsuga diversifolia* Mast. and *Picea jezoensis* var. *hondoensis* Rehde also grow at this study site (1600–2400 m a.s.l.), their densities are lower than for the two *Abies* species (Miyajima et al. 2007). Subordinate trees were all deciduous broad-leaved trees: *Betula ermanii* Cham., *Sorbus commixta* Hedland, and *Acer ukurunduense* Trantv. et Meyer. Miyajima et al. (2007), Miyajima and Takahashi (2007), and Takahashi et al. (2012) describe in detail species composition and forest structure along the altitudinal gradient of Mt. Norikura.

Mean annual temperature was estimated as 5.1, 2.7, 0.3 and −2.1 °C at 1600, 2000, 2400, and 2800 m a.s.l., respectively, from temperatures recorded at Nagawa Weather Station (1063 m a.s.l.) between 1979 and 2011 using the standard lapse rate of −0.6 °C for each +100-m altitude.

Shoot sampling

Three open-growing trees were selected for each species at each altitude to examine shoot growth and NSC concentration. A paved road for vehicles continues up to 2700 m a.s.l. (Takahashi and Miyajima 2010). Only a limited number of vehicles with a pass can use the road. Effects of the road on tree growth have not been noted so far (Ida and Ozeki 2000). Therefore, the two *Abies* species were selected near the road side to sample shoots unshaded by other trees. Even though *P. pumila* is a dwarf scrub with a unimodal size structure (Takahashi and Yoshida 2009), we selected *P. pumila* away from the road. All selected *P. pumila* trees were barely shaded by other trees. Strong wind affects the growth of *P. pumila* (Takahashi and Yoshida 2009), so sampling was not done at wind-exposed sites in order to reduce the effects of wind.

Trunk height of dwarf pine *P. pumila* was almost the same at each altitude (~2 and 1 m at 2400- and 2800-m a.s.l., respectively), so that mature trees were selected at each altitude. However, although the maximum trunk height of the two *Abies* species was ~20 m at 1600 and 2000 m a.s.l. (Miyajima and Takahashi 2007) and ~12 m at 2400 m a.sl. (Takahashi et al. 2012): trunk height of the selected *Abies* trees was limited to between 2 and 3 m to minimize the effects of tree size on shoot growth and NSC concentration.

Three 1-year-old shoots and three current-year shoots of each tree were sampled once a month from April to October in 2011. Shoot height was measured at sampling, and shoot length was measured for only 1-year-old shoots. Although shoot sampling started soon after snow had melted, the start month differed between the four altitudes because the snow-melt period was later at higher altitudes. Sampling of current-year shoots started 2 months after the start of sampling of 1-year-old shoots because current-year shoots mature a long time after the growth period begins—from early or mid-June at 1600 m a.s.l. and from mid- or late July at 2800 m a.s.l. (Takahashi and Koike 2014). Table 1 shows the timetable for sampling of current-year (198) and 1-year-old (306) shoots, respectively, for the three species combined.

Measurement of NSC concentration

Sampled shoots were brought back to the laboratory on the day of sampling and were dried at 80 °C for 24 h to kill all living cells and end changes in carbohydrate composition. The sampled shoots were stored at −40 °C after drying, and NSC concentration was measured after re-drying at 80 °C for 24 h. Gansert and Sprick (1998) observed that starch concentration was considerably lower in the bark than in the wood xylem of *Fagus sylvatica* seedlings. Therefore, in this study, the bark of shoot samples was peeled off to measure accurately NSC concentrations in branch woods. Sampled shoots were divided into: (1) 1-year-old needles, (2) 1-year-old branch woods, (3) current-year needles, and (4) current-year branch woods; branch wood and needle samples were ground into fine powder.

To determine the concentration of nonstructural carbohydrates in needles and branch woods, we followed the methods described by Kabeya and Sakai (2003). Starch was hydrolyzed to glucose with amyloglucosidase and was measured using the glucose–peroxidase method (Rufty and Huber 1983). Soluble sugar was determined using the phenol– sulfuric acid method (Dubois et al. 1956). Starch and soluble sugar concentrations were determined using a spectrophotometer (UVmini-1240, Shimadzu, Kyoto).

Statistical analysis

Shoot height possibly affects shoot growth (Takahashi et al. 2006). However, our preliminary analysis showed that shoot height barely affected shoot length and NSC concentrations. Therefore, shoot height was not considered for statistical analysis. Length of 1-year-old shoots was compared between the upper and lower distribution limits for each species using the analysis of variance (ANOVA). Differences of NSC concentrations among months and between upper and lower distribution limits were tested using the two-way ANOVA for each organ of each species. Statistical test was done for each concentration of starch, soluble sugar, and total (starch + soluble sugar). Values of NSC concentrations were arcsine transformed before statistical tests because of proportion data. All statistical analyses were done using free statistical software R 2.9.0 (R Development Core Team 2009).

Results

Shoot length was significantly lower at the upper than the lower distribution limit for each species (ANOVA, $P < 0.001$ for each; Fig. 1).

NSC concentrations of needles and branch woods of 1-year-old and current-year shoots of the three species differed greatly between months; the variable of month was statistically significant in all cases for each concentration of starch, soluble sugar, and total (starch + soluble sugar) (ANOVA, at least $P < 0.05$, Table 2). For example, NSC concentrations of needles and branch woods of 1-year-old shoots of the three species were high in spring but decreased toward summer (Fig. 2a–f). This tendency was more conspicuous for the two *Abies* species than for *P. pumila*. The reduction of NSC concentrations was mainly due to reduced starch for the 1-year-old needles (Fig. 2a–c), whereas both starch and soluble sugar concentrations decreased in the 1-year-old branch woods of the two *Abies* species (Fig. 2e, f). NSC concentrations, especially soluble sugar, of needles and branch woods of all shoots of all species increased from summer to autumn (Fig. 2). The increase in NSC concentration from summer to autumn was more evident in branch woods than in needles for both current-year and 1-year-old shoots of the three species.

Table 1 Timetable for sampling of current-year (0) and 1-year-old (1) shoots for three species at four altitudes

Species	Altitude (m)	Month							Total number of shoots	
		A	M	J	J	A	S	O	Current-year	1-year-old
Pinus pumila	2800			1	1	0/1	0/1	0/1	27	45
	2400			1	1	0/1	0/1	0/1	27	45
Abies mariesii	2400			1	1	0/1	0/1	0/1	27	45
	2000		1	1	0/1	0/1	0/1	0/1	36	54
Abies veitchii	2000		1	1	0/1	0/1	0/1	0/1	36	54
	1600	1	1	0/1	0/1	0/1	0/1	0/1	45	63

Nine shoots (3 shoots × 3 trees) were sampled for each species at each altitude and month

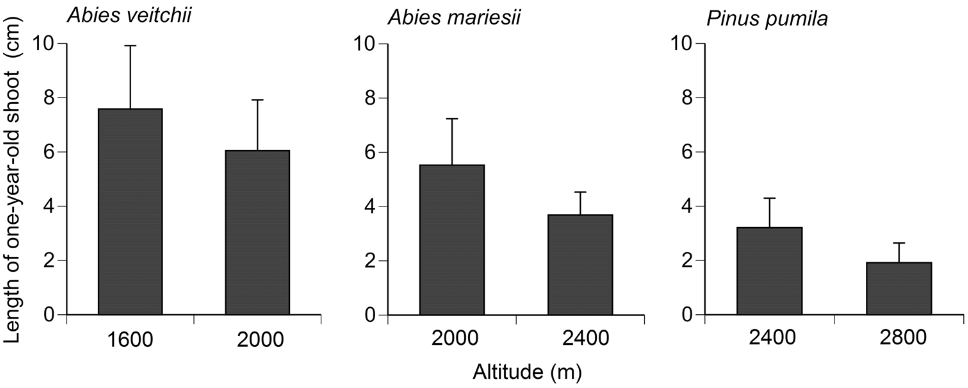

Fig. 1 Length of 1-year shoots of *Abies veitchii* (1600 m and 2000 m a.s.l.), *A. mariesii* (2000 m and 2400 m a.s.l.), and *Pinus pumila* (2400 m and 2800 m a.s.l.) at their lower and upper distribution limits on Mt. Norikura, central Japan. The mean value with positive standard deviation (SD) is shown for each species at each altitude. The number of samples was 63 for *A. veitchii* at 1600 m a.s.l., 54 for *A. veitchii* and A. *mariesii* at 2000 m a.s.l., 45 for *A. mariesii* and *P. pumila* at 2400 m a.s.l., and *P. pumila* at 2800 m a.s.l

Table 2 Results of two-way analysis of analysis of variance (ANOVA) for concentrations of nonstructural carbohydrates (total, starch, soluble sugar, percent dry matter) of *Abies veitchii*, *A. mariesii*, and *Pinus pumila*

Organ	Dependent	*Abies veitchii*				*Abies mariesii*				*Pinus pumila*			
	Variable	Altitude	Month	A × M	*n*	Altitude	Month	A × M	*n*	Altitude	Month	A × M	*n*
N1	Starch	2.2	119.8***	13.3***	115	1.6	143.5***	15.5***	99	0.6	108.0***	6.3***	90
	Sugar	5.5	8.3***	3.2*	115	0.4	8.1***	2.0	99	1.6	5.5***	2.8*	90
	Total	1.1	32.9***	7.7***	115	0.2	25.1***	9.1***	99	1.3	18.2***	3*	90
B1	Starch	0.4	9.0***	3.3**	111	1.0	10.6***	5.5***	99	5.6*	56.5***	0.5	90
	Sugar	13.7***	23.0***	5.0***	111	6.7*	44.2***	1.2	99	<0.1	26.1***	3.3*	90
	Total	9.2**	22.9***	2.9*	111	9.5**	43.4***	3.2*	99	0.6	17.2***	2.8*	90
N0	Starch	43.2***	9.5***	1.1	79	<0.1	3.2*	14.0***	63	24.4***	7.6**	2.3	53
	Sugar	7.8**	7.0***	2.8*	79	4.2*	15.1***	3.3*	63	0.5	52.0***	2.5	53
	Total	0.1	5.1***	2.0	79	1.6	9.7***	0.4	63	6.7*	40.9***	3.5*	53
B0	Starch	15.5***	10.1***	2.3	70	1.0	9.2***	10.4***	63	1.4	7.3**	4.4*	53
	Sugar	11.7**	23.0***	5.2**	70	<0.1	61.9***	4.2*	63	1.6	68.2***	1.0	53
	Total	2.7	17.3***	2.8*	70	0.3	52.9***	5.6**	63	3.2	66.3***	1.5	53

F values are shown with their statistical significance

Altitude (A), month (M) and their interaction (A × M) were used for independent variables

N1 1-year-old needles, *B1* 1-year-old branch woods, *N0* current-year needles, *B0* current-year branch woods

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Total NSC concentrations of each organ were not so different between the upper and lower distribution limits for the three species (Fig. 2). The variable of altitude was significant for only two of the 12 cases for total NSC concentrations, and the effect of altitude on total NSC concentrations of each organ was different among months for the three species because the interaction of altitude and month was significant for most cases (Table 2). For example, in the case of 1-year-old branch woods of *A. mariesii* that showed a statistical significance of altitude (Table 2), total NSC concentrations were greater at the upper than the lower distribution limit in July and August only (Fig. 2e). Although the variable of altitude was also significant for the current-year needles of *P. pumila*, the difference of total concentrations was not apparently clear between upper and lower distribution limits (Fig. 2g). Total concentrations of 1-year-old needles of *A. veitchii* in May and of *A. mariesii* in June were considerably lower at the upper than at the lower distribution limit (Fig. 2b, c). However, total concentrations of some other months were slightly greater at the upper distribution limit (Fig. 2). Therefore, no consistent difference between two altitudes was found for each organ of each species.

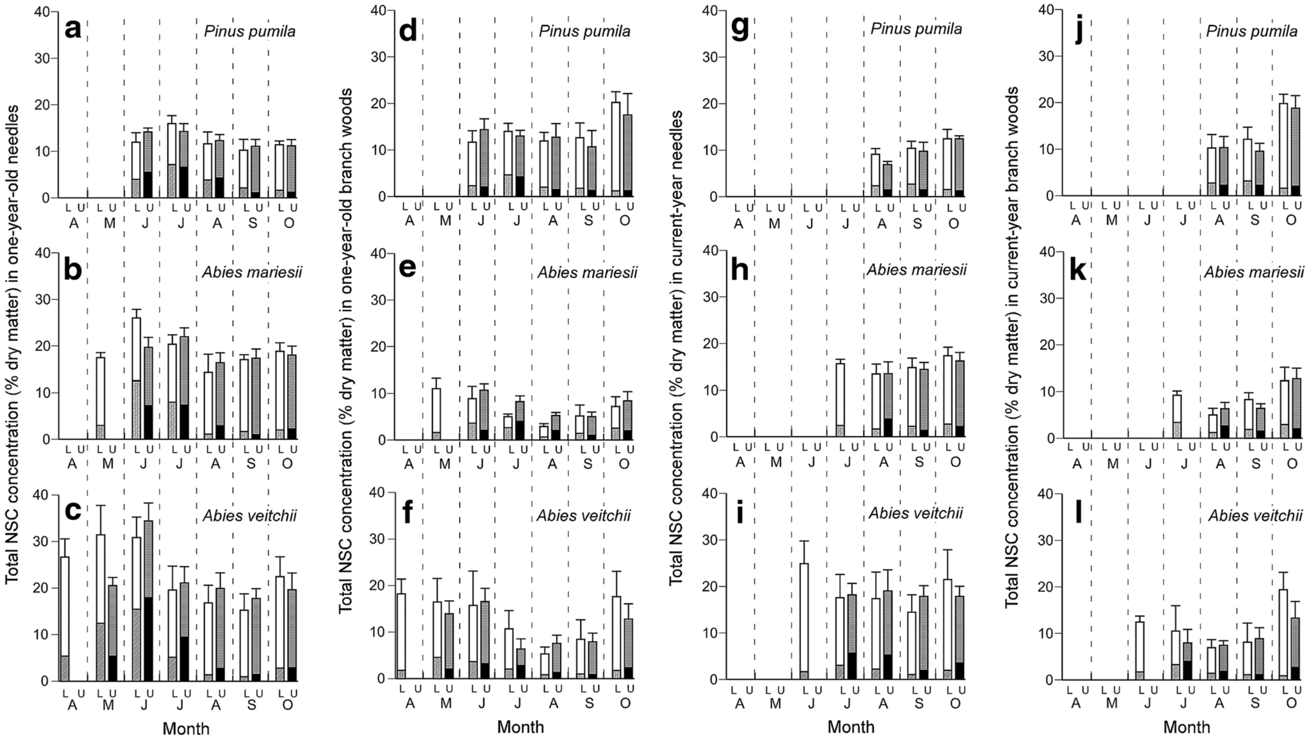

Fig. 2 Seasonal variation in nonstructural carbohydrate (NSC) concentrations in each organ of *Pinus pumila* (*U* 2800 m, *L* 2400 m a.s.l.), *Abies mariesii* (*U* 2000 m; *L* 2400 m a.s.l.), and *A. veitchii* (*U* 1600 m; *L* 2000 m a.s.l.) on Mt. Norikura, central Japan. Each three *vertical* graphs are NSC concentrations in 1-year-old needles (**a**–**c**), 1-year-old branch woods (**d**–**f**), current-year needles (**g**–**i**), and current-year branch woods (**j**– **l**). Mean values with positive standard deviations (SD) are shown. *Lower dark* and *upper light* parts indicate starch and soluble sugar concentrations, respectively, for each *bar*

Discussion

Seasonal changes in NSC concentrations were evident for the three species, i.e., decreasing from spring to summer in 1-year-old needles and branch woods, and soluble sugar concentrations increased from summer to autumn, especially in both 1-year-old and current-year branch woods. Kibe and Masuzawa (1992) also reported similar seasonal changes of NSC concentrations of *P. pumila* in the Yatsugatake mountain range in central Japan. The reduction of starch concentrations in the early and middle growing season is due to its consumption for growth in 1-year-old needles and branch woods. The reduction of soluble sugar concentrations during this period may reflect carbon translocation to the other plant organs for sink activity. Both shoot and radial growth of stems of the three species grow vigorously early in the growing season (Takahashi 2005; Takahashi and Koike 2014). Radial stem growth ceases in mid-August, irrespective of altitude, for the three species (Takahashi and Koike 2014). Shoot elongation of *P. pumila* ceases earlier than the radial growth of stems (Takahashi 2005; Takahashi and Koike 2014). Although there is no literature regarding cessation of shoot elongation of *A. mariesii* and *A. veitchii*, shoot elongation of the two *Abies* species ceases by at least mid-August (our personal observation). The minimum NSC concentrations were observed in August for 1-year-old needles and branch woods of the three species; August coincided with the period in which radial stem growth ceased for this species. Reduction of sink activity would increase NSC concentrations after August. The increase of soluble sugar concentration of branch woods in the late growing season is due to the transformation of starch to soluble sugar for the maintenance of respiration in living cells in winter (Kramer and Kozlowski 1979) and for the increase of cold tolerance by increasing osmotic potential (Yoshida and Sakai 1967; Sauter 1988; Kozlowski 1992). Therefore, seasonal changes of NSC concentrations correspond to those of growth.

NSC concentrations in 1-year-old needles were higher at the lower than at the upper distribution limit for *A. veitchii* in May and *A. mariesii* in June. Shoot growth starts from early or mid-June at 1600 m a.s.l. for *A. veitchii* and from late June or early July at 2000 m a.s.l. for *A. mariesii* (Takahashi and Koike 2014); therefore, May and June are just before shoot growth at the lower distribution limits, respectively. If temperature is high enough for photosynthesis before the start of growth in late spring or early summer, newly photosynthetic production is temporally stored in existing needles. This explains, at least partially, the higher NSC concentrations in 1-year-old needles at the lower distribution limits for the two *Abies* species. The increased concentration during this period was due to the increase of starch. Other studies also showed increased starch concentrations just before and during the time of bud break (Fischer and Höll 1991; Egger et al. 1996). Therefore, phenology is an important factor for the different NSC concentrations between upper and lower distribution limits at the beginning of the growing season.

Although shoot length was shorter at the upper than the lower distribution limit for each species, total NSC concentrations in each organ showed no clear differences between upper and lower limits, except for 1-year-old needles of *A. veitchii* in May and *A. mariesii* in June, as described previously. Length of growth period is shorter and daytime air temperature lower at higher altitudes. Annual net photosynthetic production decreases at higher altitudes because of shorter growth period and temperature dependency of photosynthesis (DeLucia and Smith 1987). Therefore, both sink (i.e., growth) and source limitation (i.e., photosynthesis) are greater at higher altitudes, which causes a lower growth rate at the upper distribution limit for the three species in this study. In the source-limitation condition, trees cannot grow because of deficiency of photosynthetic production, even if cell division and maturation are possible. By contrast, in the sink limitation condition, trees cannot grow because the temperature is too low for cell division and maturation, even if photosynthesis is possible. It is supposed that NSC concentration increases and decreases under sink and source limitation conditions, respectively (Piper et al. 2006; Shi et al. 2008). In this study, concentrations were not different between upper and lower distribution limits for each organ of each species, i.e., both sink and source activities decreased by similar degrees at upper distribution limits. Therefore, NSC concentration might not differ between upper and lower distribution limits for the three species.

This study showed that: (1) NSC concentrations of needles and branch woods changed seasonally, according to phenology of shoot growth, for the three species at the upper and lower distribution limits, and (2) NSC concentrations of each organ did not differ clearly between upper and lower distribution limits for each species during summer and autumn, although shoot elongation of each species was shorter at the upper distribution limit. Therefore, this study suggests that growth reduction at the upper distribution limit is due to reduction of both sink and source activities, with similar degrees for each species. However, further studies on sink and source activities, such as temperature-dependent photosynthesis and growth traits, are necessary to reveal clearly the cause of growth reduction in high altitudes for the three species.

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