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ORIGINAL ARTICLE

Vertical and leaf-age-related variations of nonstructural carbohydrates in two alpine timberline species, southeastern Tibetan Plateau

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Abstract Spatial pattern of nonstructural carbohydrates (NSC) was analyzed in two alpine timberline species (*Abies georgei* and *Juniperus saltuaria*) growing at the timberline in Sergyemla Mountain, southeastern Tibetan Plateau. We aimed to examine the effect of canopy height and leaf age on balance between carbon uptake and consumption of timberline tree species in extremely environmental condition. The results showed that no significantly vertical variation in sugars, starch or NSC (soluble sugars plus starch) in *A. georgei* was found for any aged needles. Also, there were no significant differences among vertical gradients in both current and last-year leaves for *J. saltuaria*. However, different-aged needles/leaves showed significant differences in NSC concentrations in both *A. georgei* and *J. saltuaria*. For *A. georgei*, needle mean NSC across vertical canopies showed a significant increase from current-year to 2-year needles ($P < 0.05$), followed by a gradual decrease from 2- to 5-year needles ($P < 0.05$), whereas for *J. saltuaria*, last-year leaves had significant higher NSC except sugars compared with current-year leaves across canopies ($P < 0.01$). The observed trends of NSC along vertical canopy heights and leaf ages suggested that, in extreme environmental condition, not only light-induced carbon acquisition in photosynthesis but also carbohydrate export from leaves should be taken into account to explain the spatial pattern of NSC.

Keywords Alpine timberline · Canopy height · Carbon balance · Leaf age · Nonstructural carbohydrates

Introduction

Plants produce, store, invest, and lose carbon compounds during growth (Würth et al. 2005). The pool size of the mobile carbon, with the greatest fraction commonly present as nonstructural carbohydrates (NSC, largely starch and sugars), reflects the balance between carbon gain by photosynthesis and demand by growth and metabolism (Chapin et al. 1990; Körner 2003; Würth et al. 2005). In recent years, NSC in plant organs has been successfully used to mirror plant overall carbon supply status (Hoch et al. 2002; Hoch and Körner 2003; Körner 2003; Shi et al. 2006). Additionally, carbon allocation to growth or storage may be determined by the trade-off between growth and survival of plants (Kitajima 1994; Poorter and Kitajima 2007), therefore NSC has been used to indicate species growth strategy (Poorter and Kitajima 2007; Bansal and Germino 2008).

Various external and internal factors, including drought (Würth et al. 2005), low temperatures (Körner 2003; Hoch et al. 2003; Shi et al. 2006), atmospheric CO_2 and pollutant concentrations (Körner et al. 2005), light availability (Würth et al. 1998; Niinemets et al. 2004, 2005; Poorter and Kitajima 2007), and reproduction or growth (Oleksyn et al. 2000), change the temporal or spatial patterns of NSC in plant species. It is well known that light availability largely varies with depth of canopy in a forest, and more light is available to upper than lower canopy (Niinemets

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and Tenhunen 1997; Tateno and Kawaguchi 2002; Prasolova et al. 2003), which leads to higher NSC in upper compared with middle and lower canopies as a result of the relative higher photosynthetic capacity in upper canopy, i.e., “more light, more NSC” (Meletiou-Christou et al. 1994; Würth et al. 1998; Herrick and Thomas 1999; Niinemets et al. 2006). However, most of these works were limited to low-elevation region; information on spatial variability of NSC in tree species under abiotic stress, such as at alpine timberline environment, is scarce, which limits the use of NSC to assess the carbon supply status or growth strategy in this area.

The Tibetan Plateau, the third pole of the world, is the ideal and an important place for exploring the structure and function of natural ecosystems and their response to climate change due to the fragility and sensitivity of ecosystems under the extreme environmental conditions driven by high-altitude climates. Alpine timberline of Sergyemla Mountain on the southeast edge of the Tibetan Plateau is one of the highest-altitude timberlines in the world, with the tree limitation near 4,500 m. *Abies georgei* and *Juniperus saltuaria* are two typical timberline species dominating in this region, and the timberline belongs to abrupt type because timberline ecotone is very narrow and the upper part of closed forest and tree limit are mostly coincident. NSC measurement of the two species will be useful to assess the carbon supply status and to indicate the balance between carbon assimilation and resumption of plants. However, no data of NSC pattern seem to be available for these two species except for a study of Shi et al. (2008), in which only altitudinal variation of mature leaf NSC was examined. To our knowledge, the current study is the first to assess the spatial pattern at the extreme alpine environment on the Tibetan Plateau. Our goal was to study how leaf NSC concentrations would vary with vertical canopy heights and leaf age in both *A. georgei* and *J. saltuaria* within the harsh timberline area, compared with plants in other environments.

Materials and methods

Study site

The study site (29°35′56″N, 94°36′38″E, 4,350 m. a.s.l.) was located on Sergyemla Mountain, located 50 km from Linzhi Town in southeast Tibet, China. The climate is characterized by high precipitation, cloudy days, cold winters, and cool summers. Mean annual temperature is −0.73°C with a minimum annual temperature of −31.6°C and maximum temperature of 24°C (Xu 1995; He et al. 2009). Average annual precipitation is 1,134.1 mm, with 75% of the annual average amount in June to September (Xu 1995; He et al. 2009). The soil is sour brown soil which is slightly acidic (mean pH 5.5). Total and available soil N are 4.5 and 0.03 mg/g, total and available soil P are 0.34 and 0.003 mg g^{-1}, and total soil C is 63.3 mg g^{-1}, respectively (Li et al. 2009). The study site is dominated by *A. georgei* and *J. saltuaria*, two typical timberline species, evergreen shrubs including *Rhododendron pingianum* and *Rhododendron wardii*, and deciduous shrubs including *Salix oritrepha*, *Ribes glaciale*, and *Sorbus rehderiana*. It is interesting to note that, although the two species grow in the same region, they are distributed on different slopes, with *A. georgei* dominating on northwest-facing slope and *J. saltuaria* on southeast-facing slope. *A. georgei* forest has a mean canopy height of 11.5 m, diameter at breast height (DBH) of 33.9 cm, and stem density of 459 stems/ha, whereas *J. saltuaria* forest has a mean canopy height of 5.9 m, DBH of 27.6 cm, and stem density of 633 stems/ha. The upper limit of *A. georgei* is 4,350 m, whereas it is 4,500 m for *J. saltuaria*. In this study, we sampled trees of *A. georgei* and *J. saltuaria* in the range of 4,300–4,350 m and 4,370–4,410 m, respectively.

Leaf sampling

Due to significant seasonal change occurring in NSC (Hoch et al. 2002; Bansal and Germino 2008), we carefully completed the leaf sampling at approximately the same time. A total of six *A. georgei* (aged 100–200 years) and six *J. saltuaria* (aged 200–300 years) trees were selected for leaf sample collection in the middle and late growing season, on 28–30 July 2005, after the completion of the current-season shoot growth. Increment cores were extracted at a height of 1.45 m in the trunk and tree age was determined by counting the annual rings in each core. Mean tree height and mean DBH for selected *A. georgei* were 13.2 ± 1.0 m and 33.5 ± 5.7 cm, respectively, whereas they were 6.1 ± 0.7 m and 30.7 ± 6.7 cm for *J. saltuaria*, respectively (data are mean ± standard deviation, SD).

At each sampling date, six needle age classes (current year, and 1-, 2-, 3-, 4-, and 5-year) were collected from the upper, middle, and lower canopy positions (13.1 ± 0.8 m, 7.8 ± 1.1 m, and 3.3 ± 0.6 m, respectively) for *A. georgei* by climbing trees. The apex of a long shoot produces a yearly growth increment that bears a single age class of needles. We counted needle age classes from shoot tips, which were validated by counting tree rings at the base of the shoots (Luo et al. 2005). For *J. saltuaria*, however, it was difficult to clearly determine leaf age, therefore we only sampled the current- and last-year leaves based on distinct differences between these two kinds of leaves. As for *A. georgei*, leaves were also sampled from three canopy positions (6.1 ± 0.7 m, 3.8 ± 0.5 m, and 1.1 ± 0.7 m, respectively). All needle/leaf samples were collected from the south side of trees. Each sample, composed of more than 200 needles/leaves, was immediately stored in a cool box and killed in a microwave oven (30 s at 600 W) within 6 h after collection. Thereafter, leaf samples were oven-dried to constant mass at 75°C and ground finely, and then subsamples were taken for nonstructural carbohydrate analysis.

Analysis of NSC

Nonstructural carbohydrates, defined here as the sum of free sugars (glucose, fructose, and sucrose) plus starch, were analyzed based on an enzymatic digest method with subsequent high-performance liquid chromatographic (HPLC) determination. Ground plant material was extracted with boiling, distilled water for 30 min. The soluble fraction was then analyzed for glucose, fructose, and sucrose using HPLC determination. In a second step, the insoluble material (including starch) was dialyzed at 4°C for 12 h immediately in order to remove mobile carbohydrate compounds, and then incubated for 20 h at 40°C with the crude enzyme (Clarase, a fungal amylase, Miles Laboratory Inc., Elkhart, Ind., USA) as described by Würth et al. (2005). After centrifugation, the supernatant plant extract was treated and analyzed in the same way as above, i.e., HPLC method. According to the glucose concentration, the starch concentration could be determined. Carbohydrates other than starch, sucrose, fructose, and glucose are not covered here, but they commonly contribute little to the total carbohydrate pool (Hoch and Körner 2003). Soluble sugars, starch, and total NSC were expressed on a dry-matter basis.

Statistical analysis

Two-way analysis of variance (ANOVA) with sampling canopy height and needle/leave age as two main factors was performed to test the differences in sugar, starch, and NSC concentrations between sampling heights as well as leaf ages. By using two-way analysis of variance the interaction between sampling canopy height and leaf age was also determined.

Results

NSC concentrations of *A. georgei* and *J. saltuaria*

We pooled the NSC data across sampling canopy heights and needle/leave ages to compare the NSC of *A. georgei* and *J. saltuaria*. The results indicated that there were large differences in the sugar, starch, and NSC concentrations between the two alpine timberline species growing in the same area ($P < 0.001$). *A. georgei* had obviously higher concentrations of sugar, starch, and NSC compared with *J. saltuaria* (7.89 ± 0.99% versus 5.25 ± 0.89% for sugars, 8.40 ± 0.74% versus 6.18 ± 0.68% for starch, and 16.29 ± 1.18% versus 11.44 ± 1.18% for NSC).

Variations of NSC concentrations along sampling heights

With the sampling height variation from upper to lower canopy, no significant difference in sugars, starch or NSC in *A. georgei* was found for current- or 1-, 2-, 3-, 4- or 5-year needles except a weakly significant difference of soluble sugars existing between upper and lower canopy (Tables 1, 2). Also, there was no significant variation between different canopies in current- and last-year leaves for *J. saltuaria* (Tables 1, 2).

Variations of NSC concentrations along needle/leaf ages

Because no significant effect with canopy heights was found, the sugar, starch, and NSC changes along needle/leaf age were reported based on the pooled data across three canopies. Needle age had significant effect on sugar, starch, and NSC concentrations for *A. georgei* (Fig. 1, Table 2). With increasing needle age, sugars showed very significant increase from current-year to 2-year needles, followed by a weak but significant decline from 2- to 5-year needles (Fig. 1a). NSC had a similar pattern as sugars with needle age (Fig. 1c). For starch, 1-year needle was significantly higher than current-year needle, with a gradual decrease from 1- to 5-year needles (Fig. 1b). There were significant differences in starch and NSC between current- and last-year leaves for *J. saltuaria* (Fig. 2, Table 2). However, no obvious difference in sugars existed between current- and last-year leaves (Fig. 2, Table 2).

Discussion

Carbon status of two alpine timberline species

Compared with the needle/leaf NSC of timberline or treeline species reported by previous studies (on average, 6.13% in Hoch et al. 2002, 8–14% in Hoch and Körner 2003, and 12.5% in Piper et al. 2006), the NSC concentrations in this study are relatively higher, especially for *A. georgei*. Although the study area is situated at one of the highest-altitude timberline in the world and the two dominant species sampled in this study almost reach their upper

Table 1 Needle/leave sugar, starch, and NSC concentrations (% dry mass) of different ages for *A. georgei* and *J. saltuaria* in relation to canopy position

Species	Needle/leaf age	Carbon components	Upper canopy	Middle canopy	Lower canopy
A. georgei	Current-year needle	Sugars	6.40 ± 0.58^{a}	6.18 ± 1.05^{ab}	5.55 ± 0.87^{b}
		Starch	7.80 ± 0.96^{a}	7.71 ± 0.80^{a}	8.04 ± 0.67^{a}
		NSC	14.20 ± 0.95^{a}	13.89 ± 1.33^{a}	13.59 ± 1.23^{a}
	1-year-old needle	Sugars	7.52 ± 1.25^{a}	7.61 ± 1.05^{a}	8.41 ± 1.17^{a}
		Starch	8.80 ± 0.81^{a}	8.79 ± 0.61^{a}	8.82 ± 0.86^{a}
		NSC	16.32 ± 1.21^{a}	16.41 ± 1.30^{a}	17.23 ± 1.51^{a}
	2-year-old needle	Sugars	9.10 ± 0.72^{a}	8.90 ± 1.06^{a}	8.71 ± 0.75^{a}
		Starch	8.69 ± 0.95^{a}	8.75 ± 0.67^{a}	8.44 ± 0.94^{a}
		NSC	17.79 ± 1.21^{a}	17.64 ± 1.31^{a}	17.16 ± 1.33^{a}
	3-year-old needle	Sugars	9.15 ± 0.95^{a}	8.89 ± 0.51^{a}	8.02 ± 0.73^{a}
		Starch	8.76 ± 0.74^{a}	8.38 ± 0.77^{a}	8.70 ± 0.82^{a}
		NSC	17.91 ± 0.80^{a}	17.27 ± 1.04^{a}	16.72 ± 0.91^{a}
	4-year-old needle	Sugars	8.17 ± 0.98^{a}	8.64 ± 0.91^{a}	8.55 ± 1.50^{a}
		Starch	8.69 ± 0.48^{a}	8.28 ± 0.64^{a}	8.35 ± 0.90^{a}
		NSC	16.86 ± 0.85^{a}	16.92 ± 0.92^{a}	16.90 ± 1.89^{a}
	5-year-old needle	Sugars	8.68 ± 1.17^{a}	7.18 ± 1.11^{a}	7.34 ± 0.79^{a}
		Starch	8.20 ± 0.54^{a}	8.12 ± 0.74^{a}	7.93 ± 0.51^{a}
		NSC	15.88 ± 0.98^{a}	15.30 ± 1.26^{a}	15.28 ± 0.82^{a}
J. saltuaria	Current-year leaf	Sugars	5.42 ± 0.42^{a}	5.14 ± 1.33^{a}	4.81 ± 0.75^{a}
		Starch	5.55 ± 0.53^{a}	6.06 ± 0.53^{a}	5.89 ± 0.87^{a}
		NSC	11.00 ± 0.82^{a}	11.20 ± 1.17^{a}	10.70 ± 1.42^{a}
	Last-year leaf	Sugars	5.42 ± 1.17^{a}	5.56 ± 0.49^{a}	5.17 ± 1.00^{a}
		Starch	6.51 ± 0.66^{a}	6.75 ± 0.81^{a}	6.34 ± 0.69^{a}
		NSC	11.93 ± 1.77^{a}	12.31 ± 0.68^{a}	11.51 ± 1.15^{a}

Data are presented mean ± SD. The same letter indicates no significant difference at the 0.05 level

Table 2 Two-way ANOVA analyses of the effects of needle/leaf age and sampled canopy on sugars, starch, and NSC concentrations in *A. georgei* and *J. saltuaria*

	Needle/leaf age		Sampling height		Needle/leaf age × sampling height	
	A. georgei	*J. saltuaria*	*A. georgei*	*J. saltuaria*	*A. georgei*	*J. saltuaria*
Sugars	30.43***	0.71^{ns}	0.80^{ns}	0.77^{ns}	1.61^{ns}	0.19^{ns}
Starch	6.16***	9.21**	0.57^{ns}	0.95^{ns}	0.95^{ns}	0.42^{ns}
NSC	36.02***	5.57*	1.25^{ns}	0.84^{ns}	0.94^{ns}	0.04^{ns}

F values are presented

ns No significance

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

limits, their needle/leaf NSC concentrations remained relatively high even under the extreme cold environment. According to the existing evidence that NSC could mirror a tree's carbon balance, the relative abundance of NSC may indicate no carbon limitation under the present CO_2 status, which is consistent with the conclusion in a study on altitudinal pattern of tree NSC in eastern Himalayas by Shi et al. (2008) and other latest studies which confirmed the growth limitation by analyzing NSC at different altitudes (Hoch et al. 2002; Hoch and Körner 2003; Piper et al. 2006; Shi et al. 2006). In addition, theoretical models suggest that carbon investment in storage (higher NSC) is an advantageous strategy in habitats with frequent stress or disturbance (Iwasa 1997; Poorter and Kitajima 2007). For plant species growing in extremely high-altitude environments characterized by frequent low temperature, allocation of carbon to storage would enhance plant endurance to harsh climate at the expense of plant growth (Kitajima 1994; Smith and Stitt 2007; Bansal and Germino 2008). The relatively higher NSC concentrations may imply a special growth strategy of these two species under extremely high-altitude timberline area compared with plant species in other environments, i.e., allocating more carbon to storage rather than using it on plant growth.

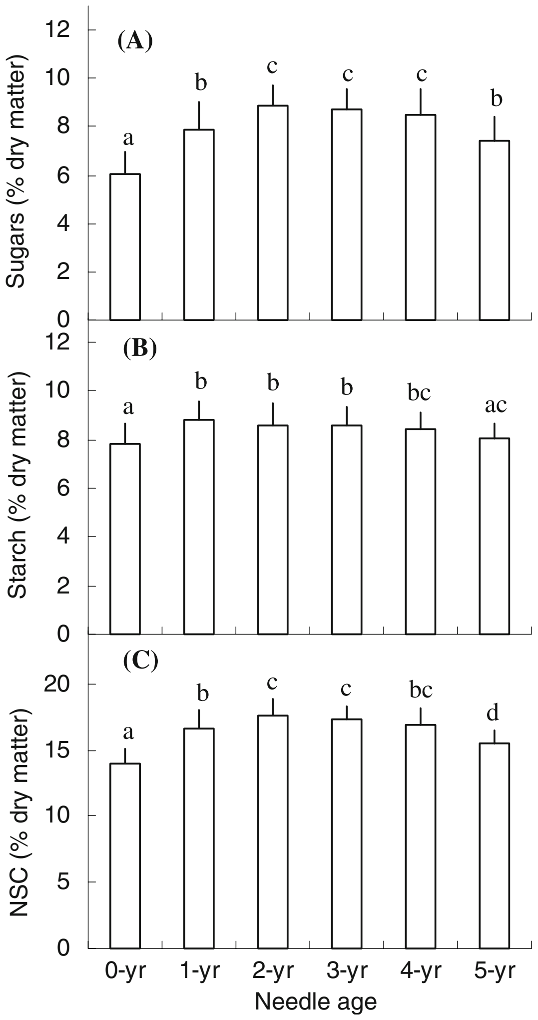

Fig. 1 Variations of sugar (**a**), starch (**b**), and NSC concentrations (**c**) with needle age of *A. georgei*. Data are presented mean ± SD. *Different letters* indicate significant differences between needle age at the 0.05 level. The value for each age needle is the pooled data across three canopies due to no significant effect of sampling canopy height

Compared with *J. saltuaria* distributed on the southeast-facing slope, *A. georgei* on northwest-facing slope had higher sugar, starch, and also NSC concentrations, which agrees with previous study on NSC measurement of Cupressacceae (*Juniperus saltuaria*, *J. tibetica*) and Pine (*Abies georgei*) (Shi et al. 2008). The difference between *J. saltuaria* and *A. georgei* in the present study may be related to the adaptation of the two species to different microclimatic conditions. Previous studies have verified that, in a harsh environment, plants prefer to store more carbon so as to increase their survival rather than spending it on growth (Iwasa 1997; Smith and Stitt 2007; Poorter and Kitajima 2007). Situated on the northwest-facing slope, where soil temperature is about 1°C lower than southeast-facing slope, as well as relatively lower light availability (unpublished data), *A. georgei* was placed in an unfavorable condition compared with *J. saltuaria*. In this case, carbohydrate reserves are especially important to enhance tolerance of harsh environment (e.g., low temperature or light on northwest-facing slope). However, it is very difficult to fully understand species-specific growth strategy under different slope environments only using the NSC concentrations of leaf samples. Further study should measure relative growth rate with associated leaf eco-physiological characteristics, NSC storage in other organs besides leaves, and also environmental factors at the two slopes to confirm specific growth strategy of these two species.

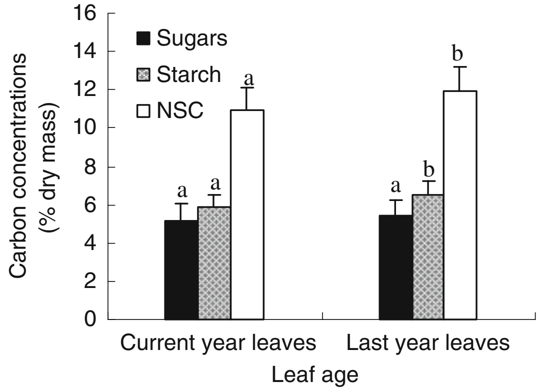

Fig. 2 Variations of sugar, starch, and NSC concentrations in last- and current-year leaves of *J. saltuaria*. Data are presented mean ± SD. *Different letters* indicate significant differences between last- and current-year leaves at the 0.05 level. The value in this figure is the pooled data across three canopies due to no significant effect of sampling canopy height

The canopy effect

Unexpectedly, no significant difference in NSC between sampling canopy heights for current- to 5-year needles was found in *A. georgei* except a weakly significant difference in sugars between upper and lower canopy. Also, there was no evident variation in NSC between canopy heights in current- and last-year leaves of *J. saltuaria*. Many previous studies have reported the vertical pattern of NSC along canopy heights and have confirmed "more light, more NSC" in tree species (Meletiou-Christou et al. 1994; Würth et al. 1998; Herrick and Thomas 1999; Niinemets et al. 2006). In accordance with our study, Li et al. (2001) found that neither slope exposure (south versus north) nor position within the crown (upper, middle, and lower part of crown) had effect on NSC in *Pinus cembra* at the alpine treeline in the Swiss Central Alps. Unfortunately, we did not measure available light for different vertical canopy heights due to the lack of efficient measuring apparatus. However, the timberlines dominated by *J. saltuaria* and *A. georgei* in this study belong to abrupt type, and even at the upper timberline, almost closed canopies are found due to the tree heights and densities. In addition, we found significant vertical variations of leaf traits (i.e., specific leaf area, N and P content per leaf area) from lower to upper canopy (Li et al. 2009), which may be determined directly by light availability (Evans and Poorter 2001; Prasolova et al. 2003). Therefore, we expect higher light availability in upper canopy, followed by middle and lower canopies. In fact, more light in upper than lower canopy has been found in many studies, especially for closed canopies (Niinemets and Tenhunen 1997; Tateno and Kawaguchi 2002; Prasolova et al. 2003).

No significant variations of NSC along canopy gradients may be as a result of different exports for canopy-dependent leaves/needles, because the leaf NSC not only reflects the net photosynthetic carbon uptake but may be related to the distribution of photosynthetic products (Chapin et al. 1990; Hoch and Körner 2003; Körner 2003; Würth et al. 2005). By measuring tissue-specific δ^{13}C, Schleser (1992) has indicated that tree organs at lower or middle canopy height were not wholly supplied by the corresponding segment leaves at the same height, which indicates that carbohydrates produced by upper-canopy leaves may be partially transferred to lower or middle organs of trees, whereas for lower or middle leaves the transfer of carbohydrates is less. Also, Li et al. (2007) confirmed the transfer of carbohydrates from upper-canopy leaves of timberline species in this area by using stable isotope methods. The vertical pattern of NSC may be related to the extremely harsh climate at the high-altitude timberline area, in which transfer of carbohydrates from upper leaves is more than for middle or lower leaves. In addition, leaves in lower canopy could decrease carbon loss by decreasing respiration (Björkman 1981). Higher carbon transfer accompanied by higher carbon loss due to respiration for upper leaves may account for the lack of difference in NSC with canopy height. However, this is a preliminary explanation, and powerful evidence for distribution pattern of photosynthetic carbon should be verified by stable isotope labeling studies such as that described by Körner et al. (2005).

Leaf/needle effect

In general, significant effect of leaf/needle age on sugar, starch, and NSC concentrations was found in *A. georgei* and *J. saltuaria*. For *A. georgei*, carbohydrates showed very significant increase from current-year to 1- or 2-year needles, followed by a weak decline. Likewise, current-year leaf had lower starch and NSC compared with last-year leaf for *J. saltuaria*. Increases in NSC concentrations with increasing needle/leaf age (current-year versus 1- or 2-year needles for *A. georgei* in Fig. 1, current-year versus last-year leaves for *J. saltuaria* in Fig. 2) have been found in previous studies for evergreen conifers and explained by decreasing activities of enzymes responsible for carbon translocation, or decreasing ability of phloem to export photosynthates with needle aging (Hendrix and Huber 1986; Egger et al. 1996; Niinemets 1997). Similarly, Cabálková et al. (2007) found that needle NSC showed significant variations with increasing needle age from current year to 2 years old and explained the differences by the fact that older needles support the development of young shoots. However, related references are mostly focused on relatively young leaves (Hendrix and Huber 1986; Niinemets 1997; Cabálková et al. 2007). In fact, NSC concentrations did not exhibit a constantly increasing pattern with needle age. For needle age greater than 2 years in this study, NSC including starch and soluble sugars showed a gradual decrease (Fig. 2). Robakidze and Bobkova (2003) also found that 1- to 2-year-old needles have higher monosaccharides than 3- to 9-year-old needles in Siberian spruce. Generally, a balance exists between carbon acquisition in photosynthesis and carbohydrate export from leaves (Hendrix and Huber 1986). With increasing leaf age, photosynthetic capacity of leaf indeed largely declines accompanied by decrease of carbon translocation (Niinemets et al. 2004, 2005). The weak decrease of NSC with needle age from 2 to 5 years may suggest that the carbon acquisition in photosynthesis may decrease more significantly than the rate of carbohydrate export as needle age exceeds 2 years. Of course, this assumption needs to be confirmed by measuring photosynthetic characteristics and activities of enzymes responsible for carbon translocation in the future. Additionally, the accumulation of structural carbohydrates (i.e., cellulose and lignin) dilutes NSC (Li et al. 2001) because lignification of the needle hypodermis proceeds until at least the third year of development (about 2 years old) (Niinemets 1997).

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References

Bansal S, Germino MJ (2008) Carbon balance of conifer seedlings at timberline: relative changes in uptake, storage, and utilization. Oecologia 158:217–227

Björkman O (1981) Responses to different quantum flux densities. In: Lange OL, Nobel PS, Osmond CB (eds) Encyclopedia of plant physiology. Springer, Berlin, pp 57–107

Cabálková J, Wahlund KG, Chmelík J (2007) Complex analytical approach to characterization of the influence of carbon dioxide concentration on carbohydrate composition in Norway spruce needles. J Chromatogr A 1148:189–199

Chapin FSIII, Schulze E, Mooney HA (1990) The ecology and economics of storage in plants. Annu Rev Ecol S 21:423–447

Egger B, Einig W, Sclereth A, Wallenda T, Magel E, Loewe A, Hampp R (1996) Carbohydrate metabolism in one- and two-year-old spruce needles, and stem carbohydrates from 3 months before until 3 months after bud break. Physiol Plantarum 96:91–100

Evans JR, Poorter H (2001) Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant Cell Environ 24:755–767

He JC, Luo TX, Xu YQ (2009) Characteristics of eco-climate at smith fir timberline in the Sergyemla Mountains, southeast Tibetan Plateau. Acta Ecol Sin 29:37–46

Hendrix DL, Huber SC (1986) Diurnal fluctuations in cotton leaf carbon export, carbohydrate content, and sucrose synthesizing enzymes. Plant Physiol 81:584–586

Herrick JD, Thomas RB (1999) Effects of CO_2 enrichment on the photosynthetic light response of sun and shade leaves of canopy sweetgum trees (*Liquidambar sryraciflua*) in a forest ecosystem. Tree Physiol 19:779–786

Hoch G, Körner Ch (2003) The mobile carbon supply of pines at the climatic treeline: a global comparison. Oecologia 135:10–21

Hoch G, Popp M, Körner Ch (2002) Altitudinal increase of mobile carbon pools in *Pinus cembra* suggests sink limitation of growth at the Swiss treeline. Oikos 98:361–374

Hoch G, Richter A, Körner Ch (2003) Non-structural carbon compounds in temperate forest trees. Plant Cell Environ 26: 1067–1081

Iwasa Y (1997) Optimal size of storage for recovery after unpredictable disturbances. Evol Ecol 11:41–65

Kitajima K (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seeding shade to tolerance of 13 tropical trees. Oecologia 98:419–428

Körner Ch (2003) Carbon limitation in trees. J Ecol 91:4–17

Körner Ch, Asshoff R, Bignucolo O, Hättenschwiler S, Keel SG, Peláez-Riedl S, Pepin S, Siegwolf RTW, Zotz G (2005) Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2. Science 309:1360–1362

Li MH, Hoch G, Körner Ch (2001) Spatial variability of Mobile Carbohydrates within *Pinus cembra* trees at the alpine treeline. Phyton 41:203–213

Li MC, Luo TX, Liu XS, Kong GQ (2007) Distribution characteristics of δ^{13}C values in different organs of *Aibes georgei* growing at alpine tree-line. Chin J Appl Ecol 18:2654–2660

Li MC, Liu DY, Kong GQ (2009) Vertical differences of nutrient resorption and use efficiency at different canopy heights of alpine tree species *Abies georgei* var smithii (viguie et gaussen) cheng, Tibetan Plateau. Pol J Ecol 57:63–72

Luo TX, Luo J, Pan YD (2005) Leaf traits and associated ecosystem characteristics across subtropical and timberline forests in the Gongga Mountains, Eastern Tibetan Plateau. Oecologia 142: 261–273

Meletiou-Christou MS, Rhizopoulou S, Diamantoglou S (1994) Seasonal changes of carbohydrates, lipids and nitrogen content in sun and shade leaves from four Mediterranean evergreen scleraophylls. Environ Exp Bot 34:129–140

Niinemets (1997) Acclimation to low irradiance in *Picea abies*: influences of past and present light climate on foliage structure and function. Tree Physiol 17:723–732

Niinemets Ü, Tenhunen JD (1997) A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species Acer saccharum. Plant Cell Environ 20:845–866

Niinemets Ü, Tenhunen JD, Beyschlag W (2004) Spatial and age-dependent modifications of photosynthetic capacity in four Mediterranean oak species. Funct Plant Biol 31:1179–1193

Niinemets Ü, Cescatti A, Rodeghiero M, Tosens T (2005) Leaf internal diffusion conductance limits photosynthesis more strongly in older leaves of Mediterranean evergreen broad-leaved species. Plant Cell Environ 28:1552–1566

Niinemets Ü, Cescatti A, Rodeghiero M, Tosens T (2006) Complex adjustments of photosynthetic potentials and internal diffusion conductance to current and previous light and leaf age in Mediterranean evergreen species *Quercus ilex*. Plant Cell Environ 29:1159–1178

Oleksyn J, Zytkowiak R, Karolewski P, Reich PB, Tjoelker MG (2000) Genetic and environmental control of seasonal carbohydrate dynamics in trees of diverse *Pinus sylvestris* populations. Tree Physiol 20:837–847

Piper F, Cavieres L, Reyes-Díaz M, Corcuera L (2006) Carbon sink limitation and frost tolerance control performance of the tree *Kageneckia angustifolia* D. Don (Rosaceae) at the treeline in central Chile. Plant Ecol 185:29–39

Poorter L, Kitajima K (2007) Carbohydrate storage and light requirements of tropical moist and dry forest tree species. Ecology 88:1000–1011

Prasolova NV, Xu ZH, Lundkvist K, Farquhar GD, Dieters MJ, Salker S, Saffigna PG (2003) Genetic variation in foliar carbon isotope composition in relation to tree growth and foliar nitrogen concentration in clones of the F1 hybrid between slash pine and Caribbean pine. For Ecol Manage 172:145–160

Robakidze EA, Bobkova KS (2003) Carbohydrate accumulation in *Siberian spruce* needles of various ages. Russ J Plant Physiol 50:509–515

Schleser GH (1992) δ^{13}C patterns in a forest tree as an indicator of carbon transfer in trees. Ecology 73:1922–1925

Shi PL, Körner Ch, Hoch G (2006) End of season carbon supply status of woody species near the treeline in western China. Basic Appl Ecol 7:370–377

Shi PL, Körner Ch, Hoch G (2008) A test of the growth-limitation theory for alpine tree line formation in evergreen and deciduous taxa of the eastern Himalayas. Funct Ecol 22:213–220

Smith AM, Stitt M (2007) Coordination of carbon supply and plant growth. Plant Cell Environ 30:1126–1149

Tateno R, Kawaguchi H (2002) Differences in nitrogen use efficiency between leaves from canopy and subcanopy trees. Ecol Res 17:695–704

Würth MKR, Winter K, Körner Ch (1998) Leaf carbohydrate responses to CO_2 enrichment at the top of tropical forest. Oecologia 116:18–25

Würth MKR, Peláez-Riedl S, Wright SJ (2005) Non-structural carbohydrate pools in a tropical forest. Oecologia 143:11–24

Xu FX (1995) Forest ecology research in Xizang. Liaoning University Press, Shenyang