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Mobile carbohydrates in Himalayan treeline trees I. Evidence for carbon gain limitation but not for growth limitation

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Summary To test whether the altitudinal distribution of trees is determined by a carbon shortage or an insufficient sugar fraction (sugar:starch ratio) in treeline trees, we studied the status of nonstructural carbohydrates (NSC) and their components (total soluble sugars and starch) in *Abies fabri* (Mast.) Craib and *Picea balfouriana* var. *hirtella* Rehd. et Wils. trees along three elevational gradients, ranging from lower elevations to the alpine treeline, on the eastern edge of the Tibetan Plateau. For comparison, we investigated a low-altitude species (*Tsuga yunnanensis* (Franch.) Pritz.) which served as a warm-climate reference because it is distributed in closed montane forests below 3100 m a.s.l. in the study area. The carbon status of *T. yunnanensis* responded to altitude differently from that of the treeline species. At the species level, total NSC was not consistently more abundant in treeline trees than in trees of the same species growing at lower elevations. Thus there was no consistent evidence for carbon limitation of growth in treeline trees. For the three treeline species studied (*P. balfouriana* and *A. fabri* in the Kang-Ding Valley and *A. fabri* in the Mo-Xi Valley), winter NSC concentrations in treeline trees were significantly lower than in lower-elevation trees of the same species, suggesting that, in winter, carbon is limited in treeline trees. However, in no case was there total overwinter depletion of NSC or its components in treeline trees. Treeline and low-altitude species had similar sugar:starch ratios of about three at their upper-elevational limits in April. We conclude that survival and growth of trees at the elevational or latitudinal climate limit depend not only on NSC concentration in perennial tissues, but also on the maintenance of an overwintering sugar:starch ratio greater than three.

Keywords: altitudinal limit, nonstructural carbohydrates, sugar:starch ratio, treeline ecology, treeline formation, winter hardening.

Introduction

The latitudinal and altitudinal treeline is one of the most conspicuous ecological boundaries (Körner 2003*a*). How the treeline is formed is the subject of debate, and several hypotheses have been proposed. These include: (1) climatic stress as a result of repeated damage by freezing (e.g., Perkins and Adams 1995), frost desiccation (e.g., Wardle 1981*a*) and phototoxic effects (e.g., Öquist and Huner 1991); (2) disturbance due to mechanical damage by wind, ice blasting, snow break and avalanches (Vangardingen et al. 1991, Grace and James 1993); (3) insufficient tissue maturation because of the short growing season (e.g., Baig and Tranquillini 1980, Wardle 1981*a*); (4) temperature-limited reproduction (Wardle 1981*b*, Sveinbjörnsson et al. 1996, Hättenschwiler and Smith 1999); (5) direct growth limitation (Däniker 1923, Körner 2003*a*); and (6) carbon limitation (Schulze et al. 1967, Stevens and Fox 1991, Wardle 1993). The first four hypotheses are related to the alpine/arctic physical environment (physical hypotheses), which varies from region to region. Only the last two hypotheses, which relate to the biophysiology of treeline trees (biophysiological hypotheses), may explain the physiological mechanism of global alpine/arctic treeline formation (Körner 1998, Li and Kräuchi 2005).

The persistence and development of treeline trees in a harsh alpine environment depends not only on the availability of mobile carbohydrates to maintain respiration and growth during the short growing season (Volenec et al. 1996, Ogren et al. 1997, Ogren 2000, Körner 2003*b*), but also on the accumulation of soluble sugars in perennial organs during winter hardening. These sugars prevent intracellular ice formation, which can severely injure trees (Levitt 1956, Sakai and Yoshida 1968, Sakai and Larcher 1987, Morin et al. 2007). The sharply reduced growth rate of trees at or close to the treeline (Tranquillini 1979, Li et al. 2003, Li and Yang 2004) has often been explained by a shortage of photo-assimilates (Schulze et al.

1967, Stevens and Fox 1991, Wardle 1993). However, classical leaf gas-exchange studies have revealed no particular impairment of photosynthesis in treeline trees compared with trees at lower altitudes (Tranquillini 1979, Körner 2003*a*), indicating that low leaf net photosynthetic rate alone cannot explain the balance between carbon gain and carbon loss at a given time or during a given period.

There is no conclusive evidence that treeline trees are carbon-limited or growth limited (Körner 1998, Li and Kräuchi 2005). The size of the nonstructural carbohydrate (NSC) pool of trees growing near the treeline may provide an answer because it reflects the balance between carbon-source and carbon-sink activity (Li et al. 2002, Körner 2003*b*). Where there is an elevational increase in carbon limitation, NSC concentration can be expected to decrease as the tree limit is approached (source limitation). In contrast, if tissue formation is limited (sink limitation hypothesis; Körner 1998), it should lead to an increasing abundance (or saturation) of mobile carbon compounds with increasing altitude because the carbon demand for tree growth is reduced.

Using mobile carbohydrates as an indicator, Hoch and Körner (2003) found that the concentrations of mobile carbohydrates were not lower in three *Pinus* species at their alpine treelines (*P. cembra* L. in the Swiss Alps, *P. sylvestris* L. in northern Sweden and *P. hartwegii* Lindl. in Mexico) than in trees at lower elevations. However, Richardson (2004) detected significant carbon limitation in *Picea rubens* Sarg. and *Abies balsamea* (L.) P. Mill growing at alpine treelines in the northeastern USA. Similarly, Cavieres et al. (2000) found clear evidence of a carbon shortage in *Espeletia neriifolia* (Kunth.) Schultz-Bipontinus ex Weddell, but not in *Podocarpus oleifolius* D. Don, both growing at the tropical alpine treeline in the Venezuelan Andes.

Such contradictory findings prompted us to test the carbon limitation hypothesis in other treeline tree species in other regions. Specifically, we investigated mobile carbohydrates in two non-*Pinus* species (*Abies fabri* (Mast.) Craib and *Picea balfouriana* var. *hirtella* Rehd. et Wils.) that grow from lower elevations to the alpine treeline in the Gongga Mountains, SW China (Table 1). We sought answers to two questions. (1) Do treeline trees in SW China have lower mobile carbohydrate concentrations than lower-elevation trees as predicted by the carbon limitation hypothesis on treeline formation? (2) To what extent can the ratio of soluble sugars to starch explain the functional mechanisms of alpine treeline formation, given the clear relationship between winter hardiness and availability of soluble sugars in perennial tissues (Ogren et al. 1997, Morin et al. 2007). For comparative purposes, we studied a low-altitude species (*Tsuga yunnanensis* (Franch.) Pritz.), distributed in closed montane forests below 3100 m a.s.l. in the study area (Table 1), as a warm-climate reference.

Materials and methods

Study sites and species

The study was conducted in two valleys, the Mo-Xi Valley, which runs south–east, and the Kang-Ding Valley, which runs north–west, located along the northwest face of Mt. Gongga (29°35′45″ N, 101°52′45″ E, 7556 m a.s.l.), southwestern China. Because of topography and the southeast monsoon, the Mo-Xi Valley is relatively warm and moist and the Kang-Ding Valley is relatively hot and dry. Mean precipitation is about 1000 mm year^{-1} in the Mo-Xi Valley (data collected at 1600 m a.s.l. from 1960 to 1989), and about 800 mm year^{-1} in the Kang-Ding Valley (data collected at 2616 m a.s.l. from 1952 to 1990). The soil in both valleys has developed from granite, classified as a mountain gray-brown soil (He et al. 2005). The forests surrounding the study sites had naturally regenerated, but were moderately disturbed during the 1970s and 1980s by selective-cutting and grazing. Since 1986, the area has been protected and no such disturbances have occurred. Characteristics of the study sites and study trees are summarized in Table 1.

The study sites in the Mo-Xi Valley are located on a northeast-facing slope, where *Abies fabri* is distributed from 2700 m (the lowest elevational distribution in this valley) to 3700 m (the alpine treeline), and *Tsuga yunnanensis* from 2300 to 3100 m (upper-elevational limit) (Table 1). The dense understory is dominated by *Bashania* sp., reflecting the warm moist environment (Table 1).

The study sites in the Kang-Ding Valley are located on a southwest-facing slope, where *A. fabri* is distributed between 3200 and 3780 m (the treeline), and *Picea balfouriana* var. *hirtella* occurs between 3270 and 3820 m (Table 1). This site has a dense understory of *Quercus aquifolioides* Rehd. et Wils., reflecting the hot dry environment (Table 1).

For brevity, we henceforth refer to the species studied (*Abies fabri*, *Picea balfouriana* var. *hirtella* and *Tsuga yunnanensis*) by their generic names, and to trees at the alpine treeline (*Picea* and *Abies*) or their upper-elevational distribution (*Tsuga*) as TT, and trees at the mid- and lowest elevations as MT and LT, respectively.

Sampling

We sampled tree tissues only twice because we were interested in the size of the mobile carbon pool in trees from lower elevations to the upper-elevational limits, and not in the seasonal variability of this pool. The first set of tissue samples was taken immediately before bud break (between April 17 and 21, 2005), to check the size of the April carbon pool, which should be relatively low as a result of winter depletion (Fischer and Holl 1991, Gonzalez 1991), and to determine if TT completely depleted the mobile carbon pool, especially soluble sugars, in winter. The second set of tissue samples was taken during peak shoot growth between July 24 and 28, 2005. The size of the July carbon pool allowed us to determine if there were enough carbohydrates for structural growth in alpine TT and how their pool sizes compared with those in trees at lower elevations.

Six healthy undamaged trees ($n = 6$) growing in stands were selected for uniformity in age to avoid possible age effects on the parameters studied and in the visible needle color of each tree species along each elevational gradient (Table 1). Because

Table 1. Characteristics of the plots and sampled trees ($n = 6$) in the Mt. Gongga region, Sichuan, SW China. Codominant species in a layer are indicated by "+", and connected layers are indicated by "–". Abbreviation: *Rh* = *Rhododendron*.

Elevation (m)	Slope exposure	Soil water classification	Mean air temperature (°C)	Age (years)	Height (m)	Diameter (cm)	Community
Abies fabri in the Mo-Xi Valley (uppermost distribution at 3700 m a.s.l.)							
3670	NE	Mesic	1.6	71 ± 8	10.4 ± 3.2	23.5 ± 6.3	*Abies fabri* – *Rhododendron* spp.
3100	NE	Mesic	4.5	64 ± 12	14.7 ± 4.1	26.3 ± 4.7	*A. fabri* – *Bashania* sp.
2750	NE	Moist	6.2	67 ± 6	18.3 ± 4.4	28.1 ± 7.8	*A. fabri* + *Tsuga yunnanensis* – *Bashania* sp.
Abies fabri in the Kang-Ding Valley (uppermost distribution at 3780 m a.s.l.)							
3750	SW	Dry	2.4	51 ± 15	15.9 ± 6.4	27.6 ± 5.6	*A. fabri* – *Quercus aquifolioides* + *Rh.* spp.
3300	SW	Dry	4.7	44 ± 7	20.3 ± 2.7	36.8 ± 2.8	*A. fabri* – *Q. aquifolioides* + *Rh.* spp.
Picea balfouriana var. hirtella in the Kang-Ding Valley (uppermost distribution at 3820 m a.s.l.)							
3800	SW	Dry	2.1	37 ± 5	8.6 ± 2.2	22.4 ± 2.7	*P. balfouriana* var. *hirtella* – *Q. aquifolioides*
3400	SW	Dry	4.2	36 ± 10	13.4 ± 2.7	26.0 ± 5.0	*P. balfouriana* var. *hirtella* – *Q. aquifolioides*
Tsuga yunnanensis in the Mo-Xi Valley (uppermost distribution at 3100 m a.s.l.)							
3100	NE	Mesic	4.5	87 ± 21	28.1 ± 7.6	35.0 ± 1.5	*A. fabri* + *T. yunnanensis* – *Bashania* sp.
2750	NE	Moist	6.2	79 ± 24	23.5 ± 4.8	35.8 ± 2.5	*A. fabri* + *T. yunnanensis* – *Bashania* sp.
2350	NE	Moist	8.2	62 ± 14	15.4 ± 4.6	32.0 ± 3.3	*A. fabri* + *T. yunnanensis* – *Rh.* sp.

the concentrations of mobile carbohydrates in needles fluctuate daily in response to photosynthetic activity (Graham et al. 2003), samples were always taken around noon to ensure the effects of sunlight and needle surface temperature on carbohydrates were relatively constant. One- (previous year), 2- and 3-year-old needles (only 1- and 2-year-old needles in *Tsuga* could be distinguished) from non-shaded leading branches on the upslope side of the tree were collected separately, to avoid age effects on needle NSC (Li et al. 2001). Two cores from each sample tree were taken on opposite sides of the stem (parallel to the slope) at 40 cm above the ground, with a 5-mm-diameter corer. The outer 3 cm of stem sapwood (xylem tissue only, about 7–10 tree-rings) of the two cores was removed and combined for each sampled tree to obtain enough ground sample for chemical analysis. Fine roots (< 2 mm in diameter, with bark) were collected from each sample tree. All samples were immediately stored in a cool box and killed in a microwave oven (40 s at 600 W) and dried to constant mass at 65–70 °C.

Chemical analysis: total soluble sugars

Dried plant material was ground to pass a 1-mm sieve. The powdered material (0.1 g) was placed in a 10-ml centrifuge tube and 5 ml of 80% ethanol added. The mixture was incubated at 80 °C for 30 min, and then centrifuged at 5000 *g* for 5 min. The pellets were re-extracted twice with 80% ethanol. The pellet was saved for starch analysis and the combined supernatants were stored at –20 °C until analyzed for soluble sugar by the anthrone method (Seifter et al. 1950). Glucose was used as a standard. Soluble sugar concentration was calculated on a dry matter basis (% dm).

Chemical analysis: starch

Starch was extracted from the ethanol-insoluble pellet after removing the ethanol by evaporation. Starch in the residue was released by boiling in 2 ml of distilled water for 15 min. After cooling to room temperature, 2 ml of 9.2 M $HClO_4$ was added and the starch was hydrolyzed for 15 min. Four ml of distilled water was added and the mixture centrifuged at 5000 *g* for 10 min. The pellet was re-extracted with 2 ml of 4.6 M $HClO_4$. The combined supernatants were adjusted to 20 ml. Starch concentration was measured spectrophotometrically at 620 nm using anthrone reagent, and calculated by multiplying the glucose concentration by the conversion factor of 0.9 (Osaki et al. 1991). Glucose was used as a standard. The starch concentration was expressed on a dry matter basis (% dm).

Data analysis

Nonstructural carbohydrate is defined as the sum of the starch plus the total soluble sugars for each tissue at each sample date. Data (NSC, starch, total soluble sugars) were tested for normality (Kolmogorov–Smirnov-Test). Two-way analyses of variance (ANOVAs) were performed with elevation and tissue type as factors to determine elevational trends in concentrations of NSC and its components for overall (the three treeline cases combined) and individual species at each sampling date. At the tissue level for each species, two-way ANOVAs were repeatedly used with elevation and sampling date as factors for fine roots and stem sapwood, and with needle age and elevation, or needle age and sampling date as factors for needles. Elevational effects on NSC and its components at different elevations within each tissue category and date were tested by single factor ANOVAs, and followed, if significant, by *t*-paired comparisons or Tukey's HSD test at $\alpha = 0.05$ to evaluate differences between pairs of means. Because we were mainly interested in the effects of elevation on mobile carbohydrates, other effects such as tissue type and the interaction between elevation and tissue type are not systematically discussed.

Results

Abies and Picea in the Kang-Ding Valley

For *Picea* grown in the Kang-Ding Valley, two-way ANOVAs indicated that the NSC concentration in TT was significantly lower than in LT in both April ($P < 0.001$) and July ($P < 0.001$) (Table 2). Starch concentration was also significantly lower in TT than in LT in both April and July ($P < 0.001$ in both cases) (Table 2). However, the concentrations of total soluble sugars did not differ between TT and LT in either April ($P = 0.90$) or July ($P = 0.58$) (Table 2).

For *Abies* trees grown in the Kang-Ding Valley, two-way ANOVAs showed that the NSC concentration was slightly higher in TT than in LT in both April ($P = 0.06$) and July ($P = 0.07$) (Table 2). The concentration of total soluble sugars was significantly higher in TT than in LT in April ($P < 0.001$), but not in July ($P = 0.43$) (Table 2). Starch concentration was significantly lower in TT than in LT in April ($P = 0.023$), but slightly higher in July ($P = 0.08$) (Table 2).

A comparison of *Picea* and *Abies* grown in the Kang-Ding Valley revealed contradictory elevational trends in NSC between species; i.e., NSC in TT < LT for *Picea* and NSC in TT > LT for *Abies* (Table 2). The differences in NSC concentrations between TT and LT were primarily a result of different starch concentrations between TT and LT *Picea* trees (Table 2, Figure 1), and to differences in concentrations of both starch and total soluble sugars between TT and LT *Abies* trees (Table 2, Figure 2).

For *Picea*, two-way ANOVA with needle age and elevation as factors indicated that only altitude significantly affected needle NSC concentrations ($P < 0.001$). The TT had a needle NSC of 15.5% dm, whereas LT had a needle NSC of 19.9% dm (Table 3). Two-way ANOVA with needle age and sampling date as factors showed that only sampling date significantly affected needle NSC ($P = 0.04$) (Table 4). Needle NSC concentration was 16.7% dm in April and 18.7% dm in July (Table 4). Two-way ANOVA detected no effects on root NSC (Tables 3 and 4). Nonstructural carbohydrates in fine roots were 9.2% dm in TT and 11.1% dm in LT (Tables 3). Only sampling date significantly affected NSC in stem sapwood ($P < 0.001$) (Tables 3 and 4), according to a two-way ANOVA with elevation and sampling date as factors. Stem sapwood had an NSC concentration of 4.5% dm in TT and 3.9% dm in LT (Table 3).

For *Abies*, two-way ANOVA with needle age and sampling date as factors revealed no effects on needle NSC (18.5–19.1% dm across sampling dates and needle ages) (Ta-

Table 2. Concentrations (% dry matter) of nonstructural carbohydrate (NSC) and its components (soluble sugars, starch) as functions of the elevation at which the trees grow (see Table 1). Two-way ANOVAs were conducted with elevation and tissue type as factors.

	Soluble sugars		Starch		NSC	
	April	July	April	July	April	July
Picea trees at 3800 m a.s.l. (treeline) and 3400 m in the Kang-Ding Valley						
3800 m	8.58	8.24	3.09	4.22	11.67	12.46
3400 m	8.63	8.39	5.50	7.36	14.14	15.74
$F_{1,29}$	0.02	0.33	68.78	23.47	30.85	26.77
P	0.90	0.58	< 0.001	< 0.001	< 0.001	< 0.001
Abies trees at 3750 m a.s.l. (treeline) and 3300 m in the Kang-Ding Valley						
3750 m	10.73	10.02	3.10	5.04	13.82	15.06
3300 m	8.56	9.73	4.07	4.06	12.63	13.79
$F_{1,29}$	33.10	0.66	6.05	3.36	4.02	3.77
P	< 0.001	0.43	0.023	0.08	0.06	0.07
Abies trees between 3670 m a.s.l. (treeline) and 2750 m in the Mo-Xi Valley						
3670 m	13.97	13.27	4.62	3.31	18.60	16.58
3100 m	14.33	13.21	4.56	2.68	18.89	15.89
2750 m	14.79	12.99	4.73	2.22	19.52	15.21
$F_{2,44}$	0.67	0.11	0.12	5.13	0.96	2.01
P	0.52	0.90	0.89	0.012	0.395	0.15
The above three treeline cases combined (Lower E trees = lower elevation trees)						
Treeline trees	11.09	10.51	3.61	4.19	14.70	14.70
Lower E trees	10.66	10.37	4.77	4.55	15.43	14.91
$F_{1,89}$	3.53	0.24	31.00	1.35	6.80	0.31
P	0.07	0.63	< 0.001	0.25	0.011	0.58
Tsuga between 3100 m a.s.l. (upper elevational limit) and 2350 m in the Mo-Xi Valley						
3100 m	7.89	6.15	2.91	4.76	10.80	10.91
2750 m	7.32	6.06	3.61	4.18	10.92	10.24
2350 m	6.90	7.38	4.72	3.08	11.62	10.45
$F_{2,35}$	1.29	3.03	5.92	7.12	0.65	0.35
P	0.29	0.07	0.008	0.004	0.53	0.71

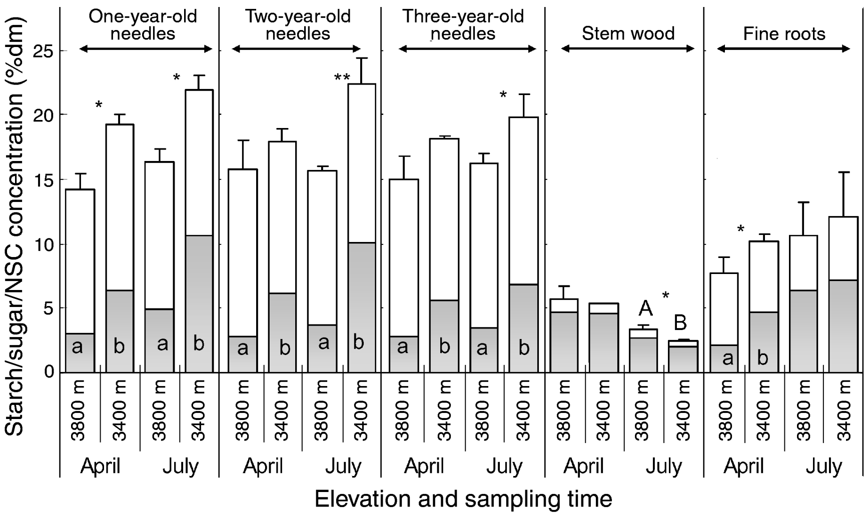

Figure 1. Mean concentrations of total soluble sugars (open bars), starch (filled bars) and NSC (soluble sugars + starch) in tissues of *Picea balfouriana* var. *hirtella* trees growing at the alpine treeline (3800 m a.s.l.) and at 3400 m a.s.l. in the Kang-Ding Valley, Mt. Gongga, SW China. Statistical differences in means between the elevations within each tissue category and date were tested by *t*-paired comparisons. Asterisks (for NSC: *, $P < 0.05$; **, $P < 0.01$; $n = 6$) and different letters (uppercase letters for sugars, and lowercase letters for starch) indicate statistically significant differences ($P < 0.05$; $n = 6$) between trees at 3400 and 3800 m a.s.l. within each tissue category and date. Standard error bars are given for NSC only.

ble 4). Only altitude significantly affected needle NSC concentration ($P = 0.006$) (Table 3) according to two-way ANOVA with needle age and elevation as factors. Needle NSCs in TT and LT were 19.6 and 18.0% dm, respectively (Table 3). Root NSC concentration was significantly affected only by sampling date ($P = 0.02$; two-way ANOVA) (Tables 3 and 4). Root NSC was 7.3% dm in April and 11.0% dm in July (Table 4). Two-way ANOVA indicated that sampling date and elevation had no effects on NSC in sapwood even when considered together (Tables 3 and 4). Stem sapwood contained an NSC concentration of 3.7% dm in TT and 3.5% dm in LT (Table 3).

According to *t*-paired comparisons between TT and LT for each tissue category within each sampling date, the concentrations of NSC and starch in *Picea* were frequently significantly lower in TT than in LT (Figure 1), whereas *Abies* showed significant differences in NSC ($P < 0.01$) and sugars ($P < 0.5$) between TT and LT only in 3-year-old needles in April (TT > LT for both), and in sugars only in July stem wood ($P < 0.05$ for TT > LT) (Figure 2).

Generally, TT had higher sugar:starch ratios than LT for both *Abies* and *Picea*, and *Abies* trees had higher ratios than *Picea* trees at each sampling date (Table 5).

Abies trees in the Mo-Xi Valley

Results of two-way ANOVAs indicated that tree elevation had no effect on NSC in either April ($P = 0.40$) or July ($P = 0.15$) (Table 2). Concentrations of total soluble sugars did not vary with tree elevation in either April ($P = 0.52$) or July ($P = 0.90$) (Table 2). The differences in starch concentrations between trees at different elevations were not significant in April ($P = 0.89$), but they were significant in July (higher-elevation trees > lower-elevation trees; $P = 0.012$) (Table 2).

Two-way ANOVA with needle age and elevation as factors showed no effects on needle NSC ($P = 0.46$ for elevation; $P = 0.29$ for needle age; $P = 0.31$ for their interaction). Needle NSC concentrations in TT, MT and LT were 24.5, 23.6 and 24.7% dm, respectively (Table 3). Two-way ANOVA with needle age and sampling date as factors indicated no age effect ($P = 0.18$), no interaction between needle age and sampling date ($P = 0.78$), but significant effects of sampling date on nee-

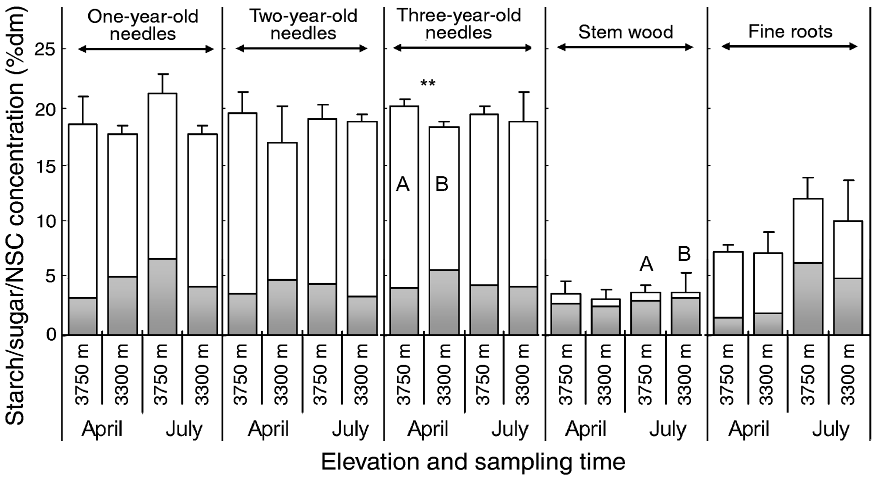

Figure 2. Mean concentrations of total soluble sugars (open bars), starch (filled bars) and NSC (soluble sugars + starch) in tissues of *Abies fabri* trees growing at the alpine treeline (3750 m a.s.l.) and at 3300 m a.s.l. in the Kang-Ding Valley, Mt. Gongga, SW China. Statistical differences in means between the elevations within each tissue category and date were tested by *t*-paired comparisons. Asterisks (for NSC: **, $P < 0.01$; $n = 6$) and different letters (uppercase letters for sugars) indicate statistically significant differences ($P < 0.05$; $n = 6$) between trees at 3750 and 3300 m a.s.l. within each tissue category and date. No significant differences were found for starch. Standard error bars are given for NSC only.

Table 3. Concentrations of nonstructural carbohydrates (NSC; % dry matter) as a function of growing site elevation (see Table 1). Two-way ANOVAs were conducted with elevation and needle age as factors for needles, and with elevation and sampling time as factors for roots and stem wood.

	Needles	Fine roots	Stem sapwood
Picea at 3800 m a.s.l. (treeline) and 3400 m in the Kang-Ding Valley			
3800 m	15.5	9.2	4.5
3400 m	19.9	11.1	3.9
F (df)	50.05 (1,35)	2.18 (1,11)	3.4 (1,11)
P	< 0.001	0.18	0.10
Abies at 3750 m a.s.l. (treeline) and 3300 m in the Kang-Ding Valley			
3750 m	19.6	9.6	3.7
3300 m	18.0	8.6	3.5
F (df)	8.63 (1,35)	0.60 (1,11)	0.12 (1,11)
P	0.006	0.46	0.74
Abies between 3670 m a.s.l. (treeline) and 2750 m in the Mo-Xi Valley			
3670 m	24.5	10.4	4.2
3100 m	23.6	11.9	4.3
2750 m	24.7	8.8	4.0
F (df)	0.78 (2,53)	9.73 (2,17)	0.15 (2,17)
P	0.46	0.003	0.87
Tsuga between 3100 m a.s.l. (upper-elevational limit) and 2350 m in the Mo-Xi Valley			
3100 m	14.1	12.1	3.1
2750 m	13.6	11.7	3.4
2350 m	13.4	11.8	5.4
F (df)	0.32 (2,35)	0.04 (2,17)	21.02 (2,17)
P	0.73	0.96	< 0.001

dle NSC concentrations ($P < 0.001$) (Table 4). Mean needle NSC concentration was 25.8% dm in April and 22.7% dm in July (Table 4). Two-way ANOVA revealed significant effects of sampling date ($P < 0.001$), elevation ($P = 0.003$) and their interaction ($P = 0.012$) on root NSC (Tables 3 and 4). Fine roots had an NSC concentration of 12.3% dm in April and 8.4% dm in July (Table 4). Root NSC in TT, MT and LT was 10.4, 11.9 and 8.8% dm, respectively (Table 3). Two-way ANOVA indicated that only sampling date significantly affected the NSC concentration of stem sapwood ($P < 0.001$) (Tables 3 and 4): it was 5.4% dm in April and 2.9% dm in July (Table 4).

Single-factor ANOVAs revealed no statistically significant differences in NSC and sugars in *Abies* trees at the different elevations within each tissue category and date, except for NSC in fine roots in July ($P = 0.02$ for MT ≅ TT > LT) (Figure 3). Single factor ANOVAs and *t*-paired comparisons indicated only two cases where starch concentrations differed significantly (Figure 3).

In April, the ratio of total soluble sugars to starch was 3.0 in TT and 3.1 in LT (Table 5). Remarkably high sugar:starch ratios were found in both TT (4.0) and LT (5.9) in July (Table 5).

Table 4. Concentrations of nonstructural carbohydrates (NSC; % dry matter) as a function of sampling time. Two-way ANOVAs were conducted with sampling date and needles age as factors for needles, and with elevation and sampling time as factors for fine roots and stem sapwood.

	Needles	Fine roots	Stem sapwood
Picea in the Kang-Ding Valley			
April	16.7	8.9	5.5
July	18.7	11.4	2.9
F (df)	4.67 (1,35)	3.62 (1,11)	65.80 (1,11)
P	0.04	0.09	< 0.001
Abies in the Kang-Ding Valley			
April	18.5	7.3	3.5
July	19.1	11.0	3.8
F (df)	1.07 (1,35)	8.20 (1,11)	0.26 (1,11)
P	0.31	0.02	0.62
Abies in the Mo-Xi Valley			
April	25.8	12.3	5.4
July	22.7	8.4	2.9
F (df)	23.75 (1,53)	46.91 (1,17)	30.16 (1,17)
P	< 0.001	< 0.001	< 0.001
Tsuga in the Mo-Xi Valley			
April	13.9	12.7	3.8
July	13.5	11.1	4.0
F (df)	0.38 (1,35)	2.75 (1,17)	0.42 (1,17)
P	0.54	0.12	0.53

Altitudinal responses of the warm-climate reference—Tsuga yunnanensis

Two-way ANOVAs indicated that the NSC concentrations in higher-elevation *Tsuga* trees were similar to those in lower-elevation trees in both April ($P = 0.53$) and July ($P = 0.71$) (Table 2). Concentrations of total soluble sugars were slightly

Table 5. Concentration ratios of total soluble sugars to starch in treeline trees (or trees at the upper-elevational distribution for *Tsuga*) and lower-elevation trees for April and July 2005. Ratios were calculated based on the mean concentrations presented in Table 2.

Treeline trees		Lowest elevation trees	
April	July	April	July
Picea in the Kang-Ding Valley			
2.8	2.1	1.6	1.1
Abies in the Kang-Ding Valley			
3.5	2.0	2.1	2.4
Abies in the Mo-Xi Valley			
3.0	4.0	3.1	5.9
The above 3 treeline cases combined			
3.1	2.5	2.2	2.3
Tsuga in the Mo-Xi Valley			
2.7	1.3	1.5	2.4

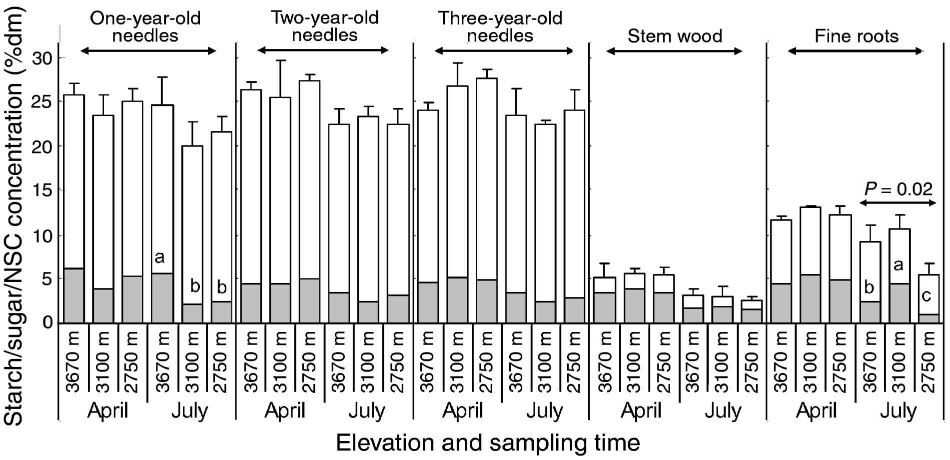

Figure 3. Mean concentrations of total soluble sugars (open bars), starch (filled bars) and NSC (soluble sugars + starch) in tissues of trees (*Abies fabri*) growing at the alpine treeline (3670 m a.s.l.) and at lower elevations in the Mo-Xi Valley, Mt. Gongga, SW China. Single factor ANOVAs were performed to test for differences among elevations within each tissue category and date. When ANOVA indicated significant elevational effects ($P < 0.05$), Tukey's HSD tests at $\alpha = 0.05$ were used to test for differences in the means between any two elevations within each tissue category and date. Values of P are given for NSC only when $P < 0.05$. Different letters (lowercase letters for starch) indicate statistically significant differences ($P < 0.05$; $n = 6$) between trees at different elevations within each tissue category and date. No significant differences were detected for sugars. Standard error bars are given for NSC only.

higher in higher-elevation trees than in lower-elevation trees in July ($P = 0.07$), but not in April ($P = 0.29$) (Table 2). However, starch concentrations were significantly lower in April in higher-elevation trees than in lower-elevation trees ($P = 0.008$), but significantly higher in the higher-elevation trees in July ($P = 0.004$) (Table 2). In contrast to the treeline species, *Abies* and *Picea*, the low-altitude species, *Tsuga*, seems to respond to altitude rather differently as can be seen in Table 2.

According to one-way ANOVAs, the only significant differences were in NSC ($P = 0.002$), sugars ($P = 0.001$) and starch ($P < 0.05$) in April stem sapwood, and in starch ($P < 0.001$) in July fine roots (data not shown). These aspects of carbon status at the tissue level are similar to those for *Abies* but differ greatly from those for *Picea*.

Two-way ANOVA with needle age and sampling date as factors revealed no effects on needle NSC (Table 4). Needle NSC was 13.9% dm in April and 13.5% in July (Table 4). Two-way ANOVA with needle age and elevation as factors did not isolate any effects on needle NSC (Table 3). Needle NSC concentrations ranged from 13.4 (LT), 13.6 (MT) to 14.1% dm (TT). Root NSC varied between 11.7 (MT), 11.8 (LT) and 12.1% dm (TT) (Table 3), and was unaffected by sampling date, elevation and their interaction (Tables 3 and 4), according to two-way ANOVAs. Two-way ANOVAs indicated that only altitude significantly affected NSC of stem sapwood ($P < 0.001$) (Tables 3 and 4). Stem-wood NSC concentrations in trees at their upper-elevational distributions, and at mid and lower elevations were 3.1, 3.4 and 5.4% dm, respectively (Table 3). Comparing the NSC concentrations among tree species within each tissue category, *Tsuga* had a much lower NSC concentration in needles than *Abies* and *Picea* but not in its other tissues (Tables 3 and 4).

Higher-elevation *Tsuga* showed a higher sugar:starch ratio in April (2.7) than in July (1.3), and lower-elevation *Tsuga* had a lower ratio in April (1.5) than in July (2.4) (Table 5). The sugar:starch ratio of *Tsuga* trees at its upper-elevational distribution was similar to that of the treeline species in April (Table 5), suggesting that trees need a sugar:starch ratio of at least three to persist at the highest elevational limit in winter, independently of whether they are treeline species or low-altitude species.

Discussion

Overall elevational trends in mobile carbohydrates

In the three treeline cases studied, the concentrations of total NSC and their components were not consistently lower or higher in trees grown at the alpine treeline than in trees of the same species grown at lower elevations, either after dormancy in April (related to winter depletion) or during the growing season in July (mainly related to growth consumption). For example, NSC concentrations in *Picea* in the Kang-Ding Valley were much lower in TT than in LT ($P < 0.001$ in both April and July; Table 2), whereas NSC concentrations in *Abies* in the Kang-Ding Valley were slightly higher in TT than in LT ($0.05 < P < 0.10$ in both April and July; Table 2). The NSC in *Abies* grown in the Mo-Xi Valley, however, did not differ between TT and LT ($P > 0.10$ in either April or July; Table 2). The NSC data for *Picea* could be considered evidence for carbon limitation of growth in TT, whereas the NSC data for *Abies* in the two valley locations seem to support the growth limitation hypothesis on treeline formation.

These contradictory results make it difficult to determine if the survival or growth of TT was limited by carbon supply. To check for an overall elevational trend in NSC concentration and its components, two-way ANOVAs (with elevation and tissue type as factors) were used to analyze the pooled data across the three treeline cases studied (*Picea* and *Abies* in the Kang-Ding Valley and *Abies* in the Mo-Xi Valley). The results of two-way ANOVAs for the pooled data indicated that the NSC concentrations were significantly lower in TT than in LT in April (14.70% dm in TT and 15.43% dm in LT; $P = 0.011$), but not in July ($P = 0.58$) (Table 2). The concentrations of total soluble sugars were slightly higher in TT (11.09% dm) than in LT (10.66% dm) in April ($P = 0.07$), but not in July ($P = 0.63$) (Table 2). In contrast, starch concentrations were significantly lower in TT (3.61% dm) than in LT (4.77%) in April ($P < 0.001$), but not in July ($P = 0.25$) (Table 2). These findings suggest that the functional mechanism of treeline formation in the three treeline cases studied is a winter carbon limitation.

Classical gas-exchange studies have yielded no evidence of photosynthetic restrictions in TT (Tranquillini 1979, Goldstein et al. 1994). However, Cavieres et al. (2000), in line with our findings, reported limited photosynthetic capacity of *Espeletia neriifolia* growing at the tropical alpine treeline in the Venezuelan Andes. Richardson (2004) also found a carbon limitation, related to significant reductions in the lignin and cellulose contents of *Picea rubens* and *Abies balsamea* growing at alpine treelines in the northeastern USA. Several studies have shown that plants subject to environmental stress tend to have lower tissue NSC concentrations than plants grown under more favorable conditions (Liu and Tyree 1997, Correia et al. 1999, Runion et al. 1999, Gwynn-Jones 2001, Polle et al. 2001). However, Bacelar et al. (2006) found that plants grown in a water-stressed environment had higher tissue NSC concentrations because environmental stress, such as drought, may restrict plant growth rather than reduce the rate of photosynthesis, leading to an accumulation of NSC (Bryant et al. 1983, Runion et al. 1999). In a field study with pine trees, Hoch et al. (2002) and Hoch and Körner (2003) found no mobile carbon depletion in TT either in winter or in the growing season, although they found that, in some cases, tissue NSC concentrations increased with altitude (Hoch et al. 2002, Hoch and Körner 2003, Shi et al. 2006).

These contradictory findings are based on limited published data. The total carbon pool size integrating tissue NSC concentrations and tissue biomass may provide better data to decide whether the alpine treeline is physiologically determined by carbon limitation. The reason for this is that TT may compensate for a decrease in the concentration of tissue NSC by producing more of that tissue (e.g., Li et al. 2003, 2006, Li and Yang 2004), if whole-tree carbon budgets are calculated (Li et al. 2002, Hoch and Körner 2003). For example, Li et al. (2006) found that needle biomass in old *Pinus cembra* trees at the alpine treeline was about three-times higher, due to the greater needle longevity, than in the same species of the same age at lowland sites. Li et al. (2002) and Hoch et al. (2002) calculated the mobile carbon pool in whole trees of *P. cembra* at the Swiss treeline and showed that needles contain the largest NSC fraction (> 60% of the total NSC pool) and roots the smallest (< 10% in May and < 20% in September) (Li et al. 2002).

Altitudinal responses of the warm-climate reference—Tsuga yunnanensis

The carbohydrate status of the warm-climate reference, *Tsuga*, was similar to that of *Abies* grown in the same valley (Table 2), but differed greatly from that in *Abies* and *Picea* grown in the Kang-Ding Valley (Table 2). This implies both species- and environment-specific responses to altitude. Species-specific responses of NSC to the same growing environment have been reported elsewhere (Kozlowski 1992, Ogren et al. 1997, Hoch et al. 2003). Previous studies have revealed long-term adaptations of the carbon physiology of trees exposed to different environmental conditions (Tranquillini 1979, Wieser 1997, Tognetti et al. 1998, Tognetti and Johnson 1999).

Does a critical sugar:starch ratio determine the alpine treeline?

There is evidence that the persistence of plants at their cold-climate limit depends largely on the accumulation of soluble sugars in perennial organs during winter hardening to prevent injury from intracellular ice formation (Levitt 1956, Sakai and Yoshida 1968, Kozlowski 1992, Ogren et al. 1997, Morin et al. 2007). In our study, the concentrations of tissue sugars in TT were not lower than those in lower-elevation trees (Table 2, Figures 1–3). Other studies have also shown no differences in foliar sugar concentrations between TT and lower-elevation trees (Hoch and Körner 2003, 2005, Shi et al. 2006). All these findings suggest that the availability of soluble sugars does not limit the persistence of TT in cold climates.

We found significantly lower tissue starch concentrations in TT in April (Table 2, except for *Abies* in the Mo-Xi Valley), especially in *Picea* (Figure 1). This result suggests that starch accumulates whenever a high level of sugars builds up, and starch is hydrolyzed to sugars when sugar concentrations are low (Kozlowski 1992). Sauter and Vancleve (1994) found that when temperatures of –5 °C persisted, most starch disappeared from poplar wood. However, our data did not show full depletion of starch in TT (Table 2, Figures 1–3).

Can the ratio of sugars to starch help explain treeline formation? It is well known that the accumulation of starch can limit photosynthesis directly. For example, a high foliar starch concentration is associated with a low assimilation rate (Nafziger and Koller 1976, Sasek et al. 1985). However, high sugar concentrations have a feedback effect on starch degradation (Trethewey and Aprees 1994, Cheng et al. 1998). Hence, it is possible that there is a starch–sugar system in trees that actively adjusts the sugar:starch ratio in response to environmental stresses (e.g., low temperature at the treeline). We found that both treeline and low-altitude tree species had similar sugar:starch ratios ranging from 2.7 to 3.5 in the late winter and early spring (Table 5). It seems that trees need a minimum sugar:starch ratio of about three at their upper-elevational limit.

Unfortunately, no experimental data were found in the literature relating sugar:starch ratios to tree persistence at high altitudes. However, in herbaceous plants, Strand et al. (2003) found that the sugar:starch ratio in leaves of *Arabidopsis thaliana* L. grown at 23 °C was much lower than in leaves of plants grown at 5 °C (< 1.5 versus up to 2.5). Patton et al. (2007) found that the sugar:starch ratio in zoysiagrass (*Zoysia* spp.) is significantly positively correlated with cold hardiness. Gwynn-Jones (2001) reported that the tissues of *Calamagrostis purpurea* (Thrin.) Thrin. under enhanced UV-B radiation had lower NSC:starch ratios than control plants. These results indicate that a plant's sugar:starch ratio actively adapts to environmental conditions. But further studies are needed to understand how and to what extent this ratio can adapt to the alpine environment in TT.

In conclusion, carbon limitation as a result of a shortage of photo-assimilates has been proposed as an explanation for the upper altitudinal or latitudinal treeline on a global scale (Schulze et al. 1967, Stevens and Fox 1991). However, the carbon limitation hypothesis may underestimate the effects of sink activities on the carbon balance. When sink or transport limitation occurs, carbohydrates accumulate in the leaf, leading to a feedback inhibition of photosynthesis (Foyer 1988, Sharkey et al. 1994), which is referred to as a growth or sink limitation (Körner 1998, 2003*a*, 2003*b*). However, we found no consistent evidence for the sink limitation hypothesis (Table 2; Figure 1 versus Figures 2 and 3). Instead, our data seem to support the carbon limitation hypothesis with a winter carbon shortage (Table 2). Hence, we suggest that winter may be the critical period for trees growing at the elevational or latitudinal climate limit, and that such trees rely not only on their total NSC concentration or their concentration of total soluble sugars, or both, but also require a sufficiently high sugar:starch ratio to overwinter successfully. This ratio depends on the relationship between starch accumulation and photosynthesis (Nafziger and Koller 1976, Sasek et al. 1985), and on sugar:starch conversion (Trethewey and Aprees 1994, Cheng et al. 1998). We conclude that a sugar:starch ratio of close to three is needed for trees, regardless of species, to survive the winter at their upper-elevational limits.

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