

# A test of the growth-limitation theory for alpine tree line formation in evergreen and deciduous taxa of the eastern Himalayas

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## Summary

1. Whether the global high elevation tree line phenomenon is associated with a low-temperature-induced limitation of sink activities (i.e. direct impact on meristems and thus, growth) or by a limitation of the trees' carbon source activities (net photosynthesis) still awaits detailed tests across a range of taxa and regions, especially for deciduous species in a short growing season.
2. Here, we test the sink limitation hypothesis in the highest tree lines of Eurasia at altitudes up to 4700 m. We assessed growth and tissue concentrations of non-structural carbohydrates (NSC) as a measure of the carbon source-sink balance in needles and branchwood of *Abies*, *Juniperus* (evergreen), *Betula* and *Larix* (deciduous).
3. The mean soil temperature in deep shade (a proxy for mean air temperature) across the growing season at tree line in this region is around 6.6 °C, which is consistent with the threshold temperature found at the natural climatic limit of the tree life-form worldwide. Mean tree height and stem diameter decreased significantly towards the upper tree line in all species studied.
4. Air temperature measurements at an inverted tree line site (valley bottom) indicate strong and rapid oscillations between nighttime freezing and mild daytime temperatures during late winter, which apparently eradicate *Abies* and select for *Juniperus*, offering potential explanations for the inferiority of *Abies* also at the upper Tibetan tree line.
5. At none of the four altitudinal transects studied did we observe a significant depletion of NSC (carbon limitation) at tree line. Instead, NSC increased in the majority of cases, suggesting direct (meristematic) low temperature constraints of growth. These results for these highest Eurasian tree lines suggest a common mechanism of alpine tree line formation for evergreen and deciduous species.

**Key-words:** altitude, carbon, source-sink balance, tree line, low temperature

## Introduction

The physiological mechanisms of alpine tree line formation have been discussed for over a century (Tranquillini 1979; Körner 2003a). However, many explanations were deduced from regional studies, often leading to an overemphasis of very local, site-specific drivers, rather than overarching mechanisms for this global phenomenon (Körner 1998a), with the inclusion of disturbances (including anthropogenic) further complicating the issue (Holtmeier & Broll 2007). In search of a functional explanation for climatic tree line positions globally, a recent model suggests a  $6.7 \pm 0.8$  °C mean growing season temperature threshold (Körner & Paulsen 2004).

The consistency of this tree line isotherm, irrespective of latitude, season length, geology, moisture regime and tree taxa suggests a common low temperature impact on plant metabolism (Hoch & Körner 2003), either related to carbon gain (source activity, photosynthesis) or sink activity (tissue formation).

A photosynthesis-related low temperature limitation of growth would translate into a depletion in tissue concentrations of mobile photo-assimilates (source-limitation hypothesis, Stevens & Fox 1991); a direct inhibition of meristematic activity at otherwise sufficient C-supply (sink- or growth-limitation hypothesis, Körner 1998a) should cause the mobile carbon pools to become larger as it gets colder. In both cases an imbalance between C-acquisition and C-processing should be mirrored by the size of the trees' non-structural carbon

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pools (i.e. concentrations of sugars, starch, neutral lipids, etc.; Chapin, Schulze & Mooney 1990; Dickson 1991). Numerous growth chamber and field studies have demonstrated soluble (low molecular weight sugars) and insoluble (starch) non-structural carbohydrates (NSC) in leaves and stems to rise when carbon supply exceeds, and to decline as carbon compounds get short relative to carbon demand (e.g. Abod & Webster 1991; Jordan & Habib 1996; Iglesias *et al.* 2002). Commonly, environmental constraints affect carbon investments (growth) long before they affect the carbon assimilation (Körner 2003b; Smith & Stitt 2007).

Mobile carbon pools in tree tissues should decline with altitude as one approaches the tree line, should carbon supply play a critical role (Hoch, Popp & Körner 2002). In contrast, a direct low temperature impact on growth should cause mobile carbon pools to increase with altitude (reduced carbon demand) at otherwise sufficient photosynthetic activity. To date, analyses of non-structural carbon reserves at tree line were restricted to a few sites and taxa (Hoch *et al.* 2002; Hoch & Körner 2003; Shi, Körner & Hoch 2006), with one location at the world's highest tree line position in the Bolivian Andes (*Polylepis*, Hoch & Körner 2005). In no case were carbons reserves depleted at tree line, but rather increased as one approaches the tree limit in support of the sink-limitation hypothesis.

In line with these observations, *Pinus uncinata* showed no stimulation of growth when exposed to elevated CO<sub>2</sub> concentrations over 4 years at tree line (Handa, Körner & Hättenschwiler 2005, 2006). In contrast, the summer-green *Larix decidua* exhibited a significant stimulation of growth by elevated CO<sub>2</sub> within the same experiment, although this effect seemed to decline after 5 years (T. Handa, pers. commun.). It thus remains unresolved whether the deciduous *Larix* is carbon limited at tree line. These experimental trees were relatively young and isolated, so they could expand their growth in any direction. This, however, is a common situation at tree line, which might facilitate CO<sub>2</sub>-sensitivity of growth (Körner 2006a). The limited number of experiments, as well as the bias of existing NSC data towards a few evergreen taxa, does not allow for a conclusive judgment about the general carbon supply status of trees at alpine tree lines.

Here, we present data for two deciduous and two evergreen tree genera at different tree line sites in the highest landmass on earth. The lift of the Himalayan chains resulted in some of the world's highest tree lines (Körner 1998a,b; Shi & Li 2000) enhanced by the 'Massenerhebungseffekt' in interior ranges (i.e. higher temperatures for a given altitude in the centre of a large mountain system compared to its front ranges (Körner 2003a). The tree line altitude climbs from 3600 m at the eastern border to 4700 m in the interior of the Tibetan Plateau. Moreover, tree lines are formed by a suite of different taxa in this region (Shi & Li 2000). Species of *Abies*, *Picea*, *Betula*, *Larix* and *Juniperus* (in the later text all addressed by genus) form increasingly higher tree lines as one moves from east to west. Here, we present data for the carbon charging of tree line trees between 4300 and 4700 m in the eastern part of the Himalayas.

## Materials and methods

### STUDY AREAS AND TREE LINE SPECIES

In the range of 30° N, tree lines are frequently situated above 4000 m a.s.l. in the eastern Himalayas (Li & Zhou 1979). Some sites are among the highest in the world, with truly arborescent trees ranging up to 4700 m a.s.l. We investigated five tree species from four different tree line sites between eastern latitudes of 90°27' and 100°24'. At each site, trees were studied along altitudinal transects, with the lowest sampling sites at the montane optimum altitude for the respective species. Intermediate samples were collected near the timberline and the uppermost samples were taken exactly at the tree line, which is defined here as the altitude above which groups of trees higher than 3 m are absent (Körner 2003a). At all tree lines, trees were sampled in mid-summer, after the completion of the current season shoot growth, but ongoing tree-ring growth and shoot maturation.

Our easternmost site was located in the Yading Nature Reserve (28°25' N, 100°24' E), Daocheng County, Sichuan Province, where *Larix potaninii* var. *macrocarpa* (Batalin) builds single species tree lines at about 4450 m a.s.l. (Liu & Zhong 1980). On 22 July 2006, we sampled a transect on a west-facing slope at 4440 (i.e. tree line), 4330 and 4200 m. In this case, additional *Larix* trees were sampled at the valley bottom at 4005 m.

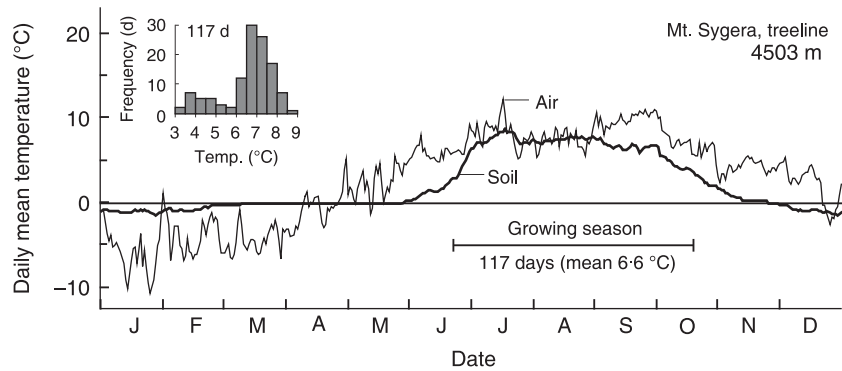
The second transect was sampled on 5 August 2005 on the eastern slope of Mt Sygera (28°37' N, 94°40' E), Nyingchi County, Tibet, where *Abies georgei* var. *smithii* (Viguie & Gaussen) and *Juniperus saltuaria* (Rehd. & Wils.); syn. *Sabina saltuaria* form tree lines at 4300 (*Abies*) and 4500 m a.s.l. (*Juniperus*), respectively (Zhang *et al.* 1988). The *Juniperus* belt above the *Abies* limit is absent on north-facing slopes, where *Abies* yields terrain to vast areas of *Rhododendron* shrub (*Rhododendron wardii*). *Juniperus saltuaria* trees were sampled at 4500 (i.e. *Juniperus* tree line), 4280 and 3980 m. *Abies* was sampled along the same slope at 4300 (i.e. *Abies* tree line), 4250 and 3980 m.

Deciduous *Betula* trees are widely distributed in montane areas of the eastern Himalayas, but only *Betula platyphylla* (Sukacev) forms arborescent tree lines at altitudes above 4300 m a.s.l. On 10 September 2006, we sampled *Betula* on the north-facing slope of Mt Mila (29°42' N, 92°05' E, Mozhugongka County, Tibet) at 4360 (i.e. tree line), 4230 and 3620 m.

*Juniperus tibetica* (Komarov); syn. *S. wallichiana* (Zhang *et al.* 1988) is naturally distributed in the middle Himalayas and forms the highest stands of truly arborescent individuals within the whole mountain range. We sampled the highest tree line of this species at 4680 m a.s.l., at a relic forest associated with a temple near Nagarze County town, Tibet (28°59' N, 90°27' E). The lower samples of the transect were taken at 4580 and 4460 m on the same slope, all collected on 21 August 2005.

### TREE MEASUREMENTS, TISSUE SAMPLING AND NON-STRUCTURAL CARBOHYDRATE (NSC) ANALYSIS

Co-dominant individual trees of 20–25 were randomly chosen at the lower, intermediate and uppermost site along each transect to measure stem diameter and tree height. At each transect the following tissues were collected from eight individual trees per altitude for NSC analysis: mature leaves, 'young branchwood' (< 4 mm diameter, c. 5 cm long) and 'older branchwood' (1–2 cm diameter, c. 3 cm long). Instead of old branchwood, stem sapwood was sampled in the case of *Betula* by cutting the tree. Bark was peeled from branch and stem samples, and only the sapwood was used for analyses. Half of the 'older branchwood' samples were used for tree-ring analyses (mean



**Fig. 1.** Daily mean temperatures at  $-10$  cm soil depth and air temperatures within tree crown of *Juniperus saltuaria* at the tree line at Mt Sygera, Nyingchi (4500 m a.s.l.). The insert shows the frequency distribution of soil temperatures during the 117 days growing season.

ring width and wood age). Samples were dried at  $80$  °C for *c.* 24 h within 15 h (mostly in  $< 8$  h) after sampling. In Yading (*Larix* tree line) where no drying oven was accessible, samples were pre-dried at  $80$  °C for about 2 h in an electric stove, and re-dried after return to the laboratory. Due to the remoteness of the *J. tibetica* tree line, samples were stored at *c.*  $15$  °C overnight and were processed and dried in the next morning. The samples were ground to fine powder for chemical analysis.

All samples were analyzed for the concentrations of NSC, defined here as the sum of free sugars (sucrose, glucose and fructose) and starch. NSC concentrations were determined photometrically after enzymatic conversions as described in Hoch *et al.* (2002). Sugar, starch and total NSC were calculated on a dry-matter basis. For *J. tibetica* wood density (in  $\text{g cm}^{-3}$ ) was determined gravimetrically in old branchwood.

#### TEMPERATURE MEASUREMENTS

Soil temperatures as well as crown-air temperatures at 2 m above the ground were monitored with data loggers ( $-20$  to  $+50$  °C, Tidbit, Onset, Cape Cod, MA). Two temperature loggers were placed at the tree line of *J. saltuaria* on Mt Sygera (4503 m a.s.l.), with one placed within a full-shaded crown at 2 m above ground, and the other buried in soil at 10 cm depth under full canopy shade, exactly following the protocol by Körner & Paulsen (2004). Temperatures at the tree line were continuously recorded at hourly intervals from 19 August 2005 to 13 September 2006.

In addition, two temperature loggers were employed to record full-shaded crown-air temperatures hourly in a valley about 350 m below tree line at Mt Sygera, from 20 August 2005 to 9 September 2006. At this site, we observed an inverted tree line, with only small *Juniperus* trees in the river plain and a distinct lower *Abies* boundary on the slopes about 40 m above the plain. This pattern suggests a local temperature inversion, which might hint at the causes of a similar situation at the tree line, where *Juniperus* reaches beyond the *Abies* tree line on SE exposed slopes. One logger was placed 2 m above ground in a *Juniperus* tree crown directly in the river plain (4150 m a.s.l.), the second logger was placed 40 m above the river plain (4190 m a.s.l.) in *Abies* crown on a N–W slope at the lower edge of the *Abies* forest.

Following Körner & Paulsen (2004), the beginning of the growing season was defined as the date at which the diurnal mean soil temperature at 10 cm depth first exceeded  $3.2$  °C (equivalent to a weekly mean of canopy air temperature of  $0$  °C). Accordingly, the end of the growing season was assumed to be the date at which the daily mean soil temperatures first dropped below  $3.2$  °C.

#### DATA ANALYSIS

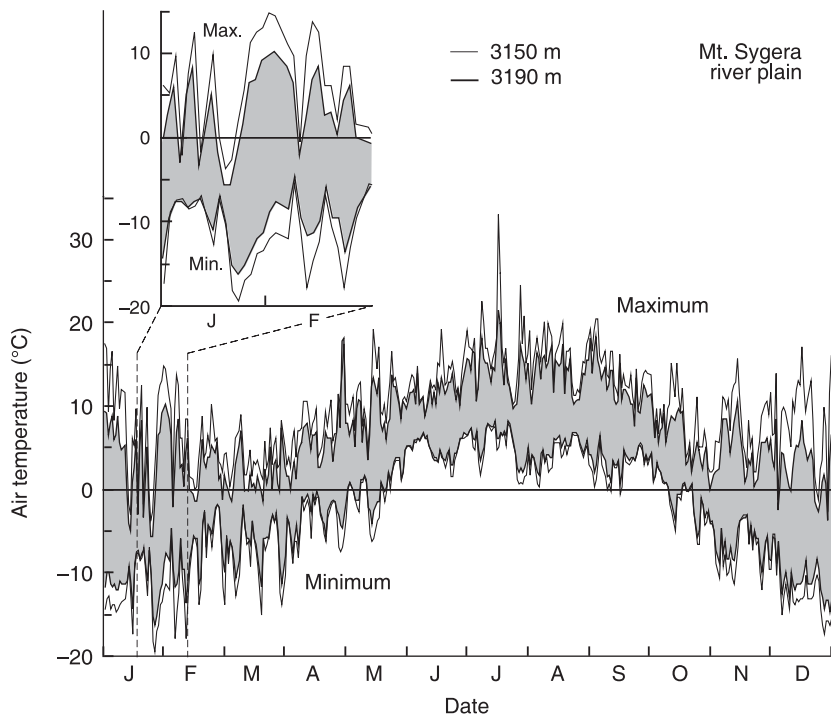
NSC concentrations were log-transformed prior to statistical analysis. All other values were computed with untransformed data. A Tukey–Kramer test was used to test for significant differences among altitudes. All statistical tests were performed with JMP 3.2.2 (SAS Institute, Cary, NC).

#### Results

##### TEMPERATURE MEASUREMENTS

At the *J. saltuaria* tree line on Mt Sygera, the growing season (as defined in Materials and methods) lasted for 117 days, from June 25, 2005 to October 19, 2006. The mean soil temperature at 10 cm depth during the growing season was  $6.6 \pm 1.3$  °C (Fig. 1). The minimum and maximum daily means during the whole measurement period were  $-1.5$  °C (January 27) and  $8.7$  °C (July 18). The extended period of unchanged soil temperatures of  $0$  °C (from March 11 to May 29) represented the period of permanent snow cover at tree line. Earlier in the year, temperatures fell below zero, due to snow-free conditions. Naturally, crown-air temperatures varied much more than  $-10$  cm soil temperatures among consecutive days, but the seasonal mean air temperature of  $6.3$  °C closely resembled the seasonal mean soil temperature. Daily means of air temperature never fell below  $-12$  °C during the entire year (absolute minimum hourly reading  $-16.4$  °C; Fig. 1). The highest daily mean air temperature of  $12.1$  °C was recorded on July 17.

The ‘inverted tree line’ site on the west slope of Mt Sygera, *c.* 350 m below the actual tree line, indeed exhibited temperature inversion conditions. Daily minimum temperatures were markedly lower at the valley bottom than at the valley flanks (Fig. 2). Especially during winter (January–March), trees were faced with strong and very rapid temperature oscillations at the bottom of the river plain (e.g. a change from  $-18$  °C to  $9.4$  °C within 12 h on 5 February, 2006, Fig. 2), with nighttime temperatures up to 5 K lower than at the lower edge of the *Abies* forest 40 m above the valley floor. The temperature oscillation is likely to be even more pronounced on open ground in gaps between trees (radiative cooling in clear nights), suggesting rather severe conditions for tree recruitment.



**Fig. 2.** Daily minimum and maximum air temperatures at an inverted tree line site at Mt Sygera with open *Juniperus saltuaria* woodland at the river plain (4150 m a.s.l.) and the lower edge of *Abies georgei* forest 40 m above (4190 m a.s.l.).

Hence, although the seasonal mean temperatures were even slightly higher at the bottom of the creek (7.0 °C) compared to the lower edge of the *Abies* forest (6.5 °C), temperature extremes were far more pronounced at the lower, *Juniperus* site, where *Abies* is absent.

#### TREE GROWTH AND REGENERATION

Average stem diameter and tree height decreased towards tree line for all investigated species (Table 1). If only the maximum readings are compared among altitudes, the reduction in height growth is more pronounced than the reduction in diameter growth. Individuals with massive stems can be found even at the uppermost sites of the studied transects (Table 1). The mean annual tree-ring increment, which was measured in older branchwood, decreased with altitude, except for the lowest elevation of the *Larix* transect (Table 1). However, only in *Abies* and *J. tibetica* was the altitudinal reduction of the mean tree-ring width statistically significant.

Across the transects, the average age of the 'old branchwood' samples was 9 years for *Larix*, 7 years for *Abies*, 22 years for *J. saltuaria* and 14 years for *J. tibetica*. The mean age of the sampled 'old branchwood' did not differ significantly among altitudes (Table 1), except for *J. tibetica*, where samples were significantly older (ANOVA  $P < 0.001$ ) at the highest site ( $20.6 \pm 2.3$  years) than at the middle ( $14.4 \pm 1.0$  years) and lowest sites ( $7.5 \pm 0.7$  years).

Tree seedlings and saplings were found in all tree line sites, but by far the most vigorous recruitment occurred at the *J. tibetica* tree line site at close to 4700 m, where masses of saplings of < 1 m height were noted along the entire transect, partly forming closed thickets. Similarly, successful regenera-

tion was frequently observed at tree lines on Mt Mila (*Betula*) and in Yading (*Larix*). Only at the Mt Sygera site (with *J. saltuaria* and *Abies*) small seedlings were very rare, but the site showed significant traces of yak grazing and trampling, which is not permitted in the sacred *J. tibetica* ecotone near Nagarze.

#### NSC ALONG ELEVATION GRADIENTS

Within each species, the NSC concentrations were highest in leaves and lowest in old branchwood (or stemwood in the case of *Betula*). All tissues had higher concentrations of starch than sugars, except for the deciduous leaves of *Betula* and *Larix* and young branchwood of *Abies*. Older sapwood in particular exhibited very low concentrations of NSC pools (Fig. 3).

The comparison of NSC along the altitudinal transects indicated no depletion of mobile carbon at tree line. In fact, for the majority of the tissues, NSC concentrations increased towards tree line, a trend which was significant for leaves of *Abies*, *J. saltuaria* and *Betula*, young branchwood of *Larix*, *J. saltuaria* and *Betula*, old branchwood of *J. saltuaria* and stem sapwood of *Betula* (Fig. 3). The only exception to this overall trend seemed to be older branchwood samples of *J. tibetica* at 4680 m, where NSC concentration were reduced significantly by c. 25% between the lowest and the highest site (Fig. 3). However, *J. tibetica* at that site was the only species that had significantly older branchwood at tree line for similar diameter samples than at the lowest site (Table 1). Because NSC concentrations decrease strongly with wood age, the measured reduction is likely to mirror the greater age of the wood samples at the upper end of this particular transect.

Table 1. Sampling sites, climate and tree growth status along the altitudinal gradients

	Yading, Sichuan	Mt Sygera, Tibet	Mt Sygera Tibet	Mt Mila, Tibet	Nagarze, Tibet
Location	28°25' N, 100°23' E	28°37' N, 94°40' E	28°37' N, 94°40' E	29°42' N, 92°06' E	28°59' N, 90°27' E
Annual mean precipitation (mm)	c. 700	c. 700	c. 700	c. 800	c. 400
Tree line altitude (m a.s.l.)	4450	4300	4503	4360	4680
Tree line species	<i>Larix potaninii</i>	<i>Abies georgei</i>	<i>Juniperus salhuaria</i>	<i>Betula platyphylla</i>	<i>Juniperus tibetica</i>
Sampling altitude (m)	4005	3980	4250	4300	4460
Stem diameter (mm)	316 <sup>a</sup> (37)	275 <sup>b</sup> (26)	290 <sup>b</sup> (24)	204 <sup>c</sup> (20)	165 <sup>b</sup> (26)
Tree height (m)	12.5 <sup>a</sup> (1.2)	8.3 <sup>b</sup> (0.6)	9.2 <sup>b</sup> (0.6)	4.4 <sup>c</sup> (0.4)	2.0 <sup>b</sup> (0.2)
Maximum diameter (mm)	710	478	490	385	400
Maximum height (m)	21.0	13.0	12.5	8.0	4.0
Tree-ring width (mm)	0.35 <sup>a</sup> (0.03)	0.46 <sup>a</sup> (0.06)	0.45 <sup>a</sup> (0.05)	0.40 <sup>a</sup> (0.05)	0.38 <sup>b</sup> (0.05)
Older branchwood age (tree-ring number)	10.4 <sup>a</sup> (0.6)	8.6 <sup>a</sup> (0.7)	7.8 <sup>a</sup> (0.8)	10.6 <sup>a</sup> (1.5)	14.4 <sup>b</sup> (2.3)
	4005	3980	4250	4300	4460
	660 <sup>a</sup> (27)	460 <sup>b</sup> (12)	260 <sup>c</sup> (2)	177 <sup>b</sup> (26)	218 <sup>b</sup> (29)
	36.0 <sup>a</sup> (0.9)	20.0 <sup>b</sup> (0.4)	13.0 <sup>c</sup> (0.3)	5.6 <sup>b</sup> (0.6)	2.4 <sup>b</sup> (0.4)
	990	780	460	500	410
	45.0	35.0	21.0	6.0	5.0
	0.94 <sup>a</sup> (0.08)	0.81 <sup>ab</sup> (0.11)	0.69 <sup>b</sup> (0.04)	0.24 <sup>a</sup> (0.03)	0.44 <sup>b</sup> (0.03)
	19.8 <sup>a</sup> (2.1)	7.5 <sup>a</sup> (0.5)	6.5 <sup>a</sup> (0.6)	20.1 <sup>a</sup> (2.4)	14.4 <sup>b</sup> (1.0)
	0.66 <sup>a</sup> (0.06)	0.66 <sup>a</sup> (0.06)	n.d.	n.d.	0.66 <sup>a</sup> (0.06)
	n.d.	n.d.	n.d.	n.d.	7.5 <sup>c</sup> (0.7)

Different letters within each species indicate significant differences at the 0.05 level among altitudes by Tukey–Kramer test. The data in parentheses are the standard error. Tree line annual mean precipitations are from nearest meteorological observatory stations (*Data source*: Chinese National Meteorological Bureau). n.d., no data.

The older branchwood of *J. tibetica* at tree line was denser than at lower elevations ( $0.64 \pm 0.03 \text{ g cm}^{-3}$  compared to  $0.60 \pm 0.01 \text{ g cm}^{-3}$ ). If expressed as mg NSC  $\text{cm}^{-3}$  volume, instead of per dry matter, the difference disappears.

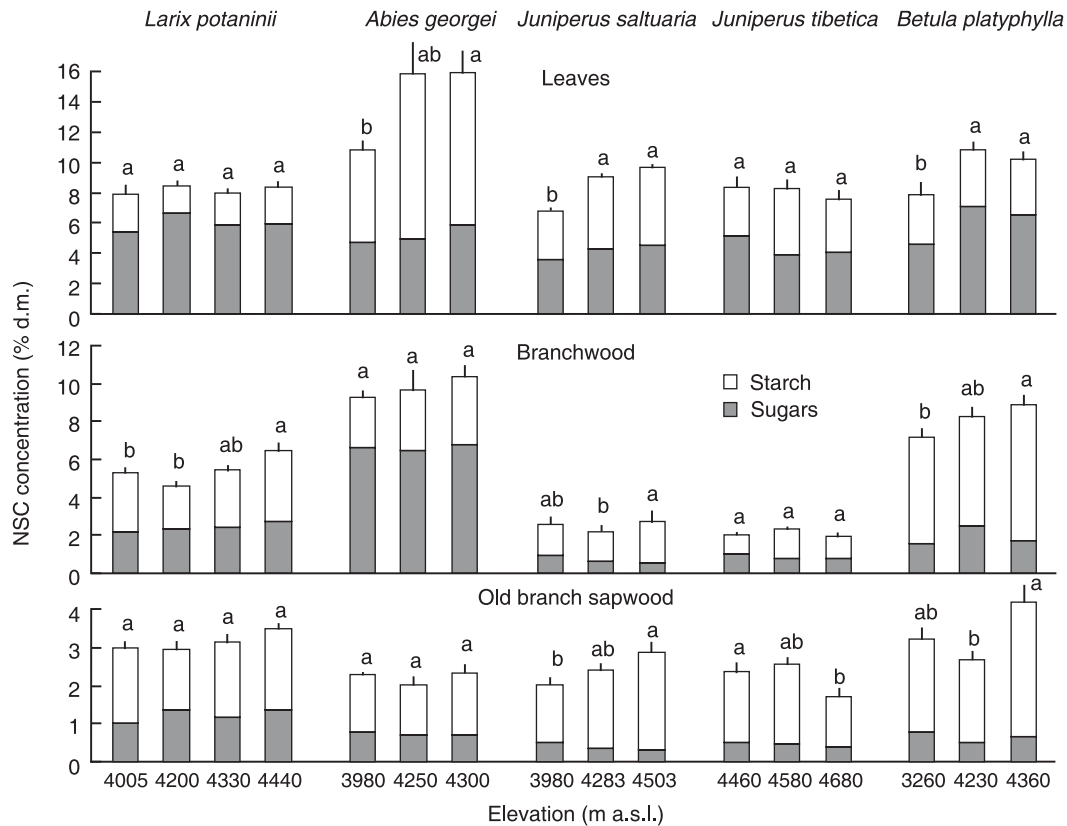
## Discussion

This broad survey of tree line ecotones in the eastern Himalayas revealed no indication of a systematic decline of carbohydrate charging of trees as they reach their upper limits. Rather, we detected a trend of increasing NSC concentrations with altitude, which was statistically significant in about half of all investigated tissues, although the investigated elevational gradients measured only a few hundred meters. Thus, based on the existing evidence that NSC mirrors a trees' carbon balance (see Introduction), carbon does not appear to be a limiting resource at tree line in these Himalayan test sites, matching observations from other tree lines (Hoch *et al.* 2002; Hoch & Körner 2003; Shi *et al.* 2006; Piper *et al.* 2006), including the highest elevation *Polylepis*-trees in the Andes (Hoch & Körner 2005).

### MEAN GROWING SEASON SOIL TEMPERATURE AND TREE LINE DISTRIBUTION PATTERNS

Although amongst the highest tree lines in the world (Shi & Li 2000), the seasonal mean soil temperature measured at the Mt Sygera tree limit matches the global mean temperature at tree lines of  $6.7 \pm 0.8 \text{ }^\circ\text{C}$ , as well as the average of  $6.5 \pm 0.7 \text{ }^\circ\text{C}$  at other warm temperate tree lines in Asia (Körner & Paulsen 2004). The narrow range of growing season temperatures at tree lines of widely differing altitudes around the globe suggests a common low temperature limit of tree growth. High carbon charging of trees even at the highest tree lines (with c. 45% lower air pressure than at sea-level, Hoch & Körner 2005) suggests that the pronounced reduction in partial pressure of  $\text{CO}_2$  does not reduce carbon assimilation to an extent that would affect tissue NSC concentrations.

Remarkably, the *Juniperus* tree line on Mt Sygera (as well as in other places) exceeds the limit of *Abies* by c. 200 m of elevation on south to east exposed slopes. This staggered altitudinal distribution of *Abies* and *Juniperus* awaits a physiological explanation. The temperature regime from the inverted tree line site (river plain) at Mt Sygera may hold the answer. Although the floodplain (with *Juniperus* only) was slightly warmer than the flanks (the lower *Abies* limit), the thermal extremes and short-term changes in temperature were more pronounced at the bottom of the valley. We therefore suggest that *Abies* is excluded from a site when severe nighttime radiative cooling is combined with pronounced daytime warming. It is well known that rapidly oscillating temperatures from very cold to warm exert more severe constraints on plants than steady deep-freezing temperatures (Strimbeck, Johnson & Vann 1993). There is evidence that *B. pubescence* in North Sweden experiences massive damage at variable, but not at stable, low temperatures (Björn Holmgren, Abisko, Sweden, pers. commun.). Mayr, Rothart & Damon (2003)



**Fig. 3.** Concentrations of non-structural carbohydrates (NSC), as the sum of free sugars and starch, in leaves, young branchwood and older branchwood (or stemwood in the case of *Betula platyphylla*). Values are given as % per dry matter ( $n = 8$  for each altitude and tissue). Different letters indicate significant differences at the 0.05 level among altitudes by Tukey–Kramer test. Note that the altitudinal decreasing trend of NSC in old branchwood of *Juniperus tibetica* is not significant; if wood density is considered and NSC concentrations are expressed as  $\text{mg cm}^{-3}$  (see text for details).

demonstrated that repeated freeze-thaw cycles induce massive xylem embolism in conifers, especially under drought-stressed conditions. *Abies* is much more sensitive to cavitation than *Juniperus*, since it experiences a complete loss of hydraulic conductivity at a much higher (less negative) needle water potential than does *Juniperus* (Larcher 2003). Hence, freezing-warming cycles of the magnitude found in this study may select for *Juniperus* and deter *Abies* from the river plain. A frost-induced inverse tree line has also been described for different *Eucalyptus* species associated with temperature inversion in a depression (Paton 1988). The higher elevation tree line of *Juniperus* at Mt Sygera and other sites in Tibet may thus also relate to late winter conditions in this monsoonal climate, with clear sky and little snow throughout most of the winter. *Juniperus turkestanica* can even survive the harsh and dry climate of the mountains of the Pamir and Karakorum, although with a short, stunted stature (Miehe 1996; Miehe & Miehe 2000).

Morphological plasticity may contribute to the relative success of *Juniperus* at such winter-dry, high elevation sites. *Larix* and *Abies* rarely form stunted, polycorm (i.e. multi-stemmed) individuals at tree line, *Juniperus*, however, does and forms shrub, krummholz, and stunted polycorm bush above the tree line. Dense thickets may have a warmer canopy climate due to decoupling from free atmospheric convection

during the growing season (Goldstein, Meinzer & Rada 1994; Körner 2003a). Seedlings and saplings of *Larix* and *Abies* may also profit from higher temperatures as long as they are nested within shrubs, especially *Rhododendron* species. But this ‘nursery’ effect by shrubs (Hättenschwiler & Smith 1999; Smith *et al.* 2003) disappears as soon as these monocormic tree species emerge from the shelter, and become exposed to low atmospheric temperatures, which restrict growth.

Enhanced solar radiation in the drier, continental part of mountain ranges (‘Massenerhebungseffekt’) is well known to facilitate tree growth at higher elevations as is the case at the Nagarze *J. tibetica* tree line (at 4680 m a.s.l.), in the driest region examined here. Higher rainfall and thus cloudiness explains the nearly 200 m lower tree line of *J. saltuaria* near Nyingchi, closer to the eastern front ranges of the Tibetan Plateau. *Polylepis* species in the Andes also reach highest elevations in very dry regions (Kessler 1995; Hoch & Körner 2005).

#### ALTITUDINAL TRENDS OF GROWTH AND SEEDLING ESTABLISHMENT

The current study documented stronger reductions in tree height than in width (tree-ring) growth towards tree line for all species, similar to previous reports on sub-alpine conifers

in the European Alps (Bernoulli & Körner 1999; Li, Yang & Kräuchi 2003), who also documented a more pronounced reduction in tree height compared to stem diameter as one approaches the altitudinal tree limit.

While seedling establishment is an unquestioned prerequisite for any plant recruitment, it does not seem to be critical for the formation of the tree lines studied here. There were crippled, multi-stemmed tree-like individuals of presumably substantial age above the tree line, but they appear to be unable to grow into upright trees. Tree seedlings above tree line in fact profit from facilitation by grasses and shrubs (Smith *et al.* 2003). But as they grow in height and gradually face increasing aerodynamic coupling, they eventually become the coldest structures and frequently face the fate of dieback in the landscape (Körner 2006b). Thus, it seems that the crucial phase is the sapling to tree transition, where trees enter a thermal regime different from that in low-stature plants.

#### ALTITUDINAL TRENDS OF NSC AND CARBON SUPPLY AT TREE LINE

The altitudinal patterns of NSC do not indicate any difference between evergreen and deciduous taxa. Both *Larix* and *Betula* had higher tissue NSC concentrations at tree lines, hence do not appear to be C-limited. This contrasts responses of (*c.* 35-years-old) *L. decidua* in the European Alps which showed enhanced radial stem growth and annual shoot increment in response to 4 years of free air CO<sub>2</sub> enrichment at tree line (Handa *et al.* 2005, 2006). From our findings, we would expect that the initial effect of CO<sub>2</sub> fertilization observed at the Swiss *Larix* tree line site is of transitory nature as has been found in many other experiments with young trees (Körner 2006a).

The exceptional pattern in branchwood NSC concentration in *J. tibetica* at 4680 m most likely resulted from the significantly older age, much narrower growth rings and thus greater wood volume density in comparison to the lower sites. NSC concentration is well known to decline from the cambium towards the pith (Fischer & Höll 1992; Magel, Einig & Hampf 2000; Hoch, Richter & Körner 2003), and smaller ring width (Yao 1970) and older cambial age (Oliva *et al.* 2006) lead to higher wood densities.

In conclusion, the observed decline in tree vigour with elevation is not associated with a depletion of tissue carbohydrate reserves in this highest tree lines of Eurasia. In most cases, NSC concentration rather increases as one approach the tree limit. This holds true for both deciduous and evergreen taxa and for the eastern (lower altitude) as well as the western (very high altitude) tree lines of the eastern Himalayan. Tree line temperatures measured at our sites match the global mean and underpin the *c.* 6 °C threshold for any significant meristematic activity, including that in roots (Alvarez-Uria & Körner 2007). While small trees and krummholz co-occur with alpine dwarf-shrubs and herbaceous vegetation at much higher altitude, upright tree crowns are at an aerodynamic disadvantage and thus are confined to lower altitudes. Our data support the sink-limitation hypothesis for tree line

formation in the worldwide largest and highest mountain system.

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