

Nitrogen and carbon source–sink relationships in trees at the Himalayan treelines compared with lower elevations

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ABSTRACT

No single hypothesis or theory has been widely accepted for explaining the functional mechanism of global alpine/arctic treeline formation. The present study tested whether the alpine treeline is determined by (1) the needle nitrogen content associated with photosynthesis (carbon gain); (2) a sufficient source–sink ratio of carbon; or (3) a sufficient C–N ratio. Nitrogen does not limit the growth and development of trees studied at the Himalayan treelines. Levels of non-structural carbohydrates (NSC) in trees were species-specific and site-dependent; therefore, the treeline cases studied did not show consistent evidence of source/carbon limitation or sink/growth limitation in treeline trees. However, results of the combined three treelines showed that the treeline trees may suffer from a winter carbon shortage. The source capacity and the sink capacity of a tree influence its tissue NSC concentrations and the carbon balance; therefore, we suggest that the persistence and development of treeline trees in a harsh alpine environment may require a minimum level of the total NSC concentration, a sufficiently high sugar:starch ratio, and a balanced carbon source–sink relationship.

Key-words: altitudinal limit; carbon limitation; C-balance; C–N ratio; growth limitation; non-structural carbohydrates; starch; sugars.

INTRODUCTION

The alpine treeline is the most studied of all distributional boundaries of trees (Korner 2003a). In more than 100 years of research on treeline phenomena worldwide, four environmental hypotheses and two biological hypotheses have been suggested for interpreting the alpine treeline formation (Tranquillini 1979; Grace & James 1993; Korner 1998; Li & Kräuchi 2005). However, none of these hypotheses

have been widely accepted (Korner 1998; Li & Kräuchi 2005). The environmental hypotheses are related to local site conditions and are limited to local treeline phenomena and cannot be applied on a global scale (Li & Kräuchi 2005; Li *et al.* 2008). Only the biological hypotheses, that is, the carbon/source limitation hypothesis (Schulze, Mooney & Dunn 1967; Stevens & Fox 1991; Wardle 1993) or the growth/sink limitation hypothesis (Däniker 1923; Korner 2003a,b), may be used to explain the functional mechanism of global alpine/arctic treeline formation (Korner 1998; Li & Kräuchi 2005; Li *et al.* 2008).

The sharply reduced growth rate of trees at or close to the alpine treeline (Tranquillini 1979; Li, Yang & Krauchi 2003; Li & Yang 2004) has been regarded as caused by a shortage of photoassimilates (Schulze *et al.* 1967; Stevens & Fox 1991; Wardle 1993). However, the photosynthetic capacity of trees has been shown to decrease or increase with increasing tree altitude depending upon the species (Goldstein, Meinzer & Rada 1994; Cabrera, Rada & Cavieres 1998; Cavieres *et al.* 2000). For example, Cavieres *et al.* (2000) found that *Podocarpus oleifolius* grown at the tropical alpine treeline did not show reductions in carbon gain because it has the capacity to adjust its photosynthetic machinery as altitude increases. In contrast, the CO₂ assimilation rate of *Espeletia neriifolia* markedly decreased with increasing altitude, and there may be a shortage of carbon gain in the treeline individuals of this species (Cavieres *et al.* 2000).

Photosynthesis is one aspect, but what is also important is the carbon balance between carbon gain and loss in a treeline tree. An indirect way to test the carbon balance is to assess the size of the non-structural mobile carbon pool in trees because non-structural carbohydrates (NSC) represent, among other reserves, a tree's capital for growth after dormancy and act as a buffer for insufficient source activity (photosynthesis) (Li, Hoch & Korner 2001, 2002; Hoch & Korner 2003; Korner 2003b). The size of this mobile carbon pool in trees growing near the treeline may provide the answer to the question of whether carbon is a limiting

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Table 1. Characteristics of the plots and the sampling trees (mean \pm 1 standard deviation; $n = 6$ trees) in the Mt. Gongga region, Sichuan, SW China

Species and plots (m a.s.l.)	Slope exposure	Soil humidity class	Mean air temperature ($^{\circ}$ C)	Age (years)	Height (m)	Diameter (cm)	Community
<i>Abies fabri</i> in the Mo-Xi Valley (uppermost distribution at 3700 m a.s.l., lowest elevational distribution at 2700 m)							
3670 m	NE	Mesic	1.6	71 \pm 8	10.4 \pm 3.2	23.5 \pm 6.3	<i>A. fabri</i> – <i>Rhododendron</i> spp.
2750 m	NE	Moist	6.2	67 \pm 6	18.3 \pm 4.4	28.1 \pm 7.8	<i>A. fabri</i> – <i>Bashania</i> sp.
<i>A. fabri</i> in the Kang-Ding Valley (uppermost distribution at 3780 m a.s.l., lowest elevational distribution at 3200 m)							
3750 m	SW	Dry	2.4	51 \pm 15	15.9 \pm 6.4	27.6 \pm 5.6	<i>A. fabri</i> – <i>Quercus aquifolioides</i> + <i>Rh.</i> spp.
3300 m	SW	Dry	4.7	44 \pm 7	20.3 \pm 2.7	36.8 \pm 2.8	<i>A. fabri</i> – <i>Q. aquifolioides</i> + <i>Rh.</i> spp.
<i>Picea balfouriana</i> var. <i>hirtella</i> in the Kang-Ding Valley (uppermost distribution at 3820 m a.s.l., lowest elevational distribution at 3270 m)							
3800 m	SW	Dry	2.1	37 \pm 5	8.6 \pm 2.2	22.4 \pm 2.7	<i>P. balfouriana</i> var. <i>hirtella</i> – <i>Q. aquifolioides</i>
3400 m	SW	Dry	4.2	36 \pm 10	13.4 \pm 2.7	26.0 \pm 5.0	<i>P. balfouriana</i> var. <i>hirtella</i> – <i>Q. aquifolioides</i>

NE, northeasterly; SW, southwesterly.

resource or not. An overabundance of carbon reserves would indicate no limitation, whereas periodic depletions of carbon reserves would suggest insufficient supplies (Li *et al.* 2002). Hoch & Korner (2003) found similar concentrations of mobile carbohydrates in three *Pinus* species at their alpine treelines (*Pinus cembra* in the Swiss Alps, *Pinus sylvestris* in northern Sweden, and *Pinus hartwegii* in Mexico) when compared with the same species at lower elevations. However, Richardson (2004) recorded a significant carbon limitation in *Picea rubens* Sarg. and *Abies balsamea* (L.) Mill grown at the alpine treelines in the northeastern USA.

Nitrogen (N) is a major component of ribulose 1-5-bisphosphate carboxylase/oxygenase (Rubisco) and other photosynthetic enzymes and structures, which regulates photosynthetic responses to environmental factors such as low temperature (Bond *et al.* 1999; Ripullone *et al.* 2003; Lewis *et al.* 2004). Korner (1989) pointed out that leaf/needle N content (% d.m.) increased with altitude in herbaceous plants, but was remarkably stable in evergreen woody plants (around 1%). However, other studies have found that the needle N content (mass basis) in *Picea rubens* and *A. balsamea* either decreased with increasing elevation of trees in the alpine ecotone (Richardson 2004), or did not vary in trees across a 1000-m-elevation range (Richardson, Berlyn & Gregoire 2001). Based on a meta-analysis covering 1280 plant species in 704 genera across 452 locations worldwide, Reich & Oleksyn (2004) proposed two contrary hypotheses. The temperature–plant physiological hypothesis states that leaf N declines with increasing temperature because cold climates may favour high leaf N to compensate for the low efficiency of physiological processes at low temperature (Reich & Oleksyn 2004; He *et al.* 2006). The biogeochemical hypothesis argues that low temperatures not only reduce N availability through suppressing decomposition and mineralization of organic matter, but also suppress root nutrient uptake, resulting in low leaf N in cold climates (Reich & Oleksyn 2004; He *et al.* 2006).

Such contradictory findings stimulated us to form and test new hypotheses that the global alpine/arctic treeline is functionally determined by (1) the needle nitrogen content associated with photosynthesis (carbon gain); or/and (2) a sufficient source–sink ratio of carbon; or/and (3) a sufficient C–N ratio. We, therefore, investigated the mobile carbohydrates and nitrogen in two species, *Abies fabri* and *Picea balfouriana* var. *hirtella*, which not only grow at low elevations, but also form the alpine treeline in the Gongga Mountains (Table 1), southwestern China.

MATERIALS AND METHODS

Study sites and species

The study was conducted in two valleys, the Mo-Xi Valley, which runs southeasterly, and the Kang-Ding Valley, which runs northwesterly, located along the northwest face of Mt. Gongga (7556 m a.s.l., 29°35'45"N and 101°52'45"E), southwestern China. The mean precipitation is approximately 1000 mm year⁻¹ in the Mo-Xi Valley (data collected at 1600 m a.s.l. from 1960 to 1989), and around 800 mm in the Kang-Ding Valley (data collected at 2616 m a.s.l. from 1952 to 1990). The soil for both valleys has developed from granite, classified as a mountain gray-brown soil (He, Liao & Zhang 2005). Other characteristics of the study sites/forests are summarized in Table 1.

The study sites in the Mo-Xi Valley are located on a northeast facing slope, where *A. fabri* (Mast.) Craib is distributed from 2700 (the lowest elevational limit on this slope) to 3700 m a.s.l. (the alpine treeline) (Table 1). The dense understorey is dominated by *Bashania* sp. and *Rhododendron* spp. (Table 1).

The study sites in the Kang-Ding Valley are located on a southwest facing slope, where *A. fabri* is distributed between 3200 (the lowest elevational limit on this slope) and 3780 m a.s.l. (the alpine treeline), and *Picea balfouriana* var. *hirtella* Rehd. et Wils. between 3270 (the lowest elevational limit) and 3820 m a.s.l. (the treeline) (Table 1). The

dense understorey is dominated by *Quercus aquifolioides* Rehd. et Wils. (Table 1).

To maximize the influence of elevation, trees of the two species studied were selected from those growing at their upper elevational limits and at their lowest distribution boundaries for comparison in this research project. The forests surrounding the study sites had naturally regenerated, but were moderately disturbed by selection-cutting and grazing during 1970s and 1980s (Li *et al.* 2008). Since 1986, the area has been protected, and no such disturbances have occurred (Li *et al.* 2008). As the upper elevational limits of the two species are mainly determined by the physical environment (e.g. low temperature, etc.), their lowest elevational limits may be mainly due to human activities (e.g. agricultural use and logging). Hence, the lowest elevational limit is probably not the naturally lowest distribution boundary of the two species in the study area.

For clarity and brevity, we henceforth refer to the species studied by their generic names, to trees at the alpine treeline as 'TT', and trees at the lowest elevational limit as 'TLL'. The ratio of mobile carbohydrate to total nitrogen is abbreviated as 'C–N ratio', and the concentration ratios of NSC and N in the source (needles) to NSC and N in the sink, which consists here of the fine roots and stem sapwood, as 'SSR–NSC' and 'SSR–N', respectively.

Sampling

We sampled tree tissues only twice because we were interested in differences in the mobile carbon pool size in trees between their lowest distribution boundaries and their upper elevational limits, and not in the seasonal variability of this pool. The first set of tissue samples was taken directly before bud break (April 17–21) to check the size of the April carbon pool, which should be relatively lower because of the winter depletion (Fischer & Holl 1991; Gonzalez 1991), and to see whether trees at the alpine treeline completely deplete the mobile carbon pool during the winter. The second set of tissue samples was taken during the peak shoot growth (July 24–28). The size of the July carbon pool allowed us to check whether there were enough carbohydrates for structural growth in treeline trees and how their pool sizes compared with those in trees at lower elevations (Li *et al.* 2008).

Six tree individuals were selected so as to be fairly similar in age (to avoid possible age effects on the parameters studied) and in the visible needle color of each tree species either at their upper elevational limit or at their lowest distribution boundary (Table 1). The selected trees were in closed-canopy stands (i.e. not isolated), healthy and undamaged. Because the levels of mobile carbohydrates in needles fluctuate daily in response to photosynthetic activity (Graham *et al.* 2003), the samples were always taken around noon, to ensure the effects of sunshine and needle surface temperature on carbohydrates were kept constant. One- (previous year), two- and three-year-old needles of each sample tree were separately collected from non-shaded leading branches on the upslope canopy side. Two cores

from each sample tree were taken on two opposite stem sides (parallel to the slope) at the stem height of 40 cm above the ground, using a 5-mm-diameter corer. The outer 3 cm of stem-wood (xylem tissue only, approximately 7–10 tree rings) of the two cores was cut down and mixed to obtain the minimum of ground sample necessary 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 were killed in a microwave oven (40 s at 600 W), and were dried to a constant weight at 65–70 °C (Li *et al.* 2008).

Chemical analyses: total soluble sugars

Dried plant material was ground to pass through a 1 mm sieve. The powdered material (0.1 g) was put into a 10 mL centrifuge tube, where 5 mL of 80% ethanol was added. The mixture was incubated at 80 °C in a water bath shaker for 30 min, and then centrifuged at 4000 rpm for 5 min. The pellets were extracted two more times with 80% ethanol. Supernatants were retained, combined and stored at –20 °C for soluble sugar determinations. The soluble sugar fraction was measured. Soluble sugars in the collected extracts were determined using the anthrone method (Seifter *et al.* 1950). An aliquot of the extract was hydrolysed in 5 mL of 0.4% anthrone solution (4 g anthrone in 1000 mL 95% H₂SO₄) in a boiling water bath for 15 min. After cooling, the sugar concentration was determined spectrophotometrically (ultraviolet-visible spectrophotometer 752S; Cany Precision Instruments Co., Ltd., Shanghai, China) at 620 nm. Glucose was used as a standard. The sugar concentration was calculated on a dry matter basis (% d.m.).

Chemical analyses: starch

The ethanol-insoluble pellet was used for starch extraction. Ethanol was removed by evaporation. Starch in the residue was released in 2 mL distilled water for 15 min in a boiling water bath. After cooling to room temperature, 2 mL of 9.2 mol L⁻¹ HClO₄ was added. Starch was hydrolysed for 15 min. Distilled water (4 mL) was added to the samples. The samples were then centrifuged at 4000 rpm for 10 min. The pellets were extracted one more time with 2 mL of 4.6 mol L⁻¹ HClO₄. Supernatants were retained, combined and filled to 20 mL. The starch concentration was measured spectrophotometrically (ultraviolet-visible spectrophotometer 752S) at 620 nm using anthrone reagent, and was calculated by multiplying glucose concentrations by the conversion factor of 0.9 (Osaki, Shinano & Tadano 1991). Glucose was used as a standard. The starch concentration was described on a dry matter basis (% d.m.).

Chemical analyses: total nitrogen

The total nitrogen content (% d.m.) was determined in finely ground oven-dried samples by the micro Kjeldahl procedure, using CuSO₄, K₂SO₄ and H₂SO₄ for digestion,

Table 2. Total nitrogen content across tissues, N in source (needles) and sink (fine roots and stem wood), and source–sink ratio of N (SSR-N = source N/sink N) in trees growing at the alpine treeline and their lowest distribution boundaries on Mt. Gongga, southwestern China

	Overall N across tissues		N in source		N in sink		Source–sink ratio	
	April	July	April	July	April	July	April	July
<i>Picea balfouriana</i> var. <i>hirtella</i> at 3800 m a.s.l. (treeline) and 3400 m in the Kang-Ding Valley								
3800 m a.s.l.	0.86	0.80	1.16	1.13	0.41	0.32	2.8	3.5
3400 m a.s.l.	0.80	0.71	1.08	0.93	0.36	0.36	3.0	2.6
<i>F</i> (d.f.)	1.95(1,29)	23.79(1,29)	1.12(1,17)	44.98(1,17)	1.35(1,11)	3.50(1,11)		
Difference (<i>P</i>)	0.18	<0.001	0.31	<0.001	0.28	0.11		
<i>Abies fabri</i> at 3750 m a.s.l. (treeline) and 3300 m in the Kang-Ding Valley								
3750 m a.s.l.	0.95	0.83	1.28	1.12	0.46	0.41	2.8	2.7
3300 m a.s.l.	0.75	0.75	1.01	1.04	0.36	0.32	2.8	3.3
<i>F</i> (d.f.)	18.68(1,29)	1.00(1,29)	12.37(1,17)	0.33(1,17)	49.61(1,11)	13.56(1,11)		
Difference (<i>P</i>)	<0.001	0.33	0.004	0.57	<0.001	0.006		
<i>Abies fabri</i> at 3670 m a.s.l. (treeline) and 2750 m in the Mo-Xi Valley								
3670 m a.s.l.	0.98	0.99	1.26	1.33	0.57	0.47	2.2	2.8
2750 m a.s.l.	0.90	0.89	1.19	1.16	0.46	0.47	2.6	2.5
<i>F</i> (d.f.)	11.43(1,29)	19.80(1,29)	6.83(1,17)	29.90(1,17)	5.08(1,11)	0.002(1,11)		
Difference (<i>P</i>)	0.003	<0.001	0.023	<0.001	0.054	0.97		
The above three treeline cases combined								
TT	0.93	0.88	1.23	1.19	0.48	0.40	2.6	3.0
TLL	0.82	0.78	1.10	1.04	0.40	0.39	2.8	2.7
<i>F</i> (d.f.)	24.54(1,89)	10.07(1,89)	14.28(1,53)	9.28(1,53)	14.76(1,35)	0.85(1,35)		
Difference (<i>P</i>)	<0.001	0.002	<0.001	0.004	<0.001	0.37		

Statistical significances were separately tested in April and July using two-way analyses of variance with elevation and tissue type as factors. Significant differences ($P < 0.05$) are highlighted in bold.

TT, trees at the alpine treeline; TLL, trees at the lowest elevational limit.

and NH_3 was determined on an auto-analyser, using the indophenol-blue colorimetric method (Allen 1989).

Data analysis

NSC is defined as the sum of the starch plus the total soluble sugars for each tissue at each sampling date. Data (NSC, starch, total soluble sugars and N content) were confirmed for normality by Kolmogorov–Smirnov-Tests. Two-way analyses of variance (ANOVAS) were repeatedly performed with elevation and tissue type as factors to find out elevational trends in the concentration of NSC, sugars, starch and N for overall (three treeline cases combined) and individual species at each sampling date. The concentrations of overall NSC and overall N across tissues, as well as NSC in sink and N in sink, were gained from two-way ANOVAS with tissue type and elevation as factors (results shown in Tables 2 & 3). At the species level, *t*-paired comparisons were performed to detect statistical differences in the parameters studied between TT and TLL within each tissue category and date (results given in Figs 1–3). The statistical test used is always mentioned in the related text. Because we are interested mainly in the altitudinal effects on the mobile carbohydrates and N in TT and TLL, other effects, such as effects of sampling time, tissue type, and the interaction between elevation and tissue type/sampling time, are not systematically discussed in the present paper.

RESULTS

Picea trees in the Kang-Ding Valley

The nitrogen content in tree tissues increased with increasing tree elevation (Table 2). No other significant differences in the source N or sink N between TT and TLL could be detected according to two-way ANOVAS, apart from a significantly higher N content in the TT source in July (1.13% d.m. in TT > 0.93% in TLL, $P < 0.001$) (Table 2). For overall N content across tissues, TT had a significantly higher N content than TLL in July ($P < 0.001$), but not in April ($P = 0.18$) (Table 2). The source N content was about three times higher than the sink N in both TT and TLL within each sampling date (Table 2). While the SSR-N decreased with increasing tree elevation in April, it increased with increasing elevation in July (Table 2).

Results of two-way ANOVAS indicated a significantly lower source NSC in TT than in TLL in both April (15.00% d.m. in TT < 18.40% in TLL, $P < 0.001$) and July (16.09% d.m. in TT < 21.39% in TLL, $P < 0.001$) (Table 3). For sink NSC, there was a slightly lower sink NSC in TT (6.68% d.m.) than in TLL (7.74%) in April ($P = 0.072$), but no difference in July ($P = 0.841$) (Table 3). Like NSC in the source tissue, across tissues TT had significantly lower overall NSC concentrations than TLL in both April (11.67% d.m. in TT < 14.14% in TLL, $P < 0.001$) and July (12.46% d.m. in TT < 15.74% in TLL, $P < 0.001$) (Table 3).

Table 3. NSC across tissues, NSC in source (needles) and sink (carbon storage organs – fine roots and stem sapwood), and source–sink ratio of NSC (SSR-NSC = source NSC/sink NSC) in trees growing at the alpine treeline and their lowest distribution boundaries on Mt. Gongga, southwestern China

	Overall NSC across tissues		NSC in source		NSC in sink		SSR-NSC	
	April	July	April	July	April	July	April	July
<i>Picea balfouriana</i> var. <i>hirtella</i> at 3800 m a.s.l. (treeline) and 3400 m in the Kang-Ding Valley								
3800 m a.s.l.	11.67	12.46	15.00	16.09	6.68	7.01	2.2	2.3
3400 m a.s.l.	14.14	15.74	18.40	21.39	7.74	7.27	2.4	2.9
<i>F</i> (d.f.)	30.85(1,29)	26.77(1,29)	26.87(1,17)	63.55(1,17)	4.29(1,11)	0.04(1,11)		
Difference (<i>P</i>)	<0.001	<0.001	<0.001	<0.001	0.07	0.84		
<i>Abies fabri</i> at 3750 m a.s.l. (treeline) and 3300 m in the Kang-Ding Valley								
3750 m a.s.l.	13.82	15.06	19.36	19.85	5.52	7.87	3.5	2.5
3300 m a.s.l.	12.63	13.79	17.58	18.37	5.21	6.93	3.4	2.7
<i>F</i> (d.f.)	4.02(1,29)	3.77(1,29)	4.19(1,17)	4.81(1,17)	0.19(1,11)	0.54(1,11)		
Difference (<i>P</i>)	0.06	0.07	0.06	0.048	0.68	0.48		
<i>A. fabri</i> at 3670 m a.s.l. (treeline) and 2750 m in the Mo-Xi Valley								
3670 m a.s.l.	18.60	16.58	25.37	23.52	8.43	6.16	3.0	3.8
2750 m a.s.l.	19.52	15.21	26.65	22.68	8.81	5.32	3.0	4.3
<i>F</i> (d.f.)	5.53(1,29)	3.51(1,29)	6.11(1,17)	0.55(1,17)	0.41(1,11)	4.21(1,11)		
Difference (<i>P</i>)	0.029	0.08	0.029	0.47	0.54	0.07		
The above three treeline cases combined								
TT	14.70	14.70	19.91	19.82	6.88	7.02	2.9	2.8
TLL	15.43	14.91	20.88	20.81	7.25	6.06	2.9	3.4
<i>F</i> (d.f.)	6.80(1,89)	0.31(1,89)	5.73(1,53)	4.11(1,53)	1.15(1,35)	2.22(1,35)		
Difference (<i>P</i>)	0.011	0.58	0.018	0.049	0.29	0.15		

Statistical significances were tested separately in April and July using two-way analyses of variance with elevation and tissue type as factors. Significant difference ($P < 0.05$) are highlighted in bold.

NSC, non-structural carbohydrates.

Tissue NSC showed a decreased trend with increasing tree elevation (Fig. 1). The NSC concentrations in tissues were frequently significantly lower in TT than in TLL, except for the stem sapwood (Fig. 1). These findings suggested that TT may suffer from a carbon limitation compared with TLL (Table 3, Fig. 1). The SSR-NSC in trees increased with decreasing tree elevation within each date. The NSC in the source tissue (ranging from 15.00 to

21.39% d.m.) was two to three times higher than that in the sink (~7–8%), resulting in an SSR-NSC of 2.2–2.9 (Table 3).

The C–N ratio in the source tissue decreased with increasing tree altitude within each sampling date (Table 4). In the carbon sink tissues, however, the C–N ratio decreased with increasing tree elevation in April, but increased with elevation in July (Table 4).

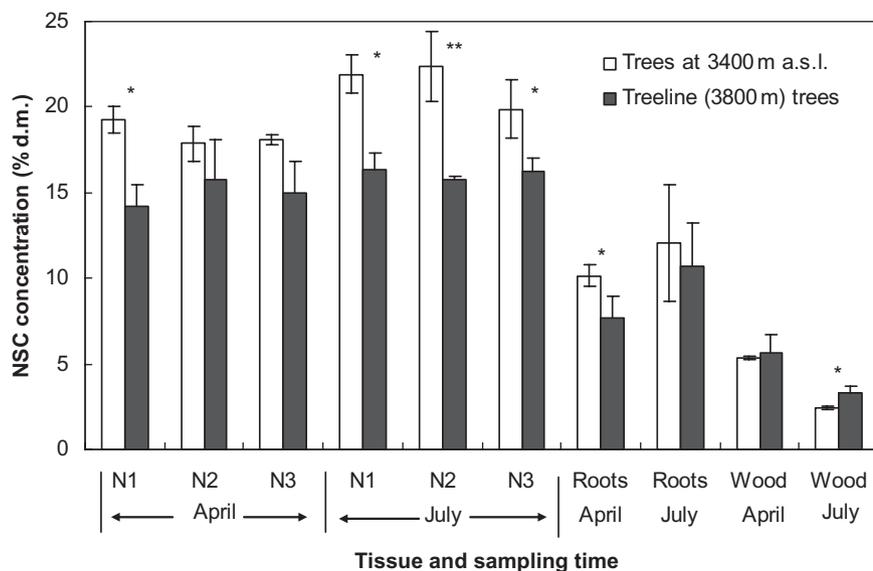


Figure 1. Mean non-structural carbohydrates (NSC) concentrations (mean \pm 1 standard deviation; % of dry matter) in the tissues of trees (*Picea balfouriana* var. *hirtella*) growing at the alpine treeline (3800 m a.s.l.) and at 3400 m a.s.l. in the Kang-Ding Valley, Mt. Gongga, southwestern China. T-paired comparisons were used for testing statistical differences in the means between the two elevations within each tissue category and date. Asterisks indicate statistically significant differences in tissue NSC concentrations ($*P < 0.05$, $**P < 0.01$; $n = 6$ trees) between trees at the two elevations within each tissue category and date. N1, N2 and N3 refer to 1-, 2- and 3-year-old needles, respectively.

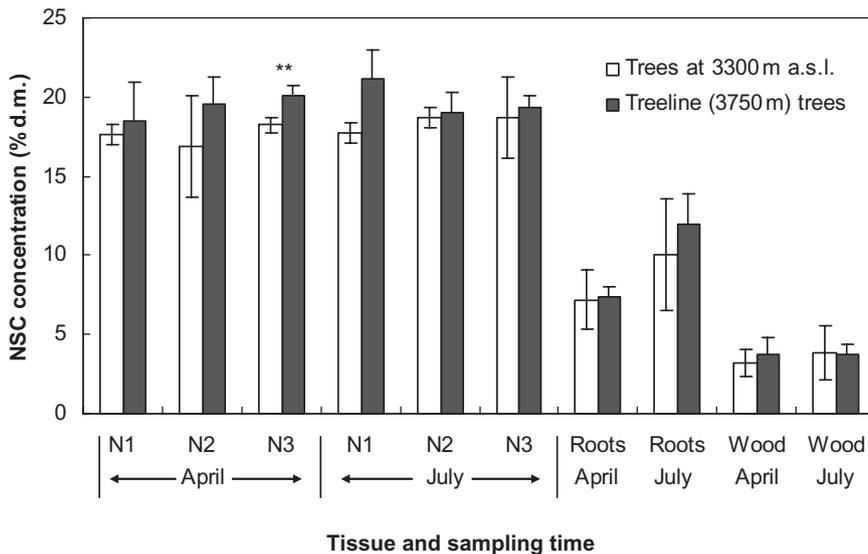


Figure 2. Mean non-structural carbohydrates (NSC) concentrations (mean ± 1 standard deviation; % of dry matter) in the tissues of trees (*Abies fabri*) growing at the alpine treeline (3750 m a.s.l.) and at 3300 m a.s.l. in the Kang-Ding Valley, Mt. Gongga, southwestern China. T-paired comparisons were used for testing statistical differences in the means between the two elevations within each tissue category and date. Asterisks indicate statistically significant differences (** $P < 0.01$; $n = 6$ trees) in the NSC concentration between trees at the two elevations within each tissue category and date. N1, N2 and N3 refer to 1-, 2- and 3-year-old needles, respectively.

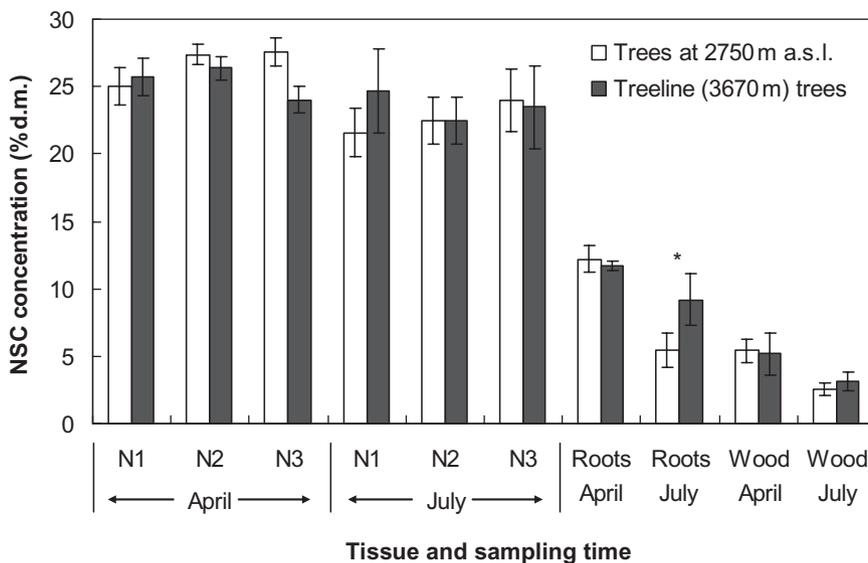


Figure 3. Mean non-structural carbohydrates (NSC) concentrations (mean ± 1 standard deviation; % of dry matter) in the tissues of trees (*Abies fabri*) growing at the alpine treeline (3670 m a.s.l.) and at 2750 m a.s.l. in the Mo-Xi Valley, Mt. Gongga, southwestern China. T-paired comparisons were used to test statistical differences in the means between the two elevations within each tissue category and date. Asterisks indicate statistically significant differences (* $P < 0.05$; $n = 6$ trees) in the NSC concentration between trees at the two elevations within each tissue category and date. N1, N2 and N3 indicate 1-, 2- and 3-year-old needles, respectively.

Abies trees in the Kang-Ding Valley

The total N content in the trees studied increased with increasing tree elevation within each sampling date

(Table 2). Two-way ANOVAS showed a significantly higher N content in the source tissue in TT (1.28% d.m.) than in TLL (1.01%) in April ($P = 0.004$), but no difference in July ($P = 0.574$) (Table 2). In the sink tissues, the difference in

Table 4. Ratio of mobile carbohydrate to total nitrogen (C–N ratio) in source (needles) and sink (fine roots and stem wood) in trees growing at the alpine treeline and their lowest distribution boundaries on Mt. Gongga, southwestern China

	<i>Picea</i> in the Kang-Ding Valley		<i>Abies</i> in the Kang-Ding Valley		<i>Abies</i> in the Mo-Xi Valley		Three treeline cases combined	
	3800 m	3400 m	3750 m	3300 m	3670 m	2750 m	TT	TLL
Source (needles)								
April	12.9	17.0	15.1	17.4	20.1	22.39	16.2	19.0
July	14.2	23.0	17.7	17.7	17.7	19.6	16.7	20.0
Sink (fine roots and stem sapwood)								
April	16.3	21.5	12.0	14.5	14.8	19.1	14.3	18.1
July	21.9	20.2	19.2	21.7	13.1	11.3	17.6	15.5

TT, trees at the alpine treeline; TLL, trees at the lowest elevational limit.

the N content between TT and TLL was statistically significant for both April ($P < 0.001$) and July ($P = 0.006$) (Table 2). Across tissues, the difference in the N content between TT and TLL was significant in April ($P < 0.001$), but not in July ($P = 0.33$) (Table 2). The SSR-N did not differ between TT and TLL in April (both 2.8), but decreased with increasing tree elevation (3.3 in TLL vs. 2.7 in TT) in July (Table 2).

The results of two-way ANOVAS showed that TT had slightly higher source NSC in April (19.36% d.m. in TT > 17.58% in TLL, $P = 0.063$), and significantly higher source NSC in July (19.85% d.m. in TT > 18.37% in TLL, $P = 0.049$) compared with TLL (Table 3). No statistical differences in the sink NSC between TT and TLL for each sampling date were detected ($P = 0.676$ for April; $P = 0.482$ for July) (Table 3). Very similar to the NSC in source, across tissues TT had slightly higher overall NSC concentrations than TLL in both April (13.82% d.m. in TT > 12.63% in TLL, $P = 0.059$) and July (15.06% d.m. in TT > 13.79% in TLL, $P = 0.067$) (Table 3). Tissue NSC showed an increased trend with increasing elevation of trees, although the difference between TT and TLL was statistically significant only in the 3-year-old needles in April ($P = 0.009$) (Fig. 2). These results, unlike the findings with *Picea*, imply that TT may not be limited by a carbon shortage (Table 3, Figs 1 & 2). The SSR-NSC did not markedly differ between TT and TLL in either April or July (Table 3). The SSR-NSC was higher in April than in July in both TT and TLL (Table 3). The C–N ratios in both source and sink tissue tended to decrease with increasing tree elevation (Table 4).

Abies trees in the Mo-Xi Valley

The total N content was higher in TT than in TLL overall (Table 2). Differences were significant or even highly significant in the source tissue in April (1.26% d.m. in TT > 1.19% in TLL, $P = 0.023$) and in July (1.33% d.m. in TT > 1.16% in TLL, $P < 0.001$), but not in the sink tissues in either April ($P = 0.054$) or July ($P = 0.97$) (Table 2). Across tissues, TT showed a significantly higher N content in April (0.98% d.m. in TT > 0.90% in TLL, $P = 0.003$) and in July (0.99% d.m. in TT > 0.89% in TLL, $P < 0.001$) than TLL (Table 2). The SSR-N decreased with increasing tree elevation in April (2.2 in TT < 2.6 in TLL), but increased with elevation in July (2.8 in TT > 2.5 in TLL) (Table 2).

Analysis of two-way ANOVAS indicated that the source NSC was significantly lower in TT (25.37% d.m.) than in TLL (26.65%) in April ($P = 0.029$), but similar in July (23.52% d.m. in TT versus 22.68% in TLL, $P = 0.47$) (Table 3). There were no statistically significant differences in sink NSC between TT and TLL in April ($P = 0.54$), but a slight difference in July (6.16% d.m. in TT > 5.32% in TLL, $P = 0.07$) was found (Table 3). Similarly to the NSC in the source, across tissues the overall NSC concentration was significantly lower in TT (18.60% d.m.) than in TLL (19.52%) in April ($P = 0.029$), but slightly higher in TT (16.58% d.m.) than in TLL (15.21%) in July ($P = 0.08$) (Table 3). *T*-paired comparisons did not identify any other

significant differences in tissue NSC between TT and TLL within each tissue category and date apart from in the fine roots in July ($P = 0.038$) (Fig. 3). Unlike the *Abies* grown at the treeline in the Kang-Ding Valley (Table 3, Fig. 2), trees of the same species growing at the treeline in the Mo-Xi Valley seem to suffer from a winter carbon shortage compared with lower-elevation *Abies* trees (Table 3, Fig. 3). The TT and TLL had the same SSR-NSC in April (both 3.0), but TT (3.8) had a lower SSR-NSC than TLL (4.3) in July (Table 3). The C–N ratio decreased with increasing tree altitude within each sampling date apart from in the July sink (Table 4).

Overall elevational trends in N, NSC and source–sink relationships

Combining the three treeline cases, the total N contents were higher in TT than in TLL overall (Table 2). This result, in combination with the results from the single species/treeline level (Table 2) described previously, indicated that nitrogen does not limit the growth and development of the trees growing at the alpine treeline. The tissue N contents were significantly lower in TLL than in TT, except in the July sink tissues, where the difference was non-significant ($P = 0.37$) (Table 2), according to two-way ANOVAS with elevation and tissue type as factors.

However, on the other hand, carbon seems to be more equivocal in determining the treeline position. As described previously, the *Picea* treeline in the Kang-Ding Valley may be physiologically determined by a carbon limitation in both winter and summer (Table 3, Fig. 1). In contrast, the *Abies* treeline in the same valley does not support the ‘carbon limitation hypothesis’ (Table 3, Fig. 2), whereas the *Abies* trees at the alpine treeline in the Mo-Xi Valley appear to suffer from a winter carbon shortage (Table 3, Fig. 3). These contradictory results led us to combine the three treeline cases to find an overall elevational trend in tissue NSC.

The results of two-way ANOVAS showed that the overall NSC concentrations were significantly lower in TT (14.70% d.m.) than in TLL (15.43%) in April ($P = 0.011$), but not in July (14.70% d.m. in TT versus 14.91% in TLL, $P = 0.581$) (Table 3), suggesting a winter carbon shortage in treelines. The source NSC was significantly lower in TT than in TLL in both April (19.91% d.m. in TT < 20.88% in TLL, $P = 0.018$) and July (19.82% d.m. in TT < 20.81% in TLL, $P = 0.049$) (Table 3). No statistical differences in sink NSC between TT and TLL in either April ($P = 0.29$) or July ($P = 0.15$) were found (Table 3). Hence, the winter carbon shortage may result from the limited source activities in treeline trees (Table 3). Combining the treeline cases, TT and TLL had the same SSR-NSC in April (both 2.9), but TT (2.8) showed a lower SSR-NSC than TLL (3.4) in July (Table 3).

The overall elevational trend in the C–N ratios showed that they were much smaller in TT source than in TLL source in both April (16.2 in TT source < 19.0 in TLL source) and July (16.7 in TT < 20.0 in TLL), as well as in

the April sink tissues (14.3 in TT sink < 18.1 in TLL sink) (Table 4). The C–N ratio in the July sink tissues was, however, higher in TT (17.6) than in TLL (15.5) (Table 4).

DISCUSSION

No nitrogen limitation in treeline trees

Microbial activity and plant metabolism are both temperature dependent, which is why cold temperatures are thought to limit nutrient availability and plant uptake (Reich & Oleksyn 2004). Thus, nutrient deficiencies may directly contribute to the location of treeline (Richardson 2004). In line with these hypotheses, Richardson (2004) and Shi, Korner & Hoch (2006) reported that needle/leaf N content tended to decrease with increasing tree elevation within the alpine ecotone, although the difference between treeline trees and lower elevation trees was not statistically significant. However, Hoch & Korner (2005) and our data have shown that the tissue N content in treeline trees was higher than in lower elevation trees. This indicates that N does not seem to be limiting tree growth and development for the alpine treelines studied. In a census of >150 plant species from nine different mountain areas of all major climatic zones, Korner (1989) found that needle/leaf N content increased with increasing altitude worldwide. Recently, a meta-analysis (covering 1280 plant species in 704 genera, across 452 locations worldwide) of leaf trait data indicated that leaf N levels increased with decreasing mean annual temperature (Reich & Oleksyn 2004). This N pattern (i.e. N increases with decreasing temperature) could be interpreted to mean that cold climates favour high leaf N to compensate for the low efficiency of physiological processes at low temperatures (Reich & Oleksyn 2004; He *et al.* 2006).

Acclimation of photosynthesis to low temperatures involves an increase in many enzymes such as Rubisco (Badger, Bjorkman & Armond 1982; Hurry *et al.* 1995). Rubisco comprises a large proportion of the nitrogen invested in the photosynthetic apparatus. Higher N contents in treeline trees (Table 2) have important implications for resource use efficiency and photosynthesis of trees in a harsh alpine environment. Experimental studies revealed that leaf photosynthetic rates of various plants/trees are positively correlated with leaf N content (Martindale & Leegood 1997; Davey *et al.* 1999; Kitajima & Hogan 2003; Diaz-Espejo, Nicolas & Fernandez 2007).

Carbon limitation in winter, but no carbon shortage in summer

The overall elevational trend in tissue NSC (Table 3) indicated that trees growing at the alpine treeline had significantly lower tissue NSC concentrations than lower elevation trees in April, but not in July. These results suggest that the treeline trees may suffer from a 'carbon limitation' during and after dormancy in winter and early spring, but not during the growing season. Such a winter carbon

limitation may lead to severe frost injuries in trees' tissues and result in high tree mortality, subsequently determining the alpine treeline position (Tranquillini 1979). Many studies have already shown that trees' hardness and ability to withstand cold are positively related to soluble carbohydrate concentrations in the tissues (Ogren 1997; Morin *et al.* 2007).

At a single species and treeline level, the three treeline cases studied did not show consistent evidence for carbon limitation for trees growing at treeline. The treeline trees of *Picea* in the Kang-Ding Valley showed an overall carbon shortage (Table 3, Fig. 1), whereas the treeline trees of *Abies* showed no carbon limitation in the same valley (Table 3, Fig. 2), but a winter carbon shortage in the Mo-Xi Valley (Table 3, Fig. 3). These findings imply that the responses of NSC to altitude are (1) species specific and (2) site dependent. Hoch & Korner (2003) did not find mobile carbon depletions in three *Pinus* species growing at the alpine treelines in either winter or summer. Shi *et al.* (2006) and Shi, Korner & Hoch (2008) recorded no summer depletion of NSC in different tree and shrub species at their upper elevational limits in southwestern China, but they did not investigate the NSC status in winter or after dormancy. In contrast, Richardson (2004) found a carbon limitation related to significant reductions in the lignin and cellulose contents in *Picea rubens* Sarg. and *A. balsamea* (L.) Mill grown at the alpine treelines in the northeastern USA. Cavieres *et al.* (2000) also reported clear evidence of a 'carbon limitation' in *Espeletia nerifolia*, but not in *Podocarpus oleifolius* growing at the tropical alpine treeline in the Venezuelan Andes.

Within a species, levels of tissue NSC vary markedly according to the site conditions under which trees grow, suggesting site-dependent responses of NSC to altitude. For example, tissue NSC concentrations were much higher in *Abies* trees in the Mo-Xi Valley (Fig. 3) than in the Kang-Ding Valley (Fig. 2, Table 3). These differences in tissue NSC concentrations may result from the differences in the site conditions, as the Kang-Ding Valley is relatively hot and dry, whereas the Mo-Xi Valley is moist and warm (see Table 1). Hot and dry conditions may induce stomatal closing, leading to a reduction in photoassimilates and NSC levels. Many experimental studies have shown that plants grown under environmental stress have lower tissue NSC concentrations than those grown under normal conditions (Liu & Tyree 1997; Correia *et al.* 1999; Runion *et al.* 1999; Gwynn-Jones 2001; Polle, McKee & Blaschke 2001). However, Bacelar *et al.* (2006) found that plants grown in a water-stressed environment had higher tissue NSC concentrations, because environmental stresses, such as water stress, may restrict plant growth rather than reducing the rate of photosynthesis, which leads to an accumulation of NSC (Bryant, Chapin & Klein 1983; Runion *et al.* 1999).

In summary, the rather contradictory findings about the carbon pool make it difficult to generalize the carbon limitation question in treeline trees. Hence, not only more case studies are needed, but also a meta-analysis, combining

the results of all studies related to the ‘carbon limitation hypotheses’, should be conducted.

Lack of evidence of adaptation of source–sink relationships and C–N ratio to a treeline environment

The tissue NSC concentration has been used to assess the balance between carbon acquisition (sources) by photosynthesis and carbon demand (sinks) for growth and respiration processes (Hoch, Popp & Korner 2002; Li *et al.* 2002, 2008; Korner 2003b; Shi *et al.* 2008). We investigated the SSR-NSC (defined as the concentration ratio of source NSC to sink NSC) and the SSR-N (defined as the ratio of source N to sink N) in a context of source–sink relationships. The SSR-NSC in treeline trees did not differ from those in lower elevation trees in April (Table 3), indicating an adaptation of source–sink relationships to altitude in winter or spring. However, the SSR-NSC in treeline trees was much lower than those in lower elevation trees in July (Table 3), probably because there was a limited source activity compared with the strong carbon sink activity in treeline trees in summer. A stronger sink imports and utilizes the photosynthetic products from sources immediately if source capacity is limited by the photosynthetic activity at treeline. The values of SSR-N were, however, smaller in trees growing at treeline than in lower elevation trees in April, but not in July (Table 2). Moreover, the C–N ratios in source tissue were always smaller in treeline trees than in lower elevation trees of the same species within each sampling date (Table 4). In sink tissues, the C–N ratios in treeline trees were consistently smaller than those in lower elevation trees in April, but not in July (Table 4). These results may also be regarded as additional evidence for limited source capacity, leading to winter carbon shortage. However, to what extent SSR-NSC, SSR-N or the C–N ratio can actually determine the alpine treeline position is still unclear as little research has been performed in this area. The present case study proposed a least ratio of source-NSC to sink-NSC of 2.2 for trees to exist at their upper elevational limits (Table 3).

Both source capacity and sink capacity are influenced by harsh environmental factors such as low temperatures at the alpine treeline. Whether trees for the alpine treeline are carbon limited depends on whether photoassimilate production or the use of photoassimilates is limited. If the use of photoassimilates is limited by the growth of a treeline tree, that is, growth limitation (Korner 1998; Li & Kräuchi 2005), then photoassimilate production should be down-regulated via feedback (Sharkey, Socias & Loreto 1994). On the other hand, NSC seems to be actively regulated by environmental stresses (Bryant *et al.* 1983; Runion *et al.* 1999). Moreover, trees growing at treeline may compensate for a decrease in the concentration of tissue NSC by producing greater amounts of that tissue when calculating whole-tree carbon budgets (Li *et al.* 2002; Hoch & Korner 2003; Hoch, Richter & Korner 2003). For example, Li, Krauchi & Dobbertin (2006) found that the needle biomass

in old *P. cembra* trees at the alpine treeline was about three times higher, because of the greater needle longevity, than in the same species with the same age at lowland sites.

CONCLUSIONS

It has been hypothesized that nutrient deficiencies may directly contribute to the location of the treeline (Richardson 2004). The present study found that nitrogen does not seem likely to limit the growth and development of trees studied at the Himalayan treelines because, contrary to the biogeochemical hypothesis (Reich & Oleksyn 2004), we found that the tissue N content increased with increasing tree elevation.

Carbon limitation because of a shortage of photoassimilates has long been regarded as the key to explain the upper altitudinal or latitudinal treeline on a global scale (Schulze *et al.* 1967; Stevens & Fox 1991). Despite the inconsistent results gained at a single species/treeline level, the results of the combined three treeline cases revealed that the treeline trees may suffer from a winter carbon shortage. On the other hand, previous studies (Li *et al.* 2002; Hoch & Korner 2003; Hoch *et al.* 2003; Korner 2003b; Richardson 2004; Shi *et al.* 2006) and the present data revealed that levels of mobile carbohydrates in trees are species specific and site dependent, and that both the source capacity and the sink capacity of a tree influence its tissue NSC concentrations and the carbon balance. We therefore suggest that the persistence and development of various treeline trees in a harsh alpine environment may depend not only on a minimum level of the total NSC concentration, but also on a sufficiently high sugar:starch ratio to overwinter successfully (Li *et al.* 2008), and on a balanced source–sink relationship to maintain a positive carbon balance.

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REFERENCES

- Allen S.E. (1989) *Chemical Analysis of Ecological Materials*, 2nd edn. Blackwell Scientific Publications, Oxford, UK.
- Bacelar E.A., Santos D.L., Moutinho-Pereira J.M., Goncalves B.C., Ferreira H.F. & Correia C.M. (2006) Immediate responses and adaptative strategies of three olive cultivars under contrasting water availability regimes: changes on structure and chemical composition of foliage and oxidative damage. *Plant Science* **170**, 596–605.
- Badger M.R., Bjorkman O. & Armond P.A. (1982) An analysis of photosynthetic response and adaptation to temperature in

- higher-plants – temperature-acclimation in the desert evergreen *Nerium-oleander* L. *Plant, Cell & Environment* **5**, 85–99.
- Bond B.J., Farnsworth B.T., Coulombe R.A. & Winner W.E. (1999) Foliage physiology and biochemistry in response to light gradients in conifers with varying shade tolerance. *Oecologia* **120**, 183–192.
- Bryant J.P., Chapin F.S. & Klein D.R. (1983) Carbon nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* **40**, 357–368.
- Cabrera H.M., Rada F. & Cavieres L. (1998) Effects of temperature on photosynthesis of two morphologically contrasting plant species along an altitudinal gradient in the tropical high Andes. *Oecologia* **114**, 145–152.
- Cavieres L.A., Rada F., Azocar A., Garcia-Nunez C. & Cabrera H.M. (2000) Gas exchange and low temperature resistance in two tropical high mountain tree species from the Venezuelan Andes. *Acta Oecologica-International Journal of Ecology* **21**, 203–211.
- Correia C.M., Areal E.L.V., Torres-Pereira M.S. & Torres-Pereira J.M.G. (1999) Intraspecific variation in sensitivity to ultraviolet-B radiation in maize grown under field conditions – II. Physiological and biochemical aspects. *Field Crops Research* **62**, 97–105.
- Däniker A. (1923) Biologische Studien über Wald- und Baumgrenze, insbesondere über die klimatischen Ursachen und deren Zusammenhänge. *Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich* **68**, 1–102.
- Davey P.A., Parsons A.J., Atkinson L., Wadge K. & Long S.P. (1999) Does photosynthetic acclimation to elevated CO₂ increase photosynthetic nitrogen-use efficiency? A study of three native UK grassland species in open-top chambers. *Functional Ecology* **13**, 21–28.
- Diaz-Espejo A., Nicolas E. & Fernandez J.E. (2007) Seasonal evolution of diffusional limitations and photosynthetic capacity in olive under drought. *Plant, Cell & Environment* **30**, 922–933.
- Fischer C. & Holl W. (1991) Food reserves of Scots Pine (*Pinus-Sylvestris* L.). 1. Seasonal-changes in the carbohydrate and fat reserves of pine needles. *Trees-Structure and Function* **5**, 187–195.
- Goldstein G., Meinzer F.C. & Rada F. (1994) Environmental biology of a tropical treeline species, *Polylepis sericea*. In *Tropical Alpine Environments* (eds P.W. Rundel, A.P. Smith & F.C. Meinzer), pp. 129–149. Cambridge University Press, Cambridge, UK.
- Gonzalez J.A. (1991) The annual TNC (total non-structural carbohydrates) cycles in two high mountain species: *Woodsia montevicensis* and *Calandrinia acaulis*. *Botanica* **42**, 276–280.
- Grace J. & James J. (1993) Physiology of trees at treeline. In *Forest Development in Cold Climates* (eds J. Alden, J.L. Mastrantonio & S. Odum), pp. 105–114. Plenum Press, New York, NY, USA.
- Graham E.A., Mulkey S.S., Kitajima K., Phillips N.G. & Wright S.J. (2003) Cloud cover limits net CO₂ uptake and growth of a rain-forest tree during tropical rainy seasons. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 572–576.
- Gwynn-Jones D. (2001) Short-term impacts of enhanced UV-B radiation on photo-assimilate allocation and metabolism: a possible interpretation for time-dependent inhibition of growth. *Plant Ecology* **154**, 67–73.
- He J.S., Fang J.Y., Wang Z.H., Guo D.L., Flynn D.F.B. & Geng Z. (2006) Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grassland biomes of China. *Oecologia* **149**, 115–122.
- He Y., Liao C. & Zhang B. (2005) A study on pedography of soil on the eastern slope of Mt. Gongga in southeastern Qinghai-Tibet Plateau. *Journal of Mountain Science* **23**, 651–656 (in Chinese).
- Hoch G. & Korner C. (2003) The carbon charging of pines at the climatic treeline: a global comparison. *Oecologia* **135**, 10–21.
- Hoch G. & Korner C. (2005) Growth, demography and carbon relations of *Polylepis* trees at the world's highest treeline. *Functional Ecology* **19**, 941–951.
- Hoch G., Popp M. & Korner C. (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. & Korner C. (2003) Non-structural carbon compounds in temperate forest trees. *Plant, Cell & Environment* **26**, 1067–1081.
- Hurry V.M., Keerberg O., Parnik T., Gardstrom P. & Oquist G. (1995) Cold-hardening results in increased activity of enzymes involved in carbon metabolism in leaves of winter rye (*Secale-Cereale* L.). *Planta* **195**, 554–562.
- Kitajima K. & Hogan K.P. (2003) Increases of chlorophyll a/b ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. *Plant, Cell & Environment* **26**, 857–865.
- Korner C. (1989) The nutritional-status of plants from high-altitudes – a worldwide comparison. *Oecologia* **81**, 379–391.
- Korner C. (1998) A re-assessment of high elevation treeline positions and their explanation. *Oecologia* **115**, 445–459.
- Korner C. (2003a) *Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems*, 2nd edn. Springer, Heidelberg, Berlin, Germany; New York, NY, USA.
- Korner C. (2003b) Carbon limitation in trees. *Journal of Ecology* **91**, 4–17.
- Lewis J.D., Lucash M., Olszyk D.M. & Tingey D.T. (2004) Relationships between needle nitrogen concentration and photosynthetic responses of Douglas-fir seedlings to elevated CO₂ and temperature. *New Phytologist* **162**, 355–364.
- Li M., Yang J. & Krauchi N. (2003) Growth responses of *Picea abies* and *Larix decidua* to elevation in subalpine areas of Tyrol, Austria. *Canadian Journal of Forest Research* **33**, 653–662.
- Li M.H. & Kräuchi N. (2005) The state of knowledge on alpine treeline and suggestions for future research. *Journal of Sichuan Forestry Science and Technology* **26**, 36–42 (in Chinese).
- Li M.H. & Yang J. (2004) Effects of microsite on growth of *Pinus cembra* in the subalpine zone of the Austrian Alps. *Annals of Forest Science* **61**, 319–325.
- Li M.H., Hoch G. & Korner C. (2001) Spatial variability of mobile carbohydrates within *Pinus cembra* trees at the alpine treeline. *Phyton-Annales Rei Botanicae* **41**, 203–213.
- Li M.H., Hoch G. & Korner C. (2002) Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. *Trees-Structure and Function* **16**, 331–337.
- Li M.H., Krauchi N. & Dobbertin M. (2006) Biomass distribution of different-aged needles in young and old *Pinus cembra* trees at highland and lowland sites. *Trees-Structure and Function* **20**, 611–618.
- Li M.H., Xiao W.F., Wang S.G., Cheng G.W., Cherubini P., Cai X.H., Liu X.L., Wang X.D. & Zhu W.Z. (2008) Mobile carbohydrates in Himalayan treeline trees I. Evidence for carbon gain limitation but not for growth limitation. *Tree Physiology* **28**, 1287–1296.
- Liu X. & Tyree M.T. (1997) Root carbohydrate reserves, mineral nutrient concentrations and biomass in a healthy and a declining sugar maple (*Acer saccharum*) stand. *Tree Physiology* **17**, 179–185.
- Martindale W. & Leegood R.C. (1997) Acclimation of photosynthesis to low temperature in *Spinacia oleracea* L. 2. Effects of nitrogen supply. *Journal of Experimental Botany* **48**, 1873–1880.
- Morin X., Ameglio T., Ahas R., Kurz-Besson C., Lanta V., Lebourgeois F., Miglietta F. & Chuine I. (2007) Variation in cold

- hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three European oak species. *Tree Physiology* **27**, 817–825.
- Ogren E. (1997) Relationship between temperature, respiratory loss of sugar and premature dehardening in dormant Scots pine seedlings. *Tree Physiology* **17**, 47–51.
- Osaki M., Shinano T. & Tadano T. (1991) Redistribution of carbon and nitrogen-compounds from the shoot to the harvesting organs during maturation in field crops. *Soil Science and Plant Nutrition* **37**, 117–128.
- Polle A., McKee I. & Blaschke L. (2001) Altered physiological and growth responses to elevated [CO₂] in offspring from holm oak (*Quercus ilex* L.) mother trees with lifetime exposure to naturally elevated [CO₂]. *Plant, Cell & Environment* **24**, 1075–1083.
- Reich P.B. & Oleksyn J. (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 11001–11006.
- Richardson A.D. (2004) Foliar chemistry of balsam fir and red spruce in relation to elevation and the canopy light gradient in the mountains of the northeastern United States. *Plant and Soil* **260**, 291–299.
- Richardson A.D., Berlyn G.P. & Gregoire T.G. (2001) Spectral reflectance of *Picea rubens* (Pinaceae) and *Abies balsamea* (Pinaceae) needles along an elevational gradient, Mt. Moosilauke, New Hampshire, USA. *American Journal of Botany* **88**, 667–676.
- Ripullone F., Grassi G., Lauteri M. & Borghetti M. (2003) Photosynthesis–nitrogen relationships: interpretation of different patterns between *Pseudotsuga menziesii* and *Populus x-euroamericana* in a mini-stand experiment. *Tree Physiology* **23**, 137–144.
- Runion G.B., Entry J.A., Prior S.A., Mitchell R.J. & Rogers H.H. (1999) Tissue chemistry and carbon allocation in seedlings of *Pinus palustris* subjected to elevated atmospheric CO₂ and water stress. *Tree Physiology* **19**, 329–335.
- Schulze E.D., Mooney H.A. & Dunn E.L. (1967) Wintertime photosynthesis of Bristlecone Pine (*Pinus aristata*) in White Mountains of California. *Ecology* **48**, 1044–1047.
- Seifter S., Dayton S., Novic B. & Muntwyler E. (1950) The estimation of glycogen with the anthrone reagent. *Archives of Biochemistry* **25**, 191–200.
- Sharkey T.D., Socias X. & Loreto F. (1994) CO₂ effects on photosynthetic end product synthesis and feedback. In *Plant Responses to the Gaseous Environment* (eds R.G. Alscher & A.R. Wellburn), pp. 55–78. Chapman and Hall, London, UK.
- Shi P.L., Korner C. & Hoch G. (2006) End of season carbon supply status of woody species near the treeline in western China. *Basic and Applied Ecology* **7**, 370–377.
- Shi P., Korner C. & Hoch G. (2008) A test of the growth-limitation theory for alpine tree line formation in evergreen and deciduous taxa of the eastern Himalayas. *Functional Ecology* **22**, 213–220.
- Stevens G.C. & Fox J.F. (1991) The causes of treeline. *Annual Review of Ecology and Systematics* **22**, 177–191.
- Tranquillini W. (1979) *Physiological Ecology of the Alpine Timberline: Tree Existence at High Altitude with Special Reference to the European Alps*. Springer, Heidelberg, Berlin, Germany; New York, NY, USA.
- Wardle P. (1993) Causes of alpine timberline: a review of the hypotheses. In *Forest Development in Cold Climates* (eds J. Alden, J.L. Mastrantonio & S. Odum), pp. 89–103. Plenum Press, New York, NY, USA.

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