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Spatial and seasonal variations in mobile carbohydrates in *Pinus cembra* in the timberline ecotone of the Central Austrian Alps

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Abstract

To test whether the altitudinal limit of tree growth is determined by carbons shortage or by a limitation in growth we investigated non structural carbohydrates and their components starch and total soluble sugars in *Pinus cembra* trees along an elevational gradient in the timberline ecotone of the Central Austrian Alps. NSC contents in needles, branches, stems, and coarse roots were measured throughout an entire growing season. At the tissue level NSC contents were not significantly more abundant in treeline trees as compared to trees at lower elevations. Along our 425 m elevational transect from the closed forest to the treeline we failed to find a stable elevational trend in the total NSC pool of entire trees and observed within season increases in the tree's NSC pool that can be attributed to an altitudinal increase in leaf mass as needles contained the largest NSC fraction of the whole tree NSC pool. Furthermore, whole tree NSC contents were positively correlated with net photosynthetic capacity. Although our observed NSC characteristics do not support the hypothesis that tree life at their upper elevational limit is determined by an insufficient carbon balance we found no consistent confirmation for the sink limitation hypothesis.

Keywords

Non structural carbohydrates; seasonal variation; elevational gradient; timberline ecotone; treeline formation; treeline limitation

Introduction

The alpine timberline is one of the most conspicuous climate driven ecological boundaries. Rather than being an abrupt boundary the upper timberline usually forms an ecotone which stretches from the forest limit i.e. the upper limit of the continuous closed forest canopy to the treeless alpine zone above (Tranquillini 1979; Wieser and Tausz 2007). Despite of its significance the cause of treeline formation, which follows a global thermal isotherm of $6.7 \pm 0.8^\circ\text{C}$ in soil temperature (Körner and Paulsen 2004), is not fully resolved. It is still under debate whether a temperature driven physiological mechanism may lead to an insufficient carbon gain (carbon limitation hypothesis; Stevens and Fox 1991) or to limitations in growth which Körner (1998, 2003) has referred to as “sink limitation hypothesis. As both hypotheses are based on alterations in the source-sink balance with increasing elevation concentrations of non structural carbohydrates (NSC) in trees near the alpine treeline have been suggested to provide an answer as they reflect a balance between carbon supply

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(source) and carbon demand (sink). It is assumed that a reduction of NSC might indicate carbon shortage (carbon limitation), and that an increase of the NSC pool would indicate restricted carbon demand due to a limited tissue formation (sink limitation) and several studies used NSC as an indicator to test for carbon source or sink limitation at the upper limit of tree growth in Europe, Asia, and America (Hoch et al. 2002; Hoch und Körner 2003, 2005; Shi et al. 2006, 2008). All these studies showed that NSC concentrations of treeline trees were not lower as compared to trees below the forest limit. Li et al. (2008) by contrast, observed that tissue concentrations of NSC tended to decrease with elevation in Himalayan treeline trees. Hence, such contradictory results provoked us to examine the seasonal course of NSC in different tissues of adult *Pinus cembra* trees along an elevational gradient within the timberline ecotone of the Central Austrian Alps in order to increase our understanding whether carbon supply (source) or carbon demand (sinks) determines treeline limitation at their upper elevational limit.

Material and methods

Study site and climatic conditions

The study was conducted in the timberline ecotone at Mt. Patscherkofel (47°12′37″N, 11°27′07″E; Tuxer Alps as part of the Central Tyrolean Alps) south of Innsbruck/Austria. In this region the *forest limit* or the upper limit of the continuous closed forest canopy is at about 1950 m a.s.l., and *tree line* i.e. the upper limit of trees; higher than 2 (Wieser and Tausz, 2007) to 3 m (Körner 2007) is located at 2180 m a.s.l. Within the timberline ecotone on Mt. Patscherkofel *Pinus cembra* is the dominating tree species and grows between 1700 and 2200 m a.s.l. The geology of the Mt. Patscherkofel region is dominated by gneisses and schist and Haplic Podzols, a soil type typical for the Central Austrian Alps (Neuwinger 1970, 1980) prevail at the study site.

A cool subalpine climate with low temperatures, a continuous snow cover from October through May, and the possibility of frost during each summer month prevails at the site. According to a 45-year record of a weather station at the forest limit (1950 m a.s.l.) the mean annual air temperature is 2.5 °C, with summer maxima up to 26.0°C and winter minima down to -28.0°C. The mean annual precipitation is of 995 mm, with the majority falling during the growing season May through October and annual soil temperature in 10 cm soil depth ranged between 2.6 and 5.7 °C during the last 15-years (summer maxima up to 15 °C and winter minima down to -6.5 °C). On Mt. Patscherkofel daily mean air temperature throughout the growing season decreases by 0.8 K per 100 m in altitude, while root zone temperature measured in 10 cm soil depth increased by 1.1 K per 100 m in altitude (Gruber et al. 2009; Wieser et al. 2009).

Tissue sampling and chemical analysis

Our four sampling sites were located at 1750, 1950, 2100, and 2180 m a.s.l. on a SW facing transect from the closed forest up to the upper tree limit. At each study plot we selected three 50 - 65 year-old *P. cembra* trees for repeated sampling (May 10, June 16, July 24, September 9, and October 11) throughout the growing season of 2008. At each sampling date we collected the following tissues: 1-year-old needles, as well as the xylem of branches (bark removed; xylem diameter about 1 cm), stems, and coarse roots (using a 5 mm diameter corer). Although the sampling location within the crown of entire trees has no effect on NSC concentrations (Li et al. 2001) needle and branch tissues were sampled almost exclusively on south to south west exposed branches within the lower third of the crown (Hoch et al. 2002). Stem and root tissue was sampled on the north-west side of the trees 1 m above ground and in 5 to 10 cm soil depth, respectively. Samples were always collected between 11:00 and 14:00 hours in order to exclude effects of irradiance and tissue temperature on

diurnal NSC contents (c.f. also Li et al. 2008). All the samples were immediately stored in a cool box and heated in a microwave oven (60 seconds; 600 W) within 4 hours after collection. Thereafter tissue samples were dried to constant weight at 80°C, ground to fine powder and stored dry at 4°C until analysis of NSC.

Thereafter 0,1mg Polyvinylpyrrolidone was added to approximately 10 mg of fine ground plant material and soluble carbohydrates were extracted twice in 80% (v/v) acetone for 15 minutes at 50°C. From the resolved residual of the soluble fraction the concentration of glucose was determined photometrical at 340nm as NADPH⁺ H⁺ formation during enzymatic conversion of glucose-6-phosphate to gluconate-6-phosphate.

Aliquots of the resolved extract were treated with hexocinae and isomerase as well as invertase, to convert fructose and sucrose into glucose, which was subsequently measured as described above. For starch measurements, starch extraction was carried out by incubating the pellet with hydrochloric acid for 2 h at 60°C. After pH adjustment, starch was hydrolyzed enzymatically to glucose and subsequently measured. The photometric analyses were conducted using test combinations from Boehringer Mannheim (Mannheim, Germany).

Scaling to the tree level

Discrete NSC measurements made on individual tissues and individual trees were “scaled up” to provide estimates of NSC contents of entire trees. Therefore, we multiplied the multiplied the organ specific NCS concentrations (needles, branches, stem, and coarse roots) with the corresponding biomass fraction, and finally summed up the results derived for each individual organ to a tree’s total.

The biomass fractions of the tree organs were calculated from carbon allocation data for 20 to 120 year old *P. cembra* trees sampled within the timberline ecotone of the central Austrian Alps (Oswald 1963, Wieser et al. 2005). As there is evidence that total tree biomass of even aged trees shows no elevational trend within the timberline ecotone (Oswald 1993; Bernoulli and Körner 1999), we used the same whole tree biomass for all altitudes in our calculations. We also took into account that across the timberline ecotone biomass allocation into needles and branches increases with elevation (Oswald 1963; Wieser and Tausz 2005). Excluding stem heartwood, which is approximately 25% of the total stem biomass for trees of comparable age and height (H. Kronfuss; personal communication) the biomass proportions adjusted to 50-year-used in our analysis and comparisons were assumed to be: 18% needles, 10% branch wood, 41 % stem sapwood and 31% coarse roots for trees at the forest limit (1950 m a.s.l.) and in the close forest below (1750 m a.s.l.). The corresponding values for treeline trees at were 19, 18, 33, and 30% for the site at 2100 m a.s.l. and 27, 28, 22, and 23% for the site at 2175 m a.s.l., respectively.

Statistical analysis

Differences in NSC, starch and total soluble sugars were evaluated by a three-way analysis of variance (ANOVA) with sampling date (May, Jun, Jul, Sep, Oct) as a measure of seasonality (S) when samples were taken, tissue (T; needles, branches, stems, coarse roots), and elevation (E, 2175, 2100, 1950, 1750 m a.s.l.) as independent factors. Effects of elevation on NSC contents within each tissue type and entire trees, at each sampling date were analyzed by one-way ANOVA. Homogeneity of variances was tested with Levene’s test and post-hoc comparisons were computed according to the LSD-test. Differences at $P < 0.05$ were regarded as statistically significant. All statistical calculations were performed using the software package SPSS 16.0 (SPSS, Inc., Chicago, USA).

Results and discussion

Seasonal changes in NSC, starch and total soluble sugar contents of needles, branches, stems and coarse roots of *P. cembra* trees along our altitudinal transect between 1750 and 2175 m a.s.l. are shown in Fig. 1. Across all sites season and tissue type significantly affected NSC, starch, and total soluble sugar contents (all $P < 0.001$; Table 1). Similar differences in NSC, starch, and soluble sugar contents with respect to season and tissue type have also been described for other conifers of the treeline ecotone (Kimura 1969; Hoch et al. 2002; Hoch and Körner 2003; Shi et al. 2006, 2008; Li et al. 2008) and are consistent with well described characteristics of conifer carbohydrate physiology (Luxmoore et al. 1995; Hansen et al. 1997; Höll 1997).

In contrast to this, elevation had no significant effect on the concentrations of NSC, starch and soluble sugars as shown in Table 1 (all $P = 0.130$). The elevation effects however, were significantly modified by season (significant interactions season $\times$ elevation; Table 1) inasmuch as there were positive and negative relationships between NSC starch and soluble sugars (Fig. 1 and 2). One-way ANOVAs revealed no statistically significant differences in NSC, starch and soluble sugar contents at the different elevations within each tissue type; except for NSC in needles in May ($P = 0.023$), and branches ($P = 0.018$) as well as stems ($P = 0.024$) in October (Table 2; Fig. 1). One-way ANOVAs indicated only one and three cases where elevation significantly affected starch and soluble sugars, respectively (Table 2). No significant differences with respect to elevation were also obtained for NSC contents in various tissues of evergreen conifers at the Himalayan treeline by Li et al. (2008). In some cases however, tissue concentrations were found to increase with elevation (Hoch et al. 2003; Hoch and Körner 2003, 2005; Shi et al. 2006, 2008).

Such contradictory findings at the tissue level prompted us to calculate the total NSC pool size of entire trees, as this may provide better data for deciding whether the alpine treeline is physiologically determined by carbon- or growth limitation (Li et al. 2008). Even when scaling up tissue specific NSC concentrations with the specific tissue biomass we failed to find a consistent elevational trend in the total carbon pool size of entire trees throughout the entire growing season of 2008 (Fig. 2). While NSC (Fig. 2), starch, and total sugar contents decreased with elevation in May (all $P = 0.004$; Table 3) NSC contents significantly increased with elevation in June and July (Fig. 2; Table 3). Starch and total sugar contents increased significantly with elevation in September ($P = 0.003$; Table 3) and in June ($P = 0.027$; Table 3), respectively. The significantly lower NSC concentrations in trees at 2100 and 2175 m a.s.l. in May are probably related to lag in bud break from low to higher elevations and hence an earlier starch accumulation prior and during bud break (Kimura 1969; Hansen and Beck 1994; Hansen et al. 1997) in needles of trees below the forest limit (Fig. 1), while treeline trees are still dormant. A delay in bud break with increasing elevation may also help explain the higher NSC and starch contents observed in June in trees at 2175 m a.s.l. as compared to trees lower down (Fig. 2). For the rest of the growing season the total NSC pool of the trees were similar or tended to increase with elevation (Fig. 2) as also found by Hoch et al. (2002) and Körner and Hoch (2003) along altitudinal transects in the treeline ecotone of Switzerland (*P. cembra*), Sweden, (*P. sylvestris*) and Mexico (*P. hartwegii*).

The growing season mean whole tree NSC pool was not significantly different across our elevational gradient and averaged 4.7 ± 1.2 , 4.0 ± 0.8 , 3.9 ± 0.9 and 3.8 ± 1.1 % dry matter in 2175, 2100, 1950 and 1750 m a.s.l., respectively. Oswald (1963) and Bernoulli and Körner (1999) found that needle biomass in *P. cembra* at the tree line was about two times-higher as compared to the forest limit. Thus, the observed increase in the whole tree's NSC pool might be attributed to an increase in leaf mass with elevation, as needles contained the largest NSC fraction of the whole tree NSC pool (Fig. 3).

Our findings of high NSC contents of trees growing within the timberline ecotone match earlier studies on gas exchange in *P. cembra* trees, which showed that carbon gain unlikely limited tree growth at high altitude (Tranquillii 1979; Wieser and Tausz 2007). For our study site at 1950 m a.s.l. (forest limit) Wieser et al. (2005) demonstrated that the respiratory carbon loss of an entire *P. cembra* tree during the dormant season from November throughout mid April can be covered by the foliage's carbon gain of one month. On the other hand, an increase in NSC concentrations of trees within the timberline ecotone has been used to support the sink limitation hypothesis for tree line formation (Körner 1998, 2003; Hoch et al. 2002; Hoch and Körner 2003). However, if higher NSC concentrations resulted from excess carbon then some indications of a feedback inhibition of elevated whole tree NSC contents on net carbon gain of the foliage might be expected (Sharkey et al. 1994; Paul and Foyer 2001; Smith and Stitt 2007). At our study sites, whole tree NSC contents were positively correlated with the net photosynthetic capacity (*sensu* Larcher 2001) obtained in a previous study by Wieser et al. (2009) at the same study sites (Fig. 4). Furthermore, NSC contents (Fig. 1 and 2), carbon gain (Wieser et al. 2005, 2009), as well as stem (Gruber et al. 2009) and root growth (Turner and Streule 1983) of *P. cembra* at the alpine timberline followed similar seasonal patterns, as also observed in conifer seedlings at timberline in the Rocky Mountains by Bansal and Geronimo (2008). These findings suggest that high whole tree NSC contents had no feedback effect on net photosynthesis. An additional consideration in interpreting NSC contents is, that stored carbon reserves are also used for coping with high elevation climate (Bansal and Germino 2008), which increasingly becomes less favourable when approaching the upper elevational limit tree growth (Holtmeier 2003), as there is evidence that the tree's intensity to counteract environmental stresses is related to the amount of stored carbohydrates (Waring 1991).

Beside foliage and woody tissues, a considerable amount of carbon is also stored in fine roots (Li et al 2008), although they comprise a small fraction of the carbon content of trees (Gower et al. 1995). Wieser et al. (2005) reported total carbon allocated into fine root growth (including respiratory losses and root exudates) of a mature *P. cembra* tree at the forest limit to be 19% of the annual net carbon gain. Gholz and Cropper (1991) reported annual maximum starch storage to be 3% of total fine root biomass, and thus matching that of the foliage in an adult *P. eloittii* stand. Furthermore, there is also evidence that up to one third of plant photoassimilates can be transferred to mycorrhizal symbionts (Nehls et al. 2007).

In addition, there is also evidence that fine root biomass increases significantly with elevation and thus also influencing the root/shoot ratio. The latter indicating a belowground shift in biomass allocation with increasing elevation as shown along elevational gradients in tropical rain forests (Kitayama and Aiba 2002; Leuschner et al. 2007) and at a latitudinal gradient in boreal forests (Helmisaari et al. 2007). Higher proportional biomass partitioning to fine roots of high altitude ecotypes (Körner and Renhardt 1987) is considered to be an adaptation to unfavorable climatic conditions (Bloom et al. 1985) where nutrient supplies are limited. Along our elevational gradient nitrogen contents in needles branches, stems, and coarse roots significantly decreases with increasing elevation (data not shown) and thus strongly suggesting decreasing nutrient availability because of low decomposition and mineralization rates due to low soil temperatures and access soil water availability (Haselwandter 2007); the latter also significantly limited soil CO₂ efflux at our study site at in 1950 m a.s.l. (Wieser 2005). Up till now however, allocation of NSC into fine roots, mycorrhizae, and root exudates is a poorly understood component in the carbon allocation of timberline associated forest trees.

In conclusion, our findings do not support the hypothesis that tree life at their upper elevational limit is determined by an insufficient carbon balance (carbon limitation

hypothesis; Stevens and Fox 1991). We also found no reliable evidence for the sink limitation hypothesis with NSC reflecting carbon saturation of growth at treeline. Thus, treeline formation may result from several interacting factors in varying combinations during an entire vegetation period. Further research however, needs to clarify the above demonstrated relationships for other tree species within the timberline ecotone of different climatic zones.

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Fig. 1. Mean concentrations of non structural carbohydrates (NSC) and its components starch (filled bars) and total soluble sugars (open bars) in needles, branches, stems and coarse roots) of *Pinus cembra* trees along an altitudinal transect between 1750 and 2175 m a.s.l. on Mt. Patscherkofel at five dates during the growing season of 2008. Values are means of 3 trees $\pm$ SD; the latter is given for NSC only.

Fig. 2.

Mean concentrations of non structural carbohydrates (NSC) and its components starch (filled bars) and total soluble sugars (open bars) in *Pinus cembra* trees along an altitudinal transect between 1750 and 2175 m a.s.l. on Mt. Patscherkofel at five dates during the growing season of 2008. Values are means of 3 trees $\pm$ SD; the latter is given for NSC only.

Fig. 3. Relative changes in growing season mean needle (open bars), branch (light dotted), stem (heavy dotted), and coarse root (solid bars) NSC accumulation patterns for *Pinus cembra* trees along an altitudinal transect between 1750 and 2175 m a.s.l. on Mt. Patscherkofel.

Figure 4.

The relationship between net photosynthetic capacity ($A_{\max}$) and whole tree nonstructural carbohydrate (NSC) contents of *Pinus cembra* during September (closed symbols) and October (open symbols) grown at 2175 (circles), 2100 (squares), and 1950 m a.s.l (triangle). Points were fit by linear regression: $y = 29.46x - 14.85$, $r^2 = 0.80$, $P = 0.016$. $A_{\max}$ data are redrawn from Wieser et al. 2009.

Table 1

ANOVA results (*P* values) for non structural carbohydrates (NSC) and its components starch, and soluble sugars in *Pinus cembra*. Samples were taken five times during the season (May, June, July, September; October) from four tissues (needles, branches, stem, roots) at four elevations (2177, 2100, 1950, and 1750 m a.s.l.). Significant differences at *P* = 0.05 are in bold and italics

ANOVA effect	NSC	Starch	Soluble sugars
Season (S)	< <i>0.001</i>	< <i>0.001</i>	< <i>0.001</i>
Tissue (T)	< <i>0.001</i>	< <i>0.001</i>	< <i>0.001</i>
Elevation (E)	0.544	0.130	0.729
S x E	< <i>0.001</i>	<i>0.004</i>	< <i>0.001</i>

Table 2

ANOVA results (*P* values) of non structural carbohydrates (NSC), starch, and soluble sugars vs. altitude in different tissues of *Pinus cembra*. Significant differences at *P* = 0.05 are in bold and italics

Tissue Type	Date	NCS	Starch	Soluble sugars
Needles	May	<i>0.023</i>	0.083	0.072
	June	0.876	0.990	<i>0.043</i>
	July	0.061	0.087	0.188
	September	0.053	<i>0.002</i>	0.740
	October	0.372	0.430	0.196
Branches	May	0.331	0.380	<i>0.028</i>
	June	0.577	0.240	0.881
	July	0.600	0.284	0.258
	September	0.615	0.404	0.768
	October	<i>0.018</i>	0.600	<i>0.011</i>
Stems	May	0.641	0.006	0.107
	June	0.629	0.717	0.452
	July	0.142	0.130	0.470
	September	0.844	0.850	0.659
	October	<i>0.024</i>	0.180	<i>0.037</i>
Roots	May	0.577	0.512	0.096
	June	0.270	0.152	0.950
	July	0.501	0.460	0.154
	September	0.549	0.380	0.748
	October	0.210	0.209	0.220

Table 3

ANOVA results (*P* values) of NSC and its components starch, and soluble sugars vs. altitude at the tree level in *Pinus cembra* trees. Significant differences at $P \leq 0.05$ are in bold and italics

ANOVA effect	NSC	Starch	Soluble sugars
May	<i>< 0.001</i>	<i>0.004</i>	<i>< 0.001</i>
Jun	<i>0.016</i>	0.968	<i>0.027</i>
Jul	<i>0.010</i>	0.088	0.088
Sep	0.067	0.003	0.672
Oct	0.081	0.090	0.329