End of season carbon supply status of woody species near the treeline in western China

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Summary
As trees and shrubs approach the high elevation tree limit, it is often assumed that they fall short in photosynthate (source limitation). Alternatively, low temperature may restrict carbon investment (growth, sink limitation). The content of mobile non-structural carbohydrates (NSC) in tissues is considered a measure of the carbon source–sink balance. To test the source vs. sink limitation hypothesis, we compared late-season NSC concentrations of various woody taxa across altitudinal gradients from the subalpine forest to the treeline at the eastern edge of the Tibetan Plateau. Since we were interested in the generality of trends, we present “community” trends across four taxa, namely \textit{Quercus aquifolioides}, \textit{Abies faxoniana}, \textit{Rhododendron} \textit{fabri} subsp. \textit{prattii} and \textit{Sorbus rufopilosa}. NSC concentrations increased significantly with altitude in branch wood, current-year and last-year leaves, while there were no significant trends in stem sapwood and root xylem. The sugar to starch ratio was roughly 1:1 in branches and evergreen leaves, while stems and roots showed a higher starch fraction. Analyses of total nitrogen in leaves and wood tissues indicated no change in the trees’ nitrogen supply with elevation. The overall altitudinal trends of NSC in this group of woody plant species revealed no depletion of carbon reserves near the tree limit, suggesting that sink limitation predominates woody plant life across this treeline ecotone community.

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Introduction

The upper limit of many plants, woody taxa in particular, is often associated with a critically low temperature during the growing season. The most prominent of these range limits is the high elevation treeline, which had shown to follow a global isotherm of 6.7 ± 0.8 °C (Körner & Paulsen, 2004). Because of its predominant thermal limitation, the treeline ecotone is considered a sensitive early warning system of global change (Neilson, 1993). Despite its significance, the cause of treeline formation is still not fully resolved. In part this is due to the fact that regional explanations had been prioritized over global patterns. Körner (1998, 2003a, 2003b) summarized five categories of hypotheses which had been favored in the past: (1) climatic stress (Perkins & Adams, 1995), (2) disturbance (Grace & James, 1993), (3) reproduction (Smith, Germino, Hancock, & Johnson, 2003; Sveinbjörnsson, Kauhanen, & Nordell, 1996; Wardle, 1981), (4) carbon balance (Schulze, Mooney, & Dunn, 1967; Stevens & Fox, 1991) and (5) growth limitation (Körner, 1998; Tranquillini, 1979). Hypotheses (1)–(3) offer explanations in a variety of local and regional situations but cannot be applied across the world’s humid mountains. Given the global occurrence of the high elevation treelines, any common treeline theory needs to account for low-latitude, tropical treeline as well (Hoch & Körner, 2003). Only hypotheses (4) and (5), the thermal limitation of either carbon uptake (photosynthesis) or carbon investment (growth) can meet the requirement of limitation by a common isotherm.

It is still not fully resolved whether carbon source or sink limitation determines the treeline. For a long time, carbon limitation through a shortage of photo-assimilates was regarded as key for explaining the upper altitudinal treeline (Schulze et al., 1967; Stevens & Fox, 1991). Carbon constraints were used to model treeline positions in global climate change scenarios (Cairns & Malanson, 1998). However, in contrast to these assumptions, classical gas exchange studies have never yielded photosynthetic restrictions (Goldstein, Meinzer, & Rada, 1994; Tranquillini, 1979), while growth is reduced sharply as one approaches the tree limit (James, Grace, & Hoad, 1994; Paulsen, Weber, & Körner, 2000; Tranquillini, 1979). This has led to the assumption that the high elevation treeline ecotone is not dominated by carbon limitation, but is driven by a thermal limitation of structural growth (Dàniker, 1923; Körner, 1998, 2003a, 2003b). One way to test the actual balance between uptake and demand of carbon for growth is to assess the size of the non-structural mobile carbon pool in trees. It is assumed that a depletion of these reserves could indicate carbon shortage, whereas an increase of mobile carbon compounds would signal a surplus as conditions become adverse (Körner, 1998).

Earlier studies have used non-structural carbohydrates (NSC) to test for carbon source or sink limitation at the upper limit of tree growth (Hoch & Körner, 2003; Hoch, Popp, & Körner, 2002). In all these cases conifers were studied at and below the treeline, and there was not a single case in which mobile carbon reserves were diminishing with elevation. In most cases tissue NSC concentrations...
increased with altitude. Even drastic measures such as partial defoliation had only caused transitory reductions, and NSC rapidly recovered (Li, Hoch, & Körner, 2002). In an ongoing CO₂ enrichment experiment at the treeline (Handa, Körner, & Hättenschwiler, 2005), the evergreen pine revealed no growth stimulation during the first 3 years. However, the deciduous larch grew faster, and so far seems to profit from elevated CO₂, suggesting the possibility that these trees are not carbon saturated. It is not clear whether different leafing patterns hold the sole explanation. There may also be principal species-specific differences. The treeline carbon balance question needs broader testing, including a wide spectrum of plant functional types and leafing strategies sharing life conditions in the treeline ecotone. Such a broader test is the purpose of this field survey.

We hypothesize that all woody plants, no matter what their architecture is, trees or treelike shrubs, broadleaved or conifer species, are growth (sink) limited, i.e. they serve for pools of NSC, which increase with altitude. In this paper, we present a comparative study of the altitudinal variation of NSC concentrations in different species from subalpine forests to treeline ecotone including four species, two life forms and two leafing strategies (evergreen vs. deciduous). The samples were collected in the Wolong Nature Reserve, Sichuan province, China, in the eastern, humid edge of Tibetan Plateau. Our study contributes to the understanding of treeline formation in the eastern Tibetan plateau, part of the largest mountain system of the world.

Materials and methods

Site description

This study was conducted along altitudinal transects at the treeline ecotone on Balang Mountain, Wolong Natural Reserve (30°51'N, 102°57'E), Sichuan Province, on the eastern edge of Qinghai-Tibetan Plateau. The vegetation in the alpine treeline ecotone is dominated by Abies faxoniana (range 2500–3700m), treelike multi-stem shrubs such as Rhododendron fabri subsp. pratii and Sorbus rufopilosa and treelike sclerophyll oak shrub, Quercus aquifolioides (more abundant on sunny slopes; range 2700–3600m). The treeline (trees of at least 3 m height) is situated at 3650–3750 m a.s.l. (Qin, 1987; Shi, Li, & Wang, 2002). The Abies understory is dominated by Rhododendron, which can grow to a height of over 10 m (multi-stemmed), but forms krummholz-type shrub in the upper treeline ecotone. Sorbus forms small trees or multi-stemmed shrub ranging from subalpine coniferous forest to the upper treeline. In the treeline ecotone, Rhododendron and Sorbus are commonly reaching a height of ca. 5 m. Oak forms small multi-stemmed trees (5–10 m) below 3000 m and 2–3 m high thickets at higher elevations.

The growing season at treeline usually lasts from May to mid-October. Annual mean air temperature at treeline is around 3 °C, and the temperature of the warmest month (July) is ca. 11 °C according to extrapolations from the Wolong meteorological station at 2700 m a.s.l. Mean root zone temperature during growing season at treeline is ca. 7.1 °C according to a 1-year measurement in the near area at Zhegushan, Min Mountains (Körner & Paulsen, 2004). Annual rainfall is around 900 mm.

Tissue sampling and chemical analysis

We collected samples of oak on a SE slope from 3150 to 3600m, and samples of Abies, Rhododendron and Sorbus were collected on a NE slope from 3180 to 3740 m. The transects included upper montane forest sites in order to compare NSC concentrations from climatically optimal environments with the conditions at the upper range limit. Samples were collected at the end of growing season, on 14 and 15 October 2004. NSC signals were found to be temporally stable by the end of the growing season and thus are most suitable for altitudinal comparisons (Hoch et al., 2002). Sampled Abies trees had a stem diameter of 15–25 cm. The other three species were sampled irrespective of stem diameter due to their multi-stem growth form. At each elevation the following tissues were collected: current-year leaves, previous-season leaves, 3-year-old branches, xylem of root with diameters between 0.5 and 1 cm, and 0.5 cm diameter cores of stem sapwood of the outer 2.0 cm. The age of leaves and branches was judged from twig nodes. Taproot of sampling species was directly identified under the trunk and appropriate sizes of roots were gradually traced following taproot through digging out the roots. For each species and altitude, two to three similarly sized trees or shrubs were sampled. Samples of the same tissue were pooled within each altitude and species before chemical analyses. Bark was removed from root, branch and stem samples. All samples were dried within 6 h at 75 °C for ca. 48 h, ground to powder and stored in well-sealed plastic vials over silica gel until analysis.
All samples were analyzed for concentrations of NSC. NSC is defined here as the sum of free sugars (sucrose, glucose and fructose) and starch. NSC commonly covers more than 90% of mobile carbon in plants except for genera such as *Pinus* and *Tilia* known for their excessive lipid storage (Hoch & Körner, 2003). NSC concentrations were determined using an enzymatic digesting technique with a subsequent spectrophotometric glucose test as described in Hoch et al. (2002). Ca. 10 mg of powdered samples were extracted with 2 ml boiling, distilled water for 30 min. An aliquot of the extract was treated with invertase and isomerase to convert sucrose and fructose to glucose and analyzed for total glucose using a hexosekinase reaction kit (Sigma Diagnostics, St. Louis, MO, USA). Starch and sugar standards as well as a laboratory standard powder were used as controls for analysis (Hoch et al., 2002; Li et al., 2002). Carbohydrates other than starch, sucrose, fructose, and glucose are not included in this assay, but commonly contribute little to the total carbohydrate pool (Hoch & Körner, 2003). Sugar, starch, and total NSC were calculated on a dry matter basis (% d. m.). Nitrogen concentrations in leaves (including current-year and last-year) and branches were measured after combustion in a CHN-elementar analyzer (Vario EL III, Elemental Analysysteme, Hanau, Germany).

**Data analysis**

Means across altitudes of free sugars, starch and NSC were calculated to compare NSC and its components in different tissues and species. The altitudinal trends of NSC and nitrogen were calculated across all species for each tissue type, since we are interested in the common trend across taxa. All values of NSC and nitrogen were log-transformed prior to statistical analysis. Linear relationships between NSC and altitude, between nitrogen and altitude of different tissues were tested. JMP 3.2.2 (SAS Institute, Cary, NC, USA) was used for all statistical tests.

**Results**

**NSC in different tissue types**

Across sites/elevations tissue types differ in their NSC concentrations per unit of dry matter (Fig. 1). In current-year leaves, last-year leaves and branches, *Rhododendron* had the highest concentrations of sugar, starch and NSC of all species. In contrast, oak had the highest concentration of sugar, starch and NSC in roots. NSC concentrations in stems were similar across species (Fig. 1a). Generally, last-year leaves had the highest concentration of NSC while stems had the lowest (Fig. 1b). At the end of the growing season, *Quercus* has more sugar than starch in current-year leaves, last-year leaves and 3-year-old branches but lower sugar than starch concentrations in stems and roots. The other species had more starch than sugar in the measured tissues (Fig. 1a).

Combining the data for all species, the average sugar-to-starch ratio was nearly 1:1 in current-year leaves, last-year leaves and 3-year-old branches. But stems and roots had much higher starch than sugar concentrations; especially roots had the highest concentrations of starch ($p<0.001$).

**Altitudinal trends of NSC and nitrogen**

NSC concentrations (and with sugars and starch) either showed an increase with elevation (leaves

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**Figure 1.** Average concentration of sugar, starch, and NSC (sugar+starch) pooled across sites, separated by species and tissue type (a), and means over all species per tissue type (b). Different letters indicate significant differences at $p<0.05$ by Tukey–Kramer HSD test. Letters underlined denote for starch, non-underlined for sugar. Species: 1, *Quercus aquifolioides*; 2, *Abies faxoniana*; 3, *Rhododendron fabri subsp. prattii*; 4, *Sorbus rufopilosa*. Tissues: A, current-year leaves; B, last-year leaves; C, branches; D, roots; E, stems.
Figure 2. Altitudinal trends of sugar, starch, and NSC concentration in different tissues. Probabilities and correlation coefficients are for linear regressions of log-transformed NSC vs. altitudes, four species from subalpine forests to alpine treeline ecotone are combined for analysis. Squares, *Abies faxoniana*; triangles, *Quercus aquifolioides*; open circles, *Rhododendron fabri* subsp. *pratti*; stars, *Sorbus rufopilosa*.

Leaf data were calculated without *Sorbus*, because this species has only current-year leaves, and these were already starting to senescent by the time of sampling. Another reason is that *Sorbus* is known to contain a lot of sorbitol (substituting sucrose as transport form) and hence exhibited low

and branches) or did not change significantly (stems and roots) with elevation (Fig. 2). Because data were not normally distributed across taxa, we log-transformed NSC, starch and sugar numbers for statistical analysis, which yielded clearly linear trends with increasing elevation (Fig. 2).
free sugar concentration in our NSC-assay. Across the considered taxa, sugar, starch and NSC concentrations clearly increase with altitude in leaves and branches. There is no significant altitudinal change of NSC and its components in stems and root xylem. We additionally analyzed total nitrogen concentrations in leaves and branches, but found no definite altitudinal trend for any tissue (Fig. 3).

**Discussion**

By the end of the growing season, NSC concentrations of the different tissues examined indicate that life at the upper limit of these taxa is not associated with a shortage in carbohydrate reserves. Branches and leaves showed a clear increase in NSC from subalpine forest to treeline; in roots and stems trends are non-significant. Hence, based on the mobile carbon pool size, the data reject the carbon limitation hypothesis for these species.

Concentrations are generally lower in stem sapwood and roots, which in part is explained by the higher densities of these tissues (dilution effect). Overall, the concentrations of NSC and its components are very similar to what had been observed previously in such temperate zone taxa (Hoch et al., 2002). Roots of woody plants from cold regions typically contain higher concentrations of reserve carbohydrates than the stems and store more starch at the end of season (Hoch et al., 2002; Loescher, McCarmant, & Keller, 1990; Pregitzer, 2003).

Across the treeline ecotone, there is not only a reduction of growth, but commonly there is also an increase in wood density (Däniker, 1923; Tranquillini, 1979). We did not measure tissue density for the current study, but NSC on a dry matter basis undoubtedly underestimated the NSC concentrations of woody tissues at higher altitudes on a volume basis (thicker cell walls and smaller cell volumes at higher altitudes). Likewise, leaves of trees generally become more scleromorphic with increasing altitude and show lower specific leaf area (SLA) at higher altitudes (Körner, 2003a, 2003b). Consequently, the increasing altitudinal NSC trends in leaves and young branches on a dry matter basis would become even steeper if corrected for such density effects (Hoch et al., 2002). In addition, sampling of roots (diameter <5 mm) and stems (outer 2 cm from phloem) inevitably mixed xylems of different ages. Differently aged tissues differ in NSC concentrations (Li, Hoch, & Körner, 2001), which will add to variability.

The total NSC pool may also be affected by allometric trends in the whole plant. Alpine woody taxa with stunted or krummholz stature have higher leaf mass fraction in contrast to lower elevation populations (Bernoulli & Körner, 1999). Our own measurements in Abies and Rhododendron in the Gongga Mountains also show a significant increase of leaf mass fraction (P. Shi, unpublished data). High concentrations of NSC in leaves as found here and high leaf mass fraction contribute to an even greater increase of the mobile carbon pool as one approaches treeline.

The present results match gas exchange and stable carbon isotope data, which generally support the view that photosynthetic rates are high and...
unlikely limiting plant growth at high altitudes (Körner & Diemer, 1994; Körner, Farquhar, & Wong, 1991; Mooney, Strain, & West, 1966; Terashima, Masuzawa, Ohba, & Yokoi, 1999; Tranquillini, Ko¨rner & Diemer, 1994; Ko¨rner, Farquhar, & Wong, 1991; Terashima, Masuzawa, Ohba, & Yokoi, 1999; Tranquillini, Ko¨rner & Diemer, 1994). Direct measurements of respiration at the treeline never revealed any problems of tree carbon balance (Wieser, 1997; Wieser & Bahn, 2004). Whole winter respiration, for example, represents a very small carbon loss in treeline conifers, and it is balanced by the photosynthetic assimilation of only one or two warm days during the growing season when air temperature exceed 6 °C (Wieser, 1997). In addition, the stunted morphology of alpine trees (Bernoulli & Körner, 1999) or krummholz shrubs (Shi, unpublished data) with their higher ratio of leaves will add to the positive carbon balance at the treeline.

The weak altitudinal trends of total nitrogen in leaves and sapwood indicate similar nitrogen supply across the whole treeline ecotone, suggesting that nitrogen availability does not constrain sink activity at higher altitudes. Moreover, treelines occur world-wide at similar seasonal mean temperatures between 5 and 7 °C (Körner, 1998), across rather different soil types with different nutrient availability. So insufficient nutrition (reduced nutrient uptake in cold soils) does not seem likely to limit tree growth at treelines at a global scale.

In conclusion, our findings do not support the hypothesis of a photo-assimilate driven limitation of growth at treeline. In line with previous altitudinal trends of an increase of NSC at different latitudes from tropical Mexico to Arctic Europe (Hoch & Körner, 2003), it could be shown here that neither an increased altitude nor a shorter growing season would cause a depletion in photo-assimilates. Li et al. (2002) reported that even massive perturbations such as drastic defoliation and pruning did never completely deplete carbon reserves in treeline pines, and NSC stores were partially recovered by the end of growing season, which also suggests ample provision with assimilates of treeline trees. In the past years of NSC studies, including the present one, there was no case of carbon reserve depletion at treeline. It thus seems that sink limitation predominates performance of woody plants at treeline. A mechanistic explanation for the sink limitation of tree growth at treeline, however, is still to be found.

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