

Mobile carbon supply in trees and shrubs at the alpine treeline ecotone

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Abstract Although the growth limitation hypothesis (GLH) is the most accepted physiological explanation for alpine treeline formation, the debate about its formation mechanisms still remains controversial due to contradictory findings from different studies. The functional difference between trees and alpine low-stature shrubs may hold answers, as shrubs grow well at higher elevations. We investigated carbon (C) storage in deciduous treeline species *Betula ermanii* (Erman's birch) and two dominant shrubs (deciduous

Vaccinium uliginosum and evergreen *Rhododendron aureum*), which naturally grow next to each other at the treeline ecotone on Changbai Mountain, Northeast China. We determined growth and non-structural carbohydrate (NSC) concentrations in organs with increasing elevation at the mid-growing season. Results showed that in the treeline ecotone soil temperature was lower than tree canopy air temperature due to unobvious aerodynamic decoupling near the ground. Species growth consistently decreased with increasing elevation, while NSC concentrations responded differently to elevation between trees and shrubs. An elevational increase and decrease in NSC were observed in leaves and woody organs, respectively, of *B. ermanii*. NSC concentrations in each organ significantly increased with increasing elevation

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for *R. aureum* but decreased for *V. uliginosum*. At the treeline, shrubs had higher values than *B. ermanii* in NSC, ratios of soluble sugars to starch in leaves, and leaf mass per area. Organ dependence of NSC with increasing elevation in *Betula* trees provided partial support for the GLH, while *R. aureum* and *V. uliginosum* provided strong support for the GLH and carbon limitation hypothesis, respectively. These imply that alpine shrubs may have evolved to maintain more advantageous C balance and functional features than did trees as an adaptation to higher-elevation climates.

Keywords Growth limitation · Carbon balance · Microenvironments · Stature · Deciduous treeline species · Functional difference

Introduction

The alpine treeline, one of the most conspicuous ecological boundaries, is generally accepted as a sensitive warning system of climate change. Although scientists have discussed the formation mechanisms of alpine treelines for more than a century, there is still no convincing conclusion (Körner 2003; Tranquillini 1979). At the global scale, low temperature has generally been considered to physiologically control alpine treelines (Jobbágy and Jackson 2000; Körner 1998). Körner and Paulsen (2004) found that alpine treelines globally follow a thermal isotherm of 6.7 ± 0.8 °C of mean root-zone temperature during the growing season. Two alternative hypotheses have been proposed as the general functional mechanisms of alpine treeline formation: (1) the carbon limitation hypothesis (CLH) and (2) the growth limitation hypothesis (GLH). The former predicts that trees may suffer from insufficient C supply due to a shortage of photoassimilates induced by low temperatures (Stevens and Fox 1991; Wardle 1993), while the latter assumes a direct low-temperature-induced inhibition of organ formation at otherwise sufficient C acquisition (Körner 1998).

The size of non-structural carbohydrates (NSC = soluble sugars + starch) pools in trees growing at the treeline may provide an answer, since it reflects the balance of C gain (e.g., photosynthesis) and C usage (e.g., respiration, metabolism, cell

division) (Körner 2003). It is assumed that NSC in all organs decreasing with elevation would support the CLH, and the reverse trend would support the GLH. The functional explanation for the GLH is that meristematic activity is much more sensitive to low temperature than is photosynthesis. Indeed, there is broad evidence that plant organs cannot be built at approximately 0 °C; growth activity (including cambial and apical meristems) is interrupted at 5 °C, while leaf light-saturated photosynthesis still reaches 50–70% of its full capacity at 5 °C (James et al. 1994; Körner 2008; Solfeld and Johnsen 2006). There is also empirical evidence for temperature limitations in organ formation (Rossi et al. 2007). Consequently, NSC concentrations increase in response to lower temperatures (Hoch and Körner 2009), suggesting that plant growth is not limited by C supply.

There is still no conclusive evidence that treeline trees are growth limited or C-limited. Most previous studies found no NSC depletion at the upper elevational limits for stands generally composed of evergreen tree species, suggesting that the GLH is the mechanism of treeline formation (Dang et al. 2015; Fajardo et al. 2013; Hoch and Körner 2003, 2012; Hoch et al. 2002; Körner 2003; Piper et al. 2016; Shi et al. 2006, 2008). On the other hand, the CLH has been observed in a growing number of studies (Dawes et al. 2011; Genet et al. 2011; Handa et al. 2005; Li et al. 2008b; Sveinbjörnsson et al. 2010), most of which are from deciduous species, e.g., *Nothofagus pumilio* at southern Andes treelines (Fajardo and Piper 2014, 2017). It may be ascribed to leaf habit that leaves of deciduous species are generally less robust with a lower leaf mass per area (LMA) than those of evergreen species, and are more prone to mechanical damage (e.g., wind and frost) (Körner 2003), consequently resulting in C limitations. These studies suggest that deciduous treeline species may function differently from evergreen species regarding C dynamics. However, C balance and reserves in deciduous tree species are still less understood, because much of what we know comes from evergreen species that most commonly form alpine treelines (Fajardo and Piper 2017).

Tree architecture (stature) and its related aerodynamics have also been proposed to explain why trees are more strongly influenced by low temperature at high elevations than low-stature plants (Körner 2012b; Li and Yang 2004). Upright trees are aerodynamically

more closely coupled to the free atmosphere, which enforces convective heat exchange even during favorable periods of the growing season (Körner 2012b), whereas low-stature plants benefit from a warmer microenvironment due to aerodynamic decoupling near the ground (Körner 2007). Additionally, tree self-shading in upright plants can result in colder root temperatures than that of low-stature plants, which constrains root growth (Alvarez-Uria and Körner 2007). According to the GLH, NSC concentrations would be higher in plants which are growing in a colder habitat. For example, a simulation experiment at the treeline region has compared C storage in seedlings of deciduous *N. pumilio* and evergreen *Pinus contorta* trees at tree canopy height (2 m) and ground level; the results showed a decrease in NSC in the former species, but the latter was not observed, supporting the CLH (Fajardo and Piper 2014). Although this study suggests that treeline-forming mechanisms may be taxon dependent, its results may be confounded by root-zone temperature fluctuations. This limitation could be avoided by studying sympatric pairs of trees and shrubs naturally growing close to each other in treeline ecotones for two reasons: (1) the woody stem of trees is neither a burden to their C balance nor does it increase the risk of freezing damage (Körner 2012a; Sakai and Larcher 1987), and (2) the physiological means of trees growing in cold climates are similar to those of shrubs, except for their stature and associated aerodynamics (Körner 2003, 2012b). However, very few studies have evaluated the difference in C storage among species of different growth forms near the treeline (Shi et al. 2008).

NSC concentrations are highly dependent on sampling time and organ types. The best sampling time should be when growth is most active (mid-summer) (Piper et al. 2016); however, previous studies generally have sampled at the end of the growing season when NSC concentration would be stable (Hoch and Körner 2012; Shi et al. 2008). The use of NSC concentrations to assess growth or carbon limitations should focus on newly developing organs (e.g., leaves), where meristematic activity is actively occurring (Piper et al. 2016); however, previous studies frequently chose to sample major storage organs (e.g., stems) (Fajardo et al. 2011, 2012; Hoch and Körner 2003; Lenz et al. 2014). Therefore, the sampling

organs and time may be crucial for evaluating carbon and growth limitations.

The present study aimed to test the C limitation and growth limitation hypotheses in deciduous tree species and to understand whether C stores of trees and low-stature shrubs responded differently to increase in elevation. To accomplish our objectives, we selected a common deciduous broadleaf treeline species *Betula ermanii* (Erman's birch) and two dominant shrubs with different leaf habits (deciduous *Vaccinium uliginosum* and evergreen *Rhododendron aureum*) that naturally grow next to each other in the treeline ecotone on Changbai Mountain, Northeast China. We monitored canopy air and soil temperature at the treeline. We sampled and determined NSC concentrations in their organs (leaves, branches, and roots) along an elevational gradient (from the timberline to the tree species line) in the middle of the growing season. Stems were not included, because the two shrubs displayed with intense branch bifurcation and no clear main stem. Assuming that aerodynamic decoupling near the ground occurs on Changbai Mountain as observed in other regions, we expected that soil temperature during the growing season would be warmer than tree canopy air temperature. We hypothesized that NSC elevational variation would occur in *B. ermanii* trees that couple lower air temperatures, but not in low-stature shrubs that supposedly decouple from air temperature. Specifically, in the context of the CLH, NSC concentrations in trees were expected to decrease with elevation, since deciduous species tend to be more susceptible to high-elevation mechanical damage, which may reduce C gain by reducing leaf area; in contrast, according to the GLH, NSC in trees increased or varied slightly with increasing elevation as predicted in the general evergreen species; furthermore, trees would have higher NSC concentrations than shrubs because they couple lower air temperatures. On the other hand, we hypothesized that NSC elevational variation would occur in both trees and low-stature shrubs, if there was no aerodynamic decoupling due to local multiple stressors, since our previous study found that local factors might also drive the current position of *B. ermanii* treeline (Yu et al. 2014).

Materials and methods

Study site

The present study was conducted on the northern slope of the Changbai Mountain Natural Reserve (41°3′–42°28′N, 127°9′–128°55′E), Jilin Province, Northeast China. This area thus far has not been affected by anthropogenic activity (Yu et al. 2007). *Betula ermanii* (Erman's birch), a common deciduous broad-leaf tree species, forms a pure forest stand above 1700 m above sea level (a.s.l.), with a treeline at 2018 a.s.l. on Changbai Mountain. The climatic treeline position was predicted at 2100 m a.s.l. (Xu and Lin 1981). Low-stature shrub species are limited at the treeline sites. The exception is the deciduous *Vaccinium uliginosum* (10–15 cm) and evergreen *Rhododendron aureum* (10–30 cm), which dominate the treeline ecotone with distinct difference in habitats. *V. uliginosum* is multibranched and mainly concentrated across the unshaded area in and above the treeline ecotone (1950–2300 m a.s.l.) because of its strong resistance to low temperature, drought, and infertility but not to shade. *R. aureum* has low lying, cushion-like branching pattern and generally grows under *Betula* trees and in microtopographical areas with shelter in the alpine tundra (1700–2300 m a.s.l.); this species favors cool and moist climates but is not tolerant to full sunlight or drought.

The climate at the treeline is characterized by severe cold, high humidity, and strong wind (Yu et al. 2014). The mean annual temperature varies from –2.3 to –3.8 °C. The duration of the frost-free period is approximately 65–70 days. Annual precipitation ranges from 1000 to 1100 mm, most of which occurs from June to September. Annual relative humidity is approximately 75%, with a long duration of fog of more than 260 days. Annual wind speed ranges from 6 to 10 m s⁻¹, and the duration of gales can even exceed 200 days.

Sampling and chemical analysis

Variations in mobile carbohydrates was investigated in three targeted species along an elevational gradient of 1976, 2018, and 2060 m a.s.l. encompassing timberlines, treelines, and tree species lines, respectively. The characteristics of each site are shown in Table 1. The sampling was performed on a sunny day

(August 3, 2011) in the middle of the growing season, referring to previous studies claiming the best sampling time to examine C balance should be when growth is most active (Piper et al. 2016). Also, the difference in weather condition along study sites was minimal on the clear summer day. At each elevation, we established a 30-m-long and 20-m-wide sample strip. We randomly selected dominant individuals for growth investigation, 15 trees and 10 shrubs with a 10-m and 2-m distance between each other, respectively. We measured individual height, diameter at the breast height for trees, and diameter at the ground for shrubs. Annual shoot increments (1–3 years old) were determined according to their twig nodes, as described in previous studies (Liu et al. 2009; Yu et al. 2014). Five of 15 individuals were randomly selected for sampling. The sampling organ included current-year mature leaves, two-year-old branches, and 0.5–1-cm-diameter roots for each species. Leaves were sampled from sun-exposed branches in the canopy to avoid the effect of age on NSC (Li et al. 2001). For branch and root samples, bark and phloem were removed with a knife, because they account for less of a tree's NSC-stock (Piper et al. 2016). Stems were not included, because shrubs had no clear main stems with too much branch bifurcation, and for trees NSC concentrations were generally lower in stems than in the other organs (Hoch et al. 2002; Martínez-Vilalta et al. 2016).

Samples were collected at approximately noon to minimize the diurnal fluctuations between samples, stored in a cool box in the field, and heated in a microwave oven for 40 s at 600 W within 6 h after sampling to denature enzymes. After transportation to laboratory, one-third of organ samples ($n = 5$) were dried to constant mass at 70 °C (ca. 48 h), ground to a fine powder, and then stored over silica gel at 4 °C. The other two-thirds of samples ($n = 10$) were used to determine leaf mass per area (LMA, g m⁻²). Leaf area was measured with a leaf area meter (CI-203, CID, USA).

Non-structural carbohydrates (NSC) were defined as the sum of starch and total soluble sugars. Concentrations of starch and total soluble sugars were measured using an enzymatic digesting technique with a subsequent spectrophotometric glucose test; more details are described elsewhere (Li et al. 2008b). Final concentrations of sample were calculated on a dry matter basis (% d.m.). Moreover, interspecific difference was of great interest for the present study. Leaf NSC concentrations were also calculated on a leaf area

Table 1 Characteristics of the sites and dominant species across the treeline ecotone on Changbai Mountain, Northeast China

| Variable | Timberline | Treeline | Tree species line |
|---|--------------|---------------|-------------------|
| Elevation (m a.s.l.) | 1976 | 2018 | 2060 |
| Aspect (°) | N117 | N236 | N260 |
| Slope (°) | 22 | 20 | 23 |
| Mean temperature during the whole year/the growing season (°C)* | | | |
| Scattered tree canopy | NA | – 2.3/9.9 | NA |
| Soil under scattered trees | NA | 3.1/9.0 | NA |
| Soil in the open place | NA | – 0.71/8.0 | NA |
| Diameter (cm)** | | | |
| <i>Betula ermanii</i> | 7.2 (0.46)a | 6.0 (0.41)ab | 4.6 (0.31)b |
| <i>Vaccinium uliginosum</i> | 0.35 (0.03)a | 0.33 (0.02)ab | 0.25 (0.02)b |
| <i>Rhododendron aureum</i> | 0.58 (0.04)a | 0.55 (0.03)a | 0.43 (0.03)b |
| Height (m) | | | |
| <i>Betula ermanii</i> | 6.5 (0.54)a | 4.5 (0.23)a | 1.3 (0.10)b |
| <i>Vaccinium uliginosum</i> | 0.26 (0.01)a | 0.17 (0.01)b | 0.11 (0.00)c |
| <i>Rhododendron aureum</i> | 0.45 (0.03)a | 0.26 (0.01)b | 0.17 (0.02)c |

Growth variables are shown as the means (s.e.), $n = 15$ for *Betula ermanii*, and $n = 10$ for *Vaccinium uliginosum* and *Rhododendron aureum*. Different letters indicate statistically significant differences ($p < 0.05$) in growth among elevations, Tukey's multiple range test

*NA indicates no temperature data at timberline and tree species line

**Diameter at the breast height for trees, and at the ground for shrubs

basis (g m^{-2}), because LMA, as an important index of species ecological strategies, generally responds to changes in environments, while wood density does slightly (Fajardo 2016).

The ratio of soluble sugars to starch (SSR) was also calculated. Soluble sugars provide immediate response (e.g., osmoregulation) against stressors (Sala et al. 2012), while starch generally acts as a reservoir function for storage and future use (Hartmann and Trumbore 2016). It is expected that a critical threshold in plant organs may be maintained near the upper elevational limits (Li et al. 2008b).

Temperature records

Canopy air and soil temperatures at the three sites were monitored using microclimatic loggers (– 30 to + 50 °C, HOBO H8 Pro Temperature Logger, USA). According to previous protocols (Körner and Paulsen 2004), two loggers were placed in the soil at a depth of 10 cm beneath trees under full canopy shade and under *V. uliginosum* vegetation, and another one was placed 2 m above the ground and avoiding direct sunlight. Both air and soil temperatures were monitored at

30-min intervals from January 1 to December 31, 2011. The beginning of the growing season was defined as the date at which the daily mean soil temperature at a – 10 cm depth first exceeded 3.2 °C, and the end of the growing season was defined as the date at which the daily mean soil temperatures first dropped below 3.2 °C accordingly (Körner and Paulsen 2004).

Data analysis

One-way ANOVA was used to assess the effect of elevation on height and diameter. Three-way ANOVA was used to assess the effects of elevation and age on annual shoot length, and the effects of elevation and species on NSC concentrations including soluble sugars and starch, the sugar/starch ratio (SSR), and LMA. The data were first tested for normality with the Kolmogorov–Smirnov test and log-transformed to meet the requirements of a normal distribution. Significant differences among elevations and species were assessed according to Tukey's multiple range test ($p < 0.05$). All statistical tests were performed using R 3.0.2.

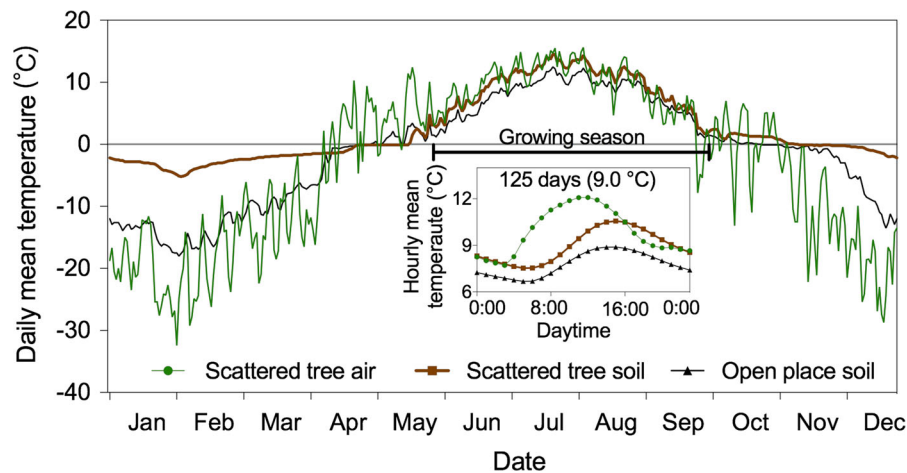


Fig. 1 Mean air and soil temperature at the treeline site. Daily mean canopy air temperature (2 m) and soil temperature (10 cm depth) at the scattered tree and open site at the treeline on Changbai Mountain. The data were collected from January 1 to December 31, 2011. The inset represents the mean hourly

temperature throughout the whole growing season. Green thin line with circle is scattered tree air temperature; brown bold line with square is scattered tree soil temperature; black intermediate line with triangle is open place soil temperature

Results

Temperature

At the *Betula* treeline on Changbai Mountain, the growing season length was 125 days from May 28 to September 30, 2011 (Fig. 1). The mean 10-cm soil temperature in the scattered tree area was 9.0 °C during the growing season and 3.1 °C during the whole year. The maximum and minimum daily mean soil temperatures were 14.5 °C (July 26) and − 5.2 °C (February 3), respectively. The period of soil temperature at approximately 0 °C indicates the days of snow cover. The canopy air temperature varied dramatically, with daily means of 9.9 and − 2.3 °C during the growing season and the whole year, respectively (Table 1; Fig. 1). The maximum and minimum daily mean air temperatures were 15.6 °C (August 8) and − 32.4 °C (February 1), respectively. The daily mean 10-cm soil temperature at the open site with *Vaccinium* vegetation (8.0 °C) followed a similar pattern as that of air temperature but with 1.9 K lower values during the growing season. The canopy air temperature was warmer than the scattered tree soil temperature from 5:00 to 15:00 but colder during the remaining hours, while soil temperature in the open was colder over the whole day.

Growth

Diameters and height of trees and shrubs significantly decreased with increasing elevation (Table 1). Annual shoot length of all species also declined dramatically with increasing elevation over the previous three years ($p < 0.05$; Fig. 2); however, it was not affected by age ($p > 0.1$). On average, shoot length decreased 29.4, 26.6, and 68.4% from the lowest to the highest elevation for *B. ermanii*, *V. uliginosum*, and *R. aureum*, respectively.

Interspecific variation in NSC

NSC concentrations varied significantly depending on species, organs, and their interaction (Table 2). The use of a leaf area basis was clearer in demonstrating interspecific difference in carbon storage (Fig. 3a), compared with the use of a dry matter basis (Fig. S1), since the former was closely related to plant light capture and carbon gain. At the lowest site, the highest NSC concentrations were observed in *V. uliginosum*. At the treeline site, NSC were lower in leaves of *B. ermanii* (12.6 g m⁻²) than those of *V. uliginosum* (17.5 g m⁻²) and *R. aureum* (21.2 g m⁻²) ($p < 0.05$), while NSC in woody organs were similar among species (Fig. 3). At the highest elevation site, *Betula* trees had consistently lower NSC than two shrubs in all organs. According to the GLH, these indicate that

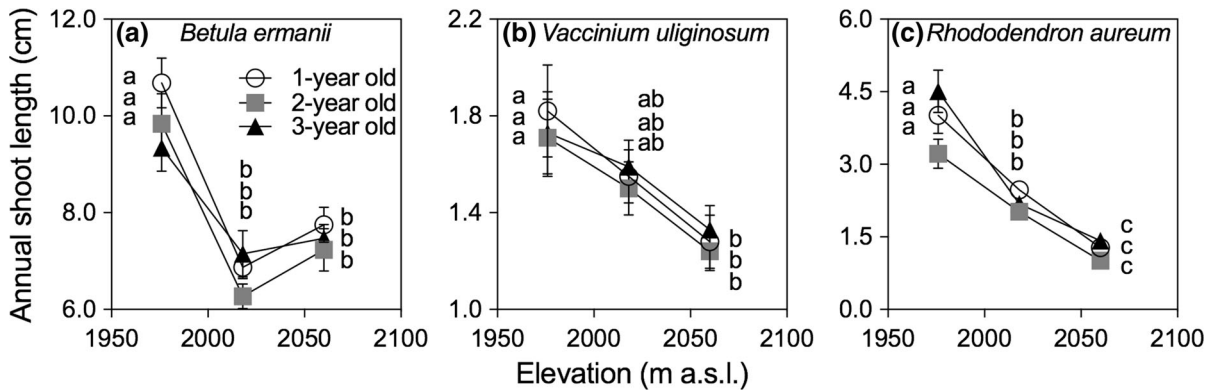


Fig. 2 Elevational variations in annual shoot length along elevation on Changbai Mountain. **a** *Betula ermanii*; **b** *Vaccinium uliginosum*; **c** *Rhododendron aureum*. Open circle, 1-year-old shoot; gray square, 2-year-old shoot; closed triangle, 3-year-old shoot. For the three species, no significant differences

($p < 0.05$) in annual shoot length were not detected among growth age, but were detected among elevation (Tukey’s multiple range test), shown in different letters (top to bottom, 1- to 3-year-old shoot). Bars represent means ($n = 10$) and vertical bars represent standard errors

Table 2 Three-way ANOVA of the effects of elevation, species, and organ on the concentrations of non-structural carbohydrates (NSC), soluble sugars, and starch and the ratio

of soluble sugars to starch (SSR) across the treeline ecotone on Changbai Mountain, Northeast China

| Factors | DF | NSC | | Soluble sugars | | Starch | | SSR | |
|---------------|----|-------|----------|----------------|----------|--------|----------|--------|----------|
| | | F | P | F | P | F | P | F | P |
| Elevation (E) | 2 | 52.35 | < 0.0001 | 67.38 | < 0.0001 | 15.61 | < 0.0001 | 0.07 | 0.9357 |
| Species (S) | 2 | 74.92 | < 0.0001 | 58.28 | < 0.0001 | 33.38 | < 0.0001 | 26.99 | < 0.0001 |
| Organ (O) | 2 | 933.5 | < 0.0001 | 2221.38 | < 0.0001 | 26.9 | < 0.0001 | 435.58 | < 0.0001 |
| E × S | 4 | 66.96 | < 0.0001 | 88.42 | < 0.0001 | 5.81 | 0.0003 | 3.24 | 0.0149 |
| E × O | 4 | 18.29 | < 0.0001 | 5.82 | 0.0003 | 18.39 | < 0.0001 | 4.94 | 0.0011 |
| S × O | 4 | 8.96 | < 0.0001 | 6.63 | < 0.0001 | 32.65 | < 0.0001 | 37.23 | < 0.0001 |
| E × S × O | 8 | 8.16 | < 0.0001 | 15.87 | < 0.0001 | 3.36 | 0.0018 | 2.19 | 0.0336 |

shrubs responded to the treeline habitat more than trees.

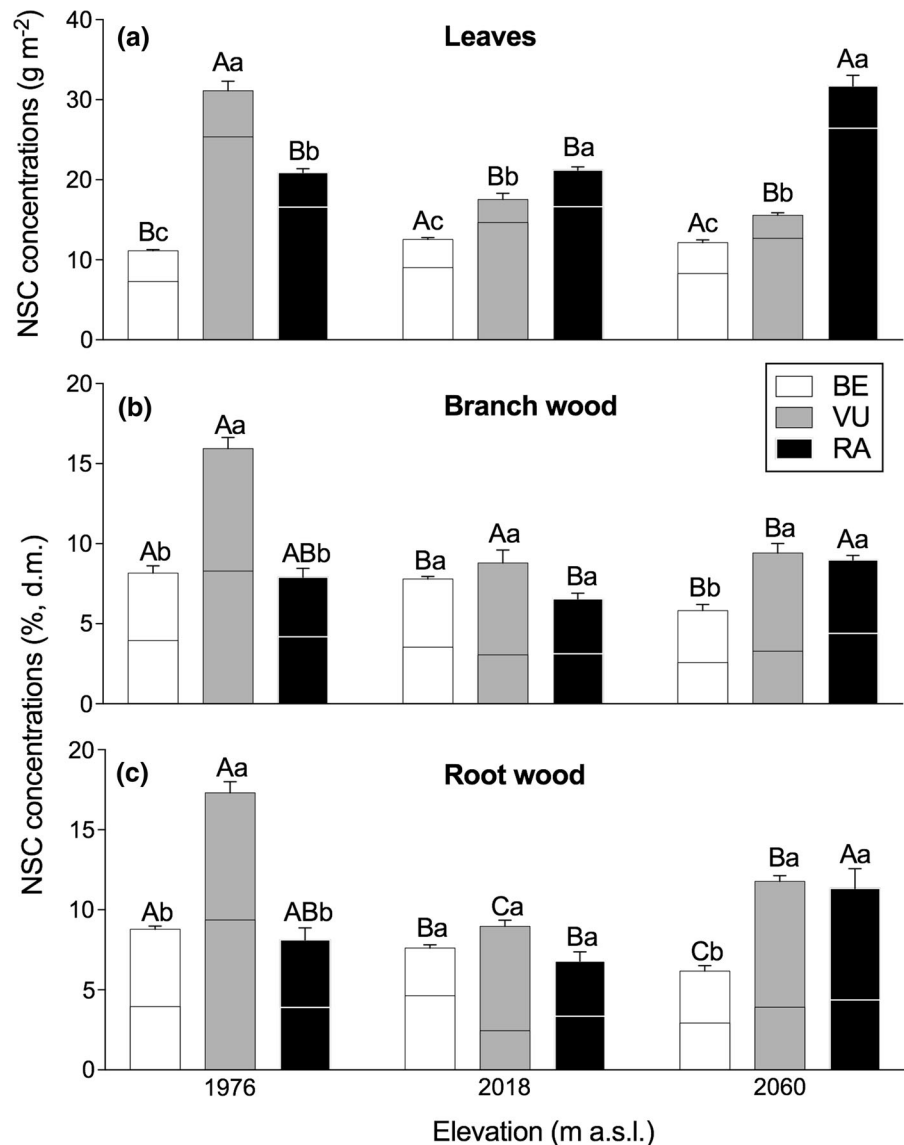
Elevational variation in NSC

NSC concentrations were significantly affected by elevation and species (Table 2). The significant interaction between elevation and species suggests that NSC elevational trends differed among species ($p < 0.0001$). At the highest elevation site, NSC significantly increased in leaves but decreased in woody organs for *B. ermanii*, and markedly decreased and increased in all organs for *V. uliginosum* and *R. aureum*, respectively (Fig. 3).

Ratio of soluble sugars to starch (SSR)

The SSR varied significantly among species, organs, and their interaction (Table 2). The SSR was not affected by elevation ($p > 0.05$), but only by the interactions related to elevation. With increasing elevation, leaf SSR tended to decrease in *B. ermanii*, and to increase in *R. aureum*, while it was nearly constant in *V. uliginosum*. The mean SSR of *B. ermanii* was much lower than that of *V. uliginosum* and *R. aureum*, particularly at the highest elevation site, with values 1.6, 5.0, and 5.1, respectively, which may represent a critical threshold (Table 3). On the other hand, SSR was relatively low in woody organs; it varied slightly with increasing elevation in *B. ermanii*

Fig. 3 Comparisons of non-structural carbohydrates (NSC) concentrations in organs among species and among elevations on Changbai Mountain. **a** Leaves; **b** branch wood; **c** root wood. Open, BE, *Betula ermanii*; gray, VU, *Vaccinium uliginosum*; dark, RA, *Rhododendron aureum*. NSC concentrations of leaves and woody organs were calculated on a leaf area basis (g m^{-2}) and dry matter basis (% d.m.), respectively. Different letters indicate significant differences ($p < 0.05$) in the NSC concentrations among elevations (uppercase) and among species (lowercase) at each elevation, determined by Tukey's multiple range test. In each bar, horizontal lines delimit starch (upper portion) and soluble sugars (lower portion). Vertical bars represent standard errors. Bars represent means ($n = 5$) and vertical bars represent standard errors



(except for roots at the treeline) and *R. aureum*, but decreased in *V. uliginosum*.

In addition, leaf mass area (LMA) had similar variation among elevations ($p > 0.05$), but significant difference among species ($p < 0.05$) (Fig. 4). The mean LMA of *B. ermanii* was 61.1 g m^{-2} , which was much lower than that of *V. uliginosum* (105.6 g m^{-2}) and that of *R. aureum* (129.2 g m^{-2}), indicating interspecific differences in stress adaptation.

Discussion

Difference in temperature between the canopy air and soil at the treeline

The present study showed that the mean soil temperature during the growing season was lower than the canopy air temperature at the treeline site (Fig. 1), in agreement with McIntire et al. (2016). It was the inverse of the expectation that temperature near the ground was generally warmer due to aerodynamic decoupling from free-air temperatures (Körner 2007). Such disappearance may be caused by multiple

Table 3 Mean ratios (s.e.) of total soluble sugars to starch (SSR) for three treeline species at different elevations in natural treeline ecotones ($n = 5$)

| Species | Leaves | Branch wood | Root wood |
|-----------------------------|-------------|-------------|------------|
| <i>Betula ermanii</i> | | | |
| 1976 m | 1.9 (0.1)ab | 1.0 (0.1)a | 0.8 (0.1)b |
| 2018 m | 2.6 (0.2)a | 0.8 (0.1)a | 1.6 (0.1)a |
| 2060 m | 1.6 (0.4)b | 0.8 (0.1)a | 0.9 (0.1)b |
| <i>Vaccinium uliginosum</i> | | | |
| 1976 m | 4.6 (0.6)a | 1.1 (0.1)a | 1.2 (0.1)a |
| 2018 m | 5.1 (0.2)a | 0.6 (0.1)b | 0.4 (0.0)b |
| 2060 m | 5.0 (0.8)a | 0.6 (0.1)b | 0.5 (0.0)b |
| <i>Rhododendron aureum</i> | | | |
| 1976 m | 3.9 (0.3)ab | 1.2 (0.1)a | 1.0 (0.1)a |
| 2018 m | 3.7 (0.2)b | 0.9 (0.1)a | 1.1 (0.2)a |
| 2060 m | 5.1 (0.4)a | 1.0 (0.1)a | 0.7 (0.1)a |

Different letters indicate significant differences ($p < 0.05$) among elevations, determined by Tukey's multiple range test

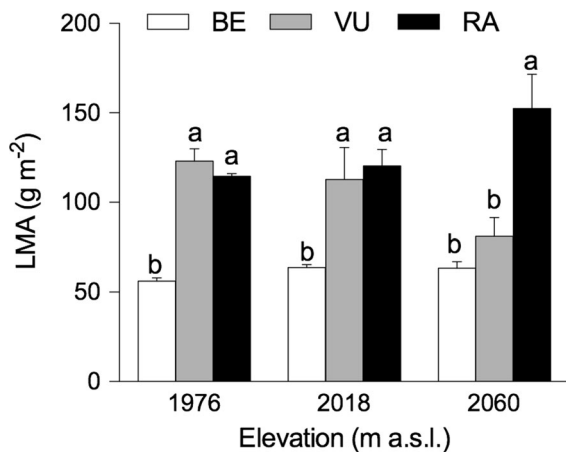


Fig. 4 Comparisons of leaf area mass (LMA) of species along elevation on Changbai Mountain. Significant differences ($p < 0.05$) in LMA were not detected among elevations but were detected among species at each elevation (Tukey's multiple range test), shown in lowercase letters. Open is BE, *Betula ermanii*; gray is VU, *Vaccinium uliginosum*; dark is RA, *Rhododendron aureum*. Bars represent means ($n = 5$) and vertical bars represent standard errors

simultaneous stressors (McIntire et al. 2016). Wind has been shown as the main factor affecting the local microclimates by influencing the aerodynamic boundary layer and thus indirectly affecting convective heat loss and evaporative cooling (Körner 2003). Indeed,

strong wind is one of most salient features on Mt. Changbai and the duration of gales even longer than 200 days per year (Yu et al. 2014), which may result in a colder soil particularly in open areas (*V. uliginosum*, 1.9 K colder). Furthermore, alpine trees as a shelter could also lead to warmer ground temperature (Bader et al. 2007). However, a shelter effect appeared only in the afternoon (after 15:00) on Changbai Mountain (Fig. 1). The lag response may be ascribed to relatively low density of *Betula* trees and simultaneous local climates, e.g., frequent clouds and precipitation (annually from 1000 to 1100 mm, mostly occurring in the growing season). Nevertheless, the mean soil temperature (9.0 °C) during the growing season was still higher than that found on average at worldwide treelines (6.7 °C) (Körner and Paulsen 2004). It implies that the current treeline position on Mt. Changbai does not reach the elevation of its climatic treeline, perhaps driven by both low temperature and other factors (e.g., water stress), as proposed by Yu et al. (2014).

Difference in NSC elevational trends between trees and shrubs

NSC concentrations in both trees and shrubs significantly responded to elevation, as indicated by the significant interaction between species and elevation (Table 2, Fig. 3). For *Betula* trees, NSC concentrations considerably increased in leaves (Fig. 3a), as observed in most of evergreen species (e.g., Hoch et al. 2012; Li et al. 2008); meanwhile, tree growth including height, diameter, and annual shoot increments consistently decrease with increasing elevation (Table 1; Fig. 2a). According to the C and growth limitation framework, these results indicate that C supply via photosynthesis in *B. ermanii* may exceed demands for growth at high elevation, suggesting there is no depletion in C storage. This is consistent with the previous findings on deciduous species, e.g., *B. platyphylla* (Shi et al. 2008), *N. pumilio* (Fajardo et al. 2013), and *B. ermanii* (Yu et al. 2014, Wang et al. 2018). On the other hand, NSC elevational decrease in branches and roots seem not to support the GLH (Fig. 3b, c), which predicts NSC concentrations should decrease with increasing elevation in all organs (Körner 2003).

The organ-dependent variation in relation to elevation may be ascribed to the foliar habit and organ

functions of deciduous species, which generally have higher C requirements than evergreens to replace leaf loss when facing stress (Hoch et al. 2003). In the treeline ecotone, multiple abiotic stresses may inversely induce trees to invest more C in defense and maintenance rather than storage (Kozlowski 1992; Wiley and Helliker 2012). In particular, *B. ermanii* trees generally produce very thin bark and are more susceptible to mechanical damage, and even exhibit krummholz-type growth patterns at sites exposed to strong wind. Moreover, alpine stress factors may also affect any period of C transport (e.g., phloem loading and unloading) (Lemoine et al. 2013); for example, Yu et al. (2014) found that water stress together with low temperature might slow the long-distance transport of photoassimilates in *B. ermanii* trees. Such high demand for C potentially leads to C starvation in storage organs. Such organ-dependent variation has been also found in other deciduous species, e.g., *N. pumilio* (Piper et al. 2016). These suggest that using NSC for distinguishing between C and growth limitations at treelines in deciduous species may be not as straightforward as in evergreen species (Fajardo and Piper 2017; Wang et al. 2018).

Interestingly, we found that NSC concentrations of all organs significantly decreased with increasing elevation in *V. uliginosum* but increased in *R. aureum* (Fig. 3). Combining elevational decrease in shrub growth (Table 1; Fig. 2b, c), these results strongly supported the CLH for *V. uliginosum*, but the GLH for *R. aureum*. Such trends are explained solely by decrease in their relative growing temperature, without any aerodynamic decoupling effect as discussed above (Fig. 1): deciduous *V. uliginosum* with a relatively short growing season may suffer from insufficient C supply at higher elevation, due to a shortage of photoassimilates limited by low temperature (Wardle 1993), while growth of evergreen *R. aureum* may be driven by elevational decrease in meristematic activity (i.e., cell and organ formation) induced by cold (Körner 1998). It is questionable whether sink activities are more sensitive than photosynthesis to low temperature in shrub species, as observed in trees (James et al. 1994; Körner 2008; Solfjeld and Johnsen 2006). On the other hand, although we found elevational decrease in annual twigs, height, and diameter, the strength of sink or source limitation in studied species may be still overestimated without direct measurements of organ

formation (i.e., cambium activity) or whole-plant biomass growth data.

Differences in C storage among organs between trees and shrubs

Alpine shrubs generally grow and cope well with colder climates at much higher elevations above the treeline. The underlying functional difference between trees and such low-stature shrubs is crucial for understanding treeline formation (Körner 2012b). In the present study, we found that trees had lower rather than higher NSC concentrations than shrubs, and such divergence became more pronounced in all organs at the highest elevation site (Fig. 3). It may be explained by C storage and demand in relation to the stature. Compared with shrubs growing near the ground, *B. ermanii* trees generally had a taller and larger canopy than the two shrubs. Although the woody stem of trees does not increase the risk of freezing damage (Körner 2012a), woody organs, as the main storage sites, account for large biomass proportion, which would significantly dilute NSC concentrations (Hartmann and Trumbore 2016). Moreover, the higher stature means the longer distance of C transport from the source to sink. It may result that trees do not reserve or make up for the reduction in C storage of woody organs as quickly as shrubs do. Furthermore, tree leaves always grow at a higher position and are less able to avoid physical damage (e.g., wind) above the treeline. As a consequence, extra C would be consumed due to leaf damage or loss. For shrubs, a high level of NSC may be also explained by lower soil temperature under shrubs than tree canopy temperature (Fig. 1), according to the GLH. Therefore, our results imply that the shortage of C storage or increased C demand for respiration in woody organs may be one of the reasons why *B. ermanii* stops at the current position. However, there are very few available data for comparison with other studies. Although Shi et al. (2006) have investigated C status in trees and shrub species at the Tibetan Plateau treeline, these species did not occur at the same elevations.

Interspecific difference in the ratio of soluble sugars to starch (SSR) may also help to interpret the treeline formation (Li et al. 2008b; Zhu et al. 2012). It has been assumed that trees growing at their elevational climate limit depend on a sufficiently high SSR to prevent stress (Li et al. 2008b). Indeed, the present study found

higher SSR in shrub leaves at higher elevations (means of 4.4 and 5.1 at 2018 and 2060 m a.s.l., respectively), while the opposite trend occurred in *Betula* trees (means of 2.6 and 1.6 at 2018 and 2060 m a.s.l., respectively) (Table 3). A lower proportion of soluble sugars may partly explain why trees could not grow and survive at higher elevation as shrubs. However, the functioning of soluble sugar, starch, and their conversion mechanisms in broadleaf deciduous species at the treeline is still incompletely understood (Yu et al. 2014).

Furthermore, LMA of *B. ermanii* was much lower than that of shrub species (Fig. 4). Although LMA is mainly explained by leaf duration (Körner 2012a), its values for shrubs in our studies were much higher than those averaged across biome (Poorter et al. 2009). Thus, our data suggest that shrubs may have adapted to higher elevations, since high-LMA leaves generally have more photosynthetic machinery per leaf area and more photosynthetic capacity in unproductive habitats (Hultine and Marshall 2000). On the other hand, *B. ermanii* that produces low-LMA leaves (less robust) may tend to reduce C investments in leaf construction, and expand leaf area to increase C assimilation at the expense of being more prone to mechanical damage.

Although shrubs were not directly sampled near their upper elevational limits, the data from the intermediate elevational ranges may still reflect differences in C storage among species. For instance, at the treeline, a 1.9 K colder soil temperature of *V. uliginosum* than tree canopy temperature corresponds to a 316-m higher elevation, based on a decrease of 0.6 K per 100 m (Körner 2003). In fact, the elevation of 2334 m is close to its elevational limit (approximately 2300 m).

As a whole, the present study detected the functional difference between trees and shrubs, but it is unnecessarily easy to conclude that results are “taxon-specific,” due to the limitation of species number and the sampling time. There is a narrow species spectrum at the Changbai treeline, including only a pure *Betula* population with two shrub species dominating the distribution in the treeline ecotone. Furthermore, the samples collected only once may also obscure the interpretations, without the consideration of C seasonal dynamics. For instance, the difference in elevational variation in *B. ermanii* may be affected by the sampling date or phenology. At high elevations, woody reserves have recently been used for the

formation of new organs, decreasing the NSC concentration; however, at low elevations, the subterranean organs and stem have higher concentration of NSC which could mean that at this stage of the growing season (mid-summer), reserves have begun to be stored. Therefore, the seasonal dynamic and a wide range of species should be considered in future investigations.

Conclusion

The present study showed different patterns of C storage between deciduous treeline species and low-stature shrubs. Aerodynamic decoupling warming was not evident at the treeline ecotone; on the contrary, soil temperature was lower than tree canopy air temperature. Growth consistently decreased with increasing elevation in all species, while NSC concentrations responded differently to elevation between *Betula* trees and shrubs. Organ dependence in *Betula* trees partly support the GLH, indicating that the deciduous treelines may be controlled by low temperature and other abiotic factors. The evidence that NSC decreased and increased in all organs of *V. uliginosum* and *R. aureum* imply strong support for the CLH and GLH, respectively. At the treeline site, NSC concentrations were lower in *Betula* trees than in shrubs, probably responding to their respective microhabitats. Furthermore, shrubs maintained more advantageous functional features than did *Betula* trees, including more C storage, higher leaf SSR, and LMA, as an adaptation to extreme climates at higher elevation. These results suggest that a greater understanding of the functional differences between trees and low-stature shrubs would contribute to our understanding of treeline formation.

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