Running title: NSC, nutrients and shrub-line

Carbon and nutrient physiology in shrubs at the upper limits: a multi-species study

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Abstract

Aims

Carbon and nutrient physiology of trees at their upper limits have been extensively studied, but those of shrubs at their upper limits have received much less attentions. The aim of this study is to examine the general patterns of non-structural carbohydrates (NSCs), nitrogen (N) and phosphorous (P) in shrubs at the upper limits, and to assess whether such patterns are similar to those in trees at the upper limits.

Methods

Across Eurasia, we measured the concentrations of soluble sugars, starch, total NSCs, N and P in leaves, branches and fine roots (< 0.5 cm in diameter) of five shrub species growing at both the upper limits and lower elevations in both summer (peak growing season) and winter (dormancy season).

Important Findings

Neither elevation nor season had significant effects on tissue N and P concentrations, except for lower P concentrations in fine roots in winter than in summer. Total NSCs and soluble sugars in branches were significantly higher in winter than in summer. There were significant interactive effects between elevation and season for total NSCs, starch, soluble sugars and the ratio of soluble sugar to starch in fine roots, showing lower soluble sugars and starch in fine roots at the upper limits than at the lower elevations in winter but not in summer. These results suggest that the carbon physiology of roots in winter may play an important role in determining the upward distribution of shrubs, like that in the alpine tree-line trees.

Keywords: non-structural carbohydrates, soluble sugars, starch, nutrient, shrub-line

INTRODUCTION

In alpine ecosystems, the distribution of tree species is confined by an upper elevation limit, i.e. a tree-line (Körner 1998). Beyond this tree-line, there commonly also exists a shrub-line consisting of low-stature shrubs or semi-shrubs (Dial *et al.* 2016; Myers-Smith *et al.* 2018). The dominance of shrubs above the alpine tree-line implies higher tolerance of shrubs than trees to cold environments. Several physiological mechanisms in relation to carbon (C) and nutrients are proposed to explain the growth and distribution of trees at the upper limits, including C source limitation (Fajardo *et al.* 2017; Li *et al.* 2008; Stevens *et al.* 1991; Zhu *et al.* 2012), sink (or growth) limitation (Hoch *et al.* 2002; Körner 2003; Piper *et al.* 2006), and nutrient limitation (Drollinger *et al.* 2017), but few studies have tested those for shrub-lines.

Understanding functional differences between trees and shrubs might shed new light on the physiological explanation of the distribution of woody plants at the upper limits (Körner 2012). Thus, to precisely project future vegetation changes in alpine ecosystems under global climate change, it is important to also understand the C and nutrient mechanisms associated with the growth and distribution of shrubs at alpine shrub-lines.

Plant survival and growth depend on not only the capacity of carbohydrate production, but also the efficiency of carbohydrate accumulation and remobilization (Simard *et al.* 2013). Non-structural carbohydrates (NSCs), including soluble sugars and starch, can accumulate in plant tissues and organs which can be used for growth and/or defense (Millard *et al.* 2010). For instance, NSCs can act as a buffer for insufficient source activity during periods of high C demands, such as in environments with a seasonally fluctuating climate or a short growing season (Hartmann *et al.* 2016; Hoch *et al.* 2003; Wurth *et al.* 2005). In addition, soluble

sugars can help adjust osmotic potential to buffer environmental stress (Charrier *et al.* 2013; Woodruff *et al.* 2011). To find out the physiological mechanisms of tree-line formation and understand the responses and dynamics of the alpine tree-line to global climate change, Körner and his colleagues (e.g. Hoch and Körner 2003; Körner 1999; Li *et al.* 2001, 2002) investigated the relationship between the production, supply, storage and use of carbohydrates in tree-line trees. So far, NSCs have been widely used as an indicator for assessing the growth potential of plants (Fajardo and Piper 2017; Guillemot *et al.* 2015; Li *et al.* 2013; Muller *et al.* 2011; Palacio *et al.* 2014).

Many studies have reported no change or even an increase in NSCs in leaves, branches, stem sapwoods or roots of tree-line trees with increasing elevation during the growing season (Dang *et al.* 2015; Hoch and Körner 2003; Hoch *et al.* 2002; Shi *et al.* 2008; Yu *et al.* 2014). Thus, a growth limitation is proposed because cell expansion (and thus growth) ceases but the production of carbohydrates are still sufficient at high elevations where temperature is low (Fajardo *et al.* 2014; Hoch *et al.* 2012). By contrast, others proposed that NSCs of tree-line trees are lower at tree-lines than at lower elevations (Fajardo and Piper 2017; Li *et al.* 2008), suggesting that carbohydrates are insufficient to compensate for respiratory losses and thus the growth of tree-line trees is limited by insufficient supply of mobile carbohydrates (i.e. the C-source limitation hypothesis) (Stevens and Fox 1991). However, most of these studies focused on the dynamics of NSCs during the growing season, but not for the dormant season (i.e. winter) (e.g. Fajardo and Piper 2017; Li *et al.* 2008). Our previous study testing multiple tree-lines across seasons and continents showed that not aboveground tissues but roots of the alpine tree-line trees suffered from a significant shortage of NSCs over winter (i.e., not during the growing season), suggesting a winter roots' carbon limitation in trees at the upper limits (Li *et al.*

2018). According to Körner *et al.* (2004), the global high altitude climatic tree-lines are associated with a seasonal mean ground temperature of 6.7°C ($\pm$ 0.8, SD). Shrub-lines are usually found hundred meters above the tree-line elevations, and thus shrubs at the shrub-lines expose to even lower temperature than tree-line trees (Li *et al.* 2016). Whether the low temperature-induced pattern of NSCs found in alpine tree-line trees exists also in shrubs at their upper limits has not been sufficiently investigated, particularly across species and regions.

Nutrients are also closely associated with tree-line formation (Drollinger *et al.* 2017; Sullivan 2016). For example, nitrogen (N) and phosphorous (P) are major components of Rubisco and rRNA, and play important roles in regulating plant growth (Chapin *et al.* 1990). Reduced soil nutrient availability with elevation could limit tree growth at tree-lines and thus is responsible for tree-line formation (McNown *et al.* 2013; Sullivan *et al.* 2015; Thébault *et al.* 2014). Nutrient availability in soils and nutrient concentrations in trees can also change asynchronously with elevation (Fajardo and Piper 2017; Wang A *et al.* 2018). However, many studies reported no change or an increase in N and P concentrations in trees at tree-lines compared to those at lower elevations (Li *et al.* 2013; Yan *et al.* 2013). Recently, a large-scale investigation covering four disparate climates and soils revealed that there was C-limitation but no N and P limitation in the alpine tree-line tree of *Nothofagus pumilio* in mid-summer (Fajardo and Piper 2017). Moreover, the end-season N and P storage, like C-storage in winter roots (Li *et al.* 2018), will greatly influence survival and early spring regrowth of trees, especially the annual growth of many woody plants relies heavily on the internal cycling of N in different organs (Millard and Grelet 2010). Therefore, it is also necessary to investigate the N and P status in shrubs at their upper limits not only during the growing season but also throughout the winter season.

While some studies have investigated patterns of NSCs in shrubs at their upper limits, these studies dealt with single shrub species on specific mountains and also took samples only in summer and thus were likely to obtain only a specific pattern (Casolou *et al.* 2020; Wang QW *et al.* 2018). To obtain a general pattern, we collected samples and measured the concentrations of NSCs (soluble sugars and starch), N and P in leaves, branches and fine roots of five shrub species at both their upper limits and their corresponding lower elevations for both summer and winter on various mountains across Eurasia. We examined whether the NSCs and nutrient status of shrubs at their upper limits depend on seasons and tissue types. We aim to test our hypothesis that the elevational patterns of tissue NSCs, N and P in shrubs at their upper limits, compared to lower elevations, are similar to those found in trees (Fajardo and Piper 2017, Li *et al.* 2018), since the former can tolerate more harsh growth conditions and thus can survive at much higher elevations than the latter. Our findings will contribute to further pointing out the common physiological mechanisms underlying the formation of the upper limits of plants, at least for woody plants.

MATERIALS AND METHODS

Site description and sample collection

We investigated five alpine shrub-lines in Chinese mountains and the Swiss Alps (Table 1). The species forming the five shrub-lines are four broad-leaved shrubs and one coniferous shrub. Of the five shrub-line species, three are deciduous and two are evergreen. The shrub-lines ranged from dry-temperate continental climate (Qilian Mts) and wet-continental climate (Changbai Mts), to humid (the Swiss Alps) and sub-tropical climate (Balang Mts) (Table 1).

Samples of each species were taken at both their upper limits and the

corresponding lower elevations (Table 1). Leaf samples were collected only in summer as three of the five shrub species studied are deciduous (Table 1), and branches and fine roots (< 0.5 cm in diameter) were sampled in both summer and winter. We collected leaves, branches and fine roots from about ten individuals and then pooled them to get a composite sample of each tissue type for each sampling date at each elevation, and the replicates were 6 ($n = 6$) for Qilian Mts and Changbai Mts, and 3 ($n = 3$) for the Swiss Alps. Mean height of each species at each elevation was measured in summer. To minimize the difference in phenology of shrubs at their corresponding upper limits and lower elevations at one site, the ‘summer’ sampling occurred during the warmest period (July-August) and the ‘winter’ sampling was carried out after the lower elevation was covered by snow. Samples of each tissue type from each plant were ground into fine powder after being oven-dried at 65 °C for 48 h.

Measurement of tissue NSCs and elements

For each sample, 0.1 g powder was put into a 10 ml centrifuge tube, and 5 ml of 80% ethanol were added. The mixture was incubated at 80 °C in a water bath-shaker for 30 min, and then centrifuged at 3500 rpm for 10 min. The pellets were extracted two more times with 80% ethanol. Supernatants were retained, combined and stored at 4 °C to determine the concentration of soluble sugars (Li *et al.* 2008).

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 being cooled to room temperature, 2 ml of 9.2 M HClO₄ was added. Starch was hydrolyzed for 15 min. Then 4 ml distilled water was added to the samples, which were then centrifuged at 4000 rpm for 10 min. The pellets were

extracted one more time with 2 ml of 4.6 M HClO₄. Supernatants were retained, combined and filled to 25 ml to determine starch. The soluble sugar and starch concentrations were measured spectrophotometrically (ultraviolet-visible spectrophotometer 752 S, Cany Precision Instruments Co., Ltd, Shanghai, China) at 620 nm using the anthrone method, and the starch was calculated by multiplying the glucose concentrations by a conversion factor of 0.9 (Li *et al.* 2008).

The concentration of N was measured by a C/N analyzer (vario MICRO cube, Elementar, Hanau, Germany). To measure P concentrations, 0.2 g plant samples were digested with a mixture of acids of nitric acid and perchloric acid (5:1) digestion and was then determined by the ammonium molybdate method (Kuo 1996). We calculated the ratio of soluble sugars to starch, which reflects the growth strategies of woody plants to environmental stress (Li *et al.* 2008).

Data analysis

Before analyses, normality of the data distribution was checked by Kolmogorov-Smirnov test. Data of starch and sugar:starch in branches and data of soluble sugars and starch in fine roots that did not meet the assumption were transformed to square root. We first examined the effects of elevation (upper limit vs. lower elevation), sampling season (summer vs. winter), tissue type (leaves, branches and fine roots), and their interactions on NSCs and nutrient status, using species (i.e. site) as random factor, and found that the levels of NSCs, N and P significantly varied with tissue type and sampling season. Therefore, we used linear mixed models to separately test the effects of season, elevation and their interaction on concentrations of soluble sugars, starch, sugar:starch, NSCs, N and P within each tissue type. In these models, season, elevation and season $\times$ elevation were treated as fixed effects, and species and its two-

way and three-way interactions with season and/or elevation (i.e. species $\times$ season, species $\times$ elevation and species $\times$ season $\times$ elevation) were included as random effects. The statistical analysis was performed using SPSS 22.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Tissue non-structural carbohydrates

For leaves, elevation had no effect on the concentration of total NSCs, soluble sugars, starch or the ratio of soluble sugar to starch in summer (Fig. 1a-d). For branches and fine roots, elevation had no significant effects on NSCs and starch concentrations and the ratio of soluble sugar to starch across seasons (Fig. 1e-l), but the concentration of soluble sugars in branches were marginally higher at the upper limits than at lower elevations (Fig. 1g). NSCs and soluble sugars in branches were significantly higher in winter than in summer (Fig. 1e, g). There was a significant ($P < 0.05$) or marginally significant ($P < 0.1$) interactive effect of elevation and season on NSCs, starch, soluble sugars and the ratio of soluble sugar to starch in fine roots: NSCs, starch and soluble sugars showed lower values at the upper limits than at lower elevations in winter but not in summer (Fig. 1i-k); The ratio of soluble sugar to starch showed higher values at the upper limits than at lower elevations in winter but not in summer (Fig. 1l).

Tissue nutrients

Elevation had no effect on the concentration of N or P in leaves in summer (Fig. 2a-b). Across seasons, elevation also did not affect N or P in branches or fine roots (Fig. 2c-f). The concentration of P in fine roots was significantly lower in winter than in

summer (Fig. 2f).

DISCUSSION

Dynamics of mobile carbohydrates of shrubs at the upper limits

In the present study, there exists large fluctuation in mobile carbohydrates across seasons, showing a significant increase in soluble sugars and NSCs in branches from summer to winter (Fig. 1e, g; $P < 0.05$). High concentrations of osmotic adjustment substances such as soluble sugars are able to help plants maintain the osmotic pressure and avoid the formation of intra- and intercellular ice crystals under cold stress (Duca 2015). This is evidenced by correlations between mobile carbohydrates and frost hardiness in woody plants, including *Juglans regia* (Améglio *et al.* 2004; Améglio *et al.* 2001), *Quercus robur*, *Q. pubescens* and *Q. ilex* (Morin *et al.* 2007), *Pinus contorta*, *P. sylvestris* and *Picea abies* (Ogren *et al.* 1997). Plants may allocate more C components into storage tissues for winter defense during the growing season. Therefore, the accumulation of NSCs in storage tissues may be an active process at the expense of growth (Chapin *et al.* 1990), demonstrating as a conservative strategy. However, low temperature may also increase the accumulation of NSCs by reducing the metabolic processes needed for growth (Piper *et al.* 2006), which indicates a passive process for C accumulation. Our previous study suggested that NSCs in treeline trees appeared to be a function of both active and passive physiological process, which was determined by a combination of environmental and intrinsic mechanisms (Li *et al.* 2018). The findings in this study supports the idea that alpine shrubs might have the ability of self-regulation to adapt to low temperature, reflecting a protective mechanism for alpine plants suffering from cold stress, and further highlight the importance of the increasing accumulation of NSCs for plants during the

growing season.

We found that tissue mobile carbohydrates of shrubs were not lower at the upper limits than at the lower elevations in summer (Fig. 1a-l). This result is consistent with findings of many previous studies on tree-line species (Dang *et al.* 2015; Fajardo *et al.* 2011; Hoch *et al.* 2002; Körner 2003; Shi *et al.* 2008; Yu *et al.* 2014), suggesting that tissue carbohydrate supply was sufficient for the growth of shrubs at the upper limits during the growing seasons. However, Lower NSCs, soluble sugars and starch concentrations, as well as higher ratio of soluble sugars to starch in fine roots at the upper limits than at the lower elevations in winter (Fig. 1i-l), suggesting that root carbohydrate storage is insufficient for winter survival and re-growth early next spring of shrubs at the upper limits. This result agrees with findings of some previous studies on tree-line tree species (Dang *et al.* 2015; Li *et al.* 2008; Li *et al.* 2018; Wang A *et al.* 2018; Zhu *et al.* 2012). Such a seasonal fluctuation of mobile carbohydrates in fine roots indicates that shrubs may allocate less NSCs into roots before winter, and/or some starch in roots was converted into soluble sugars during winter at the upper limits, thus leading to lower levels of NSCs but higher sugar-starch ratios at the upper limits. Meanwhile, an inherent greater proportion of soluble sugars compared to starch indicates a cold acclimation and a physiological mechanism contributing to cold hardiness of plants at the upper limits. The unchanged and even marginally higher concentrations of NSCs in above-ground tissues (leaves and branches) accompanied with lower levels of them in roots at the upper limits in winter suggest that the process of end-season resource re-allocation from aboveground tissues to roots may be stopped by rapid cooling or even ‘flash freezing’ temperature (Cong *et al.* 2018). Consequently, plants failed to efficiently reallocate the NSCs from aboveground tissues (especially from leaves of deciduous species prior to abscission)

to belowground roots in autumn, and thus they suffer from a root carbon shortage in winter, which has recently been proposed as a carbon physiological mechanism for the alpine tree-line formation (Li *et al.* 2018). Our results confirmed the important role of winter root storage of NSCs in determining the upper limits of shrub species distribution. These time- and tissue-dependent results emphasize that even large space scale data covering various species and different regions also cannot reach a solid conclusion concerning the physiological mechanisms for the formation of the alpine tree-line or shrub-line if the data do not cover various tissue types across time scale.

Nutrient accumulation and dynamics of shrubs at the upper limits

Nitrogen and P are the most important nutrient elements for plant growth, and higher concentrations of N and P in storage tissues indicate a higher capacity of resource supply (Chapin 1980; Chapin *et al.* 1986). From summer to winter, P in storage tissues (branches and fine roots) tended to decrease (Fig. 2d, f) but N in storage tissues did not change (Fig. 2c, e), indicating a more abundant N than P in winter. One explanation might be that less P was re-allocated from leaves to storage tissues at the end-season compared to N, whose remobilization is strongly seasonally programmed and contributes more to spring growth than current N uptake (Millard and Grelet 2010). If tissue major element concentrations of shrubs at the upper limits were lower compared to those at lower elevations, then major element limitations could at least in part influence the growth and distribution of plants at the upper limits. In addition, the uptake of N and P is supposed to be restricted at lower temperature and in shorter growing season (Liptzin *et al.* 2013). However, in our study, tissue N did not vary between shrubs at the upper limits and at lower elevations across seasons (Fig. 2d, g), but tissue P tended to be higher at the upper limits than at lower elevations across

seasons (Fig. 2e, h). Some studies demonstrated that tissue N or P levels were not lower at higher elevations (Fajardo and Piper 2017; Hong *et al.* 2014; Körner 1989; Li *et al.* 2013; Wang *et al.* 2015; Yan *et al.* 2012). For instance, increased tissue N concentrations of four tree species (*Quercus aquifolioides*, *Abies faxoniana*, *Betula ermanii*, and *Larix decidua*) with increasing elevation (Li *et al.* 2018), increased leaf N and P concentrations of a tree species (*Nothofa guspumilio*) with elevation in four different sites (Fajardo and Piper 2017), and increased leaf N and unchanged leaf P of the tree species located in seven tree-line ecotones (Mayor *et al.* 2017). One likely explanation is that the enriched tissue N and P levels of plants at the upper limits are an adaptive response to cold stress (Körner 1989; Oleksyn *et al.* 2002; Weih *et al.* 2001) as higher tissue N and P levels may protect plants from damage by low temperature (Chapin 1980). Our results suggest that neither N nor P is likely to affect the upward distribution of shrubs at the shrub-lines.

CONCLUSIONS

Our multi-species study revealed an overall decline in root soluble sugars, starch and NSC in shrubs at their upper limits compared to lower elevations in winter, and suggests that root winter storage of NSCs plays a crucial role in regulating and determining the growth and elevational distribution of shrubs. This first investigation of tissue mobile carbohydrates and nutrients in a larger set of shrub-line species shows that the determinant of the shrub distribution at the upper limits is similar to that of the tree distribution at the upper limits (Li *et al.* 2018), although shrubs and trees differ in their body size (height). This result suggests that plants, at least woody plants, irrespective of their body size, physiologically respond to the alpine low temperature similarly. Shrubs with smaller plant size are better protected by near-

surface temperature and thus can tolerate to and survive at higher elevation than the taller trees coupling with free atmosphere, as proposed by Li *et al.* (2003) and Li and Yang (2004). The time- and tissue-dependent results gained in the present study emphasize that, to arrive at a solid conclusion concerning the physiological mechanisms for the formation of the alpine tree-line and shrub-line, large space scale studies simultaneously covering both trees and shrubs, both growing and dormant season, and various tissue types are urgently needed.

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REFERENCES

- Améglio T, Decourteix M, Alves G, *et al.* (2004). Temperature effects on xylem sap osmolarity in walnut trees: Evidence for a vitalistic model of winter embolism repair. *Tree Physiol* **24**:785-93.
- Améglio T, Ewers FW, Cochard H, *et al.* (2001). Winter stem xylem pressure in walnut trees: Effects of carbohydrates, cooling and freezing. *Tree Physiol* **21**:387-94.
- Casolou V, Braidot E, Petrucci E, *et al.* (2020). Relationships between population traits, nonstructural carbohydrates, and elevation in alpine stands of *Vaccinium myrtillus*. *Am J Bot* **107**:639-49.
- Chapin FS (1980). The mineral nutrition of wild plants. *Annu Rev Ecol Syst* **11**:233-60.
- Chapin FS, Schulze E-D, Mooney HA (1990). The ecology and economics of storage in plants. *Annu Rev Ecol Syst* **21**:423-47.
- Chapin FS, Vitousek PM, Van Cleve K (1986). The nature of nutrient limitation in plant communities. *Am Ant* **127**:48-58.
- Charrier G, Cochard H, Améglio T (2013). Evaluation of the impact of frost resistances on potential altitudinal limit of trees. *Tree Physiol* **33**:891-902.
- Cong Y, Wang A, He H, *et al.* (2018). Evergreen *Quercus aquifolioides* remobilizes more soluble carbon components but less N and P from leaves to shoots than deciduous *Betula ermanii* at the end-season. *iForest* **11**:517-25.
- Dang HS, Zhang KR, Zhang QF, *et al.* (2015). Temporal variations of mobile carbohydrates in *Abies fargesii* at the upper tree limits. *Plant Biol* **17**:106-13.
- Dial RJ, Smeltz TS, Sullivan PF, *et al.* (2016). Shrubline but not treeline advance matches climate velocity in montane ecosystems of south-central Alaska. *Global*

366 *Change Biol* **22**:1841-56.

367 Drollinger S, Mueller M, Kobl T, *et al.* (2017). Decreasing nutrient concentrations in
368 soils and trees with increasing elevation across a treeline ecotone in rolwaling
369 himal, nepal. *J Mt Sci-Engl* **14**:843-58.

370 Duca M (2015). Physiology of plant resistance to unfavorable environmental factors.
371 In: "Plant physiology". Springer, Dordrecht, The Netherlands, pp. 271-308.

372 Fajardo A, Piper FI (2014). An experimental approach to explain the southern andes
373 elevational treeline. *Am J Bot* **101**:788-95.

374 Fajardo A, Piper FI (2017). An assessment of carbon and nutrient limitations in the
375 formation of the southern andes tree line. *J Ecol* **105**:517-27.

376 Fajardo A, Piper FI, Cavieres LA (2011). Distinguishing local from global climate
377 influences in the variation of carbon status with altitude in a tree line species.
378 *Global Ecol Biogeogr* **20**:307-18.

379 Guillemot J, Martin-Stpaul NK, Dufrêne E, *et al.* (2015). The dynamic of the annual
380 carbon allocation to wood in european tree species is consistent with a combined
381 source-sink limitation of growth: Implications for modelling. *Biogeosciences*
382 **12**:2773-90.

383 Hartmann H, Trumbore S (2016). Understanding the roles of nonstructural
384 carbohydrates in forest trees - from what we can measure to what we want to
385 know. *New Phytol* **211**:386-403.

386 Hoch G, Körner C (2003). The carbon charging of pines at the climatic treeline: A
387 global comparison. *Oecologia* **135**:10-21.

388 Hoch G, Körner C (2012). Global patterns of mobile carbon stores in trees at the
389 high - elevation tree line. *Global Ecol Biogeogr* **21**:861-71.

390 Hoch G, Popp M, Körner C (2002). Altitudinal increase of mobile carbon pools in

391 pinus cembra suggests sink limitation of growth at the swiss treeline. *Oikos*
 392 **98**:361-74.
 393 Hong J, Wang X, Wu J (2014). Stoichiometry of root and leaf nitrogen and
 394 phosphorus in a dry alpine steppe on the northern tibetan plateau. *PloS One*
 395 **9**:e109052.
 396 Körner C (1989). The nutritional status of plants from high altitudes - a worldwide
 397 comparison. *Oecologia* **81**:379-91.
 398 Körner C (1998). A re-assessment of high elevation treeline positions and their
 399 explanation. *Oecologia* **115**:445-59.
 400 Körner C (1999). Alpine plant life: Functional plant ecology of high mountain
 401 ecosystems. *Springer, Berlin Heidelberg*.
 402 Körner C (2003). Carbon limitation in trees. *J Ecol* **91**:4-17.
 403 Körner C (2012). Treelines will be understood once the functional difference between
 404 a tree and a shrub is. *Ambio* **41**:197-206.
 405 Körner C, Paulsen J (2004). A world - wide study of high altitude treeline
 406 temperatures. *Journal of Biogeography* **31**:713-32.
 407 Kuo S (1996). Phosphorus. In Sparks DL, Page AL, Loeppert PA, et al. (eds).
 408 Methods of Soil Analysis Part 3: Chemical Methods. Madison, WI: Soil Science
 409 Society of America and American Society of Agronomy.
 410 Li M-H, Xiao W-F, Wang S-G, *et al.* (2008). Mobile carbohydrates in himalayan
 411 treeline trees i. Evidence for carbon gain limitation but not for growth limitation.
 412 *Tree Physiol* **28**:1287-96.
 413 Li M, Yang J, Krauchi N (2003). Growth responses of picea abies and larix decidua to
 414 elevation in subalpine areas of tyrol, austria. *Can J Forest Res* **33**:653-62.
 415 Li MH, Cherubini P, Dobbertin M, *et al.* (2013). Responses of leaf nitrogen and

mobile carbohydrates in different *quercus species*/provenances to moderate climate changes. *Plant Biol* **15**:177-84.

Li MH, Hoch G, Korner C (2001). Spatial variability of mobile carbohydrates within pinus cembra trees at the alpine treeline. *Phyton-Ann Rei Bot* **41**:203-13.

Li MH, Hoch G, Korner C (2002). Source/sink removal affects mobile carbohydrates in pinus cembra at the swiss treeline. *Trees Struct Funct* **16**:331-7.

Li MH, Jiang Y, Wang A, *et al.* (2018). Active summer carbon storage for winter persistence in trees at the cold alpine treeline. *Tree Physiol* **38**:1345-55.

Li MH, Yang J (2004). Effects of microsite on growth of pinus cembra in the subalpine zone of the austrian alps. *Ann For Sci* **61**:319-25.

Li X, Rossi S, Liang E, *et al.* (2016). Temperature thresholds for the onset of xylogenesis in alpine shrubs on the tibetan plateau. *Trees Struct Funct* **30**:2091-9.

Liptzin D, Sanford Jr. RL, Seastedt TR (2013). Spatial patterns of total and available n and p at alpine treeline. *Plant Soil* **365**:127-40.

Mayor JR, Sanders NJ, Classen AT, *et al.* (2017). Elevation alters ecosystem properties across temperate treelines globally. *Nature* **542**:91-5.

McNown RW, Sullivan PF (2013). Low photosynthesis of treeline white spruce is associated with limited soil nitrogen availability in the western brooks range, alaska. *Funct Ecol* **27**:672-83.

Millard P, Grelet GA (2010). Nitrogen storage and remobilization by trees: Ecophysiological relevance in a changing world. *Tree Physiol* **30**:1083-95.

Morin X, Ameglio T, Ahas R, *et al.* (2007). Variation in cold hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three european oak species. *Tree Physiol* **27**:817-25.

Muller B, Pantin F, Genard M, *et al.* (2011). Water deficits uncouple growth from

441 photosynthesis, increase c content, and modify the relationships between c and
442 growth in sink organs. *J Exp Bot* **62**:1715-29.

443 Myers-Smith IH, Hik DS (2018). Climate warming as a driver of tundra shrubline
444 advance. *J Ecol* **106**:547-60.

445 Ogren E, Nilsson T, Sundblad LG (1997). Relationship between respiratory depletion
446 of sugars and loss of cold hardiness in coniferous seedlings over-wintering at
447 raised temperatures: Indications of different sensitivities of spruce and pine.
448 *Plant Cell Environ* **20**:247-53.

449 Oleksyn J, Reich PB, Zytkowskiak R, *et al.* (2002). Needle nutrients in geographically
450 diverse *pinus sylvestris* l. Populations. *Ann For Sci* **59**:1-18.

451 Palacio S, Hoch G, Sala A, *et al.* (2014). Does carbon storage limit tree growth? *New*
452 *Phytol* **201**:1096-100.

453 Piper FI, Cavieres LA, Reyes-Diaz M, *et al.* (2006). Carbon sink limitation and frost
454 tolerance control performance of the tree *kageneckia angustifolia* d. Don
455 (rosaceae) at the treeline in central chile. *Plant Ecol* **185**:29-39.

456 Shi P, Koerner C, Hoch G (2008). A test of the growth-limitation theory for alpine tree
457 line formation in evergreen and deciduous taxa of the eastern himalayas. *Funct*
458 *Ecol* **22**:213-20.

459 Simard S, Giovannelli A, Treydte K, *et al.* (2013). Intra-annual dynamics of non-
460 structural carbohydrates in the cambium of mature conifer trees reflects radial
461 growth demands. *Tree Physiol* **33**:913-23.

462 Stevens GC, Fox JF (1991). The causes of treeline. *Annu Rev Ecol Syst* **22**:177-91.

463 Sullivan PF (2016). Evidence of soil nutrient availability as the proximate constraint
464 on growth of treeline trees in northwest alaska: Reply. *Ecology* **97**:803-8.

465 Sullivan PF, Ellison SBZ, McNown RW, *et al.* (2015). Evidence of soil nutrient

466 availability as the proximate constraint on growth of treeline trees in northwest
 467 alaska. *Ecology* **96**:716-27.

468 Thébault A, Clément J-C, Ibanez S, *et al.* (2014). Nitrogen limitation and microbial
 469 diversity at the treeline. *Oikos* **123**:729-40.

470 Wang A, Wang X, Tognetti R, *et al.* (2018). Elevation alters carbon and nutrient
 471 concentrations and stoichiometry in *quercus aquifolioides* in southwestern china.
 472 *Sci Total Environ* **622**:1463-75.

473 Wang QW, Liu CG, Zhou W, *et al.* (2018). Mobile carbon supply in trees and shrubs
 474 at the alpine treeline ecotone. *Plant Ecol* **219**:467-79.

475 Wang Z, Xia C, Yu D, *et al.* (2015). Low-temperature induced leaf elements
 476 accumulation in aquatic macrophytes across tibetan plateau. *Ecol Eng* **75**:1-8.

477 Weih M, Karlsson PS (2001). Growth response of mountain birch to air and soil
 478 temperature: Is increasing leaf-nitrogen content an acclimation to lower air
 479 temperature? *New Phytol* **150**:147-55.

480 Woodruff DR, Meinzer FC (2011). Water stress, shoot growth and storage of non-
 481 structural carbohydrates along a tree height gradient in a tall conifer. *Plant Cell*
 482 *Environ* **34**:1920-30.

483 Wurth MKR, Pelaez-Riedl S, Wright SJ, *et al.* (2005). Non-structural carbohydrate
 484 pools in a tropical forest. *Oecologia* **143**:11-24.

485 Yan CF, Han SJ, Zhou YM, *et al.* (2012). Needle-age related variability in nitrogen,
 486 mobile carbohydrates, and $\delta^{13}\text{C}$ within pinus koraiensis tree crowns. *PloS One*
 487 **7**:e35076.

488 Yan C, Han S, Zhou Y, *et al.* (2013). Needle $\delta^{13}\text{C}$ and mobile carbohydrates in pinus
 489 koraiensis in relation to decreased temperature and increased moisture along an
 490 elevational gradient in ne china. *Trees* **27**:389-99.

491 Yu D, Wang Q, Liu J, *et al.* (2014). Formation mechanisms of the alpine erman's
492 birch (*Betula ermanii*) treeline on changbai mountain in northeast china. *Trees*
493 **28**:935-47.

494 Zhu WZ, Cao M, Wang SG, *et al.* (2012). Seasonal dynamics of mobile carbon supply
495 in *quercus aquifolioides* at the upper elevational limit. *PloS One* 7:e34213.

496

497 **Table 1** Information about the five shrub-lines in this study.

Site	Longitude (E)	Latitude (N)	Shrub-line species	Growth form	Elevation (m above sea level)	Height (cm)
Mt. Qilian, China	100°18'	38°31'	<i>Salix gilashanica</i> C. Wang et P. Y. Fu	deciduous, broad- leaved	3550	154.7±10.4
					3000	141.7±17.3
Mt. Changbai, China	128°04'	42°02'	<i>Vaccinium uliginosum</i> Linn.	deciduous, broad- leaved	2380	9.5±0.8
					1430	49.2±1.2
Chandolin, Switzerland	7°34'	46°15'	<i>Vaccinium myrtillus</i> L.	deciduous, broad- leaved	2343	40±4.5
					2000	60±7.2
Davos, Switzerland	8°37'	46°53'	<i>Juniperus communis</i> L.	evergreen, coniferous	2300	30.3±1.2
					2000	39.6±1.9
Davos, Switzerland	8°37'	46°53'	<i>Rhododendron ferrugineum</i> L.	evergreen, broad- leaved	2300	40.1±1.3
					2000	70.3±1.4

498

Figure 1 Concentrations of total NSCs (a, e, i), starch (b, f, j) and soluble sugars (c, g, k) and the ratio of soluble sugar to starch (d, h, l) in leaves, branches and fine roots of shrubs at the upper limits and the corresponding lower elevations in summer and winter. Circles and vertical lines stand for values of mean and SE of five shrubs. F values and significance levels ($^{***} P < 0.001$, $^{**} P < 0.01$, $^{*} P < 0.05$, and $^{\dagger} P < 0.1$) of ANOVAs are given. E - elevation, S - season, and $E \times S$ - the interaction of elevation and season.

Figure 2 Concentrations of N (a, c, e) and P (b, d, f) in leaves, branches and fine roots of shrubs at the upper limits and the corresponding lower elevations in summer and winter. Circles and vertical lines stand for values of mean and SE of five shrubs. F values and significance levels ($*** P < 0.001$, $** P < 0.01$, $* P < 0.05$, and $^{\dagger} P < 0.1$) of ANOVAs are given. E - elevation, S - season, and $E \times S$ - the interaction of elevation and season.