

# Carbon dynamics in the deciduous broadleaf tree Erman's birch (*Betula ermanii*) at the subalpine treeline on Changbai Mountain, Northeast China

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**PREMISE OF THE STUDY:** The growth limitation hypothesis (GLH) and carbon limitation hypothesis (CLH) are two dominant explanations for treeline formation. The GLH proposes that low temperature drives the treeline through constraining C sinks more than C sources, and it predicts that non-structural carbohydrate (NSC) levels are static or increase with elevation. Although the GLH has received strong support globally for evergreen treelines, there is still no consensus for deciduous treelines, which experience great asynchrony between supply and demand throughout the year.

**METHODS:** We investigated growth and the growing-season C dynamics in a common deciduous species, Erman's birch (*Betula ermanii*), along an elevational gradient from the closed forest to the treeline on Changbai Mountain, Northeast China. Samples were collected from developing organs (leaves and twigs) and main storage organs (stems and roots) for NSC analysis.

**KEY RESULTS:** Tree growth decreased with increasing elevation, and NSC concentrations differed significantly among elevations, organs, and sampling times. In particular, NSC levels varied slightly during the growing season in leaves, peaked in the middle of the growing season in twigs and stems, and increased continuously throughout the growing season in roots. NSCs also tended to increase or vary slightly in developing organs but decreased significantly in mature organs with increasing elevation.

**CONCLUSIONS:** The decrease in NSCs with elevation in main storage organs indicates support for the CLH, while the increasing or static trends in new developing organs indicate support for the GLH. Our results suggest that the growth limitation theory may be less applicable to deciduous species' growth than to that of evergreen species.

**KEY WORDS** Betulaceae; carbon balance; growth limitation; leaf habit; non-structural carbohydrates; organ dependence; seasonal variation; treeline formation mechanisms

The alpine treeline is one of the most striking terrestrial ecological boundaries and is thought to be caused by low temperatures (~6.7°C) that restrict growth during the growing season (Körner, 2003a; Körner and Paulsen, 2004). The existence of such a uniform isocline implies that a single common functional process underlies its formation worldwide. Studies to date have largely focused on the effects of low temperatures on the carbon (C) source–sink balance (Tranquillini, 1979; Körner, 2003b; Hoch and Körner, 2012), leading to the proposal of two hypotheses to explain the functional mechanisms involved. The C limitation hypothesis (CLH) proposes that the decrease in temperature with increasing elevation reduces C gain (i.e., photosynthesis), resulting in treeline formation at higher elevations where C gains are insufficient to compensate for

the requirements of C sinks (e.g., growth, respiration, and C losses; Wardle, 1993). Alternatively, the growth limitation hypothesis (GLH) states that low temperature constrains meristematic activity (i.e., organ formation) and drives treeline formation (Körner, 1998). Therefore, an understanding of how C dynamics in trees change with increasing elevation is essential for evaluating the mechanisms involved in treeline formation.

As the primary products of photosynthesis, non-structural carbohydrates (NSCs, mainly including starch and soluble sugars) account for most of the stored C in a plant and thus reflect the source–sink balance (Chapin et al., 1990; Körner, 2003a; Martínez-Vilalta et al., 2016). Consequently, changes in NSC concentrations in trees with increasing elevation have generally been accepted as a

proxy for testing the GLH and CLH (Shi et al., 2008; Fajardo et al., 2012; Hoch and Körner, 2012), whereby no decrease in NSC concentrations in any organ indicates support for the GLH and a decrease in NSC concentrations indicates support for the CLH. To date, many studies have found support for the GLH in various treelines, most of which were composed of evergreen species (e.g., Hoch and Körner, 2003, 2005, 2012; Shi et al., 2008; Dawes et al., 2015). However, evidence for deciduous species is mixed; for instance, *Larix potaninii* was controlled by the growth limitation at the eastern Himalayas treeline (Shi et al., 2008), whereas *L. decidua* at the Swiss treeline had great aboveground growth responses to elevated CO<sub>2</sub>, supporting the CLH (Dawes et al., 2013). Even within a given species (e.g., *Nothofagus pumilio*), treelines appear to be supportive of the GLH (Fajardo et al., 2013; Piper et al., 2016) and the CLH (Fajardo and Piper, 2014, 2017). It is questionable whether the prevalent physiological mechanism (growth limitation) most accepted to explain treeline formation applies to deciduous treeline species.

C storage of deciduous species may have higher seasonal dynamics in relation to elevation than evergreen species as a result of leaf habit (Martinez-Vilalta et al., 2016). The dominant explanation is that deciduous trees, seasonally shedding leaves, generally experience great asynchrony between supply, which occurs only in the growing season, and demand, which occurs throughout the year (Chapin et al., 1990). Accordingly, NSC concentrations would decrease to a minimum during the early growing season, increase as assimilation occurs, and finally reach a peak toward the late growing season (Schadel et al., 2009). Moreover, deciduous broadleaf species with a lower leaf mass per area (LMA) are more susceptible to frequent mechanical damage and tissue loss, making C demand high and variable (Sveinbjörnsson et al., 1992). By contrast, evergreen species can produce a stable C supply throughout the year, resulting in only a slight seasonal fluctuation in C storage (Dickson, 1989; Kozłowski, 1992; Hoch, 2015). Thus, NSC accumulation driven by growth limitation in deciduous species should clearly take place early in the growing season, when growth is more intense. Surprisingly, previous studies that have examined the GLH have measured NSCs at the end of the growing season, which may fail to reflect “growth limitations” when growth is not actually occurring (Shi et al., 2008; Yu et al., 2014). For example, Hoch and Körner (2012) investigated 13 treeline sites worldwide, composed of trees with different leaf habits (10 evergreen and four deciduous species), and found that NSCs increased with elevation in all species except the deciduous *N. pumilio* (in Chile), in which the NSC concentrations decreased in leaves but increased in branches at the end of the growing season. This also suggests that a single snapshot of changes in NSC concentrations with increasing elevation cannot be used to determine whether growth or C limitation is occurring. However, the seasonal dynamics of NSCs in deciduous treeline species are still not well understood.

C storage of deciduous species in relation to elevation may also vary differently among organs throughout the growing season (Hoch et al., 2002; Piper et al., 2016). Newly developing organs (e.g., leaves and twigs) often have greater seasonal oscillations in C storage than major storage organs (e.g., stems and roots; Martínez-Vilalta et al., 2016), due to higher meristematic activity (e.g., cell division and elongation). Although recent studies have found that developing organs could quickly become C-autonomous in the absence of any underlying stress (Keel and Schädel, 2010; El Zein et al., 2011; Landhäusser, 2011), it is unknown whether this occurs at treelines. Consequently, it has been suggested that studies using

NSC concentrations to assess C or growth limitations should focus primarily on developing organs (Piper et al., 2016). However, many previous studies have investigated NSC concentrations in major storage organs (Hoch and Körner, 2003; Fajardo et al., 2011, 2012; Lenz et al., 2014), and it has also been shown that low temperatures and other stressors may have a greater effect on NSC in major storage organs than in developing organs, due to the need to translocate C over longer distances (Pratt and Jacobsen, 2017). For instance, Piper et al. (2016) found that NSCs increased significantly in leaves with increasing elevation but tended to decrease in branches of treeline species growing in a Mediterranean climate (Chile). Therefore, consideration of the organs sampled may also be crucial for evaluating growth and C limitations in treeline species.

In the present study, we aimed to evaluate C dynamics along an elevation gradient in a common deciduous broadleaf species, Erman's birch (*Betula ermanii*), which is the predominant species at treeline on Changbai Mountain, the highest mountain in Northeast China. Although Erman's birch is the dominant treeline species in the subalpine zone in East Asia (Gansert et al., 1999), it has not been included in previous global synthesis studies on treeline formation (Harsch et al., 2009; Hoch and Körner, 2012; Martínez-Vilalta et al., 2016) because of the lack of C storage data (Yu et al., 2014). We focused on two treeline theories in relation to C balance, the GLH and CLH, by investigating tree growth and changes in NSC concentrations with increasing elevation in developing organs (leaves and twigs) and mature organs (stems and roots) of Erman's birch throughout the growing season. In support of the GLH, we expected that C storage in all organs would increase with elevation, because low temperature constrains C sinks more than C sources at higher elevations; alternatively, in support of the CLH, we expected that NSC accumulation would decrease with increasing elevation, knowing that deciduous species are more prone to tissue losses due to physical damage and, consequently, increased risks of C limitation. Furthermore, we expected that NSC concentrations would vary in all organs throughout the growing season because of the great asynchrony between C supply and demand even when the growing season is short, and that the magnitude of such change would be greater in developing organs than in mature organs because of their higher metabolic activity.

## MATERIALS AND METHODS

### Study site

The study was conducted on the north slope of the Changbai Mountain Natural Reserve (41°3′–42°28′N, 127°9′–128°55′E), Jilin Province, Northeast China. There are four defined vegetation zones along the elevational gradient in this region, including Korean pine (*Pinus koraiensis*) and broadleaved mixed forest (740–1100 m a.s.l.), conifer forest (1100–1800 m a.s.l.), Erman's birch forest (1800–2000 m a.s.l.), and alpine tundra (above 2000 m a.s.l.). For Erman's birch, the central distribution area occurs at 1900 m a.s.l.; the edge of the closed forest (hereafter “timberline”) occurs at 1950–2000 m a.s.l.; and the treeline, where tree heights are >3 m, occurs at 2018 m a.s.l. The climate at the treeline is characterized by severe cold, high humidity, and strong winds (Yu et al., 2014), with a mean annual temperature of –2.3°C to –3.8°C; a frost-free period of about 65–70 d; annual precipitation ranging from 1000 to 1100 mm, most of which occurs from June to September; and annual wind speeds ranging from 6 to

10 m s<sup>-1</sup>, with gales sometimes lasting >200 d. The growing season at the treeline generally starts at the end of May or in early June and ends when the first severe frost occurs in late September.

### Sampling

We established a 30 × 20 m sample strip at three different elevations encompassing the closed forest (1908 m a.s.l.), the timberline (1976 m a.s.l.), and the treeline (2018 m a.s.l.) for Erman's birch. We conducted sampling on three sunny days: 20 June, 3 August, and 8 September, 2010, which represented the early, middle, and late growing season, respectively. At each elevation, we selected five similarly aged trees (~7.0 cm diameter at breast height) with heights >3 m. Each tree was separated from the others by ≥10 m. On each tree, we sampled (1) mature leaves, (2) young branches (1–2 cm diameter; hereafter "twigs"), (3) stem xylem (segments 1.5 cm long, measured from the outermost stem section toward the pith at breast height), and (4) roots (0.5–1.0 cm diameter). Each organ had 10 replicates per elevation site and sampling time. To avoid light effects on NSC concentrations, we sampled leaves from nonshaded leading branches on the upslope side (Li et al., 2001). We determined twig age by counting the number of twig nodes, from which we then measured mean annual shoot growth. We removed the bark and phloem from the twig, stem, and root samples with a knife because bark accounts for little of a tree's NSC stock (Chantuma et al., 2009) and phloem mainly functions in C transport (Hartmann and Trumbore, 2016). We took two cores from opposite sides of the stem on each tree using a 0.5 mm stem corer (Suunto, Vantaa, Finland). Because it has previously been shown that starch and sugar concentrations rapidly decline from the outermost tree ring toward the pith, where concentrations are very low or even undetectable (Yu et al., 2014), we collected the outermost (youngest) 1.5 cm segments as the stem sapwood. In the case of root wood samples that were <1 cm diameter, we considered the whole xylem to be active (sapwood; Hoch et al., 2002).

We collected samples around noon to minimize the effects of diurnal fluctuations and stored them in a cool box in the field. We then heated them in a microwave oven (40 s at 600 W) within 6 h of sampling to denature the enzymes. In the laboratory, we dried half the organ samples to a constant mass at 70°C (~48 h), ground them to a fine powder, and then stored them over silica gel at 4°C. We used the remaining samples to determine the leaf mass per area (LMA, g m<sup>-2</sup>) and woody organ densities because NSC concentrations expressed in terms of mass are affected by wood density. We measured leaf area with a leaf area meter (CI-203; CID Bio-Science, Camas, Washington, USA) and calculated LMA as leaf dry mass divided by leaf area. We then dried the remaining woody organ samples to a constant mass at 70°C (~72 h) to determine their dry weight. Based on Archimedes' principle, the volume of the woody samples was measured by submerging the woody organs in water (22°C) in a glass beaker with a scale. The difference caused by submersion of the sample could be converted to volume, since water density equals the unit at the standard temperature and pressure. Finally, we calculated wood density as the ratio of dry mass of the woody organs to their volume.

### Chemical analysis

We defined NSCs as the sum of total soluble sugars and starch. The concentrations of total soluble sugars and starch were determined

using the anthrone method, as described by Li et al. (2008). Briefly, we placed the powdered material in a 10 mL centrifuge tube with 5 mL of 80% ethanol. Then we incubated the mixture at 80°C for 30 min and centrifuged it at 5000 × g for 5 min. After repeating this process twice, we spectrophotometrically measured the combined supernatants for soluble sugar (within 30 min) at 620 nm using anthrone reagent and calculated the concentrations of soluble carbohydrate from standard regression equations using glucose as a standard. Starch was released by boiling the residue in 2 mL distilled water for 15 min and then adding 2 mL of 9.2 M HClO<sub>4</sub> solution at room temperature and leaving it for 15 min to hydrolyze the starch. We then added an additional 4 mL of distilled water to the tube and centrifuged the mixture at 5000 × g for 10 min, following which we extracted the pellet again with 2 mL of 4.6 M HClO<sub>4</sub> solution. We then spectrophotometrically analyzed the combined supernatants for starch at 620 nm using anthrone reagent with glucose as a standard. We calculated starch concentration by multiplying glucose concentrations by a conversion factor of 0.9 (Osaki et al., 1991). The concentrations of soluble sugar and starch were expressed on a dry-matter basis (% dm).

### Temperature records

We monitored canopy air and soil temperatures at the three elevations using microclimatic loggers (–30 to +50 °C; HOBO H8 Pro temperature loggers; Onset, Bourne, Massachusetts, USA). One logger was placed 2 m above the ground, avoiding direct sunlight; the other was placed at a soil depth of 10 cm under full canopy shade, following a previously published protocol (Körner and Paulsen, 2004). Both loggers recorded the temperature at 30 min intervals from 1 January to 31 December 2010. The beginning of the growing season was defined as the date on which the daily mean soil temperature at a depth of 10 cm first exceeded 3.2°C, and the end of the growing season was defined as the date on which the daily mean soil temperature first dropped below 3.2°C (following Körner and Paulsen, 2004).

### Statistical analysis

We tested all data (NSC, soluble sugar, and starch concentrations) for normality with the Kolmogorov-Smirnov test and log-transformed them to meet the assumption of normality where required. We then used two-way repeated-measures analysis of variance (ANOVA) to analyze the effects of elevation and sampling date on the concentrations of NSCs and the soluble sugar and starch components for each organ type. We also used one-way ANOVA to analyze variations in annual shoot length, LMA, and wood density across all elevations. Significant differences in variables among elevations were evaluated using Tukey's multiple range test. We performed all statistical tests using SAS version 8.1 (SAS Institute, Cary, North Carolina, USA) and considered results significant at the 5% level.

## RESULTS

### Growth

The growing season was 12 d shorter at the treeline than in the closed forest, and tree diameter and height also tended to be lower at the treeline (Table 1). The annual increase in twig length over the

**TABLE 1.** Characteristics of the study sites on Changbai Mountain, Northeast China, in terms of elevation, temperature, and the growth of Erman's birch (*Betula ermanii*).

Variable	Closed forest	Timberline	Treeline
Elevation (m a.s.l.)	1908	1976	2018
Mean air/soil temperature (°C)			
Annual	-3.0/4.0	-3.0/3.1	-3.4/-1.3
Growing season	11.8/9.9	11/8.7	10.8/8.2
January	-17.5/0.4	-17.3/-0.2	-17.6/-14.2
August	12.9/12.8	13/11.6	12.6/11.0
Growth			
Length of growing season (days)	141.0	136.0	129.0
Mean diameter at breast height (cm)	8.1 (1.3)	7.2 (1.8)	6.0 (1.6)
Mean height (m)	6.4 (1.6)	6.5 (2.1)	4.5 (0.9)

Notes: Mean temperatures within the tree crown (air) and at a soil depth of 10 cm were monitored at 30 min intervals with temperature loggers (HOBO H8 Pro) from 1 January to 31 December, 2010. Values before and after the slash symbol represent mean air temperature and mean soil temperature, respectively. Growth variables are shown as means, with SE in parentheses. The beginning of the growing season was defined as the date on which the daily mean soil temperature at a depth of 10 cm first exceeded 3.2°C, and the end of the growing season was defined as the date on which the daily mean soil temperature first fell below 3.2°C (Körner and Paulsen, 2004).

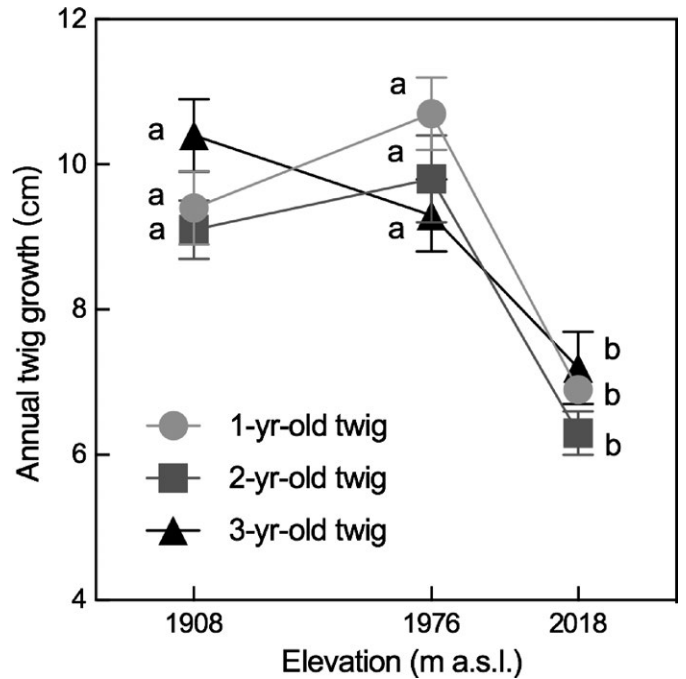
previous 3 yr decreased significantly, by an average of 29.4%, with increasing elevation ( $F_{2,27} = 43.45, P < 0.0001$ ; Fig. 1) but was not affected by age ( $F_{2,27} = 1.41, P = 0.2502$ ). In terms of functional traits, LMA increased significantly with increasing elevation ( $F_{2,42} = 30.56, P < 0.0001$ ), whereas wood density was similar across all elevations and woody organ types (Table 2).

**Variation in NSC concentrations during the growing season**

The concentrations of NSCs, soluble sugars, and starch in each organ were significantly affected by the sampling date (Table 3). There was also a significant interaction between the sampling date and elevation, indicating that the seasonal variation in NSC concentration within each organ varied among birch trees growing at different elevations. NSC concentrations in leaves varied only slightly during the growing season, due to the seasonal increase in soluble sugars being offset by the decrease in starch (Fig. 2). By contrast, the concentrations of NSCs and starch in twigs and stem wood showed significant variation during the growing season, peaking in the middle of the season (August) and then decreasing toward the end of the season (September), with this pattern being more pronounced at 1976 m a.s.l. In root wood, there was a continuous increase in the NSC and starch concentrations, though the extent of this decreased with increasing elevation. The contribution of soluble sugars to the NSC concentration was >50% at all elevations and increased with increasing elevation (Appendix S1; see Supplemental Data with this article).

**Variations in NSC concentrations with increasing elevation**

There was significant variation in the concentrations of NSCs, soluble sugars, and starch in each organ with increasing elevation, though there were some exceptions (NSCs and starch in twigs, and sugars in twigs and roots; Table 3). NSC concentrations in leaves increased significantly in June and September and increased slightly in August with increasing elevation (Fig. 2). By contrast, these trends were not observed in twigs, where the lowest values were recorded at 1976 m a.s.l. in September. For mature organs (stem wood



**FIGURE 1.** Variation in twig growth with increasing elevation: mean annual growth ( $\pm$  SE,  $n = 10$ ) of twigs of Erman's birch (*Betula ermanii*) trees growing at different elevations on Changbai Mountain, Northeast China (1908 m a.s.l. = closed forest; 1976 m a.s.l. = timberline; 2018 m a.s.l. = treeline). Growth was compared across elevations using Tukey's multiple range test. Different letters within an age group indicate significant differences among elevations ( $P < 0.05$ ).

**TABLE 2.** Variation in the leaf mass per area (LMA,  $g\ m^{-2}$ ) and wood density ( $g\ cm^{-3}$ ) of twigs, stem sapwood, and root wood in adult Erman's birch (*Betula ermanii*) trees growing at three elevations on Changbai Mountain, Northeast China (1908 m a.s.l. = closed forest; 1976 m a.s.l. = timberline; 2018 m a.s.l. = treeline).

Elevation (m a.s.l.)	Plant organ			
	LMA	Twig	Stem sapwood	Root wood
1908	46.90 (1.10) <sup>C</sup>	0.56 (0.02)	0.67 (0.02)	0.47 (0.02)
1976	56.13 (1.90) <sup>B</sup>	0.59 (0.01)	0.62 (0.01)	0.53 (0.02)
2018	63.83 (1.50) <sup>A</sup>	0.60 (0.02)	0.65 (0.01)	0.47 (0.02)
$F_{2,42}$	30.56	2.92	1.02	3.57
$P$	<0.0001	0.09	0.15	0.06

Notes: Values are means, with SE in parentheses ( $n = 15$  elevation<sup>-1</sup>). Measurements were compared across elevations using Tukey's multiple range test ( $F$  and  $P$  values are shown). Different superscript letters indicate statistically significant differences among elevations ( $P < 0.05$ ).

and root wood), NSC concentrations decreased significantly with increasing elevation. Furthermore, variations in the concentrations of sugars in stems and starch in roots tended to be consistent with those observed for NSC concentrations in each of these organs.

**DISCUSSION**

Carbon dynamics have been studied intensely in evergreen treeline species, with findings to date supporting the GLH. However, the findings about mechanisms of treeline formation in deciduous

**TABLE 3.** Two-way repeated-measures analysis of variance to test the effects of month (June, August, and September) and elevation (1908, 1976, and 2018 m a.s.l.) on the concentrations of non-structural carbohydrates (NSCs) and soluble sugar and starch components in different organs of Erman's birch (*Betula ermanii*) trees on Changbai Mountain, Northeast China. Significant differences ( $P < 0.05$ ) are in bold.

Organ	Factors	df	NSCs		Sugars		Starch	
			F	P	F	P	F	P
Leaf	Sampling date (D)	2	6.02	0.0076	318	<0.0001	229.36	<0.0001
	Elevation (E)	2	128.51	<0.0001	234.73	<0.0001	235.55	<0.0001
	D × E	4	11.52	<0.0001	137.53	<0.0001	69.66	<0.0001
Twig	D	2	38.46	<0.0001	33.03	<0.0001	19.05	<0.0001
	E	2	0.85	0.4503	1.46	0.2714	1.76	0.2138
	D × E	4	4.9	0.0049	21.77	<0.0001	3.98	0.0129
Stem wood	D	2	52.63	<0.0001	8.28	0.0018	79.31	<0.0001
	E	2	29.1	<0.0001	21.74	0.0001	5.22	0.0234
	D × E	4	2.56	0.0642	11.11	<0.0001	13.39	<0.0001
Root wood	D	2	116.33	<0.0001	67.8	<0.0001	64.67	<0.0001
	E	2	83.76	<0.0001	0.41	0.6697	78.74	<0.0001
	D × E	4	7.51	0.0005	5.13	0.0039	12.06	<0.0001

species are contradictory (i.e., Shi et al., 2008; Hoch et al., 2012; Fajardo et al., 2013, 2017; Piper et al., 2016). In the present study, we found that shoot growth in Erman's birch decreased significantly with increasing elevation, while the C dynamics of this deciduous species depended on elevation, organ type, and sampling time. NSC concentrations increased with increasing elevation in leaves, showed no variation in twigs, and decreased in stems and roots with increasing elevation. Such divergence among organs is not consistent with the results of previous studies on evergreen species, in which NSC concentrations have been found to increase with elevation in all organs (Shi et al., 2008; Hoch and Körner, 2012).

#### Growing-season dynamics in NSC concentrations among organ types

As we originally hypothesized, NSC concentrations in organs dramatically varied throughout the growing season, irrespective of elevation (Fig. 1). However, unexpectedly, we found that the highest amplitude of NSC oscillations during this period occurred not in leaves but rather in twigs and stems. These findings do not support the idea that developing organs are C-autonomous (Landhäusser, 2011) but may reflect significant amounts of mobilized C due to the large biomass of aboveground woody organs in this species. Furthermore, this pattern may not be solely explained by the source-sink framework, which accounts for imbalances between supply (photosynthesis) and demand (growth and respiration) and predicts that NSC dynamics throughout the season should be larger in leaves and roots (Martínez-Vilalta et al., 2016). This pattern may also be explained by organ functions and their roles in whole-plant C dynamics. Leaves act as the main source of carbohydrates and have high metabolic rates, high concentrations of intermediary metabolites, and large amounts of living cells requiring turgor maintenance (Sala et al., 2012); this is particularly true for deciduous broadleaf species, which need to produce and reserve enough C to meet the entire year's demand in a short time (Fajardo et al., 2013). In roots, NSC concentrations increased continuously rather than decreasing in the early season to support early growth. Thus, it is possible that (1) twig growth is supported mainly by current assimilates rather than by root C storage or that (2) roots play the main long-term storage role with intermediate osmotic and metabolic demands (Martínez-Vilalta et al., 2016), as reflected

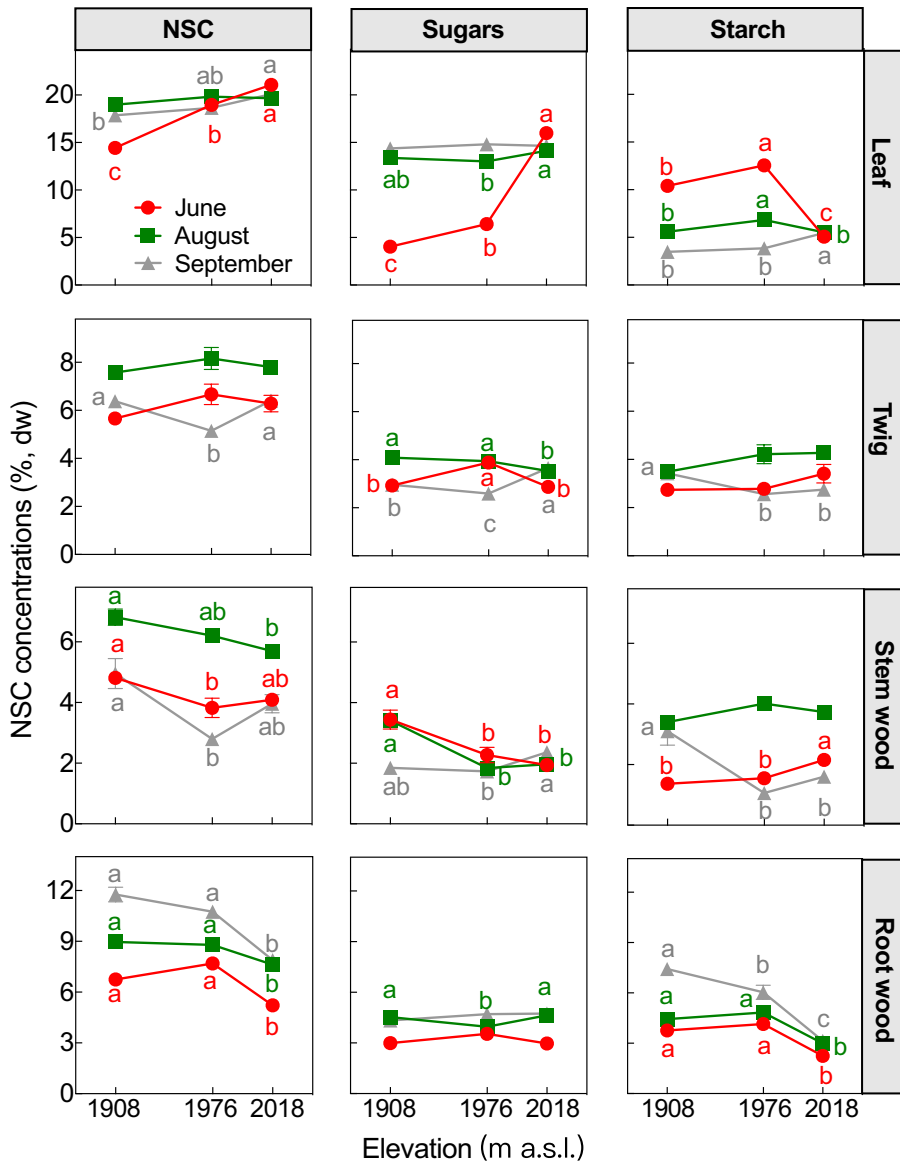
by intermediate concentrations of NSCs and the soluble sugar and starch fractions.

Twigs and stems are responsible for C translocation between the sites of C assimilation (leaves) and C storage (roots) (Hoch et al., 2002). For instance, C storage was lower in the early growing season due to the high levels of early growth, increased in the middle of the growing season when C uptake was highest, and then decreased in the late growing season as C was transported to the roots (Appendix S1). Alternatively, in the context of structural and functional relationships, it is possible that seasonal dynamics in stems are the result of selection for storage traits, which gives rise to trade-offs in transport and biomechanics traits (e.g., cavitation resistance; Pratt and Jacobsen, 2017).

Our results are inconsistent with those of previous studies on evergreen species (Hoch et al., 2002; Zhu et al., 2012; Dang et al., 2015). For instance, Hoch et al. (2002) found that NSC concentrations in the leaves and stems of *Pinus cembra* generally decreased throughout the growing season. However, our results are in line with Piper et al.'s (2016) findings for the temperate deciduous broadleaf species *N. pumilio*, suggesting that the source-sink framework cannot completely explain the growing-season dynamics of NSCs in deciduous species, though this conclusion requires further investigation at different sites.

#### Elevational trends in NSC concentrations among organ types

Changes in NSC concentrations with increasing elevation depend on organ types. There was no change in the NSC concentrations in leaves and twigs with increasing elevation, whereas stems and roots showed a decreasing trend (Fig. 2). The change in NSC concentration in leaves would have been larger had it been calculated on a volume basis, because the leaf matter area increases with increasing elevation; however, this would have had a minimal effect on woody organs, because wood densities were similar among elevations (Table 2). According to the growth limitation theory, the trends observed in leaves and twigs indicate that under low temperature, the production of carbohydrates via photosynthesis exceeds the demand for growth at high elevations in Erman's birch. Also, tree growth, including the diameter and twig length, decreased with elevation (Table 1 and Fig. 1). Thus, the results for leaves and twigs support the GLH at the treeline on Changbai Mountain. This is consistent with the findings of previous studies on deciduous species,



**FIGURE 2.** Variation in carbon storage with sampling time and elevation: mean concentrations ( $\pm$  SE,  $n = 5$ ) of non-structural carbohydrates (NSCs; circles, June), soluble sugars (squares, August), and starch (triangles, September) during the growing season in different organs of Erman's birch (*Betula ermanii*) trees growing at different elevations on Changbai Mountain, Northeast China (1908 m a.s.l. = closed forest; 1976 m a.s.l. = timberline; 2018 m a.s.l. = treeline). Statistical differences in mean concentrations among elevations were tested using Tukey's multiple range test within each sampling time and organ type, indicated by different letters ( $P < 0.05$ ). The initial statistics for these data are presented in Table 3.

such as *B. platyphylla* and *L. potaninii* (Shi et al., 2008), *N. pumilio* and *L. decidua* (Fajardo et al., 2013), and *B. ermanii* (Yu et al., 2014).

On the other hand, the decrease of NSCs in stems and roots with elevation indicates that C sources were more restricted by low temperatures than by C sinks, supporting the CLH. This is opposite of the conclusion from developing organs, perhaps because of the foliar habit and organ functions of deciduous species (Piper and Fajardo, 2014). It has been shown that deciduous species generally have higher requirements for C storage than evergreens to allow for the replacement of leaf loss when facing stress (Hoch et al., 2003; El Zein

et al., 2011; Givnish et al., 2014). Particularly at higher elevations where the abiotic stressors are more intense (Table 1), C demand would also become stronger—evidence that soluble sugars contributed to greater proportions of NSC in woody organs (especially roots) at the upper elevational limit (Appendix S1). This high demand for C may potentially lead to C starvation in storage organs (i.e., roots; Landhäusser and Lieffers, 2012; Piper and Fajardo, 2014). Furthermore, mature organs are important C storage sites that account for most NSCs in adult trees, since their biomass proportion (storage volume) is much higher than that invested in leaves or twigs (Fajardo et al., 2013). This means that although NSCs of developing organs were higher than those of mature storage organs and increased with elevation, they might not be sufficient to make up for decrease in C storage of stems and roots with elevation.

Empirical support for both the GLH and the CLH has also been found elsewhere (Shi et al., 2008; Fajardo et al., 2013; Yu et al., 2014; Piper et al., 2016), but evidence from deciduous treelines is still generally scarce and provides contradictory support for either hypothesis. For instance, a free-air CO<sub>2</sub> enrichment experiment at a Swiss treeline showed that *L. decidua* had high aboveground growth responses to elevated CO<sub>2</sub>, providing support for the CLH (Dawes et al., 2013). However, Fajardo and Piper (2014, 2017) found inconclusive support for the relative importance of either C limitation or growth limitation (Fajardo et al., 2013; Piper et al., 2016) in *N. pumilio*. These seemingly contradictory results may be partly ascribed to the sampling-time dependency and organ dependency of C storage in relation to elevation. More importantly, however, these results imply that using NSC to distinguish between C and growth limitations at treelines in deciduous species is not as straightforward as it is in evergreen species (Fajardo and Piper, 2017). Further investigations are necessary to make the definitive explanation for deciduous species clear.

It is notable that changes in NSCs with elevation were not consistent during the growing season, particularly in leaves and twigs (Table 3 and Fig. 2). If temperature is the only driver of treeline formation, seasonality should slightly affect elevational variation in NSCs. Thus, our results suggest that local factors appear to modulate C dynamics, as observed in other treelines (e.g., drought effects on Mediterranean deciduous treelines; Piper et al., 2016). Indeed, on Changbai Mountain, soils are characterized by a high content of volcanic float stone, a thin depth, and a low water-holding capacity, making them unfavorable for tree growth. Despite relatively high precipitation in this region, the radial growth of birch trees was positively affected by temperature and precipitation (Yu et al., 2007),

and shoot increment was not associated with its C status but was significantly related to high  $\delta^{13}\text{C}$  values (Yu et al., 2014). Furthermore, the mean soil temperature (8.2°C; Table 1) at the treeline was higher than that in global climate studies (6.7°C; Körner and Paulsen, 2004), indicating that the Erman's birch treeline may be located below the predicted elevation. These facts suggest that water stress may affect the treeline structure together with low temperature. However, physiological mechanisms associated with water stress remain unclear.

## CONCLUSIONS

Our results show that C dynamics in the deciduous broadleaf species Erman's birch were significantly affected by elevation, organ type, and sampling time. Although NSC concentrations varied throughout the growing season, the magnitude of such variation was greater in twigs and stems than in leaves and roots. C dynamics in relation to elevation appear to be supportive of the two explanations: developing organs support the GLH, in that there was no decrease in NSC concentrations of leaves and twigs with elevation; while main storage organs support the CLH, in that there was a decrease in C storage with increasing elevation. These findings suggest that using NSC concentration as a proxy for distinguishing the GLH and the CLH is not a straightforward way to explain treeline formation in deciduous species as it is for evergreen species. The growth limitation theory for treeline formation must be revisited. The importance of C allocation and its function needs to be emphasized in future studies.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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