Similar variation in carbon storage between deciduous and evergreen treeline species across elevational gradients

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• *Background and Aims* The most plausible explanation for treeline formation so far is provided by the growth limitation hypothesis (GLH), which proposes that carbon sinks are more restricted by low temperatures than by carbon sources. Evidence supporting the GLH has been strong in evergreen, but less and weaker in deciduous treeline species. Here a test is made of the GLH in deciduous–evergreen mixed species forests across elevational gradients, with the hypothesis that deciduous treeline species show a different carbon storage trend from that shown by evergreen species across elevations.
• *Methods* Tree growth and concentrations of non-structural carbohydrates (NSCs) in foliage, branch sapwood and stem sapwood tissues were measured at four elevations in six deciduous–evergreen treeline ecotones (including treeline) in the southern Andes of Chile (40°S, *Nothofagus pumilio* and *Nothofagus betuloides*; 46°S, *Nothofagus pumilio* and *Pinus sylvestris*) and in the Swiss Alps (46°N, *Larix decidua* and *Pinus cembra*).
• *Key Results* Tree growth (basal area increment) decreased with elevation for all species. Regardless of foliar habit, NSCs did not deplete across elevations, indicating no shortage of carbon storage in any of the investigated tissues. Rather, NSCs increased significantly with elevation in leaves ($P < 0.001$) and branch sapwood ($P = 0.012$) tissues. Deciduous species showed significantly higher NSCs than evergreens for all tissues; on average, the former had 11 % (leaves), 158 % (branch) and 103 % (sapwood) significantly ($P < 0.001$) higher NSCs than the latter. Finally, deciduous species had higher NSC (particularly starch) increases with elevation than evergreens for stem sapwood, but the opposite was true for leaves and branch sapwood.
• *Conclusions* Considering the observed decrease in tree growth and increase in NSCs with elevation, it is concluded that both deciduous and evergreen treeline species are sink limited when faced with decreasing temperatures. Despite the overall higher requirements of deciduous tree species for carbon storage, no indication was found of carbon limitation in deciduous species in the alpine treeline ecotone.

Key words: Carbon supply, elevational gradient, *Larix decidua*, *Nothofagus betuloides*, *Nothofagus pumilio*, Patagonia, *Pinus cembra*, *Pinus sylvestris*, Swiss Alps, Alpine treeline.

INTRODUCTION

Alpine treeline ecotones constitute relatively narrow and well-delineated landscape boundaries, whose maximum elevation is thought to be controlled by temperature (Tranquillini, 1979; Grace *et al.*, 2002; Körner, 2012). Globally, high elevation treelines are associated with a mean growing season isotherm of around 6·7 °C (Körner and Paulsen, 2004). Two mutually exclusive hypotheses have been proposed to explain the upper elevation limit of treelines worldwide; these are the carbon limitation hypothesis (CLH) and the growth limitation hypothesis (GLH; Fig. 1). The CLH predicts that the universal elevational reduction in tree growth at or close to the alpine treeline is a consequence of the decline in photosynthetic rates provoked by the altitudinal decrease in temperature (Stevens and Fox, 1991; Wardle, 1993). The treeline thus occurs at the elevation where annual carbon (C) gains can no longer compensate for the requirements of all the C sinks (i.e. respiration, growth and all C losses). Alternatively, the GLH claims that the processes limited by altitudinal decreases in temperature are cell and

tissue formation (Körner, 1998), indicating a low temperature threshold for growth itself rather than a limitation on C gain. The change in C storage of trees in relation to elevation has been the most accepted and used proxy to test both hypotheses (Fajardo *et al.*, 2012; Hoch and Körner, 2012). The C storage pool of trees consists of a broad variety of different C-based organic compounds (with species-specific differences in composition and relative proportion), but throughout all tree genera, non-structural carbohydrates (NSC = low molecular weight sugars + starch) are quantitatively the most important C stores, accumulating whenever C sinks (e.g. growth) are more limited than C sources (photosynthesis) (Chapin *et al.*, 1990). Thus, an elevational increase in NSCs provides support for the GLH, while an altitudinal decrease in NSCs supports the CLH.

A large body of empirical studies has found support for the GLH in a variety of treelines (e.g. Hoch and Körner, 2003, 2012; Piper *et al.*, 2006; Shi *et al.*, 2008; Fajardo *et al.*, 2011). Yet, the most important piece of evidence against the GLH was observed in a treeline *in situ* free-air CO_2-enrichment
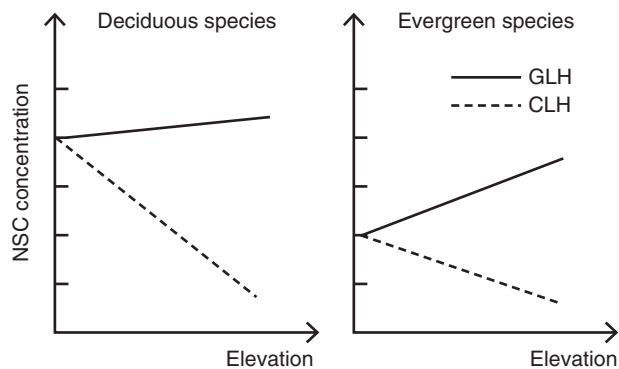
FIG. 1. Expected trends of concentrations of non-structural carbohydrates (NSC = total soluble sugars + starch) with elevation (temperature effect) according to foliar habit. First, according to empirical evidence, NSCs should be higher in deciduous than in evergreen species. Secondly, if carbon limitation (CL) is responsible for treeline formation, NSCs should invariably decrease with elevation regardless of foliar habit. Thirdly, if sink or growth limitation (GL) is responsible for treeline formation, NSCs should show an increase with elevation.

experiment, where Handa *et al.* (2005) and Dawes *et al.* (2011) found support for both the CLH in deciduous *Larix decidua* and for the GLH in evergreen *Pinus uncinata*. They determined that *L. decidua* augmented its growth as a response to the addition of CO_2, indicating that this species is potentially C limited at the treeline. One possible explanation for this contrasting response to CO_2 enrichment might be the different leaf functional types (i.e. evergreen vs. deciduous) of the two species.

Temperate winter deciduous species generally have higher levels of C reserves (NSC concentrations) than evergreens (Vanderklein and Reich, 1999; Hoch *et al.*, 2003). The classical explanation for the higher C storage pool in winter deciduous species is that these species need important amounts of NSCs to support spring growth and leaf-out, in contrast to evergreens which always have at least one photosynthetically active leaf cohort to provide the necessary C supply (Dickson, 1989; Kozlowski, 1992; Kozlowski and Pallardy, 1996). It has been found, however, that the higher NSC concentrations (particularly in older tissues) of deciduous species when compared with evergreens are maintained year-round (Barbaroux and Bréda, 2002; Hoch *et al.*, 2003; El Zein *et al.*, 2011). This supports the idea that larger pools of C reserves in deciduous tree species may reflect an adaptation to tolerate stressors by means of replacing leaf area in cases when stressors provoke losses in photosynthetic area (Pallardy, 2008). Compared with evergreens, deciduous tree species produce less robust leaves (shown by their higher specific leaf areas; SLAs), which makes them more prone to mechanical damage and eventual C limitations. What is more, in mountain ecosystems, spring frost and wind are two important causes of leaf area loss, which become more intense and frequent with elevation (Körner, 2003). Thus, the limited number of experiments, as well as the bias of existing NSC data towards evergreen taxa, does not allow for a conclusive judgement about the general C supply status of trees at alpine treelines. In light of the ambivalent findings on C relations of deciduous trees at the treeline (Handa *et al.*, 2005; Dawes *et al.*, 2011; Hoch and Körner, 2012), we may ask if the higher C storage pools and/or the differential seasonal use of C stores in deciduous vs. evergreen trees can lead to

different C relations (different net C balances) between these two functional types in response to stressors. Few studies have compared C reserves in deciduous and evergreen treeline trees (Shi *et al.*, 2008; Hoch and Körner, 2012), and none so far has worked with sympatric, phylogenetically close pairs of deciduous and evergreen species naturally growing next to each other in altitudinal treeline ecotones. Such studies should clarify whether treelines formed by deciduous tree species are determined by C limitation, as suggested by a previous study (Handa *et al.*, 2005).

The aim of this study was to assess whether the C balance of treeline tree species along elevational gradients is altered by the leaf habit of the species, i.e. if and how foliar habit strategies interact with the trees' physiological response to lower temperatures (Fig. 1). By working with deciduous–evergreen species growing next to each other in alpine treeline ecotones, we were interested in: (1) determining the magnitude of NSC concentrations per tissue and elevation for deciduous and evergreen species; and (2) testing the GLH, especially to determine whether NSC stores of deciduous species responded differently from evergreen species to changes in elevation (i.e. to decreasing temperatures). In particular, given that the requirements for C storage are higher in deciduous species, and knowing that deciduous species are more susceptible to leaf area reduction (and hence C gain) due to mechanical damage, we expect that C storage would be more reduced with elevation (i.e. lower temperature) in deciduous than in evergreen species. In the context of the CLH, this expectation would imply steeper NSC decreases with elevation in deciduous compared with evergreen species. Conversely, and in support of the GLH, deciduous species would show a smoother increase in NSCs with elevation compared with evergreen species (Fig. 1).

MATERIALS AND METHODS

Description of research sites and species

Two sites were selected in the Antillanca area within the Puyehue National Park (40°47′S, 72°11′W), southern Chilean Andes (Lake District), where deciduous *Nothofagus pumilio* (Poepp. & Endl.) Krasser (Nothofagaceae) and its evergreen congeneric *Nothofagus betuloides* (Mirb.) Oerst. grow naturally and constitute treeline species. The treeline here occurs at 1350 m a.s.l. of elevation (Table 1). Two other sites were selected in the Laguna Chiguay area within the Cerro Castillo National Reserve in the Aysén Region (45°59′S, 71°52′W Patagonia, Chile), where *N. pumilio* grows next to planted *Pinus sylvestris* L. (Pinaceae, evergreen). Seedlings of *P. sylvestris* here were produced in a nursery in the region (Tejas Verdes, Coyhaique, Corporación Nacional Forestal, pers. comm.) and seeds came from one provenance (German Forest Service at Wolfgang, Hesse, Germany). These *P. sylvestris* trees were planted in the early 1970s in an effort to cover slopes with vegetation to reduce the risk of landslides after a catastrophic human-induced forest fire. The natural treeline (*N. pumilio*) here occurs at 1300 m a.s.l. of elevation, and *P. sylvestris* was planted up to this elevation (Table 1). The last two sites were selected in the Dischma Valley near Davos (46°46′N, 9°52′E), in the Swiss Alps, where *Larix decidua* Mill. (deciduous) and *Pinus cembra* L. (evergreen) grow naturally. The treeline here occurs at 2200 m a.s.l. of elevation (Table 1).

TABLE 1. *Aspect, elevation, mean (s.e.) diameter at breast height (DBH, 1·35 m) and basal area increment (BAI: in cm² of the last 10 years) at six treeline ecotone locations in the southern Andes of Chile (Antillanca, 40°47′S and 72°11′W, 1100–1350 m a.s.l.; Cerro Castillo, 45°59′S and 71°52′W, 1000–1300 m a.s.l.) and the Swiss Alps (Davos, 46°46′ N, 9°52′E, 1800–2200 m a.s.l.)*

Site	Aspect	Elevation	DBH	BAI	DBH	BAI
Antillanca			*Nothofagus pumilio*		*Nothofagus betuloides*	
Site 1	W	1140	9·7 (1·4)	121·2 (13·9)	10·2 (0·9)	61·0 (12·1)
		1200	7·5 (0·8)	38·6 (9·9)	13·4 (1·8)	91·4 (9·8)
		1290	5·3 (0·8)	29·07 (9·9)	9·3 (1·8)	69·4 (12·1)
		1350	7·2 (1·5)	15·9 (12·1)	7·0 (1·4)	29·7 (10·8)
Site 2	NW	1150	21·2 (3·2)	123·7(14·6)	18·6 (2·7)	102·6 (11·3)
		1200	10·3 (2·0)	47·8 (10·3)	12·8 (1·6)	39·4 (10·3)
		1270	7·3 (0·6)	51·6 (10·3)	7·4 (1·1)	58·0 (12·7)
		1320	7·1 (1·0)	22·4 (11·3)	6·8 (0·9)	38·3 (10·3)
Cerro Castillo			*Nothofagus pumilio*		*Pinus sylvestris*	
Site 1	W	1040	15·1 (1·9)	137·5 (35·6)	23·4 (2·7)	117·1 (32·5)
		1100	15·5 (1·9)	67·9 (18·7)	17·6 (1·9)	78·2 (35·2)
		1180	8·4 (2·1)	27·3 (15·6)	12·3 (2·3)	99·4 (31·4)
		1290	9·4 (1·9)	18·6 (12·3)	5·0 (1·2)	36·4 (22·4)
Site 2	E	1010	17·4 (2·2)	130·5 (26·5)	25·2 (2·3)	165·6 (35·6)
		1090	12·1 (3·4)	61·0 (20·5)	23·3 (2·7)	125·1 (33·1)
		1180	15·1 (1·8)	48·3 (17·4)	15·5 (2·5)	120·2 (25·8)
		1280	5·5 (1·2)	19·5 (12·2)	8·7 (2·1)	34·1 (13·5)
Davos			*Larix decidua*		*Pinus cembra*	
Site 1	NE	1820	23·7 (3·1)	143·3 (44·6)	15·3 (2·3)	268·8 (46·4)
		1915	22·0 (3·5)	224·5 (36·4)	18·6 (2·3)	92·7 (31·9)
		2000	12·5 (2·4)	160·1 (37·5)	13·2 (2·2)	293·8 (56·2)
		2205	3·5 (1·6)	82·4 (25·3)	4·9 (1·3)	117·5 (42·3)
Site 2	SW	1830	16·9 (4·9)	338·1 (61·7)	11·4 (2·4)	210·9 (42·5)
		1950	24·9 (1·1)	237·5 (40·3)	6·8 (1·1)	112·3 (45·7)
		2100	17·0 (2·5)	263·2 (41·7)	21·5 (2·1)	168·6 (51·1)
		2215	8·0 (1·1)	51·8 (29·6)	7·8 (1·1)	45·3 (37·8)

The four elevations, in increasing order, represent low, intermediate, timberline and treeline elevations.

This sampling was done in the vicinity of the experimental *L. decidua*–*Pinus mugo* subsp. *uncinata* plantation which had also been used for an *in situ* CO_2 fertilization experiment between 2001 and 2009 (Handa *et al.*, 2005; Dawes *et al.*, 2011). While the *N. pumilio*–*N. betuloides* and *L. decidua*– *P. cembra* sites constitute mixed-species forests, i.e. the two species grow intermingled, the *N. pumilio*–*P. sylvestris* sites are formed by 38-year-old plantation stands of *P. sylvestris* growing next to natural forests of *N. pumilio*; thus, we sampled in the frontier of both. For all species, we sampled adult trees (38–120 years old), discarding juveniles (<5 cm diameter at breast height; DBH).

Field sampling and initial sample processing

Sampling was conducted in late February and early March 2008 for the Antillanca and Cerro Castillo areas, and in mid-August 2008 for Davos. Sampling took place at the end of the growing season for each site, particularly because at this time NSC concentrations in deciduous species are expected to reflect the net balance between C source and C sinks (i.e. meristematic activity) after the period of maximum demand when NSC concentrations are not biased by the different bud break dates along the elevational gradients (with later bud breaks at higher elevations; see also the respective discussion in Hoch and Körner, 2012). For all species, trees were sampled at four equidistant elevations from the treeline altitude down to the tall forest (at least 200 elevational metres below the treeline); gradients were sampled at two different slopes (sites) in each location (Antillanca, Cerro Castillo and

Davos; Table 1). Per species and sampling elevation, six trees of dominant social status, each separated by a minimum of 25 m, were selected at each site for tissue collection. Only individuals with no signs of browsing or other damage, whose growth had not been apparently suppressed or shaded, were sampled. For each individual tree surveyed, we measured DBH (1·35 m) and bark thickness. The collection of tissue samples was conducted between 1000 and 1700 h. Two stem cores were taken at the base of the tree, one 10 cm in length (shorter when young trees were considered) for chemical analysis (stem sapwood) and a second to-the-pith long core at a height of approx. 30 cm for growth determination. We used a 5·15 mm increment bore (Haglöf, Långsele, Sweden) for this purpose. Additionally, from each sampled tree, we collected sun-exposed leaves or needles (needles from the previous season) and branch sapwood (with bark and phloem removed in the field with a knife) from 2- to 5-year-old branch segments. All tissue samples were bagged and labelled, and stored in a cool box for transportation. Woody samples for NSC analyses were heated in a microwave oven at 600 W for 90 s to denature enzymes. All samples (except the ones for tree growth determination) were then oven-dried at 80 °C for 48 h. Samples necessary for NSC analyses were then ground into a fine powder and stored after being sealed with silica gel at 4 °C until analysis (Popp *et al.*, 1996).

Tree growth determination

For each site, cores were prepared following standard dendrochronological techniques (Stokes and Smiley, 1996): cores were

dried, mounted and glued firmly in grooved wooden sticks, and sanded with successively finer grades of sandpaper until optimal surface resolution allowed annual rings to be distinguished under magnification ($\times 10$). Inside-bark bole radius and annual radial increments from the last 10 years were measured to the nearest 0·01 mm using a microscope mounted on a dendrochronometer with a Velmex sliding stage and Accurite measuring system. Cross-dating accuracy was checked visually as rings were easily identified. The last 10 year basal area increment (BAI), which represents a relative measure of gains in growth, was then computed by considering the diameter at coring height and bark thickness.

Chemical analyses

Our assessment of C reserves was based on the determination of NSC concentrations, which consist of the sum of the three most abundant low molecular weight soluble sugars (glucose, fructose and sucrose) and starch. NSCs were analysed following Wong's (1990) procedure as described in detail in Hoch *et al.* (2002). About 10 mg of plant powder were extracted with 2 mL of distilled water at 100 °C for 30 min. An aliquot of the extract was used to determine low molecular carbohydrates after enzymatic conversion of fructose and sucrose to glucose. The concentration of free glucose was determined photometrically after the enzymatic conversion of glucose to gluconate-6-phosphate on a 96-well multiplate reader. Following the degradation of starch to glucose using a crude fungal amylase ('Clarase' from *Aspegillus oryzae*, Enzyme Solutions Pty Ltd, Crydon South, Victoria, Australia) at 40 °C overnight, NSC was determined in a separate analysis. The starch concentration was calculated as NSCs minus the sum of free sugars. Total soluble sugar, starch and NSC concentrations are presented on a percentage of dry matter basis for all tissues (leaves, branch wood and stem sapwood).

Statistical analysis

The influence of elevation and species foliar habit on sugars, starch and NSCs was analysed fitting linear mixed models (LMMs). Since the focus of this study is on the comparison between foliar habit types, all statistical analyses were performed across all the species considered within a leaf type (deciduous or evergreen) instead of analysing the data at the single-species level. In the modelling, we considered elevation (low, intermediate, timberline and treeline) and species foliar habit (deciduous and evergreen) as fixed factors and sites as random factors to account for among-site variation. All analyses were done in R (R Development Core Team, 2012). In testing whether there is a shift between deciduous and evergreen treeline species' C storage across the elevational gradient, we computed mean values, confidence intervals, predicted differences between deciduous and evergreen, and inference (*t*- and *P*-values) on planned contrasts using the *esticon* function in the doBy package (Højsgaard *et al.*, 2012).

RESULTS

The decrease in the tree growth rate (BAI in cm^2 for the last 10 years) with elevation was pronounced and highly significant for all species (Table 1). Elevation had a significant effect on BAI ($F = 58{\cdot}035$, $P < 0{\cdot}001$); however, foliar habit had no effect on it ($F = 3{\cdot}230$, $P = 0{\cdot}074$). On average, BAI was highest at the lowest elevation and decreased significantly towards treeline elevation for all species, with an 85·1 % decrease between the lowest and the highest elevation for *N. pumilio*, 57 % for *N. betuloides*, 74·2 % for *P. sylvestris*, 63·6 % for *L. decidua* and 67·4 % for *P. cembra* (Table 1).

Non-structural carbohydrate concentrations proved to differ significantly in relation to elevation for leaves and branch tissues; and, in relation to foliar habit, NSCs differed significantly for all tissues (Table 2). In particular, total soluble sugar, starch and NSC concentrations were always significantly higher in deciduous than in evergreen treeline species for all tissues (Tables 2 and 3, Fig. 2). The mean NSCs of deciduous species were significantly higher than those of evergreen species, by 11 % (10·5 vs. 9·4 % dry matter, $t = 4{\cdot}56$, $P < 0{\cdot}001$), 158 % (7·5 vs. 2·9 % dry matter, $t = 20{\cdot}18$, $P < 0{\cdot}001$) and 103 % (3·7 vs. 1·8 % dry matter, $t = 18{\cdot}65$, $P < 0{\cdot}001$) for leaves, branch and stem wood tissues, respectively.

In contrast to tree growth, total soluble sugar, starch and NSC concentrations revealed no depletion with elevation, but mostly increased, indicating no C shortage. In particular, the NSCs of leaves and branch tissues increased significantly with elevation (Table 2). Additionally, the elevational patterns of NSCs differed between foliar habits. Although the interaction term between elevation and foliar habit was not significant for stem sapwood tissue (Table 2), deciduous species showed a steeper increase in NSCs with elevation (21·6 %) than evergreens (no change, Fig. 2). This result was entirely caused by a significant increase in the starch concentration in deciduous species, along with a decrease in the concentration of this compound for evergreen species (Fig. 3, insert). The opposite trend occurred for leaves and branch tissues, i.e. evergreen species had higher NSC increases from the lowest to the highest elevation (leaves, 6·5 vs. 24 %; branch, 10·3 vs. 37·8 %, for deciduous and evergreen species, respectively; Fig. 3), although only the increase for leaves was significant (Table 2). In general and independent of foliar habit, higher NSCs were found in leaves (e.g. 10·67 % maximum dry matter value for deciduous and 10·7 % for evergreens) and branches (7·83 and 3·43 % for the same comparison), while the lowest values were in stem wood (4·05 and 1·88 %, Table 3).

DISCUSSION

Foliar habit strategy to face lower temperatures

For all tissues examined in this study, NSC concentrations were always higher in deciduous species. This finding corroborates classical studies (Kozlowski, 1992; Vanderklein and Reich, 1999; Hoch *et al.*, 2003). More recent studies have also found higher NSC concentrations in deciduous than in evergreen tree species. For example, Piper and Fajardo (2012) compared growth rates and functional morphological and physiological traits between *N. pumilio* and *N. betuloides* juveniles in a non-treeline mixed forest (550 m a.s.l.) in Patagonia (Chile) and found that the former had higher NSCs than the latter; the difference between them was particularly high for roots (18·6 % for deciduous vs. 11·6 % for evergreen). Richardson *et al.* (2013) working in mixed forests in the eastern USA found that

TABLE 2. *Effects of elevation, foliar habit of treeline species and the interaction of both (E × FH) on the concentration of soluble sugars, starch and non-structural carbohydrates (NSC = sugars + starch) at different elevations in natural deciduous–evergreen treeline ecotones composed of* Nothofagus pumilio *and* N. betuloides *in the southern Andes (Antillanca, Chile, 40°47′S, 72°11′W, approx. 1100–1350 m a..s.l.),* Nothofagus pumilio *and* Pinus sylvestris *in Patagonia (Cerro Castillo, Chile, 45°59′ S, 71°52′W, approx. 1000–1300 m a.s.l.) and* Larix decidua *and* Pinus cembra *in the Swiss Alps (Davos, Switzerland, 46°46′N, 9°52′E, approx. 1800–2200 m a.s.l.)*

Effect	d.f.$_{num}$, d.f.$_{den}$	Soluble sugars		Starch		NSC	
		F	P	F	P	F	P
Leaves							
Elevation	3, 275	2·42	0·066	1·65	0·178	7·24	<0·001
Foliar habit	1, 275	130·94	<0·001	57·40	<0·001	22·65	<0·001
E × FH	3, 275	1·06	0·367	0·94	0·424	2·99	0·032
Branch							
Elevation	3, 275	8·72	<0·001	1·83	0·142	3·70	0·012
Foliar habit	1, 275	11·59	<0·001	416·38	<0·001	438·78	<0·001
E × FH	3, 275	7·21	<0·001	1·43	0·234	1·41	0·887
Stem sapwood							
Elevation	3, 275	1·44	0·231	0·42	0·740	1·52	0·211
Foliar habit	1, 275	28·94	<0·001	438·49	<0·001	441·76	<0·001
E × FH	3, 275	1·14	0·332	6·19	<0·001	1·97	0·119

deciduous species (*Acer rubrum*, *Quercus rubra* and *Fagus grandifolia*) had NSC concentrations of >24 mg g^{-1}, while evergreen coniferous (*Picea rubens* and *Tsuga canadensis*) had concentrations of <20 mg g^{-1}. Finally, Shi *et al.* (2008) also observed that deciduous species (*Larix potaninii* and *Betula platyphylla*) had higher levels of NSCs than evergreen species (*Abies georgie*, *Juniperus saltuaria* and *J. tibetica*) in the eastern Himalayas (China).

The evidence showing that deciduous species have higher C storage than evergreen tree species seems to be overwhelming, and yet we still do not have an answer to the following question: for what purpose do deciduous species have more C reserves? One possibility is that higher NSC concentrations in deciduous tree species may reflect an adaptation to tolerate abiotic and biotic (e.g. herbivory) stressors by means of replacing leaf area in cases where stressors provoke loss of photosynthetic area (Pallardy, 2008; Piper and Fajardo, 2012). On the other hand, what is more certain is that higher NSC concentrations in deciduous species imply higher requirements for C storage. In line with this, we predicted different C storage trends in relation to elevation (i.e. lower temperature as a stressor) for deciduous and evergreen treeline species (Fig. 1). We found a similar increase of NSCs with elevation for both foliar habits, but these increases varied for both foliar types depending on the tissue examined (Fig. 3). This means, first, that there is no evidence of C source limitation for either of the foliar habits, and, secondly, that there is no evidence of higher C limitation in deciduous than in evergreen treeline species. Thirdly, for leaves and branch tissues, evergreen species had significantly higher NSC increases with elevation than deciduous species. Although this result supports the idea that the higher NSCs of deciduous species imply higher requirements for C storage, and are most probably an adaptation to depend on having carbohydrates when facing stress (and C source limitation), the opposite was suggested when stem sapwood was analysed, i.e. deciduous species had higher NSCs increases with elevation than evergreen species (starch increased significantly with elevation, Fig. 3).

Stem wood represents a long-term C storage site, while leaves and branches are more affected by short-term source–sink fluctuations. In addition, stem sapwood seems to be a more important site of C storage in adult trees of most tree species, as the biomass proportion invested in stem sapwood greatly exceeds that of below-ground tissue, small branches or leaves. Thus, despite the fact that the NSCs of stem sapwood were lower than those found in leaves and young branches, most NSCs in trees are stored in stem sapwood, particularly in deciduous species (Chapin *et al.*, 1990). Carbon reserves in mature leaves of evergreen species can also make up for a high proportion of the whole tree C storage pool (see fig. 6 in Hoch and Körner, 2012). If we consider that NSCs are mostly accumulated in stem sapwood, we can then assert that there may be a higher increase in NSCs with elevation for deciduous species when compared with evergreens. This result suggests that deciduous species are no more susceptible than evergreens to experience C source limitations under reduced temperature in spite of their higher requirements for C storage and their higher risk of leaf losses.

Support for the GLH

In general, growth decreased with elevation, and NSCs moderately increased or remained stable across the elevational gradient. That is, we found a similar variation in C storage (no depletion) between deciduous and evergreen treeline species across elevational gradients located in disparate mountain chains such as the southern Andes and the Swiss Alps. Our results thus provide support for the GLH for both deciduous and evergreen species. Based on the existing evidence that NSCs mirror a tree's C balance (Chapin *et al.*, 1990; Dickson, 1991), C does not appear to be a limiting resource at these alpine treeline sites, for any foliar habit type. Although empirical support for the GLH has been found elsewhere (e.g. Shi *et al.*, 2006; Fajardo *et al.*, 2011; Hoch and Körner, 2012), i.e. in no case were C reserves reduced at treeline, but rather increased as one approaches the tree limit, there is currently very limited

TABLE 3. *Mean (s.e.) tissue concentrations of soluble sugars, starch and non-structural carbohydrates (NSC = sugars + starch) for five treeline species at different elevations in natural treeline ecotones*

	Leaves				Branch				Stem sapwood			
	Low	Int	Tb	Tl	Low	Int	Tb	Tl	Low	Int	Tb	Tl
Nothofagus pumilio												
Sugars	7·44 (0·36)	7·82 (0·27)	7·48 (0·52)	7·99 (0·54)	2·18 (0·12)	2·37 (0·13)	2·45 (0·09)	2·33 (0·07)	1·39 (0·07)	1·41 (0·08)	1·40 (0·06)	1·55 (0·07)
Starch	1·70 (0·18)	1·25 (0·23)	1·38 (0·22)	1·50 (0·24)	7·20 (0·58)	7·81 (0·38)	7·30 (0·27)	8·20 (0·33)	2·32 (0·22)	2·58 (0·19)	3·22 (0·2)	3·05 (0·24)
NSC	9·13 (0·32)	9·08 (0·22)	8·86 (0·43)	9·51 (0·35)	9·36 (0·59)	10·17 (0·5)	9·77 (0·3)	10·53 (0·3)	3·7 (0·19)	4 (0·21)	4·61 (0·23)	4·61 (0·28)
Nothofagus betuloides												
Sugars	5·58 (0·21)	5·66 (0·25)	6·48 (0·15)	6·36 (0·15)	1·77 (0·16)	2·58 (0·11)	2·58 (0·18)	2·92 (0·25)	1·48 (0·14)	1·73 (0·11)	1·62 (0·11)	1·54 (0·09)
Starch	2·26 (0·16)	2·17 (0·11)	2·21 (0·14)	2·58 (0·18)	2 (0·37)	0·98 (0·27)	1·11 (0·16)	1·51 (0·29)	1·07 (0·17)	0·87 (0·18)	0·73 (0·25)	0·63 (0·11)
NSC	7·85 (0·26)	7·81 (0·24)	8·71 (0·25)	8·93 (0·2)	3·76 (0·33)	3·58 (0·32)	3·68 (0·21)	4·4 (0·38)	2·56 (0·2)	2·58 (0·2)	2·34 (0·26)	2·17 (0·13)
Pinus sylvestris												
Sugars	6·81 (0·42)	6·41 (0·45)	6·72 (0·26)	7·62 (0·46)	1·24 (0·14)	1·78 (0·21)	2·32 (0·2)	2·33 (0·2)	0·85 (0·07)	0·87 (0·12)	0·92 (0·1)	1·05 (0·13)
Starch	1·95 (0·09)	2·21 (0·19)	2·44 (0·42)	3·12 (0·42)	0·93 (0·34)	0·88 (0·18)	1·72 (0·27)	2 (0·37)	0·83 (0·08)	0·6 (0·08)	0·73 (0·09)	0·63 (0·12)
NSC	8·75 (0·48)	8·6 (0·51)	9·15 (0·55)	10·71 (0·6)	2·16 (0·45)	2·64 (0·36)	4·03 (0·44)	4·32 (0·52)	1·7 (0·11)	1·47 (0·15)	1·65 (0·17)	1·69 (0·23)
Larix decidua												
Sugars	9·21 (0·96)	10·52 (0·3)	10·78 (0·4)	10·1 (0·32)	1·21 (0·1)	1·04 (0·21)	0·99 (0·14)	0·88 (0·12)	1·02 (0·08)	0·97 (0·08)	0·9 (0·06)	0·98 (0·06)
Starch	2·59 (0·67)	2·86 (0·29)	3·49 (0·53)	2·89 (0·33)	1·39 (0·16)	1·71 (0·32)	1·43 (0·17)	1·58 (0·15)	1·55 (0·13)	1·64 (0·1)	1·73 (0·13)	1·96 (0·12)
NSC	11·8 (0·69)	13·38 (0·5)	14·27 (0·6)	12·99 (0·6)	2·59 (0·24)	2·76 (0·49)	2·43 (0·24)	2·46 (0·2)	2·58 (0·16)	2·58 (0·13)	2·61 (0·18)	2·93 (0·13)
Pinus cembra												
Sugars	4·91 (0·21)	4·95 (0·21)	5·97 (0·28)	5·77 (0·19)	0·65 (0·07)	0·72 (0·06)	0·72 (0·12)	0·71 (0·07)	0·47 (0·06)	0·62 (0·06)	0·79 (0·08)	0·68 (0·07)
Sstarch	4·39 (0·62)	5·06 (0·92)	4·87 (0·45)	6·7 (0·42)	0·91 (0·09)	1·04 (0·23)	0·65 (0·09)	0·87 (0·09)	0·88 (0·08)	0·78 (0·07)	0·83 (0·07)	0·82 (0·08)
NSC	9·3 (0·77)	10·01 (1)	10·84 (0·5)	12·46 (0·5)	1·55 (0·15)	1·77 (0·26)	1·37 (0·19)	1·58 (0·15)	1·33 (0·06)	1·4 (0·09)	1·63 (0·09)	1·48 (0·11)

Elevations are site specific (see Table 1). The four elevations, in increasing order, represent low (Low), intermediate (Int), timberline (Tb) and treeline (Tl) elevations.
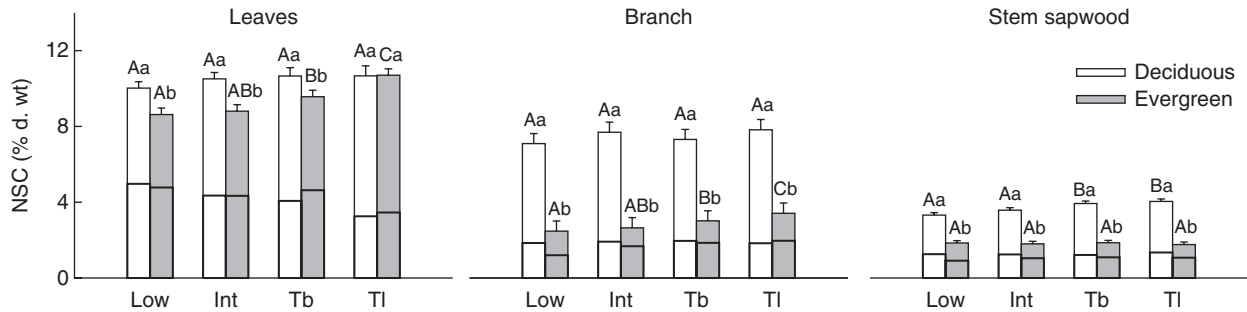
Fig. 2. General comparison between the concentrations of non-structural carbohydrates (NSCs = total soluble sugars + starch, in % dry matter) of deciduous (*Nothofagus pumilio* and *Larix decidua*) and evergreen (*N. betuloides*, *Pinus sylvestris* and *P. cembra*) treeline species for each elevation and tissue. Horizontal lines in each bar delimit soluble sugars (lower portion) and starch (upper portion). Different letters indicate significantly different ($P < 0.05$) NSC concentrations among elevations (uppercase) and between foliar habit species (lowercase) at each elevation. Elevations are site specific (see Table 1) and represent low (Low), intermediate (Int), timberline (Tb) and treeline (Tl) elevations.
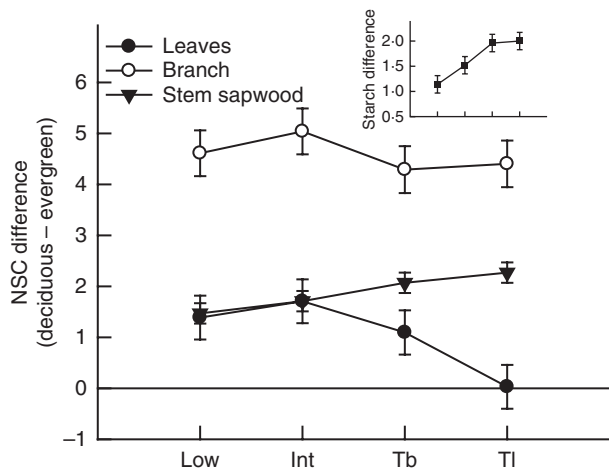

Fig. 3. Mean differences in concentration of non-structural carbohydrates (NSCs = total soluble sugars + starch) between deciduous (*Nothofagus pumilio* and *Larix decidua*) and evergreen (*N. betuloides*, *Pinus sylvestris* and *P. cembra*) treeline species for leaves, branch and stem sapwood across elevations. Inset: difference in total starch. Elevations are site specific (see Table 1) and represent low (Low), intermediate (Int), timberline (Tb) and treeline (Tl) elevations.

information on deciduous tree species at treeline. This is mostly due to the scarcity of alpine treeline sites dominated by deciduous species (aside from the scarcity of treelines where deciduous and evergreen tree species grow together). To the best of our knowledge, ours is the first study reporting C storage amounts across elevations for deciduous and evergreen treeline species growing next to one another, i.e. experiencing the same climatic conditions. Previously, only Shi *et al.* (2008) compared deciduous and evergreen tree species in the eastern Himalayas (China) – although tree species do not grow intermingled – and found support for the GLH. They also observed that the elevation pattern of NSCs did not indicate any difference between evergreen and deciduous taxa. Considering stem sapwood as a major, and most probably the main, C storage location in trees (see the discussion in the previous paragraph), the steeper increase of starch and NSC concentrations for stem sapwood in deciduous rather than evergreen species found in this study indicates that elevation leads to a more favourable C balance in the

former. Given that the leaves of deciduous species are more prone than those of evergreens to suffer from abiotic stress occurring with elevation (stronger C source limitations with elevation in deciduous), we interpret this result as a consequence of stronger C sink limitations in deciduous than in evergreen species, where the difference for sink activity appears to be higher than that for C sources (C sinks differ between leaf habits more than C sources).

In light of the previous evidence, the possibility that C limitations may drive treelines formed by deciduous species has not been totally rejected. The work of Handa *et al.* (2005) showed that *L. decidua* increased its growth (radial stem growth and annual shoot increment) at treeline elevation as a response to a 4 year addition of CO_2. Although this result clearly points to a C limitation of *L. decidua* (support for the CLH) in this treeline experiment and disagrees with our results with the same species – we sampled individuals of *L. decidua* which were in close proximity to the CO_2 experimental site – there are some aspects which can explain such a contradiction. Given that the *L. decidua* trees subjected to CO_2 fertilization were young trees (approx. 30 years old), it has been claimed that the positive effect of a C addition would be of a transitory nature (Körner, 2006). In the same vein, Dawes *et al.* (2011) found that the growth response decreased in later years and became insignificant after 8 years of increased CO_2 treatment. Alternatively, the period of CO_2 enrichment in this experiment comprised several exceptionally warm growing seasons, including the European centennial heatwave summer of 2003. Nevertheless, even if warmer than usual growing seasons might have favoured the positive CO_2 response of growth at this treeline site, it remains remarkable that *Larix*, but not *Pinus* (which was also treated with elevated CO_2 at the same site), reacted. Moreover, the initial positive growth response of *L. decidua* to the CO_2 addition was found at treeline elevation and was never compared with lower elevations. Hence, it is possible that *L. decidua* is responsive to CO_2 fertilization regardless of the elevation. In such a hypothetical case, this deciduous species would not become C limited with elevation.

Finally, the usefulness of NSC tissue concentrations as a proxy for the C supply status of a plant has recently been questioned (Sala *et al.*, 2012; Wiley and Helliker, 2012). In essence, this questioning is based on the rationale that an accumulation of C

reserves can occur despite C limitation in plants, i.e. plants that experience C shortage may actively increase their C reserve pool at the expense of their growth and other C sink activities (Wiley and Helliker, 2012). However, all experimental evidence thus far stands against this notion and NSC concentrations were found to decrease whenever plants were exposed to unequivocal situations of limiting C supply, such as low atmospheric CO_2 concentrations (Agüera *et al.*, 2006; Schädel *et al.*, 2010), defoliation (Canham *et al.*, 1999; Li *et al.*, 2002; Myers and Kitayima, 2007; Piper and Fajardo, 2012) or shade (Piper *et al.*, 2009). If low temperatures directly exerted C limitation on tree growth, one would also assume a decrease rather than an increase of C reserves in those trees. Hence, we state that in view of all existing experimental and observational evidence, increasing C reserve concentrations are not to be expected in plants faced with C limitation, and comparative analyses of NSC concentrations can indeed indicate the C supply status of plants (Fajardo *et al.*, 2012).

Conclusions

We show here that both deciduous and evergreen treeline species have similar patterns of NSCs with elevation (i.e. no decrease, but a tendency to increase concentrations), indicating that an environmental driver (temperature) inflicts a similar physiological response in treeline species regardless of their distinctive leaf phenological strategies. Overall, this supports the hypothesis that treeline species, independent of their foliar habit and requirements for C storage, have the same physiological response to decreasing temperatures. However, the more pronounced NSC increase with elevation found in stem sapwood of deciduous species might suggest that deciduous species are more growth limited by low temperatures than evergreen species.

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