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Distinguishing local from global climate influences in the variation of carbon status with altitude in a tree line species

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ABSTRACT

Aim Two alternative hypotheses attempt to explain the upper elevation limit of tree lines world-wide, the carbon-limitation hypothesis (CLH) and the growth-limitation hypothesis (GLH); the altitudinal decrease of temperature is considered the driver constraining either carbon gain or growth. Using a widely distributed tree line species (*Nothofagus pumilio*) we tested whether tree line altitude is explained by the CLH or the GLH, distinguishing local from global effects. We elaborated expectations based on most probable trends of carbon charging with altitude according to both hypotheses, considering the alternative effects of drought.

Location Two climatically contrasting tree line ecotones in the southern Andes of Chile: Mediterranean (36°54' S) and Patagonia (46°04' S).

Methods At both locations, 35–50 trees of different ages were selected at each of four altitudes (including tree line), and stem and root sapwood tissues were collected to determine non-structural carbohydrate (NSC) concentrations. NSC accumulates whenever growth is more limited than photosynthesis. An altitudinal increase in NSCs means support for the GLH, while the opposite trend supports the CLH. We also determined stable carbon isotope ratios ($\delta^{13}\text{C}$) to examine drought constraints on carbon gain.

Results NSC concentrations were positively correlated with altitude for stem tissue at the Mediterranean and root sapwood tissue at the Patagonia site. No depletion of NSC was found at either site in either tissue type. For both tissues, mean NSC concentrations were higher for the Patagonia site than for the Mediterranean site. Mean root sapwood NSC concentration values were five times higher than those of the corresponding stem sapwood at all altitudes. Values for $\delta^{13}\text{C}$ were positively correlated with altitude in the Mediterranean site only.

Main conclusions We found support for the GLH at the site without drought effects (Patagonia) and no support for the CLH at either site. It is suggested that drought moderated the effects of low temperature by masking the expected trend of the GLH at the Mediterranean site.

Keywords

Carbon balance, carbon isotope composition, Chile, drought, Mediterranean climate, non-structural carbohydrates, *Nothofagus pumilio*, Patagonia, tree line ecotone.

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INTRODUCTION

There is a general agreement that, at the global scale, low temperature during the growing season limits tree line altitude by affecting the carbon balance (Tranquillini, 1979; Körner, 1998; Wardle, 1998; Jobbágy & Jackson, 2000; Hoch & Körner, 2003; Smith *et al.*, 2003). Two alternative hypotheses have been proposed to explain the functional mechanisms behind the upper elevation limit of tree lines: the carbon-limitation hypothesis (CLH) and the growth-limitation hypothesis (GLH). The CLH predicts that the universal altitudinal reduction in tree growth at or close to the alpine tree line is a consequence of the decline in photosynthetic rates provoked by the altitudinal decrease in temperature (Stevens & Fox, 1991; Wardle, 1993). The tree line will thus occur at the elevation where annual carbon gains cannot compensate carbon losses. Alternatively, the GLH claims that cell and tissue formation are the processes being limited by the altitudinal decrease in temperature (Körner, 1998; Grace *et al.*, 2002), indicating a low temperature threshold for growth itself rather than a limitation on carbon gain (Körner & Paulsen, 2004). The carbon source–sink balance dilemma of trees at the altitudinal tree line has received increasing attention lately with respect to the role that tree line trees have in carbon sequestration and rates of carbon cycling in response to the ongoing atmospheric changes (Handa *et al.*, 2005, 2006; Hagedorn *et al.*, 2008). In this respect, a clear mechanistic understanding of tree line formation world-wide is needed in order to predict the potential impacts and changes caused by human activity and related global change in this sensitive region (Wieser, 2007).

One major limitation in the search for a universal explanation for tree line formation is the presence of local climatic factors other than temperature (Daniels & Veblen, 2003), which can also alter plant carbon balance (Körner, 1998). Drought is probably one of the most important local factors affecting tree line trees around the world (Körner, 1998; Piper *et al.*, 2006), and is expected to increase its effects due to global warming (IPCC, 2007). As with low temperature, drought may restrict both carbon gain and growth processes (i.e. cell expansion), although it is still unclear which process is more limited (Chapin *et al.*, 1990; Würth *et al.*, 2005; McDowell *et al.*, 2008; Sala *et al.*, 2010). In general, plant traits of drought and cold resistance are similar (Larcher, 2003), and so it is possible that trees of the tree line ecotone are adapted not only to cold conditions but also to drought. As a result, the highest tree lines around the world are located in semi-arid regions (e.g. northern Chile and Bolivia, the *Polylepis tarapacana* tree line); on the contrary, tree lines are depressed in humid areas such as coastal or front range mountains or in the equatorial tropics (Körner, 2003; Körner & Paulsen, 2004). Assuming that the effects of drought on the tree line ecotone will be weaker than the effects of low temperature, we can predict that drought will modulate the general trend induced by low temperatures (see below).

In the southern Andes of Chile and Argentina, *Nothofagus pumilio* (Poepping et Endlicher) Krasser (Nothofagaceae), a deciduous broadleaf species, dominates the alpine tree line, spanning a wide latitudinal range, from the Mediterranean-type

climate of central Chile (35° S) to the temperate humid–climate of Tierra del Fuego at the southernmost point of the continent (55° S). This ample latitudinal distribution provides a unique opportunity to study the mechanisms responsible for tree line formation under contrasting climates and growing season length while controlling for phylogenetic effects. To our knowledge, no study has so far examined the carbon budget of the same species at climatically different sites. Such studies would allow us to distinguish local from global influences, thus helping in the search for a universal explanation for tree line formation.

The most straightforward approach to solve the debate around tree line formation is the comparison of non-structural carbohydrate (NSC; low-weight soluble sugars plus starch) concentrations of a tree line species across an altitudinal gradient (e.g. Li *et al.*, 2002; Hoch & Körner, 2003; Körner, 2003). NSC accumulates whenever growth is more limited than photosynthesis. Accordingly, NSC compounds either accumulate or decrease depending on the source–sink balance (photosynthesis versus growth and respiration) (Chapin *et al.*, 1990). In this study, we looked at specific expectations based on the most probable trends of NSC concentration with altitude (lower temperature) according to the CLH and the GLH and considering the alternative effects of drought (Fig. 1). We expected that if photosynthesis becomes more limited with altitude than growth because of low temperatures, NSC will decrease (acquisition < demand) across the altitudinal gradient, giving support to the CLH (Fig. 1; decreasing continuous line, left panel). Conversely, if low temperatures at the tree line ultimately impede carbon-consuming processes (growth) more than carbon-accumulating processes, NSC will increase with elevation (acquisition > demand), giving support to the GLH (Fig. 1; increasing continuous line, right panel). Concomitantly, the presence of drought, as low temperature, may promote sink limitations (i.e. reduced growth and respiration; Körner, 2003; Würth *et al.*, 2005; Sala *et al.*, 2010) or carbon limitations (e.g. Bréda *et al.*, 2006; McDowell *et al.*, 2008). If drought restricts carbon gain more than carbon demand, lower NSC concentrations are expected to be found (Fig. 1, long-dashed lines). Contrary, if drought restricts carbon demand more than carbon gain, higher NSC concentrations are expected to be found (Fig. 1, short-dashed lines). Furthermore, if drought limitations operate in the same direction as low-temperature limitations (i.e. either limiting carbon gains or carbon demands) a steeper NSC–altitude slope is predicted because drought acts as an additive factor (Fig. 1, long-dashed line left panel or short-dashed line right panel). In contrast, if drought limitations are opposite to those effects imposed from low temperature (i.e. drought-limiting carbon demands and temperature-limiting carbon gain and vice versa), a more moderate response in the concentration of NSC values is expected to occur (Fig. 1, short-dashed line left side, long-dashed line right side) because drought is offsetting temperature effects. This drought-moderated response may mask the actual pattern induced by low temperature.

The aim of this study was to test whether tree line formation in the southern Andes is explained by the CLH or the GLH with the distinction of local from global effects. Specifically, we

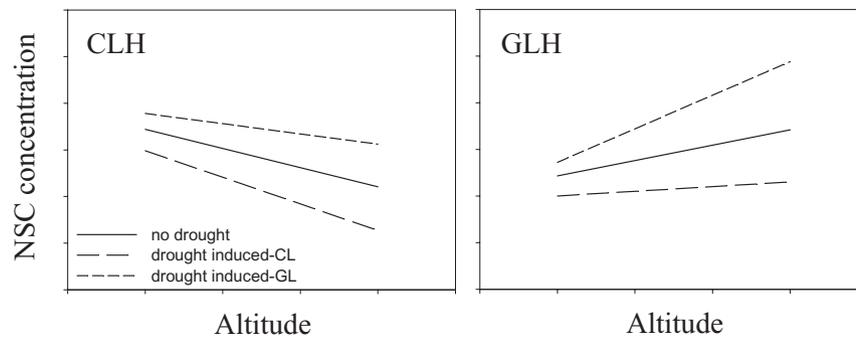


Figure 1 Expected trends of non-structural carbohydrate concentrations (NSC; sugars plus starch) with altitude (temperature effect) according to the carbon-limitation hypothesis (CLH; left panel) and the growth-limitation hypothesis (GLH; right panel) considering the alternative effects of drought. The sole effect of temperature supporting either the CLH or the GLH is depicted by a continuous line; its slope necessarily differs from 0. The inclusion of drought is depicted by dashed lines. When drought induces carbon limitation (long-dashed lines) its effect will be either additive to (slope $\ll 0$) or opposite to temperature (slope ~ 0) under the CLH or a GLH, respectively. On the contrary, when drought induces growth limitation (short-dashed lines) its effect will be either opposite to (slope ~ 0) or additive to temperature (slope $\gg 0$) under a CLH or a GLH scenario, respectively (see text for more details on the expectations).

addressed the following questions: (1) do NSC concentrations increase or decrease with altitude; and (2) how does drought alter the carbon balance in concert with the CLH or the GLH? To respond to these questions, we determined and compared current NSC concentrations in woody tissues of *N. pumilio* trees across altitudes at two climatically contrasting sites (different water availability during the growing season). From the comparison of mean NSC values between the two tree lines, we established whether drought induces carbon or sink limitations on this species. This procedure is based on the fact that tree line temperatures are quite similar around the world (Körner & Paulsen, 2004). Finally, by comparing altitudinal trends between the two tree lines we expected to quantify the bias produced by drought on the global low temperature-induced pattern (Fig. 1). We further examined our model expectations using isotopic analysis. Few studies (including Holtmeier & Broll, 2005) have examined local influences on the global pattern of causes of tree lines in a natural system.

METHODS

Research sites description

We selected tree line sites that have not been depressed by human activity or disturbed by landslides or avalanches but represent the natural climatic tree limit of the respective region. Finding such places in the southern Andes is less difficult than in Eurasia, where traditional human land use near the tree line is common (Körner, 2003). Our sites were located on upper slopes with low angles ($< 30\%$), a straight configuration and with the presence of alpine vegetation above the tree line. The study areas were selected to represent regional differences in climate between central Chile and central Patagonia. The forests of southern Chile are split between a seasonal ($36\text{--}42^\circ\text{S}$) and non-seasonal zone ($42\text{--}55^\circ\text{S}$); the difference between them is the presence of a sharp seasonal drought at latitudes lower than 42°S (Armesto *et al.*, 1995; Luebert & Plissock, 2006).

The first tree line site was located in Termas de Chillán ($36^\circ54'\text{S}$ and $71^\circ24'\text{W}$, 2100 m a.s.l.), hereafter the Mediterranean site, where *N. pumilio* is mixed with a few individuals of *Nothofagus antarctica*. This area belongs to the meso-Mediterranean belt with humid climatic conditions and a predominant drought period during summer (Almeyda & Sáez, 1958; Miller, 1976; Amigo & Ramírez, 1998). In this area most of the precipitation falls as snow from May to September (DGA, 2008). Mean annual precipitation for 1990 to 2007 at Las Trancas climate station ($36^\circ55'\text{S}$ and $71^\circ29'\text{W}$, 1200 m a.s.l.) located 12 km west from the study site was 1970 mm, being 165 mm for the five warmest months (November and March; Table 1; DGA, 2008) and *c.* 199 mm, for the same period at Diguillín climate station ($36^\circ57'\text{S}$ and $71^\circ39'\text{W}$, 710 m a.s.l.) located 30 km from the study site. The annual mean temperature for Diguillín climate station is 11.8°C and the mean temperature of the warmest month is 17.6°C , while from November to March mean temperatures are higher than 15°C ; potential evapotranspiration is 644 mm (Luebert & Plissock, 2006). The duration of the growing season at our study site can be up to 215 days (period measured January 2000 to December 2001; Körner & Paulsen, 2004). The soil is derived from andesitic rocks of volcanic origin, and ranges from 0.1 to 0.5 m in depth, decreasing strongly with elevation (Veit & Garleff, 1995).

The second study site was within the Cerro Castillo Natural Reserve ($46^\circ04'\text{S}$ and $72^\circ03'\text{W}$, 1310 m a.s.l.), hereafter the Patagonia site, where *N. pumilio* is the only tree species at the tree line. This area belongs to the supra-Temperate belt with humid climatic conditions (Almeyda & Sáez, 1958; Amigo & Ramírez, 1998). Mean precipitation for 1992 to 2007 was 416 mm for the five warmest months (November and March) at Villa Cerro Castillo climate station ($46^\circ07'\text{S}$ and $72^\circ09'\text{W}$, 340 m a.s.l.; Table 1) located 15 km south-west from the study site; most of the precipitation falls as snow from April to October (DGA, 2008). Annual mean temperatures at the two stations closest to the site are 8.6°C (Coyhaique, $45^\circ34'\text{S}$ and $72^\circ04'\text{W}$, 330 m a.s.l.) and 6°C (Balmaceda, $45^\circ54'\text{S}$ and $71^\circ42'\text{W}$, 540 m a.s.l.).

Table 1 Mean soil volumetric water content (VWC; $\text{m}^3 \text{m}^{-3}$), mean precipitation (mm) and mean temperature ($^{\circ}\text{C}$) for the seasonal growth months (November–March) for the study sites, Mediterranean ($36^{\circ}54' \text{S}$ and $71^{\circ}24' \text{W}$, 2100 m a.s.l.) and Patagonia ($46^{\circ}04' \text{S}$ and $72^{\circ}03' \text{W}$, 1300 m a.s.l.), Chile.

	November	December	January	February	March
Mediterranean					
Soil VWC	0.112 (0.006)	0.058 (0.002)	0.044 (0.002)	0.034 (0.003)	0.099 (0.006)
Precipitation	43.75 (5.25)	40.58 (4.95)	25.05 (7.43)	26.95 (5.04)	28.75 (6.06)
Temperature	13	15	17	17	16
Cerro Castillo					
Soil VWC	0.263 (0.003)	0.182 (0.004)	0.127 (0.007)	0.210 (0.001)	0.218 (0.001)
Precipitation	90.73 (6.89)	80.29 (7.43)	79.75 (7.18)	71.87 (9.92)	92.98 (7.42)
Temperature	11	12	15	15	12

Soil VWC measurements were taken at a soil depth of ~ 20 cm at both tree lines using an ECH2O Dielectric Aquameter probe (F.I.P., unpublished). Mean precipitation for the Mediterranean site was recorded between 1990 and 2007 at Las Trancas ($36^{\circ}54' \text{S}$ and $71^{\circ}29' \text{W}$, 1200 m a.s.l.) located 12 km west of the study area (DGA, 2008); for mean temperature we used data from the closer climate station (Diguillín, $36^{\circ}86' \text{S}$ and $71^{\circ}63' \text{W}$, 710 m a.s.l.). Mean precipitation for Patagonia was recorded between 1992 and 2007 at Villa Cerro Castillo ($46^{\circ}07' \text{S}$ and $72^{\circ}09' \text{W}$, 340 m a.s.l.) located 15 km south-west of the study site (DGA, 2008); for mean temperature we used data from the closer climate station (Coyhaique, 45°S $57' \text{W}$ and 72°S $02' \text{W}$, 343 m a.s.l.) (Luebert & Plissock, 2006).

Mean temperatures of the warmest month for the same two climate stations are 14.2°C (Coyhaique) and 11.5°C (Balmaceda); potential evapotranspiration for Coyhaique is 584 mm (Luebert & Plissock, 2006). The duration of growing season was 144 days for the period measured between March 2008 and April 2009 (G. Hoch, unpublished). The soil here is derived from aeolian volcanic ash deposits (Veit & Garleff, 1995). The aspects where sampling was conducted were south-west for the Mediterranean and south for the Patagonia sites.

According to the tree line paradigm (Körner, 1998; Körner & Paulsen, 2004), both tree line sites, though located at different altitudes, should show similar mean growing season temperatures. Actual soil temperatures, however, seem to differ between sites: Körner & Paulsen (2004) recorded mean growing season temperatures of 8.7 and 9.1°C for Termas de Chillán (our Mediterranean site, for the period April 2000 to December 2001), while G. Hoch (unpublished data) found a mean growing season temperature of 6.9°C for our Patagonia site (for the period March 2008 to March 2009). Moisture measurements for the first 30 cm of soil (volumetric water content, VWC) at the tree line also differed for both sites (F.I.P., unpublished). For a complete year, VWC values were on average $0.0855 \text{ m}^3 \text{ m}^{-3}$ ($\text{SE} = 0.0029$) at the Mediterranean site and $0.1659 \text{ m}^3 \text{ m}^{-3}$ ($\text{SE} = 0.0030$) at the Patagonia site. VWC values for the period November–March were on average $0.0694 \text{ m}^3 \text{ m}^{-3}$ ($\text{SE} = 0.0052$) at the Mediterranean site and $0.2001 \text{ m}^3 \text{ m}^{-3}$ ($\text{SE} = 0.0065$) at the Patagonia site (Table 1).

Field sampling and initial sample processing

As NSC levels are more stable at the end of the growing season (Hoch & Körner, 2009), the sampling was conducted during late February for the Patagonia site and mid March for the Mediterranean site. The difference in sampling times compensated for the length of growing season. *Nothofagus pumilio* trees were

studied at four different equidistant altitudes covering the upper *c.* 300 m of the tree line ecotone at each site (Table 2). For clarity and brevity, we henceforth refer to altitudes by the following acronyms: closed and mature forest (CF); intermediate stand between closed forest and timber line (IN); timber line (TB); and tree line (TL). At each altitude, we set sampling points along a sampling line every 30 m, where four to five trees belonging to different height classes (i.e. different ages) were selected for sampling. A total of 35–50 trees were selected at each altitude. Individuals with extensive browsing or other damage were excluded. Height and stem diameter at breast height (d.b.h., 1.35 m) were recorded on the selected trees, spanning the whole range of dimensions to enable us to estimate a wide range of potential ages. Tree ages were estimated using exponential models fitted to the age–d.b.h. relationship for each altitude determined from a data subset of tree cores collected at each altitude ($n = 10\text{--}15$) (A.F., unpublished). This sampling protocol covered the altitudinal gradient from closed, tall forest (absence of krummholz trees) stands below the timber line to the uppermost tree line (krummholz of *c.* 2 m height). The tree line definition adopted here refers to the transition from the uppermost krummholz forest to the treeless alpine vegetation (Körner, 1998, 2003). We were certain that we covered the entire transition given that *Nothofagus* tree lines are known to form a very sharp transition between forest and treeless alpine vegetation (Wardle, 1998; Daniels & Veblen, 2003). In Table 1 the structural characteristics of stands at each site are exhibited.

From each sampled tree, the following plant material was collected between 10:00 and 17:00 h, stem xylem cored at *c.* 30 cm height (using a 5.15-mm increment bore, Haglöf, Långsele, Sweden); and superficial fine root xylem with diameters between 5 and 10 mm (without bark), which were excavated and clipped by removing a sod of soil. All tissue samples were bagged and labelled and stored in a cool-box for transportation. In the laboratory the samples were heated in a microwave oven at

Table 2 Structural stand characteristics for the tree line *Nothofagus pumilio* study sites, Mediterranean (36°54' S and 71°24' W, 2100 m a.s.l.) and Patagonia (46°04' S and 72°03' W, 1300 m a.s.l.), Chile.

Site	Trees (<i>n</i>)	Altitude (m a.s.l.)	d.b.h. (cm)	Age (years)	Height (m)
Mediterranean					
CF	40	1750	1.5–75.0	14–344	2.0–24.9
IN	37	1860	1.5–77.0	16–335	2.0–19.6
TB	37	1950	1.8–52.0	48–206	2.0–11.0
TL	35	2080	1.5–20.5	15–122	2.0–2.5
Means			14.53 (1.24)	96 (6.87)	6.73 (0.41)
Patagonia					
CF	50	1080	0.5–94.8	16–416	1.5–19.4
IN	50	1184	1.2–56.1	14–375	1.5–16.3
TB	50	1250	1.5–30.6	45–164	2.0–12.6
TL	50	1320	2.0–21.6	19–127	1.8–2.5
Means			16.56 (1.06)	106 (5.50)	6.99 (0.39)

CF, closed forest; IN, intermediate forest; TB, timber line; TL, tree line.

Values shown for d.b.h., age and height are ranges; values with parentheses are means (\pm SE).

600 W for 90 s to denature the enzymes, and dried to a constant weight at 80 °C for 48 h. The dry mass of each organ was recorded, and the dried samples were then ground into a fine powder and stored sealed over silica gel at 4 °C until analysis. We used the outer 4 cm sections of the stem cores for NSC analysis because they contain the highest concentrations of sugar and starch (Hoch *et al.*, 2003). The whole xylem was considered active sapwood. Wood tissues increase in density with increasing altitude and NSC concentration in wood, on a dry matter basis, might be *diluted* by structural compounds (Hoch *et al.*, 2002). To solve this problem, stem wood density was estimated for each tree at each elevation as dry core mass divided by *green* core volume. Thus, all sugars and starch concentrations were expressed per unit of volume (mg cm^{-3}) for stems.

Chemical analyses

NSCs are defined here as free, low molecular weight sugars (glucose, fructose and sucrose) plus starch (Chapin *et al.*, 1990). Samples were analysed for total soluble sugars and starch using ethanol and perchloric acid to extract the compounds (Hansen & Moller, 1975). Total soluble sugars were extracted from tissue in 86% v/v ethanol at 80 °C for 1 h. The supernatant was collected after centrifugation and the concentration of total soluble sugars was determined spectrophotometrically by the resorcinol method (Roe, 1934) at a wavelength of 520 nm, using sucrose as the standard. Starch was extracted from the ethanol-insoluble fraction by agitating for 15 min with 35% v/v perchloric acid (Sutton *et al.*, 1981). The protocol for starch determination in the extract was similar to that used for sugars, but using glucose as the standard. Starch and soluble sugars in each plant component were added together to determine total NSC in mg g^{-1} dry mass. This method of extraction can yield starch values higher than those estimated by more accurate enzyme methods (e.g. Rose *et al.*, 1991; Hoch & Körner, 2003), probably as a result of hydrolysis of some cell wall components. To check for this we conducted a pilot study where we analysed a subsample ($n = 87$)

with the enzyme method to evaluate whether there was any important deviation in the soluble sugar and starch determinations. Mean soluble sugar and starch concentrations did not differ significantly between methods ($t = 8094$, $P = 0.366$ for soluble sugars and $t = 8209$, $P = 0.213$ for starch, Mann–Whitney *U*-test). Correlations between both the resulting values of methods were also significant; therefore we kept the values obtained with the resorcinol method.

The ratio between total soluble sugar (TSS) concentration and starch concentration was calculated for each tree to achieve a better comprehension of the effects of drought and altitude (low temperature) on carbon balance. Increases in the TSS/starch ratio may, for example, occur under osmotic adjustments, whereas a decrease in the TSS/starch ratio is expected when processes of accumulation of NSC become relatively more important than processes of mobilization of NSC (i.e. growth limitation).

Carbon isotope ratio analysis was conducted across altitudes for each location to examine potential effects of drought occurring concomitantly with decreasing temperatures, i.e. $\delta^{13}\text{C}$ as an indicator of tree water use efficiency (WUE; Dawson *et al.*, 2002). Although our two study sites differ in moisture availability, we cannot assume that such environmental differences will necessarily be experienced by the trees. In this regard, the $\delta^{13}\text{C}$ data may reflect the extent to which drought is being actually experienced by trees. Thus, by measuring $\delta^{13}\text{C}$ across altitudes at each tree line we distinguished the presence of local (drought) from global (temperatures) influences on the carbon balance of trees, and hence, on the NSC trend. The $\delta^{13}\text{C}$ of plant tissue along elevation gradients reflects changes physically tied to altitude, such as decreasing atmospheric pressure and temperature, and changes that are not generally altitude specific, like humidity (Körner, 2007). The influences of both atmospheric pressure and temperature on $\delta^{13}\text{C}$ have been well described in the literature and consequently can be predicted. Considering an altitudinal decrease of $\delta^{13}\text{C}$ with elevation of 0.12‰ per 100 m elevation (Körner, 2003), any variation in $\delta^{13}\text{C}$ after altitudinal

correction was mostly attributed to moisture. Although moisture increases with altitude in most of world-wide altitudinal gradients, this is not a general pattern (Körner, 2003; Körner, 2007). In February–March 2007, we collected 5.15 mm diameter increment cores from 9–11 adult trees (80–140 years old; a subsample of the trees sampled for NSC) at 0.5 m height at each altitude ($n = 76$). Cores were air dried and then sanded with successively finer grades of sandpaper to reveal annual rings. We cross-dated cores visually using narrow marker years and dissected the last 10 years of growth for isotope analysis. $\delta^{13}\text{C}$ analysis was performed with a continuous flow mass spectrometer of powder stem sapwood at the Berkeley Center for Stable Isotope Biogeochemistry, University of California, CA, USA. The stable composition of stem sapwood tissues was computed as: $\delta^{13}\text{C} = 1000 \times [(R_{\text{sample}}/R_{\text{standard}}) - 1]$, where R_{sample} indicates the ratio between the abundance of the rarest isotope (^{13}C) and the abundance of the lighter one (^{12}C) for the specific sample (Farquhar *et al.*, 1989). R_{standard} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the international Vienna Pee Dee Belemnite carbon standard.

Statistical analysis

Stem and root sapwood NSC concentrations were not normally distributed (Kolmogorov–Smirnov test for goodness of fit) and hence were log-transformed prior to any statistical analysis. As we were interested in testing the correlation between NSC concentration and altitude, we analysed our data using the model type II regression method. We could thus examine our research questions with one robust, pertinent and versatile analysis. Regression slopes were then computed as standardized major axes (SMA). For empirical correlations (trends) between NSC concentrations and altitudes for each site, SMA slopes were determined and compared with expected correlations set a priori (Fig. 1). Also, for testing for significant differences between altitudes, differences in the SMA slopes were tested following Warton & Weber (2002) using SMA tests and routines (Falster *et al.*, 2003). When common slopes between altitudes were demonstrated (homogeneity of slopes) and calculated, differences in the *elevation* of the regression slopes (y -intercept) between the four altitudes were tested using an ANCOVA-like procedure (ANOVA following transformation of the data to remove any correlation between NSC concentrations and tree age or height; Falster *et al.*, 2003). Whenever common slopes could not be demonstrated, we used two-way ANOVAs for comparing mean values between altitudes and sites. To examine altitudes and between-sites differences in NSC concentrations and $\delta^{13}\text{C}$ values, we also used this ANCOVA-like procedure. The statistical software (S)MATR (Falster *et al.*, 2003) was used for all the analyses. Tukey's honestly significant difference (HSD) *post hoc* test was applied whenever a significant difference between altitudes was found.

RESULTS

We found no depletion of NSC at the tree line at either site in either tissue type, anticipating no support for the CLH. In

Table 3 Comparisons of non-structural carbohydrate concentrations (NSC; sugars plus starch) and sugar–starch ratio of stem and root sapwood (altitudinal mean \pm SE) between four altitudes and two latitudes, with F and P (in parentheses) statistics.

	Mediterranean	Patagonia	F
Stem sapwood			
NSC (mg cm^{-3})			
CF	16.4 \pm 1.3 a	26.1 \pm 1.2 a	33.4 (< 0.001)
IN	32.7 \pm 2.4 b	22.4 \pm 1.2 a	7.5 (0.008)
TB	29.1 \pm 2.0 b	21.2 \pm 1.5 a	1.8 (0.187)
TL	32.3 \pm 1.9 b	24.4 \pm 1.6 a	2.3 (0.136)
F	13.1 (< 0.001)	1.5 (0.091)	
Ratio			
CF	0.75 \pm 0.10 a	0.2 \pm 0.006 a	37.7 (< 0.001)
IN	0.20 \pm 0.02 b	0.24 \pm 0.020 a	6.7 (0.011)
TB	0.32 \pm 0.01 b	0.58 \pm 0.016 b	1.9 (0.176)
TL	0.10 \pm 0.01 c	0.46 \pm 0.009 a	31.7 (< 0.001)
F	23.3 (< 0.001)	8.6 (< 0.0001)	
Root sapwood			
NSC (mg g^{-1})			
CF	113.3 \pm 10.3 a	143.6 \pm 5.8 a	7.4 (0.008)
IN	80.2 \pm 5.3 b	168.2 \pm 7.3 a	49.6 (< 0.001)
TB	75.3 \pm 6.0 b	180.7 \pm 9.3 b	37.6 (< 0.001)
TL	109.7 \pm 8.7 a	216.6 \pm 10.2 c	23.9 (< 0.001)
F	7.5 (< 0.001)	13.5 (< 0.001)	
Ratio			
CF	0.21 \pm 0.02 a	0.24 \pm 0.01 a	2.3 (0.130)
IN	0.25 \pm 0.09 a	0.25 \pm 0.01 a	0.1 (0.879)
TB	0.29 \pm 0.01 a	0.22 \pm 0.01 a	0.7 (0.412)
TL	0.26 \pm 0.01 a	0.21 \pm 0.01 a	9.5 (0.003)
F	1.22 (0.307)	0.83 (0.479)	

Concentration values were calculated for *Nothofagus pumilio* tree line species at two sites, Mediterranean (36°54' S) and Patagonia (46°04' S), Chile. Within sites, common slopes among elevations were demonstrated for both tissues and, depending on the tissue and tree line site, significant differences among elevations were found corroborating the trends attained above.

CF, closed forest; IN, intermediate stand; TB, timber line; TL, tree line. P -values < 0.05 in boldface.

The final row of each section contains F - and P -values for comparisons of variables between sites, determined through one-way ANCOVA-like procedures (see 'Statistical analysis' in Methods).

Means with different letters in the same column are significantly different among altitudes for each variable at each site [post hoc Tukey's honestly significant difference (HSD) test].

general, NSC concentrations were positively correlated with altitude ($P < 0.05$) for both stem and root sapwood depending on the site (Table 3). At the Mediterranean site, stem sapwood NSC increased with altitude from CF to TL (NSC = $0.109 \times \text{altitude} - 179.00$, $R^2 = 0.155$, slope differed positively from 0, $P < 0.001$), while at the Patagonia site such a trend was not found for the same organ (NSC = $-0.028 \times \text{altitude} + 104.22$, $R^2 = 0.013$, slope did not differ from 0, $P = 0.435$). In contrast, when root sapwood NSC concentrations were analysed, we found a positive and significant increase in NSC with altitude at the Patagonia site (NSC = 0.295

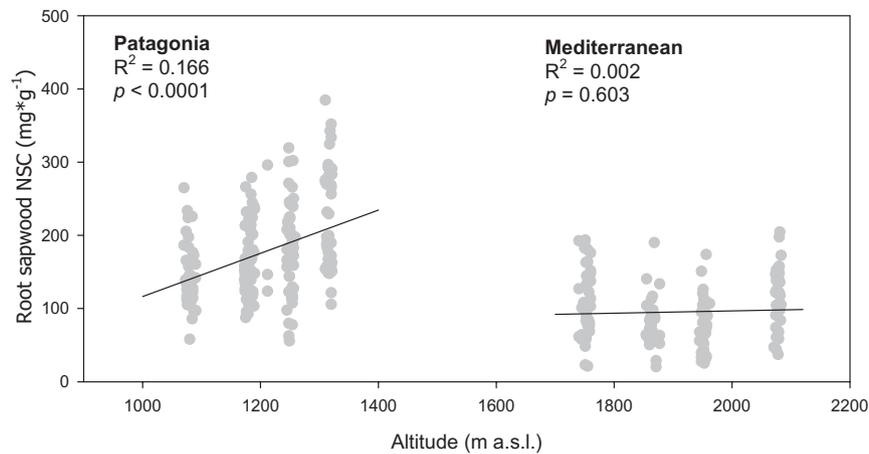


Figure 2 Concentrations of non-structural carbohydrates (NSC) in root sapwood for the tree line species *Nothofagus pumilio* across altitudes at two sites, Mediterranean (36°54' S and 71°24' W, 2100 m a.s.l.) and Patagonia (46°04' S and 72°03' W, 1300 m a.s.l.), in the southern Andes of Chile. According to the expectations of Fig. 1, the NSC–altitude slope did not differ from 0 for the Mediterranean site and did differ from 0 for the Patagonia site. For stem and root sapwood NSC concentration values, common slopes of both tree line sites could not be demonstrated [an assumption when using standardized major axes (SMA) analysis is that slopes must be homogeneous], thus we compared NSC means between sites with two-way ANOVAs.

\times altitude $- 178.60$, $R^2 = 0.117$, slope differed positively from 0, $P < 0.001$; Fig. 2), while this trend was not found at the Mediterranean site (NSC = $0.016 \times$ altitude $+ 64.83$, $R^2 = 0.002$, slope did not differ from 0, $P = 0.603$, Fig. 2). Mean root sapwood NSC concentration values were five times higher than those of the corresponding stem sapwood at all altitudes (Table 3).

Root sapwood NSC concentrations were significantly higher (more than 50%) at the Patagonia site than at the Mediterranean site at all altitudes ($P < 0.001$; Fig. 2, Table 3). For stem sapwood, NSC concentrations were, in general, lower in Patagonia than in the Mediterranean site (with the exception of CF) (Table 3). Within sites, Tukey's HSD *post hoc* tests showed that for stem sapwood, NSC values at CF were significantly lower than those for higher altitudes at the Mediterranean site, whereas no differences were found among the other three altitudes. Among altitudes, no significant differences were found for stem sapwood at Patagonia. In the Mediterranean site, NSC values for root sapwood at CF were similar to those at TL and c. 29% higher than at the other two intermediate altitudes. In Patagonia, for the same organ, NSC values at TL were 33.7% significantly higher than those obtained at CF (Table 3).

At the site and altitude levels, we found that most of the NSC concentrations were composed of starch with an approximate average ratio of 5:1 over total soluble sugars (Table 3). Thus, variation of starch concentrations with altitude for both tissues resembled the trends found for NSC concentrations (Table 4); i.e. starch mostly drove the trends. When the sugar–starch ratio was compared across the altitudinal gradient, for stem sapwood this ratio decreased with altitude at the Mediterranean site and increased at the Patagonia site (Table 2). Starch variation explained this opposite tendency (Table 4). Alternatively, increasing altitude proved to have only a very weak impact on the sugar–starch ratio when the root sapwood NSC concentrations were compared (Table 3). For root sapwood, the Patagonia

site had higher ratios when compared with the Mediterranean site at the two lowest altitudes, though differences were not significant. At the two highest elevations, in contrast, sugar–starch ratios of root sapwood were higher for the Mediterranean site, where the difference was significant only at TL (0.2583 vs. 0.2126; Table 3).

The $\delta^{13}\text{C}$ values of stem sapwood varied according to site and altitude (Fig. 3). First, while $\delta^{13}\text{C}$ values increased significantly (became less negative) with altitude at the Mediterranean site ($b = 0.0042$, $R^2 = 0.55$, $P < 0.0001$; Fig. 3) they did not vary with altitude at the Patagonia site ($b = -0.0001$, $R^2 = 0.01$, $P = 0.7422$; Fig. 3). At the Mediterranean site the $\delta^{13}\text{C}$ mean difference between TL (-26.00‰ , SE = 0.19, $n = 12$) and CF (-27.23‰ , SE = 0.15, $n = 12$) was 1.23‰, much more than what is expected due to an altitudinal decrease of 0.12‰ per 100 m elevation (e.g. decreasing pressure of gasses) as indicated by Körner (2003). The steep slope in the values of $\delta^{13}\text{C}$ at the Mediterranean site was mostly due to the exceptionally high values observed at the lower elevations. Second, $\delta^{13}\text{C}$ values at tree line altitude were on average higher at the Mediterranean site relative to the Patagonia site ($n = 21$, $F = 24.106$, $P < 0.001$), with a $\delta^{13}\text{C}$ mean of -26.00‰ (SE = 0.19, $n = 12$) for the Mediterranean site and -27.00‰ (SE = 0.18, $n = 11$) for the Patagonia site: e.g. the difference between altitude at both tree line locations is c. 800 m, suggesting that altitude, rather than soil moisture conditions, may explain the difference in $\delta^{13}\text{C}$ means.

DISCUSSION

In spite of the growing number of empirical studies that do not support the CLH, limitation of growth due to a shortage of photoassimilates is still a controversial hypothesis in the tree line debate (Smith *et al.*, 2003; Handa *et al.*, 2005; Li *et al.*, 2008). In the tree line ecotones under study, we never found a decrease of

Table 4 Comparisons of mean (\pm SE) soluble sugars and starch concentrations of stem and root sapwood across four altitudes, with *F* and *P* statistics.

	Mediterranean	Patagonia
Stem sapwood (mg cm ⁻³)		
Sugars		
CF	5.3 \pm 0.2 ab	4.9 \pm 0.2 a
IN	4.3 \pm 0.3 bc	4.5 \pm 0.3 a
TB	5.8 \pm 0.2 a	6.2 \pm 0.3 a
TL	4.2 \pm 0.4 c	6.5 \pm 0.3 a
<i>F</i>	9.0 (< 0.001)	2.4 (0.071)
Starch		
CF	11.1 \pm 1.1 a	21.7 \pm 1.0 a
IN	28.5 \pm 2.2 c	18.0 \pm 1.1 b
TB	23.3 \pm 1.9 b	14.9 \pm 1.3 b
TL	28.1 \pm 1.7 c	17.9 \pm 1.4 b
<i>F</i>	18.2 (< 0.001)	3.4 (< 0.001)
Root sapwood (mg g ⁻¹)		
Sugars		
CF	17.0 \pm 1.2 a	26.4 \pm 1.0 a
IN	11.8 \pm 1.0 a	32.9 \pm 1.6 a
TB	13.8 \pm 1.3 a	32.2 \pm 2.1 a
TL	20.1 \pm 1.4 a	26.0 \pm 1.5 a
<i>F</i>	1.3 (0.281)	1.2 (0.324)
Starch		
CF	96.8 \pm 9.2 a	117.2 \pm 5.5 a
IN	67.5 \pm 4.7 b	135.3 \pm 6.2 b
TB	61.5 \pm 5.5 b	148.4 \pm 7.5 b
TL	89.2 \pm 7.7 a	137.0 \pm 9.0 b
<i>F</i>	4.1 (0.008)	4.1 (0.007)

Concentration values were calculated for *Nothofagus pumilio* tree line species at two sites, Mediterranean (36°54' S) and Patagonia (46°04' S), Chile.

CF, closed forest; IN, intermediate stand between closed forest and timberline; TB, timber line; TL, tree line.

P-values are in parenthesis, with *P*-values < 0.05 in boldface.

F- and *P*-values are provided for comparisons of variables among altitudes within each site, determined through one-way ANCOVA-like procedures (see 'Statistical analysis' in Methods).

Means with different letters in the same column are significantly different among altitudes for each variable at each site [post hoc Tukey's honestly significant difference (HSD) test].

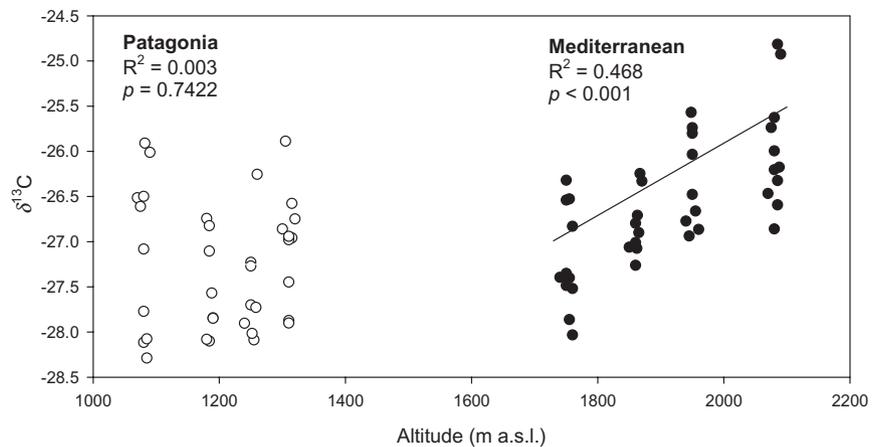
NSC concentration with altitude, clearly suggesting that tree line trees have not experienced any depletion in carbohydrate reserves during the growing season (Hoch & Körner, 2003; Körner, 2003), i.e. there is no support for the CLH. On the contrary, we observed that NSC concentrations increased significantly with altitude (i.e. in stem sapwood at the Mediterranean site and in root sapwood at the Patagonia site). If we consider that root NSC concentrations were five times higher than stem ones, we may pragmatically conclude that our data support the GLH at the site without local influence – Patagonia. Our conclusion is based on the knowledge that roots are the main organ of carbon storage in most deciduous species (Walters & Reich, 1999), and that stem–root volume ratios are

rarely higher than 5 (which is the concentration difference we found between stems and roots), e.g. 3.44 in red oak (Nadelhoffer & Raich, 1992) and 3.23 in silver birch (Uri *et al.*, 2007). Thus, if we conservatively consider that the *N. pumilio* stem–root ratio is < 5, stem volume would not offset the higher root concentration in the NSC pool.

With some exceptions, the body of studies using the NSC concentration approach has found support for the GLH. Hoch *et al.* (2002) and Hoch & Körner (2003, 2005) found no depletion of NSC in three pine species in Mexico (*Pinus hartwegii*) and Europe (*Pinus sylvestris*, *Pinus cembra*), and in *Polylepis tarapacana* in the central Andes of Bolivia. The same trend was found by Piper *et al.* (2006) in central Chile for *Kageneckia angustifolia*. In south-western China, NSC concentrations either increased or remained constant with elevation for a number of species including *Abies faxoniana*, *Larix potaninii*, *Juniperus tibetica* and *Quercus aquifolioides* (Shi *et al.*, 2006, 2008). Only Li *et al.* (2008), studying *Picea balfouriana* var. *hirtella* in the Himalayas of south-western China, found lower NSC concentrations at the tree line than at lower altitudes. However significant the finding of Li *et al.* (2008), their reports of lower NSC concentrations in tree line trees at the beginning of the growing season are biased by the temporal shift of phenology with altitude (*sensu* Hoch & Körner, 2009). The most important piece of evidence against the GLH so far has been observed in a tree line *in situ* CO₂-enrichment experiment, where Handa *et al.* (2005) found both support for the CLH in *Larix decidua* and support for the GLH for *Pinus uncinata*. They determined that *L. decidua* augmented its growth as a response to the addition of CO₂, i.e. it is carbon limited. One possible explanation for Handa *et al.*'s (2005) results is the character of deciduousness of *L. decidua*. Deciduous species differ from evergreens in the size of their carbon storage (Hoch *et al.*, 2003) and it remains uncertain if their NSC dynamic responds similarly to stressors. We found support for the GLH, suggesting that results from Handa *et al.* (2005) cannot be adduced to the leaf habit of the species.

Here, based on two approaches (model expectations and $\delta^{13}\text{C}$) we propose that carbon depletion, probably induced by drought, obscures the effect of low temperature on carbon balance, i.e. NSC accumulation. First, as we proposed specific expectations in the light of underlying mechanisms (Fig. 1) and compared them with the empirical NSC trends found, we infer that in the site with local influence (Mediterranean), drought has opposite effects on carbon balance from those imposed by low temperature, i.e. drought limits carbon gain more than carbon demands and temperature limits growth more than carbon gain (Fig. 1, long-dashed line right panel). Our thesis that drought induces carbon depletion is deduced from the comparison of NSC concentrations between the two tree lines. Then, at the Mediterranean site, the pattern of NSC resulting from the altitudinal decrease in temperature (Körner, 2007) (i.e. carbon accumulation) may have been masked by drought-induced carbon depletion (Bréda *et al.*, 2006; McDowell *et al.*, 2008; Sala *et al.*, 2010). As an aside, the decreasing stem sugar–starch ratio with altitude at the Mediterranean site may be reflecting drought-induced limitations on carbon gain as sugars

Figure 3 Correlations between altitude and carbon isotope ratio $\delta^{13}\text{C}$ (‰) in *Nothofagus pumilio* at two tree line sites, Mediterranean ($36^{\circ}54'$ S, solid circles, $\delta^{13}\text{C} = 0.0041 \times \text{altitude} - 33.91$, $R^2 = 0.468$, $P < 0.0001$) and Patagonia ($46^{\circ}04'$ S, open circles, $\delta^{13}\text{C} = 0.00004 \times \text{altitude} - 27.30$, $R^2 = 0.003$, $P = 0.7422$), in the southern Andes of Chile. For both sites, the individual standardized major axes (SMA) method was used to fit the data.



are used before starch whenever photosynthesis is curtailed (Chapin *et al.*, 1990). Second, from our carbon isotopic discrimination analysis we found that $\delta^{13}\text{C}$ increased significantly with altitude at the Mediterranean site, where the effect of elevation (300 m) only explained *c.* 0.36‰ of the *c.* 1.23‰ we found (considering an altitudinal decrease of 0.12‰ per 100 m elevation as indicated by Körner, 2003), which suggests the influence of drought.

The significant increase in $\delta^{13}\text{C}$ with elevation at the Mediterranean site may be explained by the presence of drought conditions stressed by soil structure and canopy cover, two factors known to influence soil moisture (Körner, 2003). Here, soils at the bottom of the altitudinal gradient are rich in loam and silt and have a high water retention capacity (Veit & Garleff, 1995), which could explain the exceptionally low values of $\delta^{13}\text{C}$ at this elevation. In addition, the high canopy cover at lower altitudes precludes soils from exposure to high evaporative demands (see 'Research sites description' in Methods). In contrast, in Patagonia, where mean temperatures are lower and precipitation higher, soils characteristics and canopy cover probably do not interact with altitude and have less influence on soil moisture. Differences in leaf structure between the two altitudinal gradients, and related to differences in genotypes, climate or local conditions such as soil fertility, could also be involved in the trends found for $\delta^{13}\text{C}$. Higher LMA provokes limitations of CO_2 diffusion (Syvertsen *et al.*, 1995; Wright *et al.*, 2004) and higher values of $\delta^{13}\text{C}$ (Vitousek *et al.*, 1990). Accordingly, we have found in a companion study (Fajardo & Piper, 2010) that LMA values at the Mediterranean site increased with altitude 26% more than at the Patagonia site.

$\delta^{13}\text{C}$ values did not reflect the drought difference between sites. We suggest that this result is explained by the asynchrony between the time of drought occurrence and the time of maximal growth. The isotopic composition of structural tissues reflects carbon discrimination during periods of tissue formation (*i.e.* spring). At our Mediterranean site, soil moisture during spring is probably high due to snow melt and precipitation: maximal growth (*i.e.* structural tissue production) then occurs without stomatal limitation to photosynthesis.

Finally, we think that the drought-induced carbon depletion we are proposing as a masking factor for the GLH is not related to

any generally elevation-associated environmental factor but to specific local conditions. Importantly, our study reveals for the first time that local conditions appeared to hide the trend of carbon accumulation related to the general elevation-associated temperature decrease. One implication of this is that the test of the GLH using the approach of NSC concentration along an altitudinal gradient may not apply for tree lines affected by drought. Piper *et al.* (2006), however, found support for the GLH in another drought-affected Mediterranean tree line of central Chile (33° S.), though their study was based only on the NSC concentrations in leaves that do not always reflect the NSC trend of the whole tree (Chapin *et al.*, 1990). We propose that the distinction between local (drought) and global (temperature) climate effects in mechanistic terms (carbon and growth limitations) should be considered in developing a more inclusive tree line theory when Mediterranean-type climate tree lines are considered. In this respect, Körner & Paulsen (2004) stated that the tree line in the southern Andes represents an *outlier* because its mean growing season temperature is higher (8.9°C , implying lower elevation) than the one found in most of the continental tree lines of the Northern Hemisphere ($6.7 \pm 0.8^{\circ}\text{C}$). Their generalization is based on temperature records from central Chile (Termas de Chillán), at exactly the same location as our Mediterranean site. We found lower values of NSC at the Mediterranean site relative to the Patagonia site, confirming that growth limitation due to low temperatures is lower at the Mediterranean site. This implies that the altitudinal tree line of *N. pumilio* might not be an *outlier* at the Patagonia site, where the GLH was supported and where mean growing season temperatures are actually lower, 6.9°C (Hoch, unpublished). Thus, a generalization based on a site with strong local influence seems inappropriate for the vast zone of the southern Andes.

CONCLUSIONS

Our analysis of NSC concentrations across two different climates and altitudes for the same species allows us to conclude that our results do not support the CLH. Considering that at the site without local influence (Patagonia), the main storage tissue (roots) exhibited increasing NSC concentrations across altitudes, we suggest that the effect of low temperatures on sink

activity is the mechanism responsible for tree line formation in *N. pumilio*, thus supporting the GLH. Finally, we assert that the *outlier* feature of the tree line in central Chile (*sensu* Körner & Paulsen, 2004) should be revised by considering locations without the effect of drought, which may alter the NSC–altitude approach used for distinguishing both hypotheses. The distinction between local and global causes in tree line functioning may help in building better expectations of how this system will respond to climate change.

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