

No evidence of carbon limitation with tree age and height in *Nothofagus pumilio* under Mediterranean and temperate climate conditions

Frida I. Piper* and Alex Fajardo

Centro de Investigación en Ecosistemas de la Patagonia (CIEP), Ignacio Serrano 509, Coyhaique, Chile

*Corresponding author. E-mail fpiper@ciep.cl

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- **Background and Aims** Trees universally decrease their growth with age. Most explanations for this trend so far support the hypothesis that carbon (C) gain becomes limited with age; though very few studies have directly assessed the relative reductions of C gain and C demand with tree age. It has also been suggested that drought enhances the effect of C gain limitation in trees. Here tests were carried out to determine whether C gain limitation is causing the growth decay with tree age, and whether drought accentuates its effect.
- **Methods** The balance between C gain and C demand across tree age and height ranges was estimated. For this, the concentration of non-structural carbohydrates (NSCs) in stems and roots of trees of different ages and heights was measured in the deciduous temperate species *Nothofagus pumilio*. An ontogenetic decrease in NSCs indicates support for C limitation. Furthermore, the importance of drought in altering the C balance with ontogeny was assessed by sampling the same species in Mediterranean and humid climate locations in the southern Andes of Chile. Wood density (WD) and stable carbon isotope ratios ($\delta^{13}\text{C}$) were also determined to examine drought constraints on C gain.
- **Key Results** At both locations, it was effectively found that tree growth ultimately decreased with tree age and height. It was found, however, that NSC concentrations did not decrease with tree age or height when WD was considered, suggesting that C limitation is not the ultimate mechanism causing the age/height-related declining tree growth. $\delta^{13}\text{C}$ decreased with tree age/height at the Mediterranean site only; drought effect increased with tree age/height, but this pattern was not mirrored by the levels of NSCs.
- **Conclusions** The results indicate that concentrations of C storage in *N. pumilio* trees do not decrease with tree age or height, and that reduced C assimilation due to summer drought does not alter this pattern.

Key words: Carbon isotope composition, drought, hydraulic limitation hypothesis, Mediterranean climate, non-structural carbohydrates, *Nothofagus pumilio*, ontogeny, Patagonia.

INTRODUCTION

The age-related decline in tree growth and forest productivity is one of the most conspicuous and universal natural patterns observed in forests (Yoder *et al.*, 1994; Gower *et al.*, 1996; Bond, 2000), although the mechanisms ultimately responsible for this pattern remain unknown (Ryan *et al.*, 2006; Peñuelas and Munné-Bosch, 2010). Most explanations thus far assert that the ultimate cause for age-related declining growth is insufficient carbon (C) availability to meet C demands (e.g. Yoder *et al.*, 1994; Ryan and Yoder, 1997; Magnani *et al.*, 2000; Barnard and Ryan, 2003; Martínez-Vilalta *et al.*, 2007; Zhang *et al.*, 2009) – a condition defined as C limitation (Körner, 2003; Sala *et al.*, 2010). The premise of C limitation causing an age/height-related growth decrease has been based until now on the negative association observed between tree age/height and traits tightly correlated with whole-tree net C gain, such as hydraulic and stomatal conductance (Barnard and Ryan, 2003; Mencuccini *et al.*, 2005; Martínez-Vilalta *et al.*, 2007; Zhang *et al.*, 2009), leaf nitrogen (N) concentration (Gower *et al.*, 1996; Martínez-Vilalta *et al.*, 2007), photosynthetic capacity (Niinemets, 2002), autotrophic/heterotrophic biomass ratio (i.e. specific leaf area) (Becker *et al.*, 2000; Magnani *et al.*, 2000; Barnard and Ryan, 2003; Zhang

et al., 2009) and minimum leaf water potential (Barnard and Ryan, 2003). To date, however, no single study has directly demonstrated that older/taller trees become C limited, particularly when compared with younger/shorter trees (Ryan *et al.*, 2006; Sala *et al.*, 2010). Also, it is not clear if traits related to C gain can properly predict C limitation (Ryan *et al.*, 2006).

The C balance in plants is determined by a complex interaction between whole C gain and whole metabolic C demands (Chapin *et al.*, 1990). Variations in either C gain or C demands can therefore potentially affect the C balance (Chapin *et al.*, 1990), illustrating that inferences of C limitation should not be solely based on variations in C gain. A more thorough and adequate approach for evaluating net changes in C balance is achieved by measuring the concentration of major forms of C reserves, such as non-structural carbohydrates (NSCs) (Chapin *et al.*, 1990; Hoch *et al.*, 2002, 2003; Körner, 2003; Palacio *et al.*, 2008; Sala *et al.*, 2010). If C limitation does occur, the amount of C reserves must decrease as trees mobilize carbohydrates from storage sites whenever photosynthetic production is insufficient to meet metabolic demands (Chapin *et al.*, 1990). Notably, the few studies that have examined C reserves in trees of different ages and heights did not find any decrease in C reserves (Sala and Hoch, 2009; Genet *et al.*, 2010). For example, Sala and Hoch (2009) found that

both NSC concentrations and C isotopic composition ($\delta^{13}\text{C}$) actually increased with tree height in ponderosa pine when comparing 6 and 37 m tall trees, indicating that trends in C reserves do not necessarily match trends in C assimilation. Results from these studies challenge the notion of C limitation as the ultimate process explaining the age-related slow down in tree growth, and raise the issue of whether C use, instead of C gain, may be more or similarly curtailed by ontogeny leading to either a positive or unaffected C balance, respectively.

A less studied trait with important implications for the ontogenetic variation in the tree C balance is wood density (WD). In cross-species studies, leaf gas exchange and stem water status have been found to decrease with increases in WD (Santiago *et al.*, 2004; Meinzer *et al.*, 2008), and a negative association between WD and water storage – which may be important in compensating for hydraulic limitations – has been documented (Scholz *et al.*, 2007). In support of this, Zhang *et al.* (2009) suggested that increasing WD with tree height entails a lower C return per unit of C invested. WD also has important methodological implications on the use of NSCs in assessing C balance: an increase in WD dilutes NSC concentrations expressed in terms of dry matter, whereas decreasing WD concentrates it (Niinemets, 1997; Hoch *et al.*, 2002). As WD has been found to either increase (Zhang *et al.*, 2009) or decrease (Fajardo and Piper, 2011) with tree age/height, it seems essential to account for its variation and the effect on NSCs when C limitation with tree ontogeny is being assessed.

The claimed association between the phenomenon of increasing worldwide tree mortality and climate change has added urgency to the need to understand the ontogenetic decline in tree growth and productivity (McDowell *et al.*, 2008; Zhang *et al.*, 2009; Palik *et al.*, 2011). It has been proposed that reduced C assimilation by drought stimulates the consumption of C reserves, leading to C limitation and eventually to tree death due to C starvation (McDowell *et al.*, 2008; Palik *et al.*, 2011), where taller trees are viewed as more vulnerable to death given their already limited C assimilation by hydraulic path length and gravity (McDowell *et al.*, 2008; Zhang *et al.*, 2009). Although some studies have found that drier conditions restrict the stomatal conductance, photosynthesis and hydraulic conductance relatively more in older/taller than in younger/shorter trees (Phillips *et al.*, 2002; Piper and Cavieres, 2010), there are at least two arguments against the hypothesis of drought amplifying C limitation with tree age/height. First, in some species, stomatal sensitivity to drought is relatively higher in younger size classes of trees (McDowell *et al.*, 2005; Martínez-Vilalta *et al.*, 2007), meaning that height-related differences in C assimilation are minimal under dry conditions. Secondly, processes related to both the retrieval of stored reserves (e.g. mobilization, phloem transport) and the use of available carbohydrates (i.e. respiration and growth) are sensitive to drought and potentially depressed by it (Ruehr *et al.*, 2009; Sala *et al.*, 2010).

In this study we tested the occurrence of age- and height-related C limitation in the widely distributed deciduous tree species *Nothofagus pumilio*. Furthermore, by working at two sites in southern Chile characterized by contrasting summer precipitation levels, we attempted to ascertain the effect of drought on the ontogenetic pattern of carbon storage. We address the following questions. (a) Does

N. pumilio exhibit either age- or height-related C limitations? (b) Is the ontogenetic variation in C status related to traits directly associated with C gain, such as $\delta^{13}\text{C}$, growth and WD? (c) How does the presence of summer drought influence the ontogenetic patterns of C storage? We believe that a better understanding of how the C balance varies during tree life is crucial in providing new evidence for determining the ultimate causes of the age-related declines in tree growth. Sala and Hoch (2009) previously explored the relationship between C limitation and tree age/height; however, they did not consider the effect of WD when reporting NSC levels and compared tree populations located in the same region. The present study considered the effect of WD as a diluting factor altering NSC concentration and compared two *N. pumilio* populations affected by contrasting climatic conditions and separated by 10° of latitude.

MATERIALS AND METHODS

Description of species and research sites

Nothofagus pumilio (Poepp. et Endl.) Krasser (Nothofagaceae) is a deciduous broadleaf light-demanding tree species that extends across a wide latitudinal and altitudinal range in the southern Andes of Chile and Argentina (Rodríguez and Quezada, 2003). In this study, monospecific multiaged sub-alpine forests of *N. pumilio* were sampled. The study areas were selected to represent the regional differences in climate between south central Chile and central Patagonia. Forests of southern Chile are split between a seasonal (36–42 °S) and a non-seasonal zone (42–55 °S); the difference between them is the presence of a sharp seasonal drought and warmer summers at latitudes below 42 °S (Luebert and Plissock, 2006).

The first site was located in Termas de Chillán (36°54'S and 71°24'W, approx. 1750 m a.s.l.); hereafter the Mediterranean site. This area belongs to the meso-Mediterranean belt with humid climatic conditions and a predominant drought period during summer (Miller, 1976; Amigo and Ramírez, 1998). Precipitation for 1990–2007 at the Las Trancas climate station (36°55'S and 71°29'W, 1200 m a.s.l.) located 12 km west of the study site was 165 mm for the five warmest months (November–March; Informe Meteorológico de Chile, Dirección General de Aguas, 2008). The mean annual temperature at the Diguillín climate station (36°86'S and 71°63'W, 710 m a.s.l.) is 11.8 °C and the mean temperature of the warmest month is 17.6 °C, while from November to March mean temperatures are >15 °C; potential evapotranspiration is 644 mm (Luebert and Plissock, 2006). The soil is derived from andesitic rocks of volcanic origin, ranging from 0.1 to 0.5 m deep, decreasing strongly with elevation (Veit and Garleff, 1995).

The second study site was within the Cerro Castillo National Reserve (46°04'S and 72°03'W, 1050 m a.s.l.); hereafter the Patagonia site. This area belongs to the supra-Temperate belt with humid climatic conditions (Amigo and Ramírez, 1998). Mean precipitation for 1992–2007 was 416 mm for the five warmest months (November–March) at Villa Cerro Castillo climate station (46°07'S and 72°09'W, 340 m a.s.l.) located 15 km southwest of the study site (Informe Meteorológico de Chile, Dirección General de Aguas, 2008). Mean annual

temperatures at the two stations closest to the site are 8.6 °C (Coyhaique, 45°57'S and 72°03'W, 343 m a.s.l.) and 6 °C (Balmaceda, 45°90'S and 75°72'W, 520 m a.s.l.). Mean temperatures of the warmest month for the same two climate stations are 14.2 °C (Coyhaique) and 11.5 °C (Balmaceda), while from November to March mean temperatures are <15 °C; potential evapotranspiration for Coyhaique is 584 mm (Luebert and Pliscoff, 2006). The soil is derived from aeolian volcanic ash deposits (Veit and Garleff, 1995). As a result of precipitation and temperature differences between both locations during the growing season, water balance estimations indicate a deficit in January and February for the weather stations located near the Mediterranean site but not for those near the Patagonian site (Luebert and Pliscoff, 2006). Supporting these observations, volumetric water content (VWC) of the first 30 cm of soil measured at each location also differed (HOBO® Micro station, Echo probes, F. Piper, unpubl. res.). For a complete year, VWC values were on average 0.086 m³ m⁻³ (s.e. = 0.003) at the Mediterranean site and 0.166 m³ m⁻³ (s.e. = 0.003) at the Patagonian site. VWC values for the period November–March were on average 0.070 m³ m⁻³ (s.e. = 0.005) at the Mediterranean site and 0.200 m³ m⁻³ (s.e. = 0.007) at the Patagonian site.

Field sampling and initial sample processing

Sampling was conducted during late February 2007 at the Patagonia site and mid-March 2007 at the Mediterranean site. Sampling took place at the end of the growing season for each site: at the end of the growing season NSC concentrations in deciduous species are expected to reflect the net balance between C sinks (i.e. meristems) and sources for the complete growing season, and both C sinks and sources are still active (Hoch *et al.*, 2003). Although we do not discount some seasonal variation in NSCs, we note that only a small portion of the C pool is used in spring – the most

C-demanding period – in deciduous species, i.e. small sink variation (Hoch *et al.*, 2003), and that any ontogenetic influence on C balance should in any case be expressed in the NSC concentrations irrespective of circumstantial seasonal variation. *Nothofagus pumilio* trees were studied at elevations where tree height variation was greatest. These included sub-alpine multiaged mature forests (i.e. the presence of numerous gaps where saplings and juveniles are found) located in a belt 50 m wide around 1750 m a.s.l. in the Mediterranean site and around 1050 m a.s.l. in the Patagonia site (Table 1). The difference in altitude between both sites represents the natural decline in altitude with latitude for these particular forests. We set sampling points along a sampling line every 30 m, where 4–5 trees belonging to different height classes (i.e. different ages) of dominant social status were selected for sampling. A total of 77 and 100 trees were selected at the Mediterranean and Patagonia sites, respectively. Individuals with extensive browsing or other damage, or apparently suppressed or shaded, were excluded. For each individual tree surveyed, we also measured tree height using a clinometer (Suunto, Finland), diameter at breast height (DBH, 1.35 m), and bark thickness. Mean stand variables such as DBH, tree age and height did not differ between locations, although maximum sampled height was higher at the Mediterranean site (Table 1). Tree heights ranged from 2.0 to 24.9 m at the Mediterranean site and from 1.5 to 19.4 m at the Patagonia site. It should be noted that although individuals of *N. pumilio* can reach heights of up to 30–40 m elsewhere, the trees sampled attained maximum heights recorded for the study sites (Fajardo and de Graaf, 2004); what matters with respect to the interpretation of the data is the maximum height of trees at the site being studied (see Petit *et al.*, 2011).

The collection of woody samples was conducted between 1000 and 1700 h. Two stem cores were taken at the base of

TABLE 1. Statistics of structural stand characteristics and values of non-structural carbohydrate (NSC = starch + soluble sugars) concentration, wood density (WD) and C isotopic composition ($\delta^{13}\text{C}$) of the tree species *Nothofagus pumilio* growing at two locations in southern Chile, Mediterranean (36°54'S) and Patagonia (46°04'S)

Trait	Mediterranean			Patagonia		
	Mean	Range	s.e.	Mean	Range	s.e.
DBH	18.63	1.5–77	2.19	21.15	0.5–94.8	1.94
Age	87	24–220	5.99	94	13–250	5.71
Height	9.08	2–24.9	0.68	9.19	1.5–19.4	0.60
NSC _{stem(mass)}	45.45	9.08–115.8	3.11	52.95	7.84–126.37	1.84
NSC _{stem(vol)}	24.26	5.19–59.27	1.61	25.80	3.81–70.82	0.96
NSC _{root}	95.57	20.50–193.61	5.05	155.89	57.98–279	4.77
Starch _{stem(mass)}	36.77	2.68–101.68	3.02	43.91	6.47–107.76	1.59
Starch _{stem(vol)}	19.56	1.40–51.70	1.57	21.37	3.10–60.4	0.79
Starch _{root}	82.73	4.79–331.12	5.47	126.25	45.26–224.14	4.18
TSS _{stem(mass)}	9.01	3.17–18.07	0.33	9.62	1.37–21.63	0.37
TSS _{stem(vol)}	4.88	0.18–9.8	0.18	4.71	0.7–10.9	0.18
TSS _{root}	14.45	1.7–44.83	0.82	29.64	12.72–62.47	0.97
$\delta^{13}\text{C}$	-26.93	-28.03 to -25.47	0.14	-27.44	-29.29 to -25.91	0.21
WD	0.55	0.42–0.68	0.01	0.49	0.41–0.68	0.01

DBH, diameter at breast (1.35 m) height in cm; Height, tree height in m; NSC, non-structural carbohydrates in mg g⁻¹ when based on mass and in mg cm⁻³ when based on sapwood volume; TSS, total soluble sugars in mg g⁻¹ when based on mass and in mg cm⁻³ when based on sapwood volume; WD, wood density in g cm⁻³.

the tree, one 10 cm in length (shorter when young trees were considered) for chemical analysis and WD determination, and, for a sub-sample of trees (40 per site), a second to-the-pith long core at approx. 30 cm high for tree age and growth determination. A 5·15 mm increment bore (Haglöf, Långsele, Sweden) was used for this purpose. Superficial fine roots with diameters between 5 and 10 mm (without bark) were excavated and clipped by removing a sod of soil, for NSC determination. All tissue samples were bagged and labelled, and stored in a cool box for transportation. Stems and roots were selected for NSC analyses because they represent the main storage sites in deciduous temperate species (Chapin *et al.*, 1990; Hoch *et al.*, 2003; Palacio *et al.*, 2008). Woody samples for NSC and $\delta^{13}\text{C}$ analyses were heated in a microwave oven at 600 W for 90 s to denature enzymes. All roots and wood cores (except the ones for tree age and growth determination) were then oven-dried at 80 °C for 48 h. The dry mass of cores was recorded and WD was calculated. Stem WD was estimated for each tree as dry core mass divided by green core volume, which was measured *in situ* by cutting the ends of each core section perpendicularly to the sides, measuring its length with a calliper (10^{-5} m precision), and multiplying the length by the cross-sectional area (diameter of the increment bore, 5·15 mm). Samples necessary for NSC and stem $\delta^{13}\text{C}$ were then ground into fine powder and stored sealed over silica gel at 4 °C until analysis.

Tree age and growth determination

For each site, cores were prepared following standard dendrochronological techniques (Stokes and Smiley, 1996). Tree age at coring height was determined by reading annual rings from the cores of the subsample of trees (40 per site), using a microscope mounted on a dendrochronometer with a Velmex sliding stage and Accurite measuring system. Cross-dating accuracy was checked using COFECHA v6·06P. For all trees sampled, tree ages were estimated using linear models fitted with likelihood methodologies to the age–DBH relationship determined from our data and a larger data set of tree cores collected at each site ($n = 200$) that were dated for a companion study (A. Fajardo, unpubl. res.). It was found that a power DBH–age relationship proved to be the best fit (lowest AIC_c) (slope = 0·571, $R^2 = 0·713$, s.d. = 27·604). Tree ages ranged from 24 to 220 years at the Mediterranean site and from 13 to 250 years at the Patagonia site, respectively. Inside-bark bole radius, annual radial increment of the last 10 years and sapwood depth were measured to the nearest 0·01 mm. Sapwood–heartwood boundaries were visually identified on each core in the field. The last 10 year basal area increment (BAI) was then computed by considering diameter at coring height and bark thickness, which represents a relative measure of gain in growth. Growth efficiency (GE) was also computed as an index of tree vigour. Growth efficiency has been defined as the individual tree's stemwood increment per unit of foliage or leaf area (Waring, 1983). However, sapwood is commonly used as a surrogate for leaf area, based on the established allometric relationship between these two variables (Seymour and Kenefic, 2002).

In computing GE, the BAI was divided by the current sapwood basal area.

Chemical analyses

Our assessment of C reserves was based on the determination of total low molecular weight soluble sugars (TSS = glucose, fructose and sucrose) and starch (TSS + starch = NSCs) (Chapin *et al.*, 1990) as they represent the main forms of carbon storage in broadleaf deciduous 'starch' tree species, i.e. NSCs serve as the main C storage form, in contrast to 'fatty' trees which accumulate important amounts of lipids (Hoch *et al.*, 2003; Palacio *et al.*, 2008). For stem NSC analysis, the 10 cm sections of cores were used because they contain the highest concentrations of sugars and starch in mature trees (Hoch *et al.*, 2003). Samples were analysed for TSS and starch using ethanol and perchloric acid to extract the compounds (Hansen and Moller, 1975). TSS were extracted from 0·1 g of dry mass in 86 % (v/v) ethanol at 80 °C for 1 h. The supernatant was collected after centrifugation and the concentration of TSS was determined spectrophotometrically by the resorcinol method (Roe, 1934) at a wavelength of 520 nm, using sucrose as 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 determining starch in the extract was similar to that used for sugars, but using glucose as standard. This method of extraction can yield starch values higher than those estimated by more accurate enzyme methods (Rose *et al.*, 1991), probably as a result of hydrolysis of some cell wall components. This was checked for in a companion study (Fajardo *et al.*, 2011) and no significant deviation in the soluble sugars and starch determinations was found between the enzyme method (claimed to be more precise) and our method. Starch and TSS in each plant component were added together to determine total NSCs in mg g^{-1} dry matter. Considering that WD may vary with tree ontogeny, stem NSC, TSS and starch concentrations were all expressed per unit of sapwood volume (mg cm^{-3} , WD-mediated) and per unit of dry matter (mg g^{-1}).

Although gas-exchange measurements may be considered a more direct estimate of C assimilation, they can be affected by seasonal or weather conditions at the time of measuring (Farquhar *et al.*, 1989; McDowell *et al.*, 2005). Thus, it was decided to work with stem $\delta^{13}\text{C}$ since it integrates stomatal conductance and C assimilation in the long term and can provide a good estimate of stomatal limitations on C assimilation provoked by either drought, tree height or both. The analyses were conducted on stem wood – the same sample used for NSCs – because in deciduous species sapwood $\delta^{13}\text{C}$ represents, better than leaves, the long-term photosynthetic discrimination (Michelot *et al.*, 2011). $\delta^{13}\text{C}$ analysis was performed with a continuous flow mass spectrometer in powdered 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

First, the expected growth rate (BAI) decline with tree age and height relationship was assessed by fitting models hypothesized to represent alternative trends: no decline (linear and power models), stabilization of the growth rate after increasing (logistic) and a decline of the growth rate after increasing (quadratic polynomial, Gaussian and gamma). We then compared relative fit using Akaike's information criterion for small samples (AIC_c) (Burnham and Anderson, 2002), and selected the model with the lowest AIC_c as the best fit, which stands for the simplest model extracting the maximum information from the data. Residuals of the model fitting were normally distributed. GE analyses were conducted similarly, where the expectations were associated with a gradual decline (linear, power and quadratic polynomial models). Both analyses were performed using R (R-Development, 2009) and the 'likelihood' and 'nlme' (Pinheiro *et al.*, 2009) packages.

NSC concentrations were not normally distributed (Shapiro–Wilk W test for goodness-of-fit) and hence were log-transformed prior to any statistical analysis. Distributions were

evaluated with JMP 8.0 (SAS). Bivariate relationships of NSC concentration, BAI, WD and $\delta^{13}\text{C}$ with tree age and height were analysed using the Model Type II regression method, where the regression slope is computed as the standardized major axis (SMA). The SMA method is appropriate when the primary purpose of the analysis is to estimate the relationship between two variables (i.e. a functional rather than a predictive relationship was sought). Furthermore, the SMA method provides a superior estimate of the slope summarizing the relationship between two variables to that of ordinary linear regression and residuals as the relationships are estimated and minimized in both the X and Y dimensions. The statistical software (S)MATR (Falster *et al.*, 2003) was used for all the analyses.

RESULTS

Age/height-related trends in carbon storage across locations

Tree growth rate (BAI) effectively decreased with age and height after a period of increasing BAI at both locations, and thus the gamma model provided the best fit (lowest AIC_c) to the observed trend (Fig. 1, Supplementary Data Table S1, available online). BAI was lowest in the younger/shorter

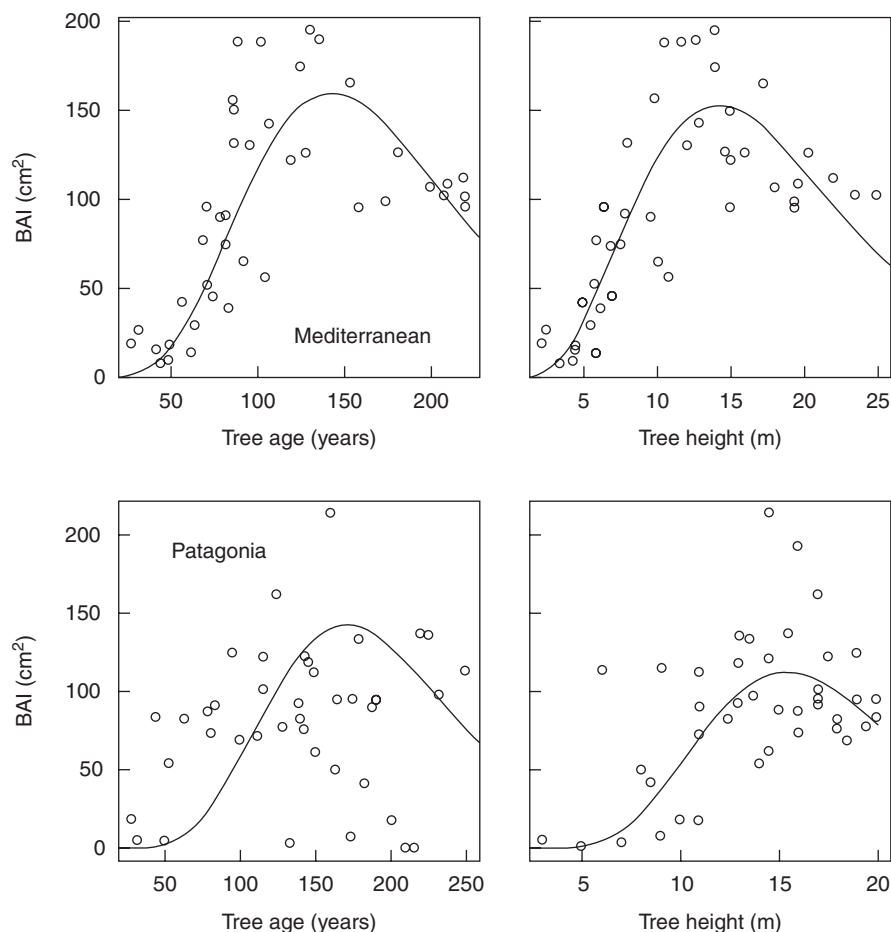


FIG. 1. Tree growth rate (basal area increment in cm^2 of the last 10 years, BAI) in relation to tree age and height of *Nothofagus pumilio* at two contrasting climatic locations (Mediterranean and Patagonia) in the southern Andes of Chile. The curves were fitted with a gamma model (best fit, Supplementary Data Table S1, available online), which best represents the decline in growth rate.

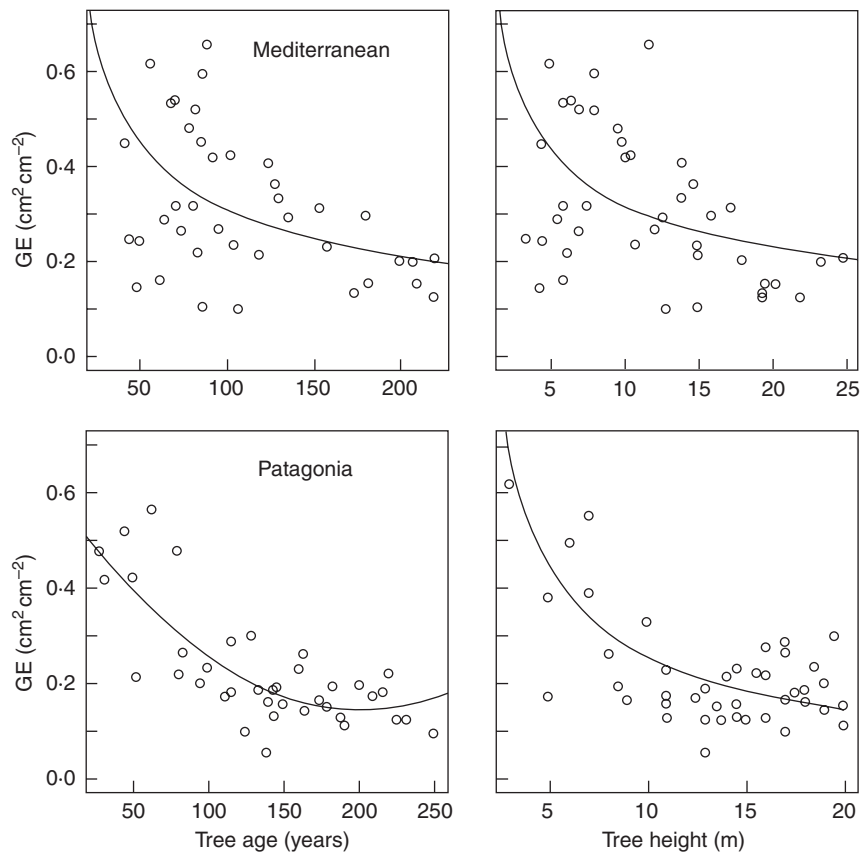


FIG. 2. Growth efficiency (basal area increment in cm^2 of the last 10 years over basal area of sapwood in cm^2 , GE) in relation to tree age and height of *Nothofagus pumilio* at two contrasting climatic locations (Mediterranean, top, and Patagonia, below) in the southern Andes of Chile. The curves were best fitted with power and quadratic polynomial models (Supplementary Data Table S1, available online).

trees at both sites, then increased up to a point (100–150 years and approx. 15 m height in Mediterranean, 150–200 years and approx. 15 m height in Patagonia), and finally declined with tree age/height (Fig. 1). Growth efficiency proved to have a different pattern from BAI; it monotonically decreased with tree age/height and the best fit was provided by the power and quadratic models (Fig. 2, Supplementary Data Table S1).

In contrast to tree growth, concentrations of stem and root sapwood NSCs never decreased with tree age/height (slopes did not differ from 0, $P > 0.05$; Tables 2 and 3, Fig. 3). At the Mediterranean site, the correlation between stem sapwood NSC concentrations per unit of dry matter with tree height and tree age was nearly significant and positive ($P = 0.054$, Tables 2 and 3). These trends disappeared, however, when stem sapwood NSC concentrations were expressed on a sapwood volume basis (Tables 2 and 3), i.e. when WD was considered. For the Patagonia site, stem and root NSC concentrations remained stable with both tree age and height ($P > 0.05$, Tables 2 and 3, Fig. 3).

Age/height-related variation in $\delta^{13}\text{C}$ and WD

The $\delta^{13}\text{C}$ values increased significantly ($P < 0.01$) with tree age (Table 2) and height (Table 3, Fig. 4) solely at the

Mediterranean site; they did not vary ($P > 0.05$) at the Patagonia site. WD significantly declined with tree age and tree height at both sites (Tables 2 and 3, Fig. 4). Between sites, these decreases had similar slopes (WD vs. tree age, $P = 0.099$, common slope = -0.0012 ; WD vs. tree height, $P = 0.300$, common slope = 0.0107) but differed in the intercepts (WD vs. tree age, $F_{1,175} = 20.00$, $P < 0.001$; WD vs. tree height, $F_{1,175} = 26.94$, $P < 0.001$), with Mediterranean trees having the highest WD. Thus, the ontogenetic trend in WD affected the NSC concentrations when they were expressed per unit of dry mass: the effect of WD on concentrations was solved by expressing compounds per unit of volume of tissue.

DISCUSSION

No evidence of carbon limitation with tree age or height in Nothofagus pumilio

No evidence of C limitation was found in *N. pumilio* across tree age and height: tree growth ultimately decreased with tree age and height, but stem and root NSC concentrations did not. A similar result was also found by Sala and Hoch (2009) and Genet *et al.* (2010), who worked with a coniferous

TABLE 2. Variations in NSC concentrations, C isotopic composition ($\delta^{13}\text{C}$) and wood density (WD) with tree age of the tree species *Nothofagus pumilio* growing at two locations with contrasting climates in southern Chile, Mediterranean ($36^{\circ}54'S$) and Patagonia ($46^{\circ}04'S$)

Trait	Mediterranean						Patagonia					
	<i>n</i>	<i>b</i>	CI _{low}	CI _{upp}	<i>r</i> ²	<i>P</i>	<i>n</i>	<i>B</i>	CI _{low}	CI _{upp}	<i>r</i> ²	<i>P</i>
NSC _{stem(mass)}	75	0.005	0.004	0.006	0.05	0.054	95	0.003	0.002	0.004	0.005	0.510
NSC _{stem(vol)}	75	0.005	0.004	0.006	0.024	0.185	94	-0.003	-0.004	-0.002	0.006	0.456
NSC _{root}	74	0.004	0.003	0.005	0.008	0.447	99	0.002	0.002	0.003	0.021	0.154
$\delta^{13}\text{C}$	20	0.018	0.012	0.027	0.346	0.006	18	-0.02	-0.03	-0.012	0.000	0.970
WD	77	-0.001	-0.002	-0.001	0.268	<0.001	99	-0.001	-0.001	-0.001	0.159	<0.001

Relationships were assessed with type II regression models. Slopes and confidence intervals of the slope (CI) are based on log₁₀-transformed data (except for $\delta^{13}\text{C}$, whose values are negative).

NSC, non-structural carbohydrates in mg g⁻¹ when based on mass and in mg cm⁻³ when based on sapwood volume; $\delta^{13}\text{C}$, carbon isotope ratio in ‰; WD, wood density in g cm⁻³.

TABLE 3. Variations of NSC concentrations, C isotopic composition ($\delta^{13}\text{C}$) and wood density (WD) with tree height of the tree species *Nothofagus pumilio* growing at two locations of contrasting climate in southern Chile, Mediterranean ($36^{\circ}54'S$) and Patagonia ($46^{\circ}04'S$)

Trait	Mediterranean						Patagonia					
	<i>n</i>	<i>b</i>	CI _{low}	CI _{upp}	<i>r</i> ²	<i>P</i>	<i>n</i>	<i>B</i>	CI _{low}	CI _{upp}	<i>r</i> ²	<i>P</i>
NSC _{stem(mass)}	75	0.045	0.036	0.057	0.053	0.046	97	0.031	0.025	0.038	0.022	0.143
NSC _{stem(vol)}	75	0.043	0.034	0.054	0.027	0.160	96	0.030	0.024	0.037	0.000	0.914
NSC _{root}	75	0.036	0.028	0.045	0.004	0.576	100	0.022	0.018	0.027	0.013	0.266
$\delta^{13}\text{C}$	20	0.162	0.113	0.233	0.442	0.001	18	0.232	0.140	0.382	0.033	0.470
WD	77	-0.012	-0.014	-0.009	0.263	<0.001	99	-0.01	-0.012	-0.008	0.162	<0.001

Relationships were assessed with type II regression models. Slopes and confidence intervals of the slope (CI) are based on log₁₀-transformed data (except for $\delta^{13}\text{C}$, whose values are negative).

NSC, non-structural carbohydrates in mg g⁻¹ when based on mass and in mg cm⁻³ when based on sapwood volume; $\delta^{13}\text{C}$, carbon isotope ratio in ‰; WD, wood density in g cm⁻³.

and two broadleaf tree species, respectively. As did they, we found that younger/shorter trees have lower stem NSC concentrations per unit of dry mass, though this trend was significant at the Mediterranean site only (Table 3). However, as WD was higher in younger/shorter trees, when considering NSC concentrations on a volume basis we found that the mass-based trend disappeared and instead the NSC concentration remained stable across tree age/height (Fig. 3). Thus, our discussion will be focused on the NSC concentration trends expressed on a volume basis since they are more reliable than mass-based NSC concentrations in reflecting variations in C balance, as the latter get diluted as tissue density increases (Niinemets, 1997; Hoch *et al.*, 2002). This trend of no change in the relationship between NSC concentration on a volume basis and tree age/height was consistent between locations with contrasting climates. Also, the trend was similar for both types of tissue scrutinized – roots and stems – which represent the main storage sites in deciduous tree species (Chapin *et al.*, 1990; Hoch *et al.*, 2003).

The WD decline with tree age/height may have a key role in preventing *N. pumilio* undergoing C limitation. WD correlates negatively with water storage in different cross-species studies (Meinzer *et al.*, 2003, 2008; Scholz *et al.*, 2007) and at the intraspecific level trees increase their reliance on water storage to compensate for size-dependent hydraulic constraints

(Goldstein *et al.*, 1998; Phillips *et al.*, 2003). Increasing water storage associated with decreasing WD with tree age/height in *N. pumilio* may have compensated for major reductions in C assimilation due to hydraulic constraints, preventing the trees from experiencing C limitation. This explanation may also work for the Mediterranean site where, in spite of decreasing $\delta^{13}\text{C}$, in the absence of WD-mediated compensation, the stomatal limitations on C assimilation could have been larger than those observed (see below). Additionally, low WD is associated with lower tissue construction costs and higher net C returns (Santiago *et al.*, 2004; Meinzer *et al.*, 2008; Zhang *et al.*, 2009) which are further advantages in terms of C economy.

Carbon limitation occurs when the whole-tree C pool cannot meet C demands, and some sink activity (e.g. growth) may be impaired (Chapin *et al.*, 1990; Körner, 2003; McDowell *et al.*, 2008; Sala *et al.*, 2010). The pool of NSCs is defined as NSC concentration in a given tissue by the size of that tissue (Chapin *et al.*, 1990; Palacio *et al.*, 2008). Although we did not estimate NSC pools (we lacked appropriate allometric equations), it is unlikely that the storage size of roots and stem sapwood decreased with tree age/height in our study species (i.e. something that could promote a decreasing NSC pool). For example, sapwood area tends to increase with tree height and age (Meinzer *et al.*, 2005, 2006) or at least it

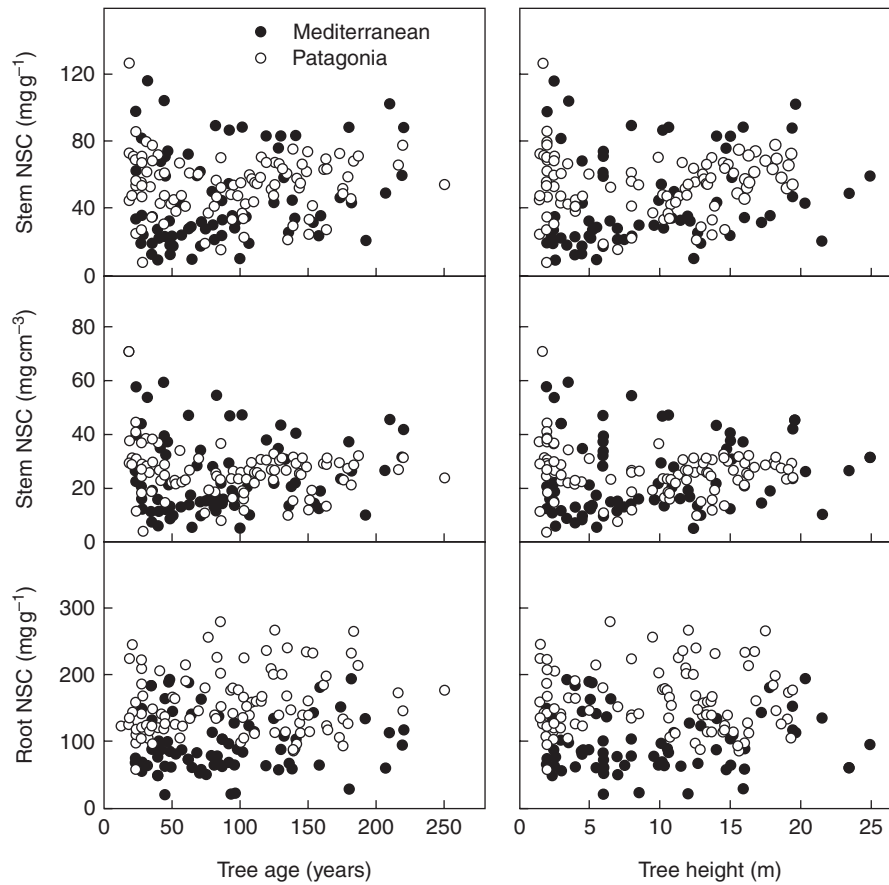


FIG. 3. Effect of tree age and tree height on concentrations of non-structural carbohydrates (NSCs) in stems and roots of *Nothofagus pumilio* at two contrasting climatic locations (Mediterranean and Patagonia) in the southern Andes of Chile. Note that stem NSC concentrations are expressed per mass unit and per volume unit. Only the stem NSC concentration on a dry mass basis at the Mediterranean site correlated significantly with tree age and tree height (see Table 2).

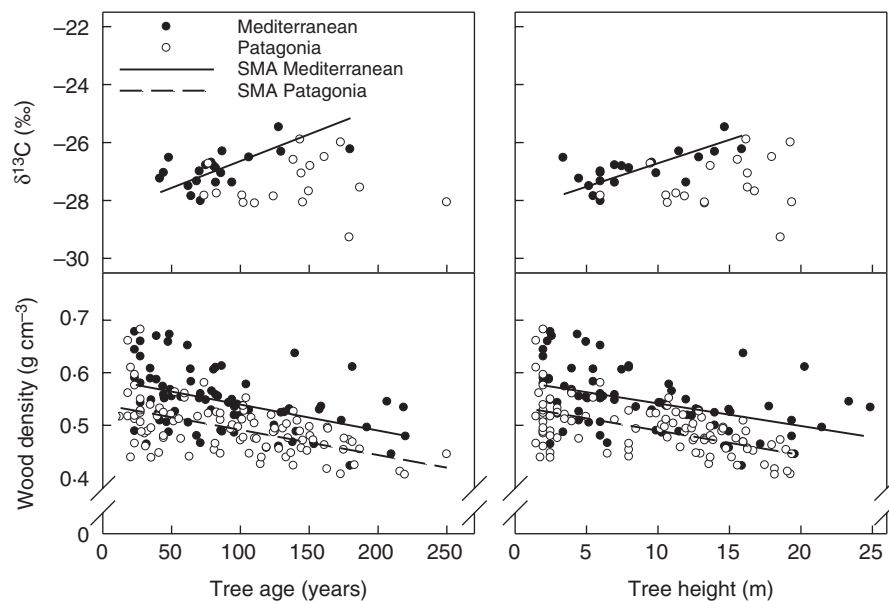


FIG. 4. Standardized major axes (SMA) relationships between carbon isotope composition ($\delta^{13}\text{C}$) and wood density (WD) of *Nothofagus pumilio* with tree age and tree height. Correlations of $\delta^{13}\text{C}$ with tree age and tree height at the Mediterranean site and of WD with tree age and tree height at both sites were statistically significant (Table 2).

does not decline drastically (Magnani *et al.*, 2000), which along with an increase in height with tree age infers higher sapwood volume. Whether such an increase in sapwood volume translates into higher sapwood NSC pools will depend on how the proportion of sapwood storing NSCs and the radial distribution of sapwood NSCs vary with ontogeny. Two studies have evaluated NSC radial distribution in two temperate deciduous broadleaf species, *Fagus sylvatica* and *Quercus petraea* (Barbaroux and Bréda, 2002; Hoch *et al.*, 2003), and found maximum NSC concentrations in the outermost part of the stem cores, regardless of the diameter differences (*F. sylvatica* 15 cm and *Q. petraea* 20 cm, Barbaroux and Bréda, 2002; *F. sylvatica* 32 cm and *Q. petraea* 34 cm, Hoch *et al.*, 2003). Accordingly, it is fair to assume that the sapwood NSC pool – like concentrations – did not decrease with ontogeny in *N. pumilio*, which provides support for our suggestion that the ontogenetic-related growth decline in this species is not caused by low C availability.

Other explanations for growth decline

Alternative explanations of C limitation accounting for the ontogenetically induced decline in growth have been proposed in recent years. Weakened sink strength at the top of tall trees (Woodruff *et al.*, 2004; Bond *et al.*, 2007) and impaired long-distance phloem transport (Sala and Hoch, 2009) are two appealing hypotheses based on the height-induced reductions in cell turgor. One important prediction of these explanations is that the concentration of mobile C compounds (NSCs) should increase with tree height, reflecting proportionally greater reductions of C use relative to C assimilation as trees grow taller (Sala and Hoch, 2009). Using NSCs, we did not find support for such an expectation. However, a reduction in C demands for growth along with reduced photosynthesis could have occurred at the Mediterranean site, which would explain the constant concentrations of NSCs. Alternatively, it has been indicated that not all the C stored is effectively able to replace the C shortage caused by photosynthetic reductions, since allocation priorities to certain plant functions may limit the use of C reserves for other, less essential functions (e.g. growth) (Millard *et al.*, 2007). Variations in C allocation between different plant functions are common during ontogeny where increased allocation to defences, reproduction and storage frequently occur at the expense of reduced growth (Becker *et al.*, 2000; Boege and Marquis, 2005; Genet *et al.*, 2010). Thus, even when accounting for sufficient C reserves to support continued growth (i.e. no C limitation) it seems likely that growth becomes relatively less important compared with other functions. In particular, *N. pumilio* is a masting species, a type of reproduction that requires important quantities of C reserves (Miyazaki *et al.*, 2002; Hoch *et al.*, 2003; Genet *et al.*, 2010). Notably, Genet *et al.* (2010) also found constant concentrations of NSCs with tree age in two other deciduous, masting species (i.e. *F. sylvatica* and *Q. petraea*), two species that belong to a family phylogenetically closely related to *N. pumilio*. Additionally, allocation to storage is higher in deciduous species when compared with evergreens throughout the different ontogenetic stages (Walters and Reich, 1999; Hoch *et al.*, 2003) and, although only a small fraction of the NSC pool is used for bud break

in spring, the real functional role of their high C reserves remains unclear (Hoch *et al.*, 2003). Hoch *et al.* (2003) suggest that high C reserves in deciduous species may be important in coping with increases in herbivore abundance since leaves are soft and palatable. However, herbivores of deciduous species may not discriminate between trees of different age classes, and under such pressure natural selection could have favoured high concentrations of NSC across different tree ages. In support of this idea, leaf mass per area – a trait associated with attractiveness for herbivores – of *N. pumilio* does not vary significantly with ontogeny (Fajardo and Piper, 2011) and insect outbreaks have been reported across the complete age range (Paritsis, 2009). A high allocation to C storage is also important in facing harsh environmental conditions, such as drought and frost, since osmotic agents are synthesized from the mobile C pool (Chapin *et al.*, 1990). In this respect, it has been proposed that temperate deciduous species of the genus *Nothofagus* are higher water consumers than other groups such as tropical deciduous or temperate evergreen *Nothofagus* (Read and Farquhar, 1991; Read *et al.*, 2010), thus they could be highly dependent on osmotic agents. Whether an ontogenetically stable allocation to storage supports these peculiarities of *N. pumilio* needs to be confirmed.

Influence of drought on the ontogenetic pattern of carbon storage

The significant increase of $\delta^{13}\text{C}$ with tree age/height found only at the Mediterranean site reflects that the photosynthetic discrimination against ^{13}C of *N. pumilio* – a surrogate of stomatal limitations – is affected by an interaction between climate and tree age/height. Mediterranean climates are characterized by dry and hot summers, with a constantly high vapour pressure deficit (VPD); all these conditions are expected to restrict C assimilation mainly due to reductions in stomatal aperture on photosynthesis. It has been hypothesized that stomatal limitations on C assimilation related to dry air or soil are amplified in taller trees due to their hydraulic limitations (Ryan and Yoder, 1997; McDowell *et al.*, 2008; Zhang *et al.*, 2009). We found support for this hypothesis, as have others (e.g. Phillips *et al.*, 2002; Sala and Hoch, 2009; Piper and Cavieres, 2010); also, our results are consistent with a study reporting higher instantaneous water use efficiency – a parameter directly linked to $\delta^{13}\text{C}$ (Michelot *et al.*, 2011) – in adult trees compared with seedlings of *N. pumilio* at a location also influenced by Mediterranean climate (Premoli and Brewer, 2007). However, the constraints on C assimilation with tree age/height, suggested by $\delta^{13}\text{C}$ data at the Mediterranean site, were not translated into decreasing NSC concentrations. One possible explanation for this result is that the stomatal limitations experienced by taller trees were not high enough to reduce C assimilation (and C availability) significantly and hence to stimulate C mobilization. As we suggested in the previous section, the decreasing WD with tree age/height may have been an effective compensatory mechanism operating in our studied trees, as it allows higher water storage (Meinzer *et al.*, 2003, 2008; Scholz *et al.*, 2007) and eventually improves C economy (Santiago *et al.*, 2004; Meinzer *et al.*, 2008; Zhang *et al.*, 2009). On the other hand, it has been highlighted that growth may be

constrained at the top of taller trees due to height-induced reduced turgor (Woodruff *et al.*, 2004; Bond *et al.*, 2007) and drought probably accentuates these turgor reductions (Sala and Hoch, 2009). Due either to effective hydraulic compensation or to reduced C demands, or a combination of both, our results show that height-related drought-induced constraints on C assimilation do not necessarily lead to a decrease in C storage.

Conclusions

No support was found for C limitation in *N. pumilio* with age- or height-mediated processes, i.e. NSC levels remained stable across tree age and height whilst growth declined. This result was consistent between two locations with contrasting summer precipitation, suggesting that dry conditions do not promote ontogenetic or height-related decreases in NSC concentrations in spite of an increase in $\delta^{13}\text{C}$ under dry conditions. Our study supports previous evidence indicating that direct measurements of C reserves appear crucial in judging whether C limitation does ultimately occur (Sala and Hoch, 2009; Sala *et al.*, 2010; Piper, 2011). The evidence presented here suggests that C limitation is not the ultimate mechanism causing the age- or height-related declining tree growth.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of Table S1: summary of model selection, including AIC_c, Pearson correlation coefficient, s.d., parameter estimates and slopes for different models fitting tree age and tree height vs. the basal area increment of the last 10 years and growth efficiency for *Nothofagus pumilio* at two locations in the southern Andes of Chile.

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