Mediterranean and temperate treelines are controlled by different environmental drivers

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Summary

1. The growth limitation hypothesis (GLH) is the most accepted explanation for treeline formation, but it has been scarcely examined in Mediterranean regions, where treelines are located at lower elevations than in temperate regions. The GLH states that low temperature is the ultimate environmental driver for treeline formation, constraining C-sinks (i.e. tissue formation) more than C-sources. The GLH predicts similar or increasing (but not decreasing) non-structural carbohydrate (NSC) concentrations with elevation throughout the course of the growing season. We hypothesized that elevational trends in growth and NSC in Mediterranean regions are not determined by low temperature alone.

2. We tested the GLH by examining the mean annual basal area and NSC concentrations in developing (new twigs) and ripened tissues (branch, stem) of trees growing at three elevations in three Mediterranean and three temperate treelines in the Chilean southern Andes (33° S, *Kageneckia angustifolia*; 36, 40 and 46° S, *Nothofagus pumilio*) and in Spain (36° N, *Pinus sylvestris*; 42° N, *Pinus uncinata*). Samples for NSC were taken at the onset of summer and autumn, which represent periods of contrasting drought intensities in Mediterranean regions.

3. Tree growth decreased significantly with elevation in temperate treelines but not in Mediterranean treelines. In Mediterranean treelines, new twig NSC concentrations increased significantly with elevation in the early summer but not in the early autumn. In temperate treelines, in contrast, no elevational or seasonal variation (or interaction between them) was observed in new twig NSC concentrations. The NSC concentrations of the branches and stems from both climates showed no elevational trends. The soluble sugars' NSC fraction increased over the season in Mediterranean treelines and decreased in temperate treelines.

4. *Synthesis*. Although we found support for the growth limitation hypothesis (GLH) in temperate and Mediterranean treelines, our study shows that the tree growth and C balance in Mediterranean treelines are not controlled by low temperature alone. We suggest that environmental factors other than temperature explain the lower global elevation of Mediterranean treelines when compared to temperate treelines.

Key-words: Carbon balance, carbon limitation, drought, *Kageneckia angustifolia*, *Nothofagus pumilio*, *Pinus sylvestris*, *Pinus uncinata*, plant–climate interactions, timberline

Introduction

One of the most significant ecological and biogeographic findings in the last 20 years is that high-elevation treelines,

defined as the upper limit of > 3 m tree distribution, occur at the elevation where the mean temperatures for the growing season are *c.* 6.4 °C (Körner 1998, 2012a; Körner & Paulsen 2004; Hoch & Körner 2012). Since this global temperature threshold for treeline elevation has been found throughout the world, it has been proposed that there is a common driver

behind treeline formation (Körner 1998). Considering that adult trees, as opposed to shrubby and alpine vegetation, are not able to decouple their aerial meristems from colder air temperatures and they self-shade their roots, Körner (1998) hypothesized that treeline formation occurs due to the limitations that low temperatures exert on meristematic activity (i.e. carbon sink activity). This hypothesis, known as the growth limitation hypothesis (GLH), establishes that colder temperatures at higher elevations limit carbon (C) sink activity (i.e. C investment in growth) more than C gain (i.e. photosynthesis), leading to maintained or increased levels of C storage (e.g. non-structural carbohydrates, NSC) with elevation. This is contrary to the carbon limitation hypothesis (CLH) which states that reduced C gain with elevation (i.e. temperature) causes treeline formation at the elevation where C gain is insufficient to compensate for respiratory losses, thus predicting decreasing NSC levels with elevation (Stevens & Fox 1991). Evidence thus far appears more supportive of the GLH than the CLH, as most tree species show no growth but still present 50% of their maximum photosynthetic capacity at treeline temperatures (Alvarez-Uria & Körner 2007; Rossi *et al.* 2007; Körner 2012b; Schenker *et al.* 2014). In addition, NSC concentrations have rarely been found to decrease with elevation and more often found to increase (Hoch, Popp & Körner 2002; Hoch & Körner 2003, 2012; Piper *et al.* 2006; Shi, Körner & Hoch 2008; Fajardo *et al.* 2012).

Alpine treelines located in the Mediterranean-type climate regions (hereafter referred to as Mediterranean treelines) are a remarkable exception to the global treeline temperature pattern. In all the cases for which temperature records are available, they indicate higher temperatures (i.e. lower elevations) than the global mean (Körner & Paulsen 2004; Hoch & Körner 2012; Körner 2012a). For example, mean temperature for the growing season at treeline elevation is 8.7 °C for *Nothofagus pumilio* in central Chile and 9.4 °C for *Pinus albicaulis* in California (Körner & Paulsen 2004); 8.0 °C for *Pinus heldreichii* in Greece, and 8.2 °C for *Fagus moesiaca* in Macedonia (Körner 2012a). The apparent depression of Mediterranean treelines has been so far explained *case by case*, indicating for example a lack of a sufficiently hardy taxon (e.g. a missing taxon) for the *Nothofagus* treeline, the exceptionally short and warm growing seasons for *P. albicaulis*, and anthropic disturbances combined with a lack of true soil for some European treelines (Körner & Paulsen 2004; Körner 2012a). Interestingly, *P. albicaulis* and *N. pumilio* treelines are depressed in Mediterranean regions, but not in temperate regions where they closely match the global temperature for treeline elevation (7.1 °C in Montana and 6.8 °C in Patagonia, respectively; Hoch & Körner 2012; Fajardo & Piper 2014). Whereas the prevailing explanations for treeline formation (e.g. the GLH and the CLH) are exclusively based on the effect of low temperatures on C balance, the previous examples could be reflecting the presence of an additional driver modulating treeline elevations at Mediterranean regions. The presence of such a driver was suggested by Fajardo, Piper & Cavieres (2011), who found a significant increase in root NSC concentrations of *N. pumilio* at a temperate treeline, but not at a Mediterranean location. Differences in elevational trends of NSC concentrations and growth between Mediterranean and temperate treelines argue against low temperature as the only climatic driver of treeline formation. However, such a comparison has been little investigated (Fajardo, Piper & Cavieres 2011).

The physiology of trees at Mediterranean treeline ecotones, and perhaps the treeline elevation, could be affected by Mediterranean climatic peculiarities. Among these peculiarities, summer drought is the most prominent (Schultz 1995). In the short term, or under low intensity, drought promotes increases in C storage because C sink activity is more sensitive to drought than photosynthesis (Muller *et al.* 2011). In the long term, drought causes reductions in NSC concentrations because respiration continues (Sala, Piper & Hoch 2010; McDowell 2011; McDowell *et al.* 2011). Therefore, Mediterranean treelines, during or after the summer drought, will unlikely exhibit NSC elevational patterns reflecting only temperature effects. How such pattern could change due to drought is difficult to predict because precipitation does not follow a universal trend with elevation, as temperature does (Körner 2007). However, precipitation appears to increase with elevation in most Mediterranean mountains (Table 1). This could result, for instance, in reduced growth in the lowest elevations along with NSC accumulation when drought is still mild. Regardless of how drought could alter the C balance along elevational gradients at Mediterranean treelines, it is first important to detect whether such change takes place. If treeline formation is driven only by low temperature, elevational patterns in NSC concentrations should remain consistent between early summer and autumn, as it has been found for temperate treelines (Hoch & Körner 2003). However, if treeline formation is affected by drought, elevational patterns in NSC concentrations will likely change during the season. Specifically, they could reflect the effects of only temperature before summer drought, but temperature plus drought effects during, or just after, the period of highest drought intensity (i.e. mid-late summer for most Mediterranean regions). As a result, temperate and Mediterranean regions should exhibit similar elevational NSC concentration patterns in early summer, but different patterns in late summer–early autumn. If drought does affect the tree C balance at Mediterranean treelines, we should also expect an increase in the NSC fraction that corresponds to soluble sugars, given the osmotic role of the main soluble sugars in plants (e.g. sucrose; Chapin, Schulze & Mooney 1990).

A recent debate centres on whether increases in NSC concentrations with elevation and drought could reflect an adaptive prioritization of C allocation to storage, rather than growth limitations (Smith *et al.* 2003; Wiley & Helliker 2012; Palacio *et al.* 2014). An adaptive prioritization of C allocation to storage could be advantageous at high elevations or under drought conditions given the critical role of storage for tree survival (Chapin, Schulze & Mooney 1990; Smith *et al.* 2003; Wiley & Helliker 2012). A potential approach to disentangle mechanisms behind NSC accumulation with respect to elevation is the assessment of NSC concentrations

Table 1. Variation in precipitation along elevation, treeline elevation and treeline species in some Mediterranean mountains of the world

Location (elevational gradient[1])	Precipitation change[2]	Treeline elevation[3]	Treeline species[4]	References
1. Southern Anatolia, Turkey, 37°33′ N 30°32′ E (500–1000)	+1.00	1800–2300	*Cedrus atlantica, Cedrus libani, Juniperus thurifera*	Fontaine *et al.* (2007)
2. Yosemite NP, California, US, 37°53′ N 119°14′ E (1200–2600)	+0.21	3200–3320	*Pinus albicaulis*	Körner (2012a)
3. Sierra Nevada, Spain, 37°12′ N 3°15′ W (1000–3478)	+0.48	2400	*Pinus sylvestris*	Messerli & Winiger (1992) and Körner, Paulsen & Pelaez-Riedl (2003)
4. High Atlas, Morocco, 31°03′ N 7°54′ W (1000–4165)	+0.17	2600	*Cedrus atlantica, Juniperus thurifera*	Messerli & Winiger (1992)
5. Farellones, Chile, 33°21′ S 70°19′ W (520–2475)	+0.14[5]	2200	*Kageneckia angustifolia*	Piper *et al.* (2006) and Luebert & Pliscoff (2006)
6. Termas de Chillán, Chile, 36°54′ S 71°24′ W (144–710)	+1.9[6]	2200	*Nothofagus pumilio, Nothofagus antarctica*	Luebert & Pliscoff (2006) and Fajardo, Piper & Cavieres (2011)

[1]In metres above sea level (a.s.l.), it is the elevational difference for which the precipitation change (second column) was estimated.
[2]In mm m^{-1}, calculated as the mean difference in precipitation between the highest and lowest elevation for which information was available. The positive sign '+' indicates that for all the gradients examined, precipitation increased with elevation. For sites 1 and 2, information was taken from the web (http://www.dmi.gov.tr/files/en-US/climateofturkey.pdf; http://www.nps.gov/archive/yose/nature/wtr_climate.html).
[3]In m a.s.l., according to information provided in the literature (Reference column) and to personal observations of B. Viñegla for site 3 and of J.J. Camarero for site 4.
[4]According to information provided in the literature (Reference column) and to personal observations of J.J. Camarero for sites 1 and 4.
[5]Precipitation given for Santiago de Chile and Embalse El Yeso.
[6]Precipitation given for Chillán and Diguillín.

in different plant tissues. Adaptive NSC accumulation should take place in plant tissues typically recognized as major storage sites (e.g. roots, stems, old branches), which are characterized by a physical protection from disturbances (e.g. externally covered by bark), and by enough space for accumulation (i.e. high proportion of sapwood; Chapin, Schulze & Mooney 1990). However, NSC accumulation driven by growth limitation should clearly take place in newly developing tissues (e.g. new twigs and leaves, buds), that is tissues where growth (cell division, elongation and differentiation) is actively occurring. Surprisingly, previous studies examining the GLH have assessed NSC concentrations in major storage tissues (like roots, stems or branches), but not in developing tissues (Hoch, Popp & Körner 2002; Hoch & Körner 2003; Fajardo, Piper & Cavieres 2011; Fajardo *et al.* 2012). Examining NSC concentrations in major storage tissues to test the GLH can also be problematic because C translocation itself is affected by drought and low temperature (Sevanto *et al.* 2014; Savage *et al.* 2015). Thus, elevational increases in the NSC concentration of ripened tissues could be a reflection of translocation impediments to the C-sinks. In contrast, NSC accumulation in or very close to the C-sinks significantly reduces the influence of impeded translocation and hence can better test for growth limitations.

In this study, we investigated whether treeline ecotones in Mediterranean and temperate regions are controlled by similar drivers. We hypothesized that, in contrast to temperate treelines, Mediterranean treelines are not determined only by low temperatures. If so, we first expect to find decreased tree growth along with increasing elevation in temperate treelines but not in Mediterranean treelines, where intense summer drought at low elevations could nullify or reverse the elevational trend in tree growth driven by temperature alone.

Secondly, we expect that elevational trends in NSC concentrations and soluble sugar fractions will not change during the growing season in temperate treelines, but they will change in Mediterranean treelines; the elevational trends should then be similar between regions in early summer. This expectation implies that Mediterranean treelines will not exhibit elevational increases in NSC concentration *during* or *after* summer drought. We tested our hypothesis by examining the elevational patterns in tree growth rates and NSC concentrations in developing tissues (new twigs), before and after summer drought, in Mediterranean and temperate treelines located in Chile and Spain (Table 1). To further investigate whether elevational variations in NSC concentrations reflect C imbalances or adaptive C allocation responses, we also examined NSC concentrations in formed (i.e. ripened) tissues (stems and branches). We anticipate that NSC accumulation driven by limited growth must take place in tissues under active growth (e.g. developing new twigs), while NSC accumulation driven by prioritized C allocation to storage must occur in major storage sites (e.g. stem, branch sapwood).

Materials and methods

SPECIES AND RESEARCH SITE DESCRIPTIONS

We selected locations to represent Mediterranean and temperate climatic treelines in Chile and Spain (Table 2). At each location, we identified the area with uppermost forest patches with upright trees > 3 m tall, representing the *actual* or *thermal* treeline according to Körner & Paulsen (2004) and selected a treeline gradient for sampling that consisted of three elevational transects from treeline elevation downslope to a mature, tall-stature tree forest. In the Chilean Andes, one Mediterranean treeline site was located in Termas de Chillán,

Table 2. Geographic location, treeline elevation, main climatic characteristics and treeline species of the studied areas

Study site	Location	Treeline elevation (m a.s.l.)	Seasonal[1]/annual precipitation (mm)	Mean temperature warmest month (°C)	Growing Season Length (days)	Treeline species
Mediterranean						
Farellones, Chile	33°21′ S; 70°19′ W	2200	19.4/445[2]	17.3[3]	180[3]	*Kageneckia angustifolia* (Rosaceae)
Termas de Chillán, Chile	36°54′ S, 71°24′ W	2100	165/2000[4]	17.6[7]	215[6]	*Nothofagus pumilio* (Nothofagaceae)
Sierra Nevada, Spain	37°04′ N, 02°01′ W	2418	172/851[9]	21.7[9]	175[13]	*Pinus sylvestris* (Pinaceae)
Temperate						
Cerro Castillo, Chile	46°04′ S, 72°03′ W	1310	250/1100[7]	9.1[7]	148	*Nothofagus pumilio* (Nothofagaceae)
Antillanca, Chile	40°47′ S, 72°11′ W	1350	454/3661[5]	10.7[5]	ND	*Nothofagus pumilio* (Nothofagaceae)
Ordesa, Pyrenees, Spain	42°38′ N, 00°02′ W	2200	100[10]/1700[11]	11[12]	165[12]	*Pinus uncinata* (Pinaceae)

[1]From November to March in Chile; from May to September in Spain and US; [2]Santibáñez & Uribe (1990); [3]L. Cavieres (unpublished data); [4]Dirección General de Aguas, Chile; [5]Daniels & Veblen (2004); [6]Körner & Paulsen (2004); [7]Luebert & Pliscoff (2006); [8]Körner (2012a); [9]Linares *et al.* (2014); [10]data from nearby 'Refugio de Góriz' station: 42°39′49″ N, 00°00′50″ E (Camarero & Gutiérrez 1999); [11]Wiegand *et al.* (2006); [12]from June 2012 to July 2013 (J.J. Camarero, unpublished data); [13]Körner, Paulsen & Pelaez-Riedl (2003).

where *N. pumilio* (Nothofagaceae) is the dominant treeline species occasionally accompanied by a few individuals of *Nothofagus antarctica*. In this area, climate is of a Mediterranean-type and January and February are typically the driest months of the year (Schultz 1995; Luebert & Pliscoff 2006). 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). Another Mediterranean treeline in Chile was located in Farellones (Table 2), where the dominant treeline species is *Kageneckia angustifolia* (Rosaceae). The soil at this location is thin (< 1 m depth) with a coarse texture derived from a mixture of intrusive volcanic rocks, assigned to a Miocene age (18–20 m.y.b.p.), alternating with sedimentary rocks of lacustrine origin (Cavieres, Peñaloza & Kalin Arroyo 2000). One of the temperate treeline sites in Chile was located within the Cerro Castillo Natural Reserve, hereafter referred to as Cerro Castillo, where *N. pumilio* is the only tree species at the treeline. Climate at this area is cold temperate (Luebert & Pliscoff 2006). Soil moisture at this site during January and February 2009 was *c.* threefold and sixfold higher than in the Termas de Chillán site (F.I. Piper, unpubl. data). A second temperate treeline site in Chile was located in the Antillanca area within the Puyehue National Park, where *N. pumilio* co-occurs with sparse individuals of *N. antarctica* and *N. betuloides* (Fajardo, Piper & Hoch 2013). In Cerro Castillo and Antillanca, most precipitation falls as snow from May to September, and the soils are derived from andesitic rocks of volcanic origin. Treelines in Chile were located on slopes with relatively low angles (< 30%), a straight configuration, and with the presence of alpine vegetation above the treeline. The slopes sampled have a south-west and north-east aspect in Termas de Chillán and Farellones, respectively, while the Cerro Castillo and Antillanca treelines have a south-east aspect. *Nothofagus* treelines constitute an abrupt transition from the forest to a treeless alpine area, whereas the *K. angustifolia* treeline in Farellones constitutes a diffuse form of treeline.

In Spain, we selected a *Pinus sylvestris* (Pinaceae) treeline in Sierra Nevada National Park (Andalucía, southern Spain), hereafter referred to as Sierra Nevada, as the Mediterranean treeline, and a *Pinus uncinata* treeline in the Spanish Central Pyrenees (Aragón, northern Spain), hereafter referred to as Pyrenees, as the non-Mediterranean or temperate counterpart (Table 2). In Sierra Nevada, sampling was carried out on a south-west aspect with a slope of *c.* 40°. The climate in Sierra Nevada is Mediterranean, with hot, dry summers. The bedrock is mainly composed of schist; the soil is typically less than 0.1 m in depth. Here, we sampled a naturalized population of *P. sylvestris* afforestations that were planted in the middle of the 20th century in an effort to cover slopes with vegetation after the abandonment of grazing activities. We are not certain if these afforestations were performed at the elevation of the *thermal* treeline, but we can fairly assume that the *thermal* elevation should have been naturally reached during this period. The study site in the Pyrenees was located in the Sierra de Cutas area, within the buffer zone of the Ordesa and Monte Perdido National Parks, on a south-face aspect with a slope of *c.* 20°. This treeline ecotone has not been affected by local, anthropogenic disturbances (logging, grazing) during the last 50 years (Camarero & Gutiérrez 2004). *Pinus uncinata* is the dominant treeline species in the area, forming open forests and an abrupt treeline ecotone (Wiegand *et al.* 2006). Soils in the area are poorly developed from sandstones and calcareous limestones (Camarero, Gutiérrez & Fortin 2000).

Soil moisture measured at each studied treeline during the growing season was lower in Mediterranean than in temperate regions (Fig. 1). We lack soil humidity data for the intermediate and low elevations. Nevertheless, given that in all of our Mediterranean study sites precipitation increases with elevation (Table 1), differences in soil humidity between temperate and Mediterranean climates are expected to increase when elevation decreases.

SAMPLING

To separate the potential influences of drought from low temperature on the tree C balance along elevation, we sampled plant tissue in both early summer (i.e. before drought, Fig. 1) and early autumn (i.e. after drought, Fig. 1). The early summer sampling was conducted between the last week of December 2011 and the first week of January 2012 for sites in Termas de Chillán, Antillanca and Cerro Castillo, during the last week of December 2013 in Farellones, and at mid-June 2012 in Sierra Nevada and Pyrenees. The early autumn sampling was carried out in late March 2012 in Ter-
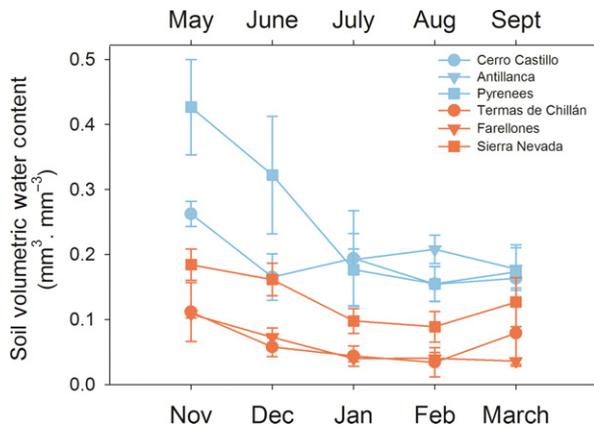
Fig. 1. Mean of monthly soil volumetric water content at treeline (expressed in mm^3 of water per mm^3 of soil) in three Mediterranean (red symbols) and three temperate (blue symbols) treelines of Chile and Spain during the growing season period (November to March in Chile; May to September in Spain). Error bars indicate 1 SD. Mean values considered the following periods: 2007–2012 in Pyrenees, 2008 and 2010 years in Antillanca, 2008–2010 in Cerro Castillo, 2006–2007 in Farellones, 2008–2009 in Termas de Chillán, and 2012–2015 in Sierra Nevada. Measurements were taken at a soil depth of −30 cm (ECH$_2$O S–SMA–M005 probes, connected to a HOBO® Micro Station logger, Onset Computer Corporation). See Table 2 for sites' description.

mas de Chillán, Antillanca and Cerro Castillo, during the last week of March 2013 in Farellones, and in the second week of September 2012 in Sierra Nevada and Pyrenees. For plant tissue collection, we selected 6–10 adult trees, separated by a minimum of 30 m from each other at three equidistant elevations (*c.* 100–200 m from one to another), located from the treeline elevation downslope to a tall, mature forest. We selected dominant trees without signs of browsing or other damages and of relatively similar size (diameter at breast height, DBH, 1.35 m, was between 15 and 30 cm). The collection of tissue samples was conducted between 11:00 and 17:00 h. For chemical analyses, we extracted a stem core using a 5.15-mm-wide

increment borer (Haglöf, Långsele, Sweden), a section of *c.* 5-year-old branch using regular pruning scissors, and a section of recently formed terminal twig. We removed *in situ* bark and phloem from the branches using a knife, and we additionally discarded heartwood from the cores. In contrast, no bark or phloem removal was possible from the new twigs as they were formed by a very tender tissue (i.e. poorly lignified). In this case, the whole tissue was used for analysis. All tissue samples were bagged, labelled and stored in a cool box for transportation. A second, to-the-pith long core was extracted for each sampled tree at < 30 cm height using the same 5.15-mm increment borer. This second core was intended for tree growth determination. (For *Nothofagus* treelines, we included additional cores from unpublished studies that were extracted the same dates, see Table 3).

Since NSC concentrations expressed in mass are affected by the tissue density, we examined the elevational trends in wood density. We were not able to perform this examination for all of our sites, but only for Termas de Chillán and Cerro Castillo in Chile and Sierra Nevada and Pyrenees in Spain. We estimated the wood density of each tissue sample as the ratio between the dry mass and the volume of that mass. Fresh volume for each tissue was determined by submerging the sample into a glass beaker on a scale. The difference caused by the introduction of the sample, which equals the volume of water displaced by the sample, was recorded and converted to volume based on the density of water as 1.0 g cm^{-3} at standard temperature and pressure. Samples were then heated in a microwave oven at 600 W for 90 s to denature enzymes (Popp *et al.* 1996) and placed to dry in a forced-air oven (Memmert GmbH, Schwabach, Germany) at 70 °C for 72 h. The sample dry mass was subsequently measured to compute wood density, and samples were finally ground into a fine powder and stored over silica gel at 4 °C until chemical analyses were conducted.

TREE GROWTH DETERMINATION

Cores were prepared following standard dendrochronological techniques (Stokes & Smiley 1996). Cores were dried, mounted and glued firmly on grooved wooden sticks and sanded with successively finer

Table 3. Elevation (m a.s.l.) and mean (±1 SE) annual basal area increment (cm^2) at six treeline ecotone locations in Mediterranean (Farellones and Termas de Chillán in Chile, and Sierra Nevada in Spain) and temperate (Antillanca and Cerro Castillo in Chile, and Pyrenees in Spain) treelines

	Mediterranean								
	Farellones *K. angustifolia*			Termas de Chillán *N. pumilio*			Sierra Nevada *P. sylvestris*		
	Elevation	*n*	BAI	Elevation	*n*	BAI	Elevation	*n*	BAI
Treeline	2200	6	5.05 (1.87)	2080	9	9.12 (2.38)	2400	8	9.81 (1.87)
Intermediate	2100	5	4.39 (1.18)	1900	14	12.15 (1.61)	2200	8	7.11 (1.01)
Low	2000	5	2.50 (0.62)	1750	7	8.31 (0.65)	2000	8	11.40 (1.70)
	Temperate								
	C. Castillo *N. pumilio*			Antillanca *N. pumilio*			Pyrenees *P. uncinata*		
	Elevation	*n*	BAI	Elevation	*n*	BAI	Elevation	*n*	BAI
Treeline	1300	8	5.36 (1.87)	1350	7	7.59 (2.47)	2200	10	3.59 (0.46)
Intermediate	1200	10	6.03 (0.55)	1200	16	9.75 (1.87)	2050	10	12.51 (1.11)
Low	1010	10	11.90 (2.06)	1100	7	14.50 (4.10)	1900	10	12.13 (1.43)

grades of sandpaper until optimal surface resolution allowed the annual rings to be distinguished under magnification (10×). Inside-bark bole radius and annual radial increments from the last 10 years were measured to the nearest 0.01 mm using a microscope mounted on a dendrochronometer with a Velmex sliding stage and Accurite measuring system (Bloomfield, NY, USA). Cross-dating accuracy was checked visually as rings were easily identified. We estimated a mean annual basal area increment (BAI) for the last 10 years, which represents a relative measure of gains in growth, by considering the diameter at coring height and bark thickness, where the latter was assumed constant. The BAI was then computed as:

$$\mathrm{BAI} = \frac{\pi(R_t^2 - R_{t-10}^2)}{10},$$

where R_t is the radius of the stem at year t, and R_{t-10} is the radius of the stem of the previous 10 years. The radius of the stem, R_t, was computed by considering the diameter at coring height, bark depth, and the distance between pith and the last year ring in the core.

CHEMICAL ANALYSES

The sampled trees' stems, branches and twigs from each location, sampling date and elevation were analysed for non-structural carbohydrates (NSC = soluble sugars + starch). In approximately 15 mg of dried powder sample, soluble sugars and starch concentrations were determined. Soluble sugars were extracted with a methanol: chloroform: water solution separated from pigments and lipids by the addition of water and chloroform (Rose *et al.* 1991), and the main sugars (sucrose, glucose and fructose) were determined by the phenol sulphuric method, using 2% phenol and reading at 490 nm (Chow & Landhäusser 2004). The residual pellet was dried overnight at 50 °C in a forced-air stove and starch was then gelatinized (Rose *et al.* 1991) and hydrolysed to glucose with amyloglucosidase (10115; Sigma-Aldrich, St. Louis, MO, USA) at 45 °C overnight. Glucose was similarly determined as the method used for soluble sugars (Chow & Landhäusser 2004). Soluble sugars and starch concentrations were expressed as mg g^{-1} of dry weight. Total NSC concentrations were calculated from the sum of soluble sugars and starch.

STATISTICAL ANALYSES

The influence of climate, elevation and the sampling date on the NSC concentrations and the soluble sugars fraction were analysed fitting linear mixed-effects models. In the modelling, we considered climate (Mediterranean and temperate), elevation (low, mid and treeline) and the sampling date (early summer, early autumn) as fixed factors, with random effects for the sites, and for the trees nested in each site and elevation, the former to take into account the among-site variation and the latter was considered because tree individuals within each elevation and site were more likely to resemble one another than tree individuals from other sites and elevation. The significance of terms was done using the approach of assessing *P*-values for main factors and interactions with the ANOVA function on lme object in R (Faraway 2006). Variables were all log–transformed to meet the requirements of normal distribution prior to analysis. Differences in NSC concentrations and soluble sugar fractions between elevations were tested for significance at the *P* < 0.05 level by Tukey–Kramer honestly significant difference (HSD) tests using the multcomp package in R (Hothorn, Bretz & Westfall 2008). All analyses were carried out in R (R Development Core Team 2013).

Results

GROWTH

Elevation, but not climate (Mediterranean versus temperate), had a significant effect on tree growth ($F = 9.223$, $P < 0.001$), measured as the mean annual BAI. The interaction term between elevation and climate proved to be significant ($F = 5.589$, $P = 0.005$); the mean annual BAI decreased significantly with elevation in temperate treelines but not in Mediterranean treelines (Fig. 2). For temperate treelines, the increase in BAI from treeline to the lowest elevation was *c.* 50% in Cerro Castillo (*N. pumilio*) and *c.* 70% in Pyrenees (*P. uncinata*; Table 3). For Mediterranean treelines, growth trends were mixed among sites: in Farellones (*K. angustifolia,*) BAI was in fact *c.* 50% higher at the treeline than at the lowest elevation, while in Termas de Chillán (*N. pumilio*) and Sierra Nevada (*P. sylvestris*), BAI was rather similar when treeline and the lowest elevation were compared (Table 3). For temperate treelines, intermediate elevations had intermediate values of BAI, whereas for Mediterranean treelines, BAI at intermediate elevations did not differ from the other two elevations (Fig. 2). At the treeline, BAI was significantly higher in Mediterranean than in temperate climates (Fig. 2).

NON-STRUCTURAL CARBOHYDRATES

We found no effect of elevation on NSC concentrations at any tissue. However, we did find a significant interaction
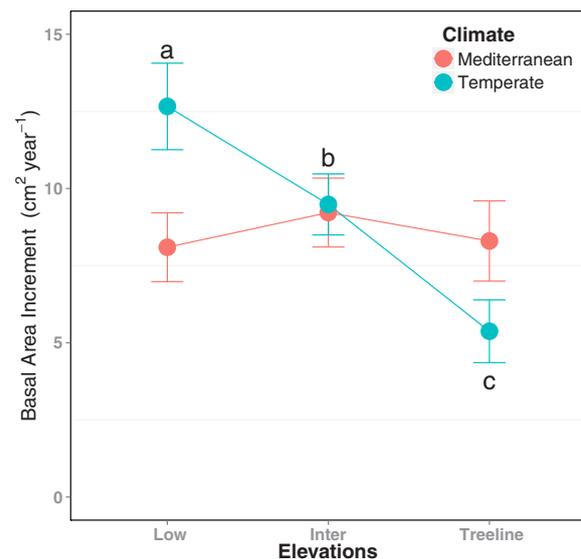
Fig. 2. Mean annual relative tree growth (basal area increment) at three Mediterranean and three temperate treelines of Chile and Spain. Mediterranean treeline species sampled were *Kageneckia angustifolia* (Rosaceae, Chile), *Nothofagus pumilio* (Nothofagaceae, Chile) and *Pinus sylvestris* (Pinaceae, Spain); temperate treeline species sampled were *N. pumilio* (Nothofagaceae, Chile) and *Pinus uncinata* (Pinaceae, Spain). See Table 2 for study sites' climatic description. Different letters indicate significant differences, at *P* < 0.05, among elevations.

Table 4. Mixed-effect model outputs when considering climate (C, Mediterranean versus temperate), elevation (E, low, mid and treeline), season (S, early summer versus early autumn), and the interaction of these factors on the concentration of non-structural carbohydrates (NSC, mg g^{-1}) and the soluble sugar fraction of NSC in recently formed twigs, branches and stem in treeline trees (TSS/NSC). Six treeline ecotone locations were considered: Farellones and Termas de Chillán in Chile and Sierra Nevada in Spain (Mediterranean climate), and Antillanca and Cerro Castillo in Chile and Pyrenees in Spain (Temperate climate)

	d.f.	Twig		Branch		Stem	
		F	P	F	P	F	P
NSC							
C	1,4	0.878	0.402	0.014	0.912	0.844	0.412
E	2,8	1.522	0.275	3.369	0.087	0.523	0.609
S	1,264	9.675	0.002	104.643	< 0.001	26.046	< 0.001
C × E	2,8	1.283	0.329	2.012	0.196	0.881	0.451
C × S	1,264	56.686	< 0.001	15.313	< 0.001	1.734	0.189
E × S	2,264	2.636	0.074	0.691	0.502	4.051	0.019
C × E × S	2,264	3.929	0.021	2.445	0.089	1.027	0.359
TSS/NSC							
C	1,4	0.726	0.442	0.331	0.596	0.669	0.460
E	2,8	0.035	0.966	0.921	0.437	0.886	0.449
S	1,264	1.471	0.226	2.136	0.145	62.363	< 0.001
C × E	2,8	0.125	0.884	0.797	0.484	0.806	0.480
C × S	1,264	20.618	< 0.001	1.709	0.192	0.315	0.575
E × S	2,264	2.717	0.068	2.030	0.133	0.064	0.938
C × E × S	2,264	3.910	0.021	0.002	0.998	1.261	0.285

effect of climate, season and elevation on new twig NSC concentrations (Table 4). In Mediterranean regions, new twig NSC concentrations significantly increased with elevation in early summer but not in early autumn. By contrast, in temperate regions, new twig NSC concentrations did not vary with elevation, neither in early summer nor in early autumn (Fig. 3). Across elevations, there was a significant effect of the interaction between sampling date and climate on the new twig and branch NSC concentrations, which decreased from early summer to early autumn in Mediterranean treelines but increased in temperate treelines (Table 4). Stem NSC concentrations increased with elevation in early summer but not in early autumn; although the climate had no significant effect, this result was only found in Mediterranean treelines (Fig. 3).

For all tissues, the NSC fraction corresponding to soluble sugars was unaffected by sampling season, climate or elevation. Interestingly, we did find a significant interaction effect of climate and season on the soluble sugar fraction of new twigs (Table 4, Fig. 4); it increased from early summer to early autumn in Mediterranean treelines, but it decreased in temperate treelines. Finally, tissue density was in general similar between the treeline and the lowest elevations in all the sites examined except in Termas de Chillán, where new twigs were significantly denser in the lowest elevation (Table 5).

Discussion

MEDITERRANEAN TREELINE FORMATION: TEMPERATURE IS NOT THE ONLY DRIVER

In global comparisons, Mediterranean treelines have been found to occur at *exceptionally* warmer temperatures (i.e.

lower elevations) than temperate treelines (Körner 2012a). Anthropogenic influences (widespread in the Mediterranean Basin), mountain chains not reaching sufficient elevation for some species, missing taxon and a long winter snow pack period (e.g. Sierra Nevada, CA, USA) have been mentioned as possible causes for Mediterranean treelines being formed at relatively low elevation (Körner & Paulsen 2004; Körner 2012a). Moisture limitation has been discarded as a cause for this depression in elevation (Körner 2012a). Rather, Mediterranean treeline formation has been hypothetized to be mainly driven by the same mechanisms than influence non-Mediterranean treelines: reduced C sink activity due to low temperature (the GLH; Körner 1998). Our results, however, are consistent with an exclusive role of temperature controlling tree growth at elevational gradients in temperate regions but not in Mediterranean regions, where we found similar basal area increment between the treeline and lower elevations. This result is evidence that growth limitations at the lowest elevations examined (*c.* 300 m below treeline) were similar in magnitude to those occurring at treeline elevation, thus revealing that factors other than temperature control the tree growth of Mediterranean treeline ecotones. Due to the global character of our assessment, these factors are most likely related to climatic features of these regions (e.g. drought) rather than to site- or species-specific differences like substrate, photoperiod or phenology.

The influence of different factors affecting temperate and Mediterranean treelines is also indicated by the contrasting seasonal effects on the elevational trends of new twigs' NSC concentrations between the two regions. When temperature is the only driver of treeline formation, seasonality has little effect on the elevational trends in NSC (Hoch & Körner
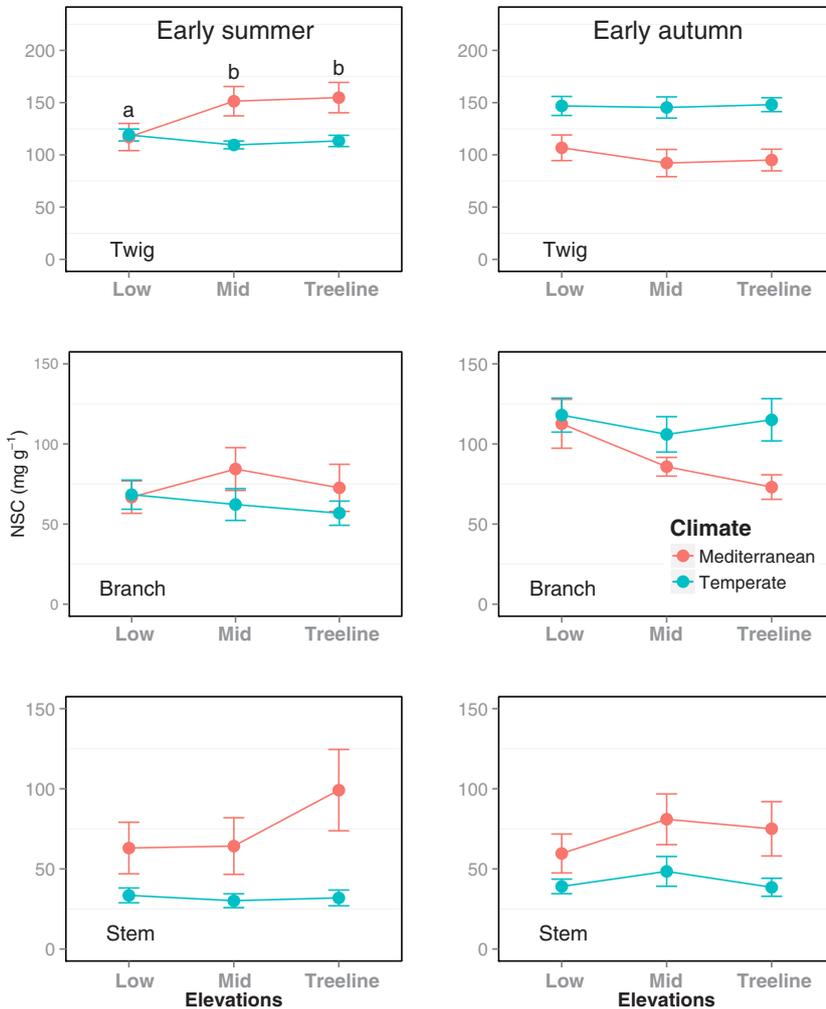
Fig. 3. Non-structural carbohydrate (NSC) concentrations per unit of dry mass of new twigs, branches and stem sapwood in treeline trees sampled in early summer and early autumn, at three Mediterranean and three temperate treelines of Chile and Spain. Error bars refer to standard errors. Different letters indicate significant differences, at *P* < 0.05, among elevations.

2003; Shi, Körner & Hoch 2008). The lack of seasonal variation that we found in the elevational trend of new twig NSC concentration for temperate regions can be thus interpreted as support for temperature as the main mechanistic driver of treeline formation. On the other hand, the contrasting elevational trend in new twig NSC concentration found in Mediterranean regions between early summer and early autumn indicates that temperature was not the only driver of the elevational trends in tree C balance. Our results for Mediterranean regions further revealed a strong elevational increase in new twig NSC concentrations during early summer, which generally coincides with a period of active growth and still high soil humidity (Fig. 1). This result indicates that growth limitations were significantly higher at the treeline than at lower elevations, consistent with an exclusive effect of temperature at this period. However, by the end of the growing season (after the occurrence of summer drought, Fig. 1), this trend vanished coincident with a decrease in new twig and branch NSC concentrations in the whole gradient, suggesting that C demand increased over the season in relation to the C gain, particularly at the treeline elevation. In the lowest elevation, the most pronounced summer drought could have anticipated the end of the growing season period leading to some

NSC accumulation (Körner 2003), which in turn could explain the vanishing of the elevational increase in new twig NSC concentrations observed in early summer. In contrast, new twig and branch NSC concentrations in temperate regions increased from early summer to early autumn, further supporting that tree C balance in temperate and Mediterranean treeline ecotones is controlled by different factors. We did not measure root NSC concentrations, which is the main storage tissue in many tree species and whose seasonal trends may be decoupled from above-ground tissues (Rosas *et al.* 2013). Thus, we cannot discard that some of the seasonal or elevational changes in NSC concentrations in above-ground tissues could reflect altered C demand by roots.

Other results of our study are consistent with a role of drought controlling tree C balance at Mediterranean elevational gradients. Wood density is known to be negatively related to temperature and precipitation (e.g. Preston, Cornwell & DeNoyer 2006). The fact that in Mediterranean treelines, the wood density of new twigs was indeed higher at the lowest elevation than at the treeline suggests a more constraining effect of drought than of low temperature on tissue formation. Likewise, that the soluble sugar fraction of new twig NSC concentrations increased over the season in
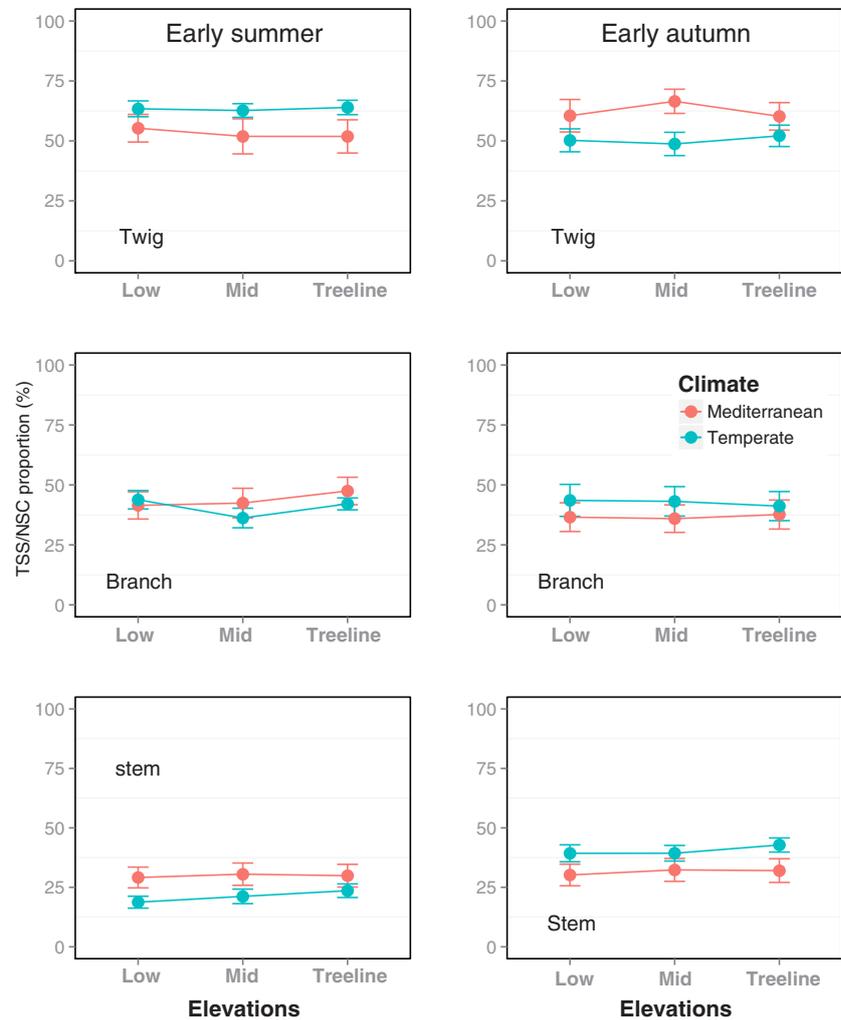
Fig. 4. Elevational variations in the soluble sugars-to-NSC fraction of new twigs, branches and stem sapwood, sampled in early summer and early autumn, at Mediterranean and temperate treelines. See Table 2 for study sites included in each climatic category.

Mediterranean regions, but decreased in temperate regions, is consistent with an increased demand of osmotic compounds during summer drought (Chaves, Maroco & Pereira 2003), and with drought-induced growth limitations (Muller *et al.* 2011).

Our study shows that temperature is not the only driver of tree growth and C balance in treeline ecotones of Mediterranean regions. This evidence opens the possibility of drought as an explanation for the elevational depression of Mediterranean treelines world-wide. One of the reasons to dismiss drought as a causal factor of treeline elevation is that some of the highest treelines in the world occur at regions with very low annual precipitation (e.g. *Polylepis tarapacana* at c. 4.7 °C in Mt. Sajama, Bolivia; *Juniperus tibetica* at c. 6.8 °C in Sygera Mountains, Tibet; Körner 2012a). It is important to note that there are noticeable differences in the rate and timing of precipitation between these two locations and the treelines located in Mediterranean climates. In Tibet, for example, annual precipitation is as low as c. 300 mm but it mostly falls during the growing season (Fang *et al.* 2004). A similar situation occurs in Bolivia, where the *invierno boliviano* brings maximum precipitation and air humidity during the summer months (Hardy *et al.* 1998). Although

annual precipitation in Mediterranean climates is usually in the range of 500–1000 mm, it falls mostly in winter, likely determining a period of water deficit and drought stress for most vegetation (Schultz 1995). Another reason to argue against drought as setting treeline elevation has been that in most Mediterranean mountains precipitation increases, rather than decreases, with elevation (Körner 2012a; Table 1). However, precipitation gradients are not necessarily correlated with drought gradients. Low temperature reduces water absorption by plants (e.g. it reduces the water movement), causing effects similar to drought (Kramer 1940; Larcher 2003). Thus, Mediterranean treelines could certainly be affected by drought driven by reduced precipitation and limited soil water absorption by plants. In fact, in a previous study, Fajardo, Piper & Cavieres (2011) found that $\delta^{13}C$ values of stem sapwood increased (i.e. became less negative) with elevation in *N. pumilio* at Termas de Chillán (Mediterranean), but not at Cerro Castillo (temperate) treeline locations. Paleo-ecological studies, indeed, have documented historical declines in the treeline elevation in Sierra Nevada (California, USA) associated with drought periods (Lloyd & Graumlich 1997). Similarly, the highest treeline elevation in Sierra Nevada (Spain) has been linked to warm and wet cli-

Table 5. Elevational variation in wood density (g cm^{-3}) of new twigs, branches and stem sapwood in adult trees of *Nothofagus pumilio* growing at two locations in southern Chile (Termas de Chillán and Cerro Castillo), *Pinus uncinata* in Pyrenees (Spain) and *Pinus sylvestris* in Sierra Nevada (Spain)

	Chile		Spain	
	Temperate Cerro Castillo	Mediterranean Termas de Chillán	Temperate Pyrenees	Mediterranean Sierra Nevada
Twig				
Low	0.44 (0.02)[a]	0.68 (0.02)[a]	0.24 (0.01)[b]	0.35 (0.03)[a]
Mid	0.52 (0.04)[a]	0.61 (0.02)[a,b]	0.30 (0.02)[a]	0.38 (0.04)[a]
Treeline	0.47 (0.03)[a]	0.59 (0.03)[b]	0.25 (0.00)[b]	0.30 (0.05)[a]
F (P-value)	1.38 (0.26)	3.37 (0.04)	12.60 (< 0.0001)	2.12 (0.13)
Branch				
Low	0.44 (0.01)[a]	0.60 (0.01)[a,b]	0.49 (0.03)[a]	0.45 (0.02)[a,b]
Mid	0.44 (0.00)[a]	0.61 (0.01)[a]	0.48 (0.01)[a]	0.44 (0.04)[a]
Treeline	0.46 (0.01)[a]	0.55 (0.02)[b]	0.52 (0.02)[a]	0.40 (0.02)[b]
F (P-value)	1.52 (0.23)	4.68 (0.01)	1.47 (0.24)	2.69 (0.08)
Stem				
Low	0.63 (0.04)[a]	0.74 (0.03)[a]	0.51 (0.02)[a]	0.52 (0.02)[a,b]
Mid	0.64 (0.03)[a]	0.70 (0.03)[a]	0.49 (0.02)[a]	0.49 (0.01)[a]
Treeline	0.70 (0.04)[a]	0.71 (0.03)[a]	0.50 (0.02)[a]	0.54 (0.01)[b]
F (P-value)	1.00 (0.37)	0.41 (0.66)	0.71 (0.50)	5.92 (0.005)

Values represent means and standard errors (in parentheses) from eight trees per elevation for *N. pumilio* and *P. sylvestris* and from ten trees for *P. uncinata*. Comparisons among elevations within each location were done using simple one-way ANOVA (*F*-ratio and *P*-value are shown). Different letters indicate a statistically significant difference among elevations within tissues and locations ($P < 0.05$, Tukey HSD *post hoc* test).

matic conditions during the Holocene (Anderson *et al.* 2011).

GROWTH LIMITATION OR PREFERENTIAL C ALLOCATION TO STORAGE?

A recent debate centres on whether NSC accumulation at the treeline reflects growth limitations or a prioritization of storage over growth (Wiley & Helliker 2012; Palacio *et al.* 2014). Wood cores and branches are the most commonly sampled tissues for NSC analyses in studies testing the GLH. They represent major C storage sites in trees, making it difficult to discern whether their NSC accumulation reflect growth limitation or prioritized allocation. By contrast, NSC accumulation in tissues recently formed or under formation, as we found in new twigs at Mediterranean regions, is more likely to reflect growth limitation than prioritized allocation. The hypothesis of preferential C allocation to storage states that increased NSC concentration with elevation may be an adaptive response to overcome future negative C balances potentially caused by low temperatures or harsh conditions (Smith *et al.* 2003; Wiley & Helliker 2012). If that were the case, trees should allocate C in their main storage tissues, which are those generally protected from disturbances and characterized by sufficient sapwood area. NSC accumulation in recently formed tissues is unlikely as an adaptive strategy for eventual C mobilization and usage, since these tissues are more exposed to harsh climate-induced damage and have a reduced sapwood volume. Our results thus support the notion that any NSC accumulation with elevation results from environmentally driven growth limitation (Fajardo *et al.* 2012).

Conclusions

This study demonstrates that although C sink limitation is the most likely process controlling treeline formation in both Mediterranean and temperate treelines (i.e. NSC concentrations did not decrease across elevations), the environmental control on tree growth and C balance along elevational gradients differs between both regions. For temperate treelines, temperature appears as the main limiting factor of growth at the treeline. For Mediterranean regions, a lack of elevational trend in tree growth demonstrates the presence of factors other than temperature. We suggest that one of these factors is drought, which by the end of the growing season could override the temperature-driven elevational increase in NSC concentrations of developing tissues observed at early summer.

Acknowledgements

Data accessibility

Data available from the Dryad Digital Repository, doi:10.5061/dryad.ks97h (Piper *et al.* 2016).

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