

AN EXPERIMENTAL APPROACH TO EXPLAIN THE SOUTHERN ANDES ELEVATIONAL TREELINE¹

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- **Premise of the study:** The growth limitation hypothesis (GLH) is the most accepted mechanistic explanation for treeline formation, although it is still uncertain whether it applies across taxa. The successful establishment of *Pinus contorta*—an exotic conifer species in the southern hemisphere—above the *Nothofagus* treeline in New Zealand may suggest a different mechanism. We tested the GLH in *Nothofagus pumilio* and *Pinus contorta* by comparing seedling performance and carbon (C) balance in response to low temperatures.
- **Methods:** At a southern Chilean treeline, we grew seedlings of both species 2 m above ground level, to simulate coupling between temperatures at the meristem and in the air (colder), and at ground level, i.e., decoupling air temperature (relatively milder). We recorded soil and air temperatures as well. After 3 yr, we measured seedling survival and biomass (as a surrogate of growth) and determined nonstructural carbohydrates (NSC).
- **Key results:** *Nothofagus* and *Pinus* did not differ in survival, which, as a whole, was higher at ground level than at the 2-m height. The root-zone temperature for the growing season was 6.6°C. While biomass and NSC decreased significantly for *Nothofagus* at the 2-m height compared with ground level (C limitation), these trends were not significant for *Pinus*.
- **Conclusions:** The treeline for *Nothofagus pumilio* is located at an isotherm that fully matches global patterns; however, its physiological responses to low temperatures differed from those of other treeline species. Support for C limitation in *N. pumilio* but not in *P. contorta* indicates that the physiological mechanism explaining their survival and growth at treeline may be taxon-dependent.

Key words: carbon source–sink balance; leaf habit; missing taxon; nonstructural carbohydrates; *Nothofagus pumilio*; Patagonia; *Pinus contorta*; plant–climate interactions; timberline.

Alpine treelines are conspicuous vegetation boundaries that characterize most mountain landscapes around the world. On a global scale, alpine treelines have been claimed to be physiologically controlled by low temperature and are hence considered one of the most responsive to global warming (Tranquillini, 1979; Körner, 1998; Jobbágy and Jackson, 2000; Grace et al., 2002; Körner 2012a). In a search for general trends, Körner and Paulsen (2004) found that most alpine treelines (particularly in the northern hemisphere) are located at an isotherm of 6.7°C (± 0.8 SD) of mean root–zone temperature for the growing season. This striking result cemented the notion that low temperatures during the growing season control the treeline elevation. The most plausible explanation for this so far has been that low temperatures affect trees' carbon (C) balance (Körner, 1998). A recent worldwide study in 13 alpine treeline regions confirmed the existence of this narrow isotherm for treelines (rectified to 6.4°C ± 0.7 SD) and concluded that the mechanism explaining this pattern is C sink limitations in trees (Hoch and Körner, 2012).

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The temperature limit of tree growth represents a threshold that markedly separates trees from other life forms that are able to grow above the treeline elevation, e.g., alpine vegetation. The discussion about the physiological mechanism for treeline formation has been focused on how low temperatures affect C acquisition or C usage. It has been affirmed that in low temperature-adapted plants, including treeline trees, light-saturated photosynthesis reaches approximately 50% its full capacity at +5°C, whereas no tree has ever been shown to exhibit significant growth (e.g., cell division) below 5°C (James et al., 1994; Solfeld and Johnsen, 2006; Alvarez-Uria and Körner, 2007; Rossi et al., 2007). As a consequence, all low temperature-adapted plants tested thus far have shown an increase in their C reserves (i.e., nonstructural carbohydrate [NSC] concentrations) when exposed to colder temperatures (e.g., Hoch and Körner, 2009), suggesting that plant growth and development are not limited by carbon supply. These observations contradict the carbon-limitation hypothesis (as proposed by Stevens and Fox (1991) and Wardle (1993)), which suggests that tree growth at cold temperatures is limited by carbon supply. The growth limitation hypothesis (GLH), on the other hand, proposes that cell and tissue formation—and not C acquisition—are the processes that are first limited by the elevational decrease in temperature (Körner, 1998). A growing number of empirical studies support the GLH, i.e., these found no decrease, and most often an increase, in NSC concentrations with elevation (e.g., Piper et al., 2006; Shi et al., 2008; Fajardo et al., 2012; Hoch and Körner, 2012). Further support for this hypothesis is found in the duration of mitosis and cell multiplication, which exponentially increases with decreasing temperatures, tending to reach infinite levels below approximately 6°C (Körner, 2003); there is

also evidence of temperature limitations in wood formation by trees (Rossi et al., 2007). Previous work has also proposed that tree architecture and its associated aerodynamics are the primary reasons why trees are more strongly affected by low temperatures at high elevations than smaller-stature plants (Körner, 2012b). With their significant height above ground, upright trees are more closely coupled to prevailing (lower) ambient temperatures than smaller plants, which profit from aerodynamic decoupling and periodic canopy warming (Wilson et al., 1987; Körner, 2012b). In addition, it has been proposed that trees (with their canopies) shade their own roots, resulting in colder root temperatures than for small-stature vegetation (Körner, 1998), limiting growth at root level (Alvarez-Uria and Körner, 2007). According to the GLH, we would expect that if a plant were moved to lower temperatures (e.g., temperature coupling between its meristems and air), we would find an increase in its NSC. The same expectation holds true for other short-stature alpine vegetation.

Different taxa dominate alpine treelines around the world; these include northern hemisphere species of the genera *Picea*, *Pinus*, *Larix*, and *Betula*. In the southern hemisphere, with few exceptions (e.g., Piper et al., 2006), species of the *Nothofagus* genus (Nothofagaceae) thrive at high elevations (Wardle, 1998; Fajardo et al., 2011; Cieraad et al., 2012; Körner, 2012a). Some authors have suggested that *Nothofagus* treelines may be climatically depressed, thus representing a genus-specific boundary, which implies the existence of an elevational gap between the current *Nothofagus* treeline elevation and a claimed potential tree limit located at lower isotherms (Wardle, 1998; Körner and Paulsen, 2004; Körner, 2012a; but see Cieraad et al., 2014). This claim is based on two facts: (1) *Nothofagus* treeline populations in both Chile and New Zealand have been documented growing at higher temperatures than the 6.7°C isotherm for treelines in the rest of the world (central Chile, 36° S, 8.9°C; New Zealand, 39° S, 8.2°C and 42° S, 10.7°C) (Körner and Paulsen, 2004). (2) Wardle (1985, 1998), and more recently Cieraad et al. (2012), have found that *Pinus contorta* (a northern hemisphere conifer commercially planted in the southern hemisphere) thrives several meters above the *Nothofagus* treeline in New Zealand. Regarding the first point, new root zone records at *Nothofagus* treelines in Chile and New Zealand have shown that they do in fact match global trends (Hoch and Körner, 2012; Cieraad et al., 2014), undermining the idea of a genus-specific treeline for *Nothofagus*. However, there is still the fact that in New Zealand *Pinus contorta* grows well 300 m above the natural *Nothofagus* treeline, which questions the existence of one taxa-independent mechanism responsible for treeline formation, suggesting instead that *Nothofagus* and *Pinus* may be differentially affected by low temperatures. It is important to consider that floras in the northern and southern Hemispheres are substantially different in terms of their evolutionary histories (Takhtajan, 1986). When Gondwana (current southern hemisphere) broke apart, its smaller component area, lower phylogenetic diversity and greater isolation compared with the northern hemisphere (Morrone, 2009) caused the flora in the southern hemisphere to come in contact with fewer plant lineages and perhaps experience less selection pressure for specific stressors such as low temperatures. Consistent with this idea, *Nothofagus* species are generally less resistant than northern hemisphere conifers to freezing (Sakai and Weiser, 1973; Sakai et al., 1981; Alberdi et al., 1985; Feild and Brodribb, 2001; Cieraad et al., 2012).

The objective of the present study was to examine the general applicability of the growth limitation hypothesis (GLH) as

a definitive mechanism to explain treeline formation. For this, we experimentally compared the growth, survival, and NSC storage of seedlings of a native treeline species (*Nothofagus pumilio*) with those of an exotic species (*Pinus contorta*) when exposed to cold temperatures. We chose *Pinus contorta* since it has proved to grow well several meters above comparable treelines, such as the one formed by *N. solandri* var. *cliffortioides* in New Zealand (Wardle, 1985, 1998; Cieraad et al., 2012). Additionally, we recorded root-zone temperatures to check whether they are in accordance with global trends. If our study shows that the growth of both species is similarly constrained by low temperatures and that C reserves accumulate, then the evidence will support the GLH as a definitive mechanism responsible for treeline formation. Alternatively, if seedlings significantly decrease in both growth and NSC with lower temperatures, then the carbon limitation hypothesis (CLH) would be supported. To accomplish our objectives, we established 2-yr-old seedlings of *N. pumilio* and *P. contorta* at treeline and above treeline elevations and allowed them to grow for three growing seasons at different temperatures, at either 2 m above ground level (detached from the presumed beneficial conditions provided by ground level and thus forcing coupling between meristematic and air temperatures) or at ground level (decoupling air temperature). This experimental arrangement aimed to mimic the low temperature effects on meristematic shoot and root tissues of a taller tree and, eventually, the temperature effects on the tree's C balance as a whole. We used seedling survival and biomass as an indication of seedling performance and NSC as the best proxy for distinguishing between C source (CLH) or C sink limitations (GLH) as a potential physiological cause for treeline formation (Fajardo et al., 2012).

MATERIALS AND METHODS

Study site and tree species—We conducted the experiment at an alpine treeline in the Cerro Castillo National Reserve (46°04'S and 72°03'W, 1300–1350 m a.s.l.), Aysén Region (Patagonia), Chile. The mean soil temperature (10 cm depth) for a growing season of 144 d (November 2008 through April 2009) was 6.9°C (Hoch and Körner, 2012), and the annual precipitation was ~1200 mm, mainly as snow—the precipitation during the growing season was ~500 mm (Informe Meteorológico de Chile, Dirección General de Aguas, Coyhaique, Chile, 2008). The site is situated on an east-facing hill, with a slope of ~25%. Although the soil in this area is derived from aeolian volcanic ash deposits, at the treeline, the ground is rather rocky. The current forest–alpine ecotone here constitutes a sharp and directional stressful gradient due to wind intensity and, possibly, radiation, from closed canopy krummholz forest down slope to treeless alpine vegetation up slope. At the study site, *Nothofagus pumilio* (Poepp. & Endl.) Krasser (Nothofagaceae) is the only tree species at the treeline (Fajardo et al., 2011). In the region, *Pinus contorta* var. *latifolia* (Engelm.) (Pinaceae) has been extensively planted, first, in an effort to cover slopes with vegetation to reduce the risk of landslides after catastrophic, human-induced forest fires and later for productive purposes. Seeds for *Pinus contorta* plantations in the Chilean Patagonia came from the Cascades Region of Oregon State (USA). Currently, nurseries producing *P. contorta* seedlings get their seeds from local plantations. These two species will be addressed by their genus name for clarity.

Temperature experimental design—We established an experiment where we exposed seedlings of both species to four temperature treatments: (1) ground level at treeline elevation (1300 m a.s.l., hereafter *treeline*); (2) 2-m height at *treeline* elevation; (3) ground level at alpine elevation (1350 m a.s.l., hereafter *alpine*); and (4) 2-m height at *alpine* elevation. To accomplish the 2-m-height temperature conditions, we hung seedlings 2 m above the ground from a pole (Fig. 1) and compared their performance and NSC (see below) to seedlings growing on the ground. In more detail, 2-yr-old seedlings of *Nothofagus* and *Pinus* were obtained from “Las Lengas” nursery (CONAF, Coyhaique, 600 m



Fig. 1. Photograph showing the coupling air temperature condition for seedlings of two species (*Nothofagus pumilio* and *Pinus contorta*) growing at the treeline elevation (1300 m a.s.l.) in pots held in wire baskets that were attached to poles 2 m above the ground. Seedlings were also grown at ground level (at the base of poles) and at the alpine elevation (1350 m a.s.l.). The experiment was conducted at a treeline location in the Cerro Castillo National Reserve (46°04'S and 72°03'W, 1300–1350 m a.s.l.), Aysén Region (Patagonia), Chile. Gray plastic wires around the pole are data loggers for monitoring air and soil temperatures. Photo credit: Alex Fajardo.

a.s.l.), where they were grown in full light conditions in 500 mL cylindrical polyethylene bags containing clay-rich forest soil. Seedlings of the same species were of uniform age and size, although *Nothofagus* (mean height = 13.4 cm \pm 1.1 SE) seedlings were larger than *Pinus* (mean height = 10.6 cm \pm 0.9 SE) seedlings. In early November 2008 (i.e., the onset of the growing season and after snow melt) seedlings in their nursery soil were brought up to treeline elevation where they were put into 1 L green plastic pots with drainage holes. We added local treeline-elevation soil to refill pots when necessary. Two seedlings of each species were placed at ground level beside poles (ground-level temperature treatment). Next to them, an impregnated wood pole (10 cm diameter) 2.6 m tall was buried to a depth of 0.4 m, and two other seedlings of each species were hung in green wire baskets at the top of the pole (2-m height temperature treatment, Fig. 1). The baskets were secured to the poles with metal braces. Seedlings on the ground and on the pole were randomly oriented; furthermore, pots were located at each of the four cardinal points (see Fig. 1). In total, eight poles were buried, four at the treeline elevation (1300 m a.s.l.) and four at the alpine elevation (1350 m a.s.l., Fig. 1). Poles at each elevation were buried \sim 100 m apart. Overall, two individuals of each species (*Nothofagus* and *Pinus*) were placed at two heights (ground level and 2 m) and at two elevations on the slope (*treeline* and *alpine*); this arrangement was replicated at four transects, totaling 64 seedlings. To avoid any water limitations, especially during bud burst, we watered all seedlings regularly to assure survival for the first 4 wk (twice a week) of each growing season. Given that the precipitation at the experiment's location can reach 500 mm during the growing season (November–March, Informe Meteorológico de Chile, Dirección General de Aguas, 2010), we were confident that water stress would not affect our experiment. In fact, only two dead seedlings (*Pinus*), which could have been attributable to water stress, were replaced in the first growing season during the early 4-wk period. Finally, the 2-m-high seedlings were brought to the ground at the end of each growing season (end of April) to avoid the possibility of falling in the winter due to heavy snow. Early in the season (i.e., first weeks of November), the same 2-m-high seedlings were returned to the tops of each pole. Thus, our experiment only considered the effects of temperature differences during the growing season.

Temperature measurements—As a means to assess treatment effects, air and soil temperatures were continuously recorded at hourly intervals throughout the three full growing seasons at ground level and 2 m for one pole at both the *treeline* and *alpine* elevations. For these measurements, we used TMC20–HD sensors, connected to a 4-channel data logger HOBO U12–006 (Onset Computer,

Bourne, Massachusetts, USA). At the treeline elevation, we installed four temperature sensors for one pole. Two sensors were attached to the pole, one at foliage height 10 cm above the ground and 2 m above the ground. These two sensors were positioned under a white, double-layered, aerated plastic shelter to prevent any exposure to direct sunlight. Two other sensors were buried in a pot at ground level and at a 2-m height. We installed another set of four temperature sensors in the same manner for one pole at the alpine elevation. The hourly, monthly, and whole-season means were calculated. In particular, mean growing season soil temperatures were computed using the Körner and Paulsen (2004) criterion, i.e., the mean growing season period began when the mean weekly root-zone temperature exceeded 3.2°C in the spring and continued until it first fell below 3.2°C in the autumn (for an alternative criterion, see Cieraad et al., 2014).

Survival and biomass measurements—The experiment was established in November 2008, and seedlings were harvested in late March 2011 (end of the third growing season). During this period, we conducted multiple censuses (for each growing season, one census in late spring and another in early autumn) in which seedlings were rated as dead or alive. At the end of the experiment, all live seedlings were harvested for biomass and NSC determinations. We used final biomass as a proxy of growth increment, given that seedlings within each species were of similar size at the beginning of the experiment. At the end of the growing season, tree NSC concentrations are expected to be more stable and thus better reflect the net balance between C source and C sinks (i.e., meristematic activity) after the period of maximum demand (Hoch and Körner, 2009). Seedlings were brought to the laboratory within 2 h after harvesting in a cooler with ice packs to reduce tissue respiration. They were then separated into leaves, shoots, and roots. Roots were thoroughly washed with tap water, gently brushed, and put back in paper bags, as were leaves and shoots. To analyze seedlings' NSC, a portion of each seedling's shoots and roots was heated in a microwave in three 20-s cycles at maximum power to stop enzymatic activity (Popp et al., 1996). Finally, all plant material was dried in a forced-air oven (Memmert GmbH, Schwabach, Germany) at 70°C for 72 h, after which tissues were weighed separately with a scale to a precision of 0.0001 g.

Nonstructural carbohydrate analysis—Our assessment of C reserves was based on the determination of nonstructural carbohydrates (NSCs), which consists of the sum of the three most-abundant low-molecular-weight soluble sugars (glucose, fructose, and sucrose) and starch. For each seedling, dry shoot and root material was ground with a coffee grinder, and the powder was passed through a 0.5 mm mesh. The tissue concentration (in milligrams per gram dry mass) of soluble sugars and starch were analyzed following the method of Marquis et al. (1997). Total soluble sugars were extracted with a methanol–chloroform–water (12:5:3) solution and separated from nonpolar pigments and lipids according to Dickson (1979) and determined by a colorimetric reaction with phenol 2% and sulphuric acid, measuring absorbance at 490 nm (Chow and Landhäusser, 2004). Starch in pellets was hydrolyzed to glucose overnight using a sodium acetate buffer and amyloglucosidase (Sigma-Aldrich 10115, St. Louis, Missouri, USA) at 45°C and determined with a phenol–sulphuric acid reaction (Marquis et al., 1997). Two replicates per sample were run; samples were re-analyzed when replicate values differed by >10%. We finally added soluble sugars and starch concentrations to obtain NSCs. We expressed NSC as concentrations per unit dry mass (mg/g) and as the total amount of NSC per tissue (pools in g). Pools of NSC were computed as the NSC tissue concentration times the tissue biomass.

Statistical analysis—To test whether seedling performance differed among treatments, we fitted linear mixed models (LMM) and generalized linear mixed models (GLMM; Pinheiro and Bates, 2001), considering a logistic link function for survival (alive vs. dead, binomial residual distribution) and a normal linear regression for biomass and NSCs (concentrations and pools). For these analyses, we used the packages *lme4* (Bates and Maechler, 2009) and *nlme* (Pinheiro et al., 2009) in the program R (R Development Core Team, 2013). Models considered random effects for transect and fixed effects for species (*Nothofagus* vs. *Pinus*), treatment (ground level vs. 2-m height), and elevation (*treeline* vs. *alpine*). In all GLMMs, the effect of fixed factors was evaluated using likelihood ratio tests (LRTs), while the random factor (transect) was included as a variance component (Bolker et al., 2009). Parameter estimates of the best-fit models are provided in Appendix S1 (see Supplemental Data with the online version of this article). Additionally, we set a priori planned orthogonal comparisons to be estimated and tested, namely to see whether there was a shift in survival between species depending on the height

from the ground or the elevation; for this, we used the *esticon* function in the *doBy* package (Højsgaard et al., 2012) in R. For biomass and NSC concentrations, we determined the significance of terms using the more traditional approach of assessing *P* values for main factor and interaction effects (ANOVA function on lme object in R). In both cases (i.e., LMM and GLMM), we also used maximum likelihood and restricted maximum likelihood (REML) solutions (for fixed and random effects, respectively) when estimating parameters and testing hypotheses because the ANOVA method is known to be more sensitive to unbalanced data and outliers. Finally, differences in mean growing season temperatures among the different thermal treatments were assessed using a simple LMM.

RESULTS

Temperature conditions—Soil temperature (10 cm below ground) at the treeline elevation for the three growing seasons was on average 6.6°C. In more detail, mean hourly, monthly, and whole-season air and soil temperatures were always warmer at the ground level than at the 2-m height (Table 1, Fig. 2); 1.5 K for air ($F_{1,34} = 5.91, P = 0.020$) and 1.4 K for soil ($F_{1,34} = 4.95, P = 0.033$) whole-season temperatures. The difference in air temperatures between ground level and 2 m matched our expectations for air-temperature coupling. Likewise, the difference in soil temperatures between the ground level and 2-m height was also effective in mimicking a colder root zone. We did not find, however, much temperature difference between treeline and alpine elevations for hourly, monthly, or whole-season periods (Table 1, Fig. 2); 0.7 K for air ($F_{1,28} = 1.15, P = 0.292$) and 0.2 K for whole-season soil temperature ($F_{1,28} = 0.07, P = 0.795$). We did not find a significant interaction for distance from the ground and elevation for mean temperatures ($F_{1,28} = 0.82, P = 0.374$). The maximum hourly air temperature difference between the 2 m height and ground level was 7 K and occurred at noon. This trend was reversed at night, where the difference was less than 2 K (Fig. 2). In general, soil mean temperatures at the ground varied little during the day.

Survival and biomass—After three growing seasons, seedling response to the different treatments in terms of survival and biomass proved to be species-specific. In particular, when the

TABLE 1. Monthly and whole-season mean (standard deviation) air and soil temperatures (°C) for each of the four treatments: ground level and 2-m height at (A) treeline (1300 m a.s.l.) and (B) alpine (1350 m a.s.l.) elevations. Air temperature at ground level was measured 10 cm above the ground.

Time	Air		Soil	
	Ground	2-m	Ground	2-m
A) Treeline				
December	5.5 (6.3)	2.9 (3.0)	5.9 (2.6)	4.2 (3.6)
January	7.3 (6.0)	4.8 (3.3)	6.8 (1.7)	5.6 (3.9)
February	7.7 (7.9)	5.5 (4.9)	6.7 (2.3)	6.0 (5.2)
March	8.6 (6.9)	7.1 (4.1)	8.3 (2.2)	7.3 (4.8)
April	3.9 (6.5)	3.6 (5.0)	4.6 (1.8)	3.6 (5.1)
Whole season	6.9 (6.1)	5.0 (4.4)	6.6 (2.4)	5.5 (4.8)
B) Alpine				
December	4.4 (5.4)	2.8 (3.2)	6.1 (2.5)	3.9 (3.9)
January	6.3 (5.1)	4.7 (3.4)	7.2 (1.7)	5.4 (4.2)
February	6.8 (6.1)	5.5 (5.0)	7.4 (2.5)	6.0 (5.8)
March	7.9 (5.9)	7.0 (4.1)	7.1 (2.2)	7.6 (5.2)
April	3.5 (6.1)	3.4 (5.1)	5.3 (1.9)	3.5 (5.3)
Whole season	6.0 (5.8)	4.9 (4.5)	6.6 (2.5)	5.5 (5.2)

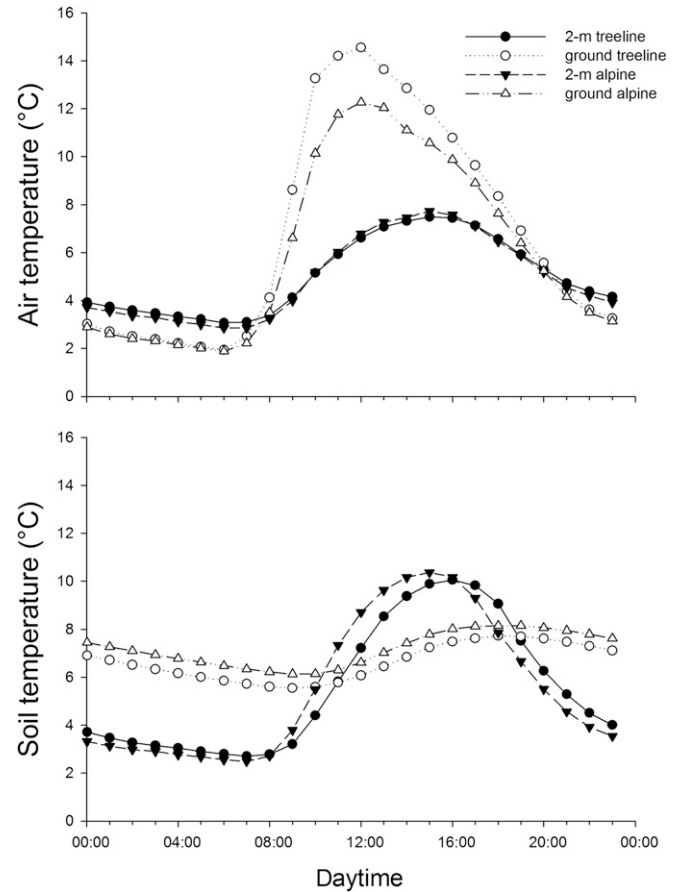


Fig. 2. Average hourly air (upper panel) and soil (lower panel) temperatures through three complete growing seasons for each experimental treatment: ground level (10 cm) at treeline elevation (1300 m a.s.l.), 2 m above the ground at treeline elevation, ground level (10 cm) at alpine elevation (1350 m a.s.l.), and 2 m above the ground at alpine elevation. Temperatures were monitored hourly using TMC20-HD sensors, connected to a 4-channel data logger HOBO U12-006 (Onset Computer).

two species were considered together, distance from the ground influenced seedling survival (Tables 2, 3), being significantly higher at the ground (91%, GLMM estimate values) than at 2 m (66%, Table 3). However, when seedling survival was analyzed separately by species, no significant differences were found between treatments (Table 3). Thus, seedling survival was not affected by elevation, distance from the ground or, more relevant for our purposes, by species ($P > 0.05$, Table 2). Additionally, the interaction term between temperature treatments and species for seedling survival was not significant (Table 2). Overall, these results show that the survival of *Pinus* was not greater than that of *Nothofagus*. Seedling biomass, on the other hand, was significantly influenced by all three factors (Table 4), elevation (lower biomass at *alpine* than at *treeline*), distance from the ground (seedlings at 2 m grew significantly less than those at ground level), and species (Table 3). The interaction between distance from the ground and species was significant (Table 4, $P < 0.05$), indicating that the response to the 2-m-aboveground treatment was species-specific. While seedling biomass of *Nothofagus* was significantly higher at ground level (7.99 g) than at 2 m (4.37 g), *Pinus* seedling biomass was no different at ground level (2.24 g) than at 2 m (1.91 g, Table 3).

TABLE 2. Summary of likelihood ratio test estimates (χ^2) and their respective inference (P value) for survival after three growing seasons based on elevation (treeline vs. alpine), distance from the ground (ground level and 2-m height), species (*Nothofagus* vs. *Pinus*), and interactions among them for seedlings of *Nothofagus pumilio* and *Pinus contorta* growing experimentally in a treeline in Patagonia, Chile. Data were analyzed using generalized linear mixed models.

Model	df	χ^2	P value
~1	2		
Elevation	3	3.386	0.066
Distance	3	6.168	0.013
Species	3	1.481	0.224
Elevation + Distance	4		
Elevation \times Distance	5	1.991	0.158
Elevation + Species	4		
Elevation \times Species	5	0.213	0.645
Distance + Species	4		
Distance \times Species	5	0.004	0.950
Elevation + Distance + Species	5		
Elevation \times Distance \times Species	9	2.292	0.682

Nonstructural carbohydrates—Concentrations and organ-specific pools of NSC were significantly affected by the distance from the ground in the temperature treatment and by species, but not by elevation (Table 4, Fig. 3). Notably, the interaction between the distance from the ground in the temperature treatment and the species was clearly significant ($P < 0.001$) for NSC concentrations and pools in all tissues (Table 4). While NSC concentrations were statistically similar for seedlings of *Pinus* at the 2-m height and at ground level ($F_{1,19} = 3.14$, $P = 0.096$), for *Nothofagus*, NSC concentrations were significantly lower in seedlings at 2 m than at ground level ($F_{1,20} = 39.40$, $P < 0.001$, Table 4, Fig. 3). We found a similar pattern when NSC pools were compared between distances from the ground: significantly different for *Nothofagus* ($F_{1,20} = 14.57$, $P = 0.001$), but not for *Pinus* ($F_{1,19} = 2.27$, $P = 0.149$, Fig. 3). The decrease in NSC concentrations in *Nothofagus* from the ground level to the 2-m height was 49%, mainly driven by a decrease in starch (ground level = 118 vs. 2-m height = 45 mg/g), which corresponded to 62%. For *Pinus*, there was a rather similar, albeit not significant, increase in starch (11%) and soluble sugars (18%) from the ground level to the 2-m height. On average, NSC concentrations and pools were significantly higher in *Nothofagus* than in *Pinus* (Table 4, Fig. 3). In general and independently of temperature treatments and species, NSC concentrations varied consistently between shoots and roots (Table 4). However, for NSC pools, higher values were generally found

in roots than in shoots, mainly driven by the higher biomass in the roots.

DISCUSSION

Species-specific responses to lower temperatures: Carbon limitations—Our results do not support a common physiological mechanism responsible for the treeline formation of *Nothofagus* and *Pinus*. In our experiment, both species were similarly resistant to low temperatures (they survived equally), and yet *Nothofagus* seedlings grew significantly less when subjected to the colder temperature treatments (~1.5 K colder), while their NSC (concentrations and pools) notably decreased (especially starch, which was reduced to half). Given that biomass (i.e., a surrogate of growth in our experiment) was heavily constrained by colder temperature in *N. pumilio*, the significant reduction in NSC concentrations and pools can certainly be attributed to C limitations. Thus, we found that the behavior of *Nothofagus* strongly supported the CLH. The behavior of *Pinus*, in contrast, did not support the GLH or the CLH, given that its seedling biomass and NSC did not differ significantly among treatments.

It is challenging to explain how these two species had contrasting growth and C reserve responses to lower temperatures given that most empirical studies on the matter have shown support for the GLH, or rather, they have certainly falsified the CLH as a mechanism explaining treeline formation. We think, however, that there are two potential explanations for the reduction in NSC with decreasing temperatures and growth (i.e., support for the CLH) in this deciduous *Nothofagus* treeline species: (1) photosynthesis and cell division are being affected by colder temperatures, and (2) other sink processes may be causing the C reduction. First, despite cell duplication or mitotic processes being more sensitive to low temperatures than leaf photosynthesis in many species (Körner, 2012a), it is uncertain whether this is true for all the treeline species. Limitation of growth due to a shortage of photoassimilates is still a controversial hypothesis in the treeline theory (Smith et al., 2003; Handa et al., 2005; Dawes et al., 2011). Although we did not measure gas exchange in our seedlings, there is scattered evidence that in some species C acquisition can become as constrained as growth at low temperature. For example, in a treeline, in situ CO₂-enrichment experiment, Handa et al. (2005) and Dawes et al. (2011) found that contrary to *Pinus uncinata* (evergreen), *Larix decidua* (a deciduous treeline species) augmented its growth in response to the addition of CO₂

TABLE 3. Mean values (and confident intervals around the mean) for (A) survival and (B) biomass growth estimates for *Nothofagus pumilio* and *Pinus contorta*, after three growing seasons in an alpine treeline in Patagonia, Chile. Seedlings were exposed to different temperature conditions: elevation (treeline, 1300 m a.s.l. vs. alpine, 1350 m a.s.l.) and distance from the ground (ground level vs. 2 m).

Growth estimates for taxa	Distance from the ground			Elevation		
	Ground level	2-m	P value	Treeline	Alpine	P value
A) Survival						
<i>Nothofagus</i>	0.94 (0.65–0.99) a	0.75 (0.48–0.91) a	0.180	0.94 (0.64–0.99) a	0.76 (0.47–0.93) a	0.188
<i>Pinus</i>	0.88 (0.60–0.97) a	0.56 (0.31–0.79) a	0.068	0.81 (0.54–0.94) a	0.63 (0.36–0.83) a	0.260
Total	0.91 (0.74–0.97) a	0.66 (0.47–0.81) b	0.026	0.88 (0.70–0.95) a	0.69 (0.50–0.83) a	0.086
B) Biomass (g)						
<i>Nothofagus</i>	7.99 (7.29–8.69) a	4.37 (3.67–5.07) b	<0.001	6.85 (5.93–7.78) a	5.50 (4.58–6.42) b	0.043
<i>Pinus</i>	2.24 (1.53–2.94) a	1.91 (1.21–2.61) a	0.510	2.41 (1.49–3.34) a	1.73 (0.80–2.65) a	0.299

Note: Different letters indicate a significant effect ($P < 0.05$) on the seedling response to treatments within each species.

TABLE 4. Effects, *F* ratio and statistical significance of elevation (treeline, 1300 m a.s.l., alpine, 1350 m a.s.l.), distance from the ground (ground level, 2 m) and species (*Nothofagus pumilio*, *Pinus contorta*) and their interactions on growth (biomass in g), concentrations (mg/g for roots and shoot, and mg/g per seedling) and total pools (mg/seedling) of nonstructural carbohydrates (soluble sugars + starch) in seedlings of two species growing experimentally in a treeline ecotone in Patagonia, Chile. Data were analyzed using linear mixed models.

Source of variation	df	Biomass	Nonstructural carbohydrates					
			Concentrations			Total pools		
			Roots	Shoot	Seedling	Roots	Shoot	Seedling
Elevation	1	11.00**	0.52	0.43	0.02	1.43	3.79	3.25
Distance	1	18.68***	11.56**	16.06***	19.81***	17.26***	5.66*	17.92***
Species	1	155.33***	16.22***	8.45**	18.14***	51.94***	75.73***	90.09***
Elevation × Distance	1	0.03	0.97	1.35	0.01	1.22	2.47	0.03
Elevation × Species	1	0.40	1.93	0.68	1.95	0.06	0.30	0.11
Distance × Species	1	5.69*	18.09***	29.25***	33.41***	14.54***	23.67***	26.12***
Elevation × Distance × Species	1	0.01	0.11	0.20	0.19	0.32	1.36	0.97

Notes: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

and to warm years, indicating that *L. decidua* benefited from extra C (i.e., it was C-limited). Second, it is possible that *Nothofagus* and *Pinus* differ in the sink processes that are responsible for C reduction under low temperatures; i.e., deciduous treeline species may follow a different C storage trend than evergreen species, such as having higher C storage requirements (Fajardo et al., 2013) or potentially larger sink capacities (Dawes et al., 2011). In addition, angiosperms are less resistant than conifers to cavitation (Feild and Brodrribb, 2001; Maherali et al., 2004) and may incur higher metabolic maintenance costs and xylem conduit repair (Johnson et al., 2012). Freeze–thaw events are expected to be common at tree-line elevation, even in summer; thus, we suggest that *Nothofagus* may use more carbohydrates in xylem repair and maintenance than *Pinus*. Further studies are needed to unveil the definitive role of foliar habit on the C dynamics in plants, particularly

when facing stressful conditions such as lower temperatures and drought.

We are confident that our experiment did effectively test, for the first time, the mechanism proposed to drive growth limitations at treeline elevation, i.e., a coupling between air and meristematic temperatures and lower root-zone temperatures. These mechanisms are thought to occur primarily in mature trees due to their stature and canopy characteristics, respectively, and not due to ontogenetically driven differences in physiology (Körner, 2012b). Therefore, by placing the meristematic tissues of seedlings of the same age at taller statures we were able to mimic the low temperature effects on meristematic tissues of a taller tree and, eventually, the temperature effects on the C balance of the tree as a whole. Furthermore, although ground-level air temperatures fluctuated during the day (with colder absolute temperatures throughout the night, Fig. 2), they were definitely

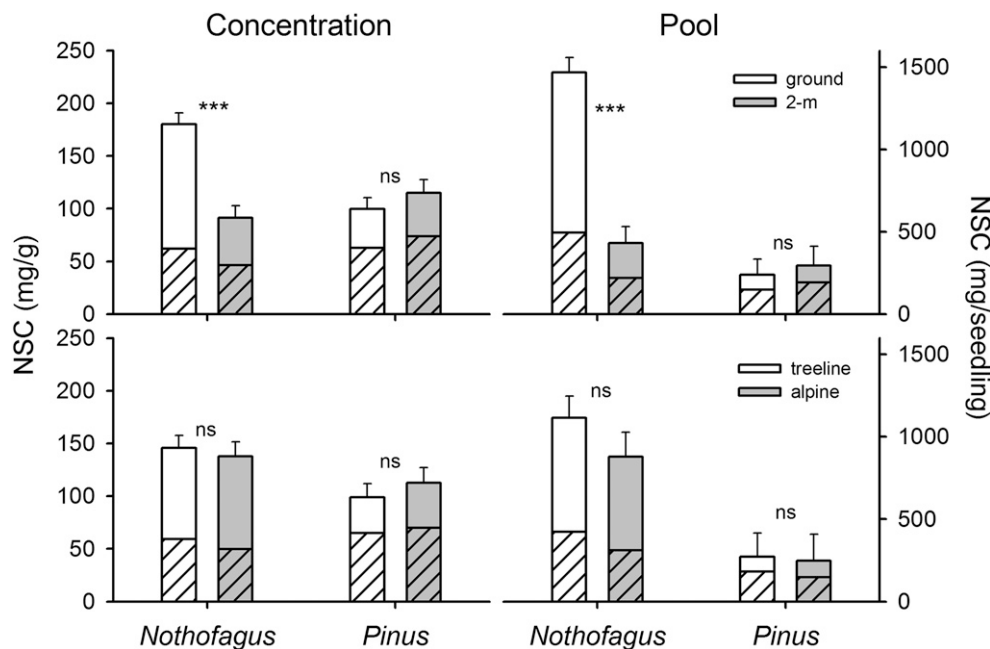


Fig. 3. Mean nonstructural carbohydrate (NSC) concentrations (left panel) and pools (right panel) in *Nothofagus pumilio* (native) and *Pinus contorta* (exotic) seedlings growing at either ground level or 2 m above the ground (upper plots) and at two elevations (treeline or alpine, lower plots). NSC = starch (hatched bar) + total soluble sugars (white bar). NS, no significant differences in NSC concentrations between treatment levels; ***, *P* < 0.001 highly significant differences (see Results).

more favorable on average (warmer mean temperatures) for growth and survival of both species than the temperatures at the 2-m height. This result encompasses two ideas: (1) to prevail as a plant in these harsh, alpine conditions, growing closer to the ground helps protect the plant from colder air temperatures (Wilson et al., 1987; Körner, 2012b), and (2) the growing season's mean temperatures seem to be a relevant measure to characterize plant performance at treeline elevation, in agreement with conclusions drawn by Körner and Paulsen (2004). Nevertheless, we note that the estimated duration of the growing season has emerged as an important factor in the determination of this mean temperature (Cieraad et al., 2014).

One potential limitation of our experimental setup is the artificial exposition of the seedlings' root system to temperature and moisture conditions 2 m above the ground; they are unlikely to be the same as those experienced by upright tree roots at the treeline. Although we compensated for potential dryness by frequently watering these seedlings during the growing season, we cannot discount the occasional occurrence of some water deficit. We think, however, that such water deficit should have had minor effects on both species' performances. *Nothofagus pumilio* seedlings have been found to grow as well under water deficit as under well-watered conditions, at least for Patagonian populations (Piper et al., 2013). Additionally, soil temperatures were warmer than those air temperatures experienced by seedling foliage (Fig. 2). How does this relative warming affect our results? First, we highlight that the exposure of seedling meristems to cold temperatures at the 2-m height was similarly achieved for both roots and shoots (no significant difference between organs; $F_{1,30} = 1.45$, $P = 0.237$) and that these temperatures were clearly distinguished from warmer temperatures at ground level. These two points support the effectiveness of our experiment in that it reduced meristematic root and shoot temperatures to values adduced to drive growth limitation. Finally, we found the same variation trend in shoot and root NSC concentrations, demonstrating that C balance was not tissue dependent. Therefore, we are positive that placing seedling roots at the 2-m height was a good approach to reduce temperatures at the foliage meristems and root zone.

Evidence against a missing taxon in the *Nothofagus* treeline—In our experiment, we found that *Pinus contorta* did not outperform *Nothofagus pumilio*, the native dominant treeline species in the southern Andes, when the seedlings grew at the same elevation or at any of the different temperature conditions. The coldest temperature treatment (e.g., air temperature at the 2-m height, alpine elevation) was on average 2 K colder than the air temperature at ground level at the treeline elevation, which corresponds to 330 m higher in elevation (0.6 K per 100 m, Körner, 2003). Thus, if both species were planted at 1630 m a.s.l., *Pinus* would not have higher survival and growth rates than *Nothofagus*. Our result agrees with previous studies demonstrating that *Nothofagus* treeline species effectively resist freezing temperatures that are much lower than those recorded at treeline elevations during the growing season (Sakai et al., 1981; Alberdi et al., 1985; Cieraad et al., 2012). In addition, we found that our 3-yr root-zone temperature records (6.6°C) fully matched those found in other worldwide treeline studies (Körner and Paulsen, 2004; Hoch and Körner, 2012), thus certainly showing that the Patagonia *Nothofagus* treeline is located at similar isotherms as those of other northern hemisphere treelines (e.g., Swiss Alps, Himalayas), mainly dominated by coniferous species. Overall, current experimental findings, along with treeline temperature

records in Patagonia (ours and those of Hoch and Körner, 2012) and new records for the *Nothofagus menziesii* treeline in New Zealand—from 6.4°C for the growing season (Alan Mark, personal communication to Ch. Körner [Körner, 2012a, 33–56]) and 7.0°C for a wider growing season (Cieraad et al., 2014)—show that there is little leeway to claim that in the southern Andes there is a missing taxon treeline, i.e., that *Nothofagus* forms a species-specific upper forest limit that is not climate-driven (c.f. Körner and Paulsen, 2004; Körner, 2012a).

Conclusions—We have experimentally shown that the survival of *Pinus contorta* was not higher than that of *Nothofagus pumilio* at the colder conditions of the treeline elevation, but that C reserves and biomass were reduced by low temperatures in only *Nothofagus*. This evidence implies no support for the GLH, also suggesting that low temperature effects on growth and C reserves are taxon-dependent. With temperature conditions that were on average 1.5 K cooler than treeline conditions, we found strong support for C limitation in *Nothofagus*. In the case of *Pinus*, our study found no support for the CLH or the GLH. Evidence for most treeline species so far has shown support for the GLH; only for *Larix decidua*, a deciduous species like *Nothofagus*, has there been evidence to support the CLH (Handa et al., 2005; Dawes et al., 2011). The formulation of the GLH represents a notable shift in understanding and explaining treeline formation worldwide, particularly by emphasizing C investment rather than C uptake as the physiological mechanism that limits plant development under low temperatures. We think, nonetheless, that a universal theory for treeline formation based on low temperatures would still need to embrace or explain (1) southern Andes deciduous *Nothofagus*, (2) deciduousness (*Larix decidua* and *Nothofagus pumilio*), and (3) Mediterranean treeline species (cf. Körner, 2012a). In all these cases, there seems to be, at least in some sites, C limitation (Dawes et al., 2011; Fajardo et al., 2011; Hoch and Körner, 2012). We claim that once we understand the concomitant processes driving these treeline outliers, we will be able to more accurately predict treeline responses to current and future climate changes worldwide.

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