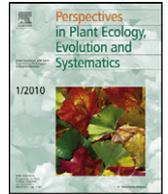




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Research article

Simulated warming does not impair seedling survival and growth of *Nothofagus pumilio* in the southern Andes

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ABSTRACT

It has been predicted that subalpine forests will be negatively affected by global warming; however, direct responses to experimental warming have been scarcely examined in these systems. In this study we evaluated the effects of higher temperatures with and without water addition on the survival and growth of recently emerged (small) and large seedlings of the widely distributed species *Nothofagus pumilio* in subalpine forests of the southern Chilean Andes. We also examined the variations in seedling traits related to carbon balance in order to infer the causal mechanisms of survival and growth responses. Treatments of open top chambers (OTCs) were combined with watering in two locations with differing climates: Antillanca (40°S, humid) and Cerro Castillo (46°S, drier). OTCs increased mean and maximum air temperatures by 0.6 °C and 2–3 °C, respectively, and decreased soil humidity by 56% in Antillanca and 30% in Cerro Castillo, fulfilling methodological expectations and climate model predictions. After two complete growing seasons, the survival, relative growth rate (RGR), biomass, and a suite of seedling traits were measured and analyzed using mixed-effects models. Warming and watering in combination with watering significantly increased large seedling survival in Cerro Castillo. In Antillanca, warmer conditions increased the height, biomass, and leaf area of small seedlings, and the RGR of large seedlings. In this location, warming also caused lower leaf carbon isotopic composition in both age classes and higher specific leaf area in small seedlings, suggesting whole-plant carbon gain improvements; warming did not produce any drought effects. Our results indicate that warming produces positive effects on the seedling establishment of *N. pumilio* in the southern Andes, highlighting the importance of site-specific effects in response to climate change in widespread species. Site-specific effects can most likely explain the discrepancies between the results of this study and the predictions outlined by previous studies for these forests.

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Introduction

Low temperatures are one of the major constraints for seedling establishment in subalpine forests (Tranquillini, 1979; Ferrar et al., 1988; Woodward et al., 1995; Cuevas, 2000; Lajzerowicz et al., 2004). Consequently, global warming may mitigate the physiological limitations imposed by cold temperatures on the survival and growth of seedlings located at high elevations, thus promoting their establishment and causing timberline infilling (Innes, 1991). Such unidirectional expected changes, however, may not occur if

warmer conditions simultaneously lead to higher water deficit in plants. An increased water deficit could offset the expected positive effects from temperature increases, limiting, for instance, seedling survival and growth (Daniels and Veblen, 2004; Oberhuber, 2004). Ultimately, this may even lead to a reduction in subalpine forest cover (Hayhoe et al., 2004).

In many regions of the world, it has been predicted that water stress will become more frequent and intense under a global warming scenario (Bates et al., 2008). Water stress driven by higher temperatures (via increased evapotranspiration) causes substantial impacts on plant communities including seedling mortality, growth cessation (Tercero-Bucardo et al., 2007), and reduction of species richness (Lloret et al., 2004). In widely distributed species, predictions on seedling responses to climate warming are difficult to make because a given species may show contrasting responses depending on the location; e.g., warming increased growth of

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Vaccinium uliginosum at arctic latitudes, but not in mid-latitude alpine ecosystems (Kudo and Suzuki, 2003). Warming-induced drought may not be significant in sites with high precipitation, simply because high soil water availability may still be sufficient to meet plant's water demands. On the other hand, local climates may entail intraspecific variations in drought and thermal resistance. Individuals located in areas where drought is infrequent are expected to be poorly adapted to drought and thus more susceptible to experience global warming-induced water stress than individuals in seasonally drought-affected locations (Benito-Garzón et al., 2011; Piper and Zúñiga-Feest, 2012). Although site-specific conditions and local adaptations are important to understand seedling responses to global warming, few experimental studies have considered these effects.

Seedlings' response to global warming is much dependent on how plant functional traits are able to vary in response to such changes. In order to help shed further light on the mechanisms involved in seedlings' adaptation to new environments it is necessary to identify which traits vary concomitantly with survival and growth in response to warming. It is known that traits related to carbon gain often show contrasting responses to higher temperatures and drought, hence it becomes critical to know the net effect that warming has on trait variation. For instance, specific leaf area (SLA, leaf area per unit of leaf mass), total leaf area, and leaf area ratio (LAR, leaf area/total biomass) are directly related to the growth rate (Poorter and Remkes, 1990; Lambers et al., 2008), and they are known to decrease in response to colder or drier conditions at the intraspecific level (Li et al., 2004; Fajardo and Piper, 2011). Leaf nitrogen (N) concentrations may increase in response to warming as higher temperatures tend to accelerate soil N mineralization (Rustad et al., 2001). Leaf N is positively related to intrinsic water use efficiency (iWUE) – the efficiency of CO₂ assimilation in relation to water loss during transpiration (Wright et al., 2001, 2004), a trait underlying drought resistance (Piper et al., 2007).

In the southern Andes of South America the deciduous broadleaf tree *Nothofagus pumilio* (Poepp. et Endl.) Krasser (Nothofagaceae) is the dominant subalpine and treeline species, and is thus widely distributed, from 35°S in central Chile to the southernmost point of the continent at 55°S (Veblen et al., 1996). The species shows significant levels of variation in morphological and physiological traits across environmental gradients, driven by both adaptation and acclimation (Barrera et al., 2000; Premoli and Brewer, 2007; Premoli et al., 2007; Fajardo and Piper, 2011; Piper and Fajardo, 2011). Climatic models for the region have predicted temperature increments of 1.7–3.9 °C for the next hundred years (0.1–0.2 °C per decade); the largest increments are predicted to occur within the northern distribution of *N. pumilio* and at high elevations (IPCC, 2007). Accordingly, several studies using different approaches have accounted for the high drought susceptibility of *N. pumilio*, warning of the deleterious effects of warming on the structure and dynamics of these forests (e.g. Lara et al., 2001; Daniels and Veblen, 2004; Villalba et al., 2005; Heinemann and Kitzberger, 2006; Tercero-Bucardo et al., 2007). Most of these studies have focused on the species central and northern distributional ranges, where survival and growth responses have been found to be variable with site location (Daniels and Veblen, 2004; Heinemann and Kitzberger, 2006). To assess the importance of site-specific conditions in modulating plant responses to global warming, a comprehensive approach to evaluate the effects of global warming on seedling establishment should contemplate *in situ* experimental manipulations of temperature and water availability in different climates where the species naturally occurs.

In the present study, we examined the net effects of simulated warming (e.g. higher temperatures and warming-induced drought) on seedling survival and growth of *N. pumilio* in two subalpine

forests differing in precipitation. Our main objective was to disentangle the effects of temperature increase from those related to concomitant drought, considering the site's influence. Hence, we set up experiments in two locations in the southern Chilean Andes: a humid site (Antillanca) and a mesic site (Cerro Castillo). In general, we predict that the response of seedlings of *N. pumilio* to simulated warming will be site-specific. Thus, warming may have negative effects and watering positive effects on populations from humid locations (e.g. Antillanca) due to poor local adaptation to concomitant drought (drought-mediated effects). On the other hand, seedlings at more mesic locations (e.g. Cerro Castillo) might benefit from warming and show no response to watering due to better adaptation to drought. Alternatively, warming may benefit seedlings and watering may have no effect in humid-climates if abundant precipitation prevents seedlings from experiencing warming-induced water deficit (temperature-mediated effects). Conversely, seedlings in more mesic locations (e.g. Cerro Castillo) could be harmed by warming and benefited by watering if precipitation becomes insufficient to fulfill plants' water requirements. We further examined effects of simulated warming and concomitant drought on a suite of traits related to carbon gain (SLA, total leaf area, LAR, leaf nitrogen concentration, carbon isotopic composition, and shoot/root ratio) as potential predictors of seedling performance.

Materials and methods

Study species and research locations

N. pumilio extends through a wide latitudinal and altitudinal range in the southern Andes of Chile and Argentina covering a diverse variety of climates (Veblen et al., 1996; Rodríguez and Quezada, 2003). This species generally occupies the coldest and driest extremes of forest along its latitudinal range, where it can be found either forming pure forests or associated with *Nothofagus antarctica* forming mixed deciduous forests (McQueen, 1976; Veblen et al., 1996). *N. pumilio* reproduces by masting events (synchronous, episodic reproduction) and extensive seedling establishment occurs in cleared land following large-scale disturbances (Veblen et al., 1996) or within canopy gaps following tree falls (Fajardo and de Graaf, 2004). Alternatively, it can also form seedling banks in the understory that may persist for up to 25 years before a gap opens (Cuevas and Arroyo, 1999). We found no germinant seedlings in the first growing season. However, as a consequence of a masting event, which occurred in both locations during the summer of 2009, we were able to evaluate how our treatments affected germinant seedlings for the last complete growing season.

The study was carried out in two locations within the southern Chilean Andes, which differ in regional climate. The first location, *Antillanca*, is located within the Puyehue National Park (40°47'S, 72°11'W). The mean annual precipitation for the 1991–97 period was 3661 ± 457 mm in a nearby meteorological station (Aguas Calientes, located 20 km from our study site) (Daniels and Veblen, 2004), although values higher than 6000 mm have been recorded at the study area (Godoy et al., 2001). Most of this precipitation falls between May and November. Precipitation for the growing period (November–March) was 454 mm during the 2009–10s period (Informe Meteorológico de Chile, Dirección General de Aguas). Mean temperatures for the warmest (January) and coldest (August) months are 10.75 °C and 0.85 °C, respectively (Hijmans et al., 2005). The soils are derived from andesitic rocks of volcanic origin. The vegetation consists of an overstory dominated by *N. pumilio* along with some individuals of the evergreen *Nothofagus betuloides* and

a sparse understory mainly composed of *Gaultheria mucronata* and *Empetrum rubrum*, two dwarf shrubs (Ericaceae), and some components of *Ovidia pillopollo* (Thymelaeaceae), *Embothrium coccineum* (Proteaceae) and *Drimys andina* (Winteraceae). The second location is found within the Cerro Castillo National Reserve (46°04'S, 72°03'W), hereafter called *Cerro Castillo*. The mean annual precipitation here is 1100 mm, and 250 mm for the growing period (December–March) (Informe Meteorológico de Chile, Dirección General de Aguas). Mean temperatures for the warmest (January) and coldest (July) months are 9.1 °C and –1.55 °C, respectively (Hijmans et al., 2005). The soil is derived from Aeolian volcanic ash deposits. The vegetation consists of *N. pumilio* in the overstory and few individuals of *G. mucronata*, *E. rubrum*, and *Berberis empetrifolia* (Berberidaceae) in the understory. In a companion study (A. Fajardo, unpublished) it was found that mean tree height, stand stem density and basal area per hectare were fairly similar in the two subalpine forest stands where the experiments were established in both locations: height, 15.5 (SE=2.6) and 16.9 (SE=2.8) m; density, 880 (SE=121) and 740 trees (SE=85) ha⁻¹; and basal area, 27.5 (SE=5.1) and 22.7 (SE=4.3) m² ha⁻¹, for Antillanca and Cerro Castillo, respectively.

The experiment was established 75 m below the treeline elevation, under the canopy of monospecific subalpine mature forests of *N. pumilio*. Specific elevations were 1275 and 1235 m a.s.l in Antillanca and Cerro Castillo, respectively. Because temperature decreases with latitude for a given elevation, and mean temperature for the growing season at the treeline is similar across latitude (Körner and Paulsen, 2004), we assumed that differences in elevation fairly compensated for temperature differences driven by latitude.

Experimental design

The experiment was set in both locations in December 2008 (late austral spring) and was continued until December 2010. Warming was passively simulated *in situ* using 114 cm-diameter, hexagonal open top chambers (OTCs) that rely primarily on trapping solar energy (Marion et al., 1997). OTCs were made of transparent Plexiglass®, with walls 34 cm high, set up at a 60° angle in relation to the soil's surface (open tops measuring 71 cm in diameter). This type of OTC has been extensively used in global warming experiments in tundra and alpine ecosystems (International Tundra Experiment, www.itex-science.net) (Molau and Mølgaard, 1996; Marion et al., 1997; Kudo and Suzuki, 2003; Sierra-Almeida and Cavieres, 2010). At each site, we selected an area which contained no noticeable canopy gaps, high *N. pumilio* seedling density in order to ensure sufficient plant material for the experiment, and low understory companion species density in order to avoid plant competition. In this area, one perpendicular-to-the-slope transect was set and plots (~120 cm-diameter) were established at 3–5 m intervals along the transect. We applied a split-plot experimental design (Sokal and Rohlf, 1995), where twelve plots in Antillanca and six in Cerro Castillo were randomly chosen for the installation of OTCs (+OTC); a similar number of plots were assigned to be controls (-OTC). Each plot (+OTC or -OTC) was split into two halves – the sample unit; one half was subjected to watering (+W) for the whole summer period (mid-December to mid-March), whereas the other half was maintained without watering (-W). Water was applied to compensate for warming-induced drought. The watering system consisted of a main water container connected to plastic hoses with auto-regulated droppers. An automatic watering control system (Model 94075, Orbit®, USA) was used to control the watering time. Three droppers were installed at each sub-plot (inside and outside OTCs), each one was closely fixed to one seedling. Each dropper provided a daily watering of 4 ml/min for 5 min at 0700

a.m. To avoid contaminating the treatments, only the lower half of each plot (slope ~5%) was watered, while the upper half was maintained without watering. The watering amount and frequency were calculated to ensure regular water provision between visits to the sites. At each visit, we refilled the containers. In both sites, water was collected from natural streams of snow-melt originating nearby. In order to provide a more realistic simulation of global warming by, for example, extending the growing season (Danby and Hik, 2007), OTCs remained in place throughout the experiment (i.e., including winter).

Environmental measurements

As a means to assess the treatments' efficacy, we monitored the following environmental variables at each location: (1) soil temperature of the upper 5 cm (TMC20-HD sensors, connected to a 4-Channel data logger HOBO® U12-006, Onset Computer Corporation), (2) soil volumetric water content of the upper 30 cm (ECH₂O S-SMA-M005 probes, connected to a HOBO® Micro Station logger, Onset Computer Corporation), for one quadruplet of treatments; and (3) air temperature and relative humidity 20 cm above ground (HOBO® U23-001 Temp/RH, Onset Computer Corporation) for one plot with OTC (i.e. inside) and one plot without OTC. All measurements were recorded hourly. Then, daily averages for the 2008–09s and 2009–10s growing seasons were calculated. The air vapor pressure deficit (VPD) was calculated from values of air relative humidity and air temperature. Finally, in order to assure that canopy openness was homogeneous between treatments (+OTC, -OTC) and across replicates, we took one hemispherical photo above each OTC (and -OTC) and estimated the percentage of canopy openness above plots using a 7-mm Nikon f 7.4 fish eye lens mounted on a Nikon Coolpix 5000 digital camera (Nikon Corporation, Tokio, Japan) and GLA (Gap Light Analyzer ver. 2) software (Frazer et al., 2000). Canopy openness proved not to differ between the treatments in both sites ($p > 0.05$).

Plant measurements

For the purpose of survival and growth determination, we selected 20–25 naturally established seedlings with heights of 5 to 10 cm (hereafter referred as *large seedlings*) within each plot in December 2008, making a total of 714 and 271 in Antillanca and Cerro Castillo, respectively. Additionally, in December 2009 we selected 15–25 germinant seedlings (hereafter referred as *small seedlings*) within each plot, making a total of 306 in Antillanca and 110 in Cerro Castillo. Seedlings located too close to OTC walls (<15 cm) were not included in the analysis.

All seedlings in each sample unit were counted at the beginning of the experiment for survival analyses. We evaluated seedling survival at the end of December 2010 (early summer of the third growing season), because sometimes seedlings may lose leaves before the end of the season, which could lead to an incorrect mortality assignment. To determine seedling growth, we randomly selected and flagged 2 seedlings per size class in each sample unit. These seedlings were also meant to be harvested at the end of the study (late December 2010) for biomass, nitrogen, and isotopic determination (see below). Large seedlings' height and relative growth rate for the third growing season (RGR_h, in cm cm⁻¹) were computed using the difference between ln(h_2) and ln(h_1), where h_1 and h_2 correspond to the height of seedlings at the beginning and end of the 2010–11 growing season, respectively. In *N. pumilio*, h_1 is easily recognizable at the end of the season due to the presence of scars that form when buds break. In small seedlings, growth was evaluated as height, biomass, and leaf area at harvest time.

After harvesting, seedlings were immediately transported in cold containers to the laboratory. Leaves were detached from the rest of the plant and laid flat separately on a white sheet and photographed with a reference square of known area using a Cyber-shot® S750 digital camera (Sony Electronics Inc., Japan). Total projected leaf area was calculated using the image-processing software SIGMAPROC (Systat Software Inc., Richmond, CA, USA). Leaves were then oven-dried at 70 °C to a constant weight, along with the remaining non-leaf tissue to determine total biomass and carbon isotopic composition. For each seedling, SLA was determined as the ratio between total leaf area and total leaf mass, and for small seedlings only LAR was calculated as the ratio between total leaf area and total biomass. Leaf N concentration was determined from dry, ground leaves by a combustion analyzer (LECO TruSpec® Micro CHN). Carbon isotope ratio analyses were conducted across treatments for each location to examine potential effects of drought occurring concomitantly with increased temperatures. The carbon isotope ratio of leaf material ($\delta^{13}\text{C}$) is a proxy of intrinsic water use efficiency and stomatal closure due to water stress. Reduction in soil humidity and increment in the leaf-to-air vapor pressure difference are predicted to cause stomatal closure and to consequently increase (less negative values) the $\delta^{13}\text{C}$ in plant biomass (Farquhar et al., 1989). $\delta^{13}\text{C}$ was analyzed using an elemental analyzer/continuous flow isotope ratio mass spectrometer (IRMS) (ANCA/SL elemental analyzer coupled with a Finnigan MAT Delta PlusXL IRMS). Isotope analyses were conducted at the Center for Stable Isotope Biogeochemistry, University of California–Berkeley. Isotope ratios are expressed in delta notation (‰) relative to an accepted reference standard: Vienna Pee Dee Belemnite for carbon isotope values.

Statistical analyses

To evaluate survival responses in the different treatments we fitted generalized linear mixed-effects models (GLMM), considering a logistic link function (alive vs. dead, binomial residual distribution) (Pinheiro and Bates, 2001) and using the package lme4 (Bates and Maechler, 2009) in R (R Development Core Team, 2009). Thus, we fitted the following model:

$$\text{logit}(N_{a,sp}, N_{d,sp}) = \beta_0 + \beta_1 Wr_p + \beta_2 Wt_p + \beta_3 Wr_p Wt_p + \alpha_p + \varepsilon_{sp},$$

where the β_s are fixed-effect parameters to estimate, α_p is the random effect (plots) to estimate, N is the abundance of alive, a , or dead, d , seedlings within each of s sub-plots (treatment; e.g. +OTC +W), and p plots (e.g. +OTC or –OTC); Wr represents the warming treatment (+OTC, –OTC); Wt is the watering treatment (+W, –W); ε is the error term, and logit (alive, dead) is the logit link used for binomial data of number of alive “successes” and the number of dead “failures”. The sampling unit is the subplot (not the seedling), with a variable number of seedlings. To assess the significance of model parameters, we used likelihood ratio tests (LRT). Additionally, we set *a priori* planned orthogonal comparisons among treatments to be estimated and tested. For this, we computed net effects of the watering by comparing survival levels (logit scale on planned contrasts).

For continuous variables (RGR_h, height, biomass, leaf area, SLA, LAR, shoot/root ratios, and $\delta^{13}\text{C}$), we fitted linear mixed-effects models (LMM), which are essentially the same models we used for the seedlings survival without the logit link (normal instead of binomial residual distribution). In this case, determining the significance of terms was done using the more traditional approach of assessing p -values for the main factor and interaction effects (ANOVA function on lme object in R). In both cases, we also used maximum likelihood and restricted maximum likelihood (REML)

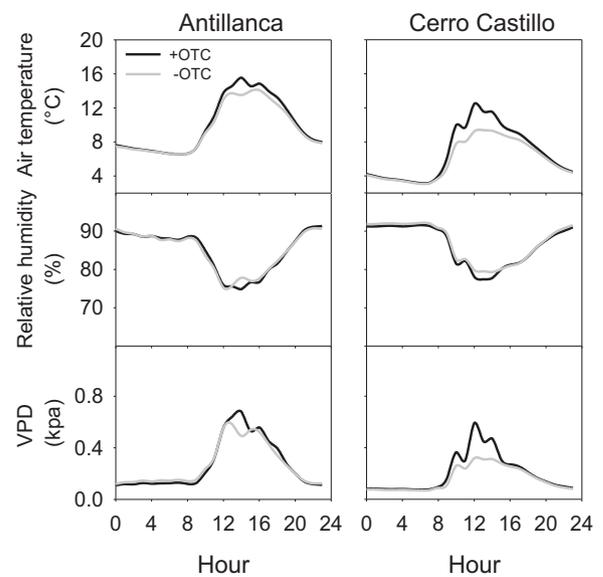


Fig. 1. Air temperature, relative air humidity, and air vapor deficit (VPD) inside (+OTC) and outside (–OTC) open top chambers (OTC) at the subalpine forest of *Nothofagus pumilio* in two locations in the southern Chilean Andes: Antillanca (40°47'S, 72°11'W) and Cerro Castillo (46°04'S, 72°03'W). Values correspond to daily averages from mid December to March 31st of the 2008–09 and 2009–10 periods.

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.

Results

Effects of OTCs on environmental conditions

In support of the efficacy of our simulated warming experiment we found that in both locations OTCs increased mean and maximum air temperatures by 0.6 °C and 2.5–3 °C, respectively, whereas they did not modify minimum temperatures. In both locations, the soil temperature inside OTCs was 0.8–0.9 °C higher than outside OTCs (Table 1). Consistent with temperature modifications, soil humidity was reduced by 56% and 30% in Antillanca and Cerro Castillo, respectively (Table 1). In more detail, OTCs increased mean maximum temperature by c. 2 °C between 12:00 and 18:00 h, whereas they did not cause any change during the rest of the day – including overnight (Fig. 1). Air relative humidity was slightly reduced by OTCs leading to higher VPD, particularly during the 12:00–18:00 h period of the day (Fig. 1). OTCs caused important reductions in soil humidity, which were compensated for by watering at about 60 and 100% in Antillanca and Cerro Castillo, respectively (Table 1).

Survival and growth responses to OTCs and watering

In general, we found that in both locations OTCs had no effect on survival, with the exception of large seedlings in Cerro Castillo. Here, seedlings growing inside OTCs had 36% higher survival than seedlings growing outside OTCs (Table 2). Watering also had no effect on seedling survival, except for large seedlings in Cerro Castillo; this was entirely due to a highly significant effect of watering inside OTCs, where large seedlings had 40–100% higher survival than seedlings growing in other conditions (a difference of 2.2 on the logit scale inside OTCs, p -value < 0.001, planned contrasts,

Table 1

Experimental environmental conditions of simulated warming (open top chamber, OTC) and watering (W) in two locations in the southern Andes of Chile: Antillanca (40° 47' S, 72° 11' W) and Cerro Castillo (46° 04' S, 72° 03' W).

	Air temperature (°C)			Soil temperature (°C)	VWC (m ³ m ⁻³)	Air humidity (%)	VPD (kPa)	Canopy openness (%)
	Mean	Max.	Min.	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
Antillanca								14.1 (±1.7)
–OTC –W	10.0 (±0.3)	16.5 (±0.6)	5.5 (±0.3)	11.6 (±0.08)	0.09 (±0.00)	83.8 (±1.2)	0.29 (±0.02)	
–OTC +W				11.5 (±0.08)	0.10 (±0.00)			
+OTC –W	10.6 (±0.3)	18.8 (±0.7)	5.7 (±0.3)	12.5 (±0.09)	0.04 (±0.00)	83.6 (±1.2)	0.32 (±0.03)	
+OTC +W				12.0 (±0.09)	0.07 (±0.00)			
Cerro Castillo								12.7 (±3.4)
–OTC –W	6.7 (±0.23)	12.1 (±0.3)	2.8 (±0.2)	5.9 (±0.02)	0.10 (±0.00)	85.8 (±0.8)	0.18 (±0.01)	
–OTC +W				5.9 (±0.02)	0.12 (±0.00)			
+OTC –W	7.3 (±0.24)	15.4 (±0.5)	2.9 (±0.2)	6.7 (±0.02)	0.07 (±0.00)	84.9 (±0.8)	0.22 (±0.02)	
+OTC +W				6.6 (±0.02)	0.09 (±0.00)			

Values shown correspond to daily averages and standard errors for the 2008–09 and 2009–10 growing seasons; SE stands for standard error.

Table 2

Likelihood ratio tests (χ^2 and p -value) for warming (OTC), watering (W) and the interaction of both, and estimates of survival probabilities (mean ± SE) for treatments based on generalized mixed-effects models (GLMM) for seedlings of *Nothofagus pumilio* at two subalpine forest locations in the southern Andes of Chile: Antillanca (40° 47' S, 72° 11' W) and Cerro Castillo (46° 04' S, 72° 03' W).

	Antillanca	Cerro Castillo
Small seedlings		χ^2 (p -value)
OTC	0.915 (0.633)	3.909 (0.142)
W	1.130 (0.568)	1.470 (0.480)
OTC * W	0.832 (0.362)	1.420 (0.233)
		Mean (±1 SE)
–OTC –W	0.798 (0.565) a	0.651 (0.601) a
–OTC +W	0.808 (0.628) a	0.595 (0.675) a
+OTC –W	0.790 (0.579) a	0.709 (0.615) a
+OTC +W	0.900 (0.687) a	0.806 (0.649) a
Large seedlings		χ^2 (p -value)
OTC	3.350 (0.187)	16.961 (<0.001)
W	3.524 (0.172)	19.250 (<0.001)
OTC * W	3.660 (0.067)	16.296 (<0.001)
		Mean (±1 SE)
–OTC –W	0.837 (0.589) a	0.675 (0.554) b
–OTC +W	0.750 (0.609) a	0.470 (0.617) c
+OTC –W	0.795 (0.564) a	0.606 (0.555) b
+OTC +W	0.890 (0.610) a	0.946 (0.676) a

p -Values < 0.05 (in bold) refer to a significant effect of a treatment factor on seedlings survival; different letters are used to differentiate a significant effect on the response among treatments.

Table 2, Fig. 2). Neither OTCs nor watering had any significant effect on the survival of small seedlings in any location.

Although there was a trend of higher growth inside OTCs, this effect was only significant in Antillanca (Table 3, Fig. 3). At this site, small seedlings grew more inside OTCs, having 40, 18, and 21% higher height, biomass, and leaf area, than seedlings

Table 3

Statistical results (F and p -values) showing the effects of warming (OTC), watering (W) and the interaction of both based on linear mixed-effect models (LMM) for seedlings of *Nothofagus pumilio* in two subalpine forest locations in the southern Andes of Chile: Antillanca (40° 47' S, 72° 11' W) and Cerro Castillo (46° 04' S, 72° 03' W).

	Small seedlings			Large seedlings
	Height (cm)	Biomass (g)	Leaf area (cm ²)	RGR (cm cm ⁻¹)
Antillanca				
OTC	9.710 (0.002)	3.950 (0.050)	5.18 (0.025)	13.00 (<0.001)
W	1.790 (0.184)	1.560 (0.217)	4.23 (0.048)	1.25 (0.27)
OTC * W	0.210 (0.651)	0.390 (0.532)	0.09 (0.077)	0.42 (0.52)
Cerro Castillo				
OTC	2.550 (0.122)	2.840 (0.103)	2.980 (0.095)	0.01 (0.98)
W	0.010 (0.924)	0.050 (0.829)	0.020 (0.882)	0.62 (0.44)
OTC * W	0.540 (0.469)	0.100 (0.750)	0.550 (0.466)	0.36 (55)

Notes: p -values < 0.05 (in bold) refer to a significant effect of a treatment factor on the response variable; biomass was not normally distributed and hence square root transformations were used to achieve normality. Standard errors of final biomass were computed on normalized data.

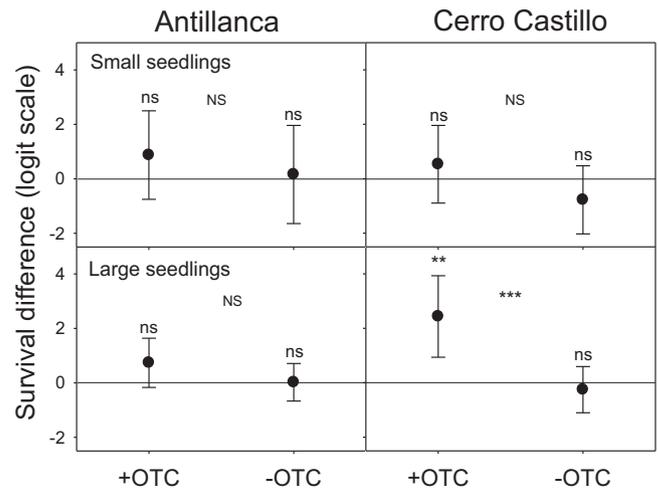


Fig. 2. Differences in survival (logit scale) between seedlings of *Nothofagus pumilio* grown with and without watering inside (+OTC) and outside (–OTC) open top chambers. For small and large seedlings, survival was recorded after one and two complete growing seasons, respectively. Where the difference is positive, growing under a watering regime is better; where the difference is negative growing without watering is better. *** indicates planned comparisons between treatments statistically significant at p -value < 0.0001; ns stands for a non-significant ($p > 0.05$) comparison. Planned comparisons were also conducted between seedlings grown inside and outside OTCs, for which statistical significance is shown in the middle of each panel.

growing outside OTCs. Likewise, OTCs significantly increased the RGR_h of large seedlings. Watering, on the other hand, had a significant positive effect on the leaf area of small seedlings in Antillanca (Fig. 3); such a positive effect was highest inside

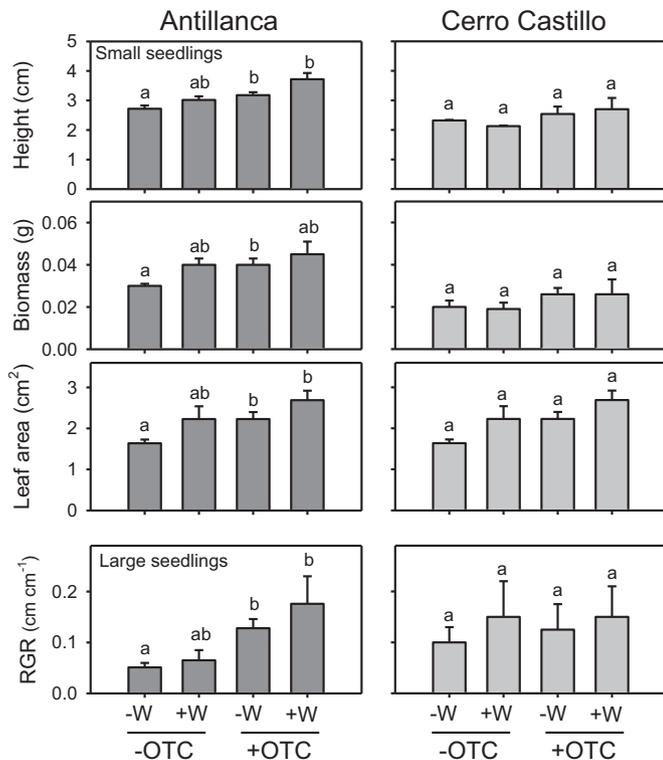


Fig. 3. Effects of simulated warming (by open top chambers, OTCs) and watering on seedling growth of *Nothofagus pumilio* at two locations in the southern Chilean Andes: Antillanca (40° 47' S, 72° 11' W) and Cerro Castillo (46° 04' S, 72° 03' W). For small seedlings, data shown correspond to height, biomass, and leaf area after one complete growing season. For large seedlings, relative growth rate in height after two complete growing seasons is shown. Different lower case letters indicate differences between treatments statistically significant at p -value < 0.05.

OTCs (Table 3). Watering had no effect on the growth of small seedlings in Cerro Castillo or on the large seedlings in any site (Table 3).

Plant traits

When small seedlings grew inside OTCs in Antillanca, they had significantly higher SLA and lower $\delta^{13}\text{C}$ and leaf N concentration than outside OTCs (Table 4). Similar results were observed for large seedlings at the same location, although there was no variation in leaf N concentration, and the maximum SLA was found inside OTCs without watering (this was the only case for a significant interaction). In contrast, OTCs had no effect on these traits in seedlings of any size class in Cerro Castillo (Table 4). Watering only caused increments in the leaf N concentration of small seedlings in Cerro Castillo (Table 4). LAR and root/shoot ratio were not affected by any treatment (Table 4).

Discussion

Warming simulation

Our simulated warming experiment caused a slight increase in mean temperature for the growing season (0.6 °C) in both sites. Although this value is lower than what general models predict for the end of the 21st century in southern South America (e.g. 1.7–3.9 °C, IPCC, 2007), it is in accordance with the real temperature increases predicted for the short term (c. 0.2 °C per decade, under a worst-case scenario of CO₂ emissions). In southern Chile's case,

c. 0–0.5 °C temperature increases are predicted for the next two decades (IPCC, 2007). As an aside, climate predictions for southern South America should be interpreted with cautions, especially because there are still extensive regions with few or no meteorological stations, in addition to a scarcity of reliable records on which to base models (Hansen et al., 2010).

Warming effects on seedling performance of *Nothofagus pumilio*

In the subalpine forests of *N. pumilio* examined in this study, simulated future conditions of warming did not decrease seedling survival or growth (Figs. 2 and 3). This general result means that warming-induced water deficit did not override the benefits caused by higher temperatures on seedling survival and growth. In fact, warming had positive effects on these responses (increased growth in Antillanca and increased large seedling survival in Cerro Castillo). Thus, the prediction that global warming could have deleterious effects on seedling establishment due to increased water deficit, pointed out by several studies in different plant communities (Millar et al., 2004; Lloret et al., 2005; Shevtsova et al., 2009), including the forests of *N. pumilio* (Daniels and Veblen, 2004; Heinemann and Kitzberger, 2006; Tercero-Bucardo et al., 2007), was not supported. Rather, our results are consistent with a central role of low temperature limiting seedling establishment at subalpine forests (Cuevas, 2000; Lajzerowicz et al., 2004), where positive responses of seedlings to experimental warming seem to be common (e.g. Xu et al., 2012). This may imply that temperature increases associated with climate change will promote forest infilling (Innes, 1991).

The lack of warming-induced negative effects on seedling survival and growth of *N. pumilio* found in this study may be explained by the small variation in mean temperature induced by the OTCs (i.e. 0.6 °C). Nevertheless, as the small change in temperature caused significant reductions in soil humidity (56 and 30% in Antillanca and Cerro Castillo, respectively), experimental conditions appeared rather adequate to test effects of warming-induced water deficit on *N. pumilio*. A better explanation for the mismatch of our results with particular predictions could be climate difference. Most studies reporting negative impacts of drought on the survival and growth of *N. pumilio* were carried out just across the Andes (east slopes) (Heinemann and Kitzberger, 2006; Tercero-Bucardo et al., 2007). Due to a rain shadow effect, precipitation in these slopes is up to 80% lower than in the west slopes (Daniels and Veblen, 2004) (where our study was conducted). Even our mesic site, Cerro Castillo, has higher precipitation than any other site located on the eastern Andes. Thus, the minimum level of soil humidity assessed in this study could have not resulted limiting for *N. pumilio*. Nonetheless, Daniels and Veblen (2004) suggested that water deficit associated with climate change could limit seedling establishment of *N. pumilio* at the treeline ecotone in Antillanca. They found time-dependent relationships between the seedling establishment of *N. pumilio* and interannual- and decadal-scale climate variation, suggesting that high temperatures promotes seedling establishment only when high precipitation co-occurs. We did not corroborate such a relationship of water stress being the major limiting factor for seedling performance at this site. Daniels and Veblen (2004), however, sampled at the treeline elevation, where soil is characterized by a coarse texture, which limits humidity retention. In contrast, we conducted our experiment 75 m below the treeline (i.e., subalpine forest), where soil texture is finer and vegetation cover is higher than at the treeline, leading to higher water retention and lower evapotranspiration. Additionally, at treeline elevation a more open canopy will certainly trigger higher water demands compared to those occurring in our experiment at the timberline elevation with some canopy cover.

Table 4

Linear mixed-effect model outputs (*F* and *p*-values) showing the effects of warming (OTC), watering (W) and the interaction of both, and estimates of seedling trait values (\pm SE) and comparisons among treatments for seedlings of *Nothofagus pumilio* at two subalpine forest locations in the southern Andes of Chile: Antillanca (40° 47' S, 72° 11' W) and Cerro Castillo (46° 04' S, 72° 03' W).

	Small seedlings				Large seedlings			
	$\delta^{13}\text{C}$ (‰)	Nitrogen (%)	SLA (cm ² g ⁻¹)	LAR	Shoot/root	$\delta^{13}\text{C}$ (‰)	Nitrogen (%)	SLA (cm ² g ⁻¹)
Antillanca					<i>F</i> (<i>p</i> -value)			
OTC	13.25 (0.005)	6.22 (0.03)	4.25 (0.04)	1.45 (0.23)	0.06 (0.80)	6.97 (0.027)	3.84 (0.08)	1.75 (0.19)
W	0.35 (0.57)	2.16 (0.16)	1.38 (0.24)	0.03 (0.87)	0.00 (0.98)	1.98 (0.193)	4.30 (0.06)	0.37 (0.55)
OTC*W	0.69 (0.43)	1.52 (0.24)	0.12 (0.73)	0.39 (0.53)	1.01 (0.32)	1.02 (0.338)	0.001 (0.98)	5.11 (0.03)
					Mean (\pm SD)			
–OTC –W	–29.74 (0.21) <i>a</i>	1.04 (0.06) <i>a</i>	206.95 (6.82) <i>a</i>	57.40 (3.00) <i>a</i>	1.75 (0.12) <i>a</i>	–30.87 (0.21) <i>a</i>	2.09 (0.06) <i>a</i>	184.43 (9.61) <i>a</i>
–OTC +W	–29.71 (0.30) <i>a</i>	1.24 (0.09) <i>a</i>	217.32 (12.68) <i>a</i>	55.28 (6.04) <i>a</i>	1.94 (0.24) <i>a</i>	–30.70 (0.11) <i>a</i>	1.93 (0.06) <i>a</i>	199.41 (15.96) <i>ab</i>
+OTC –W	–30.32 (0.21) <i>b</i>	0.95 (0.06) <i>b</i>	223.17 (7.28) <i>b</i>	60.25 (3.29) <i>a</i>	1.89 (0.13) <i>a</i>	–31.19 (0.21) <i>b</i>	1.94 (0.08) <i>a</i>	219.70 (9.21) <i>b</i>
+OTC +W	–30.63 (0.30) <i>b</i>	0.98 (0.09) <i>b</i>	240.00 (15.84) <i>b</i>	64.24 (7.77) <i>a</i>	1.71 (0.31) <i>a</i>	–31.64 (0.27) <i>b</i>	1.77 (0.08) <i>a</i>	190.21 (15.96) <i>ab</i>
Cerro Castillo					<i>F</i> (<i>p</i> -value)			
OTC	0.30 (0.59)	0.11 (0.74)	1.14 (0.29)	0.80 (0.38)	0.02 (0.89)	0.21 (0.66)	1.12 (0.31)	0.84 (0.37)
W	0.58 (0.46)	4.51 (0.05)	1.15 (0.29)	0.66 (0.42)	0.08 (0.78)	0.45 (0.52)	0.12 (0.73)	1.82 (0.19)
OTC*W	0.87 (0.38)	0.11 (0.74)	1.92 (0.18)	3.60 (0.07)	0.46 (0.50)	0.02 (0.90)	0.53 (0.48)	1.49 (0.23)
					Mean (\pm SD)			
–OTC –W	–29.83 (0.21) <i>a</i>	1.16 (0.11) <i>a</i>	242.61 (13.90) <i>a</i>	65.96 (5.83) <i>a</i>	2.79 (0.47) <i>a</i>	–30.718 (0.258) <i>a</i>	1.56 (0.14) <i>a</i>	170.10 (14.06) <i>a</i>
–OTC +W	–29.81 (0.28) <i>a</i>	1.49 (0.14) <i>b</i>	203.93 (18.27) <i>a</i>	60.81 (7.28) <i>a</i>	3.00 (0.65) <i>a</i>	–30.303 (0.515) <i>a</i>	1.51 (0.14) <i>a</i>	196.14 (15.74) <i>a</i>
+OTC –W	–29.74 (0.25) <i>a</i>	1.16 (0.13) <i>a</i>	205.52 (16.98) <i>a</i>	60.66 (6.82) <i>a</i>	3.26 (0.60) <i>a</i>	–30.555 (0.258) <i>a</i>	1.61 (0.19) <i>a</i>	173.26 (13.75) <i>a</i>
+OTC +W	–30.18 (0.28) <i>a</i>	1.40 (0.14) <i>b</i>	208.62 (19.4) <i>a</i>	75.77 (7.61) <i>a</i>	2.70 (0.70) <i>a</i>	–30.383 (0.364) <i>a</i>	1.36 (0.19) <i>a</i>	175.42 (16.21) <i>a</i>

Notes: *p*-values < 0.05 (in bold) refer to a significant effect of a treatment factor on the response variable; different letters are used to differentiate a significant effect on the response among treatments.

Seedling survival and growth responses, as hypothesized, were site-specific. Warming significantly increased large seedling survival in Cerro Castillo suggesting that these seedlings are more temperature-limited than those in Antillanca. In this regard, mean temperature for the growing season in Cerro Castillo was c. 3 °C lower than in Antillanca. Watering did not affect seedling survival in Antillanca while in Cerro Castillo large seedling survival increased in response to watering combined with warming and decreased in response to watering outside OTCs. The increased survival in response to watering combined with warming could be explained by the different compensation for warming induced-drought achieved through watering at the sites (full at Cerro Castillo; 60% at Antillanca). Additionally, seedlings growing in Antillanca may have a higher drought resistance than those of Cerro Castillo, hence being less responsive to watering. Consistent with this idea, seedlings in Antillanca grew faster in response to warming in spite of the more drastic reductions in soil moisture respect to Cerro Castillo (Table 1), and had generally lower shoot/root ratios (Table 4). From a local adaptation perspective, this result is contrary to what we had expected (i.e., lower drought resistance in seedlings from the humid location). Although Antillanca appears to be a rather humid site, there is certainly some Mediterranean influence (Daniels and Veblen, 2004) which may be driving local adaptation to resist seasonal drought. In the same line, Antillanca's volcanic soils account for very low water retention (Veblen et al., 1977), suggesting that reduced water availability may be more common than expected from precipitation records. This is supported by the generally lower values of volumetric water content in Antillanca as compared to Cerro Castillo (Table 1). The negative effects of watering on the survival of large seedlings in Cerro Castillo when it was not combined with warming could indicate that in this location *N. pumilio* has some intolerance to high soil moisture (–OTC+W treatment combination was the most humid treatment at Cerro Castillo, Table 1), as suggested by other studies (Martínez Pastur et al., 2007; Ivancich et al., 2012). Together, these findings confirm that seedling responses to simulated warming appear to be modulated by local climate and intraspecific variation, as has been previously suggested for *N. pumilio* (Barrera et al., 2000; Premoli, 2003; Premoli and Brewer, 2007; Fajardo and Piper, 2011).

Trait variation caused by warming

Along with higher growth, warming promoted higher SLA and leaf area in seedlings grown in Antillanca. In subalpine forests, SLA and leaf area decrease with elevation (Li et al., 2004; Fajardo and Piper, 2011), suggesting that they are limited by temperatures. Our results indicate that warming effectively reduces such limitations on SLA and leaf area, leading to faster growth in seedlings of *N. pumilio*. At the same time, these results show no evidence of any drought effect induced by warming, which, on the other hand, should have provoked lower SLA. Warming, unexpectedly, did not cause any increment in leaf N concentration, but instead triggered it to decrease for small seedlings in Antillanca. We think that although warming increased maximum soil temperatures, increments were small and most likely insufficient to accelerate soil mineralization as it has been found in other studies (Rustad et al., 2001). Alternatively, the lower leaf N concentrations in seedlings under warming found in Antillanca may reflect some nutrient limitation associated with faster growth. In this case, insufficient leaf N concentration could have caused the lower iWUE (i.e., more negative values of $\delta^{13}\text{C}$) of seedlings under warming regarding seedlings grown in normal conditions, if photosynthetic capacity became N limited (Wright et al., 2001). Nonetheless, the most parsimonious interpretation of the lower $\delta^{13}\text{C}$ of seedlings inside the OTCs seems to be an absence of stomatal limitations on photosynthesis, which appears consistent with the higher photosynthetic capacities and leaf area found in the seedlings of *N. pumilio* in response to experimental drought (Martínez Pastur et al., 2007; Ivancich et al., 2012). Alternatively, a “water-spending” habit characterized by high rates of stomatal conductance under water deficit has been found in South American deciduous *Nothofagus* (Read et al., 2010). In this scenario, warming could have led seedlings to experience water stress, thus being unable to regulate stomatal conductance.

Implications for community structure and dynamic

The most recent climatic models are equivocal for precipitation predictions for southern South America (<http://cmip-pcmdi.llnl.gov/cmip5/>). If no variation in precipitation is assumed, reductions in soil moisture can parsimoniously be expected to be

higher in Antillanca than Cerro Castillo, due to higher temperature increases for the former (IPCC, 2007). We note that under the temperature increases and, particularly, reductions in soil moisture achieved in this study, survival was kept high and seedling growth was favored. And yet, faced with the unprecedented 20th century warming in the southern Andes (Villalba et al., 2003) there has, surprisingly, been no recent regeneration pulses of *N. pumilio* (Cuevas, 2002; Daniels and Veblen, 2004; Fajardo and McIntire, 2012). Our results suggest that by maintaining high survival and growth rates of established seedlings global warming should promote denser seedling banks composed of larger and hence more water-demanding seedlings. Competition for water and nutrients could eventually override the initial advantages of global warming on growth and survival (e.g. Kudo and Suzuki, 2003), thus aligning our results with the documented absence of increased regeneration of *N. pumilio*.

Conclusions

We showed here that simulated warming did not hinder the survival and growth of *N. pumilio* seedlings at two climatically contrasting sites in southern Chile, despite the presence of concomitant dryness. In response to warming and watering, growth was stimulated in seedlings in Antillanca but not in Cerro Castillo, suggesting that intraspecific variation influences responses to global warming in this species, in line with what has been indicated in other studies (Benito-Garzón et al., 2011; Fajardo et al., 2011). Overall, total leaf area and SLA appeared to be good predictors of seedling performance under warming conditions. Our study, thus, concurs with general predictions about positive effects of global warming on seedling establishment in cold-humid regions (Taylor, 1995; Holtmeier and Broll, 2005; Danby and Hik, 2007).

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References

- Barrera, M.D., Frangi, J.L., Richter, L.L., Perdomo, M.H., Pinedo, L.B., 2000. Structural and functional changes in *Nothofagus pumilio* forests along an altitudinal gradient in Tierra del Fuego, Argentina. *J. Veg. Sci.* 11, 179–188.
- Bates, D., Maechler, M., 2009. lme4: Linear Mixed-effects Models using Eigen and R Package Version 0.999375-31. <http://CRAN.R-project.org/package=lme4>
- Bates, B.C., Kundzewicz, Z.W., Wu, S., Palutikof, J.P., 2008. Climate Change and Water. Technical Paper of the Intergovernmental Panel on Climate Change. IPCC Secretariat, Geneva.
- Benito-Garzón, M., Alfá, R., Robson, T.M., Zavala, M.A., 2011. Intra-specific variability and plasticity influence potential tree species distributions under climate change. *Global Ecol. Biogeogr.* 20, 766–778.
- Cuevas, J.G., 2000. Tree recruitment at the *Nothofagus pumilio* alpine timberline in Tierra del Fuego, Chile. *J. Ecol.* 88, 840–855.
- Cuevas, J.G., 2002. Episodic regeneration at the *Nothofagus pumilio* alpine timberline in Tierra del Fuego, Chile. *J. Ecol.* 90, 52–60.
- Cuevas, J.G., Arroyo, M.K., 1999. Absence of a persistent seed bank in *Nothofagus pumilio* (Fagaceae) in Tierra del Fuego, Chile. *Rev. Chil. Hist. Nat.* 72, 73–82.
- Danby, R.K., Hik, D.S., 2007. Responses of white spruce (*Picea glauca*) to experimental warming at a subarctic alpine treeline. *Global Change Biol.* 13, 437–451.
- Daniels, L.D., Veblen, T.T., 2004. Spatiotemporal influences of climate on altitudinal treeline in northern Patagonia. *Ecology* 85, 1284–1296.
- Fajardo, A., de Graaf, R., 2004. Tree dynamics in canopy gaps in old-growth forests of *Nothofagus pumilio* in Southern Chile. *Plant Ecol.* 173, 95–106.
- Fajardo, A., McIntire, E.J.B., 2012. Reversal of multicentury tree growth improvements and loss of synchrony at mountain tree lines point to changes in key drivers. *J. Ecol.* 100, 782–794.
- Fajardo, A., Piper, F.I., 2011. Intraspecific trait variation and covariation in a widespread tree species (*Nothofagus pumilio*) in southern Chile. *New Phytol.* 189, 259–271.
- Fajardo, A., Piper, F.I., Cavieres, L.A., 2011. Distinguishing local from global climate influences in the variation of carbon status with altitude in a tree line species. *Global Ecol. Biogeogr.* 20, 307–318.
- Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40, 503–537.
- Ferrar, P.J., Cochrane, P.M., Slatyer, R.O., 1988. Factors influencing germination and establishment of *Eucalyptus pauciflora* near the alpine tree line. *Tree Physiol.* 4, 27–43.
- Frazer, G.W., Canham, C.D., Lertzman, K.P., 2000. Gap Light Analyzer (GLA), Version 2.0: image processing software to analyze true-colour, hemispherical canopy photographs. *Bull. Ecol. Soc. Am.* 81, 191–197.
- Godoy, R., Oyarzún, C., Gerding, V., 2001. Precipitation chemistry in deciduous and evergreen *Nothofagus* forests of southern Chile under a low-deposition climate. *Basic Appl. Ecol.* 2, 65–72.
- Hansen, J., Ruedy, R., Sato, M., Lo, K., 2010. Global surface temperature change. *Rev. Geophys.* 48, RG4004.
- Hayhoe, K., Cayan, D., Field, C.B., Frumhoff, P.C., Maurer, E.P., Miller, N.L., Moser, S.C., Schneider, S.H., Cahill, K.N., Cleland, E.E., Dale, L., Drapek, R., Hanemann, R.M., Kalkstein, L.S., Lenihan, J., Lunch, C.K., Neilson, R.P., Sheridan, S.C., Verville, J.H., 2004. Emissions pathways, climate change, and impacts on California. *Proc. Natl. Acad. Sci. USA* 101, 12422–12427.
- Heinemann, K., Kitzberger, T., 2006. Effects of position, understory vegetation and coarse woody debris on tree regeneration in two environmentally contrasting forests of north-western Patagonia: a manipulative approach. *J. Biogeogr.* 33, 1357–1367.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., Richardson, K., 2005. WorldClim. Global Climate Data. <http://www.worldclim.org>
- Holtmeier, F.-K., Broll, G., 2005. Sensitivity and response of northern hemisphere altitudinal and polar treelines to environmental change at landscape and local scales. *Global Ecol. Biogeogr.* 14, 395–410.
- Innes, J.L., 1991. High-altitude and high-latitude tree growth in relation to past, present and future global climate change. *Holocene* 1, 168–173.
- IPCC, 2007. Climate Change 2007: Synthesis Report. Geneva, Switzerland.
- Ivancich, H.S., Lencinas, M.V., Martínez Pastur, G.J., Soler Esteban, R.M., Hernández, L., Lindstrom, I., 2012. Foliar anatomical and morphological variation in *Nothofagus pumilio* seedlings under controlled irradiance and soil moisture levels. *Tree Physiol.* 32, 554–564.
- Körner, C., Paulsen, J., 2004. A world-wide study of high altitude treeline temperatures. *J. Biogeogr.* 31, 713–732.
- Kudo, G., Suzuki, S., 2003. Warming effects on growth, production, and vegetation structure of alpine shrubs: a five-year experiment in northern Japan. *Oecologia* 135, 280–287.
- Lajzerowicz, C.C., Walters, M.B., Krasowski, M., Massicotte, H.B., 2004. Light and temperature differentially colimit subalpine fir and *Engelmann spruce* seedling growth in partial-cut subalpine forests. *Can. J. Forest Res.* 34, 249–260.
- Lambers, H., Chapin, F.S.I., Pons, L., 2008. Plant Physiological Ecology, 2nd ed. Springer-Verlag, New York.
- Lara, A., Aravena, J.C., Villalba, R., Wolodarsky-Franke, A., Luckman, B.H., Wilson, E.O., 2001. Dendroclimatology of high-elevation *Nothofagus pumilio* forests at their northern distribution limit in the central Andes of Chile. *Can. J. Forest Res.* 31, 925–936.
- Li, C., Liu, S., Berninger, F., 2004. *Picea* seedlings show apparent acclimation to drought with increasing altitude in the eastern Himalaya. *Trees* 18, 277–283.
- Lloret, F., Peñuelas, J., Estiarte, M., 2004. Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. *Global Change Biol.* 10, 248–258.
- Lloret, F., Peñuelas, J., Estiarte, M., 2005. Effects of vegetation canopy and climate on seedling establishment in Mediterranean shrubland. *J. Veg. Sci.* 16, 67–76.
- Marion, G.M., Henry, G.H.R., Freckman, D.W., Johnstone, J., Jones, G., Jones, M.H., Lévesque, E., Molau, U., Mølgård, P., Parsons, A.N., Svoboda, J., Virginia, R.A., 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. *Global Change Biol.* 3, 20–32.
- Martínez Pastur, G., Lencinas, M.V., Peri, P.L., Arena, M., 2007. Photosynthetic plasticity of *Nothofagus pumilio* seedlings to light intensity and soil moisture. *Forest Ecol. Manage.* 243, 274–282.
- McQueen, D.R., 1976. The ecology of *Nothofagus* and associated vegetation in South America. *Tuatara* 22, 38–68.
- Millar, C.I., Westfall, R.D., Delany, D.L., King, J.C., Graumlich, L.J., 2004. Response of subalpine conifers in the Sierra Nevada, California, U.S.A., to 20th-century warming and decadal climate variability. *Arct. Antarct. Alp. Res.* 36, 181–200.
- Molau, U., Mølgård, P., 1996. ITEX Manual. Danish Polar Center, Copenhagen, Denmark.
- Oberhuber, W., 2004. Influence of climate on radial growth of *Pinus cembra* within the alpine timberline ecotone. *Tree Physiol.* 24, 291–301.
- Pinheiro, J., Bates, D.M., 2001. Mixed Effects Models in S and S-PLUS. Springer, New York.

- Piper, F.I., Fajardo, A., 2011. No evidence of carbon limitation with tree age and height in *Nothofagus pumilio* under Mediterranean and temperate climate conditions. *Ann. Bot.* 108, 907–917.
- Piper, F.I., Zúñiga-Feest, A., 2012. Intraspecific variation in drought resistance of *Nothofagus antarctica* (G. Forst.) Oerst. (Nothofagaceae). *Gayana Bot.* 69, 365–368.
- Piper, F., Corcuera, L.J., Alberdi, M., Lusk, C., 2007. Differential photosynthetic and survival responses to soil drought in two evergreen *Nothofagus* species. *Ann. Forest Sci.* 64, 447–452.
- Poorter, H.C., Remkes, 1990. Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia* 83, 553–559.
- Premoli, A.C., 2003. Isozyme polymorphisms provide evidence of clinal variation with elevation in *Nothofagus pumilio*. *J. Hered.* 94, 218–223.
- Premoli, A.C.A.C., Brewer, 2007. Environmental versus genetically driven variation in ecophysiological traits of *Nothofagus pumilio* from contrasting elevations. *Aust. J. Bot.* 55, 585–591.
- Premoli, A.C., Raffaele, E., Mathiassen, P., 2007. Morphological and phenological differences in *Nothofagus pumilio* from contrasting elevations: evidence from a common garden. *Aust. Ecol.* 32, 512–523.
- R-Development-Core-Team, 2009. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, ISBN 3-900051-07-0. <http://www.R-project.org>
- Read, J., Hill, R.S., Hope, G.S., 2010. Contrasting responses to water deficits of *Nothofagus* species from tropical New Guinea and high-latitude temperate forests: can rainfall regimes constrain latitudinal range? *J. Biogeogr.* 37, 1962–1976.
- Rodríguez, R., Quezada, M., 2003. Fagales. In: Marticorena, C., Rodríguez, R. (Eds.), *Flora de Chile*. Editorial de la Universidad de Concepción, Concepción, Chile, pp. 64–76.
- Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J.H.C., Gurevitch, J., GCTE-NEWS, 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* 126, 543–562.
- Shevtsova, A., Graae, B., Jochum, T., Milbau, A.N.N., Kockelbergh, F., Beyens, L., Nijs, I., 2009. Critical periods for impact of climate warming on early seedling establishment in subarctic tundra. *Global Change Biol.* 15, 2662–2680.
- Sierra-Almeida, A., Cavieres, L.A., 2010. Summer freezing resistance decreased in high-elevation plants exposed to experimental warming in the central Chilean Andes. *Oecologia* 163, 267–276.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry. The Principles and Practice of Statistics in Biology Research*, third ed. W.H. Freeman, New York.
- Taylor, A.H., 1995. Forest expansion and climate change in the mountain hemlock (*Tsuga martensiana*) zone, Lassen Volcanic National Park, California, USA. *Arct. Alp. Res.* 27, 207–216.
- Tercero-Bucardo, N., Kitzberger, T., Veblen, T.T., Raffaele, E., 2007. A field experiment on climatic and herbivore impacts on post-fire tree regeneration in north-western Patagonia. *J. Ecol.* 95, 771–779.
- Tranquillini, W., 1979. Physiological ecology of the alpine timberline. Tree existence at high altitudes with special references to the European Alps. *Ecological Studies*, vol. 31. Springer, Berlin, Heidelberg, New York.
- Veblen, T.T., Ashton, D.H., Schlegel, F.M., Veblen, A.T., 1977. Plant succession in a timberline depressed by vulcanism in South-Central Chile. *J. Biogeogr.* 4, 275–294.
- Veblen, T.T., Donoso, C., Kitzberger, T., Rebertus, A., 1996. Ecology of Southern Chilean and Argentinean *Nothofagus* forests. In: Veblen, T.T., Hill, R.S., Read, J. (Eds.), *The Ecology and Biogeography of Nothofagus Forests*. Yale University Press, New Haven, pp. 293–353.
- Villalba, R., Lara, A., Boninsegna, J.A., Masiokas, M., Delgado, S., Aravena, J.C., Roig, F.A., Schmelter, A., Wolodarsky, A., Ripalta, A., 2003. Large-scale temperature changes across the southern Andes: 20th-century variations in the context of the past 400 years. *Clim. Change* 59, 177–232.
- Villalba, R., Masiokas, M., Kitzberger, T., Boninsegna, J.A., 2005. Biogeographical consequences of recent climate changes in the southern Andes of Argentina. In: Huber, U.M., Bugmann, H.K.M., Reasoner, M.A. (Eds.), *Global Change and Mountain Regions*. Springer, Dordrecht, The Netherlands, pp. 157–166.
- Woodward, A., Schreiner, E.G., Silsbee, D.G., 1995. Climate, geography, and tree establishment in subalpine meadows of the Olympic Mountains, Washington, U.S.A. *Arct. Alp. Res.* 27, 217–225.
- Wright, I.J., Reich, P.B., Westoby, M., 2001. Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Funct. Ecol.* 15, 423–434.
- Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., Villar, R., 2004. The worldwide leaf economics spectrum. *Nature* 428, 821–827.
- Xu, Z., Yin, H., Xiong, P., Wan, C., Liu, Q., 2012. Short-term responses of *Picea asperata* seedlings of different ages grown in two contrasting forest ecosystems to experimental warming. *Environ. Exp. Bot.* 77, 1–11.