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Research

Seed dormancy responses to temperature relate to *Nothofagus* species distribution and determine temporal patterns of germination across altitudes in Patagonia

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Summary

- Seeds integrate environmental cues that modulate their dormancy and germination. Although many mechanisms have been identified in laboratory experiments, their contribution to germination dynamics in existing communities and their involvement in defining species habitats remain elusive.
- By coupling mathematical models with ecological data we investigated the contribution of seed temperature responses to the dynamics of germination of three *Nothofagus* species that are sharply distributed across different altitudes in the Patagonian Andes.
- Seed responsiveness to temperature of the three *Nothofagus* species was linked to the thermal characteristics of their preferred ecological niche. In their natural distribution range, there was overlap in the timing of germination of the species, which was restricted to mid-spring. By contrast, outside their species distribution range, germination was temporally uncoupled with altitude. This phenomenon was described mathematically by the interplay between interspecific differences in seed population thermal parameters and the range in soil thermic environments across different altitudes.
- The observed interspecific variations in seed responsiveness to temperature and its environmental regulation, constitute a major determinant of the dynamics of *Nothofagus* germination across elevations. This phenomenon likely contributes to the maintenance of patterns of species abundance across altitude by placing germinated seeds in a favorable environment for plant growth.

Introduction

Accounting for the spatial distribution of plant species has been a subject of fundamental importance in the history of plant ecology, and it also provides valuable information for conservation planning. Many functional traits contribute to species adaptation to environment, leading to recruitment in natural ecosystems (Kempel *et al.*, 2013; Read *et al.*, 2014). Germination and seedling establishment are of extreme relevance, due to their strong environmental vulnerability (Grubb, 1977; Bykova *et al.*, 2012; Green *et al.*, 2014). The study of their ecological regulation and phenotypic variation across natural gradients therefore constitutes an attractive approach for understanding the mechanisms behind vegetation patterns.

In several species, seed germination is highly regulated by environmental factors, and constitutes one of the most dramatic changes of the plant lifecycle, involving the transition from a resistant, quiescent state to one of extreme vulnerability: the seedling. An extensive range of experiments indicate that temperature is one of the main environmental factors that regulate seed physiology across plant taxa (Bewley *et al.*, 2013), having major effects on both dormancy and on the rate of germination of nondormant seeds (Probert, 2000; Batlla & Benech-Arnold, 2015). Numerous laboratory studies have demonstrated an association between seed responsiveness to temperature and the thermic characteristics of their habitat range (Thompson, 1968, 1970; Probert, 2000; Rosbakh & Poschlod, 2015). However, at present, we still lack empirical data that explain the contribution of this behavior to the modulation of patterns of germination in existing communities distributed across contrasting thermic ranges.

In Patagonian temperate forests, it is possible to distinguish several environmental gradients, of which the pluviometric and thermal gradients are probably the main influences accounting for species distribution and abundance. In the western Andes, three dominant tree species, *Nothofagus obliqua*, *N. nervosa (= N. alpina)*, and *N. pumilio* have a clear altitudinal distribution: *N. obliqua* is frequent at 650–850 m above sea level, *N. nervosa* is more abundant *c.* 900–1000 m and *N. pumilio* is distributed above 1000 m, usually constituting the altitudinal limit of these temperate forests (Hill & Dettmann, 1997). In these ecosystems, temperature is the environmental factor that shows the strongest association with altitude (this study), suggesting the existence of different thermal niches. In the present work, by coupling the power of population threshold models (Garcia-Huidobro *et al.*, 1982; Batlla & Benech-Arnold, 2015) with field experiments in a steep elevational gradient of the Patagonia, we describe a dormancy model capable of predicting species germination dynamics across altitudes in an old-growth temperate forest, and tested two key hypotheses: seed responsiveness of the three *Nothofagus* species to temperature is linked to the thermic characteristics of the species' ecological niche; and interspecific differences in the responsiveness to temperature during seed after-ripening and germination contribute to germination dynamics of *Nothofagus* spp. across altitudes. Overall, this work shows that fine-scale differences in the temperature responses of *Nothofagus* seeds contribute to the adjustment of temporal patterns of germination across altitudes. This phenomenon may influence the maintenance of vegetation patterns in these ecosystems, by placing germinated seeds in a favorable environment for growth.

Materials and Methods

Plant material

Seeds were collected over three consecutive years (2010–2012) from natural populations located at Lanín National Park, Tromen and Chall-Huaco Valley (Supporting Information Table S1) at the time of their natural dispersal (February–March). They were used immediately in laboratory experiments. For field experiments, seeds were stored for 3 or 15 months in dry conditions at 2°C (which maintains dormancy; Table S2) until use.

Seed handling for the generation of germination time-course curves under controlled conditions

We used the average viability of each seed batch, measured by the tetrazolium test (Moore, 1966), to calculate the number of seeds to include in each replica plate in order to achieve at least 30 viable seeds per plate. Seeds were placed in 9-cm-diameter Petri dishes, on cotton moistened with 5 ml of a water solution containing 1% fungicide VITAVAX-FLO (Luján Agrícola, Argentina). Seeds were imbibed and irradiated for 1 h with far red light (calculated active phytochrome/total phytochrome ratio (Pfr/P) = 0.1). This treatment minimizes the quantities of active phytochromes formed during the development of the seed in the mother plant, decreasing the differences in seed germination due to microhabitat light quality experienced during seed development. Seeds were then wrapped in black plastic sheets and stratified in darkness for the periods indicated below. After stratification, seeds were exposed for 24 h to hourly pulses of 3 min of red light (calculated Pfr/P = 0.87) in order to bypass light requirements for germination, and then transferred to the different germination temperatures, in darkness. Germination was monitored daily for 40 d. At the end of the experiment, ungerminated seeds were tested for viability by tetrazolium and only seeds with red-stained embryos were considered for the estimation of germination percentage. Light sources were described in Arana *et al.* (2014).

Estimation of population parameters

Time-course cumulative germination curves were generated from a factorial experiment involving five periods of stratification (0, 30, 45, 60 and 110 d for *N. obliqua* and *N. nervosa*; 0, 30, 45, 60 and 100 d for *N. pumilio*) × 3 temperatures of stratification (0.5, 1.5 and 4°C) × 3 germination temperatures (12, 17 and 22°C). The final proportion of germinated seeds (pT) was used to obtain optimal dormancy population parameters ($T_{l(50)}$, σ_{Tl}, $T_{h(50)}$ and σ_{Th}) by adjusting values in Eqn 5. The concepts of 'lower limit temperature (T_l)' and 'higher limit temperature (T_h)' introduced by Washitani (1987) allowed us to deal with the temperature-dependent expression of dormancy (i.e. relative dormancy). When a seed population displays relative dormancy, final germination percentages decrease gradually as incubation temperature departs from that at which no (or less) dormancy is expressed. This gradual decrease can be regarded as a consequence of a different dormancy level in the individuals comprising the population. For this reason, it is considered that both T_l and T_h are normally distributed within the population, with mean $T_{l(50)}$ and $T_{h(50)}$, respectively, and standard deviation σ_{Tl} and σ_{Th}, respectively (Batlla & Benech-Arnold, 2003). The values of T_l and T_h define the thermal range within which seeds are able to germinate, and this range is related to the dormancy level of the seed population (i.e. the lower the dormancy level, the wider the difference between T_l and T_h). Thus, the calculation of these parameters after each stratification treatment allowed us to test the effect of temperature in dormancy alleviation. However, the effect of temperature on germination of the fraction of nondormants was evaluated by calculating the mean thermal time for seed germination of the seed population ($\theta_{(50)}$) and its standard deviation (σ_θ) (Garcia-Huidobro *et al.*, 1982), by adjusting these values to the dynamics of germination at different temperatures using Eqn 6. Calculations were performed using a nonlinear least-squares curve-fitting method (Premium Solver Platform 7.0; Frontline systems, Incline Village, NV, USA). Maximum fit between simulated and experimentally obtained data was achieved by an iterative technique using a quasi-Newton algorithm.

Estimation of the base temperature (T_b), optimum temperature (T_o) and ceiling temperature (T_c) of germination

The base temperature (T_b) and the ceiling temperature (T_c) are the temperatures below and above which germination does not occur for nondormant seeds, and the temperatures above and below which thermal time is accumulated, when seeds are incubated between T_l and T_h. The optimum temperature (T_o) is the temperature at which germination is fastest (Batlla & Benech-Arnold, 2015). For the calculation of T_b, T_o and T_c, nondormant seeds (seeds stratified at 0.5°C for 110 d for *N. nervosa* and *N. obliqua*; or 100 d for *N. pumilio*) were germinated at 12, 17, 22, 24, 26 and 30°C. Time-course germination curves were generated by counting germination every day for 40 d and fitted to Gompertz equation $Y = C \times \exp(-\exp(-B \times (X - M)))$, were C, B and M, are equation parameters (Fig. 1a–c). The rate of germination (GR) of each fraction of the population ($\mathrm{GR_g}$) was calculated from theoretical curves as:

$$\mathrm{GR_g} = 1/d_g \qquad \text{Eqn 1}$$

where d_g is the value, in days, for completion of germination of the fraction g of the population calculated as:

$$d_g = M - ((\log_e(-\log_e Y/C))/B) \qquad \text{Eqn 2}$$

In order to determine T_b, we based calculations on concepts developed by Garcia-Huidobro *et al.* (1982) in which the GR of each fraction of the population is linearly related to the incubation temperature. T_b is defined by the interception of the temperature axis when $\mathrm{GR_{(g)}} = 0$ (Batlla & Benech-Arnold, 2015).

Each *Nothofagus* species showed little variation in T_b between the different subpopulations ($T_b = 4.5 \pm 0.1$°C, 0.47 ± 0.1°C; 1 ± 0.4°C, *N. obliqua*, *N. nervosa* and *N. pumilio*, respectively). Therefore, once ranges for T_b were identified, several linear regressions were re-calculated for each species, which were constrained to pass through a common T_b for all subpopulations. For each independent calculation, we varied T_b values in 0.5°C increments and chose the temperature value that yielded the best fit (minor Σsy.x values) for the linear regression as the characteristic T_b for the species (Fig. 1d–f; Table S3). At the supra-optimal range of temperatures, T_c is defined by the interception of the temperature axis when $\mathrm{GR_1} = 0$ for the different fractions of the population. T_o is defined by the interception of Eqn 6 (results) with Eqn 3 that describes the accumulation of θ in the supra-optimal range as:

$$\theta_h = (T_c - T) \times t_g \qquad \text{Eqn 3}$$

In which θ_h is the thermal time required to be accumulated for the completion of germination in the supra-optimal range and t_g is the time required to complete germination of the fraction g of the population (Garcia-Huidobro *et al.*, 1982; Batlla & Benech-Arnold, 2015). For each species, T_o was calculated by constraining interception of segmental linear regressions in the suboptimal

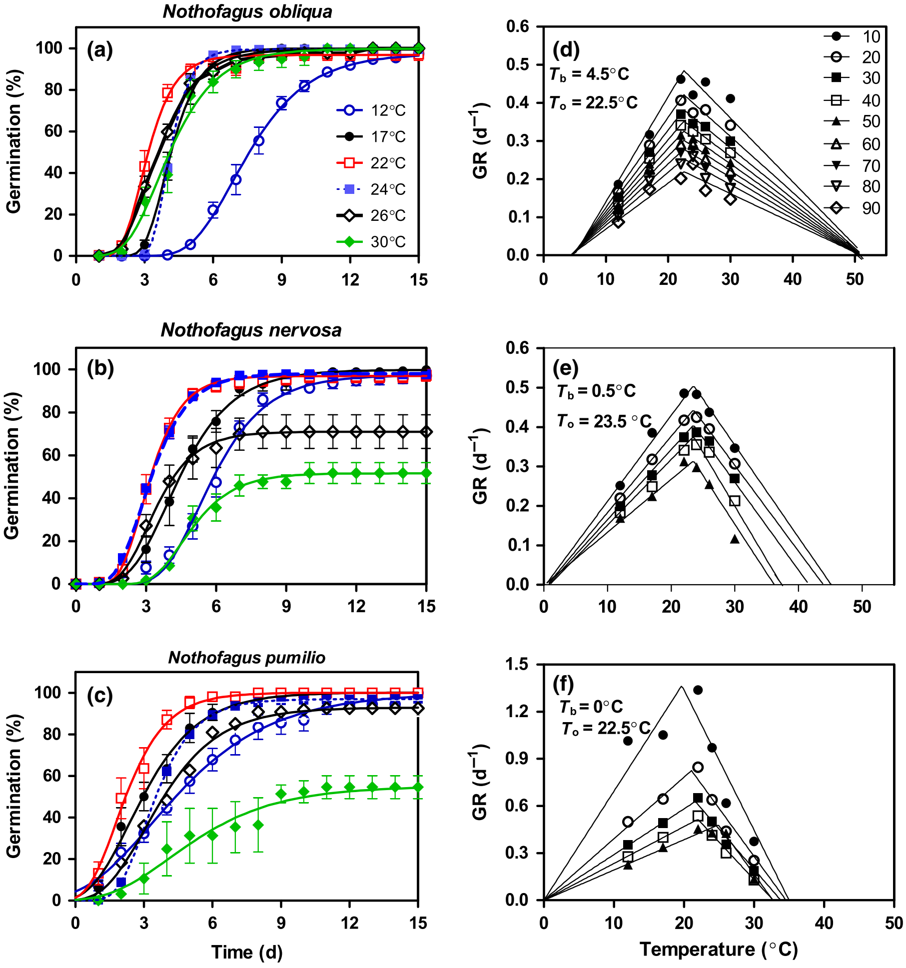

Fig. 1 Relationships between germination rate (GR) of *Nothofagus obliqua*, *N. nervosa* and *N. pumilio* seeds, and incubation temperature. Cumulative germination curves of nondormant *N. obliqua* (a), *N. nervosa* (b) and *N. pumilio* (c) at 12, 17, 22, 24, 26 and 30°C and the nonlinear adjustment to the Gompertz equation: $Y = C \times \exp(-\exp(-B \times (X - M)))$, where C, B and M are equation parameters. In (d–f) the germination rate for different population fractions (p.f.) were regressed as linear functions of temperature. Coefficient of determination (R^2) for *N. obliqua* (d) (p.f.: 10%–90% $R^2 = 0.9$), *N. nervosa* (e) (p.f.:10%–50%: $R^2 = 0.9$) and *N. pumilio* (f) (p.f.: 10%: $R^2 = 0.8$; 20%–40%: $R^2 = 0.9$). Seeds were stratified at 0.5°C for 110 d (*N. obliqua* and *N. nervosa*) or 100 d (*N. pumilio*) in order to break dormancy, and germinated at 12, 17, 22°C (suboptimal range), or 24, 26 and 30°C (supra-optimal range). Estimated base temperature (T_b) and optimal temperature (T_o) for germination are indicated inside each panel. Vertical bars indicate the standard error of the mean (SEM).

and supra-optimal germination range to pass through a common T_o for all the fractions of the population. For each independent calculation, we varied T_o values in increments of 0.5°C and chose the temperature value that yielded the best fit (minor $\Sigma sy.x$ values) for the segmental linear regression as the characteristic T_o for the species (Fig. 1d–f; Table S4).

Development of the model

We developed a model that assumed a constant T_b and T_o for the entire seed population, whereas θ_1, the thermal time required to be accumulated for the completion of germination of the population in the sub-optimal thermal range, was considered normally distributed (Covell *et al.*, 1986; Ellis *et al.*, 1986; Benech-Arnold *et al.*, 1990). We assumed that T_b and T_o did not vary with stratification. In our model, each seed of the population is capable of germinating in a temperature range within a lower (T_l) and upper (T_h) temperature threshold, each normally distributed in the population (Washitani, 1987; Grundy *et al.*, 2000). Through this approach, the proportion of germinating seeds at a given temperature was calculated as described in Eqn 5. We introduced the lower (T_l) and upper (T_h) temperature threshold modification to improve preliminary fitting to previously proposed models (Garcia-Huidobro *et al.*, 1982; Covell *et al.*, 1986; Ellis *et al.*, 1987) ($R^2 = 0.92$, 0.77, 0.88 for *N. obliqua*, *N. nervosa* and *N. pumilio*, respectively), in which germination is assumed to occur at temperatures above a common T_b for all fractions of the population, and θ is normally distributed in the population.

Model evaluation and study of seed behavior across altitude

In order to evaluate the model performance with independent data and to study the possible effects of soil temperature on the dynamics of seed dormancy/germination across altitudes, we carried out laboratory and field experiments, using seeds collected in 2011 and 2012 (Table S1). For laboratory experiments, seeds were stored for 95 d at 0.5, 1.5 and 4°C and germination time-course curves at 12, 17 or 22°C were generated as indicated above. We performed three biological replicates with a pool of collected seeds from 2011 and 2012 (30 viable seeds per plate). For testing the accuracy of the models in the prediction of seed germination in the field, *Nothofagus* seeds (30 viable seeds for *N. nervosa* and *N. pumilio,* and 60 viable seeds for *N. obliqua*) were placed inside nylon mesh bags (at least nine bags per species per altitudinal site) and buried (5 cm depth) during late autumn at 650 m. Seeds overwintered for 60 d were exhumed, washed, surface sterilized and assayed for germination in the lab at 12, 17 and 22°C. These seeds had not germinated in the field. Another group of seeds was incubated in the field for 90 d, then exhumed and germination-assessed, in order to establish the final percentage of germination (G_{max}) in the field.

In order to study seed behavior across altitudes, *Nothofagus* seeds were buried as indicated above, across three levels of an altitudinal gradient: 650, 930 and 1100 m. Seeds were exhumed after 60 and 120 d (2012) or 85 and 160 d (2013) of burial at the different altitudes. Germinated seeds in the mesh bags were counted, and data were used to establish final percentage of germination in the field.

Study site

The study site is an old-growth mixed temperate forest, with understory vegetation dominated by the native bamboo *Chusquea culeou*, Desvaux. *Nothofagus obliqua* is the dominant forest species *c.* 650 m and *N. pumilio* dominates the highest forest zones from 1100 to 1700 m. Although *N. nervosa* is distributed throughout the entire altitudinal gradient, its abundance is greatest in areas *c.* 900–1100 m. Similarly, it is possible to find *N. obliqua* trees *c.* 930 m, which comprises the altitudinal limit of its distribution.

Air temperature values across elevation were scored daily for 4 yr (2010–2014) and data showed that mean temperature at 650 m above sea level was 8.4°C, whereas at 930 and 1100 m it was 6.5 and 5.9°C, respectively. We observed that for the same day, the daily mean temperature at 650 m was always higher than at 930 and 1100 m (Fig. S1). In addition, we observed that in general, daily mean temperature at 1100 m was slightly lower than at 930 m. At low daily mean temperatures it is possible to find days with similar temperatures at 930 and 1100 m. However, at temperatures > 15°C, values at 1100 m were always lower than values at 930 m (Fig. S1).

Evaluation of climatic data

Soil temperature and moisture were monitored hourly during the period of seed incubation in the forest, using sensors connected to data loggers (http://www.cavadevices.com). Air temperature and moisture were monitored using Onset HOBO® data loggers (http://www.onsetcomp.com/). Climatic data were processed using the R package (R Development Core Team, 2008). R : FR ratios at the soil surface of the experiments were measured near midday (12:00–15:00 h) at all sampling dates using a 660/730 nm quantum sensor (Skye Instruments Ltd, Powys, UK).

Evaluation of fit between observed and predicted data

Evaluation of the fit between observed and predicted data was performed by the estimation of the coefficient of determination (R^2) through the following equation:

$$R^2 = 1 - \left[\sum (y_{obs} - y_{pred})^2 / \sum (y_{obs} - \bar{y}_{obs})^2\right] \qquad \text{Eqn 4}$$

(y_{obs}, observed values; y_{pred}, predicted data).

Results

Temperature effects on dormancy and germination

Temperature regulates germination in two main ways: by modulating seed dormancy, and by affecting the germination rate of nondormant seeds (Batlla & Benech-Arnold, 2015). We first analyzed germination behavior of naturally dispersed *Nothofagus* seeds at 12, 17 and 22°C, and observed that, whereas *N. obliqua* and *N. pumilio* did not germinate in these conditions, a large proportion of *N. nervosa* seeds completed germination at the warmer temperatures (% germination: 22°C = 86.4 ± 3.8; 17°C =75.4 ± 3.8), although only a small fraction of the population (6 ± 0.76%) germinated at 12°C. This indicates the existence of dormancy in *Nothofagus* spp. It is widely recognized that temperate species often require a period of cold stratification or moist chilling to break dormancy, representing a natural mechanism which favors germination during spring (Probert, 2000). Therefore, we tested the effect of a wide temperature range (−4 to 12°C) in alleviating seed dormancy. Dormancy of the three species was greatly reduced when seeds were stratified in a temperature range between 0.5 and 4°C (Table S5): final germination was much higher when compared with treatments with no stratification (see data brackets in this paragraph), particularly for long periods of stratification (100–110 d). Seeds stratified at −4°C did not germinate at the tested temperatures, but showed viable embryos after tetrazolium tests. By contrast, temperatures of 8 and 12°C had no effect on dormancy of *N. obliqua* and *N. pumilio* seeds, and had only a slight effect on *N. nervosa* germination. Moreover, all three *Nothofagus* species showed a strong decrease in seed viability (*c.* 80%) after long periods (100–110 d) at 8 and 12°C, indicating that stratification at warmer temperatures (≥ 8°C) induces seed ageing and subsequent loss of viability.

Once we had determined the temperature range that efficiently alleviated dormancy, we evaluated the effect of different times and temperatures of stratification on the rate of dormancy loss. For this purpose, we characterized temperature-dependent variations in dormancy levels as changes in the thermic range permissive for germination. For example, dormant seeds are expected to germinate within a narrow temperature range, characterized by a lower limit (T_l) and a higher limit (T_h), which are assumed to be normally distributed in the seed population (Washitani, 1987). As seeds lose dormancy, the range of permissible germination temperatures increases, and this can be expressed either as a decrease in T_l or an increase in T_h of the population. Experimental evidence shows that in spring-germinating species, dormancy release is generally associated with a decrease in T_l, and only minor changes in T_h have been observed (Baskin & Baskin, 1998). Therefore, these species express dormancy mainly at low incubation temperatures, and loss of dormancy can be quantified as changes in the mean lower limit temperature for germination ($T_{l(50)}$) of the seed population. To study the variation of T_l and T_h with stratification, we designed a factorial experiment that combined five periods of stratification (0, 30, 45, 60 and 110 d for *N. obliqua* and *N. nervosa*; 0, 30, 45, 60 and 100 d for *N. pumilio*) × 3 temperatures of stratification (0.5, 1.5 and 4°C) × 3 germination temperatures (12, 17 and 22°C). We generated cumulative germination curves and, taking into account the final proportion of germinating seeds *p(T)* at a given temperature *T*, we estimated the mean lower and upper temperatures for germination ($T_{l(50)}$ and $T_{h(50)}$) and their standard deviations (σ_{Tl} and σ_{Th}) as:

$$p(T) = \{\Phi[(T - T_{l(50)})/\sigma_{Tl}] - \{1 - \Phi[(T - T_{h(50)})/\sigma_{Th}]\}\} \qquad \text{Eqn 5}$$

(Φ, normal probability integral).

We found that dormancy loss of *N. obliqua*, *N. nervosa* and *N. pumilio* seeds during stratification exhibited similar trends, characterized by a progressive decrease of $T_{l(50)}$ and σ_{Tl} with the length of the stratification treatment, with little variation in $T_{h(50)}$ and σ_{Th} (Table 1). The different temperatures of stratification had little effect on the rate of dormancy loss, because $T_{l(50)}$, σ_{Tl}, $T_{h(50)}$ and σ_{Th} showed only small variations when seeds were chilled in the range between 0.5 and 4°C. Beyond these shared patterns, we detected strong differences between species in the response to stratification; in response to the same time and temperature of stratification, *N. pumilio* and *N. nervosa* seeds rapidly lost dormancy and where able to germinate at remarkably lower temperatures than *N. obliqua*. This is indicated by the interspecific differences in values of $T_{l(50)}$ after the same stratification treatment (Table 1).

Having determined the dynamics of dormancy loss, we next evaluated the dynamics of germination in relation to temperature of the fraction of seeds that had lost dormancy, and the influence of the different stratification treatments on this dynamics. We used a thermal-time approach (Garcia-Huidobro *et al.*, 1982) that establishes that a quantity of thermal time (θ), in °Cd, is required to complete germination. θ is assumed to be normally distributed with a mean ($\theta_{(50)}$) and standard deviation (σ_θ) in the population, and is accumulated within a range of temperatures characterized by a base temperature (T_b) and a ceiling temperature (T_c), when seeds are incubated in a range between T_l and T_h (Garcia-Huidobro *et al.*, 1982; Washitani, 1987; Grundy *et al.*, 2000; Batlla & Benech-Arnold, 2015). In the range between T_b and T_c, the rate of germination (GR), defined as the inverse of the time to germinate for a specific population fraction, generally increases as temperature increases, reaching a maximal value at the optimum temperature (T_o) and decreasing at temperatures above T_o (Garcia-Huidobro *et al.*, 1982). In the suboptimal thermic range ($< T_o$) the accumulation of thermal time for each population fraction ($\theta_{(g)}$) can be calculated as:

$$\theta_{(g)} = (T - T_b)t_g \quad \text{when } T > T_{lg} \qquad \text{Eqn 6}$$

($\theta_{(g)}$, thermal time to completion of germination of a fraction (g) of the population; t_g and T_{lg}, time to completion of germination, and the lower limit temperature of the fraction g of the population, respectively).

Therefore, in order to describe the rate of germination in relation to temperature, and the way in which this rate is affected during after-ripening, we estimated the changes in $\theta_{(50)}$ and σ_θ in relation to stratification. For this purpose, we first estimated T_b for nondormant fully after-ripened *Nothofagus* seeds (Fig. 1). Our results indicate that the three *Nothofagus* species showed little variation in T_b among population fractions, and a relatively variable T_c (Fig. 1), in concordance with data from other species (Garcia-Huidobro *et al.*, 1982; Covell *et al.*, 1986). Therefore,

Table 1 Estimated population parameters for *Nothofagus obliqua*, *N. nervosa* and *N. pumilio* seeds stratified at 0.5, 1.5 and 4°C

0.5°C	*N. obliqua*							*N. nervosa*							0.5°C	*N. pumilio*						
Days of stratification	$T_{l(50)}$[a]	σ_{Tl}[b]	$T_{h(50)}$[c]	σ_{Th}[d]	$\Theta_{(50)}$[e]	σ_{θ}[f]	RMSE	$T_{l(50)}$	σ_{Tl}	$T_{h(50)}$	σ_{Th}	$\Theta_{(50)}$	σ_{θ}	RMSE	Days of stratification	$T_{l(50)}$	σ_{Tl}	$T_{h(50)}$	σ_{Th}	$\Theta_{(50)}$	σ_{θ}	RMSE
0	–	–	–	–	–	–	–	15.4	2.2	36.4	1.2	184.8	76.8	18.1	0	–	–	–	–	–	–	–
30	24.2	5.2	45	5.0	141.4	51.5	8.4	13.8	2.1	36.4	1.2	143.8	67.9	32.2	30	18.0	2.9	35.9	2.4	338.9	119.8	15.2
45	23.4	4.2	45	5.0	198.6	75.1	22.4	13.6	2.1	36.4	1.2	123.3	70.6	28.4	45	17.9	2.2	35.9	2.3	284.1	76.3	33.8
60	12.3	1.0	45	0.5	117.6	42.1	45.2	10.0	1.5	36.4	1.2	115.3	42.0	46.2	60	10.3	1.7	35.9	2.3	116.2	45.0	54.0
110	8.3	2.2	45	0.5	51.9	15.3	16.1	1.6	0.5	36.4	1.2	71.9	25.8	21.1	100	0.5	0.5	40.9	0.5	52.0	20.0	41.5

1.5°C	*N. obliqua*							*N. nervosa*							1.5°C	*N. pumilio*						
Days of stratification	$T_{l(50)}$	σ_{Tl}	$T_{h(50)}$	σ_{Th}	$\Theta_{(50)}$	σ_{θ}	RMSE	$T_{l(50)}$	σ_{Tl}	$T_{h(50)}$	σ_{Th}	$\Theta_{(50)}$	σ_{θ}	RMSE	Days of stratification	$T_{l(50)}$	σ_{Tl}	$T_{h(50)}$	σ_{Th}	$\Theta_{(50)}$	σ_{θ}	RMSE
0	–	–	–	–	–	–	–	15.4	2.2	36.4	1.2	184.8	76.8	18.1	0	–	–	–	–	–	–	–
30	24.9	4.9	45	5.0	235.5	91.3	3.7	14.2	2.1	36.4	1.2	148.6	76.3	44.5	30	18.1	2.8	35.0	2.5	440.3	157.0	22.3
45	23.8	4.8	45	5.0	214.7	76.3	15.9	13.9	1.9	36.4	1.2	171.8	96.1	30.6	45	19.2	2.5	36.3	2.7	253.4	33.9	19.7
60	12.4	1.0	45	0.5	153.6	52.5	51.7	10.3	1.7	36.4	1.2	124.8	56.5	62.8	60	8.7	1.7	35.9	2.3	112.7	45.0	76.7
110	9.1	1.5	45	0.5	56.4	15.3	23.0	2.0	0.5	36.4	1.2	64.8	23.4	38.4	100	0.5	0.5	35.9	2.3	25.8	10.0	43.9

4°C	*N. obliqua*							*N. nervosa*							4°C	*N. pumilio*						
Days of stratification	$T_{l(50)}$	σ_{Tl}	$T_{h(50)}$	σ_{Th}	$\Theta_{(50)}$	σ_{θ}	RMSE	$T_{l(50)}$	σ_{Tl}	$T_{h(50)}$	σ_{Th}	$\Theta_{(50)}$	σ_{θ}	RMSE	Days of stratification	$T_{l(50)}$	σ_{Tl}	$T_{h(50)}$	σ_{Th}	$\Theta_{(50)}$	σ_{θ}	RMSE
0	–	–	–	–	–	–	–	15.4	2.1	36.4	1.2	184.8	76.8	18.1	0	–	–	–	–	–	–	–
30	27.1	5.3	45	0.5	217.0	80.1	4.6	13.4	2.0	36.4	1.2	147.1	81.5	44.8	30	19.2	4.8	35.9	2.4	489.8	141.0	18.3
45	25.1	5.9	45	0.5	116.1	42.3	11.0	13.8	1.7	36.4	1.2	147.3	79.6	26.2	45	18.7	1.7	35.8	3.1	257.8	74.8	16.2
60	12.8	2.5	45	0.5	151.2	53.9	60.0	11.0	1.5	36.4	1.2	165.4	71.6	39.6	60	13.2	3.1	35.9	2.3	235.0	80.0	60.8
110	8.8	2.1	45	0.5	56.8	16.0	16.7	4.0	1.0	36.4	1.2	63.0	20.2	48.1	100	0.5	0.5	35.9	1.9	43.0	19.0	50.9

Parameters were obtained by simulation of the germination time-course curves for seeds stratified for the indicated periods at the different temperatures and germinated at 12, 17 or 22°C. Dashed lines indicate that estimation of population thermal parameters was not possible due to zero germination in the test. RMSE, root mean-square error of empirical and theoretical data fitting.

[a] Mean lower limit temperature for germination of the seed population. The term indicates the lower temperature that yields 50% of germination of the seed population.

[b] Standard deviation of the values of lower limit temperature for germination (T_l) in the seed population.

[c] Mean higher limit temperature for germination of the seed population. The term indicates the higher temperature that yields 50% of germination of the seed population.

[d] Standard deviation of the values of higher limit temperature for germination (T_h) in the seed population.

[e] Required amounts of thermal time units (°C d) for completion of germination of the 50% of the seed population.

[f] Standard deviation of the required thermal time units (θ) for the completion of germination of the different fractions of the seed population.

for each species, we estimated a common value of T_b for all fractions of the population. We found that *N. obliqua* exhibited a higher T_b than either *N. nervosa* or *N. pumilio* (T_b *N. obliqua* = 4.5°C > *N. nervosa* = 0.5°C > *N. pumilio* = 0°C).

Once T_b was characterized, we chose a thermal time model for quantifying the effect of stratification on the rate of germination of nondormant seeds. The results described above (Table S5; Fig. 1) prompted us to choose a thermal time model characterized by the assumption of a common T_b and a normal distribution of T_c between different population fractions (Garcia-Huidobro *et al.*, 1982). In addition, we assumed that T_b was not affected by stratification, and that the seed population is capable of germinating within a thermal range between a lower limit temperature (T_l) and an upper limit temperature (T_h) (Washitani, 1987; Grundy *et al.*, 2000). Using this approach, we modeled experimental germination time-course curves from the factorial experiment described above using Eqns 5 and 6, and obtained a strong fit between the experimental and the simulated data ($R^2 = 0.99$, 0.98, 0.98 for *N. obliqua*, *N. nervosa* and *N. pumilio*, respectively: see Fig. S2 for individual fits after stratification at 0.5°C). We found an inverse relationship between germination rate and the level of dormancy of the population, as shown by the progressive decrease in $\theta_{(50)}$ and σ_θ, with the duration of stratification, for all three *Nothofagus* species (Table 1). Beyond these shared patterns, we detected strong interspecific differences in the response to stratification; strongly dormant *N. pumilio* seeds required the accumulation of relatively high quantities of thermal time units compared with *N. nervosa* and *N. obliqua*, as shown by its remarkably high $\theta_{(50)}$ values in comparison with the other two species (Table 1: 30 d of seed stratification). In addition, *N. pumilio* showed a very sharp rate of change in $\theta_{(50)}$ with the length of the stratification treatment which indicates that the effect of temperature on the rate of germination of the population is particularly dependent on the degree of seed dormancy.

Construction of dormancy models in *Nothofagus*

In order to characterize and predict seed responsiveness to temperature during dormancy loss and germination, we developed a dormancy model by regressing $T_{l(50)}$, σ_{Tl}, $\theta_{(50)}$ and σ_θ as functions of the length of stratification treatment for all temperatures tested (Fig. 2). As stated above, we observed that different stratification temperatures within the optimum range of 0.5 to 4°C had no differential effect on the physiology of the seeds, indicated by the low variation in seed thermal parameters between temperatures. However, individual species showed strong differences in the rate of dormancy loss with the duration of stratification, as indicated by interspecific differences in the rate of change in the population parameters $T_{l(50)}$ and σ_{Tl} with the duration of chilling. Therefore, for the construction of the dormancy models, changes in population parameters were predicted as a function of the time of stratification, irrespective of the temperature. The performance of the models was evaluated using empirical data from field experiments, and from an independent experiment carried out under controlled conditions. For field experiments, *Nothofagus* seeds were overwintered in the forest for 60 d. These seeds did not germinate in the field, and were assessed for subsequent germination in the laboratory. Another group of seeds was incubated in the field for 90 d, then exhumed and counted for seeds that had already germinated, in order to establish the final percentage of germination (G_{max}) in the field. Using the equations described in Fig. 2, we predicted changes in germination thermal range parameters ($T_{l(50)}$, σ_{Tl}) and in thermal time parameters $\theta_{(50)}$ and σ_θ after 60 (Fig. 3a–i) and 90 d (Fig. 3j) of incubation and estimated germination. We assumed constant values of $T_{h(50)}$ and σ_{Th} (Table S6). The predicted G_{max} ($G_{(max)p}$) in the field (Fig. 3j) was calculated using Eqn 5, as $G_{(max)p} = p(T) \times 100$. Overall, the model provided a good prediction of seed behavior, with R^2 in the 0.61–0.96 range. Laboratory experiments confirmed the accuracy of the model, with R^2 values for predicted and observed cumulative germination ≥ 0.9 (Fig. S3).

Evaluation of germination behavior of *Nothofagus* seeds across altitudes

One outcome of our models is that the germination behavior of a species was largely differentiated by the response of seeds to stratification, which yields interspecific differences in $T_{l(50)}$, whereas seed after-ripening was rather insensitive to changes in temperature in the thermic range that promotes dormancy loss (Table 1; Fig. 1). Therefore, after dispersal, dormancy of the three *Nothofagus* species would be broken during overwintering, when soil temperatures are around or below 4°C (Fig. 4a). During spring, interspecific variation in $T_{l(50)}$, in combination with natural gradients of soil temperature across altitudes, would strongly regulate the ability of seeds to germinate across the altitudinal gradient, and this phenomenon would be dependent on species type and altitude.

In order to test this prediction we conducted field experiments to measure *N. obliqua*, *N. nervosa* and *N. pumilio* seed germination in experimental field plots at different altitudes in the Argentinean Andes. Seeds were buried in Lanín National Park at three altitudes, 650, 930 and 1100 m above sea level, during late autumn of both 2012 and 2013, and germination was scored after 60, 85, 120 and 160 d of overwintering. We used the equations from Fig. 2 to estimate germination thermal range parameters and calculate $G_{(max)p}$. In this system, soil temperature showed a clear association with altitude (Fig. 4a).

We found that in the altitudinal zone where each species shows greatest abundance (650 m for *N. obliqua*, 930 m for *N. nervosa* and 1100 m for *N. pumilio*), germination of all three species temporally overlapped and was restricted to early and mid-spring (Fig. 4). By contrast, we observed large variation in the timing of germination of different species for seeds buried outside of their natural distribution zone. *Nothofagus obliqua* seeds did not germinate by late winter (85 d of overwintering), but germination at their natural elevation was nearly 100% by mid-spring (Fig. 4b: 650 m, 120 d overwintering). But at higher altitudes at this time, germination (G_{max}) of *N. obliqua* was progressively less (84 ± 1% at 930 m and 37.7 ± 12% at 1100 m, Fig. 4b). By late spring (160 d after burial), long after germination was completed at its natural elevation (Giordano *et al.*, 2009, and Fig. 4), *N. obliqua* seeds completed germination at higher altitudes (Fig. 4b)

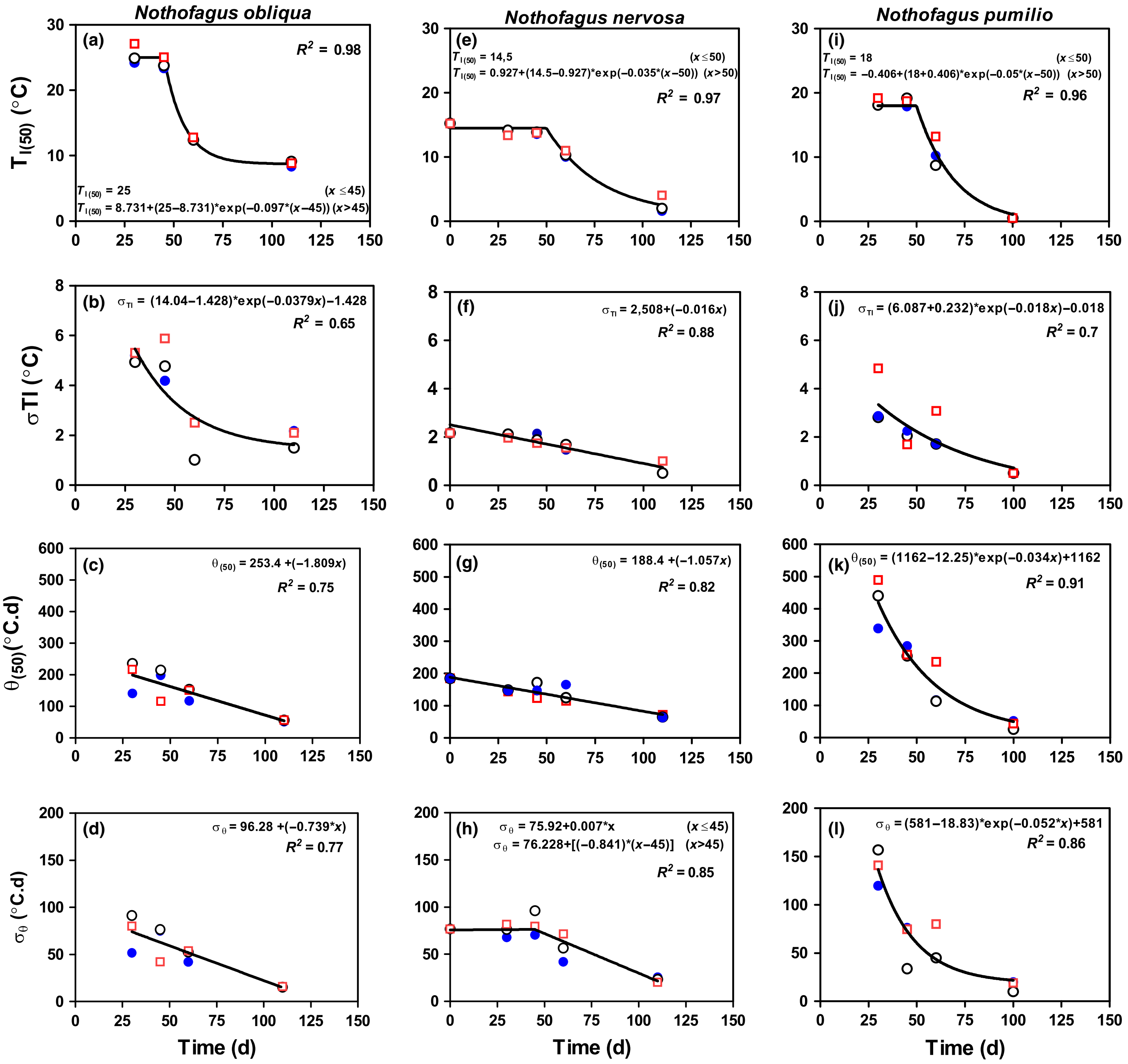

Fig. 2 Estimated values of the mean lower limit temperature ($T_{l(50)}$), standard deviation of the lower limit temperature (σ_{Tl}), mean thermal time for seed germination ($\theta_{(50)}$) and standard deviation of the thermal time (σ_{Θ}) in *Nothofagus obliqua* (a–d), *N. nervosa* (e–h) and *N. pumilio* (i–l) seeds plotted as function of stratification time. Seeds were stratified at 0.5°C (open black circles), 1.5°C (closed blue circles) or 4°C (open red squares) for the indicated time in days (d). Mathematical equations describing the relationship between the estimated thermal time parameters with time of seed stratification and R^2 are indicated inside each figure panel.

indicating a delay in germination outside its natural species range. *Nothofagus pumilio* seeds showed a different effect of altitude on germination. Overwintering for 85 d resulted in a large proportion of seeds germinating in the lower altitude zones by late winter (51 ± 6% at 650 m; 50.6 ± 15% at 930 m; 5 ± 3% at 1100 m; Fig. 4d), well before the normal time of germination in its natural distribution areas (100% germination by mid-spring at 1100 m; Fig. 4d). This result is concordant with the observed $G_{\max}$ of *N. pumilio* seeds at 650 m in late winter during 2012, after 90 d of overwintering (Fig. 3j). *Nothofagus nervosa* showed a similar temporal pattern of early germination in low-altitude habitats, although this was much less dramatic than for *N. pumilio* (Fig. 4c). Around mid-spring (120 d of seed overwintering), these two species were able to fully germinate across their natural distribution range (Fig. 4c,d). Although there was a distinction in soil hydric content between the lowest and the two highest altitude zones during late winter and spring (Fig. S4), these differences did not seem to have had an effect on timing of germination across

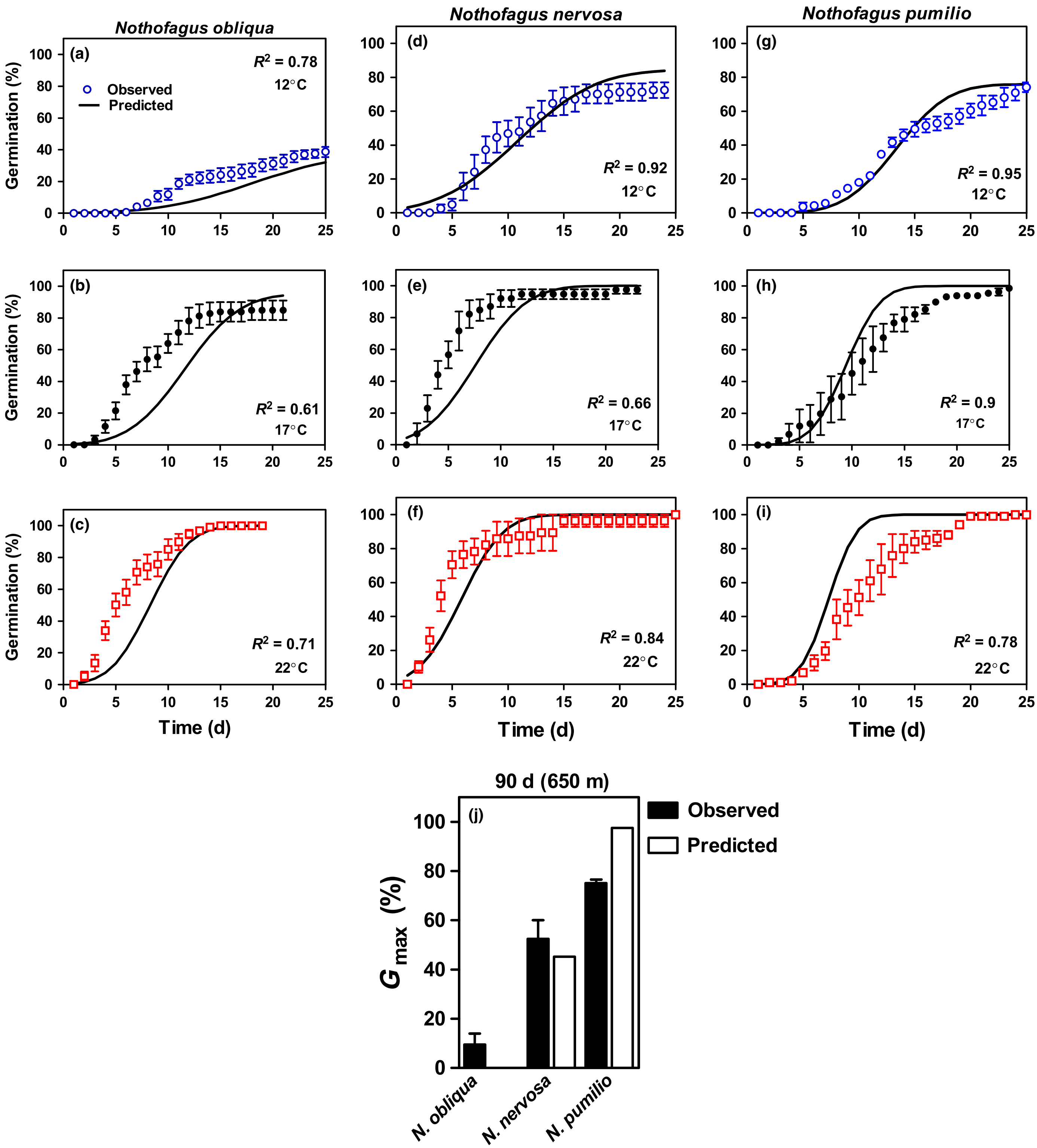

Fig. 3 Validation of thermal time models in field experiments. Predicted (solid line) and observed cumulative germination curves at 12°C (open blue circles), 17°C (closed black circles) or 22°C (open red squares) for *Nothofagus obliqua* (a–c), *N. nervosa* (d–f) and *N. pumilio* (g–i) seeds overwintered for 60 d in the forest and then germinated under controlled conditions. Seed incubation in the field started at the end of May 2012, when field temperatures were proximate to break dormancy. (j) Seed germination in mesh bags after 90 d of overwintering in the field. Predicted thermal time parameters for theoretical data calculation was performed using the equations of Fig. 2 and are listed in Supporting Information Table S6. Daily mean soil temperature at 650 m = 4.2°C. Vertical bars indicate SEM. R^2 for the observed and predicted values fitting are shown inside each panel. (j) G_{max} (%) is the final percentage of germination.

altitude because the generated dormancy-loss models based only on responses to temperature (Fig. 2) were sufficient to predict accurately the seed behavior of the three species across the altitudinal range (open bars, Fig. 4b–d).

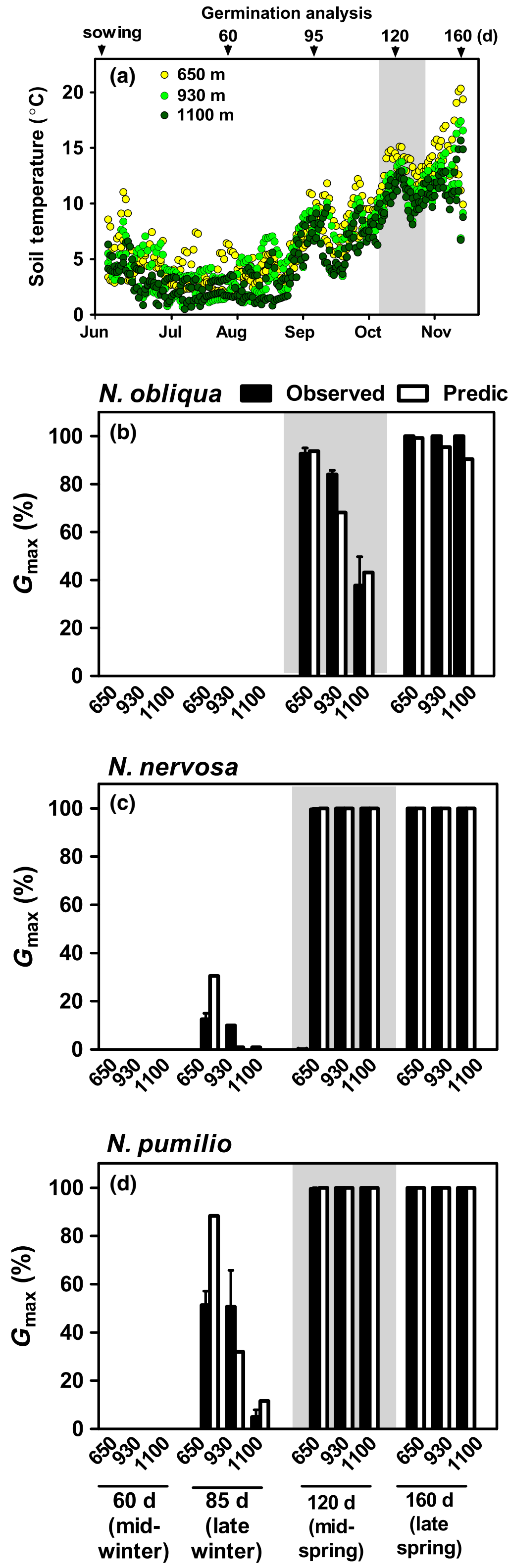

Fig. 4 Evaluation of germination behavior of *Nothofagus* seeds across altitudes. (a) Daily mean soil temperature at different altitudes during the period of seed overwintering and germination in the forest (autumn–spring). Data correspond to the daily values for 2012 and 2013 seasons. Observed (closed bars) and predicted (open bars) germination of *N. obliqua* (b), *N. nervosa* (c) and *N. pumilio* (d) seeds sown for 60 (mid-winter), 85 (late-winter), 120 (mid-spring) or 160 d (late spring) across the altitudinal gradient is also indicated. Altitude (in m above sea level), days of seed incubation in the field, and the season of seed exhumation are indicated below (d). Predicted thermal time parameters for theoretical data calculation are listed in Supporting Information Table S6. Mean soil temperature across the altitudinal gradient for the last 35 d before germination counting: 60 d, 650 m = 2.74°C, 930 m = 3.9°C, 1100 m = 2.7°C; 85 d, 650 m = 4.27°C, 930 m = 2.2°C, 1100 m = 1.6°C; 120 d, 650 m = 11.13°C, 930 m = 9.48°C, 1100 m = 8.47°C; 160 d, 650 m = 12.47°C, 930 m = 11.39°C, 1100 m = 10.77°C. Vertical bars indicate the SEM. (b–d) G_max (%) is the final percentage of germination.

Discussion

Our studies demonstrate different patterns of seed responsiveness to temperature in three *Nothofagus* species which are linked to the thermic characteristics of their favored ecological niches. Additionally, we provide direct field evidence indicating that this trait influences seasonal timing of germination, likely contributing to the dynamics of early forest regeneration processes across an elevational gradient in the Patagonian Andes. Taken together, our results provide ecologically relevant data in associating functional traits with species distribution, and offer evidence in favor of the hypothesis that thermic responses at early stages of development contribute, at least partially, to the maintenance of species distribution patterns across elevation, by placing the germinated seed in a favorable ecological context (Fig. 5).

It is challenging to evaluate the effects of temperature on seed germination behavior in a species-specific way, because temperature regulates both the level of dormancy and the rate of germination of nondormant seeds (Batlla & Benech-Arnold, 2015). The use of dormancy population parameters such as T_l and T_h (Washitani, 1987; Grundy *et al.*, 2000) in combination with thermal-time models for the estimation of θ_1 of the fraction of nondormant seeds (Batlla & Benech-Arnold, 2015), provided a flexible approach to clearly disentangle the effects of temperature on seed dormancy from those on germination. Moreover, population threshold models provide an extremely flexible framework for predicting the timing of developmental transitions in complex environments (Donohue *et al.*, 2015). Through this approach we demonstrated that the *Nothofagus* species showed marked differences in their response to stratification: similar temperatures and periods of stratification yielded large interspecific differences in the range of temperatures allowing germination of the populations, indicated by the differences in $T_{l(50)}$. For example, in response to the same stratification treatment, *N. obliqua*, which is distributed in warm zones of the altitudinal gradient showed, on average, higher $T_{l(50)}$ than *N. nervosa* and *N. pumilio* (Table 1). Moreover, once dormancy was lost, species that are more abundant at higher and colder regions of the gradient showed lower T_b and $T_{l(50)}$ (i.e. a higher capacity to germinate at lower temperatures than those inhabiting the warmer, lower zones) (Table 1;

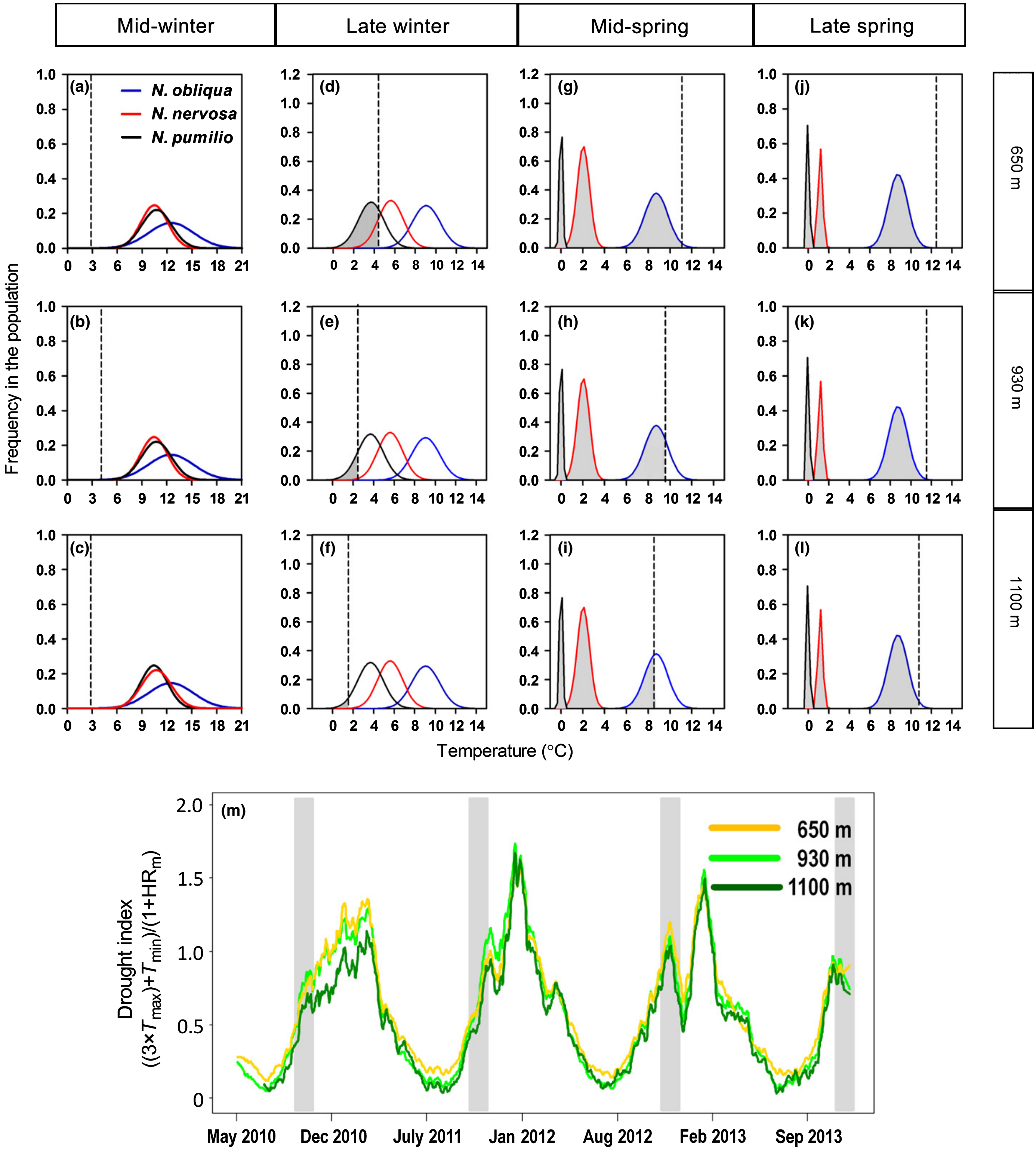

Fig. 5 Effects of the lower limit temperature of germination (T_l) and soil temperature in the seasonal timing of germination of *Nothofagus obliqua*, *N. nervosa* and *N. pumilio* across the altitudinal range. (a–l) Shaded areas indicate the population fraction predicted to germinate based on average soil temperature (interception of *x* axes by the vertical dotted line). In this model, alleviation of seed dormancy starts during late autumn, when soil temperatures are *c*. 4°C (Fig. 4a), and dormancy alleviation for each species is dependent on time of seed after-ripening in the forest, occurring equally across the whole altitudinal gradient. Germination of a certain fraction of the seed population occurs when the average value of soil temperature is above the value of T_l for that fraction. Predicted thermal time parameters and mean soil temperature across the altitudinal gradient are the same as in Fig. 4. (m) The drought index calculated as $((3 \times T_{max}) + T_{min})/(1 + HR_m)$, where T_{max} is the daily maximum temperature, T_{min} is the daily minimum temperature and HR_m is the daily average air humidity. (m) Shaded areas indicate the period of seed germination in the natural range of species distribution.

Fig. 1), suggesting a link between physiological functional traits and elevation. The relationships between germination temperature and altitude predicted by our models are largely in concordance with some (Mariko *et al.*, 1993; Cochrane *et al.*, 2011), although not all (Linington, 1979; Cavieres & Arroyo, 2000; Rosbakh & Poschlod, 2015), previous data based on laboratory assessments. Studies that, in contrast to ours, report a negative relationship between germination temperature and altitude, propose that relatively high germination temperatures are required in colder environments to prevent seedling loss in high-elevation habitats, because germination would be triggered solely by warm soil temperatures in late spring or early summer to reduce the risk of seedling frost (Billings & Mooney, 1968). Temperate forests of the Patagonian Andes have a Mediterranean climate characterized by warm and dry summers with a wet season that includes autumn, winter and mid-spring (Kottek *et al.*, 2006; Peel *et al.*, 2007; Fig. 5). Germination close to summer, as proposed in such studies, would be unfavorable for seedling establishment because of limited water availability. We observed that deeply dormant *N. pumilio* seeds showed both high values of $T_{l(50)}$ and $\theta_{(50)}$ (Table 1) – in other words, dormant seeds are able to germinate only in relatively warm temperatures and need the accumulation of relatively high amounts of thermal units for germination. These characteristics might prevent early germination at higher altitudes. For example, if winter temperatures raise transiently, the combined requirement of warm temperatures with high amounts of thermal units would prevent germination during unusual warm periods in winter, and ensure seedling emergence during spring. Hence, relationships between seed responsiveness to temperature and altitude should include the effect of temperature not only on germination but also on the rate of dormancy alleviation. They also should not be generalized for worldwide forest environments, but restricted to seasonal characteristics of local ecosystems.

We demonstrated that in their natural distribution range, germination of the three *Nothofagus* species occurred at the same time during early and mid-spring (Figs 4, 5). This timing of germination was a consequence of intraspecific differences in the rate of dormancy loss, expressed as changes of $T_{l(50)}$ with the period of stratification, in combination with the existence of natural thermic gradients across altitudes. Given that *Nothofagus* spp. do not form a persistent seed bank in natural conditions (Cuevas & Arroyo, 1999), our models provide a mechanistic framework for the prediction of seed behavior in the forest from dispersion to germination. Another relevant characteristic of *Nothofagus* spp. is their seed-masting habit (Richardson *et al.*, 2005) which represents a concentrated sporadic reproductive effort. In addition, the genus comprises relatively small-seeded species, with limited seed storage resources to help germinants survive in harsh environments. Taken together, the strict control of the timing of germination by environmental cues is a key process in order to take advantage of the brief window of opportunity between cold winters and dry summers, determining plant recruitment.

A focal point of our theoretical and experimental data is that G_{max} is highly susceptible to soil thermal changes (Figs 4, 5). Dense tree canopies lower ground layer temperatures (Chen *et al.*, 1999; Norris *et al.*, 2012; von Arx *et al.*, 2013). Thus, canopy and understory heterogeneity (Giordano *et al.*, 2009; Caccia *et al.*, 2015) might create soil thermic microhabitats that likely generate local intraspecific variations in G_{max}. We chose homogeneous forest habitats for field experiments, representative of old-growth stands and characterized by a canopy and understory cover that yields R : FR ratios ranging from 0.5 to 0.7 at the soil surface. Therefore, our predictions of seed behavior in natural conditions are restricted to the most representative natural environments. In this context, we show that the three *Nothofagus* species are able to germinate across the entire altitudinal gradient, but it is the timing of germination of each species that was strongly affected outside their natural elevation range. This is illustrated by the fact that in low-altitude environments, germination of a large proportion of *N. pumilio* seeds occurred in late winter, a period with high probability for seedling frost, whereas germination of *N. obliqua* was strongly delayed at high altitudes, and only completed close to the extreme summer period of drought (Figs 4, 5). This suggests that the species-specific responses to temperature reported here affect the timing of germination, probably contributing to fitness and therefore maintaining patterns of species distribution across the elevational gradient. The absence of adult individuals of *N. pumilio* and *N obliqua* in the low- and high-altitude zones, respectively, are in accordance with this last observation. Moreover, in the lower altitude habitats, occasionally it is possible to find young *N. pumilio* seedlings next to stream margins as a result of seed transport by water from the higher altitude habitats. However, these seedlings do not survive the first growth season. The fact that germination behavior changed when species were placed in an environment different from their natural one in the reciprocal sowings in the altitudinal gradient (Fig. 4) provides evidence for adaptation in seed behavior of *Nothofagus* spp. across altitudes. Our data also suggest that seed behavior is under mortality selection (Donohue *et al.*, 2010). In this context, it is possible that niche determines germination characters; population fractions that show extreme germination behavior would be negatively selected across generations because they would germinate out of the favorable period for growth. However, more research is needed to test this hypothesis.

Other factors that are likely to contribute towards forest regeneration dynamics, but which we have not investigated, include interactions with different organisms (Caccia *et al.*, 2006; Garibaldi *et al.*, 2011; Kempel *et al.*, 2013), heterogeneity of forest understory (Giordano *et al.*, 2009; Caccia *et al.*, 2015) and natural gradients in UV radiation (Caldwell, 1968; Körner, 2007). Their relevance might vary according to the climate characteristics of germination and growth seasons, and to the properties of forest microhabitats. Moreover, during sporadic masting of bamboo (*Chusquea culeou*), predation of *N. obliqua* but not of *N. nervosa* seeds decreased in bamboo flowered areas (Kitzberger *et al.*, 2007). This phenomenon might influence the final proportion of *N. obliqua* and *N. nervosa* germinating seeds, and therefore trigger differential species recruitment. Hence, there is not a simple framework for assessing the net contribution of seed behavior to species′ abundance in natural environments. Despite that, our model successfully predicts the dynamics of early stages of forest regeneration, and stresses the contribution of seed physiology to temporal patterns of germination. This is likely to be an important component in plant recruitment and, ultimately, the distribution of species across altitudes.

Plant phenology has been proposed to be highly sensitive to global climate change (CaraDonna *et al.*, 2014). The strong effect of changes in soil temperature across altitudes in natural environments on *Nothofagus* $G_{\max}$ provides evidence in support of this proposal. In addition, this phenomenon has important implications in the context of future climate scenarios because it indicates that that predicted shifts in temperature for the Patagonian Andes (Rusticucci & Barrucand, 2004) will strongly affect the timing of germination of *Nothofagus* spp. in their present distribution areas. Timing of germination has been demonstrated to influence fitness in the crucifer *Arabidopsis thaliana* (Donohue, 2002). The wide range of germination temperatures shown by *Nothofagus* seeds during dormancy alleviation suggests that germination behavior could adapt quickly to climate change. This may have strong implications for predicting future effects of global shift on plant distribution. Data reported here should be incorporated into programs studying species vulnerability to climate change (Pacifici *et al.*, 2015) and in prioritizing action for conservation planning of the Gondwana flora.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Altitudinal relationships between air daily mean temperatures.

Fig. S2 Cumulative germination curves of *N. obliqua, N. nervosa* and *N. pumilio* at different germination temperatures.

Fig. S3 Validation of thermal time models in the laboratory.

Fig. S4 Daily mean soil hydric content across elevation during the period of seed overwintering and germination in the forest (autumn–spring).

Table S1 Populations sampled in the study

Table S2 Final germination percentages of *N. obliqua*, *N. nervosa* and *N. pumilio* seeds freshly harvested or stored at 2°C

Table S3 Linear regressions between GR and temperature

Table S4 Linear regressions between GR and temperature

Table S5 Effect of stratification temperature on the germination of *Nothofagus* seeds

Table S6 Predicted dormancy and germination related parameters for *N. obliqua*, *N. nervosa* and *N. pumilio* seeds after 60, 85, 90, 120 and 160 d of stratification