

# Looking at the forest from below: the role of seedling root traits in the adaptation to climate change of two *Nothofagus* species in Argentina

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**Abstract** Global climate change (CC) is an evolutionary challenge for natural tree populations. Scientific experts forecast an increase in temperature and a decrease in precipitation in the Argentine Patagonian Cordillera. Knowledge of the variation of adaptive traits at the seedling stage is crucial to prediction the adaptability of forest tree species. We aim to analyze genetic variation in root and shoot quantitative traits among and within natural populations of two key North Patagonian forest species, in order to evaluate their ability to deal with CC through adaptation. We established two common garden nursery trials: (1) 81 open pollinated families of *Nothofagus obliqua* from eight natural populations, and (2) 74 families of *Nothofagus alpina* from seven populations. At the end of the first growing season we measured shoot height, diameter, root length and root and stem dry biomass; we also estimated foliage biomass. In addition the root to stem ratio, specific root length and the root to shoot ratio were calculated. For both species intra-population variation was generally low, and average differentiation was moderate, with high levels of differentiation in some traits related to drought stress, indicating low adaptation capacity but also adaptation to current stressful conditions, which means it may be possible for some populations to survive CC without changing their genetic structures. Further studies are needed, including phenotypic plasticity assays, to complete the picture.

**Keywords** Allometry · Drought resistance · Population genetics

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## Introduction

Global climate change (CC) is an evolutionary challenge for natural populations, particularly for long-lived species such as trees. In 2014 the IPCC reported a gradual rise in global temperature over recent decades, and confirmed an increase in this trend, predicting inevitable changes in global natural and economic systems. Regarding Argentina, a local panel of experts has recently reported an increase of 0.4° C in the average annual temperature of Patagonia for the period 1960–2010, and a marked decrease in average precipitation, particularly in the Andes Cordillera sector (Centro Inv. Mar & Atm 2015). This report also forecasts for the remainder of the century, and depending on the chosen CC scenario, a continuous temperature increase at least of similar magnitude as the observed in the last 60 years, and a significant decrease in precipitation in the Cordillera, from 10 to 30% of current annual averages.

In the long term, and in the absence of mitigating measures, CC is likely to overwhelm the in situ adaptive capacity of ecosystems, not due to changes in climate means, but rather due to a greater recurrence and intensity of extreme events such as droughts, heat waves, off-season frosts, torrential rains, or the effects of these physical changes, such as the occurrence of catastrophic fires or pest outbreaks. Cases of massive mortality in forest ecosystems produced by extreme drought and heat waves have already been reported in North Patagonia (Bran et al. 2001) and worldwide (Allen et al. 2010).

In the temperate forests of Patagonia two species of the emblematic tree genus *Nothofagus* stand out because of their potential for timber production: *Nothofagus alpina* (Poepp. & Endl.) Oerst. and *Nothofagus obliqua* (Mirb.) Oerst. (Tortorelli 1956). Both these species are involved in an ongoing domestication program in Argentina, conducted by the *Instituto Nacional de Tecnología Agropecuaria* (INTA). They are also key species in their ecosystems, which are mostly found in Chile but also have considerable presence on the eastern side of the Andes Cordillera. In Argentina *N. alpina* occurs from 39°20'S to 40°35'S, lying mainly within Lanín National Park, while *N. obliqua* has a wider latitudinal distribution, from 36°50'S to 40°10'S. Sympatric populations occur at the latitude of Lácar Lake; *N. obliqua* dominates from 600 to 750 m a.s.l., and *N. alpina* from 950 to 1150 m a.s.l., where *Nothofagus pumilio* (Poepp. & Endl.) Krasser appears and begins to climb forming pure stands and finally the tree line (Sabatier et al. 2011). The natural range of *N. obliqua* extends further to the east than *N. alpina*, and natural populations are found in less humid sites (Sabatier et al. 2011). Accordingly, *N. obliqua* seedlings have lower vulnerability to embolism than *N. alpina* ones (Varela et al. 2010).

The predicted CC will very probably affect the natural distribution of forest tree species in North Patagonia. This topic has been specifically addressed for *N. alpina* and *N. obliqua* by means of ecological niche modelling (Marchelli et al. 2017). According to this study, the extinction of a large number of populations of *N. alpina* is likely to occur in Argentinian sub-Antarctic forests in the next 35 years. At the same time, *N. obliqua* is likely to spread gradually replacing the current mixed forests with pure stands and colonizing sites where the species is not present today.

Considering the embolism tolerance ability of both species (Varela et al. 2010), the replacement of *N. alpina* by *N. obliqua* in the sympatric forests and in adjacent pure ones is a probable consequence of climate change. Local extinction of *N. alpina* due to drought stress would likely free *N. obliqua* from competition pressure in sites still suitable for the latter species. Evidence that this process is currently occurring has been recently reported (Sola et al. 2015). An evaluation of natural regeneration in a sympatric forest logged in

1993 showed that 20 years later the proportion of established seedlings experienced a sharp increase in *N. obliqua* and decrease in *N. alpina*, even though *N. alpina* had prevailed among the remnants.

The seedling and sapling phases of plant development are the most important and acute selective stages in the life span of a tree species (Seiwa and Kikuzawa 1991; Moles and Westoby 2004; Grossnickle 2005, 2012). Knowledge of selection drivers at these stages is crucial to our understanding of the natural processes of extinction or persistence, and also to the management of afforestation for restoration (or even commercial) purposes. Several morphological attributes such as the proportion of root to shoot mass and root depth may drive drought resistance of plants (Padilla and Pugnaire 2007; Grossnickle 2012; Matías et al. 2014; Ledo et al. 2018). In this regard, seedlings of populations adapted to drought stress are expected to present longer roots but less development of aboveground biomass (Cregg 1994; Paz 2003; Climent et al. 2004; Markesteijn and Poorter 2009); that is a greater ability to absorb water and a smaller transpiration surface area.

Natural populations can survive CC locally by means of adaptation (changes in their genetic frequencies) or phenotypic plasticity (Aitken et al. 2008), two different strategies that occur simultaneously, with unknown prevalence. The ability to adapt (adaptability) of a certain species can be assessed by estimating the additive genetic variance (Houle 1992) of their populations in adaptive meaningful traits.

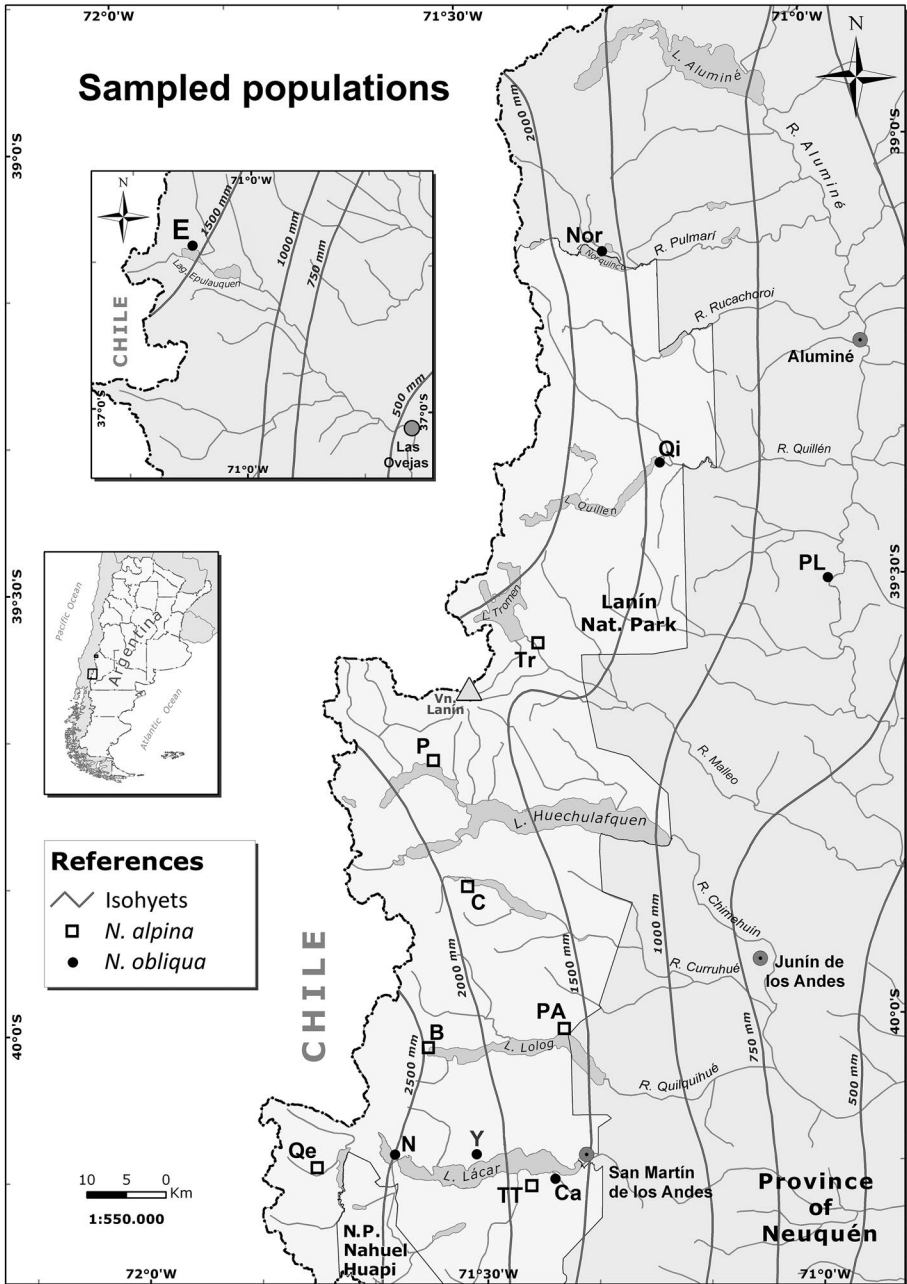
Taking into account the severe future scenario foreseen for Andean North Patagonia, we ask whether the next generation of natural populations of *N. alpina* and *N. obliqua* will be able to become established and survive. We aim to analyze genetic variation among and within natural populations of these two important *Nothofagus* species in root/shoot quantitative traits presumably involved in drought resistance, through a nursery common garden trial, in order to evaluate their ability to deal with CC through adaptation. Bearing in mind the evidence observed in sympatric forests, we expect *N. alpina* to have a lower level of intra-population genetic variation compared to *N. obliqua*. Similarly, given the wider amplitude of the ecological gradients occupied by *N. obliqua*, we expect greater inter-population genetic variation of this species in comparison to *N. alpina*.

## Materials and methods

### Plant material and measurement of variables

The intra and inter-population variation of *N. obliqua* and *N. alpina* was analyzed based on morphological root and shoot traits of seedlings at the end of their first year of growth, in two nursery common garden trials. Although planned, the high annual variation in seed production of these species (Marchelli and Gallo 1999) made it impossible to sample both of them during the same year. Unfortunately, *N. alpina* seed production was almost nil the year we collected *N. obliqua* seeds, and vice versa. Hence, the trials had to be performed separately for each species, and consequently comparisons cannot be made before making conclusions for each species independently.

In 2008 seeds of eight natural populations of *N. obliqua* (Fig. 1) were collected directly from the crowns of open-pollinated trees selected at random; a distance of at least 30 m was maintained between trees to minimize the probability of sampling related individuals. Populations were selected so as to represent the complete Argentinean range of the species. The same procedure and criteria were applied in 2009 for the collection of *N. alpina* seeds



**Fig. 1** Map of sampled populations. Empty squares represent *N. alpina* populations (Tr Tromen, P Paimún, C Curruhué, PA Puerto Arturo, B Boquete, Qe Queñi, TT Tren Tren) and full circles represent *N. obliqua* populations (E Epulauquen, Nor Noquínco, Qi Quillén, PL Pilo Lil, N Nonthué, Y Yuco, Ca Catritre)

**Table 1** Location, mean annual precipitation (MAP), mean annual UNEP aridity index (AI<sub>U</sub>), January and July mean temperatures (T °C), number of families sampled and mean weight of 100 filled seeds of the *N. alpina* and *N. obliqua* natural populations included in the common garden trials

Population	Latitude (S)	Longitude (W)	Altitude (m a.s.l.)	MAP (mm year <sup>-1</sup> )	AI <sub>U</sub>	T °C (Jan.)	T °C (July)	No. of families	100 SW (g)
<i>Nothofagus alpina</i>									
B	40°01'50"	71°34'38"	910	1600	2.88	13.07	1.37	10	1.37
C	39°50'34"	71°30'30"	1030	1400	2.45	13.16	0.89	11	1.44
P	39°42'17"	71°33'52"	930	1800	3.35	12.07	0.93	10	1.46
PA	40°01'02"	71°22'19"	910	1200	2.06	14.57	1.93	12	1.31
Qe	40°09'54"	71°45'20"	920	2400	5.03	9.67	-0.03	10	1.36
Tr	39°36'18"	71°20'57"	1110	1300	2.35	13.52	1.01	9	1.48
TT	40°11'53"	71°25'53"	1040	1400	2.36	13.81	1.61	12	1.40
Total								74	
<i>Nothofagus obliqua</i>									
CaA	40°11'01"	71°23'50"	850	1250	2.24	14.31	1.98	11	0.93
CaB	40°10'26"	71°24'10"	650	1250	2.10	15.25	2.90	12	0.83
E	36°49'14"	71°03'29"	1500	1800	3.50	12.14	0.07	12	2.68
N	40°08'46"	71°37'03"	780	1850	3.29	13.30	2.09	10	1.03
Nor	39°09'11"	71°15'38"	1100	1250	2.17	13.82	1.20	5	0.94
PL	39°31'03"	70°57'33"	900	700	1.09	17.28	3.42	12	0.90
Qi	39°21'27"	71°13'12"	1050	1150	1.97	14.37	1.55	7	1.13
Y	40°09'07"	71°30'39"	700	1500	2.69	13.70	1.75	12	0.91
Total								81	

from seven natural populations (Fig. 1). An average of more than 10 trees was sampled in each population (Table 1). Both collections were carried out in “full mast” years; thus, according to Marchelli and Gallo (1999) a good genetic representation of the sampled populations is expected. Each population was environmentally characterized by several features presented in Table 1 (climatic variables were taken from Bianchi and Cravero 2010). The mean weight of 100 filled seeds is also reported for each population. In order to estimate this parameter we sampled four repetitions of 100 seeds per family, in all the families of each population, and these were subsequently fresh weighed with 0.01 g accuracy.

Seedlings of both species were produced during the same years as seeds were collected (seeding date: 9.15.2008 and 9.28.2009 for *N. obliqua* and *N. alpina* respectively). Standard protocols were followed (Azpilueta et al. 2010), using a germination tray for each family (progeny of a single open-pollinated tree). Once an appropriate size had been reached (about 1 month after emergence) the seedlings were transplanted to 25-liter cylindrical pots (28 cm diameter, 40 cm depth) filled with pre-sifted loam soil, placing four seedlings in each pot (transplanting date: 10.20.2008 and 11.27.2009 for *N. obliqua* and *N. alpina* respectively). The potted plants were placed outdoors, with moderate sun protection (50% shade cloth transmittance) and watered by a sprinkler system two or three times a week during the growing season. Substrate fertility was supplemented with two applications of NPK fertilizer at the beginning of the growing season (0.56 g/l of NEW PLANT 18-7-17™; N=101 ppm, P=18 ppm, K=80 ppm). Micronutrients were also applied twice, through foliar fertilization.

The *N. obliqua* trial consisted of eight natural populations (two sites of contrasting altitude were sampled in the Catritre location, so we considered them as different populations), with 5–12 families per population (81 families in total) and eight seedlings per family, which resulted in a total of 648 seedlings. In the case of *N. alpina*, seven natural populations were used with 9–12 families per population (74 families in total) and eight seedlings per family, resulting in a total of 592 seedlings. The pots were first arranged in four columns, and the seedlings were then transplanted. Both trials were divided into eight blocks, following a presumed wind gradient, and within each block and pot the seedlings were randomly distributed (single-tree plot). The trial was surrounded by one line of pots to minimize edge effects. Both trials were measured on completion of the first growing season, during winter dormancy. Plants were carefully removed from the pots and roots were washed with abundant running water (date of plant harvesting: 7.31.2009 and 6.30.2010 for *N. obliqua* and *N. alpina* respectively).

For each plant we measured shoot height (SH; with 0.1 cm accuracy), root collar diameter (D; measured with a caliber, 0.01 mm accuracy) and length of longest root (RL; 0.1 cm accuracy). We then obtained the root (Rdb) and stem (Sdb) dry biomass by drying the plants for 48 h at 72° C then weighing each component separately, to 0.0001 g accuracy. The ratio between root and stem dry biomass ( $R/S = Rdb/Sdb$ ) and specific root length ( $SRL = RL/Rdb$ , in [m/g]) were subsequently calculated.

Since artificial defoliation causes changes in carbon allocation (Reichenbacher et al. 1996; Osier and Lindroth 2004; Wiley et al. 2017), we preferred not to remove the leaves before measuring biomass, but rather to let the natural process be completed. We sacrificed this leaf trait so as to have more confidence in the stem and root traits. We estimated the foliage biomass of each seedling by means of a regression equation developed for *Nothofagus pumilio* (a closely related species) seedlings and saplings, based on height and diameter data (Schmidt et al. 2009). We first tested the published equations correlating predicted and measured values of Rdb and Sdb for both species. Since these correlations were significant and high (*N. obliqua* Rdb:  $r_{pred.meas} = 0.86$ ; *N. obliqua* Sdb:  $r_{pred.meas} = 0.89$ ; *N. alpina*

Rdb:  $r_{\text{pred.meas}}=0.85$ ; *N. alpina* Sdb:  $r_{\text{pred.meas}}=0.89$ ), we utilized the following regression equation to estimate foliage biomass

$$Fdb = 0.0010272 \times D^{1.762} \times SH^{0.341}.$$

We then calculated the total aboveground biomass (A) of each seedling by adding the measured Sdb and the estimated Fdb, and finally we calculated the ratio between Rdb and A (R/A).

## Statistical analysis

In order to visualize the relationships between the traits considered, and their ability to discriminate between the populations, we performed a Principal Component Analysis (PCA) for each species with the ‘FactoMineR’ package in R 3.3.0 (Husson et al. 2017), with all traits taken as active variables and population as a supplementary variable.

In order to test differences between populations and variability among families, ANOVA for each of the traits considered was performed under the following nested mixed model:

$$y_{ijk} = \mu + \rho_i + \beta_j + \varphi_k(\rho_i) + e_{ijk}$$

where  $y_{ijk}$  is the value of the variable measured on the seedling from the  $k$ th family nested in the  $i$ th population located in the  $j$ th block;  $\mu$  is the general mean of the variable,  $\rho_i$  is the effect (fixed) of the  $i$ th population;  $\beta_j$  is the effect (random) of the  $j$ th block;  $\varphi_k(\rho_i)$  is the effect (random) of the  $k$ th family within the  $i$ th population, and  $e_{ijk}$  is the residual error  $\sim \text{NID}(0, \sigma^2)$ .

ANOVA tests were performed with the ‘lmer’ function of the ‘lme4’ package (Bates et al. 2015) in R 3.3.0., with restricted maximum likelihood (REML) estimation. For post hoc population mean comparisons, we applied simultaneous multiple Tukey contrasts with the ‘glht’ function in the ‘multcomp’ package version 1.4-5 (Hothorn et al. 2008) in R 3.3.0. The normality of the data was assessed using a histogram and qq plot for each variable. Homoscedasticity was checked by a graph of residuals versus predicted values, and in the cases of heteroscedasticity we carried out a natural log transformation (nl).

To evaluate the contribution of random effects to the model, we performed likelihood ratio tests (LRTs) for the null hypothesis that the simpler model (fitted after removing the random effect of interest from the complete model) is not different from the complete model.

In order to analyze geographical patterns of variation, we also performed correlation tests between population means of the traits considered and their main environmental features (latitude, altitude, mean annual precipitation, mean January temperature, mean July temperature and mean annual UNEP aridity index). In order to account for possible maternal effects linked to seed size, we also estimated correlations of the traits with the weight of 100 filled seeds.

## Genetic parameters

Additive genetic variance is the basic parameter for estimating quantitative genetic variation within a population, and is a function of the family variance estimated experimentally. The multiplier factor of family variance in the formula of additive variance depends on the degree of kinship between members of families used, which is 2 for full-sibs and 4 for half-sibs. Open pollinated families are usually considered as half-sibs, although they are really a mixture of half and full-sibs in unknown proportions. In a previous study (Pastorino et al. 2013)

we estimated the kinship coefficient ( $k$ ) of some Argentine populations of *N. alpina* and *N. obliqua*. Since they were not the same populations as in the present study we took those estimations as a reference, using a mean value, thus obtaining the result:  $k_{alpina} = 0.30080$  and  $k_{obliqua} = 0.27393$ . Then, the additive genetic variance was estimated as:

$$\sigma_A^2 = \frac{1}{k} \cdot \sigma_f^2$$

where  $\sigma_A^2$  is the additive genetic variance and  $\sigma_f^2$  is the family variance.

Since the value of  $\sigma_A^2$  is a function of the units used, the additive genetic coefficient of variation after Houle (1992) was calculated in order to make direct comparison possible between different variables:

$$CV_A = 100 \cdot \frac{\sqrt{\sigma_A^2}}{X}$$

where  $X$  is the variable mean.

Another useful parameter used to estimate intra-population variation is narrow-sense heritability ( $h^2$ ), which was consequently estimated for each population and variable through the components of variance analysis, and according to the equation:

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2} = \frac{\sigma_A^2}{(\sigma_f^2 + \sigma_b^2 + \sigma_e^2)}$$

where  $\sigma_P^2$  is the phenotypic variance,  $\sigma_b^2$  is the variance of the block, and  $\sigma_e^2$  is the error variance.

The standard error (SE) of the heritability was calculated using the formula:

$$SE_{h^2} = 1/k \cdot \sqrt{\frac{2 \cdot [1 + (n-1) \cdot h^2/1/k]^2 \cdot (1 - h^2/1/k)^2}{n \cdot (n-1) \cdot (f-1)}}$$

where  $f$  is the number of families and  $n$  is the number of individuals within each family (Falconer and Mackay 1996).

For each species we calculated the phenotypic correlation (Pearson's correlation coefficient:  $r_p$ ) and additive genetic correlation ( $r_A$ ) between all the possible combinations of the traits for all individuals of each species as a pool. For these parameters we preferred to pool the data of the individuals of all populations from each species to better fit the correlations since we intend to characterize the relationship between the traits instead of the populations. We utilized the following formulae:

$$r_p = \frac{Cov_{xy}}{\sqrt{\sigma_x^2 * \sigma_y^2}} \text{ and } r_A = \frac{Cov_{fxy}}{\sqrt{\sigma_{fx}^2 * \sigma_{fy}^2}}$$

where  $Cov_{xy}$  is the covariance between variables  $x$  and  $y$ , and  $\sigma_x^2$  and  $\sigma_y^2$  are the variances of the variables (Falconer and Mackay 1996), while  $\sigma_{fx}^2$  and  $\sigma_{fy}^2$  are the family variances of the



variables and  $Cov_{f_{xy}}$  is the family covariance estimated according to O'Neill et al. (2001) as:

$$Cov_{f_{xy}} = \frac{\sigma_{f_{xy}}^2 - \sigma_{f_x}^2 - \sigma_{f_y}^2}{2}$$

where  $\sigma_{f_{xy}}^2$  is the family variance of a new variable resulting from the sum of both variables. Standard errors of both correlations were calculated by:

$$SE_{r_p} = \sqrt{\frac{1 - r_p^2}{N - 2}} \text{ and } SE_{r_A} = \sqrt{\frac{(1 - r_A^2)^2}{f - 1}}$$

where  $N$  is the total number of individuals sampled and  $f$  is the number of families (Visscher 1998).

Partitioning the phenotypic variance of each variable into its components, and particularly distinguishing components of variance among and within populations, allows us to calculate differentiation among populations in quantitative traits,  $Q_{ST}$  (Spitze 1993):

$$Q_{ST} = \frac{\sigma_{pop}^2}{(\sigma_{pop}^2 + 2 \cdot \sigma_A^2)}$$

where  $\sigma_{pop}^2$  is the variance among populations estimated with the described statistical model, but considering population as a random effect.

## Results

### Variation among- and within-populations of *N. alpina*

In the *N. alpina* trial, the overall mean for shoot height (SH) was 5.4 cm, Tromen and Queñi being the populations with the shortest seedlings (4.9 cm on average) and Paimún the population with the tallest ones (6.3 cm on average). The overall mean for root collar diameter (D) was 2.04 mm, Tromen having the thinnest seedlings (1.65 mm) and Paimún the thickest ones (2.48 mm). The mean length of the longest root (RL) was 29.8 cm, which is more than five times the mean shoot height. Stem dry biomass (Sdb) and root dry biomass (Rdb) varied greatly between populations, with the highest population means having twice the value of the lowest ones, and again: Tromen was the population with the lowest values and Paimún the population with the largest ones. However, the ratio between root and stem dry biomass (R/S) was similar in all populations, with an overall mean of 3.77. Including foliage dry biomass, the ratio between the belowground and the aboveground biomass (R/A) reached a mean value of 1.62; that is, about half the average R/S. Finally, specific root length (SRL) had a mean value across populations of 1.24 m/g, with the highest population mean in Tromen (1.61 m/g), and the lowest in Paimún (0.84 m/g) (see Table 2).

In PCA, axis 1 accounted for 55.15% of the overall variation and was related to all variables except R/S and R/A, with a negative relationship between SRL and the other variables measured. Axis 2 explained 23.21% of the variance, and was associated with R/S and R/A. Populations, as supplementary variables, were separated on both axes,

**Table 2** Mean and its standard deviation (sd), heritability ( $h^2$ ) and its standard error (SE), additive genetic variance ( $\sigma^2_A$ ) and additive genetic coefficient of variation (CVA) of all traits considered in each *N. alpina* natural population included in the common garden trial

	B	C	P	PA	Qe	Tr	TT	Mean	Q <sub>ST</sub>
<i>Nothofagus alpina</i>									
SH (cm)									
Mean (sd)	5.7 (1.5)	5.0 (0.9)	6.3 (1.4)	5.6 (1.0)	4.9 (1.0)	4.9 (0.8)	5.5 (1.0)	5.4 (1.2)	0.47
$h^2$ (SE)	0 (0.02)	0.65 (0.25)	0 (0.02)	0.41 (0.17)	0.24 (0.12)	0.56 (0.21)	0 (0.01)	0.27 (0.12)	
$\sigma^2_A$	0	0.6	0	0.4	0.3	0.4	0	0.2	
CV <sub>A</sub>	0	15.03	0	11.29	10.62	12.81	0	7.11	
Tukey	cd	ab	d	c	a	ab	bc		
D (mm)									
Mean (sd)	2.21 (0.48)	2.00 (0.45)	2.48 (0.57)	1.91 (0.44)	1.94 (0.43)	1.65 (0.40)	2.05 (0.44)	2.04 (0.51)	0.35
$h^2$ (SE)	0 (0.02)	0.57 (0.22)	0.53 (0.24)	0.03 (0.03)	0.53 (0.22)	0.17 (0.08)	0.02 (0.02)	0.26 (0.12)	
$\sigma^2_A$	0	0.12	0.18	0.01	0.10	0.03	0	0.06	
CV <sub>A</sub>	0	17.12	17.00	4.04	16.26	10.28	2.89	9.66	
Tukey	cd	bc	d	ab	b	a	bc		
RL (cm)									
Mean (sd)	33.3 (9.9)	29.8 (9.0)	31.4 (9.0)	29.1 (8.2)	29.9 (7.6)	25.4 (8.8)	29.8 (8.0)	29.8 (8.8)	0.30
$h^2$ (SE)	0 (0.02)	0 (0.02)	0 (0.02)	0.15 (0.08)	0.11 (0.07)	0.62 (0.23)	0.01 (0.02)	0.13 (0.06)	
$\sigma^2_A$	0	0	0	10.2	6.2	49.3	0.6	9.5	
CV <sub>A</sub>	0	0	0	10.95	8.32	27.64	2.56	7.07	
Tukey	b	b	b	ab	b	a	b		
Sdb (g)									
Mean (sd)	0.1099 (0.053)	0.0831 (0.039)	0.1317 (0.067)	0.0863 (0.054)	0.0763 (0.036)	0.0564 (0.026)	0.0934 (0.040)	0.0912 (0.051)	0.36
$h^2$ (SE)	0 (0.02)	0.57 (0.22)	0.27 (0.14)	0.18 (0.09)	0.54 (0.23)	0 (0.01)	0.20 (0.09)	0.25 (0.12)	
$\sigma^2_A$	0	0.0009	0.0012	0.0005	0.0007	0	0.0003	0	
CV <sub>A</sub>	0	35.79	26.57	26.56	35.38	0	19.35	20.52	
Tukey	cd	b	d	b	b	a	bc		

**Table 2** (continued)

	B	C	P	PA	Qe	Tr	TT	Mean	Q <sub>ST</sub>
<b>Rdb (g)</b>									
Mean (sd)	0.4728 (0.270)	0.3113 (0.176)	0.5187 (0.318)	0.3031 (0.194)	0.2970 (0.195)	0.2184 (0.128)	0.3517 (0.202)	0.3512 (0.237)	0.37
h <sup>2</sup> (SE)	0 (0.02)	0.03 (0.03)	0.35 (0.18)	0.32 (0.14)	0.39 (0.18)	0.23 (0.10)	0 (0.01)	0.19 (0.01)	
σ <sup>2</sup> <sub>A</sub>	0	0.0009	0.0354	0.0125	0.0151	0.0038	0	0.01	
CV <sub>A</sub>	0	9.64	36.26	36.9	42.26	28.33	0	21.91	
Tukey	cd	b	d	ab	ab	a	bc		
<b>R/S</b>									
Mean (sd)	4.26 (1.46)	3.69 (1.10)	3.80 (1.08)	3.53 (1.39)	3.68 (1.25)	3.90 (1.92)	3.68 (1.27)	3.77 (1.37)	-
h <sup>2</sup> (SE)	0.47 (0.22)	0.03 (0.03)	0.12 (0.08)	0.10 (0.06)	0.32 (0.15)	0.34 (0.14)	0 (0.01)	0.20 (0.10)	
σ <sup>2</sup> <sub>A</sub>	1.02	0.04	0.14	0.20	0.50	1.29	0	0.46	
CV <sub>A</sub>	23.75	5.33	9.90	12.62	19.21	29.07	0	14.27	
Tukey	a	a	a	a	a	a	a		
<b>SRL(m/g)</b>									
Mean (sd)	0.92 (0.52)	1.27 (0.73)	0.84(0.62)	1.39 (0.94)	1.47 (0.8)	1.61 (1.34)	1.17 (0.80)	1.24 (0.90)	0.63
h <sup>2</sup> (SE)	0 (0.02)	0.07 (0.05)	0 (0.02)	0 (0.02)	0.05 (0.04)	0.98 (0.36)	0 (0.01)	0.16 (0.07)	
σ <sup>2</sup> <sub>A</sub>	0	0.04	0	0	0.10	1.90	0	0.30	
CV <sub>A</sub>	0	17.4	0	0	18.7	107.6	0	20.5	
Tukey	a	ab	a	b	b	b	ab		
<b>R/A</b>									
Mean (sd)	1.88 (0.63)	1.58 (0.48)	1.62 (0.47)	1.54 (0.59)	1.56 (0.54)	1.62 (0.76)	1.62(0.55)	1.62 (0.58)	0.43
h <sup>2</sup> (SE)	0.39 (0.18)	~0 (0.02)	0.03 (0.03)	0.12 (0.07)	0.31 (0.15)	0.37 (0.19)	0 (0.01)	0.17 (0.09)	
σ <sup>2</sup> <sub>A</sub>	0.16	<0.01	<0.01	0.04	0.09	0.22	0	0.07	
CV <sub>A</sub>	29.05	~0	6.07	16.83	23.96	36.92	0	16.12	
Tukey	b	ab	ab	a	a	ab	ab		

Tukey tests results are presented (different letters indicate significant differences)

SH shoot height D; root collar diameter; RL length of the longest root, Sdb stem dry biomass, Rdb root dry biomass, R/S ratio between root and shootdry biomass, SRL specific root length, R/A ratio between root and aboveground biomass

but particularly on axis 1 (Fig. 2). It is clear in this graph how different the populations Tromen and Paimún are, as they lie at the extreme ends of opposite quadrants.

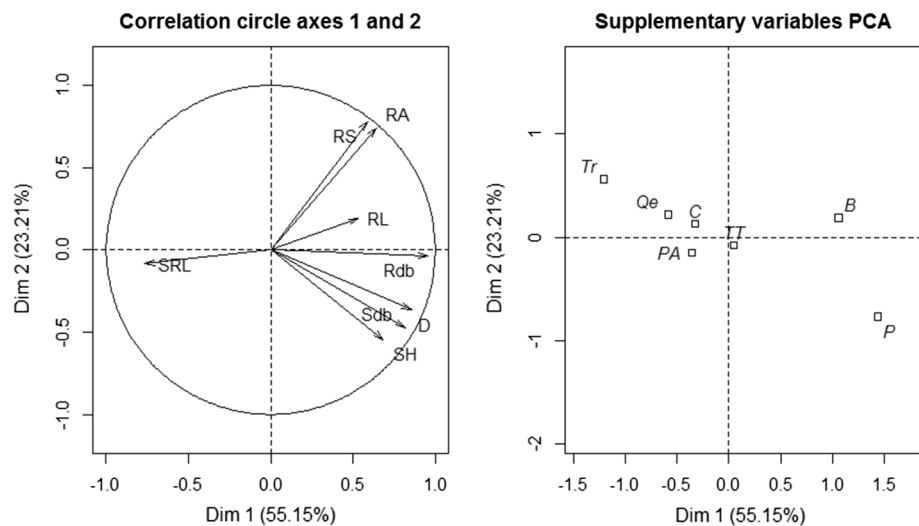
Differences between populations were shown to be significant for all variables except the ratio between root and stem dry biomass ( $P_{R/S}=0.1249$ ). According to the post hoc Tukey tests, Tromen, with the smallest seedlings, was the most different population, followed by Paimún, which produced the largest seedlings (Table 2).

Considering the complete statistical model, the family factor was significant only for D, Sdb and Rdb, and even in these cases it explained just 8.13, 6.60 and 7.22% of the total variance, respectively. Intra-population variation was quite variable among populations and traits, but in general tended to be low (mean heritability and mean additive genetic coefficients of variation of all populations for all variables:  $h^2=0.20$  and  $CV_A=14.65$ ). Tromen showed the highest inter-trait mean  $h^2$  and  $CV_A$  ( $h^2_{Tr}=0.42$ ;  $CV_{A,Tr}=30.82$ ), while  $h^2$  and  $CV_A$  were estimated as zero for several populations and traits. In terms of heritability, the highest intra-population variation was estimated in Tromen for SRL ( $h^2_{SRL}=0.98$ ).

All correlations were significant except for the phenotypic correlations between SH and R/S, and SH and R/A. Phenotypic correlations were in general moderate to high, the highest being between R/S and R/A ( $r_{P,R/S,R/A}=0.96$ ) (Table 3). SRL phenotypic correlations with all the other traits (except RL) were negative and moderate. Additive genetic correlations were generally high, the highest estimated value being even higher than 1 ( $r_{A,D-RL}=1.14$ ). Root length was highly genetically correlated with all traits. A positive correlation was found between Shoot height and SRL, but these traits were inversely correlated with all the other traits.

Differentiation between populations was moderate on average ( $Q_{STmean}=36\%$ ), with the lowest estimates in RL ( $Q_{STRL}=30\%$ ) and the highest in SRL ( $Q_{STSRL}=63\%$ ) (R/S was the only trait with non-significant differences between populations, thus assuming  $Q_{ST}=0\%$ ).

Regarding the correlation tests performed to analyze geographical patterns of variation, no relationship could be shown between any environmental variable of the *N. alpina*



**Fig. 2** *Nothofagus alpina* PCA graphics; on the left: correlation circle of axes 1 and 2; on the right: supplementary variables on the principal components plane

**Table 3** Phenotypic (above diagonal) and additive genetic (below diagonal) correlations between morphological traits of *N. alpina* seedlings and their standard errors (in brackets)

	Shoot height (SH)	Diameter (D)	Root length (RL)	Stem dry biomass (Sdb)	Root dry biomass (Rdb)	Rdb-Sdb ratio (R/S)	Specific root length (SRL)	Below/above ground biomass (R/A)
SH (cm)	1.00	0.67 (0.03)	0.23 (0.04)	0.74 (0.03)	0.65 (0.03)	0.01 (n.s.)	-0.45 (0.04)	0.08 (n.s.)
D (mm)	-0.03 (0.12)	1.00	0.38 (0.04)	0.84 (0.02)	0.83 (0.02)	0.23 (0.04)	-0.64 (0.03)	0.23 (0.04)
RL (cm)	-0.74 (0.05)	1.14 (0.03)	1.00	0.35 (0.04)	0.47 (0.04)	0.38 (0.04)	-0.14 (0.04)	0.41 (0.04)
Sdb (g)	-0.06 (0.12)	0.96 (0.01)	0.89 (0.02)	1.00	0.82 (0.02)	0.10 (0.04)	-0.53 (0.04)	0.19 (0.04)
Rdb (g)	-0.18 (0.12)	0.92 (0.02)	1.01 (0.003)	0.93 (0.02)	1.00	0.54 (0.04)	-0.64 (0.03)	0.60 (0.03)
R/S	-0.44 (0.09)	0.38 (0.1)	0.94 (0.01)	0.21 (0.11)	0.52 (0.09)	1.00	-0.50 (0.04)	0.96 (0.01)
SRL (m/g)	0.13 (0.12)	-0.54 (0.08)	-1.03 (0.01)	-0.39 (0.1)	-0.43 (0.09)	-0.77 (0.05)	1.00	-0.54 (0.04)
R/A	-0.29 (0.11)	0.37 (0.1)	0.89 (0.02)	0.29 (0.11)	0.59 (0.08)	0.97 (0.01)	-0.78 (0.05)	1.00

n.s. not significant

populations and the means of the considered seedling traits. Neither were significant correlations found with seed weight.

### Variation among- and within-populations of *N. obliqua*

The overall mean height (SH) of seedlings in the *N. obliqua* trial was 6.8 cm, the Epulauquen population presenting the lowest mean (4.8 cm) and the Catritre Alto population the highest mean (8.6 cm, which is almost double the former). Overall root collar diameter (D) was 1.61 mm, with small differences between population means. The average length of the longest root (RL) was 30.2 cm (that is, almost five times the mean SH), varying between 25.8 cm in Yuco and 34.0 cm in Pilo Lil. Dry biomass was also quite different between aerial and radical portions, since the mean root dry biomass (Rdb: 0.2785 g) was almost three times greater than the mean stem dry biomass (Sdb: 0.0971 g). The ratio between root and stem dry biomass (R/S) varied from 2.55 in Catritre Bajo to 3.50 in Epulauquen, with an overall average of 2.97 (Table 4). The average R/A across populations was almost half of the mean R/S value. Finally, the mean ratio between root length and root biomass (SRL) was 1.86 m/g (Table 4).

In the PCA, axis 1 accounted for 54.87% of the overall variance and was related to all traits except R/S and R/A. This axis showed a negative relationship between SRL and the other traits. Axis 2 described 25.31% of the variance and was specifically related to R/S and R/A. Populations, as supplementary variables, were separated by axis 2 into two main groups, with Epulauquen, Quillén and Ñorquinco above and Catritre B, Yuco and Nonthué below, Catritre A and Pilo Lil being positioned between these two groups (Fig. 3).

Significant differences between populations were shown for all variables except D ( $P_D=0.1944$ ) and Sdb ( $P_{Sdb}=0.4066$ ). The Tukey tests showed that few populations determined these significant differences. Epulauquen was one of the most differentiated populations, but did not differ from Ñorquinco and Quillén for any trait (Table 4). It is remarkable that although the seedlings of Epulauquen were the shortest, they were among those with the longest roots, and in fact presented the highest root dry biomass, thus resulting in the population with the highest R/S and R/A. Similarly, there was another set of populations which did not differ for any trait: Catritre Bajo, Catritre Alto, Yuco and Nonthué, which was reflected in the PCA grouping.

For both species there is remarkable consistency in the discriminating power of the traits in the PCA, with all but two traits explaining the variation on the principal axis, which accounts for ~55% of the variance. The remaining two traits (R/S and R/A, which are highly correlated) explained ~25% of the variance on the secondary axis. Thus, the ratio between root and aerial biomasses, although accounting for a small part of the variance, did explain the part that was not explained by the other six traits. Consequently, this secondary axis contributed decisively to the separation of the populations studied, especially in *N. obliqua*.

Analyses of variance showed that the family factor was significant only for SH and Rdb, and even in these two cases it explained a minimal part of the total variance (6.85 and 8.27% respectively). Despite some moderate heritability values, intrapopulation variation was rather low (average of all populations for all variables:  $h^2=0.16$ ;  $CV_A=21.09$ ), with the exception of the Epulauquen population, which more than doubled these mean values, with  $h^2=0.38$  and  $CV_A=45.08$  (Table 4).

Only a small number of phenotypic correlations were not significant (D vs. R/S; D vs. R/A; Sdb vs. R/S and Sdb vs. R/A). The highest correlations were the same as for *N.*

*alpina* ( $r_{P,D-Sdb}=0.81$ ;  $r_{P,D-Rdb}=0.83$ ;  $r_{P,Rdb-Sdb}=0.88$ ;  $r_{P,R/S-R/A}=0.85$ ) plus two more ( $r_{P,D-SH}=0.80$ ;  $r_{P,SH-Sdb}=0.83$ ). SRL was significantly, negatively and moderately correlated with all traits. Additive genetic correlations were high, even greater than 1 in several cases. Correlations greater than the theoretical upper limit of 1 are rare but possible values, since the procedure used is an estimation of the real correlations, where the non-additive genetic effects could be included in the estimates of genetic variance and covariance (Lynch and Walsh 1998). Correlations involving RL were a special case because estimated family variance was nil, so it was not possible to calculate additive genetic correlations (Table 5).

Inter-population variation was moderate as an average of all traits (mean  $Q_{ST}=0.31\%$ ). However, worthy of note is the high differentiation estimated in R/A and R/S ( $Q_{ST,R/A}=89\%$ ;  $Q_{ST,R/S}=61\%$ ) (Table 4).

With respect to analysis of the geographical patterns of variation, significant correlations were shown for *N. obliqua* between the altitude of their populations and the traits SH, D, R/S, SRL and R/A; and also between the weight of 100 filled seeds (100 SW) and SH.

## Discussion and conclusions

The family factor was significant in just a few traits, and even in those cases represented a low proportion of the total variability. In terms of  $CV_A$ , within-population variation was low in both species, but slightly lower in *N. alpina*. This population genetics parameter is a proxy for evolvability (Houle 1992), namely the ability of an organism to evolve, and mainly to adapt. Therefore, it seems that the analyzed populations of both species, but especially those of *N. alpina*, have a low capacity for adaptation; that is, a low ability to vary the frequency of their genetic variants in order to survive in situ. This is the main result of the present study. Of course, this low intra-population variation pattern is not constant but on the contrary varies between populations and traits.

In a previous *N. obliqua* nursery trial performed with 4 of the 8 populations used in the present study, Barbero (2014) found even lower  $CV_A$  values for several growth, morphological and phenological traits in seedlings (across traits average:  $CV_{A,E}=9.31$ ;  $CV_{A,PL}=5.50$ ;  $CV_{A,Y}=6.93$ ;  $CV_{A,CaB}=4.59$ ). Similar low values were estimated for *Fagus sylvatica* and Swedish populations of *Quercus robur* (Baliuckas et al. 2001), and for *Betula pendula*, *Alnus glutinosa* and *Quercus robur*, measured at ages 4–9 (Baliuckas and Pliura 2008) in bud flushing, autumn leaf coloring and height traits,  $CV_A$  rarely exceeding 25 in both studies. However, these results contrast with those of *Quercus robur* from six Lithuanian populations growing in two different sites, where the  $CV_A$  of adaptive traits reached a mean value of 18.52 at one of the sites and 31.74 at the other (Baliuckas and Pliura 2003).

Since additive genetic correlations are in general greater than phenotypic correlations, environmental correlations should consequently be low (Lynch and Walsh 1998), which highlights the possibility of using a combination of several of these traits as early selection criteria. In addition to these general trends, a surprising contrast arises between the two species: shoot height showed positive and high or moderate genetic correlations with the other traits in *N. obliqua* (except with SRL, which is negative), but negative and low or moderate genetic correlations in *N. alpina* (except with SRL, which is positive), although this contrast is not verified with respect to phenotypic correlations. This result indicates that: (1) the association between shoot height and the other traits is poorly inherited in *N. alpina*, while the opposite is true for *N. obliqua*; this could be evidence of differential

**Table 4** Mean and its standard deviation (sd), heritability ( $h^2$ ) and its standard error (SE), additive genetic variance ( $\sigma_A^2$ ) and additive genetic coefficient of variation ( $CV_A$ ) of all traits considered in each *N. obliqua* natural population included in the common garden trial

	CaA	CaB	E	N	Nor	PL	Qi	Y	Mean	Q <sub>ST</sub>
<i>Nothofagus obliqua</i>										
SH (cm)										
Mean (sd)	8.6 (5.8)	7.6 (5.6)	4.8 (2.9)	7.3 (5.5)	6.4 (4.1)	7.2 (4.9)	5.5 (3.3)	6.6 (4.2)	6.8 (4.8)	0.09
$h^2$ (SE)	0.38 (0.17)	0.21 (0.10)	0.30 (0.13)	0.38 (0.18)	0 (0.07)	0.14 (0.07)	0.64 (0.33)	0.09 (0.05)	0.27 (0.14)	
$\sigma_A^2$	13.4	6.8	2.6	11.5	0	3.4	7.4	1.6	5.9	
$CV_A$	42.57	34.63	33.72	46.29	0	25.61	49.06	19.27	31.39	
Tukey	c	bc	a	bc	ac	bc	ab	ac		
D (mm)										
Mean (sd)	1.67 (0.66)	1.51 (0.70)	1.74 (0.71)	1.49 (0.56)	1.79 (0.80)	1.62 (0.69)	1.58 (0.58)	1.53 (0.61)	1.61 (0.67)	–
$h^2$ (SE)	0 (0.02)	0.25 (0.11)	0.38 (0.16)	0.06 (0.05)	0.01 (0.08)	0.03 (0.03)	0.22 (0.15)	0.09 (0.05)	0.13 (0.08)	
$\sigma_A^2$	0	0.13	0.20	0.02	0.01	0.02	0.07	0.03	0.06	
$CV_A$	0	23.53	25.59	9.35	5.51	7.72	17.14	12.02	12.61	
Tukey	a	a	a	a	a	a	a	a		
RL (cm)										
Mean (sd)	32.0 (15.2)	27.8 (15.9)	32.5 (12.6)	28.3 (13.3)	31.6 (13.3)	34.0 (15.0)	29.8 (13.2)	25.8 (14.9)	30.2 (14.5)	0.38
$h^2$ (SE)	0.04 (0.04)	0.11 (0.06)	0.48 (0.19)	0 (0.02)	0.06 (0.01)	0 (0.02)	0.26 (0.17)	0 (0.02)	0.12 (0.08)	
$\sigma_A^2$	9.1	27.9	76.7	0	9.8	0	45.8	0	21.2	
$CV_A$	9.43	19.01	26.94	0	9.90	0	22.76	0	11.01	
Tukey	ab	ab	b	ab	ab	b	ab	a		
Sdb (g)										
Mean (sd)	0.1069 (0.129)	0.1032 (0.129)	0.0991 (0.111)	0.0963 (0.107)	0.1073 (0.107)	0.0997 (0.102)	0.0916 (0.121)	0.0780 (0.084)	0.0971 (0.111)	–
$h^2$ (SE)	0 (0.02)	0.25 (0.11)	0.44 (0.18)	0.14 (0.08)	0 (0.07)	0 (0.02)	0.03 (0.06)	0 (0.02)	0.11 (0.07)	
$\sigma_A^2$	0	0.0042	0.0055	0.0016	<0.0001	0	0.0005	0	0.0015	
$CV_A$	0	63.19	75.08	41.57	0	0	23.74	0	25.45	
Tukey	a	a	a	a	a	a	a	a		



**Table 4** (continued)

	CaA	CaB	E	N	Nor	PL	Qi	Y	Mean	Q <sub>ST</sub>
<b>Rdb (g)</b>										
Mean (sd)	0.2864 (0.251)	0.2860 (0.437)	0.3589 (0.447)	0.2275 (0.236)	0.3444 (0.317)	0.2867 (0.281)	0.2357 (0.200)	0.2006 (0.209)	0.2785 (0.324)	0.03
h <sup>2</sup> (SE)	0 (0.02)	0.26 (0.12)	0.56 (0.22)	0.23 (0.12)	0 (0.07)	0 (0.02)	0 (0.04)	0 (0.02)	0.13 (0.08)	
$\sigma^2_A$	0	0.0505	0.1154	0.0131	0	0	<0.0001	0	0.0224	
CV <sub>A</sub>	0	78.57	94.64	50.24	0	0	0	0	27.93	
Tukey	ab	ab	b	ab	ab	ab	ab	a		
<b>R/S</b>										
Mean (sd)	2.87 (0.87)	2.55 (0.86)	3.50 (1.17)	2.63 (0.86)	3.43 (1.04)	3.12 (1.31)	3.25 (1.08)	2.54 (0.98)	2.97 (1.10)	0.61
h <sup>2</sup> (SE)	0 (0.02)	0 (0.02)	0.17 (0.08)	0.65 (0.27)	0 (0.07)	0 (0.02)	0.17 (0.13)	0.04 (0.03)	0.13 (0.08)	
$\sigma^2_A$	0	0	0.23	0.50	0	0	0.21	0.04	0.12	
CV <sub>A</sub>	0	0	13.61	27.02	0	0	14.3	7.87	7.85	
Tukey	ad	a	e	abc	de	cde	bde	ab		
<b>SRL(m/g)</b>										
Mean (sd)	1.64 (1.05)	2.30 (2.26)	1.61 (1.02)	1.95 (1.11)	1.38 (0.85)	1.98 (1.26)	1.74 (0.90)	2.02 (1.25)	1.86 (1.33)	0.49
h <sup>2</sup> (SE)	0.48 (0.20)	0.15 (0.08)	0.31 (0.13)	0.25 (0.13)	0.57 (0.39)	0.11 (0.07)	0 (0.04)	0.06 (0.04)	0.24 (0.14)	
$\sigma^2_A$	0.55	0.84	0.34	0.32	0.42	0.19	0	0.10	0.35	
CV <sub>A</sub>	57.8	60.3	46.0	40.7	55.4	30.8	0.0	22.8	39.24	
Tukey	ab	a	b	ab	ab	ab	ab	ab		
<b>R/A</b>										
Mean (sd)	1.40 (0.46)	1.22 (0.43)	1.77 (0.67)	1.26 (0.44)	1.68 (0.47)	1.46 (0.56)	1.55 (0.64)	1.17 (0.46)	1.43 (0.57)	0.89
h <sup>2</sup> (SE)	0.10 (0.06)	0 (0.02)	0.03 (0.03)	0.44 (0.02)	0 (0.07)	0.06 (0.05)	0.20 (0.15)	0.32 (0.14)	0.14 (0.09)	
$\sigma^2_A$	0.02	0	0.01	0.09	0	0.02	0.08	0.07	0.04	
CV <sub>A</sub>	12.14	0	8.36	26.19	0	11.88	23.28	24.11	13.25	
Tukey	abc	ab	d	ab	cd	bc	bd	a		

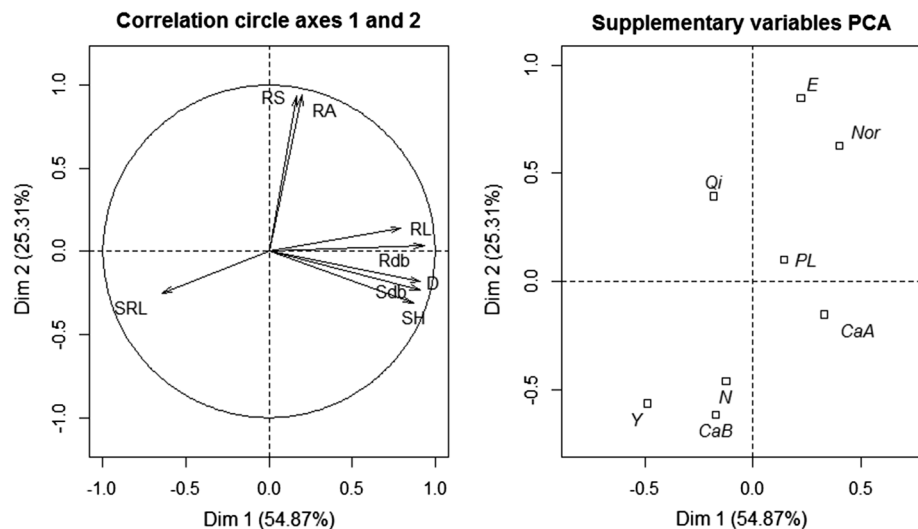
Tukey tests results are presented (different letters indicate significant differences)

SH shoot height, D root collar diameter, RL length of the longest root, Sdb stem dry biomass, Rdb root dry biomass, R/S ratio between root and shoot dry biomass, SRL specific root length, R/A ratio between root and aboveground biomass

adaptation to the contrasting natural ranges of the two species; and (2) *N. alpina* appears to be better adapted for light competition than *N. obliqua*, since taller seedlings will not be the ones with a larger biomass or a better ratio between belowground and aboveground biomasses.

Inter-population variation was moderate as an average of all traits in *N. alpina* and *N. obliqua* (mean  $Q_{ST\ N.alpina}$  = 36%;  $Q_{ST\ N.obliqua}$  = 31%). Comparison between this quantitative differentiation parameter and the  $F_{ST}$  estimated with neutral markers can shed light on the role of adaptation in modelling the genetic variation patterns of natural populations (Merilä and Crnokrak 2001). We did not analyze our seedlings with neutral markers, but there are studies of Argentinian populations (including the ones we sampled) of both species that reported  $F_{ST}$  values estimated with neutral markers, and these can be considered as a general reference. For *N. obliqua* a  $F_{ST}$  of 5% was estimated with isozymes (Azpilicueta et al. 2009), and with SSRs (Azpilicueta et al. 2013). For *N. alpina*,  $F_{ST}$  was estimated at 5% with isozymes (Marchelli and Gallo 2004) and at 6% with SSRs (Azpilicueta et al. 2013). Given that the  $Q_{ST}$  values we estimated are higher than published  $F_{ST}$  values, we could consequently recognize a probable adaptation effect to the present environmental conditions in the quantitative genetic variation patterns controlling the traits studied.

Two groups of undifferentiated populations were revealed through the Tukey tests and the PCA in the analysis of *N. obliqua* seedlings: a northern group formed by Epulauquen, Ñorquinco and Quillén, and a southern group comprising Catritre Bajo, Catritre Alto, Yuco and Nonthué (these four populations are distributed in the Lácar lake basin). The first group is characterized by higher altitude, ranging from 1050 to 1500 m asl, while the Lácar group grows at an altitude between 650 and 850 m asl. Pilo Lil population, with its 900 m asl, only differs from the populations of the northern group in shoot height. Besides having an intermediate altitude, this population stands out as being the driest, which is an environmental condition with an effect close to that of high altitude (e.g. Yang et al. 2010). Consequently, altitude (and relatedly, drought stress) could be the environmental driver



**Fig. 3** *Nothofagus obliqua* PCA graphics; on the left: correlation circle of axes 1 and 2; on the right: supplementary variables on the principal components plane

**Table 5** Phenotypic (above diagonal) and additive genetic (below diagonal) correlations between morphological traits of *N. obtiqua* seedlings and their standard errors (in brackets)

	Shoot height (SH)	Diameter (D)	Root Length (RL)	Stem dry biomass (Sdb)	Root dry biomass (Rdb)	Rdb-Sdb ratio (R/S)	Specific root length (SRL)	Below/Above ground biomass (R/A)
SH (cm)	1.00	0.80 (0.03)	0.63 (0.03)	0.83 (0.02)	0.74 (0.03)	-0.13 (0.04)	-0.48 (0.04)	-0.10 (0.04)
D (mm)	0.79 (0.04)	1.00	0.67 (0.03)	0.81 (0.03)	0.83 (0.02)	0.04 (n.s.)	-0.55 (0.04)	-0.05 (n.s.)
RL (cm)	n.e.	n.e.	1.00	0.63 (0.03)	0.70 (0.03)	0.24 (0.04)	-0.32 (0.04)	0.29 (0.04)
Sdb (g)	1.12 (0.03)	1.51 (0.14)	n.e.	1.00	0.88 (0.02)	-0.08 (n.s.)	-0.48 (0.04)	0.01 (n.s.)
Rdb (g)	0.59 (0.07)	1.02 (0.004)	n.e.	1.29 (0.07)	1.00	0.18 (0.04)	-0.51 (0.04)	0.24 (0.04)
R/S	0.31 (0.1)	0.45 (0.09)	n.e.	1.24 (0.06)	1.56 (0.16)	1.00	-0.27 (0.04)	0.85 (0.02)
SRL (m/g)	-0.76 (0.05)	-0.93 (0.01)	n.e.	-1.44 (0.12)	-0.90 (0.02)	-1.29 (0.08)	1.00	-0.32 (0.04)
R/A	0.12 (0.11)	0.33 (0.1)	n.e.	0.29 (0.1)	0.63 (0.07)	1.43 (0.11)	-0.78 (0.04)	1.00

*n.s.* not significant, *n.e.* cannot be estimated due to the nil value of one trait's family variance

of an adaptation process that would have led to the differentiation of these two groups of populations.

An alternative hypothesis could be proposed, based on information obtained through selective-neutral genetic markers. An analysis of molecular variance carried out with isozymes showed a latitudinal structure with two main groups, north and south of Lanín volcano (39°40'S) (Azpilicueta et al. 2009); the same two groups as in the present study were reported, according to cpDNA haplotypes (Azpilicueta et al. 2013). Moreover, by means of SSRs markers, the same four populations included in our northern group were shown to belong to a single genetic zone (Azpilicueta et al. 2013). These north and south groups were interpreted as having originated from two different glacial refugia (Azpilicueta et al. 2009), that is, ancient differentiation due to demographic causes.

Considering the predicted impact of climate change, R/S, R/A and SRL are probably the most relevant traits among those analyzed in the present study (Padilla and Pugnaire 2007; Grossnickle 2012; Matías et al. 2014; Ledo et al. 2018). As mentioned previously, differentiation between *N. obliqua* populations was moderate on average, but high in R/A, R/S and SRL (in decreasing order of importance). The population with the highest R/A and R/S mean values was Epulauquen, which must be highlighted in this respect, since it is probably largely responsible for this differentiation (the distinctive character of this population has been reported in a previous study by Azpilicueta et al. 2014). Thus, some populations produce seedlings with high R/A and R/S ratios, probably adapted to drought conditions, and others produce seedlings with low R/A and R/S ratios, probably adapted to competition for light. This result is in accordance with the low intra-population variation of this species in these traits, with the remarkable exception of the Nonthué population. Hence, in relation to R/A and R/S, local adaptation seems a likely response to climate change only in Nonthué, and the persistence of *N. obliqua* could depend on the already-adapted populations. A recent study on *N. obliqua* resprouting capacity supports this idea, suggesting that plastic responses and migration may also play a role in species persistence (Aparicio et al. 2015). Since the populations supposedly adapted to drought are quite distant (some hundred kilometers across the mountains) from those not adapted, natural migration is uncertain, so an assisted migration strategy (e.g., Benito-Garzón and Fernández-Manjarrés 2015) could be the key to conservation of the threatened populations in a scenario of extreme drought.

According to the post hoc comparisons, Tromen was the most different *N. alpina* population. This is consistent with previous studies based on Chloroplast DNA markers, (Marchelli et al. 1998; Marchelli and Gallo 2006), where groups of north and south populations were proposed according to different haplotypes, Tromen being the only population in our study that belonged to the north group. Likewise, based on SSRs, Azpilicueta et al. (2013) could not cluster Tromen with other *N. alpina* populations. Finally, Tromen also differs from other populations in seed traits (Marchelli and Gallo 1999).

Differentiation between *N. alpina* populations was moderate on average, and in R/A in particular, but null in R/S and relatively high in SRL (Tromen was the population with the highest SRL mean value). This relatively high differentiation in SRL could also be interpreted as a consequence of adaptation to different environments.

Two points should be mentioned in particular. Two sites of a single population were sampled in *N. obliqua* (Catritre Alto and Bajo). In spite of their contrasting altitudes they could not be differentiated by any of the traits considered (according to the Tukey tests). We could only mention a certain differentiation trend visualized in the PCA graphic. Another detail is related to seed weight. It was only possible to demonstrate that this parameter is correlated with shoot height in *N. obliqua*, and probably this case is related

to the high altitude of the population with the heaviest seeds (Epulauquen). Therefore, evidence of maternal effects cannot be reported. On the other hand, the altitude of the sampled populations was correlated with several traits in *N. obliqua*, but not in *N. alpina*. This could simply reflect the contrasting ranges of these species.

In conclusion, the two species presented similar levels of intra- and inter population variation for the seedling traits considered, some of which are likely to be related to adaptation to drought stress. The level of intra-population variation was low, indicating general low adaptability of the natural populations, thus representing an important handicap to respond to CC by means of a selection process. At the same time, differentiation was moderate in general and high in some adaptively relevant traits, indicating that those populations adapted to current harsh environments possibly manage to survive CC without changing their genetic structures.

Notwithstanding, we analyzed only one of the possible strategies of these species to persist in situ. Phenotypic plasticity should also be considered. Field trials with both species essayed together under different environmental conditions would enable us to complete the picture.

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