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Thinking in the sustainability of *Nothofagus antarctica* silvopastoral systems, how differ the responses of seedlings from different provenances to water shortage?

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Abstract *Nothofagus antarctica,* is the most important species of the silvopastoral systems of southern Patagonia. However, there is limited information on the ecophysiological response mechanisms of seedlings for survival under different resource availability regimes, and whether such processes may differ between provenances. In this study, we evaluated the functional response to water shortage of seedlings of four *N. antarctica* provenances grown under controlled conditions. After an initial full irrigation period, seedlings were subjected to a water shortage cycle. Measurements of the diameter at the base of the stem, net photosynthesis (A_n), stomatal conductance to water vapour (g_s), predawn water potential (Ψ_{pd}), osmotic potential at maximum and zero turgor (Π_{100}; Π_0), relative water content at zero turgor (RWC_0) and maximum modulus of elasticity (E_{max}) were carried out during the experimental period. Differences between provenances concerning An and g_s rates were recorded under full irrigation conditions, but were not detected in association with drought stress. All provenances had similar osmotic

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potential values (Π_{100} and Π_0) at full irrigation, but water shortage led to significant osmotic potential differences between provenances. During the whole experimental period, no significant differences on RWC_0 and E_{max} values were recorded among provenances. Under full irrigation conditions, all *N. antarctica* provenances had a similar physiological performance which suggests that environmental conditions may be the main driver for phenotypic differences in this species. From the osmotic adjustment results gathered, it is derived that a major osmoregulation capacity in response to water shortage is a common feature of all the *N. antarctica* provenances evaluated.

Introduction

The *Nothofagus* (Fagaceae) genus is one of the most representative tree genera of the southern hemisphere, being mainly distributed in temperate forests of Australia, New Zealand, and southern Chile and Argentina (Tanai 1986). In the last two countries, the deciduous *Nothofagus antarctica* (G. Forster) Oerst. (commonly known as "ñire" or "ñirre") is the species with the broadest ecological amplitude, thriving in a latitudinal range from 36°30′ to 56°00′ S and an altitudinal gradient from 0 to 2000 m above sea level (Veblen et al. 1996). With an extension of 431,000 ha, the distribution of ñire forests in Argentinean southern Patagonia occupies the ecotone zone between the more productive *N. pumilio* forests, and the steppe where water availability conditions severely limit growth of forest trees (Collado 2001; Peri and Ormaechea 2013). This area includes contrasting environmental conditions such as poorly drained sites with high precipitations, or drier sites exposed to strong winds in the limit with the Patagonian steppe (Veblen et al. 1996). Furthermore, several studies in *N. antarctica* forests showed that stands located at different environmental conditions and under distinct use (e.g., unmanaged forests vs. thinned stands for silvopastoral use) have variable responses in terms of ecological and productive parameters (Peri et al. 2010; Ladd et al. 2014; Bahamonde et al. 2015; 2016a, b; Gargaglione et al.

2013). Approximately 70% of ñire native forests in Patagonia have been used as silvopastoral systems, also involving the removal of trees to increase forage and consequently meat production (Peri et al. 2016). However, according to the new Argentinean National Law of Minimum Environmental Standards for Native Forests (Law No 26331), silvopastoral systems, must ensure the continuity of the tree stratum. Related to this affair, studies carried out in *N. antarctica* forests in continental Patagonia showed that higher seedling installation occurred in forests with silvopastoral use compared to primary unmanaged forests (Bahamonde et al. 2016a). Nevertheless, the authors reported that at the end of the growing season the survival rate was often quite low in the first year and close to zero at the second year after seed germination. In contrast, in Tierra del Fuego (a large island of Southern Patagonia) seedling installation was reported to occur both in primary unmanaged and silvopastoral-used forests, creating a well-established seedling bank, where after 4 years, the amount of seedlings in silvopastoral use almost doubled that of primary forests (Peri et al. 2016). In this context, management of regeneration has been suggested as a strategy to promote the continuity of the arboreal stratum (Bahamonde et al. 2016a). However, there is limited knowledge on the ecophysiological mechanisms of seedling survival under different resource availability situations, also concerning the performance of different provenances. On the other hand, in the next decades an increase in temperatures and a decrease in precipitations are expected in Patagonia (3rd report of the Ministry of Environment and Sustainable Development of Argentina, MESP 2015), being the response of forest species to such future scenario totally uncertain. In general, seedlings of *N. antarctica* require high levels of light and soil moisture to survive with a more secondary role of site quality (Bahamonde et al. 2013; Soler et al. 2013). Previous reports indicate that seedlings may develop different strategies depending on the origin of the populations. This species has been observed to be sensitive to water and light constraints (Peri et al. 2009). Decreased photosynthetic net assimilation (A_n) rates have been recorded when growing *N. antarctica* seedlings from northern Patagonia at low levels of light and soil moisture (Peri et al. 2009). Such study was however focused on analyzing the phenotypic response of seedlings

Table 1 Main characteristics of *Nothofagus antarctica* forests from four provenances in southern Patagonia

Provenance	Geographical coordinates	Altitude (m a.s.l.)	MAP (mm year^{-1})	MAT (°C)	DH (m)	Evtp (mm year^{-1})
CC2	51°13′21″S–72°15′44″W	295	520	5.9	11.7	696
CC4	51°17′11″S–72°15′00″W	250	450	5.5	8	718
TM	51°19′05″S–72°10′47″O	452	400	5.1	5	736
TDF	54°20′03″S–67°52′04″W	90	640	5.1	13.6	571

MAP mean annual precipitation, *MAT* mean annual temperature, *DH* dominant height of mature trees, *Evtp* potential annual evapotranspiration

from a single provenance. Nevertheless, information about intra-specific variability in *N. antarctica* is scarce. This intra-specific variability in response to water stress in gas exchange parameters has been assessed for other genus and species of Fagaceae, such as *Quercus sp.* (Arend et al. 2011), *Fagus sp.* (Sánchez-Gómez et al. 2013) or *Nothofagus pumilio* (Premoli and Brewer 2007).

Some investigations analyzed inter-specific differences in the *Nothofagus* genus focussed on traits related to leaf water relations. Varela (2010) evaluated the osmotic adjustment of *N. nervosa* and *N. obliqua* (closely related species to *N. antarctica*) through pressure—volume curves, but no significant differences were recorded between treatments (well-watered vs. water stressed plants). Variations in hydraulic properties of leaves and shoots, and xylem anatomy of different *Nothofagus* species have been recently assessed (Bucci et al. 2013; Dettmann et al. 2013). However, few studies examined the functional response of *N. antarctica* to water shortage comparing plants from different provenances.

Considering that: (i) the use of *Nothofagus antarctica* forests under silvopastoral systems must ensure the continuity of arboreal stratum; (ii) management of regeneration is necessary to reach the previous goal; and (iii) increased temperatures and decreased precipitations are expected in Patagonia in the near future, it proves essential to assess the response of seedlings to environmental changes (Peri et al. 2016). Therefore, the aim of this investigation was to evaluate the functional responses of *N. antarctica* seedlings from different provenances of southern Patagonia to water shortage by analyzing gas exchange parameters and osmotic adjustment capacity. Our main hypothesis was that the population native to the area having the most severe environmental conditions (i.e., lower precipitation,

higher evapotranspiration and lower temperature) would be able to display a better response to the water shortage.

Materials and methods

Plant material

Seeds of *N. antarctica* from four provenances (CC2, CC4, TM and TDF) of southern Patagonia were collected at different locations during March (2014). These provenances are a combination of contrasting geographical, climatic and local environmental conditions, which results in contrasting phenotypic trees in the field. The major contrasting conditions between provenances are mainly related to the TM and TDF sites, the first (TM) being located at a higher altitude, with a lower annual precipitation and higher potential evapotranspiration than the last (TDF) one (Table 1). Such different environmental conditions are consistent with the major differences observed in the height of mature dominant trees, having 5 m in the TM provenance, 13.6 m in the TDF provenance (Table 1).

Experimental design

Seeds from the four provenances were germinated as described by Bahamonde et al. (2011) and planted in 100 cc trays. The number of seedlings obtained was 9, 10, 6 and 12 for CC2, CC4, TM and TDF provenances, respectively. In a first initial growth phase of two months, seedlings were well watered and kept in a greenhouse with 25/18 °C (day/night) temperature and a mean of 60% relative humidity. Thereafter, seedlings were carefully transplanted to 2 L pots and grown for 6 months in a climatic chamber under a photoperiod of 14/10 h of light/darkness, a

temperature range of 25 °C day/20 °C night, 65% relative humidity and a PPFD (photosynthetic photon flux density) at the top of 800 μmol m^{-2} s^{-1}. After 5 months of maintaining plants well-watered, seedlings were submitted to a water stress cycle for one additional month by decreasing watering on a weekly basis. Thus, plants were kept from field capacity (FC) to 70% FC, 50% FC, 40% FC and 33% FC, over the next 4 weeks, respectively. Plant water status was established by measuring both predawn water potential (Ψ_{pd}) with a Scholander pressure chamber (Soil Moisture Equipment Corp, Santa Barbara, CA, USA), and gas exchange rates (T_0 and T_F, see next section). The soil water content in the pots was recorded (SWC) at different times during the drought stress cycle by capacitance/frequency domain sensors (10HS Large Soil Moisture sensor, connected to ProCheck data logger, Decagon Devices).

Leaf functional and morphological parameters measurements

The diameter at the base of seedling stems was measured at the fourth month of growth at the climatic chamber (hereafter T_0), after 30 days (i.e., at the end of full irrigation cycle, $T_{0.5}$) and by the end of the experimental period (i.e., after 6 months, by the end of water stress cycle, hereafter referred to as T_F). The relative diameter growth at the base of stem seedling was subsequently calculated between T_0 and $T_{0.5}$ (well-watered period) and between $T_{0.5}$ and T_F (water stressed period). The gas exchange parameters measured with a Li-Cor 6400 portable photosynthesis system equipped with the chamber LFC-40 (LICOR Inc., Lincoln, NE, USA) were: net photosynthesis (A_n), transpiration rate (E), chlorophyll fluorescence and stomatal conductance to water vapour (g_s) at T_0 and T_F. Leaves were exposed to a fixed CO_2 concentration of 400 ppm using the built-in Li-Cor 6400-01 CO_2 mixer (Li-Cor, Inc.), a PPFD of 1200 μmol m^2 s^{-1} (15% blue light), using the Li-Cor 6400-40 fluorescence chamber (Li-Cor, Inc.), a temperature of 24° C, a leaf-to-air vapor pressure deficit of 1.1 kPa and a RH of 60–65%. Measurements were carried out at T_0 (well watered period) and T_F (end of the water stress cycle). The intrinsic water use efficiency (WUE_i) was calculated as A_n/g_s in μmol fixed CO_2 per mmol transpired H_2O. Once gas exchange measurements were completed, the projected leaf area was estimated by cutting

the section of leaves that was enclosed into the chamber, scanning them, and analyzing the images using the ImageJ software (Rasband 1997–2004). This procedure was employed because the leaves were too small to occupy the entire area of the measuring chamber. The calculated measuring leaf area was used to recalculate the gas exchange results with the standard algorithms provided by the Li-Cor 6400 simulator. At each date of measurement, fresh leaves from all the treatments without the petiole were scanned and leaf areas were estimated by image analyses as indicated above. Leaves were subsequently dried at 65 °C for three days and weighted for estimating the specific leaf area (SLA). In addition, one leaf per plant was sampled, and the following water relation parameters were inferred by building P–V curves (Corcuera et al. 2002): osmotic potential at maximum and zero turgor (Π_{100}; Π_0), relative water content at zero turgor (RWC_0), relative water content of the apoplast (RWC_a), maximum modulus of elasticity (E_{max}), and dry/full-hydrated weight ratio (DW/TW). Plant water status and soil water availability were recorded from measuring predawn water potential (Ψ_{pd}) at the beginning (well watered period) and the end of the experiment (water stress).

To evaluate the phenotypic response of seedlings to water stress, the phenotypic plasticity index (PPI, Valladares et al. 2006) was calculated as follows:

PPI = (Maximum mean X − Minimum mean X)/ Maximum mean X, where X represents the parameter to be evaluated.

Data analysis

Exploratory testings were carried out to verify the compliance with the assumptions of normality, homoscedasticity and independence of data for each evaluated situation. While the Shapiro–Wilk test was performed to verify the normality of the data, the Levene test was used to verify homoscedasticity. The independence was verified by analysing residuals from graphs. Leaf functional and morphological parameters of seedlings were analysed with ANOVA for repeated measures with provenances as between-subject factor and each measuring date as within-subject factor. This analysis was performed because the values are not independent of time. Tukey tests were carried out to test differences between factors when F-values were significant ($P < 0.05$). The
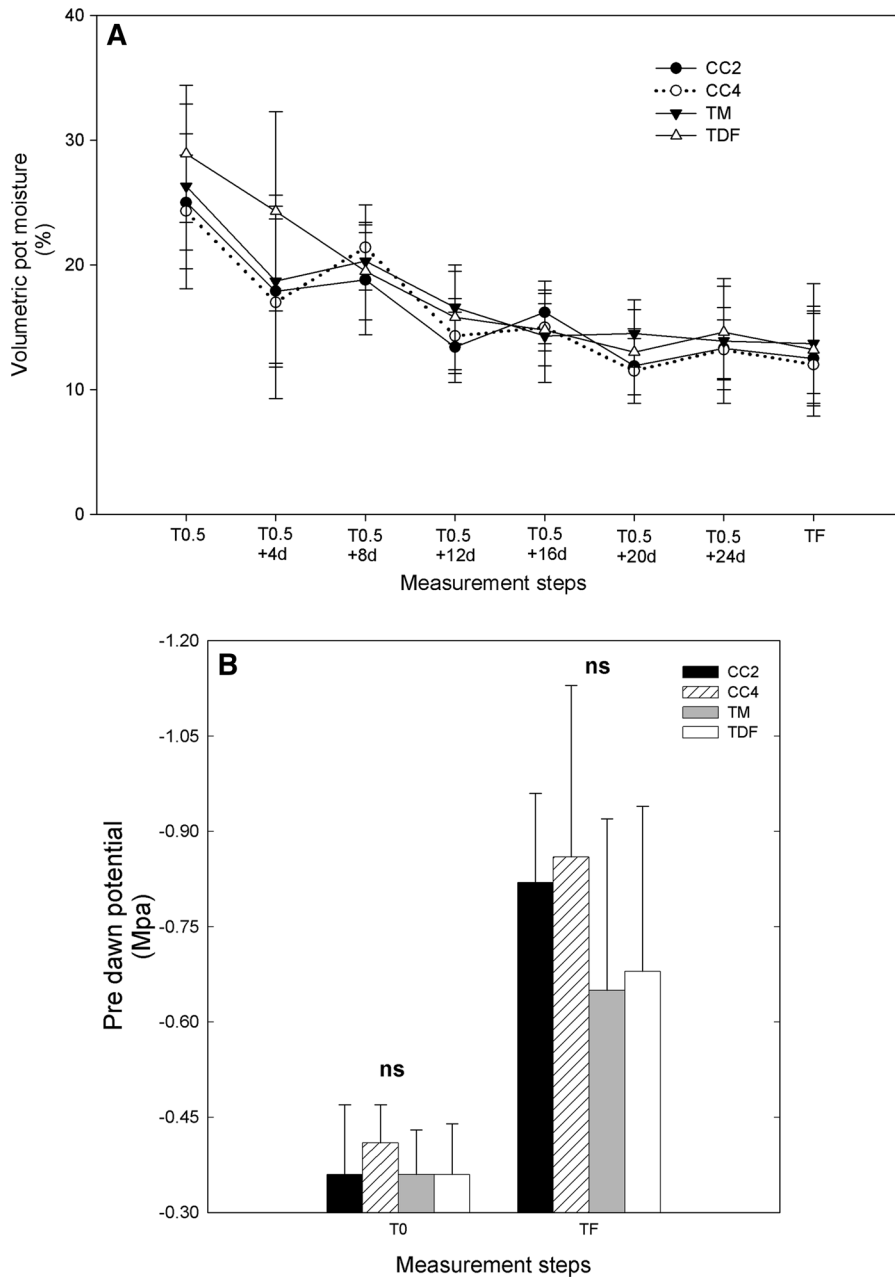
Fig. 1 Volumetric pot moisture (**a**) and pre-dawn potential (**b**) in *Nothofagus antarctica* seedlings from four provenances at different dates. The bars are the standard deviation of the mean. T_0 well-watered plants, $T_{0.5}$ end of well-watered period, T_F water shortage, d days. Different letters in a same date indicate significant differences ($P < 0.05$) between provenances; *ns* not significant

mean values of phenotypic plasticity index, considering all parameters, between provenances were compared with ANOVA.

Results

The volumetric soil water content at the end of the regular watering period ($T_{0.5}$) averaged 28% and
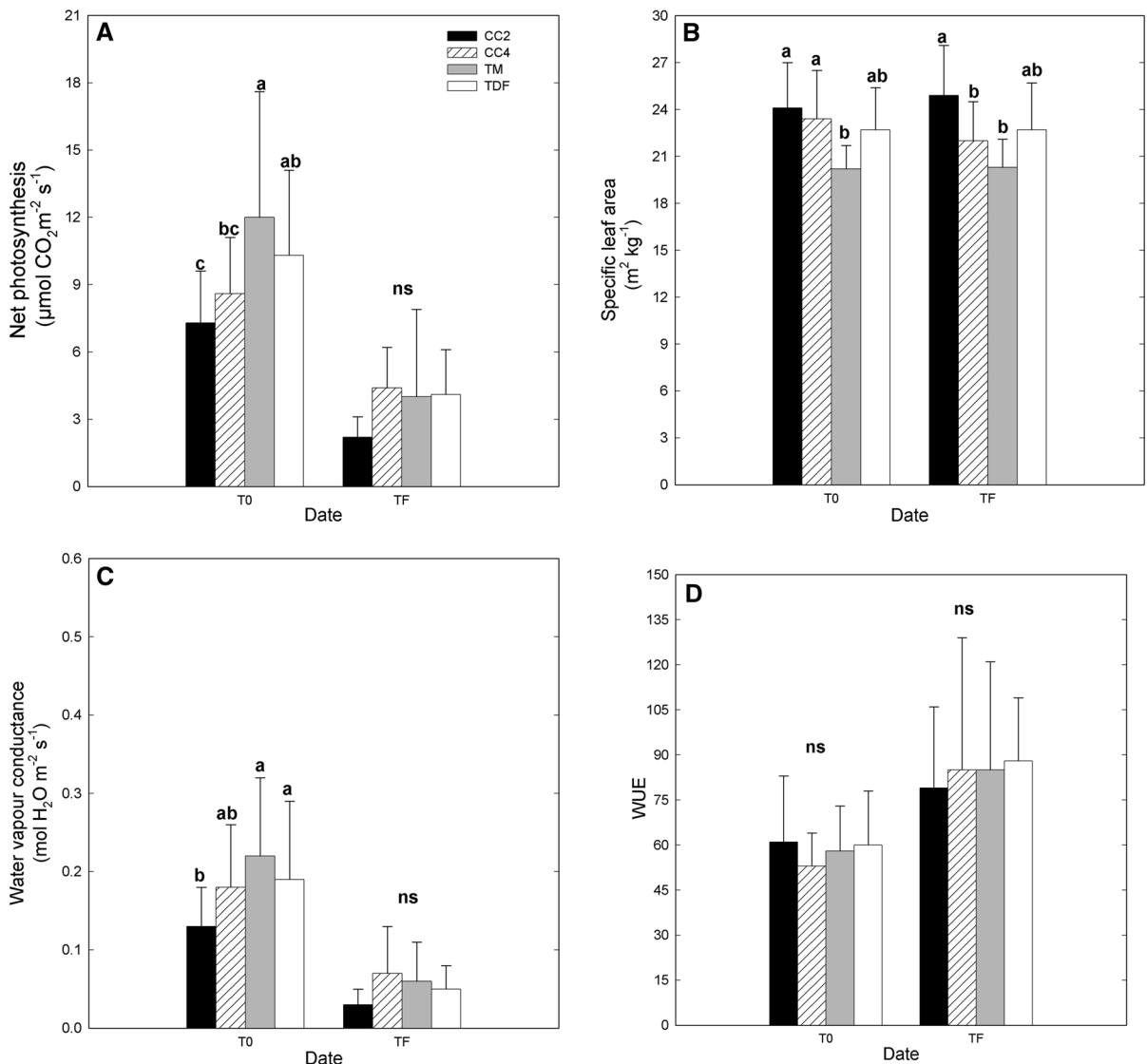
Fig. 2 Net photosynthesis (**a**), specific leaf area (**b**), stomatal water vapour conductance (**c**) and intrinsic water use efficiency (**d**) in *Nothofagus antarctica* seedlings from four provenances at different dates. The bars are the standard deviation of the mean. T_0 well watered plants, T_F water shortage. Different letters in a same date indicate significant differences ($P < 0.05$) between provenances; *ns* not significant

decreased to 14% at the end of water shortage period (Fig. 1a), with no differences being recorded between provenances ($P > 0.05$). Similarly, the pre-dawn potential of plants varied from -0.38 to -0.76 MPa, for well-watered and water-stressed plants, respectively (Fig. 1b). Non-significant differences were observed between provenances within each treatment. The net photosynthetic rate (An) was different between provenances when well-watered plants were compared (T_0), but these differences vanished at the

peak of the water shortage period (T_F, Fig. 2a). When we compared the percentage of An reduction with water shortage according to provenances, the higher decrease was measured in CC2 and TM with an average of 66%, and significantly higher ($P < 0.05$) than to CC4 (46%) and the TDF provenance (55%). Specific leaf area (SLA) values were lower for the TM provenance regardless of the irrigation regime, with no differences being observed among sampling dates ($P > 0.05$) at all (Fig. 2b). Significant stomatal
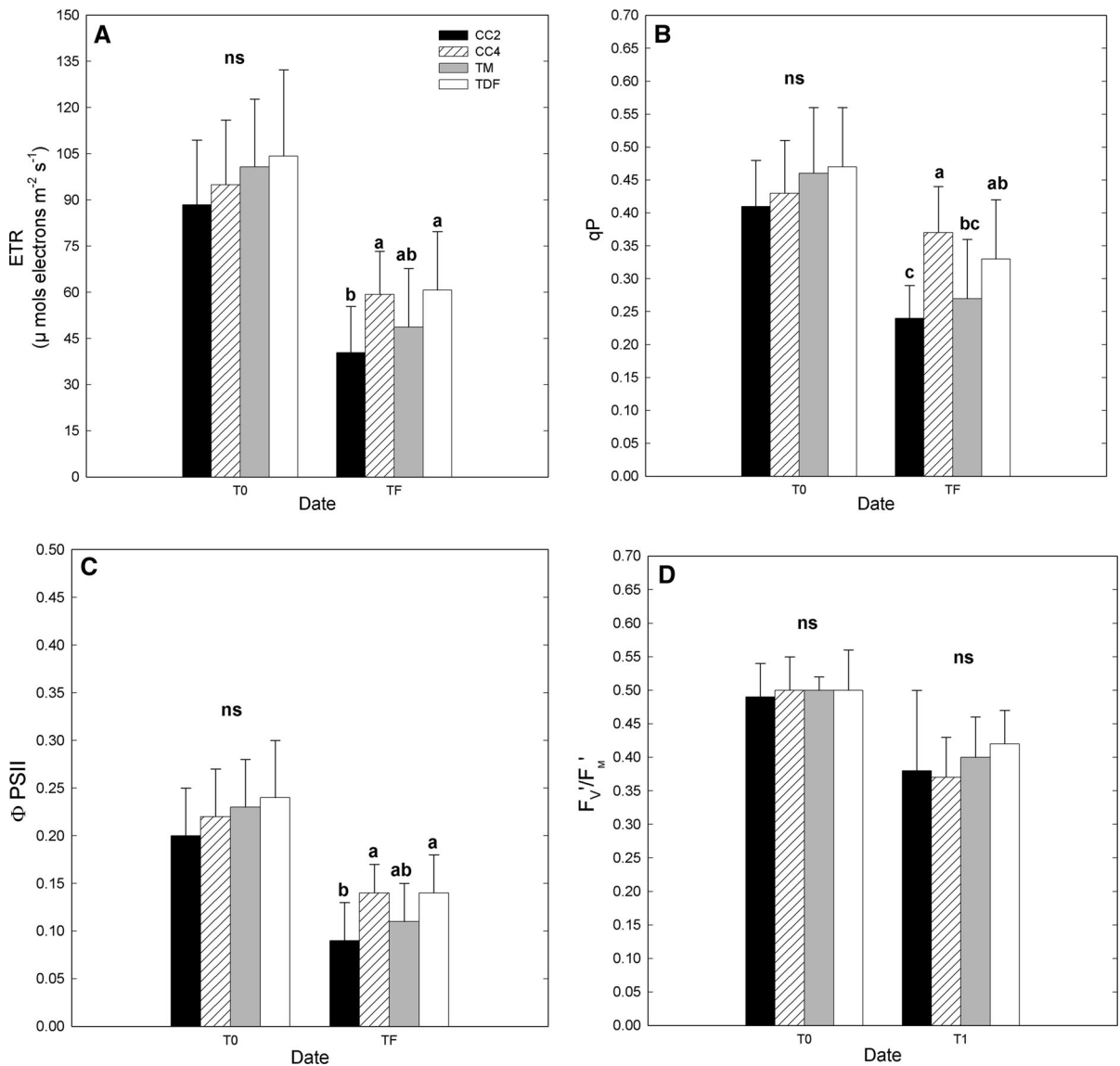
Fig. 3 Electrons transport rate (**a**), photochemical quenching (**b**), quantum yield of photosystem II (**c**) and intrinsic quantum yield of photosystem II (**d**) in *Nothofagus antarctica* seedlings from four provenances at different dates. The bars are the standard deviation of the mean. T_0 well-watered plants, T_F water shortage. Different letters in a same date indicate significant differences ($P < 0.05$) between provenances; *ns* not significant

conductance (g_s) and An differences were observed between provenances when the plants were well watered, being the CC2 values the lowest recorded (Fig. 2c). The reduction of leaf stomatal conductance after water shortage (TF) was significant ($P < 0.001$) for all provenances, but there were no differences between them. The intrinsic water use efficiency was similar between provenances under the two irrigation regimes (i.e., well-watered and water shortage) (Fig. 2d). Comparing between dates and for all

provenances, the WUE was higher at T_F (peak of water shortage), but the increase was only significant ($P < 0.05$) in CC4.

The electron transport rate (ETR), photochemical quenching (qP) and the quantum yield of photosystem II (Φ PSII) did not vary between provenances at the beginning of the trial (T_0), but decreased significantly ($P < 0.05$) with water shortage depending on the provenance, being CC2 the lowest value recorded under water stress conditions (Fig. 3a–c). On the

Table 2 Average values ($\pm$ standard deviation) of P–V curves parameters evaluated in *Nothofagus antarctica* seedlings from four provenances at different water levels

Prov.	Π_{100} (Mpa)		Π_0 (Mpa)		RWC_0		DW/TW		E_{max} (Mpa)	
	Well watered	Water shortage	Well watered	Water shortage	Well watered	Water shortage	Well watered	Water shortage	Well watered	Water shortage
CC2	-1.1 ± 0.2a	-1.4 ± 0.2ab	-1.5 ± 0.2a	-2.0 ± 0.2ab	0.82 ± 0.08a	0.80 ± 0.06a	0.27 ± 0.02ab	0.32 ± 0.03a	4.2 ± 1.7a	5.9 ± 2.2a
CC4	-1.2 ± 0.2a	-1.4 ± 0.2ab	-1.6 ± 0.1a	-2.1 ± 0.5a	0.83 ± 0.04a	0.81 ± 0.06a	0.27 ± 0.02ab	0.32 ± 0.02a	4.9 ± 1.7a	5.9 ± 2.3a
TM	-1.2 ± 0.2a	-1.6 ± 0.2a	-1.5 ± 0.2a	-1.8 ± 0.4ab	0.85 ± 0.06a	0.76 ± 0.13a	0.29 ± 0.02a	0.35 ± 0.02a	5.8 ± 2.4a	6.3 ± 3.7a
TDF	-1.2 ± 0.1a	-1.3 ± 0.2b	-1.6 ± 0.2a	-1.8 ± 0.3b	0.80 ± 0.07a	0.82 ± 0.05a	0.26 ± 0.03b	0.29 ± 0.03b	5.7 ± 1.9a	5.6 ± 2.2a

Different letters in a same column indicate significant differences (P < 0.05) between provenances

Prov. provenances, Π_{100} osmotic potential at maximum turgor, Π_0 osmotic potential at zero turgor, RWC_0 relative water content at zero turgor, *DW/TW* dry/full hydrated weight ratio, E_{max} maximum modulus of elasticity of wall cell

other hand, the intrinsic quantum yield of photosystem II (F_V'/F_M') was not different between provenances (neither at the beginning nor the end of the experiment, Fig. 3d), but decreased significantly (P < 0.05) between dates in all cases excepting TM.

Osmotic potentials at maximum (Π_{100}) and zero turgor (Π_0) were not different between provenances when plants were well watered, but after water shortage there was a general decrease and differences between provenances were significant (Table 2). When comparing Π_{100} in seedlings of each provenance among dates, only the plants from TM showed a significant decrease in response to water shortage (P < 0.05). In the case of Π_0, there were significant differences among dates, and the lowest values were recorded after water shortage with no clear differences between populations (Table 2). Both the relative water content at zero turgor (RWC_0) and maximum modulus of elasticity (E_{max}), did not vary between provenances and no differences were detected among dates. The Dry to full-hydrated weight ratio (DW/TW) was different between plants from TM and TDF, being the values lower in TDF before and after water shortage. Values were significant higher (P < 0.05) after water shortage in all provenances.

Although differences in the diameter at the base stem were minimal between provenances at T_0, after one month ($T_{0.5}$) such differences were not detectable during the remaining experimental period (T_F) (Fig. 4a). The relative growth rate did not vary between origins during the well-watered period (T_0–$T_{0.5}$) or during the water shortage period ($T_{0.5}$–T_F) (Fig. 4b). However, comparing among periods, the relative growth rate was significantly lower (P < 0.05) during the water shortage period in all the provenances.

The calculated phenotypic plasticity indexes varied between provenances depending of each parameter, however average values of all parameters were not significantly different between provenances (Table 3).

Discussion

In this study, we recorded variable responses to soil water deficits by comparing four different *N.*
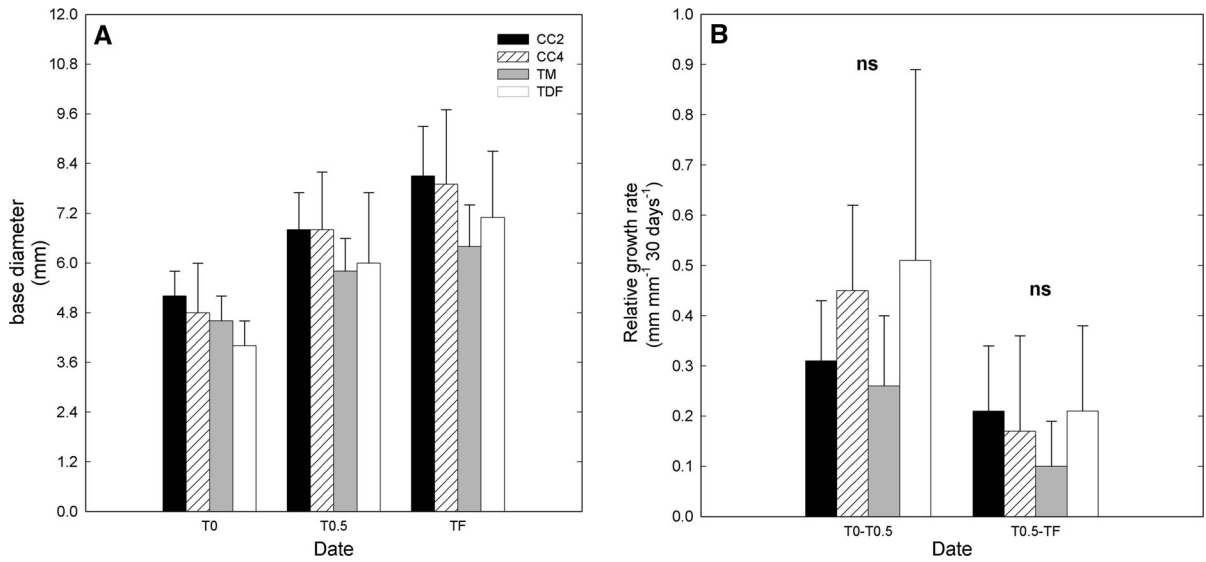
Fig. 4 Base diameter of the stem (**a**) and relative growth rate (**b**) in *Nothofagus antarctica* seedlings from four provenances at different dates. The bars are the standard deviation of the mean. T_0 well watered plants, $T_{0.5}$ end of well watered period, T_F water sortage. Different letters in a same date indicate significant differences ($P < 0.05$) between provenances; *ns* not significant

Table 3 Phenotypic plasticity index of functional and physiological parameters evaluated in *Nothofagus antarctica* seedlings from four provenances

Provenance	An	Swvc	WUEi	SLA	RGR	ETR	qP	FPSII	F′V/F′M	OP_100	OP_0	RWC₀	DW/TW	E_max	Average[a]
CC2	0.70	0.77	0.29	0.03	0.63	0.54	0.41	0.55	0.22	0.27	0.33	0.02	0.19	0.40	0.38
CC4	0.49	0.61	0.62	0.06	0.75	0.38	0.14	0.36	0.26	0.17	0.31	0.02	0.19	0.20	0.33
TM	0.67	0.73	0.46	0.01	0.74	0.52	0.41	0.52	0.20	0.33	0.20	0.11	0.21	0.09	0.37
TDF	0.60	0.74	0.47	0.01	0.74	0.42	0.30	0.42	0.16	0.08	0.13	0.02	0.12	0.02	0.30

An net photosynthesis, *Swvc* stomatal water vapour conductance, *WUEi* intrinsic water use efficiency, *SLA* specific leaf area, *RGR* relative growth rate of seedlings, *ETR* electrons transport rate, *qP* photochemical quenching, *FPSII* quantum yield of photosystem II, *F′V/F′M* intrinsic quantum yield of photosystem II, *OP_100* osmotic potential at maximum turgor, *OP_0* osmotic potential at zero turgor, *RWC₀* relative water content at zero turgor, *DW/TW* dry/full hydrated weight ratio, *E_max* maximum modulus of elasticity of wall cell

[a] Not significant differences were found in the average between provenances

antarctica provenances. The leaf net photosynthesis rates (An) measured in the four *N. antarctica* populations are similar to those reported by Peri et al. (2009) for two-year-old seedlings of this species in northern Patagonia, which reached maximum values of 11.1 μmol CO_2 m^{-2} s^{-1} and near to zero in well-watered and water-stressed plants, respectively. However, these values are lower than those recorded for other *Nothofagus* spp. of this region. Peri et al. (2009) reported values of 16.8 μmol CO_2 m^{-2} s^{-1} and Varela (2010) measured 13.6 μmol CO_2 m^{-2}

s^{-1} for well-watered *N. nervosa* and *N. obliqua* seedlings. In our study, the highest An rate recorded for the provenance native of the highest altitude (TM) was coincident with the results of Premoli and Brewer (2007), who also measured higher An values in plants of *N. pumilio* from higher altitudes in a common garden experiment. Nevertheless, this should be interpreted with caution because altitude is not the only environmental difference between the provenances analysed, and the number of populations was low to infer a relationship between maximum

photosynthetic capacity and altitude, or growing season´s length as observed in other studies (Lajtha and Getz 1993; Hovenden and Brodribb 2000).

The negative effect of drought stress on photosynthesis is well documented (Bréda et al. 2006). In our study, the origin of the seedlings affected their photosynthetic responses to water shortage according to the observed differences in the reduction of An when comparing well-watered and water-stressed plants. This implies that the differences between provenances when seedlings were well watered, were not maintained after water shortage, and may reflect a different performance in terms of the phenotypic plasticity of the studied provenances, as corroborated by the phenotypic plasticity index determined. There is no preliminary information on tree inter-provenance variability in response to water shortage of *N. antarctica*. However, several studies carried out with trees and other woody species reported inter-provenance variability in response to drought stress (e.g., García-Plazaola and Becerril 2000; Atzmon et al. 2004; Ramírez-Valiente et al. 2010; Sánchez-Gómez et al. 2013).

The SLA values recorded in this study are higher than those measured by Calabria and Puntieri (2008) for *N. dombeyi* growing in northern Patagonia at two different light levels, and are also above the values of *N. cunninghamii* measured at different altitudes and two light levels in Tasmania (Hovenden and Vander Schoor 2006). Although changes in this foliar trait have been often associated with different light levels, investigations carried out with other species recorded SLA changes in response to drought (e.g., Liu and Stützel 2004). However, in this work we did not observe an effect of water availability on SLA, which is coherent with the *N. antarctica* results gathered by Bahamonde et al. (2014), where SLA was sensitive to light, but not to water availability. The transpiration rate (E) followed a similar trend to photosynthesis, both between different provenances and measuring dates. The values of E determined in this study for well-watered and water-stressed seedlings were higher and similar, respectively to those reported by Piper et al. (2007) for *N. nitida* and *N. dombeyi* seedlings growing at water potentials higher (less negative) than − 1.5 Mpa. Concerning WUE_i, our values were higher than those reported by Piper et al. (2007) at water potentials > − 1.5 Mpa, and similar to those determined for seedlings subjected to severe drought stress (water potential < − 2.5 Mpa). Although it might be expected an increase of WUE_i in response to drought (Ogaya and Peñuelas 2003; Yin et al. 2005), we observed that water shortage did not affect WUE_i, which was only significantly higher in the CC4 provenance, may be due to a genetic effect as suggested by Read and Farquhar (1991).

Reduction in ETR in response to drought has also been reported for several species, and has been suggested as a mechanism to protect leaf photochemistry from reactive oxygen species production (Flexas et al. 1999; Golding and Johnson 2003). Photochemical quenching (qP) and Φ PSII followed a similar decreasing trend with water shortage, which agrees with the results obtained by Piper et al. (2007) with two different *Nothofagus* spp. and other species (Golding and Johnson 2003). Our results suggest that regardless of the provenance, water shortage affected (decreased) the quantum yield of PSII. This is interpreted as a consequence of a decrease in qP and the intrinsic quantum yield of PSII in most provenances, with the exception of TM, for which the reduction on Φ PSII was only due to decreased of qP.

The stomatal conductance (g_s) values measured in this study were lower than those reported by Peri et al. (2009) for *N. antarctica* and *N. pumilio* seedlings at similar water potentials, but these measurements were performed in two-year-old seedlings. The decrease of gs recorded in our investigation was similar to the previously found in this (Peri et al. 2009) and other *Nothofagus* spp. (Piper et al. 2007).

Read et al. (2010) evaluated twenty-three *Nothofagus* spp. from tropical and temperate forests, including evergreen and deciduous species as *N. antarctica*. Most of the parameters derived from P–V curves in our study were very close to those of Read et al. (2010) for deciduous *Nothofagus* spp, but the E_{max} was lower in our study. Read et al. (2010) suggested that deciduous *Nothofagus* spp. appear to have lower physiological drought resistance in comparison to temperate and tropical evergreen species. According to these findings, *N. antarctica* seedlings from all the provenances evaluated, did not show osmotic adjustment in response to the water shortage. Only when all the provenances were considered together as a whole, it was observable an osmotic adjustment at the species level. Similarly, Varela (2010) did not find evidence for an osmotic

adjustment when seedlings of *N. obliqua* and *N. nervosa* were subjected to water stress in Northern Patagonia. Variability in osmotic adjustment in response to drought has been found across species from different water regimes, and also between populations within a species with contrasting water availability. This mechanism of drought tolerance may not be well developed in the genera, as it has been frequently observed for other Fagaceaes such as *Quercus* spp. (Aranda et al. 2004). These authors found osmotic adjustment in *Quercus pyrenaica* and *Q. petraea* seedlings when exposed to three consecutive water stress cycles, being the osmoregulatory capacity higher in *Q. pyrenaica*. This finding is interpreted by the authors in terms of higher adaptative capacity to drought of *Q. pyrenaica* than *Q. petraea*. Similarly, Nguyen-Queyrens and Bouchet-Lannat (2003) evaluated the osmotic adjustment in response to water stress in *Pinus pinaster* seedlings from five provenances with contrasting soil water availability. These authors reported significant differences in osmotic adjustment among provenances and a significant negative relationship between the level of osmotic adjustment and the precipitation of the origin of the provenances.

High correlations between stem base diameter and aerial or total biomass have been found in seedlings of different species (Curt et al. 2005; Achten et al. 2010). In this study, water shortage had a similar effect on all provenances, decreasing the relative growth rate of the stem diameter. Similarly, Varela (2010) reported a reduction in leaf and shoot biomass, and height relative growth in water-stressed seedlings of *N. nervosa* and *N. obliqua* that are close related species to *N. antarctica*.

Final considerations

As a general trend, the four provenances of *N. antarctica* evaluated had a similar performance during the well-watered period, which suggests that environmental conditions may be the main driver for phenotypic differences of this species (e.g., dominant height of mature trees). Similarly, Premoli and Brewer (2007) reported that differences in eco-physiological traits of *N. pumilio* found under field conditions were not observed in a common garden experiment, suggesting a major effect of the

environment. Furthermore and contrary to our preliminary hypothesis, the water shortage did not trigger different apparent physiological responses between provenances. It is concluded that further field and common garden studies are necessary to improve our understanding of the eco-physiological response of these species to different stress factors. Regarding osmotic adjustment and in agreement with previous reports, it is concluded that either higher water deficits may be necessary to generate a response, or that a true capacity of osmoregulation in response to drought is a common feature of all the provenances evaluated. The results obtained provide information on response of *Nothofagus antarctica* seedlings to drought but future studies should be carried out for understanding of *N. antarctica* water economy in a context of climate change and as tool for improving forest silvopastoral use in southern Patagonia.

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