

Differential photosynthetic and survival responses to soil drought in two evergreen *Nothofagus* species

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Abstract – We asked if differences in distribution between *Nothofagus nitida* and *N. dombeyi* were associated with differences in drought tolerance. Survival, gas exchange and chlorophyll fluorescence were measured on seedlings subjected to a gradual drought. At a predawn leaf water potential (Ψ_m) of -2.7 MPa, survival of *N. nitida* was 50%, compared to 100% in *N. dombeyi*. Under well-watered conditions, the two species displayed similar stomatal conductance (g_w) and transpiration (E), but net photosynthesis (A) and instantaneous water-use efficiency (WUE_i) were slightly higher in *N. nitida*. A , E and g_w declined in *N. nitida* along the gradual drought but increased in *N. dombeyi* at a Ψ_m between -1.5 and -2.5 MPa, and declined then drastically at a Ψ_m below -2.5 MPa. As *N. dombeyi* was able to maintain A at higher levels despite declining g_w , this species displayed significantly increased WUE_i at Ψ_m below -2.5 MPa. Photochemical efficiency of PSII in the light ($\Delta F/F_m'$) and photochemical quenching (q_P) were always lower in *N. nitida* and along with the photochemical efficiency in the dark (F_v/F_m) they declined in both species. Non-photochemical quenching (NPQ) increased slowly in *N. dombeyi* along with the gradual drought, whilst it decreased in *N. nitida*. These results show that differences in drought tolerance are in agreement with sorting of *Nothofagus* species along moisture gradients in south-central Chile.

drought tolerance / gas exchange / *Nothofagus* / water use efficiency / soil water potential

Résumé – Différence de réponse de la photosynthèse et de la survie en situation de sécheresse édaphique dans deux espèces à feuilles persistantes de *Nothofagus*. Nous nous sommes demandés si des différences de distribution entre *Nothofagus nitida* et *N. dombeyi* sont associées à des différences de tolérance à la sécheresse. La survie, les échanges gazeux et la fluorescence de la chlorophylle ont été mesurés sur de jeunes plants soumis à une sécheresse croissante. Lorsque le potentiel hydrique de base (Ψ_m) atteignait -2.7 MPa la survie était de 50 et de 100 % pour *N. nitida*, et *N. dombeyi*, respectivement. Dans des conditions d'alimentation hydrique suffisante, les deux espèces ont présenté des valeurs voisines de conductance stomatique (g_w) et de transpiration (E) mais la photosynthèse nette (A) et l'efficacité instantanée d'utilisation de l'eau (WUE_i) étaient légèrement plus élevées pour *N. nitida*. A , E et g_w ont diminué pour *N. nitida* au cours d'une sécheresse croissante mais ont légèrement augmenté pour *N. dombeyi* pour des valeurs de Ψ_m comprises entre -1.5 et -2.5 MPa, puis diminué fortement à des valeurs de Ψ_m inférieures à -2.5 MPa. Par conséquent, *N. dombeyi* a présenté des valeurs de WUE_i plus élevée que *N. nitida* à des niveaux de Ψ_m inférieurs à -2.5 MPa. L'efficacité photochimique du PSII à la lumière ($\Delta F/F_m'$) et le quenching photochimique (q_P) étaient toujours inférieurs pour *N. nitida*. L'efficacité photochimique à l'obscurité (F_v/F_m) ainsi que $\Delta F/F_m'$ ont diminué dans les deux espèces. Le quenching non-photochimique (NPQ) a légèrement augmenté pour *N. dombeyi* avec la sécheresse, alors qu'il diminuait pour *N. nitida*. Ces résultats montrent que des différences de tolérance à la sécheresse correspondent à la distribution d'espèces de *Nothofagus* le long de gradients d'humidité dans le centre sud du Chili.

tolérance à la sécheresse / échanges gazeux / *Nothofagus* / efficacité d'utilisation de l'eau / potentiel hydrique du sol

1. INTRODUCTION

The evergreen trees *Nothofagus nitida* (Phil.) Krasser and *N. dombeyi* (Mirb.) Blume are important physiognomic dominants of the temperate forests of south-central Chile [4, 26]. These two closely-related species are very similar in life-history and morphology [15] but differ in geographic distribution [28]. *N. nitida* is restricted to highly oceanic climates with > 2500 mm annual precipitation and high humidity throughout the year [27, 28]. Its congener *N. dombeyi* occupies a broader range of habitats, including sites with annual precipitation < 1500 mm and several weeks of summer

drought [1, 4, 26]. Although differences in drought tolerance seem likely to at least partly explain the distributional differences described above, to date this idea has not been evaluated experimentally.

Water use efficiency is thought to play an important role in drought tolerance [5]. In the short term, water use efficiency is represented by the ratio of carbon gain (A) to water transpired (E) – i.e. the instantaneous water use efficiency ($WUE_i = A/E$, [10]). Enhanced WUE is a common response in plants exposed to drought [17, 24, 29] although genotypic effects have also been indicated [18]. In fact, it has been suggested that genotypic and environmental effects affect WUE responses of plants in opposite directions [12, 18], with wet

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Table I. Physical and chemical properties of soils at sites where seedlings were obtained. Mean values (%) with standard errors in parentheses.

Characteristics	Nd Site	Nn Site
Silt	20.7 (0.65)	39.5 (0.5)
Clay	21 (4.6)	20.9 (0.5)
Sand	58 (3.9)	38.1 (0.5)
pH	4.9 (0.12)	4.5 (0.12)
Organic matter	2.15 (0.7)	10.5 (5.8)
Nitrogen	0.055 (0.015)	0.26 (0.05)
C/N	23.4 (0.1)	21.8 (8.9)

site species showing larger long term WUE than more mesic species.

Reduced stomatal conductance during drought limits a plant's ability to use light for photosynthesis, because of reduced CO₂ concentrations inside leaves [20]. The excess energy is often dissipated thermally, although severe exposure to high PFD (photon flux densities) can damage photosystem II [3] – either of these conditions reducing the efficiency of photosystem II.

We measured survival, gas exchange and fluorescence parameters of *Nothofagus nitida* and *N. dombeyi* seedlings subjected to gradual drought. Here we show that differences in drought tolerance are associated with differences in geographic distribution between the two species, but that the underlying differences in photosynthetic physiology are manifested only during drought, and not under well-watered conditions.

2. MATERIALS AND METHODS

2.1. Study sites and plant material

The experiment was carried out between November 2004 and March 2005 in a greenhouse at the Universidad Austral, Valdivia, Chile. During June 2004, 100 two-three years old seedlings of *N. dombeyi* and *N. nitida* were collected from semi-shade in second-growth stands in the Coast Ranges south of Valdivia, and transplanted to 3-L 20-cm high pots. The two species were obtained from separate stands on the seaward (west-facing) slopes of the range, the *N. dombeyi* stand located at 80 m elevation a.s.l (39° 58' 02" S, 73° 33' 39" W), and the *N. nitida* stand at about 350 m a.s.l (39° 59' 40", 73° 34' 12" W). As the soils of the two sites differed in texture and nutrient content (Tab. I), and considering that *N. nitida* is the more sensitive of the two species to transplanting (M. Alberdi, unpublished) plants were potted in original soil of the *N. nitida* site, in order to standardise experimental conditions for the two species. Plants were installed in the greenhouse and acclimated to the new soil environment for five months prior to the start of the experiment. During this period, pots were regularly well-watered, and their positions on the greenhouse bench rotated weekly. Photosynthetic photon flux density (PPFD) inside the greenhouse was 50% of external PPFD. Almost all plants produced new leaves during the acclimation period after transplanting, the few that did not being discarded. All measurements were carried out on these new leaves.

2.2. Experimental design

On 18 November 2004 we selected the 90 most even-sized plants of each species, with heights of 20–40 cm. Watering was suspended for 80 randomly-chosen individuals of each species, whereas the remaining 10 were kept well watered. Beginning three weeks after the suspension of watering, every 8–10 days a different group of 10 drought-stressed individuals of each species was randomly chosen for sampling. Soil water content of the selected pots was measured, and gas exchange and fluorescence parameters recorded for each surviving individual. Thus, time-courses of soil volumetric water content, gas exchange and fluorescence were established, starting from well-watered conditions. Pots were then re-watered to check for survival of wilted seedlings, and returned to the same watering regime as the controls until the end of the experiment. Measurements on the controls, repeated at each sample date to discard possible phenological effects on the studied parameters, were also included in the data set.

2.3. Soil Water Content

Soil moisture was registered as volumetric water content (VWC) using a Time Domain Reflectometry (TDR) soil moisture meter (Trimellog, Germany). The value for one pot was the average of three measurements made over a depth of 18 cm (the length of the guides of the TDR' probes). Values of VWC (water vol/soil vol × 100) were converted to gravimetric water content by a calibration curve constructed with a battery of soil samples with different VWC which were dried at 60 °C and weighed. Values of gravimetric water content were converted to soil water potential (Ψ_m) by means of a calibration curve established at the Soil Analysis Laboratory, Faculty of Agronomy, Universidad de Concepción.

2.4. Chlorophyll fluorescence

Fluorescence measurements were carried out on attached leaves, dark-adapted for 30 min before measurements, of five seedlings per group. Light pulses were generated and signals recorded using a pulse-amplitude modulated fluorometer (FMS 2, Hansatech Instruments Ltd., UK). Minimal fluorescence (F_o) was determined by applying a weak modulated light ($0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$) and maximal fluorescence (F_m) was induced by a short pulse (0.8 s) of saturating light ($8000 \mu\text{mol m}^{-2} \text{s}^{-1}$). After 10 s, actinic light ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$) was turned on to obtain fluorescence parameters during steady-state photosynthesis. Saturating pulses were applied after steady-state photosynthesis had been reached in order to determine maximal fluorescence in light-adapted leaves (F_m') and steady-state fluorescence (F_s). Maximal photochemical efficiency F_v/F_m , (where variable fluorescence $F_v = F_m - F_o$), and effective photochemical efficiency ($\Delta F/F_m'$) (where $\Delta F/F_m' = F_m' - F_s/F_m'$), were used as indicators of the potential and effective quantum yield (photochemical efficiency) of PSII, respectively [7]. Photochemical quenching (qP) and non photochemical quenching were calculated following Schreiber et al. [21].

2.5. Gas exchange

Net photosynthetic rate (A), stomatal conductance (g_w), and transpiration rate (E) were determined with a portable gas exchange

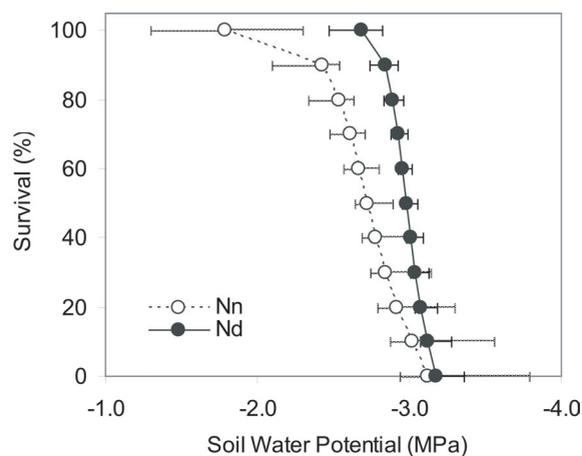


Figure 1. Effect of soil water potential (Ψ_m) on survival *Nothofagus dombeyi* and *Nothofagus nitida* seedlings, modelled by logistic regression. Nd: $R^2 = 0.58$, $p < 0.0001$. Nn: $R^2 = 0.42$, $p < 0.0001$. Horizontal bars represent the limits of the prediction intervals.

system (LCi, ADC Bioscientific Ltd., UK). A , g_w and E were measured on attached leaves on the same five plants of each group that were used for fluorescence measurements, between 09.00 and 11.00 AM. At the time of measurements air temperature was 10–15 °C, PAR varied between 100 and 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and the relative humidity between 30 and 40% (the effect of PPFD variability on photosynthesis was not significant; $p = 0.60$). The instantaneous water use efficiency (WUE_i) was calculated as A/E in μmol fixed CO_2 per mmol transpired H_2O [10].

2.6. Statistical Analyses

Logistic regression was used to assess the effect of Ψ_m (continuous variable) on the binary survival variable (dead = 1; alive = 0). Soil Ψ_m expected for a given survival rate was estimated by inverse prediction. Additionally, a logistic model was fitted to test for inter-specific differences in the effect of water stress on survival, with species and Ψ_m as effects. Model fit was calculated using likelihood ratio statistics, and effect significance was estimated with the Wald test [22].

Data of gas exchange and fluorescence were ordered and analyzed in three ranges of Ψ_m : < -2.5 MPa, -2.5 to -1.5 MPa and > -1.5 MPa. These ranges were selected because they constitute groups of similar sample size. Differences between means of measured parameters inside each range of Ψ_m were tested by Student's t -test [22]. When data did not show normal distribution, the medians were compared by a Mann-Whitney Rank Sum Test.

3. RESULTS

3.1. Survival

Survival of both *Nothofagus dombeyi* and *N. nitida* decreased as Ψ_m declined after suspension of watering, but this response differed between the two species (Fig. 1, Tab. II). Mortality began at much higher Ψ_m in *N. nitida* than in *N.*

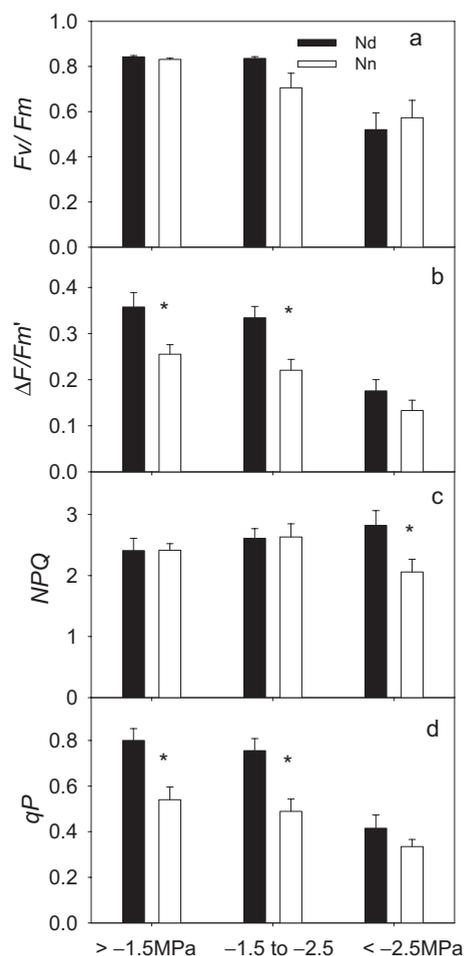


Figure 2. Effect of soil water potential (Ψ_m) on fluorescence parameters in seedlings of *N. dombeyi* and *N. nitida* (a): Photochemical efficiency of PSII in the dark (F_v/F_m), (b): Photochemical efficiency of PSII in the light ($\Delta F/F_m'$), (c): Non-photochemical quenching (NPQ), (d): Photochemical quenching (qP).

dombeyi. Thus, at -2.7 MPa, for example, although survival of *N. nitida* had fallen to 50%, that of *N. dombeyi* was still at 100%. Nonetheless, inter-specific differences in survival decreased when drought intensity increased and reached 0% at -3.1 MPa in both species.

3.2. Fluorescence

F_v/F_m , $\Delta F/F_m'$ and qP declined gradually with increasing drought in *N. nitida*, remaining close to optimal values in *N. dombeyi* down to -2.5 MPa (Fig. 2). At $\Psi_m > -1.5$ MPa, $\Delta F/F_m'$ and qP were lower in *N. nitida* than in *N. dombeyi*, reflecting the higher sensitivity of this species to drought (Fig. 2). NPQ was similar between species at $\Psi_m > -2.5$ MPa but was larger for *N. dombeyi* at $\Psi_m < -2.5$ MPa ($p = 0.035$).

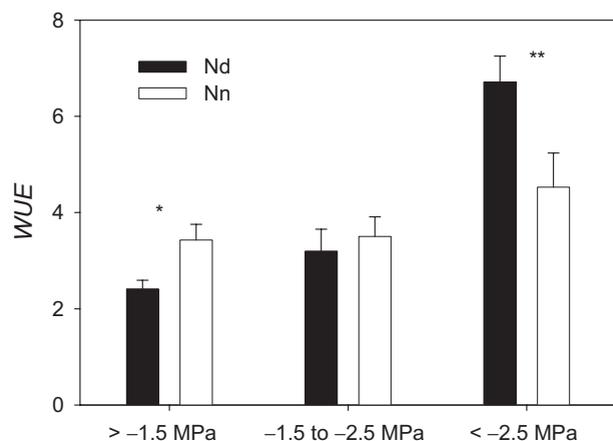


Figure 3. Effect of soil water potential (Ψ_m) on water use efficiency (WUE) in seedlings of *N. dombeyi* and *N. nitida*.

Table II. Wald Test showing the effect of Ψ_m , species and their interaction on survival of seedlings of *Nothofagus dombeyi* and *N. nitida*.

Source	Nparm	DF	Wald ChiSquare	Prob > ChiSq
Ψ_m	1	1	26.5	0.0000
Species	1	1	8.33	0.0039
$\Psi_m \times$ species	1	1	4.16	0.0413

3.3. Gas exchange

WUE_i increased with drought intensity in *N. dombeyi*, but remained invariable in *N. nitida* (Fig. 3). Under well-watered conditions (≥ -1.5 MPa) *N. nitida* had significantly higher WUE_i than *N. dombeyi* (Mann-Whitney Rank Sum Test, $p = 0.032$) but this was reversed at $\Psi_m < -2.5$ MPa ($p < 0.007$). These opposing trends in WUE_i were driven by differences in A , which was higher in *N. nitida* at $\Psi_m \geq -1.5$ MPa ($p = 0.009$), but higher in *N. dombeyi* at $\Psi_m < -2.5$ MPa ($p < 0.002$). The decline of A with Ψ_m was gradual in *N. nitida* whereas it increased at a Ψ_m between -1.5 and 2.5 MPa in *N. dombeyi* declining only at a Ψ_m below -2.5 MPa. E and g_w were similar between species and declined also similarly with Ψ_m in both species (Fig. 4). Thus, the cross-over of species' relative WUE_i resulted from *N. dombeyi* maintaining higher A during drought than *N. nitida*, rather than from differences in E or g_w (Figs. 3, 4).

4. DISCUSSION

Nothofagus dombeyi seedlings showed evidence of larger drought tolerance than those of *N. nitida*, in agreement with the respective distribution patterns of the two species. The lower drought tolerance of *N. nitida* could explain this species' virtual exclusion from the rain shadow created by the Chilean Coast Ranges [1, 4, 27]. Drought tolerance differences could also be involved in distribution patterns on the western (coastal) slopes of the range, since soils at the *N. dombeyi*

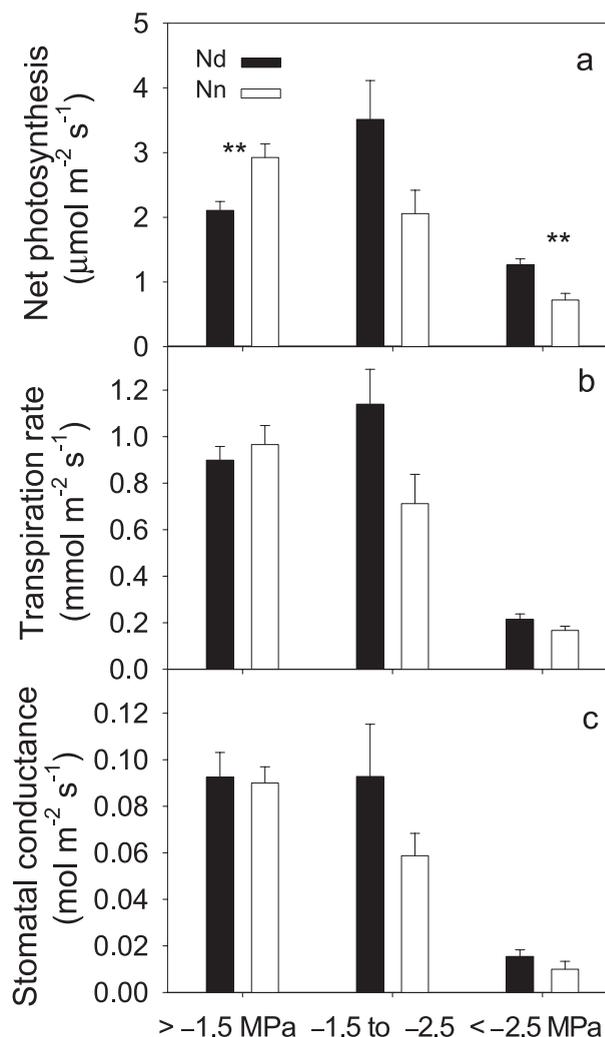


Figure 4. Effect of soil water potential (Ψ_m) on gas exchange parameters of *N. dombeyi* and *N. nitida* seedlings. (a): Net CO_2 assimilation (A), (b): Transpiration (T) (g_w), (c): Stomatal conductance.

site displayed a larger sand fraction than those at the *N. nitida* site (Tab. I) and are therefore likely to retain less moisture in summer.

Relative WUE_i of the two species depended on soil moisture content. Well-watered *N. dombeyi* had lower WUE_i , E and A than *N. nitida* under the same conditions, but WUE_i of the latter was unaltered by drought, whereas that of the former increased, as A of *N. dombeyi* declined slower than g_w and E (Figs. 3, 4). Differences in WUE appeared thus to be driven primarily by differences in photosynthetic rate (Figs. 3, 4), in agreement with other studies [2, 9, 23] and in contrast to others that attributed variations in WUE by differences in stomatal conductance [8, 18, 30]. Severe drought stress thus gave rise to a cross-over in WUE of the two species which occurred at a soil water potential of -2.0 MPa. Survival differences between the species began to become manifest at a similar Ψ_m value, consistent with the idea that increased WUE could be one mechanism underlying the higher drought

tolerance of *N. dombeyi* seedlings. Other factors that we did not quantify (e.g. morphological adaptations [5, 20], osmotic adjustment [20] or lower flux density [11]) may of course also contribute to drought tolerance differences. More experiments including in vivo measurements of leaf water potential are needed for a more complete understanding of the distribution of *Nothofagus* spp. in south-central Chile.

Several factors might underline the patterns of *WUE* we observed under well-watered conditions. It has been previously suggested that species' constitutive differences in *WUE* under well-watered conditions show quite different patterns to those seen in the short-term response of plants to drought [8, 12, 16]. For example, Read and Farquhar [18] found that Australasian *Nothofagus* species from sites with low rainfall in summer had surprisingly low *WUE* under well-watered conditions, associated with high maximum stomatal conductance. They attributed this to selection for opportunistic carbon gain under conditions of mild to low drought stress, accompanied by morphological adaptations ensuring adequate hydraulic supply of leaves, e.g., high root to shoot ratios. This could also apply to our comparison of *N. dombeyi* and *N. nitida*. It is also possible that the maximum PPFD used in this experiment ($400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was insufficient to saturate photosynthesis of well-watered *N. dombeyi*, since this species is shade-intolerant with a relatively high light saturation point [13, 19]. In contrast, we have found that photosynthesis of *N. nitida* seedlings saturates around this value (F. Piper, unpublished). This could have caused underestimation of the maximum WUE_i of well-watered *N. dombeyi*. Despite this doubt, it is clear that *WUE* of the two species responds differently to drought, and our findings add to a body of evidence that the physiological basis of inter-specific variation in drought tolerance is best understood by measurements made under actual drought stress, rather than under well-watered conditions. Brodrribb and Hill [2] showed that instantaneous and long-term water use efficiency under well watered conditions did not give any indication of the relative drought tolerance of a group of southern hemisphere conifers. Valladares et al. [25] found that "water-saving" *Quercus* spp. had higher *WUE* than "water-spending" *Pistacia* spp., but only during drought.

Photoinhibition, represented by a decrease in Fv/Fm [14], was gradual in *N. nitida*, whereas in *N. dombeyi* occurred only at a Ψ_m lower than -2.5 MPa, in agreement to its higher drought tolerance. Consistently, lower $\Delta F/Fm'$ and qP in *N. nitida* reflect the down-regulation of PSII induced indirectly by stomatal closure (Fig. 2) [14]. As *N. dombeyi* has a relatively high photosynthetic saturation point, it is possible that the maximum PPFD used in this experiment was insufficient to saturate its photosynthesis, possibly contributing to a lower energy excess in this species. By the same token, energy excess may have been insufficient to active thermal dissipation at Ψ_m above than -2.5 MPa (Fig. 2). However, as the photosynthetic saturation point declined with drought intensity [6] the same PPFD became probably saturating at a Ψ_m lower than -2.5 MPa, producing the increase of NPQ in *N. dombeyi* and the concomitant decrease in Fv/Fm , $\Delta F/Fm'$ and qP .

In conclusion, this study provides experimental evidence that differences in drought tolerance may at least partly ex-

plain sorting of *Nothofagus* species along environmental gradients in south-central Chile. The greater drought tolerance of *N. dombeyi* compared to *N. nitida* was associated with higher *WUE* and photosynthesis under severe drought stress.

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