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Research paper

Hydraulic differences along the water transport system of South American *Nothofagus* species: do leaves protect the stem functionality?

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Hydraulic traits were studied for six *Nothofagus* species from South America (Argentina and Chile), and for three of these species two populations were studied. The main goal was to determine if properties of the water conductive pathway in stems and leaves are functionally coordinated and to assess if leaves are more vulnerable to cavitation than stems, consistent with the theory of hydraulic segmentation along the vascular system of trees in ecosystems subject to seasonal drought. Vulnerability to cavitation, hydraulic conductivity of stems and leaves, leaf water potential, wood density and leaf water relations were examined. Large variations in vulnerability to cavitation of stems and leaves were observed across populations and species, but leaves were consistently more vulnerable than stems. Water potential at 50% loss of maximum hydraulic efficiency (P^{50}) ranged from -0.94 to -2.44 MPa in leaves and from -2.6 to -5.3 MPa in stems across species and populations. Populations in the driest sites had sapwood and leaves more vulnerable to cavitation than those grown in the wettest sites. Stronger diurnal down-regulation in leaf hydraulic conductance compared with stem hydraulic conductivity apparently has the function to slow down potential water loss in stems and protect stem hydraulics from cavitation. Species-specific differences in wood density and leaf hydraulic conductance (K_{Leaf}) were observed. Both traits were functionally related: species with higher wood density had lower K_{Leaf} . Other stem and leaf hydraulic traits were functionally coordinated, resulting in *Nothofagus* species with an efficient delivery of water to the leaves. The integrity of the more expensive woody portion of the water transport pathway can thus be maintained at the expense of the replaceable portion (leaves) of the stem–leaf continuum under prolonged drought. Compensatory adjustments between hydraulic traits may help to decrease the rate of embolism formation in the trees more vulnerable to cavitation.

Keywords: congeneric species, hydraulic conductivity, leaf hydraulic conductance, vulnerability to cavitation, water relations.

Introduction

Most studies on hydraulic architecture of plants have focused on the characteristics of roots and stems and more recently on

the hydraulic characteristics of leaves, whereas relatively few studies have examined simultaneously stem and leaf hydraulics (Hao et al. 2008, Chen et al. 2009, Johnson et al. 2011).

Resistance to water transport in leaves may account for 30–80% of the total hydraulic resistance along the whole-plant water transport pathway (Becker et al. 1999, Nardini 2001, Sack et al. 2002), even though the water transport pathway in leaves represents a very small fraction of the total distance that must be traversed as water moves from the soil to leaves. Furthermore, leaves appear to be more vulnerable to embolism than the stems to which they are attached, often losing a substantial fraction of their hydraulic conductance at higher water potentials (Brodribb and Holbrook 2004, Woodruff et al. 2007). However, embolism formation and repair usually occur more readily in leaf xylem than in stem xylem (e.g., Bucci et al. 2003, Hao et al. 2008). These findings suggest that leaves can be both a hydraulic bottleneck and a safety valve and thus traits related to water movement in leaves may be critical to the success of plants in water-limited environments.

Thirty-five extant species of *Nothofagus* are found in temperate forests of the southern hemisphere, including south-east Australia, Tasmania, New Zealand, New Guinea, New Caledonia and southern South America. South America has nine of those species grouped within three subgenera (Swenson et al. 2001). The South Pacific disjunct distribution of the *Nothofagus* genus has intrigued biologists for over a century. A simple vicariance model for explaining the evolution of *Nothofagus* from the breakup of the worldwide Fagales ancestors is currently a topic of debate (e.g., Swenson et al. 2001, Knapp et al. 2005, Head 2006). However, regardless of the mode of geographic evolution, it is assumed that the fruits are incapable of trans-oceanic dispersal, and consequently, the recent introduction of new *Nothofagus* species into South America by long-distance dispersal is unlikely. This implies that the nine South American species have probably evolved independently and radiated into the several environments where they actually grow.

Little information exists on the ecophysiological characteristics of the *Nothofagus* species from Chile and Argentina, despite their important contribution to the structure and functioning of temperate forests in South America (e.g., Martinez Pastur et al. 2007, Peri et al. 2009; Read et al. 2010, Varela et al. 2010, Jimenez-Castillo et al. 2011). The nine South American *Nothofagus* species occupy different habitats along precipitation and temperature gradients and are the dominant

species of nearly all the Patagonian forests on both Andean slopes in southern Chile and Argentina. Some species grow in relatively dry habitats (e.g., *Nothofagus glauca* (Phil.) Krasser, *Nothofagus pumilio* (Poepl. & Endl.) Krasser), while others grow in relatively wet habitats (e.g., *Nothofagus betuloides* (Mirb.) Oerst, *Nothofagus dombeyi* (Mirb.) Oerst). Regardless of the annual precipitation, all species experience a substantial seasonal decrease in soil water availability during the summer. The objective of this research was to study hydraulic traits of six species of the *Nothofagus* genus from temperate Argentinean and Chilean forests to determine if functional coordination exists between stems and leaves in terms of their water transport capacity. In addition, we evaluated the variability in vulnerability to cavitation and other hydraulic traits between two populations of three *Nothofagus* species growing in sites with different mean annual precipitation (MAP). We hypothesized that stem hydraulic architecture is coordinated with leaf hydraulic and functional traits linked to plant drought tolerance in *Nothofagus* species. Our second hypothesis was that stems should be less vulnerable to cavitation than leaves, consistent with the theory of hydraulic segmentation of trees along the vascular system. Our third hypothesis was that vulnerability to cavitation of stems and leaves is lower in populations of *Nothofagus* species growing in drier sites.

Materials and methods

Field sites

Field measurements were made between February and March 2005 (summer in the southern hemisphere) at five sites of *Nothofagus*-dominated forests in southern Argentina and Central Chile. Four sites were located in Argentinean Patagonia: the Baggilt Lake National Reserve, the La Hoya Ski Center, the Huemules Valley and Trevelin. The precipitation in all sites is highly seasonal, with a high proportion falling during winter. The driest site was located in Los Ruiles National Reserve in Region VII of Central Chile. Information about latitude, altitude, MAP, mean annual temperature (MAT) and maximum air temperature, minimum relative humidity, soil water content in the upper soil (0–25 cm depth) and pH NaF measured during the study period are indicated in Table 1. Air temperature and relative humidity during the study were measured with sensors

Table 1. Latitude, altitude, MAP, MAT, maximum air temperature (T_{max}), minimum relative humidity (RH_{min}), soil pH NaF and soil water content (SWC) in the 0–25 cm layer during the study period for five study sites in Argentina and Chile.

Site	Latitude	Altitude (m)	MAP (mm)	MAT (°C)	T_{max}	RH_{min} (%)	pH NaF ^a	SWC (%)
Baggilt Lake	43°16'S, 71°41'W	1200	>1500	3.6	24.8	39.4	10.1–12.2	47.2 ± 3.1
Trevelin	43°07'S, 71°33'W	380	1032	10.0	25.17	31.3	9.2–9.5	14.6 ± 2.8
Huemules Valley	42°55'S, 71°25'W	700	1000	9.6	28.7	30.2	10.5–11.6	16.2 ± 1.7
La Hoya	42°54'S, 71°18'W	900	900	7.2	25.56	28.5	8.5–11.0	11.5 ± 1.2
Los Ruiles	35°50'S, 72°30'W	224	671	15.3	23.51	35.0	–	14.3 ± 0.5

^apH NaF values >9.2 indicate presence of allophane in volcanic soils (Irisarri 2000).

connected to dataloggers (HOBOs Pro series; Onset Computer Corporation, Pocasset, MA, USA). In each site, soil samples were obtained following a systematic sampling in order to determine the water content and pH in sodium fluoride (NaF). pH NaF is an indicator of the presence of amorphous clays (imogolite, allophane) in volcanic soils. Amorphous clays in combination with soil organic matter generate stable aggregates that improve soil water availability (Warkentin and Maeda 1980, Wada 1985). In this study, we did not measure soil water availability; however, based on the soil water content and the presence or absence of clays we may have a non-quantitative estimate of the relative differences in water availability to plants in each site. The four sites located in Argentina corresponded to volcanic soils. The Baggilt and Huemules sites exhibited the presence of allophane all along the soil profiles; allophanic soils are characterized by high water availability (Warkentin and Maeda 1980). On the other hand, soils of Trevelin showed a transition imogolite/allophane, whereas soils in La Hoya Ski Center were shallow, rocky and amorphous clays were not always present. According to reference values for volcanic soils (Lanciotti and Cremona 1999), water contents <15%, as those found in Trevelin and La Hoya, suggest that these sites had a lower soil water availability than the other sites during the study period, at least compared with Argentinean sites.

Study species

Of the nine species of *Nothofagus* (Nothofagaceae) occurring in South America, six species were selected for this study. In three of the selected species with a wide geographical and ecological distribution, two different populations were included. The following species were studied at each site: at Baggilt Lake, *N. pumilio* (Poepp. et Endl.) Krasser and *Nothofagus antarctica* (G. Forster) Oerst; at La Hoya, *N. pumilio*; at Huemules, *N. antarctica*; at Trevelin, *N. dombeyi* (Mirb.) Oerst; at Los Ruiles, *N. dombeyi*, *Nothofagus alessandrii* Espinosa, *Nothofagus obliqua* (Mirb.) Oerst, and *N. glauca* (Phil) Krasser.

At each site, five tall mature individuals with an unobstructed crown were selected per species and population. Table 2

Table 2. Species name, common name, latitudinal and altitudinal range and leaf phenology of the *Nothofagus* species studied.

Species name	Common name	Latitude south	Altitude (m)	Phenology
<i>N. antarctica</i> (N)	Nire	35–56°	200–2000	Deciduous
<i>N. pumilio</i> (N)	Lenga	36–55°	0–1800	Deciduous
<i>N. dombeyi</i> (N)	Cohiue	39–45°	400–1200	Evergreen
<i>N. obliqua</i> (L)	Hualo	34–38°	100–350	Deciduous
<i>N. glauca</i> (L)	Roble	33–36°	150–500	Deciduous
<i>N. alessandrii</i> (F)	Ruil	35°	150–500	Deciduous

Within parentheses and after the species name (first column) is the subgenera: N, *Nothofagus*; L, *Lophozonia*; and F, *Fuscopora* (from Swenson et al. 2001).

shows the species names, altitudinal and latitudinal distribution and leaf phenology of the six *Nothofagus* species studied.

Leaf water potential

Predawn and midday leaf water potential (predawn Ψ_{Leaf} and midday Ψ_{Leaf}) were measured with a pressure chamber (PMS1000, PMS Instrument Co., Corvallis, OR, USA). Leaf samples were taken between 06:00 and 07:00 h and between 12:00 and 14:00 h, respectively. For each of the species and populations, 15 terminal branches from five different individuals were cut with a sharp razor blade and sealed immediately in small plastic bags with moist paper towels in them and kept briefly in a cooler until balancing pressures were determined in the laboratory. Measurements were made during the end of the dry season and during days with maximum air temperature and low relative humidity higher and lower, respectively, than the mean annual values (Table 1). Consequently and although the measurements were made during one day in each site, we consider that predawn Ψ_{Leaf} and midday Ψ_{Leaf} measured represent seasonal minima or the largest amounts of water deficits that each species is subjected to, in accordance with results of Jimenez-Castillo et al. (2011) and M. Jimenez-Castillo (unpublished data) who measured similar midday Ψ_{Leaf} in *Nothofagus* species after 3 or 4 months rainless.

Wood density, leaf mass per area and leaf water content

Wood density (ρ) was measured by the water-displacement method on five terminal stems from each species and population. After removal of the bark and pith, ρ was calculated as

$$\rho = M / V$$

where M is the dry mass of the sample (oven-dried at 60 °C for 72 h) and V is the sample volume. Volume was estimated by submerging the sample in a container with distilled water resting on a digital balance with a 0.001 g precision. The sample was kept submerged during measurements until saturation with the help of a very small needle, without touching the walls of the container. The mass (M) registered on the balance represents the volume of water displaced by the sample, times the density water. The sample volume is then calculated as $V_{\text{sample}} = M / \rho_{\text{H}_2\text{O}}$.

Hydraulic conductivity

Stem hydraulic conductance (k_h) was measured on five branches per species and population, taken from five different individuals. Samples were collected early in the morning by cutting branches from the canopy in air, and then re-cutting under water in order to relax xylem tensions and prevent the formation of non-native embolism during transport. The cut ends were kept under water and the entire branches were tightly covered with plastic bags and transported to the

laboratory. Then the stem segments were recut immediately under water and attached to the hydraulic conductivity apparatus. Maximum vessel length estimated using the method described by Zimmerman and Jeje (1981) varied from 20 to 40 cm (data not shown). Stem segments much longer than the maximum vessel length were used to avoid open vessels. Distilled water was used as the perfusion fluid. Relatively low hydrostatic pressure generated by a constant hydraulic head of 32 cm was applied to avoid refilling of embolized vessels. Hydraulic conductivity ($\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) was calculated as the ratio of the flow rate through the segment (J_v ; kg s^{-1}) and the pressure gradient across the segment ($\Delta P/\Delta L$; MPa m^{-1}). Sapwood conducting area (A_S) and distal leaf area (A_L) were measured to calculate the ratio $A_L : A_S$. Specific hydraulic conductivity (k_S ; $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) was calculated as the ratio of k_h to A_S and leaf-specific hydraulic conductivity (k_L ; $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) was calculated as the ratio of k_h to A_L .

Stem xylem vulnerability

The air injection method was used to measure the vulnerability of stem xylem to water-stress-induced embolism (Sperry and Saliendra 1994). Five terminal branches from five different trees per species and/or population were collected in the field as described above. The segments were inserted into the air-injection chamber with both ends protruding and attached to the tubing system for measuring k_h as described above. Air emboli were removed by flushing the samples at 100 kPa for 15 min with filtered water and the k_h maximum ($k_{h\text{max}}$) was calculated. A vulnerability curve was generated by first pressurizing to 0.5 MPa for 15 min. After equilibration, $k_{h(0.5)}$ was measured. The k_h was measured for pressures ranging from 0.5 to 7.0 MPa or until the conductivity of the segment was negligible. The percentage of loss of conductivity (PLC_{stem}) was then calculated as

$$\text{PLC}_{\text{stem}} = 100(k_{h\text{max}} - k_h) / k_{h\text{max}}$$

Three to five curves were generated for each species and population by plotting the PLC_{stem} against increasing applied pressure. The xylem pressure that induced a 50% loss of stem conductivity (P_{stem}^{50}) and the PLC stem at midday leaf water potential were estimated from vulnerability curves. Vulnerability curves shown in Figure 1 were obtained by pulling all data of the three to five curves obtained per species and population.

Leaf vulnerability and pressure–volume curves

Leaf hydraulic vulnerability curves were determined by measuring leaf hydraulic conductance (K_{Leaf}) using the partial rehydration method described by Brodribb and Holbrook (2003). The measurement is based on the analogy between rehydration of desiccated leaves and charging of a capacitor through a resistor as follows:

$$K_{\text{Leaf}} = C \ln(\Psi_0 / \Psi_t) / t$$

where C is the leaf capacitance, Ψ_0 is the leaf water potential before rehydration and Ψ_t is the leaf water potential after rehydration for t seconds. A range of leaf water potentials was attained through slow bench drying of leafy branches collected from the field. Leaf water potentials for K_{Leaf} calculations were then measured after 0.5–1 h equilibration inside black plastic bags with slightly wet paper towels. Leaf vulnerability curves were plotted as PLC_{Leaf} against initial Ψ_{Leaf} before rehydration.

Capacitance values both before and after turgor loss point (π^0) were calculated from leaf pressure–volume relations (Tyree and Hammel 1972) and expressed in absolute terms and normalized by leaf area using the following equation:

$$C = \Delta \text{RWC} / \Delta \Psi_L \times (\text{DM} / \text{LA}) \times (\text{WM} / \text{DM}) / M$$

where RWC is the leaf relative water content, DM is leaf dry mass (g), LA is leaf area (m^2), WM (g) is mass of leaf water at 100% RWC (WM = fresh mass – dry mass) and M is molar mass of water (g mol^{-1}).

Pressure–volume curves were determined for four or five terminal branches from each species and population. Terminal branches for pressure–volume analyses were cut in the field at predawn, re-cut immediately under water and covered with black plastic bags with the cut end in water for ~2 h until measurements began. After each determination of balancing pressure with the pressure chamber, terminal branches were immediately weighed to the nearest 0.001 g and, until the next measurement, left to transpire freely on the laboratory bench. After all balancing pressure–weight measurements were completed, the terminal branches were oven-dried at 70 °C to a constant mass and weighed (Tyree and Richter 1981). Pressure–volume curves were analyzed using a program developed by Schulte and Hinckley (1985) which allows estimating osmotic potential at the turgor loss point (TLP).

Safety margin

To assess the degree of stomatal control of evapotranspiration losses before substantial run-away embolism can occur in the stems, a safety margin between stomatal closure and the depression of stem hydraulic conductivity was defined. The safety margin was quantified as the difference between osmotic potential at the TLP and the xylem pressure resulting in a 50% loss of stem conductivity.

Statistical analysis

All data were tested for normal distribution and homogeneity of variance. Differences in physiological traits between the two populations of *N. pumilio*, *N. antarctica* and *N. dombeyi* were analyzed using a *t*-test. The significance of species effect was

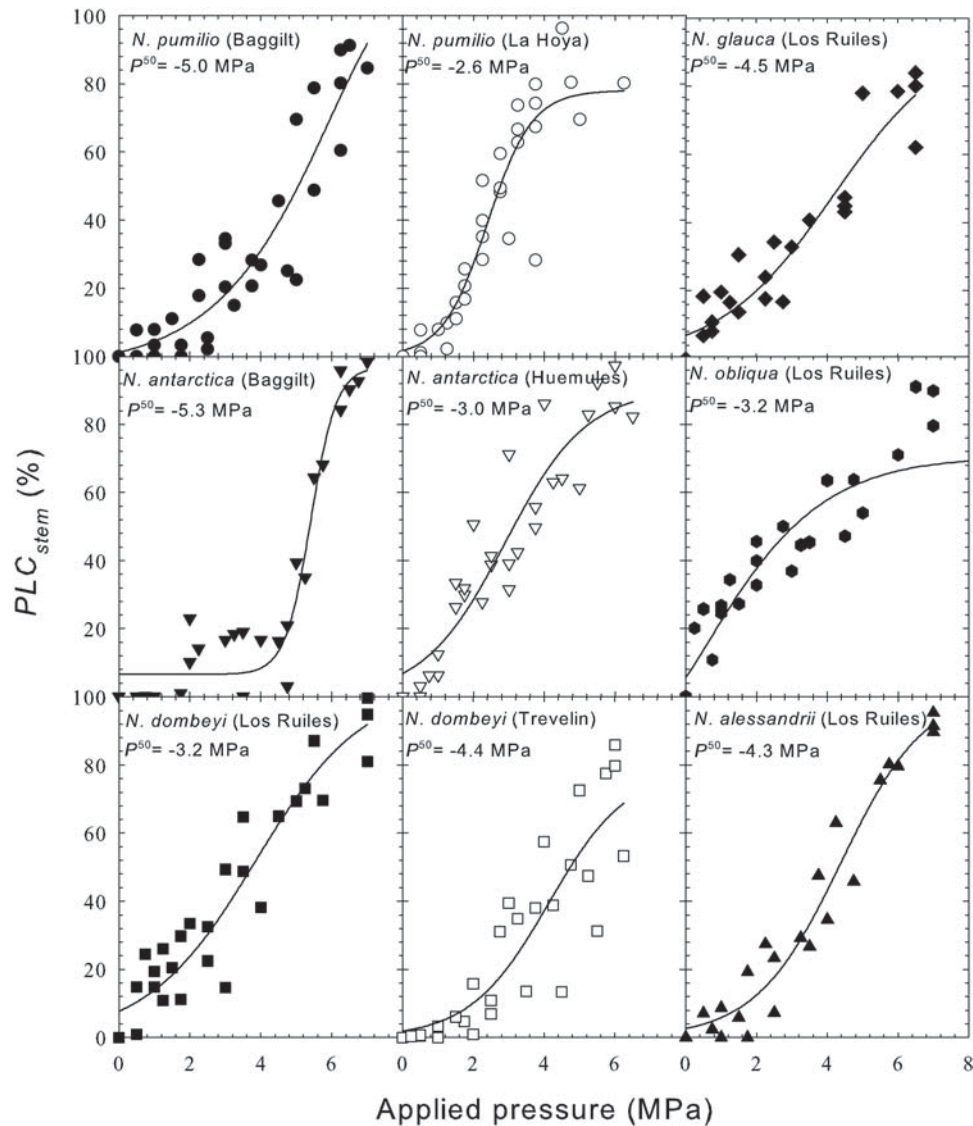


Figure 1. Stem vulnerability curves of all *Nothofagus* species and populations. Sigmoid function curves were fitted to the data ($R^2 > 0.8$; $P < 0.05$). In each panel the water potential at 50% loss of stem hydraulic conductivity (P^{50}) is indicated.

determined by one-way analysis of variance and a Tukey's HSD (honestly significant difference) test. Evaluation of significant differences in the leaf vulnerability to cavitation was not possible with only one vulnerability curve per species and population. Stem and leaf vulnerability curves were best described by sigmoid functions that resulted in $R^2 > 0.80$. Correlations between two variables were examined by linear or non-linear regression analyses.

Results

The studied sites differed in MAP and mean annual temperature (MAT) (Table 1). Los Ruiles was the driest and warmest of the six sites, whereas Baggilt Lake was the wettest and coldest one. Within one species, the two populations were in sites with different MAP and MAT. The driest site for *N. pumilio* was La

Hoya, for *N. antarctica* was the Huemules Valley and for *N. dombeyi* was Los Ruiles. During the study period (end of the dry season) the maximum air temperature was substantially higher than MAT and the soil water content in the shallowest layers (0–25 cm) varied between 45% in the Baggilt site and 11% in the La Hoya site.

The *Nothofagus* species exhibited significant variations in stem vulnerability to cavitation ($F = 68$; $P < 0.001$; Figure 1). Water potential at 50% loss of maximum stem hydraulic conductivity (P^{50}_{stem}) at species level varied between -3.2 MPa in *N. obliqua* to -4.5 MPa in *N. glauca*. This range was extended when the populations (-5.3 MPa in *N. antarctica* to -2.6 MPa in *N. pumilio*) were included in the analysis (Figure 1). While *N. pumilio* was the species with the largest variation in P^{50}_{stem} between populations (2.4 MPa; $P < 0.001$), *N. dombeyi* exhibited the lowest variations in P^{50}_{stem} across sites (1.2 MPa;

$P < 0.05$). There were also species and population-specific variations in leaf vulnerability to cavitation (Figure 2) but the magnitude of the variation was lower than for P^{50}_{stem} . The pressure at 50% loss of maximum leaf conductance (P^{50}_{Leaf}) across species and populations ranged between -0.93 and -2.44 MPa (Figure 2). Species growing at Los Ruiles had higher (values closer to zero) P^{50}_{Leaf} (-1.24 ± 0.19 MPa) than species or populations from the other sites (-1.98 ± 0.14 MPa; $P < 0.05$).

The largest species-specific difference between P^{50}_{Stem} and P^{50}_{Leaf} was 3.56 MPa in *N. glauca* (Figures 1 and 2). Within one species, *N. pumilio* exhibited both the smallest and the largest differences between P^{50}_{Stem} and P^{50}_{Leaf} depending on the populations (2.9 MPa at the Baggilt site and 0.77 MPa at the La Hoya site; Figures 1 and 2). There was no significant

relationship between P^{50}_{stem} and P^{50}_{Leaf} (data not shown; $R^2 = 0.04$ $P = 0.6$). Nevertheless, the predicted percent loss of hydraulic conductance in leaves (PLC_{Leaf}) at midday Ψ_{Leaf} across species and populations was positively correlated with the predicted percent loss of hydraulic conductance in stems (PLC_{Stem}) at midday Ψ_{Leaf} (Figure 3). In nearly all cases PLC_{Leaf} was higher than PLC_{Stem} with the exception of a population of *N. pumilio*, which had similar PLC_{Leaf} and PLC_{Stem} . The stem water potential is usually substantially higher than the Ψ_{Leaf} and consequently PLC_{stem} should have been even lower and further apart from the species-specific PLC_{Leaf} if we had used stem water potential, instead of Ψ_{Leaf} , to develop the PLC_{stem} vs. PLC_{Leaf} relationship of Figure 3.

Species- and population-specific wood density ranged between 0.49 and 0.62 g cm^{-3} (Figure 4). There were signifi-

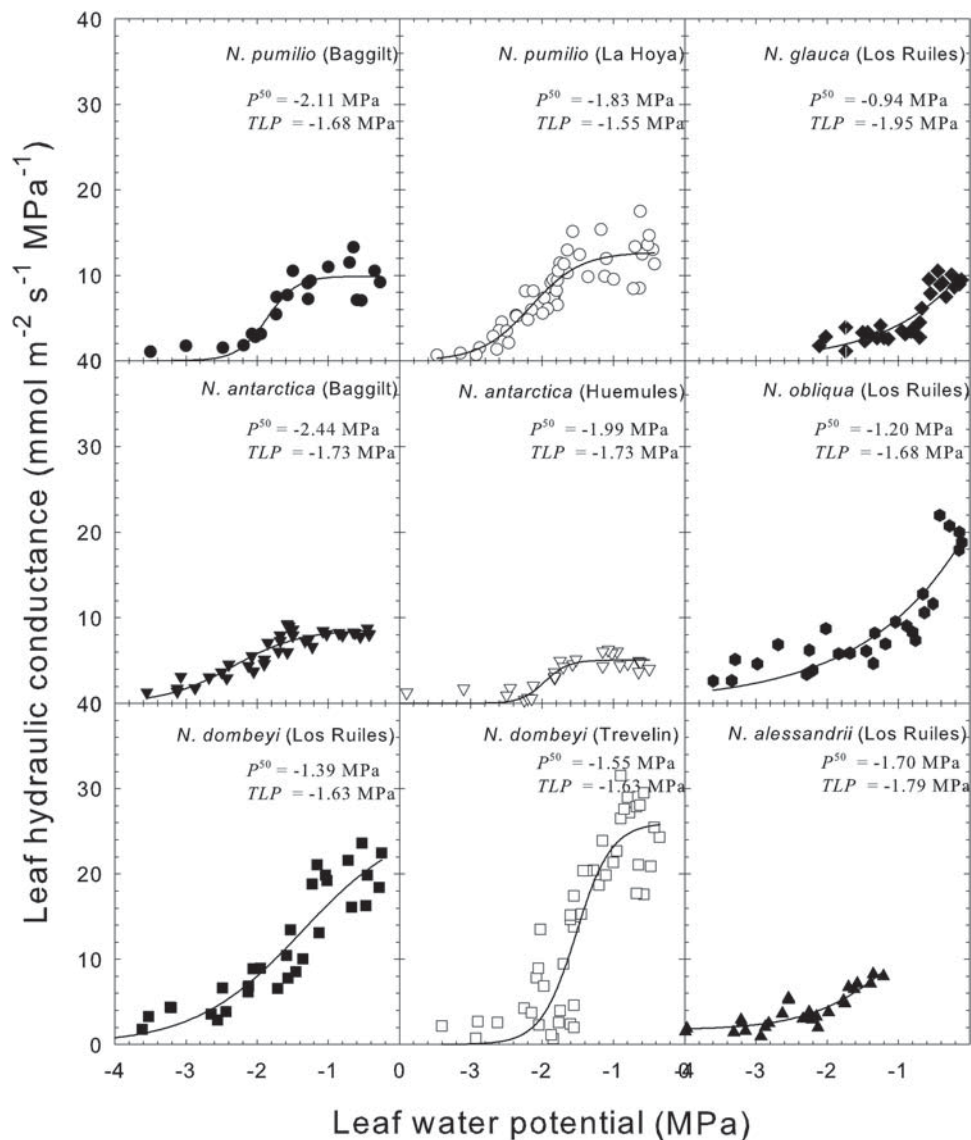


Figure 2. Leaf vulnerability curves of all *Nothofagus* species and populations. Sigmoid function curves were fitted to the data ($P < 0.05$). In each panel the water potential at 50% loss of maximum leaf hydraulic conductance (P^{50}) and TLP are indicated.

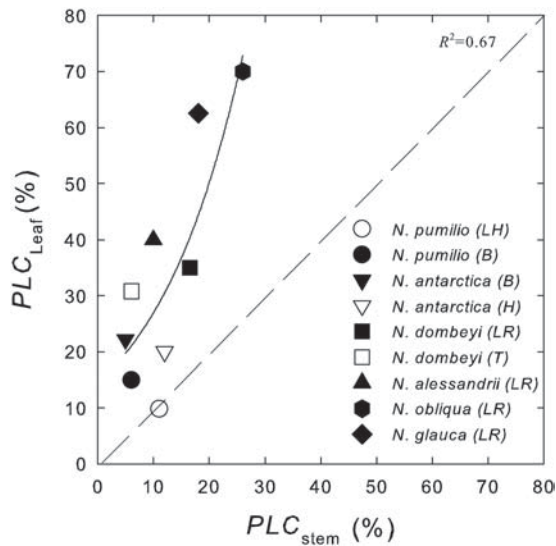


Figure 3. Relationship between percent loss of stem hydraulic conductivity (PLC_{stem}) at midday leaf water potentials (Ψ_{Leaf}) and percent loss of leaf hydraulic conductance (PLC_{Leaf}) at midday Ψ_{Leaf} obtained from stem and leaf vulnerability curves of all *Nothofagus* species and populations. The continuous line is the growth exponential function fitted to the data ($y = 14 \exp(0.06x)$; $P < 0.01$). The dashed line is the 1 : 1 relationship between PLC_{stem} and PLC_{Leaf} .

cant differences in wood density between species ($F = 2.7$; $P < 0.05$). The highest wood density was found in *N. alessandrii* (0.62 g cm^{-3}). Within one species there was a tendency for lower wood density in the populations of the driest sites, but only *N. pumilio* had significant differences in wood density between populations ($P < 0.01$) with the La Hoya population having the lowest wood density. The safety margin ($P_{\text{stem}}^{50} - \text{TLP}$) and P_{stem}^{50} were positively and negatively correlated, respectively, to wood density across species and populations, suggesting that high wood density was associated with high resistance to embolism formation (Figure 4). Wood density explained $\sim 50\%$ of the variation in this safety margin and in the vulnerability to cavitation across populations and species. The population of *N. pumilio* at La Hoya was at the lower end of these functional relationships and therefore had a safety margin of only 1 MPa and a stem P_{stem}^{50} of -2.6 MPa , implying that the terminal stems of this species were fairly vulnerable to cavitation. *Nothofagus pumilio* and *N. antarctica* exhibited larger variation in the safety margin between populations ($\sim 2.3 \text{ MPa}$) than *N. dombeyi* (0.4 MPa ; $P < 0.05$).

Maximum specific hydraulic conductivity (k_s) measured in the early morning varied between species ($F = 2.40$; $P = 0.05$), whereas leaf-specific hydraulic conductivity (k_L) was independent of the species (Figure 5). Nevertheless, significant differences in k_s and k_L were found within one species. For example, the populations of *N. pumilio* at La Hoya and of *N. antarctica* at Huemules had significantly higher k_s and k_L than Baggilt populations ($P < 0.05$). Leaf-specific and specific hydraulic conduc-

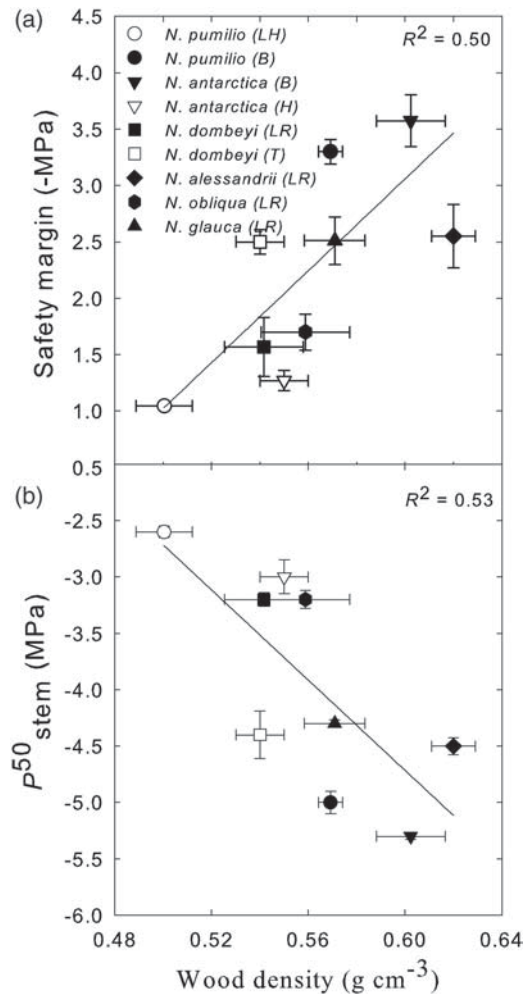


Figure 4. Relationship between (a) the stem safety margin ($P_{\text{stem}}^{50} - \text{TLP}$) and wood density and (b) P_{stem}^{50} and wood density of six species of *Nothofagus* and two populations of three species in southern Argentina and Central Chile. Each point in (a) represents the mean value of three to five trees for TLP ($n = 3-5 \pm 1 \text{ SE}$) and $n = 5$ for wood density, and the line represents the linear regression fitted to the data ($y = -7.7 + 17.2x$; $P < 0.05$). The line in (b) is the linear regression fitted to the data ($y = 7.3 - 20.0x$; $P < 0.1$).

tivity were linearly and positively correlated across species and populations (Figure 5). Both k_s and k_L increased exponentially with decreasing wood density (Figure 6). The population of *N. pumilio* at Los Ruiles had the lowest wood density and highest k_s and k_L . There was no significant relationship between P_{stem}^{50} and k_s ($R^2 = 0.03$; $P = 0.64$) across species and populations (results not shown).

The *Nothofagus* species exhibited significant variations in predawn and midday leaf water potential (Ψ_{Leaf}) measured at the end of the dry season (predawn Ψ_{Leaf} $F = 54.3$; $P < 0.001$; midday Ψ_{Leaf} $F = 14$; $P < 0.001$). Predawn Ψ_{Leaf} varied between $-0.22 \pm 0.02 \text{ MPa}$ in *N. obliqua* and $-1.26 \pm 0.10 \text{ MPa}$ in *N. alessandrii* (data not shown) and midday Ψ_{Leaf} ranged from $-1.3 \pm 0.04 \text{ MPa}$ in *N. dombeyi* to $-1.96 \pm 0.04 \text{ MPa}$ in *N. alessandrii* (Figure 7a). Populations of three species of

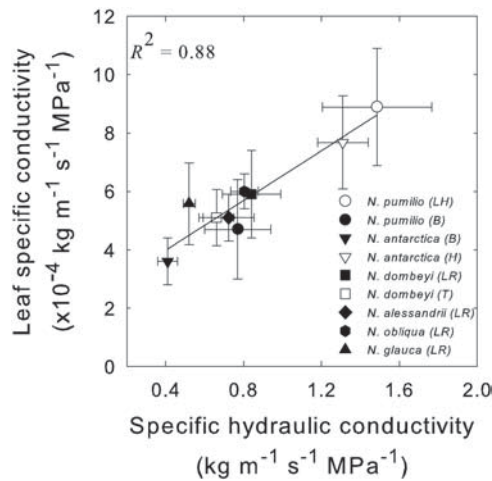


Figure 5. Relationship between leaf hydraulic conductivity and specific hydraulic conductivity of six species of *Nothofagus* and two populations of three species in southern Argentina and Central Chile. Each point represents the mean value ± 1 SE of five trees per species and population ($n = 5$). The line is the linear regression fitted to the data: $y = 2.27 + 4.27x$; $R^2 = 0.88$, $P < 0.001$.

Nothofagus had higher (less negative) predawn and midday Ψ_{Leaf} in the driest sites than in the wettest sites, but only populations of *N. antarctica* and *N. dombeyi* exhibited significant differences in predawn Ψ_{Leaf} ($P < 0.001$) and only *N. antarctica* exhibited also differences in midday Ψ_{Leaf} ($P < 0.001$) between populations. Leaf water potentials at TLPs varied between species (Figure 2; $F = 2.63$; $P < 0.05$) and were more negative than midday Ψ_{Leaf} in all species or populations ($P < 0.05$), except in *N. glauca*, in which both values were similar (indicating that all the species except *N. glauca* maintained cell turgor in their leaves).

Leaf capacitance showed significant differences between species ($F = 4.32$; $P < 0.05$) with *N. alessandrii* having the lowest leaf capacitance (Figure 7b). Differences between populations were not significant, although the populations of two species tended to have higher leaf capacitance in the driest sites. While leaf capacitance ranged from 0.18 to 0.7 mol m⁻² MPa⁻¹ across species and populations, leaf hydraulic conductance (K_{Leaf}) estimated at midday Ψ_{Leaf} varied 5-fold (from 4 to 18.5 mmol m⁻² s⁻¹ MPa⁻¹) (Figure 7). *Nothofagus dombeyi* exhibited the highest midday K_{Leaf} among all species (between 15.2 and 18.5 mmol m⁻² s⁻¹ MPa⁻¹). Leaf hydraulic conductance measured in the morning (morning K_{Leaf}) also varied significantly between species (from 23.01 \pm 0.11 mmol m⁻² s⁻¹ MPa⁻¹ in *N. dombeyi* to 6.25 \pm 0.90 mmol m⁻² s⁻¹ MPa⁻¹ in *N. antarctica*; $F = 56$; $P < 0.001$; data not shown) but within one species there were no significant differences between populations. Leaf hydraulic conductance (K_{Leaf}) at Ψ_{Leaf} corresponding to the TLP was relatively high (close to or >10 mmol m⁻² s⁻¹ MPa⁻¹) in most species (e.g., *N. pumilio* and *N. dombeyi*; data not shown). There was

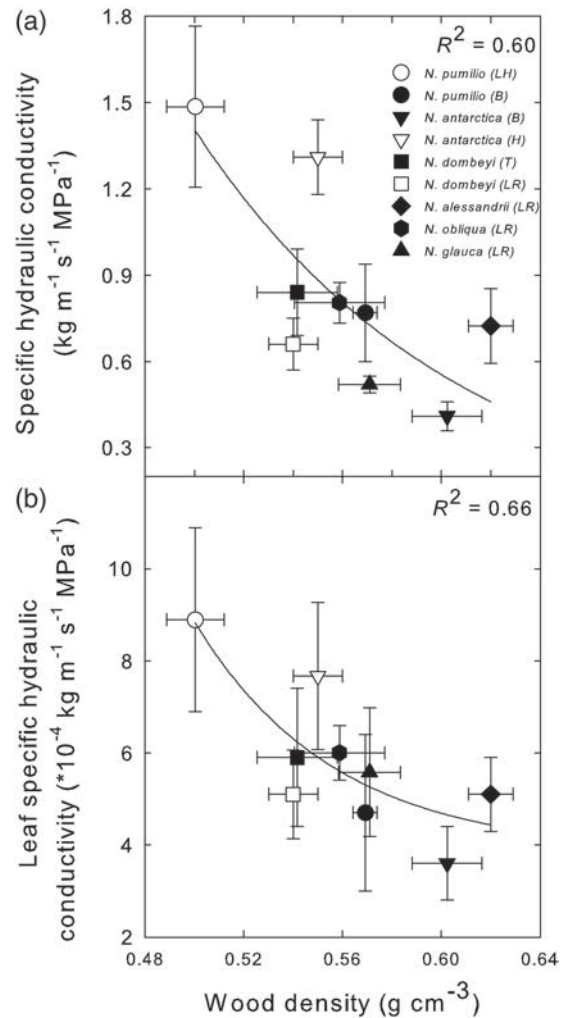


Figure 6. (a) Specific hydraulic conductivity (k_s) and (b) leaf-specific hydraulic conductivity (k_L) as a function of wood density. Each point represents the mean value ± 1 SE of five trees per species and population for k_s , k_L and wood density ($n = 5$). The lines are the linear regressions fitted to the data: (a) $y = 0.48 + 33900 \times \exp(-20.87x)$; $P < 0.05$; and (b) $y = 3.83 + 35258 \times \exp(-17.7x)$; $P < 0.05$.

no significant relationship between P_{Leaf}^{50} and K_{Leaf} across species and populations ($R^2 = 0.2$; $P = 0.21$; results not shown). Leaf capacitance was positively correlated with midday K_{Leaf} ($R^2 = 0.67$, $P < 0.05$; data not shown).

These leaf properties were also associated with traits of the stems such as wood density (Figure 7). Midday Ψ_{Leaf} , leaf capacitance and midday K_{Leaf} were all linearly and negatively correlated to wood density across species and populations (Figure 7). Species and populations with the lightest wood were able to maintain higher midday Ψ_{Leaf} and K_{Leaf} and had higher leaf capacitance. *Nothofagus alessandrii* was the species with the lowest leaf capacitance and K_{Leaf} and the highest wood density, while *N. pumilio* had the highest leaf capacitance.

There was no relationship between maximum k_L and Huber values ($A_S:A_L$) and between maximum k_L and K_{Leaf} ($R^2 = 0.09$

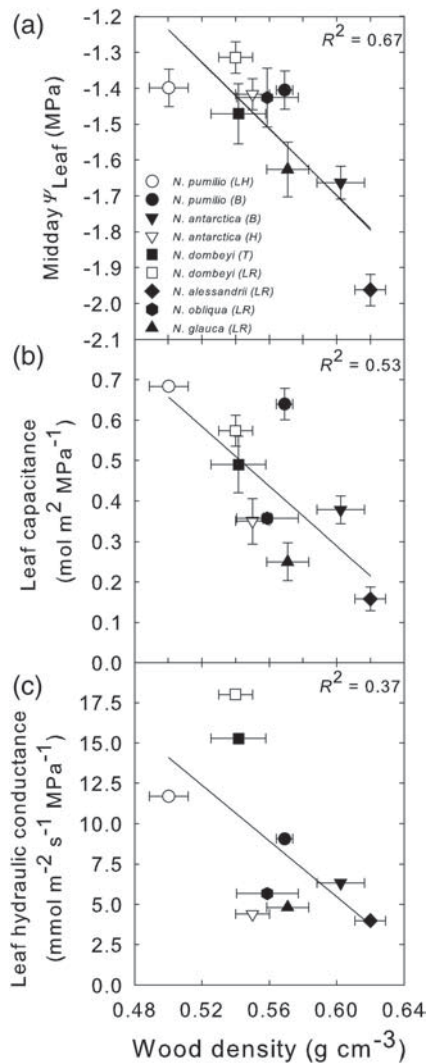


Figure 7. Relationship between (a) midday Ψ_{Leaf} and wood density, (b) leaf capacitance and wood density and (c) midday leaf hydraulic conductance (K_{Leaf}) and wood density in six species of *Nothofagus* and two populations of three species in southern Argentina and Central Chile. The lines are the linear regressions fitted to the data: (a) $y = 1.1 - 4.6x$; $P < 0.01$; (b) $y = 2.5 - 3.7x$; $P < 0.05$; (c) $y = 57 - 86x$; $P < 0.1$. Each point represents the mean value of five trees per species/population ($n = 5 \pm 1\text{SE}$) and five leaves or branches per tree for midday Ψ_{Leaf} and wood density and $n = 3-5$ per species/population for leaf capacitance.

and $R^2 = 0.03$, respectively). Nevertheless, the $A_L:A_S$ ratio increased asymptotically with increasing midday K_{Leaf} across species and populations (Figure 8a). While K_{Leaf} is a leaf trait, $A_L:A_S$ is a morphological variable reflecting the water transport capacity of stems in relation to potential water demand of leaves. According to this relationship, the lower stem hydraulic capacity to supply water to the leaves observed in some species and populations was compensated for by a higher leaf hydraulic efficiency. The difference between midday Ψ_{Leaf} and predawn Ψ_{Leaf} decreased exponentially with increasing K_{Leaf} estimated at midday Ψ_{Leaf} (Figure 8b).

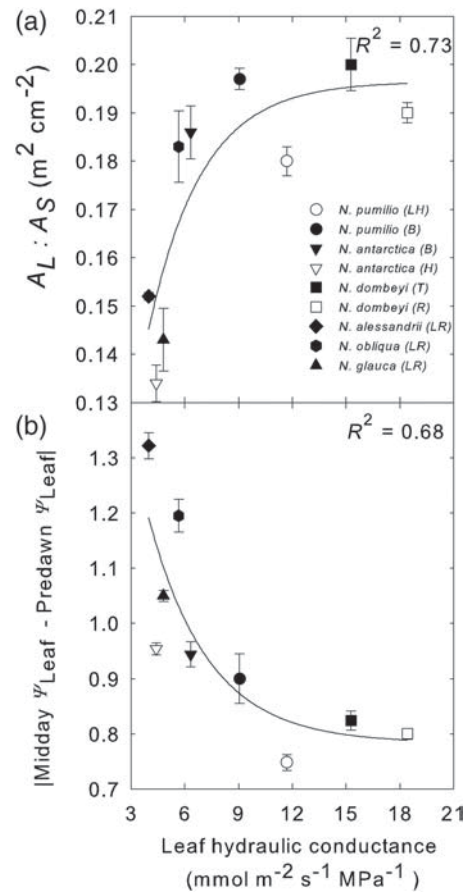


Figure 8. (a) The ratio of leaf surface area to the cross-sectional area of active xylem and (b) the difference between midday Ψ_{Leaf} and predawn Ψ_{Leaf} (midday $\Psi_{\text{Leaf}} - \text{predawn } \Psi_{\text{Leaf}}$) in relation to leaf hydraulic conductance measured at midday for all species and populations studied. The line in (a) is the exponential rise to the maximum function fitted to the data ($y = 0.19 \times (1 - \exp(-0.33x))$; $P < 0.005$) and the line in (b) is the exponential decay function fitted to the data ($y = 1.95 + 237 \times \exp(-3.89x)$; $P < 0.05$).

Discussion

Intra-specific variation in vulnerability to cavitation

Significant intra-specific variations in stem resistance to cavitation were found in two *Nothofagus* species (*N. antarctica* and *N. pumilio*) growing in sites with different MAP. The differences in vulnerability between populations could be the consequence of phenotypic plasticity driven by environmental conditions or by population-specific genetic differences. Studies in a common garden with populations of *N. pumilio* growing along altitudinal gradients close to our study site in Argentina (Premoli and Brewer 2007, Premoli and Mathiasen 2011) found that photosynthesis and morphological characteristics associated with carbon assimilation are under genetic control, whereas ecophysiological traits related with water use have plastic environmental-induced responses. Thus, regardless of the origin of the differences we assumed that environmental factors are involved in the observed variations in resistance to cavitation between populations.

Our findings are contrary to some studies suggesting a lack of adjustment in vulnerability to cavitation at intra-specific level (e.g., Martínez-Vilalta and Piñol 2002, Martínez-Vilalta et al. 2009), while they are in line with the results obtained for populations of *Fagus sylvatica* L. (Herbette et al. 2010, Wortemann et al. 2011) and other woody species (e.g., Choat et al. 2007, Beikircher and Mayr 2009, Barnard et al. 2011) which show a high plasticity in stem P^{50} . However, the magnitude of variation in stem P^{50} between populations of *Nothofagus* was very large (up to 2.4 MPa) and much larger than that in those studies. The degree of phenotypic plasticity in stem P^{50} seems to be related with the ecological distributional range (geographic and altitudinal). For example, *N. pumilio* and *N. antarctica* with 2.4 and 2.3 MPa of difference in stem P^{50} between populations are the species with the broader altitudinal and longitudinal distribution compared with *N. dombeyi* that had a difference of only 1.2 MPa between populations.

Similar to other studies (Martínez-Vilalta et al. 2009, Barnard et al. 2011) and contrary to our expectations, higher vulnerability to embolisms in stems and leaves occurred in populations at the driest sites with lower MAP. Nevertheless, this is consistent with the midday Ψ_{Leaf} measured at the end of the dry season which tended to be less negative in the dry sites, suggesting that these species should have adaptations to maintain relatively high water potentials at low water availability. Barnard et al. (2011) found that two conifers had higher sapwood capacitance to compensate for the lower resistance to cavitation in the more arid conditions. Although we did not estimate the sapwood capacitance, differences in wood density (a proxy of the sapwood capacitance; Meinzer et al. 2003, 2006, Scholz et al. 2007) between populations, with lower wood density in the driest sites, suggest that a higher water storage capacity could avoid morphological changes (e.g., conduit diameter, cell wall reinforcement, pit properties) that would increase resistance to cavitation. In addition, the higher water transport efficiency at xylem branch and leaf level (k_S , k_L and K_{Leaf}) might allow for sufficient acquisition of water for trees with higher hydraulic vulnerability, preventing the drop of Ψ_{Leaf} and avoiding embolism in populations experiencing lower soil water availability. These hydraulic adjustments would limit also the need for energetic expenditure for osmotic adjustments as we did not find changes in TLPs between populations. However, the compensatory responses to high vulnerability to cavitation could not be enough under severe drought, in particular for species with lower phenotypic plasticity such as *N. dombeyi* that experienced dieback during the most severe drought of the 20th century (1998–1999, Suárez et al. 2004).

On the other hand, the differences in vulnerability to cavitation between populations could be the consequence of the differences in MAT. In this study, wetter sites (Baggilt and

Trevelin) have also lower MATs (3.6 and 10 °C, respectively) and are subjected to more freeze–thaw events per season than the more arid sites (Huemules, La Hoya and Los Ruiles). In agreement with this, the lower P^{50} (more negative) found in populations of *N. antarctica*, *N. pumilio* and *N. dombeyi* in the wettest and coldest sites could represent a strategy to avoid freeze-induced embolisms because they are subjected to longer periods of below freezing temperature. Although cavitation resistance apparently depends mainly on pit membrane properties (Tyree and Zimmermann 2002, Hacke et al. 2004, Pittermann et al. 2006, Cochard et al. 2009), the conduit diameter has also been observed to be strongly correlated with the vulnerability to cavitation (Davis et al. 1999, Feild and Brodrigg 2001, Pittermann and Sperry 2003) and, in agreement with the Hagen–Poiseuille law, with the water transport efficiency. In our case, the lower k_S in the wettest sites indicates that the populations in those sites have smaller conduit lumen diameter, thus reducing the risk of freeze–thaw embolism formation compared with populations from the driest sites.

Differences in vulnerability to cavitation between leaves and stems

Leaves were more vulnerable to embolism than the stems to which they were attached, with P^{50} of leaves being substantially higher than P^{50} of stems in all *Nothofagus* species and populations studied. Thus, under prolonged drought, the integrity of the more expensive woody portion of the water transport pathway can be maintained at the expense of the more replaceable and terminal portions (leaves) of the stem–leaf continuum. The leaves can be shed if leaf water potential reaches the tree-specific threshold value and consequently water loss is restricted (total tree transpiration decreases) maintaining the functionality of the stems. Thus, the leaf embolism would act as a safety valve to protect the hydraulic pathway between the soil and the stem (Chen et al. 2010). However, this is not the case for *N. pumilio* at La Hoya which had P^{50} stems close to P^{50} leaves (−2.6 and −1.83 MPa for stems and leaves, respectively). Stems of this species were only 0.5 MPa more resistant to drought-induced cavitation than leaves. This species had the lowest wood density and the highest k_S and k_L across all populations and species studied. With the exception of *N. pumilio* at La Hoya, stem xylem was operating far from the point of catastrophic dysfunction *sensu* Tyree and Sperry (1988). Similar relative differences in vulnerability to cavitation between stems and leaves were observed in other studies (e.g., Brodrigg and Holbrook 2004, Woodruff et al. 2007, Hao et al. 2008, Zhang et al. 2009, Chen et al. 2009, 2010, Johnson et al. 2011). However, this pattern was not observed in sympatric deciduous trees of the Euphorbiaceae family where P^{50} of stems and leaves was similar (Chen et al. 2009) as well as in

canopy tree species in the subtropical Atlantic Forest of Northern Argentina (M. Villagra, unpublished results). It is difficult to predict how general this pattern of comparatively high vulnerability to cavitation of leaves compared with stems will be when additional information across a broader range of species and environments is obtained, but so far these findings suggest that leaves are one major hydraulic bottleneck for plant water transport. Thus, traits related to water movement in leaves should be critical to the success of plants in water-limited environments.

Based on the morning K_{Leaf} and leaf vulnerability curves in this study with *Nothofagus* species, substantial loss in K_{Leaf} is predicted to occur on a daily basis for all species and populations at midday Ψ_{Leaf} . For example, *N. obliqua* with a midday Ψ_{Leaf} of -1.42 MPa loses >50% of its maximum K_{Leaf} . This predicted midday K_{Leaf} decline was also consistent with the results of previous studies that showed that K_{Leaf} can be depressed by relatively moderate environmental stresses, such as high irradiance and low relative humidity at midday (Bucci et al., 2003, Brodribb and Holbrook 2004). Down-regulation at midday of K_{Leaf} and k_L varied across species; however, it was stronger in most of the species (with the exception of *N. pumilio*) in leaves compared with stems. Midday K_{Leaf} depression may constitute a hydraulic signal for stomatal regulation to prevent further water loss and therefore to reduce embolism formation in stems (Brodribb and Holbrook 2003, Zhang et al. 2009). Consistent with this, the average percent loss of midday hydraulic conductivity across the stems of all *Nothofagus* trees studied was low (i.e., only 12.3%). Depressions in hydraulic conductivity associated with dehydration have been partially explained by xylem embolism formation (Bucci et al. 2003, Johnson et al. 2011), requiring daily repair to avoid accumulation of non-functional conduits and total loss of conductivity. Embolism refilling is a function of the internal pressure imbalance produced by an increase in osmotically active solutes in cells outside the vascular bundles in leaves during the day and by the consequent increase in tissue water volume which is constrained by the cortex (Bucci et al. 2003). Consequently, embolized conduit repair in portions of the water transport pathway distant from the sites of carbohydrate synthesis may not be advantageous in energetic terms and low loss of hydraulic conductivity at stem level may constitute a mechanism to avoid the cost involved in embolism repair.

Hydraulic coordination between leaves and stems

Hydraulic coordination in this study was revealed by correlated changes in hydraulic traits of stems and leaves, which were consistent with an efficient delivery of water to the transpiring leaves. For example, across *Nothofagus* species and populations, higher K_{Leaf} and higher k_L were correlated with lower wood density. Leaf-specific hydraulic conductivity is a

measure of the efficiency of water supply in relation to potential transpirational demand and a determinant of stomatal control of leaf gas exchange and water balance. Indeed, a number of studies have reported a close coordination between leaf gas exchange and k_L , both within (Meinzer and Grantz, 1990, Hubbard et al. 2001) and across (Meinzer et al. 1995 Mencuccini 2003, Santiago et al. 2004, Campanello et al. 2008) species. Hydraulic coordination was also revealed by the positive relationship across species between PLC_{Stem} and PLC_{Leaf} indicating that when the stems are less vulnerable to cavitation, leaves are also less vulnerable to embolisms and vice versa. Several studies have revealed broad convergence in the coordination of plant functional traits. Many of these studies have focused on coordination within individual organs (e.g., leaves) in which traits such as maximum photosynthetic rates (A_{max}), leaf mass per area, leaf N content and leaf life span are related to each other in a universal manner across many plant species (e.g., Reich et al. 1997, 1999, Roderick et al. 1999, Wright et al. 2004). In this study we emphasized the coordination between leaf and stem traits. In addition to roots, leaves represent the greatest resistance to water flow from soil to leaves (Nardini and Salleo 2000; Pratt et al. 2010). The stem hydraulics has been studied more extensively than leaf hydraulics (e.g., K_{Leaf}) even though in the aerial part of the plant a large portion of the hydraulic resistance resides in the leaves (Sack and Holbrook 2006). The stems are, however, more expensive to build and less replaceable than leaves. Selective pressures for coordination between leaf and stem traits should operate in such a manner to ensure efficient delivery of water to the evaporative surface of the plant and to maintain the safety of the woody parts of the long-distance water-conductive system.

Another measure of the integrity of the water-conductive pathway is the safety margin ($P_{\text{stem}}^{50} - \text{TLP}$) reflecting the degree of stomatal control of evapotranspiration losses before substantial run-away embolism can occur in the stems (Choat et al. 2007). *Nothofagus* species with higher relative vulnerability of leaves compared with terminal stems have a larger safety margin for the stems. This implies that these species exhibit a wider range of water potentials at which stomatal conductance begins to down-regulate the pressure at which 50% of embolism occurs.

Another fundamental feature of the hydraulic architecture of plants is wood density. Biophysical properties of the active xylem and wood density, in particular, are expected to constrain water balance of the plant. Variations in wood density are a strong predictor of concomitant variations in a suite of traits related to stem water storage capacity, efficiency of xylem water transport and avoidance of turgor loss (Meinzer 2003, Bucci et al. 2004, Gartner and Meinzer 2005, Preston et al. 2006, Scholz et al. 2007, Bucci et al. 2009). In this study, wood density was directly related to the stem safety margin and

was indirectly related to P_{Stem}^{50} , k_S and k_L across *Nothofagus* species and populations. According to the k_L and wood density relationship, species with low wood density had higher k_L , and consequently a more efficient water transport per unit of leaf surface area, than species or populations with high wood density. Also species-specific midday leaf water potential, leaf capacitance and K_{Leaf} were negatively related to wood density. Despite the narrow range of wood density variation across *Nothofagus* species and populations, the strong predictive power of this biophysical trait points to properties of the xylem itself as one of the key functional differences among species.

In conclusion, our research on *Nothofagus* species demonstrates that hydraulic traits of leaves and stems are functionally related in a coordinated manner resulting in a hydraulic architecture design with an efficient water transport from the soil sources to the leaf sinks. Species and populations differed in several hydraulic properties, with larger differences found in stem hydraulic than in leaf hydraulic properties. On the other hand, we found that leaves were much more vulnerable to cavitation than stems, consistent with the idea that the more vulnerable leaves helps to avoid that hydraulic failure extends to the organs of the plant that have higher construction costs such as the stems. The phenotypic variation in resistance to cavitation was higher at the within-species level than between species. Surprisingly, populations in the driest sites appeared less vulnerable than those grown in the wettest sites but there were species- and population-specific compensatory mechanisms such as higher water storage and hydraulic efficiency that reduced the water potential drop and thus the probability of embolism formation in stems.

Conflict of interest

None declared.

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