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## Research paper

# Removal of nutrient limitations in forest gaps enhances growth rate and resistance to cavitation in subtropical canopy tree species differing in shade tolerance

Mariana Villagra<sup>1,2,3,6</sup>, Paula I. Campanello<sup>1,2,3</sup>, Lia Montti<sup>1,2,3,4</sup> and Guillermo Goldstein<sup>1,3,5</sup>

<sup>1</sup>Laboratorio de Ecología Funcional, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias, Exactas y Naturales, Universidad de Buenos Aires Ciudad Universitaria, Pabellón II, 2 piso, Ciudad de Buenos Aires (C1428EHA), Argentina; <sup>2</sup>Instituto de Biología Subtropical (IBS), Facultad de Ciencias Forestales, Universidad Nacional de Misiones, Bertoni 85, Puerto Iguazú (3370), Misiones, Argentina; <sup>3</sup>Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina; <sup>4</sup>Instituto de Ecología Regional (IER), Universidad Nacional de Tucumán, Yerba Buena, Tucumán, Argentina; <sup>5</sup>Department of Biology, University of Miami, PO Box 249118, Coral Gables, FL 33124, USA; <sup>6</sup>Corresponding author (marian.villagra@gmail.com)

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A 4-year fertilization experiment with nitrogen (N) and phosphorus (P) was carried out in natural gaps of a subtropical forest in northeastern Argentina. Saplings of six dominant canopy species differing in shade tolerance were grown in five control and five N + P fertilized gaps. Hydraulic architectural traits such as wood density, the leaf area to sapwood area ratio (LA : SA), vulnerability to cavitation (P50) and specific and leaf-specific hydraulic conductivity were measured, as well as the relative growth rate, specific leaf area (SLA) and percentage of leaf damage by insect herbivores. Plant growth rates and resistance to drought-induced embolisms increased when nutrient limitations were removed. On average, the P50 of control plants was  $-1.1$  MPa, while the P50 of fertilized plants was  $-1.6$  MPa. Wood density and LA : SA decreased with N + P additions. A trade-off between vulnerability to cavitation and efficiency of water transport was not observed. The relative growth rate was positively related to the total leaf surface area per plant and negatively related to LA : SA, while P50 was positively related to SLA across species and treatments. Plants with higher growth rates and higher total leaf area in fertilized plots were able to avoid hydraulic dysfunction by becoming less vulnerable to cavitation (more negative P50). Two high-light-requiring species exhibited relatively low growth rates due to heavy herbivore damage. Contrary to expectations, shade-tolerant plants with relatively high resistance to hydraulic dysfunction and reduced herbivory damage were able to grow faster. These results suggest that during the initial phase of sapling establishment in gaps, species that were less vulnerable to cavitation and exhibited reduced herbivory damage had faster realized growth rates than less shade-tolerant species with higher potential growth rates. Finally, functional relationships between hydraulic traits and growth rate across species and treatments were maintained regardless of soil nutrient status.

**Keywords:** herbivory, hydraulic conductivity, LA : SA, nutrient addition, semideciduous Atlantic Forest, xylem vulnerability to cavitation, trade-off.

## Introduction

Water transport along the soil–plant–atmosphere continuum plays an important role not only in the water economy of plants but also in the energy balance of leaves and in the delivery of

nutrients and growth regulators to different plant tissues. As leaf water potential decreases because of transpirational water loss, the tension increases in the xylem, which may compromise the capacity of plants to transport water. The loss of

hydraulic conductivity is attributed to embolization of the conduits by air that is introduced through pit membrane pores between adjacent vessels (Zimmermann 1983, Tyree and Sperry 1989). Embolisms decrease the volume flow of water through the conduits, reducing the supply of water to the sites of evaporation in the leaves and green stems (Meinzer et al. 2001). The loss of hydraulic function has serious consequences for plant performance because photosynthesis and growth depend on water transport to the sites of carbon fixation (Hubbard et al. 2001, Brodribb and Holbrook 2007). Thus, cavitation-induced embolism of stem xylem has the potential to affect the growth, reproduction and survival of plants (Blackman et al. 2009, Brodribb and Cochard 2009).

Rapid growth results in stems with low wood density (Muller-Landau 2004, Nascimento et al. 2005, Chao et al. 2008, Novaes et al. 2009), which has been correlated with low resistance to embolism (Hacke et al. 2001, Jacobsen et al. 2005, Chave et al. 2009, Markesteijn et al. 2011). High wood density is not only associated with low growth rates but also with high biomechanical safety, resistance to physical damage and shade tolerance of tree species (Putz et al. 1983, Augspurger and Kelly 1984, Kitajima 1994, Hacke et al. 2001, van Gelder et al. 2006, Alvarez-Clare and Kitajima 2007, Zanne et al. 2010). Wood density decreases with the addition of N by fertilization, probably due to increased growth rates and the development of larger vessels (Hacke et al. 2010). Xylem with larger vessels may be more vulnerable to cavitation than xylem with narrower ones. In their study, Hacke et al. (2010) found a negative relationship between specific conductivity and resistance to embolism, supporting the hypothesis that a trade-off between water transport efficiency and safety exists (Zimmermann 1983). The trade-off between xylem safety and xylem transport efficiency can be explained by the air-seeding hypothesis (Wheeler et al. 2005), in which species with more efficient xylem are more susceptible to embolisms because wider and longer conduits tend to have larger pitted wall areas, increasing the probability that pit membrane pores are more prone to air seeding compared with narrower conduits. However, this trade-off has not always been observed (Maherali et al. 2004, Choat et al. 2007, Jacobsen et al. 2007, Pratt et al. 2007, Campanello et al. 2011, Plavcová and Hacke 2012).

The hydraulic architecture of plants can be modified by nutrient availability due to allometric changes (e.g., the leaf area to sapwood area ratio, LA : SA) and/or direct effects on the biophysical properties of the conductive system. These properties include changes in vessel density and size, pit membrane characteristics and spatial arrangement of conduits in the xylem tissue (Choat et al. 2008, Sperry et al. 2008, Zanne et al. 2010), all of which can alter the vulnerability of xylem to embolism. Despite the large number of studies on the vulnerability of the water transport pathway (e.g., Tyree and Sperry 1989, Maherali et al. 2004, Markesteijn et al. 2011), there is little

information about the influence of nutrient availability on vulnerability to cavitation. For example, fertilization with nitrogen (N) caused P50 to decrease (increased the resistance to cavitation) in stems of trees in a Brazilian savanna (Bucci et al. 2006), to increase in *Populus* (Salicaceae) species (Hacke et al. 2010) and had no effect on the vulnerability of *Pinus taeda* L. (Pinaceae) stems (Ewers et al. 2000).

At the southern limit of the semideciduous Atlantic Forest in Argentina, short dry spells and freezing temperatures can both affect plant performance and may induce dysfunction in the water pathway from soil to leaves. Furthermore, in this forest ecosystem, soil nutrient availability, especially phosphorus (P), is very low (Montti et al. 2011), implying that some nutrients may be an important chronic constraint to plant growth. We studied the hydraulic architecture and growth of six canopy species differing in shade tolerance. Saplings were grown in a 4-year fertilization experiment with N and P in a homogeneous old growth forest of the semideciduous Atlantic Forest inside the Iguazú National Park, Argentina. Measurements were made in plants growing in forest gaps, which helped to reduce light limitation effects. We examined (i) the relationship between species-specific growth, hydraulic conductivity, wood density, vulnerability to drought-induced embolism and average specific leaf area (SLA), and (ii) the trade-off between water transport efficiency (specific hydraulic conductivity,  $K_S$ ) and xylem safety (water potential at which 50% loss of stem conductivity occurs). We predicted that nutrient addition would increase the hydraulic conductivity of the saplings to compensate for increases in growth, and that fast growth in fertilized plants would result in the development of lighter wood, which would increase water transport efficiency. We also expected high-light-requiring species to have higher  $K_S$ , lower wood density and faster growth rates compared with shade-tolerant species in the forest gaps and the changes (acclimation to removal of nutrient limitation) to be larger in light-requiring species.

## Materials and methods

### Study site, experimental design and species

Research was carried out in a highly protected area inside the Iguazú National Park (25°37'S, 54°20'W), Misiones, Argentina. The study site receives an average annual rainfall of 2000 mm, evenly distributed throughout the year. During short dry spells (i.e., 20–30 days), however, soil water potentials can attain values as low as –2 MPa (Campanello et al. 2007). The annual mean air temperature is 21 °C. Freezing temperatures may occur from 2 to 9 days per year during the winter season, depending on proximity to rivers and the altitude of the site (Ligier et al. 1990, Gatti et al. 2008). The soils are deep Ultisols containing high concentration of Fe, Al and Si (Ligier et al. 1990).

Ten permanent 15 × 15 m plots were established during 2004 in the center of natural gaps with similar incoming solar radiation (34 ± 4% of total radiation) estimated with hemispherical photographs. These gaps are representative of those found in the study area (Campanello et al. 2007). Forest canopy height in the study area was between 25 and 30 m (M. Villagra, personal observations).

Before the treatments began, four soil samples per plot were collected at 0–10 cm depth and mixed to form a composite sample; samples were analyzed for available P using a modified Olsen method, and N by Kjeldahl. Soil N concentration was 10.5 ± 1.6 µg g<sup>-1</sup> N. Phosphorus concentrations before the treatment were not determined but it is assumed in this study that the P levels in the control plots were similar to P levels before the treatment began. After 18 months of nutrient addition, soil N and P concentrations in the control treatment were 12.8 ± 2.0 µg g<sup>-1</sup> N and 3.0 ± 0.3 µg g<sup>-1</sup> P, respectively, and those in the N + P treatment were 10.3 ± 1.2 µg g<sup>-1</sup> N and 9.3 ± 1.0 µg g<sup>-1</sup> P. Subsequent analysis showed similar N and P values. Analyses were performed by the soil laboratory at the Instituto Nacional de Tecnología Agropecuaria (INTA), Castelar, Argentina.

The experiment included two treatments with five replicates in a randomized complete design. The treatments were: the control (no added fertilizer) and N plus P (100 kg N ha<sup>-1</sup> year<sup>-1</sup> as ammonium nitrate and urea + 100 kg P ha<sup>-1</sup> year<sup>-1</sup>, calcium triple superphosphate). The fertilizers were applied (sprinkled) in granular form on the organic soil surface three times during each year, from February 2004 to December 2008. A buffer area of 10 m surrounding the plots was also fertilized.

Six abundant tree species, common in tropical seasonally dry forests across the Neotropics (Pennington et al. 2000) and of importance in the study area, that vary in regeneration light requirements and wood density (Carvalho 2003, Campanello et al. 2011) were selected for the study. In order of decreasing shade tolerance these species were: *Balfourodendron riedelianum* (Engl.) Engl. (Rutaceae), *Cordia americana* (L.) Gottschling & J.S. Mill. (Boraginaceae), *Maclura tinctoria* (L.) Steud. ssp. *tinctoria* (Moraceae), *Cedrela fissilis* Vell. (Meliaceae), *Cordia trichotoma* (Vell.) Arráb. ex Steud. and *Peltophorum dubium* (Spreng.) Taub. (Fabaceae). Seeds were collected between August 2002 and February 2003 from 5 to 10 parent trees and germinated in nursery flats filled with local top-soil. When the first set of leaves had expanded, the seedlings were transferred to plastic seedling bags filled with local soil and placed under 80% shade cloth in a nursery. Seedlings were transplanted to the field in June 2004. Initial sizes were different: the mean height (cm ± SE) was 17.8 ± 1.8 for *B. riedelianum*, 61.4 ± 6.2 for *C. fissilis*, 24.3 ± 1.6 for *C. americana*, 29.1 ± 3.8 for *C. trichotoma*, 54.8 ± 2.8 for *M. tinctoria* and 65.7 ± 9.8 for *P. dubium*. Seedlings were planted randomly in the plots along five transects spanning an area of 5 × 15 m, spaced 1 m apart from each other. Between 11 and 15 individuals per species were planted in each plot, except for

*C. trichotoma* with only three plants per plot planted. The death of some individuals of this species precluded the measurement of some physiological variables. Growth in diameter (measured at a marked section of the stems 2 cm above the soil), height and total leaf area was measured at the start and at the end of the experiment (March 2005–March 2009) on three to five individuals per species from each treatment. Relative growth rate (RGR) was expressed as the increment in stem diameter between March 2005 and March 2009, divided by the initial value of the stem diameter. Accumulative growth rate over the 4-year period was preferred instead of the average annual growth rate because of the high variability in growth during this initial stage in the life cycle of the plants.

### Stem hydraulic conductivity

Stem hydraulic conductivity was measured on sun-exposed terminal branches of five individuals per species and treatment during February 2009. The segments used were 12–25 cm long depending on the species, and were longer than the longest xylem vessel measured according to Zimmermann and Jeje (1981). The species with the shortest maximum vessel length (9 cm) was *C. americana* and the species with the longest vessel length (20 cm) was *M. tinctoria*. Samples were collected early in the morning, cut under water and transferred immediately to the laboratory with the cut end immersed in water and tightly covered with black plastic bags. The stems were re-cut under water and connected to a hydraulic conductivity apparatus (Sperry et al. 1988). Distilled and de-gassed water was used as the perfusion fluid. Water flow generated by a constant hydraulic head of 40 cm was volumetrically measured using pipettes connected to the downstream end of the stems. Following a short equilibration period, three consecutive flow measurements were obtained. Hydraulic conductivity ( $K_H$ ; kg m s<sup>-1</sup> MPa<sup>-1</sup>) was calculated as

$$K_H = J_v / (\Delta P / \Delta L)$$

where  $J_v$  is flow rate through the segment (kg s<sup>-1</sup>) and  $\Delta P / \Delta L$  is the pressure gradient across the segment (MPa m<sup>-1</sup>).

Both ends of the segments were then smoothed with a clean razor blade and perfused with methyl blue dye. Sapwood area (SA) was determined at ~1 cm from each segment end by measuring the stained cross-sectional area. Specific hydraulic conductivity ( $K_S$ ; kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup>) was calculated as the ratio of  $K_H$  to average SA, and leaf-specific hydraulic conductivity ( $K_L$ ; kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup>) was calculated as the ratio of  $K_H$  to LA, where LA corresponds to the leaf area distal to each segment measured.

### Stem vulnerability to cavitation

Hydraulic vulnerability curves were generated using the bench drying method (Sperry et al. 1988). Terminal branches were

excised before dawn from 3–5 plants of each species and were immediately re-cut under water, covered with black plastic bags and transported to the laboratory for measurements. In the laboratory, branches were left to freely transpire for different time intervals to obtain a wide range of water potentials. Then they were stored in black plastic bags to equilibrate for at least 1 h. The water potential ( $\Psi_L$ ) of two leaves was then measured using a pressure chamber (PMS, Albany, OR, USA) and the corresponding segment was cut under water and attached to the apparatus to measure the hydraulic conductivity as described previously. Maximum  $K_H$  ( $K_{max}$ ) was determined after initial  $K_H$  measurements by flushing the segments with the perfusion fluid (distilled and de-gassed water) at a pressure of 0.1 MPa for 20 min, to remove air from embolized vessels. The initial  $K_H$  measurement ( $K_i$ ) was expressed as a percentage of  $K_{max}$ , and percent loss of conductivity (PLC) was calculated as  $(100(1 - K_i/K_{max}))$ . Stem hydraulic vulnerability curves were obtained by plotting PLC against  $\Psi_L$ . A sigmoid curve was fitted to the data and the xylem water potential at 50% loss of stem hydraulic conductivity ('P50') was calculated (see Figure S1 available as Supplementary Data at *Tree Physiology* Online).

### Wood density

Terminal branches used for stem vulnerability curves were also used for wood density estimates. A 2-cm-long stem section was cut and both bark and pith were removed from the segment. The volume of sapwood was measured using the water-displacement method. The sapwood was then oven-dried at 70 °C for 72 h to determine the dry mass. Wood density was obtained as the ratio of dry mass to volume.

### Specific leaf area and leaf insect herbivory

Five fully sun-exposed leaves were collected from five trees per treatment in the same plots where hydraulic properties were measured. Fresh leaf area was determined using a scanner and the Leaf Area Measurement software (V.1.3. Copyright 2003, Sheffield University; www.shef.ac.uk). Leaves were then oven-dried at 60 °C until constant weight, and their dry weight was used to calculate SLA.

For assessing leaf herbivory damage five plants per species and treatment were measured. During February 2006, when insects were highly abundant, five recently initiated leaves per plant were tagged. After 1 month these leaves were harvested to determine leaf area removed by herbivores using a scanner. Later, the same image was digitally modified to simulate the leaf area without herbivory and to determine the percentage of leaf area damaged.

### Statistical analysis

All analyses were performed using a two-way analysis of variance (ANOVA) with individual values used as replicates. The following linear model was used to fit the measuring data:

$$\text{var} = \text{nutrient} + \text{species} + (\text{nutrient} \times \text{species})$$

where 'var' refers to the tested variable (e.g., LA : SA,  $K_L$ , or  $K_S$ ), 'nutrient' refers to the nutrient treatment effect considered as fixed (control or N + P), 'species' refers to the species effect considered as fixed and '(nutrient × species)' refers to the interaction term. To assess the assumption of homogeneity of variances, the data were modeled with homogeneous and heterogeneous variances and the resulting models were compared using the Akaike information criterion to select the most parsimonious model that would best explain the data. Results of the models are given in Table S1 available as Supplementary Data at *Tree Physiology* Online. A Fisher's least significant difference (LSD) test was used to examine differences between the mean values of each treatment when the interaction term was statistically significant. All variables were log-transformed. Values of P50 were estimated from vulnerability curves. To test differences in P50 between treatments a *T*-test was used with species values as replicates. To assess the effect of fertilization for each species the residuals of P50 obtained from the vulnerability curve were also compared through a *T*-test. Pearson's correlation analysis was used to examine interspecific trait relationships. To rule out the potential effect of ontogenetic differences due to plant size in the control and N + P treatment, we performed correlations between height, diameter, total leaf area and physiological variables and none was significant. Significant differences were considered if  $P < 0.05$ . The InfoStat 2011 statistical package (Di Rienzo et al. 2011) was used for statistical analysis. The ANOVAs were performed using an interface to R (R Developing Core Team 2011) implemented in InfoStat software (Di Rienzo et al. 2011).

## Results

### Growth and leaf damage

After 4 years of fertilization, the final size of the plants growing with the addition of N + P was greater than in plants growing in control plots (Table 1). The differences were significant in all the species except *C. trichotoma*. The total leaf area was doubled with the addition of N + P in species such as *C. fissilis*, *M. tinctoria* and tripled in *C. americana*. A significant correlation between the RGR in total leaf area and the RGR in diameter was found ( $r = 0.69$ ,  $P < 0.05$ ). The relative growth rate in diameter varied among species and between treatments (Figure 1a), but the degree of the response differed among the species (i.e., the interaction species × treatment was significant,  $P < 0.001$ ). While *M. tinctoria* increased its diameter by 75% with nutrient addition compared with the control treatment, *C. fissilis* increased <25%.

Herbivory was studied for a short time during leaf expansion, when most of the damage occurs (Coley and Barone 1996). Percentage of leaf damage did not respond to fertilizer

Table 1. Diameter, height, total leaf area per plant and leaf damage for the six studied species in the control (C) and in the fertilized (N + P) treatments. Means  $\pm$  SE are shown. Different letters indicate significant differences between treatments per species (LSD-Fisher,  $P < 0.05$ ).

	Diameter (mm)	Height (cm)	Total leaf area (m <sup>2</sup> )	Leaf damage (%)
	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
<i>B. riedelianum</i>				
C	31.40 $\pm$ 1.50a	295.20 $\pm$ 12.25a	2.26 $\pm$ 0.20	7.28 $\pm$ 0.83
N + P	39.40 $\pm$ 1.17b	408.20 $\pm$ 13.66b	2.72 $\pm$ 0.19	5.52 $\pm$ 1.20
<i>C. americana</i>				
C	37.80 $\pm$ 1.69a	421.60 $\pm$ 21.07a	1.79 $\pm$ 0.14a	5.25 $\pm$ 0.99
N + P	59.60 $\pm$ 1.25b	604.00 $\pm$ 15.68b	6.83 $\pm$ 0.48b	3.30 $\pm$ 1.11
<i>M. tinctoria</i>				
C	48.60 $\pm$ 2.16a	548.00 $\pm$ 23.54a	9.10 $\pm$ 0.74a	10.09 $\pm$ 2.32
N + P	82.40 $\pm$ 1.89b	697.00 $\pm$ 18.81b	20.66 $\pm$ 0.65b	7.42 $\pm$ 2.70
<i>C. fissilis</i>				
C	34.20 $\pm$ 1.53a	171.80 $\pm$ 10.26	1.66 $\pm$ 0.16a	1.40 $\pm$ 0.23
N + P	48.40 $\pm$ 4.11b	215.40 $\pm$ 17.79	3.63 $\pm$ 0.08b	0.68 $\pm$ 0.42
<i>C. trichotoma</i>				
C	46.00 $\pm$ 1.53	345.67 $\pm$ 24.70a	2.97 $\pm$ 0.34	13.14 $\pm$ 0.93
N + P	52.33 $\pm$ 6.77	434.00 $\pm$ 41.53b	3.86 $\pm$ 0.28	15.10 $\pm$ 0.67
<i>P. dubium</i>				
C	58.20 $\pm$ 3.18a	511.40 $\pm$ 18.36a	2.49 $\pm$ 0.16a	19.25 $\pm$ 1.66a
N + P	71.40 $\pm$ 3.30b	632.00 $\pm$ 30.07b	4.06 $\pm$ 0.31b	9.50 $\pm$ 1.00b

addition (Table 1), except for *P. dubium* which showed a significantly lower percentage of leaf damage in fertilized plants than in control plants. The largest percentage of leaf damage was observed in *P. dubium* and was twice that observed in *M. tinctoria* and three times higher than in the other species. The lowest percentage of leaf damage was observed in *C. fissilis*, with <5% of the leaf area removed by insect herbivores. Forty percent of the saplings of this species in both treatments had *Hypsipyla grandella* Zeller (Lepidoptera: Pyralidae) attacks in apical buds, resulting in leaf death, malformation of stems and probably permanent stunting. Thus, for *C. fissilis*, 40% leaf damage would be a more realistic estimation of herbivory assuming that the total leaf area was similar among plants.

### Growth and hydraulic architecture

The addition of N and P influenced several hydraulic architectural traits. Specific hydraulic conductivity ( $K_S$ ) and leaf-specific hydraulic conductivity ( $K_L$ ) differed among species, but the direction and magnitude of the differences between the control and the N + P treatment were not consistent across species (Figure 1b, c). The addition of nutrients increased  $K_S$  in *B. riedelianum* and *C. americana* while *P. dubium* showed a 75% decrease in  $K_S$ . Consistent with changes in  $K_S$ , adjustments in  $K_L$  were also observed (i.e., the addition of N + P resulted in increases in  $K_L$  in *B. riedelianum* and *C. americana*, whereas *P. dubium* showed a significant decrease in  $K_L$ ). A significant positive relationship between  $K_S$  and  $K_L$  (Table 2) across species and treatments was observed. Among the species, *M. tinctoria* and *B. riedelianum* displayed the lowest values of  $K_S$  and  $K_L$  in both control and fertilization treatments (Figure 1b,

c). The LA : SA differed significantly among species, as well as between treatments, resulting in decreased LA : SA with fertilization (Figure 1d). Although this study found that  $K_S$  scaled with  $K_L$ , neither of these two attributes was correlated with LA : SA (Table 2).

There was a positive correlation between the RGR and total leaf area per plant across species (Figure 2a). The RGR in diameter increased with decreasing LA : SA across species, including both the unfertilized and fertilized plants (Figure 2b); that is, the higher the species-specific relative growth, the larger the capacity for water transport in relation to potential demand for water by the leaves.

### Wood density and vulnerability to xylem cavitation

The addition of nutrients resulted in a lower wood density when considering all the species (Figure 1e). There was a significant negative correlation between species-specific wood density and  $K_S$  regardless of the treatment (Figure 3). Although  $K_L$  tended to decrease with an increase in wood density, the correlation between these two variables was not significant (Table 2).

Nutrient addition increased xylem resistance to embolism (Figure 4). The analysis showed that among species, P50 was significantly lower (i.e., more negative) in *B. riedelianum*, *M. tinctoria* and *C. americana* growing in fertilized plots compared with control plots. The most vulnerable species were *C. fissilis* and *P. dubium* and the least vulnerable was *C. americana* (Figure 4). There was no relationship between P50 and  $K_S$  or between P50 and wood density (Table 2), but a positive correlation between LA : SA and P50 was found (Figure 5a).

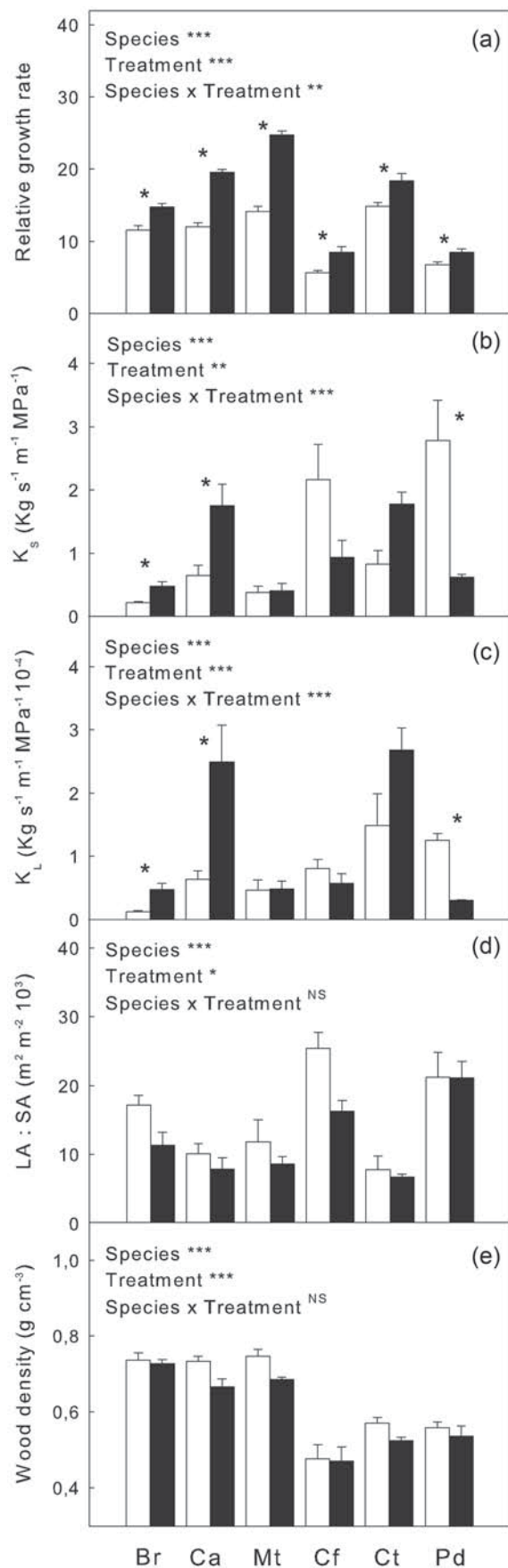


Table 2. Pearson's correlation analysis between specific hydraulic conductivity ( $K_S$ ;  $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ), leaf-specific conductivity ( $K_L$ ;  $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1} 10^{-4}$ ), wood density (WD;  $\text{g cm}^3$ ), the leaf area to sapwood area ratio (LA : SA,  $\text{m}^2 \text{m}^{-2} 10^3$ ), xylem water potential at which 50% loss of conductivity occurs (P50; MPa), relative growth rate in diameter (RGR) and specific leaf area (SLA;  $\text{m}^2 \text{g}^{-1}$ ).

	$K_S$	$K_L$	WD	LA : SA	P50	RGR
$K_S$						
$K_L$	0.61*					
WD	-0.59*	-0.30				
LA : SA	0.33	-0.46	-0.43			
P50	0.10	-0.51	-0.41	0.69*		
RGR	-0.34	0.31	0.48	-0.84***	-0.69*	
SLA	0.54	-0.19	-0.56	0.74**	0.81**	-0.67*

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

The RGR in diameter was negatively correlated with P50 across all species and treatments (Figure 5b), indicating that plants with more negative P50 values (less vulnerable to cavitation) had higher RGRs. Both P50 and LA : SA were positively correlated with SLA across species and treatments (Figure 6).

## Discussion

### Impact of N + P addition on growth and hydraulic traits

In this study, we tested whether the hydraulic architecture and growth of trees in subtropical forest gaps changed after N and P limitations were reduced by a 4-year fertilization treatment. The experimental design used in this study attempted to maintain the environmental conditions as close as possible to those that the plants face during the initial part of their life cycle in the forest. Factors such as herbivore pressure and mechanical damage from falling tree debris were not controlled. The use of gaps similar in size to those existing in a natural forest was intended to partially remove the limiting effect of low solar radiation on plants in the understory. The results showed a clear effect of nutrient addition on sapling growth and strongly suggest that N and/or P are important limiting resources (when light is not limiting) in this ecosystem. Total leaf area, stem diameter and maximum height were significantly higher in plants growing with N + P addition. In a review paper (Lawrence 2003) analyzing a large data set on tropical seedlings growing mostly under controlled conditions, it was concluded that most of the species exhibited a positive growth response to fertilization and the magnitude of the response of high-light-requiring species was more than twice that of

Figure 1. Stem hydraulic traits and RGRs in diameter of the six studied species in the control (C, white bars) and in the fertilized (N + P, black bars) treatments. Values are species means ( $\pm$ SE). Also shown are the results from two-way ANOVAs (\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ). Asterisks above bars of each species indicate significant differences between C and N + P ( $P < 0.05$ , LSD Fisher). Br, *Balfourodendron riedelianum*; Ca, *Cordia americana*; Mt, *Maclura tinctoria*; Cf, *Cedrela fissilis*; Ct, *Cordia trichotoma*; and Pd, *Peltophorum dubium*.

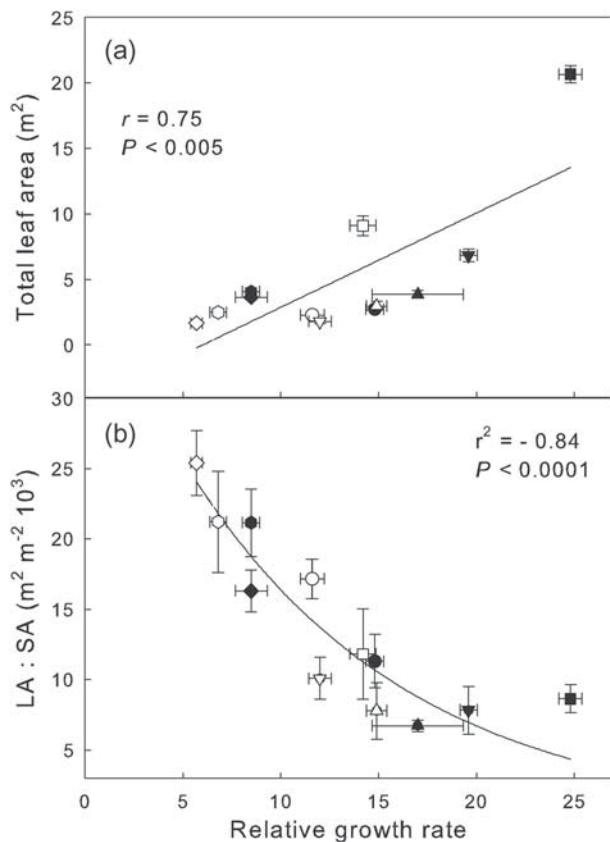


Figure 2. Relationship between total leaf area and RGR measured as relative increment in stem diameter (a) and the leaf to sapwood area ratio (LA:SA) and RGR (b) for the species studied in the control (white symbols) and N + P treatments (black symbols). Symbols are mean values ( $n = 4-5$ ). Bars indicate standard error of the means. The  $r$  value in (a) corresponds to the Pearson correlation coefficient and  $r^2$  in (b) corresponds to the determination coefficient of an exponential decay function that was fitted to the data:  $y = 41.9 \times \exp(-0.09x)$ . *Balfourodendron riedelianum* (○, ●); *Cordia americana* (▽, ▼); *Maclura tinctoria* (□, ■); *Cedrela fissilis* (◇, ◆); *Cordia trichotoma* (△, ▲) and *Peltophorum dubium* (◊, ●).

shade-tolerant species. In this study, on the other hand, the shade-tolerant species exhibited the highest growth rates regardless of treatment. The growth rates of high-light-requiring species were relatively low because herbivory pressure in those species was very high.

In fertilized plots, the SA increased more than the total leaf surface area per plant, and consequently species-specific LA:SA was lower in fertilized saplings. The LA:SA reflects the pattern of carbon allocation to leaves and xylem tissue. Several studies have observed changes in carbon allocation patterns with the addition of N + P, with greater increases in aboveground primary production (i.e., total leaf area) than in belowground carbon allocation (Giardina et al. 2003, Coyle and Coleman 2005, Stape et al. 2008, Novaes et al. 2009). Long-term fertilization with N + P in five Brazilian savanna trees resulted in a significant increase in total leaf area and a decrease in midday leaf water potential ( $\Psi_L$ ) in all the species,

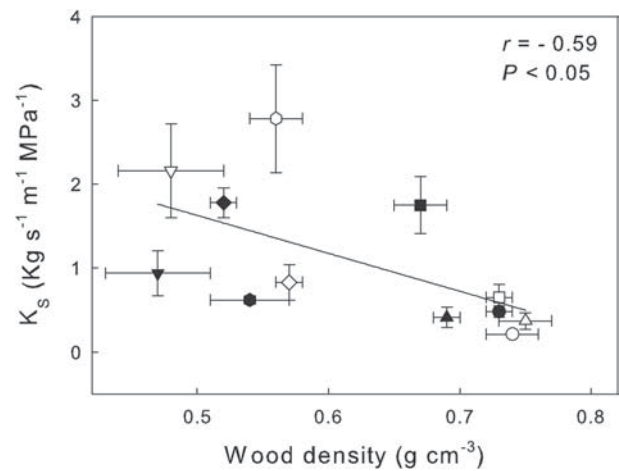


Figure 3. Relationship between specific hydraulic conductivity ( $K_S$ ) and wood density in the study species in the control (white symbols) and N + P treatments (black symbols). Symbols are the mean value of each species and treatment ( $n = 4-5$ ). Bars indicate standard error of the means. The  $r$  value corresponds to the Pearson correlation coefficient. Symbols are the same as in Figure 2.

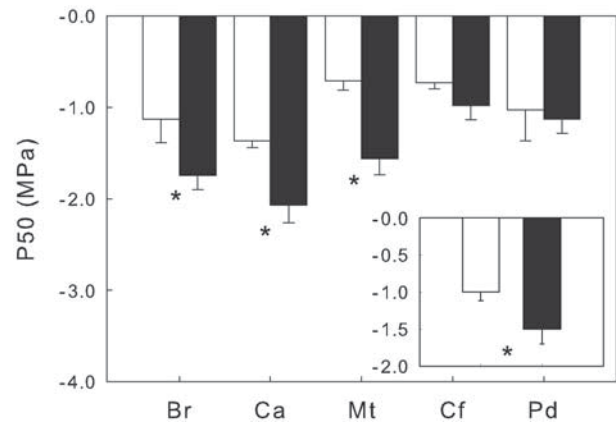


Figure 4. Water potential at which 50% loss of stem conductivity occurs (P50) in the control (C, white bars) and in the fertilized (N + P, black bars) treatments. Values are species means ( $\pm$ SE). The standard errors in this case were estimated from the vulnerability curves. In the inset, the results from the  $T$ -test comparing P50 between C and N + P with species values used as replicates are shown. Values are treatment means ( $\pm$ SE). Asterisks below bars indicate significant difference between P50 values ( $P < 0.05$ ,  $T$ -test). Abbreviations are the same as in Figure 1.

probably as a consequence of the lack of a compensatory increase in root biomass (Bucci et al. 2006). Similarly, in this study we found significant changes in the total leaf area of the trees, which could potentially have caused an imbalance between water uptake and supply to the leaves if the belowground biomass remained constant. Root biomass was not measured in our study but it is possible that high nutrient availability did not result in increased allocation to roots as shown in other studies (e.g., Huante et al. 1995, 1998, Paz 2003, Santiago et al. 2012).

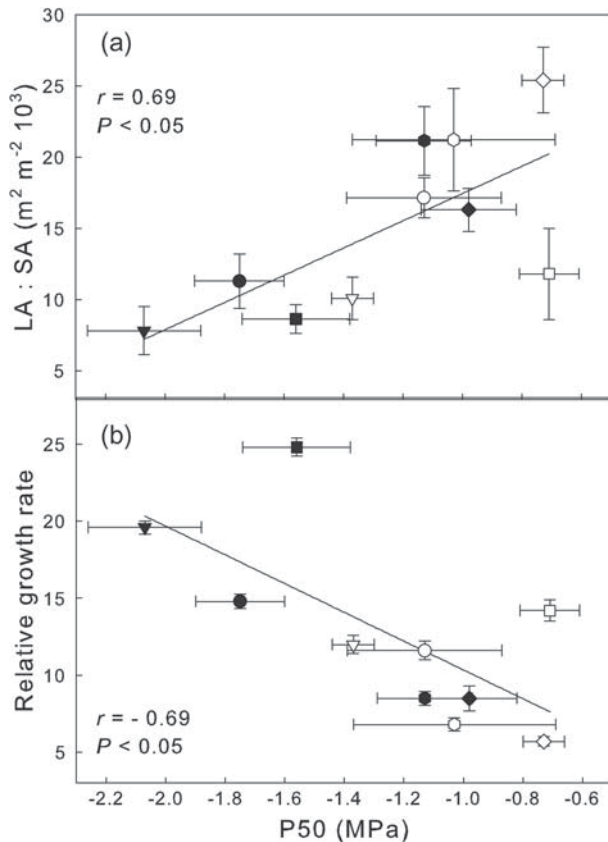


Figure 5. Relationship between the leaf to sapwood area ratio (LA : SA) and the water potential at which 50% loss of stem conductivity occurs (P50) (a), and relative growth rate (RGR) measured as relative increment in stem diameter and P50 (b) for five species studied in the control (white symbols) and N + P treatments (black symbols). Each point represents the estimated value in the vulnerability curve for P50 and the mean value for RGR ( $n = 4-5$ ). Bars indicate standard error of the means. The  $r$  value corresponds to the Pearson correlation coefficient. Symbols are the same as in Figure 2.

Fertilization did not have the same effect on  $K_s$  and  $K_L$  across all species. Leaf-specific conductivity ( $K_L$ ) values were relatively low for *B. riedelianum* and *M. tinctoria*, consistent with their limited ability to colonize high-light environments (Carvalho 2003, Campanello et al. 2011) compared with the fast-growing and more light-requiring species, such as *C. trichotoma* and *P. dubium*, especially in control plots. This result is consistent with a study in another neotropical forest showing that  $K_L$  is related to tree successional stage (McCulloh et al. 2011). However, growth rates were not correlated with water transport efficiency (i.e.,  $K_L$ ) as previously found by Campanello et al. (2008) for plants growing in shade-houses where herbivory was prevented. Herbivory in field conditions may have obscured the potential growth of the species in control plots as well as in fertilized plots (Andersen et al. 2010, Santiago et al. 2012). For example, *M. tinctoria* was one of the species with the lowest  $K_L$  and the highest growth in both diameter and total leaf area and *C. trichotoma* showed a relatively low growth

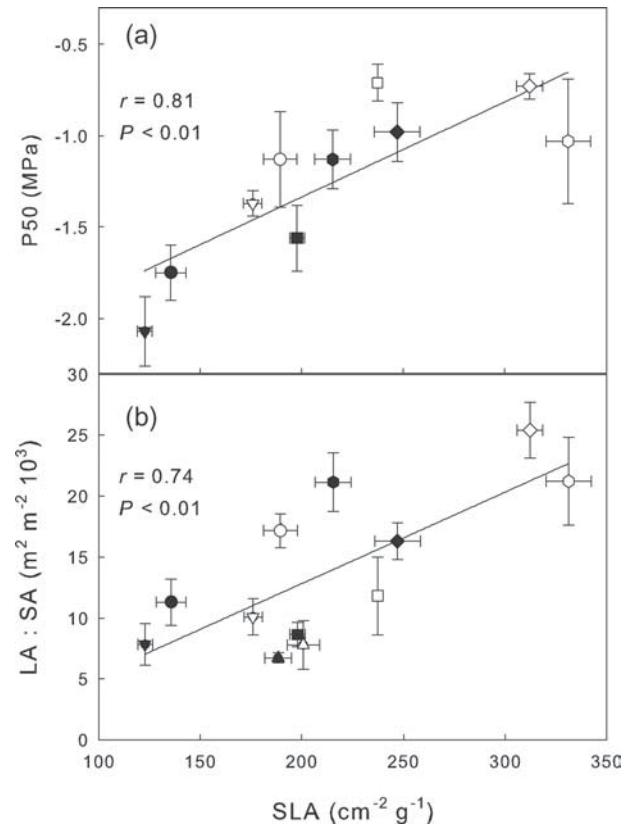


Figure 6. Relationship between the water potential at which 50% loss of stem conductivity occurs (P50) (a), the leaf to sapwood area ratio (b) and the specific leaf area (SLA). Symbols correspond to the species growing in the control (white symbols) and N + P treatments (black symbols). Each point represents the estimated value in the vulnerability curve for P50 stem and the mean value for LA : SA and SLA ( $n = 4-5$ ). Bars indicate standard error of the means. The  $r$  value corresponds to the Pearson correlation coefficient. Symbols are the same as in Figure 2.

rate but high  $K_L$ . The other shade-tolerant species, *B. riedelianum*, also had relatively low  $K_L$  and  $K_s$  but higher growth rates than *C. fissilis* and *P. dubium*, both gap-colonizing species.

The addition of nutrients resulted in lower wood density across species similar to the patterns observed in other studies (e.g., Bucci et al. 2006, Hacke et al. 2010). This suggests that there were changes in sapwood anatomy of terminal branches that led to increased porosity in plants of those species growing in N + P fertilized gaps (i.e., increased vessel size when nutrient limitation was removed).

Saplings of *B. riedelianum*, *C. americana* and *M. tinctoria* grown under N + P addition exhibited a lower xylem water potential at 50% loss of stem hydraulic conductivity (P50) and thus were more resistant to xylem cavitation than saplings growing in unfertilized gaps. Among species, P50 values varied between  $-0.71$  and  $-2.07$  MPa, and fell within the range of P50 values observed for tropical rain forest species (Maherali et al. 2004). The increase in cavitation resistance in fertilized plants probably enabled them to avoid xylem dysfunction they



could have experienced during drought periods and as a consequence of increases in the total leaf area per plant without an apparently concomitant increase in belowground biomass. Higher total leaf area per plant should have increased evaporative losses, resulting in more negative leaf and stem water potentials. Wood anatomy and wood density acclimation may have occurred, which could have resulted in hydraulic properties of the water transport system better adjusted to the more frequent drought periods. Xylem vulnerability to embolism has been shown both to decrease (Bucci et al. 2006) and increase (Hacke et al. 2010) with N or N + P addition, which may reflect different species-specific responses to different environments. It is important to note that contrary to the nutrient effects observed here, a more limited plastic response of P50 to changes in growth irradiances in plants grown in a greenhouse was observed in another study in the same ecological area (Campanello et al. 2011). The results of our study contribute to the current debate on the acclimation capacity of the vulnerability to cavitation in vascular plants (Choat et al. 2012).

#### Correlations between hydraulic architectural traits

There was no evidence of a trade-off between hydraulic efficiency and vulnerability to cavitation across all the species. Many studies have found no evidence of a trade-off between these two hydraulic properties (e.g., Maherali et al. 2004, Choat et al. 2007, Pratt et al. 2007, Jacobsen et al. 2007, Campanello et al. 2011, Plavcová and Hacke 2012). Moreover, a recent study indicates that these two characteristics of the hydraulic system could be genetically independent (Lauri et al. 2011). Since there is ample evidence that this trade-off is not universal, it is necessary to analyze potential trade-offs considering the hydraulic traits at the whole-plant level, rather than just within specific organs, because there may be alternative mechanisms that contribute to the efficiency and safety of the hydraulic pathway (e.g., high capacitance and the ability to repair embolized vessels on a daily basis) (Meinzer et al. 2010).

We found a significant correlation between wood densities and stem-specific hydraulic conductivity. The species with higher wood density had a lower capacity to transport water through the xylem. Some studies suggest that anatomical traits, such as vessel diameter or vessel density, are involved in hydraulic efficiency but not in hydraulic safety. Results showing that drought-induced embolism is more related to microscopic anatomical traits (e.g., the size of pit pores and pit membrane area, pit membrane porosity and wall reinforcement) than to macroscopic anatomical ones (Tyree and Sperry 1989, Hacke et al. 2001, Choat et al. 2008, Fichot et al. 2010) are likely to explain in part why a lack of trade-off between xylem efficiency and xylem safety is often shown in stems, as observed in our study, and why a stronger correlation between wood density and hydraulic efficiency than between wood density and vulnerability to cavitation should be expected.

#### Leaf area to sapwood area ratio, SLA and growth performance

The LA : SA decreased significantly with nutrient additions. This trait is considered a morphological index of water transport capacity in relation with transpirational demand, with lower LA : SA implying a higher water transport capacity per unit leaf surface area (Goldstein et al. 2002). We found a strong negative relationship between LA : SA and the RGR across species, suggesting that plants with faster growth rates had a higher water supply capacity per unit leaf area. However,  $K_L$ , a direct measure of water transport efficiency to leaves, was not correlated with growth rate. Indeed, the plants with the fastest growth rates tended to have the lowest water transport capacity. A possible explanation, as suggested by Buckley and Roberts (2006), is that during the initial growth phase of trees there is a high risk of trunk rupture, and the selective pressure for improved radial stem growth, and therefore the production of sapwood (i.e., a lower LA : SA) would be advantageous in terms of plant biomechanics regardless of wood density.

The species that were less vulnerable to cavitation in this study grew faster, as evidenced by the negative correlation between P50 and the RGR in diameter. This finding appears to be contrary to the idea that xylem cavitation resistance is mainly achieved at the expense of low growth rates under the assumption that the degree of vulnerability to cavitation of a species is associated with a given cost in terms of carbon allocation (Wikberg and Ögren 2004, Cochard et al. 2007). An alternative explanation is that the more cavitation-resistant species (more negative P50) may have avoided hydraulic dysfunction during short drought periods, thereby preventing interruption of their growth.

Both LA : SA and P50 were strongly correlated with SLA. The SLA is a measure of the area used for energy capture per unit dry mass invested in leaf construction. More shade-tolerant species are expected to have lower values of SLA (Kitajima 1994; Lusk and Warton 2007, Lusk et al. 2008) due in part to a large proportion of fibers, epidermis and cell walls in tissues (Alvarez-Clare and Kitajima 2007, Onoda et al. 2008), resulting in leaves more resistant to herbivory (Coley and Barone 1996). Leaf and stem characteristics conferring biomechanical safety were functionally related as shown by the significant correlations of SLA with P50 and LA : SA across species. This agrees with the general pattern that more shade-tolerant species have leaves and stems that are more resistant to herbivory and experienced less mechanical damage increasing the survivorship of seedlings and saplings in the forest understory (Kitajima 1994). According to these results, species such as *C. fissilis*, *C. trichotoma* and *C. americana* that had the highest potential growth rates and water transport efficiency, when water stress and herbivore damage were prevented in shade houses (Campanello et al. 2008), had lower realized growth rates in the forest in this study. These species were probably more affected by water deficits during dry spells

(because of their less negative P50) and by biomechanical and herbivory damage because of high LA : SA and SLA.

## Conclusions

The results showed a clear effect of nutrient addition on plant growth and strongly suggest that N and/or P are important limiting resources in the subtropical forests of northeastern Argentina when light is not limiting. The results also showed that the studied species partially adjusted their hydraulic architecture to fertilization and consequently were able to make compensatory changes such as an increase in water transport capacity and in resistance to water stress-induced embolisms in the fertilized plots. We found a significant correlation between wood density and the water transport efficiency of the xylem across species and treatments. More shade-tolerant species had higher wood density, were less damaged by insect herbivores and were less vulnerable to hydraulic dysfunction. This combination of traits may allow these plants to have higher RGRs than light-requiring species. Our results highlight the importance of biomechanical resistance enhancement during the initial phase of tree establishment in gaps and also show the importance of performing experiments in the field where some of the conditions (e.g., herbivory) are not controlled, to understand and predict growth patterns and functional relationships of the tree species in their natural habitat.

## Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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## Conflict of interest

None declared.

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