



Tree Physiology 00, 1–11  
doi:10.1093/treephys/tpt098



## Research paper

# Functional relationships between leaf hydraulics and leaf economic traits in response to nutrient addition in subtropical tree species

Mariana Villagra<sup>1,2,3,6</sup>, Paula I. Campanello<sup>1,2,3</sup>, Sandra J. Bucci<sup>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, Argentina; <sup>3</sup>Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina; <sup>4</sup>Grupo de Estudios Biofísicos y Ecofisiológicos, Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco, Argentina; <sup>5</sup>Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, FL 33124, USA; <sup>6</sup>Corresponding author (marian.villagra@gmail.com)

Received June 20, 2013; accepted October 8, 2013; handling Editor Roberto Tognetti

Leaves can be both a hydraulic bottleneck and a safety valve against hydraulic catastrophic dysfunctions, and thus changes in traits related to water movement in leaves and associated costs may be critical for the success of plant growth. A 4-year fertilization experiment with nitrogen (N) and phosphorus (P) addition was done in a semideciduous Atlantic forest in north-eastern Argentina. Saplings of five dominant canopy species were grown in similar gaps inside the forests (five control and five N + P addition plots). Leaf lifespan (LL), leaf mass per unit area (LMA), leaf and stem vulnerability to cavitation, leaf hydraulic conductance ( $K_{\text{leaf\_area}}$  and  $K_{\text{leaf\_mass}}$ ) and leaf turgor loss point (TLP) were measured in the five species and in both treatments. Leaf lifespan tended to decrease with the addition of fertilizers, and LMA was significantly higher in plants with nutrient addition compared with individuals in control plots. The vulnerability to cavitation of leaves ( $P50_{\text{leaf}}$ ) either increased or decreased with the nutrient treatment depending on the species, but the average  $P50_{\text{leaf}}$  did not change with nutrient addition. The  $P50_{\text{leaf}}$  decreased linearly with increasing LMA and LL across species and treatments. These trade-offs have an important functional significance because more expensive (higher LMA) and less vulnerable leaves (lower  $P50_{\text{leaf}}$ ) are retained for a longer period of time. Osmotic potentials at TLP and at full turgor became more negative with decreasing  $P50_{\text{leaf}}$  regardless of nutrient treatment. The  $K_{\text{leaf}}$  on a mass basis was negatively correlated with LMA and LL, indicating that there is a carbon cost associated with increased water transport that is compensated by a longer LL. The vulnerability to cavitation of stems and leaves were similar, particularly in fertilized plants. Leaves in the species studied may not function as safety valves at low water potentials to protect the hydraulic pathway from water stress-induced cavitation. The lack of rainfall seasonality in the subtropical forest studied probably does not act as a selective pressure to enhance hydraulic segmentation between leaves and stems.

**Keywords:** leaf hydraulic conductance, leaf lifespan, leaf mass per unit area, semideciduous Atlantic Forest, turgor loss point.

## Introduction

Nitrogen (N) deposition has been predicted to increase over the next century by as much as 250% as a result of global change (Galloway et al. 2004, Lamarque et al. 2005). The

overall effects of N deposition on the growth, physiology and chemistry of plants may depend on other factors such as soil water availability and on the scale of the process observed. However, the globally increasing trends in intensity and severity of droughts (IPCC 2007) may impair the effects of more

available N. In this global change context, the morphological and physiological plasticity of plants in key functional attributes will determine the species-specific responses to environmental changes (Nicotra et al. 2010). Investment in carbon and nutrients in leaves have been shown to vary substantially between species worldwide (Wright et al. 2004). The species-specific economic spectrum reflects the balance between investments and returns in nutrients and carbon and should be linked to leaf hydraulic properties. For example, capturing more soil N and increasing foliar N concentration should result in photosynthetic enhancement and fix more carbon at the cost of larger rates of water loss. Cost and benefits not only involve leaf traits related to carbon gain but also hydraulic traits in leaves. Nutrients in tropical soils affect growth and seedling establishment and shape plant community composition and the distribution of individual species (e.g., Fyllas et al. 2009, Patiño et al. 2009, Condit et al. 2013). Both N and phosphorous (P) possibly co-limit productivity and growth in tropical soils (Elser et al. 2007, Vitousek et al. 2010).

During drought, plants can close their stomata to slow down transpiration and water consumption, while avoiding rapid declines in plant water potential and the risk of xylem cavitation as a result of air-seeding or vessel implosion (Zimmermann 1983, Sperry and Tyree 1988, Hacke et al. 2001, Johnson et al. 2012). Embolisms can lead to vessels becoming completely dysfunctional, a loss of plant hydraulic conductivity and ultimately death under extreme water deficits (Tyree and Sperry 1988, Davis et al. 2002, Engelbrecht et al. 2005). When comparing different vegetative organs of trees, leaves appear to be more vulnerable to cavitation than stems (Hao et al. 2008, Johnson et al. 2011, Bucci et al. 2012, 2013), suggesting that the hydraulic pathway is segmented, which constrains cavitation to leaves before the effects of rapid pressure drops can spread into the stems (Zimmermann 1983, Tyree and Ewers 1991).

The inter-specific relationships between leaf traits associated with plant carbon economy and hydraulic architecture have not yet been well established. Leaf lifespan (LL) is a major trait related to leaf lifetime carbon gain and its economic return (Westoby et al. 2000, Falster et al. 2012). Recently, Simonin et al. (2012) found that LL is negatively correlated with leaf hydraulic conductance ( $K_{\text{leaf}}$ ) across species. Nardini et al. (2012a) found a negative correlation between  $K_{\text{leaf}}$  and xylem resistance to cavitation and leaf mass per unit area (LMA), suggesting that species with a conservative behaviour in relation to leaf carbon investment also show a similar strategy in relation to water transport efficiency and xylem embolism. In the latter two studies,  $K_{\text{leaf}}$  was expressed on a mass basis ( $K_{\text{leaf\_mass}}$ ), which provides a better proxy for the efficiency of the leaf hydraulic system in terms of water supply to the bulk of leaf tissues or in economic terms to better represent its carbon costs.

The mechanisms underlying the link between nutrient availability and plant hydraulics entail the increment in growth and its effects on the carbon allocation patterns, wood anatomy, water relations and hydraulic architecture (Goldstein et al. 2013). In a previous work, we showed that nutrient addition enhances growth rate, decreases wood density and stem vulnerability to cavitation of tree saplings growing in gaps of the semideciduous Atlantic Forest in Northeastern Argentina (Villagra et al. 2013). In this study, we answer the questions: (i) does leaf hydraulic vulnerability change in response to increase in nutrient availability? (ii) Are stem and leaf hydraulics traits functionally coordinated with each other as well as with leaf economic traits such as LL and LMA? Removal of nutrient limitations was achieved by repeated N and P fertilizations. Tree saplings of similar age from five species growing in fertilized and control (non-fertilized) gaps were selected for this study. Gaps of similar size were used to maintain similar solar radiation levels on the study plants.

## Materials and methods

### Study site, experimental design and species

This study was conducted on field-grown saplings of five species common in the semideciduous Atlantic Forest at Iguazú National Park (25°37'S, 54°20'W), Misiones, Argentina. This forest region receives an even distribution of precipitation throughout the year; with an average annual rainfall of 2000 mm. Soil water potentials can attain values as low as −2 MPa (Campanello et al. 2007) during short dry spells. The soils are deep Ultisols containing high concentrations of Fe, Al and Si (Ligier et al. 1990). The annual mean air temperature is 21 °C, but freezing temperatures occasionally occur in winter (Ligier et al. 1990, Gatti et al. 2008). The forest has mature trees ranging from 20 to 45 m in height, and abundant lianas, epiphytes and bamboos species in the understory. The canopy is mostly dominated by trees of the genus *Cordia* L. (Boraginaceae), *Ocotea* Aubl. and *Nectandra* Rol. ex Rottb. (Lauraceae), *Parapiptadenia* Brenan and *Peltophorum* (Bogel) Benth. (Fabaceae), *Cedrela* P. Browne and *Trichilia* P. Browne (Meliaceae), and *Plinia* L., *Eugenia* L. and *Myrciaria* O. Berg. (Myrtaceae) (Srur et al. 2009). A significant percentage of the tree species (25–50%) are deciduous (Leite and Klein 1990). Leaf loss occurs between April and September depending on the species.

The experiment included one fertilization treatment and a control with five replicates each in a randomized complete design (Villagra et al. 2013). Ten permanent 15 × 15-m plots were established during 2004 in natural gaps. A 5-m buffer zone was delimited around each plot to minimize edge effects. The treatments were: control (no fertilizer added) and N + P (100 kg N ha<sup>−1</sup> year<sup>−1</sup> + 100 kg P ha<sup>−1</sup> year<sup>−1</sup>). The fertilizers were sprinkled in granular form on the organic soil surface in three equal doses during the year from February 2004 to December 2008. The buffer areas surrounding the plots were also fertilized.

Before fertilization, N concentration in the top 10 cm of the control plot soils was  $10.5 \pm 1.6 \mu\text{g g}^{-1}$  Kjeldahl extractable N (Villagra et al. 2013). The concentration of P was not determined, but we assumed that the P levels in the control plots were similar before and after the treatment began. After 18 months of nutrient addition there were  $12.8 \pm 2.0 \mu\text{g g}^{-1}$  Kjeldahl extractable N and  $3.0 \pm 0.3 \mu\text{g g}^{-1}$  Olsen extractable plant available P. In the N+P treatment the values were  $10.3 \pm 1.2 \mu\text{g g}^{-1}$  N and  $9.3 \pm 1.0 \mu\text{g g}^{-1}$  P (Villagra et al. 2013). Foliar N concentration did not change with nutrient additions, but there was an increase in foliar P concentration (Montti et al. 2013). Fertilized plants with N+P had higher total leaf area than control plants (Montti et al. 2013, Villagra et al. 2013).

Five common tree species at the site were selected (Table 1). The species varied in regeneration light requirements and wood density (Carvalho 2003, Campanello et al. 2011, Villagra et al. 2013). Seeds were collected between August and February 2003 from five to 10 parent trees growing at Parque Nacional Iguazú and were germinated in local topsoil in nursery flats. 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. Five- to 10-month-old seedlings were planted in June 2004. Plants were randomly distributed in the plots spaced 1 m apart each other along five transects, spanning an area of  $5 \times 15$  m. Between 11 and 15 individuals per species were planted on each plot. The seedlings of the different species differed in initial sizes: mean height (cm  $\pm$  1 SE) was  $17.8 \pm 1.8$  for *Balfourodendron riedelianum*,  $61.4 \pm 6.2$  for *Cedrela fissilis*,  $24.3 \pm 1.6$  for *Cordia americana*,  $54.8 \pm 2.8$  for *Maclura tinctoria* and  $65.7 \pm 9.8$  for *Peltophorum dubium*. All physiological measurements were performed during February–March 2009. Sun-exposed trees (1.70–6.40 m height) were used to perform the measurements.

### Stem vulnerability to cavitation

Data for stem vulnerability to cavitation were obtained from a published study performed on the same field experiment (Villagra et al. 2013). Briefly, three to five branches per species (one per plot) were sampled on the control and N+P

treatments and hydraulic vulnerability curves were generated using the bench drying method (Sperry et al. 1988). The water potential at which 50% loss of hydraulic conductivity occurs ( $P50_{\text{stem}}$ ) was derived from those curves and used as an index of the resistance to xylem cavitation.

### Leaf vulnerability curve

Leaf hydraulic vulnerability curves were developed for each species by measuring the percentage loss of  $K_{\text{leaf}}$  from  $K_{\text{leaf\_max}}$  in leaves rehydrated from a range of leaf water potentials ( $\Psi_{\text{leaf}}$ ). The  $K_{\text{leaf}}$  was measured by assessing the kinetics of  $\Psi_{\text{leaf}}$  relaxation upon leaf rehydration (Brodribb and Holbrook 2003). Branches from three individuals of each species per treatment were cut at predawn and immediately covered by black plastic bags and transported to the laboratory. The branches were allowed to desiccate slowly until a wide range of  $\Psi_{\text{leaf}}$  was obtained. Three adjacent leaves were chosen per branch. The  $\Psi_{\text{leaf}}$  of two leaves were determined using a pressure chamber (PMS Instrument Company, Albany, OR, USA). Leaves with similar values (i.e., if the difference was  $<0.1$  MPa) were used and the average  $\Psi_{\text{leaf}}$  was used as the initial data. The third leaf was then cut under water and allowed to rehydrate for a period of 12–15 s depending on the species and the degree of rehydration (time required for rehydration was previously determined). The  $K_{\text{leaf}}$  was calculated as:

$$K_{\text{leaf}} = C_{\text{leaf}} \ln \frac{\Psi_o / \Psi_f}{T},$$

where  $\Psi_o$  is initial  $\Psi_{\text{leaf}}$  (MPa),  $\Psi_f$  is the final  $\Psi_{\text{leaf}}$  (MPa),  $T$  is the duration of rehydration (s) and  $C_{\text{leaf}}$  is the leaf capacitance ( $\text{mmol m}^{-2} \text{MPa}^{-1}$ ).

Leaf capacitance was determined from pressure–volume relationships. If the initial  $\Psi_{\text{leaf}}$  was higher than the turgor loss point (TLP), then  $K_{\text{leaf}}$  was calculated using  $C_{\text{leaf}}$  obtained before the TLP; otherwise  $C_{\text{leaf}}$  after the TLP was used (Brodribb and Holbrook 2003). Leaf capacitance was normalized by leaf area or leaf dry weight as suggested by Nardini et al. (2012b) and Simonin et al. (2012), thus obtaining  $C_{\text{leaf\_area}}$  and  $C_{\text{leaf\_mass}}$ . Leaf vulnerability was determined by plotting  $K_{\text{leaf}}$  against  $\Psi_o$ . The water potential at which 50% loss of leaf hydraulic conductance occurs ( $P50_{\text{leaf}}$ ) and the  $K_{\text{leaf\_max}}$  were estimated from a four-parameter logistic function fitted to the data.

### Pressure–volume relations

The leaves were cut at predawn at the base of the petiole, re-cut immediately under water and the entire leaves covered with black plastic bags with the cut end in water until measurements began within 2 h. For *C. americana* small terminal shoots were sampled because of its reduced leaf petioles, and for species with large and compound leaves (*B. riedelianum*, *C. fissilis* and *P. dubium*), leaflets were used to determine water potential with

Table 1. Family and leaf phenology of the study species.

Species	Family	Leaf phenology
<i>Balfourodendron riedelianum</i> (Engl.) Engl.	Rutaceae	Brevidciduous
<i>Cordia americana</i> (L.)	Boraginaceae	Evergreen
<i>Maclura tinctoria</i> (L.) Steud. ssp. <i>Tinctoria</i>	Moraceae	Deciduous
<i>Cedrela fissilis</i> Vell.	Meliaceae	Deciduous
<i>Peltophorum dubium</i> (Spreng.) Taub.	Fabaceae	Deciduous

the pressure chamber (PMS Instrument Company). Immediately following  $\Psi_{\text{leaf}}$  measurement leaves were weighed on a precision balance (0.001 g). Between readings the leaves were left to transpire freely for a short period of time. Subsequent measurements of weight and balancing pressure continued until very negative  $\Psi_{\text{leaf}}$ . Then the leaves were oven-dried at 70 °C for 48 h. Linear and non-linear functions were fitted to the data at both sides of the TLP. The pressure–volume curves were generated from five to seven leaves of three to four individuals per species and treatment and used to estimate the TLP, leaf osmotic potential at full turgor ( $\pi^{100}$ ) and  $C_{\text{leaf}}$ .

#### Wood saturated water content

Terminal branches were used to measure wood saturated water content (WSWC). A 2-cm-long stem section was cut, and both bark and pit were removed from the segment. The sapwood was kept in water overnight to saturate the sample. After the surface was dried with tissue paper, the sample was weighed to the nearest milligram. Then the sapwood was oven-dried at 70 °C for 72 h to determine dry mass. Wood saturated water content was determined as:

$$\text{WSWC} = \frac{\text{SM} - \text{DM}}{\text{DM}},$$

where SM is the saturated mass (g) and DM is the dry mass (g).

#### Leaf mass per unit area and LL

Five fully sun-expanded leaves were collected from five trees per treatment in plots where hydraulic properties had been measured. The leaf area was scanned and analysed with Leaf Area Measurement (V.1.3, copyright 2003, Sheffield University; [www.shf.ac.uk](http://www.shf.ac.uk)). Leaves were then oven-dried (60 °C), and their dry weight used to calculate LMA.

Mean LL was obtained from a published study performed on the same field experiment (Montti et al. 2013) except for *C. fissilis*. To assess LL, five leaf buds exposed to full sunlight on five saplings of each species from all control and N + P plots were marked at the beginning of the growing season. The leaf bud represented a cohort of leaves of the same age. Census of individuals was taken monthly. The Kaplan–Meier product limit method (Kleinbaum and Klein 2005) was used to estimate mean LL. The sample sizes for leaf demography ranged from 125 to 285 leaves per species.

#### Statistical analysis

The InfoStat 2011 statistical software (Di Rienzo et al. 2011) was used for statistical analysis. Pearson correlations were used to examine key inter-specific trait relationships. A *t*-test for dependent samples was used to test for the treatment effect on variables on pooled species. Data from vulnerability curves were analysed with GraphPad Prism version 6.0

software using a sigmoid function and the parameters  $P50_{\text{leaf}}$  and  $K_{\text{leaf\_max}}$  obtained for each species were compared between treatments with the Extra sum-of-squares *F* test (GraphPad Software, Inc., San Diego, CA, USA). To compare LL between treatments, a log-rank test was performed (Kleinbaum and Klein 2005).

## Results

The LMA increased significantly in plants growing with the addition of N + P (Table 2). All species increased the LMA at least 20% with nutrient addition. The mean LL decreased across all species in fertilized plants compared with control plants and the difference was marginally significant (Table 2).

The  $K_{\text{leaf}}$  of each species decreased as  $\Psi_{\text{leaf}}$  became more negative (Figure 1). The  $K_{\text{leaf\_max}}$  remained relatively constant despite N + P addition (Table 2). The difference between treatments for  $K_{\text{leaf\_max}}$  was 0.3 mmol m<sup>-2</sup> s<sup>-1</sup> MPa<sup>-1</sup> for *M. tinctoria* and 3 mmol m<sup>-2</sup> s<sup>-1</sup> MPa<sup>-1</sup> for *C. fissilis* (Figure 1 and Table 2). There was no difference in  $K_{\text{leaf\_max}}$  between control and N + P treatments within each species. The  $K_{\text{leaf}}$  values corresponding to the TLP ranged between 13 and 65% of  $K_{\text{leaf\_max}}$  (inferred from vulnerability curves, Figure 1). The average  $P50_{\text{leaf}}$  across all species did not vary between control and N + P treatments (Table 2). Three species increased their  $P50_{\text{leaf}}$  and became more vulnerable to drought-induced embolisms with N + P fertilization (*B. riedelianum*, *C. fissilis* and *M. tinctoria*), while the other two species decreased their  $P50_{\text{leaf}}$  (*C. americana* and *P. dubium*). No statistical differences were found for *C. fissilis* (Figure 1 and Table 2).

There was no significant relationship between  $P50_{\text{stem}}$  and  $P50_{\text{leaf}}$  across species and treatments ( $r_{\text{Pearson}} = 0.36$ ,  $P = 0.30$ ), although  $P50_{\text{leaf}}$  of fertilized plants were similar to  $P50_{\text{stem}}$ , indicating that stems and leaves of fertilized plant were equally vulnerable to cavitation (Figure 2). The largest species-specific difference between  $P50_{\text{stem}}$  and  $P50_{\text{leaf}}$  was found in *B. riedelianum* growing in control plots (1.06 MPa). The only species that had slightly higher vulnerability in the leaves than in the stems was *M. tinctoria* in the N + P treatment (Figure 2).

Both LL and LMA were negatively correlated with  $P50_{\text{leaf}}$ ; that is, both leaf traits decreased when leaves became more vulnerable to cavitation (less negative  $P50_{\text{leaf}}$ ) (Figure 3a and c, Table 3). A single linear relationship was fitted to all species and treatments, suggesting that  $P50_{\text{leaf}}$  was related to leaf carbon economy. The  $K_{\text{leaf\_mass}}$  decreased with increasing LL and LMA across the species regardless of treatments (Figure 3b and d, Table 2). The  $K_{\text{leaf}}$  on leaf area basis was not significantly correlated either with LL or LMA or  $P50_{\text{leaf}}$ ; nevertheless,  $K_{\text{leaf\_area}}$  was positively correlated with WSWC (Table 3).

The WSWC increased linearly with decreasing wood density ( $r_{\text{Pearson}} = -0.83$ ,  $P < 0.005$ , data not shown). The WSWC



Table 2. Leaf functional traits for the studied species in the control (C) and N + P plots. Units: LMA ( $\text{g m}^{-2}$ ), mean LL (days),  $\text{P50}_{\text{leaf}}$  (MPa),  $K_{\text{leaf\_max}}$  ( $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ).

	LMA		LL		$\text{P50}_{\text{leaf}}$		$K_{\text{leaf\_max}}$	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<i>B. riedelianum</i>								
C	53.1	2.3	183	4	-2.19	0.09	3.6	0.2
N + P	74.2	3.1	167	4	-1.86	0.04	2.8	0.1
<i>C. americana</i>								
C	56.9	1.4	213	4	-1.63	0.03	6.4	0.3
N + P	83.0	3.7	207	3	-2.04	0.05	5.6	0.2
<i>M. tinctoria</i>								
C	41.7	0.6	126	3	-1.61	0.05	2.6	0.1
N + P	50.6	0.9	116	3	-1.34	0.04	2.9	0.2
<i>C. fissilis</i>								
C	32.0	0.5	136	19	-1.70	0.17	5.1	0.6
N + P	40.6	1.3	92	18	-1.28	0.30	8.1	1.9
<i>P. dubium</i>								
C	30.5	1.0	146	5	-0.94	0.10	2.3	0.3
N + P	46.8	1.3	133	6	-1.37	0.03	3.2	0.1
<i>T</i>	-4.91		-2.63		-0.19		-0.74	
<i>P</i>	0.0080		0.0579		0.8573		0.4994	

Means  $\pm$  SE are shown. SE for  $\text{P50}_{\text{leaf}}$  and for  $K_{\text{leaf\_max}}$  were derived from vulnerability curves. Results of a paired *t*-test are also shown.

was asymptotically related to the TLP and linearly related to  $K_{\text{leaf\_mass}}$  across species and treatments (Figure 4). Thus, species with higher WSWC lost turgor at higher (less negative)  $\Psi_{\text{leaf}}$ . The  $\text{P50}_{\text{leaf}}$  and  $K_{\text{leaf\_mass}}$  were linearly and exponentially correlated to the TLP, respectively (Figure 5, Table 3). Species with the highest TLP exhibited higher  $\text{P50}_{\text{leaf}}$  and  $K_{\text{leaf\_mass}}$ . The  $K_{\text{leaf\_mass}}$  was also positively correlated to the osmotic potential at full turgor across species (Figure 5b inset).

## Discussion

Traits related to leaf carbon economy in the studied species changed with the removal of nutrient limitations. The mean LL in the studied species tended to decrease and the LMA was significantly higher in plants with nutrient addition compared with individuals in control plots. The  $\text{P50}_{\text{leaf}}$  either increased or decreased with the nutrient treatment depending on the species, but the average  $\text{P50}_{\text{leaf}}$  did not change with nutrient addition. Similar lack of response to fertilization was observed for  $K_{\text{leaf\_max}}$ .

### Hydraulic segmentation

The vulnerability of stems and leaves were similar, suggesting that leaves do not act as safety valves at low water potentials to protect the hydraulic pathway from water stress-induced cavitation. The lack of rainfall seasonality in the subtropical forest studied probably is not a selective pressure to enhance hydraulic segmentation between leaves and stems. All species in fertilized plots were at or very near to the 1 : 1 linear relationship between  $\text{P50}_{\text{leaf}}$  and  $\text{P50}_{\text{stem}}$ , indicating that leaves

and stems are equally vulnerable to cavitation. Comparative studies of the vulnerability to cavitation of stems and leaves in ecosystems with strong seasonality in precipitation show that leaves are more vulnerable than stems (Hao et al. 2008, Zhang and Cao 2009, Chen et al. 2010, Johnson et al. 2011, Bucci et al. 2012, 2013). Less expensive tissues, such as leaves, can be discarded or shaded to prevent water loss and to maintain the functionality of the stems (Johnson et al. 2011, Bucci et al. 2012).

At the study site, drought periods are short and unpredictable; thus stems might be rarely affected by embolisms caused by drought. However, in a recent study, dead branches were observed for some species after an infrequent dry spell in the Iguazú National Park during the summer of 2012. Most of the study species were deciduous or brevidciduous (only *C. americana* was evergreen). Deciduousness is a strategy of plants to avoid drought or freezing temperatures; consequently, it may not be necessary to invest in traits that increase resistance to cavitation in leaves. Deciduous species in this study had higher  $\text{P50}_{\text{leaf}}$  (most vulnerable) than the evergreen and the brevidciduous species. Thus the dynamics of leaf production may play an important role in determining the hydraulic architecture of the species in the semideciduous Atlantic Forest.

Some components of the water transport system of plants, e.g., the stems and leaves, serve as transient sources of water for leaf transpiration (Meinzer et al. 2001). For example, the stem water reservoirs help to maintain the efficiency of the transport system and to reduce daily and seasonal fluctuations in  $\Psi_{\text{leaf}}$  (Goldstein et al. 1998, Scholz et al. 2007, 2012,

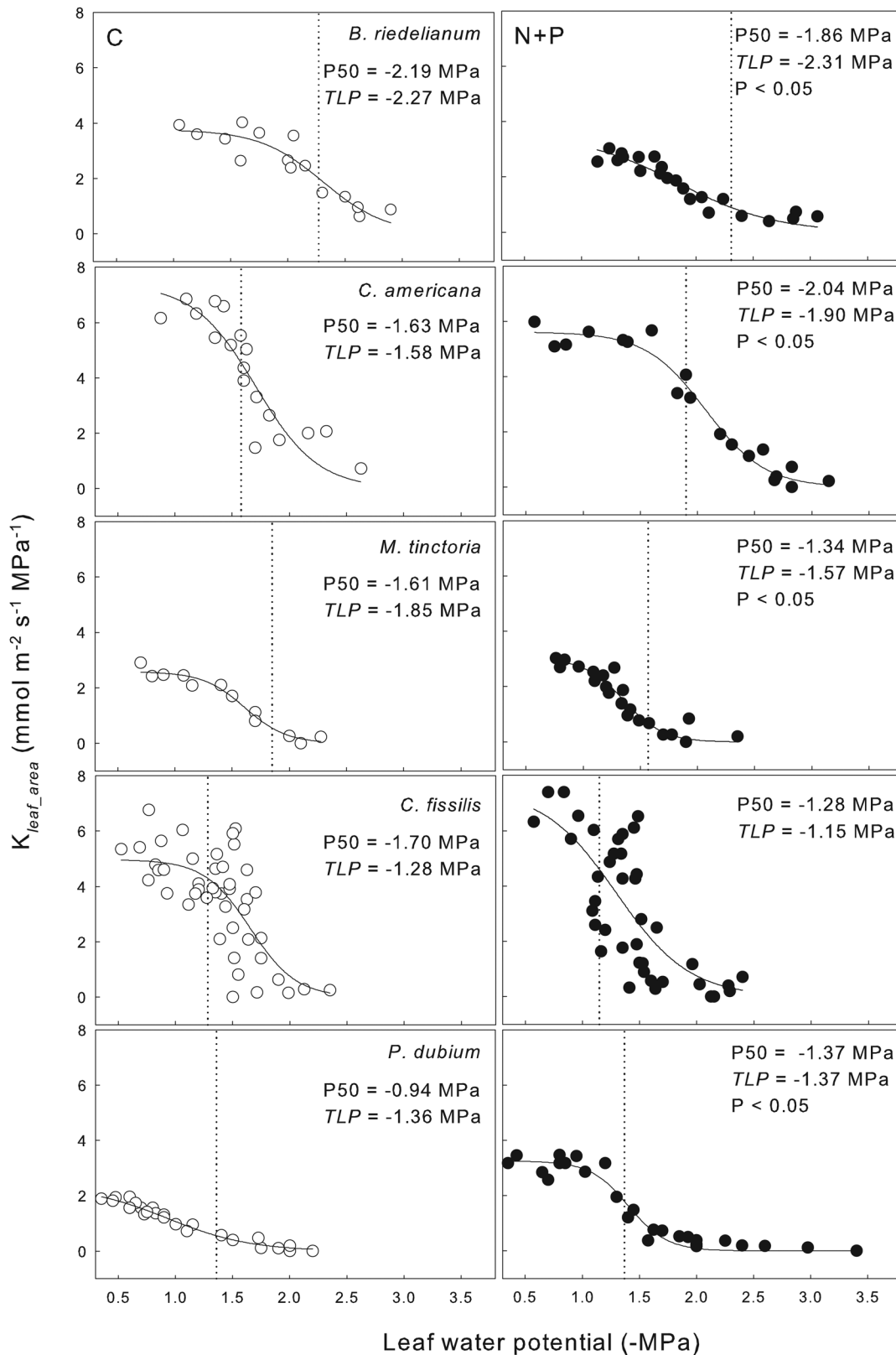


Figure 1. Leaf hydraulic conductance on a leaf area basis ( $K_{\text{leaf\_area}}$ ) as a function of  $\psi_{\text{leaf}}$  for the study species in control plots (right panels) and N + P plots (left panels). Each point represents the average  $K_{\text{leaf}}$  from two leaves of a single branch. A sigmoid function was fitted to the data ( $P < 0.05$  in all cases). The  $\psi_{\text{leaf}}$  at which 50% loss of  $K_{\text{leaf}}$  occurs is indicated as P50, and the  $\psi_{\text{leaf}}$  at the TLP is indicated as TLP. Dashed vertical lines represent the TLP. The  $P$  values are from an Extra sum-of-squares  $F$  test evaluating significant differences of P50 for each species between control and N + P plots.

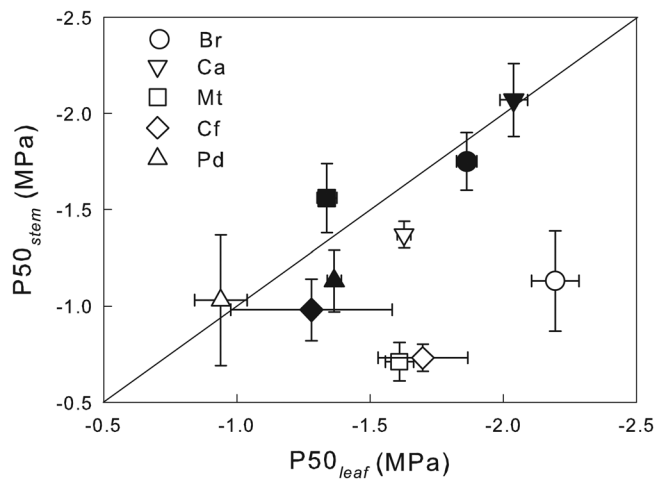


Figure 2. Relationship between the water potential at which 50% loss of hydraulic conductivity occurs in stems ( $P50_{stem}$ ) and leaves ( $P50_{leaf}$ ) for the five species studied in control plots (open symbols) and N + P plots (filled symbols). Each point represents mean values ( $\pm$  SE) for single species in each fertilization treatment. The SE were obtained from the vulnerability curves. The line is the 1:1 relationship between  $P50_{stem}$  and  $P50_{leaf}$ : Br, *B. riedeliana*; Ca, *Co. americana*; Mt, *M. tinctoria*; Cf, *C. fissilis*; Pd, *P. dubium*.

Meinzer et al. 2008, Zhang et al. 2013). However, in leaves, water stored in the epidermis, mesophyll, and in specialized cells or in the extracellular mucilage (Sack et al. 2003) contributes only to a small fraction of the daily transpiration and does not seem to help maintain the water balance of a plant (Scholz et al. 2011). In this study, we observed that species with higher WSWC had a higher water transport efficiency and TLP. These results suggest that water stored in the stems may buffer daily fluctuations in plant water status and extend carbon gain on a daily basis. Although the saturated water content of a tissue cannot be considered as equivalent to its capacitance, a measure of the ability to release water to the xylem in response to variations in water status (Scholz et al. 2011), both variables are positively correlated (Scholz et al. 2007). Pineda-García et al. (2013) suggested that sapwood water reserves have a high adaptive value during the initial stages of plant development, since the ability of roots to obtain water can be limited.

### Leaf economy and hydraulic traits

Leaf economy was related to leaf hydraulic traits, as described by the negative relationships observed between LL, LMA and

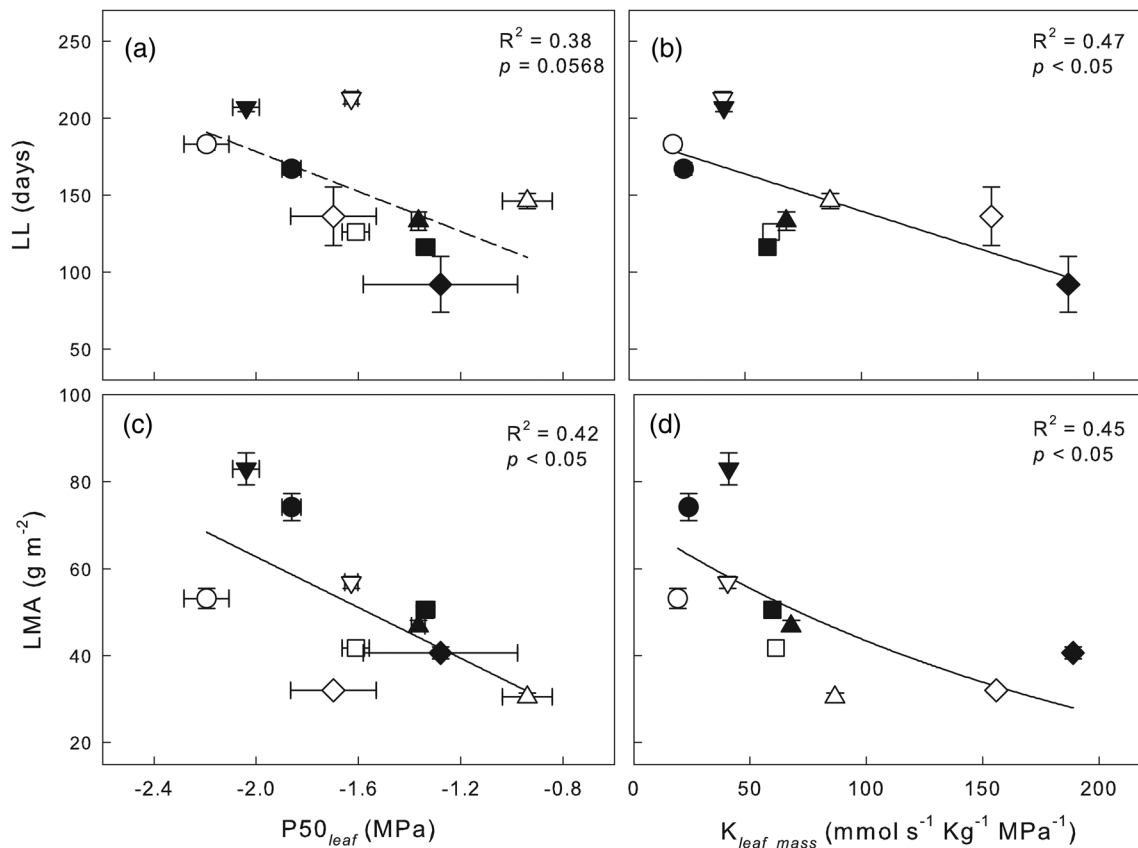


Figure 3. Relationships between  $P50_{leaf}$  and (a) mean LL and (b) LMA, as well as between  $K_{leaf\_mass}$  and (c) LL (c) and LMA (d) for five species studied in control plots (open symbols) and N + P plots (filled symbols). Each symbol represents mean values ( $\pm$  SE) for single species. Species symbols are as in Figure 2. The solid lines represent the regressions fitted to the data: (a)  $y = 48 - 65x$ ; (b)  $y = 188 - 0.05x$ ; (c)  $y = 4 - 29x$ ; and (d)  $y = 71 \times \exp(-0.0005x)$ .

Table 3. A Pearson correlation matrix showing  $r$  values for correlations between WSWC,  $\pi^{100}$ ,  $K_{\text{leaf\_area}}$  or  $K_{\text{leaf\_mass}}$ ,  $\Psi_{\text{leaf}}$ ,  $P50_{\text{leaf}}$ , LMA and mean LL.

	WSWC	TLP	$\pi^{100}$	$K_{\text{leaf\_area}}$	$K_{\text{leaf\_mass}}$	$P50_{\text{leaf}}$
TLP	0.75*					
$\pi^{100}$	0.76*	1.00*				
$K_{\text{leaf\_area}}$	0.70*	0.39*	0.37			
$K_{\text{leaf\_mass}}$	0.94*	0.80*	0.81*	0.54		
$P50_{\text{leaf}}$	0.30	0.76*	0.75*	-0.09	0.49	
LMA	-0.49	-0.67*	-0.67*	0.08	-0.63*	-0.65*
LL	-0.42	-0.55	-0.55*	0.05	-0.68*	-0.62*

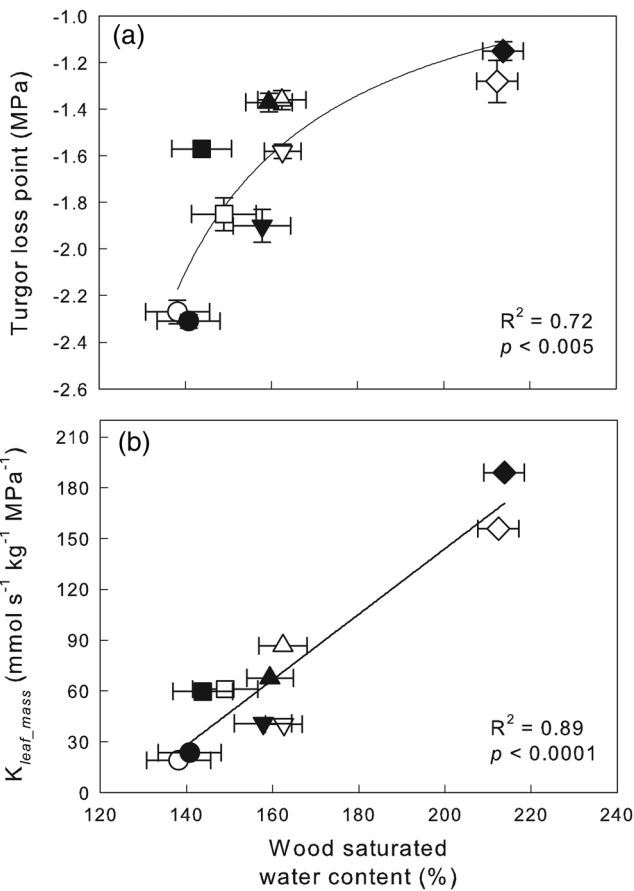


Figure 4. Relationships between WSWC and (a) the  $\Psi_{\text{leaf}}$  at the TLP, and (b)  $K_{\text{leaf\_mass}}$  for five species studied in control plots (open symbols) and N + P plots (filled symbols). Each symbol represents mean values ( $\pm$  SE) for single species. Species symbols are as in Figure 2. The solid lines represent: (a) an hyperbola function fitted to the data,  $y = -0.59 \times x / (-101 + x)$ ; (b) the linear regression fitted to the data  $y = -24 + 19x$ .

$P50_{\text{leaf}}$ , as well as  $K_{\text{leaf\_mass}}$ . These results suggest that species with less vulnerable leaves have associated a higher carbon investment, thus implying a higher cost for supplying water to leaves (leaves more resistant to cavitation and with lower  $K_{\text{leaf\_mass}}$ ). Functional relationships between some of the study traits were not affected by fertilization. Research examining leaf hydraulic and leaf economic traits has shown contrasting

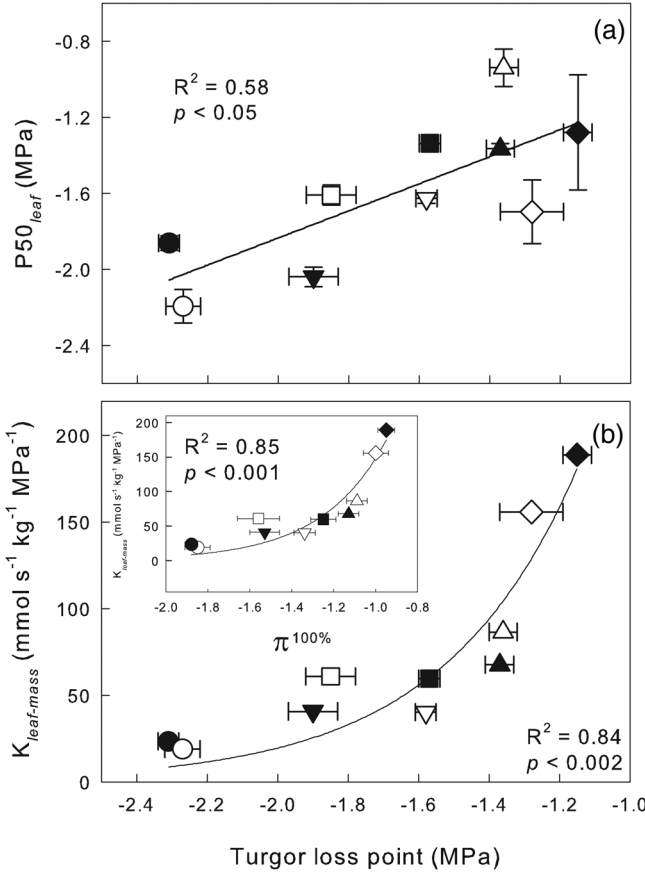


Figure 5. Relationships between the  $\Psi_{\text{leaf}}$  at the TLP and (a)  $P50_{\text{leaf}}$ , (b)  $K_{\text{leaf\_mass}}$  for five species studied in control plots (open symbols) and N + P plots (filled symbols). Each symbol represents mean values ( $\pm$  SE) for single species. In the inset, the relationship between the  $\pi^{100}$  and the  $K_{\text{leaf\_mass}}$  is shown. Species symbols are as in Figure 2. The solid lines represent: (a) the linear regression fitted to the data,  $y = -0.41 + 0.71x$ ; (b) the exponential growth function fitted to the data  $y = 36,403 \times 14^x$ . In the inset, the solid line represents the exponential growth function fitted to the data  $y = 40,155 \times 27^x$ .

results; some studies found significant correlations between  $K_{\text{leaf}}$ ,  $P50_{\text{leaf}}$  and LMA (Nardini et al. 2012a, Simonin et al. 2012), while other studies failed to reveal such relationships (Tyree et al. 1999, Sack et al. 2003, Blackman et al. 2010, Bucci et al. 2013). Also, no trade-off was found between  $K_{\text{leaf\_area}}$  and  $P50$  in other studies (Sack and Holbrook 2006, Blackman et al.



2010, Scoffoni et al. 2011, Nardini et al. 2012a). The primary focus of most of these studies has been to examine the relation of leaf economic traits with the  $K_{\text{leaf\_area}}$  rather than  $K_{\text{leaf\_mass}}$ . As such, some of the reported results may have been biased to processes occurring at the leaf–atmosphere interface and not in the whole leaf tissues as suggested by Nardini et al. (2012a). Recently, other studies found that  $K_{\text{leaf\_mass}}$  was negatively correlated with LL or LMA, while  $K_{\text{leaf\_area}}$  was not (Nardini et al. 2012a, Simonin et al. 2012). In our study we extend these functional patterns to subtropical tree species. Despite the narrow range of LL across the study species, negative correlations were found not only between  $K_{\text{leaf\_mass}}$ , LL and LMA, but also between LL and  $P50_{\text{leaf}}$  and between  $K_{\text{leaf\_mass}}$  and  $P50_{\text{leaf}}$ . These trade-offs have a large functional significance because a plant with more expensive and less vulnerable leaves retains them for a longer period of time.

We found a linear relationship between  $K_{\text{leaf\_mass}}$ , TLP and  $n100$  for the species studied. The species with a low TLP (more negative) have lower supply of water to leaf tissues (low  $K_{\text{leaf\_mass}}$ ) and are more resistant to drought-induced cavitation. Alternatively, a high water transport efficiency ( $K_{\text{leaf\_mass}}$ ) may allow for sufficient acquisition of water for tree species, preventing the drop in the  $\Psi_{\text{leaf}}$  and avoiding embolisms, while keeping stomata open and thus preventing carbon assimilation to decrease. A slow-growing species, *B. riedelianum*, had the lowest values of  $P50_{\text{leaf}}$  and TLP, while *C. fissilis* and *P. dubium*, both fast-growing species, had the highest ones. The latter two species are common in forest gaps, where water availability is commonly higher than under a closed canopy (Ashton 1992, Camargo and Kapos 1995).

## Conclusions

There were no consistent differences in leaf hydraulic traits between control and fertilized plants, suggesting that leaf hydraulics is not critical in the adaptation of these species to environments with different soil nutrient availability. However, the fertilization treatment affected traits related to the leaf economic spectrum such as LL and LMA, which were correlated with leaf hydraulic traits. The  $K_{\text{leaf\_mass}}$  was negatively correlated with LMA and LL, indicating that there is a carbon cost associated to increased water transport that is compensated by a longer LL. Contrary to our expectations, the leaves of the studied species do not act as safety valves of the hydraulic system. Hydraulic segmentation does not seem to be an adaptation for mitigating the effects of short dry periods in the studied plants.

## Acknowledgments

We thank Diego Varela, M. Rosa Olivera, M. Genoveva Gatti and Lía Montti for their helpful assistance with field data collection. We are also grateful to the Administración de Parques Nacionales (APN), CIES, DTRNEA and the park rangers who

provided logistic support and facilities at the Iguazú National Park field sites.

## Conflict of interest

None declared.

## Funding

This research was supported by the Agencia Nacional de Promoción Científica y Tecnológica (grant PICT2000 08341) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina.

## References

- Ashton PMS (1992) Some measurements of the microclimate within a Sri Lankan tropical rainforest. *Agric For Meteorol* 59:217–235.
- Blackman CJ, Brodribb TJ, Jordan GJ (2010) Leaf hydraulic vulnerability is related to conduit dimensions and drought resistance across a diverse range of woody angiosperms. *New Phytol* 188: 1113–1123.
- Brodribb TJ, Holbrook NM (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiol* 132:2166–2173.
- Bucci SJ, Scholz FG, Campanello PI et al. (2012) Hydraulic differences along the water transport system of South American Nothofagus species: do leaves protect the stem functionality? *Tree Physiol* 32:880–893.
- Bucci SJ, Scholz FG, Peschiutta ML, Arias NS, Meinzer FC, Goldstein G (2013) The stem xylem of Patagonian shrubs operates far from the point of catastrophic dysfunction and is additionally protected from drought-induced embolism by leaves and roots. *Plant Cell Environ* 36:2163–2174.
- Camargo JLC, Kapos V (1995) Complex edge effects on soil moisture and microclimate in central Amazonian forest. *J Trop Ecol* 11: 205–221.
- Campanello PI, Gatti MG, Ares A, Montti L, Goldstein G (2007) Tree regeneration and microclimate in a liana and bamboo-dominated semideciduous Atlantic Forest. *For Ecol Manag* 252:108–117.
- Campanello PI, Gatti MG, Montti LF, Villagra M, Goldstein G (2011) Ser o no ser tolerante a la sombra: economía de agua y carbono en especies arbóreas del Bosque Atlántico (Misiones, Argentina). *Ecol Austral* 21:285–300.
- Carvalho PER (2003) Especies arboreas brasileiras. Ministerio da Agricultura, Pequaria e Abastecimento, Brasília, Brazil.
- Chen JW, Zhang Q, Li XS, Cao KF (2010) Gas exchange and hydraulics in seedlings of *Hevea brasiliensis* during water stress and recovery. *Tree Physiol* 30:876–885.
- Condit R, Engelbrecht BM, Pino D, Pérez R, Turner BL (2013) Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. *Proc Natl Acad Sci USA* 110:5064–5068.
- Davis SD, Ewers FW, Sperry JS, Portwood KA, Crocker MC, Adams GC (2002) Shoot dieback during prolonged drought in *Ceanothus chaparral* of California: a possible case of hydraulic failure. *Am J Bot* 89:820–828.
- Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2011) InfoStat versión 2011. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

- Elser JJ, Bracken MES, Cleland EE et al. (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol Lett* 10:1135–1142.
- Engelbrecht BMJ, Kursar TA, Tyree MT (2005) Drought effects on seedling survival in a tropical moist forest. *Trees* 19:312–321.
- Falster DS, Reich PB, Ellsworth DS, Wright IJ, Westoby M, Oleksyn J, Lee TD (2012) Lifetime return on investment increases with leaf lifespan among 10 Australian woodland species. *New Phytol* 193:409–419.
- Fyllas NM, Patiño S, Baker TR et al. (2009) Basin-wide variations in foliar properties of Amazonian forest: phylogeny, soils and climate. *Biogeosciences* 6:2677–2708.
- Galloway JN, Dentener FJ, Capone DG et al. (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry* 70:153–226.
- Gatti MG, Campanello PI, Montti LF, Goldstein G (2008) Frost resistance in the tropical palm *Euterpe edulis* and its pattern of distribution in the Atlantic Forest of Argentina. *For Ecol Manage* 256:633–640.
- Goldstein G, Andrade JL, Meinzer FC, Holbrook NM, Cavelier J, Jackson P, Celis A (1998) Stem water storage and diurnal patterns of water use in tropical forest canopy trees. *Plant Cell Environ* 21:397–406.
- Goldstein G, Bucci SJ, Scholz FG (2013) Why do trees adjust water relations and hydraulic architecture in response to nutrient availability? *Tree Physiol* 33:238–240.
- Hacke UG, Sperry JS, Pockman WT, Davis SD, Mcculloh KA (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126:457–461.
- Hao GY, Hoffmann W, Scholz FG, Bucci SJ, Meinzer FC, Franco A, Cao KF, Goldstein G (2008) Stem and leaf hydraulics of congeneric tree species from adjacent tropical savanna and forest ecosystems. *Oecologia* 155:405–415.
- IPCC (2007) Summary for policymakers. In: Solomon S, Qin D, Manning M et al. (eds) *Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge/New York.
- Johnson DM, Mcculloh KA, Meinzer FC, Woodruff DR, Eissenstat DM (2011) Hydraulic patterns and safety margins, from stem to stomata, in three eastern US tree species. *Tree Physiol* 31:659–668.
- Johnson DM, Mcculloh KA, Woodruff DR, Meinzer FC (2012) Evidence for xylem embolism as a primary factor in dehydration-induced declines in leaf hydraulic conductance. *Plant Cell Environ* 35:760–769.
- Kleinbaum DG, Klein M (2005) *Survival analysis. A self-learning text*. Springer, NY.
- Lamarque JF, Kiehl JT, Brasseur GP et al. (2005) Assessing future nitrogen deposition and carbon cycle feedback using a multimodel approach: analysis of nitrogen deposition. *J Geophys Res* 110:D19303.
- Leite PF, Klein RM (1990) *Vegetação*. In: Mesquita OV (ed) *Geografia do Brasil: Região Sul, Vol. 2*. Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro, pp 113–150.
- Ligier HD, Matteio HR, Polo HL, Rosso JR (1990) *Provincia de Misiones*. In: Secretaría de Agricultura, Ganadería y Pesca (eds) *Atlas de suelos de la República Argentina. Tomo II. Centro de Investigaciones de Recursos Naturales, INTA, Argentina*, pp 109–154.
- Meinzer FC, Clearwater MJ, Goldstein G (2001) Water transport in trees: current perspectives, new insights and some controversies. *Environ Exp Bot* 45:239–262.
- Meinzer FC, Woodruff DR, Domec JC, Goldstein G, Campanello PI, Gatti MG, Villalobos-Vega R (2008) Coordination of leaf and stem water transport properties in tropical forest trees. *Oecologia* 156:31–41.
- Montti L, Villagra M, Campanello PI, Gatti MG, Goldstein G (2013) Functional traits enhance invasiveness of bamboos over co-occurring tree saplings in the Semideciduous Atlantic Forest. *Acta Oecol* (in press) doi: j.actao.2013.03.004.
- Nardini A, Pedà G, Rocca NL (2012a) Trade-offs between leaf hydraulic capacity and drought vulnerability: morpho-anatomical bases, carbon costs and ecological consequences. *New Phytol* 196:788–798.
- Nardini A, Pedà G, Salleo S (2012b) Alternative methods for scaling leaf hydraulic conductance offer new insights into the structure–function relationships of sun and shade leaves. *Funct Plant Biol* 39:394–401.
- Nicotra AB, Atkin OK, Bonser SP et al. (2010) Plant phenotypic plasticity in a changing climate. *Trends Plant Sci* 15:684–692.
- Patiño S, Lloyd J, Paiva R et al. (2009) Branch xylem density variations across the Amazon Basin. *Biogeosciences* 6:545–568.
- Pineda-García F, Paz H, Meinzer FC (2013) Drought resistance in early and late secondary successional species from a tropical dry forest: the interplay between xylem resistance to embolism, sapwood water storage and leaf shedding. *Plant Cell Environ* 36:405–418.
- Sack L, Holbrook NM (2006) Leaf hydraulics. *Ann Rev Plant Biol* 57:361–381.
- Sack L, Cowan PD, Jaikumar NJ, Holbrook NM (2003) The ‘hydrology’ of leaves: coordination of structure and function in temperate woody species. *Plant Cell Environ* 26:1343–1356.
- Scholz FG, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm F (2007) Biophysical properties and functional significance of stem water storage tissues in Neotropical savanna trees. *Plant Cell Environ* 30:236–248.
- Scholz FG, Phillips NG, Bucci SJ, Meinzer FC, Goldstein G (2011) Hydraulic capacitance: biophysics and functional significance of internal water sources in relation to tree size. In: Meinzer FC, Lachenbruch B, Dawson TE (eds) *Size- and age-related changes in tree structure and function*. Springer, New York, pp 341–361.
- Scholz FG, Bucci SJ, Arias NS, Meinzer FC, Goldstein G (2012) Osmotic and elastic adjustment in cold desert shrubs differing in rooting depth: coping with drought and subzero temperatures. *Oecologia* 170:885–897.
- Scoffoni C, Rawls M, McKown A, Cochard H, Sack L (2011) Decline of leaf hydraulic conductance with dehydration: relationship to leaf size and venation architecture. *Plant Physiol* 156:832–843.
- Simonin KA, Limm EB, Dawson TE (2012) Hydraulic conductance of leaves correlates with leaf lifespan: implications for lifetime carbon gain. *New Phytol* 193:939–947.
- Sperry JS, Tyree MT (1988) Seasonal occurrence of xylem embolism in sugar maple (*Acer saccharum*). *Am J Bot* 75:1212–1218.
- Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ* 11:35–40.
- Srur M, Gatti F, Benesovsky V, Herrera J, Melzew R, Camposano M (2009) Los tipos de vegetación y ambientes del Parque Nacional Iguazú y su distribución en el paisaje. In: Carpinetti B (ed) *Parque Nacional Iguazú, Conservación y desarrollo en la Selva Paranaense de Argentina*. Administración de Parques Nacionales, Buenos Aires, Argentina, pp 99–118.
- Tyree MT, Sperry JS (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress?—answers from a model. *Plant Physiol* 88:574–580.
- Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. *New Phytol* 119:345–360.
- Tyree MT, Salleo S, Nardini A, Lo Gullo MA, Mosca R (1999) Refilling of embolized vessels in young stems of laurel. Do we need a new paradigm? *Plant Physiol* 120:11–21.
- Villagra M, Campanello PI, Montti L, Goldstein G (2013) Removal of nutrient limitations in forest gaps enhances growth rate and resistance to cavitation in subtropical canopy tree species differing in shade tolerance. *Tree Physiol* 33:285–296.
- Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecol Appl* 20:5–15.

- Westoby M, Warton D, Reich PB (2000) The time value of leaf area. *Am Nat* 155:649–656.
- Wright IJ, Reich PB, Westoby M et al. (2004) The worldwide leaf economics spectrum. *Nature* 428:821–827.
- Zhang JL, Cao KF (2009) Stem hydraulics mediates leaf water status, carbon gain, nutrient use efficiencies and plant growth rates across dipterocarp species. *Funct Ecol* 23:658–667.
- Zhang YJ, Meinzer FC, Qi JH, Goldstein G, Cao KF (2013) Midday stomatal conductance is more related to stem rather than leaf water status in subtropical deciduous and evergreen broadleaf trees. *Plant Cell Environ* 36:149–158.
- Zimmermann MH (1983) Xylem structure and the ascent of sap. Springer, Berlin, Germany.