



Tree Physiology 31, 1–12
doi:10.1093/treephys/tpu087



Research paper

Water storage dynamics in the main stem of subtropical tree species differing in wood density, growth rate and life history traits

Laureano Oliva Carrasco^{1,2,3}, Sandra J. Bucci^{2,4}, Débora Di Francescantonio^{1,2}, Oscar A. Lezcano¹, Paula I. Campanello^{1,2,8}, Fabián G. Scholz^{2,4}, Sabrina Rodríguez^{1,2,3}, N. Madanes³, Piedad M. Cristiano³, Guang-You Hao^{5,6}, N. Michele Holbrook⁶ and Guillermo Goldstein^{2,3,7}

¹Laboratorio de Ecología Forestal y Ecofisiología, Instituto de Biología Subtropical (IBS), Facultad de Ciencias Forestales (FCF), Universidad Nacional de Misiones, Bertoni 85, Puerto Iguazú (3370), Misiones, Argentina; ²Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina; ³Laboratorio de Ecología Funcional, Departamento de Ecología Genética y Evolución, Instituto IEGEBA (CONICET-UBA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina; ⁴Grupo de Estudios Biofísicos y Ecofisiológicos, Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco, Comodoro Rivadavia, Argentina; ⁵State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, Liaoning 110016, China; ⁶Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA; ⁷Department of Biology, University of Miami, PO Box 249118, Coral Gables, FL 33124, USA; ⁸Corresponding author (pcampanello@gmail.com)

Received January 31, 2014; accepted September 29, 2014; handling Editor Kathy Steppe

Wood biophysical properties and the dynamics of water storage discharge and refilling were studied in the trunk of canopy tree species with diverse life history and functional traits in subtropical forests of northeast Argentina. Multiple techniques assessing capacitance and storage capacity were used simultaneously to improve our understanding of the functional significance of internal water sources in trunks of large trees. Sapwood capacitances of 10 tree species were characterized using pressure–volume relationships of sapwood samples obtained from the trunk. Frequency domain reflectometry was used to continuously monitor the volumetric water content in the main stems. Simultaneous sap flow measurements on branches and at the base of the tree trunk, as well as diurnal variations in trunk contraction and expansion, were used as additional measures of stem water storage use and refilling dynamics. All evidence indicates that tree trunk internal water storage contributes from 6 to 28% of the daily water budget of large trees depending on the species. The contribution of stored water in stems of trees to total daily transpiration was greater for deciduous species, which exhibited higher capacitance and lower sapwood density. A linear relationship across species was observed between wood density and growth rates with the higher wood density species (mostly evergreen) associated with lower growth rates and the lower wood density species (mostly deciduous) associated with higher growth rates. The large sapwood capacitance in deciduous species may help to avoid catastrophic embolism in xylem conduits. This may be a low-cost adaptation to avoid water deficits during peak water use at midday and under temporary drought periods and will contribute to higher growth rates in deciduous tree species compared with evergreen ones. Large capacitance appears to have a central role in the rapid growth patterns of deciduous species facilitating rapid canopy access as these species are less shade tolerant than evergreen species.

Keywords: hydraulic safety margin, sap flow, sapwood capacitance, stored water use, volumetric water content.

Introduction

Internal water storage in trees is known to play an important role in the water economy of trees by acting as an intermediate

source of water for transpiring leaves (e.g., Waring and Running 1978, Nobel and Jordan 1983, Goldstein et al. 1984, Meinzer et al. 2001, Scholz et al. 2008). The contribution of stored water

from the main stems of trees to total daily transpiration ranges from 10 to 50% (Holbrook and Sinclair 1992, Loustau et al. 1996, Goldstein et al. 1998, Steppe and Lemeur 2004, Scholz et al. 2007, 2008). Daily utilization of stored water in trunks for transpiration has been estimated from lags between the crown and basal sap flow and from lags between stem sap flow and air saturation deficits (ASD, Schulze et al. 1985, Goldstein et al. 1998). Water storage calculation can also be derived from measurements of gravimetric water content of trunk tissue samples and thermodynamic estimates of tissue water availability (Goldstein et al. 1984). Recently, frequency domain reflectometry (FDR) with high precision, high temporal resolution and low temperature sensitivity has been used to monitor continuously the volumetric water content (VWC) in the main stems of living trees (Hao et al. 2013, Oliva Carrasco et al. 2013). Whether these different techniques would provide similar results on the patterns of plant internal water storage use needs to be tested by simultaneous measurements on the same trees.

It is important to distinguish between water storage capacity (WSC) and hydraulic capacitance (C). The first term expressed in units of mass or volume of water is an extensive property and thus is a function of the integrated volume of available water in the storage compartment, while capacitance reflects intrinsic differences in the biophysical properties of the storage tissues and their potential ability to release water to the xylem in response to variations in the water status of different plant organs (Scholz et al. 2011). Sapwood capacitance can be calculated from pressure–volume relationships of the sapwood tissue sample using water content estimates and psychrometric measurements of stem water potential (Scholz et al. 2007). Although WSC and capacitance are fundamentally different properties, both influence whole-tree water transport properties and tree water relations. In this study, techniques that assess capacitance and WSC were used simultaneously to improve our understanding of the functional significance of internal sources of water in tree trunks. The abundance and spatial distribution of xylem parenchyma, its connections to the phloem and other living bark cells, as well as osmotic adjustments in those living cells and changes in cell turgor (Borchert and Pockman 2005, Scholz et al. 2008, Steppe et al. 2012), have been used to understand fine-scale mechanisms of water transfer between water storage compartments and the long distance water transport pathway. However, this level of resolution provides relatively little information in terms of the total amount of water moving from reservoirs to the transpiring leaves in large forest trees.

Tropical and subtropical humid forests are notable for their high species diversity as well as for high diversity in tree architecture, light requirements and growth rates. Subtropical forests in northeast (NE) Argentina are characterized by little seasonality of rainfall; however, dry spells of several weeks can occur any time of the year (Campanello et al. 2007). During

the summer, forest trees on average transpire >100 mm per month (P. Cristiano, unpublished data). The ASD is very high at midday in these subtropical forests, particularly during the summer when ASD can reach 4.5 kPa. Physiological traits related to the regulation of water utilization and water balance not only on a daily basis but also during dry spells should be important adaptations to the environment. The semideciduous forests of southern Brazil, eastern Paraguay and NE Argentina have been considered to be part of the Atlantic Forest that runs along the coast of Brazil down to Misiones Province in Argentina. The analysis of patterns of geographical distribution of some species that are conspicuous in seasonal dry neotropical forests has led to the understanding that these subtropical semideciduous forests are part of a group of unconnected areas across Central and South America regarded as seasonally tropical dry forests (Pennington et al. 2009, Werneck et al. 2011). The subtropical forest in NE Argentina contains deciduous, brevideciduous and evergreen species with deciduous ones accounting for 25–50% of the tree species (Leite and Klein 1990). Compared with seasonally dry forests, seasonality in precipitation does not exist (except for short dry spells) and, because of its subtropical location, only mild frosts occur during the cold season (Gatti et al. 2008).

The major objective of this study was to determine the extent to which species-specific differences in trunk water storage and sapwood capacitance had impacted the life history and other ecophysiological traits of trees, such as main stem, branches and leaf water potential, growth rates and deciduousness, and to assess the potential adaptive value of trunk sapwood capacitance and water storage compartments for keeping adequate water supply to the canopy during the day and during short drought periods. With this aim the dynamics of water storage discharge and refilling in the trunk of canopy trees in a subtropical semideciduous forest in NE Argentina was monitored. Trunk sapwood capacitance was characterized on 10 dominant tree species differing in growth rates, wood density and leaf phenology. Another objective was to assess if diurnal discharge and refilling of the main stem water storage was consistent with the changes in sapwood VWC, and contraction and expansion of the trunk diameter. Species with high growth rates were expected to have lower wood density and higher capacitance and to exhibit an efficient use of stem water storage to maintain a suitable water balance compared with species having low growth rates and high wood density.

Materials and methods

Study area and plant species

The research was carried out in a native forest inside the Iguazú National Park (25°31′–25°43′S, 54°08′–54°32′W). The forest has mature canopy trees ranging from 20 to 45 m in height with abundant lianas and epiphytes. The canopy is mostly dominated

by trees of the genus *Cordia* L. (Boraginaceae), *Ocotea* Aubl. and *Nectandra* Rol. ex Rottb. (Lauraceae), *Parapiptadenia* Brenan, *Peltophorum* (Bogel) Benth. (Fabaceae), *Cedrela* P. Browne, *Trichilia* P. Browne (Meliaceae), *Plinia* L., *Eugenia* L. and *Myrciaria* O. Berg. (Myrtaceae). Mean annual precipitation in the area is ~2000 mm, evenly distributed throughout the year. Mean annual air temperature is 21 °C with monthly means of 25 °C in January and 15 °C in July, the warmest and coldest months of the year, respectively. Ten canopy tree species differing in wood biophysical properties, growth rates and leaf habits were studied. All studied species are diffuse-porous mature trees with diameter at breast height (DBH) ranging from 34 to 68 cm (Table 1). The deciduous and brevideciduous species studied are also found in seasonally tropical dry forests in South America (Pennington et al. 2009, Werneck et al. 2011).

Trunk sapwood density and capacitance

Wood density (ρ) was measured by the water-displacement method on tree core samples of the main stems with the bark removed. Two types of wood samples were obtained from the outer sapwood (next to the cambium) and the inner sapwood (next to the outer sapwood). The wood density was calculated as

$$\rho = \frac{M}{V},$$

where M is the dry mass of the sample (oven dried at 60 °C for 72 h) and V is the sample volume. Volume was estimated by submerging the sample in a container with distilled water resting on a digital balance with a 0.001 g precision. The sample was kept submerged during measurements until saturation with the help of a very small needle, without touching the walls of the container.

Cylinders of the sapwood were obtained with a 5-mm increment borer in the main stem of the 10 studied species, sealed in glass vials and transported to the laboratory where they were allowed to hydrate in distilled water for <2 h to avoid oversaturation. After hydration samples were quickly blotted

to remove excess water, placed in the caps of thermocouple psychrometer chambers (JRD Merrill Specialty Equipment, Logan, UT, USA), weighed and then sealed inside the chamber for determination of water potential isotherms. The psychrometer chambers were then placed in an insulated water bath and allowed to equilibrate for at least 3 h before measurements with a dew point microvoltmeter (HR-33T, Wescor, Logan, UT, USA). Measurements were repeated at frequent intervals until the water potential values stabilized. The chambers were opened and the samples were allowed to dehydrate for different time intervals, reweighed in the psychrometer caps, resealed inside the psychrometer chambers and allowed to equilibrate for another determination of water potential. Moisture release curves were generated by plotting the water potential against the relative water content. The relationships between sapwood water potential and relative water content were used to calculate sapwood capacitance as described in Meinzer et al. (2003). Capacitance was normalized by the sapwood tissue volume to facilitate comparison of absolute amounts of water released per unit decline in water potential. Sapwood capacitance (C ; kg m⁻³ MPa⁻¹) was calculated as follows:

$$C = \frac{dW}{d\Psi}$$

where W is the weight of water per unit volume of tissue and Ψ is the water potential of the tissue (Scholz et al. 2007).

Sap flow and main stem water storage calculations

Sap flow was measured with the heat dissipation method (Granier 1985, 1987) between September and December 2013. Briefly, a pair of 20-mm long, 2-mm diameter hypodermic needles containing a copper-constantan thermocouple inside a glass capillary tube and a heating element of constantan coiled around the glass tubes were inserted into the sapwood near the base of the main stem and in a branch in each plant. The upper (downstream) probe was continuously heated at a constant power by the Joule effect, while the unheated upstream probe served as a temperature reference. Temperature differences between the upstream and downstream probes were

Table 1. Family, species name and authority, DBH (means \pm SE) and leaf phenology of the trees used in the study.

Family	Species	DBH (cm)	Leaf phenology
Rutaceae	<i>Balfourodendron riedelianum</i> (Engl.) Engl.	47 \pm 8	Brevideciduous
Meliaceae	<i>Cabralea canjerana</i> (Vell.) Mart.	45 \pm 8	Evergreen
Meliaceae	<i>Cedrela fissilis</i> Vell.	43 \pm 10	Deciduous
Malvaceae	<i>Ceiba speciosa</i> (A.St.-Hil., A.Juss. and Cambess.) Ravenna	41 \pm 13	Deciduous
Sapotaceae	<i>Chrysophyllum gonocarpum</i> (Mart. and Eichler)Engl.	34 \pm 8	Evergreen
Boraginaceae	<i>Cordia trichotoma</i> (Vell.) Arráb. Ex Steud.	42 \pm 11	Deciduous
Fabaceae	<i>Holocalyx balansae</i> Micheli	68 \pm 8	Evergreen
Fabaceae	<i>Lonchocarpus muehlbergianus</i> Hassl.	38 \pm 6	Brevideciduous
Lauraceae	<i>Ocotea diospyrifolia</i> (Meisn.) Mez.	52 \pm 21	Evergreen
Fabaceae	<i>Parapiptadenia rigida</i> (Benth.) Brenan	65 \pm 23	Brevideciduous

recorded every 10 s and 10 min averages were stored in solid-state storage modules (SM192, Campbell Scientific, Logan, UT, USA) connected to data loggers (CR 10X, Campbell Scientific). Sap flux density was calculated from the temperature difference between the two probes using an empirical calibration (Granier 1985, 1987) and validated more recently for diffuse-porous tree species (e.g., Clearwater et al. 1999). Temperature differences between both the probes were corrected for natural temperature gradients between the probes (Do and Rocheateu 2002). Probes were left in the trees for a short period of time (from 7 to 15 days) after installation to minimize wound effects. Even if the absolute values of sap flow could be misleading without calibrations as indicated by Steppe et al. (2010), on a comparative basis and within the same individual at a different height in the tree, the sap flow estimates are undoubtedly valid for assessing the dynamics of water transfer from and into the reservoirs. Comparisons between the branch and the main stem sap flow within the same tree were used to calculate the trunk WSC as explained below. Mass flow of sap per individual was obtained by multiplying flux density by sapwood cross-sectional area. The sapwood cross-sectional area was obtained by the dye-injection method near the base of the main trunk and in a branch from each individual of each species. After few hours, cylinders of the sapwood were obtained with a 5-mm increment borer and the portion dyed was determined.

Branch sap flow rates were used to obtain whole-crown transpiration according to Goldstein et al. (1998). In the current study, a representative branch next to the main stem at ~20 m above the ground level, instead of terminal branches, was used for logistic reasons, which may have resulted in smaller amounts of calculated water stored. Whole-crown transpiration was estimated by normalizing the branch sap flow with respect to the average daily maximum value. We used one branch per tree after the first bifurcation (with similar hydraulic hierarchy). We chose trees with branches in the same position within the crown. The number of branches in each tree ranged between two in *Chrysophyllum gonocarpum* and four in *Cedrela fissilis*. In three of the species, the branches in the first bifurcations were of similar size to those where sensors were installed, with the exception of *C. gonocarpum* with one of the two branches having a slightly smaller diameter compared with the main stem (23 and 30 cm, respectively). Based on the law of conservation of mass, the amount of water circulating on a daily basis at the base of the main stem is the same amount that is transported through all the branches, assuming that the tree trunk has zero daily net water storage change. Because only one branch was measured on each tree, sap flow in the branch was normalized in order to compare its diurnal pattern with that measured at the base of the tree (Goldstein et al. 1998). Normalized branch sap flow was then divided by the daily sum of the normalized 10 min averages divided by 6, then multiplied by the total daily sap flow measured at the base of the tree trunk which was

assumed to be equal to the total daily transpiration. This procedure yielded rates of crown transpiration on an hourly basis. The total diurnal stem WSC was estimated by subtracting 10 min averages of basal sap flow from whole-crown sap flow when basal sap flow was less than whole-crown flow, summing the differences and then dividing by six. Time lags between the sap flow at the base of the main stem and the sap flow in canopy branches were calculated by finding the highest cross-correlation between both the sap flow time series.

Trunk VWC

Moisture sensors based on the FDR technology (Model GS3, Decagon Devices, Inc., Pullman, WA, USA) were installed in the main stem sapwood at breast height. The details of sensor installation and calibration have been described by Hao et al. (2013). Because laboratory and factory calibrations were very similar, factory calibration was used in this study. Data were obtained using EM50 data loggers (Decagon Devices, Inc.) every 10 min for different time periods. Main stem VWC was measured simultaneously with sap flow measurements.

Trunk diameter variations

At the same time, diurnal microvariations in stem radius were continuously monitored with point electronic dendrometers with very low temperature sensitivity ($0.27 \mu\text{m } ^\circ\text{C}^{-1}$) (ZN11-T-WP; Zweifel Consulting, Hombrechtikon, Switzerland). The electronic displacement sensor (linear motion potentiometer) was in contact with the bark (only a small portion of dead bark was shaved) and consequently diurnal radius variations mainly reflected changes in water content in both the xylem and active bark tissues. Other factors such as turgor drive growth may be involved (Steppe et al. 2006) but the magnitude of diurnal variations should be relatively small compared with changes in water content in both xylem and living cells in the bark.

To determine monthly variations in stem diameter band dendrometers were used. Dendrometers were made manually and consisted of a stainless steel tape encircling a tree stem, with one end passing through a collar (which is attached to the other end) and connected back to itself with a stainless steel spring (Cattellino et al. 1986). Three months after dendrometer installation (allowing for stem–dendrometer adjustment), a permanent mark was made on the metal band next to the collar. As the stem diameter increases, the mark moves away from the collar and the spring is stretched, keeping the dendrometer tight. A flexible ruler was used to measure stem diameter changes with an accuracy of 0.5 mm. Changes in stem circumference were recorded monthly from February 2012 to February 2013.

Leaf and main stem water potential

Minimum leaf water potential was measured with a pressure chamber (PMS, Albany, OR, USA) during a 30-day dry spell in

January 2012. Leaf water potentials of three to four leaves from each of the three different individuals per species were measured at midday (13:00 h). Minimum main stem water potential was considered to be the water potential at which the amount of released water in the moisture release curves stopped increasing linearly and began to increase asymptotically (Meinzer et al. 2003). This estimated value of stem water potential was intermediate between predawn (water potential at thermodynamic equilibrium between soil and leaves) and midday leaf water potentials.

Branch vulnerability curves

The air-injection method was used to measure vulnerability of stem xylem to water-stress-induced embolism (Sperry and Saliendra 1994). Terminal branches from three different trees per species were collected in the field and immediately transported to the laboratory. The segments were inserted into the air-injection chamber with both ends protruding and attached to the tubing system for measuring hydraulic conductance (k_h). We used the longest possible segments to try to avoid open-vessel artifacts, which may lead to overestimation of vulnerability to cavitation (Cochard et al. 2013). Air emboli were removed by flushing the samples at 0.1 MPa for 10 min with filtered water and k_h maximum (k_{hmax}) was calculated. A vulnerability curve was generated by first pressurizing to 0.5 MPa for 5 min. After equilibration, $k_{h(0.5)}$ was measured. This process was repeated for pressures ranging from 0.5 to 4 MPa, or until water flow through the stem segment was negligible. The percentage loss of conductivity (PLC_{stem}) was then calculated as:

$$PLC_{stem} = 100 \times \frac{k_{hmax} - k_h}{k_{hmax}}$$

A vulnerability curve was generated for each species by plotting the PLC_{stem} against increasing applied pressure. Sampling methods can generate artifacts in PLC patterns by inducing a degree of embolism that is itself a function of xylem tensions at the moment of sample excision (Wheeler et al. 2013). In our study, the branches were excised at dawn when water potentials were close to 0 MPa, thus decreasing the risk of artifacts in PLC determinations. Xylem pressure that induced 50 and 88% loss of stem conductivity (P_{50} and P_{88} , respectively) and the stem PLC at midday leaf water potential were estimated from vulnerability curves. The need to study hydraulic architecture traits in different portions of the root–stem–leaf continuum is of importance during studies of plants' hydraulic architecture (Meinzer et al. 2010) and in particular the assessment of vulnerability to cavitation in the main stem of trees, which may be different compared with branches.

Results

There was a wide range of sapwood capacitance across 10 canopy tree species (Figure 1). Mean sapwood capacitance

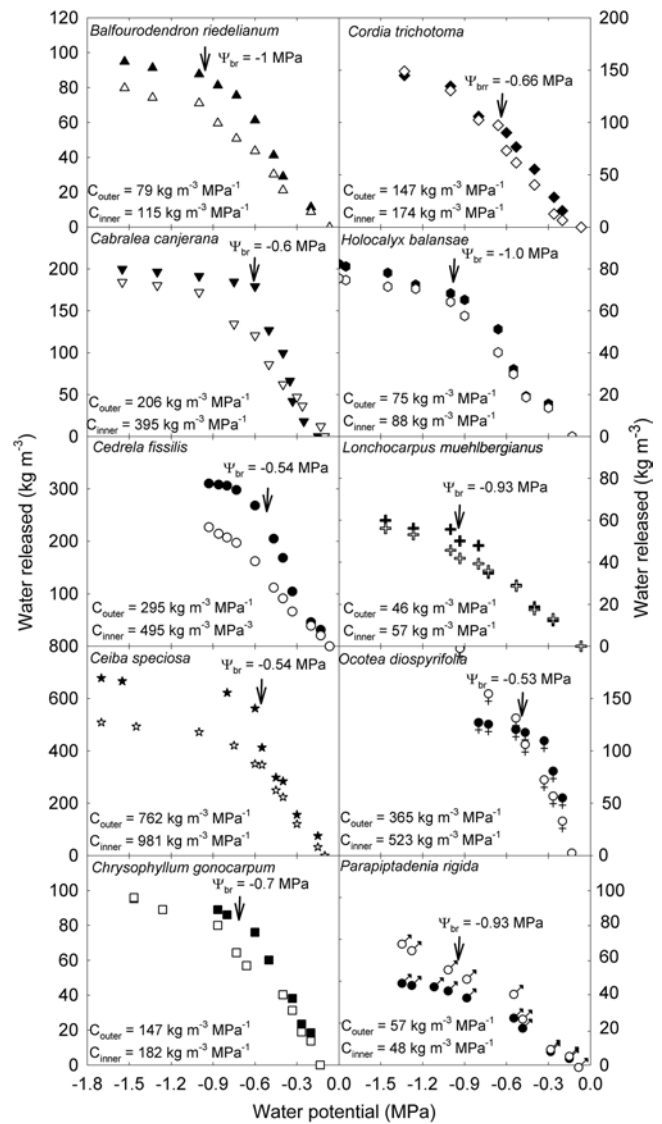


Figure 1. Cumulative amount of water released per unit trunk sapwood volume as a function of sapwood water potential in 10 subtropical tree species. Values of trunk sapwood capacitance were estimated from the slopes of the lines fitted to the linear portions of the water release curves. The arrows indicate mean daily minimum water potential of main stems. Open symbols correspond to values of outer sapwood (next to the cambium) and filled symbols correspond to values of inner sapwood. In each panel the outer and inner capacitance are indicated.

(outer and inner capacitance) varied between 51 $kg\ m^{-3}\ MPa^{-1}$ in *P. rigida* and 870 $kg\ m^{-3}\ MPa^{-1}$ in *C. speciosa*. Outer capacitance (capacitance of the part of the sapwood adjacent to the cambium) was lower than the inner capacitance in all species except in *P. rigida* (Figure 1). In species such as *C. canjerana* and *C. fissilis* the inner capacitance was approximately twice as high as the outer capacitance. Similar to the species-specific capacitance differences, the mean sapwood density also varied across species from 0.3 to 0.86 $g\ cm^{-3}$ (Figure 2) and it was higher in the outer part of the sapwood (data not shown). There was a negative exponential relationship between

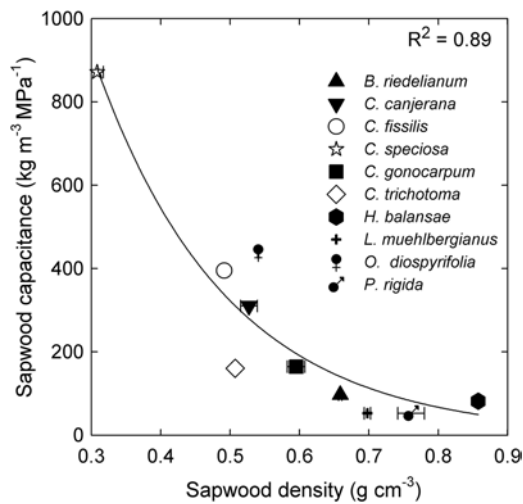


Figure 2. Mean trunk sapwood capacitance (outer and inner capacitance) in relation to mean trunk sapwood density (outer and inner wood density) for 10 tree species. The line is the negative exponential regression fitted to all the data ($y = -4.7 + 2513\exp(-4.2x)$; $P < 0.0001$). Open symbols correspond to deciduous species and filled symbols correspond to evergreen and brevideciduous species.

sapwood capacitance and density across species (Figure 2). *Ceiba speciosa* was the species with the highest capacitance and the lowest density, while *P. rigida* had the lowest capacitance and one of the highest wood densities. Species also differed in sapwood VWC ($0.39 \text{ m}^3 \text{ m}^{-3}$ in *B. riedelianum* to $0.58 \text{ m}^3 \text{ m}^{-3}$ in *C. speciosa*) and sapwood capacitance was linearly and positively related to the daily average VWC across species ($R^2 = 0.71$, $y = 0.39 + 0.0002x$; $P < 0.0001$).

The minimum leaf water potential measured during a dry period varied between -2.75 MPa in *C. trichotoma* and -1.1 MPa in *C. speciosa* (Figure 3a). Similarly, the minimum stem water potential varied between -0.53 and -1 MPa (Figure 3b). Both the water potentials increased (became less negative) with increasing capacitance, but while leaf water potentials increased linearly with increasing sapwood capacitance stem water potential did not vary substantially across species when the capacitance was higher than $400 \text{ kg m}^{-3} \text{ MPa}^{-1}$ (Figure 3). All the study species exhibited a negative safety margin between the minimum leaf water potential and the xylem pressure at 50% loss of hydraulic conductivity (P_{50}) (Figure 4a). This safety margin using minimum stem leaf water potential indicated that all species, except *L. muehlbergianus* and *P. rigida*, operated at xylem pressures above P_{50} (data not shown). When a more conservative safety margin was used (the difference between minimum leaf or stem water potential and the xylem pressure at the catastrophic hydraulic dysfunction level, or 88% of loss of hydraulic conductivity; P_{88}), leaves and stems of all species exhibited positive safety margins, with the exception of the leaves and stems of *L. muehlbergianus* and leaves of *P. rigida* (Figure 4b). Both the safety margins (except stem $\Psi_{\min} - P_{50}$, data not shown) were related to sapwood

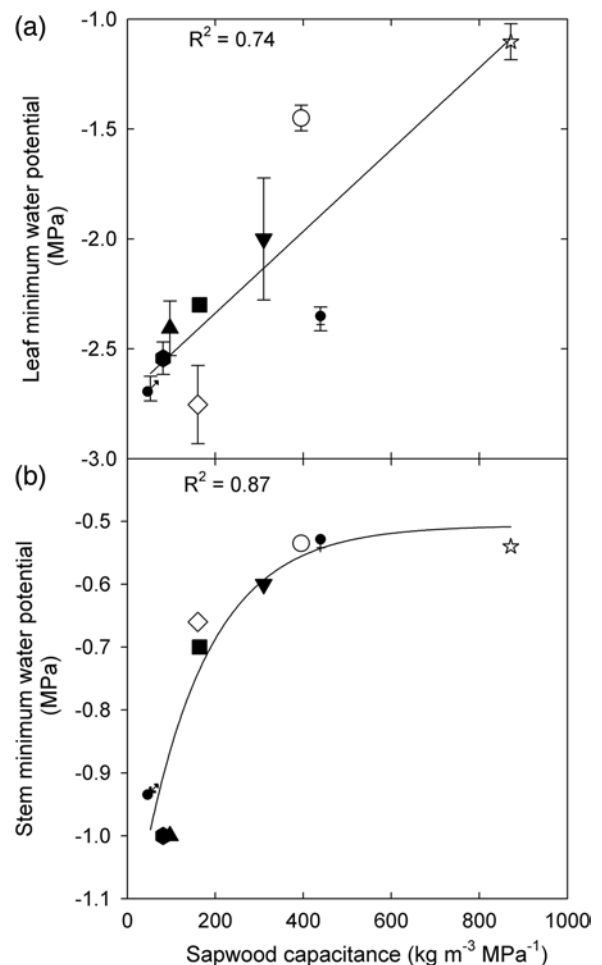


Figure 3. Leaf minimum water potential (a) and main stem minimum water potential (b) as a function of mean trunk sapwood capacitance for 10 tree species. The line in (a) is the linear regression fitted to the data ($y = -2.67 + 0.0017x$; $P < 0.0001$) and the line in (b) is the exponential rise to maximum function fitted to the data ($y = -1.2 + 0.69(1 - \exp(-0.007x))$; $P < 0.001$). The values of leaf minimum water potentials are averages for 3 days depending on the species \pm SE. Symbols as in Figure 2.

capacitance with a rapid increase at low sapwood capacitance and an asymptotical value of safety margins at capacitances $>200 \text{ kg m}^{-3} \text{ MPa}^{-1}$ when safety margins became independent of species-specific capacitance variations (Figure 4).

The diurnal patterns of sap flow at the base of the trunk and in branches follow a typical pattern of increase in the early morning and a decrease in the afternoon (Figure 5, left panels). In species with high capacitance, such as *O. diospyrifolia*, sap flow in the branches started earlier than in the base of the trunk ($\sim 1 \text{ h}$), and the maximum sap flow was reached sooner in the crown than at the base of the main stem. In a species with relatively low capacitance such as *B. riedelianum* both flows increased simultaneously (Figure 5, left panels). Differences in the time course of basal sap flow and crown sap flow (the integration of sap flow for all branches in a tree) were used to determine the period of discharge and recharge of water

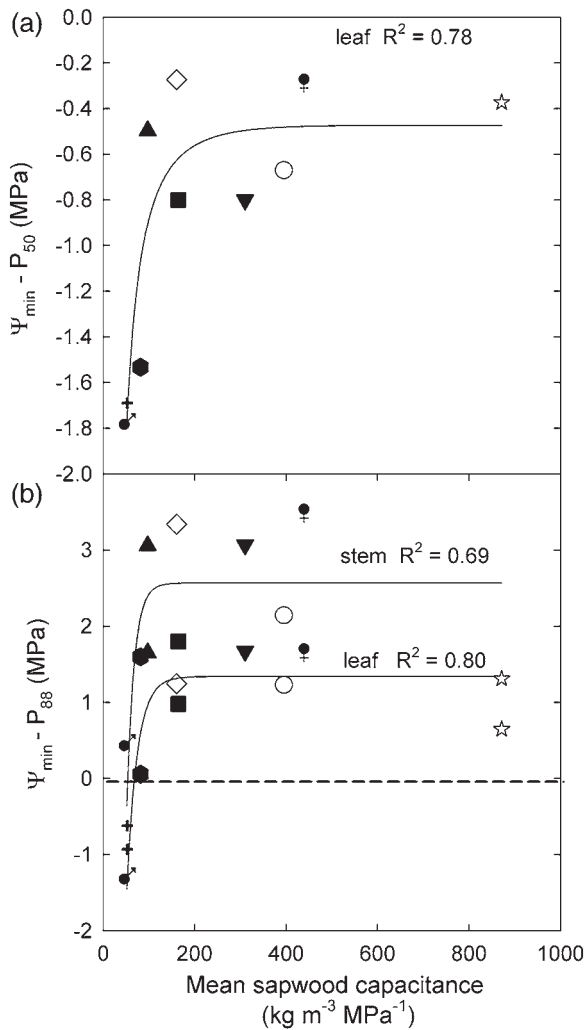


Figure 4. (a) Difference between leaf minimum water potential and branch xylem pressure at 50% of loss of hydraulic conductivity versus trunk sapwood capacitance for 10 subtropical tree species. The line is the function fitted to the data ($y = -0.5(1 - \exp(-0.001x))^{1.6}$; $P < 0.01$). (b) Difference between leaf minimum water potential or main stem minimum water potential and branch xylem pressure at 88% of loss of hydraulic conductivity versus trunk sapwood capacitance. The lines are the exponential functions fitted to the points (leaf: $y = -0.05 + 2.2/1 + \exp(-(x - 81)/0.13)$; $P < 0.001$); stem: $y = -68 + 71(1 - \exp(-0.06x))$; $P < 0.05$). Symbols as in Figure 2.

from internal stem storages (Figure 5, right panels). The discharge of water from internal water storage occurred between 7:30 and 13:00 h depending on the species (positive values of crown–basal sap flow) and the recharge occurred in the afternoon (negative values of crown–basal sap flow; Figure 5, right panels). Estimates of the contribution of stored water in stems of trees to total daily transpiration for the species depicted in Figure 5, right panels, are indicated in each panel.

Consistent with the use and recharge of stored water, diurnal variations in VWC were observed for all species (Figure 6). Water content decreased during the morning when the sap flow increased, and increased during the afternoon when the

sap flow decreased. As a consequence of the use of water stored and recharged, the stem diameter decreased in the morning and increased in the afternoon (Figure 6). Stem contraction followed the VWC decrease in the morning with a lag of ~3 h. In three of the four species stem expansion followed VWC increase in the late afternoon with a small lag of a few hours (Figure 6). Species-specific stored water use (determined by comparing sap flow patterns in the crown and at the stem base) increased exponentially with increasing sapwood capacitance and decreased asymptotically with increasing sapwood density (Figure 7). Species-specific stored water used ranged from 5.9 to 28% and tended to be higher for species with higher capacitance. Stem water use from *C. speciosa* was the highest across all the species. *Ceiba speciosa* is a stem-succulent tree species that develop very large stems in dry sites where they look like baobab trees. The large quantities of discharge water into the transpirational stream coming from the internal stem storage are consistent with the high capacitance and the very low wood density of this species.

Species-specific cumulative stem growth rates measured with band dendrometers during the 12 consecutive months (from February 2012 to February 2013) were linearly related to sapwood density when an outlier (*P. rigida*, a species with high sapwood density and high cumulative growth rate) was removed from the analysis (Figure 8). Growth rates decreased linearly across species with increasing sapwood density. The deciduous species tended to exhibit higher stem growth rates than evergreen species.

Discussion

Wood capacitance seems to be a key functional trait that strongly affects many aspects of plant functioning in the studied species. It showed a strong correlation with short-term water balances in large trees, including the operating minimum stem and leaf water potentials and the ‘safety margin’ between minimum water potential and water potential causing catastrophic run-away embolisms. These correlations are likely mediated by sapwood capacitance and percentage of daily water use drawn from the trunk internal water storages, resulting in a buffering effect for maintaining tree water balance in species with relatively low wood density and high growth rates.

Capacitance, WSC and sapwood density

The results of this study showed that although WSC (measured as the VWC of the sapwood, VWC) and capacitance are different properties, both traits influence whole-tree water use patterns and water relations and they were positively correlated. Species with higher capacitance (higher capacity to release water to the transpiration stream at a given water potential gradient) had higher stem VWC too. In previous studies using FDR to assess changes of VWC

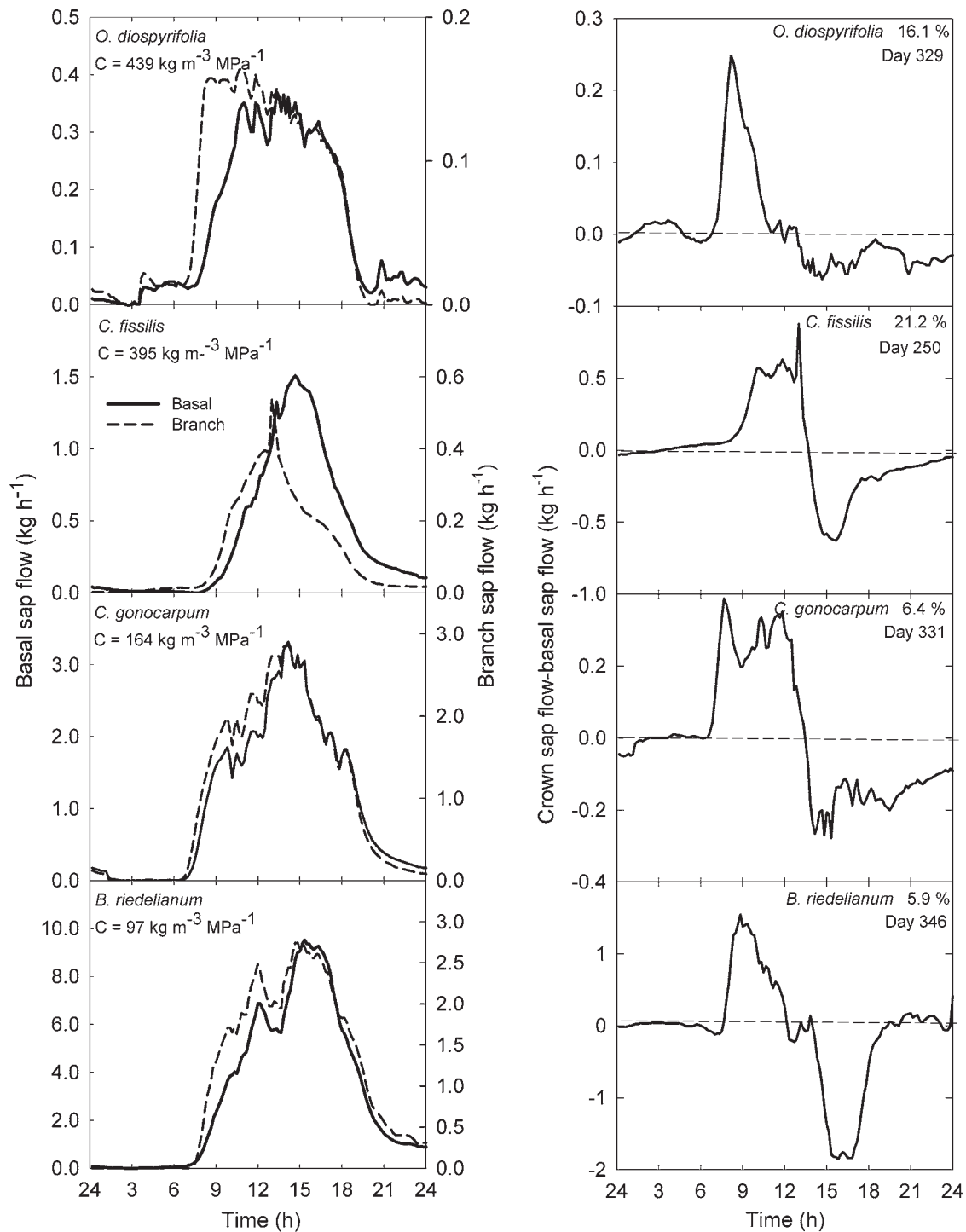


Figure 5. Left panels: typical diurnal patterns of volumetric sap flow measured at the base of the main stem (dashed line) and in a branch (solid line) for four tree species differing in trunk sapwood capacitance. Right panels: differences between the crown and the basal sap flow during the course of 1 day for the trees shown in left panels. Positive values of the difference between the crown and the basal sap flow indicate time periods when water was principally discharged from the stem water storage, and negative values indicate time periods when water from the soil was refilling the water storage. Basal sap flow and sap flow of branches are depicted at different scales.

in *Betula papyrifera* and in subtropical canopy species, the recorded diurnal fluctuations in VWC indicated the importance of stem water storage in the water balance of large trees (Hao et al. 2013, Oliva Carrasco et al. 2013).

Species with higher WSC and capacitance may thus have an enhanced ability to buffer temporary canopy water use peaks at midday and during periods with low soil water availability. Although the method used to estimate sapwood capacitance

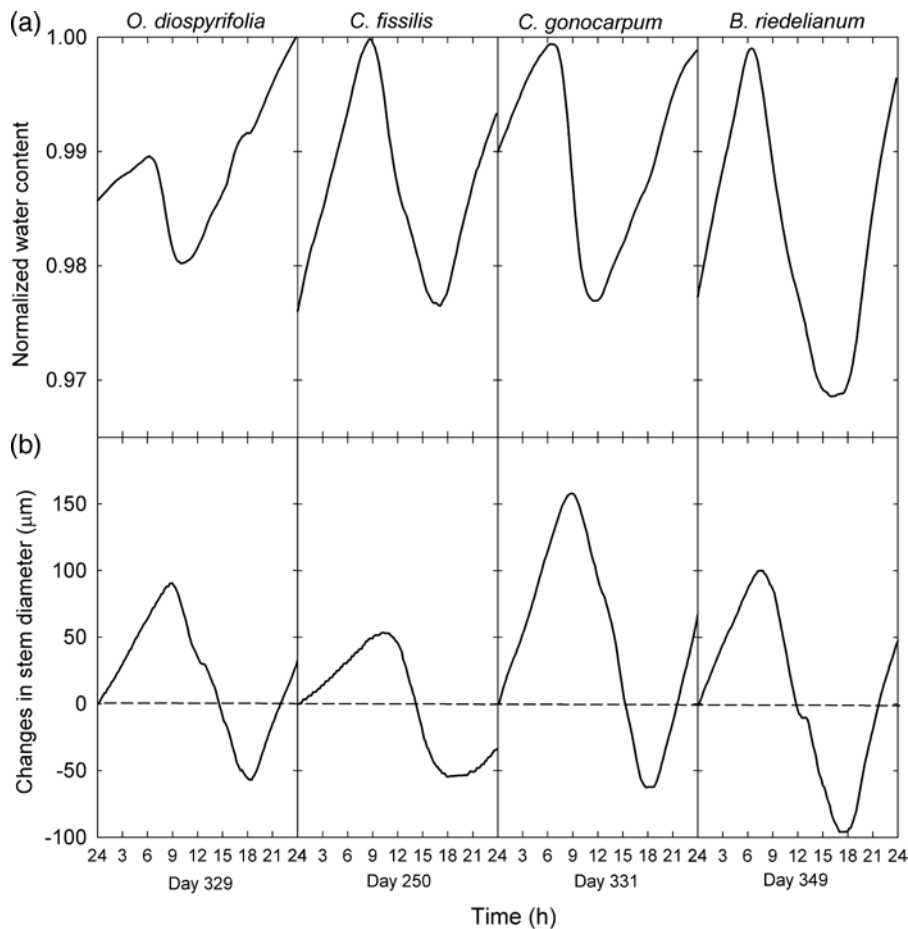


Figure 6. Diurnal changes in normalized main stem VWC (a) and changes in the main stem diameter (b) in the same four tree species as in Figure 5. Positive and negative values of stem expansion indicate time periods of stem water discharge and recharge, respectively.

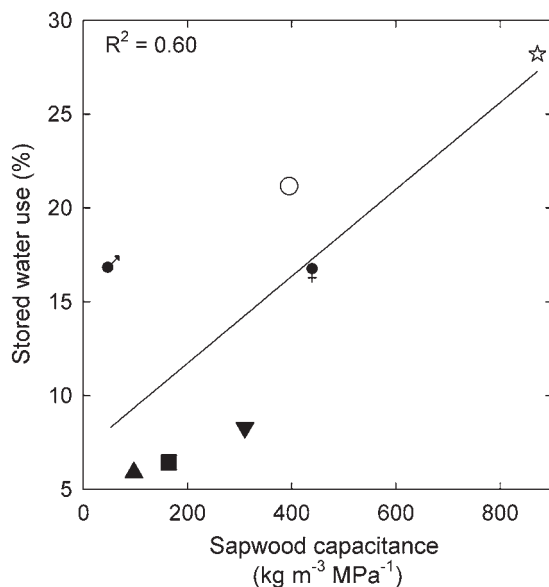


Figure 7. Percentages of main stem stored water used in relation to total daily transpiration as a function of species-specific mean trunk sapwood capacitance. The line is the linear regression fitted to the data ($y = 7.09 + 0.02x$, $P = 0.041$). Symbols as in Figure 2.

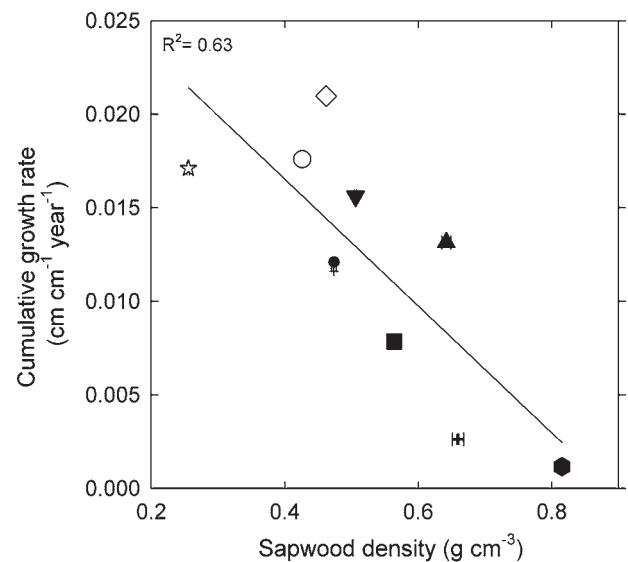


Figure 8. Species-specific cumulative growth rate during a year (from Feb 2012 to Feb 2013) versus mean trunk sapwood density for nine tree species (one outlier species was removed from the graph). The line is the linear regression fitted to the data. $y = 0.03 - 0.03x$, $P < 0.05$). Symbols as in Figure 2.

gives this value per unit pressure and not all species reach the same water potential range, sapwood capacitance taking into account the in situ operational range in water potential of the reservoir was highly correlated with sapwood capacitance per pressure unit as shown in Figure 1 ($y = 2x - 81.7$; $R^2 = 0.98$, $P < 0.0001$), indicating that the species-specific patterns are maintained.

Dynamic measurements of VWC observed in the four species showed a consistent pattern that decreased in the morning during stem water storage depletion and increased during the afternoon when sap flow decreased and the storage was refilled. The large daily amplitude of the variation in whole-stem diameter (e.g., 200 μm) in our studied tree species was similar to whole-stem diameter variations in Cerrado trees (Scholz et al. 2008). As a consequence of the use of stored water and its recharge, the stem diameter decreased in the morning and increased in the afternoon. Stem contraction, however, followed the VWC decrease in the morning with a lag of ~ 3 hours. The lag between the decrease in VWC and stem contraction was expected because the xylem is a relatively rigid tissue and water movement from the living tissues in the bark to the xylem should occur after a relatively large water potential gradient favoring water movement between the bark and the sapwood is developed. All the studied species reached their maximum water content sooner than maximum stem diameter ranging from 90 min in *C. gonocarpum* to 170 min in *O. diospyrifolia*. This behavior seems logical as the stem water content was obtained from main stem sapwood samples, whereas the stem diameter was measured on the whole trunk (including the bark) and the magnitude of the lags could be a consequence of the differences in hydraulic connections between the xylem and phloem, thickness of bark and the elastic traits of the tissues. It has recently been suggested that there is significant radial water movement between the bark and the xylem tissue, which is facilitated by aquaporins (Steppe et al. 2012).

The wood density of the tree species in the subtropical forest studied exhibited a large variation that was within the range of wood densities measured in several neotropical forests (Muller-Landau 2004). Consistent with the wood density variations, there was a wide range of sapwood capacitance. Deciduous species with high growth rates tended to have a higher capacitance than evergreen and brevideciduous species. In particular, the deciduous species *C. fissilis* and *C. speciosa* had a capacitance in the 495–981 $\text{kg m}^{-3} \text{MPa}^{-1}$ range for the inner sapwood. Species-specific sapwood capacitance across the 10 studied species exponentially decreased with increasing sapwood density. While species-specific density increased from 0.2 to $\sim 0.9 \text{ g cm}^{-3}$, capacitance decreased from 981 to 46 $\text{kg m}^{-3} \text{MPa}^{-1}$, indicating strong structure-based connections between sapwood density and capacitance.

Daily patterns of sap flow measured at the base of the stem and in canopy branches in four tree species indicated diurnal discharge and refilling of stem water storage, which was consistent with diurnal VWC and stem diameter fluctuations. Similarly, branch–basal sap flow lag was positively correlated with sapwood capacitance across species, with larger lags occurring in species with larger capacitances, which is consistent with patterns observed for savanna species (Phillips et al. 2008). Estimates of the contribution of stored water in stems of trees to total daily transpiration were higher for species with higher capacitance. Species-specific stored water use was positively correlated with sapwood capacitance and negatively correlated with sapwood density. This is the first time that concurrent measurements of water utilization estimated by sap flow measurements, diurnal variations in VWC, mean sapwood capacitance and mean sapwood density have been carried out in situ in adult trees.

Capacitance, minimum water potentials and safety margins

Capacitance helped to buffer the changes in minimum leaf and stem water potentials during short dry periods in the studied species, but it was not sufficient to prevent the leaves from losing $>50\%$ of their hydraulic conductance. However, the air-seeding pressure P_{50} , an index widely used to compare xylem hydraulic resistance between species, does not reflect the threshold water potential leading to leaf shedding. Particularly in this study, we observed that deciduous species with the largest capacitance were those with minimum leaf water potentials closer to P_{50} compared with the evergreen species that experienced larger water deficits (P_{50} more negative), while species such as *H. balansae* and the brevideciduous *P. riedelianum* that remains without leaves during a short time period exhibited a very large safety margin (leaf $\Psi_{\text{min}} - P_{50}$). Even considering a more conservative safety margin (leaf $\Psi_{\text{min}} - P_{88}$), these brevideciduous species had water potentials below the pressure inducing 88% loss of hydraulic conductivity, which could trigger the senescence and dropping of leaves during sporadic dry periods. Using three different types of hydraulic safety margins, Meinzer et al. (2009) found that there is a diminishing role of stem capacitance in slowing fluctuations in xylem pressure.

Apparently sapwood capacitance is overdimensioned for the studied deciduous species growing in this subtropical forest as both the leaf and stem water potentials appear to be similar across a range of species exhibiting sapwood capacitance $>200 \text{ kg m}^{-3} \text{MPa}^{-1}$. Probably high stem capacitance has an adaptive value in these species that are dominant in seasonally tropical dry forests, where the risk of embolism formation is high, but was conserved in the less seasonal subtropical forest where they also occur. Although previous studies in this forest had indicated a lack of hydraulic segmentation between leaves

and stems (Villagra et al. 2013), the safety margin ($\Psi_{\min} - P_{88}$) observed in this study, which represents a more realistic index for comparison of the hydraulic risk between plant organs or between species, indicates that leaves are more vulnerable to near total hydraulic failure than stems. Nevertheless, this difference between leaves and stems may not be important since the safety margins are substantially large (>1 MPa for the leaves and 1.5 MPa for the stems of most of the studied species) even during severe short dry periods. These safety margins are similar to the values reported in a survey of angiosperm trees (Choat et al. 2012).

Allometric relationships between growth rate, stem capacitance and stem density

Consistent with high capacitance and low wood density, the growth rates of the studied deciduous species were relatively high compared with the evergreen species. A linear relationship between wood density and growth rate across species was observed. Deciduous species tended to have lower wood density and higher growth rate compared with evergreen and brevideciduous species. Negative correlations between mean growth rate per species and wood density have been observed in a number of tropical forests (Burslem and Whitmore 1999, Enquist et al. 1999, Muller-Landau 2004). In diffuse-porous species, variation in wood density should constrain stem hydraulics (Stratton et al. 2000) because wood density is an index of the balance between solid material and the relative cross-sectional area available for water transport (Meinzer et al. 2008). In species with lower wood density, a combination of higher xylem hydraulic conductivity (Stratton et al. 2000, Bucci et al. 2004, 2009, Santiago et al. 2004) and larger internal water storage buffering temporary water potential decreases (Bucci et al. 2004, 2012, Santiago et al. 2004) may likely reduce the effects of stomatal limitations to photosynthetic carbon assimilations during periods with water deficits (Bucci et al. 2004, Santiago et al. 2004), which result in higher growth rates.

The subtropical forest studied is characterized by the coexistence of deciduous species with high capacitance and rapid growth rates and evergreen species with slower growth rates and low capacitance. The deciduous species are leafless during the mild winter season and thus the evergreen trees can use the relatively high incoming solar radiation when the climatic conditions are more favorable for growth (temperatures closer to the optimum for CO₂ assimilation) and the evaporative demand is relatively low, minimizing the risks of water deficits.

Conclusions

The results of this study indicate a strong impact of sapwood properties on stem water storage, which influences the water economy of canopy trees even in humid forests without obvious rainfall seasonality. Species-specific trunk water storage

and sapwood capacitance were strongly coordinated with life history and ecophysiological traits such as stem and leaf water potential, growth rates and deciduousness. The large sapwood capacitance in deciduous species helped to avoid a large percentage of embolism formation in the xylem tissue beyond which it would be very difficult to recover from hydraulic dysfunction. Conspicuous capacitance may be a low-cost adaptation to avoid water deficits during the day and under short but intense drought periods as the carbon allocation in the sapwood of deciduous species is low (low wood density). The maintenance of the water status and transport capacity was crucial in deciduous species which grow fast during relatively short time periods compared with evergreen species. Large capacitance and stored water use in the sapwood appear to have a central role in the rapid growth patterns of deciduous species, facilitating rapid canopy access as these species are less shade tolerant than the evergreen species.

Acknowledgments

The authors are grateful to Patricio MacDonagh, the Aglomerado Productivo Forestal Misiones y Norte de Corrientes (APF), 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. They also thank K. Steppe and two anonymous reviewers for comments that greatly improved the original manuscript.

Funding

This research was supported by the Agencia Nacional de Promoción Científica y Tecnológica (PITEC project Consolidación del APF and PICT 2011-01860) and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina.

References

- Borchert R, Pockman WT (2005) Water storage capacitance and xylem tension in isolated branches of temperate and tropical trees. *Tree Physiol* 25:457–466.
- Bucci SJ, Goldstein G, Meinzer FC, Scholz FG, Franco AC, Bustamante M (2004) Functional convergence in hydraulic architecture and water relations of savanna trees: from leaf to whole plant. *Tree Physiol* 24:891–899.
- Bucci SJ, Scholz FG, Goldstein G, Meinzer FC (2009) Soil water availability as determinant of the hydraulic architecture in Patagonian woody species. *Oecologia* 160:631–641.
- 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.
- Burslem DFRP, Whitmore TC (1999) Species diversity, susceptibility to disturbance and tree population dynamics in tropical rain forests. *J Veg Sci* 10:767–776.

- 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.
- Cattellino PJ, Becker CA, Fuller LG (1986) Construction and installation of manmade dendrometer bands. *North J Appl For* 3:73–75.
- Choat B, Jansen S, Brodribb TJ et al. (2012) Global convergence in the vulnerability of forests to drought. *Nature* 491:752–756.
- Clearwater MJ, Meinzer FC, Andrade JL, Goldstein G, Holbrook NM (1999) Potential errors in measurement of no uniform sap flow using heat dissipation probes. *Tree Physiol* 19:681–687.
- Cochard H, Badel E, Herbette S, Delzon S, Choat B, Jansen S (2013) Methods for measuring plant vulnerability to cavitation: a critical review. *J Exp Bot* 64:4779–4791.
- Do F, Rocheateu A (2002) Influence of natural temperature gradients on measurements of xylem sap flow with thermal dissipation probes. 1. Field observations and possible remedies. *Tree Physiol* 22:641–648.
- Enquist BJ, West GB, Charnov EL, Brown JH (1999) Allometric scaling of production and life-history variation in vascular plants. *Nature* 401:907–911.
- 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 Manag* 256:633–640.
- Goldstein G, Meinzer FC, Monasterio M (1984) The role of capacitance in the water balance of Andean giant rosette species. *Plant Cell Environ* 21:397–406.
- Goldstein G, Andrade JL, Meinzer FC, Holbrook NM, Cavalier 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.
- Granier A (1985) Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des arbres. *Ann Sci Forest* 42:193–200.
- Granier A (1987) Evaluation of transpiration in a Douglas fir stand by mean of sap flow measurement. *Tree Physiol* 3:309–320.
- Hao G-H, Wheeler JK, Holbrook NM, Goldstein G (2013) Investigating xylem embolism formation, refilling and water storage in tree trunks using frequency domain reflectometry. *J Exp Bot* 64:2321–2332.
- Holbrook NM, Sinclair TR (1992) Water balance in the arborescent palm, *Sabal palmetto*. II. Transpiration and stem water storage. *Plant Cell Environ* 15:401–409.
- Leite PF, Klein RM (1990) Vegetação. In: IBGE (eds) Geografia do Brasil: Região Sul. Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro, pp 113–150.
- Loustau D, Berbigier P, Roumagnac P, Arruda-Pacheco C, David JS, Ferreira MI, Pereira J, Tavares R (1996) Transpiration of a 64-year-old maritime pine stand in Portugal. 1. Seasonal course of water flux through maritime pine. *Oecologia* 107:33–42.
- 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, James SA, Goldstein G, Woodruff D (2003) Whole-tree water transport scales with sapwood capacitance in tropical forest canopy trees. *Plant Cell Environ* 26:1147–1155.
- Meinzer FC, Woodruff DR, Domec J-C, Goldstein G, Campanello PI, Gatti MG, Villalobos R (2008) Coordination of leaf and stem water transport properties in tropical forest trees. *Oecologia* 156:31–41.
- Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DR (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. *Funct Ecol* 23:922–930.
- Meinzer FC, McCulloh KA, Lachenbruch B, Woodruff DR, Johnson DM (2010) The blind men and the elephant: the impact of context and scale in evaluating conflicts between plant hydraulic safety and efficiency. *Oecologia* 164:287–296.
- Muller-Landau HC (2004) Interspecific and Inter-site variation in wood specific gravity of tropical trees. *Biotropica* 36:20–32.
- Nobel PS, Jordan PW (1983) Transpiration stream of desert species: resistances and capacitances for a C3, a C4, and a CAM Plant. *J Exp Bot* 34:1379–1391.
- Oliva Carrasco L, Bucci SJ, Scholz FG et al. (2013) Water storage discharge and refilling in the main stems of canopy tree species investigated using frequency domain reflectometry and point electronic dendrometers. *Acta Hort* 991:19–24.
- Pennington RT, Lavin M, Oliveira-Filho A (2009) Woody plant diversity, evolution, and ecology in the tropics: perspectives from seasonally dry tropical forests. *Annu Rev Ecol Evol Syst* 40:437–457.
- Phillips N, Scholz FG, Bucci SJ, Goldstein G, Meinzer FC (2008) Using branch and basal trunk sap flow measurements to estimate whole-plant water capacitance: a caution. *Plant Soil* 305:5–13.
- Santiago LS, Goldstein G, Meinzer FC, Fisher JB, Machado K, Woodruff D, Jones T (2004) Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia* 140:543–550.
- 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, Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Miralles-Wilhelm F (2008) Temporal dynamics of stem expansion and contraction in savanna trees: withdrawal and recharge of stored water. *Tree Physiol* 28:469–480.
- Scholz FG, Phillips NG, Bucci SJ, Meinzer FC, Goldstein G (2011) Hydraulic capacitance; biophysical 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. Springer, New York, pp 341–361.
- Schulze E, Eennik D, Matyssek R, Penka M, Zimmennann R, Vasicek F, Gries W, Kueera J (1985) Canopy transpiration and water fluxes in the xylem of the trunk of *Larix* and *Picea* trees—a comparison of xylem flow, porometer and cuvette measurements. *Oecologia* 66:475–483.
- Sperry JS, Saliendra NZ (1994) Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. *Plant Cell Environ* 17:1233–1241.
- Steppe K, Lemeur R (2004) An experimental system for analysis of the dynamic sap-flow characteristics in young trees: results of a beech tree. *Funct Plant Biol* 31:83–92.
- Steppe K, De Pauw DJW, Lemeur R, Vanrolleghem PA (2006) A mathematical model linking tree sap flow dynamics to daily stem diameter fluctuations and radial stem growth. *Tree Physiol* 26:257–273.
- Steppe K, De Pauw DJW, Doody TM, Teskey RO (2010) A comparison of sap flux density using thermal dissipation, heat pulse velocity and heat field deformation methods. *Agric For Meteorol* 150:1046–1056.
- Steppe K, Cochard H, Lacoite A, Ameglio T (2012) Could rapid diameter changes be facilitated by a variable hydraulic conductance? *Plant Cell Environ* 35:150–157.
- Stratton L, Goldstein G, Meinzer FC (2000) Stem water storage capacity and efficiency of water transport: their functional significance in a Hawaiian dry forest. *Plant Cell Environ* 23:99–106.
- Villagra M, Campanello PI, Bucci SJ, Goldstein G (2013) Functional relationships between leaf hydraulics and leaf economics in response to nutrient addition in subtropical tree species. *Tree Physiol* 33:1308–1318.
- Waring RH, Running SW (1978) Sapwood water storage: its contribution to transpiration and effect upon water conductance through the stems of old-growth Douglas-fir. *Plant Cell Environ* 1:131–140.
- Werneck FP, Costa GC, Prado DE, Sites JW (2011) Revisiting the historical seasonally dry tropical insights based on palaeodistribution modelling and palynological. *Glob Ecol Biogeogr* 20:272–288.
- Wheeler JK, Huggett BA, Tofte AN, Rockwell FE, Holbrook NM (2013) Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. *Plant Cell Environ* 36:1938–1949.