



## HOW DROUGHT AND DECIDUOUSNESS SHAPE XYLEM PLASTICITY IN THREE COSTA RICAN WOODY PLANT SPECIES

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### ABSTRACT

This paper explores the phenotypic plasticity of xylem in tropical trees in order to test the hypothesis that different leaf phenological patterns influence levels of xylem plasticity. Wood anatomy was studied in the wet-deciduous species *Cordia alliodora* (Ruiz & Pav.) Oken, the dry-deciduous species *Tabebuia rosea* (Bertol.) DC., and the evergreen species *Ocotea veraguensis* (Meisn.) Mez., collected from seasonally dry forest and tropical cloud forest in Costa Rica. Xylem plasticity and trait conservatism were examined by analysing the coefficient of variation (*CV*) and the relative distance plasticity index (*RDPI*) of xylem anatomical traits. The two deciduous species exhibited wider vessels, lower wood density, and higher Huber values than the evergreen species. Furthermore, intervessel connectivity was highest for the two deciduous species in seasonally dry forest compared to cloud forest, whereas the opposite was found for the evergreen species. Overall highest trait variability was found for all plants at the seasonally dry site. The evergreen species *O. veraguensis* had the highest plasticity values compared to the deciduous species. Highest plasticity was found for vessel composition index (vessel area/vessel number), while strongest trait conservatism was found for vessel diameter. In conclusion, our data indicate that evergreen *O. veraguensis* does not show less xylem plasticity than the two deciduous tree species studied.

**Keywords:** Cloud forest, drought, seasonally dry forest, tropical trees, wood anatomy.

### INTRODUCTION

Water transport in plants is driven by transpiration at the leaf level, which generates a tension that pulls up water from the roots to the leaves via the xylem tissue (Dixon & Joly 1895). Since the water column inside the xylem tissue is usually under negative pressure, air can be drawn into the conduits through small pores and cracks in conduit walls or by nucleation processes (Tyree & Zimmermann 2002). This cavitation

process, *i.e.* embolism formation inside a conduit, may lead to a blockage of the hydraulic pathway and will frequently result in a more negative stem water potential, which can be partly avoided by stomatal closure and leaf shedding (Brodribb *et al.* 2003). Nevertheless, increased drought levels will inevitably lead to high levels of embolism formation, which eventually result in plant desiccation and partial or complete dieback. Because future climate change scenarios predict an increase in temperature, shifts in rainfall, and more severe and frequent drought events worldwide (IPCC 2013), distribution patterns of many species are fundamentally influenced by drought (Pockman & Sperry 2000; Engelbrecht 2012), and many tree species living on the brink of their hydraulic safety margins may suffer under predicted climate conditions (Choat *et al.* 2012).

Apart from physiological and anatomical adaptations of leaves for dealing with drought stress (Bartlett *et al.* 2012), plants have evolved different xylem anatomical strategies as a trade-off between optimal growth and protection against risk of hydraulic failure. These strategies are clearly reflected in the enormous variation in xylem anatomical features described for many woody species over the last 100 years (Fichtler & Worbes 2012; Fonti & Jansen 2012). Especially changes in vessel properties such as a reduced vessel diameter, an increase in intervessel wall thickness and vessel density, may help to cope with increasing drought stress and prevent embolism formation.

The ability of plants to grow in spatially and temporally heterogeneous environments by modifying their xylem anatomy has been suggested to be closely associated with their distribution patterns and survival success (Darwin 1859; Reich & Wright 2003; Sánchez-Gómez *et al.* 2008).

Because most plants exhibit strong plasticity when exposed to environmental changes such as drought stress (Couso & Fernández 2012), climate change may favour high species plasticity (Parmesan 2006). In contrast, some plants keep individual trait values related to their ecotypes (Rozendaal *et al.* 2006), suggesting that plasticity is not always essential (Valladares *et al.* 2007). For instance, an increase in plasticity of one trait may decrease the performance of another. This “maladaptivity” can lead to a decrease in survival rate when extreme conditions favour two contrary traits, *e.g.* increasing vessel diameter while decreasing cell wall thickness simultaneously (Sánchez-Gómez *et al.* 2008; Couso & Fernández 2012). As a result, even little or no plasticity might be adaptive (Ghalambor *et al.* 2007) and may even provide a successful stress tolerance strategy (Grime & Mackey 2002), such as slow growing, evergreen species (Wright *et al.* 2004). In contrast, deciduous species tend to show an overall higher plasticity in morphological and physiological traits (Böhnke & Bruelheide 2013).

It is known that stem and leaf traits are integrated at a number of levels (Sack & Holbrook 2006). However, little attention has been paid to the influence of leaf phenology on the plasticity of stem xylem under varying levels of water availability. In this study we aim to investigate the potential trade-off between deciduousness and xylem plasticity. More specifically, we explore the impact of variability in rainfall on the xylem anatomy and plasticity in one evergreen and two deciduous broad-leaved tree species. Vulnerability to cavitation of leaf and the stem xylem is affected by the xylem water potential, transpiration rate (Litvak *et al.* 2012) and therefore leaf

phenology. Deciduous broad-leaved species exhibiting large seasonal differences in hydraulic conductivity and transpiration rates (Sobrado 1993) are therefore expected to show a highly plastic xylem. Evergreen species, on the other hand, are hypothesized to be more conservative in their xylem anatomy, maintaining relatively low conductivity during wet and dry seasons (Ishida *et al.* 2010; Fu *et al.* 2012), and may exhibit a higher resistance to cavitation than deciduous species (Fu *et al.* 2012). We hypothesize that this conservative strategy is reflected in reduced xylem plasticity of the evergreen *Ocotea veraguensis*.

## MATERIALS AND METHODS

### *Field sites*

Samples were collected in September and October 2010 at two sites in Costa Rica. The Santa Rosa National Park (10° 50' N, 85° 37' W, elevation 100–300 m asl) is located within a seasonally dry forest in the northwest of Costa Rica and represents a dry, warm and low elevation site. Mean annual precipitation (MAP) is 1200 mm and mean annual temperature (MAT) is 27 °C. This site is characterized by a constant temperature throughout the year, but has a strong seasonal change in precipitation. Highest precipitation with around 95 percent of the total rainfall occurs during the rainy season from June until November, whereas almost no rain falls during the dry season between December and May.

The Monteverde Cloud Forest Reserve (10° 16' N, 84° 49' W, elevation 800–1400 m asl) is a tropical cloud forest with a MAP of 4,000 mm and a MAT of 17.5 °C. Despite the steady rainfall throughout the year, a distinct seasonal pattern occurs, including a drier period from December to April and a wetter period from May to November. Also, the impact of a short summer with alternating sunny and rainy days, called “veranillo”, is a common occurrence at the Pacific slope of Costa Rica and creates a difference in climate parameters from June to August.

### *Plant material*

Three tree species common to Costa Rica were investigated: *Tabebuia rosea* (Bertol.) DC. (Bignoniaceae), *Cordia alliodora* (Ruiz & Pav.) Oken (Boraginaceae), and *Ocotea veraguensis* (Meisn.) Mez (Lauraceae).

*Tabebuia rosea* is the tallest of the three species, with a height up to 35 m (Gentry 1996). In seasonally dry habitats, shoot growth starts at the beginning of the rainy season. A second growth period is possible in mesic environments. *Tabebuia rosea* is dry-deciduous with leaf abscission at the beginning of the dry season. A second leaf shedding is possible around July (Croft 1978). The seasonal phenology, however, is highly variable. In dry lowland habitats, leaf shedding tends to occur up to three months earlier than at wetter, higher elevations (Borchert 1983).

*Cordia alliodora* is a pioneer species with a diverse morphology, up to 30 m tall in rain forests but usually smaller, more bushy growth forms in deciduous forests (Janzen 1983). Besides the symbiosis of *C. alliodora* with various ant species living in domatia of the branches, leaf phenology of this species is characterized by its wet-deciduousness. Plants lose their leaves at the beginning of the rainy season (May), remain leafless for

1–2 months and produce new leaves by the end of August (Croat 1978). Being a successful, fast growing and well adapted competitor, *C. alliodora* serves as an optimal species for agroforestry (Vindas & Pablo 1983).

*Ocotea veraguensis* is an evergreen species, reaching a maximum height of c. 13 m. The distribution of *O. veraguensis* is limited to Central America, with its southernmost occurrence in Colombia and Ecuador.

Plant samples were mainly collected in open or easily accessible areas. In Santa Rosa, nearly all samples were collected near roads, whereas plants sampled at Monteverde were mainly growing on meadows. At each site, five individuals per species were selected, with a minimal tree height of 3 m. Five sun-exposed branches, with a south to south-west orientation, a similar height (c. 3 m) and diameter (c. 2 cm) were collected per specimen. One foliated branch per specimen was cut off in the field. After cutting, samples were wrapped up in a plastic bag and brought to the laboratory.

### **Morphology**

Tree height ( $H_{Tr}$ ), diameter at breast height ( $DBH$ ), and branch height ( $H_{Br}$ ) were measured in the field. The branches were defoliated and cut off equidistantly along each branch. Cross-sectional images were analysed to determine the amount of potential xylem conductive tissue (sapwood) and the Huber value ( $HV$ ), *i.e.* the ratio of potential xylem conductive area to total leaf-area of a branch, was determined using image analysis software (Schneider *et al.* 2012). Huber value measurements were conducted for Monteverde, but not for Santa Rosa.

### **Xylem anatomy**

Wood samples collected in the field were air-dried and boiled in water for at least 4 h before sectioning. Transverse sections (c. 30  $\mu\text{m}$ ) of branches were made using a sliding microtome (Sledge microtome GSL 1, Swiss Federal Research Institute WSL, Birmensdorf, Switzerland). Samples were bleached (Danklorix), stained in a mixture of safranin and alcian blue (20 : 80, v/v), dehydrated with an ethanol series (50%, 70%, 96%), treated with the clearing agent Neo-Clear and permanently mounted with Neo-Mount (Merck chemicals, Darmstadt, Germany).

Light microscopy images were recorded with a Leica DFC 300 FX camera mounted on a Leica DMR BE microscope (Leica Microsystems, Wetzlar, Germany). Some samples showed semi-ring porosity, especially those from Santa Rosa. In order to include as much vessel variability as possible, the complete sector of a transverse section from bark to pith, including at least 100 vessels per sample, was used to measure xylem anatomical characters. Therefore, images recorded at a 40x magnification were stitched together using a stitching plugin for ImageJ (<http://fly.mpi-cbg.de/~preibisch/software.html>). The stitched images were then analysed with Image Pro Plus (v. 7.0, Media cybernetics, Silverspring, MA, USA).

Several anatomical characters were measured and calculated (Table 1) for five branches of each specimen according to Scholz *et al.* (2013a). Cross sections were used to measure vessel density ( $V_D$ ), vessel grouping ( $V_G$ ) (Carlquist 2001), and inter-vessel contact fraction ( $F_C$ ; Jansen *et al.* 2011). Vessel lumen was measured as the

Table 1. Overview and definition of the wood anatomical and morphological characters measured in three tree species (*Cordia alliodora*, *Tabebuia rosea*, *Ocotea veraguensis*) sampled at two study sites (Santa Rosa and Monteverde) in Costa Rica.

Character	Unit	Description / Formula
$D$	$\mu\text{m}$	Average equivalent circle diameter
$DBH$	m	Tree diameter measured at breast height
$D_{\text{MAX}}$	$\mu\text{m}$	Maximum vessel diameter
$F$	–	Vessel lumen fraction; $F = V_{\text{D}} * V_{\text{A}}$
$F_{\text{C}}$	–	Intervessel contact fraction, <i>i.e.</i> portion of vessel wall in contact with other vessels
$H_{\text{Br}}$	m	Height at the base of a branch collected
$H_{\text{Tr}}$	m	Tree height
$HV$	–	Huber value = Area of sapwood / Total leaf area
$K_{\text{T}}$	$\text{kg/m} * \text{MPa} * \text{s}$	Theoretical hydraulic conductance Hagen-Poiseuille-Equation: $(\pi * D^4) / (128 \eta)$
$S$	–	Vessel composition index; $S = V_{\text{A}} / V_{\text{D}}$
$TD^2$	–	Theoretical vessel wall implosion resistance index $= (T_{\text{VW}} / D_{\text{MAX}})^2$
$T_{\text{VW}}$	$\mu\text{m}$	Intervessel wall thickness measured as the double intervessel wall between two adjacent vessels
$V_{\text{A}}$	$\mu\text{m}^2$	Vessel area
$V_{\text{D}}$	/mm <sup>2</sup>	Vessel density, <i>i.e.</i> number of vessels per mm <sup>2</sup>
$V_{\text{G}}$	–	Vessel grouping index, <i>i.e.</i> ratio of total vessel number to total number of vessel groupings
$WD$	$\text{g/cm}^3$	Wood density

equivalent circle diameter ( $D$ ), the maximum vessel diameter ( $D_{\text{MAX}}$ ), and average vessel area ( $V_{\text{A}}$ ) using transverse sections. The hydraulically weighted diameter and the vessel diameter corresponding to the average lumen conductivity ( $D_{\text{H}}$ ) were derived from  $D$ . Intervessel wall thickness ( $T_{\text{VW}}$ ) was measured to calculate the vessel implosion resistance index ( $TD^2$ ; Hacke *et al.* 2001). Further parameters derived from  $D$ ,  $V_{\text{A}}$ , and  $V_{\text{D}}$  were: the vessel lumen fraction ( $F = V_{\text{D}} * V_{\text{A}}$ ; Zanne *et al.* 2010), and the vessel composition index ( $S = V_{\text{A}} / V_{\text{D}}$ ; Zanne *et al.* 2010). The theoretical hydraulic conductance ( $K_{\text{T}}$ ; Tyree & Zimmermann 2002) was calculated using the Hagen-Poiseuille equation (Table 1).

Wood density ( $WD$ ) was measured using the water displacement method (Hacke *et al.* 2000). Fresh debarked wood samples were immersed in water placed on a high precision balance (CPA-255-D, Sartorius AG, Göttingen, Germany). The weight of the displaced water was converted into volume. The samples were dried for at least 48 h until weight was constant.  $WD$  ( $\text{g/cm}^3$ ) was expressed as the ratio of dry weight to fresh volume.

### Statistics

Differences between species and sites were tested using a two-way ANOVA and compared post hoc via a Tukey-HSD test. An ANCOVA was used to compare the regression models between the species and sites.

To test for trait plasticity among species at two different sites, the coefficient of variation (CV) was calculated for all anatomical characters measured. For a detailed analysis of the trait variability, anatomical characters were divided into vessel specific traits, *i.e.*  $D$ ,  $F_C$ ,  $T_{VW}$ ,  $TD^2$ , and  $V_G$ , and tissue specific traits, *i.e.*  $F$ ,  $K_T$ ,  $S$ , and  $V_D$ .

The relative distance plasticity index (RDPI) was calculated to test for overall plasticity of species according to Valladares *et al.* (2006). RDPI calculates the distance of one trait between two individuals: individual  $j$  of a particular species  $x$  growing under condition  $i$  with a second individual  $j'$  of the same species  $x$  growing under another condition  $i'$ . The sum of one pair is then divided by the total number of compared pairs or distances  $n$  (Eq. 1). We calculated the distances among trait values,  $d_{ij} \rightarrow i'j'$ , for all pairs of species,  $i$  and  $i'$  represent the sites Santa Rosa and Monteverde. RDPI ranged from 0 (no plasticity) to 1 (maximal plasticity).

$$RDPI = \sum [d_{ij} \rightarrow i'j' / (x_{i'j'} + x_{ij})] / n$$

Eq. 1 – Equation for the relative distance plasticity index (RDPI).

All statistical analyses were conducted in R (R v2.15.2; R Development Core Team, 2012). We applied a Shapiro-Wilk test to test for normality of distribution, and a Levene's test to test for homoscedasticity. Non-normally distributed characters were Log-transformed. Collinearity between anatomical and morphological traits were tested via a correlation matrix and significance values were adjusted via a Bonferroni correction.

### RESULTS

We found significant differences in wood anatomy, morphology and plasticity between species and sites (Fig. 1). The evergreen species showed on average a higher wood density, smaller vessels and a higher vessel density than the two deciduous species.

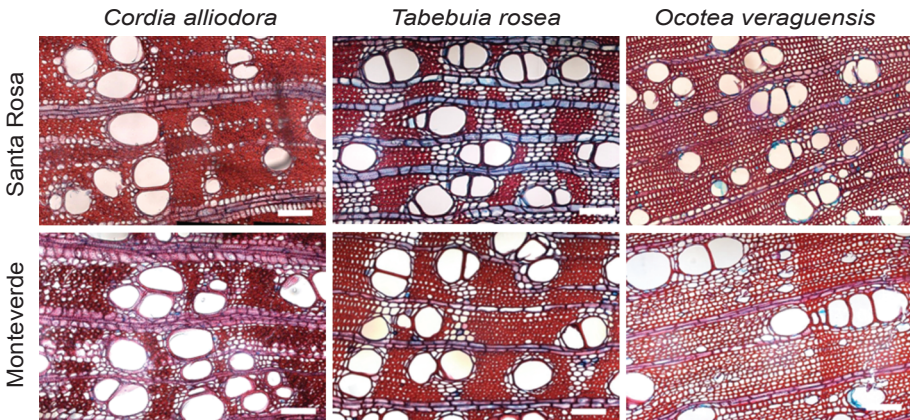


Figure 1. Light microscopic images of the xylem structure of *Cordia alliodora*, *Tabebuia rosea* and *Ocotea veraguensis* growing at Santa Rosa and Monteverde. — Scale bars represent 100  $\mu\text{m}$ .

Table 2. Anatomical features measured for *Cordia alliodora*, *Tabebuia rosea* and *Ocotea veraguensis* at Santa Rosa and Monteverde. Given are mean values with standard error in parentheses. Letters calculated via a post-hoc Tukey-HSD indicate significant differences between means. Sample size N are five individuals per species.

Site / Species	D [ $\mu\text{m}$ ]	F	F <sub>C</sub>	HV /104	K <sub>T</sub> [kg/m * MPA * s]	S	T <sub>VW</sub> [ $\mu\text{m}$ ]
<b>Santa Rosa</b>							
<i>C. alliodora</i>	54.8 (5.6) ab	0.13 (0.02) a	0.14 (0.04) ab	n.a.	11.40 (4.59) ab	7.41 (2.12) a	12.09 (1.76) b
<i>T. rosea</i>	54.4 (1.8) ab	0.13 (0.01) a	0.17 (0.02) ab	n.a.	10.55 (1.41) ab	5.8 (0.72) a	7.66 (0.69) a
<i>O. veraguensis</i>	45.3 (4.2) a	0.12 (0.01) a	0.15 (0.02) ab	n.a.	6.93 (1.15) a	3.44 (0.98) a	7.52 (0.18) a
<b>Monteverde</b>							
<i>C. alliodora</i>	56.4 (2) ab	0.11 (0.01) a	0.12 (0.01) b	5.81 (2.38) a	9.45 (0.81) ab	7.47 (1.15) a	10.64 (0.9) ab
<i>T. rosea</i>	57.7 (2.2) ab	0.13 (0.02) a	0.14 (0.02) ab	3.00 (0.588) a	12.06 (2.28) ab	7.33 (0.84) a	8.52 (0.39) ab
<i>O. veraguensis</i>	53.3 (5.1) ab	0.11 (0.01) a	0.23 (0.02) a	2.63 (0.161) a	9.40 (2.51) ab	6.67 (2.11) a	7.78 (0.64) a
Site / Species	TD <sup>2</sup>	V <sub>D</sub> [ $\mu\text{mm}^2$ ]	V <sub>G</sub>	WD [g/cm <sup>3</sup> ]			
<b>Santa Rosa</b>							
<i>C. alliodora</i>	0.036 (0.006) b	48.5 (10.2) a	2.27 (0.98) a	0.53 (0.06) bc			
<i>T. rosea</i>	0.014 (0.002) a	48.9 (5.7) a	1.64 (0.12) a	0.50 (0.01) ab			
<i>O. veraguensis</i>	0.022 (0.005) ab	78.5 (22.8) a	1.52 (0.07) a	0.57 (0.01) bc			
<b>Monteverde</b>							
<i>C. alliodora</i>	0.026 (0.005) ab	38.7 (4.0) a	1.38 (0.06) a	0.34 (0.03) a			
<i>T. rosea</i>	0.016 (0.001) a	42.5 (4.9) a	1.49 (0.1) a	0.41 (0.02) ac			
<i>O. veraguensis</i>	0.015 (0.001) a	45.8 (5.5) a	2.3 (0.15) a	0.55 (0.05) bc			

### Morphology

The highest trees were found for *Cordia alliodora* ( $H_{Tr} = 7.2 \text{ m} \pm 1.65$  standard error) and the smaller individuals for *Tabebuia rosea* ( $H_{Tr} = 5.4 \text{ m} \pm 1.5$ ) and *Ocotea veraguensis* ( $H_{Tr} = 4.8 \text{ m} \pm 1.0$ ), resulting in an equivalent decrease in the height of the branches that we collected ( $H_{Br}$ ).

Huber values ( $HV$ ) did not differ significantly between species (Table 3), but *C. alliodora* ( $HV = 5.8/10^4 \pm 4.3$ ) showed slightly higher values than *T. rosea* ( $HV = 2.8/10^4 \pm 1.2$ ) and *O. veraguensis* ( $HV = 3.0/10^4 \pm 1.0$ ).

### Xylem anatomy

Equivalent circle diameters ( $D$ ) differed significantly between species but not sites (Table 3), although it tended to be larger for each species in Monteverde than in Santa Rosa (Table 2). Vessel wall thickness ( $T_{VW}$ ) was significantly different between species ( $df = 2, F = 8.86, P < 0.001$ ; Table 3), but not between sites. Thickest vessel walls were found for *C. alliodora* ( $T_{VW} = 11 \mu\text{m} \pm 3$ ). The other two species both showed average  $T_{VW}$  values of  $8 \mu\text{m} \pm 1$ . The vessel implosion resistance index  $TD^2$  was significantly different between species ( $df = 2, F = 7.80, P < 0.01$ ; Table 3), but not between sites.  $TD^2$  was found to be twice as large in *C. alliodora* ( $0.03 \pm 0.01$ ) than in *O. veraguensis* ( $0.015 \pm 0.009$ ) and *T. rosea* ( $0.015 \pm 0.003$ ; Table 2). All other characters were not significantly different between sites or species. However, we detected differences in overall vessel connectivity: vessel grouping index ( $V_G$ ) and intervessel contact fraction ( $F_C$ ; Table 2) was lower in Monteverde than in Santa Rosa for the deciduous plants, whereas the opposite was found for the evergreen species *O. veraguensis*.

Table 3. Differences in anatomical and morphological characters between species, sites and the species  $\times$  site interaction.

Significant interactions are bold. Levels of significance:  $P < 0.05$  \*,  $P < 0.01$  \*\*,  $P < 0.001$  \*\*\*, n.a. - Huber values ( $HV$ ) were only measured for Monteverde.

Character	Species		Site		Species $\times$ Site	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<i>D</i>	3.81	<b>0.03*</b>	1.67	0.20	1.69	0.19
<i>F</i>	1.36	0.27	1.28	0.29	1.80	0.17
<i>F<sub>C</sub></i>	5.56	<b>&lt; 0.01**</b>	1.28	0.29	3.04	<b>0.04*</b>
<i>HV</i>	1.63	0.17	n.a.	n.a.	n.a.	n.a.
<i>K<sub>T</sub></i>	3.11	0.06	1.27	0.29	2.28	0.10
<i>S</i>	3.03	0.06	1.72	0.20	1.00	0.41
<i>TD<sup>2</sup></i>	7.97	<b>&lt; 0.01**</b>	3.12	0.06	1.92	0.15
<i>T<sub>VW</sub></i>	13.19	<b>&lt; 0.001***</b>	0.87	0.43	0.66	0.58
<i>V<sub>D</sub></i>	1.46	0.25	2.33	0.12	0.83	0.49
<i>V<sub>G</sub></i>	0.84	0.44	0.77	0.47	1.44	0.25
<i>WD</i>	5.39	<b>0.01*</b>	7.89	<b>&lt; 0.01**</b>	4.03	<b>0.01*</b>



$WD$  was significantly different between all species occurring at Santa Rosa ( $0.53 \text{ g/cm}^3 \pm 0.08$ ) and Monteverde ( $0.45 \text{ g/cm}^3 \pm 0.11$ ). The site differences had stronger impact on  $WD$  than interspecific differences (Site:  $df = 3, F = 8.43, P = 0.008$ ; Species:  $df = 23, F = 5.28, P = 0.01$ ; Table 3). However, an apparent difference in  $WD$  for all species was found, with *O. veraguensis* having on average denser wood ( $0.56 \text{ g/cm}^3 \pm 0.07$ ) than *C. alliodora* ( $0.46 \text{ g/cm}^3 \pm 0.15$ ) and *T. rosea* ( $0.46 \text{ g/cm}^3 \pm 0.06$ ; Table 2).

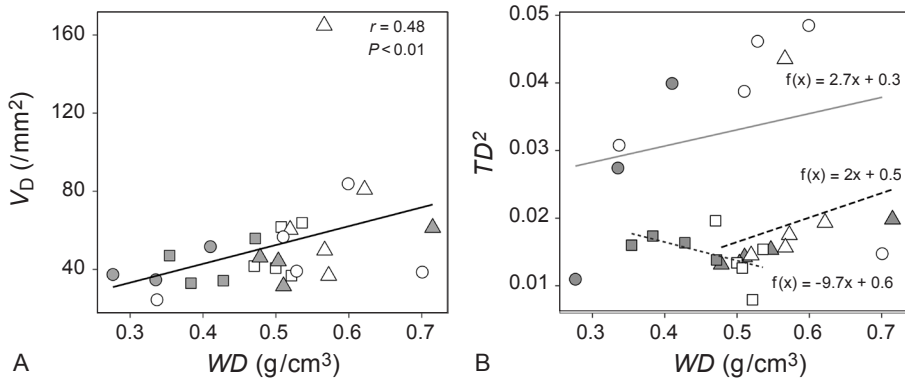


Figure 2. Correlations between (A) wood density ( $WD$ ) and vessel density ( $VD$ ) and (B) wood density and vessel implosion resistance  $TD^2$ . – Symbols represent different species (*Cordia alliodora* = circles, *Tabebuia rosea* = squares, *Ocotea veraguensis* = triangles) and sites (open symbols = Santa Rosa, grey symbols = Monteverde). – Lines indicate species regression slopes. – Fig. 2A: solid black = regression of all species; Fig. 2B: solid grey = *C. alliodora*; dashed grey = *T. rosea*; dashed black = *O. veraguensis*.

### Relationship among the traits measured

Vessel density ( $V_D$ ) was strongly correlated with most of the characters measured.  $V_D$  was positively correlated with contact fraction ( $F_C, r = 0.39, P = 0.02$ ) and wood density ( $WD, r = 0.48, P = 0.01$ ) (Fig. 2A).  $WD$  was also highly correlated with  $TD^2$  (Spearman rank correlation coefficient;  $r = 0.83, P = 0.008$ ), but only for *O. veraguensis* and *C. alliodora* (Fig. 2B). Vessel wall thickness ( $T_{VW}$ ) was correlated with morphological parameters, e.g. tree height ( $H_T, r = 0.41, P = 0.01$ ; Fig. 3) and branch height

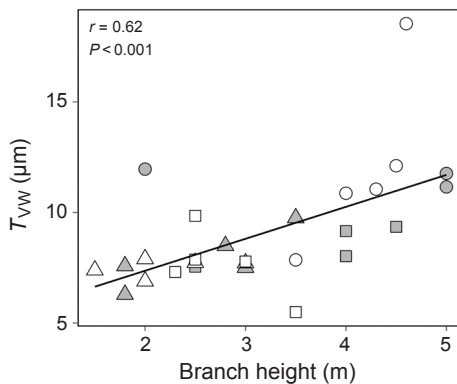


Figure 3. Correlations between branch height ( $H_{Br}$ ) and intervessel wall thickness ( $T_{VW}$ ) of *Cordia alliodora*, *Tabebuia rosea* and *Ocotea veraguensis* growing at Santa Rosa (SR) and Monteverde (MV). Symbols represent different species and sites; *C. alliodora* = circles, *T. rosea* = squares, *O. veraguensis* = triangles; open symbols = Santa Rosa, grey symbols = Monteverde.

( $H_{Br}$ ,  $r = 0.62$ ,  $P < 0.001$ ).  $T_{VW}$  was also significantly correlated with diameter ( $D$ ,  $r = 0.51$ ,  $P < 0.001$ ) and theoretical hydraulic conductivity ( $K_T$ ,  $r = 0.40$ ,  $P = 0.02$ ). Furthermore, the connectivity parameters vessel grouping index ( $V_G$ ) and intervessel contact fraction ( $F_C$ ) were highly interrelated ( $r = 0.94$ ,  $P < 0.001$ ).

**Levels of phenotypic variation**

All species had low intraspecific coefficients of variation (CV) for vessel specific traits at Monteverde (MV;  $CV < 25\%$ ) and higher CV values at Santa Rosa (SR;  $CV > 25\%$ ). For vessel specific traits (Fig. 4A), most variability was found in vessel implosion resistance index ( $TD^2$ ) and contact fraction ( $F_C$ ), and less variability was found in vessel diameter ( $D$ ), vessel wall thickness ( $T_{VW}$ ), and vessel grouping ( $V_G$ ). The highest overall vessel specific trait variability was found for *C. alliodora*. Considering tissue specific traits (Fig. 4B), an overall higher variation was also found for species at Santa Rosa, with the theoretical hydraulic conductance ( $K_T$ ) and vessel composition index ( $S$ ) being the most variable traits. The least variable and therefore most conservative character was vessel lumen fraction ( $F$ ) across all sites and species. *Ocotea veraguensis* had the largest variation among both sites in tissue specific traits. The most conservative species was *T. rosea*: neither vessel specific traits nor tissue specific traits varied more than 50 percent in CV for this species.

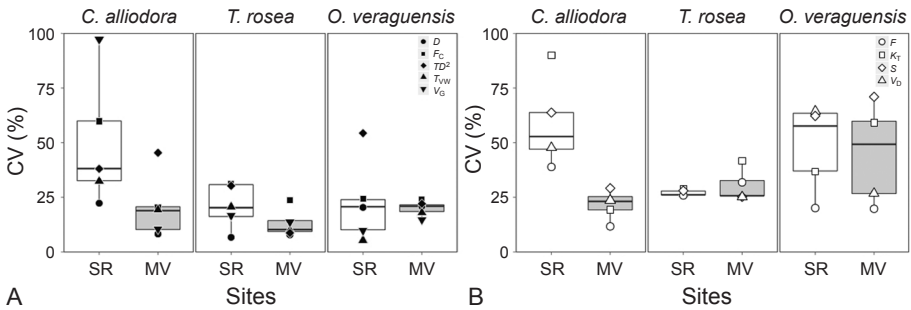


Figure 4. Coefficient of variation for (A) vessel specific and (B) tissue specific traits of *Cordia alliodora*, *Tabebuia rosea* and *Ocotea veraguensis* growing at Santa Rosa (SR) and Monteverde (MV). Acronyms are according to Table 1.

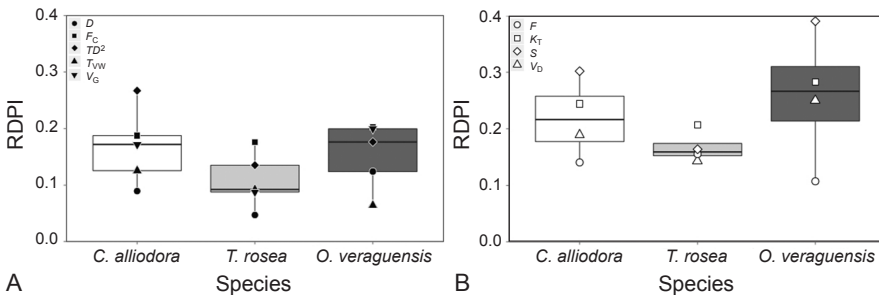


Figure 5. Relative plasticity indices (RDPI) for (A) vessel specific and (B) tissue specific traits of *Cordia alliodora*, *Tabebuia rosea* and *Ocotea veraguensis*. Acronyms are according to Table 1.

Moderate relative distance plasticity index values (RDPI < 0.5) were observed for all traits. *Ocotea veraguensis* was the most plastic and *Tabebuia rosea* the most conservative species (Fig. 5).

Vessel specific RDPI-values were on average below 0.2, with  $F_C$  and  $TD^2$  being the most plastic and  $D$  and  $T_{VW}$  the most conservative traits (Fig. 5A). High overall plasticity was found for tissue specific traits (Fig. 5B). Vessel size to number ratio ( $S$ ) and theoretical hydraulic conductance ( $K_T$ ) were the most plastic and vessel density ( $V_D$ ) and vessel lumen fraction ( $F$ ) the most conservative traits among all species.

## DISCUSSION

### *Morphological and anatomical differences across species and sites*

The results show considerable differences in plasticity values of xylem characters of the deciduous and evergreen species studied, reflecting differences across the two sites and interspecific variation.

Wood density ( $WD$ ), a trait negatively correlated with mean annual precipitation in tropical zones (Fan *et al.* 2012), varied significantly between sites and species. Highest densities were found at Santa Rosa, especially in the evergreen species *O. veraguensis*. Crous *et al.* (2012) demonstrated that high wood density, which is determined in some species by high vessel density and small vessel diameters (Martínez-Cabrera *et al.* 2011; Ziemińska *et al.* 2013), helps evergreen species to deal with excessive water stress in dry environments (Markesteyn *et al.* 2011). In contrast, deciduous species had a lower  $WD$ . These species are capable of shedding leaves during the dry season, avoiding embolism formation in the stem and thereby safeguarding the stem hydraulic system during drought stress (Sobrado 1997). Therefore, in line with our results, deciduous plants can maintain wider vessels and exhibit lower wood density in dry environments than evergreen broad-leaved plants without an increased risk of hydraulic failure (Choat *et al.* 2005; Chen *et al.* 2009a, b; Fu *et al.* 2012).

Vessel diameter is among the most important wood anatomical characters determining plant adaptation to drought. According to the Hagen-Poiseuille-Equation, the hydraulic conductivity increases proportionally to the vessel diameter raised to the fourth power (Tyree & Zimmermann 2002). In line with our expectations, diameters of all species were lower at Santa Rosa than at Monteverde, with the most pronounced difference in *O. veraguensis*. Besides this difference in vessel diameter, all three species reacted in different ways to optimize hydraulic efficiency and safety.

### *The evergreen species Ocotea veraguensis*

Various anatomical characters may contribute to hydraulic safety in *O. veraguensis*. We found a decrease in vessel diameter ( $D$ ) and an increase in vessel implosion resistance ( $TD^2$ ) from the wetter Monteverde toward the drier Santa Rosa site. An increase in  $TD^2$  may help to prevent theoretical vessel implosion under drought stress (Hacke *et al.* 2001; Crous *et al.* 2012). Along with a decrease in  $D$ , we found a decline in theoretical hydraulic conductance ( $K_T$ ), which is not surprising considering that hydraulic safety is generally assumed to be accompanied by a decrease in efficiency (Sperry *et al.* 2008).

Also, overall  $K_T$  values of the evergreen *O. veraguensis* were significantly below the values of the deciduous species as shown by data of Sobrado (1993).

The high vessel density ( $V_D$ ) found at Santa Rosa does not seem to compensate the negative effect of decreasing vessel size on  $K_T$ , even though a high number of narrow vessels is much safer in terms of cavitation resistance compared to a few large vessels (Abrantes *et al.* 2012). The impact of cavitation within one small vessel is marginal in comparison to that of an embolized large vessel (Baas *et al.* 2004). The changes in diameter and amount of vessels are clearly reflected by the difference in vessel composition index ( $S$ ) between Santa Rosa and Monteverde (Table 2). Apart from vessel size and abundance, we found lower vessel contact fraction ( $F_C$ ) and vessel grouping ( $V_G$ ) values for Santa Rosa than for Monteverde for *O. veraguensis*. Low vessel connectivity may limit air-seeding throughout the hydraulic network and therefore provide an adaptation to dry environments (Loepfe *et al.* 2007; Scholz *et al.* 2013b).

#### **Deciduous species – *Cordia alliodora* and *Tabebuia rosea***

For the two deciduous species, *C. alliodora* and *T. rosea*, no pronounced differences in vessel diameter and theoretical hydraulic conductance were found between trees growing at Santa Rosa and Monteverde.

Moreover, these species showed different adaptations to the two environments studied. For instance, vessel wall thickness ( $T_{VW}$ ) along with vessel implosion resistance ( $TD^2$ ) differed notably between Monteverde and Santa Rosa for *C. alliodora*, but not for *T. rosea*.

Since *C. alliodora* is still leaf-bearing at the beginning of the dry season (November) this species should be able to withstand a relatively high xylem tension of its xylem sap. The mean  $P_{50}$  value reported for this species by Lopez *et al.* (2005) was -3.2 MPa. Not surprisingly,  $T_{VW}$  and  $TD^2$  are higher at Santa Rosa than at Monteverde. Furthermore, significantly higher contact fraction ( $F_C$ ) and vessel grouping ( $V_G$ ) values were exhibited for both deciduous species in Santa Rosa as compared to Monteverde.

In evergreen species growing in dry habitats and coping with constant water stress throughout the season, high vessel connectivity could be associated with an increased risk of air-seeding. In contrast, deciduous species without conductive ground tissue

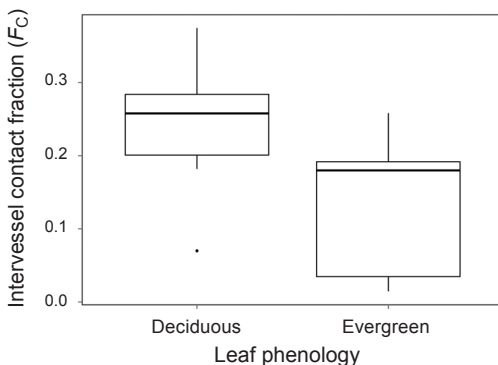


Figure 6. Boxplot representing the variation in intervessel contact fraction ( $F_C$ ) among deciduous and evergreen species based on the Xylem Functional Traits Database (SJ, BC, unpublished data). Study species are not included in the dataset. Number of all species,  $n = 24$ .

(tracheids) such as *C. alliodora* and *T. rosea* can drop their leaves to prevent embolism formation. The risk of embolism formation could be outbalanced by a high vessel connectivity, because this would increase the number of alternative hydraulic pathways in case some vessels become embolized (Carlquist 1984).

This finding of higher vessel connectivity in deciduous species compared to evergreen species is also supported by the Xylem Functional Traits Database (SJ, BC, unpublished data; Fig. 6; Wilcoxon rank sum test;  $W = 97.5$ ,  $P = 0.02$ ).

Furthermore, a well-developed vessel network may help to refill cavitated vessels in a fast and efficient way (Vogt 2001; Christman *et al.* 2012). Faster refilling was indeed found for grouped vessels of *Vitis vinifera* (Brodersen *et al.* 2010) compared to more solitary vessels. This possible refilling may be facilitated in deciduous species because they are capable of holding a certain amount of water inside the stem during the dry season (Borchert & Pockman 2005). Furthermore, Ishida *et al.* (2010) found minimal conductance values in twig xylem of already defoliated trees during the dry season, supporting the idea of constant water flow retaining a substantial portion of xylem function (Brodrribb *et al.* 2002). Additionally, Borchert and Pockman (2005) demonstrated a shorter rehydration phase in tropical deciduous species compared to evergreen plants. This maintenance of the hydraulic capacitance may partly facilitate early leaf flushing and help to refill embolized vessels.

However, the mechanisms behind embolism refilling processes, which range from root pressure to novel refilling, are far from being fully understood (Brodersen & McElrone 2013).

### **Morphological and anatomical relationships**

By testing for collinearity between the anatomical variables, two obvious relationships were found. First, a pronounced correlation between vessel contact fraction ( $F_C$ ) and vessel grouping index ( $V_G$ ) was detected, as suggested by Lens *et al.* (2011). Second, a strong negative correlation between vessel size and vessel density could be demonstrated (Carlquist 1975; Sperry *et al.* 2008; Zanne *et al.* 2010).

Strong interrelations of wood anatomical characters and tree morphology have also been shown in previous studies (Martínez-Cabrera *et al.* 2011; Fichtler & Worbes 2012). For instance, strong correlations have been found between vessel implosion resistance ( $TD^2$ ), and wood density ( $WD$ ) (Hacke *et al.* 2001; Crous *et al.* 2012). Interestingly, we found a strong correlation between  $TD^2$  and  $WD$  for *O. veraguensis* and *C. alliodora*, but not for *T. rosea*. The relationship between wood anatomical traits and  $WD$  in angiosperms remains unclear (Preston *et al.* 2006; Schuldt *et al.* 2013). Wood density is not always influenced by vessel characters (*e.g.*  $D$ ,  $TD^2$ ), but also by characters of other cell types, such as rays and fibres (McCulloh *et al.* 2011; Zheng & Martínez-Cabrera 2013; Ziemińska *et al.* 2013). Finally, a strong correlation was found for vessel wall thickness ( $T_{VW}$ ) and branch height ( $H_{Br}$ ). This could be explained by an increase in tension of the xylem water column from the root to the crown and from the trunk to branches (Tyree & Zimmermann 2002), assuming that thicker walls are required in higher xylem tissues to withstand a more negative water potential.

### ***Differences in trait plasticity between evergreen and deciduous species (CV and RDPI)***

To our surprise, the overall plasticity was fairly similar for evergreen and wet-deciduous species ( $RDPI > 0.2$ ). This rejects the hypothesis that the evergreen *O. veraguensis* is more conservative in its overall trait values than the deciduous species studied.

By separating the traits into vessel and tissue specific groups, we were able to draw a clearer picture of xylem plasticity among the species. Interestingly, vessel diameter ( $D$ ), which is negatively correlated with embolism resistance (Chen *et al.* 2009a, b) but positively with hydraulic conductivity (Carlquist 1975; Zheng & Martínez-Cabrera 2013), was shown to be highly conservative among all species. The two deciduous species did not differ significantly in vessel diameter between the sites studied, supporting previous findings of conservative vessel diameters in *C. alliodora* (Choat *et al.* 2007).

Overall, the vessel lumen fraction ( $F$ ), which was strongly correlated with vessel diameter, was the most conservative tissue related character for all species. In contrast, the vessel composition index ( $S$ ) was highly plastic in *O. veraguensis* and *C. alliodora*, but not in *T. rosea*.  $S$  is strongly linked to the amount of sapwood and stem specific conductivity (Zanne *et al.* 2010), which is clearly influenced by leaf transpiration. While evergreen leaves are exposed to seasonal changes throughout the year, their xylem must resist larger fluctuations in xylem water potential than deciduous species. This is reflected in the variability of the vessel composition index ( $S$ ) of *O. veraguensis* between Santa Rosa and Monteverde. In contrast, deciduous species tend to exhibit a lower resistance to cavitation ( $P_{50}$ ) and shed their leaves before reaching critical values (Edwards & Diaz 2006). According to the Xylem Functional Trait Database (SJ, BC, unpublished data), deciduous angiosperm plants exhibit an average  $P_{50}$  of  $-2.14 \text{ MPa} \pm 1.26$ . In comparison, evergreen species can reach more negative  $P_{50}$  values (average  $P_{50} = -4.1 \text{ MPa} \pm 2.60$ ). Interestingly, *C. alliodora* is more resistant to cavitation than the average deciduous species ( $P_{50} = -3.2 \text{ MPa}$ ; Lopez *et al.* 2005). *Cordia alliodora* had the thickest vessel walls ( $T_{VW} = 11 \mu\text{m}$ ) and the highest vessel implosion resistance ( $TD^2 = 31$ ) across all sites, which enables it to maintain leaves during the dry period. Also, *C. alliodora* plants that grow in dry areas show leaf shedding at the end of the dry season, towards February or March (Borchert 1994), while specimens at wetter sites shed their leaves only at the beginning of the rainy season in May (Croat 1978). In addition to its variable wood anatomy, *C. alliodora* is highly variable in growth form, leaf size and other morphological parameters (Choat *et al.* 2007), supporting its overall pioneer status.

### ***Phenotypic plasticity***

Phenotypic plasticity in long-living sessile organisms such as trees, will enable plants to withstand varying environmental conditions on a seasonal and annual basis (Bradshaw 2006). To what extent does this account for the characters found in our three species investigated?

*Ocotea veraguensis* has the most limited abundance and distribution, occurring mainly in Central America, with a few occurrences at the east coast of Mexico and its

southernmost occurrence in Colombia and Ecuador (Global Biodiversity Information Facility; <http://www.gbif.org>).

*Ocotea veraguensis* primarily alters its vessel anatomy via changing its vessel diameter. Vessel diameter was found to show a strong phylogenetic conservatism for oak species and *Metrosideros polymorpha* (Cavender-Bares *et al.* 2004; Fisher & Goldstein 2007). *Ocotea veraguensis* may be genetically determined limited in changing its vessel diameter. This phylogenetic restriction in hydraulic adaptability is compensated by altering vessel density (Carlquist & Hoekman 1985), leading to a trade-off between vessel diameter and vessel density.

In contrast, forest tree species with a broad distribution range, like *C. alliodora*, often consist of various regionally adapted populations, covering a high degree of genetic variability and adaptive solutions to changing environments. The concept of high genetic variability in *C. alliodora* is supported by significant genetic differences between populations growing at the Pacific and Atlantic coasts of Costa Rica (Chase *et al.* 1995).

## CONCLUSIONS

Anatomical and morphological differences were found for the three species studied between Santa Rosa and Monteverde, but also species specific variability in vessel and tissue specific properties were observed. Our data do not support the hypothesis that the evergreen species *O. veraguensis* shows higher xylem conservatism than the two deciduous species. The relative distance plasticity index of the evergreen species was similar to that of the wet-deciduous species, although the variability in underlying anatomical characters was different. The drought-deciduous tree, *T. rosea*, showed the least plasticity.

The highly conservative vessel diameter, which we found in all three species studied, was compensated by adapting other vessel structures or tissue specific properties, such as vessel density, vessel composition index, and vessel connectivity. Overall, this paper shows clear differences in xylem plasticity and adaptation strategies of trees with different leaf phenology. Beside the apparent phenotypic plasticity, future studies should also consider the genetic inheritability of a species when quantifying plasticity. Therefore, controlled greenhouse experiments (Pita *et al.* 2003), shelter exclusion experiments (Matías *et al.* 2012), or provenance tests (Richardson *et al.* 2013) on a low number of species but high specimen number should help us with quantifying the plasticity thresholds of anatomical characters.

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## REFERENCES

- Abrantes J, Campelo F, García-González I & Nabais C. 2012. Environmental control of vessel traits in *Quercus ilex* under Mediterranean climate: relating xylem anatomy to function. *Trees* 27: 655–662.
- Baas P, Ewers FW, Davis SD & Wheeler EA. 2004. Evolution of xylem physiology. In: Hemsley AR & Poole I (eds.), *The evolution of plant physiology*: 273–295. Elsevier Academic Press, Oxford, UK.
- Bartlett MK, Scoffoni C & Sack L. 2012. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. *Ecology Letters* 15: 393–405.
- Böhnke M & Bruehlheide H. 2013. How do evergreen and deciduous species respond to shade? – Tolerance and plasticity of subtropical tree and shrub species of South-East China. *Environmental and Experimental Botany* 87: 179–190.
- Borchert R. 1983. Phenology and control of flowering in tropical trees. *Biotropica* 15: 81–89.
- Borchert R. 1994. Water status and development of tropical trees during seasonal drought. *Trees* 8: 115–125.
- Borchert R & Pockman WT. 2005. Water storage capacitance and xylem tension in isolated branches of temperate and tropical trees. *Tree Physiology* 25: 457–466.
- Bradshaw AD. 2006. Unravelling phenotypic plasticity – why should we bother. *New Phytologist* 170: 644–648.
- Brodersen CR & McElrone AJ. 2013. Maintenance of xylem network transport capacity: A review of embolism repair in vascular plants. *Frontiers in Plant Science* 4: 1–11.
- Brodersen CR, McElrone AJ, Choat B, Matthews MA & Shackel KA. 2010. The dynamics of embolism repair in xylem: *in vivo* visualizations using high-resolution computed tomography. *Plant Physiology* 154: 1088–1095.
- Brodribb TJ, Holbrook NM, Edwards EJ & Gutiérrez MV. 2003. Relations between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. *Plant, Cell & Environment* 26: 443–450.
- Brodribb TJ, Holbrook NM & Gutiérrez MV. 2002. Hydraulic and photosynthetic co-ordination in seasonally dry tropical forest trees. *Plant, Cell & Environment* 25: 1435–1444.
- Carlquist S. 1975. *Ecological strategies of xylem evolution*. University of California Press, Berkeley, CA, USA.
- Carlquist S. 1984. Vessel grouping in dicotyledon wood: significance and relationship to imperforate tracheary elements. *Aliso* 10: 505–525.
- Carlquist S. 2001. *Comparative wood anatomy – Systematic, ecological, and evolutionary aspects of dicotyledon wood*. Ed. 2. Springer Verlag, Berlin, Germany.
- Carlquist S & Hoekman DA. 1985. Ecological wood anatomy of the woody southern Californian flora. *IAWA Bull. n.s.* 6: 319–347.
- Cavender-Bares J, Kitajima K & Bazzaz F. 2004. Multiple trait associations in relation to habitat differentiation among 17 Floridian oak species. *Ecological Monographs* 74: 635–662.
- Chase M, Boshier D & Bawa K. 1995. Population genetics of *Cordia alliodora* (Boraginaceae), a neotropical tree. 1. Genetic variation in natural populations. *Amer. J. Bot.* 82: 468–475.
- Chen J-W, Zhang Q & Cao K-F. 2009a. Inter-species variation of photosynthetic and xylem hydraulic traits in the deciduous and evergreen Euphorbiaceae tree species from a seasonally tropical forest in south-western China. *Ecological Research* 24: 65–73.
- Chen J-W, Zhang Q, Li X-S & Cao K-F. 2009b. Independence of stem and leaf hydraulic traits in six Euphorbiaceae tree species with contrasting leaf phenology. *Planta* 230: 459–468.



- Choat B, Ball MC, Luly JG & Holtum JAMM. 2005. Hydraulic architecture of deciduous and evergreen dry rainforest tree species from north-eastern Australia. *Trees* 19: 305–311.
- Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S, Bhaskar R, Bucci SJ, Feild TS, Gleason SM, Hacke UG, Jacobsen AL, Lens F, Maherali H, Martínez-Vilalta J, Mayr S, Mencuccini M, Mitchell PJ, Nardini A, Pittermann J, Pratt RB, Sperry JS, Westoby M, Wright IJ & Zanne AE. 2012. Global convergence in the vulnerability of forests to drought. *Nature* 50: 10–12.
- Choat B, Sack L & Holbrook NM. 2007. Diversity of hydraulic traits in nine *Cordia* species growing in tropical forests with contrasting precipitation. *New Phytologist* 175: 686–698.
- Christman MA, Sperry JS & Smith DD. 2012. Rare pits, large vessels and extreme vulnerability to cavitation in a ring-porous tree species. *New Phytologist* 193: 713–720.
- Couso LL & Fernández RJ. 2012. Phenotypic plasticity as an index of drought tolerance in three Patagonian steppe grasses. *Annals of Botany* 110: 849–857.
- Croat TB. 1978. *Flora of Barro Colorado Island*. Stanford University Press, Stanford, CA, USA.
- Crous CJ, Jacobs SM & Esler KJ. 2012. Wood anatomical traits as a measure of plant responses to water availability: invasive *Acacia mearnsii* De Wild. compared with native tree species in fynbos riparian ecotones, South Africa. *Trees* 26: 1527–1536.
- Darwin C. 1859. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. Oxford University Press, London, UK.
- Dixon HH & Joly J. 1895. On the ascent of sap. *Philos. Trans. Royal Soc. B: Biological Sciences* 186: 563–576.
- Edwards EJ & Diaz M. 2006. Ecological physiology of *Pereskia guamacho*, a cactus with leaves. *Plant, Cell & Environment* 29: 247–256.
- Engelbrecht BM. 2012. Forests on the brink. *Plant Ecology* 491: 675–677.
- Fan Z-X, Zhang S-B, Hao G-Y, Slik FJW & Cao K-F. 2012. Hydraulic conductivity traits predict growth rates and adult stature of 40 Asian tropical tree species better than wood density. *J. Ecology* 100: 732–741.
- Fichtler E & Worbes M. 2012. Wood anatomical variables in tropical trees and their relation to site conditions and individual tree morphology. *IAWA J.* 33: 119–140.
- Fisher JB & Goldstein G. 2007. Wood vessel diameter is related to elevation and genotype in the Hawaiian tree *Metrosideros polymorpha* (Myrtaceae). *Amer. J. Bot.* 94: 709–715.
- Fonti P & Jansen S. 2012. Xylem plasticity in response to climate. *New Phytologist* 195: 734–736.
- Fu P-L, Jiang Y-J, Wang A-Y, Brodribb TJ, Zhang J-L, Zhu S-D & Cao K-F. 2012. Stem hydraulic traits and leaf water-stress tolerance are co-ordinated with the leaf phenology of angiosperm trees in an Asian tropical dry karst forest. *Annals of Botany* 110: 189–199.
- Gentry AH. 1996. *A field guide to the families and genera of woody plants of northwest South America (Colombia, Ecuador, Peru): with supplementary notes on herbaceous taxa*. University of Chicago Press, Chicago.
- Ghalambor CK, McKay JK, Carroll SP & Reznick DN. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* 21: 394–407.
- Grime J & Mackey J. 2002. The role of plasticity in resource capture by plants. *Evolutionary Ecology* 16: 299–307.
- Hacke UG, Sperry JS & Pittermann J. 2000. Drought experience and cavitation resistance in six shrubs from the Great Basin, Utah. *Basic and Applied Ecology* 41: 31–41.
- 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.

- IPCC. 2013. Summary for Policymakers. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V & Midgley PM (eds.), *Climate change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press, Cambridge, UK and New York, NY, USA.
- Ishida A, Harayama H, Yazaki K, Ladpala P, Sasrisang A, Kaewpakasit K, Panuthai S, Staporn D, Maeda T, Gamo M, Diloksumpun S, Puangchit L & Ishizuka M. 2010. Seasonal variations of gas exchange and water relations in deciduous and evergreen trees in monsoonal dry forests of Thailand. *Tree Physiology* 30: 935–945.
- Jansen S, Gortan E, Lens F, Lo Gullo MA, Salleo S, Scholz A, Stein A, Trifiló P & Nardini A. 2011. Do quantitative vessel and pit characters account for ion-mediated changes in the hydraulic conductance of angiosperm xylem? *New Phytologist* 189: 218–228.
- Janzen DH. 1983. *Costa Rican natural history*. Ed. 3. University of Chicago Press, Chicago, MI, USA.
- Lens F, Sperry JS, Christman MA, Choat B, Rabaey D & Jansen S. 2011. Testing hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in the genus *Acer*. *New Phytologist* 190: 709–723.
- Litvak E, McCarthy HR & Pataki DE. 2012. Transpiration sensitivity of urban trees in a semi-arid climate is constrained by xylem vulnerability to cavitation. *Tree Physiology* 32: 373–388.
- Loepfe L, Martinez-Vilalta J, Piñol J & Mencuccini M. 2007. The relevance of xylem network structure for plant hydraulic efficiency and safety. *J. Theoretical Biol.* 247: 788–803.
- Lopez OR, Kursar TA, Cochard H & Tyree MT. 2005. Interspecific variation in xylem vulnerability to cavitation among tropical tree and shrub species. *Tree Physiology* 25: 1553–1562.
- Markestijn L, Poorter L, Paz H, Sack L & Bongers F. 2011. Ecological differentiation in xylem cavitation resistance is associated with stem and leaf structural traits. *Plant, Cell & Environment* 34: 137–148.
- Martínez-Cabrera HI, Schenk HJ, Cevallos-Ferriz SRS & Jones CS. 2011. Integration of vessel traits, wood density, and height in angiosperm shrubs and trees. *Amer. J. Bot.* 98: 915–922.
- Matías L, Quero JL, Zamora R & Castro J. 2012. Evidence for plant traits driving specific drought resistance. A community field experiment. *Environmental and Experimental Bot.* 81: 55–61.
- McCulloh KA, Johnson DM, Meinzer FC, Voelker SL, Lachenbruch B & Domec J-C. 2011. Hydraulic architecture of two species differing in wood density: opposing strategies in co-occurring tropical pioneer trees. *Plant, Cell & Environment* 35: 116–125.
- Parnesan C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* 37: 637–669.
- Pita P, Gascó A & Pardos JA. 2003. Xylem cavitation, leaf growth and leaf water potential in *Eucalyptus globulus* clones under well-watered and drought conditions. *Functional Plant Biology* 30: 891.
- Pockman WT & Sperry JS. 2000. Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *Amer. J. Bot.* 87: 1287–1299.
- Preston KA, Cornwell WK & Denoyer JL. 2006. Wood density and vessel traits as distinct correlates of ecological strategy in 51 California coast range angiosperms. *New Phytologist* 170: 807–818.
- Reich PB & Wright IJ. 2003. The evolution of plant functional variation: traits, spectra, and strategies. *International J. Plant Sciences* 164: 143–164.
- Richardson SJ, Allen RB, Buxton RP, Easdale TA, Hurst JM, Morse CW, Smissen RD & Peltzer DA. 2013. Intraspecific relationships among wood density, leaf structural traits and environment in four co-occurring species of *Nothofagus* in New Zealand. *PLoS one* 8: e58878.

- Rozendaal DMA, Hurtado VH & Poorter L. 2006. Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. *Functional Ecology* 20: 207–216.
- Sack L & Holbrook NM. 2006. Leaf hydraulics. *Annual Review of Plant Biology* 57: 361–381.
- Sánchez-Gómez D, Zavala MA & Valladares F. 2008. Functional traits and plasticity linked to seedlings' performance under shade and drought in Mediterranean woody species. *Annals of Forest Science* 65: 1–15.
- Schneider C, Rasband W & Eliceiri K. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671–675.
- Scholz A, Klepsch M, Karimi Z & Jansen S. 2013a. How to quantify conduits in wood? *Frontiers in Plant Science* 4: 1–11.
- Scholz A, Rabaey D, Stein A, Cochard H, Smets E & Jansen S. 2013b. The evolution and function of vessel and pit characters with respect to cavitation resistance across 10 *Prunus* species. *Tree Physiology* 33: 684–694.
- Schuldt B, Leuschner C, Brock N & Horna V. 2013. Changes in wood density, wood anatomy and hydraulic properties of the xylem along the root-to-shoot flow path in tropical rainforest trees. *Tree Physiology* 33: 161–174.
- Sobrado MA. 1993. Trade-off between water transport efficiency and leaf life-span in a tropical dry forest. *Oecologia* 96: 19–23.
- Sobrado MA. 1997. Embolism vulnerability in drought-deciduous and evergreen species of a tropical dry forest. *Acta Oecologica* 18: 383–391.
- Sperry JS, Meinzer FC & McCulloh KA. 2008. Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. *Plant, Cell & Environment* 31: 632–645.
- Tyree MT & Zimmermann MH. 2002. Xylem structure and the ascent of sap. Ed. 2. Springer-Verlag, Berlin, Heidelberg, New York.
- Valladares F, Gianoli E & Gómez JM. 2007. Ecological limits to plant phenotypic plasticity. *New Phytologist* 176: 749–763.
- Valladares F, Sanchez-Gomez D & Zavala MA. 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology* 94: 1103–1116.
- Vindas S & Pablo E. 1983. *Flórula del Parque Nacional Cahuita: manual de campo para la identificación de los principales árboles y palmas*. Ed. 1. Editorial Universidad Estatal a Distancia, San José, Costa Rica.
- Vogt UK. 2001. Hydraulic vulnerability, vessel refilling, and seasonal courses of stem water potential of *Sorbus aucuparia* L. and *Sambucus nigra* L. *J. of Experim. Bot.* 52: 1527–1536.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ & Villar R. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821–827.
- Zanne AE, Westoby M, Falster DS, Ackerly DD, Loarie SR, Arnold SEJ & Coomes DA. 2010. Angiosperm wood structure: Global patterns in vessel anatomy and their relation to wood density and potential conductivity. *Amer. J. Bot.* 97: 207–215.
- Zheng J & Martínez-Cabrera HI. 2013. Wood anatomical correlates with theoretical conductivity and wood density across China: evolutionary evidence of the functional differentiation of axial and radial parenchyma. *Annals of Botany* 112: 927–935.
- Ziemińska K, Butler D, Gleason SM, Wright IJ & Westoby M. 2013. Fibre wall and lumen fractions drive wood density variation across 24 Australian angiosperms. *AoB Plants* 5: 1–32.