

# Vulnerability and hydraulic segmentations at the stem-leaf transition: coordination across Neotropical trees

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#### Introduction

#### Global climate change leads to increasing frequency of extreme drought events in tropical rainforests (Duffy et al., 2015; Hilker et al., 2014; Gloor et al., 2015). Tropical rainforests host the highest biodiversity world-wide and play a disproportionate role in terrestrial ecosystem functions, such as hydrological (Mu et al., 2011; Schlesinger & Jasechko, 2014) and carbon (C) cycles (Bonan, 2008; Pan et al., 2011). Extreme drought events endanger these ecosystem functions, particularly by inducing tree mortality (Phillips et al., 2009; Allen et al., 2010; Zuleta et al., 2017; Aleixo et al., 2019), leading to large release of stored C and altering species and functional community composition (Esquivel-Muelbert et al., 2019). Therefore, drought-induced tree mortality requires a better understanding of tree drought resistance in order to predict future biodiversity and global C dynamics. However, understanding drought-induced tree mortality and drought resistance requires the identification of physiological thresholds and plant traits associated with drought resistance (Choat et al., 2018; McDowell et al., 2018).

#### **Summary**

Hydraulic segmentation at the stem–leaf transition predicts higher hydraulic resistance in leaves than in stems. Vulnerability segmentation, however, predicts lower embolism resistance in leaves. Both mechanisms should theoretically favour runaway embolism in leaves to preserve expensive organs such as stems, and should be tested for any potential coordination.
We investigated the theoretical leaf-specific conductivity based on an anatomical approach to quantify the degree of hydraulic segmentation across 21 tropical rainforest tree species. Xylem resistance to embolism in stems (flow-centrifugation technique) and leaves (optical visualization method) was quantified to assess vulnerability segmentation.

• We found a pervasive hydraulic segmentation across species, but with a strong variability in the degree of segmentation. Despite a clear continuum in the degree of vulnerability segmentation, eight species showed a positive vulnerability segmentation (leaves less resistant to embolism than stems), whereas the remaining species studied exhibited a negative or no vulnerability segmentation.

• The degree of vulnerability segmentation was positively related to the degree of hydraulic segmentation, such that segmented species promote both mechanisms to hydraulically decouple leaf xylem from stem xylem. To what extent hydraulic and vulnerability segmentation determine drought resistance requires further integration of the leaf-stem transition at the whole-plant level, including both xylem and outer xylem tissue.

According to the cohesion-tension theory (Dixon & Joly, 1895; Dixon, 1914), water is transported under tension through the plant, from the soil to the atmosphere. The evaporation of water at the air-liquid interface close to stomata creates a driving force for water movements from roots to leaves. The water transport system of plants is not impermeable to gas, and gas bubbles can be formed in xylem sap within conduits, which is more likely to occur under drought stress. Embolism within conduits impedes water flow, such that the accumulation of embolism leads to the loss of hydraulic conductivity, the loss of transpiration, and finally dehydration (Brodribb & Cochard, 2009; Urli *et al.*, 2013; Blackman *et al.*, 2019a). Therefore, there is a selective pressure on plants to be embolism resistant, which is affected by environmental water availability (McAdam & Cardoso, 2018).

Plant drought resistance is usually defined as the dehydration time between the onset of water stress with stomatal closure and various stages of tissue dehydration, and eventually mortality (Blackman *et al.*, 2016; Volaire, 2018). According to this definition, drought resistance involves various mechanisms that delay the critical occurrence of embolism in xylem conduits. The leaf 'safety-valve' mechanism, as first proposed by Zimmermann (1983), predicts that drought-induced embolism events should be confined to disposable organs, such as leaves, in favour of more expensive and perennial organs, such as branches and trunk. Then, embolism formation and complete dehydration of leaves should limit the upstream water demand and would decrease the effective evaporative crown surface area (Blackman *et al.*, 2019b), at the cost of C assimilation. Two hypotheses have been proposed to highlight this safety-valve mechanism: the hydraulic segmentation hypothesis and the vulnerability segmentation hypothesis.

First, based on Zimmermann (1983), the hydraulic segmentation hypothesis predicts a 'hydraulic constriction' at the stem-leaf junction, which drives the leaves to be more hydraulically resistant (or less conductive). This differentiation of hydraulic resistance should drive a larger, steeper water potential gradient at the stem-leaf transition than along the stem for instance (Zimmermann, 1978, 1983). Even if embolism resistance is similar in leaf and stem xylem, maintaining lower water potentials in leaves should result in xylem embolism in the leaf veins prior to stem xylem. Hydraulic segmentation patterns have been repeatedly demonstrated, mainly in the 1980s and 1990s, based on leafspecific conductivity measurements (hydraulic conductivity of an organ divided by the supplied leaf area; kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup>) and on comparison between leaves and stems (Zimmermann, 1978; Tyree et al., 1991; Tyree & Alexander, 1993; Joyce & Steiner, 1995; Nardini & Pitt, 1999; Cruiziat et al., 2002; Eisner et al., 2002; however, see Pivovaroff et al., 2014). However, there is no clear demonstration of a link between hydraulic segmentation, the appearance of embolism within leaves, and the time to dehydration.

Second, the vulnerability segmentation hypothesis (Tyree & Ewers, 1991; Tyree & Zimmermann, 2002) predicts leaves to be less resistant than stems to the drought-induced loss of conductance. Though the loss of conductance in stems is only driven by xylem embolism, the loss of conductance in leaves could be driven by xylem embolism and/or the loss of functionality of the outside-xylem compartment (Scoffoni et al., 2017a). With the development of new methods for measuring xylem resistance to embolism in stems and leaves, and the loss of leaf conductance, the vulnerability segmentation hypothesis has gained renewed interest since the 2000s. Positive segmentation (leaf less resistant to the loss of conductance than stem) has been shown frequently (Hao et al., 2008; Chen et al., 2009; Johnson et al., 2011, 2016; Bucci et al., 2012; Nolf et al., 2015; Charrier et al., 2016; Hochberg et al., 2016; Rodriguez-Dominguez et al., 2018; Skelton et al., 2018; Losso et al., 2019). However, there are also records of negative segmentation (leaf more resistant to the loss of conductance than stem; Klepsch et al., 2018), or the absence of segmentation (leaf and stem equally resistant to the loss of conductance; Chen et al., 2009; Nolf et al., 2015; Skelton et al., 2017, 2018; Klepsch et al., 2018; Losso et al., 2019). A recent meta-analysis suggests that the degree of vulnerability segmentation is correlated with biome aridity (Zhu et al., 2016), with a larger positive segmentation degree found in dry regions.

Therefore, a lack of vulnerability segmentation has been suggested for tropical rainforest trees (Zhu *et al.*, 2016), but this suggestion requires more evidence. Moreover, more data on embolism resistance of leaves and stems are desirable, since we do not know how segmentation might be linked with leaf longevity or leaf phenology (i.e. winter and drought deciduousness, evergreen, marcescent).

Most studies investigating the vulnerability segmentation hypothesis were based on measurements of the whole leaf conductance, integrating both the xylem pathway and the outsidexylem pathway (Hao *et al.*, 2008; Chen *et al.*, 2009, 2010; Johnson *et al.*, 2011, 2016; Bucci *et al.*, 2012; Nolf *et al.*, 2015; Scoffoni *et al.*, 2017b), whereas water transport in the stem relies on xylem only. But owing to long-standing methodological concerns, differences between leaf and stem xylem in embolism resistance have only recently been investigated (Charrier *et al.*, 2016; Hochberg *et al.*, 2016; Skelton *et al.*, 2017, 2018; Klepsch *et al.*, 2018; Rodriguez-Dominguez *et al.*, 2018), and not yet on a broad set of species.

Here, we combine anatomical and physiological measurements to test for hydraulic and vulnerability segmentation at the stem-leaf transition and the potential coordination between both types of segmentation. We studied trees from the Amazon rainforest spanning a wide taxonomic range of species, phylogenetic diversity (magnoliids, rosids, asterids), and functional diversity. For 21 species and 53 trees, we measured xylem embolism resistance in both leaf veins and stem wood to test the vulnerability segmentation hypothesis. Verifying the hydraulic segmentation hypothesis was based on theoretical conductivity measurements. We specifically addressed the following two questions:

First, do tropical rainforest trees exhibit hydraulic and/or vulnerability segmentation at the stem-leaf transition? We hypothesize that there is a continuum of hydraulic and vulnerability segmentations across species.

re hydraulic and vulnerability segmentation correlated across species? Indeed, even if hydraulic and vulnerability segmentation should theoretically lead to the same effect—that is, a safety-valve pattern—these two types of segmentation have not been studied together on a broad set of species.

Regarding the possible coordination of both types of segmentation, we can posit three hypotheses. First, we can hypothesize that the different types of segmentation negatively trade off with each other if they are both associated with costs and benefits;, that is, the combination of both segmentation types in a pronounced way may not be compatible by severely impeding water flow, gas exchange, and C assimilation. Second, we can hypothesize that the safety-valve pattern is always under strong selection, which leads to a positive coordination between hydraulic and vulnerability segmentation to maximize the safety-valve effect. Third, an independence of the type of segmentation could be hypothesized for two reasons: first, depending on the phylogenetic position and the various levels of canalization (i.e. a strong genetic control limiting phenotypic variability; Waddington, 1942) among anatomical traits, hydraulic segmentation could be easily selected and achieved rather than the vulnerability segmentation, or

inversely; second, the hydraulic segmentation could not be a proper way to achieve a safety-valve pattern.

#### **Materials and Methods**

#### Study site and species

The experiment was conducted in French Guiana, at the Paracou experimental station (https://paracou.cirad.fr/; 5°16′26″N, 52°55′26″W), in a lowland tropical rainforest (Gourlet-Fleury *et al.*, 2004). The warm and wet tropical climate of French Guiana is highly seasonal due to the north–south movement of the intertropical convergence zone. Average annual rainfall (2004–2014) at the study site was  $3102 \pm 70$  mm, and the average annual air temperature was  $25.7 \pm 0.1^{\circ}$ C (Aguilos *et al.*, 2019). There is a long dry season, lasting from mdi-August to mid-November, during which rainfall is < 100 mm month<sup>-1</sup>.

Only canopy, dominant, adult trees were sampled. A total of 21 tree species and 53 trees were sampled, with three trees per species for a total of 14 species. For seven species, only one to two trees were sampled. The species covered a large phylogenetic diversity, with the main clades of the flowering plants represented; that is, magnoliids, rosids, and asterids (Table 1).

There is very little information on the foliar phenology of tree species in French Guiana (growth, leaf shedding). Based on the studies of Loubry (1994a,b) and personal observations, we can state that five of the species we studied are deciduous and five are evergreen (Table 1). Following discussions with several botanist colleagues, we believe that the 14 remaining species are evergreen, although we cannot support this hypothesis with growth monitoring information (D. Sabatier, J.-F. Molino, J. Engel, personal communication)

#### Stem embolism resistance

The field sampling procedure for the measurements of stem embolism resistance was held between January and July 2017 (after the dry season). Canopy branches 2-3 m in length were sampled by professional tree climbers. Sun-exposed branches were sampled as much as possible, within the safety limits of the climbers. Just after the cut, the branches were defoliated and wrapped in wet tissue. On the same day of collection, branches were recut under water to a length of 1.5 m. Then, branches were totally wrapped in wet paper towels, with the stem ends closed off using gaffer tape. Vulnerability curves were obtained with the flow-centrifugation technique, using a 1 m diameter rotor to avoid open-vessel artefact during stem embolism resistance measurements (CAVI1000 technology; DGMeca, Gradignan, France; Lamarque et al., 2018; Lobo et al., 2018). This method was developed for and validated on many long-vesselled species, such as Quercus sp. (Lobo et al., 2018). Vessel length was also controlled by measuring maximum vessel length following the

Table 1 List of species studied, their family classification, leaf phenology, and presence of exudates at the leafy shoot level.

Species	Family	Phenology	Month*	Exudates <sup>†</sup>
Bocoa prouacensis	Fabaceae	Evergreen?		No
Chaetocarpus schomburgkianus	Peraceae	Evergreen (Loubry, 1994)		No
Chrysophyllum sanguinolentum	Sapotaceae	Evergreen (Nicolini & Heuret, pers. obs.)		Yes
Dicorynia guianensis	Fabaceae	Deciduous (Loubry, 1994)	Any month of the year	No
Eperua falcata	Fabaceae	Deciduous (Loubry, 1994)	Jan–Feb–Mar–Apr–May– Jun–Jul–Aug–Sep–Dec	No
Eperua grandiflora	Fabaceae	Evergreen?		No
Eschweilera coriacea	Lecythidaceae	Evergreen?		No
Eschweilera sagotiana	Lecythidaceae	Evergreen?		No
Goupia glabra	Goupiaceae	Evergreen (Loubry, 1994)		No
Gustavia hexapetala	Lecythidaceae	Evergreen?		No
Iryanthera sagotia	Myristicaceae	Evergreen?		No
Lecythis persistens	Lecythidaceae	Evergreen?		No
Lecythis poiteauii	Lecythidaceae	Deciduous (Loubry, 1994)	Jan-Apr	No
Licania membranacea	Chrysobalanaceae	Evergreen?		No
Manilkara bidendata	Sapotaceae	Evergreen?		Yes
Moronobea coccinea	Clusiaceae	Deciduous (Loubry, 1994)	Feb–Mar–Apr–May–Jun– Jul–Aug–Sep–Oct	Yes
Pradosia cochlearia	Sapotaceae	Deciduous (Loubry, 1994)	Sep-Oct-Nov	Yes
Protium opacum	Burseraceae	Evergreen?		Yes
Protium sagotianum	Burseraceae	Evergreen?		Yes
Protium subserratum	Burseraceae	Evergreen?		Yes
Qualea rosea	Vochysiaceae	Evergreen (Loubry, 1994)		No
Symphonia sp1	Clusiaceae	Evergreen?		Yes
Tachigali melinonii	Fabaceae	Evergreen?		No
Virola michelii	Myristicaceae	Evergreen (Loubry, 1994)		No

'Evergreen?' refers to a lack of certitude if or not the species is evergreen.

\* Refers to the month when deciduousness has been observed.

† Here, we only refer to species displaying exudates hindering repeated measurements of leaf water potential with the pressure chamber.

air-injection method (see Ziegler *et al.*, 2019). Branches were sent from Kourou to Bordeaux by priority transport and arrived there within 3 d. There, branches were recut to 1 m length under water and debarked at both ends before flow-centrifugation measurements were conducted. Vulnerability curves were fitted with a sigmoid function (Pammenter & Van der Willigen, 1998).  $P_{12}$ , stem,  $P_{50,\text{stem}}$ , and  $P_{88,\text{stem}}$  (megapascals) were extracted from vulnerability curves, such that  $P_{x,\text{stem}}$  corresponds to the water potential for which x% of the maximum conductivity is lost. The data relative to the diversity of stem embolism resistance are discussed by Ziegler *et al.* (2019), where the reader can find the precisely described method with the flow-centrifuge method (Cochard *et al.*, 2013).

#### Leaf embolism resistance

Measurements of leaf embolism resistance were held between November 2018 and March 2019, on the same trees that were sampled for the measurements of stem embolism resistance. We generally sampled three trees per day, during the morning and before solar midday, in order to avoid low leaf water potentials. Branches similar to the one previously collected were cut (1 m long, sun-exposed branches with c. 50 leaves or leaflets for monitoring of water potential as described later). Once arrived on the forest ground, the proximal stem of the branch was recut under water to avoid artefactual embolism. This recut stem extremity was then placed in a 51 water-filled plastic bottle, and all the leaves were packed in a black plastic bag. This system should minimize dehydrating of the branches and allow the water potential to homogenize across the branch (Brodribb et al., 2016b). Bagged branches were brought back to the laboratory after field work and allowed to rehydrate overnight.

To measure embolism resistance of leaf xylem, we relied on an optical light transmission method (Brodribb et al., 2016a,b). Embolism is detected and quantified, during the sample dehydration, by monitoring changes in light transmission through the xylem, since a water-filled and an air-filled (i.e. embolized) conduit do not exhibit the same transmittance. A high spatial resolution was provided by a 6400 dpi-imaging scanner (Epson Perfection V800; Epson America Inc., Long Beach, CA, USA). The sampled branch was put on a bench and a fully developed and undamaged leaf was tapped with gaffer tape on the scanner to avoid movements with dehydration-induced shrinkage. The tape was placed on the leaf margins to monitor the veins, not to prevent leaf water loss. The drying leaf was imaged every 4 min until all the leaves of the branch were fully dried up. The scanning process was automatized with a custom computer program. The leaf was illuminated from the abaxial face side and scanned on the adaxial face side to create an image of transmitted light. The scanned image was cropped to  $15 \text{ mm} \times 30 \text{ mm}$  (according to the memory mass storage), encompassing all the vein orders, including the midrib.

Identification and quantification of embolism events were carried out using a custom IMAGEJ macro (https://imagej.nih.gov/ij/ ). All the macros required for image analyses, as also the detailed description of all steps for image analyses, are provided at www.

opensourceov.org. Briefly, image subtraction between successive images in the stack was run, highlighting changes in light transmission between two images in case an embolism event occurred during the 4 min time window. The amount of embolism was quantified by calculating the number of embolized pixels scanned out of the total number of embolized pixels at the end of the drydown period. Artefacts caused by slow movements induced by leaf shrinkage were filtered. For N raw images, we get N-1'subtraction-result' images. The 'subtraction-result' images containing embolism were isolated: a threshold was applied to highlight the embolism events, and the remaining noise was cleaned. The number of pixels per 'subtraction-result' image was quantified with the IMAGEJ 'analyse particle' function. Knowing the date and the time of capture of each image, we knew the cumulated number of 'embolized pixels' according to dehydration time. In order to get vulnerability curves, time was converted in water potential by monitoring the water potential during sample dehydration and the imaging process.

For most species, leaf water potential was monitored with a Scholander pressure chamber (Model 1505D; PMS Instrument Co., Albany, OR, USA). Leaf water potential was measured every 1-3 h, depending on the speed of the water potential drop. We used three leaves per measurement, with the leaves sampled at different positions within the branch sample. The imaging process and water potential monitoring started together, at 08:00 h, after one night of rehydration. The first water potential measurement was then done just after a leaf was placed and tapped on the scanner. In this way, we generally had five or six leaf water potential measurements per day. For every species, the first measured leaf water potential was above -0.5 MPa, confirming that the branch rehydration with plastic bags was effective. The drop in water potential between the first and the second measurements was generally high, but the drop per unit of time stabilized rapidly and remained linear, indicating stomatal closure, and a drop of water potential only driven by minimum leaf conductance (Brodribb et al., 2016a,b). After stomatal closure, the leaf water potential vs time curve followed a highly linear phase, allowing estimation of water potential during the night, when measurements were not feasible. Knowing the cumulated embolism vs time curve and the leaf water potential vs time curve, it was possible to plot the cumulated embolism vs water potential curve, known as the vulnerability curve.

For species producing exudates, we could not easily and repeatedly measure leaf water potential with the pressure chamber (Table 1). For these species, water potential was monitored with a psychrometer (ICT International, Armidale, NSW, Australia) attached to the stem, providing a continuous measure of water potential. We assumed equilibrated water potentials between leaf and stem because samples spent a night in a plastic bag, and because of closed stomata during most of the time of the branch dehydration (Rodriguez-Dominguez *et al.*, 2018). For the eight species with exudates, we could not easily and repeatedly measure leaf water potential with the pressure chamber (Table 1). In a first step (October 2017), we validated on species without exudates that the use of pressure chamber and a psychrometer (ICT International) attached to the stem gave comparable results for the measurement of the water potential (errors < 0.3 MPa). Then, for species with exudates, water potential was monitored with the psychrometer, providing a continuous measure of water potential. Vulnerability curves were fitted with a sigmoid curve (Pammenter & Van der Willigen, 1998) using the 'fitplc' function of the FITPLC package in R (Duursma & Choat, 2017). Embolism was expressed as the percentage of the total 'embolized pixels' across images. Vulnerability curves were expressed as the percentage of 'embolized pixels' according to the water potential.  $P_{12,\text{leaf}}$ ,  $P_{50,\text{leaf}}$ , and  $P_{88,\text{leaf}}$  (megapascals) were extracted from vulnerability curves, such that  $P_{x,\text{leaf}}$  corresponds to the water potential for which x% of the maximum number of 'embolized pixels' is reached. For half of the species studied, embolism could not be observed in thick and lignified midribs. Therefore, our vulnerability curves were based on embolism events occurring only in second, third, and higher vein orders.

#### Assessment of vulnerability segmentation

The vulnerability segmentation degree was assessed as  $P_{50,\text{leaf}}$  –  $P_{50,rem}$ . The larger the positive difference was between  $P_{50,leaf}$  and P50,stem, the larger was the segmentation degree (Tyree & Zimmermann, 2002). We therefore refer to positive segmentation when leaves are more vulnerable than the stem and to negative segmentation if the opposite case occurs. The two measures of embolism resistance for leaves and stems were assessed with two different methods: one was based on optical measurements, and one was based on hydraulic measurements. Whereas the optical method directly quantifies the amount of embolism (as the percentage of embolized particles), the flow-centrifuge method quantifies loss of hydraulic conductivity (PLC, %), and thus quantifies embolism indirectly. The validity of our  $P_{50,\text{stem}}$  measurements has been discussed by Ziegler et al. (2019). However, Brodribb et al. (2016b) found a strong linear relationship between a hydraulic  $P_{50,\text{leaf}}$  and an optical  $P_{50,\text{leaf}}$ . Brodribb *et al.* (2017) also found a strong linear relationship for stem measurements between a hydraulic  $P_{50,\text{stem}}$  based on the flow-centrifugation technique and an optical  $P_{50,stem}$ . These results confirm that cumulated 'embolized pixels' can be compared to the cumulated loss of hydraulic conductivity. Moreover, all our vulnerability curves were S-shaped, indicating that most of the embolism events occurred abruptly, in a small range of water potential, thereby indicating that most of the loss of conductivity should have also occurred within this water potential range.

#### Assessment of hydraulic segmentation

Most previous studies investigating hydraulic segmentation are based on hydraulic flow measurements (Zimmermann, 1978; Tyree *et al.*, 1991; Tyree & Alexander, 1993; Joyce & Steiner, 1995; Nardini & Pitt, 1999; Cruiziat *et al.*, 2002; Eisner *et al.*, 2002). Here, the hydraulic segmentation was assessed only based on anatomical measurements, allowing for the calculation of theoretical hydraulic conductivities based on the Hagen–Poiseuille law (Tyree & Zimmermann, 2002). To our knowledge, only a few studies have directly compared theoretical hydraulic conductivity with flow-measurement conductivity in flowering plants for stems (Hargrave et al., 1994; Choat et al., 2007) or leaves (Martre et al., 2000). Theoretical conductivity is logically overestimated, because end-wall resistivity in vessels due to pits and perforations is not taken into account in theoretical calculations (Sperry et al., 2005; Wheeler et al., 2005; Christman & Sperry, 2010). However, the relationship between conductivities measured with the two techniques is strong ( $R^2 = 0.74$  in Hargrave et al. (1994), Martre et al. (2000), and Choat et al. (2007)), conserving the ranking of species or samples and supporting the use of theoretical conductivity in a relative, comparative perspective. Moreover, several studies suggest that the end-wall resistivity is strongly correlated to lumen resistivity or vessel diameter (0.82  $< R^2 < 0.96$ ; Sperry *et al.*, 2005; Wheeler *et al.*, 2005; Hacke et al., 2006; Christman & Sperry, 2010). This further supports the interest of vessel diameter for estimating hydraulic conductivity without taking into account end-wall resistivity, and above all the use of theoretical hydraulic conductivity to compare species.

Hydraulic segmentation was assessed at the shoot level. In the context of our study, a shoot was defined as a single unbranched stem supporting the longest succession of intact leaves, with no missing leaves at nodes between the youngest and the oldest leaf (Fig. 1). Hydraulic segmentation was assessed as the difference between the leaf-specific conductivity (LSC, kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup>) at the shoot level (LSC<sub>shoot</sub>) and the LSC at the leaf level (LSC<sub>leaf</sub>), following that:

$$E = \frac{F}{A_L}$$
 and  $LSC = \frac{K_{tb}}{A_L}$ 

(*E*, evapotranspiration, kg; *F*, water flux, kg s<sup>-1</sup>;  $A_L$ , downstream leaf area, m<sup>2</sup>;  $K_{th}$ , hydraulic conductivity, kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup>). Thus:

$$\frac{F}{K_{\rm th}} = \frac{E}{\rm LSC}$$
 and  $K_{\rm th} = \frac{\rm dP}{\rm dx}$ 

(dP/dx, pressure gradient, MPa m<sup>-1</sup>; Tyree & Ewers, 1991). Thus, LSC is inversely proportional to the pressure drop between the base of the considered organ and the site of transpiration. Finally, the difference between  $LSC_{shoot}$  and  $LSC_{leaf}$  (i.e. the hydraulic segmentation degree,  $LSC_{shoot} - LSC_{leaf}$ ) is the theoretical pressure drop between the shoot and the leaf, or the segmentation degree. We had seven compound-leaved species in our data set. Thus, to be rigorous, we considered that  $LSC_{leaf}$  for compound-leaved species was the LSC of a single leaflet (Fig. 1).

For measurements of stem embolism resistance, we sampled leafy shoots of branches used for the CAVI1000 measurements. In the laboratory, we selected the most appropriate shoot defined as vigorous, with undamaged leaves, no inflorescences or infructescences, and with the longest succession of leaves for a given species. We cut all leaves and measured their total area with a scanner and the IMAGEJ program. We measured the length of the shoot stem with a ruler. We sampled a 1 cm long stem section of the shoot stem at the very base of the shoot for anatomical observations. We sampled the petiole of a leaf situated in the



**Fig. 1** Schematic representation of the sampling procedure for assessing the hydraulic segmentation degree. For simple-leaved species (left panel), leaf-specific conductivity (LSC) at the leaf level ( $LSC_{leaf}$ ) is the theoretical hydraulic conductivity of the petiole divided by the area of the entire supplied simple leaf (in red, right panel). For compound-leaved species (right panel),  $LSC_{leaf}$  is the theoretical hydraulic conductivity of the petiolule divided by the area of supplied leaflet (in red, right panel). In both cases, LSC at the shoot level ( $LSC_{shoot}$ ) is the theoretical hydraulic conductivity at the base of the shoot divided by the entire shoot leaf area. In both cases (simple leaf and compound leaf), the hydraulic segmentation degree is assessed as the difference between  $LSC_{shoot}$  and  $LSC_{leaf}$ .

middle part of the shoot, and we measured the length of this leaf with a ruler. For compound-leaved species, we sampled the petiolule of a leaflet situated at the middle part of the leaf. Petioles and petiolules were sampled at the leaf and leaflet basis, respectively. For compound leaves, we measured the length of this leaflet, plus the rachis path length between the sampled leaflet and the leaf petiole.

We measured one shoot for each individual tree. For each shoot we included a stem sample from the base of the shoot and a petiole sample (or a petiolule sample for compound leaves). From these samples, we made 8 µm thickness cross-sections by paying attention to having a complete section of the xylem. Samples were embedded in paraffin, and cross-sections were realized with a rotary microtome (Microm HM 355 S; Thermo Fisher Scientific, Waltham, MA, USA). Anatomical sections were coloured with a FASGA (safranin + Alcian blue) staining (Tolivia & Tolivia, 1987). Images of each cross-section were digitized with an optical microscope (Olympus BX60; Olympus Corp., Tokyo, Japan) with ×1000 magnification and a Canon EOS 500D camera (lens Olympus U-TVI-X; F 0.0; ISO 100; speed 1/25<sup>th</sup>s). We measured the number of vessels and the crosssectional area of each vessel with a light microscope and IMAGEJ. Knowing the number of vessels and vessel surface area, we calculated the theoretical hydraulic conductivity  $K_{\rm th}$  of the stem at the base of the shoot and of the petiole or petiolule. Stem and petiole Kth served to finally calculate LSCshoot and LSCleaf, respectively, as Kth divided by the shoot leaf area and individual leaf area (or leaflet area for compound leaves), respectively.

As the vessel diameter increases axially, basipetally, and predictably from leaf tip to the trunk base to mitigate the hydrodynamic resistance with the hydraulic path length (West *et al.*, 1999; Olson *et al.*, 2014; Lechthaler *et al.*, 2019a), we standardized the effect on vessel diameter for this path length by dividing LSC<sub>shoot</sub> by the stem length between the apex and the base of the shoot and by dividing  $LSC_{leaf}$  by leaf length (Lechthaler *et al.*, 2019b). Finally, the standardized LSC segmentation is our estimate of stem–leaf hydraulic segmentation throughout this study.

#### Statistical analyses

All statistical analyses were performed with the R software (http://CRAN-R-project.org). For the trait-by-trait analysis, we used standardized major axis regression (Warton *et al.*, 2006), which allows measurement of the error on both *x*-axis and *y*-axis (Harvey & Pagel, 1991), with the R package SMATR (Falster *et al.*, 2006). We analysed the average per species. We used comparison tests to compare all traits between the shoot or stem and the leaf across all species. Comparison tests were conducted with Student, Welch, or Mann–Whitney–Wilcoxon tests, depending on the parameters of the samples (effectives, normality of distribution, variance).

#### Results

The  $P_{50,leaf}$  varied from -4.61 to -1.35 MPa, and the  $P_{50,stem}$  varied from -7.63 to -1.83 MPa across the species studied (Fig. S1). The mean  $P_{50,leaf}$  across species was  $-3.29 \pm 0.90$  MPa, the mean  $P_{50,stem}$  across species was  $-3.87 \pm 1.58$  MPa.  $P_{50,leaf}$  and  $P_{50,stem}$  were on average not significantly different across species (Fig. 2a).  $P_{50,leaf}$  was positively related to  $P_{50,stem}$  (Fig. 2b), with a slope of 0.31, significantly lower than 1 (P < 0.001). One species (*Iryanthera sagotiana*) was outlying from this trend with the highest  $P_{50,leaf}$  (less embolism resistant; -1.35 MPa). Nine species showed a positive ( $P_{50,leaf} > P_{50,stem}$ ) vulnerability segmentation based on confidence intervals (Fig. S2; *Chrysophyllum sanguinolentum, Eperua grandiflora, Eschweilera sagotiana, Goupia glabra, Gustavia hexapetala, Manilkara bidendata, Pradosia cochlearia, Virola michelii*). Four species showed a negative ( $P_{50}$ ,



Fig. 2 Vulnerability segmentation at the stem-leaf transition for 21 species in French Guiana. (a) Comparison between P<sub>50,leaf</sub> and  $P_{50,\text{stem}}$  across all species. (b)  $P_{50,\text{leaf}}$ according to  $P_{50,\text{stem}}$  in comparison with the 1 : 1 line. H0 (b = 1) is the null hypothesis for the slope being not different from 1. (c) Comparison between P<sub>88,leaf</sub> and P<sub>88,stem</sub> across all species. (d)  $P_{88,\text{leaf}}$  according to  $P_{88,\text{leaf}}$ stem in comparison with the 1:1 line. The slope is significantly different from 1.  $P_{50}$  and  $P_{88}$ : water potential inducing 50% and 88% loss of conductance, respectively. Colours indicate botanical families (green, Burseraceae; brown, Chrysobalanaceae; orange, Clusiaceae; red, Fabaceae; cyan, Goupiaceae; grey, Lecythidaceae; pink, Myristicaceae; yellow, Peraceae; blue, Sapotaceae; purple, Vochysiaceae). SDs are plotted around the mean for each species.

 $l_{eaf} < P_{50,stem}$ ) vulnerability segmentation based on confidence intervals (Fig. S2; *Bocoa prouacensis, Dicorynia guianensis, Protium opacum, Qualea rosea*). Six species showed no ( $P_{50,leaf} = P_{50,stem}$ ) vulnerability segmentation based on confidence intervals (Fig. S2; *Chaetocarpus schomburgkianus, Eperua falcata, Lecythis poiteauii, Licania membranacea, Symphonia* sp1, *Tachigali melinonii*). The three other species were based on only one individual measured, with two species with positive vulnerability segmentation (Fig. S2; *I. sagotiana, Protium sagotianum*) and one species with negative vulnerability segmentation (Fig. S2; *Protium subserratum*).

The  $P_{88,\text{leaf}}$  varied from -5.52 to -1.65 MPa, and the  $P_{88,\text{stem}}$  varied from -10.45 to -2.50 MPa across species. The mean  $P_{88,\text{leaf}}$  across species was  $-3.90 \pm 1.06$  MPa, the mean  $P_{88,\text{stem}}$  across species was  $-4.98 \pm 2.03$  MPa.  $P_{88,\text{leaf}}$  was on average significantly different from  $P_{88,\text{stem}}$  across species (Fig. 2c).  $P_{88,\text{leaf}}$  was positively related to  $P_{88,\text{stem}}$  (Fig. 2d), with a slope of 0.27, significantly lower than 1 (P < 0.001).

The mean LSC<sub>leaf</sub> across species was  $(1.24 \pm 1.55) \times 10^{-4}$  kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup>, the mean LSC<sub>shoot</sub> across species was  $(0.342 \pm 2.50) \times 10^{-4}$  kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup>. The mean standardized LSC<sub>leaf</sub> across species was  $(1.01 \pm 1.20) \times 10^{-5}$  kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup>, and the mean standardized LSC<sub>shoot</sub> across species was  $(3.02 \pm 2.94) \times 10^{-5}$  kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup> (Fig. 3a). The standardized LSC<sub>leaf</sub> was on average lower than the LSC<sub>shoot</sub> across species (Fig. 3a). The standardized LSC<sub>leaf</sub>

was positively related to the standardized LSC<sub>shoot</sub> (Fig. 3b), with a slope significantly lower than 1 (P < 0.001). One species (*C. schomburgkianus*) was highly outlying from this trend with the highest standardized LSC<sub>leaf</sub> (5.71 × 10<sup>-5</sup> kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup>). One species displayed a negative LSC segmentation (*I. sagotiana*; Fig. 3b).

The  $P_{50}$  vulnerability segmentation ( $P_{50,\text{leaf}} - P_{50,\text{stem}}$ ) ranged from -1.58 to 3.18 MPa across species, with a mean of 0.65 ± 1.40 MPa (Fig. S2). The  $P_{88}$  vulnerability segmentation ( $P_{88,\text{leaf}} - P_{88,\text{stem}}$ ) ranged from -1.41 to 5.16 MPa across species, with a mean of  $1.24 \pm 1.77$  MPa. The standardized LSC segmentation ranged from  $0.23 \times 10^{-5}$  to  $7.63 \times 10^{-5}$  kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup> across species, with a mean of  $(2.01 \pm 2.21) \times 10^{-5}$  kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup>. The standardized LSC segmentation was weakly and positively related to the  $P_{50}$  and  $P_{88}$  vulnerability segmentation (Fig. 4a,b).

#### Discussion

#### Stem-leaf vulnerability segmentation

A third of the tropical tree species studied here (eight species) displayed a positive vulnerability segmentation (i.e. the leaf xylem being more vulnerable to embolism than that of the stems) and showed a very broad range of values up to a maximum degree of 3 MPa between leaf and stem xylem (Fig. 4a), contrary to earlier



**Fig. 3** Hydraulic segmentation at the stem–leaf transition for 21 species in French Guiana. (a) Comparison between standardized leaf-specific conductivity (LSC) at the leaf level (LSC<sub>leaf</sub>) and shoot level (LSC<sub>shoot</sub>) across all species. (b) Standardized LSC<sub>leaf</sub> according to standardized LSC<sub>shoot</sub> in comparison with the 1 : 1 line. The slope is significantly different from 1. Standardization refers to the division of LSC by the conductive path length. Colours indicate botanical families (green, Burseraceae; brown, Chrysobalanaceae; orange, Clusiaceae; red, Fabaceae; cyan, Goupiaceae; grey, Lecythidaceae; pink, Myristicaceae; yellow, Peraceae; blue, Sapotaceae; purple, Vochysiaceae). SDs are plotted around the mean for each species.



**Fig. 4** (a)  $P_{50}$  segmentation degree and (b)  $P_{88}$  segmentation degree according to the standardized leaf-specific conductivity (LSC) segmentation degree.  $P_{50}$  and  $P_{88}$  are the water potentials inducing 50% and 88% loss of conductance, respectively. Standardization refers to the division of LSC by the conductive path length. Colours indicate botanical families (green, Burseraceae; brown, Chrysobalanaceae; orange, Clusiaceae; red, Fabaceae; cyan, Goupiaceae; grey, Lecythidaceae; pink, Myristicaceae; yellow, Peraceae; blue, Sapotaceae; purple, Vochysiaceae). SDs are plotted around the mean for each species.

suggestions (Zhu *et al.*, 2016). This segmentation degree is as large as has been reported for *Eucalyptus* species, which are known to be drought-affiliated species (Blackman *et al.*, 2019b). Our results evidenced that some species exhibit vulnerability segmentation and others do not. This is in agreement with past studies showing positively segmented (Hao *et al.*, 2008; Chen *et al.*, 2009; Johnson *et al.*, 2011, 2016; Bucci *et al.*, 2012; Nolf *et al.*, 2015; Charrier *et al.*, 2016; Hochberg *et al.*, 2016; Rodriguez-Dominguez *et al.*, 2018; Skelton *et al.*, 2018; Losso *et al.*, 2019), negatively segmented (Klepsch *et al.*, 2018), or unsegmented species (Chen *et al.*, 2009; Nolf *et al.*, 2015; Skelton *et al.*, 2017, 2018; Klepsch *et al.*, 2018; Losso *et al.*, 2019). Although the anatomical, ecological, and physiological mechanisms that underlie this variation remain poorly understood, the available evidence seems to indicate that vulnerability segmentation represents a functional trait in its own right, with a continuum across species, and with a strong variation across species, even at a local scale. This also evidences that some species have evolved vulnerability segmentation, whereas some others have not (no or negative vulnerability segmentation).

The case of the four species that are negatively segmented is of interest. For these species, our results suggest that leaf vein xylem is more resistant to embolism than stem xylem is. Theoretically, when leaves incur hydraulic failure, this means that the upstream system, including stem xylem, has already become highly prone to embolism propagation. Therefore, these species seem not to protect perennial organs as positively segmented species do, assuming that stem embolism occurs in the field and that survival and growth of apical and lateral meristems are affected by embolized stem conduits. The negative vulnerability segmentation is probably due to high embolism resistance of leaf xylem, with a mean of -3.79 MPa across species exhibiting a negative segmentation degree, which is close to the mean  $P_{50,stem}$  of -3.93 MPa across the all trees measured (Ziegler *et al.*, 2019). Thus, these species with a negative vulnerability segmentation are not particularly vulnerable to embolism at all. It can also be hypothesized that this low  $P_{50,leaf}$  is enough to cope with the local seasonal water deficit, or that these species rely on other drought resistance mechanisms, such as low leaf minimum conductance.

Recently, Blackman et al. (2019b) demonstrated for eight *Eucalyptus* species that the vulnerability segmentation degree was strongly and linearly related to the time of plant dehydration in a glasshouse experiment. The results of Blackman et al. (2019b) suggest that vulnerability segmentation has a functional significance as a drought resistance mechanism. Moreover, several facts support the importance of vulnerability segmentation for drought resistance (i.e. maximization of dehydration time). First, embolism should theoretically impede leaf water supply (Hochberg et al., 2016), reducing leaf conductance, residual water loss, and a water potential drop of the plant. Second, some studies suggest that vulnerability segmentation could be associated with leaf shedding (Tyree et al., 1993; Hochberg et al., 2017) and that leaf shedding may prevent the reduction of a plant's water potential (Wolfe et al., 2016). Therefore, it would be useful in future research to investigate the functional value of segmentation by directly quantifying the branch and whole-plant plant dehydration time along a gradient of vulnerability segmentation.

With respect to the potential relationship between vulnerability segmentation and deciduousness (Tyree et al., 1993; Hochberg et al., 2017), there is no evidence that deciduousness is associated with the onset of the dry season for Guianese rainforest trees (Table 1; Loubry, 1994a,b), and thus that deciduousness would be a drought resistance mechanism in this context. This further suggests that vulnerability segmentation is not a 'routine trigger' of leaf shedding for Guianese trees. It would also be interesting to know the temporal frequency of leaf xylem embolism in the field, and whether or not vulnerability segmentation actually affects the lifespan of tropical rainforest tree leaves. Leaves could also represent large C investment, especially for leaves with high leaf lifespan (Wright et al., 2004; Reich, 2014). This also questions the relevance of vulnerability segmentation for species with a high leaf lifespan, and how vulnerability is related to the leaf economics spectrum (Wright et al., 2004).

#### Stem-leaf hydraulic segmentation

We found that most species were positively hydraulically segmented based on our theoretical-LSC-based approach, displaying a four-fold variation. These results are consistent with previous studies following the hypothesis of Zimmermann (1983) on hydraulic segmentation (Zimmermann, 1978; Tyree *et al.*, 1991; Tyree & Alexander, 1993; Joyce & Steiner, 1995; Nardini & Pitt, 1999; Cruiziat *et al.*, 2002; Eisner *et al.*, 2002). Surprisingly, we found that one species (*I. sagotiana*) exhibited negative segmentation, with the stem being more hydraulically resistant than leaves. However, this finding of negative segmentation seems really idiosyncratic, or even incorrect, since a negative segmentation seems highly unlikely. It would mean that a plant has invested C in a stem structure that is hydraulically inefficient, with water hardly reaching leaves. However, our study strongly suggests that the hydraulic segmentation degree can be considered as a functional trait in its own right, with a strong variation across species at a local scale. Our results may inspire future work on the anatomical determinants and physiological effects of hydraulic segmentation across species based on hydraulic measurements such as *in situ* comparison of water potentials between leaves and stems, or comparison of flow measurements of leaf-specific conductance between leaf and shoot levels.

Recent findings challenge hydraulic segmentation as a mechanism for determining a safety-valve pattern as hypothesized by Zimmermann (1984). Martin-StPaul et al. (2017) demonstrated that stomatal closure occurs before stem embolism, which may also hold for leaf xylem embolism (Hochberg et al., 2017; Creek et al., 2020). Stomatal closure should theoretically lead to equilibration; that is, homogenization of water potentials across a branch or a plant, reducing any hydraulic segmentation pattern. However, during periods of water stress, residual water losses are never zero, even if stomata are closed, due to both leaky stomata and leaf cuticular conductance. which may drive the leaf minimum conductance (Duursma et al., 2019). The question, therefore, is whether or not residual transpiration could maintain a significant water potential gradient between the leaf and the stem, which may lead to embolism formation in leaves first. In a recent meta-analysis, Duursma et al. (2019) showed that the mean leaf minimum conductance across 221 species is c. 4.9 mmol  $m^{-2} s^{-1}$ , which is far lower than the 'routine' or maximum stomatal conductance by hundreds of millimoles per square metre per second (Brodribb & Holbrook, 2003; Klein, 2014). However, hydraulic segmentation could also be seen as a strategy selected by species that are unable to strongly reduce their leaf minimum conductance and leaf residual water losses. Stem flow measurements at stomatal closure, or measurements of stem and leaf water potentials at stomatal closure, would be useful to properly quantify potential hydraulic segmentation at stomatal closure.

If stomatal closure occurs before embolism propagation occurs in the xylem (Hochberg *et al.*, 2017; Martin-StPaul *et al.*, 2017; Creek *et al.*, 2020), cancelling any safety-valve pattern, the stem–leaf hydraulic segmentation currently suffers from a misinterpretation. Indeed, it can be argued that hydraulic segmentation allows for an equi-resistance principle within the tree. The whole-tree hydraulic system is formed of multiple root-to-leaf pathways, with different path lengths (e.g. due to different leaf heights), and therefore potentially different axial hydraulic resistances (Bettiati *et al.*, 2012). If we assume that all leaves perform at the same metabolic rate, they should be equally supplied with water (Pittermann *et al.*, 2018; Echeverría *et al.*, 2019). Therefore, root-to-leaf pathways with a variable length in a tree should be more or less equally resistant, with no leaves being poorly supplied. As stem–leaf hydraulic segmentation implies that most of the total xylem hydraulic resistance is concentrated in leaves, this also favours water movement along all root-stem xylem pathways. In other words, the bottleneck at the stem-leaf transition will allow root-stem pathways upstream of the bottleneck to be filled with water. This equi-resistance interpretation was already sensed by Zimmermann on an early study of LSC distribution (Zimmermann, 1978, p. 2294): 'Resistances are higher along the path of water from roots to lower lateral leaves than along the path to the leaves at the top of the stem. This should at least partly compensate for the disadvantage of height to which water has to be brought to the top leaves'.

From the equi-resistance point of view, the stem-leaf hydraulic segmentation is just another perspective on the welldescribed universal axial vessel-widening pattern (Olson et al., 2014, 2018). Indeed, theoretical models and empirical data confirm that the axial basipetal widening in vessel diameter reduces the increase of hydrodynamic resistance with conductive path length (West et al., 1999; Becker et al., 2000; Enquist, 2003; Anfodillo et al., 2013; Olson et al., 2014). However, several studies demonstrate that the vessel widening rate is not constant along a tree, increasing towards leaves, and being the highest within leaves (Petit et al., 2008; Bettiati et al., 2012; Lechthaler et al., 2019a; Levionnois et al., 2020). This pattern of increasing widening rate towards leaves is in agreement with the idea that most of the total hydraulic resistance is concentrated in leaves (Sack & Holbrook, 2006). Moreover, the axial vessel widening pattern has already been investigated within the equi-resistance perspective (Bettiati et al., 2012). Therefore, the measurement of the stem-leaf segmentation would just be a discretization of the continuous axial vessel widening pattern. Along the continuous axial vessel widening, there is certainly a superimposition of discrete rupture in the variation of vessel diameter and LSC at branch and leaf junctions (Isebrands & Larson, 1977; Larson & Isebrands, 1978; Zimmermann, 1978, 1983). In this regard, Zimmermann's view on hydraulic segmentation was tightly related to the idea of 'hydraulic constrictions' at branch and leaf junctions. The hydraulic constriction at the branch-leaf junction, particularly around the petiole, has also been viewed to allow or facilitate leaf abscission. Indeed, leaf shedding has already been shown to prevent water loss and a rapid decrease in plant water potential, which contributes to the stem hydraulic integrity (Wolfe et al., 2016; Hochberg et al., 2017), but not demonstrated for tropical rainforests. Then, drought-induced leaf shedding could be related to leaf abscission, which is associated with hydraulic constriction (André et al., 1999). The implication of the petiole structure for leaf shedding could be investigated in future experiments.

#### Coordination among segmentations

Hydraulic and vulnerability segmentations were positively related, even if the relationship was weak. However, this may not be unusual based on the number of traits included, their degree of integration, and their variability, which drastically increases the degree of freedom of errors (LSC<sub>leaf</sub>, LSC<sub>shoot</sub>,  $P_{50,leaf}$ ,  $P_{50}$ , stem). If hydraulic segmentation functions as safety-valve pattern,

coordination between hydraulic and vulnerability segmentation supports the hypothesis that the safety-valve pattern is under strong selection, favouring at the same time both hydraulic segmentation and vulnerability segmentation. It is remarkable to see that species highly resistant to stem embolism are also species with high hydraulic and vulnerability segmentation, potentially maximizing their dehydration time (Blackman *et al.*, 2016, 2019b), and suggesting a strong selection of drought-related traits for these species. These findings further suggest that hydraulic segmentation and vulnerability segmentation are mechanisms favouring drought resistance.

Our understanding of how both types of segmentation are coordinated would also benefit from hydraulic (instead of theoretical) measurements to assess hydraulic segmentation, because intervessel pits provide considerable hydraulic resistance (Hacke et al., 2006; Sperry et al., 2006) and strongly control drought-induced embolism (Lens et al., 2011; Li et al., 2016; Kaack et al., 2019). Therefore the variation in pit characteristics between leaves and stems should be a key parameter in the relation between hydraulic segmentation and vulnerability segmentation (Klepsch et al., 2018; Kotowska et al., 2020). Future research directions should address how hydraulic and vulnerability segmentation are related to other well-known mechanisms that affect the drought-resistance spectrum (Bartlett et al., 2016; Pivovaroff et al., 2016; Santiago et al., 2018), such as minimum leaf conductance (Duursma et al., 2019), hydraulic safety margin (Choat et al., 2012), stomatal closure (Martin-StPaul et al., 2017), capacitance (Gleason et al., 2014), and rooting depth (Brum et al., 2017).

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#### **Author contributions**

SL, PH and SJ conceived and designed the study; SL, EC, CZ, SC, CStahl, CG and PH collected field samples; SL, PH and CSalmon performed anatomical sections and image analyses; SL and EC performed leaf embolism resistance measurements; CZ and SD managed stem embolism resistance measurements; SL and EC performed data analysis; SL, PH and SJ wrote the manuscript; all authors discussed the results and contributed valuable comments on the manuscript.

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### Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Leaf optical vulnerability curves for all 21 species showing the percentage of observed embolism (%) in the leaf xylem according to xylem water potential (MPa).

Fig. S2 Mean optical vulnerability curves for leaves and mean hydraulic vulnerability curves for stems for all 21 species showing the percentage of observed embolism or loss of conductivity (%) in the xylem according to xylem water potential (MPa).

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