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The hydraulic architecture of the plants:
study of the allometric relations in stem and leaves.

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L'architettura idraulica nelle piante:
Studio delle relazioni allometriche di fusto e foglie.

Coordinatore: Prof. Davide Matteo Pettenella
Supervisore: Prof. Tommaso Anfodillo

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*"Everything you can imagine,
nature has already created."*

Albert Einstein

"La revolución es la armonía de la forma y el color,
y todo está y se mueve, bajo una misma ley
–la vida–."

Frida Kahlo

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SUMMARY

The xylem in plants is formed by interconnected dead cells that allow the flow of water from the roots to the leaves. The ascent of sap is mainly passive and it is driven by water evaporation from the mesophyll cell walls in the leaf. The water evaporation generates capillary suction on the menisci at the micro-porous of cell walls, causing negative hydrostatic pressure that propagates down the water column in the xylem. Due to plants grow in height the length of the hydraulic path increases progressively posing the question whether the hydraulic resistance increases accordingly. There is evidence that plants have evolved xylem structures that compensate the possible increase of the hydraulic resistance imposed by path length, namely the tip-to-base conduits widening. Conduits widening has been reported in several species, both angiosperms and conifers, showing that the degree of widening from tip to the base of the stem is very similar among species, or in other words, that plants converge towards a universal xylem structure. Nevertheless, several points on the hydraulic architecture of plants remain to be elucidated.

A largely debated point is whether xylem anatomical traits (e.g. the absolute cell size) change with climatic conditions. Moreover, whether and how the conduits widening in the stem may affect the xylem anatomy of the leaf is still not fully understood. This Ph.D. project aims to widen our understanding of the allometric relations of leaves and stem xylem, considering how the environmental conditions and the height of the plant affect the hydraulic architecture of the water transport system.

A methodological study (**Study 1**) has been performed on the xylem tissue of stems of Acacia trees grown in different water availability conditions. The main result was that, once the anatomical data were standardized for the tree height, the hydraulic architecture of the xylem did not change in relation to the environmental conditions.

Two studies have been performed on the hydraulic architecture of leaves. The main focus was on the anatomical traits of the xylem conduits in relation to the leaf dimensions and/or the position in the tree crown (height from the base of the stem). The main results were that the xylem traits scaled with the leaf area independently by the position in the crown (**Study 2**). A fine analysis of the leaf midrib (i.e. major leaf vein) has shown a rigid hydraulic architecture and tissues coordination (**Study 3**) that was well predicted by the distance from the leaf tip.

Both studies showed that the dimensions of the terminal veins were conserved among leaves and within leaf suggesting that the hydraulic architecture of the xylem in the leaf evolved in a way to guarantee an equal distribution of the hydraulic resistances (and thus of the water) among leaves and within the leaf lamina.

Finally, we implemented the anatomical data of both stem and leaf into a hydraulic model to assess the distribution of resistances along the hydraulic path to evaluate how the anatomy of the transport system affects the physiology of the entire tree (**Study 4**).

This thesis has highlighted that the path length (i.e. the height of the plant and the dimensions of the leaf) is the main factor affecting the hydraulic architecture of the tree. The conduit dimension in both stem and leaf are determined by the distance from the terminal parts, stem apex or leaf tip respectively. Climatic conditions resulted to have marginal (non-significant) effect on the stem anatomical traits. In the leaf, the dimensions of the xylem conduits are statistically invariant with changes in plant size. This rigid hydraulic architecture of the tree, from the stem to the leaf, allows minimizing the effect of the path length on the hydraulic resistance, confining nearly the whole gradient of water potential within the leaves.

SOMMARIO

Lo xilema nelle piante è formato da cellule morte interconnesse che consentono il flusso di acqua dalle radici alle foglie. L'ascesa della linfa è principalmente passiva ed è guidata dall'evaporazione dell'acqua dalle pareti cellulari del mesofillo nella foglia. L'evaporazione dell'acqua genera un'aspirazione capillare sui menischi a livello dei micro-pori delle pareti cellulari, causando una pressione idrostatica negativa che si propaga lungo la colonna d'acqua nello xilema. A causa dell'aumento in altezza delle piante, la lunghezza del percorso idrico aumenta progressivamente ponendo la domanda se la resistenza idraulica aumenta di conseguenza. Vi è evidenza che le piante hanno evoluto strutture xilematiche che compensano il possibile aumento della resistenza idraulica imposta dall'aumento della lunghezza del percorso, come ad esempio l'allargamento dei condotti dalla punta alla base. L'allargamento dei condotti è stato osservato in diverse specie, sia angiosperme sia conifere, dimostrando che il grado di allargamento dalla punta alla base dello stelo è molto simile tra le specie, o in altre parole, che le piante convergono verso una struttura xilema universale. Tuttavia, restano da chiarire diversi punti sull'architettura idraulica delle piante.

Un punto largamente dibattuto è se tratti anatomici dello xilema (ad esempio la dimensione assoluta delle cellule) cambiano con le condizioni climatiche. Inoltre, se e come i condotti che si allargano nello stelo possano influenzare l'anatomia dello xilema della foglia non è ancora completamente compreso. Il progetto di questo dottorato mira ad ampliare la nostra comprensione delle relazioni allometriche nello xilema delle foglie e del fusto, considerando come le condizioni ambientali e l'altezza della pianta possano influenzare l'architettura idraulica del sistema di trasporto dell'acqua.

Uno studio metodologico (**Studio 1**) è stato eseguito sul tessuto xilematico di fusti di alberi di acacia cresciuti in diverse condizioni di disponibilità idrica. Il risultato principale è stato che, una volta che i dati anatomici sono stati standardizzati per l'altezza dell'albero, l'architettura idraulica dello xilema non è cambiata in relazione alle condizioni ambientali.

Sono stati eseguiti due studi sull'architettura idraulica delle foglie. L'obiettivo principale degli studi riguardava i tratti anatomici dei condotti dello xilema in relazione alle dimensioni della foglia e / o alla posizione nella chioma dell'albero (altezza dalla base del fusto). Dai risultati si evince che i tratti dello xilema si ridimensionano in base all'area fogliare indipendentemente

dalla posizione nella chioma (**Studio 2**). Un'analisi fine della nervatura principale della foglia ha mostrato una rigida architettura idraulica e la coordinazione dei tessuti (**Studio 3**), ben predetta dalla distanza dalla punta della foglia. Entrambi gli studi hanno dimostrato che le dimensioni delle vene terminali sono conservate tra le foglie e all'interno della stessa foglia, suggerendo che l'architettura idraulica dello xilema si è evoluta in modo da garantire distribuzione omogenea delle resistenze idrauliche (e quindi dell'acqua) tra le foglie e lungo la lamina fogliare.

Infine, abbiamo implementato i dati anatomici di fusto e foglia in un modello idraulico per stimare la distribuzione delle resistenze lungo il percorso idraulico per valutare in che modo l'anatomia del sistema di trasporto influisca sulla fisiologia dell'intero albero (**Studio 4**).

Questa tesi ha evidenziato che la lunghezza del percorso (vale a dire l'altezza della pianta e le dimensioni della foglia) è il fattore principale che influenza l'architettura idraulica dell'albero. La dimensione del condotto sia nel fusto che nella foglia è determinata dalla distanza dalle parti terminali, rispettivamente l'apice del fusto o la punta della foglia. Le condizioni climatiche risultano avere un effetto marginale (non significativo) sui tratti anatomici del fusto e nella foglia, le dimensioni dei condotti dello xilema sono statisticamente indipendenti rispetto alle variazioni nelle dimensioni della pianta. Questa rigida architettura idraulica dell'albero, dal fusto alla foglia, consente di minimizzare l'effetto della lunghezza del percorso sulla resistenza idraulica, confinando quasi l'intero gradiente del potenziale idrico all'interno delle foglie.

INTRODUCTION

The water transport is a vital process common to all plants. In trees, the water is transported mostly passively throughout the xylem, a tissue composed mainly of dead cells that functions as conduits allowing the water to flow from the roots to the leaves. The water flows following a water potential gradient, from less negative (in the soil) to more negative (in the atmosphere), and the flow is inversely proportional to the hydraulic resistance of the medium passed through, in this case, the xylem tissue. The evolution of height in plants has involved mechanisms that compensate the hydraulic resistance imposed by increasing path length, such as the xylem conduit widening from the tip to the base of the stem.

Intrinsic patterns of xylem conduit variation within trees have been observed in the late 1800's by Sanio (Sanio, 1872) highlighting that xylem conduit diameter increases from the stem apex to base and from pith to bark. In recent years, these patterns have been theoretically predicted (West *et al.*, 1999), empirically investigated, and shown to relate strongly to the principles of optimization of water transport capacity and carbon costs of cell wall construction (Mencuccini *et al.*, 2007). The diameter of xylem conduits formed in a given year increases along the tree stem from the tree apex to the base following a highly conserved pattern, well described by a power function (i.e., the rate at which conduits widen is greater at the apex). The exponent of this axial trend varies little among species and environments (from 0.15 to 0.33) (Anfodillo *et al.*, 2013b; Olson *et al.*, 2014, 2018).

Despite the universality of this anatomical pattern, the variability in xylem conduits dimensions among trees is often associated with climatic conditions. The xylem conductivity scales with the diameter of the conduits to the fourth power (according to the Hagen-Poiseuille law: Tyree and Ewers 1991), and thus conduit size regulates the capacity of xylem to conduct water, and provide the canopy with the water needed for key plant functions. However, hydraulic efficiency (i.e., high conductance of wide conduits) is widely regarded as being a trade-off with safety (i.e. the vulnerability of the xylem system to embolism formation; Hacke and Sperry 2001). For instance, when less soil water is available, xylem vessels are exposed to lower water potentials and thus at greater risk of embolism (Cochard, 2006). This has led to the widespread notion that water availability could represent a strong

selective force, favouring trees with narrower vessels under drier conditions (Pfautsch *et al.*, 2016; Warwick *et al.*, 2017; Larter *et al.*, 2017).

The conduit size-tree height relationship implies that the meaning of "conduit size" within an evolutionary perspective can be evaluated only after having standardized conduit size (for a given sampling height) according to the distance from the apex (Rosell *et al.*, 2017). Such standardization (i.e. comparing conduits at the same tree height) allows removing seeming differences among trees/species of different heights, even across wide ranges of water availability (Olson *et al.*, 2014).

The predictions of the model proposed by West et al (West *et al.*, 1999, WBE model), seem largely in agreement with the empirical data, (Anfodillo *et al.*, 2006; Lintunen *et al.*, 2010; Olson & Rosell, 2012; Anfodillo *et al.*, 2013b; Olson *et al.*, 2014; Petit *et al.*, 2014), although some observations have been unexpected. In the work of Olson et al. (Olson *et al.*, 2014), conducted on 257 species, the widening of the conduits at the base of the stem was reported to scale with the tree height with an exponent of about 0.45 across. However, mean diameter of the vessels at the tips of the terminal twigs (i.e. stem apex), also increased in diameter with plant height, scaling with an exponent of about 0.23. Thus, as conduits widen with height at the stem base, they also do so at the stem tip. Taken together, these exponents resulted in an overall widening exponent is ca. 0.2 as predicted (Olson *et al.*, 2014). The widening observed in the twig tips is potentially explained as the result of selection favouring constant per-leaf area sapwood volume (Olson *et al.*, 2014; Givnish *et al.*, 2014; Drake *et al.*, 2015; Rosell *et al.*, 2017). As stem length increases, the widening of terminal conduits would lower total path-length resistance, potentially permitting constant construction costs of conduits per leaf over very substantial height increases (see Rosell *et al.*, 2017 for more detail). Whatever the cause of the apparently very widespread pattern of terminal twig conduit diameter increase with plant height (Olson *et al.*, 2014; Rosell & Olson, 2014), a relevant question is how leaf conduits contribute to achieving stem tip conduit widening (Coomes *et al.*, 2008). Specifically, it is not clear whether the pattern of terminal twig vessel widening with plant height affects the vein network of the leaves.

In the leaf, the conduits have been reported to change from the midrib (i.e. the principal vein) to the most terminal narrow veins (McCulloh *et al.*, 2004; Scoffoni *et al.*, 2012; Carvalho *et al.*, 2017).

The most common hydraulic models to predict the variation of conduits dimension in the leaf are based on a hierarchical vein pattern. The veins are grouped in orders: the 1st order veins that run from the base of the lamina to the leaf apex, the 2nd veins that branch from it, the 3rd and higher orders that decrease in size and form the reticulated network that transport the water close to the stomatal cavities (Hickey, 1973; Ellis *et al.*, 2009; Sack *et al.*, 2012). The anatomical traits are expected to change among vein orders, but to remain constant within the same order. Recent studies have highlighted how conduits size influences the susceptibility to embolism in leaf venations. Larger conduits resulted more vulnerable to embolism than narrower ones, suggesting a correlation between embolism resistance and vein orders (Brodribb *et al.*, 2016b,a; Scoffoni *et al.*, 2017b). However, the few studies considering the axial variation of conduit diameter along the major veins have reported significant changes in anatomical traits (Coomes *et al.*, 2008; Petit & Anfodillo, 2013), suggesting that the length of the vein, rather than the vein order, should be considered in leaf architectural models. Detailed information on the variation of the anatomical traits of the leaf venation would allow predicting the distribution of water potential, and thus, how embolism events might spread throughout the whole hydraulic network.

Understand the anatomical pattern of the hydraulic system, from the stem to the leaf, is of crucial importance to evaluate the functionality of the water transport system. The dimension and the degree of widening of the conduits have a pivotal effect on the distribution of the hydraulic resistances along the pathway. Thus, identifying hydraulic models that predicts the anatomical pattern of the water transport system might be helpful to investigate essential physiological processes, such as the distribution of the water potential gradient. In this work, we analysed the variation of the anatomical traits of the xylem in respect to the length of the path, namely the distance from the tree apex when considering the stem, or the distance from the leaf tip when considering the leaves. Finally, the anatomical data of both stem and leaves have been implemented into a hydraulic model to assess the role of the anatomical pattern in shaping the leaf-to-root gradient of xylem water potential.

AIMS & HYPOTHESES

The aim of this thesis is to better understand the allometric relations of stem and leaves xylem, considering how the environmental conditions and the height of the plant affect the hydraulic architecture of water transport system.

STEM	LEAF	
QUESTION 1:	**QUESTION 2:**	**QUESTION 3:**
In light of the anatomical variation with height, do climatic factors affect anatomical traits in the stem?	How tree height and leaf dimension affect anatomical traits in the leaf?	How the xylem anatomical traits vary along the midrib in relation with the distance from the leaf tip?
HYPOTHESIS 1:	**HYPOTHESIS 2:**	**HYPOTHESIS 3:**
Once the anatomical traits are standardized for the tree height, the effect of climatic factor would be insignificant.	The conduits are expected to increase with leaf area to guarantee a suitable water supply, and with tree height to compensate for the hydraulic resistance.	The conduits in the midrib widen from the leaf tip to the base of the petiole with a high degree of widening, coupled with a degree of conduit merging towards the petiole.
STUDY 1:	**STUDY 2:**	**STUDY 3:**
A standardization method to disentangle environmental information from axial trends of xylem anatomical traits	*Petiole base vessel diameter is predicted by leaf area, not by height in a tree*	*Axial anatomy of the leaf midrib provides new insights into the hydraulic architecture and embolism patterns of Acer pseudoplatanus leaves*

WHOLE TREE

QUESTION 4:

How the anatomical patterns influence the water potential gradient in the tree?

HYPOTHESIS 4:

The widening of xylem conduits minimizes the path length resistance and confines nearly the whole gradient of water potential within the leaves

STUDY 4:

Negligible tension gradient in long stems: leaves account for the majority of the whole hydraulic path's resistance

OUTLINE OF THE THESIS

This thesis consists of 4 articles coming out from my PhD activity.

The first paper, "A standardization method to disentangle environmental information from axial trends of xylem anatomical traits" has been published in Tree Physiology (Chapter 1). It is a Method paper that proposes a new standardization method for disentangling environmental information from axial trends of xylem anatomical traits, and thus fundamental to rigorously assessing effects of climate or other edaphic variables on xylem anatomy.

A second paper entitle "Invariant features of leaf vasculature with position in the crown", is a manuscript to be submitted soon to a peer-reviewed journal (Chapter 2). It aims to evaluate how the pattern of terminal twig vessel widening with plant height affects the vein network of the leaves. It assesses how the diameter of petiole conduits and of the terminal veins conduits change in relation with tree height and leaf dimension.
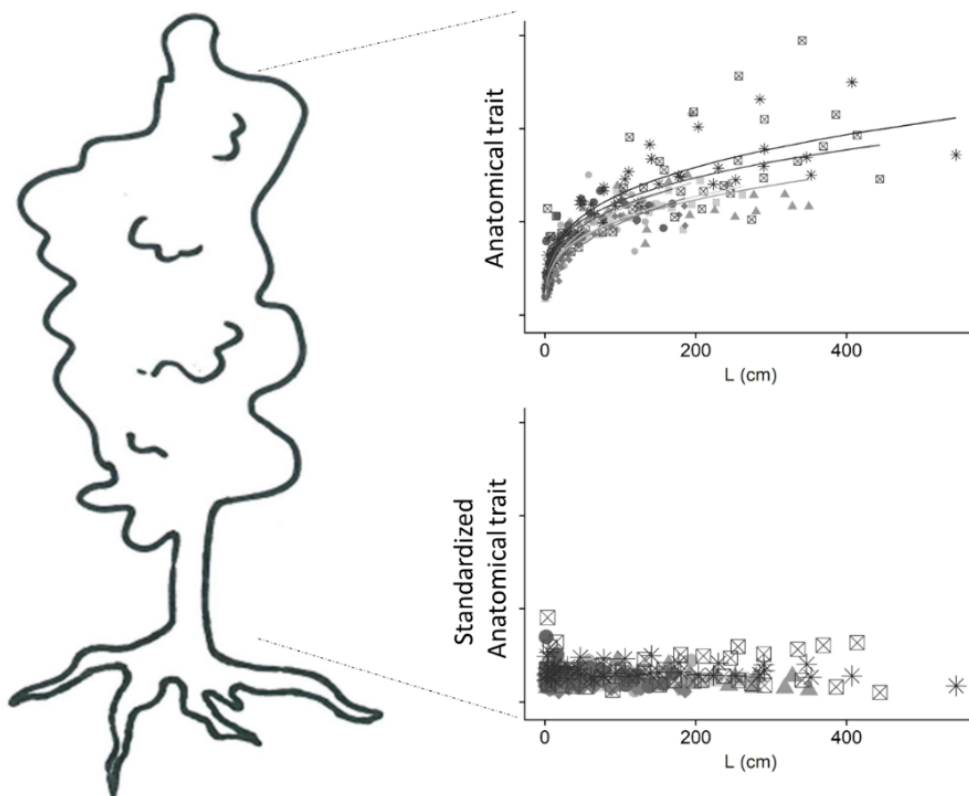
A third paper, "Axial anatomy of the leaf midrib provides new insights into the hydraulic architecture and embolism patterns of *Acer pseudoplatanus* leaves", has been submitted to Journal of Experimental Botany (Chapter 3). It presents a detailed analysis of the xylem anatomy along the midrib with the hypothesis that xylem conduits, even within the same vein order, might change considerably, with consequences on the resistance to embolism of the specific vein.

A fourth paper, "Negligible tension gradient in long stems: leaves account for the majority of the whole hydraulic path's resistance", to be submitted soon to a peer-reviewed journal (Chapter 4). It shows how the basipetal conduit widening along both leaf/needle and stem plays a pivotal role in shaping the leaf-to-root xylem water potential gradient.

The thesis ends with a chapter with general conclusions.

A standardization method to disentangle environmental information from axial trends of xylem anatomical traits

A standardization method to disentangle environmental information from axial trends of xylem anatomical traits

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SL: analysis and interpretation of the data, drafting and revising of the manuscript, TLT and YG: conception and design of the study, acquisition of data, revising of the manuscript, FP: conception and design of the study, analysis and interpretation of the data, revising of the manuscript, AT and MA: conception and design of the study, revising of the manuscript, GP: analysis and interpretation of the data, drafting and revising of the manuscript.

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Summary

Anatomical traits such as xylem conduit diameter and vessel connectivity are fundamental characteristics of the hydraulic architecture of vascular plants. Stem xylem conduits are narrow at the stem apex, and this confers resistance to embolisms that might otherwise be induced by large, negative water potentials at the top of tall trees. Below the apex, conduits progressively widen and this characteristic minimizes effects of path length on total hydraulic resistance. While interconnections among xylem vessels have been noted for decades, their role(s) are not fully clarified. For example, we do not know if they allow water to bypass embolised vessels, or increase the risk of spread of embolisms, or how their arrangement varies within a tree. Here we demonstrate the benefit of removing the independent effect of stem length, on assessment of effects of external (e.g., climatic) factors on such xylem traits. We measured the hydraulic diameter (Dh) and vessel conductivity index (VCI) along the stem of 21 shrubs/trees of similar height (1.19 <H<5.45 m) belonging to seven *Acacia* species, across a wide aridity gradient in Australia. All trees showed similar scaling exponents of Dh (b=0.33) and VCI (b=0.53) *vs.* axial distance from the apex (L), thus conforming with general patterns in woody plants. After de-trending for L, neither Dh (P=0.21) nor VCI (P=0.109) differed across the aridity gradient. We found that across a wide gradient of aridity, climate had no effect on xylem anatomy of *Acacia* spp, that was instead dictated by axial distances from stem apices. We argue that the use of standardization procedures to filter out intrinsic patterns of vascular traits is an essential step in assessing climate-driven modifications of xylem architecture.

Introduction

Water flows from roots to leaves through a network of xylem vascular elements. Such a network must, on one hand, be sufficiently conductive to sustain leaf transpiration, and on the other hand, limit the risk of formation and spread of embolisms. Axial organization of vascular elements has a great many similarities across species (Anfodillo *et al.*, 2013b) and is largely stable during ontogeny (Prendin *et al.*, 2018). At the stem apex, where the xylem water potential is more negative (see Venturas et al. 2017), conduits are the narrowest. Wider elements embolize more easily at low tensions (Nardini *et al.*, 2017; Olson *et al.*, 2018). Below the apex, conduits progressively increase in diameter towards the stem base, following a pattern common to all vascular plants (Anfodillo *et al.*, 2013b; Olson *et al.*, 2014, 2018). The widening pattern is well approximated by a power function ($a \sim x^b$) where a is the conduit diameter, x is the path length, and b is a scaling exponent, typically ranging from ~0.15 to ~0.33. Nevertheless, diameter variation can be pronounced within a few meters of the apex, and can become negligible towards the base of tall trees (Becker *et al.*, 2003; Petit *et al.*, 2010). This pattern helps minimize increases in total hydraulic resistance (*Rh*) with increasing path length (i.e., tree height) (West *et al.*, 1999; Petit & Anfodillo, 2009; Petit *et al.*, 2010) and has been suggested as representing the "least bad" combination of hydraulic efficiency and safety, and carbon construction costs (Mencuccini *et al.*, 2007).

A large body of empirical evidence now confirms that conduit diameters widen basally, especially in the first few meters down from the stem apex. While total distance from the apex clearly co-varies with age for much of ontogenic development, it is the former that drives conduit diameter (Petit *et al.*, 2008; Anfodillo *et al.*, 2012). Nevertheless, neglecting such an important pattern can lead to arguable conclusions in studies addressing the effects of external conditions on xylem anatomy. For example, it is often reported that trees growing in wetter conditions have larger diameter conduits in branchwood and stemwood than trees in drier conditions (e.g. McCulloh et al. 2015, Pfautsch et al. 2016, Hacke et al. 2017). But are these differences due directly to climate, or to indirect effects of climate via growth, tree height and path length (which do not infinitely increase)? The difficulties of separating proximal and distal drivers of xylem anatomy are illustrated by recent studies showing that fewer but wider conduits were produced under reduced soil water availability (Petit *et al.*, 2016; Kiorapostolou *et al.*, 2018).

Vessel connectivity can also affect both hydraulic efficiency and safety (Carlquist, 1984; Loepfe *et al.*, 2007; Lens *et al.*, 2011; Zhao, 2016). Spatial distributions of vessels vary according to species and environment. In some species, single vessels are isolated from one another by a dense matrix of less conductive and thick-walled fibres. In others, vessels are arranged in more or less numerous groups or clusters. Not only is variation in vessel arrangement along stems and branches largely undescribed, its importance for hydraulic efficiency and safety is mostly unknown and readily debatable (Scholz et al. 2013). One hypothesis is that larger grouping provides greater safety against the risk of embolism formation and characterizes species growing under reduced water availability (Robert *et al.*, 2009; Fonti *et al.*, 2013; Trifilò *et al.*, 2014). The rationale is that presence of adjacent vessels increases redundancy and safety, since there are alternative pathways for water should some vessels embolize (Loepfe *et al.*, 2007; Trifilò *et al.*, 2014). The opposite view is that greater vessel grouping might increase vulnerability of the system to embolism, since air bubbles could spread between neighbouring vessels through pit pores (Scholz *et al.*, 2013b), and plants with reduced vessel grouping might have advantages in xeric environments (Zanne *et al.*, 2006).

The broad aim of this study was to test if xylem traits of *Acacia* spp. conform to established patterns of axial variation. We describe a method for disentangling these patterns from climatic information. We selected individuals of comparable size (ranging in height from 1.19 m to 5.45 m) of seven species of the genus *Acacia* growing as understory to dominant eucalypts across a wide climatic gradient. Our driest site had a ratio of precipitation (P) to potential evapotranspiration (PET) of 0.24, while at the wettest P/PET was 1.24. We first assessed variation in anatomical traits along the stem, and then standardized the data to remove effects of hydraulic path length (i.e., distance from the apex). We then assessed the effect of aridity on the standardised xylem traits.

Material and methods

Study area

The study area is located in SE Australia. Seven different sites were selected across a gradient in aridity, assessed by the computed aridity index (ratio of precipitation to potential

evapotranspiration) (Fig. 1, Table 1). Climate data from weather stations located near each site were obtained from the Australian Government's Bureau of Meteorology (bom.gov.au) (Fig. 2). The total annual precipitation ranges from 330 mm in the driest site to 1100 mm in the wettest site, while mean annual temperatures ranged from 16 to 23 °C (Table 1).
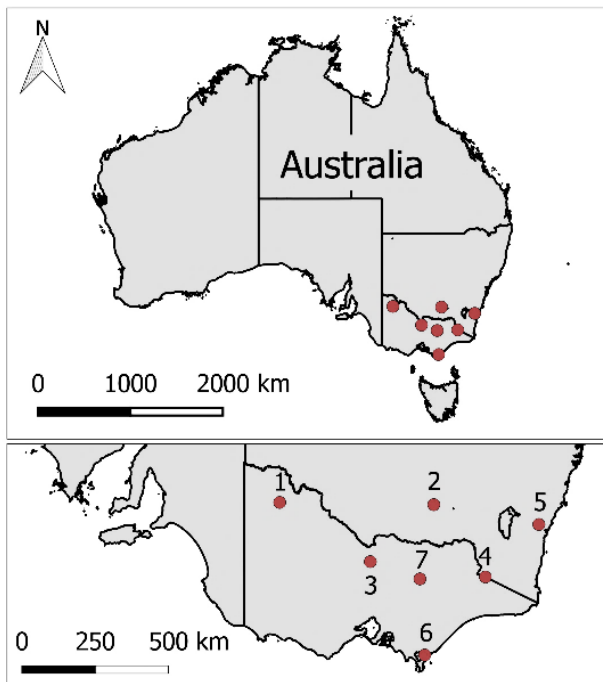

Figure 1: Study area in Australia: the red dots indicate the different sites where the sampling has been performed.

Table 1: Information on the sampling sites

| Site	Annual Prec (mm)	Mean Max Temp range (°C)	Soil type	Aridity index (P/PE)	Species	Average tree height (cm)
1	330.1	15.2 - **23.8** - 32.4	Sandy	0.24	*Acacia bivenosa* (DC.)	208 sd. 54
2	572.6	12.7 – **22.2** - 31.7	Sandy	0.35	*Acacia decora* (Reichb.)	149 sd. 40
3	506.2	12.5 - **21.15** - 29.8	Loam	0.51	*Acacia pycnantha* (Benth.)	271 sd. 118
4	788.8	9.5 - **16.7** - 23.9	Clay loams/ light clay	0.69	*Acacia amoena* (H.L. Wendl.)	183 sd. 4
5	1108.2	16.3 – **20.25** - 24.2	Loam	0.85	*Acacia falciformis* (DC.)	155 sd. 16
6	1055.0	12.2 - **16.4** - 20.6	Sandy	1.02	*Acacia verniciflua* (A. Cunn.)	377 sd. 91
7	1034.6	11.9 – **21.05** - 30.2	Clay loams/ light clay	1.24	*Acacia dealbata* (Link)	415 sd. 113
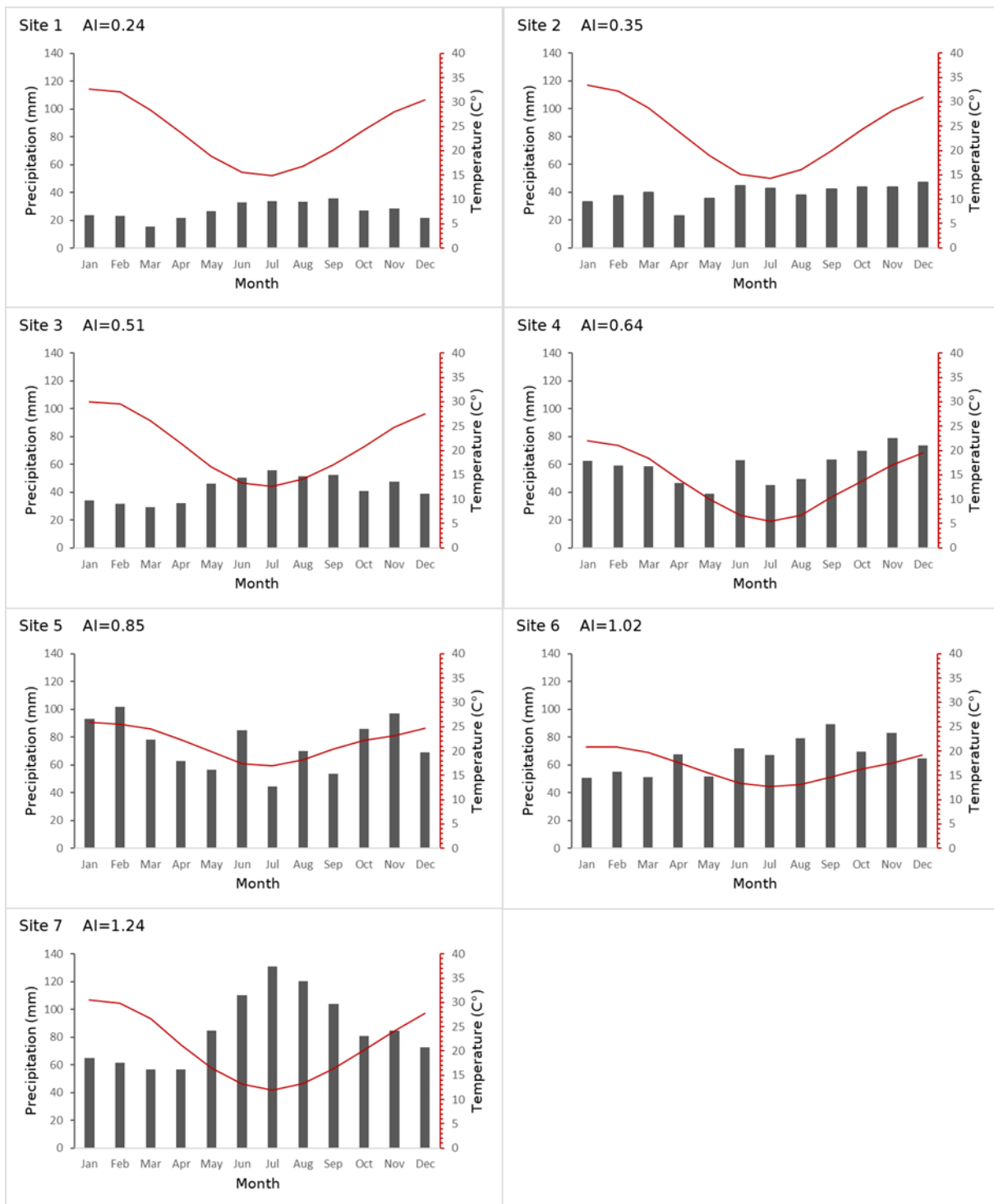
Figure 2: Climatic diagrams for the different sampling areas. Monthly mean of the least 30 years for precipitation (grey bars) and temperature (red line) are reported.

Plant material, sample preparation and measurements

Seven species belonging to the genus *Acacia*, one characteristic of each site, were selected for analysis (Table 2). All studied *Acacia* spp. were understorey so as to eliminate, as far as possible, light as a driver of the traits in question (e.g. Kunstler et al. 2009, Bourdier et al. 2016). Three individuals per species were felled at each site and hemispherical pictures were taken with a fisheye lens placed at the root collar of the felled stem. The images were analysed with a gap light analyser (GLA, Version 2.0.4, image processing software) (Frazer et al., 1999) and the canopy openness was estimated as the percentage of open sky seen from beneath the forest canopy using (Table 2).

Table 2: Information on the sampled trees

Site	Species	Average DBH (mm)	Average tree height (cm)	Average canopy openness (%)
1	*Acacia bivenosa* (DC.)	32.99 sd. 16.29	208 sd. 54	60.79 sd. 10.25
2	*Acacia decora* (Reichb.)	20.39 sd. 9.69	149 sd. 40	41.12 sd. 3.26
3	*Acacia pycnantha* (Benth.)	18.7 sd. 5.88	271 sd. 118	38.76 sd. 2.35
4	*Acacia amoena* (H.L. Wendl.)	19.8 sd. 1.46	183 sd. 4	35.32 sd. 1.14
5	*Acacia falciformis* (DC.)	12.78 sd. 2.39	155 sd. 16	13.63 sd. 0.62
6	*Acacia verniciflua* (A. Cunn.)	33.13 sd. 6.44	377 sd. 91	26.33 sd. 0.70
7	*Acacia dealbata* (Link)	39.04 sd. 15.04	415 sd. 113	28.71 sd. 2.88

Wood samples were stored and transported in thick-walled sealed plastic containers and conserved in ethanol (40%). Afterwards, the samples were sliced with a rotary microtome (Leica RM2255) at 20 μm, and cross sections were stained with a mix solution of Safranin (1% in water) and Alcianblue (0.5% in water), washed with increasing concentrations of ethanol (50%, 70%, 99.5%) to remove stain residual, and finally closed with covermounts fixed with few drops of Eukitt® (Bioptica, Milan, Italy). Digital images from the last annual ring were captured with a high-resolution digital camera mounted on a light microscope (Leica DM2500) set at the magnification of 100x. A minimum of 30 xylem vessels were always included in each sample. Digital images were then analysed with ImageJ (Abramoff *et*

al., 2004) for the assessment of area, perimeter and local coordinates of each vessel. The hydraulic diameter (*Dh*) was calculated, considering the element as cylindrical, as (Kolb & Sperry, 1999):

$$Dh = \Sigma d^5 / \Sigma d^4$$

eq. 1

where *d* is the diameter of a given vessel.

Statistical analyses

The spatial arrangement of vessels at each position along the stem was assessed by using two different indices. We performed a point pattern analysis with the "nndist" function available from in R in the *spatstat* package (Baddeley & Rolf, 2014) for the assessment of a vessel connectivity index (*VC*I) and Clark and Evans uniformity index (UI). VCI is computed as the average of the simple Euclidean distance of the centroid of each vessel to that of its nearest neighbor, corrected by the radius of the vessels, so as to quantify the actual distance between the cell walls of two adjacent cells. Low values of VCI indicate an increase in vessel connectivity, whereas higher values denote more isolation of vessels with respect to neighboring vessels. The Clark and Evans uniformity index (UI) is the ratio of the observed mean nearest neighbor distance to that expected for a Poisson point process of the same intensity (Clark & Evans, 1954). UI discerns the aggregation of the vessels in random distributed (UI=1), uniform (UI<1) and clustered (UI>1).

Log10-transformation of data, in order to meet normality and homoscedasticity assumptions, were made prior to further statistical analyses. For each tree, we assessed a type-II linear regression with reduced major axis (RMA) for the variation of both *Dh* and *VCI* with *L,* in order to account for potential errors in both *x* and *y* variables. The slope (*b*) of the resulting function was used to standardize the *Y* trait (either *Dh* and *VCI*) by removing the effect of the axial path length (*L*), as:

$$Y_{std} = Y / L^b \quad \text{eq. 2}$$

Anova was performed to test the effect of the aridity index (*AI*), mean annual temperature (*MAT*) and total annual precipitation (*TAP*) on the standardised vessel hydraulic diameter (*Dh_{std}*) and the standardised vessel conductivity index (*VCI_{std}*), with tree identity being used as blocking factor.

Results

The analysed *Acacia* species presented a diffuse porous wood, with mainly solitary vessels or vessels arranged in pairs or triplets. Vessels were associated with varying amounts of paratracheal parenchyma, usually more abundant than scanty. The vessels were surrounded by axial parenchyma and fibres of varying thickness. Parenchyma rays were generally uniseriate or biseriate, short and narrow.
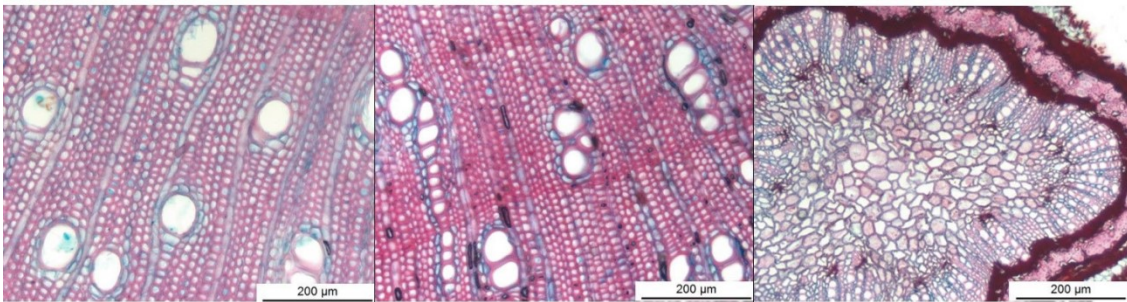

Figure 3: Anatomical sections of Acacia bivenosa stem sampled at different distances from the apex. From leaf to right: 155 cm (base), 74 cm, and 2 cm from the stem apex.

Vessel size

In all analysed trees, we found that vessel hydraulic diameter (*Dh*) increased basally from the most apical point to the base (Fig. 3, Fig. 4). *Dh* varied from a minimum of ~ 9 µm in the most apical sections, to a maximum of 147 µm at the stem base. These axial trends were well described by power functions, relating *Dh* to the distance from the apex (*L*), with scaling exponents (*b*) ranging from 0.18 to 0.43 with an average of 0.33. The trends were not statistically different as shown by the confidence intervals (Fig. 4, Table 3).

When *Dh* was standardized for the distance from the apex according to eq. 2 (Dh_{std}), its axial variation was no longer significant (Fig. 5). According to our statistical analysis, none of the climatic parameters had a significant effect on Dh_{std} (*AI*: P=0.21; *TAP*: P=0.306; *MAT*: P= 0.344).

Table 3: Intercept (a) and exponent (b) of the regression line obtained using RMA. The line describes the Dh variation with the distance from the apex for each plant (ID) and Site. The data were log transformed. R2, confidence interval at 95% and p-value are given.

ID	a	b	R2	a_95%CI	b_95%CI	p-value
		LOG10_Dh ~ LOG10_Dist from apex				
0.24_1	1.019	0.354	0.930	0.921; 1.101	0.298; 0.421	2.63E-08
0.24_2	1.195	0.262	0.946	1.138; 1.244	0.225; 0.304	5.42E-09
0.24_3	1.116	0.296	0.966	1.047; 1.177	0.261; 0.335	1.95E-09
0.35_1	1.032	0.357	0.480	0.391; 1.302	0.170; 0.800	0.008648
0.35_2	1.177	0.289	0.706	0.972; 1.303	0.193; 0.446	0.000167
0.35_3	0.850	0.514	0.877	0.66; 0.996	0.401; 0.662	2.43E-06
0.51_1	0.984	0.373	0.858	0.779; 1.141	0.283; 0.490	5.47E-06
0.51_2	1.093	0.316	0.901	0.996; 1.17	0.257; 0.391	2.21E-07
0.51_3	1.196	0.249	0.822	1.021; 1.327	0.176; 0.345	4.73E-05
0.69_1	0.853	0.433	0.936	0.715; 0.964	0.361; 0.523	2.66E-07
0.69_2	0.859	0.408	0.963	0.781; 0.926	0.359; 0.465	3.00E-09
0.69_3	0.963	0.382	0.957	0.892; 1.023	0.330; 0.444	3.50E-08
0.85_1	1.190	0.308	0.697	0.985; 1.328	0.192; 0.479	0.000386
0.85_2	1.044	0.392	0.908	0.928; 1.139	0.320; 0.479	1.40E-07
0.85_3	1.219	0.266	0.854	1.115; 1.3	0.204; 0.346	2.36E-06
1.02_1	0.967	0.433	0.824	0.678; 1.187	0.321; 0.580	7.39E-06
1.02_2	1.432	0.184	0.490	1.091; 1.625	0.087; 0.357	0.00529
1.02_3	1.151	0.295	0.787	0.949; 1.301	0.210; 0.410	2.33E-05
1.24_1	1.184	0.294	0.944	1.098; 1.259	0.252; 0.343	7.32E-09
1.24_2	1.121	0.364	0.922	0.986; 1.232	0.303; 0.438	5.28E-08
1.24_3	1.333	0.244	0.873	1.214; 1.422	0.193; 0.313	1.02E-06
Site						
A_0.24	1.104	0.308	0.924	1.059; 1.146	0.28; 0.338	2.14E-23
B_0.35	1.02	0.385	0.684	0.893; 1.128	0.306; 0.48	4.76E-11
C_0.51	1.091	0.311	0.852	1.016; 1.157	0.271; 0.358	6.63E-17
D_0.69	0.907	0.398	0.939	0.858; 0.952	0.365; 0.435	6.84E-23
E_0.85	1.16	0.315	0.783	1.085; 1.224	0.265; 0.374	1.66E-14
F_1.02	1.171	0.311	0.657	1.022; 1.298	0.244; 0.389	7.81E-11
G_1.24	1.211	0.303	0.877	1.144; 1.273	0.268; 0.341	8.39E-20

Vessel connectivity

The Clark and Evans uniformity index (UI) suggested a change in spatial distribution of vessels when moving from the apex to the base of the stem. UI < 1 were recorded for samples close to the apex, while UI~ 1 for the rest of the stem. Vessels were more clustered near the apex, and become progressively more randomized toward the base.

An index of connectivity between vessels (*VCI*) increased basally from the apex to the base in all trees. *VCI* varied from a minimum of ~ 7 µm close to the stem apex to a maximum of 271 µm at the stem base. Variation in *VCI* with distance from the apex (*L*) was well described by power functions ($0.48 \leq R^2 \leq 0.97$), with scaling exponents (*b*) ranging from 0.30 to 0.71 (Fig. 4, Table 4). When *VCI* was standardized for *L* according to eq. 2 (*VCI$_{std}$*), its axial variation was no longer significant (Fig. 5). According to our statistical approach, none of the climatic parameters had a significant effect on *VCI$_{std}$* (AI: P=0.109; TAP: P= 0.244; MAT P= 0.889).
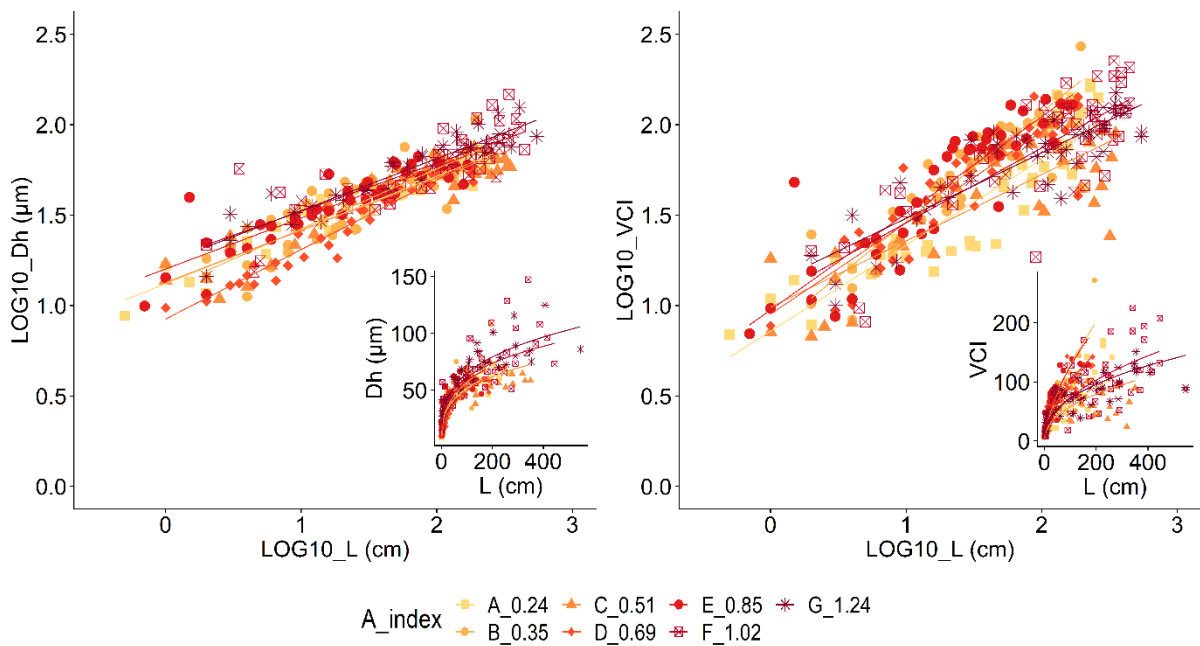

Figure 4: Axial variation of the hydraulic diameter (Dh) and vessel nearest-neighbour distance (VCI) with distance from the apex (L) for log–log transformed data and non-transformed data in the bottom right. The trees are grouped per aridity index from the drier

Table 4: Intercept (a) and exponent (b) of the regression line obtained using RMA. The line describes the VCI variation with the distance from the apex for each plant (ID) and Site. The data were log transformed. R2, confidence interval

			LOG10_VCI ~ LOG10_Dist from apex			
ID	**a**	**b**	**R2**	**a_95%CI**	**b_95%CI**	**p-value**
0.24_1	0.760	0.528	0.830	0.499; 0.952	0.396; 0.707	5.80E-06
0.24_2	0.847	0.464	0.750	0.572; 1.045	0.317; 0.669	6.25E-05
0.24_3	0.703	0.552	0.610	0.039; 1.089	0.347; 0.906	0.000353
0.35_1	0.796	0.617	0.772	0.39; 1.071	0.427; 0.897	7.80E-05
0.35_2	0.802	0.662	0.947	0.661; 0.922	0.570; 0.770	5.35E-09
0.35_3	0.764	0.700	0.800	0.407; 1.026	0.496; 0.978	3.68E-05
0.51_1	0.731	0.441	0.645	0.227; 1.051	0.258; 0.729	0.000943
0.51_2	0.908	0.513	0.872	0.726; 1.052	0.401; 0.653	1.03E-06
0.51_3	0.960	0.394	0.797	0.655; 1.184	0.271; 0.562	9.27E-05
0.69_1	0.769	0.600	0.645	0.115; 1.177	0.336; 1.022	0.001663
0.69_2	0.766	0.608	0.913	0.581; 0.921	0.499; 0.737	9.89E-08
0.69_3	1.016	0.521	0.929	0.887; 1.122	0.428; 0.633	4.62E-07
0.85_1	0.921	0.605	0.738	0.544; 1.159	0.406; 0.919	0.000169
0.85_2	0.683	0.711	0.844	0.376; 0.905	0.544; 0.942	3.47E-06
0.85_3	1.026	0.521	0.915	0.881; 1.145	0.430; 0.632	8.87E-08
1.02_1	0.503	0.679	0.687	-0.09; 0.933	0.474; 0.962	1.15E-05
1.02_2	1.250	0.314	0.778	1.013; 1.431	0.224; 0.432	1.38E-05
1.02_3	0.930	0.465	0.645	0.453; 1.248	0.288; 0.730	0.000312
1.24_1	1.275	0.301	0.786	1.076; 1.433	0.216; 0.409	1.07E-05
1.24_2	0.879	0.456	0.759	0.585; 1.105	0.343; 0.604	5.70E-07
1.24_3	0.941	0.481	0.914	0.768; 1.086	0.400; 0.579	2.67E-08
Site						
A_0.24	0.776	0.529	0.814	0.641; 0.892	0.454; 0.617	3.53E-16
B_0.35	0.793	0.654	0.834	0.655; 0.915	0.564; 0.757	2.22E-16
C_0.51	0.874	0.44	0.683	0.693; 1.019	0.349; 0.552	8.98E-11
D_0.69	0.88	0.557	0.819	0.747; 0.993	0.475; 0.654	6.61E-15
E_0.85	0.886	0.605	0.799	0.748; 1.001	0.515; 0.713	3.48E-15
F_1.02	0.887	0.493	0.647	0.654; 1.083	0.394; 0.611	3.39E-12
G_1.24	1.039	0.406	0.762	0.905; 1.155	0.344; 0.477	1.45E-16

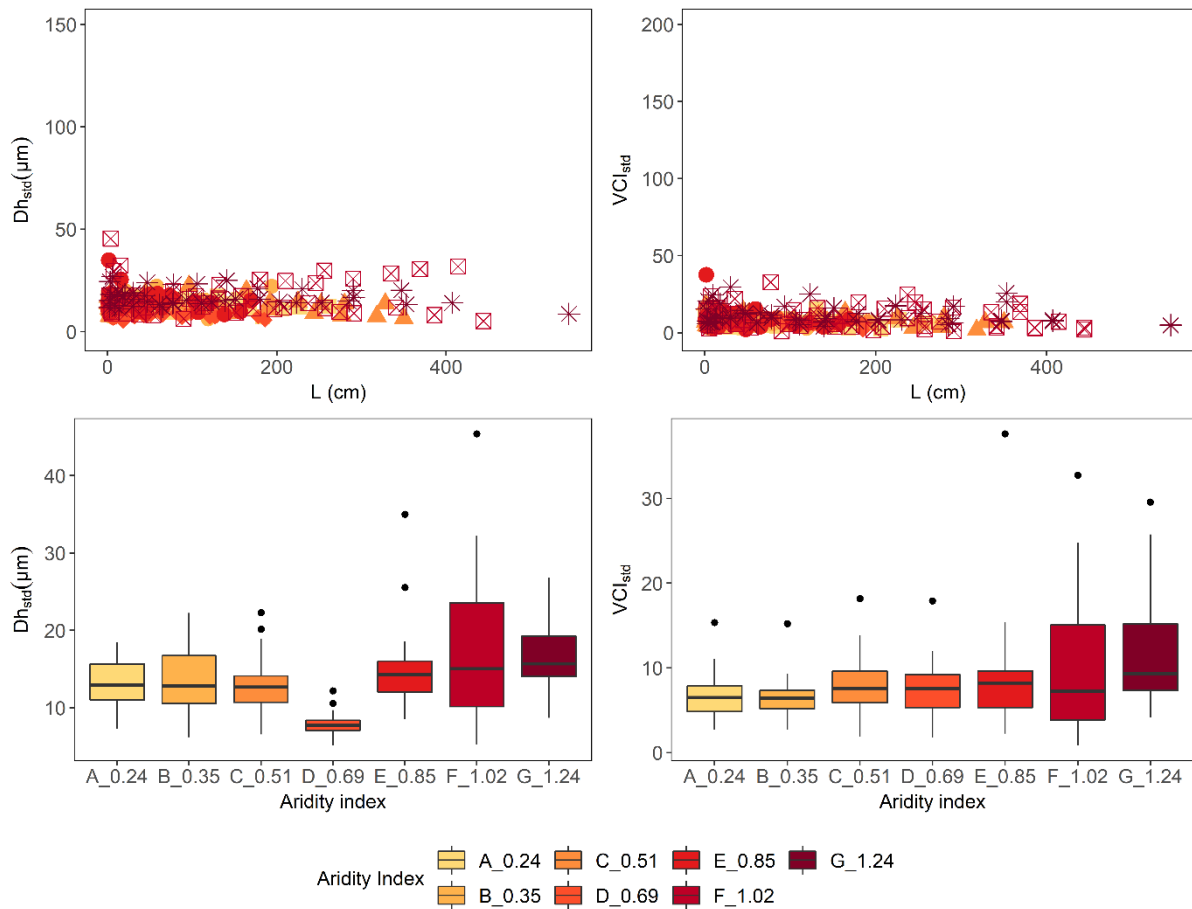*Figure 5: Axial variation of the standardized hydraulic diameter (Dhstd) and vessel nearest-neighbour distance (VCIstd) with distance from the apex (L) and frequency distribution of Dhstd and VCIstd per aridity gradient below. The trees are grouped per aridity.*

Discussion

The data reveal clear axial variation in the size and connectivity of vessel conduits of *Acacia* spp. The influence of hydraulic path length (i.e., distance from the apex, *L*) on vessel attributes was strong and standardizing for *L* effects helped clarify the influence of climate on xylem structure. All studied *Acacia* spp. were understorey so as to eliminate, as far as possible, light as a driver of the traits in question (e.g. Kunstler et al. 2009, Bourdier et al. 2016).

Axial scaling of anatomical traits

We evaluated axial variation in vessel hydraulic diameter (*Dh*) and vessel conductivity index (*VCI,* i.e. the average distance between the cell walls of two adjacent vessels). All our data

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suggest common axial scaling of both traits within stems, according to which both *Dh* and *VCI* progressively increase from the apex towards the base (Fig. 3). These axial scalings were conserved across a gradient in aridity, where the Aridity Index (*AI*) increased five-fold, highlighting that xylem architecture is highly conserved - arguably an unavoidable requirement for survival.

The observed axial trend of *Dh* (widening) is in agreement with previous studies, i.e. consistent with the universality of this scaling pattern (with an exponent *b* in the range 0.15-0.4) found in woody shrubs and trees of different species in different environments (Anfodillo *et al.*, 2006; Petit *et al.*, 2008, 2010, 2011, 2014; Petit & Anfodillo, 2009; Fan *et al.*, 2009; Lintunen *et al.*, 2010; Bettiati *et al.*, 2012; Olson *et al.*, 2018). Collectively, these studies also support the hypothesis that scaling of vessel traits with *L* is essential for the functionality and efficiency of the water transport system (West *et al.*, 1999; Anfodillo *et al.*, 2006; Mencuccini *et al.*, 2007; Petit & Anfodillo, 2009; Olson *et al.*, 2018). Progressive enlargement of vessels from the apex to the base ensures that most of the path length resistance lies within a short distance from the apex, thus facilitating the maintenance of total xylem hydraulic resistance irrespective of absolute tree height (West *et al.*, 1999; Petit & Anfodillo, 2009).

Our observation that axial variation in *VCI* is similar among our investigated *Acacia* species in different environments is highly novel, although the collected data were insufficient to test for possible convergent scaling across species. It highlights the strong role played by the path length, and thus by the plant size, in shaping the hydraulic architecture of the xylem. The distance between adjacent vessels (*VCI*) was smallest at the tree apex, where vessels were more clustered (*UI*<1), and progressively increased towards the stem base, where vessel distribution became more randomized (*UI*=1). The spatial arrangement of vessels has often been proposed to be important for their reciprocal interconnections, with potential important implications for the efficiency and safety of the water transport system. The smaller inter-vessel distance (*VCI*) close to the apex supports the hypothesis that pathway redundancy allows water to more easily bypass embolized vessels (Loepfe *et al.*, 2007; Trifilò *et al.*, 2014). Here too, we acknowledge that separating hydraulic benefits of smaller VCI from those of smaller *Dh* is not straightforward.

Standardization of anatomical traits

Standardizing data is common practice in other fields that draw upon structural features of wood. In dendrochronology, for example, data are normally standardized to remove non-

stationary processes (such as radial growth) and clarify effects of climatic variation (Helama *et al.*, 2004; Carrer *et al.*, 2015). Although the axial trend in conduit diameter has been widely demonstrated (Fan *et al.*, 2009; Lintunen *et al.*, 2010; Petit *et al.*, 2011; Anfodillo *et al.*, 2013b; Olson *et al.*, 2014; Petit *et al.*, 2014; Olson *et al.*, 2018), standardization procedures are often neglected. When coupled with the difficulties of precisely defining how known drivers of rates of tree growth (e.g. water, light, nutrients, temperature) affect cell dimensions, and of how these same drivers provide limits to tree structures (height especially), it is not surprising that there are strongly held but sometimes opposing views. A persistent dichotomy is that either path length (i.e., tree height) (Olson *et al.*, 2014; Rosell *et al.*, 2017) or climatic conditions (Pfautsch et al. 2016; Hacke et al. 2017) drive variability in xylem anatomy. Since all studied *Acacia* trees presented strong axial variations of *Dh* and *VCI*, the effect of path length on *Dh* and *VCI* was removed to facilitate statistical analysis of the effects of climate. We removed the effects of path length (*L*) via a simple standardization method. Our approach can also be used more widely. Conservative standardization of anatomical traits can be achieved using Eq. 2, with the factor *b* being the average scaling exponent for the axial trend of a given trait (e.g. *b≈0.2* for the hydraulic diameter of xylem conduits).

Anatomical responses to climate

Standardized *Dh* and *VCI* showed no variation with climate. In this study, none of the sampled *Acacia* were > 5.4m in height, while the shortest was ~1m. Nonetheless, that xylem architecture among species of *Acacia* from contrasting environs is conserved, reinforces the dominant effect of axial path length.

Our results raise a separate question as to whether there are genus-specific or species-specific differences in adaptation to climate. A recent study of *Eucalyptus* trees across a similar gradient, reported different conclusions (Pfautsch *et al.*, 2016), albeit tree heights ranged up to almost an order of magnitude greater than in the present study. Climate strongly affected vessel diameters, with narrower vessels reported for drier environments, but almost no widening along the stem of the sampled trees. No data were reported for apical parts, where changes in diameter with path length are usually greatest. For example, less than 20% of *Dh* variation is found between 8 and 69 m from the stem apex in tall eucalypts (e.g., *E. regnans*) (Petit *et al.*, 2010). When considering large ranges in tree height, accounting for or removing path length effects becomes even more important. For example, Olson et al. (2014) reported significant increases in apical *Dh* with total tree height, and interpreted these as necessary attributes for growing taller. As noted above, limits to tree height and control of rates of

growth are still open (and complex) questions. Further studies are needed to resolve whether variability in vessel size (as reflected in distance from the apex) may also be due to climate, independent of the effects of climate on tree size/height (Givnish *et al.*, 2014).

Several studies have reported stronger vessel grouping in xeric environments, suggesting a possible role of this trait in increasing the safety of the water transport system (Robert *et al.*, 2009; Fonti *et al.*, 2013; Trifilò *et al.*, 2014). Conversely, some studies have reported strong links between vessel grouping and vulnerability to drought induced cavitation (Scholz *et al.*, 2013b). We demonstrated an axial trend in the spatial organization of vessel elements. Again, after standardization, *VCI* showed little variability with climate. We presently lack sufficient comparable studies and data to make definitive conclusions. Nonetheless, our data suggest that environment applies only weak selective pressure on this trait.

Standardizing to remove (or otherwise accounting for) axial trends is fundamental to rigorously assessing effects of climate or other edaphic variables on xylem anatomy. Reconciling this and other studies of the effects of climate on xylem architecture is important to implement climate models aiming to predict the contribution of vegetation to global carbon and water cycles. Such reconciliation will require careful application of both analytical methods and statistical analysis.

Acknowledgements

CHAPTER 2

Invariant features of leaf vasculature with position in the crown
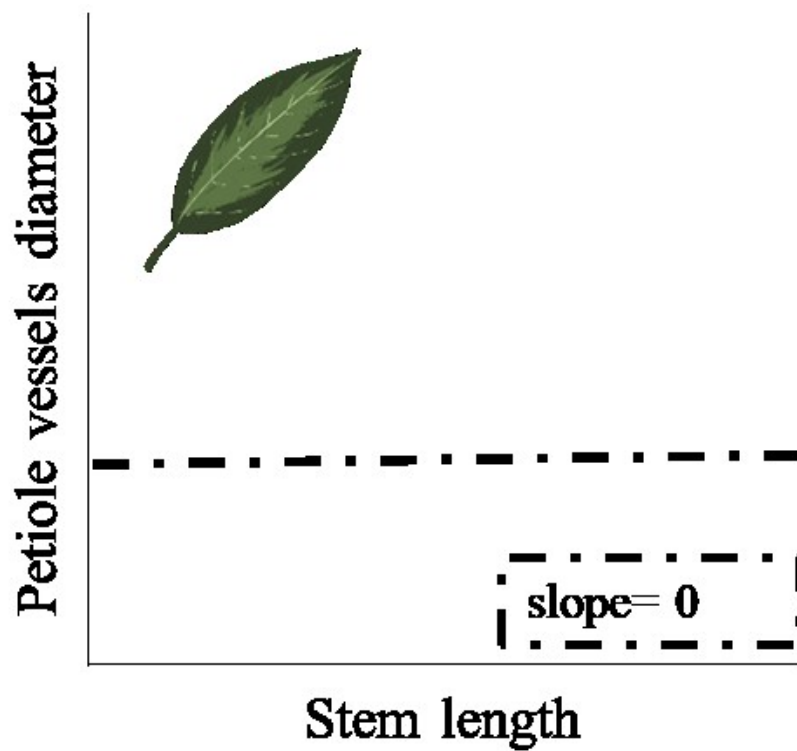
Invariant features of leaf vasculature with position in the crown

Running head:

Invariant features of leaf vasculature

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Author Contributions:

SL: samples collection, analysis and interpretation of the data, drafting and revising of the manuscript; JAR: samples collection, analysis and interpretation of the data, revising of the manuscript; MC, AE, DS, samples collection; CBW, TW, TA: conception and design of the study, MO: conception and design of the study, samples collection, analysis and interpretation of the data, drafting and revising of the manuscript.

Keywords: petiole vessels diameter, terminal veins diameter, distance from the tree base, hydraulic residence.

In preparation

Summary

Hydraulic optimality models predict widening of xylem conduits with tree height in a way that compensates the hydraulic resistance imposed by increasing pathlength with height growth. Empirical data confirm this prediction, showing that not only do vessels widen with plant height at the stem base (slope = 0.42), but vessels at the twig tips also widen with height (slope=0.2). A missing detail in understanding whole plant widening is how leaf conduits contribute to achieving stem tip conduit widening. We measured petiole vessel diameter and vessel diameter in minor terminal veins of 239 leaves of 49 angiosperm species with diverse leaf morphologies. Leaves were collected at different distances from the stem base, from 10 cm to over 30 meters. Our results showed that, standardizing for leaf area, petiole base vessel diameter does not scale with plant height, in contrast to terminal twig vessel diameter. Terminal leaf vein vessel diameter was independent of both leaf area and distance from the stem base. Leaves, once area is accounted for, are invariant with respect to their position in the crown, and thus to the pathlength from the stem base to the terminal twig they are connected, as regards their conduit diameters. Predictability of leaf conduit diameters, together with terminal twig conduit widening with height, are consistent with the idea that, controlling for leaf size, hydraulic resistance is similar in leaves regardless of position in the tree and as trees grow taller.

Introduction

Height growth in plants involves mechanisms that compensate for the hydraulic resistance imposed by increase in conductive path length, and while it seems clear that tip-to-base conduit widening is part of the mechanisms involved, numerous outstanding questions remain (Rosell *et al.*, 2017). If a conduit of fixed diameter becomes longer, as when a plant stem grows longer, the increase in conduit wall area imposes increasing friction on the conductive stream, leading to drops in conduction (Vogel, 2013). Instead of remaining of constant diameter, plant conduits widen quickly from the narrow terminal ones found in leaves, to the wider ones at the petiole base (Sack *et al.*, 2012), and from the stem tip to the stem base (Anfodillo *et al.*, 2006; Olson *et al.*, 2014). This widening appears sufficient to buffer the increase in resistance that would in its absence be imposed by increasing conductive pathlength. This buffering is likely the result of natural selection: leaf water supply is tightly linked to photosynthesis (Meinzer & Grantz, 1990; Sperry, 2000; Brodribb & Feild, 2000), and given variation in a population of a species of plant, individuals with per-leaf area photosynthetic rates that remain constant with height growth (or at least decrease as slowly as possible, cf. Enquist, 2003; Niklas *et al.*, 2007; Savage *et al.*, 2010) will be favoured over those that experience a drop in rate with growth (or experience steeper drops). As part of this selection favoring relatively constant per-leaf area photosynthetic rates with height growth (Stephenson *et al.*, 2014; Michaletz *et al.*, 2014), tip to base conduit widening likely plays a key role. Various optimality models predict the rate of tip-to-base conduit widening that should permit constant per-leaf area photosynthetic rates while minimizing conduit construction costs and embolism vulnerability (West *et al.*, 1999). While data seem largely in agreement with these predictions, (Anfodillo *et al.*, 2006; Lintunen *et al.*, 2010; Olson & Rosell, 2012; Olson *et al.*, 2014, 2018; Petit *et al.*, 2014), some empirical observations have been unexpected.

The principal unexpected observation, not predicted in any optimality model, is that in flowering plants, not only do vessels widen with plant height at the stem base, but vessels at the twig tips also widen with height. In the largest study to date, mean vessel diameter at the stem base was found to scale with an exponent of about 0.45 across 257 species (mean species values of 609 samples) (Olson *et al.*, 2014), more than double the rate predicted by optimality models (ca. 0.2) (Anfodillo *et al.*, 2006; Petit & Anfodillo, 2009; Olson *et al.*, 2014, 2018).

However, mean diameter of the vessels at the tips of the terminal twigs, distalmost from the stem base, also increased in diameter with plant height, scaling with a slope of about 0.23 (Fig.1). Thus, as vessels widen with height at the stem base, they also do so at the stem tip.
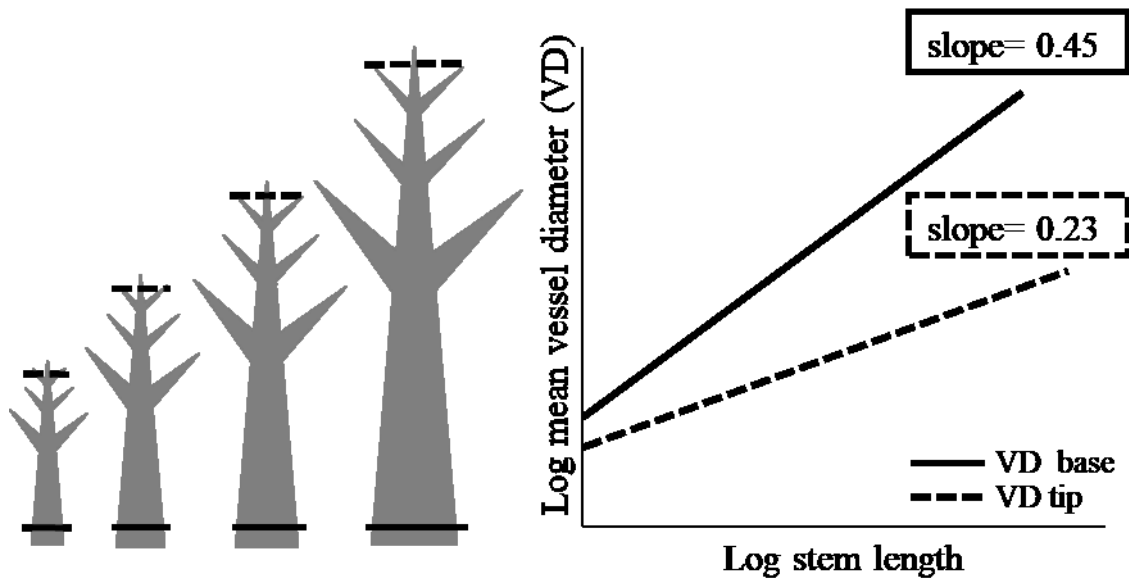

Figure 1: Not only do vessels at the base scale with height, but vessel diameter of the treetop terminal twigs does as well. This diagram illustrates that vessels widen with height at both the stem base (black line), with a slope of about 0.45, as well as at the twig tips, with a slope = 0.23, (dashed line) (cf. Olson et al., 2014). Vessel widening at the stem tip with height likely reduced per-vessel resistance, meaning that the same leaf area can be supplied with the same amount of water at a minimal increase in conduit carbon cost as a plant grows taller.

Together, these exponents mean that the overall widening exponent is ca. 0.2 as predicted (Olson et al., 2014, 2018). This widening observed at the twig tips is potentially explained as the result of selection favoring constant (or at least minimizing the increase in) per-leaf area sapwood volume with height growth (Olson *et al.*, 2014; Givnish *et al.*, 2014; Drake *et al.*, 2015; Rosell *et al.*, 2017). As stem length increases and the constant volume of sapwood available for deployment of conduits per leaf becomes longer and thinner, widening of terminal conduits would lower total path-length resistance, potentially permitting constant construction costs over very substantial height increases (see Olson et al., 2014; Rosell et al., 2017 for more detail). Whatever the cause of the apparently very widespread pattern of terminal twig vessel diameter increase with plant height (Olson *et al.*, 2014; Rosell & Olson, 2014), an outstanding question is how leaf conduits contribute to achieving stem tip conduit widening (Coomes et al., 2008).

While it is clear that in general leaf vessels increase in diameter between the distalmost vessels (analogous to capillaries, from which water diffuses into the mesophyll) and the widest vessels at the petiole base (Sack et al., 2012), whether leaf vessels widen in tandem with terminal twig vessels with height growth is unknown. Considering the assumptions of hydraulic optimality models and empirical results showing that terminal twig vessels increase in diameter with height (Fig. 1), we predicted that terminal twig vessel-vessel widening in the leaf would follow one of two patterns. One possibility was the rate of vessel widening from terminal leaf veins to the petiole base remains constant with height, but vessels in terminal veins increase in diameter with height. Given a constant rate of vessel widening, wider terminal leaf vessels would mean wider petiole base vessels and thus, potentially, wider vessels in the terminal twigs. Our alternative prediction was that vessels in terminal leaf veins do not increase predictably in diameter with height in a tree or leaf area, as assumed by optimality models (West et al., 1999), and as seems to be the case in empirical studies (Sack et al., 2012), but that within-leaf vessel widening rates are faster in leaves that are higher in the canopy. Such a situation would result in wider vessels at the petiole base in leaves that are farther from the tree base and therefore wider terminal twig vessel diameters. Testing these predictions required data from leaves taken at different distances from the stem base and in different part of the crown from phylogenetically and morphologically disparate species.

Our data rejected both of the alternative patterns we postulated. We rejected the notion that vessels in terminal leaf veins widen predictably with distance from the stem base or with leaf area, as well as rejecting the notion that greater distance from the stem base should be associated with faster terminal leaf vein-to-petiole base vessel widening. Our data showed that the only factor predicting the diameter of the petiole vessels was leaf area (the proxy we used for within-leaf conductive path length, Cho *et al.*, 2007; Kandiannan *et al.*, 2009; Rouphael *et al.*, 2010), and that the diameters of the vessels in terminal leaf veins were not predicted by distance from the stem base, plant height, leaf area, or any other variable we measured. Our results suggest that, standardizing for area, leaves are interchangeable with regard to their patterns of vessel diameter variation, no matter their distance from the stem base.

Material and methods

Plant material

We gathered two datasets with differing levels of detail to test our hypotheses regarding how leaf vessel widening might be involved in terminal twig vessel widening with height. The first aimed for generality and sampled very broadly across the vessel bearing "dicots," including the "basal" dicots (Austrobaileyales, Chloranthales, Laurales, Magnoliales, Piperales), monocots (Alismatales), and across the core eudicots (Table S1). We sampled leaves from the uppermost twig of the canopy (the twig tip farthest from the stem base), as well as wood samples from the base of the trunk (wedges of the outermost xylem), from 3 individuals per species from 43 species, 32 families, and 28 orders. The species were morphologically disparate, including trees, shrubs, and lianas, and spanned a wide array of habitats, from tropical dry forest, savannah, temperate rainforest, and tropical rainforest. We used these samples to examine whether, as terminal twig mean vessel diameter does, vessels in terminal vein or mean petiole base vessel diameters scale with height (taking into account leaf area).

The other dataset sampled leaves taken from twig tips at differing distances from the stem base within six species of angiosperms. This intraspecific dataset allowed us to examine the same questions as the interspecific dataset but within individuals. We wished to examine whether distance from the stem base, and thus conductive pathlength, was associated with differing terminal vein vessel or petiole base vessel diameters within individuals. For the intraspecific dataset we selected Albizia niopoides (Chodat) Burkart (Fabaceae: Mimosoideae), Cordia dodecandra A. DC. (Cordiaceae), Illicium mexicanum A. C. Smith (Illiciaceae), Manilkara zapota (L.) P. Royen (Sapotaceae), Moringa stenopetala (Baker f.) Cufod. (Moringaceae), and Swartzia cubensis (Britton & Wilson) Standl. (Fabaceae: Papilionoideae) (Table 1). Illicium is a member of Austrobaileyales, which, excepting Amborella, is the clade sister to all of the rest of the angiosperms. It has vessels with long scalariform perforation plates and tracheids as the ground tissue (tracheids are conductive imperforate tracheary elements). It grows in cool highland cloud forests in central Mexico. Albizia and Swartzia are legumes, with Albizia having large, feathery bipinnate leaves with minute leaflets, whereas Swartzia has once-pinnate leaves with large leaflets. Both have vessels with simple perforation plates surrounded by axial parenchyma and libriform fibers, which are non-conductive, as the ground tissue. Albizia was collected in a lowland tropical

dry forest with an 8 month dry season, whereas Swartzia is from moist subdecidous lowland tropical forest. Cordia is a member of Boranginales, whereas Manilkara is a member of Ericales. Both have relatively sclerophyllous leaves, are evergreen, and while both have simple perforation plates and libriform fibers as the ground tissue, Cordia has exceptionally dense wood (used as a replacement for ebony) with very thickwalled libriform fibers, and Manilkara has wood of relatively low density with thinwalled libriform fibers. Both are evergreen elements in subdecidous lowland tropical forest. Finally, Moringa stenopetala is a member of Brassicales. It is a deciduous tree with large (>1 m) long 2-3 pinnate leaves with large, very thin leaflets. Moringa stenopetala has a pachycaul water-storing trunk with simple perforation plates and very thinwalled libriform fibers replete with water and starch. It is native to the Rift Valley of northwestern Kenya and southwestern Ethiopia but the individuals sampled were cultivated in an area of dry tropical forest with a long dry season and low rainfall. Like our interspecific dataset, our intraspecific dataset sampling spanned a very wide range of leaf morphologies and phenologies, and wood anatomical modes. This sampling allowed us to ask whether terminal vein ("capillary") or mean petiole base vessel diameters scale with height, standardizing for leaf area across and within species.

Sample preparation and measurements

For 43 species we collected 1-3 leaves per species taken at the tree top from individuals of different height (0.32-38 meters, table S1). For the six species selected for the intraspecific dataset, tall individuals were selected to obtain a wide range of leaf distances from the base (Table 1). Twenty to twenty-five leaves per species were sampled from different individuals (3 to 12). Leaves were collected at different distances from the base (0.08 to 33.8 meters) throughout the tree height. Leaves were stored in 70% aqueous ethanol. We measured the distance from the petiole base to the base of the trunk for each leaf collected, following the branches, to approximate the conductive path length experienced by each leaf. For brevity, we refer to this variable as the "height" of each leaf. We measured leaf traits and anatomical features from these samples. We measured leaf area with an Epson Perfection V700 Photo scanner and WinFOLIA software (Regent Instruments Inc, Ste-Foy, Canada). We cut transverse anatomical sections of the petioles with a sliding microtome (GSL Microtome, WSL, Birmensdorf, Switzerland) at 15-20 μm. We stained the sections with a solution of safranin (1% in water) and astra blue (0.5% in water), washed them with water and alcohol to remove residual stain, dehydrated them in clearing agent, and mounted them in Entellan

mounting medium (Merck KGaA, Germany). We made paradermal sections of the leaf lamina to measure vessels in terminal leaf veins ("capillaries"). For leaf clearing, we immersed the leaves in a solution of sodium hydroxide (10%) 1-12 hours depending on the leaf thickness, abrading the lamina with a razor blade to remove layers of mesophyll in thick leaves. We then washed the leaves and placed them in bleach until they became transparent. For both petiole anatomical sections and lamina paradermal sections, we measured the diameters of 25 randomly selected vessels with the ocular micrometer of a light microscope at 100x magnification. We calculated mean values per leaf of petiole base vessel (Dpet) and terminal vein vessel (Dterm) diameters. The mean values were calculated dividing the sum of the vessel diameters by the number of vessels measured.

Table 1: *Information on species and collecting site selected for the intraspecific dataset.*

Species	Order, Family	Collection locality	Forest type
Albizia niopoides (Benth.) Burkart	Fabales, Fabaceae	Tuxtla Gutiérrez, Chiapas, Mexico	Lowland tropical dry forest
Cordia dodecandra A. DC.	Lamiales, Boraginaceae	Felipe Carrillo Puerto, Quintana Roo, Mexico	Subdecidous lowland tropical forest
Illicium mexicanum A. C. Sm	Austrobaileyales, Schisandraceae	Tlanchinol, Hidalgo, Mexico	Cool highland cloud forest
Manilkara zapota (L.) P. Royen	Ericales, Sapotaceae	Felipe Carrillo Puerto, Quintana Roo, Mexico	Subdecidous lowland tropical forest
Moringa stenopetala (Baker f.) Cufod.	Capparales, Moringaceae	Chamela area, Jalisco, Mexico	Dry tropical forest
Swartzia cubensis (Britton & Wilson) Standl.	Fabales, Fabaceae	Felipe Carrillo Puerto, Quintana Roo, Mexico	Moist subdecidous lowland tropical forest

Statistical analyses

There is a positive relation between leaf area and mean vessel diameter at the petiole base (Coomes et al., 2008), a relationship expected if vessels widen predictably with distance from the terminal veins to the petiole base. Given the striking pattern of terminal stem conduit diameter scaling with plant height (Fig. 1), it is unclear whether the distance of the leaf from the stem base may have any effect on petiole base vessel diameter or on tip-to-petiole base vessel widening within individual leaves. We tested two predictions to account for the scaling exponent of 0.2 observed in terminal twig vessel diameter with height across angiosperms

(Olson et al., 2014, 2018). In both cases we took leaf area into account because higher leaf area implies longer conductive path length, and therefore wider vessels, and so testing the effect of height on conduit diameters needed to standardize for leaf area. The first was that mean petiole base vessel diameter should scale with height in a tree and that terminal vein vessel ("capillary") diameter should remain unchanged, implying changes in tip-to-base vessel widening rate within leaves with height. The second assumed a constant tip-to-base vessel widening rate, and that mean petiole base diameter should scale with height as the result of predictable scaling with height of terminal vein vessel ("capillary") diameters.

To examine these possibilities, we log10-transformed data to meet normality and homoscedasticity assumptions, and fit linear regressions predicting mean petiole base vessel diameter or mean terminal vein vessel diameter based on leaf area and distance from the base at which each leaf was collected ("height"). We tested the significance of the interaction term between leaf area and height. For the intraspecific dataset, we fit linear mixed-effects models predicting mean petiole base vessel diameter or mean terminal vein vessel diameter based on height, leaf area, and their interaction. We fit models using the "nlme" package (Pinheiro *et al.*, 2014) in R (version 3.3.1, R Core Team, 2016), with species as the random effect (random intercept models). We compared models with and without this random effect to determine which best described the variance structure of the data. Models with random effects fit better than those without them, based on likelihood ratio tests and AIC. Non-significant effects or interactions were removed and we refitted the models. We calculated the $R2$ of the mixed effects model using the approach of Nakagawa and Schielzeth (Nakagawa & Schielzeth, 2013) with the r2glmm package (Jaeger, Byron. 2017. r2glmm: Computes R Squared for Mixed (Multilevel) Models. R package version 0.1.2. https://CRAN.R-project.org/package=r2glmm).

Results

Across all the analyzed leaves (236), mean petiole base vessel diameter varied from 9 to 66 μm and terminal vein vessel diameter varied from 3 to 15.5 μm. Leaf area spanned from 4 to 1271 cm2 across species. Rejecting both of our predictions, the linear models obtained for the interspecific dataset and the linear mixed-effects models obtained for the intraspecific dataset

showed that leaf area (as proxy for the leaf pathlength) was the only significant predictor of petiole vessel diameter (Fig. 2, Fig. 3).
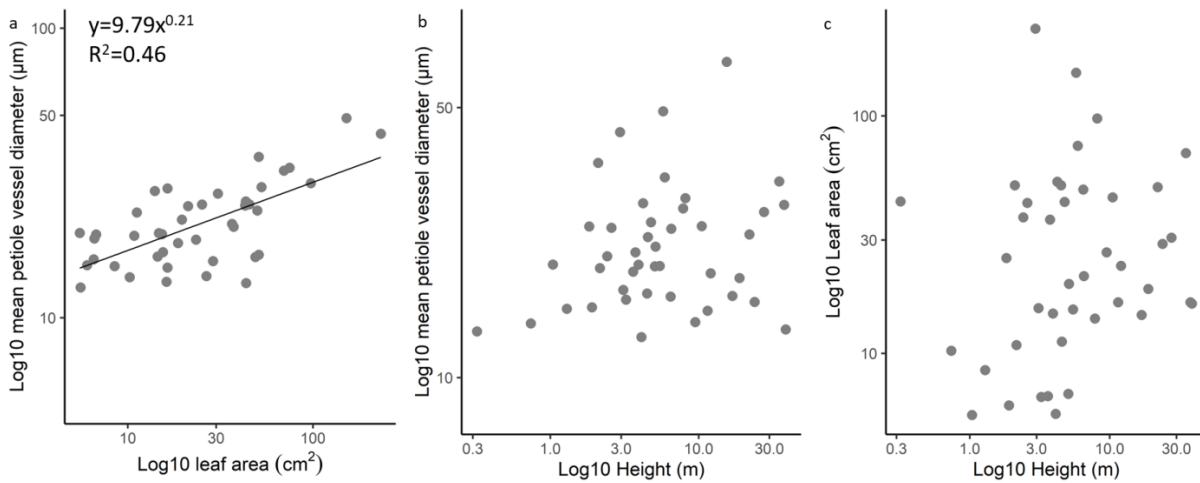

Figure 2: *Across species, mean petiole base vessel diameter is predicted by leaf area but not by height. (a) Leaf area strongly predicts mean petiole base vessel diameter, presumably because greater leaf area implies greater distance from the terminal leaf vessels. If vessels widen predictably with distance from the terminal end of the conductive stream, then greater leaf area should lead to predictably wider vessels. (b) Unlike the terminal twig vessels to which they are adjacent (Fig. 1), mean petiole base vessel diameter does not scale with height. (c) Leaf area does not scale with height, rejecting the possibility that wider leaf areas with height would lead to wider mean petiole base vessel diameter and therefore wider terminal twig vessel diameters. Axes log–log transformed. Points are species mean values from 43 species (Table S1).*
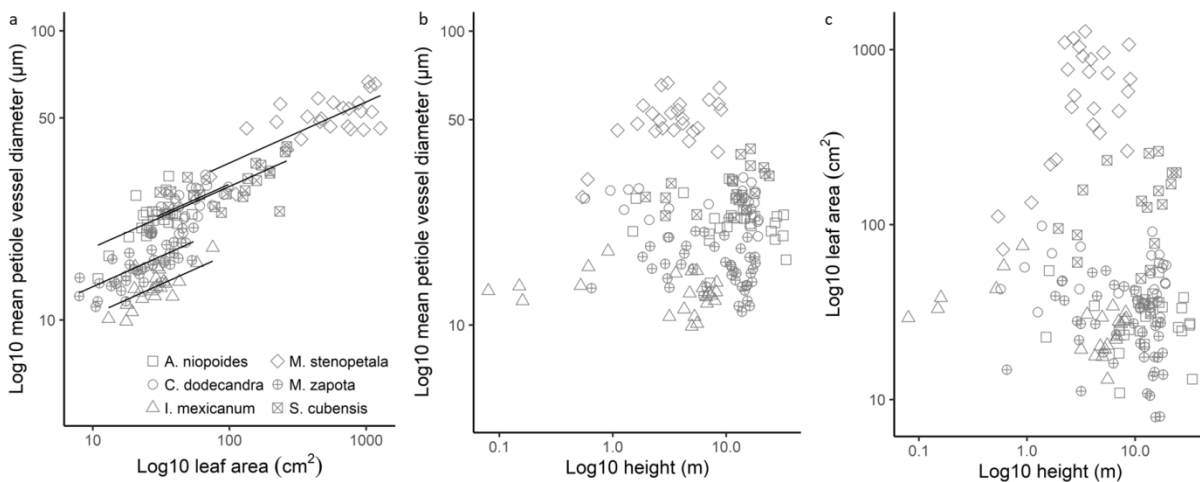

Figure 3: *As with the across-species dataset, within species, mean petiole base vessel diameter is predicted by leaf area but not by height. (a) Mean petiole base vessel diameter is strongly predicted by height across species with a similar slope by leaf area, though with slightly differing intercepts (Table 2, 2S). (b) Rejecting one of our main predictions, petiole base vessel diameter is not predicted by height in a tree. (c) As in the across species dataset (Fig. 2), leaf area does not scale with height within trees. Axes log–log transformed. Points are leaf mean values from 6 species, different symbols represent different species.*

In the model fit for the interspecific dataset, which predicted mean petiole base vessel diameter based on leaf area, the height at which the leaf was collected, and a leaf area height interaction term, the interaction term was not significant (P=0.282). In the model without the interaction term, height was non-significant (P=0.705), leaving the final model with leaf area as the only predictor and with a R2 of 0.42 (Table 2, Fig. 2). Likewise, in the mixed model fit for the intraspecific dataset, which predicted mean petiole base vessel diameter based on leaf area, the height at which the leaf was collected within the tree, a leaf area height interaction term, and the species random effect, the interaction term was not significant (P=0.468). In the model without the interaction, height was non-significant (P=0.998), leaving the final model predicting mean petiole base vessel diameter based only on leaf area and the species random effect (Table 2, Fig. 3). The Nakagawa and Schielzeth R2 was 0.46 for this model (Table 2, Fig. 3a). Species random intercepts are given in Table S2. Correlation between height and leaf area was very low in both datasets (interspecific dataset: -0.036 P<0.05; intraspecific dataset-0.18, P<0.05), excluding the possibility that height lost significance in the multiple regression model because of collinearity with leaf area (Fig 2b, Fig 3b)

Table 2: *Linear models predicting petiole base vessel diameter or terminal vein diameter based on leaf area.*

Linear models for the interspecific dataset				
	log_{10} *mean petiole base vessel diameter*		Log_{10} *terminal vein diameter*	
	N=42, R^2=0.42		N=41, R^2=-0.015	
Fixed effect	Coefficient (95% CI)	P-value	Coefficient (95% CI)	P-value
Intercept	0.997 (0.874, 1.120)	<0.0001	0.871 (0.738, 1.003)	<0.0001
Log_{10} leaf area	0.235 (0.149, 0.321)	<0.0001	-	-
Linear mixed- effect models for the intraspecific dataset				
	log_{10} *mean petiole base vessel diameter*		Log_{10} *terminal vein diameter*	
	N=159, R^2=0.46		N=143, R^2=0.001	
Fixed effect	Coefficient (95% CI)	P-value	Coefficient (95% CI)	P-value
Intercept	0.991 (0.885, 1.098)	<0.0001	0.847 (0.721, 0.974)	<0.0001
Log_{10} leaf area	0.212 (0.176, 0.248)	<0.0001	-	-

CI: confidence intervals.

We then predicted mean terminal vein vessel diameter for the 43 species (interspecific dataset), based on leaf area, the height at which the leaf was collected, and a leaf area height interaction term. The interaction term was not significant (P=0.882). In the model without the interaction, neither height (P=0.160) nor leaf area (P=0.522) were significant (Table 2, Fig. 4). Similar results were obtained for the intraspecific dataset, where the height leaf area interaction term was also non-significant (P=0.520). In the model without the interaction, neither height (P=0.329) nor leaf area (P=0.287) were significant (Table 2, Fig. 5). The models indicated that terminal leaf vein vessel diameter can be effectively regarded as invariant with leaf area and with the height at which a leaf was borne in the canopy (Table 2, Fig. 4, Fig. 5).
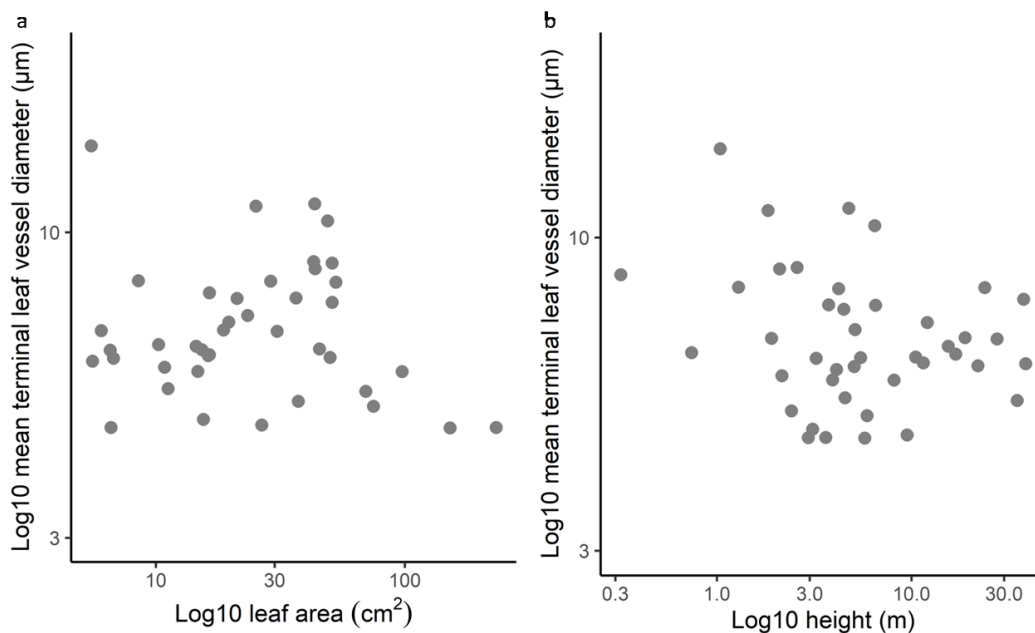

Figure 4: *Terminal leaf vein vessel diameter is invariant with leaf area or height. (a) Mean terminal leaf vein vessel diameter showed no pattern of predictable variation with leaf area. (b) Likewise, and rejecting one of our predictions, mean terminal leaf vein vessel diameter also did not vary predictably with distance from the stem base. That terminal leaf vein vessels are for all practical purposes invariant is consistent with assumptions of metabolic scaling theory. Axes log–log transformed. Points are species mean values from 43 species (Table S1).*
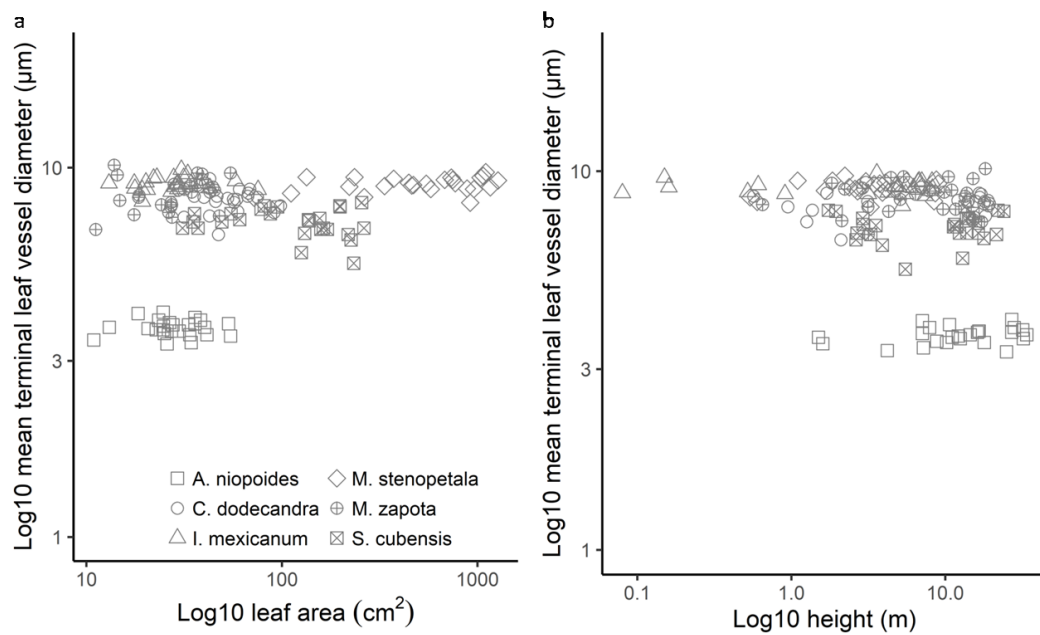
Figure 5: As with the across-species dataset, within species terminal leaf vein vessel diameter is invariant with leaf area or height. (a) Mean terminal leaf vein vessel diameter showed no pattern of predictable variation with leaf area and also (b) did not vary predictably with height (i.e. distance from the stem base). As in the across species dataset (Fig. 3), the invariance of terminal leaf vein vessels is consistent with assumptions of metabolic scaling theory. Axes log–log transformed. Points are leaf mean values from 6 species, different symbols represent different species.

Discussion

Though the leaves of a tree are often situated at vastly different distances from the stem base, sufficient water needs to reach all leaves, so identifying how the xylem pathway is involved in water supply to leaves is crucial. If all conduits were the same diameter, the leaves with shorter conductive pathlengths would experience lower resistance and therefore receive disproportionate amounts of water, while leaves farther from the base would be situated atop pathlenghts of greater resistance and would receive less water. In the stem, increase in conduit diameter with height has been reported in several studies, not only for vessel diameter at the stem base but also for mean vessel diameter in terminal twigs (Olson et al., 2014, 2018). These results come from studies of species-level mean vessel diameter from the topmost twigs of individuals across species. Studies within individuals are needed, but the comparative pattern implies that terminal twigs high in a plant, with longer pathlengths, have wider vessels compared to twigs closer to the stem base, with shorter pathlengths. It is plausible that wider terminal twig vessels would be favored at greater heights because the increase in diameter

could compensate for the increase in hydraulic resistance that accompanies increase in pathlength. Moreover, individuals that maintain an invariant amount of carbon invested in the active conduit network per unit leaf area during growth should be favored by selection over individuals whose carbon investment increases. If more distant leaves are supplied with conduits that are wider over their entire length, and thus of lower resistance than narrower conduits, then leaves higher in a tree could in principle be supplied by fewer conduits than leaves lower in the tree while minimizing per-leaf increases in carbon investment in the xylem with height growth. This hypothesis predicts that a given amount of leaf area lower in the tree should be supplied by more numerous, narrower conduits, and the same amount of leaf area should be supplied by fewer, wider conduits. Such a within-plant scenario would be consistent with findings of across-species scaling of topmost twig vessel diameter with height across species (Olson et al., 2014, 2018), as well as with predictions of metabolic scaling theory that selection should minimize the increase in sapwood carbon cost per-leaf area with height growth (Olson et al., 2018). Therefore, it is important to understand how leaves are involved in the scaling of terminal twig vessel diameter with height.

In contrast to our expectation that the predictable increase in terminal twig vessel diameter (Olson et al., 2014, 2018) with height (Fig. 1) would be reflected in vessel widening in leaves, our results showed no predictable pattern in mean petiole base vessel diameter or in terminal vein vessel diameter with distance of the leaf from the stem base. The variation in petiole vessel diameter was largely explained by leaf area (Fig. 2a, Fig. 3a, Table 2) (presumably because leaf area reflects conductive path length) and the diameter of the terminal vein vessels was invariant with respect to both leaf area and distance from the stem base (Fig. 4, Fig. 5, Table 2). These findings rejected both our hypotheses, and showed instead that the predictable widening of terminal twig vessel diameters across species is not paralleled in the leaves. Our data therefore suggest the following points with regard to the deployment of conduit diameters in tree crowns.

Our results suggest that selection has favored, just as in the stem, vessel widening from the narrow terminal leaf vein vessels to the wider vessels at the petiole base. If leaves with a larger leaf area had the same vessel diameter at the petiole base than leaves with smaller leaf area, they would experience higher hydraulic resistance and, since water flows in paths with lower hydraulic resistance, small leaves would receive more water. The observation of mean petiole vessel diameter scaling predictably with leaf area (Fig 2a, Fig, 3a, Table 2) is

consistent with predictions regarding selection favoring compensation of the increase in conductive path and thus in hydraulic resistance (Price & Enquist, 2007; Coomes *et al.*, 2008; Price *et al.*, 2009; Scoffoni *et al.*, 2012; Carvalho *et al.*, 2017; Gleason *et al.*, 2018). Because the diameters of vessels is one of the factors strongly influencing hydraulic resistance (as per the Hagen-Poiseuille law), vessels widening seems to buffer the increase in resistance imposed by the increase in leaf area (used as proxy for within-leaf conductive path length) and leaves of different leaf area in the crown would experience similar hydraulic resistance and would receive similar amount of water per unit of leaf area.

With regard to terminal leaf veins, neither leaf area nor height predicted terminal vein vessel diameter (Fig 4, Fig, 5, Table 2). In almost all life system, natural selection has favored hierarchical systems with invariant terminal units. Being vessels in terminal veins the terminal unit of the xylem from where the water diffuses out of the conductive system to the surrounding mesophyll, natural selection would favor individuals having invariant terminal vein vessels with maximal surface area for exchange of water. From the point of view of our results, terminal leaf vein vessels are for all practical purposes invariant as predicted by the assumptions of metabolic scaling theory for terminal units (West *et al.*, 1999; West & Brown, 2005).

Models of plant vascular architecture assume leaves to be hydraulically invariant with changes in plant size (West et al., 1999; McCulloh et al., 2003). These models predict that variation in vessel diameter among leaves and within leaves are not expected to change systematically as a function of plant height. This prediction underlies the practice of modeling the productivity of a branch, a tree, or a whole forest by simply summing leaf area (West *et al.*, 1999; Anfodillo *et al.*, 2013a; Jucker *et al.*, 2015). This practice implies that leaves are equivalent in terms of their performance regardless of size and height in a tree, and this equivalence of performance is only possible if leaves are supplied with similar amounts of water per unit leaf area regardless of position in the tree and as trees grow taller. Our results help point to the anatomical characteristics of the hydraulic system that allow leaf performance to remain constant (or at least minimize drops in performance) with height growth. Our results showed that leaf conduit diameters were not predicted by distance from the stem base, though they were predicted by leaf area. From the point of view of our results, leaves would seem, in terms of their conduit diameters, equivalent and essentially indifferent with respect to their position in the crown and thus to the pathlength from the stem base to the

terminal twig they are connected, consistent with the assumptions of hydraulic architectural models (Fig 6) (West et al., 1999).
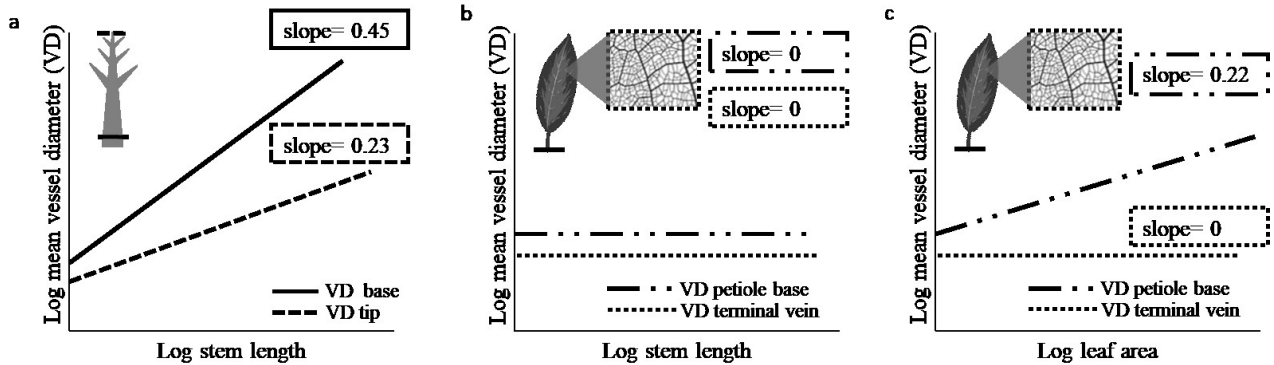

Figure 6: *Vessel diameter scale with height at the base of the stem and at the treetop, whereas vessels diameter in the leaf (both at petiole base and at terminal veins) are invariant with height and only mean petiole base vessel diameter is predicted by leaf area. This diagram illustrates how stem length predicts vessel diameter in the stem (a, see Fig 1). (b) Petiole base vessel diameter and terminal vein vessel diameter do not scale with height (i.e. stem length). Leaves would seem, in terms of their vessel diameters, equivalent and essentially indifferent with respect to their position in the crown and thus to the pathlength from the stem base to the terminal twig they are connected. (c) The mean petiole base vessel diameter is strongly predicted by leaf area presumably because greater leaf area implies greater distance from the terminal leaf vessels. Terminal leaf vein vessels do not showed any predictable variation with leaf area and, from the point of view of our results, they are invariant as predicted by the assumptions of metabolic scaling theory for terminal units.*

Recent studies focused on the predictability of leaf trait variation rejected the assumption of leaves as independent units with plant size, reporting a significant relationship between leaf area with plant height (Fonseca *et al.*, 2000; Price *et al.*, 2014; Gleason *et al.*, 2018). In these works, leaf area was studied with respect to the height of the plant, finding that taller plants have on average larger leaves. The focus of these studies was slightly different than the one taken here, because they did not examine the distance of each leaf from the stem base (our "height" variable), and thus it is not possible to evaluate whether there was a significant relationship between leaf size or vessel dimensions and their position with respect to the stem base. Our results using "height" showed that vessel dimensions of the leaves, controlling for leaf area, were invariant with respect to their distance from the stem base, consistent with the assumptions of hydraulic models.

The position of the leaf in the crown is often associated to different morphologies. Leaf exposed to high light irradiation (sun leaf) are smaller and thicker than leaves less exposed to

light (shade leaf) that are wider and thinner. Also differences in water hydraulic conductance and carbon assimilation have been reported, with sun leaves having higher leaf conductance and assimilation than the shade leaves (Sack & Holbrook, 2006; Sellin *et al.*, 2008; Scoffoni *et al.*, 2012). Leaf conductance is affected by mesophyll anatomy, biochemistry of the leaf and leaf xylem anatomy (such as the diameter and the number of the vessels) (Niinemets, 2010; Scoffoni *et al.*, 2015; Brocious & Hacke, 2016; Carins Murphy *et al.*, 2016). Our results suggest that with regard to the hydraulic system of the leaf, standardizing for leaf area, there should be no reason to expect variation in conduit diameter. If variations in petiole vessel diameter of leaves of same size are found to be predicted by light exposition, it will be important to investigate the causes in light of our results.

Selection has favoured individuals with a xylem system that widens from the tree top to the stem base and leaf xylem conduit diameters that vary independently of height. The comparative pattern of vessel diameter widening of both stem base and terminal twigs is well predicted by the height of the plant (Fig. 1), and a similar pattern could be expected within individuals, with terminal twigs with shorter pathlengths having narrower vessels than terminal twigs with longer pathlengths. This pattern in the stem suggests that natural selection favours a rate of vessel widening that compensates resistance and maintains constant sapwood carbon cost per unit leaf area. In our data, leaf area was the main, indeed only, driver of petiole vessel widening, consistent with the expectation that greater leaf area is associated with greater conductive path length and therefore wider vessels basally. With regard to the terminal leaf vein vessels, we found no ability of either leaf area or height within the plant to predict capillary diameter, consistent with the assumption of terminal units as invariant in metabolic scaling theory (West et al., 1999; Sack et al., 2012). Further research must focus on testing the pattern of within-individual terminal twig vessel diameter widening, and evaluate whether the described patterns of both stem and leaf are also observed in gymnosperms.

Supporting Information

Table S1: Information on species and collecting site selected for the interspecific dataset.

Species	Order, Family	Collection locality	Vegetation type	Habit	Average height/ stem length (m)
Annona coriacea Mart	Magnoliales, Annonaceae	Botucatu, São Paulo, Brazil	Savannah	tree	2.10
Anthurium scandens (Aubl.) Engl	Alismatales, Araceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	liana	1.04
Aralia excelsa (Griseb.) J.Wen	Apiales, Araliaceae	Chamela, Jalisco, Mexico	Dry tropical forest	tree	15.48
Aristolochia jaliscana Hook. & Arn.	Piperales, Aristolochiaceae	Chamela, Jalisco, Mexico	Dry tropical forest	liana	5.48
Bauhinia rufa (Bong.) Steud.	Fabales, Fabaceae	Botucatu, São Paulo, Brazil	Savannah	shrub	4.22
Byrsonima coccolobifolia Kunth	Malpighiales, Malpighiaceae	Botucatu, São Paulo, Brazil	Savannah	Tree	1.83
Byrsonima intermedia A.Juss.	Malpighiales, Malpighiaceae	Botucatu, São Paulo, Brazil	Savannah	shrub	1.29
Calatola costaricensis Standl.	Icacinales, Icacinaceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	tree	18.85
Campomanesia adamantium (Cambess.) O.Berg	Myrtales, Myrtaceae	Botucatu, São Paulo, Brazil	Savannah	tree	2.16
Cissus gossypiifolia Standl.	Vitales, Vitaceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	liana	37.7
Cochlospermum vitifolium (Willd.) Spreng.	Malvales, Bixaceae	Chamela, Jalisco, Mexico	Dry tropical forest	tree	8.14
Couepia grandiflora (Mart. & Zucc.) Benth. ex Hook.f.	Malpighiales, Chrysobalanaceae	Botucatu, São Paulo, Brazil	Savannah	tree	2.58
Crispiloba disperma (S.Moore) Steenis	Asterales, Alseuosmiaceae	Daintree, Queensland, Australia	Tropical rainforest	shrub	4.49
Crossopetalum uragoga (Jacq.) Kuntze	Celastrales, Celastraceae	Chamela, Jalisco, Mexico	Dry tropical forest	straggling shrub	1.91
Davilla elliptica A.St.-Hil.	Dilleniales, Dilleniaceae	Botucatu, São Paulo, Brazil	Savannah	liana	5.07
Eupomatia bennettii F.Muell.	Magnoliales, Eupomatiaceae	Daintree, Queensland, Australia	Tropical rainforest	shrub	0.32
Eupomatia laurina R.Br.	Magnoliales, Eupomatiaceae	New South Wales, Australia	Temperate rainforest	tree	11.50
Guaiacum coulteri A.Gray	Zygophyllales, Zygophyllaceae	Chamela, Jalisco, Mexico	Dry tropical forest	tree	4.12
Hedyosmum mexicanum C.Cordem.	Chloranthales, Chloranthaceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	tree	10.50
Lippia balansae Briq.	Lamiales, Verbenaceae	Botucatu, São Paulo, Brazil	Savannah	shrub	3.24
Liquidambar styraciflua L.	Saxifragales, Altingiaceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	tree	23.84
Machaerium cobanense Donn.Sm.	Fabales, Fabaceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	liana	22.00
Mansoa verrucifera (Schltdl.) A.H.Gentry	Lamiales, Bignoniaceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	liana	38.70
Miconia albicans (Sw.) Steud.	Myrtales, Melastomataceae	Botucatu, São Paulo, Brazil	Savannah	tree	3.75
Miconia sp	Myrtales,	Botucatu, São Paulo, Brazil	Savannah	shrub	0.74

|	Melastomataceae				
Myriocarpa longipes Liebm.	Rosales, Urticaceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	tree	5.76
Otopappus tequilanus (A.Gray) B.L.Rob.	Asterales, Compositae	Chamela, Jalisco, Mexico	Dry tropical forest	liana	6.54
Pennantia cunninghamii Miers	Apiales, Pennantiaceae	New South Wales, Australia	Temperate Rainforest	tree	2.42
Piper	Piperales, Piperaceae	Chamela, Jalisco, Mexico	Dry tropical forest		2.95
Piptocarpha rotundifolia (Less.) Baker	Asterales, Compositae	Botucatu, São Paulo, Brazil	Savannah	tree	3.10
Plenckia populnea Reissek	Celastrales, Celastraceae	Botucatu, São Paulo, Brazil	Savannah	tree	3.62
Pyrostegia venusta (Ker Gawl.) Miers	Lamiales, Bignoniaceae	Botucatu, São Paulo, Brazil	Savannah	liana	7.85
Rinorea guatemalensis (S. Watson) Bartlett	Malpighiales, Violaceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	tree	9.50
Siparuna thecaphora (Poepp. & Endl.) A.DC.	Laurales, Siparunaceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	shrub	4.77
Sparattanthelium amazonum Mart.	Laurales, Hernandiaceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	liana	12.07
Stryphnodendron adstringens (Mart.) Coville	Fabales, Fabaceae	Botucatu, São Paulo, Brazil	Savannah	tree	4.55
Styrax ferrugineus Nees & Mart.	Ericales, Styracaceae	Botucatu, São Paulo, Brazil	Savannah	tree	5.12
Tabebuia ochracea A.H. Gentry	Lamiales, Bignoniaceae	Botucatu, São Paulo, Brazil	Savannah	tree	5.91
Trichostigma octandrum (L.) H.Walter	Caryophyllales, Phytolaccaceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	liana	27.60
Trimenia moorei (Oliv.) Philipson	Austrobaileyales, Trimeniaceae	New South Wales, Australia	Temperate rainforest	liana	16.90
Vatairea lundellii (Standl.) Record	Fabales, Fabaceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	tree	35.00
Vernonanthura phosphorica (Vell.) H.Rob.	Asterales, Compositae	Botucatu, São Paulo, Brazil	Savannah	shrub	3.93
Vochysia guatemalensis Donn. Sm.	Myrtales, Vochysiaceae	Los Tuxtlas, Veracruz, Mexico	Tropical rainforest	tree	6.47

Table S1. Random intercepts per genus for the model predicting mean petiole base vessel diameter based on leaf area and species as a random effect.

Species	Intercept
Albizia niopoides	0.932
Cordia dodecandra	0.939
Illicium mexicanum	0.699
Manilkara zapota	0.796
Moringa stenopetala	1.012
Swartzia cubensis	0.931

CHAPTER 3

Axial anatomy of the leaf midrib provides new insights into the hydraulic architecture and embolism patterns of *Acer pseudoplatanus* leaves.

Distance from the leaf tip (L)
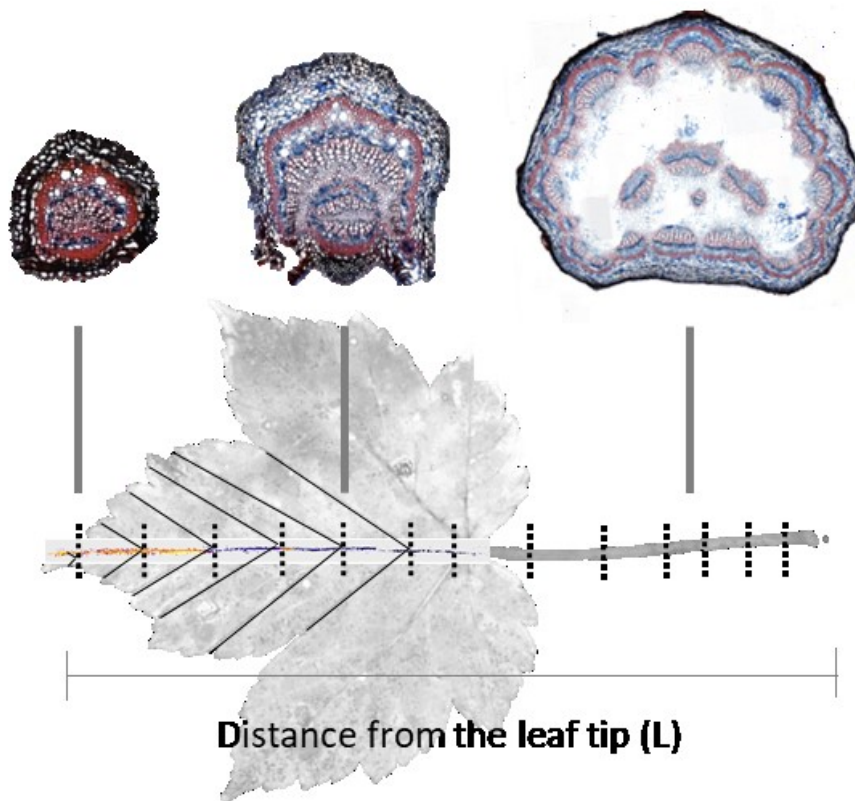
Axial anatomy of the leaf midrib provides new insights into the hydraulic architecture and embolism patterns of *Acer pseudoplatanus* leaves

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Author contribution:
SL designed the study and coordinated sample collection and data analyses, and prepared the first manuscript draft. PG and MG contributed to measurements and analyses. TA designed the study. All authors contributed to discussion and critically contributed to the final manuscript version.

Summary

The structure of leaf veins is described with a hierarchical scheme (e.g. midrib, 1st order, 2nd order), which may overlook the axial variation in the xylem conduits from leaf tip (or margin) to the base of each single vein of a given order. We hypothesise that xylem conduits within the same vein order change considerably, with consequences on the water potential gradient and resistance to embolism.

We measured the hydraulic diameter (*Dh*), and number of vessels (*VNo*) along the midrib and petioles of *Acer pseudoplatanus* leaves. We estimated the leaf area supplied (LA$_{sup}$) at different points of the midrib and how the variation in anatomical traits affected embolism resistance.

Our results showed that the *Dh* scales with leaf length (L) with a power of 0.42, and the VNo scales hypo-allometrically with LA$_{sup}$ (0.66). The total conductive area scales isometrically with the LA$_{sup}$. Embolism events along the midrib occurred first in the basipetal part and afterwards at the leaf tip where vessels are narrower.

In all measured leaves, xylem conduit traits vary greatly within the same vein order, making it possible to predict the progress of embolism within the leaf blade.

Introduction

The leaf is the terminal part of the water transport system in plants and is where the largest amount of hydraulic resistance is located. The physiological mechanisms and morphological traits of leaves largely determine how the plant deals with water deficit. The control of stomatal conductance induced by leaf dehydration is the primary mechanism involved to avoid (or limit) embolism events (Scoffoni *et al.*, 2017a). However, in prolonged conditions of soil water shortage, the leaf veins are prone to embolism. Major veins are reported to be more vulnerable to embolism than minor veins (Brodribb *et al.*, 2016b,a; Scoffoni *et al.*, 2017b), suggesting that the xylem conduit lumen relevantly affects the transport system safety. If the size of conduits in the veins drives the susceptibility to embolism, with wider conduits more vulnerable to embolism, then a detailed pattern of conduit size variation within the leaf blade would allow a prediction of how embolism events might spread throughout the hydraulic network.

Different hydraulic models have been proposed to explain the variation in conduits diameter and conductive area in the leaf. For angiosperms, the models are based on the hierarchical vein pattern that is grouped by branching level from the principal veins (i.e. midrib) (Hickey, 1973; Ellis *et al.*, 2009; Sack *et al.*, 2012) (Fig 1a). Models based on Murray's law would predict an increase in the total conductive area moving from the midrib to higher vein orders (terminal thin veins) (McCulloh *et al.*, 2003, 2004; Gleason *et al.*, 2018), whereas models based on da Vinci's rule (i.e. the pipe model) would predict a preservation of the conductive area (Price *et al.*, 2013; Carvalho *et al.*, 2017). Both models predict a decrease in the conduits diameter towards the leaf tip, but with a different degree of tapering. Moreover, the variation in diameter is predicted to occur only when the branch rank changes implying a hierarchical network.

One alternative option for dealing with continuous tubes (i.e. not exclusively a branched network) is to apply the approach developed by West *et al.* (1999) for the hydraulic system in plant stems and branches. This approach predicts a widening of the conduits from the stem apex towards the base that follows a pattern that is well fitted by a power law with exponent $\approx$ 0.2 (West *et al.*, 1999; Anfodillo *et al.*, 2006). This degree of widening can almost compensate for the increase in hydraulic resistance due to plant growth in height (i.e. increase

of path length). Empirical data have shown that the degree of widening is universal in the stem xylem of all plants and always falls close to 0.2 (Anfodillo *et al.*, 2006; Olson *et al.*, 2014, 2018). It is therefore possible to apply the same approach for predicting how conduit diameter (*Dh*) widens with length of the vein in a leaf (*L*), by using the equation:

$$Dh = aL^b$$

Eqn 1

Where *a* is the cells diameter measured at the most distal veins, L the distance from the leaf tip and *b* is the experimental degree of widening (the exponent).

The few available data relative to the axial variation in conduit diameter along the major veins would suggest that in leaves the degree of widening is higher (i.e. twofold) than that in conduits of stems and branches (Coomes *et al.*, 2008; Petit & Anfodillo, 2013). This observation suggested two alternative hypotheses on the architecture of the vascular network. If the network of leaf veins was structured following a simple pipe model (i.e. with an isometric relationship between number of stomata and number of conduits) there would be an over-compensation for the increase in resistance with leaf length. Moreover, the high degree of widening would result in wide vessels at the base of the petiole, more sensitive to embolism. Alternatively, it is possible to hypothesise that the number of conduits is not constant along the leaf, but that the narrow conduits at the leaf tip would progressively merge in wider conduits towards the leaf petiole. In this case, we would expect a hypo-allometric relationship between the number of conduits and number of stomata (or leaf area) that, combined with a higher degree of vessels widening, would possibly lead to preservation of the theoretical flow as predicted by a widened pipe model.

To test if the hydraulic system is essentially a pipe model or if it is instead composed of a series of tubes that can merge towards the petiole, it is necessary to measure the number of conduits from leaf tip to base of the petiole and to test the scaling with the supplied (downstream) leaf area.

Here, we tested the hypothesis that conduits in the leaf veins widen from leaf tip to base of the petiole with a high degree of widening (compared to the one reported for the stem) coupled with a degree of conduit merging towards the petiole. Furthermore, considering that wider vessels may be more vulnerable to embolism, we hypothesise that embolism along the midrib should always initiate at the base of the leaf where conduits are wider.
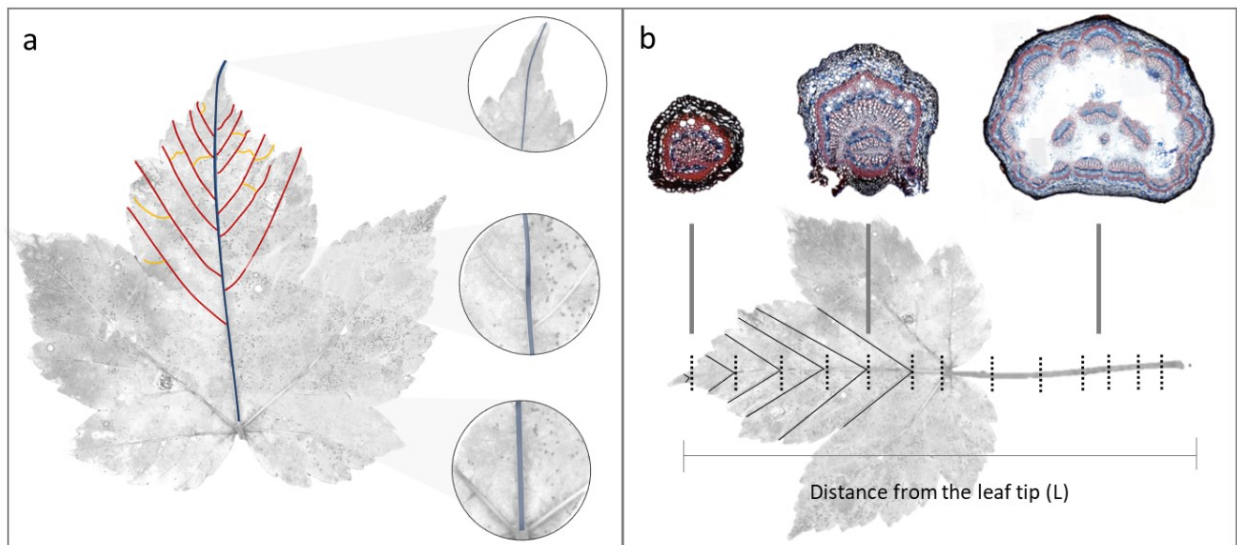
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Figure 1: (a) Schematic representation of the hierarchical subdivision of veins in orders: midrib in blue, examples of second order veins in red and examples of third order veins in yellow. A zoom in of the leaf midrib at different distance from the leaf tip highlight the difference in vein diameter. (b) Schematic representation of the different measurements taken along the midrib. Dashed lines represent the points at which the anatomical sections have been performed. Solid lines represent the estimation of leaf area supplied for each section.

Material and Methods

Plant material

We collected five apical shoots of young *Acer pseudoplatanus* plants growing in a wooded area of San Vito di Cadore (North of Italy). The sample collection was performed at the end of the growing season (August 2016) to ensure that leaves were fully developed. The leaves were preserved in 50% alcohol. Seven healthy leaves were selected for the measurements. For each leaf we measured the leaf area (*LA*) and we prepared several anatomical sections along the midrib (ca. 10-16 depending on leaf size) (Fig. 1).

Anatomical sections were obtained at different distances from the leaf tip. Hypothesising a steep change in the anatomical trait mostly in the distal part, we performed sections at each centimetre for the first 3 cm and every 2 for the rest of the leaf lamina and petiole (Fig.1b). The sections were embedded in paraffin and cut at 14 μm with a rotary microtome Leica RM 2125 (Leica Biosystems, Nussloch, Germany). We used a solution of safranine (1%) and astra blu (0.5%) to stain the preparations for highlighting the lignified cells and better distinguishing the xylem vessels, and then we permanently fixed them on slides with Eukitt

(BiOptica, Milan, Italy). Slides were scanned with a D-Sight 2.0 scanner (A. Menarini diagnostic, Firenze, Italy) at 100x magnification, and images were analysed with ROXAS software (von Arx,www.wsl.ch/roxas). High magnification and the use of ROXAS allow for a fine analysis of the cells and accurate information to be obtained on the number of vessels (*VNo*), total conductive area of the section (*CTA*, calculated as the sum of the cell areas) and hydraulic weighted diameter (*Dh*) measured as:

$$Dh = \Sigma d^5 / \Sigma d^4$$
Eqn 2

We estimated the diameters of the vain bundle sheath for each cross section (*DV*).

Leaf area (*LA*) was obtained by scanning the leaf lamina (1000 dpi) and analysing the image with imageJ (Rasband, 2018, https://imagej.nih.gov/ij/). The high quality scans allowed for the estimation of the supplied leaf area (*LA$_{sup}$*) at different distances from the leaf tip. Considering the position of the anatomical sections we divided the leaf area following the 2nd and 3rd order veins and we cumulated the area sectors when moving from leaf tip to petiole (Fig. 1b). For this estimation, we assumed that the water flow is unidirectional from the base of the lamina to the tip and that the distal part of the midrib serves only the distal part of the leaf lamina following principles of hydraulic resistance. We estimated the vessels density (*VD*) per unit of leaf area along the midrib as the number of conduits divided by the supplied leaf area.

Vulnerability to embolism

Three young (1or 2 years old) healthy saplings of *Acer pseudoplatanus* were sample grown in a wooded area of San Vito di Cadore (North of Italy) in middle of August. The plants were cut from the base of the root collar at predawn and, immediately after excision, placed in black plastic bags with damp paper towels to prevent further water loss and transported to the laboratory. To avoid potential artefacts associated with open vessels, we collected the entire saplings.

For each sapling we selected one healthy and fully developed apical leaf and used the optical method proposed by Brodribb *et al.* (Brodribb *et al.*, 2016b,a) to capture embolism events in the midrib. Images were taken with a reflex camera every minute for 24 hours in control laboratory conditions (temperature 22°-24° C, 40-50% relative humidity). Due to the

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thickness of the midrib we performed the measurement in reflective mode (see http://www.opensourceov.org).

Image sequences were analysed with ImageJ (National Institutes of Health, Bethesda, MD, USA). to identify embolism events, seen as changes in the reflection of the midrib. We used the toolbox "OSOV" (https://github.com/OpenSourceOV) to perform image subtraction of subsequent images to reveal rapid changes in light reflection. Embolism events were thresholded, allowing automated counting of each event using the analyse-stack function. Noise was removed using the outlier removal. The temporal colour maps of embolism events were created by colouring the embolism area in each sequence using a colour scale over time, with the first event of embolism at time 0.

Statistical analysis

Data were Log10-transformed in order to meet normality and homoscedasticity assumptions. We used the lmoled2 package in R (version 3.3.1, R Core Team, 2016) to assess type-II linear regressions with reduced major axis (RMA) in order to account for potential errors in both x and y variables. We tested how the anatomical traits varied with distance from the leaf tip (L), and/or the supplied leaf area (LA_{supp}).

Results

The anatomical traits of the xylem along the midrib varied markedly. At the distal part of the leaf lamina (leaf tip), the xylem is composed of narrow vessels with high density per unit of leaf area. Moving from leaf tip to petiole base the general observed pattern is that xylem conduits widen in diameter and decrease in density (Fig. 2a, 3). No major changes in anatomical traits were observed at the junction between leaf lamina and petiole.

The vessels diameter widened rapidly from leaf tip to the base of the petiole by more than a factor 4 (thus more than a factor 16 in vessel area). At the leaf tip the *Dh* was 5.5-6.5 µm and in the petiole c.a. 20-25 µm. The widening pattern was well fitted by a power law with exponent 0.42 if plotted vs. distance from the leaf tip (L) (R^2=0.88, CI exponent=0.39;0.45, Table 1) (Fig. 2a).

Vein diameter also widened with distance from the leaf tip, with an exponent of 0.59 (R^2=0.87, CI exponent=0.51; 0.67, Table 1), significantly higher than the one found for the

relationship between *Dh* and distance from the leaf tip (Fig. 2). The *Dh* scaled with vein diameter with an exponent of 0.81 (R^2=0.91, CI exponent=0.72;0.89, Table 1) (Fig. 2c).
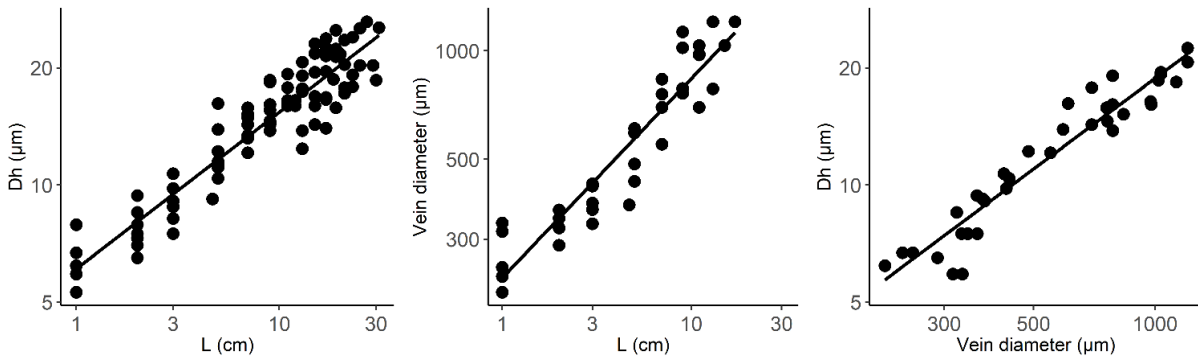

Figure 2: Variation of (a) the hydraulic diameter (Dh) of the vessels with distance from the leaf apex (L), vein diameter with distance from the leaf apex (L) and Dh with the vein diameter. The data were log–log transformed data. The equation of the functions, the R2, p-values and 95% CI are reported in table 1.

The number of conduits increased from a few tens to a hundred when moving from the first centimetre of the leaf blade to the petiole. VNo scales with LA_{sup} with an exponent of 0.66 (Fig. 3a) significantly different from 1 (slope C.I.=0.62; 0.70, R^2=0.92). The vessels number per unit of leaf area (i.e. vessels density, *VD*) varied along the midrib. Higher density was observed in the firsts cm (*VD* >50/cm^2) of the leaf tip and steeply decreased towards the leaf lamina (*VD*< 12/cm^2) reaching a plateau along the petiole (Fig. 3b).
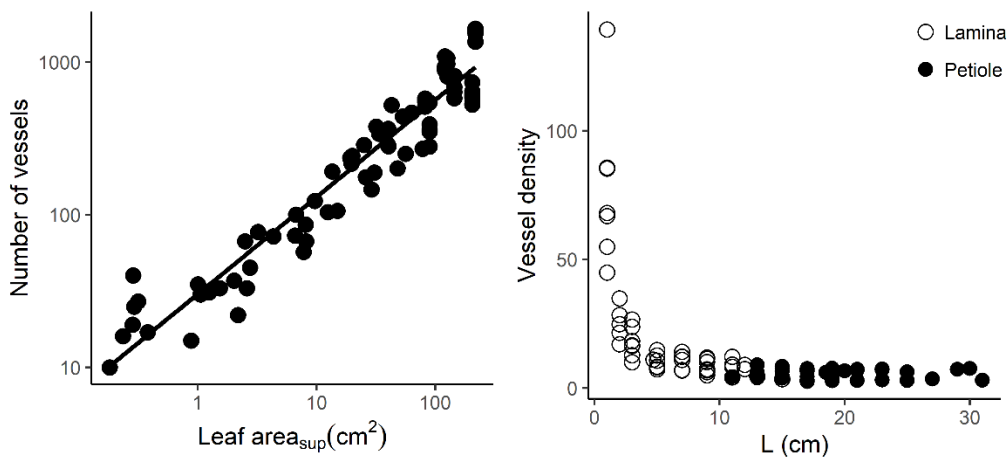

Figure 3: (a)Variation of the number of vessels (VNo) with the supplied leaf area (LA$_{sup}$) for log–log transformed data. The equation of the function is y=27.54x$^{0.66}$. The regression has a R2 of 0.92, (CI exponent =0.61;0.70) (Table 1). (b) variation of the vessels density (VD) with the distance from the leaf apex. Empty circles correspond to measurement on the lamina whereas filled circles measurements on the petiole.

The total conductive area (mean vessel area multiplied by number of vessels) scaled isometrically with the supplied leaf area with an exponent of 0.98, not different from 1 (slope C.I. = 0.94; 1.03) (R^2 of 0.97, Table 1) (Fig. 4).
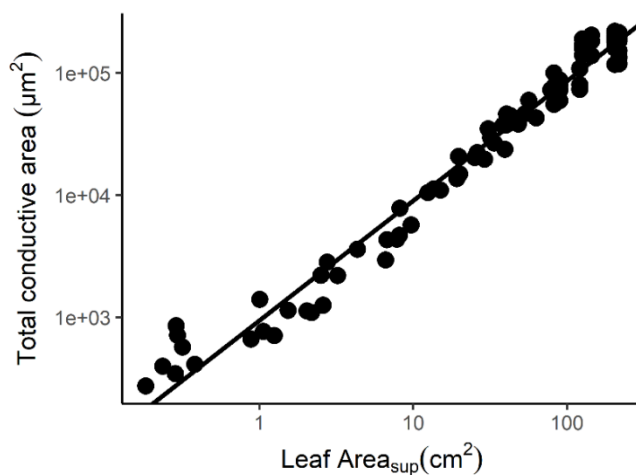

Figure 4: Variation of the total conductive area (CTA) with the supplied leaf area (LA_{sup}) for log–log transformed data (CTA= cumulative area of all vessels for each section). The equation of the function is $y=933x^{0.98}$. The regression has a R2 of 0.97 (Table 1).

The first embolism event always occurred in the basal part of the midrib at about 7-10 hours after the sapling was excised (time=0). Embolism at the leaf tip occurred about 150-200 minutes later, and continued for about 6-7 hours (500-540 minutes) (Fig. 5).
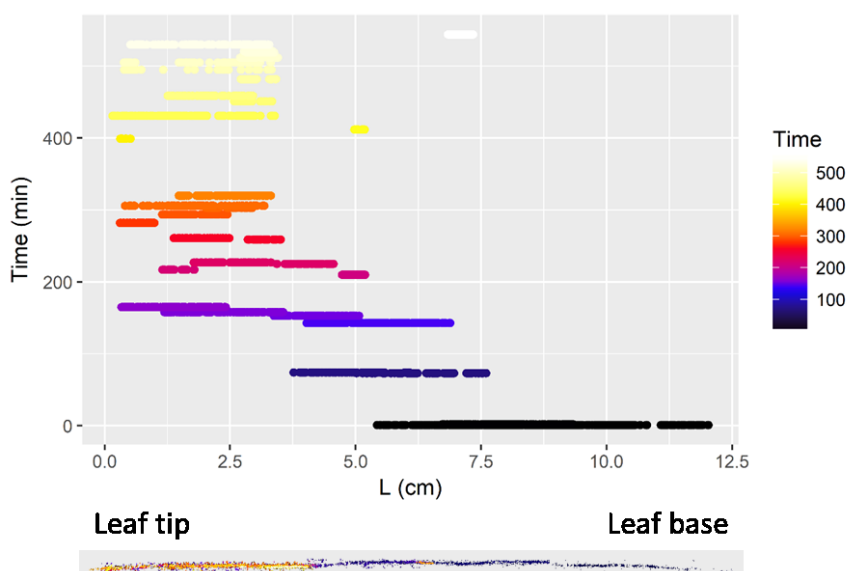

Figure 5: Time at which embolism events occurred (Time) along the midrib (L, leaf tip = 0). The first embolism event was set at time = 0. Below the graph is reported a colour map of all embolisms recorded along the midrib during the desiccation.

Discussion

In this paper, we propose a different approach for studying the structure and functionality of veins that is based on distance from the leaf tip instead of on pure hierarchical schemes. This approach allows us to find strict allometric relationships that might be successfully used for making predictions on the main traits of the vascular structure of the midrib, namely vessel diameter, vessel number and, as a consequence, progress of the embolism within the leaf. Such predictions are difficult to obtain from a hierarchical approach, which prioritises the branching order of the veins instead of the length of the vein itself. Commonly, veins are of different length and this, rather than their order, may affect the anatomy and thus physiology of the leaf vascular network.

We observed a clear variation in vessels diameter within the same vein order (i.e. the midrib) of a sycamore maple leaf. The diameter of the conduits increased along the midrib and petiole with a degree of widening more than double that reported for the stem xylem (Anfodillo *et al.*, 2006; Petit *et al.*, 2008; Olson *et al.*, 2014), but similar to those reported in the literature for leaves of ash species (Petit & Anfodillo, 2013). The variation is not coupled with any change in the hierarchical vein order, suggesting that midrib widening is mainly driven by the length of the vein itself or, in other words, by the distance from the terminal part of the veins.

The vessels widening is necessary for guaranteeing a constant hydraulic conductance for all possible paths, which may differ in length, similarly to what has been predicted to occur in trees (West *et al.*, 1999). Leaves located at different heights within the crown will be supplied uniformly thanks to vessel widening along the stem and roots that isolates the majority of resistance in the narrower terminal parts of the transport system. The fact that all parts of the leaf blade must be supplied uniformly was implicitly demonstrated by the observation that the stomatal density did not differ systematically in different parts of the leaf (e.g. proximal vs. distal) (Fiorin *et al.*, 2016). Moreover, the sizes of the conduits found at the leaf tip are similar to those reported for minor veins (Carvalho *et al.*, 2017), suggesting that conduit size is mainly determined by the path length and not constrained by vein order. Data from species distributed globally found no relation between the size of minor veins and leaf area (Sack *et al.*, 2012). The quite conserved size of minor veins guarantees an equal distribution of hydraulic resistance within the leaf lamina and among leaves, allowing for an equal distribution of water in the crown.

This result is further supported by the observation on embolism events. The terminal part of the midrib embolizes after the central and basal parts indicating that the terminal vessels are more resistant to embolism, due to their narrow diameters. Similar results were found for leaves of different species, where embolism initially occurred in the main larger vessels of the midrib followed by second order veins and minor veins (Brodribb *et al.*, 2016a). The anatomical variation we found along the midrib affected the embolism resistance within the same vein order, again supporting the hypothesis that distance from the terminal veins is a good predictor for understanding the leaf from both structural and functional perspectives.

Since longer veins must also have a larger difference in vessel size (i.e. wider elements at the base compared to the tip) we could predict that susceptibility to embolism differs in relation to vein length. Brodribb *et al.* (2016a) showed that the amplitude of temporal evolution of embolism (i.e. the time between the beginning and end of embolism events) in the first order veins is much wider than in veins of the third order. This could be explained by the fact that third order veins are generally shorter and with narrower amplitude of sizes, inducing more synchronous embolism.

As hypothesised, the variation in number of conduits per supplied leaf area was hypo-allometric, implying that the midrib architecture does not follow the widened pipe model prediction. The change in vessels density suggests that the narrow distal vessels merged into fewer larger vessels along the midrib and that these remain rather constant in number along the basal part of the lamina and along the petiole where there are no vein junctions as in the leaf blade. The variation in vessels density may be related to the diverse role of the conduits along the leaf lamina. As shown in the study of Price *et al.* (2013), the vein network passes from a Murray's law-like system in the minor veins, to an area preserving system in the larger main veins. If a higher number of conductive elements may be favoured in the terminal part of the lamina to implement water distribution to the terminal unit (i.e. vein loops and stomata), a decrease in vessel number along the basal part of the midrib may compensate for the carbon cost.

The combination of a high degree of widening and the degree of vessels merging from the leaf tip to petiole base resulting in a strong isometric relationship between the total conductive area and supplied leaf area highlights the importance of coordination in leaf tissues. This conformation might allow the best trade-off between safety and efficiency since narrower conduits showed higher resistance to embolism than wider ones, also in accordance with previous findings (Hacke *et al.*, 2006; Brodribb *et al.*, 2016b,a; Scoffoni *et al.*, 2017b; Olson

et al., 2018). The high degree of widening isolates narrow conduits at the terminal part of the water transport path, where the majority of total hydraulic resistance is confined, whereas the number of conduits supports water flow to the terminal units.

Our results suggest that, when the entire vein length is considered, the variation in anatomical and functional traits in the midrib is as great as among orders and that leaf length and/or supplied leaf area should be considered in leaf architectural models. Awareness of the widening pattern of conduits in the veins might help in predicting the evolution of embolism in the leaf blade in relation to water stress. Further studies are needed to evaluate if the results found for the leaves of *Acer pseudoplatanus* are consistent with other species and in the different vein orders.

Negligible tension gradient in long stems: leaves account for the majority of the whole hydraulic path's resistance
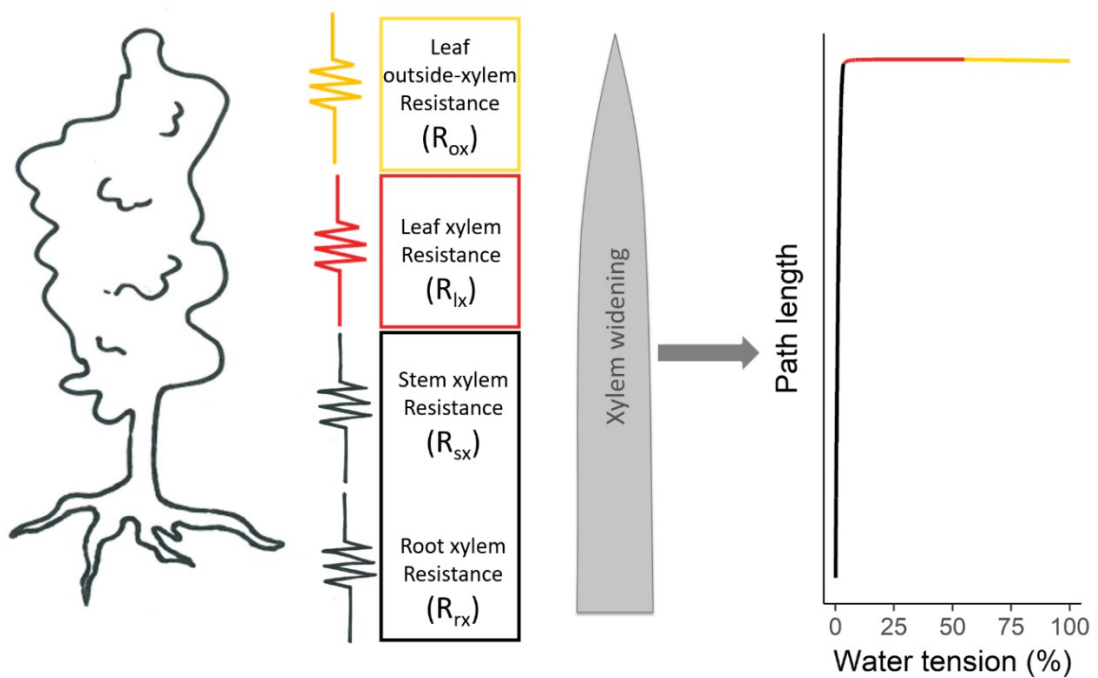
Negligible tension gradient in long stems: leaves account for the majority of the whole hydraulic path's resistance

Running head:

Negligible tension gradient in long stems

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Author contribution:
SL and NK collected and prepared sampled, carried out measurements and analyses and prepared the first manuscript draft. GP designed the study and coordinated sample collection and contributed to data analyses. All authors contributed to discussion and critically contributed to the final manuscript version.

In preparation

Summary

Xylem conduits widen in diameter from leaves to roots, but the role of this anatomical pattern in shaping the leaf-to-root gradient of xylem water potential (Ψxyl) is still unknown.

We used data of conduit diameter variation along the leaf/needle and stem axes for two angiosperms (*Acer pseudoplatanus*, *Fagus sylvatica*) and one gymnosperm (*Picea abies*) to model the axial variation of the total resistance (R_{CUM}) and Ψxyl along the leaf-stem hydraulic path.

The majority of R_{CUM} (from 79 to 98%) is confined within the leaf/needle, where conduits are the narrowest. Consequently, a steep gradient of water potentials theoretically develops from the mesophyll cells until the leaf/needle insertion into the stem, from where it becomes rather flat, with water potentials in the range of those of roots.

Our analyses would suggest that the Ψxyl developing along stems and branches is less negative (up to one order of magnitude) than the general belief. Incorporating a more correct development of Ψxyl along the longitudinal plant axis into hydraulic models might help to better elucidate on mechanisms and dynamics of embolism spread and recovery, the development of positive xylem pressures and, in general, the plants' resistance to water stress.

Introduction

According to the cohesion-tension theory for the plant water relations, the water within the xylem flows against gravity from the soil to the canopy due to the lower water potential developed in the transpiring leaves (Dixon & Joly, 1895; Angeles *et al.*, 2004). The evaporation of water in the mesophyll and the consequent vapor diffusion in the atmosphere (i.e. leaf transpiration) is the mechanism driving the water uplift. The surface tension at the liquid/vapour interface does not overcome the adhesion forces in the nanopores of the mesophyll cell walls inside the substomatal cavities (estimated in the range of 5-10 nm, (Tyree & Zimmermann, 2002)), determining the formation of water menisci inside these cavities and the transmission of subatmospheric pressures along the whole hydraulic path until the rootlets. The water lost with transpiration is replenished by the bulk water flowing from roots to leaves through a network of dead and hollow conduits (i.e. the xylem) in a metastable liquid phase (Nobel, 2012; Brown, 2013).

According to the Darcy's law, the water flow is determined by the ratio between the difference in water potential between two points along the hydraulic path ($\Delta\Psi$) and the total hydraulic resistance (R) of the medium passed through (Reid *et al.*, 2005):

$$F = \Delta\Psi/R \hspace{4cm} \text{Eq. 1}$$

The $\Delta\Psi$ between leaves and soil (Ψ_{leaf} - Ψ_{soil}) is strongly influenced by the surrounding environmental conditions. When the atmosphere is dry and hot, the transpiring leaves loose more water from the mesophyll cell walls, with even lower water potential (i.e. higher tensions) being transmitted down along the xylem path. Instead, when soil gets drier, their water potentials lowers accordingly, determining a reduction in $\Delta\Psi$ between leaf and soil.

The total resistance of the plant's hydraulic system is given by cumulating the resistances along the path length (R_{CUM}) (Fig. 1). At the extremes of the hydraulic path (i.e. in the leaf mesophyll and rootlets), water moves along a very short symplastic path characterized by a high hydraulic resistance (Cochard *et al.*, 2004; Sack & Holbrook, 2006). For the nearly entire path length, water flows via the apoplastic path of xylem conduits. The whole path resistance of a given xylem hydraulic pipe connecting roots to leaves is determined by the size of the conduits, and their pits' properties (e.g., density and pore size) controlling the radial movement of water between adjacent conduits. According to the Hagen-Poiseuille's law, the hydraulic resistance (R) of a single xylem conduit's lumen can be assimilated to that of a

capillary tube, and as such being proportional to its length (l) and inversely proportional to the fourth power of its diameter (d):

$$R = \frac{128 \cdot \eta \cdot l}{\pi \cdot d^4}$$ Eq. 2

where η is the dynamic viscosity of water (10^{-9} MPa at 20°C). Because R scales with d^4, the diameter variation of the conduits connected in series in a hydraulic pipe can have a great effect on its total hydraulic resistance. Despite xylem conduits are known to vary in size (both diameter and length) along branches, stem, and roots (Sanio, 1872; Tyree & Zimmermann, 2002), and pit number and size have been reported to be strongly correlated to conduit dimensions (Becker *et al.*, 2003; Sperry *et al.*, 2006; Lazzarin *et al.*, 2016), yet the effects of these anatomical patterns on the axial gradient of xylem water potential (Ψxyl) along the SPAC have been rarely considered. Most often the total xylem resistance has been considered as a sort of "unit resistance", so that the water potential is assumed to vary linearly between the two extremes of the hydraulic path (i.e., Ψxyl scales isometrically to the path length) (e.g., Venturas *et al.*, 2017). Applying such an approach implicitly assumes that xylem does not vary in conductivity along the longitudinal plant axis although variation in water potential along the stem have been reported in previous studies (Tyree *et al.*, 1991; Tyree, 1997).

Conversely it has been widely demonstrated that the diameter of xylem conduits continuously varies along the stem, being rather narrow at the tree top and becoming progressively wider towards the stem base (Anfodillo *et al.*, 2006, 2013b, Olson *et al.*, 2014, 2018). The scaling of conduit diameter with the distance from the stem apex commonly follows a trend well approximated to a power function ($Y=a \cdot X^b$), with the rate of conduit enlargement per unit of path distance being steeper close to the tree apex and gradually reaching a plateau towards the stem base. The scaling exponent (b) of the basipetal widening of stem's xylem conduits has been commonly reported to converge to the value of 0.2-0.25 irrespective of species, tree size or environmental conditions (Anfodillo *et al.*, 2006, 2013b, Olson *et al.*, 2014, 2018). The hydraulic consequence of such an axial configuration is that wider conduits basally add progressively lower contribution to the total resistance. As a consequence, R_{CUM} will progressively increase with the distance from the stem apex (L) (Mäkelä & Valentine, 2006), but much less than linearly, so that most of R_{CUM} remains confined within a very short distance from the stem apex (West *et al.*, 1999; Petit & Anfodillo, 2009; Petit *et al.*, 2010).

The elements of the stem xylem are directly connected to the elements composing the leaf xylem network, where conduits are on average narrower than those in the stem, and decrease

in diameter from the petiole to the narrow minor veins (Coomes *et al.*, 2008; Sack *et al.*, 2012; Petit & Anfodillo, 2013). Moreover, recent studies have highlighted that the total leaf's hydraulic resistance is substantially determined in equal proportion by the resistances in series of the leaf xylem and of the outside-xylem paths in the mesophyll (Cochard *et al.*, 2004; Trifiló *et al.*, 2016; Scoffoni *et al.*, 2017a).

This work aims to evaluate the effect of the axial variation in conduit diameter along the whole leaf-to-root hydraulic path in shaping the root-to-leaf gradient of water potential (Ψx). We combined our own measurements on axial variation in conduit diameter along the main leaf/needle axial vein with published data on the axial variation from the stem apex to base for two angiosperms (*Acer pseudoplatanus* L. and *Fagus sylvatica* L.) and one gymnosperm species (*Picea abies* (L.) Karst.). We implemented these data into a simple hydraulic model to assess the effect of distribution of resistances along the hydraulic path in shaping the gradient of water potential along the entire vascular path from the ultimate (i.e., distal) element of the leaf/needle venation network to the rootlets (Fig. 1).
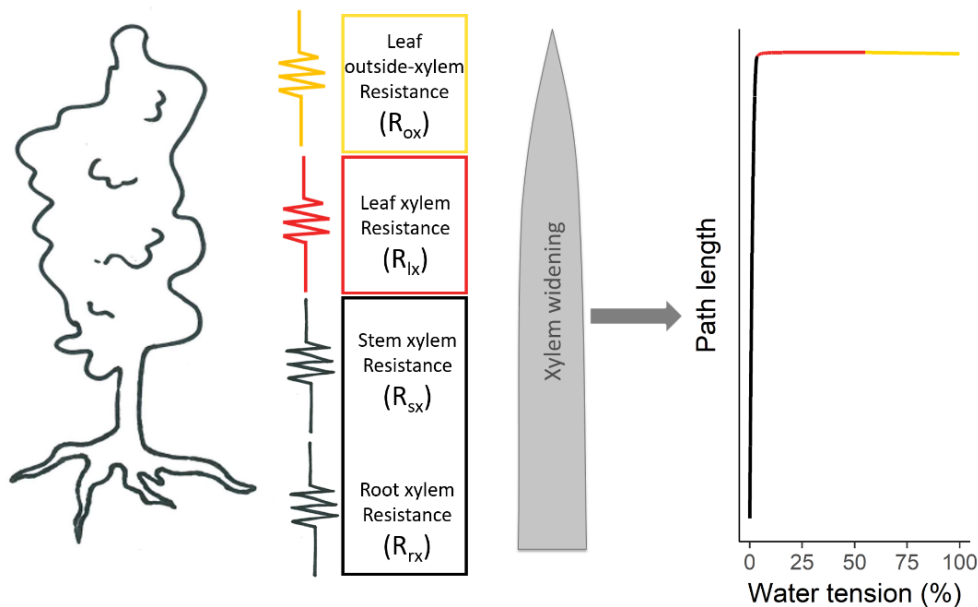
Fig. 1: Graphic representation of the distribution of the different hydraulic resistances connected in series along the hydraulic path from the roots to the leaf mesophyll. Due to the axial variation of conduit diameter along the axes of leaves (yellow) and stems (black) and to the magnitude of the outside-xylem resistance in the leaves (red), almost the total water tension gradient is confined within the leaf (red, yellow lines) whereas the variation in the stem is almost negligible (black line).

Material and methods

Anatomical analyses

We focused our analyses on two angiosperms (*Acer pseudoplatanus* L. and *Fagus sylvatica* L.) and one gymnosperm (*Picea abies* (L.) Karst).

At the end of the growing season 2017 (to ensure that the leaves were fully developed), we collected three to seven leaves/needles per species from trees growing at the botanical garden of the University of Padova (http://www.ortobotanicopd.it/en). For each leaf/needle, we cut 3-10 segments at different distances from the leaf/needle tip, and embedded them in paraffin (Anderson & Bancroft, 2002). Micro-sections were then cut with a rotary microtome Leica RM 2245 (Leica Biosystems, Nussloch, Germany) at 14-15 μm. For the leaves, we performed sections every 2 cm along the midrib and petiole whereas needles of *P. abies* were cut along the longitudinal axis at every ~1mm. Sections were stained with a solution of safranin and Astra blu (1% and 0.5% in distilled water, respectively) and permanently fixed on glass slides with Eukitt (BiOptica, Milan, Italy). Slides of leaf sections were scanned with a D-Sight 2.0 scanner (A. Menarini diagnostic, Firenze, Italy) at 100x magnification and relative images analyzed with ROXAS v. 3.0.139 (von Arx & Dietz, 2005; von Arx & Carrer, 2014) for the automated measurement of the xylem vessel diameters. Slides of the needle sections were observed under a light microscope (Nikon Eclipse80i; Nikon, Tokyo, Japan) connected to a digital camera at 400x magnification, and the diameter of the six biggest tracheids was manually measured using a linear measurement tool. For each leaf/needle section, the hydraulic diamter (*Dh*) was calculated as:

$$Dh = \Sigma d^5 / \Sigma d^4 \qquad\qquad\qquad \text{Eq. 3}$$

where *d* is the diameter of a given vessel.

For the same four species, we obtained from published and unpublished data the estimated *Dh* at different distances from the stem apex.

Statistical analyses

Data of *Dh* and distance from the leaf/needle tip (*L*) were Log10-transformed in order to meet the normality and homoscedasticity assumptions, and fitted with a linear regression to

estimate the *y*-intercept and the widening coefficient (i.e., the slope *b*) for each species and organ (leaf/needle or stem) (Table 1).

Hydraulic model

For each species, the estimated basal widening pattern of conduit diameter along the leaf midrib/needle axis and further below along the stem were implemented into a hydraulic model for the assessment of the axial variation in the cumulative path resistance and in the xylem water potential along the longitudinal hydraulic path of a theoretical tree of 30 meters height.

We calculated the hydraulic resistance (*r*) of each conductive elements of the hydraulic path according to Hagen-Poiseuille (Eq. 2), assuming a fixed conduit length of 1 mm. The cumulative path resistance (R_{CUM}) was calculated as the sum of all conduits starting from the apical (i.e., distal) element and moving down until the stem base.

R_{CUM} was calculated according to three different compositions of the axial hydraulic path: (i) considering only the xylem conduits of the stem (R_{sx}) (cf. West *et al.*, 1999; Becker *et al.*, 2003; Petit & Anfodillo, 2009); (ii) considering the xylem conduits of the stem and leaf midrib/needle ($R_{sx} + R_{lx}$); (iii) considering the stem, the leaf and also the outside-xylem (R_{ox}) resistance as the most distal element of the hydraulic path. We estimated the R_{ox} equal to 50% of the total leaf/needle resistance, as reported in literature (Trifiló *et al.*, 2016; Scoffoni *et al.*, 2017) ($R_{sx} + R_{lx} + R_{ox}$).

At each mm along the hydraulic path, the water potential was estimated according to Eq. 1 and the estimation of water flow (*F*), under the assumption of the conservation of mass. The total path resistance was calculated as the sum of the single element resistances and an arbitrary $\Delta\Psi$ was applied between the most distal and the proximal element of the hydraulic path. The water potential at the base of a given xylem element (Ψ_{X1}, considered cylindrical) was calculated as:

$$\Psi_{X1} = F * r + \Psi_{X0}$$
<div align="right">Eq. 4</div>

where *r* is the conduit resistance and Ψ_{X0} is the water potential at the base of the next xylem element.

We then expressed Ψ_X as relative variation in tension between the most apical (distal) element of the hydraulic path (i.e., 100% of tension) and the stem base (0% of tension).

We also ran a model simulation to assess the theoretical effects of different soil ($\Psi soil$ from 0 to -2 MPa) and air dryness (Ψair from -40 to -80 MPa) (Brown & van Haveren, 1972) on the variation in the water potential (Ψ_X) along the whole hydraulic path from the substomatal

cavities until the soil/root interface. The water potential calculated for the apical element of the hydraulic path, (i.e., at the outside xylem level) accounted also for the leaf osmotic adjustment to compensate for the gravitational pressure drop of 0.01 MPa/m.

Results

The diameter of xylem conduits in the angiosperm leaves widen along the midrib from the leaf tip to the petiole (Fig. 2, 3) following a power trajectory characterized by a scaling exponent of b=0.41 (CI 95%: 0.37; 0.44, Table 1) for *A. pseudoplatanus* and b=0.33 for *F. sylvatica* (CI 95%: 0.18; 0.47, Table 1), which represent widening factors approximately twice as large as those reported in literature for the species' stem (Table 1). At the junction between petiole and stem, the dimensions of xylem conduits were similar (Fig. 3). Instead, in the analysed conifer *P. abies* the needle tracheids did not significantly vary in diameter axially and were around 3.5 µm in diameter (Fig. 2, 3, Table 1). Comparing the tracheid size of needles *vs*. those at the stem apex (Fig. 3, Table 1), it resulted that at the junction between needle's petiole and the stem, the variation in tracheid diameter was rather steep.
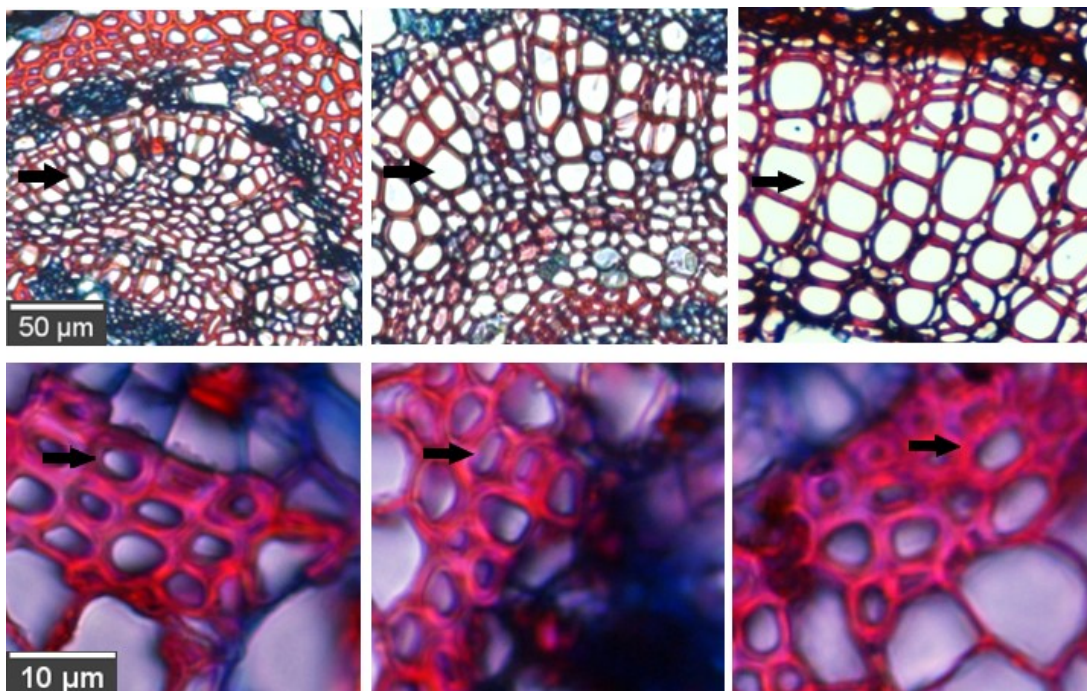

Figure 2: *Anatomical sections taken at 20, 60, 180 mm from the leaf tip for Acer pseudoplatanus (upper images) and at the tip, middle and base of a Picea abies needle of 15 mm in length. The black arrows indicate the xylem conduits.*

Table 1: Parameters (y-intercept, a; slope, b; their 95% confidence intervals; R² and p-value) of the linear regressions of Log10Dh vs. Log10L describing the conduit widening along the leaf/needle or stem longitudinal axis, where L (in mm) is the distance from the apical element of the leaf/needle or stem, respectively. Data of Dh along the stem were taken from literature (Anfodillo et al., 2006; Petit et al., 2008).

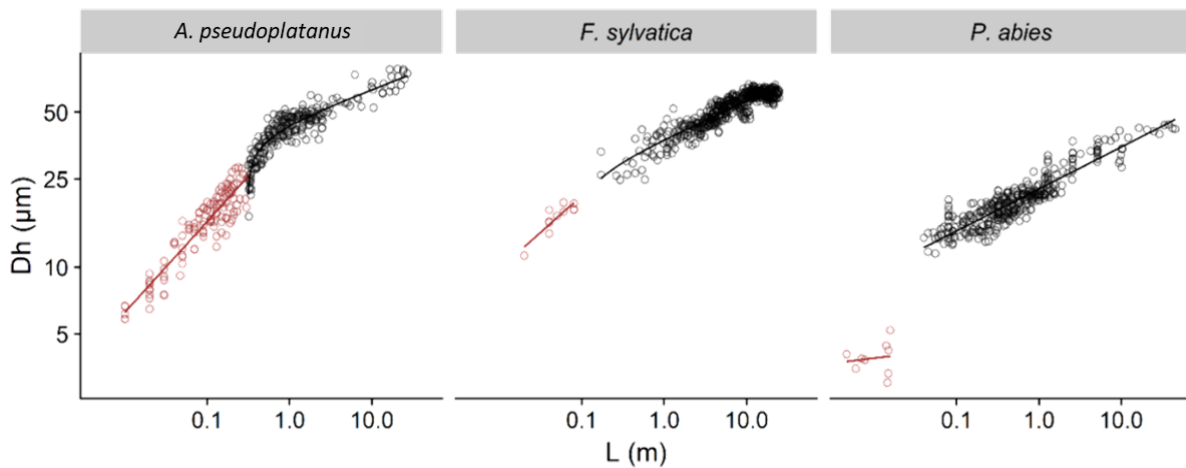| *Species*		*a*	*b*	R^2	*p-value*	*a 95% CI*	*b 95% CI*
Acer pseudoplatanus	*Leaf*	0.39	0.41	0.85	**9.32e-49**	0.32; 0.45	0.37;0.44
	Stem	1.22	0.14	0.85	**4.68e-94**	1.20; 1.25	0.13; 0.15
Fagus sylvatica	*Leaf*	0.66	0.33	0.75	**2.4e-6**	0.41; 0.90	0.18;0.47
	Stem	1.34	0.13	0.79	**3.3e-164**	1.32; 1.35	0.13;0.14
Picea abies	*Leaf*	0.54	0.04	0.02	0.36		
	Stem	0.85	0.16	0.82	**7.1e-140**	0.83;0.87	0.16;0.17

Figure 3: Variation of the hydraulic diameter (Dh) with the distance from the apex of leaf/needle (L) along the leaf/needle (red symbols) and stem (black symbols, data from Anfodillo et al., 2006; Petit et al., 2008) axes. Power scaling parameters of the relationship between Dh and axial length assessed separately for leaves and stems are reported in table 1.

The estimated variation of the cumulative resistance (R_{CUM}) with the increasing path length (L) showed that the hydraulic resistances are not homogenously distributed along the hydraulic path (Fig. 4), but elements of progressively highest resistance are located towards the apical regions.

Along the longitudinal axis of the stem, R_{CUM} increased from the apex downwards at rates progressively decreasing towards the stem base (Fig. 4a). When the contribution of leaf/needle conduits (Rlx) to the total path resistance was added to that of stem's conduits (Rsx), the model predicted that 65% and 95% of the total hydraulic resistance was confined within the needle in conifers and within the leaf midrib in angiosperms, respectively (Fig. 4b). When also outside xylem resistance (Rox) was considered in the total path resistance (i.e. 50% of the total leaf/needle resistance), almost the entire R_{CUM} (98% for *A. pseudoplatanus*, 92% for *F. sylvatica* and 85% for *P. abies*) was confined at the leaf level (Fig. 4c).

Accordingly, the same trends were predicted for the variation of the percentage of water potential from the leaf tip to the base of the stem, with the steepest gradients been confined within the leaf/needle (Fig. 4d).
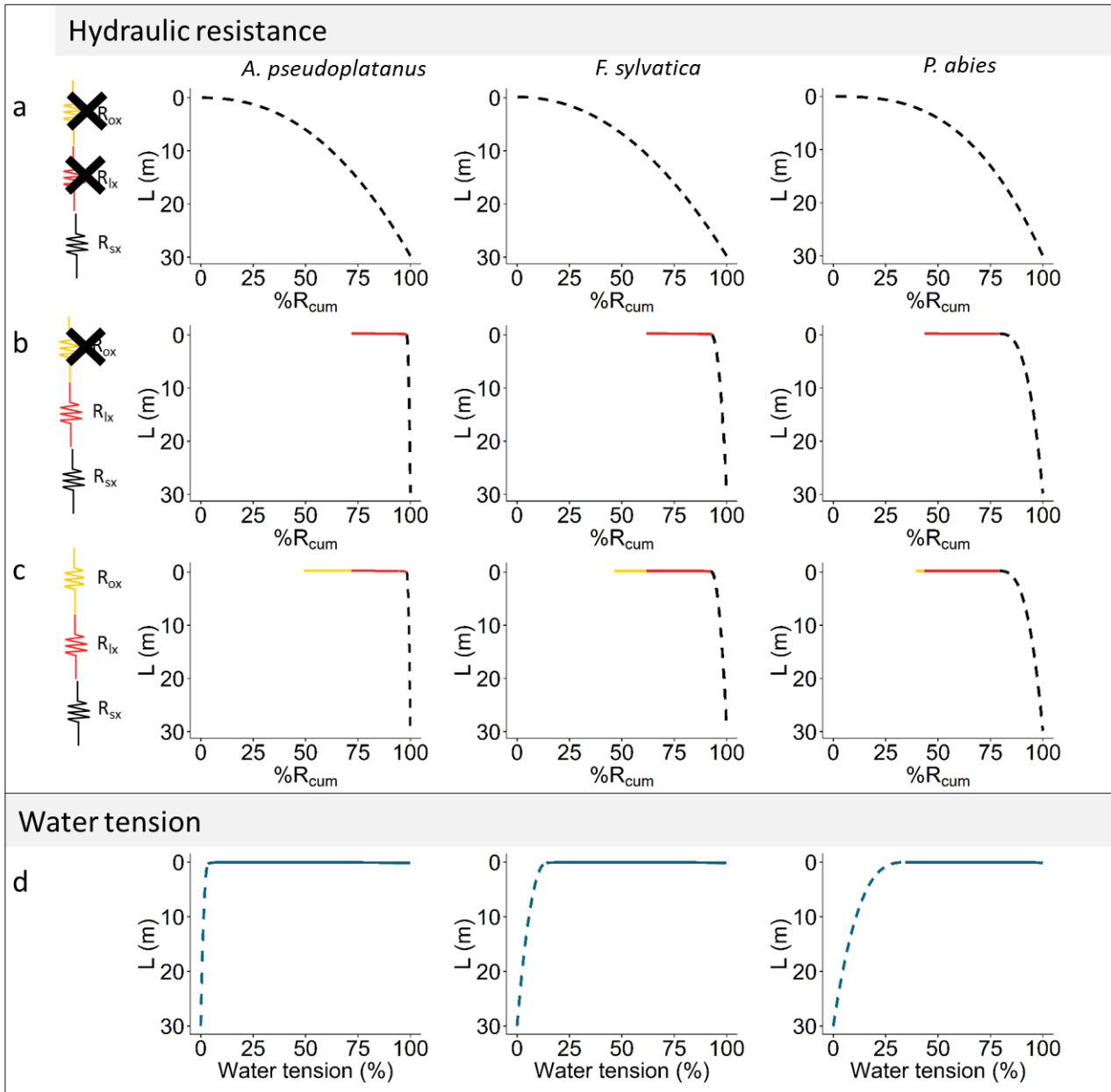
Figure 4: *Relative axial variation of the cumulated resistance (R_{cum}) with the path length (L) considering (a) only the stem vasculature (R_{sx}, black dotted line), (b) the integrated vasculatures of stem and leaves/needles (R_{sx} + R_{lx}, black + red line), and (c) the total pathway, i.e. accounting also for the outside-xylem resistance in the leaves/needles (R_{sx} + R_{lx} + R_{ox}, black + red+yellow line). (d) Relative variation in tension between the most apical (distal) element of the hydraulic path (i.e., the air/liquid interface on the mesophyll cell walls, 100% of tension) and the stem base (0% of tension). The dotted and solid lines represent the hydraulic path along the stem and leaf/needle, respectively.*

By running a series of simulations for considering all the resistances along the hydraulic path (*Rsx+Rlx+Rox*), and applying virtually possible conditions of soil water potentials ($\Psi soil$=0 to -2 MPa) and air water potentials in the substomatal cavities (Ψair=-40 to -80 MPa), the model predicted that the gradient of xylem water potential rapidly increases along the leaf midrib from the tip to the petiole (Fig 5, dotted line, simulation made for *A. pseudoplatanus*). Notably, at the petiole base, the Ψx is predicted not to reach very low values, and is mostly dependent on $\Psi soil$ and much less influenced by the air dryness.
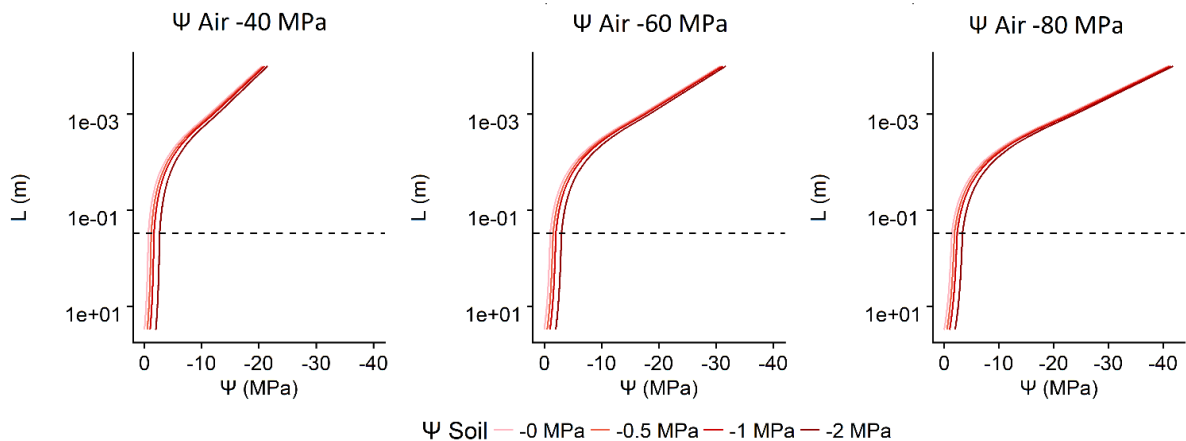
Figure 5: Theoretical effects of different soil ($\Psi soil$ from 0 to -2 MPa) and air dryness (Ψair from -40 to -80 MPa) on the variation in the water potential (Ψ) along the whole hydraulic path from the most apical (distal) element of the hydraulic path (i.e., the air/liquid interface on the mesophyll cell walls) until the soil/root interface (L = 30 meters). The dotted line indicates the separation between the hydraulic paths along leaf and stem.

Discussion

Our results provided insights on how the integrated axial organization of the xylem vascular system of stem and leaves/needles may affect the distribution of resistances along the hydraulic path, with relevant consequences for the shape of the water potential gradient from the terminal element of the leaf/needle down to the stem base, and for both the hydraulic safety and efficiency of the transport system.

Our anatomical analyses of the xylem vascular system support the hypothesis that the size of conduit elements substantially depends on their position along the hydraulic path (Anfodillo *et al.*, 2006, 2013b). At the leaf level, we encountered a clear difference between the two angiosperms and the gymnosperm species. While in the conifer the tracheid elements did not significantly increase in diameter along the longitudinal axis of the short needles, angiosperm vessels progressively increase in diameter along the main leaf venation from the leaf tip until the petiole. According to the comparison between our measurements and the anatomical data reported in literature for the same species (Anfodillo *et al.*, 2006; Petit *et al.*, 2008), at the junction between leaf/needle and the xylem of the stem, the diameter difference between stem's and needle's tracheids in *P. abies* seemed to be higher than that between stem and petiole vessels in angiosperms. Moreover, the rate of basal conduit widening (i.e., the scaling exponent *b*) was higher along the leaf venation than that reported for the stem alone, in agreements with previous reports (Coomes *et al.*, 2008; Petit & Anfodillo, 2013).

Our data of conduit diameter were limited to only three species, but were consistent with the hypothesis of stable scaling patterns of conduit diameter with the path length in leaf venations (Coomes *et al.*, 2008; Petit & Anfodillo, 2013) and stem axis across species (Anfodillo *et al.*, 2006; Olson *et al.*, 2014). Furthermore, it is worth mentioning that the contribution of the end-wall resistance was estimated to be a constant fraction (56-64%) of the total lumen resistance in both angiosperms and gymnosperms (Sperry *et al.*, 2006), in agreement with the reported isometry between pit aperture and conduit diameter (Becker *et al.*, 2003; Lazzarin *et al.*, 2016). Moreover, our simulations did not imply any capacitive effects such as those of structures developing along the hydraulic path (e.g., ray parenchyma, phloem, bark, etc.).

In our simulations, it appeared clear that the total hydraulic resistance (R_{CUM}) progressively cumulates from the stem apex towards the base. Even though this increase is much less than linear (Petit & Anfodillo, 2009), yet the resistance is not completely stabilized with increasing

path length (Mäkelä & Valentine, 2006) (Fig. 4a). When we considered together both the leaf/needle and stem vascular systems in our simulations, R_{CUM} increased sharply above 80% for a distance corresponding to the leaf/needle length suggesting that the stem's contribution to the total hydraulic resistance is nearly negligible. Leaf/needle length, its conduit widening, and the variation in conduit size at the junction between leaf/needle and stem were the main factors affecting the increase in R_{CUM} from the leaf/needle tip down along the hydraulic path to the stem base. We encountered a difference between our angiosperms and the *P. abies* in the xylem anatomical design at the junction between leaf/needle and the stem. We found no basal tracheid widening along the short conifer needles, associated to a large difference in tracheid diameter between needle and stem at their junction. Instead, anatomical data of our angiosperms revealed a continuous increase in xylem conduit diameter from the apical midrib's element downwards along its longitudinal axis and further down along the stem until its base. In both cases, the consequence is a hydraulic segmentation of the foliar element, along which most of R_{CUM} would remain concentrated, thus minimizing the contribution of the stem's xylem to R_{CUM}. Notably, such a contribution became even weaker when we accounted also for the contribution of the living cells of the outside-xylem hydraulic pathway to R_{CUM}. In our simulations we implemented a conservative approximation of the outside-xylem resistance (R_{OX}), being equal to 50% of the total leaf/needle hydraulic resistance in both angiosperms and conifer. However, R_{OX} had been reported to vary between 40 to 88 % of the total leaf resistance across angiosperm species (Cochard *et al.*, 2004; Trifiló *et al.*, 2016; Scoffoni *et al.*, 2017a), but no data are available for conifer needles. However, these results indicate in general that the higher is the contribution of R_{OX} to the total leaf resistance, than the lower is the contribution of the stem's xylem to the total hydraulic resistance of the whole transport system (R_{CUM}).

Steep variations in hydraulic resistivity are suggested to determine hydraulic segmentations of different portions of the hydraulic path, with important consequences for the axial gradient of water potential and for the spread of gas emboli (Tyree & Zimmermann, 2002). According to the reported axial anatomical pattern of xylem conduit diameter along the whole leaf-to-roots path, a hydraulic segmentation would originated as leaves/needles becomes nearly hydraulically "uncoupled" from the stem, with the former containing nearly the total gradient of negative water potential, whereas down along the latter the gradient would become rather flat, with water potentials approximating that at the root/soil interface. Consequently, our model would predict that, in case of water saturation of soil ($\Psi_{SOIL} \approx 0$ MPa), the water

potential developing along the stem would not be much low, even if air dryness is very high (i.e. high vapor pressure deficit - VPD), thus avoiding high risks of embolism formation, irrespective of stomatal control of transpiration (Martin-StPaul *et al.*, 2017).

The prediction of our model that the difference in water potential between the stem apex and base is very low conflicts with the common view that the water potential along the stem increases linearly from leaves (Ψ_{LEAF}) to soil (Ψ_{SOIL}) (e.g.,Venturas *et al.*, 2017). Moreover, the prediction of a very steep tension gradient along the leaf venation network is likely hard to be properly tested. However, it is worth mentioning that Ψ_{LEAF} measured with the Scholander's pressure chamber represents only an average water potential of the different leaf tissues (Scholander *et al.*, 1964), thus preventing the possibility of a precise assessment of the water potential gradient at different positions along the leaf venations, and perhaps estimating a more negative water potential than what would precisely be at the sample base. Nevertheless, it has been demonstrated that a steep gradient of water potential developing during the day along the midrib of compound leaves can be appreciated by simply measuring the water potential of the apical leaflet (more negative Ψ_{LEAF}) vs. that of the whole compound leaf at the level of the midrib's petiole (less negative Ψ_{LEAF}), thus supporting our hypothesis (Petit & Anfodillo, 2013).

Further studies are expected to explore more in details whether different combinations of conduit widening/no widening along the leaf/needle axis and the absolute difference (higher/lower) in conduit diameter at the junction between the stem axis and the leaf/needle petiole determine different degrees of segmentation/integration between leaf and stem hydraulics. High segmentation (nearly all the hydraulic resistance confined within the leaf) would prevent the stem xylem to develop extremely negative water potentials due to increasing transpiration streams, and would link the water potential of all the hydraulic path of roots, stem and branches to that of rootlets. In such a way, solute concentration in storage compartments could more easily develop positive pressures in the xylem and/or favor the recovery of embolised conduits. To conclude, the vascular segmentation between leaves and stem might represent a strategy for safeguarding the hydraulic system from undesired losses of conductance due to embolism in the stem, and perhaps potential species-specific differences in leaf-to-stem hydraulic segmentation can help to better understand why some species are more vulnerable than others to environmental stresses.

FINAL CONCLUSIONS

With this study, I presented a series of experiments aiming to further understand the allometric relations of stem and leaves xylem, and their effect on the physiology of the water transport system.

The study highlighted that the hydraulic architecture of the trees is mainly determined by the height of the plants and the dimensions of the leaves. These rigid patterns allow for an efficient water transport guaranteeing an equal distribution of the hydraulic resistances among leaves, compensating, at the same time, for the hydraulic resistance imposed by growth in height of the plant.

The rigidity of the anatomical pattern in the stem over a range of climatic conditions has been shown by the standardization method presented. It has been demonstrated how the tree height is the main driver not only for conduits diameter but also for vessel conductivity, highlighting the benefit of removing the independent effect of stem length, on assessment of effects of climatic factors on such xylem traits. In the light of climatic change, an accurate assessment of the effect of the environments on tree anatomy is becoming of central importance and standardization methods in wood anatomy and plant ecology might be useful to improve the existing climatic models.

The approach used for the studies conducted on the leaves has major elements of novelty. The majority of the studies on the hydraulic architecture of the xylem rarely consider how the variations in the stem anatomy may or may not affect the hydraulic architecture of the leaf. Moreover, the strong relationship between the anatomical traits of the midrib and the leaf dimension, shows how the existing model should be implemented by considering not only the hierarchical distributions of the veins, but also their length.

The general hydraulic architecture of the plant, considering both stem and leaf, is shaped predominantly by physical constraints related to path length. In previous studies, the total xylem resistance has often been regarded as a sort of "unit resistance" with the xylem water potential increasing linearly from the tree top to the base. However, once the basipetal conduit widening is accounted for, almost all the hydraulic resistance and all the gradient of water potential are confined in the leaves, while in the stem are mostly negligible. In the context of the relationships between structural and hydraulic properties of the xylem, this study outlines

how the architectural design of the xylem network, from the stem xylem to the leaf venations, determine that the hydraulic status of the stem is negligibly affected by air dryness but substantially depend on soil conditions.

In conclusion, this study represents an important contribution to the general understanding of the mechanisms determining a functional water transport throughout the xylem. The hydraulic architecture of the plant evolved in a way to guarantee a homogenous distribution of the hydraulic resistances in the terminal part of the water transport system (i.e. the leaves) and at the same time allowing for the tree growth in height.

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