Functional xylem anatomy: intra and interspecific variation in stems of herbaceous and woody species

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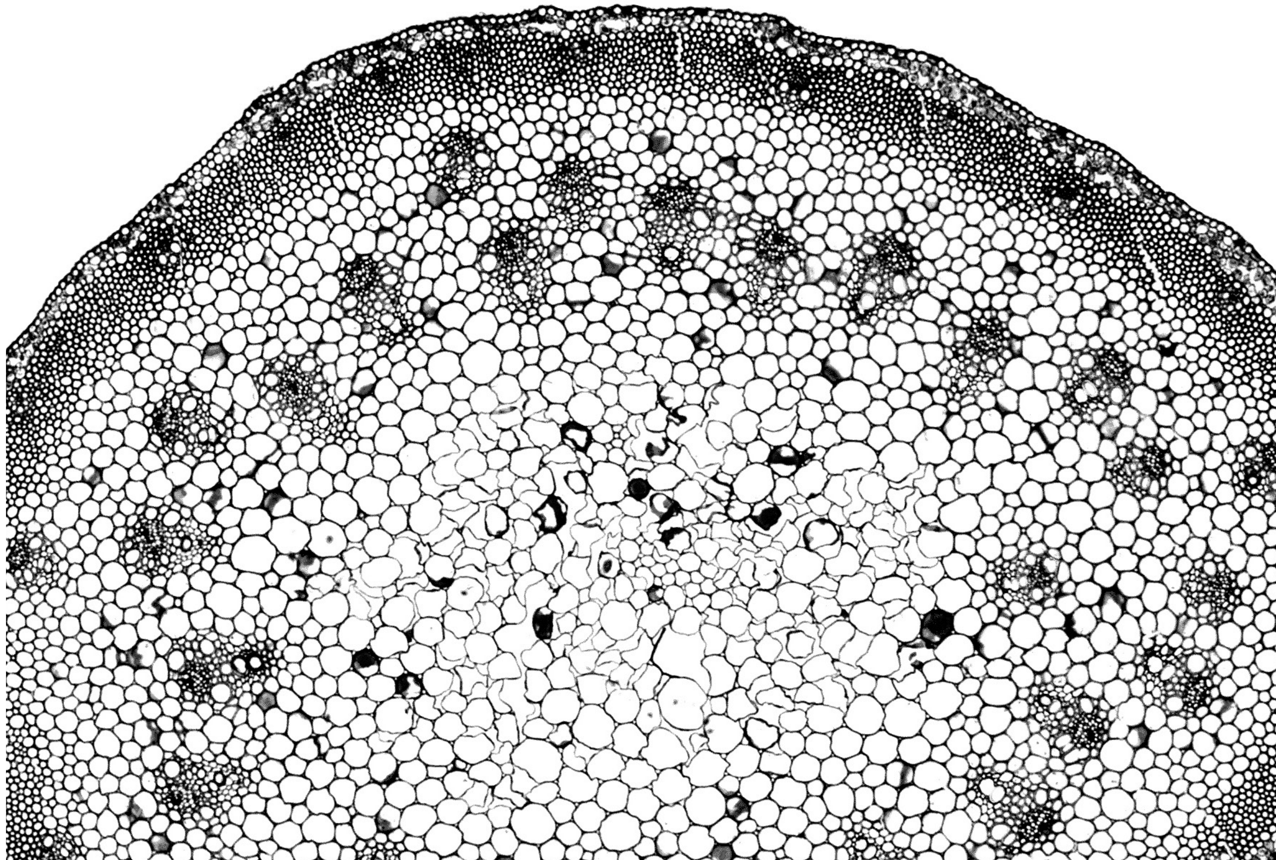
Chapter 6

DISCUSSION AND GENERAL CONCLUSION



Frequent episodic droughts and heat waves due to the ongoing climate change are increasing drought-induced forest mortality in a vast range of forest ecosystems (Allen *et al.* 2010; Dai 2013). This also applies to grassland and crop productivity that will undergo severe yield loss under this climate scenario since water limitation is one of the most important constraints for agriculture (Ciais *et al.* 2005; Brookshire & Weaver 2015). Under these water limiting conditions, plant hydraulics and plant anatomy have become important traits to predict species' vulnerability and its impact on plant distribution and survival under increasing climatic stresses (Anderegg *et al.* 2013). Understanding how mortality mechanisms work in plant species is challenging, and approaches used so far to construct realistic mortality vegetation models vary in terms of species, life stage studied and variables measured (Anderegg *et al.* 2013; Anderegg 2015). Therefore, efforts on investigating plant mortality mechanisms still remain insufficient (McDowell *et al.* 2008; Choat *et al.* 2012; Urli *et al.* 2013; Adams *et al.* 2017).

Plants will respond to changes in availability of abiotic natural resources through environmentally-induced shifts in phenotype (phenotypic plasticity), thereby explaining that hydraulic and wood traits vary across different zones (O'Brien *et al.* 2017). Along this line, investigating plastic responses about structure-function relationships on drought-induced embolism is crucial for increasing the predictability of modeling climate impacts on vegetation (Soudzilovskaia *et al.* 2013).

The results of my PhD thesis contribute to the comprehension of the ecological significance of embolism resistance and the functional aspects of xylem anatomical traits related to water conductivity. An overview of the main findings of the thesis can be seen in Fig. 1. In chapter 2 I show that abiotic differences between the Brazilian cerrado and caatinga habitats are important to explain the variation in wood anatomical characters in the co-occurring species *Tabebuia aurea* and *Tocoyena formosa*, and that soil differences - especially high aluminum concentrations and low nutrients in cerrado soils - are likely to explain the intraspecific wood anatomical differences between individuals from different sites. In chapter 3, I investigate the intraspecific wood anatomical variation in the same population of the two co-occurring Brazilian species, demonstrating wood trait variation along the main stem to deal with constraints related to increasing tree height, especially increasing hydraulic resistance. This intraspecific variation along the vertical axis of the main trunk outcompetes site as predictor for wood trait variation. In chapter 4 I show the (indirect) link between embolism resistance and lignification via the thickness of intervessel pit membranes, reflected in higher embolism resistance of insular woody stems of the Canary Island *Argyranthemum* (Asteraceae) species compared to the stems of their herbaceous continental relatives. This result matches with the abundance of insular woody species in the drier areas of the Canary Islands (Lens *et al.* 2013b) and with the ongoing observation of the distribution of derived woody species in continental areas that experience a few consecutive months of drought per year (F. Lens, global derived woodiness dataset, personal communication). Finally, in chapter 5 I investigate the often neglected field of embolism resistance in stems of herbaceous (Brassicaceae and Asteraceae) species occurring in different vegetation zones of Tenerife (Canary Islands, Spain). I demonstrate that also herbaceous eudicot stems can vary considerably in embolism resistance. Additionally, I emphasize that the difference in mean annual precipitation across the habitats of the herbaceous species is strongly linked to their ability to withstand embolism formation in stems as well as their ability of adapt specific anatomical features of their hydraulic system, both at inter and intraspecific level.

Below, I explain the main findings of the chapters 2-5 with their major implications in the integrated field of plant hydraulics and wood anatomy. I finalize the chapter with a personal view about future directions that have great potential to boost this research field.

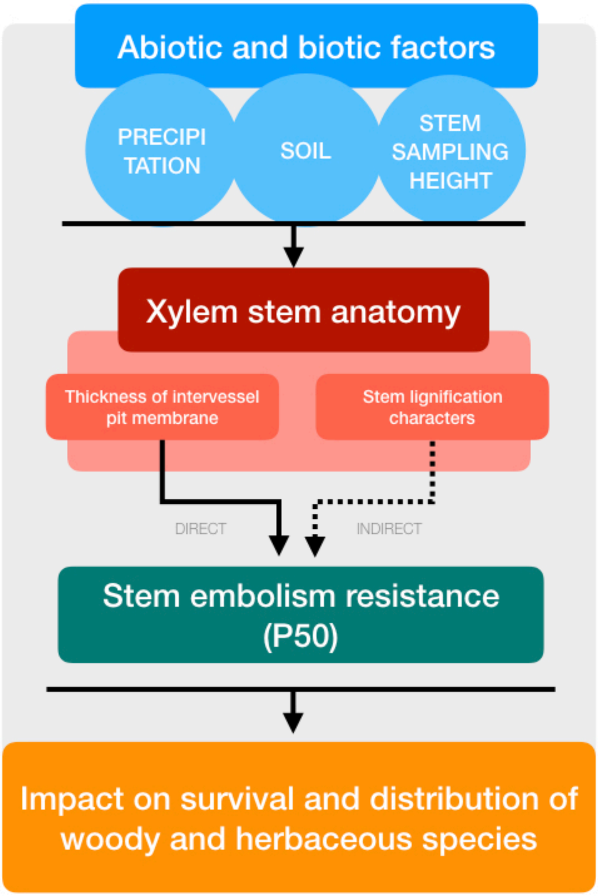


Figure 1 • Schematic representation of the main findings of my thesis.

Site and tree sampling height as predictors of xylem anatomical plasticity and its functional adaptive meaning in water conductance, survival and distribution of species

Xylem forms the bulk tissue in woody plants, and enables long distance water transport towards the tree canopy, where transpiration via stomata induces water loss and CO2 uptake to photosynthesis (Brodribb & Feild 2000). The different xylem functions are integrated to maximize plant fitness, but the environmental constraints have a major role in determining the different adaptive solutions to the structure/function demands (Ackerly 2004; Baas *et al.* 2004).

A remarkable example of how the environment plays an important role in the plasticity of wood anatomical characteristics was shown in chapter 2. The variation in xylem anatomical traits of two common co-occurring species (*Tabebuia aurea* and *Tocoyena formosa*) of the two main seasonally dry environments in Brazil, cerrado and caatinga, is explained by differences between sites. Since the two species belong to two unrelated families (Bignoniaceae and Rubiaceae, respectively), the factor species was found to be stronger than site to explain wood anatomical variation, and thereby emphasizing the importance of variation determined by evolutionary history. The impact of site (cerrado and caatinga) on the plasticity of wood anatomy was clearly demonstrated in the paired comparisons of each wood anatomical character between individuals from the same species growing in both sites, emphasizing the intraspecific species adaptation.

Rain seasonality is typical of both the cerrado and caatinga, although both biomes have unique rainfall patterns. In caatinga, the irregularity of rainfall is remarkable throughout the years and the mean precipitation is confined to only three months. The cerrado, despite its marked dry season, is not considered a xerophytic vegetation in contrast to caatinga (Oliveira & Marques 2002), especially due to certain general aspects of the vegetation. For instance, most of the cerrado plants develop large green leaves during the entire year and also flourish during the dry period (Rivera *et al.* 2002). Additionally, they also develop deep rooting systems enabling plants to access water deeply stored in the soil during the dry periods, maintaining transpiration and carbon fixation (Oliveira *et al.* 2005). However, the cerrado plants have to deal with edaphic adversities: the cerrado soils are nutrient-poor and aluminum-rich, which leads to toxic conditions that plants need to overcome (Coutinho 2002). Based on that, I suggested that the xeromorphic wood features associated with the individuals from cerrado in chapter 2 might be a response for the chronic low availability of edaphic mineral nutrients (oligotrophism) and the high aluminum concentration (aluminum toxicity). Accordingly, the oligotrophic and aluminum toxic soils have been reported as the main causes of xeromorphic features in cerrado plants, such as sclerophylly in leaves (Coutinho 1983; Oliveira *et al.* 2003; Souza *et al.* 2015), and it has been conceptualized as the

Theory of Oligotrophic Scleromorphism (Arens 1958; Arens *et al.* 1958; described in English in Salatino 1993), or peinomorphism (sensu Walter 1973). The results of chapter 2 corroborate the findings of higher lignification (higher sclerophylly) in cerrado plants, showing thicker fiber walls in individuals of *T. aurea* from cerrado. Additionally, the reduced size of individuals of *T. formosa* from cerrado might also be explained by the higher aluminum concentration as well as by the lower concentration of essential micronutrients, such as manganese in the cerrado soils. Aluminum is a strong plant growth reducing element in acid soils (Kochian 1995), and the deficiency of any essential micronutrient can cause disorders in physiological and biochemical processes, resulting in reduced plant growth (Kozlowski *et al.* 1991).

Besides the influence of environmental factors on the plasticity of wood anatomy, it is also known that plant height has an important effect due to necessary xylem adjustments to overcome increasing resistance in hydraulic conductance (Petit *et al.* 2010; Andofillo *et al.* 2013; Olson *et al.* 2018; Pfautsch *et al.* 2018). In chapter 3 I showed how sampling height influences the variation of wood traits along the main trunk of the same two species and sites described in chapter 2. The approach of using two species occurring in two different environments was important because it enabled us to test to which extent the factor site would also influence the axial trait variation in wood, since I had already studied the impact of site to explain species specific adaptation for each environment. I found that site was negligible to explain the variation of wood traits, since out of 13 wood traits assessed, only three had shown to be influenced by site and for only one species.

There was much variation in wood traits for the two species along the tree trunk in vessels, fibers as well as axial and ray parenchyma. Some of the variation can be functionally interpreted as a way of counterbalancing the increased resistance in water conductivity with increased tree height, such as the widening of vessels downwards and the increase in vessel density and vessel fraction upwards in both species. Additionally, both species showed that the largest vessels are linked to the thinnest intervessel pit membranes, which makes sense from a hydraulic point of view since more efficient, wider (and presumably also longer) vessels towards the trunk base of taller trees should have thinner intervessel pit membranes in order to synergistically reduce the resistance to long distance water transport (Hacke *et al.* 2006; Choat *et al.* 2008; Rosell *et al.* 2017).

Analogous to the particular adaptations of each species to deal with the adversities of the two environments shown in chapter 2, there were particular wood trait adaptations along the main trunk of the trees to deal with mechanical and hydraulic constraints between the two species in chapter 3. For instance, the axial variation of wood density in *T. aurea* is linked to fiber wall thickness, while in *T. formosa* wood density is related to vessel and ray characters. Likewise, rays seem to have different functions for each species, linked to conductance in *T. aurea* and to mechanics in *T. Formosa*, and this could be related to contrasting ray compositions in both species. In summarize, the two first chapters of my thesis showed that the two species have species-specific trade-offs to deal with environment constraints and with trade-offs associated with axial variation.

The functional significance of P_{50} as an adaptive trait to plant survival and distribution

The functional significance of stem P_{50} as an adaptive trait responding to environmental changes and plant distribution is shown in chapters 4 and 5. In chapter 4, this relationship was observed at the genus level, amongst five species of the insular woody *Argyranthemum* on Tenerife, Canary Islands. The most vulnerable species was the evergreen A. broussonetii from the wet laurel forests of Tenerife. Accordingly, all the other species studied of *Argyranthemum* are more resistant to embolism and native to drier areas of Tenerife. The functional significance of these findings are supported by the observations of chapter 5, where I showed that the difference in mean annual precipitation along different vegetations zones of Tenerife explains the variation in stem anatomy and embolism resistance amongst herbaceous species of Brassicaceae and Asteraceae. The data proved that the most vulnerable species were collected in wetter environments while the most resistant ones were sampled in drier vegetation types, thereby emphasizing the ecological value of P_{50} in the distribution and survival of herbaceous species. Additionally, I also showed this same functional relevance of stem P_{50} at the intraspecific level within both *Sisymbrium orientale* and *Hirschfeldia incana* (Brassicaceae) occurring in different areas with contrasting mean annual precipitation. While the majority of studies assess interspecific differences in P_{50} (Oliveira *et al.* in press; Zhang *et al.* 2018), the intraspecific variation is less understood, and only studied in a few woody and herbaceous species showing no variation in some species and considerable variation in others (Choat *et al.* 2007; Martínez-Vilalta *et al.* 2009; Lamy *et al.* 2014; Cardoso *et al.* 2018; Volaire *et al.* 2018). In chapter 5, a significant intraspecific difference in stems of the two Brassicaceae species was observed and was found to be explained by precipitation: for both species, the more embolism resistant populations occur in drier areas. This positive correlation between drought and embolism resistance in stems has been demonstrated in the literature for woody trees (Maherali *et al.* 2004; Bouche *et al.* 2014; Blackman *et al.* 2012; Trueba *et al.* 2017), and it also seems to be valid for herbaceous species (Lens *et al.* 2016).

The thickness of intervessel pit membrane in angiosperms is a major anatomical character explaining variation in embolism resistance

As expected from the air-seeding hypothesis, the intervessel pit membrane has a key role in avoiding the spread of embolism between adjacent conduits, since the thickness of the pit membrane corresponds to the size and shape of the pit pores through which the air-water menisci cross during the air-seeding process (Choat *et al.* 2008; Lens *et al.* 2013a; Li *et al.* 2016). In chapter 4, the thickness of intervessel pit membrane (T_{PM}) was found to be the most important anatomical trait explaining stem P_{50} variation of the insular woody species of *Argyranthemum* and their five herbaceous relatives. The more resistant, woody species have thicker intervessel pit membranes compared to the herbaceous, less resistant relatives, occurring on the European continent. Another noteworthy finding of this study is that T_{PM} was positively linked with the degree of lignification in stems. This leads us to the main conclusion of our chapter 4: the increase in woodiness of insular woody species explains indirectly the increase in embolism resistance via the thickness of intervessel pit membrane.

Despite all the strong evidence that T_{PM} plays a major role in explaining embolism resistance, in chapter 5 the degree of woodiness (P_{LIG}) provided a higher explanatory power for differences in embolism resistance amongst herbaceous Brassicaceae and Asteraceae. It might be that the functional relevance of T_{PM} in herbs is less important compared to woody species, for a reason that is not known. A previous study investigating the P_{50} - T_{PM} relationship in grasses also did not find any relationship (Lens *et al.* 2016). Moreover, in my study of chapter 5, the $P_{50} – T_{PM}$ relationship disappeared when only assessing the Brassicaceae species dataset, and in chapter 4, the $P_{50} – T_{PM}$ relationship was true only when the herbaceous data set was combined with the woody data set. Nevertheless, studies investigating the relationship between stem $P_{50} – T_{PM}$ amongst herbaceous species remain very scarce, so it is too soon to make any conclusion about the functional relevance of T_{PM} in herbs. That being said, when analysing the two populations of *Sisymbrium orientale*, a significant difference in P_{50} was observed, matching the significant difference in T_{PM}, with the more embolism resistant population having thicker intervessel pit membranes.

The strong but indirect link between lignification and embolism resistance

Water transport in plants is driven by a gradient in negative pressures inside the water conducting cells, which generates an inner xylem mechanical stress (Bittencourt *et al.* 2016). The tensions inside conduits generate imploding forces and therefore cell walls, or even entire stems (Lens *et al.* 2016) need to be reinforced to avoid a potential collapse of conduits (Hacke *et al.* 2001a). In this line, collapse of xylem conduits has only been observed in cells that lack a robust support of the fibre matrix, for instance in leaves (Cochard *et al.* 2004; Brodribb & Holbrook 2005; Zhang *et al.* 2016) and in low-lignin stems of poplar mutants (Kitin *et al.* 2010). Additional to imploding forces, positive pressures can also occur inside embolized conduits (Pereira *et al.* 2017). The interaction between these opposite forces requires the presence of a mechanical system in wood that would mitigate the costs associated with high tensions (Hacke *et al.* 2001a). In this context, a stronger mechanical system due to thicker-walled conduits or fibers leads to higher wood density and is linked to water transport safety rather than water transport efficiency (Jacobsen *et al.* 2005, 2007; Chave *et al.* 2009). In addition, more lignified stems are interpreted as a mechanism to avoid the formation of microcracks through which embolism nucleation may occur or air could be sucked in (Jacobsen *et al.* 2005; Zwieniecki & Secci 2015). Accordingly, the formation of cracks in wood does occur during the drying process of wood material (Hanhijarvi *et al.* 2003) and the likelihood of cracks formation decreases with wood density (Ilic 1999). Therefore, numerous studies have reported the relationship between higher wood density (which could also be linked with higher stem lignification) and higher absolute values of P_{50}, both in woody (Hacke *et al.* 2001a; Jacobsen *et al.* 2005; Jansen *et al.* 2009; Pereira *et al.* 2017) and in herbaceous lineages (Lens *et al.* 2012b, 2013, 2016; Tixier *et al.* 2013).

In my study, I corroborated these findings in chapter 4 showing that the insular woody stems of *Argyranthemum* species are more embolism resistant than those of their herbaceous relatives. The proportion of lignified area per total stem area (P_{LIG}) as well as the proportion of xylem fiber wall per fiber ($P_{FW}F_X$) explained the variation in embolism resistance in this clade of daisies. The increment of a xylem mechanical support against implosion is the reason for the hydraulic-mechanical trade-off, which can result from either an increase in vessel wall to lumen ratio (Hacke *et al.* 2001a; Jacobsen *et al.* 2007; Cardoso *et al.* 2018) or an increase in fibre matrix support (more and thicker walled xylem fibres) (Jacobsen *et al.* 2005, 2007; Pratt & Jacobsen 2017; Dória *et al.* 2018). Interestingly, we also found a positive correlation between lignification and increasing embolism resistance amongst the different individuals of *Cladanthus mixtus* (Asteraceae). This species showed the highest range in the degree of stem lignification amongst the individuals studied, coupled to the highest degree in embolism resistance.

Besides the hydraulic-mechanical trade-off, the link between increased wood formation and embolism resistance in the daisy lineage matches the observation that the majority of insular woody species native to the Canary Islands are often distributed in the dry coastal areas (Lens *et al.* 2013b). Additionally, it also agrees with an ongoing global derived woodiness database at the flowering plant level, comprising almost 7,000 species of which most of them are native to regions with a marked drought period such as (semi-)deserts, savannas, steppes and Mediterranean-type habitats (F. Lens, global derived woodiness dataset, personal communication). The abundance of derived woody species in (periodically) dry areas supports the hypothesis that drought might have driven wood formation in many derived woody lineages (Dória *et al.* 2018).

Also amongst the herbaceous Brassicaceae and Asteraceae species studied (chapter 5), the link between lignification and embolism resistance was retrieved. Surprisingly, the proportion of lignified area per total stem area (P_{LIG}), which is mainly defined by the amount of woodiness, was the character that best explained the variation in embolism resistance in stems: the higher the stem lignification, the more resistant to embolism formation the species is. The same trend was found between the two populations of *Sisymbrium orientale*: the more resistant population showed thicker intervessel walls and higher P_{LIG} compared to the more vulnerable population.

Despite the overwhelming evidence for the mechanical-hydraulic link, it is hard to functionally explain why more lignified species are better adapted to drought. At first sight, there seems to be no evidence for a direct functional link between increased wood formation and increased embolism resistance. As commented above, the embolism spreading via air-seeding occurs at the intervessel pit membrane level, which is more likely to affect the existence of high levels of embolisms. Therefore, the reported correlations between higher lignification and thicker intervessel pit membranes, such as thicker vessel walls and thicker membranes (Jansen *et al.* 2009; Li *et al.* 2016), might indicate that mechanically reinforced stems are indirectly correlated with embolism resistance via air-seeding. This hypothesis was highlighted in chapter 4, where intervessel pit membrane thickness (T_{PM}) was found to be the functional missing link explaining the correlation between stem lignification and embolism resistance in the daisy clade. In chapter 5, the link T_{PM} - lignification was also found at the intraspecific level in the herbaceous *Sisymbrium orientale* collected in contrasting environments: the more resistant population showed a higher proportion of lignified area in the stem, thicker intervessel wall, and thicker intervessel pit membranes. However, the T_{PM} – lignification correlation disappeared in our entire data set (including all the herbaceous Asteraceae and Brassicaceae species), showing that increased lignification characters are not necessarily linked to thicker intervessel pit membranes.

Future perspectives

Our understanding about relationships between xylem structure and long-distance water transport has made great progress during the last decades. This progress is particularly due to novel non-invasive techniques available to examine water flow and air bubble formation in xylem conduits, such as nuclear magnetic resonance imaging, high-resolution computed tomography and the optical technique (Brodersen *et al.* 2013, 2018; Knipfer *et al.* 2015; Torres-Ruiz *et al.* 2015; Brodribb *et al.* 2016). Likewise, progress in identification of artefacts during hydraulic measurements (long vessels generating r-shaped curves) and artefacts due to incorrect lab protocols (proper fixation of fresh samples to preserve the pit membranes), has led to more reliable measurements (Plavcová *et al.* 2013; Li *et al.* 2016).

As a result of this progress, the direct functional link between embolism formation and pit structure has been demonstrated in several studies, although the chemical composition of the pit membranes and the 3D structures of their micropores remain poorly known. Likewise, detecting the presence of hydrophobic surfaces at the pit membrane area, which would affect the contact angle between air bubbles and the pit membrane micropores, would help to elucidate how the first embolisms are formed and the detailed mechanisms behind drought-induced embolism formation and spread via air-seeding. Moreover, a better understanding about the presence and variety of chemical components in living xylem parenchyma and dead water conducting cells would increase our knowledge about the effect of sap flow content on surface tension and pit membrane function, which is important specially under the light of new discoveries of stable, surfactants-coated nanobubbles (Jansen & Schenk 2015; Schenk *et al.* 2015, 2017, 2018). Furthermore, these fine-scale observations in pits and xylem sap should be carried out in different organs, along different height positions within the tree, and throughout different time periods in the year to better understand the importance of interconduit pits in long-distance water transport of plants.

Given the importance of P_{50} as an ecological trait influencing the distribution of species and a potential driver for plant diversification and species coexistence (Larter *et al.* 2017; Oliveira *et al.* in press), a promising venue for future studies is investigating whether generalists exhibit higher phenotypic plasticity in P_{50} than specialists in their stems, roots and leaves. Additionally, integrating hydraulic traits with molecular phylogenies will be relevant to better understand evolution of the hydraulic system, especially in these lineages that show low morphological variation, but clear habitat and soil preferences. Furthermore, considering that embolism resistance is linked to drought-induced mortality, using hydraulic data in models for predicting tree mortality during climate extremes will help to better elucidate the physiological processes and mechanisms underlying drought mortality. In this line, gathering data from underrepresented forests, such as rainforest species - represented by only 59 tree species out of 226 species from 81 sites worldwide in a meta-analysis of Choat *et al.* 2012 - would be of utmost importance to accurately predict which trees/forests are more vulnerable to climate change. Consistent with a better prediction of plant response to climate change, assessing plant height in relationship with hydraulic traits across species and environments will also be valuable to understand the distribution of tree species across different biomes, since plant height is the main driver for vessel diameter (Olson *et al.* 2018).

In conclusion, it is clear that the boundary between plant hydraulics and wood anatomy has great potential to generate crucial information about plant survival and adaptation, geographic distribution, and evolution. However, xylem hydraulic and anatomical traits represent only one element of an entire range of adaptive strategies that plants employ to survive and compete. Therefore, these hydraulic and anatomical observations in stems should be combined with leaf and root measurements in order to obtain a global plant approach. Features of special interest are (1) stomata sensitivity, which determine transpiration rate and xylem pressure, (2) sapwood to leaf area ratios, which have an influence in the safety and efficiency of water conductance, (3) root distribution and depth, which influence the access to water source, and (4) and xylem capacitance, helping to maintain the integrity of the water transport. This more integrative approach will definitely help us to understand inter and intraspecific variation in hydraulic and anatomical traits with respect to drought tolerance across diverse plant lineages.