FUNCTIONAL BALANCES IN FOREST CANOPY TREES AND LIANAS

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Functional balances in forest canopy trees and lianas

Lan Zhang

Thesis

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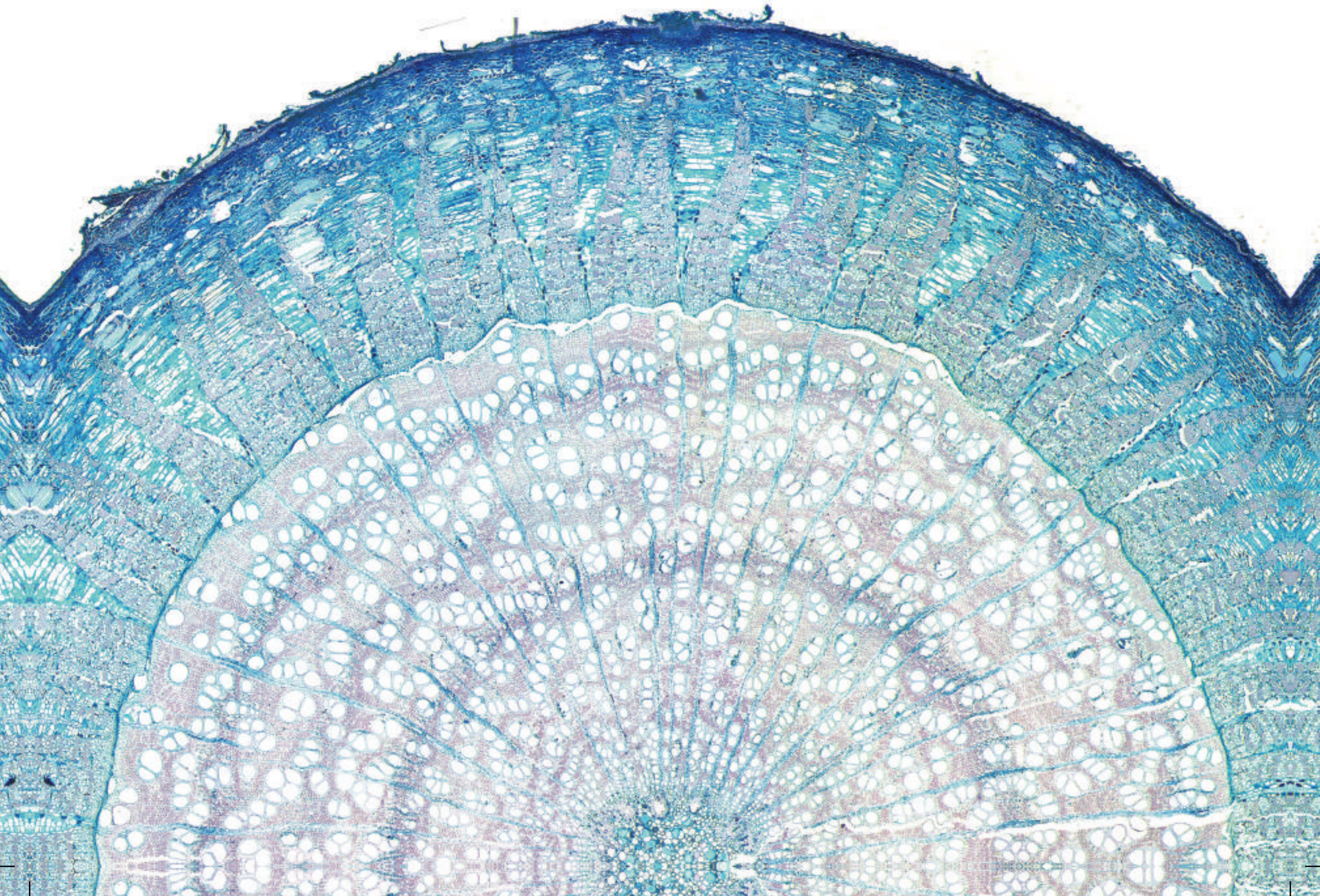
TABLE OF CONTENTS

CHAPTER 1

General introduction



Forests cover approximately 30% of global land area (FAO, 2016) and are the major terrestrial carbon sink (Pan *et al.*, 2011). The majority of forest above-ground biomass (carbon sink) is in canopy woody plants. These woody plants form crowns by extending their upper terminal branches to display their leaves for light capture. All the crowns that cover the forest surface form the forest canopy (Bongers, 2001). The forest canopy serves as the functional interface between terrestrial biomass and the atmosphere (Ozanne *et al.*, 2003) and is especially responsible for water loss and carbon fixation, but can be vulnerable to drought and cold (Olson *et al.*, 2018). Canopy branches under high light levels potentially fix most carbon through leaf gas exchange (Santiago *et al.*, 2004; Sellin & Kupper, 2005; Sellin *et al.*, 2019), but face high irradiation and temperature during the day resulting in high transpiration demands (Meinzer *et al.*, 1997; Meinzer, 2003; Sellin & Kupper, 2007). These conditions thus determine the water and carbon cycle between soil, forest and atmosphere and ultimately influence the carbon sequestration and water use of entire forests. Canopy branches thus act as bottleneck for the hydraulic integrity and carbon gain of canopy woody plants and, in turn, the whole forest. However, due to the difficulties to access the forest canopy, studies are more focused on saplings and juveniles (Zwieniecki *et al.*, 2004; Poorter, 2009; Sellin *et al.*, 2019), or species within planted stands (Rust & Roloff, 2002; Sellin & Kupper, 2005). Though several canopy studies reveal hydraulic performance of canopy trees (Santiago *et al.*, 2004; Santiago *et al.*, 2018), the water-carbon cycles in relation to the physiology of canopy branches is poorly studied (Tor-ngern *et al.*, 2015; Woodruff *et al.*, 2016). Therefore, our study focuses on the carbon uptake and water loss related issues within the branches of canopy plants in the upper canopy of forests and thus aims to provide more information on this knowledge gap.

WOODY PLANTS IN DIFFERENT CLIMATES

Woody plants occur in many climate zones ranging from hot tropical forests to cold temperate forests. Tropical forests have the most biomass and biodiversity, but face the most degradation (FAO, 2016). Temperate forests are important wood sources for industrial purpose, and the total area is worldwide stable (FAO, 2016). Tropical forests and temperate forests clearly differ in temperature, precipitation and soil fertility which essentially influence plant physiology (Rees *et al.*, 2001; Wright *et al.*, 2004; Schuldt *et al.*, 2016).

Temperate forests normally have cold winters compared to tropical forests and sometimes have severe frost that hampers tree growth (Vanoni *et al.*, 2016). Woody plants that adapt to temperate climates are more resistant to freezing induced hydraulic failure than tropical species (Sperry & Sullivan, 1992; Zanne *et al.*, 2014). In temperate forest, conifer and broadleaved tree species are two major plant types that form the forest canopy and distinctly differ in appearance and wood anatomy. Conifers are the gymnosperms that are most common at high latitudes and elevations and can be well adapted to cold and nutrient poor soils (Bond, 1989; Tyree & Sperry, 1989; Sperry & Sullivan, 1992; Davis *et al.*, 1999). Conifers normally have lower water transport efficiency in the stem, and lower photosynthesis in the leaves as well as lower storage capacity of water and carbon than broadleaved trees. If these fundamental differences cause distinct water-carbon cycles between conifers and broadleaved trees is unknown. Our study aims to fulfil this knowledge gap.

In tropical forests, canopy woody plants face high radiation level and temperatures which in turn cause high evapotranspiration that requires large amounts of water supply (Santiago *et al.*, 2004; Badgley *et al.*, 2017). Liana and tree species are the major large canopy plants that represent most of above-ground forest biomass in tropical forests. Lianas cannot support themselves while trees do support their own body weight. They both belong to the angiosperms but differ in growth form, and anatomical, physiological and chemical attributes (Schnitzer & Bongers, 2002; Zhu & Cao, 2009; Angyalossy *et al.*, 2015; Asner & Martin, 2015). In general, lianas take growth advantage under dry and stressful soils while trees are found to increase with increasing mean annual precipitation (Putz & Mooney, 1991; Schnitzer, 2005; Schnitzer, 2018; Schnitzer & van der Heijden, 2019). As tropical trees need to afford the weight of themselves plus the weight from lianas, these trees require higher mechanical stability. In contrast, lianas climb adjacent trees to reach the forest canopy, and develop long-twisted stems which may elongate water transport pathways. Though studies have been done on comparing the differences of hydraulic and mechanic traits in saplings of lianas and trees (van der Sande *et al.*, 2013), integrity of these traits in canopy studies are rare (De Guzman *et al.*, 2016; Santiago *et al.*, 2018). Our study thus targets on understanding if the distinct design differences between trees and lianas result in trade-offs between hydraulic and mechanical traits.

Thus, our study focusses on conifer and broadleaved tree species in temperate forests to understand the water-carbon circles in their canopy branches. Besides, our study discusses the co-existing liana and tree species in tropical forests to study if their different designs cause divergence in hydraulic and mechanical properties in canopy branches. This knowledge will contribute to better understanding possible future forest community shifts under climate change.

SIMPLE STRUCTURE, DIVERSE LIVES

Although woody plants widely differ in leaf characters and growth forms, they share a similar anatomical structure with pith, xylem and phloem in the stem (Fig. 1.1). Xylem and phloem are spatially connected to each other and transport water from roots to leaves and sugar from leaves to required places, respectively. Conifer's xylem is mainly composed of tracheid cells which transport water and provide mechanical stability. Broadleaved lianas and trees have differentiated vessels that transport water, and separate fibres that provide mechanical support (Evert, 2006).

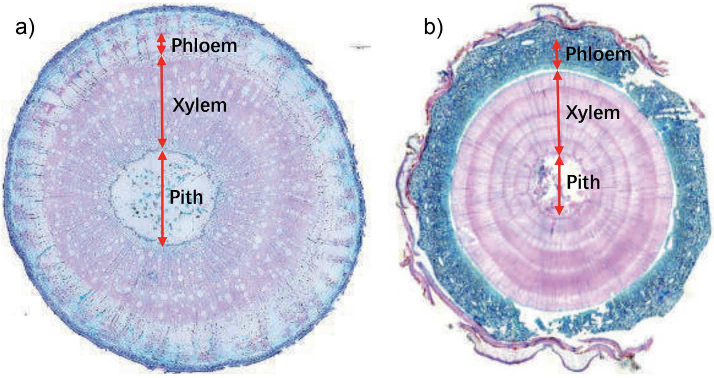


Figure 1.1 Illustration of cross-sectional anatomical structure of a) angiosperm, *Byttneria aspera* Collebr. ex Wall. (a liana species) and b) gymnosperm, *Picea koraiensis* Nakai (a conifer species) woody plants.

In conifers, each tracheid is a 2-4 µm long tube with pits along tangential and radial walls, and tracheid's diameter seldomly exceeds 30-50 µm (Bond, 1989). These tiny and short tubes allow conifers to resist freeze/thaw-induced embolism and thus adapt to cold environments (Cochard *et al.*, 1992a; Sperry & Sullivan, 1992). However, the small size of tracheids limits the water transport ability of conifers. In angiosperms, vessels are much longer and bigger conduits compared to tracheids. Vessel diameter can reach 500 µm in some broadleaved species and one single vessel length can exceed 2 m in some lianas (Bond, 1989; Jacobsen *et al.*, 2012). The long and big vessels allow broadleaved lianas and trees to transport water much more efficiently, but this comes with a greater risk for hydraulic failure in vessels (Bittencourt *et al.*, 2016; Venturas *et al.*, 2017).

Ray parenchyma is a cell type that appears in the xylem of both conifers and broadleaved lianas and trees (Fig. 1.2). Ray parenchyma interconnects other cell types in the xylem and phloem radially. Axial parenchyma occurs in xylem and phloem scattered or aggregated, depending on the species (Crivellaro and Schweingruber 2013). Ray and axial parenchyma can store water and sugars (Salleo *et al.*, 2004; Plavcová & Jansen, 2015). Sieve cells are sugar conducting cells in gymnosperms, whereas in angiosperms sieve-tube members are present. Sieve cells of conifers are smaller but longer compared to the sieve tubes in broadleaved lianas and trees (Liesche & Schulz, 2018).

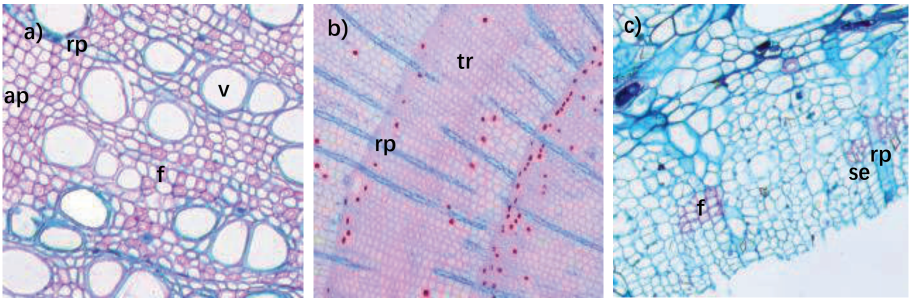


Figure 1.2 Illustration of the cross-sectional anatomical structure in xylem of a) an angiosperm, *Pterospermum menglunense* (a broadleaved tree species); b) a gymnosperm, *Larix gmelinii* (a conifer species), and in phloem of c) an angiosperm, *Semecarpus reticulata* (a broadleaved tree species). v, vessel; f, fibre; tr, tracheid; ap, axial parenchyma; rp, ray parenchyma; se, sieve elements.

With this basic structure, woody plants generate a wide variation of species that are broadly distributed in different forest biomes. How this basic structure makes such a diverse plant kingdom is fantastic and worth to study. Especially the basic anatomical structure implies an important role of all tissues in whole plant performance and in controlling the water and carbon cycles within plants, but this has not yet been clearly studied (Meinzer, 2003). Our study aims to understand how anatomical features influence the species adaptations to different climates.

CONCEPTUAL MODEL INTEGRATING TISSUE FUNCTIONS

In woody plants, water is upward transported from root to leaves for photosynthesis and evapotranspiration. Meanwhile, sugar is transported away from the leaves for plant body construction and other functions, such as maintaining metabolism and defence, keeping osmotic balances in the phloem and repairing embolism (Canny, 1997; Bucci *et al.*, 2003; McCulloh *et al.*, 2010). Ray and axial parenchyma function as water and carbon storage reserves (Salleo *et al.*, 2004; Plavcová & Jansen, 2015) for vessel refilling (Canny, 1997), defence against pathogens (Deflorio *et al.*, 2008; Morris *et al.*, 2016a) and in some species as mechanical support (Burgert *et al.*, 1999; Reiterer *et al.*, 2002). The interconnected anatomical structure allows water and sugar dynamically cycling in the plant body under osmotic pressure (Hölttä *et al.*, 2009; De Schepper *et al.*, 2013; Pfautsch *et al.*, 2015). In my study, I start from a conceptual model that integrates the functions of these tissues with the above described plant structure (Fig. 1.3). This conceptual model is inspired by Cannell and Dewar (1994) who proposed functional relationships between water loss by transpiration and water transport in stems, carbon gain, storage and nutrient uptake, but we only focus on the functions relating to water loss, carbon gain and storage. In addition to the proposed functions, we add carbohydrate transport (the function of phloem) as a new function to reveal the functional relation between carbon acquisition by leaves and carbon (sugar) transport in the phloem.

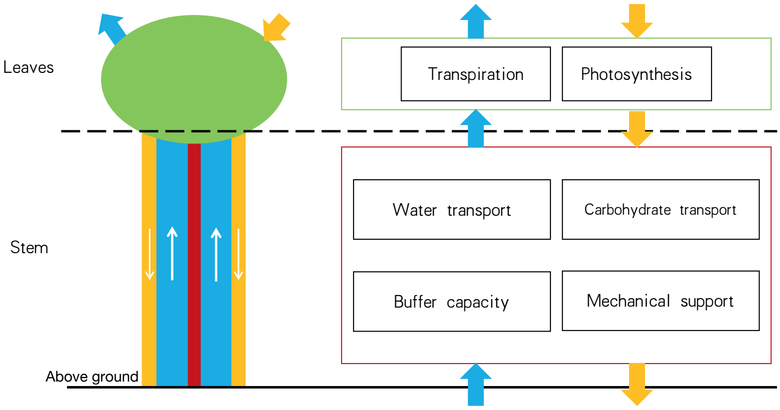


Figure 1.3 A conceptual model of a woody plant. The woody plant is simplified into two parts: leaves and stem. In the stem, the brown bar represents pith area and the blue bars represent the xylem with upward arrows, and the blue arrows, showing the water transport direction. The outer yellow bars represent the phloem with downward arrows, and the yellow arrows, indicating the sugar transport direction.

In this conceptual model, we only consider the leaves and stem of a woody plant. The leaves are responsible for gas exchange (transpiration and photosynthesis) and the stem for transport (hydraulic and carbohydrate conductivity) and storage of water and sugar (buffer capacity), as well as mechanical support (mechanical safety). The water transpires from the leaves (water loss) and carbon is fixed in the leaves. In the stem, water is upward transported to the leaves while sugars are downward transported into the stem. During these processes, light can importantly influence the water loss and carbon gain because of the stronger gas exchange rate under high light condition (Givnish, 1988; Davi *et al.*, 2008).

FUNCTIONAL BALANCES AND TRADE-OFFS

Woody plants balance investments in different tissues or organs to maximize their functioning while not breaking down carbon budget. These plants thus maintain functional balances between leaves , stem and roots (Dickson, 1989; Cannell & Dewar, 1994; Lacointe, 2000). Following Cannell and Dewar (1994) that reviewed several balances in a plant body, in my study, I focus on the balances between water loss and water support/storage, and between carbon gain and carbon translocate/storage (as shown in Fig. 1.3), and reveal these balances by functional ratios between tissue areas (Trugman *et al.*, 2018; Trugman *et al.*, 2019).

Woody plants transpire large amounts of water from the leaves and compensate these water losses by sufficient water supply through the stem (Cannell & Dewar, 1994; Lacointe, 2000; Marcelis & Heuvelink, 2007). This process requires carbon investment in both leaves and stems with limited carbon input (Trugman *et al.*, 2018). Thus, plants need to balance investment on leaves (transpiration) and stem xylem (hydraulic conductivity and mechanical support) to reach more gas exchange while not causing drought-induced cavitation by insufficient water supply in xylem, or breakage of a stem or branch that is insufficiently stable.

Meanwhile, the produced sugar needs to be translocated to required plant tissues, or for temporary storage. Carbohydrate storage is important when plants have to persist periods of water stress, and when leaves have not flushed yet or fully expanded. This storage capacity allows plants to buffer the shortage/excess of carbon or water temporarily (Cannell & Dewar, 1994). Thus, carbon investment in leaves (photosynthesis), phloem (carbohydrate conductivity) and ray parenchyma (buffer capacity) needs to be balanced as well. These balances ultimately influence the carbon flux into plants and ecosystems (Cannell & Dewar, 1994; Savage *et al.*, 2016).

Within the transport and support system of the stem, carbon investment needs to be balanced between tissue types. Water conductivity closely relates with carbohydrate conductivity (Hölttä *et al.*, 2006; Hölttä *et al.*, 2009). A higher water and sugar transport ability may benefit from a higher buffer capacity, i.e. storage area, since this capacity allows for short water or carbon shortage (Plavcová & Jansen, 2015; Savage *et al.*, 2016). In angiosperms, with more space for transport in vessels and storage in parenchyma, less space is left for mechanical support by fibres. In gymnosperms, trancheids contribute to both hydraulic conductivity and mechanical support, but come at the cost of storage in parenchyma. Overall, plants are thus supposed to balance between functions of water transport, carbohydrate transport, buffer capacity and mechanical support.

Beside these tissue functions, hydraulic safety is an important function for transporting water safely in plants' conduits. Hydraulic safety is often defined as the ability to resist embolism due to drought or frozen (Sperry *et al.*, 1988). Drought-induced cavitation is the mechanism that a functional xylem conduit becomes air-seeded due to the neighbouring cavitated conduit through a pit membrane. Freeze-thaw induced cavitation means that after thawing gas bubbles emerge, which cause embolism in the conduit. Hydraulic safety can be more critical to tall woody plants which have more negative water potentials in branches than smaller ones to maintain the passive water flow from roots to leaves (Sterck & Zweifel, 2016). Despite this, tall woody plants should maintain a safe water transport system and thus avoid drought-induced cavitation and mechanical failure. Possible trade-offs between hydraulic safety, hydraulic conductivity and mechanical safety are expected, but the patterns are still ambiguous in the literature (Gleason *et al.*, 2016).

THE OBJECTIVES

The main objective of my study is to gain insight in how canopy co-existing tall woody plants balance the acquisition of carbon and loss of water in the leaves with the transport and storage of water and carbon in the stem. I therefore studied functional balances in exposed branches in these canopy woody plants. More particularly, I focus on the balance between water loss from leaves via transpiration versus water transport into the leaves through the stem xylem conduits and water storage in stem ray parenchyma; the balance between carbon gain in leaves via photosynthesis versus carbon transport away from the leaf through phloem and partly into storage of carbon in ray parenchyma; and possible trade-offs between hydraulic safety, hydraulic conductivity and mechanical safety. I therefore studied canopy trees of species that coexist in temperate forests and trees and lianas of coexisting species in tropical forests to show whether and how species converge or diverge in structures and functions. More specifically, I address the following questions in 3 subsequent chapters (2-4):

1. How do tree species differ in their functional ratios between leaf area, xylem area and phloem area across deciduous tree species in a temperate forest? In this study (Chapter 2), I compare 10 deciduous tree species in a Dutch 45-yr-old forest, and discuss the functional ratios in the light of the carbon and water economy

of trees, and relate possible differences to species differences in shade-tolerance.

2. How do conifer and broadleaved tree species differ in their functional balances between water and carbon related functions in a temperate forest? In this study (Chapter 3), I compare conifer tree species with deciduous broadleaved tree species in a Chinese temperate forest. I discuss how conifers and broadleaved tree species differ in the way they coordinate their functional balances in the light of the carbon and water economy of trees.
3. How do liana and tree species coordinate trade-offs between hydraulic conductivity (water transport efficiency), hydraulic safety (drought resistance) and mechanical safety? In this study (Chapter 4), I compare 12 liana species with 10 tree species in a tropical evergreen forest in China. I discuss possible trade-offs between these functions and their underlying anatomical traits difference between lianas and trees.

THESIS OUTLINE

This thesis consists of five chapters: the general introduction (this chapter), three research chapters (chapter 2-4) ordered from temperate (chapter 2-3) to tropical (chapter 4) forests, and the general discussion (synthesis, chapter 5).

In ***chapter 2***, we investigate the first question how trees differ in their functional ratios between leaf area, xylem area and phloem area across deciduous trees, and how these ratios differ between species differing in shade-tolerance as well as between sun-exposed and shaded branches. Thus, in this chapter, we focus on the functional balances between water loss and water support, and between carbon gain and carbon transport, as well as buffer capacity. We explore these functions and ratios with shade-tolerance (the ability to adapt to shade condition) and sun-exposed or shaded branches. We analyse 10 deciduous broadleaved tree species from an even-aged Dutch temperate forest locating in the Netherlands.

In ***chapter 3***, we focus on the same functions but move one step forward. We test if co-existing conifer and broadleaved tree species differ in the relation between sugar transport (carbohydrate conductivity) and storage (buffer capacity) with water transport (hydraulic conductivity), and if these differences ultimately influence xylem growth. We analyse 14 canopy trees including evergreen/ deciduous conifers and deciduous broadleaved trees in a temperate mixed forest locating in NE-China. We discuss the second question how conifer and broadleaved tree species differ in their functional balances between water and carbon related functions.

In the end, we move to a tropical forest where the highest irradiation levels and temperatures occur. Under such circumstances, tall woody plants in tropical forests have stronger evapotranspiration than in temperate forests (Bonan, 2008). Meanwhile, canopy trees in tropical forests have higher tree height than those in temperate forests (Tao *et al.*, 2016), and these trees need to afford extra weight from co-existing lianas. Therefore, the hydraulic and mechanical stress maybe high in tropical canopy trees. We thus examine the possible trade-offs between hydraulic conductivity, hydraulic safety and mechanical safety in ***chapter 4***. We analyse 22 co-existing evergreen broadleaved species including lianas and trees emerging in the forest canopy in a monsoon tropical forest locating in SW-China. We discuss the last question how liana and tree species coordinate the trade-offs between hydraulic conductivity, hydraulic safety and mechanical safety.

With all these questions being investigated, in ***chapter 5***, I summarise our findings and discuss the generality of our results. In the end, I discuss the limitations and further implications of canopy branch studies.

STUDY AREAS

In total, we have three plots including two temperate forests - Hollandse Hout (NL) and Changbai Mountain (CB) (Fig. 1.4), and one tropical forest - Xishuangbanna (BN). Except NL, the other two plots have a canopy crane allowing to reach the forest canopy.

Hollandse Hout forest is situated in the centre of the Netherlands (52°28′N, 5°26′E). This forest, which covers c. 900 ha, was established in the 1960s and 1970s (Bijlsma & Verkaik, 2008), when over 15 deciduous and coniferous tree species were planted in monospecific stands. The site is characterized by a temperate marine climate with mild summers (average temperature in June–August: 15.9°C) and winters (average temperature in December–February: 2.5°C), and an annual precipitation of c. 800 mm (KNMI, 2015). The forest soil is a calcareous, nutrientrich, clay soil of uniform texture (Bijlsma & Verkaik, 2008), and groundwater tables range between 1.6 and 1.2 m below the soil surface (TNO-NITG, 2015).

Changbai forest locates in Changbaishan Nature Reserve, Jilin Province, NE China (41°15’-42°35’N, 127°15’-129°00’E). The site is a typical representative of conifer-broadleaved mixed forest (Zhou & Dai, 2012). The site is situated at an altitude of around 800 m and has a canopy crane installed with a height of 60 m and a turning arm with length of 30 m. The area is temperate continental climate and affected by the monsoon with a long and cold winter, and a warm, rainy but short summer. Average annual temperate is 3.6℃ and average annual precipitation is around 700 mm.

Xishuangbanna is a seasonal tropical lowland rain forest located in the core area of National Nature Reserves in the Bubeng region, Xishuangbanna (XTBG), Yunnan Province, SW China (21° 36’N 101°35’E). The altitude of the crane site is about 780 m. The area has a typical monsoon climate with distinguishable wet and dry seasons. A wet season starts from May and lasts till October and a dry season characterizes the period from November till April. The average annual temperature is 21.8 ℃ with mean monthly temperature ranging from 15 ℃ in December to 25 ℃ in June. The average annual precipitation is 1493 mm of which 1256 mm (84%) falls between May and October (Cao *et al*. 2006).

NL, Hollandse Hout
Broad leaved trees (10 spp.)
Traits measured:
Leaf area
Xylem area
Phloem area
Vessel area
Ray parenchyma area

BN, Xishuangbanna
Liana (12 spp.) vs. trees (10 spp.)
Traits measured:
Vessel area/percentage
Ray parenchyma area
Mean vessel dimeter
K_S, P_{50}, MOR

CB, Changbai Mountain
Conifer (5 spp.) vs. broadleaved trees (9 spp.)
Traits measured:
Leaf area
Xylem area
Phloem area
Ray parenchyma area
Xylem growth rate
K_S

Figure 1.4 Illustration of three studied sites including plant types and measured traits.

CHAPTER 2

Functional ratios among leaf, xylem and phloem areas in branches change with shade tolerance, but not with local light conditions, across temperate tree species

Lan Zhang, Paul Copini, Monique Weemstra and Frank J. Sterck

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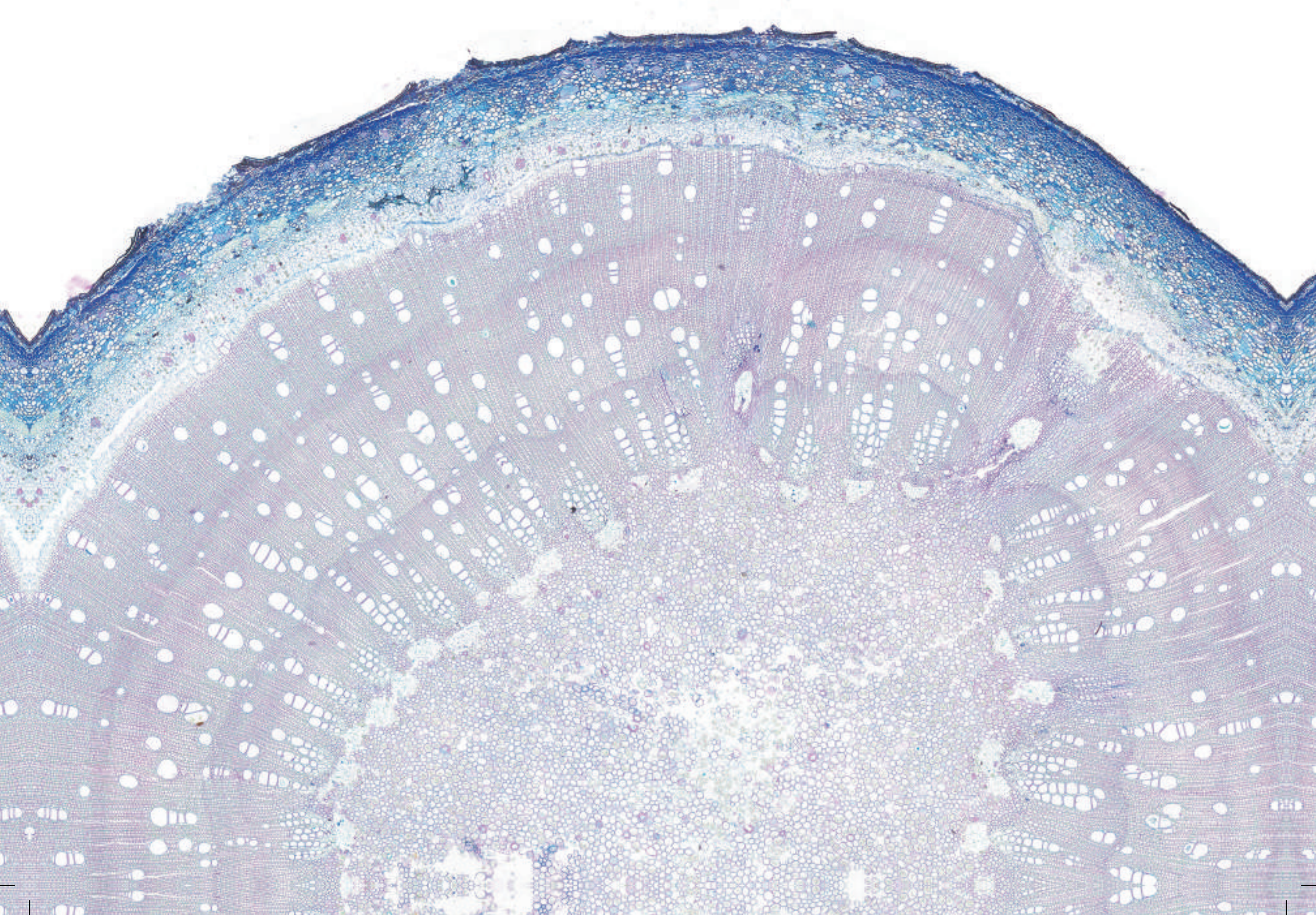

ABSTRACT

- Leaf, xylem and phloem areas drive the water and carbon fluxes within branches and trees, but their mutual coordination is poorly understood. We test the hypothesis that xylem and phloem areas increase relative to leaf area when species are selected for, or branches are exposed to, higher levels of light intensity.
- Trees of 10 temperate, broadleaved and deciduous, tree species were selected. Fifty-centimetre-long branches were collected from shaded and exposed conditions at a height of 3–4m. We measured the total leaf area, xylem area, phloem area and leaf traits, as well as the area of the constituent cell types, for a stem section at the branch base.
- Xylem area : leaf area and phloem area : leaf area ratios did not differ consistently between sun and shade branches, but, as expected, they decreased with species' shade tolerance. Similar trends were observed for conductive cell areas in xylem and phloem.
- Trees of light-demanding species maintain higher water loss and carbon gain rates per leaf area by producing more xylem area and phloem area than shade-tolerant species. We call for more comparative branch studies as they provide an integrated biological perspective on functional traits and their role in the ecology of tree species.

INTRODUCTION

The success of trees largely depends on the distribution of leaves for light capture and carbon gain, which ultimately determine tree growth and survival (Horn, 1971; Sterck & Schieving, 2007). Trunks and branches allow trees to position leaves at more favourable locations for light capture. Trees may minimize investments in cross-sectional stem areas of trunks and branches to enable more investment in leaf area and stem extension and, in turn, to more effectively compete for space and better light conditions. The stem areas should nevertheless be sufficient to maintain the stability of the branch itself (Sterck *et al.*, 2006) and the entire tree (Sellier & Fourcaud, 2009), and to guarantee the transport of water through stem xylem (Whitehead *et al.*, 1984) to the leaves and the export of sugars from the leaves through stem phloem (De Schepper & Steppe, 2011). The allocation principles that determine leaf area and xylem area within trees, as well as within branches, have been extensively discussed in the literature and are remarkably well in line with pipe model theory (i.e. constant leaf area : sapwood area ratio, Whitehead *et al.* 1984 and Ogawa 2015). Yet, such relationships between leaf area and phloem area have - as far as we know - never been presented. An overall understanding of allocation principles amongst these three key tissues is required because they largely control water and carbon fluxes in trees and ultimately influence tree growth (Cannell & Dewar, 1994; Weiner, 2004; Steppe *et al.*, 2015). Although these principles might be largely similar for branches and whole trees (at least for leaf area vs sapwood area, Togashi *et al.* 2015), the study of branches rather than stems allows for more effective broad-scale comparisons across species and light environments.

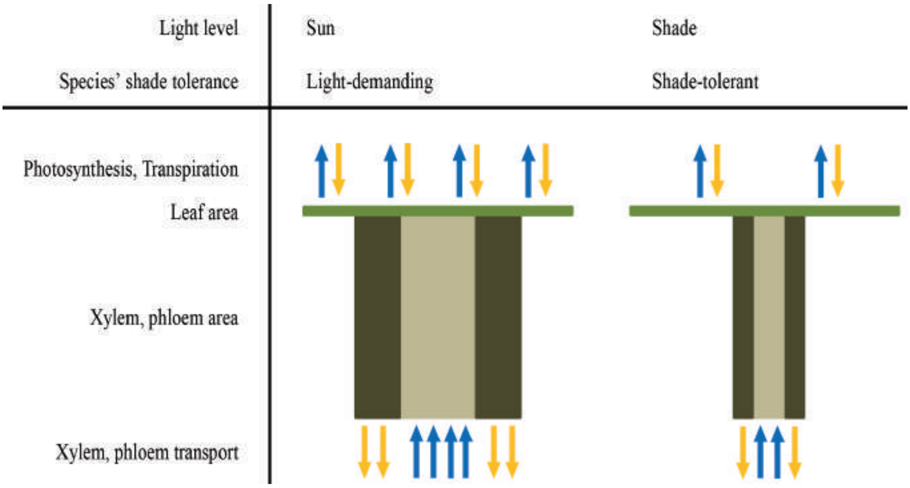

Figure 2.1 Conceptual model for the water and carbon fluxes per unit leaf area in branches or trees. Sun and shade conditions refer to local light levels of branches. Alternatively, they may refer to the light levels that species are selected for, where more shade-tolerant species are assumed to be selected for lower light levels than are more light-demanding tree species. Blue arrows represent the water fluxes from leaves or in the xylem, and yellow arrows represent carbon gain by leaves and sugar export in the phloem. The high water and carbon flux densities per leaf area in high light or of light-demanding species require larger functional xylem and phloem areas to maintain these fluxes in the branches than are necessary in the shade, or for shade-tolerant species.

Allocation to leaf area in relation to xylem and phloem areas in branches may depend on the prevailing light conditions (Fig. 2.1). Branches exposed to high light levels develop sun leaves with more mesophyll layers (Niinemets & Sack, 2006), higher stomatal density (Givnish, 1988; Abrams & Kubiske, 1990) and higher photosynthetic capacity (Givnish, 1988; Reich *et al.*, 1998; Niinemets & Sack, 2006; Davi *et al.*, 2008) than shade leaves on branches in low light conditions. Sun leaves may thus have higher carbon uptake rates per leaf area than shade leaves, but this comes with higher leaf respiration rates and more rapid water loss per leaf area (Givnish, 1988). Therefore, we hypothesize that light levels impact the allocation to leaf, xylem and phloem areas in branches: in high light, fast assimilation on a leaf area basis leads to a faster production of sugars, which need to be rapidly exported through phloem, and coinciding rapid water loss by transpiration, which needs to be matched by fast water supply through xylem. Consequently, sun branches with high photosynthetic rates on a leaf area basis may require a larger xylem area per leaf area, as well as a larger phloem area per leaf area, than shade branches (Fig. 2.1).

However, the literature only partially supports this hypothesis. Sun-grown woody plants have been found to have higher xylem area : leaf area ratio than plants grown in the shade (Schultz and Matthews 1993, for grapevine; Feild *et al.* 2001, for woody shrubs). Similar outcomes have been reported from within-crown comparisons: Schultz and Matthews (1993) found higher xylem area : leaf area ratios in sun branches than in shade branches in tropical trees, and Yoshimura (2011) studied oak trees and observed higher xylem area : leaf area ratios on exposed branches in the top of the crown than on branches in the shaded lower crown part. These differences between sun and shade branches may be attributed to the hydraulic properties that change from the lower to the upper canopy (see also McDowell *et al.* 2002) and not necessarily to light only (Yoshimura, 2011). It seems plausible, however, that light has an important effect in itself for the reasons explained earlier, but we did not come across an unambiguous test for this hypothesis in the literature. The hypothesized response in the phloem area to leaf area is functionally just as important. Indirectly, the accumulation of sugars that halts photosynthesis and limits growth

(Nikinmaa *et al.*, 2013) implies that the investments in phloem area relative to leaf area are critical for the maximization of carbon fluxes and growth at high light. Yet, we are unaware of any empirical evidence in support of the hypothesized high phloem area : leaf area ratio in response to high light levels.

Across tree species, leaf properties are largely explained by differences in the shade tolerance of species, irrespective of the light conditions to which the leaves are exposed (e.g. Janse-Ten Klooster *et al.* 2007). Leaves of light-demanding tree species are characterized by higher rates of photosynthesis, respiration and transpiration per leaf area than are leaves of shade-tolerant tree species (Niinemets & Valladares, 2006). In analogy with the leaf responses to local light conditions, we argue that leaves of tree species differing in shade tolerance require differential allocation to xylem and phloem. More light-demanding tree species are expected to have more xylem area per leaf area to match their higher transpiration rates per leaf area, and more phloem area per leaf area to export the rapidly acquired sugars more effectively, and *vice versa* for more shade-tolerant species (Fig. 2.1). As far as we know, such comparisons of functional ratios between tissue areas across tree species are scarce for leaf area and xylem area (but see, for example, Wright *et al.* 2006), and even absent for phloem area and leaf area (but, for xylem vs phloem area, see Hölttä *et al.* 2013).

In this study, we examine the functional ratios between xylem area and leaf area, and between phloem area and leaf area, in branches of 10 temperate, broadleaved tree species that differ in their shade tolerance (Table 2.1). First, we examine light effects on the functional ratios, and hypothesize that sun branches have a higher xylem area : leaf area ratio and phloem area : leaf area ratio than shade branches of similar length. Analogously, second, we hypothesize that light-demanding species have a higher xylem area : leaf area ratio and phloem area : leaf area ratio than more shade-tolerant species.

To test these hypotheses, we compared sun and shade branches from similar heights within the tree, thus minimizing confounding hydraulic factors (Yoshimura, 2011). We focused on branches rather than whole trees because branches can more easily be standardized for size, which is important as many xylem and phloem functional properties (e.g. conduit size) vary with distance from the apex (Olson *et al.*, 2014; Petit & Crivellaro, 2014). Moreover, a branch approach allowed us to control the sun and shade conditions of the collected branches relatively easily. We also examined different cell types with different functions present in xylem and phloem to provide further insights into the hypothesized differences in xylem and phloem area.

Table 2.1 Tree species studied, with their English names, shade-tolerant index (ST index; Niinemets & Valladares, 2006) and diameter at breast height (DBH) range of selected trees, as well as organ and tissue areas and functional ratios in sun and shade branches.

Species	English Name	ST index	DBH (m)	Leaf area (cm^2)		Xylem area (cm^2)		Phloem area (cm^2)		Xylem area : leaf area (10^{-5} cm^2 cm^{-2})		Phloem area : leaf area (10^{-5} cm^2 cm^{-2})	
				Sun	Shade	Sun	Shade	Sun	Shade	Sun	Shade	Sun	Shade
Populus trichocarpa Torr. & A. Gray	Black Cottonwood	1.27	0.54-0.76	1463	1161	0.15	0.13	0.09	0.07	11	12	6.6	6.9
Salix alba L.	White Willow	1.99	0.40-0.69	544	321	0.10	0.10	0.04	0.03	21	38	8.9	14.2
Betula pendula Roth.	Silver Birch	2.03	0.19-0.38	419	358	0.07	0.06	0.02	0.02	17	19	4.3	4.5
Quercus robur L.	Common Oak	2.45	0.24-0.46	1674	945	0.23	0.14	0.08	0.04	15	29	5.1	8.1
Fraxinus excelsior L.	Common Ash	2.66	0.32-0.65	2762	864	0.38	0.25	0.10	0.07	19	44	5.2	11.6
Alnus glutinosa (L.) Gaertn.	European Alder	2.71	0.24-0.48	1016	577	0.17	0.12	0.06	0.02	18	25	5.3	4.9
Prunus avium L.	Wild Cherry	3.33	0.24-0.54	1422	636	0.13	0.10	0.05	0.03	9	18	3.3	5.8
Acer pseudoplatanus L.	Sycamore	3.73	0.21-0.57	2087	1136	0.26	0.13	0.06	0.03	13	14	3.0	3.4
Tilia cordata Mill.	Small-leaved Lime	4.18	0.21-0.66	1409	1039	0.18	0.10	0.12	0.06	15	11	9.1	6.2
Fagus sylvatica L.	European Beech	4.56	0.31-0.56	1010	984	0.11	0.07	0.03	0.01	12	6	3.0	1.6

Standard errors of the means are presented in Fig.3.

MATERIALS AND METHODS

Study species and site description

Trees of 10 broadleaved, deciduous tree species, which varied in their shade tolerance (Table 2.1) and correspondingly in their leaf traits (i.e. transpiration and photosynthetic rate per leaf area, Supporting Information Table S2.1), were selected in the Hollandse Hout forest, located in the centre of the Netherlands (52°28′N, 5°26′E). This forest, which covers c. 900 ha, was established in the 1960s and 1970s (Bijlsma & Verkaik, 2008), when over 15 deciduous and coniferous tree species were planted in monospecific stands. The site is characterized by a temperate marine climate with mild summers (average temperature in June-August: 15.9°C) and winters (average temperature in December–February: 2.5°C), and an annual precipitation of c. 800 mm (KNMI, 2015). The forest soil is a calcareous, nutrient rich, clay soil of uniform texture (Bijlsma & Verkaik, 2008), and groundwater tables range between 1.6 and 1.2 m below the soil surface (TNO-NITG, 2015).

Sampling and sample preparation

For each species, we collected branches from nine healthy, 43-53yr-old trees from at least three different forest stands per species. The selected trees were all located at the edge of the forest stand and were fully exposed on one side, but shaded on the other. We cut two branches of 50 cm in length, one from the sun side and one from the shade side of each tree. Cutting branches at a fixed 50-cm distance from the apex minimizes the impact of distance to apex on the phloem and xylem cell sizes in the branch section (Olson *et al.*, 2014; Jyske & Hölttä, 2015). Branches were sampled between 20 September and 29 October 2013 at the end of the growing season, just before leaf discoloration and abscission, to ensure that the formation of xylem and phloem had ceased. As leaf, xylem and phloem ratios may differ throughout the vertical crown (e.g. Jyske and Hölttä 2015), all branches were cut at a similar height of 3-4 m above the forest floor. Per branch, all leaves were collected to determine the total leaf area, leaf mass and specific leaf area (SLA, the leaf area : leaf mass ratio). The basal 5-cm stem section was used to cut 20-25-µm-thick cross-sections using a G.S.L.-1 microtome (Gärtner *et al.*, 2014) for xylem and phloem measurements. Because heartwood was not yet formed, total xylem areas were equal to the sapwood areas in the branches of all species. All cross-sections were stained with a safranin/astrablue solution to colour unlignified cells blue and lignified cells red. Following dehydration (50-95-100% ethanol), the samples were rinsed with Roticlear® (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) and fixed in Canada Balsam. Photographs of entire cross-sections were taken with a digital camera (DFC 320; Leica, Cambridge, UK) mounted on a microscope (DM2500; Leica) using Leica imaging software (v.3.6.0). To obtain a photograph of the entire cross-section, sequential photographs were stitched using PTGUIPRO (v.9.1.8; New House Internet Services BV, Rotterdam, the Netherlands).

Sample measurements

We first measured the total leaf area of all 180 branches using a LI3100 Area Meter (Lincoln, NE, USA). Then, the leaves were dried at 80°C for 48h and weighed. Next, SLA was calculated per branch. Second, we estimated the total pith, xylem and phloem areas of all branches by measuring the pith, xylem and phloem width over four radii in opposite positions over the cross-section (Fig.2) using IMAGEJ software (v.1.44; http://rsb.info.nih.gov/ij; developed by W. Rasband, National Institutes of Health, Bethesda, MD, USA). In our xylem area measurements, we corrected for the pith area to reduce confounding effects of the pith area on leaf area : xylem area ratio which occur in many branch studies (Togashi *et al.*, 2015). Subsequently, the xylem area : leaf area ratio and phloem area : leaf area ratio were calculated. Next, branch age was determined by counting the number of tree rings in the cross-sections. We constructed a sub-dataset including 60 2-3-yr-old branches, that is, three sun and three shade branches of all 10 species, to minimize the possible effect of branch age on the xylem area : leaf area and phloem area : leaf area ratios.

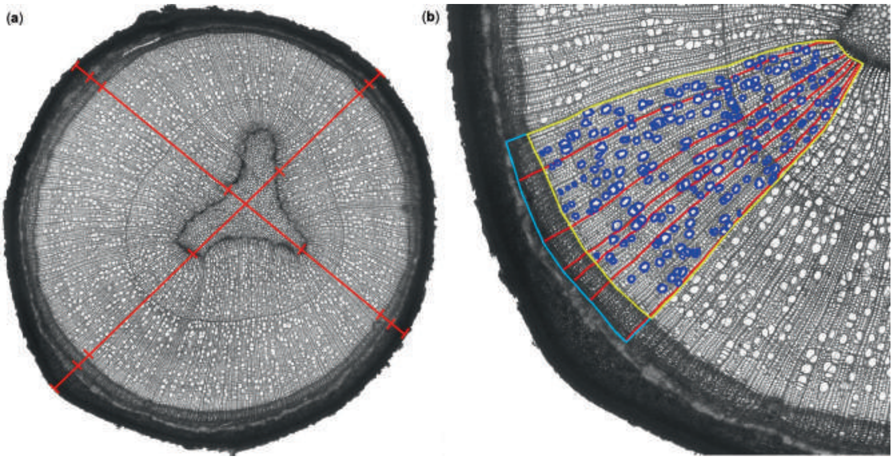

Figure 2.2 An example of a cross-section of a 2-yr-old branch of white willow (*Salix alba*) sampled at the shaded side of a tree, that is, low light level. (a) The borders between pith, xylem, living phloem and cortex are indicated along two orthogonal axes which cross the pith in the centre. (b) An example of a radial subsection, four of which were selected in each cross-section. Borders of xylem (yellow line) and adjacent phloem (light blue line) are indicated. In addition, ray parenchyma (red lines) and vessels (dark blue) are coloured. The vessel area, ray area and fibre areas were determined. In the phloem, we measured the ray area and calculated the area of cells involved in axial translocation of photosynthetic products, that is, the area containing sieve elements, companion cells and axial parenchyma (excluding phloem fibres and sclereids).

In addition, we used this subset for more detailed wood anatomical measurements employing IMAGEJ v.1.44n. For this, we created four subsections on different sides of the cross-section. The areas of the four subsections summed to c. 10% of the total cross-sectional area. Anatomical measurements on the subsections were scaled to the entire cross-sectional area according to the percentage of the total surface area covered by the subsections (Fig. 2.2). For the xylem, we measured the area covered by rays, vessels and fibre tissue relative to the xylem area. The xylem ray area was calculated from the length and width of the rays. The xylem vessel area was measured using a semi-automatic routine in which the vessel edges were detected, and both the total area and the mean of the five largest vessels were calculated. The xylem fibre area (including both fibre cells and axial parenchyma) was calculated by subtracting the ray and vessel area from the total xylem area. For the phloem, we determined the area covered by rays, similar to the xylem. In addition, the area covered by phloem fibres and sclereids was determined. Next, we subtracted the phloem ray, sclereids and fibre area from the total phloem area, resulting in the tissue that is important for axial phloem transport, that is, axial parenchyma, sieve elements and companion cells. This is referred to in the text as axial phloem. Ultimately, the xylem area : leaf area, phloem area : leaf area, vessel area : leaf area and axial phloem area : leaf area ratios were calculated. We also calculated the xylem vessel area, total ray area in xylem and phloem, total fibre area in xylem and phloem and axial phloem area relative to the total cross-sectional area of the stem.

Statistical analyses

Statistical analyses were performed in IBM SPSS STATISTICS 19 (SPSS, Inc., Chicago, IL, USA). All variables were checked for normality and $\log_{10}$ transformed if needed. For our first hypothesis, we tested whether sun branches had more xylem, phloem and leaf area, and a higher xylem area : leaf area ratio and phloem area : leaf area ratio, than shade branches, across both the full dataset (including all 180 branches) and the subset (including 60 similar-aged branches). We applied a general linear model with the light level (high vs low light) and tree species (n=10) as fixed factors, the interaction term of these fixed factors and branch age as a covariate. For our second hypothesis, we tested whether species' shade tolerance indices were significantly correlated with the ratios between leaf, xylem and phloem areas using the Spearman rank correlation test. In addition to our two hypotheses, we also tested the correlation between cell type proportions and species' shade tolerance.

RESULTS

For both the full dataset and the similar-aged subset, sun branches had overall larger leaf, xylem and phloem areas than shade branches (Table 2.2; Fig. 2.3a, c, e). Accordingly, we observed larger vessel, fibre, ray and axial phloem areas in the sun branches than in the shade branches in the more closely examined subset (Table S2.2; Fig. S2.1). The functional ratios between leaf, xylem and phloem areas did not differ consistently between the sun and shade branches across the full species set (Table 2.2), but the significant species-light interaction effect on these ratios (Table 2.2) implies species-specific responses (see Fig. 2.3b, d). Moreover, the functional ratios between different cell type areas and leaf area did not differ between sun and shade branches (Table S2.3).

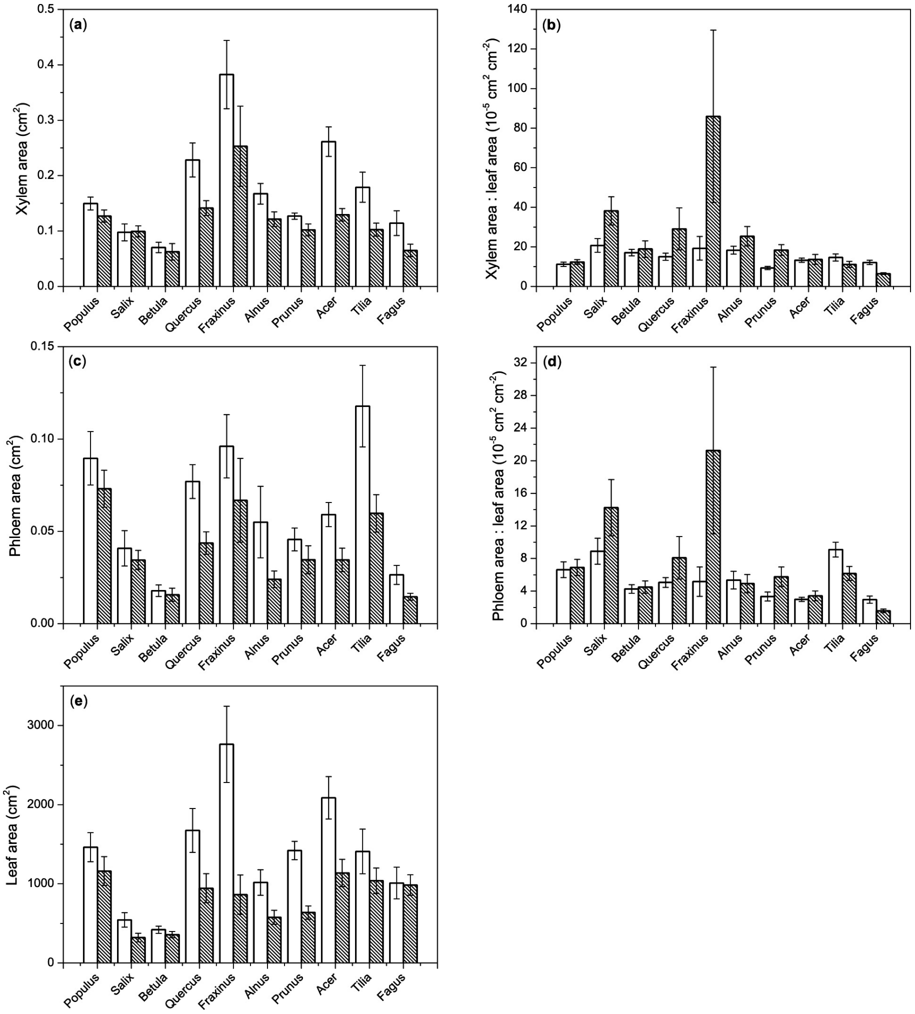

Figure 2.3 Mean tissue area and functional ratios (±2SE) for branches of 10 broadleaved tree species. For each species, nine branches were exposed to high light (open bars) and nine to low light (hatched bars) (total n = 180).

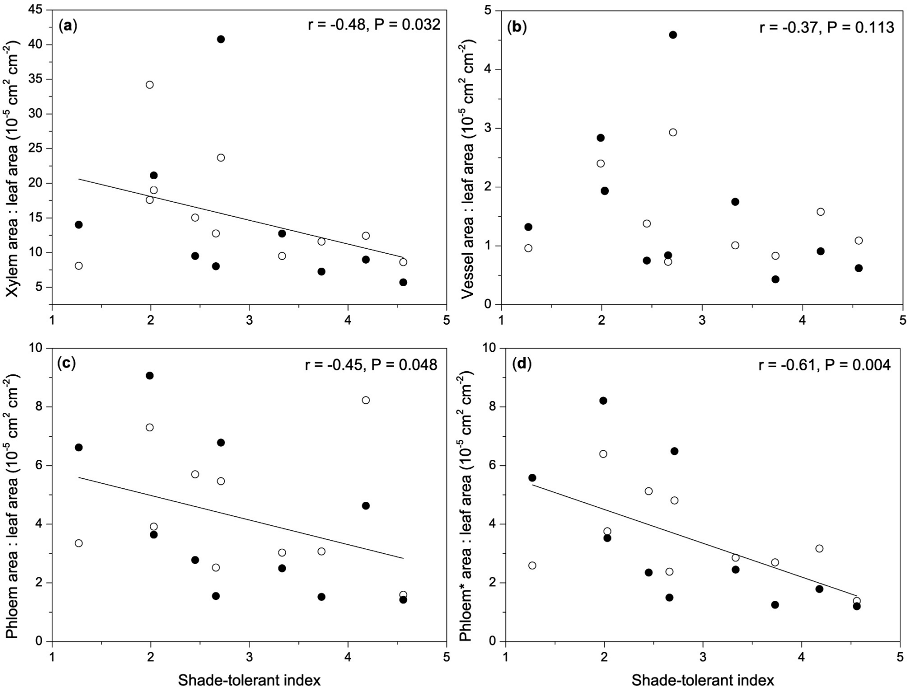

Figure 2.4 Functional ratios between (a) xylem area, (b) vessel tissue area, (c) phloem area and (d) axial phloem area and leaf area in high light (open dots) and low light (closed dots) branches of 10 temperate tree species of different shade-tolerant index (Table1). Significant trends between these percentages and the shade tolerance of species (tested with a Spearman rank correlation test) are indicated by trend lines.

Species differed in their leaf, xylem and phloem areas, as well as in their functional ratios between these variables (Table 2.2; Fig. 2.3). The differences in leaf, xylem and phloem area were not significantly related to the species' shade-tolerant index (results not shown). However, as expected, the different functional ratios did relate to species' shade tolerance: both the xylem area : leaf area ratio and the phloem area : leaf area ratio decreased with increasing shade tolerance of the species (Fig. 2.4a, c). Similar trends were observed for the vessel area : leaf area ratio and axial phloem area : leaf area ratio, although the former ratio was not significant (Fig. 2.4b, d).

The xylem and phloem surface area occupied by the cell types varied across the species (Table S2.4). These differences were partially related to species' shade tolerance: more shade-tolerant species had a larger ray parenchyma area, but a smaller axial phloem area and a marginally smaller fibre area, than more light demanding species (Fig. 2.5).

Table 2.2 Mean branch tissue areas, functional ratios and specific leaf areas (SLA) of sun and shade branches across 10 tree species.

	Mean		Light		Species		Species*Light		Age	
Variable	Sun	Shade	F	P	F	P	F	P	F	P
Areas (cm^2)										
Leaf	1380	802	42.01	**<0.001**	10.19	**<0.001**	3.65	**<0.001**	0.61	0.436
Xylem[1]	0.18	0.12	46.49	**<0.001**	21.76	**<0.001**	1.65	0.105	16.83	**<0.001**
Phloem[1]	0.06	0.04	47.70	**<0.001**	23.64	**<0.001**	0.80	0.617	26.21	**<0.001**
Ratios ($10^{-5}cm^2\ cm^{-2}$)										
Xylem area : leaf area[1]	15.1	25.9	3.46	0.065	8.23	**<0.001**	3.65	**<0.001**	8.16	**0.005**
Phloem area : leaf area[1]	5.37	7.68	0.15	0.696	10.53	**<0.001**	2.61	**0.008**	16.17	**<0.001**
SLA ($cm^2\ g^{-1}$)	146	224	155.61	**<0.001**	11.13	**<0.001**	5.42	**<0.001**	4.08	**0.045**

Species, light and interaction effects on these areas are shown for the full dataset (total n = 180). We used a general linear model, with the 10 species and two light levels (n = 9 per species per light level) as fixed factors, species-light interaction and branch age as covariate.

[1] Data were $\log_{10}$ transformed.

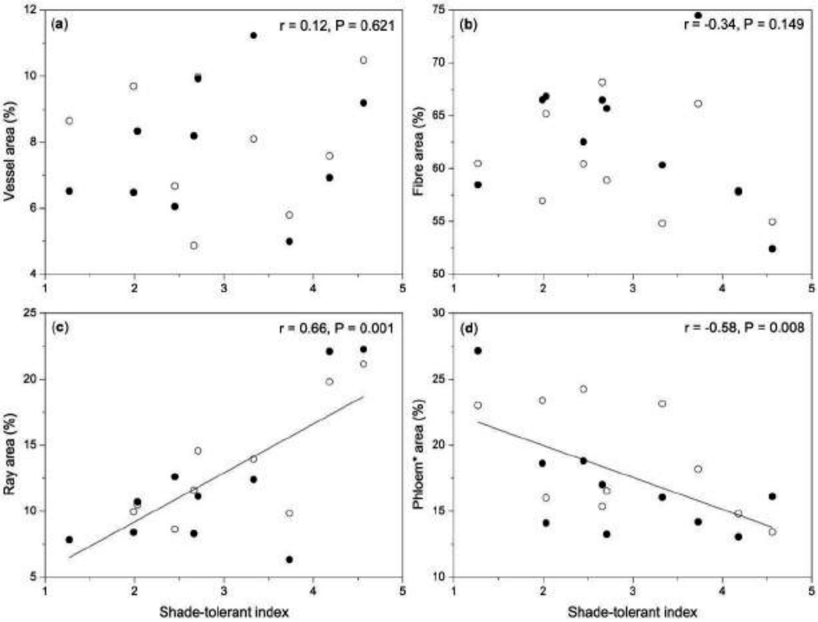

Figure 2.5 Cross-sectional areas of different cell types as a percentage of the total xylem or phloem area (see Materials and Methods section) for branches in high light (open dots) and low light (closed dots) of 10 temperate tree species of different shade-tolerant index (Table1). Significant trends between these percentages and the shade tolerance of species (tested with a Spearman rank correlation test) are indicated by trend lines.

DISCUSSION

Our study shows how trees of 10 temperate, broadleaved species coordinated leaf, xylem and phloem area in their sun and shade branches. Here, we discuss our two major results: functional ratios (i.e. xylem area : leaf area and phloem area : leaf area) did not differ consistently between sun and shade branches across the full species set, but did vary with the shade tolerance of different species. In addition, we evaluate possible trade-offs between investments in different cell types in the xylem and the phloem in relation to the shade tolerance of species.

Functional ratios did not respond to local light conditions

Leaf, xylem and phloem areas were larger in sun branches than in shade branches of equal length; both the full dataset consisting of 180 branches and the subset of 60 similar-aged branches revealed significant light effects (Tables 2, S2). However, in contrast with our expectation, the functional ratios did not differ consistently between sun and shade branches across the species set as a whole (Tables 2, S1). We hypothesized that sun branches would require a higher xylem area : leaf area ratio than shade branches to supply more water to leaves that transpire more rapidly, and a higher phloem area : leaf area ratio to export sugars from leaves that produce sugars more rapidly. As expected, sun leaves had a lower SLA and higher rates of stomatal conductance, transpiration and photosynthesis per unit leaf area than did shade leaves (Table S2.1), which agrees with the literature on many other species (Givnish, 1986; Reich *et al.*, 1998; Davi *et al.*, 2008; Houter & Pons, 2014). The higher photosynthetic capacity results from stacking mesophyll with increasing light levels (Niinemets & Sack, 2006), and the higher stomatal conductance probably results from increasing stomatal density with irradiance, as observed for 26 temperate broadleaved tree species (Abrams & Kubiske, 1990). Considering that sun branches had faster assimilation and transpiration per leaf area, but not larger xylem or phloem area per leaf area, other xylem and phloem variables driving transport rates might differ between sun and shade branches.

In theory, xylem transport rates depend on the water pressure gradient, vessel size and sap viscosity. In this study, the five largest vessels in a stem cross-section were significantly larger in the sun branches than in the shade branches (two-way ANOVA with light and species as fixed factors, light effect: $F_{1,18} = 9.9$, $P = 0.003$). Given the fourth power contribution of vessel diameter to xylem conductivity (Tyree & Zimmermann, 2002; Fonti *et al.*, 2010; Zanne *et al.*, 2010), sun branches probably increased the conductivity of the xylem, and thus transported water more rapidly to meet the higher transpiration demands, compared with shade branches. As high specific xylem conductivity mitigates the need for a steep water pressure gradient for water transport, sun branches may avoid too low water potentials in leaves and stem. Our results agreed with studies on beech (Cochard *et al.*, 2002; Lemoine *et al.*, 2002), which demonstrated that sun branches had larger vessels than shade branches. Remarkably, these two studies showed that sun branches were less vulnerable to cavitation than were shade branches. Trade-offs between cavitation vulnerability and specific xylem conductivity, as observed across species (Hacke *et al.*, 2001), may thus not always occur within species (but see Sterck *et al.* 2012) for such a trade-off within a coniferous species). In summary, branches may maintain homeostasis for the xylem area : leaf area ratio at different light levels, as they balance transpiration demand vs water supply by modifying vessel size, possibly without affecting their risk of cavitation.

Phloem transport rates are determined by the conductive phloem area, turgor pressure difference between phloem sap close to the sources of sugar (leaves) and sinks (respiring tissues, for example, in the stem), sugar concentration that drives the viscosity of the phloem sap, and specific phloem permeability and conductivity (De Schepper *et al.*, 2013; Hölttä *et al.*, 2014). If a high assimilation rate in sun leaves increases sap sugar concentrations and consequently viscosity in the phloem, this would slow down the sugar flux in the sun branches compared with shade branches. Turnbull *et al.* (2002) indeed observed that leaf soluble sugar content was higher in sun leaves compared with shade leaves. Variation in specific phloem conductivity will largely depend on hydraulic phloem tube diameters (Hölttä *et al.*, 2014), but this was not quantified in our study, and we are unaware of any study showing how phloem tube diameters respond to local light conditions. Nevertheless, there is some indirect support for the idea that wide xylem vessels are associated with relatively wide phloem tubes: phloem tubes increase in hydraulic diameter from apex to stem base, as do xylem conduits (Petit & Crivellaro, 2014; Jyske & Hölttä, 2015). If xylem vessel and phloem cell sizes are indeed related through cambial processes, sun branches could facilitate the transport of phloem sap by having wider phloem cells than do shade branches, but this speculation still lacks a direct test.

Functional ratios varied with species' shade tolerance

We hypothesized that shade-tolerant species would require less xylem and phloem area per leaf area because of their lower capacity to transpire and gain carbon on a leaf area basis compared with light-demanding species (Melis & Harvey, 1981; Lambers *et al.*, 2008), which was supported by our data (Fig. 2.4; Table S2.1). Wright *et al.* (2006) showed that the sapwood area : leaf area ratio decreased with increasing wood density across evergreen shrub and tree species. Considering that wood density is generally used as a proxy for shade tolerance (Wright *et al.*, 2006), these results are in line with our study on deciduous tree species (Fig. 2.4). We did not encounter any other study comparing phloem area : leaf area ratios with shade tolerance differences, or proxies for shade tolerance, across species. The parallel decrease in phloem area : leaf area ratio and xylem area : leaf area ratio with increasing shade tolerance implies how tissue allocation at branch level (and plant level, when driven by the same principles, as suggested by, for example, Togashi *et al.* 2015) allows species to specialize for different environments. It also shows that winter-deciduous, shade-tolerant tree species minimize their carbon costs for leaf area in two ways: at the leaf level by increasing their SLA (Walters & Reich, 1999; Walters & Reich, 2000), and at the crown level by producing branches with smaller xylem and phloem cross-sectional areas relative to the leaf area they support.

Ray parenchyma increased with shade tolerance

Branch anatomical properties varied with species' shade tolerance. In branches of shade-tolerant species, the ray parenchyma occupied a greater share of the stem cross-sectional area at the expense of axial phloem area compared with more light-demanding species (Fig. 2.5). This might have many ecological implications, but most are still highly speculative. Our result implies that branches of shade-tolerant species store more carbon, water and nutrients or proteins (Kobe, 1997; Spicer, 2014). These resource reserves can be used for respiration and enhance survival under persisting shaded forest understorey conditions (Carter & Klinka, 1992; Van Hees & Clerkx, 2003; Fownes & Harrington, 2004), and allow fast growth responses when light does become available, for example, when a nearby tree falls (Canham, 1988; Hoffmann & Schweingruber, 2002). Although speculative, these different roles of the ray parenchyma are in line with the typical survival-growth trade-off, as observed between shade-tolerant and light-demanding species (Kobe *et al.*, 1995; Janse-Ten Klooster *et al.*, 2007).

CONCLUSIONS

We showed how trees coordinated their allocation to leaf area, xylem area and phloem area in their branches, and discussed implications for carbon and water fluxes. We demonstrated that this coordination was unrelated to prevailing light conditions across the species set as a whole, but was associated with the shade tolerance differences across 10 broadleaved tree species. We argue that the integration of architectural, morphological and anatomical plant traits in branches allows for an understanding of differences in tree species from an entire plant perspective.

ACKOWLEDGEMENTS

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SUPPORTING INFORMATION

Fig. S2.1 Mean cell type surface area (±2 SE) of three branches per species per light level of ten temperate, broadleaved tree species for a similar-aged subset of branches at high light (white bars) and low light (hatched bars) (total N = 60).

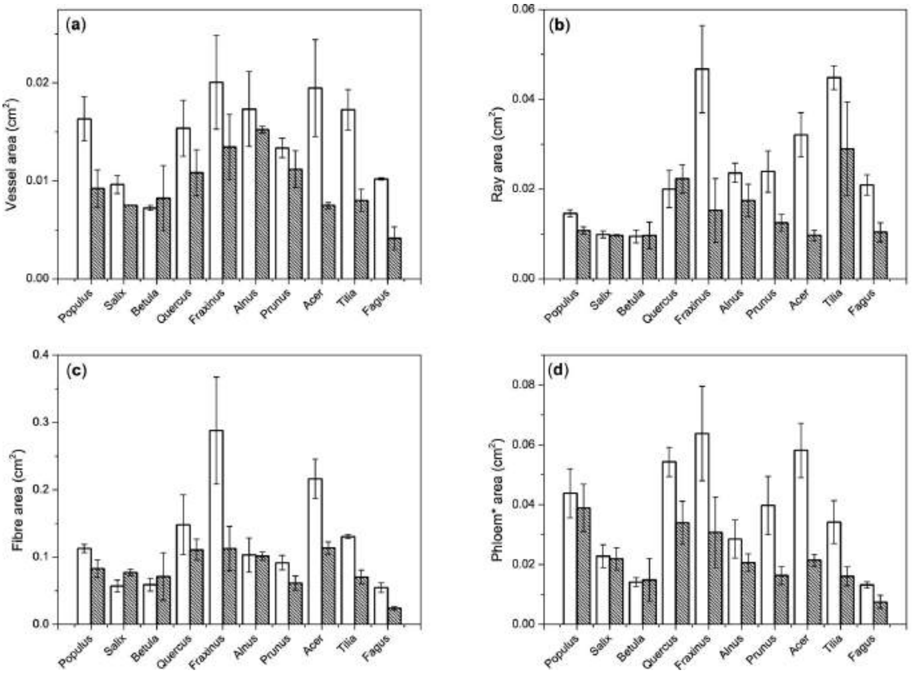

Table S2.1 Mean branch tissue and cell type area of sun and shade branches across ten temperate, broadleaved tree species, and species, light and their interaction effects on these areas for the similar-aged subset (total N = 60). (Two-way ANCOVA, species (N = 10) and light (sun versus shade branches, N = 3 per species per light level) as fixed factors, branch age as covariate.)

Areas	Mean		Light		Species		Species*Light		Age	
(cm^2)	Sun	Shade	F	P	F	P	F	P	F	P
Leaf	1293	823	19.20	<0.001	11.65	<0.001	2.98	0.009	2.11	0.154
Xylem[1]	0.1554	0.1015	20.14	<0.001	9.53	<0.001	2.03	0.062	0.45	0.508
Phloem	0.0472	0.0275	26.16	<0.001	8.49	<0.001	1.88	0.084	0.03	0.855
Phloem*	0.0373	0.0223	22.65	<0.001	6.48	<0.001	1.77	0.106	0.19	0.663
Pith[1]	0.0244	0.0257	0.07	0.800	37.46	<0.001	1.44	0.205	4.95	0.032
Cortex[1]	0.0495	0.0339	14.77	<0.001	10.45	<0.001	1.06	0.412	0.00	0.993
Vessel[2]	0.0146	0.0095	25.40	<0.001	4.45	<0.001	1.25	0.297	0.58	0.451
Ray[2]	0.0246	0.0196	29.30	<0.001	8.29	<0.001	3.02	0.008	0.07	0.787
Fibre[1]	0.1261	0.0825	17.20	<0.001	11.06	<0.001	1.96	0.071	0.35	0.560

[1] Data were $\log_{10}$ transformed; [2] data were square root-transformed.

Table S2.2 Mean functional ratios of branch tissue and cell type area relative to leaf area, across ten temperate, broadleaved tree species, and species and light effects on these functional ratios for the similar-aged subset (total N = 60). Species effects: N = 10, Kruskal-Wallis test; light effects: sun versus shade branches, N = 3 per species, per light level, Mann-Whitney U test.

	Mean		Light		Species	
Ratios (10^{-5} cm^2 cm^{-2})	Sun	Shade	Z	P	H	P
Xylem area : leaf area	13.8	29.2	-0.488	0.626	32.2	<0.001
Vessel area : leaf area	1.49	2.74	-0.510	0.610	32.9	<0.001
Phloem area : leaf area	4.42	7.37	-0.591	0.554	30.2	<0.001
Phloem* area : leaf area	3.52	6.59	-0.769	0.442	27.3	<0.001

Table S2.3 Mean cell type area relative to total (xylem or phloem) area of sun and shade branches across ten temperate, broadleaved tree species, and species, light and their interaction effects on these cell type areas for the similar-aged subset (total N = 60). (Two-way ANOVA, species (N = 10) and light (sun versus shade branches, N = 3 per species, per light level) as fixed factors).

	Mean		Light		Species		Species*Light	
Percentages (%)	Sun	Shade	F	P	F	P	F	P
Vessel area	8.01	7.78	0.28	0.601	5.11	<0.001	2.19	0.043
Ray area[1]	12.78	12.21	1.61	0.212	21.08	<0.001	1.81	0.097
Fibre area	60.39	63.17	4.79	0.035	6.21	<0.001	1.24	0.301
Phloem* area	18.81	16.84	3.76	0.059	5.38	<0.001	1.33	0.254

[1] Data were square-root transformed.

CHAPTER 3

Conifer and broadleaved trees differ in branch allometry but maintain similar functional balances

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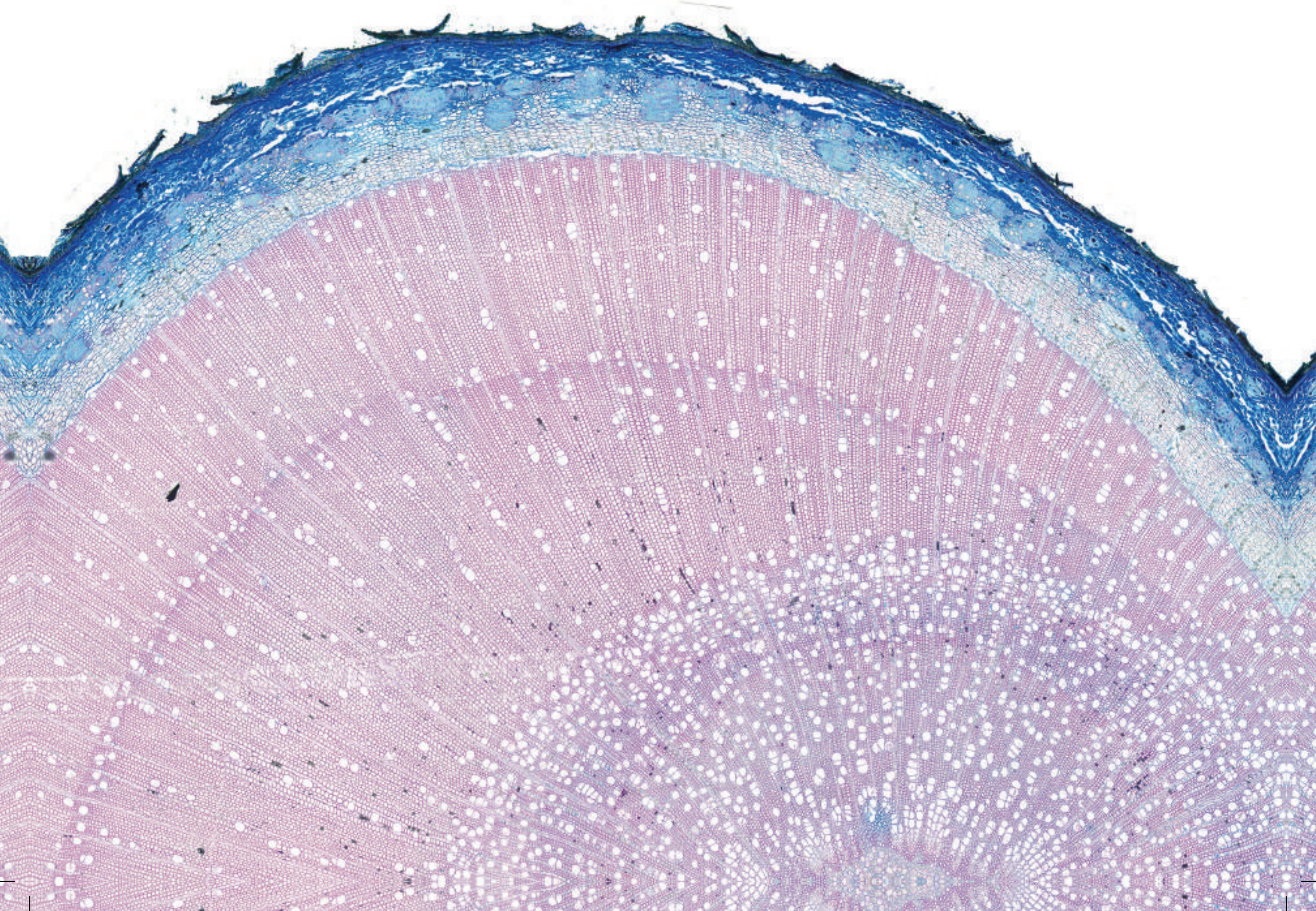

ABSTRACT

- Conifers and broadleaved trees coexist in temperate forests and are expected to differ in functional relations between water and sugar transport and storage. We compare water transport and sugar storage related traits and associate these with xylem growth to better understand how they contribute to these lifeform strategies.
- We sampled distal branches from a temperate forest canopy in northeast China using a canopy crane, and measured xylem area, axial phloem area, ray area, and leaf area, and determined ray percentage, dry wood density, specific leaf area, xylem conductivity and xylem growth rate.
- Conifers and broadleaved trees did not differ significantly in tissue areas, xylem growth rate and the relation between axial phloem area with leaf area. Conifers had lower wood density, ray percentage, and higher xylem area but lower ray area for amount of leaf area. For the same xylem conductivity, axial phloem area and ray parenchyma area did not differ between conifers and broadleaved trees. Xylem growth rate was similar for the amount of leaf area and axial phloem area.
- Conifers tend to develop more xylem area per leaf area, probably to compensate for the low water transport ability. Broadleaved trees tend to develop more water and sugar storage capacity to support yearly produced new leaves. Conifers tend to consume rather than store carbon to achieve a similar xylem expansion per year as co-existing broadleaved trees.

INTRODUCTION

Conifers and broadleaved trees co-exist in many temperate forests (Schmitt *et al.*, 2009), despite fundamental differences in morphology, anatomy and functioning (Bond, 1989; Tyree & Ewers, 1991; Becker *et al.*, 1999). Broadleaved trees are angiosperms, flowering plants, have broad leaves, are often deciduous in temperate forests, and have specialized cells for water transport (vessels) and strength (fibres) in the wood. Conifers are gymnosperms, cone-bearing seed plants, with needle-liked stereotyped shape and size leaves, and use the same cells (tracheids) for water transport and strength. Conifers have a less efficient transport system in the stem xylem and lower gas exchange rates in the leaves (Becker *et al.*, 1999; Brodribb & Feild, 2000; Brodribb *et al.*, 2005), resulting in lower maximum growth rates in well lit, well-watered habitats compared to broadleaved trees (Waring, 1983; Bond, 1989; Waring *et al.*, 2016). Meanwhile, conifers are expected to have less efficient sugars transport in phloem (Hölttä *et al.*, 2009; Petit & Crivellaro, 2014; Jyske & Hölttä, 2015), though empirical data are scarce (Srivastava, 1975; Thompson *et al.*, 1979; Helfter *et al.*, 2007; Liesche *et al.*, 2015). Despite of their low transport efficiency and photosynthesis rate, some conifers attain a higher productivity than co-occurring broadleaved trees by accumulating several cohorts of leaves with a higher total leaf biomass (Schulze *et al.*, 1977). Conifer species thus differ from broadleaved trees in their branch tissues and are less efficient in transport and gas exchange, but the resultant means for the way they coordinate biomass allocation is poorly explored.

Trees compete with one another to display their leaves at favourable high light conditions in the upper canopy. Their leaves can achieve high gas exchange rates, but this comes with high demands for water supply within the xylem and for sugar transport downward in the phloem of the stem. While trees grow larger, they balance their increasing leaf area with increasing supporting xylem area (Duursma & Falster, 2016; Kiorapostolou & Petit, 2018) and also with phloem area, though data for this are scarce (Giaquinta, 1978; Nikinmaa *et al.*, 2013). More indirect, a relationship can be expected between leaf area and ray parenchyma area, which serves for storage of water and sugars (Holbrook, 1995; Borchert & Pockman, 2005; Plavcová & Jansen, 2015). Ray parenchyma is known to be less in conifers than in broadleaved trees (Morris *et al.*, 2016b), which probably links to a lower stem capacitance in conifers (Martínez-Cabrera *et al.*, 2009; Fu *et al.*, 2019). In this study, we explore functional relations between leaf area where carbon and water exchanges between plant and atmosphere versus xylem area, phloem area and ray parenchyma area as proxies for water transport, sugar transport and water and sugar storage, respectively. We will show how conifer trees and broadleaved trees organize their tissues in different ways, and explore the consequences for growth.

Our study compares branches of canopy trees of conifer and broadleaved tree species that co-exist in a natural, temperate forest. We aim to investigate how these species coordinate their leaf area with the xylem area, phloem area, ray parenchyma area and xylem conductance in the stem section supporting the leaf area using allometric trends. We expect that conifers differ from broadleaved trees by having: a larger xylem area for a given leaf area, which compensates for a lower sapwood specific conductivity (conductivity per sapwood area); less phloem area, ray parenchyma area and xylem conductivity for a given leaf area, and a lower phloem area and ray parenchyma area for a given xylem conductivity because their leaves produce assimilates at lower rates and therefore they have lower capacity required for transport and storage. Deciduous broadleaved trees normally have limited time to conduct photosynthesis for branch development and carbon storage in order to survive dormancy during winter and produce new leaves during the next growing season (Eamus, 1999; Catovsky & Bazzaz, 2000; Lusk *et al.*, 2003). Therefore, they require a more efficient carbon translocation. We expect this to be indicated by a higher xylem growth for the amount of leaf area and phloem area in broadleaved trees.

Instead of addressing entire individuals, we focus on exposed branches in the upper canopy: (i) with the high irradiation and temperature during the day, these branches potentially gain most carbon but face high transpiration demands and can thus act as bottlenecks for the hydraulic integrity and carbon gain of the entire individual; (ii) in canopy branches, standardized branch length can be easily controlled for diminishing the tapering effects of vessels and their related functionalities (Becker *et al.*, 2000; Petit & Anfodillo, 2009); and finally (iii) it is practically more feasible to study branches than entire individuals, certainly the large ones that dwell in the forest canopy.

MATERIALS AND METHODS

Study site

The study was carried out from June to July, 2017, in Changbaishan Nature Reserve, Jilin Province, NE China (41°15’-42°35’N, 127°15’-129°00’E). The site is a typical representative of evergreen conifer (except *Larix*) and deciduous broadleaf mixed forest (Zhou & Dai, 2012). The site has an altitude of around 800 m and has a canopy crane installed with a height of 60 m and a radius of 30 m. The climate is temperate continental climate affected by the monsoon with long and cold winter, and warm, rainy and short summer. Annual temperate is 3.6℃ and annual precipitation is around 700 mm.

Field work

We sampled of total of 14 species that were in reach of the canopy crane. We thus collected branches from 4 evergreen and 1 deciduous conifer species and 9 broadleaved tree species (Table S3.1). For each species, 3 individuals (except *Tilia mandshurica* for which only 2 were in reach of the crane) were selected. From each individual tree a 0.5 m long sun-exposed canopy branch was cut, and the basal 10 cm branch segment was cut and preserved into FAA solution for anatomical analysis. The stem segment of the branch was taken at a fixed distance to apex (0.5 m) to control for the effects of tapering in xylem and phloem transport cells (Anfodillo *et al.*, 2013; Petit & Crivellaro, 2014).

In addition, branches were collected to determine the specific conductivity of the stem: for this purpose, branches were cut at 0.5 m length from the apex for conifers or at 1.5 m for broadleaved trees for hydraulic measurements. All branches were immediately treated: 1) side twigs were cut off, but ca. 10 cm base was left in order to avoid cavitation propagation to the main stem by cutting; 2) fresh water was showered on the whole branch; 3) each branch was put into a black plastic bag and wrapped.

Leaf traits measurements

All leaves on the branch were collected into a Ziplock bag. For all broad leaves, their total leaf area was scanned, and for conifers, ca. one tenth of their fresh leaves were scanned (CannonScan LiDE 220). ImageJ software (v.1.44; http://rsb.info.nih.gov/ij; developed by W. Rasband, National Institutes of Health, Bethesda, MD, USA) was used to measure areas (LA, cm^2). As our studied 5 conifer species had round or flat leaves, in order to standardize our estimation, we doubled the scanned area of needle leaves as the measured leaf area. Leaves were then oven (PH240A) dried at 65 ℃ for 48 hours and weighted (LD, g). For conifers, total leaf area was estimated by measured leaf area divided by the proportion of measured leaf dry weight to total leaf dry weight. Specific leaf area (SLA, cm^2 g^{-1}) was calculated as:

$$SLA = LA / LD$$

Anatomical measurements

Permanent cross-sections were made using the paraffin embedding method for further anatomical measurements. One 1-1.5 mm thick branch piece was cut with a razor blade from each 10 cm stem segment that embedded in the FAA solution. These pieces were dehydrated with a series of 50-96-100% ethanol. After this, they were infiltrated with limonene at air temperature and then with melted paraffin at 65 ℃ in an oven (PH240A). Afterwards, we embedded these pieces into paraffin blocks using a Leica EG1150H Paraffin Embedding Station (Leica Biosystems Nussloch GmbH, Nussloch, Germany). After the paraffin blocks got hard, we cut a 6-10 um thick cross-section per piece using a Leica RM2235 rotary microtome (Leica Biosystems Nussloch GmbH, Nussloch, Germany). Then we removed the paraffin from the cross-sections with limonene and dehydrated the pieces again by 50-96-100% ethanol series. Following dehydration, all cross-sections were stained in a safranin/astrablue solution and fixed in Neutral Balsam. After the Neutral Balsam was air dried, photographs of entire cross-sections were taken with a digital camera (Nikon DS-Ri1) mounted on a microscope (Nikon ECLIPSE 80i) using Nikon Imaging Software, NIS Elements (Nikon, Tokyo, Japan). To obtain a photograph of the entire cross-section, sequential photographs were stitched using PTGUIPRO (v.9.1.8; New House Internet Services BV, Rotterdam, the Netherlands). Tissue areas of the pith, xylem and phloem in the cross-sections were measured firstly by ImageJ software (v.1.44; http://rsb.info.nih.gov/ij; developed by W. Rasband, National Institutes of Health, Bethesda, MD, USA). Then ray parenchyma areas of cross-sections were measured using pie-observe method describing in Zhang *et al.* (2016): we selected four pie-shaped areas in four directions of a cross-section along rays, including a ray along a side and excluding it on the other. In the end, mean xylem growth rate was calculated by xylem area dividing by branch age.

Hydraulic measurements

In the laboratory, we measured sapwood-specific hydraulic conductivity (K_S, kg m^{-1} s^{-1} MPa^{-1}) which is the effective water flow per sapwood area standardized by length. We calculated xylem conductance using sapwood-specific hydraulic conductivity times xylem area. Before we started those measurements, we did a preliminary survey on the maximum vessel length (MVL) to determine the branch length to be taken from broadleaved trees for all the species collected in this study. We used an air injection method (Ewers, F. W. & Fisher, J. B., 1989) to estimate the MVL. Briefly, long branches were harvested in the field and delivered to the lab. The distal of branches were cut and connected with a 100 kPa compressed air, the upstream of the branch segments were immersed underwater. Segments were shortened at a 1-cm-interval until the first air bubble emerged. The MVL amounted to the remaining length of the segment plus 0.5 cm.

We determined K_S using a water flow meter system (Sperry *et al.*, 1988). Branch samples were shorted to ca. 1.5 times as length of MVL (if possible) under water in the lab. The remaining branches were flushed with 10 mM degassed KCl solution at 100 kPa pressure for 1 h to reach the maximum flow rate. The upstream were connected to ca.

5 kPa KCl solution. The distal of segment was connected to a pipette. A steady flow rate was obtained after ca.3-5 min equilibrium. The K_S was then calculated as:

$$K_S = F\,L / (\Delta P\, A_S),$$

Where F (kg s^{-1}) is the water flux, L is segment length (m), ΔP is pressure difference between the two end of the segment (MPa).

Dye staining method was followed to determine the actively conducting sapwood area (A_S, cm^2). Safranine solution was perfused into one end of the segment until a consistent standard colour of outflow was observed with low hydrostatic pressures. After staining, c. 5 cm from both ends of the segment were cut and the conducting area was measured using an electronic calliper (DANIU 150 mm 6-inch LCD digital stainless). The obtained two side conducting area were averaged to get branch conducting sapwood area (Hietz *et al.*, 2008).

Wood density measurements

After hydraulic measurements, one 10 cm branch segment was cut and fresh volume (V, cm^{-3}) was determined using the water displacement method. The branch segment was then dried in an oven (PH240A) at 65℃ for 48 hours and weighted (DW, g), and dry wood density (WD, g cm^{-3}) was calculated as:

$$WD = DW / V$$

Statistics

Differences between broadleaved tree and conifer species in tissue sizes, xylem growth rate, sapwood-specific conductivity, ray parenchyma percentage were tested using a linear mixed model with 41 individuals from 14 species. Lifeform was set as a fixed factor to test the mean differences between broadleaved tree and conifer species. Age was set as a fixed factor to control its effect, and species was set as a random factor to account for multiple observations within species.

We tested allometric trends using a linear mixed model as well. In the model, we compared areas of two tissues types (usually leaf area versus a stem tissue area), and tissue areas with xylem growth rate. We included lifeform as a fixed factor to examine the intercept difference between two lifeforms. Species was set as a random factor to account for multiple observations within species. Xylem conductivity, leaf area and ray parenchyma area were $\log_{10}$ transformed.

All statistical tests were conducted using RStudio (Version 0.99.903) packed with R 3.3.3 (R Core Team 2016). Linear regressions were performed with the *lm* function in the *lme4* package (Bates *et al.*, 2007).

Phylogeny

To examine how plant traits may have been influenced by phylogeny, we constructed a phylogenetic tree using the program Phylomatic (http://phylodiversity.net/phylomatic, accessed 22 February, 2019). We constructed a species-level tree with trait value being averaged within species. Phylogenetic conservatism in all traits were then analysed using Blomberg's *K* statistic which compares the observed signal in a trait to the signal under a Brownian motion model of trait evolution on a phylogeny (Blomberg *et al.*, 2003). *K* values closer to zero correspond to a random or convergent pattern of evolution, while *K* values greater than 1 indicate strong phylogenetic signal and conservatism of traits. To examine possible coordinated divergences in water- and sugar-related traits, we analysed phylogenetic independent contrast values (PICs) using Pearson product–moment correlations (Zanne *et al.*, 2010). Phylogenetic analyses were performed using

RStudio (Version 0.99.903) packed with R 3.3.3 (R Core Team 2016) including packages APE (Paradis *et al.*, 2004) and Picante (Webb *et al.*, 2010).

RESULTS

Conifers and broadleaved trees had a similar pith area, xylem area and phloem area in their branches. Mean xylem growth rate did not significantly differ between conifers and broadleaved trees. Conifers had lower ray percentage, SLA, sapwood-specific conductivity and dry wood density (Table 3.1).

Xylem area, axial phloem area, ray area and xylem conductivity increased with leaf area in both conifers and broadleaved trees (Fig. 3.1). Conifers had a higher intercept in the linear relation between xylem area and leaf area, but a lower intercept in the linear relation between ray area and xylem conductivity with leaf area. Axial phloem area and ray area increased with xylem conductivity in both conifers and broadleaved trees (Fig. 3.2). Xylem growth rate increased with leaf area and axial phloem area (Fig. 3.3).

Table 3.1 Canopy branch traits of conifers and broadleaved tree species in Changbai forest. Pith area, xylem area, phloem area, wood density and mean xylem growth rate for conifer species (N=5) and broadleaved tree species (N=9) are shown. Group differences were tested with a linear mixed model using 41 individuals from 14 species. Age was set as a fixed factor and species was set as a random factor to account for multiple observations within species. All variables were $\log_{10}$ transformed in statistic. Means of each variable and their SE were given.

Variables	Unit	Means				Age		Lifeform	
		Conifer	SE	Broadleaf	SE	t	*P*	t	*P*
Pith area	cm^2	0.023	0.006	0.026	0.008	-3.452	**0.002**	-0.042	0.967
Xylem area	cm^2	0.29	0.11	0.27	0.16	0.153	0.879	-0.604	0.557
Phloem area	cm^2	0.10	0.04	0.13	0.09	1.055	0.300	0.174	0.865
Ray percentage	%	5.03	0.27	12.46	0.59	1.570	0.125	6.420	**<0.001**
SLA	$cm^2\ g^{-1}$	60.29	9.75	137.74	11.72	0.297	0.769	4.144	**0.001**
K_S	$kg\ m^{-1}\ s^{-1}\ MPa^{-1}$	0.84	0.13	3.81	0.60	-0.654	0.517	3.774	**0.003**
Wood density	$g\ cm^{-3}$	0.40	0.009	0.47	0.013	-1.375	0.179	2.301	**0.040**
Xylem growth	$cm^2\ y^{-1}$	0.066	0.036	0.072	0.068	-4.705	**<0.001**	-0.401	0.696

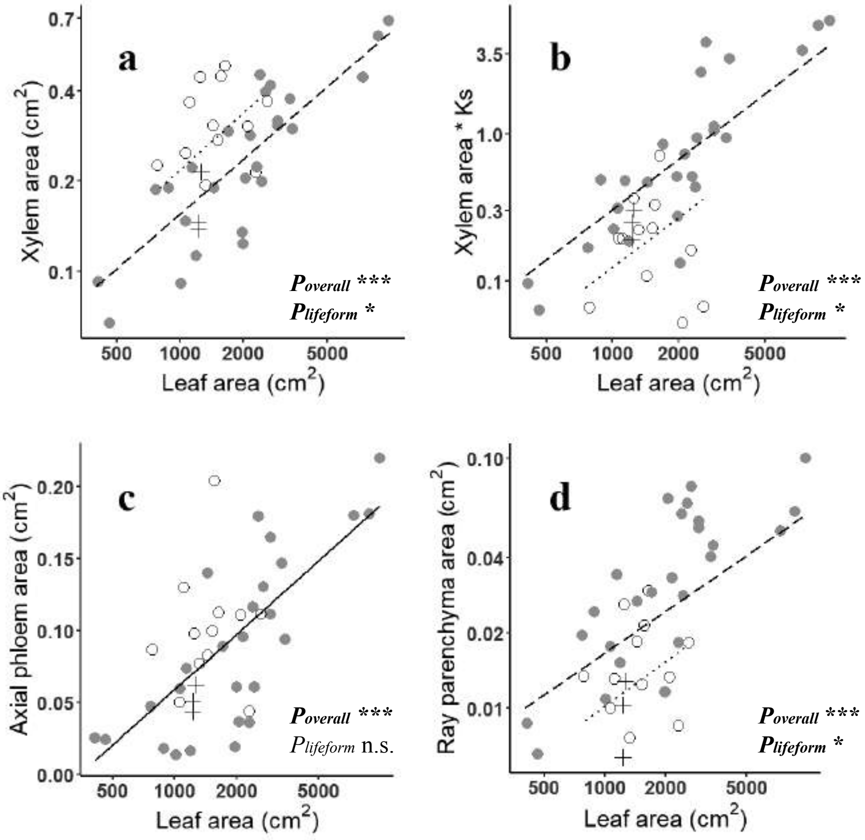

Figure 3.1 Relations of xylem area, axial phloem area, ray area and xylem conductivity (xylem area * K_S) with leaf area for temperate tree species in Changbai forest. Grey dots represent broadleaved trees and open dots represent conifers, but the deciduous conifer *Larix* was represented by crosses. Overall significant effects were indicated by solid lines. Dash lines represent broadleaved trees and dotted lines represent conifers. * means P<0.05, ** means P<0.01, *** means P<0.001. n.s. not significant.

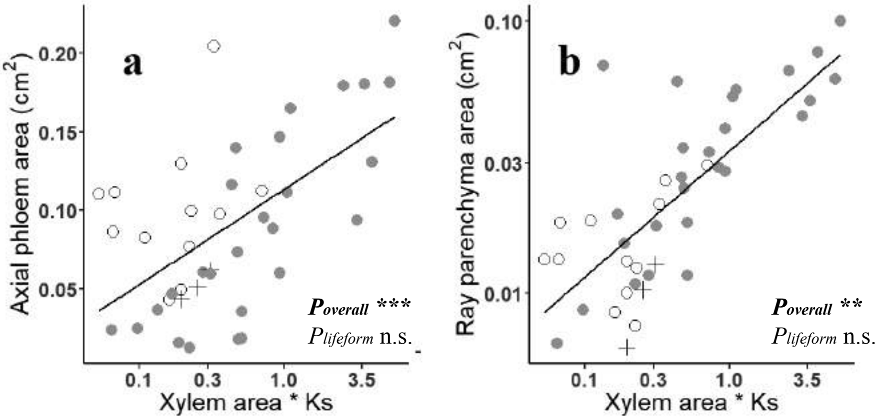

Figure 3.2 Relations of axial phloem area and ray area with xylem conductivity (xylem area * K_S) for temperate tree species in Changbai forest. Grey dots represent broadleaved trees and open dots represent conifers, but the deciduous conifer *Larix* was represented by crosses. Significant correlations effects are indicated by solid lines. * means $P<0.05$, ** means $P<0.01$, *** means $P<0.001$. n.s. not significant.

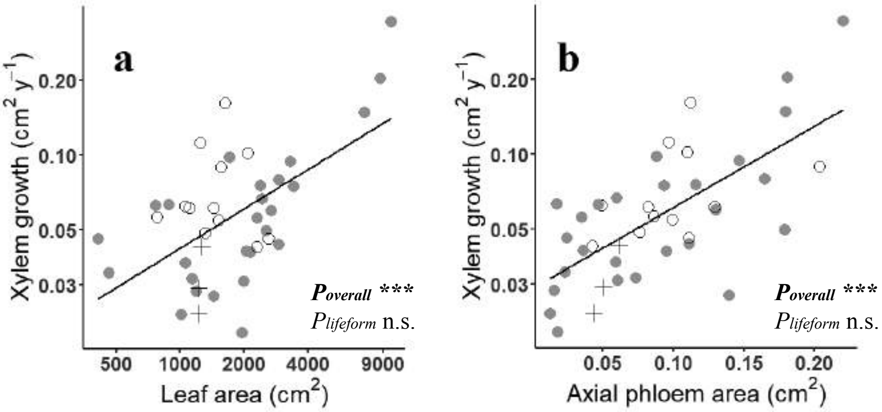

Figure 3.3 Relations of leaf area and axial phloem area with xylem growth rate for temperate tree species in Changbai forest. Grey dots represent broadleaved trees and open dots represent conifers, but the deciduous conifer *Larix* was represented by crosses. Significant correlations were indicated by solid lines. Dash lines represent

broadleaved trees and dotted lines represent conifers, when lifeform was significant. * means P<0.05, ** means P<0.01, *** means P<0.001. n.s. not significant.

Phylogenetic signal *K*-values were less than 1 for pith area, xylem area, phloem area, axial phloem area, ray area, ray percentage, leaf area, sapwood-specific hydraulic conductivity, xylem growth rate and xylem conductivity with corresponding *P*-values larger than 0.05 (Table S3.2). All relations in Fig. 3.1-3.3 were significantly correlated when considered phylogenetic divergences, using independent contrasts (Table S3.3)

DISCUSSION

In this study, we compared canopy branches between conifers and broadleaved trees in the same, natural, temperate forest. We studied functional balances within branches by quantifying allometric relationships between the leaf area and stem tissue areas. These balances represent the coordination of water loss in the leaves versus water transport in the xylem and storage in the ray parenchyma in the stem; and the coordination between carbon gain in the leaves versus sugar transport in the phloem and storage in the ray parenchyma. In addition, we related these tissue sizes to growth for both conifers and broadleaved trees. In phylogenetic analysis, only ray percentage was phylogenetically controlled (Table S3.2). All the allometric relationships did arise repeatedly and widely throughout the evolutionary history (Table S3.3) (Zheng *et al.*, 2019).

Tissue allocation in conifers and broadleaved trees

As expected, conifers had more xylem area for the amount of supported leaf area (Fig. 3.1a, Fig. S3.1a). This is consistent with results from surveys in the temperate zone (Brouat *et al.*, 1998; Becker, 2000) and a scaling study across Europe (Petit & Kiorapostolou, 2018) comparing evergreen conifers with deciduous broadleaved trees. Given that conifers have a lower xylem conductivity (Fig. 3.1b), this confirms that conifers compensate their less efficient water conducting system by producing more xylem area for a given leaf area.

However, no such differences were observed when comparing (evergreen) conifers with evergreen angiosperms of first year shoots in temperate and Mediterranean areas (Brouat *et al.*, 1998; Becker, 2000; Lusk *et al.*, 2007). This is remarkable because evergreen angiosperms tend to have a higher branch hydraulic conductivity than co-existing evergreen conifers (Lusk *et al.*, 2007). These studies however focused on saplings or very small branches (diameter < 7.5 mm) with lower water supply demands. Possibly, the high light levels and high transpiration demands for the exposed branches of our studied canopy trees resulted in the divergence between conifers and broadleaved trees (Thomas & Winner, 2002), whereas such divergence is less clear for sapling in the forest understory (Meinzer *et al.*, 1999; Mencuccini & Comstock, 1999; Brodribb & Feild, 2000).

Conifer and broadleaved tree species did not differ in axial phloem area for a given leaf area (Fig. 3.1c, Fig. S3.1b). Because broadleaved trees in general have a higher carbon gain rate per leaf area, they might require a more efficient phloem transport system to move sugars to carbon sinks elsewhere in trees. This would be consistent with Dannoura *et al.* (2011) who found a higher velocity of carbon transfer in oak and beech compared to pine in a temperate forest. Moreover, this is in line with Hölttä *et al.* (2014) and Jyske and Hölttä (2015) who showed that the xylem hydraulic efficiency is closely coupled to phloem transport efficiency, which can be expected from the coordination of xylem conduit sizes and phloem cell sizes from the same cambium (Petit & Crivellaro, 2014). Yet, we lack an empirical test whether a higher specific conductivity of phloem in broadleaved trees compensates for the phloem area that is similar to that area in conifers.

As expected, conifers have a lower ray percentage and ray area per unit leaf area than broadleaved trees (Table 3.1, Fig. 3.1d). This is in line with Morris *et al.* (2016b) who found lower ray percentage in conifers, and in line with the strong phylogenetic effect that we found for traits related to ray parenchyma. These results suggest that broadleaved trees have a higher water and sugar storage capacity than co-existing conifers. One reason for evergreen conifers to have lower storage capacity could be their longer leaf life span (Reich *et al.*, 1995; Lusk, 2001)1995; Lusk, 2001, which may lower the carbon storage requirements for crown maintenance during winters. In contrast, deciduous broadleaved trees may require a high storage capacity for carbon to survive winter and renew leaves every spring (Epron *et al.*, 2012). However, the conifer species *Larix* is deciduous too and did not have a higher ray area per leaf area than the other conifer species. As conifers' xylem is mainly built up with tracheids and ray parenchyma cells, tracheid area is directly suppressed by larger ray parenchyma area. This can lower the water transport ability in xylem. However, the vessel area of broadleaved trees is independent of ray area (Fortunel *et al.*, 2014). Thus, conifers tend to maximize water transport ability by minimizing the occupation of ray parenchyma to meet water transport demand.

Ray and phloem development with xylem growth rate

Out of our expectation, conifers and broadleaved trees tend to achieve similar xylem growth rates when scaled with leaf area or phloem area (Fig. 3.3). Though it is widely known that leaf area positively relates with stem growth (Waring *et al.*, 1980; Waring, 1983), and that phloem has important effects on plant growth (Savage *et al.*, 2016), the allocation comparisons on canopy branches between co-existing conifers and broadleaved trees have not yet been done. The convergent xylem growth rate reveals that conifers may alter other traits to compensate the low carbon fixation rate in leaves and slow mass flow in phloem (Epron *et al.*, 2012; Liesche & Schulz, 2018) to achieve a similar xylem growth rate as broadleaved trees have. In addition, xylem growth rate is not phylogenetic regulated (Table S3.2) suggesting that it indeed can be affected by other traits. Producing low-cost wood (lower wood density, Table 3.1) can be one of the strategies across conifers to overcome these disadvantages. Besides, the similar allocation of phloem area and ray area relative to the amount of xylem conductivity (Fig. 3.2) indicates that conifers and broadleaved trees may actually have comparative allocation of sugars into transport and storage area which causes similar xylem growth rate.

CONCLUSIONS

Most of our expectations on the allocation differences between conifers and broadleaved trees were not supported by our data for the studied temperate forest in NE China. Conifers maintain functional water balance between stem and leaves by compensating their low hydraulic conductivity with a wider xylem area. Broadleaved trees maintain functional carbon balance not by creating a larger phloem area per leaf area, but possibly by having larger conductive cells in phloem. The large ray area in broadleaved trees may hint to their role in carbon storage during winter, while the low ray area in conifers may be a compromise of maximizing water transport. In summary, conifers tend to facilitate carbon gain (produce more xylem area per leaf area) and consume carbon for xylem development rather than preserve them in storage tissue as broadleaved trees do. We suggest that the allocation between leaf area (carbon gain) with phloem (carbon transport) and ray (carbon and water storage) area could be important indicators in understanding adaption of different lifeforms or phenology. More studies are required to draw firm conclusions on this.

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SUPPORTING INFORMATION

Fig. S3.1 Group differences between evergreen conifers (N=4) and deciduous broadleaved trees (N=9) of sapwood-specific hydraulic conductivity (K_S), ray percentage in xylem, xylem-leaf ratio and axial phloem-leaf ratio in Changbai forest were tested with a linear mixed model using 38 individuals from 13 species. *Larix gmelinii*, the only deciduous conifer, was excluded in the analysis, but the values are included in each graph as open dots. Species was set as a random factor to account for multiple observations within species. K_S and xylem-leaf ratio were $\log_{10}$ transformed. 2SE were indicated.

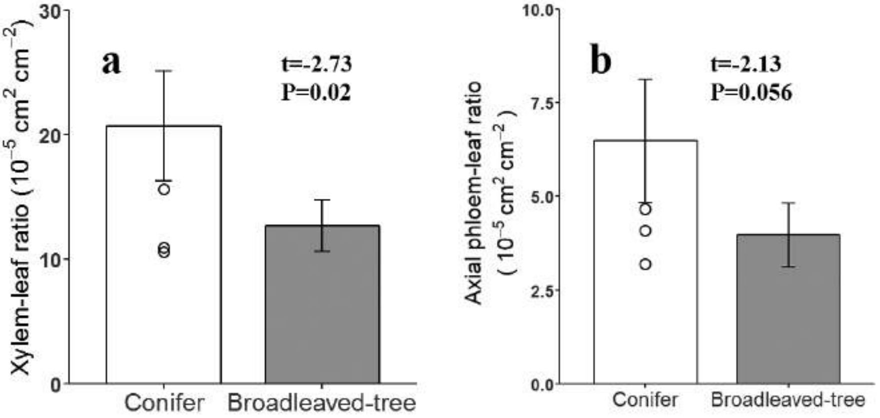

Table S3.1 Species list of sampled trees in Changbai forest. Their lifeform and phenology are given. Species names are standardized by The Plant List (http://www.theplantlist.org).

Species name	Family	Lifeform	Phenology
Abies nephrolepis (Trautv. ex Maxim.) Maxim.	*Pinaceae*	Conifer	Evergreen
Acer pictum Thunb.	*Sapindaceae*	Broadleaf	Deciduous
Betula platyphylla Sukaczev	*Betulaceae*	Broadleaf	Deciduous
Fraxinus mandshurica Rupr.	*Oleaceae*	Broadleaf	Deciduous
Juglans mandshurica Maxim.	*Juglandaceae*	Broadleaf	Deciduous
Larix gmelinii (Rupr.) Kuzen.	*Pinaceae*	Conifer	Deciduous
Picea koraiensis Nakai	*Pinaceae*	Conifer	Evergreen
Pinus densiflora Siebold & Zucc.	*Pinaceae*	Conifer	Evergreen
Pinus koraiensis Siebold & Zucc.	*Pinaceae*	Conifer	Evergreen
Populus tremula var. davidiana (Dode) C.K.Schneid.	*Salicaceae*	Broadleaf	Deciduous
Quercus mongolica Fisch. ex Ledeb.	*Fagaceae*	Broadleaf	Deciduous
Tilia amurensis Rupr.	*Malvaceae*	Broadleaf	Deciduous
Tilia mandshurica Rupr. & Maxim.	*Malvaceae*	Broadleaf	Deciduous
Ulmus laciniata (Trautv.) Mayr	*Ulmaceae*	Broadleaf	Deciduous

Table S3.2 Phylogenetic signal analysis of tissue areas, ray percentage, xylem conductivity and xylem growth rate. *K*-value and corresponding P-value for each trait was given.

Species	Pith area	Xylem area	Phloem area	Axial phloem area	Ray area	Ray %	Leaf area	K_S	Xylem area*K_S	Xylem growth
K	0.264	0.286	0.284	0.271	0.433	1.501	0.270	0.452	0.320	0.264
P	0.871	0.887	0.933	0.933	0.566	**0.001**	0.821	0.450	0.752	0.808

Table S3.3 Phylogenetically independent contrast values (PICs) of relationships between leaf area with xylem area, axial phloem area and xylem area*K_S, between xylem area*K_S with axial phloem area and ray area, and between xylem growth rate with leaf area, ray area and axial phloem area were tested for the. r^2 and *P*-value were given. "+" indicates positive correlation.

Trait 1	Trait 2	Sign	r^2	*P*-value
Leaf area	Xylem area	+	0.831	**<0.001**
Leaf area	Axial phloem area	+	0.712	**<0.001**
Leaf area	Xylem area*K_S	+	0.904	**<0.001**
Leaf area	Ray area	+	0.671	**<0.001**
Xylem area*K_S	Axial phloem area	+	0.741	**<0.001**
Xylem area*K_S	Ray area	+	0.731	**<0.001**
Xylem growth rate	Leaf area	+	0.929	**<0.001**
Xylem growth rate	Ray area	+	0.536	**<0.01**
Xylem growth rate	Axial phloem area	+	0.674	**<0.001**

CHAPTER 4

Fully exposed canopy tree and liana branches in a tropical forest differ in mechanical traits but are similar in hydraulic traits

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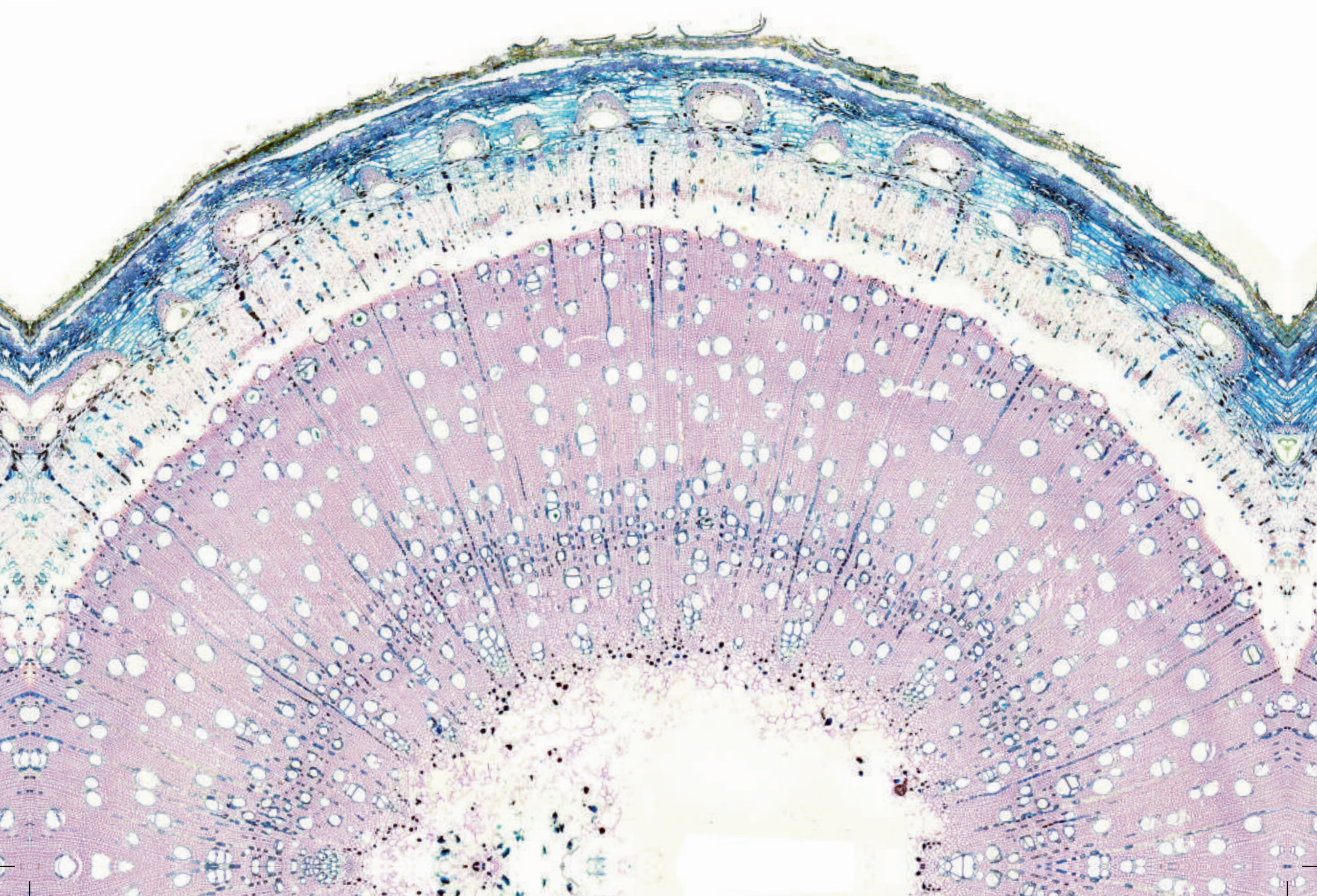


ABSTRACT

- Large lianas and trees in the forest canopy are challenged by hydraulic and mechanical failures and need to balance hydraulic conductivity, hydraulic safety and mechanical safety. Our study integrates these functions in canopy branches to understand the performance of canopy trees and lianas, and their difference.
- We sampled and measured branches from 22 species at a canopy crane in the tropical forest at Xishuangbanna, SW China. We quantified the hydraulic conductivity from the xylem-specific hydraulic conductivity (K_S), hydraulic safety from the cavitation resistance (P_{50}) and mechanical safety from the modulus of rupture (MOR) to evaluate trade-offs and differences between lianas and trees. We also measured a number of anatomical features that may influence these three functional traits.
- Trade-offs between hydraulic conductivity, hydraulic safety and mechanical safety are weak or absent; Liana branches better resist external mechanical forces (higher MOR) than tree branches; Liana and tree branches were similar in hydraulic performance (K_S and P_{50}). The anatomical features underlying K_S, P_{50} and MOR may differ between lianas and trees.
- We conclude that canopy branches of lianas and trees diverged in mechanical design due to fundamental differences in wood formation, but converged in hydraulic design.

INTRODUCTION

In tropical forests, trees and lianas grow to tall statures to display their leaves for light capture in the exposed upper forest canopy. Lianas are known to increase at the costs of trees in many tropical forests (Schnitzer & Bongers, 2011), but mechanisms related to the canopy branches are poorly explored (Schnitzer, 2018; Schnitzer & van der Heijden, 2019). These branches can achieve high carbon gain under full light exposure, but this comes with risks for structural stability and high water losses via transpiration (Givnish, 1988), especially under dry conditions. Tall woody plants have more negative water potentials in branches and leaves, and bigger conduits, than smaller plants to maintain the passive water flow driven by a water potential gradient from roots to leaves (Petit *et al.*, 2016; Sterck & Zweifel, 2016), but this comes at the risk of drought-induced cavitation (Hacke *et al.*, 2000; Olson *et al.*, 2018). Tall woody plants then should maintain a water conductive system that is safe against both cavitation and mechanical failure. Possible trade-offs between these functions have been put forward in the literature but remain highly uncertain (Pittermann *et al.*, 2006; Gleason *et al.*, 2016, Table 4.1) and are hardly studied for tall individuals of trees and lianas (van der Sande *et al.*, 2013; De Guzman *et al.*, 2016). Here we fill this knowledge gap by comparing hydraulic conductivity, hydraulic safety and mechanical safety between canopy dwelling trees and lianas in a tropical lowland rainforest. We aim to show whether trade-offs between these functions are manifested amongst species, and whether lianas and trees differ as groups in such functions. Such group differences are expected for mechanical stability since trees are standing alone while lianas are not, but the possible implications of such mechanical differences for hydraulic conductivity and safety are not clear.

Hydraulic conductivity and hydraulic safety are two functions that reflect the hydraulic performance of plants. Hydraulic conductivity can be quantified by specific hydraulic conductivity (K_S), which is the water transport ability standardised by branch length and xylem area (Sperry *et al.*, 1988). The hydraulic safety is often quantified by proxies for cavitation resistance (the occurrence of air bubbles in the transport system) such as P_{50}, which is the xylem tension that causes 50% conductivity loss by cavitation. Several studies suggest that plants cannot maximize both hydraulic conductivity (high K_S) and hydraulic safety (negative P_{50}) because large conduits that provide more efficient water transport (Tyree *et al.*, 1994) contain larger pit pores and are therefore more sensitive to air-induced cavitation through those pores (Tyree *et al.*, 1994; Hacke *et al.*, 2000; Pockman & Sperry, 2000; Martínez-Vilalta *et al.*, 2002). As lianas are known to have wider vessels (Ewers & Fisher, 1991; Gartner, 1991a; Gartner, 1991b; Wagner *et al.*, 1998), we expect that lianas have a higher K_S but a lower cavitation resistance (less negative P_{50}) than trees (Zhu & Cao, 2009; Johnson *et al.*, 2013).

Trees and lianas obviously differ in mechanical requirements, since the former are stable while standing alone and the latter use and hang on trees (and/or on other lianas) to reach the canopy. Mechanical safety can be quantified by the modulus of rupture (MOR), which is the force required for breaking a branch or stem standardised by length and cross-sectional area (Sterck *et al.*, 2006). Mechanical safety is expected to be positively related to hydraulic safety because strong, dense wood, known to convey greater mechanical safety (van Gelder *et al.*, 2006; Chave *et al.*, 2009), can contribute to avoid conduit collapse under negative pressures (Hacke *et al.*, 2001; Sperry *et al.*, 2006; Domec *et al.*, 2009; Arbellay *et al.*, 2012). In line with this, mechanical safety is expected to decrease with higher hydraulic conductivity, also because stronger wood requires denser wood with less and/or narrower vessels, which reduces hydraulic conductivity. From these among functional trait relations, we predict that lianas have a lower mechanical stability (MOR) than trees.

Differences in these three functional traits (K_S, P_{50} and MOR) across species or species groups (such as lianas versus trees) are expected to result from differences in anatomical features that can be observed in the stem or branch cross-sections (Fig. 4.1). K_S depends on the total transporting area and vessel diameter. K_S may increase with the area occupied by ray and axial parenchyma since parenchyma provides capacity for water storage and may contribute to K_S (Angyalossy *et al.*, 2012; Pratt & Jacobsen, 2017), but may also decrease when parenchyma area comes at the cost of the area occupied by vessels. Cavitation resistance may increase (P_{50} becomes more negative) with a higher wall-lumen ratio and wood density (Hacke *et al.*, 2001, but see Powell *et al.*, 2017 and Rosas *et al.*, 2019) because the resultant stronger cell wall strength lowers the risk of wall micro-fractures, which causes air-seeding and cavitation (Sterck & Bongers, 1998; Jacobsen *et al.*, 2005; van Gelder *et al.*, 2006). Species that are more vulnerable to cavitation (less negative P_{50}) may require more ray and axial parenchyma to store sugars that contribute to embolism repair (Brodersen *et al.*, 2010; Ogasa *et al.*, 2013; Klein *et al.*, 2018; Zeppel *et al.*, 2019). Finally, MOR depends on wood density (van Gelder *et al.* 2006), wall-lumen ratio (Hacke *et al.* 2001) and the total area of fibres, with higher MOR resulting from higher values of such traits. Overall, relations between these three focal functional traits and underlying anatomical traits involve possible trade-offs, but those are still poorly tested for canopy trees and lianas.

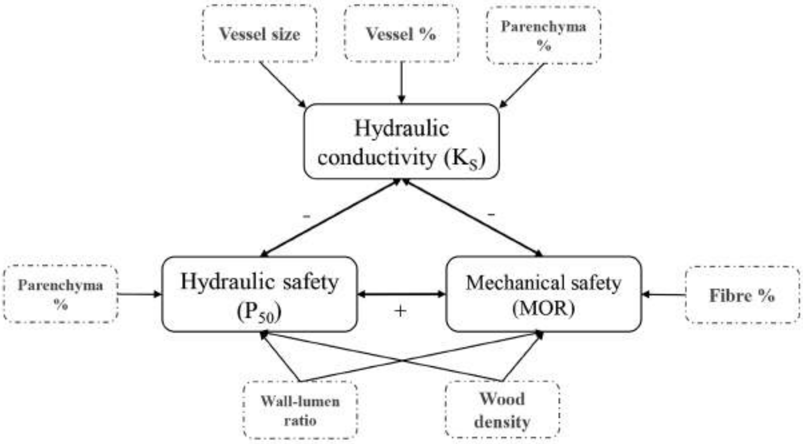


Figure 4.1 Conceptual model of trade-offs between hydraulic conductivity (quantified by the xylem-specific hydraulic conductivity, K_S), hydraulic safety (quantified by the cavitation resistance, P_{50}) and mechanical safety (quantified by the modulus of rupture, MOR) in branches of woody plants, and their underlying anatomical features. Parenchyma includes ray and axial parenchyma in xylem. Tissue percentages (Vessel%, Fibre%, Parenchyma%) are the relative areas occupied in the xylem cross-sectional areas. Double arrows represent expected correlations between two functional traits. Single arrows represent expected causal factors. ‘+’ means positive effects or correlations, and ‘-’ means negative effects or correlations.

Here we evaluate the existence of hydraulic and mechanical trade-offs between liana and tree species dwelling in the same tropical forest upper canopy. Instead of addressing entire individuals we focused on exposed branches in the upper canopy: (i) with the high irradiation and temperature during the day, these branches potentially gain most carbon but face high transpiration demands and can thus act as bottlenecks for the hydraulic integrity and carbon gain of the entire individual; (ii) in canopy branches, standardized branches length can be easily controlled for diminishing the tapering effects of vessels and their related functionalities (Becker *et al.*, 2000; Petit & Anfodillo, 2009); and finally (iii) it is practically more feasible to study branches than entire individuals, certainly the large ones that dwell in the forest canopy. In our study, we addressed three main questions: 1) how do canopy branch traits differ between lianas and trees? 2) do lianas and trees show trade-offs between hydraulic conductivity, hydraulic safety and mechanical safety in canopy distal branches? 3) How do underlying anatomical features influence trait values and trade-off amongst traits?

Table 4.1 Selected studies of trade-offs between hydraulic conductivity (K_S), hydraulic safety (drought-induced cavitation, P_{50}) and mechanical safety (MOR) in angiosperms. Only studies focused on one (or more) of our studied trade-offs are shown. Total species and/or family number used in corresponding studies are given. '?' refers to unknown total species or family numbers. '√' represents studies with a significant correlation found between two traits. '**X**' represents studies that found no significant correlation between two traits. 'weak' represents studies that found a significant trade-off under certain conditions or within a certain species group. Ranges of variables in each work are given when the units are the same as our study.

Literature	K_S-P_{50}	P_{50}-MOR	MOR-K_S	Lifeform	Species no. (family no.)	Location	Condition	Trait range
Adult/branch								
Sperry and Sullivan (1992) Tyree et al. (1994)	X			Broadleaves, conifer	5 (5)	W. USA	Temperate zone Elevation: 1370–2825 m	K_S: 0, 20 P_{50}: -10, 0
Pockman and Sperry (2000)	√			Tree, shrub	15 (8)	S. USA	Elevation: 1036 m MAP: 310 mm MDTmax: 32 °C	K_S: 0, 4 P_{50}: -12, 0
Jacobsen et al. (2005)		√		Shrub	6 (4)	W. USA	Elevation: 480 m	MOR: 0, 260 P_{50}: -10, 0
Jacobsen *et al.* (2007)			X	Shrub	17 (9)	South Africa	Elevation: 540-1160 m MAP: 315-600 mm	K_S: 0. 8 MOR: 200, 700
Choat et al. (2007)	X			Tree, shrub	9 (1)	USA Panama Costa Rica	MAP: 1250-4200 mm	K_S: 0, 14 P_{50}: -4, 0
Zhu and Cao (2009)	√			Tree, liana	6(5)	SW China	Tropical forest MAP: 1379 mm MAT: 21.4 °C	K_S: 0, 4 P_{50}: -2, 0

Continued Table 4.1

Literature	K_S-P_{50}	P_{50}-MOR	MOR-K_S	Lifeform	Species no. (family no.)	Location	Condition	Trait range
Adult/branch								
De Guzman *et al.* (2016)	✓			Tree, liana	12 (11)	Panama	MAP: 1865 mm	K_S: 0, 12 P_{50}: -1.5, 0
Gleason et al. (2016)	weak			Angiosperm Gymnosperm	335 (10)	Global	-	K_S: 0, 16 P_{50}: -14, 0
Zhang et al., this study	X	X	X	Tree, liana	22 (17)	SW China	Tropical forest MAP: 1493 mm MAT: 21.8 °C	K_S: 0, 10 P_{50}: -3, 0 MOR: 0, 170
Adult/stem								
Martínez-Vilalta et al. (2002)	✓			Tree, shrub	9 (8)	NE Spain	Mediterranean MAP: 537 mm MAT: 10 °C	K_S: - P_{50}: -7, 0
Pratt et al. (2007)	X	✓		Shrub	9 (1)	W. USA	Elevation: 480 m	K_S: 0, 2.5 P_{50}: -10, 0 MOR: 0, 275
Jacobsen *et al.* (2009)		✓		Shrub	15 (9)	W. USA	Elevation: 540-660 m; 850-1020 m MAP: 315 mm; 420 mm	MOR: 200, 700 P_{50}: -11, 0
Adult/sapling/branch/stem								
Maherali et al. (2004)	X			Angiosperm Gymnosperm	167 (50)	Global	-	K_S: 0, 8 P_{50}: -16, 0
Sapling								
van der Sande et al, unpublished	✓			Tree, liana	55 (?)	Panama	MAP: 2311-3203 mm	K_S: - P_{50}: -5, 0

MATERIALS AND METHODS

Study site

The study was carried out in a crane site in a seasonal tropical lowland rain forest located in the core area of National Nature Reserves in the Bubeng region, Xishuangbanna (XTBG), Yunnan Province, SW China (21° 36’N 101°35’E). The altitude of the crane site is about 780 m. The area has a typical monsoon climate with distinguishable wet and dry seasons. A wet season starts from May and lasts till October and a dry season characterizes the period from November till April. The average annual temperature is 21.8 ℃ with mean monthly temperature ranging from 15 ℃ in December to 25 ℃ in June. The average annual precipitation is 1493 mm of which 1256 mm (84%) falls between May and October (Cao *et al.*, 2006).

Field work

Canopy branches of trees and lianas were collected using a canopy crane with a height of 80 m and a radius of 60 m. A total of 44 individuals of 22 species (10 tree and 12 liana species, all evergreen broad-leaved angiosperm species) were randomly selected. Species information is given in Table S4.1. All the sampling work was conducted in the early morning before sunrise (during 07:00-09:00) in the wet season of 2016. Three 1.5 m long (from branch tip) canopy branches from each tree individual, and three 3 m long (from branch tip) branches from each liana individual were cut for subsequent hydraulic and mechanical measurements. All tree branches selected were self-supporting ones without hanging on other branches. All liana branches selected were non-self-supporting ones hanging on other branches. All branches were immediately treated: 1) side twigs were cut off leaving ca. 10 cm base in order to avoid cavitation by cutting; 2) the whole remaining branch was wrapped with wet towels to reduce desiccation; 3) each branch was put into a black plastic bag that was closed to avoid further water loss. Afterwards, the branches were transported to the XTBG lab for subsequent hydraulic and mechanical measurements. Additionally, for all individuals, a 50 cm long sun-exposed canopy branch was cut of which the basal 10 cm branch segment was cut and preserved in FAA solution for subsequent anatomical analysis.

Hydraulic measurements

In the laboratory, we measured xylem-specific hydraulic conductivity (K_S) and the vulnerability curve to get cavitation resistance (P_{50}). K_S is the maximal water flow per area standardized by length. P_{50} is the xylem water potential at which 50% of maximum hydraulic conductivity is lost (as defined by Domec & Gartner, 2001). Before we started those measurements, we did a preliminary survey on the maximum vessel length (MVL, values for each species are provided in Table S4.1) to determine the branch length to be taken from trees and lianas for all the species collected in this study. We used an air injection method (Ewers, Frank W & Fisher, Jack B, 1989) to estimate the MVL. Long branches were harvested in the field and brought to the lab. The distal parts of branches were cut and connected with a 100 kPa compressed air, while the upstream parts of the branch segments were immersed under water. Segments were shortened at a 1-cm-interval until the first air bubble emerged. The MVL amounted to the remaining length of the segment plus 0.5 cm.

Xylem-specific hydraulic conductivity (K_S, kg m^{-1} s^{-1} MPa^{-1}). We determined K_S using a water flow meter system (Sperry *et al.*, 1988). Branch samples were shorted to ca. 1.5 times the length of MVL under water in the lab. The remaining branches were flushed with 10 mM degassed KCl solution at 100 kPa pressure for 1 h to reach the maximum flow rate. The upstream parts were connected to ca. 5 kPa KCl solution (same as above). The distal part of a segment was connected to a pipette. A steady flow rate was obtained after ca. 3-5 min equilibrium. The K_S was then calculated as:

$$K_S = F\,L / (\Delta P\, A_x),$$

Where F (kg s^{-1}) is the water flux, L is segment length (m), and ΔP is pressure difference between the two ends of a segment (MPa). Xylem area (A_x, cm^2) was then measured by using a micro-calliper.

Cavitation resistance (P_{50}, -MPa). To obtain P_{50}, a xylem vulnerability curve was constructed at species level. This curve shows the loss in branch hydraulic conductivity with decreasing negative pressure. We used the air pressurization method to induce embolism (Cochard *et al.*, 1992b). Branches were flushed under 100 kPa pressure with degassed KCl solution for 1 h to reach a maximal conductance (K_{max}) firstly. Then a series of imposed, increasing pressures were applied in 8 to 12 steps of 0.5 MPa or 1.0 MPa (Fig. S4.3) using a pressure chamber (PMS1505D-EXP, Albany, OR, USA) until the branch was fully cavitated. Each pressurization was maintained for 15 min (see (Cochard *et al.*, 1992b) and then the pressure was released for 20 to 240 min until a stable conductance (K_i) was reached. The percentage loss of hydraulic conductivity (PLC, %) at each pressurization step was calculated as:

$$PLC = 100\% \times (K_{max}-K_i)/K_{max}$$

We used a three-parameter exponential function to fit the vulnerability curves:

$$PLC = 100/(1 + \exp(a\times(\Psi_x - X_0))$$

where a is the maximum slope of the curve, X_0 is the pressure at the 50% loss of hydraulic conductivity (P_{50}).

Mechanical and density measurements

To estimate mechanical safety, we measured the modulus of rupture (MOR) by using a laboratory technique that is used to measure the maximum load-carrying capacity in bending (Green *et al.*, 1999). We used a three-point measurement with a span length (L, mm) : diameter (D, mm) ratio of 15 to estimate MOR. A 30 cm long branch segment cut from the above described long branch was used. The branch segment was horizontally placed on two supports (span length, L). Subsequently, a vertical, downward-orientated load was applied under a constant speed (ca. 1mm s^{-1}) at the midpoint of the branch using a SUNDOO SJY-500 test stand. A portable electronic scale was used to measure the load (P_{max}, N) at the moment of branch breakage. MOR (N mm^{-2}) was then calculated as (Sterck *et al*. 2006):

$$MOR = (M_{max} \cdot R)/ I = (1/4\ P_{max} \cdot L \cdot R)/(1/4\ R^4)$$

Where M_{max} is the maximum bending moment, I is the second order moment of area, and R is the radius in a circle cross-section.

After measuring MOR, one 10 cm branch segment was cut and fresh volume (V, cm^{-3}) was determined using the water displacement method. The branch segment was then dried in an oven (PH240A) at 65℃ for 48 hours and weighted (DW, g), and dry wood density (WD, g cm^{-3}) was calculated as:

$$WD = DW / V$$

Anatomical measurements

Permanent cross-sections were made using the paraffin embedding method. One 1-1.5 mm thick branch piece was cut with a razor blade from each 10 cm branch segment, after being embedded in an FAA solution. These pieces were dehydrated with a series of 50-96-100% ethanol. After this, they were infiltrated with limonene at air temperature and then with melted paraffin at 65 ℃ in an oven (PH240A). Afterwards, we embedded these pieces into paraffin blocks using a Leica EG1150H Paraffin Embedding Station. After paraffin blocks got hard, we cut a 6-10 µm thick cross-section per piece using a Leica RM2235 rotary microtome. Then we removed the paraffin from the cross-sections with limonene and dehydrated the pieces again by 50-96-100% ethanol series. Following dehydration, all cross-sections were stained in a safranin / astrablue solution and fixed in Neutral Balsam. After the Neutral Balsam was air dried, photographs of entire cross-sections were taken with a digital camera (DFC 320; Leica, Cambridge, UK) mounted on a microscope (DM2500; Leica) using Leica imaging software (v.3.6.0). To obtain a photograph of the entire cross-section, sequential photographs were stitched using PTGUIPRO (v.9.1.8; New House Internet Services BV, Rotterdam, the Netherlands). Pith area, xylem area and phloem area of cross-sections were measured firstly by using IMAGEJ software (v.1.44; http://rsb.info.nih.gov/ij; developed by W. Rasband, National Institutes of Health, Bethesda, MD, USA). Their fractions were calculated. Then detailed tissue areas were measured using the pie-method (Zhang *et al.*, 2016): we selected four pie-shaped areas in four directions of a cross-section along rays, including a ray along one side and excluding it on the other. Vessel lumen area, ray area and axial parenchyma area were measured. Mean vessel lumen diameter was calculated. From these areas, we calculated the surface percentages in the xylem for each of these tissues per pie and for the whole cross-section. In addition, the largest 20 vessels were selected and their lumen area, lumen diameter and vessel wall thickness were measured for each cross-section. Wall-lumen ratio was calculated by dividing wall thickness by vessel lumen diameter. We added those measures for the largest vessels because they are expected to contribute strongest to hydraulic conductivity and also cause the greatest risks for cavitation and mechanical failure. Tracheids were not considered in our study because our colouring method did not allow to separate tracheids from fibers. In our species samples, Carlquist (1985) mentioned the presence of vasicentric tracheids in *Combretum latifolium* and *Strychnos angustiflora*, but no such information was provided for any other species.

Statistics

Full names of tested variables, their abbreviations and units are given in Table 4.2. Differences between lianas and trees in tissue sizes, functional traits and underlying anatomical traits were tested using a linear mixed model with 44 individuals from 22 species. K_S, MOR, WD, MVD, BVD, WLR, Parenchyma%, A_{pi}, A_x and A_{ph} were $\log_{10}$ transformed to achieve normality. Species was set as a random factor to account for multiple observations within species.

Standardized major axis (SMA) regression was used to test trade-offs between K_S, P_{50} and MOR, as well as relationships among Vessel%, Parenchyma% and Fibre%. SMA regression allows us to test differences in slopes among different groups. To meet the normality assumption for these regressions, K_S and MOR were $\log_{10}$ transformed. Lifeform was firstly set as a group factor in the SMA regressions to test for differences between lianas and trees. If lifeform had no significant effect, it was removed from the model to show a single common trend for lianas and trees. Since it is impossible to add a random factor in SMA, we averaged branch data at species level (Table S4.2, S4.6). For the correlation between K_S and P_{50}, and between MOR and P_{50}, trends were mainly driven by the extreme values of two tree species (*Allophylus cobbe* and *Balakata baccata*) respectively (Table S4.3, S4.4). We thus excluded these two species from the analysis to check for a common trend without such outliers.

Table 4.2 The list of plant variables measured for this study. We present abbreviations used in the text and their units.

Variables	Abbreviations	Unit
Functional traits		
Xylem-specific conductivity	K_S	kg m^{-1} s^{-1} MPa^{-1}
Cavitation resistance	P_{50}	-MPa
Modulus of rupture	MOR	N mm^{-2}
Tissue properties		
Dry wood density	WD	g cm^{-3}
Mean vessel lumen diameter	MVD	µm
Mean biggest vessel lumen diameter	BVD	µm
Wall-lumen ratio	WLR	µm μm^{-1}
Tissue proportions		
Vessel lumen area percentage	Vessel%	%
Ray and axial parenchyma area percentage	Parenchyma%	%
Fibre area percentage	Fibre%	%
Tissue areas		
Pith area	A_{pi}	cm^2
Xylem area	A_x	cm^2
Phloem area	A_{ph}	cm^2

Starting from the working hypothesis (see Fig. 4.1), we built regression models and used model averaging to test for the effects of anatomical features on each functional trait (K_S, P_{50} and MOR) for lianas and trees, separately. In these analyses, K_S, MOR, WD, Parenchyma% and WLR were $\log_{10}$ transformed to achieve normality. Then all variables were scaled to be able to directly compare their effects. In line with our working hypothesis (Fig. 4.1), BVD, Parenchyma% and Vessel% were set as the explanatory variables of K_S; WLR, WD and Parenchyma% were set as the explanatory variables of P_{50}; and WLR, WD and Fibre% were set as the explanatory variables of MOR. In all cases, species was set as a random factor. Multicollinearity was checked (VIF<5) but played no role in our statistical models.

Afterwards, an information theory-based approach was applied to detect the best model (Burnham & Anderson, 2003; Nakagawa & Freckleton, 2011). The best model was generated from the smallest bias-adjusted Akaike's Information Criterion (AICc). Then other models were compared to the best model by calculating the AICc difference (ΔAICc), and models with ΔAICc < 2 were used to perform model averaging (Burnham & Anderson, 2003).

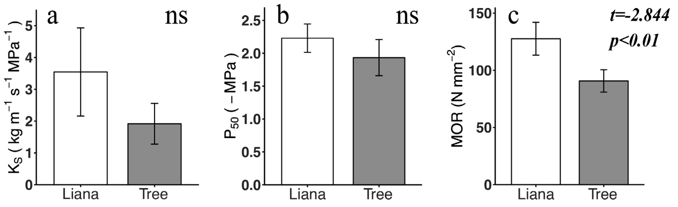
All statistical tests were conducted using RStudio (Version 0.99.903) packed with R 3.3.3 (R Core Team 2016). SMA regression was performed with the *sma* function in the *smatr* package (Warton *et al.*, 2012). Global model comparison and model averaging were performed with the *dredge* and *model.avg* function using the *MuMIn* package (Barton, 2009).

Phylogeny

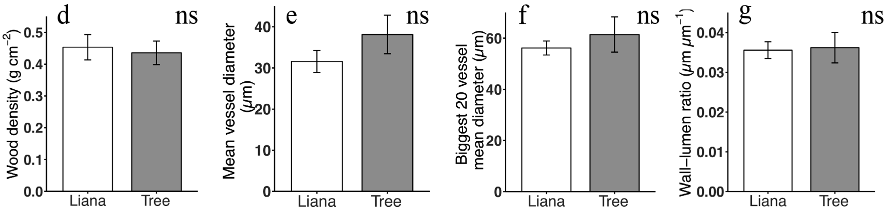
To control for possible phylogenetic effects on plant traits, we constructed a phylogenetic tree using the program Phylomatic (http://phylodiversity.net/phylomatic, accessed on February 22, 2019). We constructed a species-level tree with trait values being averaged within species. Phylogenetic conservatism in all traits were then analysed using Blomberg's *K* statistic which compares the observed signal in a trait to the signal under a Brownian motion model of trait evolution on a phylogeny (Blomberg *et al.*, 2003). *K* values closer to zero correspond to a random or convergent pattern of evolution, while *K* values greater than 1 indicate increasing strong phylogenetic signal and conservatism in trait values. To examine possible coordinated divergences in hydraulic conductivity, hydraulic safety and mechanical safety, we analysed phylogenetic independent contrast values (PICs) using Pearson product–moment correlations (Zanne *et al.*, 2010). Phylogenetic analyses were performed using RStudio (Version 0.99.903) packed with R 3.3.3 (R Core Team 2016) including the packages APE (Paradis *et al.*, 2004) and Picante (Webb *et al.*, 2010).

RESULTS

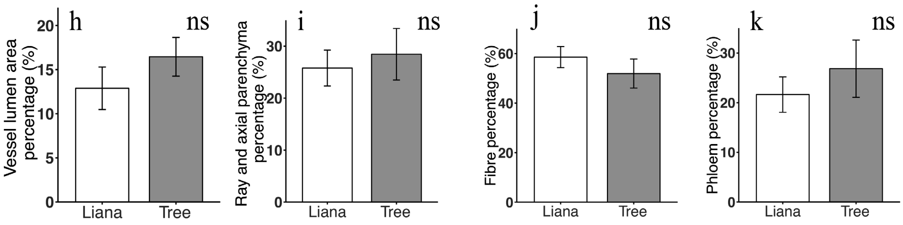
Functional traits



Tissue properties



Tissue proportions



Tissue areas

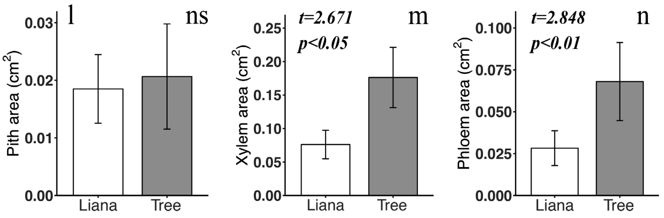


Figure 4.2 Branch property differences between lianas (N=12 species) and trees (N=10 species) in a Xishuangbanna seasonal rain forest. Differences were tested using linear mixed models with species as a random factor to estimate the overall variation. K_S, MOR, WD, MVD,

BVD, WLR, Parenchyma%, A_{pi}, A_x and A_{ph} were $\log_{10}$ transformed. Error bars represent 2SE. *t*-values and *P*-values were given for significant differences. Relations without significance are indicated by "ns".

In contrast to our expectation, MOR was significantly higher in lianas than in trees (Fig. 4.2c). Trees and lianas did however not significantly differ in K_S or P_{50} (Fig. 4.2a, 4.2b) though mean K_S was almost twice as high in lianas than in trees, but high variance occurred across liana species (Fig. 4.2a). Trees and lianas did not differ significantly in tissue properties and neither in the tissue proportions within the branch cross-sections (Fig. 4.2d-4.2k). Only xylem and phloem cross-sectional area was, as expected, higher in trees than in lianas (Fig. 4.2m, 4.2n). Thus, apart from these latter size differences, lianas and trees did unexpectedly not differ in tissue properties and proportions, and neither in their functional traits except MOR (Fig. 4.2). For all traits, *K*-values were less than 1 with corresponding *P*-values larger than 0.05 (Table S4.2). In addition, trees and lianas did not differ in anatomical structure, except for one liana species *Strychnos angustiflora*, which had included phloem (Table S4.1).

We found no evidence for trade-offs between hydraulic conductivity, hydraulic safety and mechanical safety, not across liana and tree species nor within liana and tree species separately (Fig. 4.3, Table S4.3, S4.4). Phylogenetic relatedness among the species evaluated, as measured using independent contrasts (Table S4.5), had no significant effect on correlations between hydraulic conductivity, hydraulic safety and mechanical safety.

Lianas and trees partially differed in the underlying anatomical features that explained most variation in K_S, MOR and P_{50} across species (Table 4.3). Vessel% explained variation in K_S, but the coefficient and importance value were higher for lianas. BVD contributed in explaining K_S differences across liana species, but only weakly. WLR or WD partially explained variation in MOR across liana and tree species, but only for trees the effect of WD was larger (Table 4.3). WLR and Parenchyma% partially explained the variation in P_{50} in lianas while WD partially explained the variation in P_{50} in trees, but their coefficients and importance values were rather low.

Table 4.3 Model averaging results from multiple regressions of K_S, MOR and P_{50} against a combination of possible, potential causal variables (see Figure 1). Coefficients (b) are the model averages from models with ΔAICc < 2; S.E. = standard error; importance = the proportion of models in ΔAICc < 2 set that include the causal effects.

Functional	Variable	Liana		Tree	
Trait		b ± S.E.	Importance	b ± S.E.	Importance
K_S	BVD	-0.19±0.15	0.28	-	-
	Parenchyma%	-	-	-	-
	Vessel%	0.49±0.18	**1.00**	0.39±0.22	0.45
MOR	WLR	0.20±0.15	0.23	-0.12±0.12	0.21
	WD	0.45±0.20	0.36	0.71±0.19	**1.00**
	Fibre%	-	-	-	-
P_{50}	WLR	0.05±0.13	0.22	-	-
	WD	-	-	-0.07±0.18	0.26
	Parenchyma%	-0.06±0.14	0.25	-	-

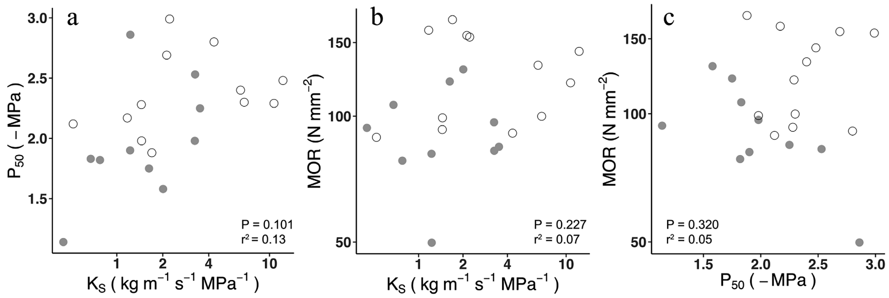


Figure 4.3 Relationships between hydraulic conductivity (K_S), hydraulic safety (P_{50}) and mechanical safety (MOR) tested by standardized major axis (SMA) regression. Lianas and trees are pooled together to examine overall correlations. K_S and MOR were $\log_{10}$

transformed. *P*-values and r^2 are given. Open dots represent lianas while grey dots represent trees.

DISCUSSION

In this study, we compared hydraulics and mechanics of liana and tree branches in an upper tropical forest canopy in China. Since none of the hydraulic, mechanical and anatomical traits showed phylogenetic conservatism (Table S4.2), we discuss our results here in the light of possible trade-offs in hydraulic and mechanical functional traits, the differences between lianas and trees as groups and the link with underlying anatomical traits, but irrespective of phylogenetic relations.

Do lianas and trees differ in functional traits?

As expected, liana branches were slenderer than those of trees and therefore were characterized by smaller tissue areas in the branch stem cross-section. Of the functional traits, only the MOR of branches significantly differed between lianas and trees: it was significantly higher in lianas than in trees implying a higher mechanical safety in liana branches. As lianas and trees did not significantly differ in their branch WD, Vessel%, Parenchyma%, Fibre% and Phloem% (Fig. 4.2), these results suggest that lianas produce stronger branch stems than trees by using similar wood density and similar shares of different tissues in stem cross-sections. This is consistent with the similar stem density shared by vines and shrubs within the same species (Gartner, 1991c), but not with the higher stiffness observed for shrubs relative to vines. Thus, lianas should have a more efficient way, at least in their canopy branches, to build up their wood to be able to grow longer with the same investment as tree branches, while still maintaining strong wood. A possible trait that may help lianas achieve this is microfibril orientation which may cause a high flexibility and strength of liana branches (Hoffmann *et al.*, 2000). This possibly unique anatomy and structure allow lianas to forage at low carbon costs (slender stems) without increasing risks of mechanical failure.

In contrast to our expectation, K_S did not differ significantly between lianas and trees (Fig. 4.2a). This is consistent with results of a sapling study by van der Sande *et al.* (2013), and is maybe partially due to the large variance within lianas. As K_S depends both on the area occupied by vessels and vessel diameter (Bittencourt *et al.*, 2016), the overlapping Vessel% in lianas and trees (Fig. 4.2h) suggests that the similar K_S is induced by similar vessel space allocation and a similar vessel diameter. This is supported by our results that BVD and MVD did not differ significantly between lianas and trees (Fig. 4.2e, 4.2f) in the studied exposed canopy branches. Though we commonly accept that lianas generate wider vessels compared to trees in cross-sections (Gartner, 1991b; Chiu & Ewers, 1992; Angyalossy *et al.*, 2012), this may not be true if we scale vessel diameter by stem length (Rosell & Olson, 2014). Thus, vessel diameter may be mainly driven by a general tip-to-base widening effect, rather than lifeform control. Since vessel fraction contributes to explaining K_S across liana branches, but not in tree branches (Table 4.3), our study suggests that some liana species have a higher hydraulic efficiency in their canopy branches than other species by allocating more space for vessels rather than widening their vessels at branch tip.

We did not find significant differences in the hydraulic safety between lianas and trees (Fig. 4.2b). The weak role of WLR and WD in both lianas and trees suggests that differences in cavitation resistance may weakly be induced by vessel wall micro-fractures in canopy branches. P_{50} differences across species were however only poorly explained by these selected anatomical features (Table 4.3) suggesting that other traits largely drive the variation in P_{50}. Inter-conduit connection and pit geometry are important for canopy branches against cavitation (Brodersen *et al.*, 2014; Tixier *et al.*, 2014; Woodruff *et al.*, 2016), but those properties were not included in our study. However, in a sapling study it was shown that trees had a higher cavitation resistance compared to lianas (van der Sande *et al.*, 2013). Our canopy trees had a P_{50} that was similar to those of tree saplings, whereas canopy lianas had a more negative P_{50} compared to those of liana saplings (van der Sande *et al.*, 2013). Possibly, lianas change cavitation resistance from sapling to adults. This suggests that lianas reduce xylem vulnerability at branch tips when they are tall and can thus compensate for the non-linear pressure gradient with increasing height (Couvreur *et al.*, 2018). Thus, hydraulic strategy may shift during plant development under different mechanisms in lianas and trees. More studies are needed to understand the mechanisms that drive the difference between sapling and adult in cavitation resistance in tall woody plants (Olson *et al.*, 2018).

Our branch study reveals the upper crown physiology and related anatomy, but the situation may change when considering stem base (Couvreur *et al.*, 2018) since the function of the stem base is more focused on mechanical support for the whole plant body. We call for more studies to compare canopy branch with stem base to enlarge our understanding of the physiological and underlying anatomical difference between lianas and trees.

Do trade-offs between hydraulic conductivity, hydraulic safety and mechanical safety exist in lianas and trees?

We hypothesized that hydraulic conductivity would trade-off against hydraulic safety. This was neither the case across both lianas and trees together, nor within lianas or trees separately (Fig. 4.3a, Table S4.4), suggesting that a high hydraulic conductivity does not necessarily connect to a low hydraulic safety in canopy branches. Support for the existence of such a trade-off varies much across studies (Table 4.1). Branch studies of Pockman and Sperry (2000) and De Guzman *et al.* (2016) showed a trade-off between K_S and P_{50} in a temperate dry vegetation and tropical seasonal dry forest. These results suggest that drought may play a role in controlling the balance between water transport and safety across species growing in the same condition. However, Choat *et al.* (2007) (a branch study) did not find such trade-off in the genus *Cordia* growing at contrasting precipitation levels, and neither Pratt *et al.* (2007) in a Rhamnaceae shrub stem study. This implies that phylogenetic control may weaken the response to drought leading to the absence of such hydraulic trade-off. Two global surveys (Maherali *et al.*, 2004; Gleason *et al.*, 2016) showed no evidence for a strong trade-off between K_S and P_{50} across stems and branches. Possibly, these weak relationships, and the lack for support of a trade-off in our study, result from species differences in underlying traits that were beyond the scope of our study, such as xylem vessel network topologies and network features, cellular arrangements, the presence of other cell types, and the degree of xylem modularity (Choat *et al.*, 2003; Wheeler *et al.*, 2005; Loepfe *et al.*, 2007).

Against our expectation, we did not find a trade-off between hydraulic conductivity and mechanical safety across lianas and trees, nor in lianas and trees separately (Fig. 4.3b, Table S4.4). For shrub branches in a Mediterranean-type climate region, there was neither a trade-off between hydraulic conductivity and mechanical safety observed (Jacobsen *et al.*, 2007). These observations imply that in both dry and wet conditions, there may be no trade-off between mechanical safety and hydraulic conductivity in branches. In angiosperms, xylem tissues differentiate into vessel, parenchyma and fibre tissues, thus implying trade-offs between those tissues. Fibre%, the main supporting tissue, was however not correlated with Vessel% (Fig. S4.1a, Table S4.4), suggesting that vessel area does not trade-off against fibre area in stem cross-sections. This is coherent with Fortunel *et al.* (2014) who found that fibre traits appeared to be independent of vessel traits. However, more studies are needed to confirm the separate development of fibre traits and vessel traits, especially in stems of tall woody plants which may experience huge mechanical stress, and fully understand the mechanism that drive the relation between hydraulic and mechanical properties in angiosperm species.

Mechanical safety and hydraulic safety were decoupled as well (Fig. 4.3c). In line with this, the underlying variables WLR in lianas and WD in trees only weakly influenced MOR and P_{50}. This contrasts with results from shrub studies (Table 4.1, Jacobsen *et al.*, 2005; Pratt *et al.*, 2007; Jacobsen *et al.*, 2009) showing a positive correlation between MOR and P_{50} in stems/branches. These shrub studies however included a much wider variation in P_{50} going down to minimum values close to -11 MPa compared to -3 MPa in our study. In their studies, they also found a negative correlation between P_{50} and WD whereas we did not find such correlation (Fig. S4.2b). Therefore, the negative water potential in our study may not have been harsh enough to cause the structural change leading to stronger wood to avoid vessel wall micro-fractures. If the range of P_{50} shown by Jacobsen *et al.* (2009) is narrowed to -4 MPa, the trade-off disappears.

The lack of trade-offs between hydraulic conductivity, hydraulic safety and mechanical safety did not change when considering phylogenetic dependence (Table S4.5), confirming that canopy branches may reflect adaptations to the environment rather than phylogenetic constraints.

CONCLUSIONS

Most of our expectations on the differences between lianas and trees were not supported by our data for the studied tropical seasonal forest in SW China. The expected trade-offs between hydraulic conductivity, hydraulic safety and mechanical safety were not found. The anatomical features only partially explained the variation in functional traits. Consequently, this study mainly shows that: 1) there are no clear trade-offs between hydraulic conductivity, hydraulic safety and mechanical safety across exposed canopy distal branches in trees and lianas; (2) liana canopy distal branches better resist branch breakage against mechanical forces than tree canopy branches; (3) lianas and trees are as groups similar in hydraulic performance; and 4) the mechanisms that drive K_S, P_{50} and MOR may differ between lianas and trees. We conclude that liana and tree branches diverged in mechanical design due to fundamental differences in wood formation, but converged in hydraulic design.

FUNDINGS

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We thank Alan Crivellaro, Padova University, and Ute Sass-Klaassen, Wageningen University, for advices on anatomical observation and methods such as sample preparation and tissue recognition of cross-sections. We thank Lourens Poorter and Masha van der Sande for statistical advice on the SMA analysis; Yingying Wang for advice on model comparison; and Zexin Fan for support on the phylogenetic analysis. We thank José Medina Vega for advices on literature; Song Lv and Xi Ai for field assistance; and the anatomical lab of Xishuangbanna Botanical Garden for providing equipment to make cross-section of branch samples. We thank the Xishuangbanna Station for Tropical Rain Forest Ecosystem Studies (XSTRE) for assistance in the sample collection.

SUPPORTING INFORMATION

Fig. S4.1 Relationships between vessel lumen area percentage, ray and axial parenchyma area percentage and fibre area percentage tested by standardized major axis (SMA) regression. *P*-value and r^2 are given in significant correlations. Open dots represent lianas, and dotted line indicates significant correlation in lianas. Grey dots represent trees, and long dash indicates significant correlation in trees. *P*-value and r^2 are given with significant correlation. ns, not significant.

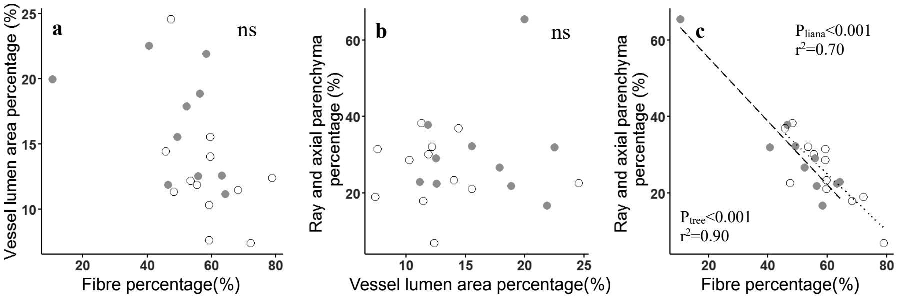


Fig. S4.2 Relations between functional traits and dry wood density of lianas (open dots) and trees (grey dots) in Xhishuangbanna seasonal rain forest. A linear mixed model was used with 44 individuals in 22 species. Lifeform was included as an interaction term to examine if lianas and trees differed in their relations among traits. Species was set as a random factor. Xylem-specific conductivity (K_S) and dry wood density were $\log_{10}$ transformed. ns, not significant.

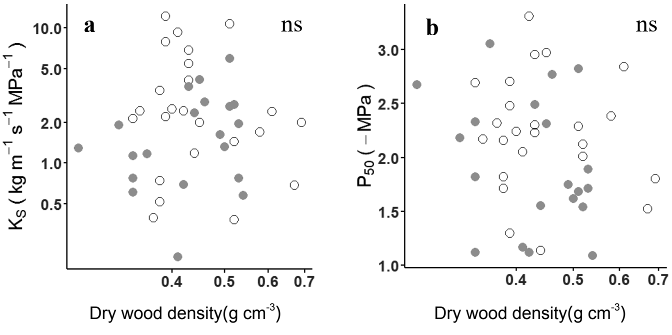
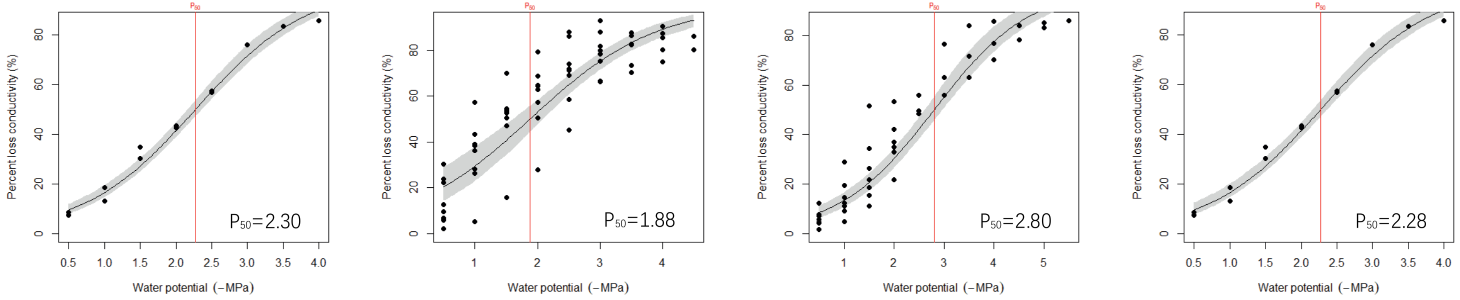
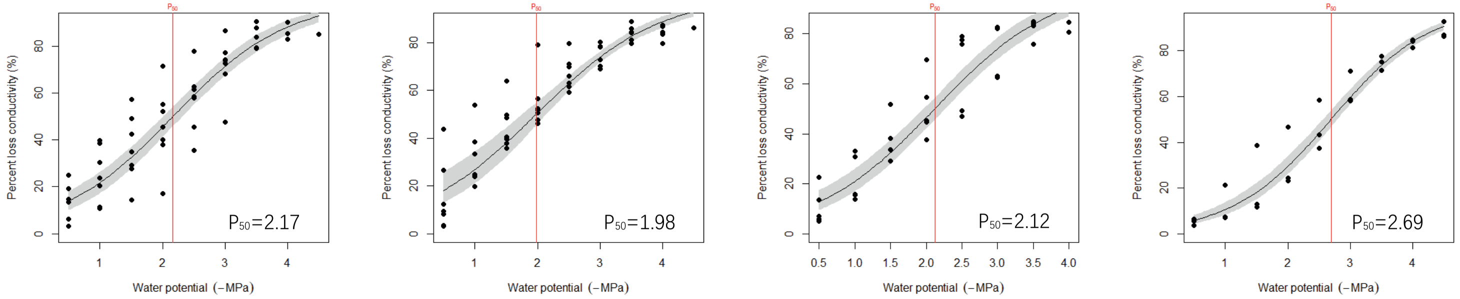


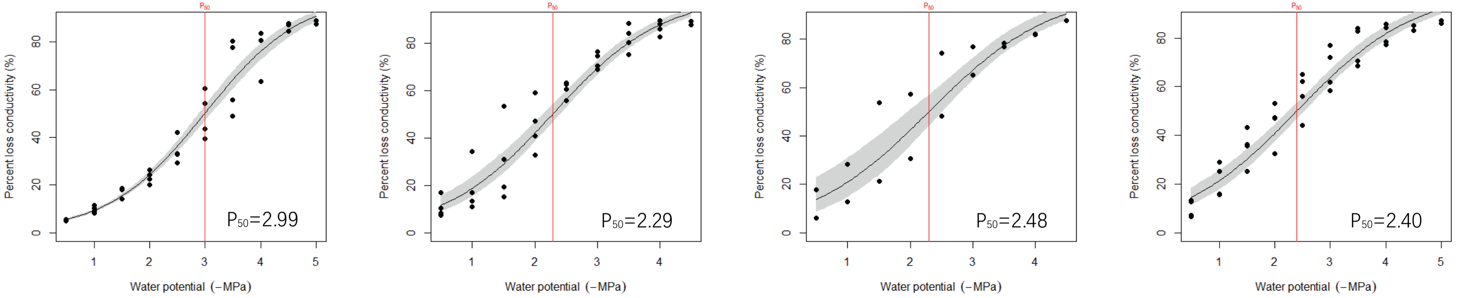
Fig. S4.3 Vulnerability curves of all species. Red lines showed cavitation resistance (P_{50}) at 50% loss of conductivity.



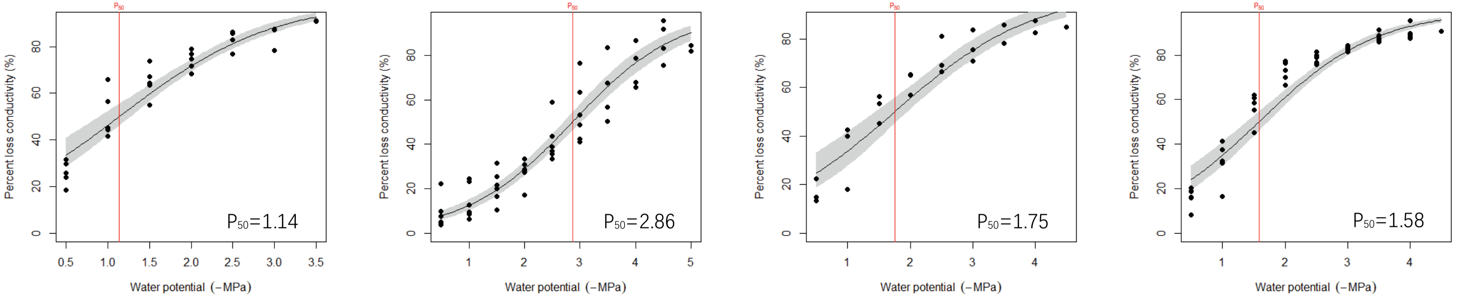
Acacia megaladena *Bauhinia aurea* *Bauhinia yunnanensis* *Byttneria aspera*



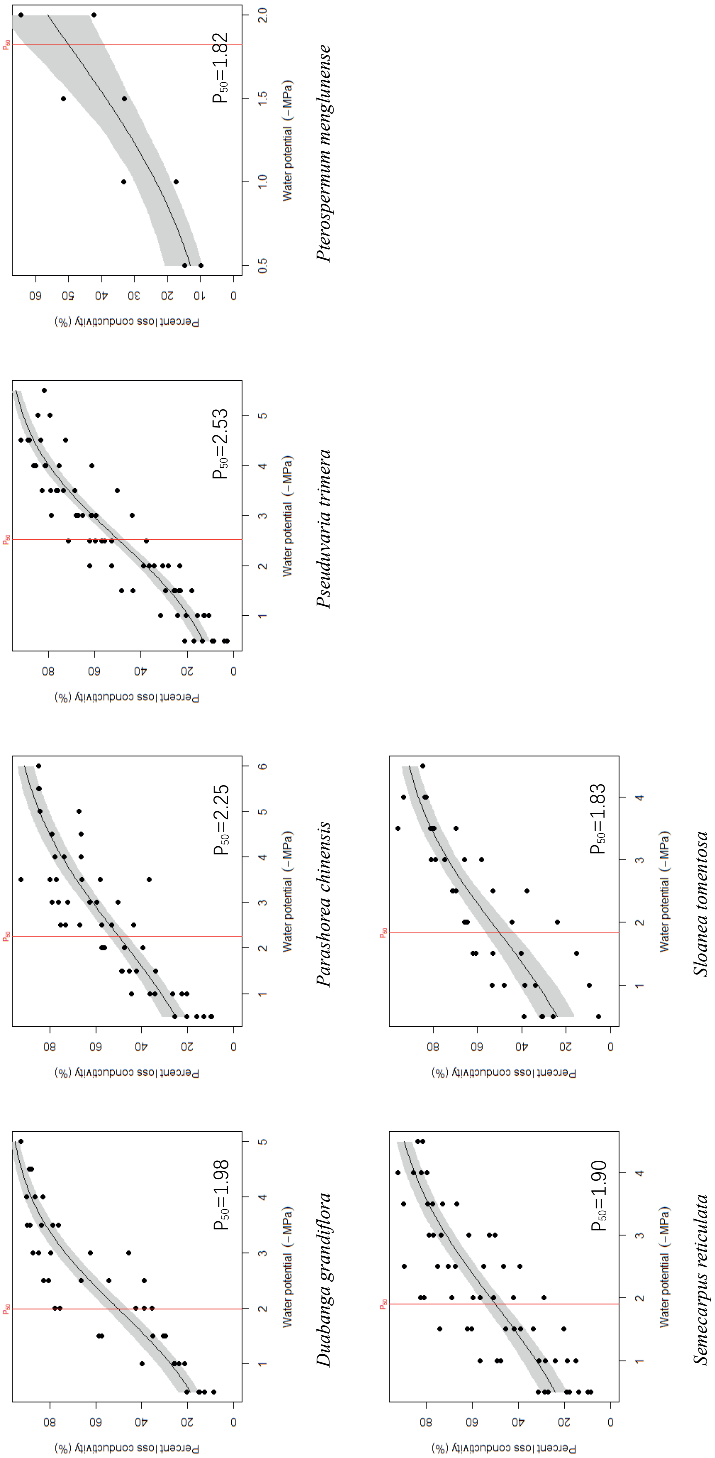
Combretum latifolium *Ficus sagittata* *Fissistigma maclurei* *Mallotus repandus*



Premna scandens *Strychnos angustiflora* *Uncaria laevigata* *Uncaria macrophylla*



Allophylus cobbe *Balakata baccata* *Castanopsis hainanensis* *Castanopsis indica*



Pterospermum menglunense

Pseuduvaria trimera

Parashorea chinensis

Duabanga grandiflora

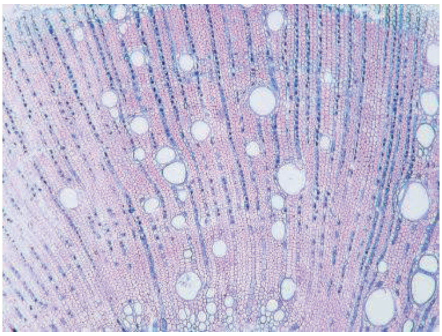
Sloanea tomentosa

Semecarpus reticulata

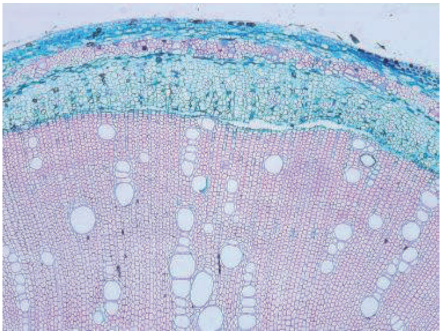
Table S4.1 Illustration of studied wood anatomy with species information of lianas and trees. Species names and family (in brackets) were standardized using The Plant List (www.theplantlist.org). All photos have the same magnification and scale bar is shown in first photo. Tree height (m), DBH (cm) and maximum vessel length (MVL, cm) are given if applicable.

Lianas

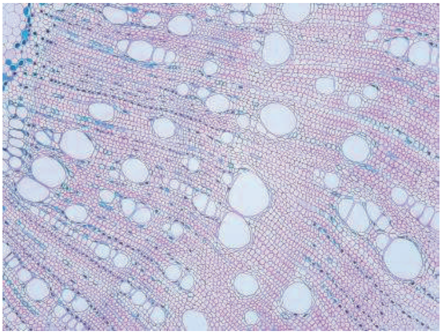
Acacia megaladena Desv. (*Leguminosae*)
MVL: ca. 170



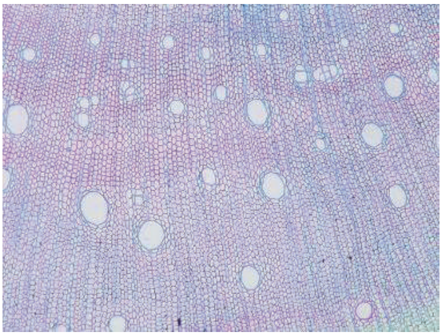
Bauhinia aurea H.Lev. (*Leguminosae*)
MVL: ca. 94



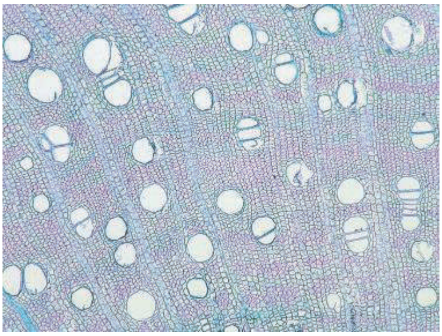
Bauhinia yunnanensis Franch. (*Leguminosae*)
MVL: ca. 202



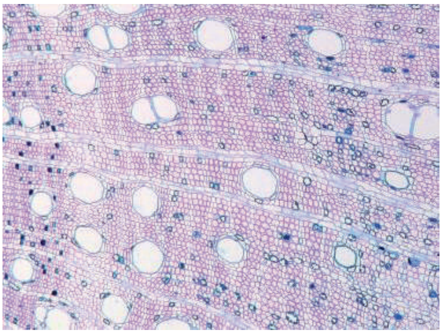
Byttneria aspera Collebr. ex Wall. (*Malvaceae*)
MVL: ca. 42

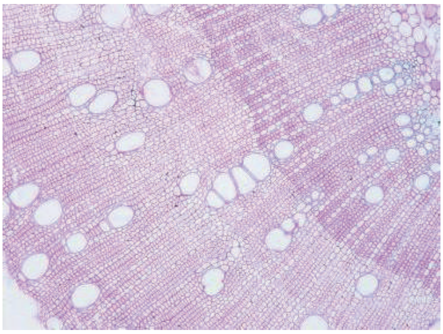


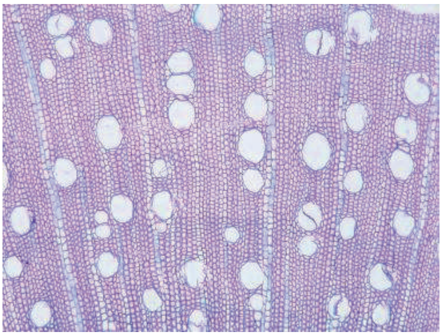
Combretum latifolium Blume (*Combretaceae*)
MVL: ca. 126

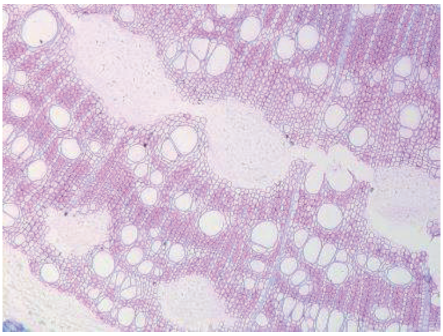


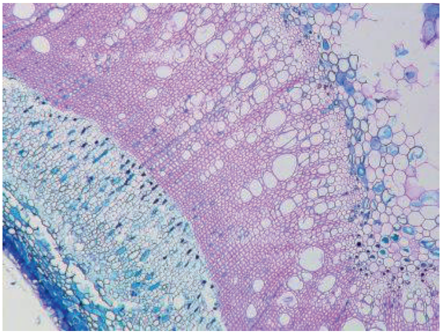
Ficus sagittata Vahl (*Moraceae*)
MVL: 62

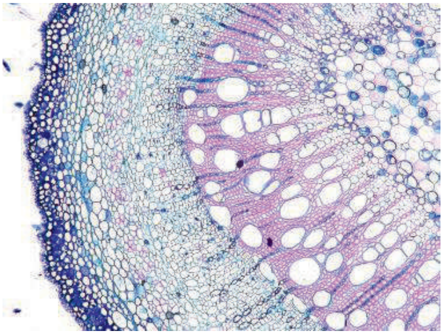

Fissistigma maclurei Merr. (*Annonaceae*)
MVL: ca. 49

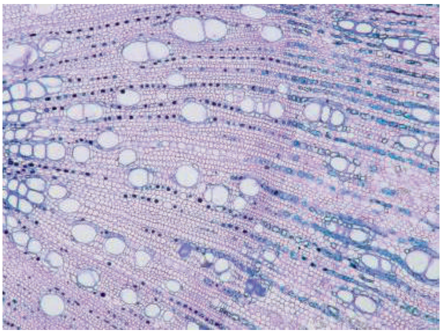

Mallotus repandus (Willd.) Müll.Arg. (*Euphorbiaceae*)
MVL: ca. 56

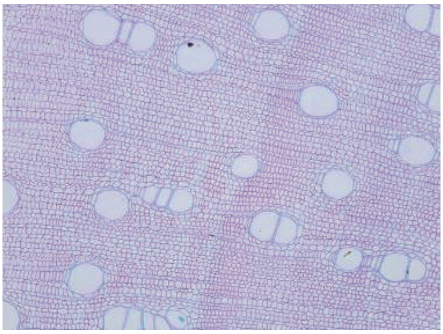

Premna scandens Roxb. (*Lamiaceae*)
MVL: ca. 119

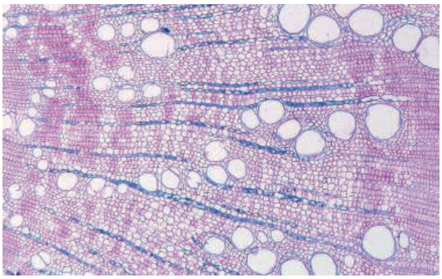

Strychnos angustiflora Benth. (*Loganiaceae*)
MVL: ca. 133


Uncaria laevigata Wall. ex G.Don (*Rubiaceae*)
MVL: ca. 162

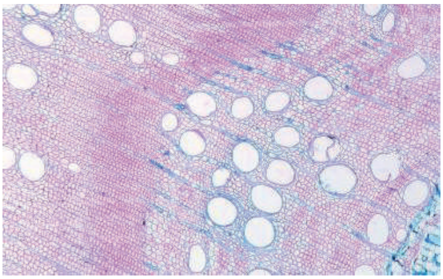

Uncaria macrophylla Wall. (*Rubiaceae*)
MVL: ca. 150


Allophylus cobbe (L.) Raeusch. (*Sapindaceae*)
MVL: ca. 57
Height: 29.8-43.2
DBH: 49.5-99.5

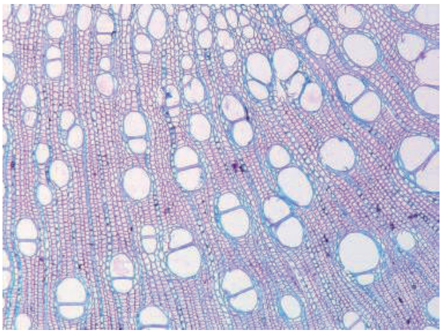

Balakata baccata (Roxb.) Esser (*Euphorbiaceae*)
MVL: 36
Height: 16.5-42.9
DBH: 49.6-96.7



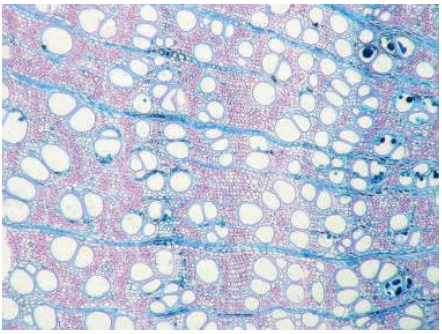
Castanopsis hainanensis Merr. (*Fagaceae*)
MVL: 77
Height: 21.2
DBH: 40



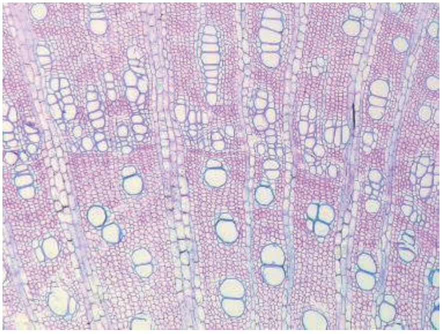
Castanopsis indica (Roxb. ex Lindl.) A.DC. (*Fagaceae*)
MVL: ca. 66
Height: 19.4-29.5
DBH: 48.1



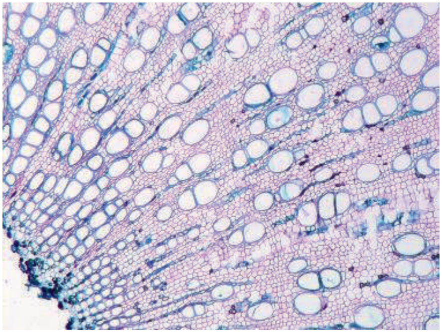
Duabanga grandiflora (DC.) Walp. (*Lythraceae*)
MVL: ca. 83
Height: 34.1-38.4
DBH: 79.8



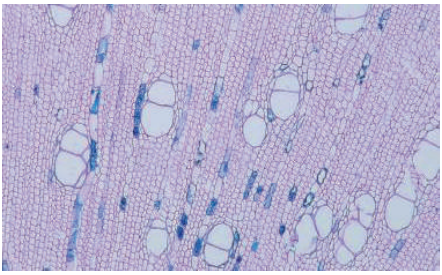
Parashorea chinensis Hsie Wang (*Dipterocarpaceae*)
MVL: ca. 61
Height: 56-68.5
DBH: 105.2-126.7



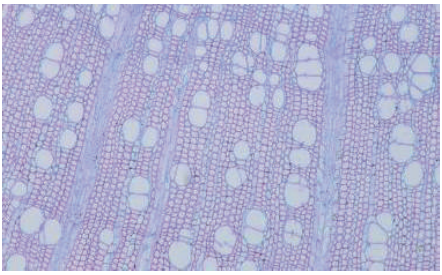
Pseuduvaria trimera (W. G. Craib) Y.C.F. Su & R.M.K. Saunders (*Annonaceae*)
MVL: ca. 33
Height: 20.6-30.5
DBH: 22.3-27.1



Pterospermum menglunense H.H. Hsue (*Malvaceae*)
MVL: ca. 67
Height: 40.1
DBH: 36.9



Semecarpus reticulata Lecomte (*Anacardiaceae*)
MVL: ca. 39
Height: 37.4-51.3
DBH: 58.7-95.2



Sloanea tomentosa (Benth.) Rehder & E.H. Wilson (*Elaeocarpaceae*)
MVL: ca. 45
Height: 39.4-42.9
DBH: 80.3-89.4

Table S4.2 Mean values of measured traits per species of lianas and trees: N, number of individual sample(s) per species, xylem-specific conductivity (K_S, kg m^{-1} s^{-1} MPa^{-1}), cavitation resistance (P_{50}, -MPa), modulus of rupture (MOR, N mm^{-2}), wood density (WD, g cm^{-3}), 20 biggest vessel lumen diameter (BVD, μm^2), mean vessel lumen diameter (MVD, μm^2), wall-lumen ratio (WLR, μm μm^{-1}), vessel percentage (Vessel%, %), ray and axial parenchyma percentage (Parenchyma%, %) and fibre percentage (Fibre%, %). Phylogenetic signal *K*-value and corresponding *P*-value for each trait was given separately.

Species	N	K_S	P_{50}	MOR	WD	BVD	MVD	WLR	Vessel%	Parenchyma%	Fibre%
Lianas											
Acacia megaladena	1	6.83	2.30	99.90	0.43	59.53	44.08	0.029	12.39	6.80	78.90
Bauhinia aurea	3	1.70	1.88	170.15	0.66	53.78	26.88	0.040	7.61	31.45	59.36
Bauhinia yunnanensis	3	4.33	2.80	91.07	0.41	46.73	26.04	0.041	15.53	20.97	59.73
Byttneria aspera	2	1.45	2.28	92.93	0.39	59.81	27.25	0.031	11.87	30.05	55.44
Combretum latifolium	3	1.17	2.17	160.61	0.54	58.28	31.24	0.035	7.42	18.86	72.21
Ficus sagittata	3	1.45	1.98	99.08	0.39	61.63	35.05	0.035	11.33	38.16	48.30
Fissistigma maclurei	1	0.52	2.12	88.98	0.38	66.85	42.56	0.032	12.18	32.03	53.61
Mallotus repandus	1	2.12	2.69	156.02	0.34	56.98	40.21	0.036	10.31	28.59	59.32
Premna scandens	2	2.22	2.99	154.70	0.44	61.60	36.20	0.034	11.46	17.81	68.33
Strychnos angustiflora	1	10.71	2.29	120.13	0.51	50.24	32.14	0.035	14.41	36.87	45.74
Uncaria laevigata	1	12.25	2.48	142.93	0.39	50.44	26.54	0.035	14.04	23.20	59.64
Uncaria macrophylla	3	6.46	2.40	132.43	0.40	53.13	29.40	0.036	24.53	22.47	47.48
Trees											
Allophylus cobbe	2	0.45	1.14	93.78	0.42	60.10	37.48	0.041	17.88	26.55	52.27
Balakata baccata	2	1.23	2.86	49.84	0.32	78.45	50.59	0.030	11.16	22.84	64.29
Castanopsis hainanensis	1	1.63	1.75	121.05	0.49	62.43	36.23	0.033	15.54	32.13	49.32
Castanopsis indica	2	2.01	1.58	129.37	0.51	61.87	43.26	0.031	18.84	21.73	56.36
Duabanga grandiflora	2	3.24	1.98	96.71	0.45	81.66	52.20	0.029	21.89	16.62	58.36
Parashorea chinensis	3	3.51	2.25	84.53	0.52	61.30	35.13	0.042	22.51	31.84	40.60

Continued Table S4.2

Species	N	K_S	P_{50}	MOR	WD	BVD	MVD	WLR	Vessel%	Parenchyma%	Fibre%
Pseuduvaria trimera	2	3.26	2.53	82.65	0.45	35.88	18.93	0.046	11.85	37.72	46.57
Pterospermum menglunense	1	0.78	1.82	78.26	0.34	47.58	31.25	0.037	19.96	65.34	10.56
Semecarpus reticulata	3	1.22	1.90	81.30	0.33	72.22	43.13	0.028	12.57	22.34	63.28
Sloanea tomentosa	2	0.68	1.83	106.44	0.54	40.92	27.71	0.045	12.55	29.02	55.80
PhyloSignal	*K*	0.83	0.72	0.70	0.55	0.64	0.64	0.60	0.53	0.66	0.66
	P	0.075	0.149	0.239	0.633	0.341	0.324	0.500	0.721	0.329	0.297

Table S4.3 Results of standardized major axis (SMA) regression were given for trade-offs between hydraulic conductivity (K_S) with hydraulic safety (P_{50}), and between hydraulic safety with mechanical safety (MOR) across liana and tree species. The slope, *P*-value and r^2 were given separately with excluding and including outliers.

Trait 1	Trait 2	Outlier excluded			Full data		
		Slope	*P*-value	r^2	Slope	*P*-value	r^2
K_S	P_{50}[1]	1.04	0.101	0.13	1.13	**0.022**	0.24
P_{50}	MOR[2]	3.97	0.320	0.052	-3.47	0.942	0.00027

[1] One tree species *Allophylus cobbe* was excluded.

[2] One tree species *Balakata baccata* was excluded.

Table S4.4 Results of standardized major axis (SMA) regression was given. Trade-offs between hydraulic conductivity (K_S), hydraulic safety (P_{50}) and mechanical safety (MOR), as well as relationships between vessel lumen area percentage (Vessel%), ray and axial parenchyma area percentage (Parenchyma%) and fibre area percentage (Fibre%) in lianas and trees were tested. The slope, *P*-value and r^2 were given in different lifeforms. The likelihood-ratio (LR) which indicating slope difference between lianas and trees was given with corresponding *P*-value.

Trait 1	Trait 2	Lianas			Trees			Slope difference	
		Slope	*P*-value	r^2	Slope	*P*-value	r^2	LR	*P*-value
K_S	P_{50} outlier excl.[1]	0.78	0.314	0.10	1.54	0.513	0.064	1.90	0.169
K_S	P_{50}	0.78	0.314	0.10	1.56	0.132	0.26	2.43	0.119
K_S	MOR	3.94	0.733	0.012	2.68	0.857	0.0043	0.63	0.428
P_{50}	MOR outlier excl.[2]	3.06	0.726	0.013	-4.99	0.250	0.183	1.02	0.312
P_{50}	MOR	3.06	0.726	0.013	-4.18	**0.022**	0.50	0.56	0.454
Vessel%	Parenchyma%	-0.49	0.755	0.010	0.31	0.709	0.018	0.86	0.353
Vessel%	Fibre%	-0.44	0.135	0.21	-0.28	0.204	0.19	1.11	0.292
Parenchyma%	Fibre%	-0.89	**<0.001**	0.70	-0.88	**<0.001**	0.90	0.0029	0.957

[1] One tree species *Allophylus cobbe* was excluded.

[2] One tree species *Balakata baccata* was excluded.

Table S4.5 Relationships between hydraulic conductivity (K_S), hydraulic safety (P_{50}) and mechanical safety (MOR) were tested for the phylogenetically independent contrast values (PICs). r^2 and *P*-value for the correlation were given. "+" indicates positive correlation while "-" indicates negative correlation.

Trait 1	Trait 2	Sign	r^2	*P*-value
K_S	P_{50}	+	0.053	0.315
K_S	MOR	-	0.014	0.612
P_{50}	MOR	-	0.068	0.256

Table S4.6 Individual values of measured traits of lianas (L) and trees (T): xylem-specific conductivity (K_S, kg m^{-1} s^{-1} MPa^{-1}), modulus of rupture (MOR, N mm^{-2}), wood density (WD, g cm^{-3}), 20 biggest vessel lumen diameter (BVD, μm^2), mean vessel lumen diameter (MVD, μm^2), wall-lumen ratio (WLR, µm μm^{-1}), vessel percentage (Vessel%, %), ray and axial parenchyma percentage (Parenchyma%, %) and fibre percentage (Fibre%, %).

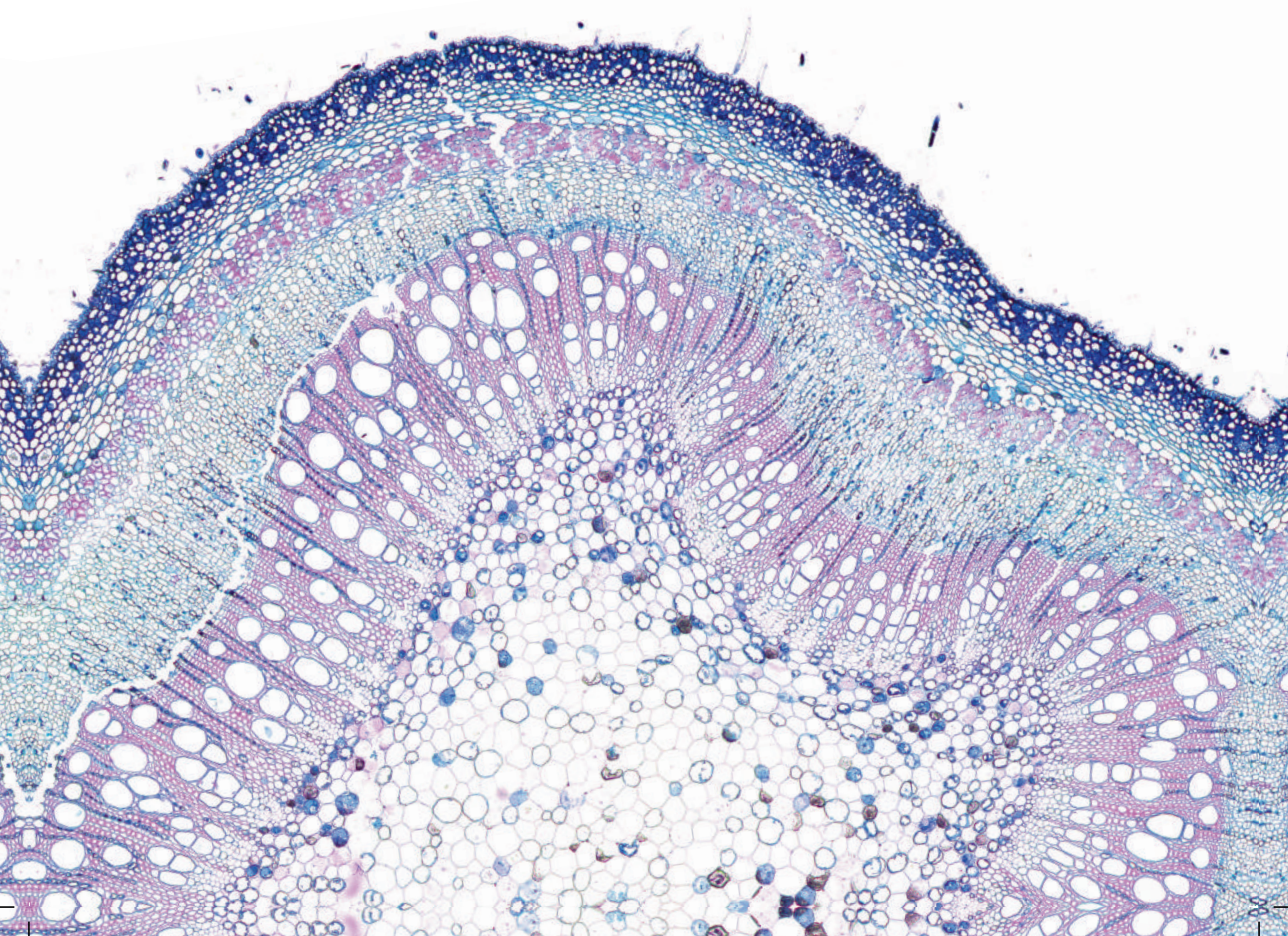
No	Species	lifeform	K_S	MOR	WD	BVD	MVD	WLR	Vessel%	Parenchyma%	Fibre%
1	*Acacia megaladena*	L	6.83	99.90	0.43	59.53	44.08	0.029	12.39	6.80	78.90
2	*Bauhinia aurea*	L	2.00	113.24	0.69	63.38	32.39	0.032	6.43	30.99	61.39
3	*Bauhinia aurea*	L	0.69	207.17	0.67	47.75	23.41	0.039	5.61	34.35	58.53
4	*Bauhinia aurea*	L	2.40	190.03	0.61	50.22	24.84	0.047	10.80	29.00	58.16
5	*Bauhinia yunnanensis*	L	4.11	93.20	0.43	48.15	24.14	0.043	16.54	23.95	55.26
6	*Bauhinia yunnanensis*	L	5.43	80.77	0.43	38.92	20.21	0.045	14.35	20.05	62.00
7	*Bauhinia yunnanensis*	L	3.46	99.23	0.38	53.12	33.84	0.036	15.71	18.92	61.92
8	*Byttneria aspera*	L	0.39	101.09	0.37	62.48	26.34	0.031	17.03	29.72	49.42
9	*Byttneria aspera*	L	2.50	84.76	0.40	57.15	28.16	0.030	6.72	30.38	61.46
10	*Combretum latifolium*	L	1.44	167.20	0.52	63.02	37.34	0.029	8.07	18.41	72.28
11	*Combretum latifolium*	L	0.38	156.44	0.52	55.98	24.04	0.041	5.81	20.78	71.79
12	*Combretum latifolium*	L	1.69	158.18	0.58	55.84	32.35	0.035	8.37	17.40	72.57
13	*Ficus sagittata*	L	1.18	103.14	0.44	61.37	41.03	0.038	10.76	32.43	55.05
14	*Ficus sagittata*	L	2.44	99.82	0.35	63.61	33.61	0.035	11.39	37.57	48.68
15	*Ficus sagittata*	L	0.74	94.28	0.38	59.90	30.50	0.032	11.83	44.47	41.18
16	*Fissistigma maclurei*	L	0.52	88.98	0.38	66.85	42.56	0.032	12.18	32.03	53.61
17	*Mallotus repandus*	L	2.12	156.02	0.34	56.98	40.21	0.036	10.31	28.59	59.32
18	*Premna scandens*	L	2.44	152.85	0.42	59.03	36.25	0.040	14.26	20.20	62.36
19	*Premna scandens*	L	1.99	156.54	0.45	64.17	36.15	0.029	8.65	15.43	74.29
20	*Strychnos angustiflora*	L	10.71	120.13	0.51	50.24	32.14	0.035	14.41	36.87	45.74
21	*Uncaria laevigata*	L	12.25	142.93	0.39	50.44	26.54	0.035	14.05	23.20	59.64
22	*Uncaria macrophylla*	L	7.88	118.25	0.39	56.19	32.76	0.033	28.62	18.63	46.21
23	*Uncaria macrophylla*	L	9.29	152.51	0.41	54.01	28.28	0.034	27.87	25.63	39.90
24	*Uncaria macrophylla*	L	2.21	126.53	0.39	49.19	27.15	0.040	17.12	23.15	56.34
25	*Allophylus cobbe*	T	0.20	92.72	0.41	56.65	34.59	0.048	11.93	28.22	57.08
26	*Allophylus cobbe*	T	0.69	94.83	0.42	63.56	40.37	0.034	23.82	24.88	47.45
27	*Balakata baccata*	T	1.16	53.96	0.36	80.71	58.61	0.030	9.99	19.78	68.87
28	*Balakata baccata*	T	1.29	45.71	0.27	76.20	42.58	0.029	12.32	25.90	59.71
29	*Castanopsis hainanensis*	T	1.63	121.05	0.49	62.43	36.23	0.033	15.54	32.13	49.32
30	*Castanopsis indica*	T	1.31	125.60	0.50	60.97	43.32	0.032	19.58	24.39	52.88
31	*Castanopsis indica*	T	2.70	133.13	0.52	62.77	43.20	0.030	18.09	19.07	59.85
32	*Duabanga grandiflora*	T	2.35	107.94	0.44	70.10	49.20	0.032	20.67	17.33	58.81
33	*Duabanga grandiflora*	T	4.14	85.47	0.45	93.21	55.20	0.025	23.11	15.90	57.90
34	*Parashorea chinensis*	T	2.63	77.19	0.51	55.64	31.13	0.044	22.44	36.42	35.44

Continued Table S6

No	Species	lifeform	K_S	MOR	WD	BVD	MVD	WLR	Vessel%	Parenchyma%	Fibre%
35	*Parashorea chinensis*	T	5.94	89.74	0.51	64.73	38.64	0.046	23.18	25.45	46.48
36	*Parashorea chinensis*	T	1.95	86.66	0.53	63.53	35.61	0.037	21.91	33.66	39.88
37	*Pseuduvaria trimera*	T	3.69	78.58	0.43	37.67	19.38	0.042	11.41	40.65	44.48
38	*Pseuduvaria trimera*	T	2.83	86.71	0.46	34.08	18.48	0.050	12.29	34.78	48.67
39	*Pterospermum menglunense*	T	0.78	78.26	0.34	47.58	31.25	0.037	19.96	65.34	10.56
40	*Semecarpus reticulata*	T	0.61	84.13	0.34	74.58	41.60	0.031	11.13	24.21	62.80
41	*Semecarpus reticulata*	T	1.92	76.94	0.32	71.39	43.09	0.026	14.04	21.71	62.38
42	*Semecarpus reticulata*	T	1.14	82.83	0.34	70.70	44.70	0.027	12.54	21.09	64.65
43	*Sloanea tomentosa*	T	0.58	115.17	0.54	46.04	30.22	0.034	12.48	34.92	50.36
44	*Sloanea tomentosa*	T	0.77	97.70	0.53	35.79	25.19	0.055	12.61	23.11	61.25

CHAPTER 5

General discussion



INTRODUCTION

Forests play an important role in the water and carbon cycle between soil and atmosphere across the globe (Ozanne *et al.*, 2003). This role however differs between climate zones because tree growth is very responsive to climate variation. Across all types of forest, tropical forests sequester the largest amounts of carbon but lose the largest amounts of water. They also harbour more biodiversity per area than temperate forests (Bonan, 2008). Tropical forests are severely threatened by deforestation nowadays while temperate forests occupy relative stable areas across the world when including the planted forests (FAO, 2016). However, both tropical and temperate forests face an uncertain future under climate change.

Canopy branches as a core to study

The future of forests will largely depend on the fate of large, adult trees - the trees that build the forest canopy (Bongers, 2001). In those canopy trees, the loss of water and the gain of carbon, as well as the transport of water and carbon in the stem, are intimately linked with growth (Sterck & Schieving, 2011; Steppe *et al.*, 2015). Woody plants transport water through the stem from fine roots to leaves and transpire the water while fixing carbon in the leaves. Tall woody plants thus supply large amounts of water to the large crown for transpiration, but also require a strong structure to support their heavy woody body and large crown. Within these tall woody plants, canopy branches can be considered as bottlenecks in the functioning of entire trees, because they potentially fix most carbon under fully exposed conditions, but also deal with high transpiration demands due to higher irradiation and temperatures than branches that occur at lower, more shaded positions, or than small plants in the understorey. The role of these canopy branches is therefore considered essential for the acquisition of carbon and loss of water and, indirectly, for the transport of both water and carbon within canopy trees, probably with strong implications for the water and carbon cycle between soil, forest and atmosphere.

Woody plants in different forest biomes and their anatomy

In temperate forests, conifers and broadleaved trees can be common and occur sometimes mixed. These two lifeforms distinctly differ in leaves, stem and tree architecture. In tropical forests, lianas and trees are the major lifeforms that differ in growth form. Lianas are woody climbers that have slender stems, and need to climb on tall trees to reach the forest canopy. Despite their differences, these lifeforms from temperate to tropical forests have stems that consist of a pith, xylem and phloem. The xylem transports water up from fine roots to leaves and is largely responsible for the support of the plant body and the phloem in the inner bark translocates carbon from leaves to other parts of a plant.

Within these lifeforms, conifers are gymnosperms that have a very different wood anatomy. Conifers' xylem is mainly composed of narrow and short conduits (transport cells); these tracheids transport water and provide mechanical support. In contrast, the broadleaved lianas and trees are angiosperms that have a more efficient water transport system than conifers because they have bigger and longer conduits, the vessels, for water transport, and they use separate fibres that provide mechanical support. Both conifers and broadleaved species have ray and axial parenchyma where they store water and carbon in xylem (Evert, 2006) (see Fig. 1.2 in introduction). In addition, conifers have narrower but longer sieve cells for carbon transport, in the form of sugars, in the phloem than broadleaved species.

The main objective of my study was to quantify how canopy co-existing tall woody plants balance the acquisition of carbon and loss of water in the leaves with the transport and storage of water and carbon in the stem. I addressed the following questions in 3 subsequent chapters (2-4):

1. How do tree species differ in their functional balances between leaf area, xylem area and phloem area across deciduous tree species in a temperate forest?
2. How do conifer and broadleaved tree species differ in their functional balances between water and carbon related functions in a temperate forest?
3. How do liana and tree species coordinate trade-offs between hydraulic conductivity (water transport efficiency), hydraulic safety (drought resistance) and mechanical safety?

In this final chapter (***chapter 5***), I synthesize the results of this thesis and other studies in order to answer my questions. I discuss the raised questions and highlight the similarities and differences between species, lifeforms and forest biomes in the hydraulic, mechanic and carbon functions of canopy branches.

CONCEPTUAL MODEL ILLUSTRATING FUNCTIONAL BALANCES

In order to answer my research questions, I start from the conceptual model presented in ***chapter 1***. Cannell and Dewar (1994) proposed functional relationships between roots, stem and leaves based on the acquisition, transport and use of water, carbon and nutrients. I adjusted his conceptual model for my study for branches in canopy trees by considering leaves responsible for carbon gain and water loss and the stem responsible for the transport and storage of both carbon and water as well as for the mechanical support of the entire branch. I extended the original model of Cannell and Dewar (1994) by relating the transport and storage functions to different cell types within the xylem and phloem in the branch stem (Fig. 5.1). Woody plants are assumed to develop these tissue and cell types in a way that the tissue functions are guaranteed, or even maximized, without breaking down the carbon budget. Overall I expect that the carbon investments in these organs (leaves versus stem), tissues (xylem versus phloem) and cell types (e.g. conduits versus parenchyma) are balanced conform the so called "functional balances": In other words, branches are thus supposed to "balance" the water transport, water loss and water storage; balance the carbon gain, carbon transport and carbon storage; and keep branches mechanically stable.

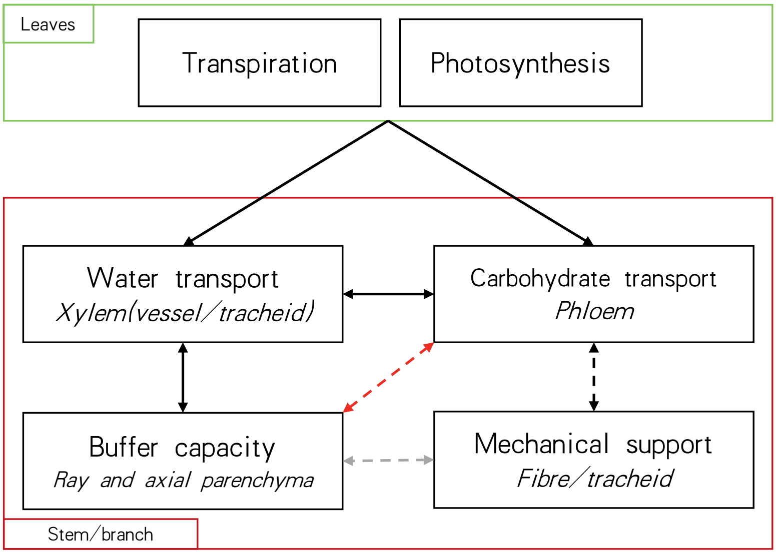


Figure 5.1 The conceptual model with studied (solid arrows) and speculated (dash arrows) relations in my study. The black arrows represent positive relations while the red arrows represent negative relations. The grey arrow represents uncertain relation. The *italics* indicate the tissues or cell types driving each function.

FUNCTIONAL BALANCES IN TALL WOODY PLANTS IN TEMPERATE FORESTS

In my study, I investigated the functional balances in two temperate forests. I compared these balances between 10 different deciduous broadleaved tree species differing in shade tolerance in one temperate forest (***chapter 2***) and between deciduous broadleaved tree species and conifer species in another temperate forest (***chapter 3***). Below I discuss the role of functional balances in temperate forests, i.e. the water loss/transport balance, carbon gain/transport balance, the carbon gain/storage balance and water loss/storage balance.

Functional balance between water loss and water transport

The water loss - transport balance is defined by the balance between the efficiency by which water is lost from the leaves and water is supplied by the conduit cells in the xylem in the stem. As light is the essential factor that provides energy for photosynthesis, I expected sun branches to produce more xylem area to support the same amount of leaf area than shade branches because their higher assimilation rates come with rapid water loss. I also expected shade-tolerant species to produce less xylem area per leaf area than light-demanding species due to their lower assimilation and transpiration rates per leaf area. I explored these expectations by comparing leaf area : xylem area ratios between sun and shade branches across 10 tree species (***chapter 2***) in a temperate forest in the Netherlands. I found that xylem area : leaf area ratio decreased with the shade-tolerance of the species, which is in line with my expectation and a study across evergreen shrub and tree species (Wright *et al.*, 2006). The decrease of xylem area : leaf area ratio with increasing shade-tolerance indicates that winter-deciduous, shade-tolerant tree species minimize their carbon costs per leaf area by producing branches with smaller xylem areas relative to the leaf area they support at the crown level.

However, the leaf area : xylem area ratio in the branches was not related to the position of branches in sun or shade. It is well known that sun leaves normally have higher rates of stomatal conductance, transpiration and photosynthesis per unit leaf area than shade leaves (Givnish, 1986; Reich *et al.*, 1998; Davi *et al.*, 2008; Houter & Pons, 2014). These higher rates in sun leaves would require more efficient water support per leaf area. My studied deciduous broadleaved tree species allow these higher rates not by expanding the xylem area per leaf area, but by producing wider vessels in sun branches. Such wider conduits effectively increase the hydraulic efficiency (Cochard *et al.*, 2002; Lemoine *et al.*, 2002) without cost of more carbon in xylem.

For temperate mixed forest, I explored if the water loss/transport balance differs between conifers and broadleaved trees (***chapter 3***). Conifer and broadleaved tree species often co-exist in temperate forests and grow to tall stature and can reach the upper forest canopy as adults. Conifers belong to the gymnosperms and produce needle-liked leaves. They are well adapted to cold and nutrient poor conditions (Sperry & Sullivan, 1992; Davis *et al.*, 1999) and can be found at high latitudes and elevations. Conifers are known to have less efficient water transport in their stem and lower photosynthetic rates in their leaves. In ***chapter 3***, I hypothesized that conifers need more xylem area to the amount of leaf area than co-existing broadleaved trees to compensate their low water transport efficiency in xylem. We sampled canopy branches from 5 conifer species and 9 deciduous broadleaved tree species in a temperate forest in NE-China.

We found that conifers indeed produced more xylem area for a given amount of leaf area compared to co-existing deciduous broadleaved trees. Moreover, conifer branches had a lower wood density than broadleaves branches. Conifers thus tend to enlarge water transport area to compensate for their low water transport efficiency in the stem, and the low wood density suggest that they do so at limited costs. However, this ratio was similar between evergreen conifers and evergreen angiosperms in other studies (Brouat *et al.*, 1998; Becker, 2000; Lusk *et al.*, 2007). These results suggest that the leaf habit, either evergreen or deciduous, may pose different constraints on branches.

Functional balance between carbon gain and carbon transport

One of the new steps in my study is to quantify the carbon gain - transport balance in co-existing tree species in temperate forests. This balance is driven by the carbon gain in the leaves on the one hand and the carbon transport in the phloem in stem on the other hand. For deciduous tree species (***chapter 2***), I predicted that sun branches have a higher phloem area : leaf area ratio than shade branches because sun branches have stronger photosynthesis which in turn fix more carbon, and need more phloem area to transport carbon. I also predicted that phloem area : leaf area ratio decreased with increasing shade-tolerance due to lower photosynthesis per leaf area in more shade-tolerant species.

I observed that sun and shade branches did not differ in phloem area : leaf area ratio, but this ratio decreased with increasing shade-tolerance. These results for the phloem area : leaf area ratio are similar as for the xylem area : leaf area ratio in sun and shade branches, and with increasing shade-tolerance. These may result from the cambial processes that determine the related vessel and phloem cell sizes (Hölttä *et al.*, 2013). Thus, sun branches could produce wider phloem cells than shade branches to facilitate the transport of carbon in phloem, but empirical data is missing. Shade-tolerant species seem to reduce phloem area in line with the lower carbon gain in leaves than light-demanding species. Thus, similar to xylem area : leaf area ratio, the phloem area : leaf area ratio does not respond to local light, but to shade-tolerance. In addition to xylem area regulation, winter-deciduous, shade-tolerant tree species thus minimize their carbon costs by producing smaller phloem areas relative to the leaf area they support at the crown level.

Moreover, in ***chapter 3***, I compared this balance between co-existing conifers and deciduous broadleaved tree species that co-occur in a Chinese temperate forest. I predicted that conifers have a lower phloem area : leaf area ratio than deciduous broadleaved tree species due to their lower assimilation rates. However, I found no difference of phloem area : leaf area ratio between conifers and deciduous broadleaved trees. Deciduous broadleaved trees with higher assimilation rates than conifers (Becker *et al.*, 1999; Brodribb & Feild, 2000) may have a more efficient carbon transfer in phloem (Dannoura *et al.*, 2011; Hölttä *et al.*, 2014; Jyske & Hölttä, 2015). Such a more efficient carbon transport in the phloem may compensate the higher carbon gain rates in leaves so that there is no need to invest more carbon into phloem to facilitate phloem sap flow in deciduous broadleaved trees. Yet, the empirical test for more efficient sap flow rates in phloem in deciduous broadleaved trees compared to conifers is still missing.

Functional balance between water loss/carbon gain and storage

The water loss/carbon gain - storage balance is determined as the balance between gas exchange that causes water loss and carbon gain in the leaves and the water and carbon stored in ray parenchyma. I tested for possible differences in this balance between conifers and co-existing deciduous broadleaved tree species in a temperate forest (***chapter 3***). I hypothesized that deciduous broadleaved trees required more ray parenchyma relative to the amount of leaf area than evergreen conifers to supply the carbon that is required for producing new branches and leaves in spring. Indeed, deciduous broadleaved tree species had a higher ray parenchyma area relative to the amount of leaf area than conifer species. This is in line with Morris *et al.* (2016b) who also found a lower ray percentage in conifers. However, the deciduous conifer species *Larix* had a ray parenchyma area per leaf area that was similar to the evergreen conifer species. This does not support our hypothesis suggesting that the observed difference between conifers and broad-leaf trees is not related to their difference in leaf habit.

HYDRAULIC AND MECHANICAL TRAITS, AND THEIR TRADE-OFFS

Tall woody plants face fundamental trade-offs between hydraulic and mechanical functions. I investigated such possible trade-offs for trees and lianas in a tropical forest located in SW China. Tall woody plants normally have more negative water potentials in branches and leaves than smaller plants (Petit *et al.*, 2016; Sterck & Zweifel, 2016) and thus face higher cavitation risks with drought (Hacke *et al.*, 2000; Olson *et al.*, 2018). They also need to maintain mechanical stability of their branches for maintaining functions such as the transport of water in the stem. Accordingly, I explored the existence of trade-offs between hydraulic conductivity, hydraulic safety and mechanical safety. Since lianas (woody climbers) and trees (self-support) differ in mechanical design, I expected this to lead to different hydraulic and mechanical traits and trade-offs between lianas and trees.

I collected sun-exposed canopy branches from 22 species (12 liana species vs. 10 tree species). I did not find differences in hydraulic and mechanical traits between lianas and trees, nor trade-offs between these traits. Thus, though lianas have much wider vessels in their stem base (Ewers & Fisher, 1991; Wagner *et al.*, 1998), they may have similar conduit sizes and similar ability to avoid cavitation in their distal branches as trees. Such drought resistance may help lianas to increase their abundance in dry and stressful conditions (Schnitzer *et al.*, 2014). The lack of trade-offs may imply that canopy branches of lianas and trees converge in their traits because they face similar constraints imposed by the environment and their large stature.

SIMILARITIES ACROSS TREE SPECIES IN FUNCTIONAL BALANCES

Though broadleaved tree species and conifer species differ in their appearance, phylogeny and wood anatomy, they will all coordinate their xylem area with their leaf area (Brouat *et al.*, 1998; Lusk *et al.*, 2007; Petit & Kiorapostolou, 2018) and phloem area (Kiorapostolou & Petit, 2018), regardless of the species (see corresponding functions and the relations in Fig. 5.1). This is because species maintain the functional balances between water transport and water loss and between water transport and carbohydrate transport by coordinating the biomass allocation between leaves and stem, and the xylem and phloem within the stem. In line with this, phloem area increased not only with leaf area, but also with the xylem area and water conductivity (***chapter 3***). While a higher water transport may be associated with bigger sieve tubes that have a more efficient mass transport in phloem (Hölttä *et al.*, 2009; Petit & Crivellaro, 2014), my results imply that canopy branches expand their phloem area to increase the carbohydrates transport ability with increasing water transport ability (xylem area). Between lifeforms, the phloem area relative to the amount of leaf area converged between evergreen conifers and deciduous broadleaved tree species (***chapter 3***). This illustrates that branches of species from different lifeforms may be rather similar in their biomass allocation to phloem area versus leaf area, regardless of their differences in leaf traits and phloem properties (Liesche *et al.*, 2015).

Despite favourable light conditions, branches of tall woody plants cannot infinitely increase their leaf area. In turn, the height of the entire plant is also limited. This is because hydraulic limitations, i.e. water stress (lower water potentials), in branches become more severe due to gravity and an increasing path length resistance (Ryan & Yoder, 1997; Niklas, 2007). Such water stress may directly limit the growth of the leaves and stem xylem and phloem, even with ample soil moisture (Koch *et al.*, 2004). Beside hydraulic constraints, mechanical constraints can be another factor that limits the growth of branches. As trees grow bigger and bigger, they need to support their own weight to avoid the gravity-induced instability, which may come at the cost of investments in canopy branches (Niklas, 2007).

In their branch stems, both conifer and broadleaved species increased ray parenchyma with increasing water conductivity (***chapter 3***). There is hardly other literature on such relationships, except Fortunel *et al.* (2014) showing a positive correlation between parenchyma fraction with vessel lumen fraction across branches in a rainforest. This indicates that species maintain functional balances between water transport and storage by adapting larger storage area with increasing water transport, probably to avoid water shortage (Morris *et al.*, 2016b). This can be one of the reasons that species in tropical forests have the largest percentage of parenchyma area in their stem cross-section, because they transpire more water (Schlesinger & Jasechko, 2014) than species in temperate and subtropical forests (Morris *et al.*, 2016b).

DIFFERENCES ACROSS TREE SPECIES IN FUNCTIONAL BALANCES

Though all tree species increase their xylem area with leaf area, lifeforms differed in the way they balanced the xylem area with leaf area. Evergreen conifers had more xylem area relative to the amount of leaf area than deciduous broadleaved tree species, but the difference disappeared when comparing with evergreen broadleaved tree species (Becker, 2000; Lusk *et al.*, 2007). This may be partially due to the higher mass density of evergreen leaves which requires more mechanical support. Moreover, in order to avoid embolism-induced loss of conductance capacity during their longer lifetimes, they may build up greater redundancy of transport tissue (Brouat *et al.*, 1998). Both of these possibilities could lead to increased xylem area per amount of leaf area in conifers. Thus, the functional balance between water loss and water transport seems to be mainly associated with species phenology (evergreen vs. deciduous) rather than lifeforms (conifer vs. broadleaved tree). In this case, under future climate change, the composition change of evergreen species versus deciduous species may affect the carbon sequestration in stems due to the inherent differences in the xylem area : leaf area ratio between evergreen and deciduous species.

The xylem area : leaf area ratio did not respond to local light environments across broadleaved tree species (***chapter 2***), but this was not the case for conifers in another study (Sancho-Knapik *et al.*, 2014). Branches under high light condition required more water transport in xylem because of the higher transpiration and photosynthesis on leaves than branches with less light availability (Givnish, 1988; Houter & Pons, 2014). Broadleaved tree species produce larger vessels rather than expanding their xylem to amount of leaf area to increase their water transport ability in high-light exposed branches (***chapter 2***, Cochard *et al.*, 2002) while conifers enlarge their xylem area to meet the higher water transport demand of those branches (Sancho-Knapik *et al.*, 2014). It thus seems that conifers enlarge the xylem area instead of producing bigger tracheids to enhance their water transport ability under exposed conditions. These findings indicate that broadleaved trees and conifers have different strategies in regulating xylem traits to adapt to varying light environments. Empirical tests about how branches adjust their phloem to light in association with leaf area have not yet been done (Amiard *et al.*, 2005).

In addition to adjustments to light conditions, species differing in shade tolerance tended to differ in the xylem area : leaf area ratio and phloem area : leaf area ratio. Light-demanding species were found to have a larger xylem area and phloem area relative to the amount of leaf area than shade-tolerant species (***chapter 2*****,** Wright *et al.*, 2006 with xylem area : leaf area ratio). As light-demanding species have higher gas exchange rates on a leaf area basis (Lambers *et al.*, 2008), the higher xylem area and phloem area per unit leaf area reveals how species allocate biomass to transporting tissues in different ways in relation to their resource niche. Remarkably, however, we did not find any response in those functional balances in relation to the local light condition of a branch. Whether conifers show the same responses remains unknown. These findings indicate that light-demanding, pioneer species produce wider stems which may contribute to carbon sequestration, but their lower wood density may off-set this effect.

Both the amount of ray parenchyma area relative to the amount of leaf area as well as the ray parenchyma percentage in stem cross-sections are lower in conifer species than broadleaved species (***chapter 3***, Morris *et al.*, 2016b). Thus, the capacity to store water and carbohydrates seems lower in conifers. One of the possible reasons can be that conifers need less water storage than broadleaved trees to deal with water shortage because of their lower efficiency of water transport in the xylem. Moreover, this may be partially because most conifers are evergreen species and require less carbon storage than deciduous broadleaved trees. The carbon gain by the older leaves can thus largely fuel the production of new leaves every spring. However, when compared to evergreen broadleaved tree species, evergreen conifers still had less ray parenchyma which does not follow this speculation. Another possibility is that since conifers' xylem is mainly composed of tracheids and ray parenchyma cells, more ray parenchyma area will directly suppress tracheid area which in turn lowers the water transport ability. This is in contrast with broadleaved trees, where the more parenchyma area in xylem is not at the cost of vessel area (***chapter 4***, Fortunel *et al.*, 2014), but of fibre area. Conifers thus seem to maximize the tracheid area for water transport at the cost of ray parenchyma area to guarantee the functional balance between water loss and water transport.

FUNCTIONAL BALANCES ARE MISSING OR UNCLEAR IN SOME CASES

There is no support for the hypothesized relationships between water transport and mechanical support in broadleaved species and conifer species (***chapter 4***, Domec & Gartner, 2002; Jacobsen *et al.*, 2007). In broadleaved species, water transport is mainly driven by vessels while mechanical support is mainly associated with fibres. Vessel and fibre traits appear to be relatively independent of each other (Fortunel *et al.*, 2014), and trade-offs between the associated functions are almost absent. In conifer species, water transport and mechanical support are both associated with tracheid traits. In the stem, water transport is determined by the tracheid size while mechanical support is more driven by the double-wall thickness per lumen diameter, i.e. thickness-span ratio, and the second moment of area (expressed as $I = 0.25\pi r^4$ for a solid circular cross section; r, stem/branch radius, Sterck *et al.*, 2006). As the thickness-span ratio is more determined by tracheid lumen diameter rather than tracheid wall thickness (Sperry *et al.*, 2006), the mechanical support becomes a function of lumen diameter. Consequently, the increasing of tracheid diameter decreases mechanical strength. However, the lack of a relationship between water transport and mechanical support indicates that the stem/ branch radius that raises to the fourth power can override the impact of thickness-span ratio on mechanical strength of a stem/branch in conifers.

Ray and axial parenchyma are commonly known as water and sugar reservoirs and thus contribute to buffering water transport and carbon supply deficiencies. Yet, the role of parenchyma for the mechanical function is commonly underestimated in broadleaved species (Burgert & Eckstein, 2001). There is evidence showing that ray and axial parenchyma play a positive role in the mechanical support of woody plants (Burgert *et al.*, 1999; Burgert *et al.*, 2001; Carlquist, 2007). However, though they add strength to mechanical stability of a stem/ branch, the occupation of ray and axial parenchyma area comes at the cost of fibre area in the xylem (***chapter 4***, Fortunel *et al.*, 2014). Overall, the role of parenchyma and its different functions are very much understudied and deserve more attention in future studies to unravel their role in different functional balances.

There are – as far as I know – no studies on the possible functional balance between sugar storage, sugar transport and carbon acquisition. My study shows indirect evidence that with increasing shade-tolerance, species increase the ray parenchyma area percentage while they decreased the phloem area percentage (***chapter 2***). Thus, shade-tolerant species have more water and sugar storage capacity, but a lower sugar transport capacity than light-demanding species. It thus seems that the buffer (storage) capacity negatively relates to the carbohydrate conductivity. Overall, my study implies functional relationships between carbon acquisition in leaves with the storage by parenchyma and sugar transport in phloem, but these relations are much understudied and require more attention in future studies.

UNCERTAIN TRADE-OFFS BETWEEN HYRAULIC CONDUCTIVITY, HYDRAULIC SAFETY AND MECHANICAL SAFETY

Species are expected to maintain a water conductive system while being protected against cavitation and mechanical failure. I therefore studied the possible relationships between hydraulic conductivity, hydraulic safety and mechanical safety but found only poor support for trade-offs between these functions (Table 5.1). In this synthesis, I select the most commonly used trait to present each function: xylem-specific conductivity (K_S, the maximal water flow per area standardized by length) to represent hydraulic conductivity, cavitation resistance (P_{50}, the xylem water potential at which 50% of maximum hydraulic conductivity is lost) to represent hydraulic safety, and the modulus of rupture (MOR, the maximum load-carrying capacity in bending) or wood density (WD) to represent mechanical safety. The higher the absolute trait value, the better the function is performed.

Globally, the trade-off between hydraulic conductivity and hydraulic safety was found to be absent or weak in stems (Maherali *et al.*, 2004) and in branches (Gleason *et al.*, 2016). Locally, this trade-off seems to occur more in dry conditions, both in stems and branches (several studies shown in Table 5.1). Though there is one study conducted in tropical forest, this is a seasonally dry semi-deciduous forest (De Guzman *et al.*, 2016). In the studies that found no trade-off, two studies were conducted in tropical forests without extremely dry period (***chapter 4***, Santiago *et al.*, 2018). These studies suggest that drought may play an important role in affecting the balance between water transport and safety. When plants are exposed to water stress, the demand of low hydraulic safety risks and sufficient water transport come in conflict. Interestingly, in studies under phylogenetic control within family, some show no support for a possible trade-off (Pratt *et al.*, 2007) for species across a precipitation gradient (Choat *et al.*, 2007), while others do show support for the trade-off (Pinol & Sala, 2000). Thus, some species or families may have a better ability to balance water transport and safety than others.

Trade-offs between hydraulic and mechanical safety were found in several shrub studies and one survey including conifers and broadleaves (Table 5.1). However, two canopy studies in tropical forests (***chapter 4***, Santiago *et al.*, 2018) did not find such a trade-off. Pittermann *et al.* (2006) shows such a trade-off in Pinaceae/Cupressaceae species, but not in Podocarpaceae/Araucariaceae species. Thus, shrub species with dense stems are always coupled with high drought resistance whereas tropical species that produce dense wood do not always have a higher drought resistance. This may be due to the ample water available in the soil of tropical wet forests. Besides, tall trees are proven to be more resilient to drought than short trees in tropical forests (Brando, 2018). These findings suggest that the balance between hydraulic and mechanical safety can be more important in shrub species than in canopy trees.

The trade-off between water transport efficiency and mechanical safety was only found in one sapling study across broadleaved tree species (Bucci *et al.*, 2004). There was no such trade-off found in studies across mature plants (Table 5.1). Thus, these two functions may largely depend on plant life stage. In angiosperm saplings, the vessel size and length can be constrained due to the small size of a plant (Sperry *et al.*, 2006; Anfodillo *et al.*, 2013). As a result, vessel density becomes the driving factor that controls hydraulic efficiency. The denser the wood is, the less vessels the wood has (Preston *et al.*, 2006), and less hydraulic efficiency is. When plants grow up, vessel length becomes longer, which mitigates the impact of vessel density on hydraulic efficiency. With the increasing water transport ability, shade-tolerant species can support higher assimilation rates in leaves, and consequently facilitate growth while not supressing wood density. This may explain why shade-tolerant species can also have efficient water transport and similar growth rate as pioneer species during their mature phases (Gutiérrez *et al.*, 2008; Baptista, 2017). However, this should not be the case for conifers since their short tracheids have less variation in length than broadleaved trees from sapling to mature. Domec and Gartner (2002) did not find this trade-off in Douglas-firs indicating mature conifers probably do not have this trade-off.

Table 5.1 Published studies of trade-offs between hydraulic conductivity, hydraulic safety and mechanical safety. Only direct studies between two traits were included. Total species and/or family number used in corresponding studies are given. '?' refers to unknown total species or family numbers. "Tree" indicates broadleaved tree species. Ranges of variables in each work are given when the units are the same as our study.

Lifeform	Species no. (family no.)	Life stage	Sample	Location	Condition	Trait range (min, max)	Literature
K_S vs. P_{50} has trade-off							
Tree Shrub	15 (8)	Adult	Branch	S. USA	MAP: 310 mm MDTmax: 32 °C	K_S: 0, 4 P_{50}: -12, 0	Pockman and Sperry (2000)
Tree Shrub	9 (8)	Adult	Stem	NE Spain	Mediterranean MAP: 537 mm MAT: 10 °C	K_S: - P_{50}: -7, 0	Martínez-Vilalta *et al.* (2002)
Conifer	? (4)	Adult	Stem	NE. USA	Mesic	K_S: 0,10 P_{50}: -12, 0	Pittermann *et al.* (2011)
Tree Liana	12 (11)	Adult	Branch	Panama	Tropical dry forest MAP: 1865 mm	K_S: 0, 12 P_{50}: -1.5, 0	De Guzman *et al.* (2016)
K_S vs. P_{50} has no trade-off							
Angiosperm	128 (50)	Adult	Stem	Global	-	K_S: 0, 8 P_{50}: -16, 0	Maherali *et al.* (2004)
Broadleaves Conifer	5 (5)	Adult	Branch	W. USA	Temperate zone	K_S: 0, 20 P_{50}: -10, 0	Sperry and Sullivan (1992) Tyree *et al.* (1994)
Shrub	9 (1)	Adult	Stem	W. USA	Mediterranean MAP: 379 mm MAT: 18.9 °C	K_S: 0, 2.5 P_{50}: -10, 0	Pratt *et al.* (2007)

Continued Table 5.1

Lifeform	Species no. (family no.)	Life stage	Sample	Location	Condition	Trait range (min, max)	Literature
Tree Shrub	9 (1)	Adult	Branch	USA Panama Costa Rica	MAP: 1250-4200 mm	K_S: 0, 14 P_{50}: -4, 0	Choat *et al.* (2007)
Tree	14 (7)	Adult	Branch	South America	Tropical forest MAP: 3041 mm MAT: 25.6 °C	K_S: 0, 8 P_{50}: -4, 0	Santiago *et al.* (2018)
Tree Liana	22 (17)	Adult	Branch	SW China	Tropical forest MAP: 1493 mm MAT: 21.8 °C	K_S: 0, 10 P_{50}: -3, 0	***Chapter 4***
K_S vs. P_{50} has trade-off under certain condition or within certain species group							
Angiosperm Gymnosperm	335 (?)	Adult	Branch	Global	-	K_S: 0, 16 P_{50}: -14, 0	Gleason *et al.* (2016)
Conifer	6 (1)	Adult	Branch	NW. USA	MAP: 340 mm MAT: 7 °C	K_S: - P_{50}: -5, 0	Pinol and Sala (2000)
P_{50} vs. MOR or WD has trade-off							
Conifer Broadleaves Shrub	48 (15)	Adult	Branch	-	-	P_{50}: -12, 0	Hacke *et al.* (2001)
Shrub	6 (3)	Adult	Stem	W. USA	-	MOR: 0, 260 P_{50}: -10, 0	Jacobsen *et al.* (2005)
Shrub	15(9)	Adult	Stem	W. USA	MAP: 315 mm; 420 mm	P_{50}: -11, 0 MOR: 200, 700	Jacobsen *et al.* (2009)

Continued Table 5.1

Lifeform	Species no. (family no.)	Life stage	Sample	Location	Condition	Trait range (min, max)	Literature
P_{50} vs. MOR or WD has trade-off							
Shrub	9 (1)	Adult	Stem	W. USA	-	P_{50}: -10, 0 MOR: 0, 275	Pratt *et al.* (2007)
P_{50} vs. MOR or WD has no trade-off							
Tree	14 (7)	Adult	Branch	South America	Tropical forest MAP: 3041 mm MAT: 25.6 °C	P_{50}: -4, 0 WD: 0.3, 0.8	Santiago *et al.* (2018)
Tree Liana	22 (17)	Adult	Branch	SW China	Tropical forest MAP: 1493 mm MAT: 21.8 °C	P_{50}: -3, 0 MOR: 0, 170	***Chapter 4***
P_{50} vs. MOR or WD has trade-off under certain condition or within certain species group							
Conifer	18 (4)	Young Adult	Branch	New Zealand New Caledonia USA	MAP: 500-2550 mm MAT: -5.4-25.8 °C	P_{50}: -8, 0	Pittermann *et al.* (2006)
K_S vs. MOR or WD has trade-off							
Tree	6 (6)	Sapling	Branch/stem	Brazil	Savanna MAP: 1500 mm MAT: 20 °C	K_S: 0, 1.2 WD: 0.3, 0.8	Bucci *et al.* (2004)
K_S vs. MOR or WD has no trade-off							
Shrub	17 (9)	Adult	Stem	South Africa	MAP: 315-600 mm	K_S: 0. 8 MOR: 200, 700	Jacobsen *et al.* (2007)

Continued Table 5.1

Lifeform	Species no. (family no.)	Life stage	Sample	Location	Condition	Trait range (min, max)	Literature
K_S vs. MOR or WD has no trade-off							
Conifer	1 (1)	Adult	Branch/stem	W. USA	MAP: 1080 mm	K_S: - WD: 0, 0.6	Domec and Gartner (2002)
Tree Liana	22 (17)	Adult	Branch	SW China	Tropical forest MAP: 1493 mm MAT: 21.8 °C	K_S: 0, 10 MOR: 0, 170	***Chapter 4***
Tree	14 (7)	Adult	Branch	South America	Tropical forest MAP: 3041 mm MAT: 25.6 °C	K_S: 0, 8 WD: 0.3, 0.8	Santiago *et al.* (2018)

FROM CANOPY BRANCH TO THE ENTIRE TREE

Conceptually, canopy branches can be considered small saplings that ramify from the large branches or main stem in large trees. With the high irradiation and temperature during the day, these branches potentially gain most carbon but face high transpiration demands. Consequently, these branches can act as bottlenecks for the hydraulic integrity and carbon gain of the entire individual. By studying branches of even-distance to apex, the tapering effects of vessels and their related functionalities can be controlled (Becker *et al*. 2000; Petit and Anfodillo 2009). Besides, branches are more easily measured than entire individuals including many important physiological traits, such as for example hydraulic traits (e.g. K_S, P_{50} and vessel length), biomass and total leaf area.

Although there are many advantages to study branches, we should realize that the branch studies are not the same as stem or whole individual tree (or liana) studies. To scale up branch traits to stem, even to the entire individual, we need more work on collecting the same type of data from branch, stem and the whole individual, if possible. As stems are key in the mechanical support of the whole plant body, the functional balances may shift from balancing more on hydraulic supply in branches to mechanical support in stems. I call for new studies that consider both hydraulic and mechanical functions in branches and entire trees (including their stem) to show how hydraulic and mechanical functions change from branches to entire large canopy plants, and also to evaluate to what extent branches can act as models of entire individuals.

ROOTS, DO THEY FOLLOW THE SAME RULE?

Roots were neglected in this study on branches but play a unique role in the functioning of plants (Fig. 5.2): they absorb water and nutrients from the soil and anchor a tree in the soil to keep it standing. Lianas' roots may have less demand on anchoring since they rely on other trees to reach the canopy.

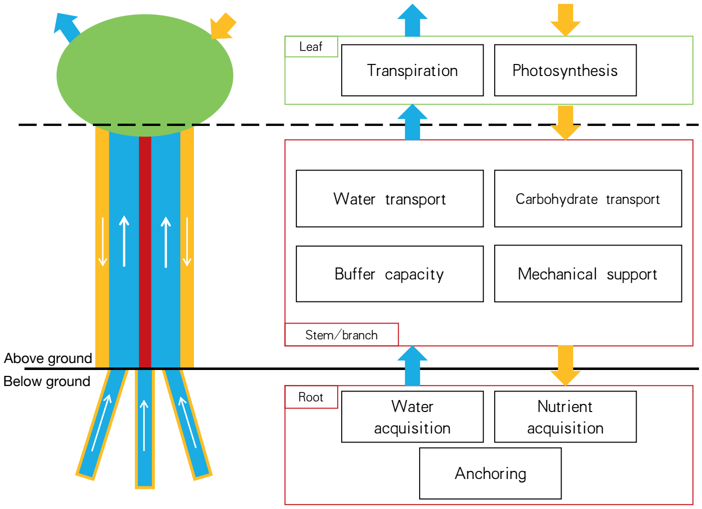


Figure 5.2 The conceptual model of an entire woody plant. The blue arrows represent water transport direction while the orange arrows represent sugar transport direction. Main functions of leaf, stem/branch and root are shown in boxes.

Roots have a complex structure and function differently depending on the order of the roots (Wachsman *et al.*, 2015; Wang *et al.*, 2017). In this synthesis, I mainly discuss the coarse roots (the third- and higher order roots) which are the main organs for water and nutrient transport and anchoring. Since it's impossible to correlate a certain root area with amount of leaf area, the root mass : leaf mass ratio has been used to reveal the functional balance between water acquisition (transport) and water loss. Similar as the pattern between leaf area and xylem area, species increase root mass with leaf mass, regardless of light condition (Freschet *et al.*, 2015).

Comparing to the stem or branch, coarse roots may have higher mechanical requirements not only to anchor the whole tree, but also for avoiding structural collapse because of the pressure from soil. Roots are found to have a similar range of cavitation resistance as stems or branches, and tradeoff between hydraulic efficiency and cavitation resistance (Pittermann *et al.*, 2006; Sperry *et al.*, 2006). However, tracheids in root were bigger than tracheids in stem in conifer species. Thus, roots tend to maintain similar drought resistance even with bigger conduits.

Roots have some similarities to stems and branches, but more studies are expected to seek for the functional divergent induced traits divergent between roots and stems/branches.

CONCLUSIONS

In my study, I propose a tissue-function-based conceptual model to understand the water and carbon cycles in the soil, plant, atmosphere continuum (Fig. 5.1). The results of my study increase the understanding of the variation and trade-offs in architectural, anatomical and eco-physiological characteristics of different life forms, i.e. lianas, broadleaved trees and conifers, in different forest biomes. This knowledge will contribute to better understanding of how woody plants coordinate their organs, tissues and cells to maintain different functions and ultimately grow and survive.

The main objective of my study was to gain insight in how canopy co-existing tall woody plants balance the acquisition of carbon and loss of water in the leaves with the transport and storage of water and carbon in the stem, and keep branches mechanically stable. I showed that coexisting woody plants sometimes converged in traits and functioning, but sometimes diverged. In relation to the first question, I showed that deciduous tree species of a temperate forests adjusted their functional balances between leaf area, xylem area and phloem area in relation to their tolerance to shade. In relation to the second question, I showed that conifers and broadleaved trees largely diverge in branch architecture. Conifers have larger transport tissue area probably to compensate for the lower transport efficiency. They may thus converge with broadleaved tree species for the balance between carbon gain and water loss in leaves versus transport capacity of water and sugar in the stem. Conifers also have much less parenchyma and thus lower storage potential for carbon and water, but the reason is not yet clear. These differences across species imply that global warming may affect species of temperate forests differently, and thus ultimately change the species composition and related carbon and water cycling in temperate forests. In relation to my third question, I showed that trees and lianas remarkably converged in most hydraulic functions, but not in their mechanical traits, in a tropical forest. It is not clear whether or not such similarities are shared among many other tropical forests, since only few studies report such results. Possible trade-offs between hydraulic and mechanical functions were thus weak and make it hard to speculate on possible consequences of climate change for the species composition and water and carbon cycle of these forests. In order to answer how these forests will change I call for studies that link the observed functional differences to overall tree performance properties, such as tree growth rates and survival probability, which was beyond the scope of my thesis.

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SUMMARY

Forests are the major terrestrial carbon sink and are critically important for the global carbon cycle. Woody plants in forests are the major elements that contribute to the water and carbon cycle between soil, forest and atmosphere. Their roots absorb water from the soil, the stems transport the water to the leaves where >98% of the acquired water transpires back to the atmosphere. Trees fix carbon via photosynthesis in the leaves and use the carbon for maintaining metabolic processes and growth. Plants are supposed to maintain functional balances for water and carbon. For water, they are expected to balance the acquisition of water via the roots with the transport of water in the stem xylem, the storage of water in parenchyma and the loss of water through the leaf stomata. For carbon, they balance the carbon gain in the leaves with the transport of sugars in the phloem within the bark and the storage of carbon in stem parenchyma. In addition, the large woody plants such as trees and lianas are expected to coordinate their stem and crown in such a way that they do not break and maintain a stable plant body. These balances are driven by structural and physiological properties.

In this study, I focus on large woody plants – trees but also lianas – and investigate how they coordinate their functional balances. The main objective of my study is to quantify how canopy co-existing tall woody plants balance the acquisition of carbon and loss of water in the leaves with the transport and storage of water and carbon in the stem. I therefore study their canopy branches, which are probably most important for the carbon gain and water loss of the entire woody plant. I compare deciduous tree species that differ in shade tolerance but co-exist in a temperate Dutch forest, conifer and broadleaved tree species that co-exist in a Chinese temperate forest, and tree and liana species

that co-exist in a Chinese tropical rain forest.

In ***chapter 2***, I address the question how trees differ in their functional ratios between leaf area, xylem area and phloem area across deciduous species in a temperate forest. I present a study on 10 deciduous tree species co-existing in an even-aged Dutch forest. I found that the area-based functional ratios did not differ consistently between sun and shade branches, but light-demanding species produced more xylem area and phloem area per leaf area than shade-tolerant species probably to compensate for their higher water loss rates and carbon gain rates in the leaves. This study thus shows that tree species differ in their branch structure to maintain similar functional balances of carbon gain in the leaves versus carbon transport in the phloem, and of water loss in leaves and water transport in the xylem.

In ***chapter 3***, I question how conifer and broadleaved tree species differ in their functional balances between water and carbon related functions in a temperate forest. I compare 5 conifer tree species with 9 deciduous broadleaved tree species in a Chinese temperate forest. Conifers are tracheid-based gymnosperms that have a lower water transport efficiency than vessel-based broadleaved angiosperms. I evaluated if this difference in the water transporting tissue causes a divergence in the functional balances between conifers and broadleaved trees. I therefore studied the ratios in xylem area to leaf area and in phloem area to leaf area between conifers and broadleaved trees. I found that conifers tend to increase xylem area to the amount of leaf area, probably to compensate for the low water transport efficiency in xylem, while phloem area to the amount of leaf area did not differ between conifers and broadleaved trees. Thus, in line with the results of ***chapter 2***, these results indicate that trees tend to enlarge their xylem area to increase their water supply to leaves when those leaves are more active in terms of high water loss rates and high carbon gain rates.

In ***chapter 4***, I question how liana and tree species coordinate possible trade-offs between hydraulic conductivity (water transport efficiency), hydraulic safety (drought resistance) and mechanical safety. I compared 12 liana species with 10 tree species in a tropical evergreen forest in China. Lianas differ from trees by relying on adjacent trees to reach the forest canopy whereas trees support themselves, with possible implications for their mechanical and hydraulic properties. Unexpectedly, I found that lianas have stronger wood but similar wood density compared to trees, and that lianas and trees did not differ in hydraulic traits. Besides, no trade-offs were found between hydraulic traits and mechanical traits, against my expectation. This lack of trade-offs may imply that these adult woody plants, exposed to similar atmospheric conditions, converged in their traits. In contrast, other species communities sometimes show trait differences and trade-offs.

Woody plants thus seem to coordinate their ratios between leaf area, xylem area and phloem area in different ways, since those balances differed in relation to the shade-tolerance of deciduous trees, the tree type (conifers versus broadleaved species), and leaf habit (evergreen vs. deciduous). These differences across species imply that global warming may affect species of temperate forests differently, and thus ultimately change the species composition and related carbon and water cycling in temperate forests. For the tropical forest, I showed that trees and lianas remarkably converged in most hydraulic functions, but not in their mechanical traits. It is not clear whether or not such similarities are shared among many tropical forests, since only few studies report such results. The weak trade-offs between hydraulic and mechanical functions make it hard to speculate on consequences of climate change for the species composition and water and carbon cycle of these forests. In order to answer how these forests will change, I call for studies that link the observed functional differences to overall tree performance properties, such as tree growth rates and survival probability.

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In the end, I should really thank my family. Thank you mom and dad for your understanding and unconditional love. Especially you mom, for supporting me with every decision I make. I thank Jia Zhang for your support during my PhD study and for the thesis design. With all the support from my family, I will move towards my dreams.

I hope everybody will be successful now and in the future!

SHORT BIOGRAPHY

I was born on 31st January 1987 in Beijing, China, a modern city without much nature left and famous for its huge population. However, I love nature and all the creatures in it. It took me quite some time to know what I would like to do: spend the rest of my life bringing nature back and creating new homes for its inhabitants. I hope to do this because it’s my passion and the thing I feel is the most meaningful and thus I would pursue it throughout my life.

Here is my story…

Although I initially studied Environmental Engineering from 2005-2009, I really started working with nature when I was 22 years old. Right after my bachelor degree, I went to Guangxi to study and protect the white-headed langur, an endangered species in the IUCN red list. The two years in Guangxi, I really felt close to nature and all those lovely creatures. I studied the behaviour of the white-headed langurs, and in the morning and evening I heard melodic bird songs. I realized in those moments that as long as we could provide a habitat for wildlife, they would find their way back home.

In order to know more about forests - home to many wild creatures - I started to learn English and finally came to Wageningen University to pursue a master’s degree in “Forest and Nature Conservation” in 2012-2014. My new student life at WUR was extremely tough, and I’m proud that I managed to finish the programme successfully. I then started my PhD in a sandwich programme from 2015-2019 funded by Wageningen University. This funding supported the collaboration between Wageningen University and the Institute of Botany of the Chinese Academy of Sciences. For my PhD I did fieldwork during the hot summers in China and came back to Wageningen during cloudy and rainy winters. With financial support from other funds, I had a very great time doing field work in the Xishuangbanna tropical forest and Changbai temperate forest. I could easily feel the different climates, and clearly saw the different forest biomes.

During my PhD programme, I participated in two international ecological conferences in Brussels and Beijing, and one national conference in Beijing. I gave oral presentations during those conferences and had nice discussions with other participants. I kept on moving towards my dream.

Now, that I finished my PhD, I keep at heart the shiny memories with my friends at Wageningen and the amazing time I had with the FEM group. Though they are not mentioned in my biography, they are the force behind these words and behind my success.

Biography to be continued in the future…

PUBLICATIONS

Meng Yao, Lijie Yin, Lan Zhang, Lijun Liu, Dagong Qin and Wenshi Pan. 2012. Parturitions in Wild White-Headed Langurs. *International Journal of Primatol* 33: 888-904.

Lan Zhang, Paul Copini, Monique Weemstra, Frank Sterck. 2016. Functional ratios among leaf, xylem and phloem areas in tree branches change with shade-tolerance, but not with local light conditions, across temperate tree species. *New Phytologist*, 209: 5566–1575.

Lan Zhang, Yajun Chen, Keping Ma, Frans Bongers, Frank J Sterck. 2019. Fully exposed canopy tree and liana branches in a tropical forest differ in mechanical traits but are similar in hydraulic traits. *Tree Physiology*, tpz070, https://doi.org/10.1093/treephys/tpz070

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)

- An overview of functional traits study among leaf, xylem and phloem worldwide – done and missing

Writing of project proposal (4.5 ECTS)

- Comparing canopy trees from boreal, temperate and tropical forests: understanding performance from scaling architecture, allocation, physiology and anatomy to whole plants

Post-graduate courses (5.4 ECTS)

- Wood anatomy workshop; Padova University, Italy (2017)
- Structural equation modelling; PE&RC (2018)
- R and Big data; PE&RC (2019)

Deficiency, refresh, brush-up courses (1.5 ECTS)

- Basic statistic; PE&RC (2015)

Competence strengthening / skills courses (4.5 ECTS)

- Scientific publishing; WGS (2015)

- Writing grant proposal; WGS (2019)
- Scientific integrity; WGS (2019)
- Career perspectives; WGS (2019)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.8 ECTS)

- PE&RC First year weekend (2015)
- PE&RC Day (2015)
- PE&RC Last year weekend (2019)

Discussion groups / local seminars / other scientific meetings (7.4 ECTS)

- Ecological theory and application (2015)
- Managing the climate–water–forest nexus for sustainable development: are we on the right track? (2015)
- Plant form and function discussion group (2015-2016)
- Wageningen Evolution and Ecology Seminars (WEES) (2015-2019)
- Journal club (2015-2019)
- REDD+ Discussion group (2016)
- Biodiversity and ecosystem function discussion group (2016-2018)
- Plant community mechanism discussion group (2016-2018)

International symposia, workshops and conferences (6.6 ECTS)

- The tenth cross-strait forest dynamic sample workshop seminar; Beijing, China (2016)
- European conference of tropical ecology and annual meeting of society for tropical ecology; Brussels, Belgium (2017)
- International congress of ecology; Beijing, China (2017)

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