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# The structure and function of ray and axial parenchyma in woody seed plants




## Dissertation

Zur Erlangung des Doktorgrades *Dr. rer. nat.*

der Fakultät für Naturwissenschaften der Universität Ulm

vorgelegt von

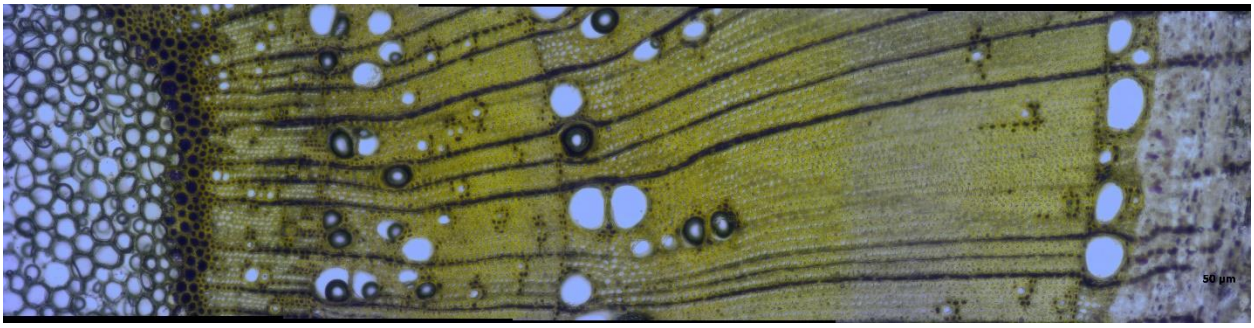
**Hugh Robert Morris**

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**Cover picture:** A stitched example I made of a transverse section of *Fraxinus excelsior* stained for starch in Lugol's solution

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**Trees**

I think that I shall never see
A poem lovely as a tree.

A tree whose hungry mouth is prest
Against the earth's sweet flowing breast;

A tree that looks at God all day,
And lifts her leafy arms to pray;

A tree that may in summer wear
A nest of robins in her hair;

Upon whose bosom snow has lain;
Who intimately lives with rain.

Poems are made by fools like me,
But only God can make a tree.

**By Joyce Kilmer, 1913**

# Part I

# -

# Introduction and Summary

# Introduction

### *General definition and function of parenchyma cells in plants*

In plants, parenchyma cells (Gr. *para*, "beside" + *enchyma*, "an infusion") make up one of the three ground tissues, alongside collenchyma and sclerenchyma cells. In general, parenchyma cells differ in having a thin primary cell wall (rarely secondary) and were the first eukaryotic cells to have evolved (Stewart, 1983). Early non-vascular plants, such as green algae (Chlorophytes and Charophytes), are made up entirely of unspecialised parenchyma with the sole function being photosynthesis, as support from other types of ground tissue is not required, unlike with the land plants. Parenchyma cells, for the most part, resemble the undifferentiated cells produced by division of meristematic cells (Simpson, 2006). They vary in shape from elongate to isodiametric, although they can also be square or rectangular, as found frequently in the ray parenchyma of secondary xylem and phloem (Panshin and Zeeuw, 1980). Parenchyma cells are alive at maturity and have the ability to dedifferentiate (totipotency), which is particularly important during events such as injury (Heplar and Newcomb, 1963; Wargo, 1977; Shigo, 1984; Aloni and Plotkin, 1985). In a typical plant, this versatile tissue can be found in every organ, from the cortex and pith of stems to the cortex of roots, the mesophyll of leaves, the pulp of fruit, and finally the endosperm of seeds. This cell type has a very diverse suite of functions, ranging from metabolic processes such as respiration, digestion and photosynthesis, together with storage of NSCs, water, wound healing and regeneration (Evert, 2006).

### *The contents of parenchyma in relation to function*

In the leaf, parenchyma forms the mesophyll (leaf middle section), which are referred to as chlorenchyma (parenchyma with chloroplasts) owing to its special role in photosynthesis. Chlorenchyma cells also have plentiful ribosomes and Golgi-bodies and a well-developed endoplasmic reticulum, all crucial in a post-photosynthetic role (Evert, 2006). Where, in the absence of light, when chloroplasts are not present, they can be replaced by amyloplasts, another specialist organelle that accumulates starch, as found in potato tubers. Amyloplasts, unlike chloroplasts, tend to take up most of the allotted space within the cytoplasm. Aerenchyma, which forms channels of air-filled cavities, is another specialised parenchyma cell type. It is a spongy tissue that makes up the mesophyll in leaves, and is also found in the roots of plants in hypoxic soils, such as with aquatic plants (Jung *et al*., 2008). A study by Wang and Cao (2012)

showed that flooding stimulates aerenchyma in the roots, stems and leaves of bald cypress (*Taxodium distichum*) and in just the roots of the Chinese tallow tree (*Triadica sebifera*), which allows for increased enhancement of $O_2$ diffusion. Parenchyma cells also have a major role as water reservoirs (Holbrook, 1995), especially where vacuoles are large and abundant (Evert, 2006). Specialist water conserving cells (where chloroplasts are absent) in succulent plants typical of mesic sites (e.g. Cactaceae, *Aloe*, *Agave*, *Peperonia*) are notably large with thin cell walls, and have large vacuoles with dense cytoplasm (Koller and Rost, 1988; Evert, 2006). A study by Liese and Grover (1961) found a clear association between parenchyma amount and water storage in the culms of bamboos, although the method used was not clear.

Mechanically, the strength of parenchyma is dependent on its turgor-pressure derived from the cell's hydraulic properties (Romberger *et al*., 1993). The thin primary cell walls of parenchyma allow for structural changes to the cell shape, where they become rigid (gauged by stiffness and the modulus of elasticity) upon the swelling of the central vacuole after an influx of fluids, or flaccid when the organelle loses pressure (Virgin, 1955). Proteins referred to as aquaporins are responsible for regulating the flow of water into and out of the vacuole, which is carried out via active transport. When the flow of water is disrupted, plasmolysis occurs upon a significant decline in turgor pressure, resulting in cell collapse. According to Niklas (1992), the mechanical properties of a group of parenchyma and the density of the packing arrangement largely dictate how effective they will be as a whole, rather than just at the individual cell level.

***Parenchyma of the secondary xylem: an overview***

Parenchyma is also found in the secondary xylem of "woody" plants (trees and shrubs), a collective term for a range of tissues formed by the vascular cambium to its interior. Lignin biosynthesis and deposition in the compound middle lamella upon completion of the secondary cells wall of mature tracheary elements and fibres, is what differentiates the secondary xylem from more juvenile non-woody tissues (Wardrop and Bland; 1959; Donaldson, 1992; Schuetz *et al*., 2013). Lignin is a polymer that acts as a stiffening agent conceived as 'chimney-like Brickwork' of 'lignin bricks', embedded in a cellulose matrix; these are useful analogies from a solely mechanical perspective (Mattheck and Breloer, 1994; Schwarze *et al*., 2000). Although, secondary xylem can be found in the members of the gymnosperm groups Ginkgophyta and Gnetophyta and in most of the division Cycadophyta, the two key groups

where it can be found are the Pinophyta (conifers) and the Angiospermae (angiosperms or flowering plants). All known species of conifers have secondary xylem, whereas its presence in the angiosperms depends on the plant's status. Many herbaceous plants of the dicotyledons flower and die quickly before secondary xylem can form, whereas it is seldom found in the monocotyledon plant group (Mauseth, 1988; Dickison, 2000). The secondary xylem (known simply as wood), is separated from the phloem and outer bark by the meristematic tissue, referred to as the vascular cambium. It is made up of three major tissues: the fibres, the tracheary elements and the parenchyma (ground tissue). The latter of these is the only living component, with the exception of 'living' fibres, where in a number of taxa (e.g. Araliaceae, Salicaceae) these 'so called' fibres can replace axial parenchyma and act as a functional substitute (Wheeler *et al*., 2007). Parenchyma of the secondary xylem usually have a secondary cell wall, although the definition has been challenged in this case, where upon lacking the complex laminar structure belonging to the tracheary elements and fibres, it has been more likened to a thickened primary cell wall (Panshin and de Zeeuw, 1980). Also, parenchyma of the secondary xylem are usually lignified, a trait unique to wood parenchyma when compared to those elsewhere in the plant, including the phloem. The lignified thickened cell walls often make it difficult to distinguish them from typical sclerenchyma cells (i.e. the fibres) when examined in cross-section (Evert, 2006). However, not all woody plants have lignified parenchyma in their wood, including many lianas (Carlquist, 1991; Angyalossy *et al*., 2012), and some tropical tree species of the Urticaceae family; for example, *Myriocarpa longipes* and *Urera glabriuscula* (Cocoletzi *et al*., 2013).

### *Symplastic pathways between the secondary phloem and xylem*

Parenchyma cells tend to intergrade with surrounding tissues of the secondary xylem, as opposed to forming masses, as it commonly does in other regions of the plant, such as in the pith, leaf, or cortex. The parenchyma cells of both the secondary xylem and the secondary phloem are further divided into ray parenchyma (RP) and axial parenchyma (AP), with the former developing from the ray initials and the latter from the fusiform initials. Both xylem and phloem are often focussed upon in isolation, without considering the connectivity between the two regions (Spicer, 2014). In fact, literature goes as far as stating that transport in the phloem is a completely active process involving living cells, where water and mineral transport in the xylem is a completely passive process without any parenchyma involvement, as continues to be acknowledged by the cohesion-tension theory (Dixon and Joly, 1894; Dixon, 1914; Askenasy, 1895; Tyree, 1997;

Stiller and Sperry, 1999). Although numerous studies have been made, along with extensive laboratory work, into the potential role of parenchyma in long distance water transport, their considerable contribution is still not valued enough to make any alteration to the original theory published more than 120 years ago. The considerable list of publications that are speculated to support parenchyma participation in long-distance water uptake has a long history from 1904 to the present day (e.g., Ursprung, 1926; Braun, 1994; Canny, 1997, 1998; Smith, 1994; Zimmermann, 1994, Zimmermann *et al*., 1995; Zwieniecki *et al*., 2001; Clearwater and Goldstein, 2005; Salleo *et al*., 2009; Brodersen *et al*., 2010; Jansen *et al*., 2011; Nardini *et al*., 2011; Brodersen and McElrone, 2013; Santiago *et al*., 2013; Johnson *et al*., 2012; Trifilo *et al*., 2014).

Rather than view regions of a woody plant as isolated, we should hold the vision that there is a three-dimensional dynamic continuum of both symplastic and apoplastic pathways all the way through the plant. For instance, RP act as a bridge between both the phloem and the xylem, where water and metabolites must pass through the meristematic cells of the vascular cambium to flow from one side to the other. Numerous studies show evidence of symplastic pathways from the phloem through to the xylem (e.g., van der Schoot and van Bel, 1990; Salleo, 2004; Sokołowska and Zagorska-Marek, 2012; Pfautsch *et al*., 2015). In an interesting experiment, Salleo *et al.* (2004) ring-barked saplings of bay laurel (*Laurus nobilis*), which, in the process, isolated the phloem from the xylem causing a lack of recovery in the xylem after stem embolisms were induced. This showed that the phloem was integral to hydraulic recovery at the time of embolism, speculating that phloem pressures must be maintained for continued radial transport and hydraulic maintenance. The ray cells are connected to each other via numerous strands of secondary plasmodesmata, symplastic (via the cytoplasm) connections that allow for active transport of materials and communication between living cells. The outer layer of ray cells on the xylem side of the cambium have, in turn, plasmodesmatal continuity with the AP as well as half-bordered pit connections with the vascular conduits, where materials coming from the phloem can pass through into both, albeit by different means (Chaffey and Barlow, 2001; Sokołowska, 2013; Spicer, 2014).

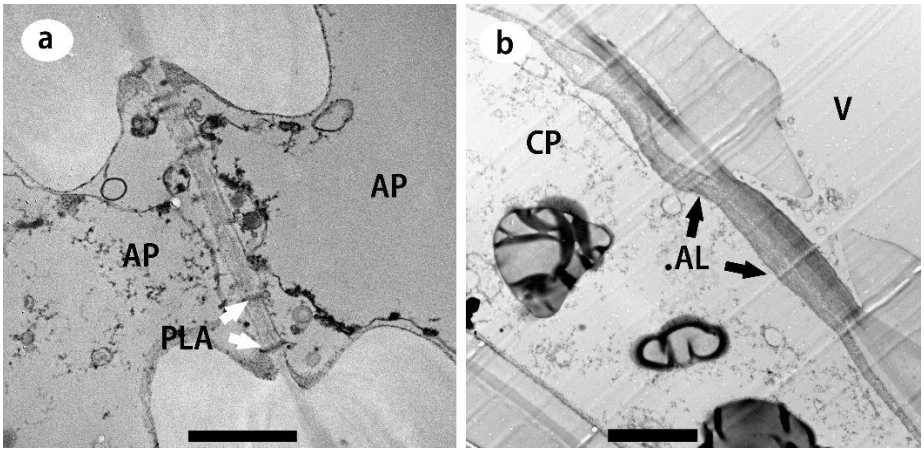



**Fig. 1** TEM (transmission electron microscopy) images of the transverse sections of **(a)** *Robinia pseudoacacia* showing two adjoining axial parenchyma cells with bordered pits (AP), showing the plasmodesmata (PLA) connections between them, which function in symplastic movement of metabolites (scale bar:1 μm), and **(b)** *Acer pseudoplatanus* with contact parenchyma (CP) adjoined to a vessel (V) via half-bordered pits, which are separated by the amorphous layer (AL) located to the inside of the contact parenchyma between the plasma membrane and the adjacent vessel-parenchyma pit membrane.(scale bar: 2 μm).

***Ray and axial parenchyma of the secondary xylem***

With exceptions, RP cells are aligned horizontally where, like 'spokes of a wheel,' they begin at the cambium and run centripetally towards the pith. Living cells make up the ray system in all angiosperms with rays, while ray-tracheids, which are dead at maturity, form an integral part of the ray system in a number of conifers (Singh *et al*., 2006). Commonly occurring along the margins of the ray system, ray-tracheids can be found in the Pinaceae (except *Abies*, *Keteleeria* and *Pseudolarix*), and in most of the Cupressaceae (Phillips, 1948). By contrast, AP cells are oriented vertically and are spatially arranged in varied patterns, the latter being a characteristic that is frequently used in the positive identification of hardwoods (IAWA Committee, 1989; Wheeler *et al*., 2007); see figure 1 in chapter 1, and table 1 and 2 in chapter 3 for a thorough assessment of AP arrangements in the secondary xylem. Conifers, by contrast, have a very uniform pattern of tracheids, with usually uniserate rays and infrequent AP cells that are often involved in resin synthesis. Conifers do not have the complexity of wood as can be found in the angiosperms, especially with regard to AP spatial arrangements and conduit form, size and patterns, with the exception of vesselless families (Wheeler *et al*., 1989; Wheeler and Baas, 1998).

Both RP and AP form a highly interconnected three-dimensional system, a detail that we are only truly beginning to embrace now, owing to advances in technology and computer software (e.g., alignment and stitching tools; Huggett and Tomlinson, 2010; Brodersen, 2013). However, past studies have shown how interconnectivity between RP, AP and vessels occurs in a range of angiosperm species through the process of serial-sectioning (Zimmermann and Tomlinson, 1966; Zimmermann, 1971), and through carefully crafted drawings (Kedrov, 2012).

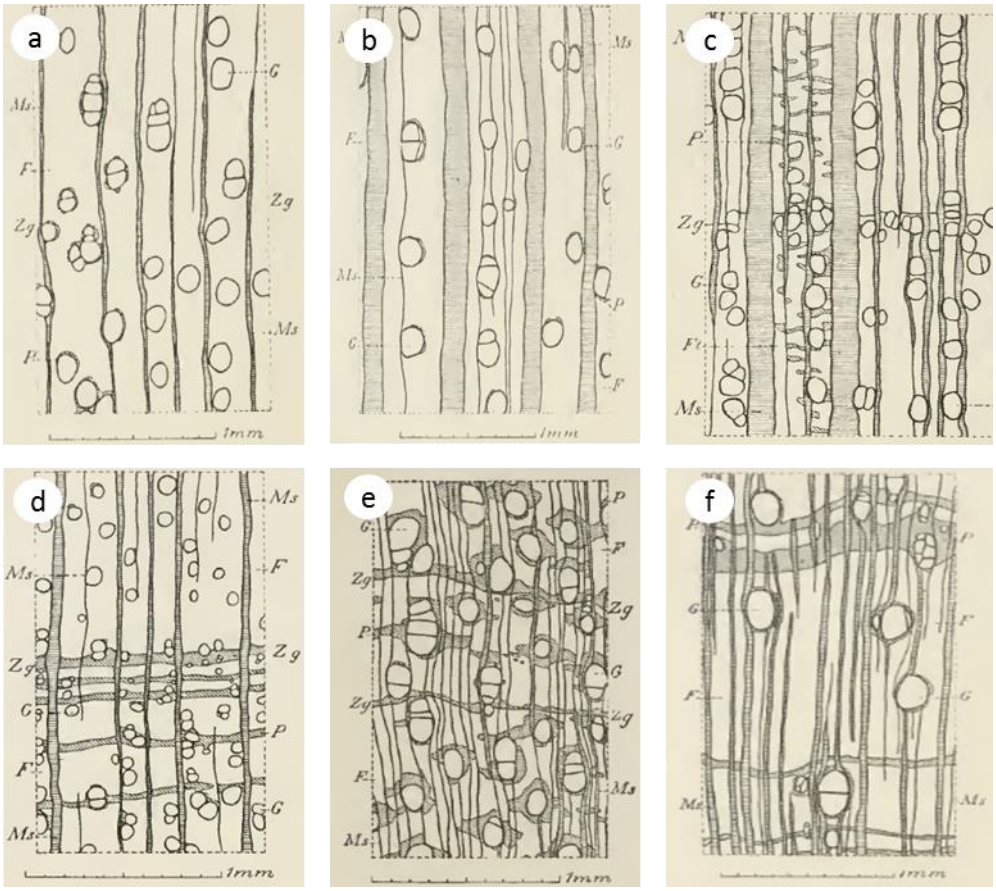



**Fig. 2** Old German drawings of cross sections from woody plants via light microscopy made over 100 years ago showing clearly the presence or absence of parenchyma between species, along with AP spatial distributions. Although these drawings were made without the advanced software we have today, they are no less accurate, where RAP fractions could be measured from these images using image J. **(a)** *Canarium hirsutum* with absent AP and narrow rays in contact with vessels, **(b)** *Leea angulata* with AP rare and wide rays, **(c)** *Platea latifolia* with scanty paratracheal AP around the vessels and both narrow and wide rays, **(d)** *Micromelum pubescens*, **(e)** *Ziziphus jujuba* with aliform, confluent and narrow banded AP arrangements together with narrow closely spaced rays AP arrangements, **(f)** *Bouea macrophylla* with wide and narrow banded AP and narrow rays. ZG Zonengtenze (zone boundary); G Gefäbe (vessels); F Libriform fibres; Ms Markstrahlen (medullary rays); P Holzparenchym (axial parenchyma). (Scale bar: 1 mm). Figures adapted from Moll JW and Janssonius HH, 1908. Mikrographie des Holzes: der auf Java vorkommenden Baumarten. Leiden: EJ Brill.

There are two kinds of RAP, those that have contact with conduits and those that are isolated from the conduits. In multiseriate rays, the horizontally elongate (procumbent) cells that are sandwiched between the layers positioned to the outside are referred to as 'isolation cells' (Braun, 1984), whereas those positioned to the outside, or all ray cells of uniseriate strands, are called 'contact cells'. Similar to the isolation cells, both in function and spatial distance from the vessels, are the vessel-distant cells found in paratracheal (mass of cells surrounding vessels) AP. Contact cells (of RP and AP) share half-bordered pits with the neighbouring conduits (Czaninski, 1977; Murakami *et al*., 1999). In angiosperms, the contact RP may be oriented vertically, and are so-called 'upright' cells. The upright RP, when in contact with AP cells, share more plasmodesmatal connections, presumably due to an increased surface area contact (Chaffey and Barlow, 2001). Also, the upright (or 'erect') RP, when in association with vessels, may be more specialised in the exchange of substances between them (Höll, 1975; Sauter and Kloth, 1986).

The composition of the cell wall is of particular interest in the contact cells of angiosperms, where at the point of contact they have a material known as the amorphous layer (also referred to as the protective layer, but only in reference to RP) (Chafe, 1974; Fujii *et al*., 1980, 1981; Murakami *et al*., 1999). Science is still unclear about its functional role at the interface between the contact cells and the vessel (Spicer, 2014). It was once considered that its function was to protect the contact cells from high osmotic pressure coming from within the vessel (van Bel and van der Schoot, 1988). The current thinking is that the layer can increase the surface area of the symplast/apoplasm interface, thus allowing for enhanced efficiency of cellular exchange (Barnett *et al*., 1993). Although the function of the amorphous layer in a defence role has been challenged (Chafe 1974; Chafe and Chauret 1974), more recent research into the role of pectins in tyloses (balloon-like extensions from contact cells that clog vessels) states otherwise (Rioux *et al*. 1998). The latter study demonstrated that the pectins accumulate in the amorphous layer and extend into the vessel, where the pectin's are then speculated to expand the surface area of the tylosis or gel, and consequencely blocking the vessels. Pectins may also have a role in the supercooling effect, where pectin is thought to help form a barrier, influencing both water loss and the development of ice crystals at freezing temperatures (Wisniewski and Davis, 1995).

***The longevity of living cells in the secondary xylem and the formation of heartwood***

RP can occupy anywhere in the region of between 8 and 25% of total sapwood area (Ghouse and Yunus, 1974; Gregory, 1977), whereas AP are much more variable and can range anywhere from < 1 to 25% (Spicer, 2014), with the latter in some succulent and pachycaul species exceeding 50% (Mauseth, 1997). The longevity of RAP cells in the secondary xylem are relatively long-lived compared to other plant regions (Evert, 2006), where they can live anywhere from two to 150 years and remain alive for up to 20 cm into the stem (Spicer and Holbrook, 2005). In *Rhododendron lapponicum*, for instance, parenchyma cells were observed to live up to 200 years old, which is extremely long-lived (Spicer and Holbrook, 2007; Schweingruber, 2013). Interestingly, AP cells in *Fraxinus* were found to be alive 45+ years after vessels stopped functioning in water uptake, where they may have changed their primary function in hydraulic maintenance to a role in storage or defence (Spicer and Holbrook, 2007). However, a study by Chapotin *et al*. (2006) showed that in the pachycaul *Adansonia* vessel-associated AP died before RP, indicating that a similar trend may also be found in other species.

Heartwood is defined as an inactive but histologically similar zone of wood to sapwood and is located to the inside of the physiologically active sapwood. As the tree ages, the parenchyma cells die where their reserve substances are released, becoming a key ingredient in heartwood formation, where the polyphenols give an overall stability to the tree's core along with giving the wood a normally darker appearance (Pinto *et al*., 2004). Regarded with particular importance during this transition are the RP cells for their chemical syntheses and resulting accumulation (Pandalai *et al*., 1985; Nakaba *et al*., 2008), and in tyloses production (Chattaway, 1949). Parenchyma cell death during the transformation to heartwood appears to be largely under genetic control (Nakada 2007, Bito *et al*., 2011, Bush *et al*., 2011), although studies into the effects of seasonal changes (phenology) on heartwood formation remain limited (Imai, 2012). A decline in sapwood respiration in a centripetal direction is suggested by many workers (e.g., Goodwin and Goddard, 1940; Higuchi *et al*., 1967; Pruyn *et al*., 2002; Pruyn *et al*., 2003; Pruyn *et al*., 2005); however, this has been disputed by some, where no change was found, even at the transition zone between sapwood and heartwood (Bowman *et al*., 2005; Spicer and Holbrook, 2007). Also, there may be a difference between parenchyma age and respiration rate when comparing angiosperms to conifers, where, interestingly, no difference was found in the two conifers studied (*Pinus strobus* and *Tsuga canadensis*), while for angiosperms the respiration

rate was reduced by half in the oldest sapwood and all parenchyma of the latter remained alive up to that point (Spicer and Holbrook, 2007). Oxygen levels also gradually deplete from the cambium towards the inner core of the tree where oxygen deprivation has been speculated to be the cause of parenchyma death (Panshin and Zeeuw, 1980; Eklund and Klintborg, 2000); however, in a later study by Spicer and Holbrook (2005) the reduced amounts of $O_2$ seem not to affect parenchyma vitality, and therefore unlikely to cause cell death or reduce respiration at the sapwood-heartwood boundary.

The parenchyma cells of the heartwood, although dead, still have very important posthumous functions, which range from an increase in durability, resistance against microbes and overall structural support (Bamber, 1976; Bamber and Fukazawa, 1985; Hillis, 1987). Aside from extractives having fungicidal properties, there is evidence that these, along with antioxidants, may together play a dual defensive role with the latter removing free radicals from the heartwood (Schulz and Nicholas, 2000). Of course, the effectiveness of the heartwood as an antimicrobial substance depends on species, moisture content, and the time of the year (Taylor *et al*., 2006), with the heartwood of some species having a much higher resistance to rot than others (Kortelainena and Viitanena, 2009).

Extractives from RAP include the following broad classifications, which all play a role in heartwood durabilty: (1) flavonoids (Harborne, 1973; Harborne and Williams, 2000), (2) quinones (Lukmandaru and Takahashi, 2009), (3) stilbenes (Schultz *et al*., 1995), and (4) tannins (Haslam, 1989).

***The functions of RAP in secondary xylem***

Although the roles of RAP have been briefly discussed in all three chapters in the context of our findings; here, the focus will be on some of the more important functions where a more detailed insight into the role of RAP will be given. The role of non-structural carbohydrates in growth, repair and in hydraulic maintenance will not be discussed in any great detail here, as it is adequately covered in chapter 2 of this thesis. However, a recent and excellent review also covers the topic in great detail (Hartmann and Trumbore, 2016).

Many of the functions of both RP and AP are shared, while others are taken over by specialised living cell types of either the ray or axial system. For instance, the lignification of the cell walls in RAP provides biomechanical support while also playing a role in defence against fungal

pathogens, as lignin is a highly durable polymer (Eriksson *et al*., 1990; Lundquist and Brunow, 2004). Both 'isolation cells' of the radial system and vessel-distant AP cells function in the storage of NSCs (non-structural carbohydrates), often just referred to as 'storage cells' (Czaninski, 1977). Smaller in size than both isolation cells and vessel-distant cells, the contact cells (contact with the vessel in vessel-bearing angiosperms or the tracheids of conifers) of RP and AP both share a 'not yet' fully understood role in maintaining the hydraulic system (Nardini *et al*., 2011; Secchi and Zwieniechi, 2011) and both tend not to store NSCs on a long term basis (Essiamah and Eschrich, 1985; Alves *et al*., 2001). Contact cells can also lower the pH of xylem sap (Fromard *et al*., 1995), which has been found to be more acidic in early spring during bud-burst, showing that the pH varies with the annual cycle in temperate tree species (Essimah, 1980; Ferguson *et al*., 1983; Sauter, 1988). Although it is not clear what function this serves, it could, however, facilitate the easier movement of ions and organic nutrients between contact cells and vessels along a pH gradient (Larsson and Moller, 1990).

Storage of carbohydrates and the storage of water in RAP are also closely affiliated, meaning that the functional role of RAP in water storage cannot be uncoupled from their role in the storage of carbohydrate reserves, with the latter having an important function in growth (primary metabolites) and tissue repair (secondary metabolites). Recent research into the role of NSCs showed that stored carbohydrates contributed significantly to drought resistance in a range of tropical and temperate tree seedlings (O' Brien *et al*., 2014; O' Brien *et al*., 2015). In summary, stored NSCs may be critical for the retention of water, as inorganic and organic solutes of the living cells acquire it from the xylem when under tension, which is in turn used to stave off potential drought by protecting the plant from dehydration (Borchert, 1994).

***Long distance water transport and RAP***

The mystery of the role of RAP in long distance water transport still remains largely unsolved; however, considering the connections between the living cells and the dead conducting cells through a continuum expanding from the secondary phloem through to the secondary xylem, along with the sophisticated anatomy and physiology of the contact cell-conduit pits (Zimmermann, 1978; van Ieperen *et al.*, 2000), the involvement of RAP in hydraulic maintenance seems unquestionable. Where its involvement is not in dispute, the mechanisms remain unclear (Nardini *et al*., 2011; Secchi and Zwieniechi, 2011). For instance, the

mechanisms behind embolism repair are among the most contentious, especially in light of recent developments over the procedures used in investigating embolisms in stems of plants (Wheeler *et al*., 2013; Sperry, 2013; Torres-Ruiz *et al*., 2015). However, the evidence for seasonal embolism repair remains convincing. Through X-ray tomography, visual evidence of water droplets derived from contact parenchyma have been shown to form and expand and coalesce in the vessels of grapevine (Brodersen *et al*., 2010), which confirms previous studies using cryo-SEM (Canny, 1997; Canny *et al*., 2007). There is also evidence that stimulating the activity of $H^+$-ATPase can promote embolism repair (Salleo *et al*., 2004). The plasma membrane has been reported to be activated by pressure from the conduit side (Zingarelli *et al*., 1999), which might release sugars from the contact parenchyma into the embolised vessel through a 'kind of' active secondary transport (proton pump mechanism) (Alves *et al*., 2004). Where seasonal embolism repair is likely to occur in a number of temperate species, there is no evidence for it in tropical species (Brodersen and McElrone, 2013). As a result of increased $H^+$-ATPase activity in the contact cells, the sugars released into the xylem stream after hydrolysis can directly increase the sap osmolarity and xylem pressure (Améglio *et al*., 2001, 2004). How an embolism activates the living cells into repair of the conduit remains speculative. It could be caused via wall vibrations originating from within the conduit, which mechanically trigger the living cells into a response (Salleo *et al*., 2008) or through changes in osmotic concentrations between contact cells and conduits (Secchi and Zwieniecki 2011, 2012). Another important factor is the point at which the living cells are triggered into the repair of the conduit or, alternatively, into the production of tyloses, where the latter is surely a last resort, as it results in the death of the contact cell(s) and the complete blockage and dysfunction of the vessel(s) (Klein, 1923; Zimmermann, 1978; Canny, 1997; Salleo *et al*., 2002; Sun *et al*., 2007). An excellent review on the subject of embolism repair was written by Clearwater and Goldstein (2005).

Hydraulic capacitance, which involves the release of stored water reserves from the bark (including the phloem) and secondary xylem, could buffer against increasing tensions in the xylem sap while avoiding embolisms in the process (Cruiziat *et al*., 2002; Meinzer *et al*., 2009; McCulloh *et al*., 2014; Pfautsch *et al*., 2015). This allows photosynthesis to continue for longer durations both diurnally and seasonally, where it has been confirmed that sapwood capacitance is higher in species of high rainfall sites (Richards *et al*., 2013), indicating that this mechanism may

be more common in the lowland tropics rather than, for instance, in temperate zones. Also, in numerous reports, capacitance was shown to be higher in species with low wood density, which are more prone to embolism formation as a consequence (Pratt *et al*., 2007; Sperry *et al*., 2008). The parenchyma of the secondary xylem, particularly AP, are likely to play a large role in capacitance, especially where wood density is low, which is found to be the case in succulent growth forms (Borchert and Pockman, 2005). However, with normal growth forms, wood density and AP fractions were found to be negatively correlated (Zheng and Martinez-Cabrera, 2013; Ziemińska *et al*., 2015). More work is needed to ascertain the role of AP cells in capacitance, particularly in tropical trees, as they have not been quantified separately in available literature, where they are generally included among a suite of other possible sources involved in capacitance (Meinzer *et al*., 2009; Pfautsch *et al*., 2015).

Besides hydraulic capacitance, there is also evidence of the involvement of RAP in long distance water uptake via the ionic effect, where sap flow is altered by changes in the ionic composition and pH of the xylem sap (Fromard *et al*., 1995; De Boer and Volkov, 2003). Ionic solutes, deriving from the contact cells through physico-chemical processes have been shown to increase flow rates in the xylem by up to 2.5 times (Zwieniecki *et al*., 2001; Gascó *et al*., 2006); however, not all solutes contribute equally with NaCl found to be significantly lower than KCl (Gascó *et al*., 2006). Although the scientific basis behind the ionic effect is not challenged (Nardini et al., 2007; Nardini *et al*., 2011; Nardini *et al*., 2012), the explanation behind the mechanism is still largely unknown, regardless of various hypotheses put forward. For instance, the hydrogel nature of pectins found in the pit membrane, is speculated to be involved in hydraulic conductance (Zwienicki *et al*., 2001). According to this study, the pores of the pit membranes physically change, where the cations cause the shrinkage of pectins (i.e. polysaccharidic polyelectrolytes), thus enlarging the pores, which in turn could increase the hydraulic conductance of the xylem (Ryden *et al*., 2000; Willats *et al*., 2001). However, available evidence shows that pectins are lacking in intervessel pit membranes, so this mechanism has received little or no acceptance in explaining the ionic effect (van Doorn *et al*., 2011, Klepsch *et al*. submitted).

A recent proposition to help explain long-distance water uptake and support the cohesion-tension theory is the surfactant-coated nanobubble hypothesis (Schenk *et al*., 2015). Although surfactants are thought to reduce xylem sap surface tension, which could increase the chances of embolism

formation under negative pressure (Christensen-Dalsgaard *et al*., 2011; Domec, 2011), the concentrations found under natural conditions are considered too low to do so. These surfactants, along with dissolved ions such as KCl, $CaCl_2$ and $MgCl_2$, all originating from the contact cells, are speculated to keep these nano-bubbles from coalescing to form large bubbles through having a stabilising effect (Craig, 2004), which could prevent the formation of embolisms.

***Defence against pathogens and RAP***

The role of RAP in defence against pathogenic fungi and bacteria is considerable and greatly underestimated, where the work carried out on this important subject has largely been the focus of plant pathologists and not plant anatomists or physiologists per sé. However, there have been a number of important papers where parenchyma, in particular the contact AP, has been the focus, albeit, in many cases, indirectly, and mostly from within the primary plant tissues. These include: in fungal-plant interactions (El Mahjoub *et al*., 1984; Street *et al*., 1986; Shi *et al*., 1992; Cooper and Williams, 2004; Schwarze *et al*., 2003; Pouzoulet *et al*., 2014), and in bacterial-plant interactions (Goodman and White, 1981; Hilaire *et al*., 2001; Basha *et al*., 2010; Schumann and D'Arcy, 2010).

To help explain pathogen-plant interactions in the secondary xylem, the CODIT model, an acronym for Compartmentalisation of Decay in Trees, was developed by Shigo (1970, 1976, 1979, 1984) and further developed by Boddy and Rayner (1983). It is made up of four walls formed of two parts: wall 1-3 form the first part of the model where the walls are in place at the time of wounding, where as wall 4, the second part of the model, forms after wounding has occurred. A change to the model has been proposed and generally accepted, where the 'D' of CODIT is replaced by 'Damage' or 'Dysfunction', simply because decay in itself is not the only mechanism to initiate physico-chemical responses by the living cells, but rather any biomechanical stimulus can, which could in turn pave the way for decay spread (Liese and Dujesiefken, 1989, 1996). RAP play a large role in all four walls. In wall 1, the AP cells act to prevent decay movement up and down the stem in an axial direction, mostly through the production of tyloses from contact cells into the neighbouring vessels (Schmitt and Liese, 1990; Sun *et al*., 2007). Wall 2 is made up of a largely static barrier composed of highly lignified fibres at the annual ring boundary where decay is prevented from moving in a radial direction (Shigo and Marx, 1977). However, studies show how this marginal parenchyma laid down at

either side on the annual growth ring plays a role in defence, along with the intermittent RP cells that break up the continuity of the annual ring along its circumference (Biggs, 1987; Schwarze and Fink, 1998; Schwarze *et al*., 2000, 2003, 2007). Wall 3, the strongest wall in place at the time of injury, is composed of the rays, where RP act to inhibit the movement of decay in a lateral direction (Shigo, 1984). Wall 4, the final instalment of the CODIT model (the only wall of part 2 of the model), also referred to as the 'barrier zone,' is laid down by the cambium and completely formed of highly suberised axial parenchyma cells intermeshed among weakly lignified vessels (Pearce and Holloway, 1984; Rademacher *et al*., 1984; Pearce, 1990).

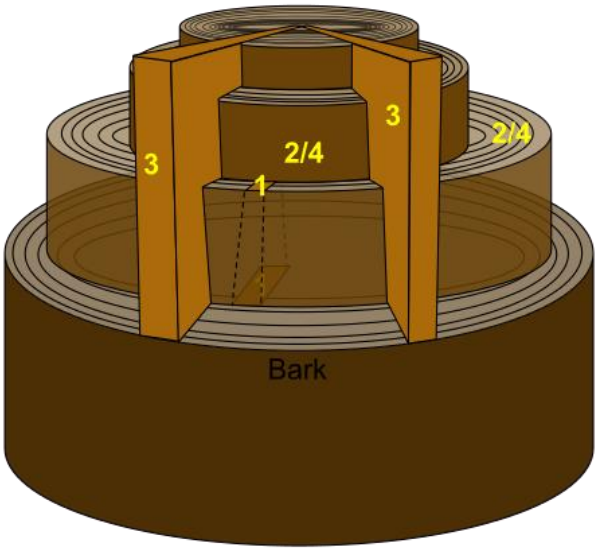



**Fig. 3** The CODIT model (compartmentalisation of decay in trees). **Wall 1:** prevents the up and downward movement of decay through tyloses development via the contact AP. **Wall 2:** prevents the radial movement of decay at the annual ring boundary (lignified fibres and marginal apotracheal AP) in temperate species, or possibly through wide-banded AP in tropical species. **Wall 3:** prevents the movement of decay laterally via the ray system (RP). **Wall 4:** prevents decay from entering new wood developed by the fusiform initials of the cambia after wall 4 is laid down. The walls run in order of 'most effective' with wall 4 being the strongest. Walls 1-3 are present at the time of injury (part 1), while wall 4 is formed after injury occurs (part 2). Image sourced from: https://commons.wikimedia.org/wiki/File:CODIT_Model.svg. Licence CC0-BY 3.0 SA https://creativecommons.org/licenses/by-sa/3.0/us/

The physico-chemical nature of RAP, along with their amount and spatial arrangement, all together play a crucial role in the battle against pathogens. Tyloses arising from the contact parenchyma can partially or wholly occlude the vessels, where the trigger seems to come from the vessel side (Klein, 1923; Zimmermann, 1978; Schmitt and Liese, 1990, 1993; Sun *et al*., 2008), although the mechanism behind how it occurs remains unclear. Research by Sun *et al*.

(2007) demonstrated that wound-induced ethylene, and not embolism formation, is required to trigger tylosis development in grapevines, which goes against previous hypotheses (Klein, 1923; Zimmermann, 1978; Canny, 1997).

Also, when the pit aperture falls below a certain diameter to the point where it can no longer accommodate tyloses due to a size restriction, the contact cells can secrete gels into the vessel lumina in their place (formerly referred to as gums) (Bonsen and Kučera, 1990; Rioux *et al*., 1998). In an interesting study by Rioux *et al*. (1998), pectin, formed in the amorphous layer of the contact cells, is secreted into the vessel alongside the gel, where, through water-induced expansion, can help the gel clog the vessel's outermost peripheries. However, counterintuitive to these findings, where the pit aperture size is critical to the release of tyloses or gels, is the study by Hillaire *et al*. (2001) whom demonstrated that pathogen related proteins induce secondary wall thickening, which in turn decreases the pit size, preventing or limiting bacterial spread from the vessels into the contact cells. The contact cells when compared to vessel-distant parenchyma, have high cytoplasmic activity (Sauter, 1973; Essiama and Eschrich, 1985; Shi *et al*., 1992; Alves *et al*., 2001), thus accumulate and release several anti-microbial materials, such as lipoidal, phenolic and terpenoid compounds (Cooper *et al*., 1996; Clérivet *et al*., 2000). Suberin, a fatty hydrophobic sealant as well as being an anti-microbial compound, normally found in the bark and produced by the living cells, has also been detected in the vessel lumina of numerous angiosperm species during all stages of CODIT (Biggs, 1987).

Conifers have adapted differently to pathogenicity and herbivory owing to their contrasting anatomical makeup, with their more uniform tracheid arrangement, mostly uniseriate rays, and sparce AP (Sperry *et al*., 2006; Carnicer *et al*., 2013). However, similar to angiosperms, the 'so-called' polyphenolic parenchyma cells (abbrev. PPC), a term associated with just conifers, accumulate and release toxic anti-microbial compounds that act against both pathogens and insects (Franceschi *et al*., 2005; Bohlmann, 2008; Kolosova and Bohlmann, 2012). Alongside polyphenols, terpinoids are a critical defence component of conifers, where their viscosity can disable insects and trigger the formation of specialised parenchyma known as traumatic resin ducts in some families (Phillips and Croteau, 1999; Martin *et al*., 2002; Byun McKay *et al*., 2003; Keeling and Bohlmann, 2006). Another factor to include here is the unique mechanism of

conifers called the torus-margo bordered pit, which can localise both embolism and decay (Choat *et al*., 2008; Fuhr *et al*., 2013; Bouche *et al*., 2014).

***RAP and mechanical properties of the secondary xylem***

A number of recent important studies have focussed on the trade-off between RAP, vessels and fibre fractions, the three main constituents of angiosperm wood (Jacobsen *et al*., 2007; Martinez-Cabrera *et al*., 2009; Zheng and Martinez-Cabrera, 2013; Ziemińska *et al*., 2013; Ziemińska *et al.*, 2015). A study by Jacobsen *et al*. (2007) found that total RAP was inversely related to the modulus of elasticity (MoE) while Martinez-Cabrera showed in a study of 61 shrubs these two variables to be independent of each other. This latter study also showed a trade-off between RP and AP, where they each had opposite correlation patterns with wood density (AP – negatively, RP – positively), demonstrating some evidence for functional partitioning between these two parenchymatous types. For instance, rays are known to have strong biomechanical properties due to their radial orientation and cell wall lignification (Mattheck and Kubler, 1995; Burgert *et al*., 1999; Burgert and Eckstein, 2001), while the opposite was found to be true for AP, where higher amounts are speculated to weaken stem longitudinal strength, due to the finding that AP is negatively correlated with wood density (Zheng and Matinez-Cabrera, 2013). In contrast, a study by Ziemińska *et al*. (2013) found the opposite trend where RP and AP were found to have no correlation with each other, and where the former was negatively correlated with wood density, AP was found to positively correlate with this variable. However, this study was carried out on twigs rather than more mature stem wood, so caution must be taken in interpreting these results.

## Aims of the thesis

The thesis is divided into three chapters (part II), with each chapter focusing on different elements concerning the amount, spatial arrangements and functions of radial and axial parenchyma in the secondary xylem of woody plants. Chapter 1 and 2 are research focussed, whereas chapter 3 is an opinion paper discussing the use of terminology in describing secondary xylem parenchyma from a historical perspective. Each of the three chapters in this thesis is an 'already' fully published article in the following Journals: New Phytologist, the American Journal of Botany, and the International Association of Wood Anatomists journal, in that order. The thesis is not laid out in the original format of the publications, due to differences in publication layout. This decision was made in order to maintain consistency in style throughout the text and references. The wording and graphs of the original articles have been strictly adhered to, with the exception that the American spelling for the article in the American Journal of Botany has been changed to British spelling, in maintaining a consistent format.

## The research topics and permissions

### **Chapter 1** - *A global analysis of parenchyma tissue fractions in secondary xylem of seed plants*

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**Title:** A global analysis of parenchyma tissue fractions in secondary xylem of seed plants

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### **Chapter 2** - *The amount of parenchyma and living fibres affects storage of non-structural carbohydrates in young stems and roots of temperate trees*

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### **Chapter 3** – *Secondary xylem parenchyma – from classical terminology to functional traits*

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## Summary of chapters

**Chapter 1:** ***A global analysis of parenchyma tissue fractions in secondary xylem of seed plants***

Knowing the mount of parenchyma cells in the secondary xylem of woody plants, together with their spatial distribution across a range of plant species, growth forms, and climates, is a very valuable basis for helping scientists to underpin the functions of living cells and how they contribute to overall plant physiology. For instance, in determining to what extent the amount of living cells might be related to defence against herbivory and pathogens, or in hydraulic maintenance of the stem and drought stress, or in the storage of non-structural carbohydrates used for growth and repair, or, lastly, in the mechanical properties of the plant. Also, this study might help to explain the degree of functional partitioning in parenchyma between plants (between both ray and axial parenchyma separately and also total RAP together), where two plants could have similar amounts of parenchyma, but a trade-off may exist in determining what function(s) the parenchyma cells of each plant are used for, whether it be a more primary role in capacitance to maintain hydraulic conductivity, or in more of a defensive role against pathogens. This trade-off in parenchyma function would be importantly connected to the life history of that particular species; for example, whether it is long or short-lived. This chapter only investigated actual parenchyma percentages across a large dataset of 1727 records (1439 species) and did not focus on their spatial arrangements, where the latter is a separate project for a follow-up study.

The primary aim of this study was to elucidate the effects of climate on ray and axial parenchyma (RAP) fractions, where we looked at a range of climate parameters in relation to RAP fractions, including: temperature, precipitation, latitude, altitude, etc. We also categorised all the species into three broad climate zones: temperate, subtropical and tropical, allowing us to explore the relationship between climate type and RAP fractions. The work also allowed us to: (1) examine the links between parenchyma and growth form, (2) to investigate differences in RAP between the parts of a woody plant, *viz*. the roots, trunks and branches, (3) to support previous works on the anatomical divergence between the angiosperms and conifers and (4) to explore differences between axial parenchyma (AP) and ray parenchyma (RP) fractions, which would help to explain how conservative or versatile these two parenchyma tissues are, together with the functional explanations behind this.

For this study, the majority of records for ray and axial parenchyma were obtained from papers and books (55 separate literature sources). We also investigated axial parenchyma and radial parenchyma separately where we had the data, as well as total RAP fractions between woody plant groups (i.e. conifers vs. angiosperms) growth forms and climates. The climate data was collected via two approaches: (1) the exact locations approach, where we had the longitudinal and latitudinal coordinates for 411 species from 68 sampling locations, and (2) the GBIF approach (Global Biodiversity Inventory System), which allowed us to gather climate information for 619 species from 612 different locations.

In conclusion, the key finding showed that temperature was the biggest driver of RAP in plants, followed by precipitation, with the latter showing a significant but negative relationship with RAP, demonstrating an increase in RAP towards drier sites. Interestingly, AP, rather than RP, was found to be the most versatile parenchyma type, with the sharp rise of RAP in tropical species being mostly due to AP, while RP remained relatively constant. This was both supported by the climate type plots and through the GAM model; however, the one anomaly was the high RP fraction found in lianas, which is speculated to be related to the biomechanical properties of the ray structure in this growth form. Also, regarding biomechanical properties, a ternary plot showed by way of a visualisation how an increased RAP fraction from temperate through to tropical climate zones occurs at the expense of fibres, the tissue type most commonly associated with wood density. Another important finding was the difference in RAP between growth forms, with succulents (including pachycauls) and lianas having much higher RAP fractions than non-succulent angiosperm trees and conifers, with the latter having the lowest RAP fractions, as expected.

**Chapter 2:** ***The amount of parenchyma and living fibres affects storage of non-structural carbohydrates in young stems and roots of temperate trees***

This important research was conducted under the premise that the amount of non-structural carbohydrates (NSCs) in the secondary xylem of wood (total carbon), is directly related to the amount of living cells, both radial and axial parenchyma (RAP), where RAP provides the uppermost limit for NSC storage in plants. The role of living fibres in the storage of NSCs are also studied here in *Clematis vitalba* and *Acer pseudoplatanus*, as they are known to be a functional replacement in the absence of AP. NSCs include monosaccharides, disaccharides and polysaccharides (i.e. soluble sugars and starch). NSCs are critical in plants, especially in growth and dormancy in woody plants of seasonal climates; however, this could be due to a bias, where far fewer studies have been conducted on NSCs in tropical climate locations where seasonality is not a factor. Of late, much attention has been given to carbohydrates in relation to growth limitation and drought induced tree mortality, where, with regarding the latter, it forms part of a continuing debate with the plant hydraulics community as to which factor is the primary cause of tree mortality under drought conditions, carbon starvation or embolism formation (Mc Dowell, 2011; Anderegg *et al*., 2012; Urli *et al*., 2013; Sevanto *et al*., 2014; Rowland *et al*., 2015; Salmon *et al*., 2015).

For this study, the roots and stems of twelve temperate tree species (11 broadleaved deciduous and one conifer) and four tropical trees species were investigated. Both NSC analyses were carried out alongside the measurements of RAP fractions (and living fibres) and the starch distribution using a potassium-iodine solution (Lugol's solution). The presence of starch alone cannot explain whether or not NSC's are present or absent from the cell, as often it is hydrolysed into sucrose when temperatures fall below zero, where in this form it may remain present in the cell and unused, quickly shifting back to starch when conditions become favourable, such as during early spring (Elle and Sauter, 2000; Hoch *et al*., 2002; Charrier *et al*., 2015). For this reason, the tropical species used for this research make for an interesting contrast.

In conclusion, the study showed a strong relationship between RAP/living fibre fraction and NSC concentration in the roots and stems of temperate trees and the only climber in the study, *C. vitalba*, where RAP and/or living fibre fraction provides the uppermost limit for NSC storage, an important contribution to our understanding of xylem-structure dynamics. Interestingly, the

amount of RAP was, in most cases, greater in the roots of temperate trees than in the stems, which also meant a greater NSC accumulation in this plant part. The study also revealed, through starch analyses, that both RP and AP/living fibres, provide equally to NSC storage in temperate species, in that both 'living cell' types were observed to be full of starch grains. Also, there was no observable difference between starch accumulations in the contact cells compared to the vessel-distant cells (referred to as isolation cells in the ray system). Starch grains were found to be less abundant in the RAP of tropical trees, however, where no correlation was found between RAP amount and NSCs in these trees. Furthermore, although RAP fractions in the tropical species were, on average, higher than in their temperate counterparts, NSC levels were even lower in the former than in the latter. As the number of tropical species studied were low, caution must be taken in interpreting these results, while the general trends shown in the temperate species are a more useful indicator of the seasonal maxima for NSC concentrations in the northern hemisphere at the onset of winter dormancy.

**Chapter 3:** ***Secondary xylem parenchyma – from classical terminology to functional traits***

The chapter was written as an opinion paper to give clarity to both functional and anatomical terms used to describe parenchyma, explaining how these terms evolved and currently relate to functional traits and/or their use in the identification of different woods. While many terms are still in use, other terms have become redundant due to being used less and less frequently as we change our functional interpretation of particular types of cells overtime. This opinion paper makes the case for a critical review of terminology, as how we use and interpret them is of utmost importance, especially for those outside the field of anatomy, in disciplines such as plant physiology, plant phytology, dendrochronology, and evolutionary biology, etc. Also, not only is the consistent application of terms important, we must consider the dynamic continuum between different anatomical tissues and refocus on the plant as a three-dimensional interconnected system, rather than viewing it as single planes through a light microscope.

## Conclusions and future prospects

The aim of this thesis was to further our understanding of the role of parenchyma in the secondary xylem of 'woody' plants, a critically important facet of plants that still remains poorly understood owing to a difficulty factor in obtaining accurate measurements until relatively recently, and a more 'industry focussed' preoccupation with other cell types, such as the vessels and fibres. This work investigated the variation in parenchyma fractions between three broad climate types, finding significantly higher fractions of both radial and axial parenchyma (RAP) in tropical locations, compared to both temperate and subtropical, particularly in the levels of axial parenchyma (AP). This was supported by examining the relationship between RAP fractions and a range of climate parameters, where temperature was found to be the main driver, in particular AP, while ray parenchyma (RP) remained relatively unchanged. There are a number of possible explanations for this. Cold temperatures place great energy demands on living cells, observed by increased respiration rates (Sperling *et al*., 2015), and through avoidance of freezing via the supercooling effect (Quamme, 1991; Neuner, 2014), which may explain why plants of cooler regions have lower RAP levels. Also, pathogens are more abundant and more virulent in the tropics (Bagchi *et al*., 2014), where greater RAP levels may help provide better armoury against pathogens (Schwarze *et al*., 2003; Romera and Bolker, 2008).

Another interesting result is that RAP increases towards drier areas, a finding that supports the high parenchyma levels found in low wood density pachycauls and other succulents, where the paratracheal (surrounding the water conducting conduits) parenchyma function as a kind of 'giant' reservoir (Borchert and Pockman, 2005; Scholz *et al*., 2007; Hearn *et al*., 2013). Paratracheal parenchyma around the vessels of pachycauls was found to be positively correlated with high capacitance (water release into the vessels), where the opposite trend was found in deciduous hardwoods with vessels enclosed by imperforate tracheary elements (Borchert and Pockman, 2005). There are two drought strategies used by plants: drought tolerance vs. drought avoidance, where species with large amounts of paratracheal AP fall into the latter category. Drought avoiders, the former of the two strategies, are speculated to have few RAP cells, but are able to tolerate low water potentials (Borchert *et al*., 1994; Goldstein *et al*., 1998). However, more capacitance measurements of both bark and xylem are required from both temperate and tropical species while also measuring RAP values, particularly AP, and their contact fractions

(percentage contact) with vessels. This important anatomy-function approach could reveal important insights into plant strategies under different climatic conditions.

The high levels of RAP found in lianas, particularly RP, is of notable interest regarding two key functions: plant biomechanics and dedifferentiation (a form of totipontency). Lianas were shown to have increased elasticity enabling a high resistance to torsion (twisting) (Gartner, 1988; Putz and Mooney, 1991), which is likely to be attributable to the high RP levels, with, in addition, having often non-lignified and thin cell walls (Schnitzer *et al*., 2015). Also, asexual reproduction via cloning is a common strategy deployed by lianas, where dedifferentiation of RAP could allow for rapid plantlet establishment (Putz, 1984; Yorke *et al*., 2014), as well as enabling fast recovery from injury when breakage occurs (Fisher and Ewers, 1991).

We tested one of the functional hypotheses put forward in chapter one, which was to see if a relationship existed between the levels of RAP and the amount of non-structural carbohydrates that can be stored in the secondary xylem, and found this to be the case. Total RAP in both stems and roots of a range of temperate species investigated provide the uppermost limit for NSC storage. This important finding could have implications for species with low or high levels of RAP under drought conditions, where species with the former could have more difficulty with keeping hydrated. Studies by O'Brien *et al*. (2014, 2015) have built a strong case for the benefits of stored NSC during times of short term drought in a range of both tropical and temperate tree species. Longer drought periods, however, may trigger a decline in NSC storage resulting in a reduction of allocation into defence compounds (Steele *et al*., 1995; Hartmann and Trumore, 2016), which could have a knock-on effect.

In summary, these findings demonstrate the importance of combining anatomy with function, paving the way for many more studies, including the investigation of various functional hypotheses. One key area that needs to be explored is the spatial arrangements of AP, where at present only qualitative data is available through the plant database, InsideWood (InsideWood; Wheeler *et al*., 2007). Also, our data was from cross-sections of wood only, where we know little about the three-dimensional aspects of living cell patterns in wood, another key area that should be given further exploration. Another crucial field of plant science to help support our microscopy analyses is phylogenetics, which could be applied to the species for which we have

RAP values, thus revealing how genetically or environmentally inclined a large spectrum of woody plants with a hugely diverse global distribution might be.

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# Part II

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# Publications

## Chapter 1

# A global analysis of parenchyma tissue fractions in secondary xylem of seed plants

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***Abstract***

Parenchyma is an important tissue in secondary xylem of seed plants, with functions ranging from storage to defence and with effects on the physical and mechanical properties of wood. Currently, we lack a large-scale quantitative analysis of ray parenchyma (RP) and axial parenchyma (AP) tissue fractions.

Here, we use data from the literature on AP and RP fractions to investigate the potential relationships of climate and growth form with total ray and axial parenchyma fractions (RAP). We found a 29-fold variation in RAP fraction, which was more strongly related with temperature than with precipitation. Stem succulents had the highest RAP values (mean ± SD: 70.2 ± 22.0%), followed by lianas (50.1 ± 16.3%), angiosperm trees and shrubs (26.3 ± 12.4%), and conifers (7.6 ± 2.6%). Differences in RAP fraction between temperate and tropical angiosperm trees (21.1 ± 7.9% vs. 36.2 ± 13.4%, respectively) are due to differences in the AP fraction, typically three times higher in tropical than in temperate trees, but not in RP fraction.

Our results illustrate that both temperature and growth form are important drivers of RAP fractions. These findings should help pave the way to better understand the various functions of RAP in plants.

**Key-words**: *angiosperms, axial parenchyma, growth form, conifers, mean annual precipitation, mean annual temperature, ray parenchyma, secondary xylem.*

### *Introduction*

Parenchyma tissue in secondary xylem is composed of living cells variable in their morphology and physiology, which usually have thin walls and are rectangular or square in shape. They are produced by fusiform and ray initials of the vascular cambium, which develop into axial parenchyma (AP) strands and ray parenchyma (RP), respectively, and run perpendicular to each other (Fig. 1). Besides the occurrence of so-called living fibres (Wolkinger 1970, 1971), ray and axial parenchyma (RAP) tissue represents the bulk of living cells in wood. Parenchyma plays multiple functions, as seen in Fig. 2 (green boxes). These functions range from storage and transport of non-structural carbohydrates (NSCs) (Hoch *et al*., 2003; Salleo *et al.*, 2004; O'Brien *et al*., 2014; Plavcová & Jansen, 2015) to defence against pathogens (Shigo, 1984; Biggs, 1987; Schmitt and Liese, 1993; Deflorio *et al.*, 2008), water storage and xylem hydraulic capacitance (Holbrook, 1995; Borchert and Pockman, 2005), storage of mineral inclusions, the transition of functional sapwood to heartwood (Pinto *et al.*, 2004; Spicer, 2005; Nawrot *et al.*, 2008), and mechanical contributions, particularly by RP (Burgert *et al*., 1999; Burgert & Eckstein, 2001; Reiterer *et al.*, 2002). Two additional functions of RAP speculated to be involved in long distance water transport are poorly understood, the first being embolism repair (Clearwater & Goldstein, 2005; Salleo *et al.* 2009; Brodersen *et al.* 2010), with the second involving the ion-mediated enhancement of xylem hydraulic conductance via the release of inorganic compounds such as $K^+$ and $Ca^{2+}$ into the transpiration stream (Zwieniecki *et al*., 2001; Jansen *et al.*, 2011; Nardini *et al.*, 2011; Santiago *et al.*, 2013). Radial transport via RAP also needs more exploration. Rays provide means for interactions between phloem and xylem (Van Bell, 1990; Spicer and Holbrook, 2007; Hearn *et al*., 2013; Pfautsch *et al*., 2015), as they stretch from the inner bark across the cambium and into the xylem.

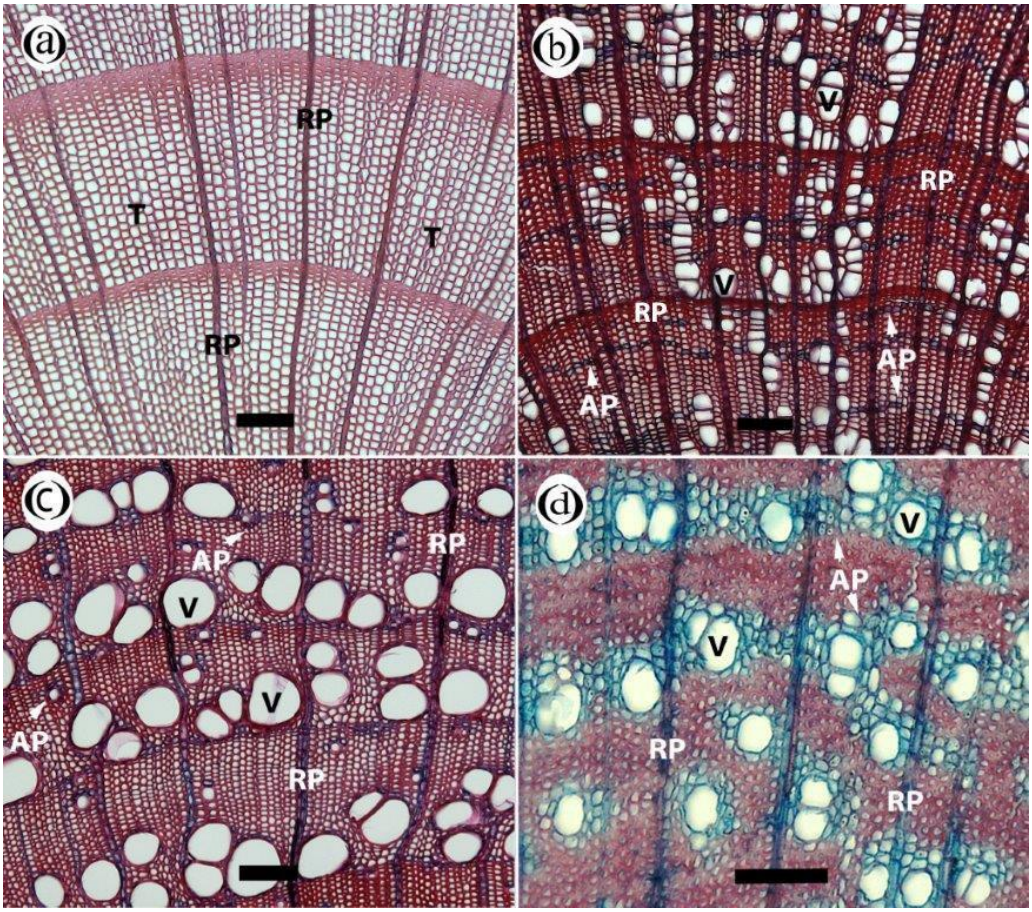



**Fig. 1** Light microscopy images of transverse sections of conifer and angiosperm stem wood with different parenchyma fractions and spatial distribution, showing a gradient from low to high ray and axial parenchyma percentages, with a conifer (a) *Picea abies*, a species with no axial parenchyma (AP) present, (b) *Carpinus betulus*, a diffuse-porous species with narrow bands of AP (white arrows), (c) *Fraxinus excelsior*, a ring-porous species with both scanty paratracheal AP (white arrows) and marginal bands, and (d) *Crescentia cujeje*, a tropical diffuse-porous species with aliform and confluent bands of AP (white arrows). The sections were stained with a combination of safranin and alcian blue, resulting in a red colour for strongly lignified cell walls (tracheids (T), vessels (V), and fibres) and a blue to purple colour for both AP and RP (radial parenchyma). All scale bars represent 100 µm.

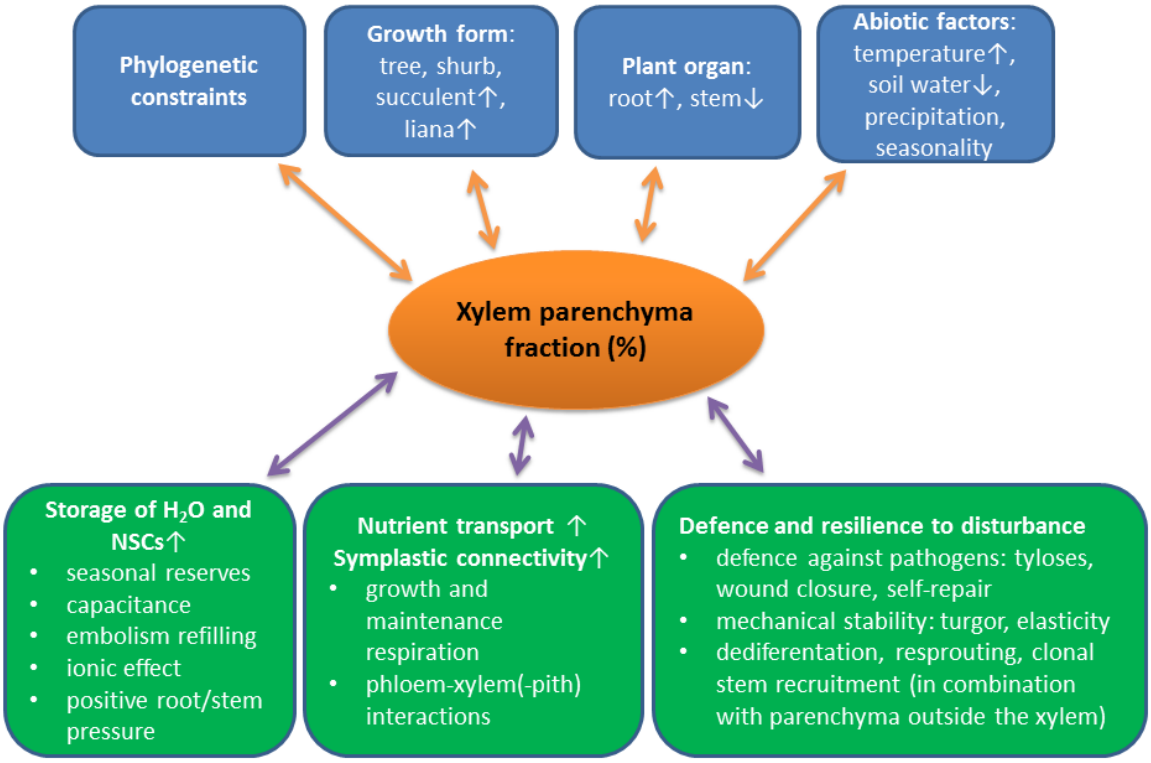



**Fig. 2** Diagram of variables (in blue) hypothesised to affect xylem parenchyma (including ray and axial parenchyma) tissue fractions in wood and the functions (in green) hypothesised to be related to parenchyma fractions. Arrows pointing upwards or downwards may indicate whether or not particular causes may lead to a speculative increase or decrease of parenchyma fraction, or that a functional process is either positively or negatively scaled with parenchyma fraction. Potential variation in parenchyma quantity due to developmental changes and patterns of spatial distribution within the xylem tissue are omitted in this framework.

RAP shows a large variability in its quantitative and qualitative anatomical characteristics across species (e.g., Kribs, 1935, 1937; Barghoorn, 1940, 1941; Normand and Chatelet, 1951; Braun and Wolkinger, 1970; Braun, 1984, Panshin and de Zeeuw, 1980; Koch, 1985). In fact, this variability has been used for wood identification and systematic purposes (e.g., IAWA Committee, 1989, 2004; Wagenführ, 2007); however, we lack a thorough understanding of the functional meaning of this variability. Recent advances in image acquisition and analysis techniques have made possible a more accurate and thorough examination of tissue percentages along with the design of studies investigating patterns of variation in total parenchyma fraction (e.g. Martínez-Cabrera *et al.*, 2009; Ziemińska *et al.*, 2013, 2015; Zheng and Martínez-Cabrera, 2013).

RAP amount responds to both phylogenetic and environmental factors (Fig. 2). Also, RAP, with the exclusion of rayless species (Carlquist, 2001), are typically higher in angiosperm than in conifer wood for both RP (15 to 20% compared to 4 to 8%) and AP (≤ 1% to ≥ 30% compared to ≤ 1%), respectively (Koch, 1985; Spicer, 2014). The level of RAP also depends on growth forms, with a surprisingly high level occurring in woody succulents and lianas (Hearn, 2009). RAP abundance might also change with climate, showing a trend for higher RAP fractions in tropical than in temperate species (Baas, 1982; Wheeler and Baas, 1991). Interestingly, the amount of AP seems to vary considerably between different eco-regions, both at the intra- and interspecific level (Wheeler and Baas, 1991; Segala-Alves and Angyalossy-Alfonso, 2002), while no general trends have been reported for RP as a whole (Fahn *et al*., 1986). As far as we know, there is no published literature on actual comparisons between AP and RP amounts across climatic regions. Different RP compositions may not have any functional advantages across a wide range of climates (Baas, 1982). If this is so, then substantial changes in the overall RP fraction might not be significant, as this paper aims to elucidate. However, RP in *Juniperus thurifera* has been shown to vary in size and abundance annually, suggesting that RP formation in this species is sensitive to interannual changes in precipitation and temperature (Olano *et al.*, 2013).

When considering the valuable contributions from earlier studies, major ecological trends in the RAP levels remain unclear. The studies that have investigated the association between RAP and climate have either relied on qualitatively classifying species into broadly defined categories based on their RAP abundance (Baas, 1982; Wheeler *et al.*, 2007) or were designed as isolated case studies, which were limited in the number of species and sites investigated (Martinez-Cabrera *et al*., 2009; Olano *et al.*, 2013; Ziemińska *et al*., 2015). As far as we are aware, the largest datasets providing detailed information on both climate-related parameters and RAP levels encompass 61 species growing on eight different sites across South and North America (Martínez-Cabrera *et al.*, 2009) and 69 species collected on three sites along the east coast of Australia (Ziemińska *et al.*, 2015). In order to better understand the global variation in RAP levels, which could point to various functional consequences of low vs. high RAP fraction, we assembled an extensive dataset that includes many hundreds of species from different climatic zones while using more rigorous quantitative methods to characterise potential trends in RAP fractions. Our main objective was to investigate the association between climate and RAP

fraction across a broad range of species and environments. We expect there to be a strong relationship between both temperature and precipitation with RAP, and potentially higher RAP levels in tropical and subtropical species than in temperate species. Also, species that experience drought are hypothesised to show high levels of RAP because of potential correlations of RAP with drought resistance mechanisms such as xylem capacitance and refilling of embolised vessels (Brodersen and McElrone, 2013; Trifilò *et al.*, 2014).

We also aimed to elucidate the links between parenchyma fraction and growth form, and to explore differences in RAP amount between organs *viz*. roots, trunks and branches. We postulate that total RAP fractions are higher in lianas and succulents than in trees due to an increased demand for mechanical elasticity in climbing plants and the importance of water storage in succulents. Across different plant organs, higher RAP and especially RP levels can be expected in roots when compared to stems because of potential differences in storage capacity and mechanical properties (Gasson and Cutler, 1990; Stokke and Manwiller, 1994; Pratt *et al*., 2007). Finally, by way of a large quantitative analysis, we aim to support previous studies (e.g., Panshin and de Zeeuw, 1980) that total RAP fraction shows a divergence between angiosperms and conifers. A more detailed phylogenetic investigation of RAP fractions at lower taxonomic levels is beyond the scope of this study and was addressed recently in a range of Chinese species (Zheng and Martínez-Cabrera, 2013).

## *Materials and Methods*

### *Compilation of the global parenchyma dataset*

A total of 1,727 records of wood parenchyma percentages for 1,439 separate species were obtained from 55 different literature sources (Notes S1). The majority of these records reported values for trunks and branches of trees and shrubs. However, data from small branches of woody climbers and succulents together with data from woody roots were also included. If available, the total fractions of vessels and fibres, representing the other main cell types found in wood, were also collected.

Data on RP and AP were available for 1,268 angiosperm records, with 144 and 225 additional records for RP and total RAP (total of 1,637 records), respectively. In conifers, only 14 out of a total of 90 records reported values for both AP and RP. This is because AP is sparse in conifers

and therefore rarely measured. For this reason, it can be assumed that the total fraction of RP in conifers is equal to the total parenchyma content without introducing substantial bias to the total parenchyma estimate. Orthography and synonymy of species names were checked using the Plant List (version 1.1; www.plantlist.org). In 14 instances we were unable to match the species name reported by the author to any recognised taxon name, and these entries were omitted. Upon pooling together data for trunks and branches reported in the same publication (see the Results section), a core dataset of 1,727 entries (1,439 species and 127 families) was obtained and used for our analyses. The compiled dataset and corresponding reference list is provided in Table S1, except for the nearly 800 species from Zheng and Martínez-Cabrera (2013), which are accessible via the TRY Plant Trait Database (https://www.try-db.org/TryWeb/Home.php; Kattge *et al.*, 2011).

*Validation of the dataset*

High data variability is inherent to large datasets compiled from different literature sources, probably due to different methods used to quantify parenchyma. For example, RAP fractions were based on thin transverse wood sections using light microscopy (e.g., Martínez-Cabrera *et al.*, 2009; Ruelle *et al.*, 2006; Ziemińska *et al.*, 2013, 2015), or on polished wood surfaces using stereomicroscopy (Poorter *et al.*, 2010; Fichtler and Worbes, 2012). The relative fraction of parenchyma tissue can be analysed by measuring the entire area covered by the tissue or by estimating this area using a grid overlay (Huber and Prütz, 1938; Smith, 1967). In older books and wood atlases, the method used was not explicitly stated (Panshin and de Zeeuw, 1980; Koch, 1985; Wagenführ, 2007). Another complication is that it can be difficult to distinguish between AP and vasicentric tracheids or thin-walled living fibres in angiosperms.

As a check of accuracy we compared data taken from the literature to our own data, where we measured RAP fractions for 16 species, out of which 10 species and 14 genera were in our literature-based dataset. Transverse sections of woody branches 0.5 to 1cm in diameter were prepared with a sliding microtome, dehydrated in an ethanol series, stained with a mixture of safranin and alcian blue and mounted in Neo Mount (Merck KGaA). Digital images were taken with a stereo zoom microscope (Axio Zoom V16, Zeiss, Germany). A wedge-shaped region spanning a total area of about 0.5-1.5mm$^2$ from the cambium to the pith was outlined and individual areas taken up by the four principal wood tissues (RP, AP, vessels, and imperforate

tracheary elements, i.e. fibres and tracheids) within this region were segmented manually in Photoshop with the aid of a graphic tablet (Wacom, Cintiq Companion, model DTH-W1300). The areas were then measured with ImageJ (Rasband, 1997–2012) and converted to percentage proportions. For 12 temperate species, which were accessible on the Ulm University campus, three small branches from the same individual were measured for each species. For four tropical species, which were available in the greenhouses of the Botanical Garden of Ulm University, one branch could be harvested for measurements, and two radial transects were measured on a transverse section. In total, 16 species (two conifers, nine temperate angiosperms, four tropical angiosperms and one temperate climber) were measured. Our data were then matched to data for 10 species from the compiled dataset. Another comparison was made at the generic level for 14 genera.

*Climate data*

To investigate correlations between climate and the amount of RAP, AP and RP, we assigned the species into three broadly designated climatic zones: temperate, tropical, and subtropical. We used the climatic classification system devised by Köppen (1936), where temperate includes both maritime and continental types, with subtropical ranging from permanent wet to summer dry and winter dry, and tropical including permanent wet, summer dry, winter dry, and monsoonal.
To complement the categorical classification we looked up the spatial coordinates for species in our dataset to serve as proxies for species distribution. We used climate data by way of two different approaches: (1) based on exact locations from the literature, climatic data were obtained for 68 different sampling locations, including 461 different records and 411 species (including both angiosperms and conifers), and (2) where exact locations were not available from the literature, we used the Global Biodiversity Inventory Facility (GBIF), which allowed us to obtain climatic information for 619 species from 612 different locations, covering a wide range of latitudes, longitudes, and altitudes (Fig. S1).

For the approach based on exact locations, climatic data for each geographical location were extracted from layers of two major climatic databases using ArcGIS (Version 10.0.4.4, ESRI, California, USA). The layers of the mean annual temperature (MAT, °C) and mean annual precipitation (MAP, mm) were sourced from Bioclim layers based on the World Clim Global Climate Database (Hijmans *et al*. 2005) for the years 1950 to 2000. The potential evapotranspiration (PET) dataset for each month and the aridity index (AI, which is MAP

divided by PET) and mean precipitation of the driest quarter (MPDQ, which is the sum of the average precipitation in the three driest successive months) was taken from the Consortium for Spatial Information (CGIAR CSI). For the GBIF approach, the following criteria were used: (1) the record was not a duplicate according to the spatial coordinates of the sample, (2) we applied a cut-off at a minimum of 10 records per species for calculating the median location and corresponding climatic computations, and (3) the record was not located within 50 km of the GBIF headquarters in Copenhagen (55.68°N, 12.59°E) to minimise the chance that a record was given a coordinate that corresponded to where the data were housed, but not where the plant was actually collected.

*Statistical analyses*

Potential trade-offs in angiosperm trees between total RAP fraction and the percentage of vessels and fibre (including tracheids) fractions were analysed by plotting these three major xylem tissue fractions on a ternary axis, including a total number of 1,302 individual specimens (394 temperate, 428 tropical, and 480 subtropical).

We used non-parametric tests due to the lack of data normality. In particular, AP fractions were skewed towards smaller values. The paired-sample Wilcoxon signed rank test was used for evaluating the differences in parenchyma fractions between roots and stems (i.e., any part above soil level) within the same species. A Kruskal-Wallis and a pairwise Wilcoxon test were performed to detect differences in RAP fractions between conifers, angiosperm trees, the two specialized angiosperm growth forms (climbers and succulents), and between angiosperm trees from different climatic zones. Spearman's rank correlation coefficients were calculated to analyse the correlation between the tissue fractions of the three main xylem cell types: RAP, vessels, and fibres (including tracheids).

The parenchyma data for which exact locations were known was analysed separately to the data for which only GBIF-derived climate data was known. We analysed the effect of MAT, MAP and altitude on the proportion of parenchyma using a general additive model (GAM) with a binomial distribution using the mgcv package (Woods 2006). No further GAM analyses were carried out on PET, AI, and MPDQ due to a high co-linearity between these variables with MAT and MAP (Fig. S2). Each explanatory variable was fitted with a smoother and the maximum effective degrees of freedom (edf, which determines the amount of smoothing) were limited to

three partitions. All smooth terms are centred when fitting a GAM in order to ensure model identifiability (Woods 2006). GAM models were carried out on angiosperms only since the sample size was insufficient for conifers. All statistical analyses were performed using R (R Development Core Team, 2010).

## *Results*

### *Overview of the core dataset*

Within the core dataset of 1,727 entries, there were 36 records for woody roots and 1,691 records for woody stems. The latter can be further subdivided into 1,520 records of trunks or branches of angiosperm trees, 89 records of trunks or branches of conifer trees, 32 records of stems from woody climbers, and 50 records of stems from woody succulents (see Table 1 for an overview). In general, there was a 29-fold variation in RAP fractions, with total fractions varying from 3.4% in *Thuja occidentalis* (a coniferous tree) to 99% in *Adenia glauca* (a pachycaul succulent from the Passifloraceae family).

### *Validation of the dataset*

A close agreement between the literature data and our measurements was found when comparing 10 tree species ($r^2 = 0.571$) and 14 genera ($r^2 = 0.920$) for total RAP percentages (Fig. S3). The agreement at the genus level was lower for the individual RP and AP data, but still significant ($r^2 = 0.39$, $P < 0.05$, n = 15, and $r^2 = 0.777$, $P < 0.0001$, n = 12 for RP and AP, respectively). However, no significant correlation occurred when comparing the RP and AP fraction data from our measurements with literature data for the same species (n = 14 and 8 for RP and AP, respectively), indicating that there were either potentially important concerns with AP and RP fractions reported in literature for any given species due to varying methodologies, intraspecific differences, or interspecific variation. The latter two could be due to developmental age, the organ, or sampling position. AP in particular seems to be the most problematic to quantify because identifying AP on transverse sections can be difficult due to anatomical similarities with thin-walled living fibres or tracheids (Stokke and Manwiller, 1994; Carlquist, 2014). Therefore, most of our analyses focussed on the more robust RAP data, while conclusions about the relative contribution of RP and AP should be interpreted with caution.

**Table 1.** Data summary of the global xylem parenchyma dataset compiled from the literature with respect to the total number of entries, literature sources, taxa (including angiosperms and gymnosperms) and parenchyma tissue fractions in wood RP, ray parenchyma; AP, axial parenchyma; RAP, ray and axial parenchyma; cv, coefficient of variation.

| | **RP** | **AP** | **RAP** |
|---|---|---|---|
| *n* entries | 1502 | 1282 | 1582 |
| *n* resources | 48 | 38 | 50 |
| *n* species/genera/families | 1265/542/119 | 1142/518/113 | 1364/596/123 |
| Mean (%) | 17.4 | 7.2 | 27.2 |
| Median (%) | 16.4 | 3.3 | 22.6 |
| Min (%) | 2.3 | 0 | 3.4 |
| Max (%) | 68.4 | 74 | 99 |
| Cv | 45.4 | 129.8 | 57.9 |

***Differences between organs, growth forms, and angiosperms vs conifers***

The differences in parenchyma percentage between roots and stems (including both trunks and branches) were not profound. Slightly higher RP fractions were found in roots than in trunks and branches (paired-sample Wilcoxon signed rank test, V = 205, $P = 0.04$, n = 23), whereas the difference in AP and total RAP was not significant (V = 91.5 and 292, $P > 0.05$, n = 22 and 31, respectively).

Data for both trunks and branches showed no significant difference in RP, AP and RAP fractions (V = 237-298.5, $P > 0.05$, n = 33-34). Therefore, trunks and branches were pooled together and their average was used for further analyses.

Significant differences in RAP fractions were detected between conifer and angiosperm trees, and between specialised growth forms (stem succulents and lianas), within the angiosperm group, using stem (i.e., trunk or branch) data only (Kruskal-Wallis test, $\chi^2 = 118.6$, $P < 0.001$, df = 3, Fig. 3). Stem succulents showed the highest values of RAP (mean ± SD: 70.2 ± 22.0%, n =

50), followed by lianas (50.1 ± 16.3%, n = 28), and angiosperm trees and shrubs (26.3 ± 12.4%, n = 1,384), whereas conifers exhibited the lowest fraction of RAP (7.63 ± 2.6%, n = 89, Fig. 3a). In angiosperm trees, there were many entries with rather high RAP, e.g. 136 entries (118 species) showed total RAP fractions above 50%.

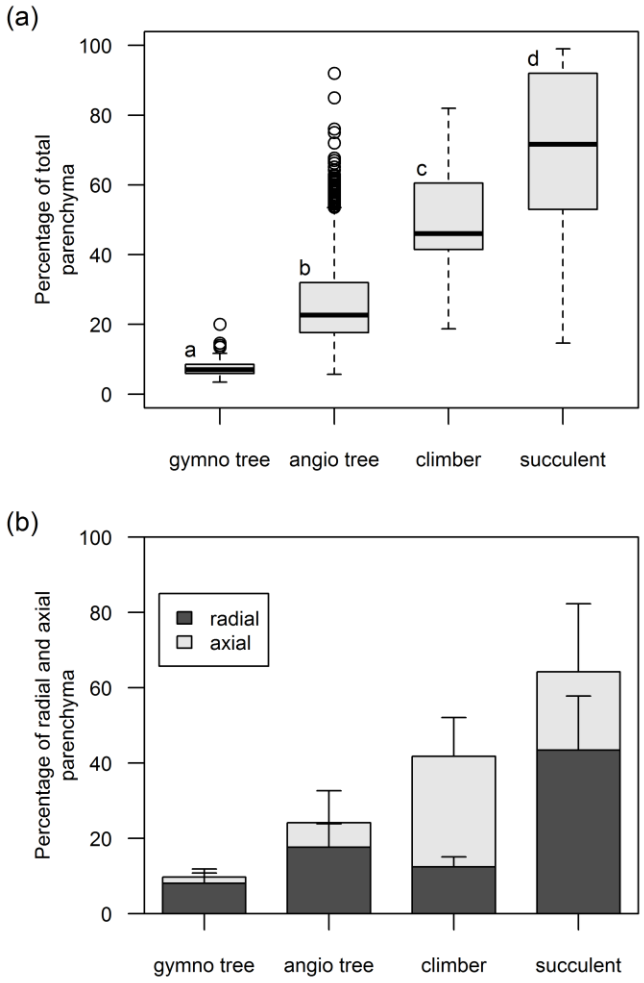



**Fig. 3** Parenchyma tissue fractions (%) across different woody plant groups and growth forms. Because of inherent differences in wood anatomy between conifer and angiosperm trees, both groups are analysed separately. The lowest parenchyma fractions are found in conifers, followed by angiosperm trees (including some shrubs), climbers and succulents. The percentage of total parenchyma (including axial and ray parenchyma) is shown in Fig. 3a, with the ray and axial parenchyma contribution for the same groups given in Fig. 3b. The box plot in Fig. 3a shows the median, 25th and 75th percentiles, error bars show 10th and 90th percentiles, and open circles show outliers. Different letters above boxes indicate statistical significance between groups (Kruskal-Wallis test, $P \leq 0.05$). Bars in Fig. 3b represent mean values ± SD. The number of specimens for Fig. 3a (with the number of specimens for Fig. 3b between brackets) is: n gymno tree = 89 (14), n angio tree =1,384 (1,205), n climber = 28 (9), and n succulent = 50 (32).

In addition to the total RAP percentage, the contribution of RP and AP was analysed (Fig. 3b), although these data should be interpreted with caution as mentioned above. The information on RP and AP fractions was available for stems of 14 conifer species, 1,205 angiosperm tree species, nine climbers, and 32 succulents. Again, conifers showed much lower fractions of both RP and AP (RP: 8.1 ± 2.7%; AP: 1.7 ± 2.2%) than angiosperm trees (RP: 17.7 ± 6.3%; AP: 6.6 ± 8.5%, Fig. 3b). Climbers in our dataset had the highest fraction of AP (29.3 ± 10.2%), while RP was relatively low (12.5 ± 2.7%). Succulents showed a high fraction of both RP and AP (RP: 43.5 ± 14.3%, AP: 20.8 ± 18.1%, Fig. 3b).

*Climate and RAP fractions*

Differences in RAP fractions in angiosperm trees (including some shrubs) growing in various climatic zones were analysed for 399 temperate, 442 tropical and 543 subtropical specimens (Kruskal-Wallis test, $\chi^2 = 224.9$, $P < 0.001$, df = 2), with mean values of 21.1% (± 7.9), 22.2% (± 9.3 and 36.2% (± 13.4) for temperate, subtropical and tropical angiosperm specimens, respectively (Fig. 4a). The amount of AP appeared to be the main driver of this difference. The total AP fraction was 13.8% (±11.0) in tropical trees, whereas it was between 4 to 5% in temperate and subtropical trees (see Fig. 4b). In contrast, average RP fractions spanned a narrow range from 16.4% (± 5) in temperate to 19.4% (± 6.8) in tropical trees.

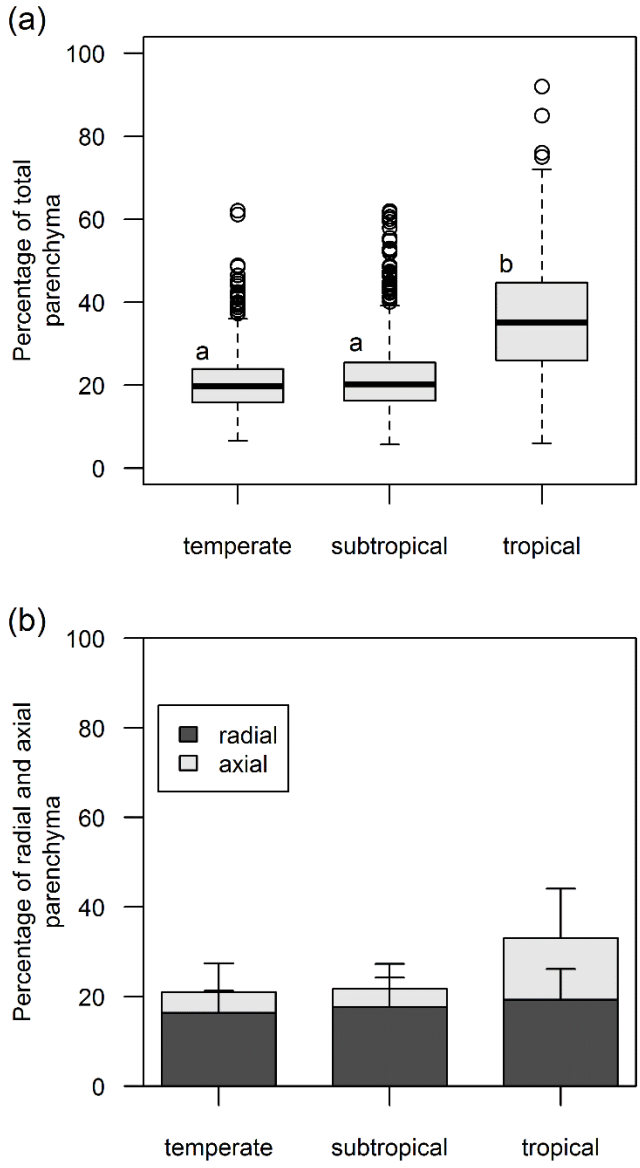



**Fig. 4** Parenchyma tissue fractions (%) in angiosperm trees classified according to the temperate, subtropical and tropical biome, showing the total parenchyma fraction (Fig. 4a) and the individual contributions of ray and axial parenchyma (Fig. 4b). The box plot in Fig. 4a shows the median, 25th and 75th percentiles, error bars show 10th and 90th percentiles, and open circles show outliers. Different letters above boxes indicate statistical significance for tropical species compared to temperate and subtropical trees (Kruskal-Wallis test, $P \leq 0.05$). Bars in Fig. 4b represent mean values ± SD. The number of specimens for Fig. 4a (Fig. 4b): n temperate = 399 (399), n tropical = 442 (287), n climber = 28 (9), and n succulent = 50 (32).

The ternary axis (Fig. 5) revealed that a higher contribution of RAP occurred mainly at the expense of fibres, particularly in tropical and subtropical trees, while total vessel fractions were typically between 5 to 20%, and on average 14.6%. A strongly negative correlation was found between the tissue fractions for RAP and fibres (including tracheids) for all biomes, especially the tropical climate (Spearman r = -0.75, $P < 0.001$ for all biomes; Table 2). Fibre tissue fractions (F) were most negatively correlated with vessel tissue fraction (V) in temperate and subtropical species (r = -0.66, and -0.59, respectively; $P < 0.001$), while a negative relationship between RAP and V was only weakly significant for the temperate and tropical climate (r = -0.21 and -0.18, respectively; $P < 0.001$; Table 2). In some temperate trees, the relatively high vessel fractions represented ring-porous species with narrow growth rings that have a high proportion of early-wood and, therefore, many wide vessels.

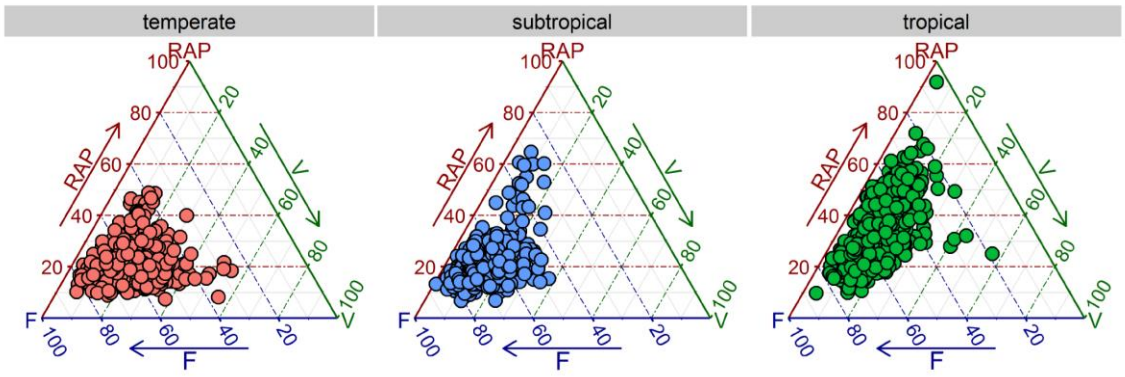



**Fig. 5** Ternary plot showing the contribution of the three principal tissues in wood: the total amount of ray and axial parenchyma (RAP, %), vessels (V, %), and fibres (including tracheids) (F, %). Each dot represents a specimen. The trade-off between RAP and F tissue fractions shows a strongly negative correlation for all data and is especially clear in the tropical biome (see Table 2). Specimens are grouped according to three major climatic zones, which follow Köppen (1936).

**Table 2.** Spearman's rank correlation coefficients showing the trade-offs between the tissue fractions of three major xylem cell types, which were analysed for three major biomes (temperate, subtropical, tropical). See Fig. 5 for a visual plot. RAP, ray and axial parenchyma; F, fibres (including tracheids); V, vessels. ***, $P \leq 0.001$.

| Correlation | All biomes | Temperate | Subtropical | Tropical |
|---|---|---|---|---|
| RAP vs F | −0.752*** | −0.480*** | −0.688*** | −0.856*** |
| RAP vs V | −0.153*** | −0.210*** | −0.066 | −0.176*** |
| F vs V | −0.427*** | −0.661*** | −0.591*** | −0.279*** |

There was a strong agreement between the climatic data derived from the sampling locations and those derived from the GBIF locations (Fig. S4). There was a clear difference between angiosperms and conifers, as we found only significant correlations between RAP and MAT, and between RAP and MAP for angiosperms. Moreover, due to a low sample number (90 records, 61 species) and limited number of locations, no climatic GAM analyses were performed on conifers.

The GAM models showed that MAT was the main driver for RAP in angiosperms ($F_{1.94, 267} = 37.21$, pseudo-$R^2 = 21.05\%$, P-value < 0.001; for exact locations, and $F_{1.95, 218} = 48.22$, pseudo-$R^2 = 31.65\%$, $P < 0.001$ for GBIF locations, Fig. 6a). However, the effect of MAT on RAP was non-linear, with RAP fractions increasing with MAT values in hot environments (> ± 17°C), but not for species in colder environments. When analysing the effect of MAT on AP and RP fractions separately, GAM models revealed significant associations for both when controlling for altitude and precipitation (Fig. S5a, Fig. S6a; Table S2, S3).

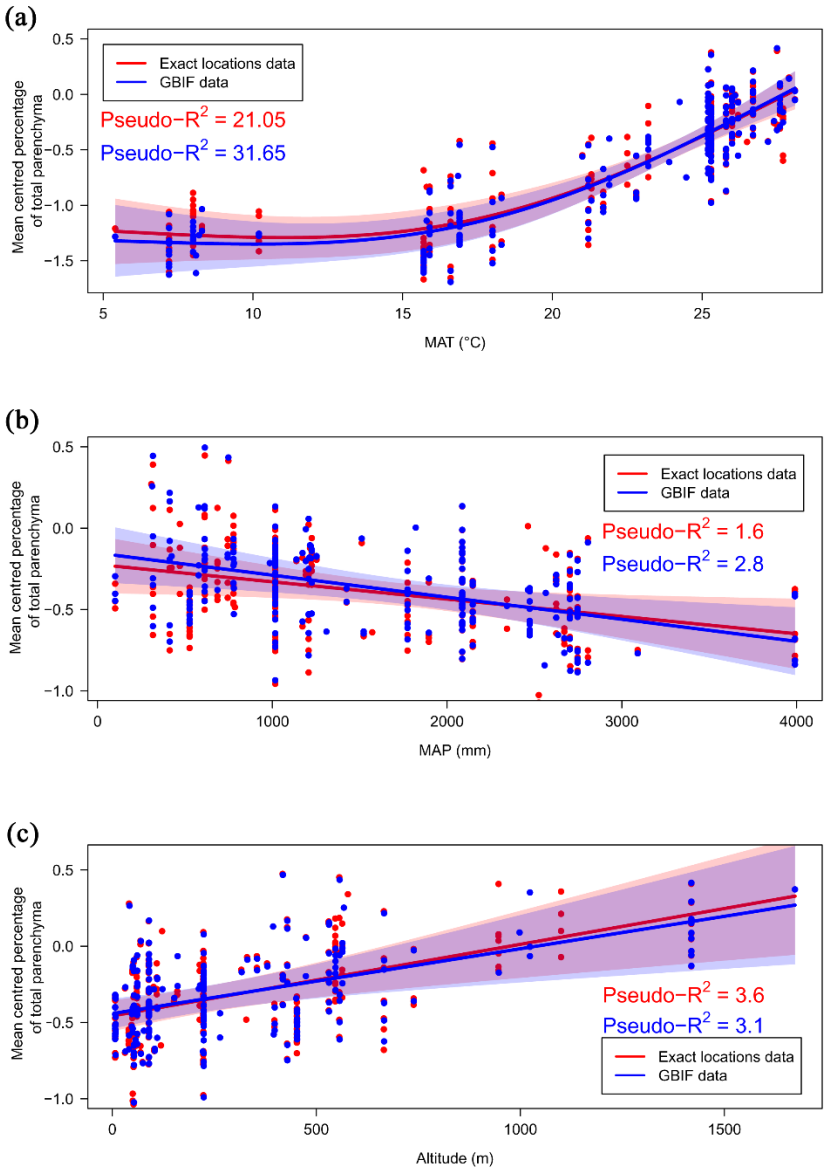



**Fig. 6** The effect of MAT (a), MAP (b), and altitude (c) on the proportion of ray and axial parenchyma (RAP) in angiosperm wood based on a general additive model with a binomial distribution for the exact location dataset (red) and the GBIF derived climate data (blue). Each climate variable was limited to three effective degrees of freedom (e.d.f.). The solid line represents the fitted smoother; 95% confidence intervals are shown as coloured tinted areas. Each dot represents a specimen for which the sampling location was reported in literature, or climate data obtained from the WorldClim database. Pseudo-$R^2$ measures the approximate deviance explained by each explanatory variable.

The relationship between MAP and RAP was also significant, although the pseudo-$R^2$ values were very low compared to those of MAT (Fig. 6b; Table S2, S3). While significance was found for the relationship between MAP and RP (Fig. S6b), this was not the case for AP (Fig. S5b; Table S2, S3). When controlling for MAP and MAT, RAP increased significantly with altitude (Fig. 6c), but these altitudinal trends were non-significant or significant for AP and RP, depending on whether the data were based on the exact locations or GBIF (Fig. S5c, Fig. S6c).

A separate GAM model between total RAP fraction and temperature also supported a quadratic latitudinal trend (Fig. 7), for both exact locations ($F_{1.95, 267} = 27.01$, pseudo-$R^2 = 18\%$, $P < 0.001$) and species from the GBIF locations ($F_{1.96, 218} = 31.81$, pseudo-$R^2 = 24.1\%$, $P < 0.001$). The latter suggests that RAP increased significantly the closer the sampling location was to the equator for both datasets.

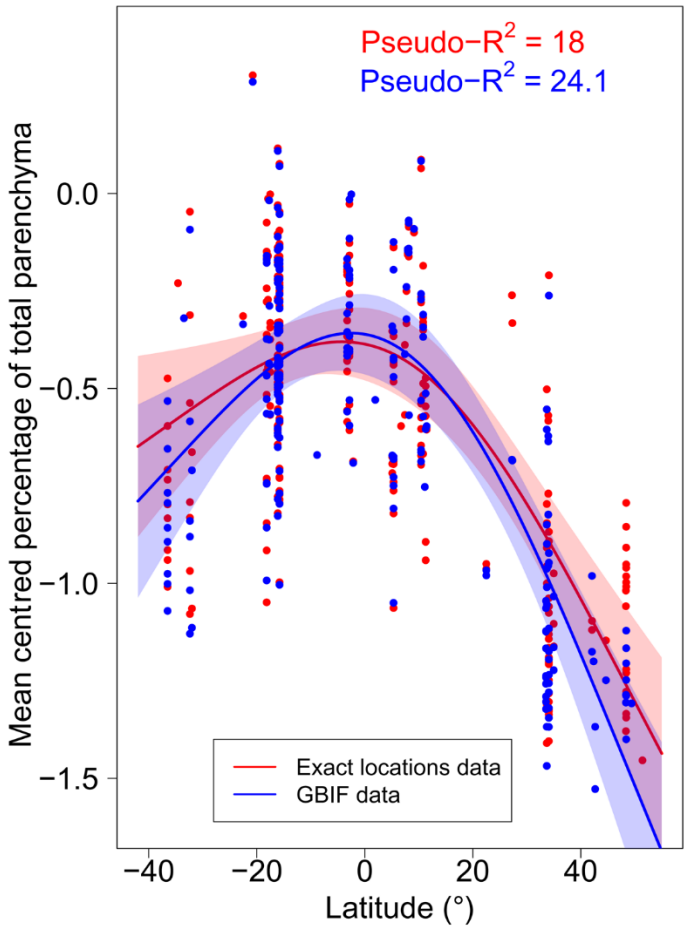



**Fig. 7** The effect of latitude on the proportion of ray and axial parenchyma (RAP) in angiosperm wood based on a general additive model with a binomial distribution for the exact location dataset (red) and the GBIF derived climate data (blue). The latitude variable was limited to three partitions. The solid line represents the fitted smoother; 95% confidence intervals are shown as coloured tinted areas. Each dot represents a specimen for which the sampling location was reported in literature, or climate data obtained from the WorldClim database. Pseudo-$R^2$ measures the approximate deviance explained by latitude.

### *Discussion*

The global RAP dataset compiled demonstrates that RAP tissues show a 29-fold variation in abundance, which agrees with previous reports across seed plants (Fujiwara *et al.*, 1991; Martínez-Cabrera *et al.*, 2009; Ziemińska *et al.*, 2013, 2015; Zheng and Martínez-Cabrera, 2013). A key finding is that RAP amounts in wood, especially AP, are driven by temperature (Fig. 6a). Albeit, precipitation also showed a significant relationship with RAP in the GAM models when controlling for altitude and MAT, although far less so than temperature (Fig. 6b; Table S2, 3). MAP showed a negative trend in the GAM models, with increasing amounts of RAP towards drier environments, which is what we expected. The temperature effect is also reflected in the latitudinal trends for RAP, especially in the northern hemisphere. However, our analyses did not support the expected difference between subtropical and temperate species (Fig.4b). Also, no significant difference was found in the RAP tissue fraction between tropical wet environments and tropical, seasonally dry areas (data not shown).

Other drivers of RAP tissue fractions include growth forms. It is clear that lianas and succulents represent two growth forms that show higher fractions of RAP than self-supporting angiosperm trees. Within the tree and shrub growth form, tropical plants have higher levels of RAP than temperate and subtropical ones (Fig. 4, 5), supporting previous studies based on qualitative (Baas, 1982; Segala-Alves and Angyalossy-Alfonso, 2002; Wheeler *et al.*, 2007) and quantitative approaches (Martinez-Cabrera *et al*., 2009). A novel finding is the fact that RAP levels in tropical plants are mainly due to an increase in AP, while RP levels remain more conservative in trees across the three major biomes analysed (Fig. 4b). This finding is not as transparent in the GAM models (Figs. S4, S5), as both RP and AP are highly associated with temperature. However, where RP amount gradually increases with temperate, AP remains unwavering until approximately 17ºC and then sharply rises exponentially with temperature. This is the reason AP is so high in the tropics compared to both temperate and subtropical regions (Fig. 4b).

Explaining why some factors may drive the RAP level while others have little or no influence requires a more detailed understanding of RAP functions (Fig. 2), especially those related to storage capacity, resistance to drought stress, frost resistance, defence mechanisms and mechanical properties.

*RAP fractions in relation to NSC storage capacity*

Storage of NSCs (non-structural carbohydrates) is arguably one of the most widely accepted functions of RAP in wood. As far as we know, however, the hypothesis that high amounts of RAP correspond with higher storage capacity of NSCs has not been tested yet in a direct, quantitative way. The assumption that higher amounts of RAP, and therefore a higher NSC capacity, should occur in roots when compared to stems could only partly be confirmed. We found no significant differences between stems and roots in total RAP and AP fractions, but did find higher levels of RP in root wood than in stem wood. As this finding came from a small sample set (n = 21-30), it is premature to make generalisations. A higher fraction of RP in roots than stems has been observed previously (Gasson and Cutler, 1990; Stokke and Manwiller, 1994; Machado *et al.*, 2007) and may be associated with a reduced need for mechanical cells such as fibres in addition to increased storage capacity of roots. Aside from RP fractions, different cell dimensions of RP have been reported in roots, especially a general increase in width of the entire ray and the individual ray cells, and a tendency towards more heterocellular rays in roots than in stems (Patel, 1965; Gasson and Cutler, 1990; Denne and Gasson, 2008).

When considering the storage capacity of RAP we could also expect to find higher RAP fractions in plants from temperate seasonal climates. However, we did not find much support for this hypothesis due to lower RAP fractions in trees from temperate compared to tropical biomes. RAP are not the only wood tissue storing NSCs; septate or living fibres also do so (Webber, 1936; Yamada et al., 2011; Carlquist, 2014).

*RAP fractions and drought stress*

It is possible that high RAP fractions in wood benefit plants in dry conditions by conferring high hydraulic capacitance, which could prevent embolism formation, or facilitate embolism refilling (Fig. 6b). Support for a drought related function of RAP is provided by the high levels of RAP in stem succulents. With an average of 70.3% RAP in succulents, it can be speculated that wood parenchyma not only stores a considerable amount of water but also provides symplastic connections with bark and pith that both serve as important water reservoirs (Borchert and Pockman, 2005; Scholz *et al*. 2007; Hearn, 2009, Hearn *et al*., 2013; Pfautsch *et al*., 2015). However, the large RAP variation in dry and seasonally dry environments suggests that plants have various strategies to survive these conditions, with stem succulents having large

parenchyma fractions while other species may show comparatively few RAP cells. It has been suggested that vessel-associated RAP may be involved in the embolism refilling process by releasing sugars and water into embolised conduits (Bucci *et al.*, 2003; Salleo *et al.*, 2004; Brodersen *et al.*, 2010; Brodersen and McElrone, 2013). While embolism repair remains controversial and poorly understood, refilling on a daily basis may not occur in conifers, which could be owing to their low RAP fractions (Choat *et al.*, 2014).

*RAP fractions and temperature*

Temperature is associated with RAP fractions in the secondary xylem, much more so than rainfall. This finding agrees with Moles *et al.* (2014), who found that 15 out of 21 plant traits are more strongly correlated with temperature than with precipitation. The high levels of RAP in tropical plants could be linked to the greater plant diversity in the tropics and the dominance of various families with high RAP levels (e.g., Fabaceae, Moraceae). Alternatively, particular functions associated with RAP (e.g., defence against pathogens, hydraulic capacitance) could be more important in tropical environments than in temperate regions. It is possible that protection against cold, including tolerance to extracellular freezing and freeze dehydration, or freeze avoidance by super-cooling, is an energy-demanding process (Quamme, 1991; Neuner, 2014), which could therefore be an important factor in reducing the RAP fraction in woody plants exposed to frost or freezing events. Also, two conifer genera growing in the tropical/subtropical mountains of the southern hemisphere (*Podocarpus* spp. and *Dacrydium* spp.) have higher RAP fractions than conifers from cool temperate regions (Braun, 1984). Similarly, *Pinus canariensis* from the warm islands of Tenerife and La Palma have RAP averages of 14.5%, with AP values accounting for 3% of this (Climent *et al.*, 1998).

*RAP fractions as a defence system*

RAP fractions might also play a large role in defence against the spread of decay via pathogenic fungi, viruses, and bacteria. The presence of RP may prevent the lateral spread of fungi, while AP does the same for axial movement (Shigo, 1984; Boddy and Rayner, 1983; Biggs, 1986). Because both RP and AP may accumulate anti-microbial compounds such as phytoalexins, phenolic compounds and suberin, which all act to inhibit fungal spread (Biggs, 1987), trees with high RAP fractions might be more resistant to brown rot fungi and therefore be better overall compartmentalisers of decay than trees with lower RAP fractions (Schwarze *et al.*, 2003).

In agreement with the defence role, the amount of RAP is higher in the sapwood of trees that have recovered from pathogenesis (Tippett and Shigo, 1981; Schmitt and Liese, 1990; Arbellay *et al.*, 2010, 2012). Interestingly, another study across seven tree species from the Amazon, have found that high parenchyma abundance and wide dilating rays were associated with poor compartmentalisation of decay, but was offset by fast wound closure (Romero and Bolker, 2008). While in angiosperms, parenchyma cells in contact with vessels can seal off conduits by way of tyloses or gum deposits to avoid the spread of decay (Biggs, 1987; Bonsen and Kučera, 1990; Schmitt and Liese, 1992; Sun *et al.*, 2007), defence in gymnosperms mainly lies in the occlusion of tracheids via aspiration of the torus-margo bordered pits (Fuhr *et al.*, 2013) and the production of abundant polyphenolic compounds and traumatic resin ducts in Pinaceae and Cupressaceae (Phillips, 1948, Hudgins *et al.*, 2004). Such a strategy is therefore consistent with a lower RAP fraction in conifers. In contrast, a higher fraction of RAP in the tropics might be driven by a greater incidence of biotic stress when compared to temperate environments (Bagchi *et al.*, 2014). The evolutionary 'arms race' with pathogens and insect herbivores may require the deployment of more RAP or the synthesis of a more diverse suite of secondary compounds by RAP in order to enhance tree defence abilities. On the other hand, the trade-off between RAP and fibre tissue fractions (Fig. 5) may also suggest that high RAP fractions could equally decrease the defence capacity.

*RAP and mechanical properties*

Since the amount of RAP occurs mainly at the expense of fibres, one would also expect important effects on wood mechanical properties. It has long been assumed that parenchyma cells often have larger lumina and thinner cell walls than fibres, so high RAP levels should theoretically result in lower wood density together with a reduced stiffness, but retain a higher elasticity. However, rays in *Liquidambar* were found to have a higher specific gravity than surrounding tissues because the ratio of cell wall to lumen was relatively high (Taylor, 1996). Moreover, in a study of 69 angiosperm species it was found that the main driver of the modulus of elasticity (defined as the ratio of tensile stress to tensile strain) was fibre wall fraction rather than RAP fraction (Ziemińska *et al.*, 2015). However, that study only looked at species within a small wood density range and the results should be treated with caution.

Several authors have suggested that wide rays, which are common in lianas, allow vessel-bearing segments to twist without rupturing, which may also explain the occurrence of non-lignified or

less lignified ray parenchyma cells in climbing plants (Schenck, 1893; Haberlandt, 1914; Sieber and Kučera, 1980; Gartner, 1991; Putz and Holbrook, 1991). Additional explanations for the high amount of RAP in lianas can be linked with dedifferentiation of parenchyma, allowing rapid recovery from injury, especially after tree fall (Dobbins and Fisher, 1986; Fisher and Ewers, 1991; Busch *et al*., 2010), and their ability to clone readily when detached from the parent plant (Putz, 1984; Yorke *et al*., 2013). A mechanical role of RAP dependant on their turgor pressure has also been suggested in *Adansonia* (Chapotin *et al.*, 2006), with RAP in this species occupying as much as 90%.

### *General Conclusion*

The 29-fold variation in the parenchyma fraction is associated with temperature-driven differences between tropical, subtropical and temperate woody plants, as well as with different growth forms such as succulents and lianas. The ecological trends discussed suggest ways for further research into how RAP plays a role in woody plant function in the storage of NSCs and water, defence against pathogens, and resilience to damage. Various functions of RAP in wood have been suggested and it is generally not clear which function takes precedence in a given situation. Based on the available evidence, this may depend on climate, plant organ, and the potential partitioning in the functional roles of RP and AP (Zheng and Martínez-Cabrera, 2013). Also, the total RAP percentage does not reflect the spatial, three-dimensional arrangement of the parenchyma network and its connectivity to other xylem tissues. More research is also needed to test the within tree variation of RAP %, which would involve labour intensive studies with careful and well-planned sampling. Further research based on observational evidence is needed to investigate the role of parenchyma in more detail, such as the spatial patterns of parenchyma networks and, along with this, to test the hypotheses presented in this paper.

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### *Author contributions*

H.M., L.P. and S.J. led the initial data compilation and coordinated the writing. H.M., L.P., S.J., E.F., H.I.M.-C., J.Z. and K.Z. contributed data and ideas. L.P. and M.A.F.G. analysed data. K.Z. and E.W. assisted with writing the final manuscript. H.M., P.C. and D.J.MG. extracted GBIF locations and climate data.

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## Supporting Information for *New phytologist*

# A global analysis of parenchyma tissue fractions in secondary xylem of seed plants

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The following Supporting Information is available for this article:

**Fig. S1** Distribution map of the species for which parenchyma fraction values were compiled.

**Fig. S2** Poly-co-linearity matrix for the parameters analysed in relation to wood anatomy, plant organ, geography and climate.

**Fig. S3** Comparison of total parenchyma fractions in wood based on our own measurements and literature.

**Fig. S4** Comparison of mean annual temperature and mean annual precipitation for species for which both sampling locations and GBIF locations were available.

**Fig. S5** The effect of MAT, MAP and altitude on the proportion of axial parenchyma in angiosperms.

**Fig. S6** The effect of MAT, MAP and altitude on the proportion of ray parenchyma in angiosperms.

**Table S1** The Global Wood Parenchyma Database (see separate file).

**Table S2** Summary of statistics for the general additive models (GAM) based on the exact locations dataset.

**Table S3** Summary of statistics for the general additive models (GAM) based on the GBIF

locations dataset.

**Notes S1** Published references from which data were extracted for analyses.

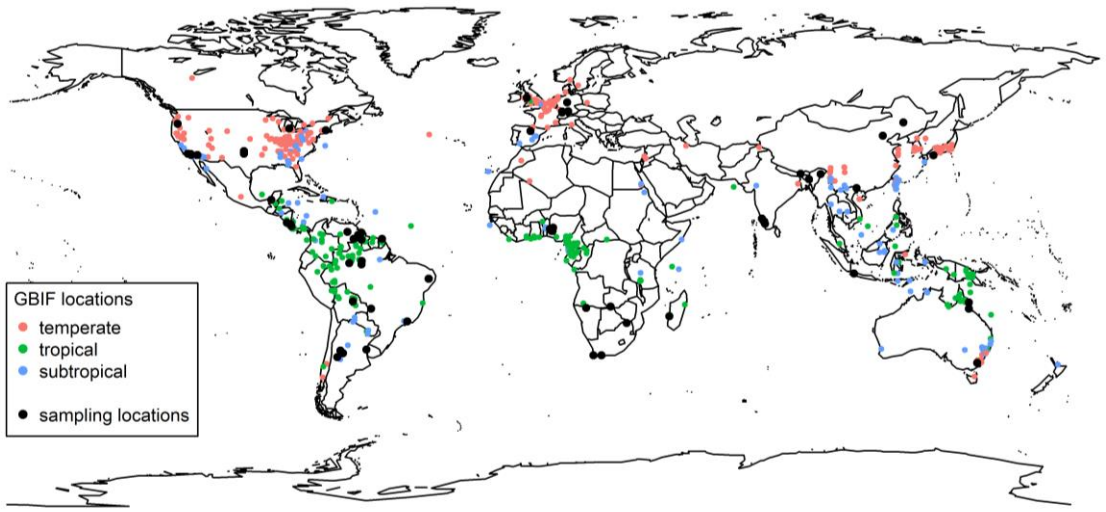



**Fig. S1** Distribution map of 612 GBIF locations and 68 exact sampling locations for angiosperm and conifer species for which parenchyma fraction values were compiled in a global xylem parenchyma dataset (see Table S1).

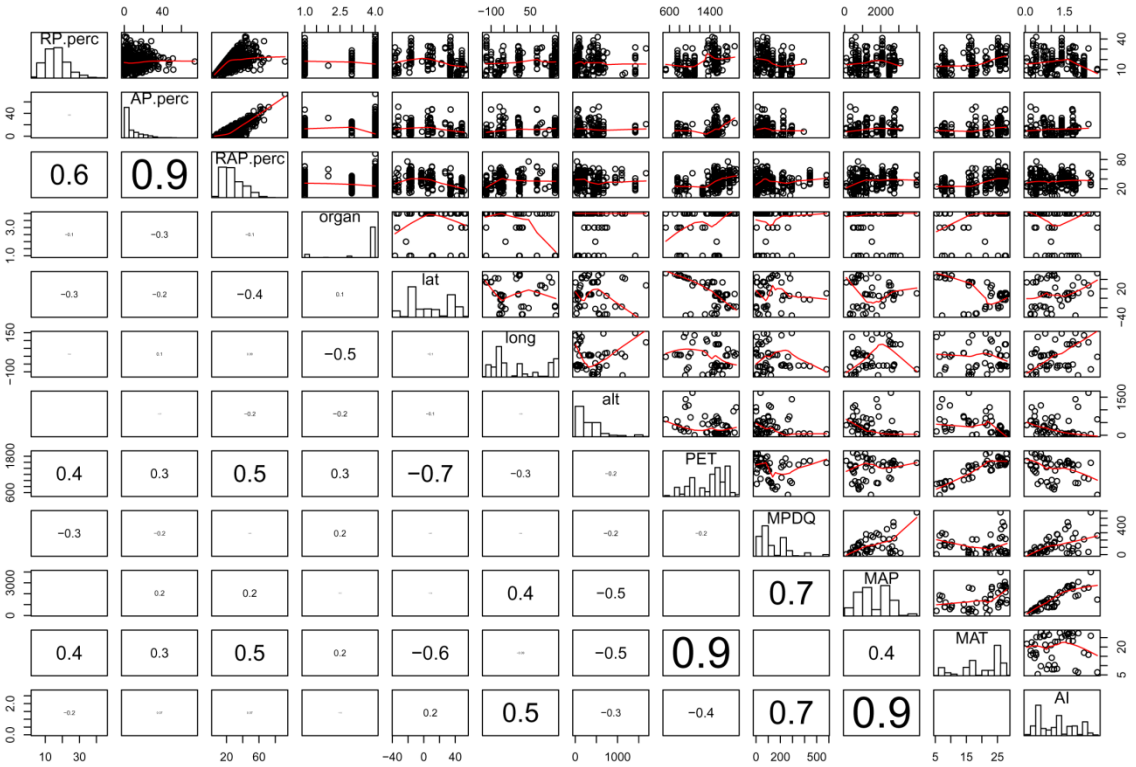



**Fig. S2** Poly-co-linearity matrix based on the GBIF dataset for the following parameters: ray parenchyma (RP.perc), axial parenchyma (AP.perc), ray and axial parenchyma (RAP.perc), organ (root, trunk, branch), latitude (lat), longitude (long), altitude (alt), potential evapotranspiration (PET), mean precipitation during the driest quarter (MPDQ), mean annual temperature (MAT), aridity index (AI).

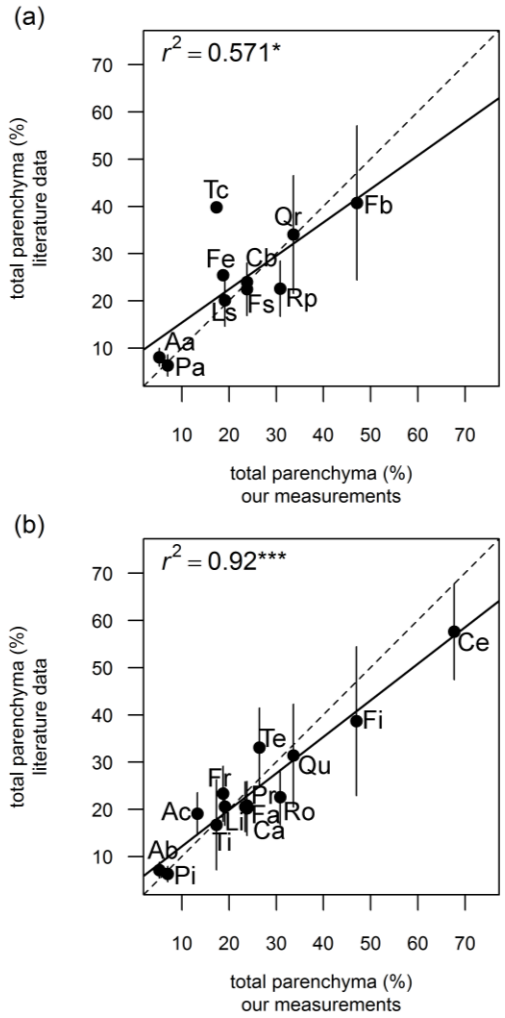



**Fig. S3** Comparison of total parenchyma fractions in wood based on our own measurements and data from literature for 10 species (a) and 14 genera (b). The following species were included in Fig. S3a, with the number of specimens obtained from literature between brackets: Aa = *Abies alba* (3), Cb = *Carpinus betulus* (1), Fs = *Fagus sylvatica* (5), Fb = *Ficus benjamina* (2), Fe = *Fraxinus excelsior* (2), Ls = *Liquidambar styraciflua* (3), Pa = *Picea abies* (4), Qr = *Quercus robur* (3), Rp = *Robinia pseudoacacia* (5), and Tc = *Tilia cordata* (1). The genera included in Fig. S3b (with reference to the number of species/specimens from literature) are: Ab = *Abies* (6/9), Ac = *Acer* (14/19), Ca = *Carpinus* (8/8), Cei = *Ceiba* (2/5), Fa = *Fagus* (4/8), Fi = *Ficus* (29/31), Fr = *Fraxinus* (7/12), Li = *Liquidambar* (3/5), Picea (5/8), Pr = *Prunus* (9/9), Qu = *Quercus* (39/60), Ro = *Robinia* (1/5), Te = *Terminalia* (9/12), and Ti = *Tilia* (9/9). The regression (solid) line and the 1:1 (dashed) line are shown. *** indicates $P \leq 0.001$, *indicates $P \leq 0.05$.

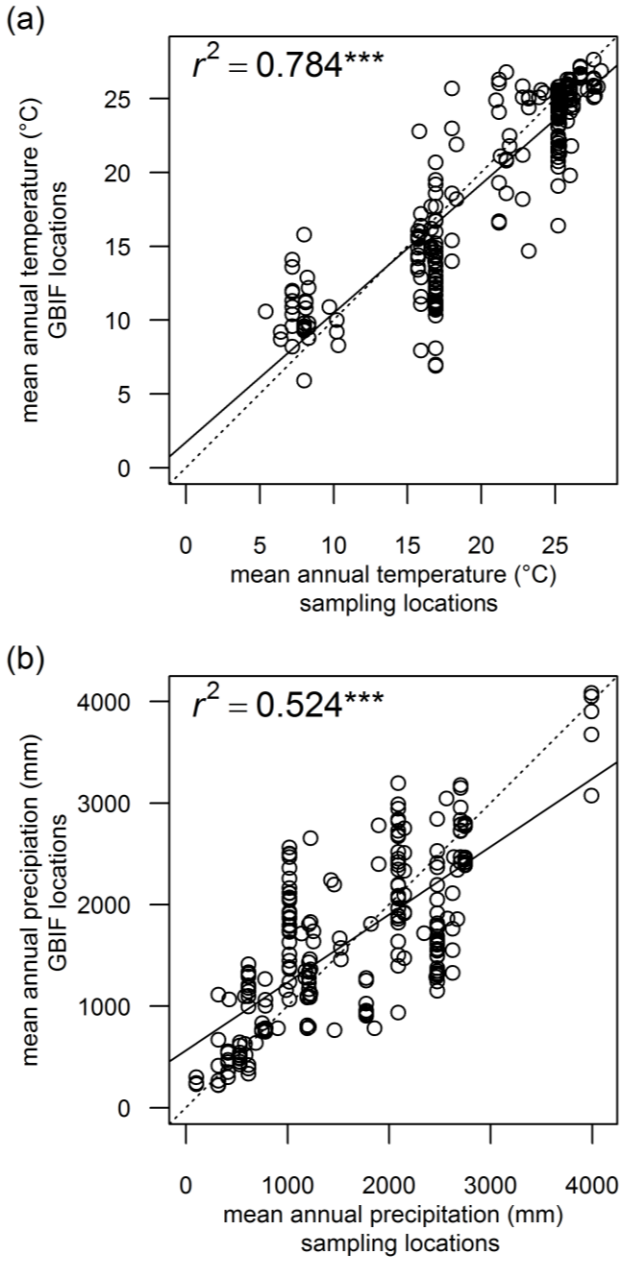
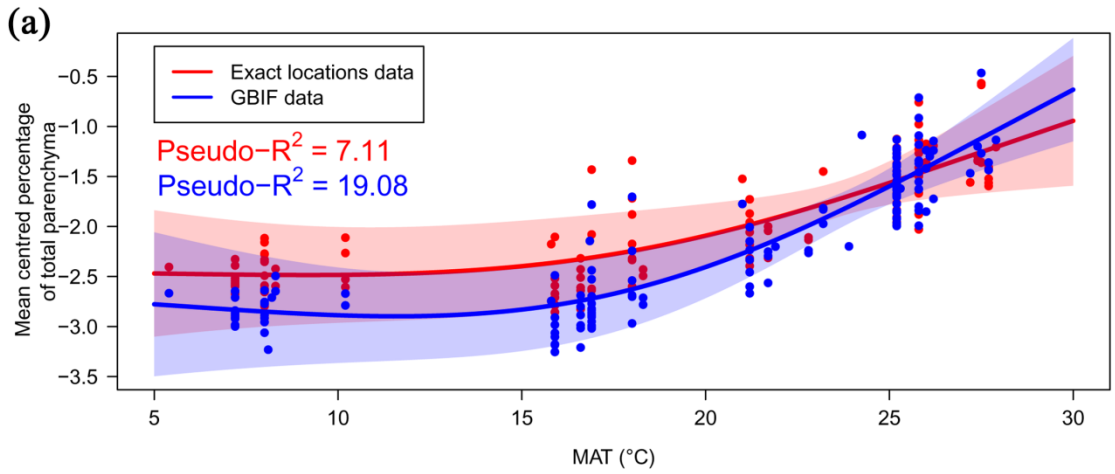
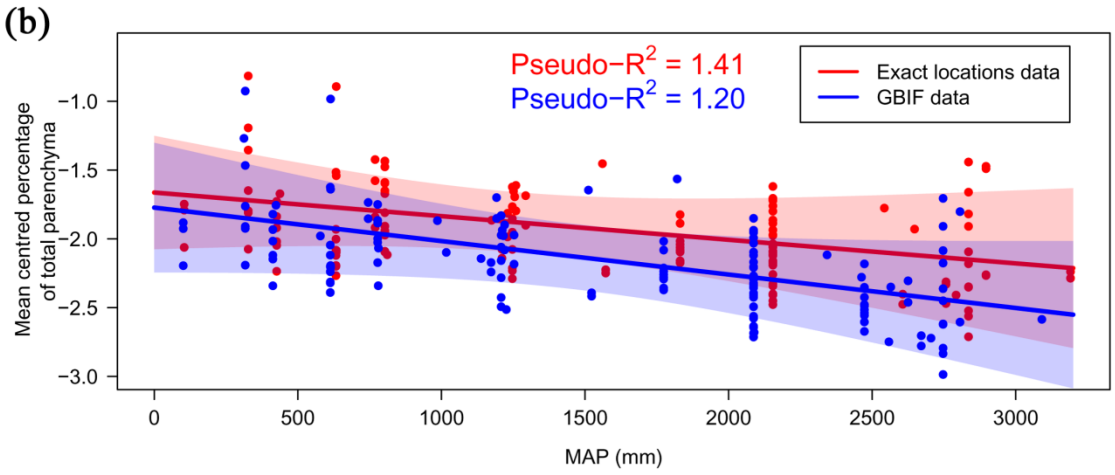



**Fig. S4** Comparison of mean annual temperature (a) and mean annual precipitation (b) values for 244 species from the global xylem parenchyma dataset for which both sampling locations and GBIF locations were available. The regression (solid) line and the 1:1 (dashed) line are shown. *** indicates $P \leq 0.001$.


(a)
Exact locations data
GBIF data
Pseudo−R2 = 7.11
Pseudo−R2 = 19.08
Mean centred percentage of total parenchyma
−0.5
−1.0
−1.5
−2.0
−2.5
−3.0
−3.5
5
10
15
20
25
30
MAT (°C)


(b)
Pseudo−R2 = 1.41
Pseudo−R2 = 1.20
Exact locations data
GBIF data
Mean centred percentage of total parenchyma
−1.0
−1.5
−2.0
−2.5
−3.0
0
500
1000
1500
2000
2500
3000
MAP (mm)

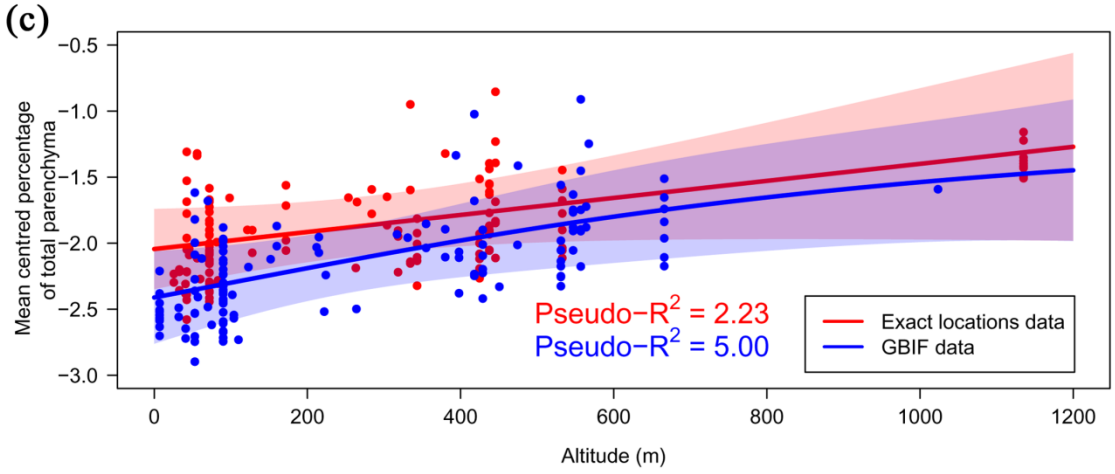

(c)
Pseudo−R2 = 2.23
Pseudo−R2 = 5.00
Exact locations data
GBIF data
Mean centred percentage of total parenchyma
−0.5
−1.0
−1.5
−2.0
−2.5
−3.0
0
200
400
600
800
1000
1200
Altitude (m)

**Fig. S5** The effect of MAT (a), MAP (b), and altitude (c) on the proportion of axial parenchyma (AP) in angiosperm wood based on a general additive model (GAM) with a binomial distribution for the exact location dataset (red) and the GBIF derived climate data (blue). Each climate variable was limited to three partitions. The solid line represents the fitted smoother; 95% confidence intervals are shown as coloured tinted areas. Each dot represents a specimen for which the sampling location was reported in literature, or climate data were obtained from the WorldClim database. Pseudo-$R^2$ measures the approximate deviance explained by each explanatory variable.

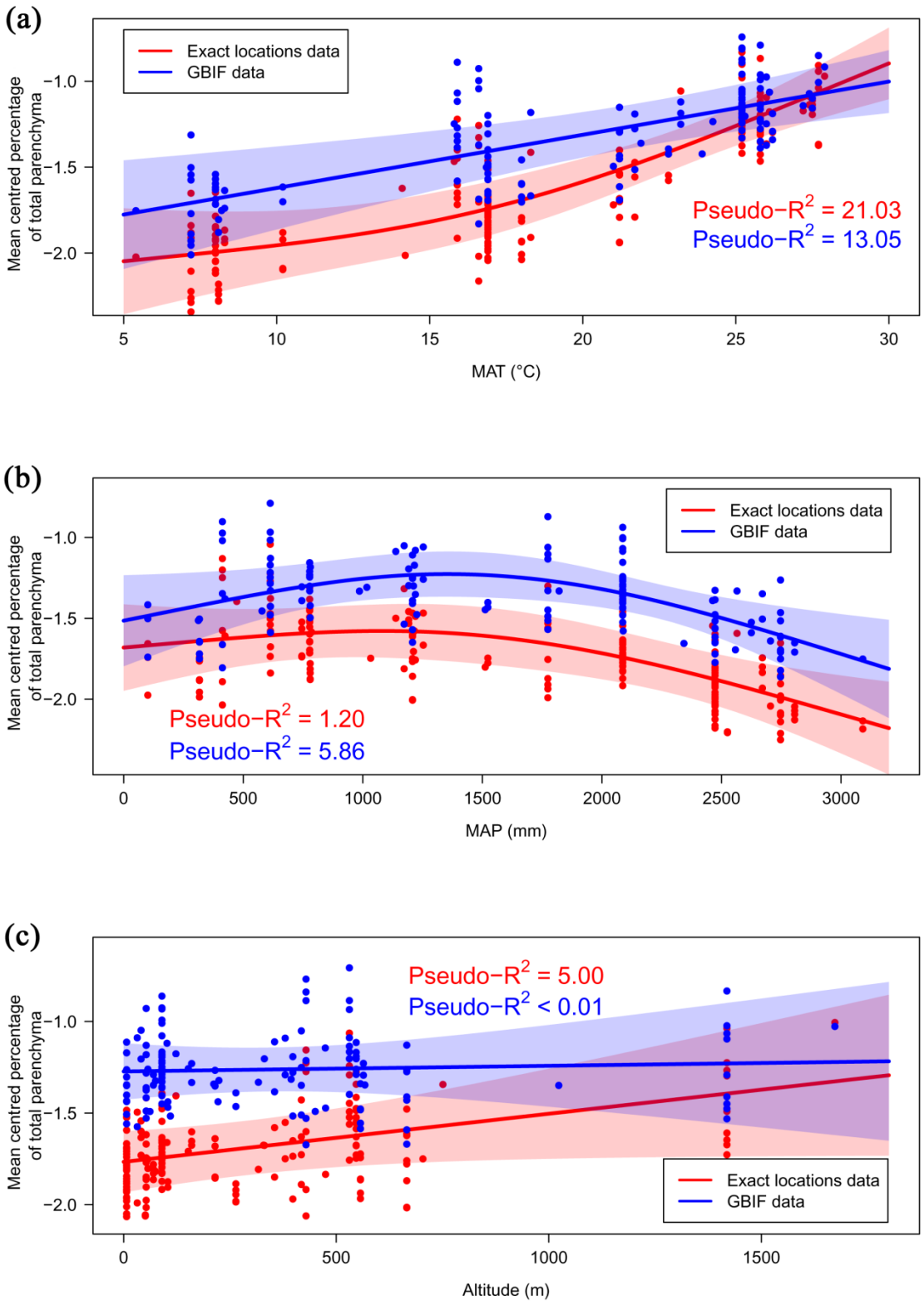

(a)
Exact locations data
GBIF data
Pseudo−R2 = 21.03
Pseudo−R2 = 13.05
Mean centred percentage of total parenchyma
−1.0
−1.5
−2.0
5
10
15
20
25
30
MAT (°C)
(b)
Exact locations data
GBIF data
Pseudo−R2 = 1.20
Pseudo−R2 = 5.86
Mean centred percentage of total parenchyma
−1.0
−1.5
−2.0
0
500
1000
1500
2000
2500
3000
MAP (mm)
(c)
Pseudo−R2 = 5.00
Pseudo−R2 < 0.01
Exact locations data
GBIF data
Mean centred percentage of total parenchyma
−1.0
−1.5
−2.0
0
500
1000
1500
Altitude (m)

**Fig. S6** The effect of MAT (a), MAP (b), and altitude (c) on the proportion of ray parenchyma (RP) in angiosperm wood based on a general additive model with a binomial distribution for the exact location dataset (red) and the GBIF derived climate data (blue). Each climate variable was limited to three partitions. The solid line represents the fitted smoother; 95% confidence intervals are shown as coloured tinted areas. Each dot represents a specimen for which the sampling location was reported in literature, or climate data were obtained from the WorldClim database. Pseudo-$R^2$ measures the approximate deviance explained by each explanatory variable.

**Table S1** The Global Wood Parenchyma Database, including 961 records, but excluding Zheng and Martínez-Cabrera (2013). Data were extracted from 55 sources and include species name, plant organ, growth form, and xylem tissue fractions (%) of ray parenchyma, axial parenchyma, and total parenchyma. Specimens were grouped according to three major climatic zones, which follow Köppen (1936).

**Köppen W. 1936.** Das geographische System der Klimate. In: Köppen W, Geiger R, eds. *Handbuch der Klimatologie.* Berlin, Germany: Gebrüder Borntraeger, 1-44.

**Zheng J, Martínez-Cabrera HI. 2013.** Wood anatomical correlates with theoretical conductivity and wood density across China: evolutionary evidence of the functional differentiation of axial and radial parenchyma. *Annals of Botany* **112**: 927-35.

**Table S2** Summary of statistics for the general additive models (GAM) fitted with a binomial distribution for the exact locations dataset. The response variables were RAP, AP and RP. Each explanatory variable was fitted with a smoother with a maximum of three effective degrees of freedom (e.d.f). P-values are approximate (Wood, 2006). Pseudo-$R^2$ measures the approximate deviance explained by each explanatory variable.

| **Response variable** | **Explanatory variable** | **e.d.f** | **F-value** | **P-value** | **Pseudo-$R^2$** |
|---|---|---|---|---|---|
| **RAP** | MAT | 1.945 | 37.210 | < 0.001 | 21.05% |
| (*n* = 270) | MAP | 1.000 | 5.219 | 0.023 | 1.6% |
| | Altitude | 1.000 | 12.933 | < 0.001 | 3.6% |
| **AP** | MAT | 1.728 | 5.108 | 0.008 | 7.11% |
| (*n* = 137) | MAP | 1.000 | 1.471 | 0.227 | 1.41% |
| | Altitude | 1.000 | 2.946 | 0.088 | 2.23% |
| **RP** | MAT | 1.000 | 21.954 | < 0.001 | 21.03% |
| (*n* = 182) | MAP | 1.876 | 4.964 | 0.008 | 1.2% |
| | Altitude | 1.000 | 3.584 | 0.060 | 5.4% |

**Wood SN. 2006.** *Generalized Additive Models: An Introduction with R.* London, UK: Chapman and Hall.

**Table S3** Summary of statistics for the general additive models (GAM) fitted with a binomial distribution for the GBIF locations dataset. The response variables were RAP, AP and RP. Each explanatory variable was fitted with a smoother with a maximum of three effective degrees of freedom (e.d.f). P-values are approximate (Wood, 2006). Pseudo-$R^2$ measures the approximate deviance explained by each explanatory variable.

| **Response variable** | **Explanatory variable** | **e.d.f** | **F-value** | **P-value** | **Pseudo-$R^2$** |
|---|---|---|---|---|---|
| **RAP** | MAT | 1.950 | 48.234 | <0.001 | 31.65% |
| (*n* = 221) | MAP | 1.000 | 8.848 | 0.003 | 2.8% |
| | Altitude | 1.000 | 10.433 | 0.001 | 3.1% |
| **AP** | MAT | 1.922 | 14.615 | <0.001 | 19.05% |
| (*n* = 142) | MAP | 1.000 | 2.955 | 0.088 | 1.2% |
| | Altitude | 1.426 | 14.615 | <0.001 | 5% |
| **RP** | MAT | 1.000 | 12.988 | <0.001 | 13.05% |
| (*n* = 142) | MAP | 1.876 | 3.842 | 0.024 | 5.86% |
| | Altitude | 1.000 | 0.044 | 0.834 | <0.01% |

**Wood SN. 2006.** *Generalized Additive Models: An Introduction with R.* London, UK: Chapman and Hall.

**Notes S1** Published references from which data were extracted for analyses.

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# Part II

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# Publications

## Chapter 2

# The amount of parenchyma and living fibres affects storage of non-structural carbohydrates in young stems and roots of temperate trees

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***Abstract***

Concentrations of non-structural carbohydrates (NSC) are used as proxies for the net carbon balance of trees and as indicators of carbon starvation resulting from environmental stress. Woody organs are the largest NSC-storing compartments in forest ecosystems; therefore, it is essential to understand the factors that affect the size of this important storage pool. In wood, NSC are predominantly deposited in ray and axial parenchyma (RAP), however, direct links between nutrient storage and RAP anatomy have not yet been established. Here, we tested if the NSC storage capacity of wood is influenced by the amount of RAP.

We measured NSC concentrations and RAP fractions in root and stem sapwood of twelve temperate species sampled at the onset of winter dormancy and in stem sapwood of four tropical trees growing in an evergreen lowland rainforest. The patterns of starch distribution were visualised by staining with Lugol's solution.

The concentration of NSC in sapwood of temperate trees scales tightly with the amount of RAP and living fibers (LFs), with almost all RAP and LFs being densely packed with starch grains. In contrast, the tropical species exhibited lower NSC concentrations despite their higher RAP and LFs fraction and showed considerable interspecific differences in starch distribution.

The differences in RAP and LFs abundance affect the ability of sapwood to store NSC in temperate trees, whereas a more diverse set of functions of RAP might be pronounced in species growing in a tropical environment with little seasonality.

**Key-words:** *axial parenchyma, carbohydrates, living fibres, rays, starch, storage, wood, xylem*

### *Introduction*

Non-structural carbohydrates (NSC, including mono-, di-, oligo-, and polysaccharides) are the direct products of plant photosynthesis that can be stored in various plant tissues and used as substrate for growth and various metabolic processes. The amount of NSC available in a plant is considered to represent an important indicator of the net carbon balance in woody plants, although the functional role and the active or passive regulation of NSC storage is not fully understood (Kozlowski, 1992; Hoch, 2015). NSC levels have traditionally been studied with respect to seasonal cycles of growth and dormancy (Drossopoulos and Niavis, 1988; Saranpää and Höll, 1989; Sauter and van Cleve, 1994). More recently, they have been investigated in relation to growth limitation (Körner, 2003; Sala and Hoch, 2009; Wiley and Helliker, 2012), altitudinal boundaries for forests (Hoch *et al*., 2002), plant survival under limited resource conditions, increased atmospheric $CO_2$ levels, and drought-induced tree mortality. Although developmental shifts are likely between seedlings and mature trees, wood provides the largest NSC-storing compartment among all tree tissues (Hoch *et al*., 2003; Würth *et al*., 2005), with an additional role for parenchyma cells in the pith and bark (Essiamah and Eschrich, 1985).

In wood, NSC are predominantly deposited in ray and axial parenchyma (RAP). Ray parenchyma (RP) represents a series of procumbent, square or upright cells produced by ray initials that extend radially from the cambium. Besides storage, RP facilitates radial conduction of NSC, water and other substances, and enables their exchange between phloem and xylem, although the mechanisms of this trafficking remain poorly understood (Van Bel, 1990; Sokolowska and Zagórska-Marek, 2012; Pfautsch *et al*., 2015). Axial parenchyma (AP) is made up of axially elongated cells, which are often organized in strands. AP has been suggested to be involved in NSC storage and transport, similar to RP (Braun and Wolkinger, 1970; Baas, 1982; Braun, 1984; Carlquist, 2001). Abundant AP may not only increase the physical space available for storage but could also enhance connectivity between RP and AP.

While NSC storage and dynamics appear tightly coupled to RAP anatomy and physiology (reviewed in Plavcová and Jansen, 2015), direct links between structural and functional RAP traits have not yet been established. Elucidating firm connections between structural and functional traits has a long tradition in the field of functional and ecological wood anatomy. While most research to date focuses on the functional anatomy of xylem conduits, providing us

with a better understanding of xylem water transport (Baas, 1982; Carlquist, 1984; Hacke and Sperry, 2001; Jansen *et al*., 2009), the current study uses a similar approach to shed more light on the role of RAP in NSC storage. More specifically, we test whether the amount of RAP found in wood is a structural proxy for the NSC storage capacity of wood.

As most of the wood biomass consists of dead, protoplasm-free fibres and conduits, the total volume of RAP should place a limitation on the amount of NSC that can be stored. The proportion of RAP in wood, usually expressed as the percentage area of RAP relative to the total wood cross-sectional area, varies greatly across species. In gymnosperms, the RAP area fractions are commonly between 5 and 10% (Panshin and Zeeuw, 1980), while in angiosperms, the RAP proportions are higher, typically between 20 and 50% (Martínez-Cabrera *et al*., 2009; Fichtler and Worbes, 2012; Zheng and Martínez-Cabrera, 2013; Ziemińska *et al*., 2013). Fractions higher than 80% have also been observed in some woody succulents and climbers (Mauseth and Plemons-Rodriguez, 1997; Hearn, 2009). Interestingly, RAP fractions tend to be higher in tropical than temperate angiosperm trees (Morris *et al*., 2015). This might be surprising at first sight when considering that the storage function is likely to be less active in tropical environments with a weak seasonality. However, RAP has multiple functions beyond storage of NSC, such as storage of water (capacitance), embolism refilling, symplastic connectivity, and various functions related to defense and resilience to disturbance (for an overview, see Fig. 2 in Morris *et al*., 2015). Moreover, the storage capacity provided by RAP may not always be fully utilized. For instance, it is well known that NSC content of wood fluctuates seasonally in both temperate (Sauter and van Cleve, 1994; Barbaroux *et al*., 2003) and tropical environments (Newell *et al*., 2002; Würth *et al*., 2005). Besides seasonal variation, there can be differences in NSC distribution between vessel-associated RAP cells and the RAP cells that are not in direct contact with vessels (Braun and Wolkinger, 1970; Czaninski, 1977; Braun, 1984).

In many species, living fibres (LFs) accumulate starch and hence contribute to NSC storage in addition to RAP. LFs, defined as fibres that retain living protoplasts, are either septate or non-septate, and vary interspecifically in their lumen diameter to wall thickness ratio (Fahn and Leshem, 1963; Wolkinger, 1970; Carlquist, 2014). The occurrence of LFs is, however, poorly documented in woody plants and their anatomical and functional distinction can be difficult to make due to a structure-function continuum with AP (Carlquist, 2014). Storage function has

been suggested for septate fibres (Carlquist, 2001), which are twice as common in tropical as in temperate trees (Wheeler *et al*., 2007). Similar to AP, septate fibres often have thin secondary walls that distinguish them from thick-walled living and non-living fibres. In contrast, non-septate LFs, which are found, for instance, in maples, often have thick secondary walls (Carlquist, 2001, 2014). Providing that the thicker cell wall occurs at the expense of cell lumen volume, it is reasonable to expect that thick-walled LFs are less efficient in storage than septate fibres or AP.

The main goal of this study was to test if the amount of NSC stored in wood is linked with RAP and LFs abundance, and to quantify the relative contribution of RP, AP and LFs to storage function. To achieve this goal we measured NSC concentration in root and stem sapwood of 12 temperate species, including ten angiosperm trees, one conifer tree and one woody climber. Samples were collected at the onset of winter dormancy when the NSC concentration is expected to be at its maximum. We measured the area fractions of RP, AP and LFs and conducted in-situ visualisation of starch. We expected that species with a relatively high amount of RAP, such as pedunculate oak (*Quercus robur*) and black locust (*Robinia pseudoacacia*), would show higher concentrations of NSC in wood than species with a low RAP fraction, for example in horse chestnut (*Aesculus hippocastanum*) and Norway spruce (*Picea abies*). We also hypothesized that more NSC would be stored in woody roots than stems and that this pattern will be paralleled by a higher RAP abundance in the roots (Pate *et al*., 1990). To complement the data obtained for temperate trees, measurements of NSC content, the presence of starch, together with RAP fractions, were conducted in the stems of four tropical tree species growing in a lowland rainforest in Costa Rica. We expected to find higher RAP proportions in tropical species (Morris *et al*., 2015), although with NSC levels not necessarily as high as those of temperate species, as the former are always actively growing, whereas the latter were sampled at the onset of winter dormancy. Nevertheless, we anticipated a positive correlation between the amount of NSC and the amount of RAP across the four tropical species.

## *Materials and Methods*

### *Plant material*

Young woody roots and stems of 12 temperate species (Table 1) were collected from mature trees (more than 3 m tall) growing in a forested area surrounding the Ulm University campus, which included the arboretum of the Botanical Garden of Ulm University (48° 25’ N, 9° 58’ E), and a forest strip along the Iller river, 2 km south of Ulm (48° 22’ N, 9° 60’ E). These areas experience a temperate climate with a mean annual temperature of 8.9 °C and mean annual precipitation of 726 mm (climate-data.org). The roots were collected from a depth of 10-50 cm and a distance of between 1 to 2 m from the root collar. The stem samples were removed from the end regions of sub-canopy lateral branches cut at a height of 2 to 3 meters with the aid of a telescopic pruning pole. All roots and stems were between 0.5 and 1.5 cm in diameter, which corresponded to a root or stem age of 3 to 8 years. All samples consisted of juvenile sapwood with no heartwood formation apparent on the cross-section after visual inspection. By avoiding heartwood, we were sure that all RAP cells in our samples were living, and therefore capable of storing NSC. During the last week of October 2014, six root and stem samples from three separate individuals of each species were collected and prepared for NSC analyses. Samples for wood anatomy and wood density measurements were collected from the same population of trees, were of a similar size, and came from the same position on the tree as those used for the NSC analyses. For logistical reasons, the sampling of material for anatomical and wood density measurements was conducted ad hoc throughout the year 2014, which has no effect on the structural parameters measured. Anatomical measurements were carried out on three different root and stem samples per species, while wood density measurements were made on six different root and stem samples collected from three individuals of each species.

**Table 1:** List of the species studied with reference to their family and acronym.

| Species | Family | Acronym |
|---|---|---|
| temperate | | |
| *Aesculus hippocastanum* L. | Sapindaceae | Ah |
| *Acer pseudoplatanus* L. | Sapindaceae | Ap |
| *Carpinus betulus* L. | Betulaceae | Cb |
| *Clematis vitalba* L. | Ranunculaceae | Cv |
| *Fraxinus excelsior* L. | Oleaceae | Fe |
| *Fagus sylvatica* L. | Fagaceae | Fs |
| *Liquidambar styraciflua* L. | Altingiaceae | Ls |
| *Prunus avium* (L.) L. | Rosaceae | Pa |
| *Quercus robur* L. | Fagaceae | Qr |
| *Robinia pseudoacacia* L. | Fabaceae | Rp |
| *Tilia cordata* Mill. | Malvaceae | Tc |
| *Picea abies* (L.) H.Karst. | Pinaceae | Piab |
| tropical | | |
| *Brosimum* sp. | Moraceae | Bros |
| *Ceiba pentandra* (L.) Gaertn. | Malvaceae | Cepe |
| *Pentaclethra macroloba* (Willd.) Kuntze | Fabaceae | Pecl |
| *Warszewiczia coccinea* (Vahl) Klotzsch | Rubiaceae | Waco |

Stem samples from four tropical species (Table 1) were collected in the vicinity of the Reserva Biológica Tirimbina, Heredia Province, Costa Rica (10° 24' N, 84° 6' W) following the same protocol as described for the temperate trees. The climate of Tirimbina is described as wet tropical showing little seasonality, with a mean annual temperature of 25.3 °C and mean annual precipitation of 3,777 mm (Lapinski and Tschapka, 2013). All samples of tropical trees were collected on 12$^{th}$ September 2014.

*NSC analyses*

Root and stem samples prepared for NSC analyses (n=6 roots and 6 stems for each species) were taken to the laboratory where they were then processed immediately or kept on ice and processed later on the same day. In the laboratory, the root and stem samples were cut into 3-cm-long segments and their bark and pith were carefully removed. The wood samples were heated in a microwave oven at 600 W for 40 seconds to deactivate the NSC-modifying enzymes (Popp et al., 1996). They were then dried to a constant weight at 80 °C for two days and sent to the Plant Ecophysiology Laboratory at the University of Basel for NSC content analyses. NSC, defined here as the sum of starch together with the three most abundant low molecular weight sugars (i.e., sucrose, glucose and fructose), were analysed photometrically following a slightly modified protocol described in Hoch et al. (2002). While various protocols can be used for NSC concentration measurements, the method of Hoch et al. (2002) is well-established and broadly agrees with other methods based on a recent comparison among 29 different laboratories (Quentin et al. 2015). Approximately 10 mg of plant powder was extracted with 2 ml distilled water at 100 °C for 30 min. An aliquot of the extract was used for the determination of low molecular carbohydrates after enzymatic conversion of fructose and sucrose to glucose. The concentration of total free glucose was then determined photometrically after enzymatic conversion of glucose to gluconat-6-phosphate using a glucose hexokinase (GHK) assay reagent (G3292, Sigma-Aldrich, St. Louis, MO, USA). Following the degradation of starch to glucose with amyloglucosidase at 49 °C overnight, NSC was determined in a separate analysis. The concentration of starch was calculated as NSC minus the free low molecular carbohydrates. Tissue concentrations were given as % dry matter.

The gravimetrically based concentrations (in mg $g^{-1}$) were also converted to volume based values (in mg $cm^{-3}$) using data on wood density. Wood density was measured by the water displacement

method. Root or stem segments about 3 cm in length (n=6 roots and 6 stems per species) were cut and debarked. Stem segments were additionally split in half and the pith was removed. Each segment was then immersed, with the aid of a dissecting needle, into a water-filled beaker resting on an electronic balance, where upon the weight of the displaced water was recorded. All segments were then oven-dried at 80 °C to a constant weight and wood density was calculated by dividing the wood dry weight by the volume of the water displaced.

*Wood anatomy and starch distribution*

Root and stem segments (n=3 roots and 3 stems for each species) collected in the field were cut into 1-cm-long pieces and stored in 70% ethanol at 4 °C until sectioned for anatomical observations. Upon rehydration in distilled water, transverse sections about 40 µm thick were prepared with a sliding microtome. The sections were immersed in a mixture of 0.35% safranin and 0.65% alcian blue for 2 min, thoroughly washed with distilled water, then gradually dehydrated through an ethanol series before being mounted on a slide in Neo-Mount (Merck Millipore, Germany). Digital images were captured at a 150x magnification using an Axio Zoom V16 microscope (Carl Zeiss Microscopy GmbH, Jena, Germany). The relative proportions of ray and axial parenchyma (RP + AP = RAP) and living fibres (LFs) were measured on wedge-shaped transects spanning from the root or stem center to the cambium. Axially oriented thick-walled cells with apparent cellular content were typically considered LFs, while axial cells with a protoplasm and a thin secondary wall were always identified as AP. To aid with distinguishing between LFs and thick-walled AP, radial sections of wood were also inspected to assess if the axial cells have a spindle-shaped morphology typical for fibres or are rectangular and arranged in strands as typical for AP. The different cell types were outlined manually with the aid of a graphical tablet (Cintiq Companion, Wacom Co Ltd, Japan) in Photoshop Elements (v12.1, Adobe Systems Incorporated, San Jose, CA, USA) and their area proportions were measured in Fiji/ImageJ (Schindelin *et al*., 2012). To obtain the relative proportions of RAP and LFs, the total surface area taken up by each cell type was divided by the total surface area of the entire transect measured, which typically was about 1 $mm^2$.

To visualise the distribution of starch, wood sections were incubated in a drop of 5% Lugol's iodine for 2 min. The samples were then briefly washed in distilled water, mounted in glycerol and observed immediately with the Axio Zoom V16 microscope.

**Table 2:** Fixed effects estimates (means ± SE) for organ (root and stem) and climate (temperate and tropical) on concentrations of total non-structural carbohydrates (NSC), starch and soluble sugars and on the amount of ray and axial parenchyma (RP + AP = RAP) and living fibres (LFs). Statistical significance was tested by comparing full and reduced models using the likelihood ratio test. Test statistics (L), degrees of freedom (df) and corresponding *P* values are shown. Statistically significant results ($P \leq 0.05$) are indicated in bold. To corroborate the results of the likelihood ratio tests, ΔAIC values were calculated, which represent the difference in Akaike information criteria values between the reduced and full model.

| | Organ (stem relative to root) | | | | | Climate (tropical relative to temperate) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mean ± SE | L | df | *P* value | ΔAIC | mean ± SE | L | df | *P* value | ΔAIC |
| NSC | -10.5 ± 2.2 | 13.1 | 1 | **$<10^{-3}$** | 11.1 | -6.3 ± 1.7 | 10 | 1 | **0.002** | 4 |
| starch | -9.8 ± 2.1 | 12.2 | 1 | **$<10^{-3}$** | 10.2 | -6.7 ± 1.7 | 10.5 | 1 | **0.001** | 4.9 |
| sugars | -0.7 ± 0.2 | 12 | 1 | **$<10^{-3}$** | 10 | 0.4 ± 0.4 | 0.7 | 1 | 0.391 | -1.2 |
| RAP + LFs | -11.1 ± 1.8 | 17.2 | 1 | **0.007** | 15.2 | 9.5 ± 6.7 | 2 | 1 | 0.158 | 0.3 |
| RAP | -8.9 ± 1.8 | 13.8 | 1 | **$<10^{-3}$** | 11.8 | 12.9 ± 5.2 | 5.4 | 1 | **0.020** | 3.8 |
| RP | -5 ± 0.9 | 24.9 | 1 | **$<10^{-3}$** | 22.9 | 3.8 ± 2.5 | 2.2 | 1 | 0.136 | 2 |
| AP | -3.9 ± 1.9 | 3.6 | 1 | 0.058 | 1.6 | 9.1 ± 5.6 | 2.6 | 1 | 0.110 | 0.6 |

*Statistical analyses*

Mean differences in NSC and RAP between organs (roots and stems) and between climate (temperate or tropical) were tested using linear mixed effects models, where organ and climate represented the fixed factors and species were treated as a random factor. The models were constructed in R using the lme function available in the nlme package (Pinheiro et al. 2015). The maximal likelihood method was used to fit the models. Statistical significance of fixed factors was assessed using the likelihood ratio tests by comparing the full models to reduced models from which the fixed factors were eliminated. The validity of the models was checked by visual inspection of residual plots. The Akaike information criteria for the full and reduced models were also calculated to confirm the appropriateness of the model selection. Relationships between RAP + LFs and NSC were analysed by ordinary least squares regression on species- and organ-level means. All analyses were conducted in R (R Core Team 2013). Results were considered statistically significant at $P \leq 0.05$.

## *Results*

*NSC concentrations*

The concentrations of NSC and their two main fractions (i.e. starch and soluble sugars) were significantly higher in roots than stems (Table 2, Fig. 1A). Overall, the highest NSC concentration of 39.1 ± 2.7% (mean ± SD) was measured in the roots of *Acer pseudoplatanus*, while the lowest concentration (0.9 ± 0.1%) was observed in the stems of *Picea abies*. In all species and organs, NSC were present mainly in the form of starch, whereas the soluble sugar fraction was less abundant, typically falling between 1 and 3%. The NSC and starch concentrations were not significantly linearly correlated between roots and stems of the same species, largely because of the relatively low NSC and starch concentrations in *Robinia pseudoacacia* roots (Fig. 2A). In contrast, there was a significant correlation between the concentrations of soluble sugars in both organs, which was principally driven by the high soluble sugar fraction found in *Tilia cordata* (Fig. 2B).

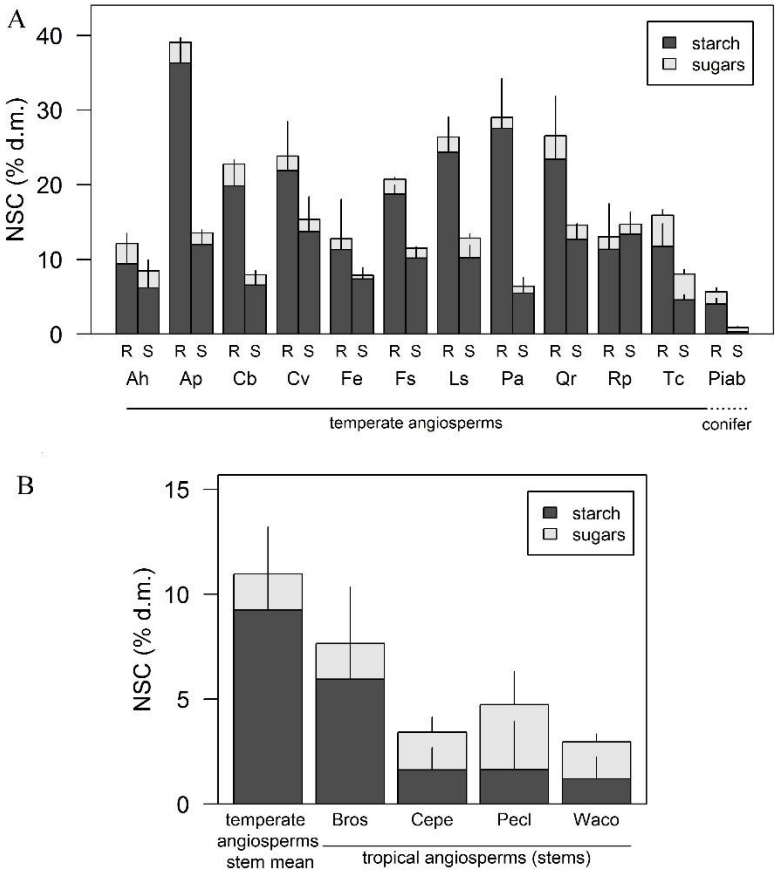



**Fig. 1:** Concentration of non-structural carbohydrates (NSC) and their division between starch (dark bars) and soluble sugar (lighter bars) fractions in the root (R) and stem (S) sapwood of twelve temperate tree species (A) and in the stems sapwood of four tropical species (B). Means ± standard deviations (n = 6) of concentrations expressed as percent dry matter (% d. m.) are shown. Acronyms of the species names follow Table 1.

Stems of tropical trees exhibited on average less than half the amount of NSC than stems of temperate angiosperms (Table 2, Fig. 1B). The difference in NSC concentrations was driven by lower starch levels, while the soluble sugar fractions were not significantly different between temperate and tropical trees (Table 2). However, NSC concentrations varied markedly among the four tropical species studied. *Brosimum* sp. showed a NSC concentration of 7.7 ± 3.7%, which is comparable to the lower-end values observed in the stems of temperate angiosperms. In contrast, *Ceiba pentandra*, *Pentaclethra macroloba* and *Warszewiczia coccinea* had lower NSC concentrations than any of the temperate angiosperm species (Fig. 1B).

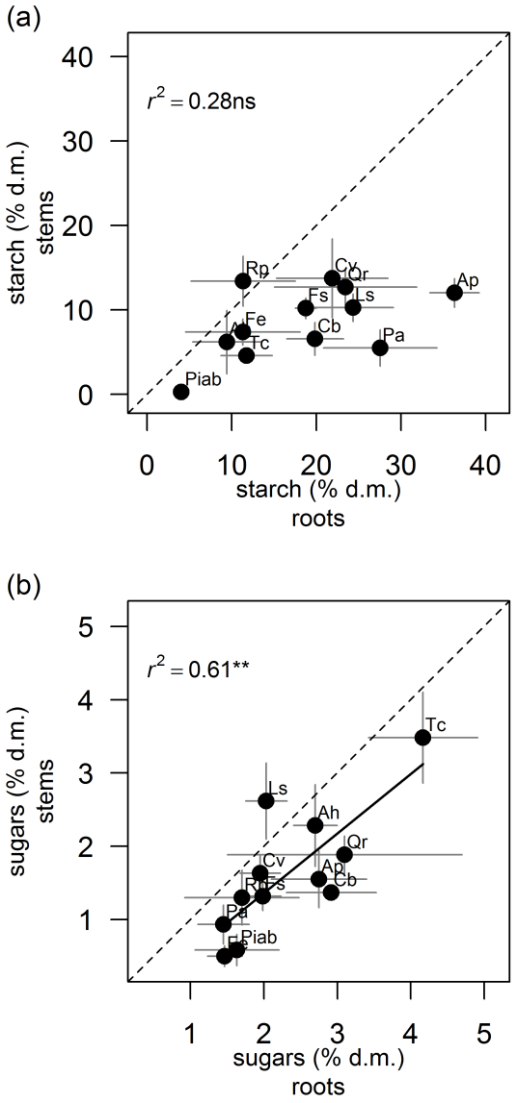



**Fig. 2:** Correlation between root and stem concentrations of starch (A) and soluble sugars (B) in twelve temperate species. Means ± standard deviations (n = 6) are shown. Abbreviations of the species names are listed in Table 1. ns = non-significant relationship ($P > 0.05$), ** = significant relationship with $P \geq 0.01$. Acronyms of the species names follow Table 1. Reference lines showing a theoretical 1:1 relationship are plotted as dashed lines. Solid line represents the linear regression to the data.

*RAP and LFs anatomy*

RP was present in all species investigated with a mean area fraction of 15.9 ± 5.4%. AP was found in all specimens (mean area fraction 10.0 ± 9.0%) except in the stems of *Picea abies* and *Warszewiczia coccinea*. Non-septate LFs occurred in *Clematis vitalba* and *Acer pseudoplatanus*, while septate LFs were found in *Warszewiczia coccinea*. In all three species, sparse simple pitting was observed on the walls of LFs. Overall, roots of temperate species exhibited significantly higher fractions of storage cells (i.e. RAP + LFs) than stems (34.9 ±

14.8% vs. 23.8 ± 11.9%, Table 2) with the exception of *Robinia pseudoacacia* and *Quercus robur* where the amount of RAP was comparable between roots and stems, averaging around 33% (Fig. 3A). In most species, both types of wood parenchyma (i.e., RP and AP) were higher in roots than in stems (Table 2). However, two ring-porous species, *Quercus robur* and *Robinia pseudoacacia*, exhibited lower AP area fractions in roots compared to stems, and *Clematis vitalba* and *Fraxinus excelsior* showed an increase in AP only (Fig. 3A). RP represented the dominant fraction of the total RAP, with the contribution of AP being approximately one-third of the total RAP. On average, the relative contribution of AP to the total RAP was about 5% higher in roots than stems. The presence of LFs was particularly noticeable in *Clematis vitalba*, resulting in very high LFs area fractions of 49.1% and 34.4% in roots and stems, respectively, and virtually all fibres were alive and filled with starch (Fig. 4A, E).

The amount of living cells (RAP + LFs) tended to be higher in tropical than in temperate trees (means 34.9 vs. 25.4%, Table 2), although the difference was not statistically significant, presumably due to the high LFs fraction in *Clematis vitalba*. Nevertheless, the four tropical species showed significantly higher fractions of RAP than those measured in the stems of temperate angiosperms (mean difference ± SE: 12.9 ± 5.2, Table 2). *Ceiba pentandra* had the highest amount of RAP reported in this study. The RAP area fraction for the latter reached 56 ± 8.6% and was driven mainly by a high AP content (AP fraction of 38.4 ± 5.3%; Fig. 3B). In contrast, *Warszewiczia coccinea* exhibited a particularly high RP fraction of 25.9 ± 1.8% (Fig. 3B). This value is almost twice as high as the mean RP fraction of all other stems measured in this study.

*Starch localisation*

Staining with Lugol's solution was used to visualise the starch distribution in RP, AP and LFs (Fig. 4). In temperate species, almost all RAP + LFs cells were filled with starch that completely occupied the cell lumen with tightly packed grains (Fig. 4 A-C, E-G). *Tilia cordata* and *Picea abies* (Fig. 4 D, H) were two exceptions to this pattern, with starch grains being packed more loosely. In most species, starch grains were present in both vessel-associated RAP cells and the cells that were not in contact with vessels, although a tendency for lower starch accumulation in vessel-associated RAP cells was sometimes apparent (Appendix S1, see Supplemental Data with online version of this article). In the case of *Clematis vitalba*, a positive staining reaction was found in the lumina of all fibres, leaving vessels and tracheids as the only cell types void of starch (Fig. 4A, E, Appendix S1). Besides

RAP and LFs, starch grains were also abundant in parenchyma cells of the perimedullary region (i.e. the region surrounding the pith) and in some species (*Quercus robur*, *Carpinus betulus* and *Liquidambar styraciflua*) were directly within the pith. In these species, the amount of starch found in the pith tended to decline as the branch age increased. In roots, the central region with primary xylem appeared sometimes enriched in starch (e.g. *Carpinus betulus*, *Aesculus hippocastanum*). In *Picea abies*, starch was also present in the epithelial cells associated with the resin ducts (Fig. 4D). While starch was generally less abundant in tropical (Fig. 4I-L) than temperate trees (Fig. 4E-H), interspecific differences in starch distribution were observed among the tropical species. In *Ceiba pentandra* starch grains were present predominantly in RP (Fig. 4I), which was in contrast to *Pentaclethra macroloba* where they were mostly present in the AP (Fig. 5K). Starch grains were also present in the LFs of *Warszewiczia coccinea* (Fig. 4L), while in *Brosimum* sp. they appeared abundant in both RP and AP, similar to that of temperate species (Fig. 4J).

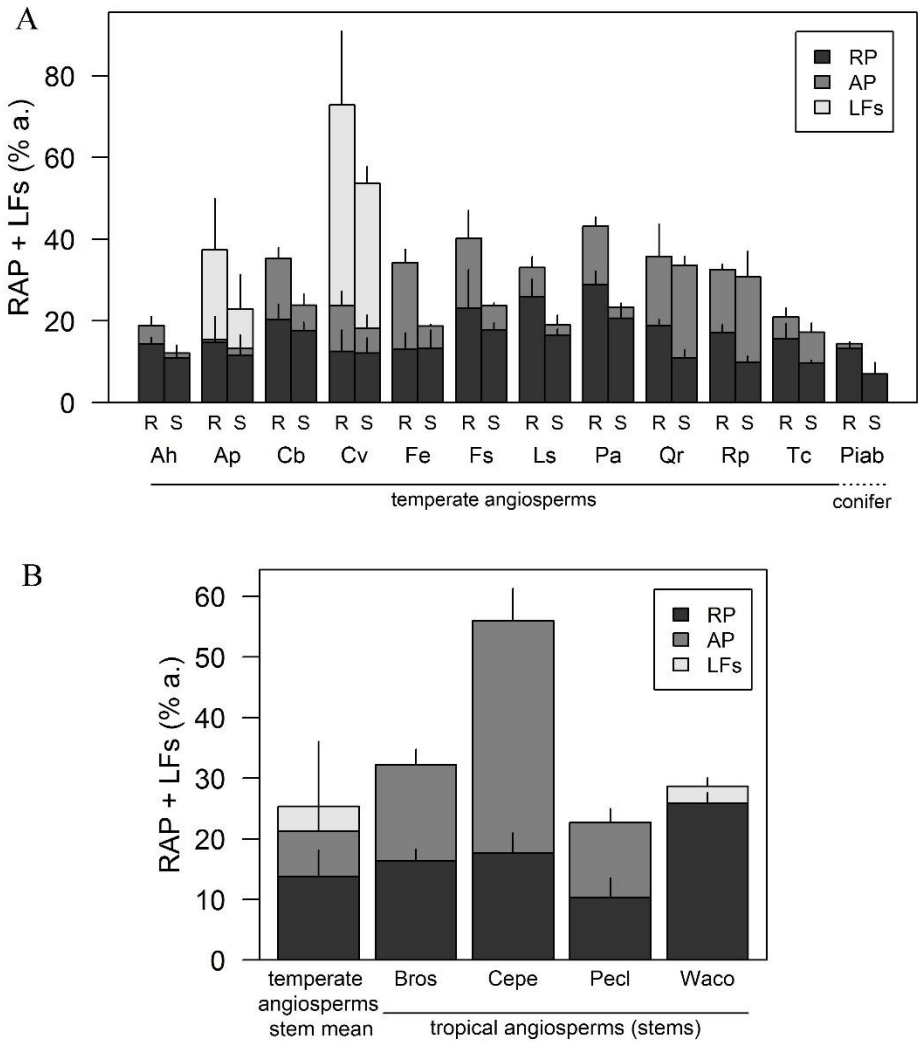



**Fig. 3:** Percent area (% a.) of ray and axial parenchyma (RP + AP = RAP) and living fibres (LFs) in root (R) and stem (S) wood of twelve temperate tree species (A) and in stem wood of four tropical species (B). Means ± standard deviations (n = 3) are shown. Acronyms of the species names follow Table 1.

*Relationship between NSC and RAP*

There was a strong positive correlation ($P < 0.001$, $r^2 = 0.64$) between the NSC concentration and the RAP + LFs fraction in temperate species when the data for *Clematis vitalba* were excluded (Fig. 5). The contrasting relationship between NSC and RAP + LFs in *C. vitalba* compared to the temperate trees was likely due to the lower storage capacity of thick-walled LFs that were particularly prominent in *C. vitalba* (Fig. 5C). The relationship between NSC and RAP + LFs was significant with $r^2 > 0.5$ when either the data for roots and stems were analysed together or when analysed separately (Fig. 5A). This relationship became even

stronger when the NSC concentrations were expressed per unit wood volume rather than on a gravimetric basis, i.e., when the differences in wood density between species and organs were taken into account (Fig. 5B). In contrast to temperate trees, NSC concentrations in the stems of the four tropical species were independent of the respective RAP + LFs content (Fig. 5C, white squares).

### *Discussion*

In this study, we showed that the differences in NSC concentrations observed in the young roots and stems of temperate species at the onset of winter dormancy were strongly related to the abundance of RAP (Fig. 5), with LFs contributing to storage in *Clematis vitalba* and *Acer pseudoplatanus*. Thus, our data provide evidence that the amount of RAP can serve as a suitable proxy for the capacity of wood to store NSC. In this regard, our results expand on the list of apparent structure-function relationships identified in xylem, which help us to better understand the vast diversity in wood anatomical traits that evolved in plants.

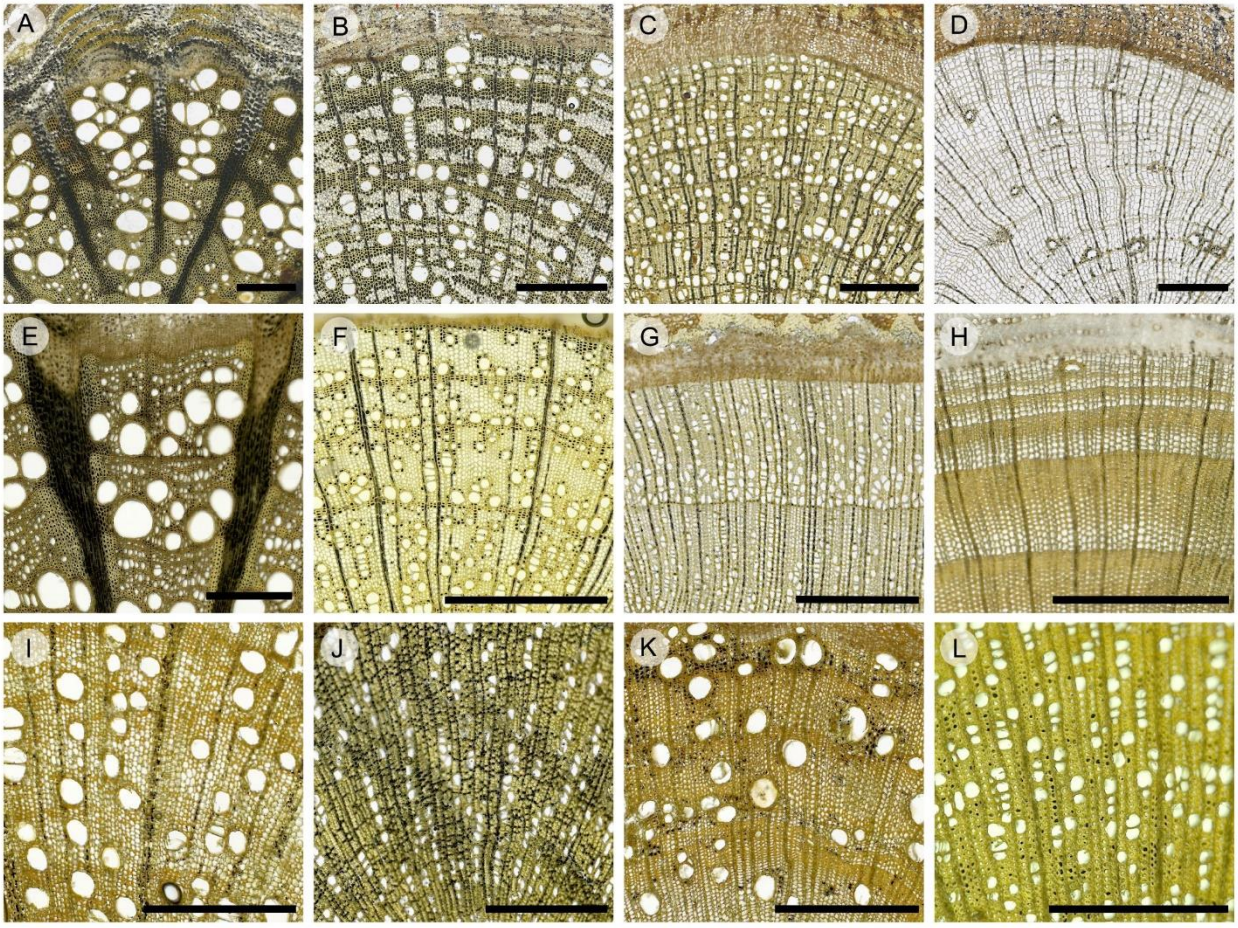



**Fig. 4:** Transverse sections of roots (A-D) and stems (E-L) stained with Lugol's solution showing the spatial distribution of starch in wood (stained black). Four temperate species, *Clematis vitalba* (A, E), *Acer pseudoplatanus* (B, F), *Aesculus hippocastanum* (C, G) and *Picea abies* (D, H), and four tropical species, *Ceiba pentandra* (I), *Brosimum* sp. (J), *Pentaclethra macroloba* (K) and *Warszewiczia coccinea* (L) are shown. Starch was present in ray and axial parenchyma of all species, although the staining intensity varied from high (A, B, E, F, J) to low (D, G, H, I, K, L). In some species, starch was also present in living fibres (A, B, E, F, L). Scale bars = 500µm.

The visual assessment of starch distribution revealed that virtually all RAP and LFs of temperate trees were filled with starch (Fig. 4A-H), indicating that the entire space available for storage in wood was utilized at the onset of winter dormancy. Contrary to earlier observations (Braun and Wolkinger, 1970; Braun, 1984), there was no clear distinction in starch accumulation between vessel-associated RAP cells and the RAP cells that are not in direct contact with vessels (Appendix S1). Our results also showed that both types of interconnected wood parenchyma, i.e. ray (RP) and axial parenchyma (AP) as a whole, contribute greatly to NSC storage. While RP represented the dominant part of the total RAP in most of the temperate species investigated in this study, abundant AP resulted in a substantial increase in the total RAP of some species, for example in *Quercus robur* and

*Robinia pseudoacacia*. AP fractions, often accounting for more than 40% of the total RAP, were higher in the roots than in the stems of the temperate species (Fig. 3A). It is possible that an increased need for mechanical support requires a strong fiber matrix that may limit the occurrence of thin-walled AP in self-supporting stems.

Alternatively, thick-walled LFs could take on the storage function without compromising wood mechanical strength (Carlquist, 2014, 2015). Among temperate species included in this study, *Clematis vitalba* showed particularly high (up to 50% of the total cross-sectional area) proportions of LFs in both roots and stems (Fig. 3A, Fig. 4 A, E, Appendix S1). Considering its high RAP + LFs fraction, the NSC levels measured in *Clematis vitalba* were relatively low (Fig. 5C). The lower NSC storage efficiency (defined as the NSC concentration per unit RAP + LFs area) in this species might be attributed to a lower ratio between cell lumen and cell wall fractions in storage tissue composed of LFs instead of AP. On the other hand, the storage capacity of LFs in *Acer pseudoplatanus* appeared to be in line with that of the other temperate species relying solely on parenchyma (Fig. 5). Whether or not LFs may provide a storage potential comparable to AP should be tested for a larger number of species. There is evidently a continuum in the dimensions and wall-to-lumen ratios of AP and LFs across species (Carlquist, 2014) and the anatomical variability of these cells, including their pit dimensions (especially pit size and density), should be more thoroughly documented in order to better understand its influence on the storage and mechanical properties of wood. Investments in thicker cell walls together with the occurrence of less extensive pitting in LFs, thus resulting in a lower storage capacity and lower accessibility and mobility of stored nutrients, might be factors that favor the occurrence of the typically thin-walled AP.

Due to the higher RAP fraction, roots were able to store more NSC than stems (Fig. 1A, Table 2), with the difference being about 2.5-fold on average and 6.5-fold at maximum. This finding highlights the important contribution of below-ground biomass to the overall NSC storage pool. The NSC concentrations of roots and stems of the same species were only partially correlated (Fig. 2), showing that the division of NSC reserves between below- and above-ground woody biomass is variable across species. The variation observed might relate to species- and organ-specific differences in phenology and subsequent growth dynamics. While little is known about the coordination between root and shoot phenology (Steinaker *et al*., 2009), large intraspecific variation in the timing and rates of root growth has recently been observed in a dozen temperate trees (McCormack *et al*., 2014). It is possible that the availability of NSC reserves could play a major role in driving these differences in root growth.

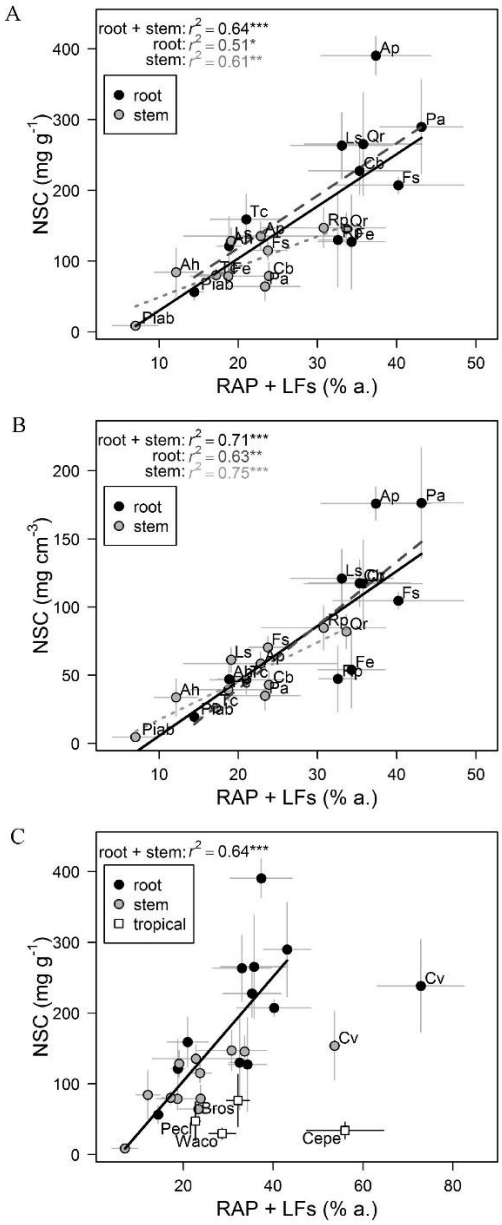



**Fig 5:** Relationship between the amount of wood parenchyma (RAP) and living fibres (LFs) and the concentration of non-structural carbohydrates (NSC) in root (black circles) and stem (grey circles) sapwood of temperate trees (A, B). Sapwood NSC concentrations were expressed on a gravimetric (A) or a volumetric (B) basis. Means ± standard deviations (n=3-6) are shown. Linear regression lines through all (solid black line), root (dashed dark grey line) and stem (dotted light grey line) data and the corresponding regression coefficients ($r^2$) are indicated. *, **, or *** denotes that the linear relationship was significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Acronyms of the species names follow Table 1. The same data as in (A) are presented in (C) together with the data for the temperate liana, *Clematis vitalba* (Cv) and four tropical species (white squares). Both *Clematis vitalba* and the tropical species are excluded from the correlation because they show lower NSC concentrations than the temperate trees in spite of their high amount of RAP and LFs.

In this study, we focused on NSC storage in young woody organs that consist of juvenile sapwood located in close proximity to the apical meristems. Mature wood in tree trunks also represents important sites of NSC storage and it can be assumed that the NSC stored in trunks provide the primary source of energy and material for the resumption of cambial activity at the beginning of the growing season (Hill *et al*., 1995). In tree trunks, not only the RAP content determines the total NSC storage potential, but so does the amount of sapwood, as the death of RAP cells associated with heartwood formation makes these cells unavailable for NSC storage (Magel *et al*., 1994). Mature wood from tree trunks may also show quantitative differences in RAP as compared to juvenile wood in branches, although no significant differences were found in AP, RP, and RAP between trunks and branches of 34 tree species (Morris *et al*., 2015).

Compared to temperate trees, the NSC levels of the four tropical species included in this study were significantly lower (Fig. 1B) in spite of their on average higher RAP + LFs content (Fig. 3B). This finding is not surprising considering that the wood tissue of the tropical trees used for NSC analyses was collected during active growth in the case of tropical trees, whereas the samples of the temperate trees were taken at the onset of winter dormancy. In fact, the tropical trees studied here never experience dormancy in their native habitat and thus may not require large reserves for the resumption of growth.

The finding that NSC and RAP data were not correlated in the tropical trees (Fig. 5C) may indicate that, in some cases, RAP plays a less important role in NSC accumulation, but might have various alternative functions. Interestingly, the abundant AP in *Ceiba pentandra* was essentially void of starch grains (Fig. 4I), and the soluble sugar fraction was not noticeably higher either (Fig. 1B). This suggests that the accumulation of NSC is not the primary function of AP in *C. pentandra*. It is possible that AP serves as an important water-storage reservoir in this fast-growing tropical pioneer that often colonizes forest gaps and copes with increased evaporative demands. In support of this idea, the role of RAP in stem water storage has already been proposed (Borchert and Pockman, 2005) and a high sapwood hydraulic capacitance was recently measured in another representative of the same genus (*C. speciosa*; Carrasco *et al*., 2015). Besides water storage, RAP is also known to play a part in defense against pathogens (Schwarze, 2007), which is a function that might be particularly important in diverse tropical ecosystems (Bagchi *et al*., 2014). An additional explanation for the higher levels of RAP in tropical compared to temperate species may be that the tropical trees do not need to protect their living cells from cold-induced damage. Since protection against cold is

energetically costly, it may limit the RAP abundance in environments where frost frequently occurs (Morris *et al*., 2015). However, more research across a broader selection of tropical species is needed to establish firm links between NSC accumulation, functional traits of RAP and plant ecological strategies.

### *Conclusion*

In conclusion, the results of this study demonstrate that the amount of RAP and LFs is strongly related to the NSC storage capacity in the wood of temperate-deciduous trees. Because virtually all RAP and LFs of temperate trees were filled with starch, the values of NSC concentrations measured here at the onset of winter dormancy provide estimates of the seasonal maxima. In contrast to temperate trees, a more diverse set of functions of RAP might be inherent in species growing in a tropical environment with little or no seasonality. Future research should move beyond a static approach of studying NSC and focus on the functional questions related to NSC transport, conversion and use (Richardson *et al*., 2013, 2015; Klein and Hoch, 2015). It can be expected that these processes are tightly coupled to RAP connectivity and the activity of various carbohydrate-modifying enzymes and transporters within the RAP cells (Sauter *et al*., 1973; Decourteix *et al*., 2006, 2008).

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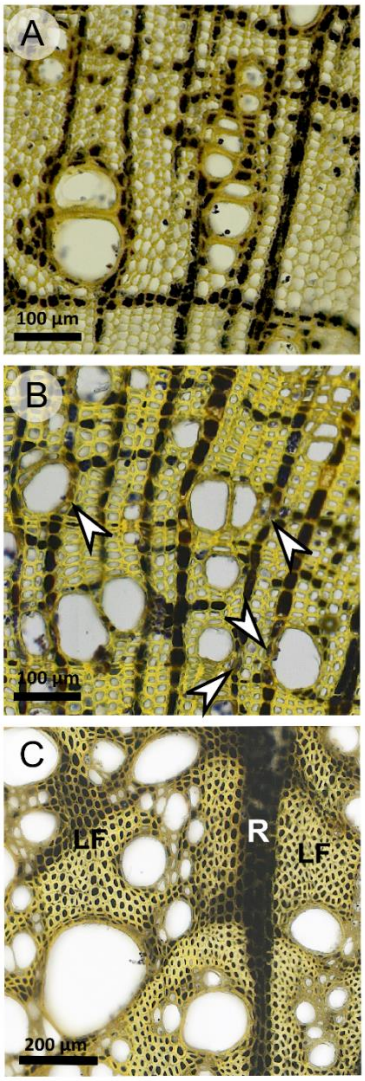
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**Supporting Information for *American Journal of Botany***

# The amount of parenchyma and living fibres affects storage of non-structural carbohydrates in young stems and roots of temperate trees

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**Appendix S1.** Light microscopy images taken at high magnification showing transverse sections of *Robinia pseudoacacia* (A), *Carpinus betulus* (B) and *Clematis vitalba* (C) stained with Lugol's solution. In *Robinia pseudoacacia*, starch grains were present in both vessel-associated RAP cells and RAP cells that are not directly adjacent to a vessel (A). In contrast, a tendency for reduced starch accumulation in vessel-associated cells is apparent in *Carpinus betulus* (B, arrowheads). Abundant living fibres (LF) and ray parenchyma (R) were filled with starch in *Clematis vitalba* (C).

# Part II

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# Publications

## Chapter 3

**Opinion Paper**

# Secondary xylem parenchyma – from classical terminology to functional traits

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## Introduction

Terminology plays a crucial role in describing and understanding the morphological and anatomical variation in living organisms. The huge diversity in plant species and their many morphological and physiological adaptations to a wide range of ecosystems is reflected in an enormous anatomical variation of woody tissues. This is perhaps somewhat surprising given that angiosperm wood consists of essentially three cell types only: imperforate tracheary elements (fibres and tracheids), vessel elements, and living parenchyma cells. However, variation in the dimensions and the arrangement of these cells provide a challenge to anyone who aims to describe and understand quantitative and qualitative differences between wood samples. The challenge lies not only in the consistent application and interpretation of terms (Lens *et al*., 2012), but also in how we deal with a dynamic continuum (i.e., fuzzy morphology *sensu* Agnes Arber and Rolf Sattler) that includes intergradations, intermediate forms, analogous and homologous features (Sattler and Rutishauser, 1997).

While the International Association of Wood Anatomy (IAWA) lists (IAWA Committee 1933, 1964, 1989, 2004) have been successful for identification and classification of angiosperm and gymnosperm wood, there is a lack of an anatomical glossary that goes beyond identification, covering the broad fields related to wood anatomy such as functional and ecological xylem anatomy, evolutionary and developmental wood anatomy, dendrochronology, etc. It is clear that achieving such a general glossary will never be perfect and will require a collaborative effort from many experts in various wood-related disciplines.

This opinion paper attempts to provide a critical review of terminology for wood parenchyma. It is especially concerned with the overlap between the descriptive terms used in systematic wood anatomical treatments and terms with functional implications. Most terminology for ray and axial parenchyma (RAP) has been defined based on the microscopy of transverse and longitudinal sections for wood identification purposes. A number of terms, especially for axial parenchyma, have changed in their usage or gone out of fashion; some of these are discussed here.

### *Parenchyma in secondary xylem*

Parenchyma tissue in the secondary xylem of woody plants represents the living symplastic tissue that is intermeshed with the apoplast (the dead tissues, which include the fibres [except living, septate ones] and conductive elements). It plays a crucial but perhaps underrated role in plant physiology. The interest in the subject from a functional perspective has been variable, with a surge of interest during the mid to late 20$^{th}$ century (Braun, 1964, 1984;

Braun and Wolkinger, 1970; Czaninski, 1977; Sauter and Kloth, 1986) followed by a decline and a resurgence of late (Salleo *et al*. 2004, Martinez-Cabrera *et al*., 2009, Zheng and Martinez-Cabrera, 2013; Ziemińska *et al*., 2013, 2015, Spicer, 2014; Morris *et al*., 2015). This renewed interest in ray and axial parenchyma (RAP) coincides with advances in image analysis and measurement techniques. Up to this point, a far greater focus has been on the dead conducting cells of wood (vessel elements and tracheids) and the mechanically important fibres of angiospermous wood (Carlquist, 2015). As more papers report on wood, it is important to address the terminology used for RAP, as there is a tendency by those outside the discipline of wood anatomy to misuse the terms and provide incorrect information. An overview of the terms related to axial and ray parenchyma are presented in Table 1 and 2.

### *Functions of RAP*

The terminology for describing RAP is important because disciplines outside of traditional wood anatomy are now paying more attention to their function e.g., plant physiology, functional ecology, plant-ecophysiology, plant pathology, and plant evolutionary-biology. The functions of RAP include: (1) storage and transport of non-structural carbohydrates (NSCs) (e.g., Hoch *et al*. 2003; Salleo *et al*., 2004; O' Brien *et al*., 2014; Plavcová and Jansen, 2015), (2) defence against pathogens (e.g., Shigo, 1984; Biggs, 1987; Schmitt and Liese, 1993; Deflorio *et al*., 2008), (3) water storage and capacitance (e.g. Holbrook, 1995; Borchert and Pockman, 2005; Pfautsch *et al*., 2015), (4) storage of minerals, such as calcium oxalate (Trockenbrodt, 1995), (5) the transition of sapwood into heartwood (Pinto *et al*., 2004; Spicer, 2005; Nawrot *et al*., 2008), and (6) biomechanical contributions, particularly by RP (e.g. Mattheck and Kubler, 1995; Burgert and Eckstein, 2001; Reiterer *et al*. 2002).

### *Contact and isolation cells of the ray system*

Secondary xylem parenchyma are living cells and are composed of two major cell types, ray parenchyma (RP) and axial parenchyma (AP), which are oriented perpendicular to each other. RP is aligned centripetally and formed from the ray initials of the cambium; AP is aligned axially and formed from the fusiform initials of the cambium (Metcalfe and Chalk, 1983). The cells in wide rays of angiosperms have been further categorised into two sub-types, 'contact' cells and 'isolation' cells, terms used by various authors to describe differing functions between the two (Braun, 1964, 1984; Braun and Wolkinger, 1970; Sauter and Kloth, 1986, Murikami *et al*., 1999; Spicer, 2014). Contact cells are in direct association with non-living conducting cells and have the ability to produce tyloses or gums. Isolation cells, which do not make any contact with conductive tissue, might be more specialised for

radial transport (Sauter and Kloth, 1986). Apart from their function, they also differ in form. Isolation cells are in many cases procumbent, and often have ray cells along their side, which are frequently ‘upright cells’. The ‘upright’ cells, or ‘erect’ ray cells, have the same orientation as the axial parenchyma, and may perform the same function as diffuse axial parenchyma in forming contacts between conduits and parenchyma, or simply contact between other parenchyma only (Höll, 1975; Carlquist, 2001). Conifers, on the other hand, have predominantly uniseriate rays, and therefore have contact cells only in their ray structure, where all ray parenchyma cells have some contact with tracheids.

**Table 1.** Anatomical descriptions for terms related to axial parenchyma (AP), along with the definition and source(s) of the term.

| ***Anatomical (descriptive) terms for AP*** | | ***Definition/comment*** | ***Sources for terms*** |
|---|---|---|---|
| ***Apotracheal****: where AP is distant or does not appear to be in contact with a vessel* | *Subcategories* | | |
| | **Diffuse** | AP scattered randomly, often sparse (e.g. *Carpinus* spp, *Crataegus* spp., *Cornus mas*). | IAWA Committee, 1933 |
| | **Diffuse-in-aggregates** | AP grouped in short tangential lines. No more than 2 cells wide (e.g. *Tilia* spp.). | Kribs 1937 |
| | **Reticulate** | This is a term for very regular networks of parenchyma bands and multiseriate rays as seen in transverse section. | Record, 1944<br>IAWA Committee 1989 |
| | **Banded AP** | From one to many cells wide; up to three cells wide – narrow banded, more than three – wide banded. This refers to apotracheal banded AP, as you can also have banded AP that is paratracheal. | Kribs, 1937 |
| | | *Concentric* - Many bands. Term no longer in use. | Jane, 1956 |

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|---|---|---|---|
| | | *Scalariform* - Successive fine lines or bands arranged horizontally or in arcs. Important point is that the distance between the rays is greater than the distance between bands (e.g. in Annonaceae), and that the rays are wider than the parenchyma bands. | Wagenführ 1961; IAWA Committee, 1989 |
| | **Marginal AP** | Bands of AP at a growth ring boundary. These are mainly apotracheal, though can be paratracheal.<br>*Initial* - rarer. Occurs at the beginning of a growth ring (examples: *Cedrela odorata. Tectona grandis*).<br>*Terminal* - most common type. Occurs at the end of a growth ring (e.g. *Magnolia* spp.). | Jane, 1934; Hess, 1950 |
| | **Ray-adjacent AP** | Diffuse AP clustered along ray margins (e.g. *Staphylea* spp.). | Hess, 1950; Carlquist & Hoekman, 1985 |
| ***Paratracheal**: where AP is in association with a vessel* | **AP Scanty Paratracheal** | A term agreed upon by the IAWA committee (1964) as preferred to vasicentric scanty (Kribs, 1937). An incomplete sheath of AP surrounding a vessel or occasional AP cells touching the vessel (e.g., *Fraxinus* spp., *Laurus nobilis*). | IAWA Committee, 1964, 1989 |

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|---|---|---|---|
| | **AP Vasicentric** | Where paratracheal parenchyma form a complete oval or circular sheath around a vessel or vessel group. | Jane, 1934; IAWA Committee, 1964, 1989. |
| | **AP Aliform** | Paratracheal AP with lateral extensions, often wing-like, forming a diamond shaped outline referred to as lozenge-aliform. It can either completely surround the vessel or can be to one side (e.g., *Inga* spp. and *Brosimum* spp.). Both winged-aliform and lozenge-aliform were designated as sub-types by the IAWA Committee, 1989. | Kribs, 1937; IAWA Committee, 1989 |
| | **AP confluent** | Where vasicentric or aliform arragements coalesce. It can completely surround or form to one side of a vessel or vessel group, forming irregular tangential or diagonal bands (e.g., *Parkia pendula*, *Chlorophora tinctoria*). A special case is banded-confluent, where many vessels are united by tangentially confluent parenchyma. | Kribs, 1937; Metcalfe & Chalk, 1950; IAWA Committee, 1964 |
| ***AP absent/extremely rare*** | | AP absent or extremely rare intergrades with both apotracheal (diffuse) or scanty paratracheal. In order to make sure that the AP is absent or extremely rare it is necessary to view longitudinal as well as transverse sections (e.g. *Salix, Populus*, *Scottellia*). Septate fibres are often present where AP is rare or absent (Wheeler *et al.* 2007). | IAWA Committee, 1989 |

**Table 2.** Functional descriptions for terms related to ray and axial parenchyma (RAP), along with the definition and source(s) of the term.

| ***Functional terms for RAP*** | ***Definition/comment*** | ***Sources for terms*** |
|---|---|---|
| | *Subcategories* | |
| | **Accessory tissues** Parenchymatous tissues in association with the conductive system (vessels and tracheids) via pit membranes (half-bordered). Through these close pit contacts a functional unity exists between the accessory tissues and the conductive system. Concerns both angiosperms and gymnosperms. | Braun, 1984 |
| | **Vessel-associated cells** Specialised RAP that are in contact with the vessels, as per term. Czaninski (1977) coined the term in preference to contact cells; however, both are used interchangeably today. | Czaninski, 1977 |
| | **Paratracheal contact** Concerns only AP in actual contact with the vessels. Concerns angiosperms only. Braun (1984) coined this term as a means of specifically referring to angiosperms, where the term paratracheidal was coined in reference to only conifers. Not in use today. | Braun, 1970, 1984 |

| | | | |
|---|---|---|---|
| | **Paratracheidal** | AP in direct contact with tracheids. Gymnosperms only (not including those with vessels). The use of this term has been discontinued. | Braun and Wolkinger, 1970 |
| | **Contact cells** | These are specialised RAP in direct contact with the vessels, a term that is in wide use today and used interchangeably with the term 'vessel-associated-cells'. Connected to vessels via half-bordered. | Braun and Wolkinger, 1970<br>Braun, 1984<br>Sauter, 1972, 1988<br>Sauter and Kloth, 1986 |
| | **Isolation cells** | Confined to the ray system, and used to describe cells that have no contact with any conductive tissues. Probably, more specialised for radial transport. | |
| ***Vessel-distant AP*** | **Storage cells (storage parenchyma)** | RAP cells that have a common function in the storage of NSCs. These are not classified as specialised cell types. These can also contain tannins and crystals, such as calcium oxylate and tend to have more numerous plasmodesmata connections (Bonnemain and Fromard, 1987; Czaninski, 1987). | Czaninski, 1977 |

### *Arrangements of the axial parenchyma system*

*Paratracheal AP*

AP is situated between the rays and is aligned vertically. These cells may be highly grouped around the vessels, which is termed 'paratracheal', or alternatively, the AP cells may completely surround the vessel as regular layers, a condition referred to as 'vasicentric' (e.g. in *Phoebe porosa*, *Enterolobium cyclocarpum*, *Olea europaea*). However, more commonly, the AP is scanty paratracheal, or may be either aliform or confluent in arrangement. The scanty paratracheal arrangement is very frequent, occuring in up to 28% of all hardwoods (Wheeler *et al*., 2007). Aliform is wing-shaped with lateral extensions and is further sub-divided into 'lozenge-aliform' (e.g. *Microberlinia brazzavillensis*, *Qualea rosea*) or 'winged-aliform' (e.g. *Jacaranda copaia*, *Terminalia superba*, *Brosimum* spp.). The term 'confluent' describes paratracheal arrangements that coalesce or where aliform types completely surround or are to one side of two or more vessels together (e.g. *Parkia pendula*, *Peltogyne confertiflora*). Both aliform and confluent have been considered a sub-set of abundant vasicentric (Kribs, 1937; Carlquist, 2001); however, this usage is not very common. The term 'scanty paratracheal' (see *Catalpa ovata*; fig.1, C) describes a situation where the AP does not completely sheath the vessel or a vessel group, or where they are just occasionally making contact with the vessel (e.g. *Pistacia vera*, *Laurus nobilis*, *Fraxinus excelsior*) (Hess 1950, Metcalfe and Chalk 1950; Plavcová and Jansen, 2015). Unilateral paratracheal parenchyma is of rare occurrence, and if present often found alongside other paratracheal arrangements. It describes paratracheal AP that forms to one-side of the vessels and is shaped in a semi-circular fashion (e.g. *Peltogyne confertiflora*). The terms abaxial and adaxial describe paratracheal AP arranged to either side of a vessel and have been fused into the term 'unilateral' and generally discontinued as descriptors because in most wood samples or sections of mature stems the orientation with respect to the cambium and pith is not obvious (IAWA Committee, 1989).

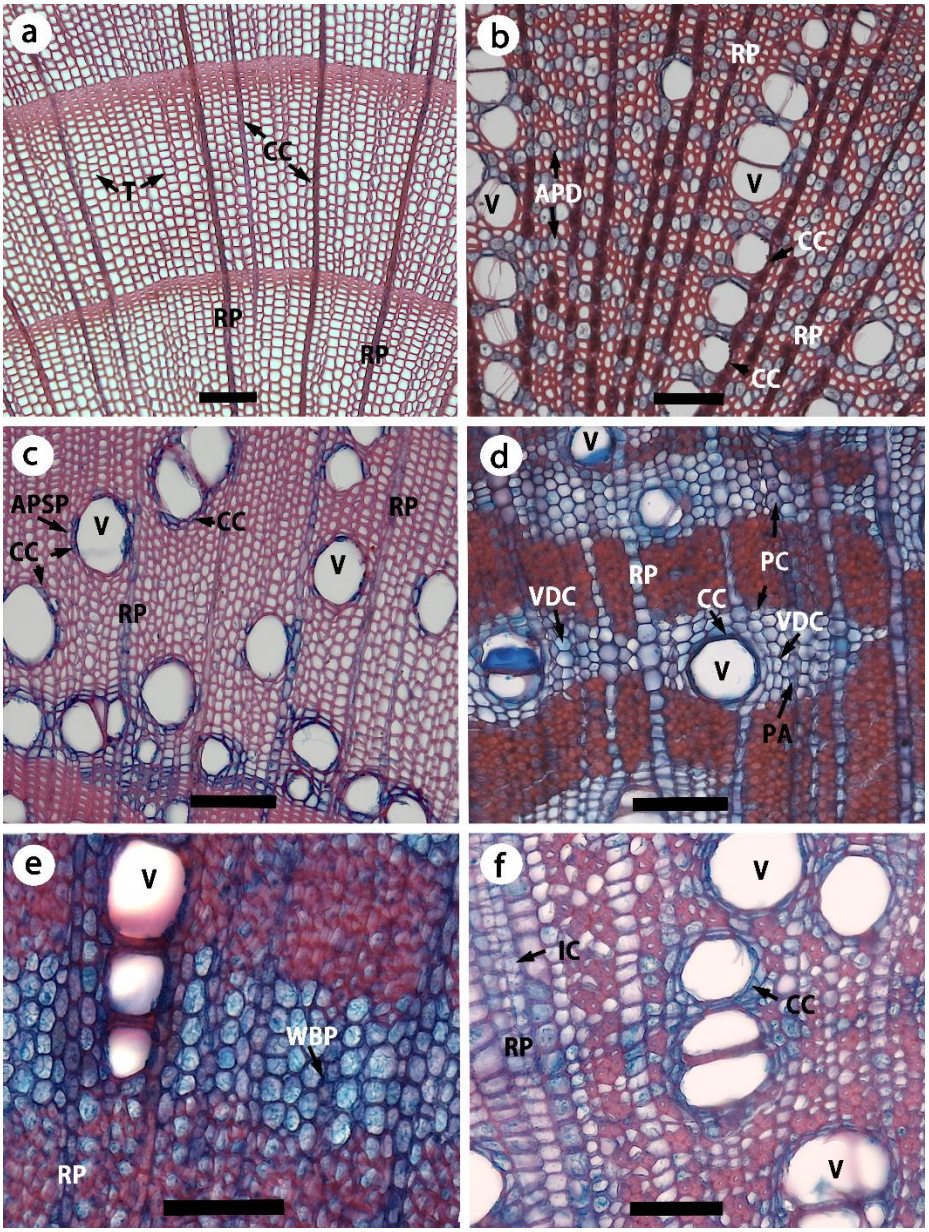



**Fig. 1** Light microscopy images of the transverse sections of one conifer and five angiosperm species showing different axial parenchyma (AP) and ray parenchyma (RP) cell arrangements where both functional and anatomical terms are shown for (a) *Picea abies* (Pinaceae), a temperate species with no axial AP present, and where all the uniserate RP are contact cells (CC) having direct contact with the tracheids (T), (b) *Quercus robur* (Fagaceae) a temperate species with axial parenchyma diffuse (APD), (c) *Catalpa ovata* (Bignoniaceae) a temperate species with axial parenchyma scanty paratracheal (APSP) shown here as contact cells, (d) *Amherstia nobilis* (Fabaceae), a tropical species with AP in paratracheal arrangements called paratracheal aliform (AP) and paratracheal confluent (PC), (e) *Caesalpinia mexicana* (Fabaceae), a subtropical species with wide-banded parenchyma (WBP) and (f) *Ceiba pentandra* (Malvaceae) , a tropical species with wide muti-seriate rays showing the isolation cells (IC) between the two most outers layers. All are from stem wood, except *Q. robur*, which was taken from the roots. The sections were stained with a combination of safranin and alcian blue, where the red colour of the former highlights strongly lignified cell walls of the tracheids, vessels and fibres, while the blue stain of the latter highlights both AP and RP. All scale bars represent 100 µm.

There is a complication between the commonly used anatomical definition of paratracheal (Metcalfe and Chalk, 1983) and Braun's (1984) functional definition. From a functional point of view, it is essential to have such a term that describes AP in immediate contact with vessels rather than also including vessel-distant AP that is part of a complete paratracheal arrangement. Braun termed vessel-distant paratracheal parenchyma 'interfibrous parenchyma', and devised the more specific term paratracheal-contact cells for the "accessory tissue" of his "hydrosystem", in analogy with the contact cells of the rays. The use of the term 'interfibrous parenchyma' was unfortunate, as it encompassed an array of arrangements that are in themselves quite distinct

Terms can be essential for the identification of wood; however, from a functional perspective, many of the terms are less valuable because of a morphological continuum. For instance, the difference between a unilateral and a lozenge-aliform arrangement may be functionally negligible, as the only separation between the two is that the latter has a diamond-shaped outline and is present around the whole vessel, an important difference for wood identification purposes. However, the broader terms, such as apotracheal, paratracheal and even scanty paratracheal are certainly important terms for functional studies, as these arrangements have an important association with the vessel in relation to capacitance and defence (Brodersen *et al*., 2010; Plavcová and Jansen, 2015; Morris *et al.* 2015). For instance, the lack of such AP arrangements in most conifer species may explain why they require narrow, "safe" tracheids as conductive tissue (Choat *et al*. 2012).

*Apotracheal AP*

The term 'apotracheal' describes AP cells that are not associated or contiguous with vessels in transverse section. Some random contacts may exist, although these are infrequent (Evert, 2006). Diffuse, diffuse-in-aggregates, and marginal AP essentially describes three subcategories of AP that are not in association with vessels. The descriptive terms for AP are when the AP appears distributed in transverse sections. For instance, in the case of diffuse there are a number of sources that describe AP as being isolated and irregularly dispersed, which is true when viewed as a single transverse section. However, from a functional point of view, this is not the case, as such cells cannot be isolated from surrounding symplastic tissue because all parenchyma cells are to some degree interconnected with each other (Zimmermann and Tomlinson, 1966; Zimmermann, 1971). All diffuse parenchyma types are networking with both the vessels and the ray system. This was demonstrated by Zimmerman

(1971) who showed that an isolated group of AP will sooner or later contact other AP and/or RP.

Alternatively, AP might act as a transport bridge between rays, thus by-passing the vessels altogether, as is the case with the AP pattern diffuse-in-aggregates or the narrow bands of axial parenchyma. The latter term 'diffuse-in-aggregates', describes where they form tangential lines, forming no more than one cell wide between rays, although in transverse section one may or may not see diffuse-in-aggregates contacting rays (Metcalfe and Chalk, 1950; Carlquist, 2001). Examples of diffuse-in-aggregates include *Tilia* spp. (Malvaceae) and *Dalbergia stevensonii* (Fabaceae). Diffuse AP are more commonly associated with temperate species. In tropical tree species where AP is absent or rare, there is a higher incidence of septate fibres (Wheeler *et al*., 2007). Here, septate fibres are presumably taking over the functional role of AP, where examples include: Acharaceae, Araliaceae, Burseraceae and Salicaceae. Both Araliaceae and Salicaceae are found in temperate and tropical biomes. *Kalopanax septemlobus* (Araliaceae) is an excellent example of a temperate species with absent or extremely rare AP, but with an abundance of septate fibres taking over the functional role.

The term 'banded' applies to AP that is one to many cells wide, and this is further subdivided into narrow-banded and wide-banded apotracheal AP (see *Caesalpinia Mexicana*; fig.1, E). Although banded arrangements are mostly apotracheal, they can be paratracheal, where they are clearly associated with the vessels. Under the IAWA Committee (1989) it forms a separate group of arrangements that includes the arbitrary, overlapping sub-categories (1) axial parenchyma bands more than three cells wide, (2) axial parenchyma in narrow bands or lines up to three cells wide, (3) axial parenchyma reticulate, (4) axial parenchyma scalariform and (5) axial parenchyma in marginal or in apparently marginal bands. Wide bands (more than three cells wide) are much more common in tropical trees, perhaps acting as a dynamic barrier against the inward advance of decay, thus replacing the growth ring of temperate species, a static barrier where highly lignified fibres slow the spread of decay; this is Wall 2 of the CODIT model, an acronym for Compartmentalisation of Decay in Trees (Shigo, 1984). Although wide banded AP are more common in the trees of the tropics than in temperate regions (e.g. Meliaceae and Moraceae), they still have a rare overall occurrence with only a 9% of the worlds' woods having this characteristic (Wheeler *et al*. 2007). A quick check of 834 species with wide bands (InsideWood); Wheeler *et al*., 2007) showed a much higher incidence of wide parenchyma bands with growth rings absent or indistinct (748 species out

of the total) indicating that in the absence of growth rings, parenchyma may be taking on a defensive role. When the wide bands run via vessels, they intergrade with confluent AP, a pattern under the umbrella of paratracheal. A term was in use to describe this as 'banded confluent' (Jane, 1970), which acknowledged that bands are not always circumferentially continuous, but can be as a result of there being a considerable amount of confluent parenchyma that form together to appear as bands. There are, of course, gradations in the continuity of the bands and they can appear independent of vessels or extend from vessel to vessel where then the distribution of the bands appears related to the position of the vessel.

Marginal AP, coined by Hess (1950) is banded, but laid out at the beginning or at the end of a growth ring, termed, respectively, initial and terminal. Usually only one type is present in a species, although some species have both, for example *Robinia pseudoacacia;* however, this rarely occurs (Carlquist, 1980). We do know that in a number of temperate species, apotracheal terminal parenchyma participate actively in defence, particularly against brown rot fungi (Schwarze and Fink, 1998, Schwarze *et al.* 2000, 2003, 2007). However, this anatomical trait has a worldwide distribution, from temperate to subtropical and tropical biomes, and studies conducted were biased to species of temperate origin. The InsideWood database (Wheeler *et al*., 2007) reports 1782 from a total of over 5000 species to have marginal parenchyma.

***From a descriptive to a functional terminology***

Braun (1970, 1984) tried to include both gymnosperms (with the exception of Gnetales) and angiosperms (not including vesselless genera) by referring to RAP in association with either tracheids or vessels as 'accessory tissues' of his hydrosystem, a term now mostly discontinued in modern publications. This was a very specific functional term used by Braun (1984), where he makes a distinction between tropical and temperate trees. Braun suggested that in tropical trees accessory tissue tends to have little or no starch because the sugars are in a state of constant remobilisation. By contrast, temperate trees have starch in the accessory tissues, but it is broken down earlier than in surrounding parenchyma (Sauter, 1966; Braun, 1984; Essiamah and Essiamah, 1985). The accessory tissues further included the functional terms 'paratracheidal parenchyma' and 'paratracheal contact parenchyma', two terms referring specifically to AP of both gymnosperms and angiosperms, respectively. Braun's (1984) usage of the term 'paratracheidal' was perhaps to make a distinction between the tracheids of conifers and the vessels of angiosperms, particularly for tropical conifers, which have, on average, more AP than their temperate counterparts, such as in many genera of the

Podocarpaceae (Morris *et al*. 2015). The contact cells of the rays were also included in Braun's accessory tissues (Braun 1964, 1970, 1984).

The term 'contact cell' (see fig. 1), was also coined for any parenchymatous cell in contact with a vessel (Sauter, 1966, 1973). More recent papers have reverted back to the original Sauter (1966) application of the term 'contact cell' (Bonsen and Kučera 1990, Améglio and Cruiziat, 1992; Sokołowska, 2013; Zwieniecki and Holbrook, 2009; Secchi and Zwieniecki, 2012). Isolation cells, from a functional and spatial perspective, not only apply to the ray structure; vessel-distant cells (see *Amherstia nobilis*; fig. 2, A) within a large group of paratracheal AP (surrounding a vessel) are in effect isolated to the same degree as those of the ray system, and may have similar functions.

Irrespective of the difference in cambial origin between RP and AP, there is a striking analogy between RP and AP as both have contact cells and vessel-distant cells, and many common functions are shared. For instance, contact cells from RP and AP both have (1) different pits, which are either half-bordered or simple, possibly functioning in the hypothetical release of sugar into vessels as opposed to those that just store NSCs (Sauter 1972, 1973; Braun 1984), (2) both can form tyloses during heartwood formation when the pit aperture exceeds 10 μm, or gums when the width falls below this figure, although tyloses occur largely from the RP during the transition to heartwood (Chattaway, 1949), while elsewhere in the sapwood, in response to pathogens etc., tyloses can occur where pits exceed 3 μm (Bonsen and Kučera 1990), (3) both have a protective layer (Schmid, 1965), also referred to as the amorphous layer when referring to vessel-associated RP and AP (Fujii *et al*., 1980, 1981), and (4) both have similar enzymatic activity marked by high mitochondrial counts, and carbohydrate storage/mobilisation cycles (Sauter, 1973; Essiamah and Eschrich, 1985; Alves *et al*., 2001). While, on the other hand, isolation cells (see *Ceiba pentandra*; fig. 1, F) of the ray structure, and paratracheal vessel-distant AP have: (1) no pit-mediated direct contact associations with vessel elements, (2) high plasmodesmatal densities, as no plasmodesmata are at the interface between a contact cell and a vessel (Bonnemain and Fromard, 1987; Czaninski, 1987), and (3) long term storage capacities (large vacuoles) with generally slow mobilisation cycles, most evidently in temperate species (Braun, 1984). In fact, during autumn, RAP that does not have contact with vessels accumulate oxalate, tannins, or large quantities of starch, a trend not shown in contact cells (Buvat, 1989).

Czaninski (1964, 1977, 1987) in the 1960s coined the term 'vessel-associated cells' (VACs) as a term for AP and RP in contact with vessels that share common functions. It was first used in the case of *Robinia pseudoacacia* for the small 'specialised cells' surrounding the vessels, and represented a more precise description than just 'contact cells'. The VACs were used as a means to further divide cells from those with a common function, such as storage of NSCs, into those with more 'specialised' functions, like the production of tyloses, or the secretion of defensive compounds into the vessel. Czaninski (1970) also coined the term 'storage cells', for vessel-distant AP, which are distinguished from VACs (or contact cells) by their larger lumen and vacuole size.

Another term 'transfer cells' as coined by Gunning *et al*. (1968, 1974) and normally used in unison with the phloem, had its use extended to xylem parenchyma in association with vessels. Chafe (1974) stated that the contact cells might function as transfer cells owing to a similar structure as those in the phloem. While as useful an analogy as it may have sounded, the term used in this context seems to have expired, because vessel-contact cells lack the elaborate wall invaginations typical of true transfer cells. These days, the terms VACs and contact cells can be used interchangeably for angiosperms, while contact cells is a suitable term when also referring to conifers.

***Conclusion and outlook***

Terms we coin and define for RAP are critically important for how we interpret them, and therefore use them in the right context, especially in relation to xylem function. Rather than a revision of terms, the main message here is that it is important to be mindful of what term to use and where correctly to use it, whether it be from a functional or structural perspective. More attention should be paid to the three dimensional structure and arrangement of RAP, which remains poorly explored despite the development of novel techniques such as high resolution computed tomography and image analysis tools (e.g., alignment and stitching tools; Huggett and Tomlinson, 2010; Brodersen, 2013). Moreover, precise descriptions in combination with exact quantification are highly desired to make progress in the field of functional and ecological wood anatomy (Scholz *et al*., 2013; von Arx *et al*., 2013, 2015). We also hope that training of new students will be offered in such a way that they will develop a special eye for detail and accuracy, which is required to capture the maximum information available in wood. Many of us would indeed be surprised by how many details are presented in old literature such as the works by Moll and Janssonius (1909 – 1936).

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west of Ireland and listen to that Jazz, the great jazz musicans, many of whom he knew, and all my cares would just drift away, just for that moment. Thanks you Gerry.

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**Date of Birth 25.01.1977**

## Professional profile

A dynamic, enthusiastic, and personable individual with a strong educational background along with a very varied experience throughout a range of disciplines, including, horticulture, arboriculture, and botany. Currently I am a doctor student at the Ulm University, Germany, investigating the role of xylem parenchyma in long distance water transport, embolism repair, defence against fungi, capacitance etc., in woody plants, under the supervision of Prof. Dr Steven Jansen. My role includes teaching on practical's, namely tropical ecology, and tropical field work supervision, including in places such as Costa Rica.

## Qualifications

| | |
|---|---|
| **2013- 2016** | **PhD (expected) in plant biology at Ulm University, Germany** |
| **2011- 2012** | **Post Graduate in Higher Education, at the University of Brighton** |
| **2006 – 2007** | **MSc in the Biodiversity and Taxonomy of Plants, The University of Edinburgh/Royal Botanic Gardens Edinburgh (Researched at the Royal Botanic Gardens, Kew)** |
| **2001 - 2005** | **BSc (Hons) Arboriculture, Forestry Department, University of Central Lancashire/Myerscough, Preston, Lancs, (OPM 68.27%)** |
| **2001 – 2003** | **NPTC (chainsaw use and tree climbing) (CS 30, CS 31, CS 31a, CS 31b, CS 36, CS39, CS 41), Myerscough/RHS** |
| **2002 – 2003** | **Certificate in Arboriculture and Estate Management, Royal Horticultural Society, Wisley** |
| **1998 – 2001** | **Diploma in Amenity Horticulture, An Grianan, Termonfeckon, Droheda, Co Louth** |
| **1996** | **Leaving certificate, St Jarlath's College, Co Galway**. |

## Previous occupations

| | |
|---|---|
| 2013 – 2016 | PhD student in plant biology at the Ulm University (current employment) |
| 2010 – 2013 | Associate lecturer, Forestry and Arboriculture Department University of Brighton |
| 2007 – 2010 | Tree officer of open spaces, Test Valley Borough Council, Andover, Hampshire |
| 2005 - 2006 | Assistant manager of Evenley Wood Garden (plant collection), Evenley, Northants |
| 2003 – 2003 | Consultant to the Royal Horticultural Society to set up a tree hazard evaluation system |
| 2002 – 2003 | Student worker at the Wisley Gardens RHS in Arboriculture and Estate Management |
| 1999 - 2000 | Student worker at Hillier Tree Nursery, Hillier Tree Farm, Andler's Ash, Liss, Hants |

**Current projects**

Currently managing a large project which brings parenchyma and vessels together using a multidisciplinary approach, in particular the relationship between axial parenchyma and vessel diameter. Here, we also focus on climate influences on vessels together with height and DBH of woody plants. We are also preparing a review paper to investigate the role of living cells in response to pathogenic fungi.

**Memberships include:** International Society of Arboriculture, the Arboricultural Society, the Institute of Chartered Foresters, and the International Dendrology Society, Arnoldia, Linnean Society (Fellow), IAWA (International Association of Wood Anatomy). Higher education Academy, UK (fellow).

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9. Plavcová L, Hoch G, **Morris H,** Ghiasi S, Jansen J. 2016. The amount of ray and axial parenchyma drives storage of non-structural carbohydrates in wood of temperate trees. *American Journal of Botany* **103:** 1-10.
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**II. Papers presented at national conferences – only published as Abstract**

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- **Morris H**, Plavcová L, Jansen S. 2015. A global analysis of parenchyma tissue fractions in secondary xylem of seed plants. *Bordeaux, France. 7-9 September, 2015.*
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# Erklärung

Ich versichere hiermit, daß ich die Arbeit selbständig angefertigt habe und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt sowie die wörtlich oder inhaltlich übernommenen Stellen als solche kenntlich gemacht habe.

Ulm, den 21.April. 2016.