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Influence of Cambial Age and Axial Height on the Spatial Patterns of Xylem Traits in *Catalpa bungei*, a Ring-Porous Tree Species Native to China

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Abstract: Studying how cambial age and axial height affects wood anatomical traits may improve our understanding of xylem hydraulics, heartwood formation and axial growth. Radial strips were collected from six different heights (0–11.3 m) along the main trunk of three Manchurian catalpa (*Catalpa bungei*) trees, yielding 88 samples. In total, thirteen wood anatomical vessel and fiber traits were observed using light microscopy (LM) and scanning electron microscopy (SEM), and linear models were used to analyse the combined effect of axial height, cambial age and their interaction. Vessel diameter differed by about one order of magnitude between early- and latewood, and increased significantly with both cambial age and axial height in latewood, while it was positively affected by cambial age and independent of height in earlywood. Vertical position further had a positive effect on earlywood vessel density, and negative effects on fibre wall thickness, wall thickness to diameter ratio and length. Cambial age had positive effects on the pit membrane diameter and vessel element length, while the annual diameter growth decreased with both cambial age and axial position. In contrast, early- and latewood fiber diameter were unaffected by both cambial age and axial height. We further observed an increasing amount of tyloses from sapwood to heartwood, accompanied by an increase of warty layers and amorphous deposits on cell walls, bordered pit membranes and pit apertures. This study highlights the significant effects of cambial age and vertical position on xylem anatomical traits, and confirms earlier work that cautions to take into account xylem spatial position when interpreting wood anatomical structures, and thus, xylem hydraulic functioning.

Keywords: wood anatomy; vertical and radial variation; earlywood; latewood; growth ring width; tyloses; pit membrane diameter; vessel lumen diameter; fibre length

1. Introduction

The xylem structure is a complex and delicate multicellular network, which simultaneously fulfils three key functions, namely water transport, mechanical support, and water and nutrient storage [1]. The anatomical traits of xylem are thus intriguing to further understand the related functional implications in trees. Spatial patterns of wood anatomical traits within trees, both radial and axial, as well as inter-tree wood anatomical variations, are therefore not only important for wood manufacture, but also for plant hydraulics and other interdisciplines.

In angiosperm tree species, vessels and fibres account for the largest fractions of vascular structures, while vessels function as the main water conducting cells of the xylem hydraulic architecture [2]. Within individual trees, the largest vessels are found in roots and vessel lumen diameters become acropetally tapered, i.e., small conduits tend to distribute in distal parts of trees such as the apical positions. According to the hydraulic segmentation hypothesis [2], distal positions in plants are more embolism-resistant, and given that larger conduits are more vulnerable to embolism [3], smaller conduits in apical parts might be a biological compensation for increased hydraulic resistance with axial height [4,5].

Vessel dimension has been investigated in many studies due to its significant contribution to hydraulic architecture and water transport [6–9]. As a key parameter of vessel dimension, vessel lumen diameter has received much attention in recent years [10,11]. Vessel lumen diameter was previously reported to decrease with axial height in a few tree species [4,5]. However, the effect of both axial height and cambial age on vessel lumen diameter and other vessel-related traits has not been fully emphasized so far. Specifically, radial patterns of vessel-related traits, as well as the effect of cambial age on these traits, have mainly been addressed in dendrochronological studies [12,13]. Previous studies commonly included wood anatomical traits of different cambial ages at the stem level [14–17], and analyses of vertical patterns of wood anatomical traits have mainly been restricted to the outer growth rings [18,19]. Accordingly, within-tree variation, i.e., axial or radial variation in xylem traits, has frequently been documented [12,13,19]; however, reports on both axial and radial variation in wood anatomical traits, as well as their functional implications, are limited to a few species so far [20–29]. As a consequence, the simultaneous effect of axial height and cambial age, which both might affect vascular differentiation, has not been well investigated.

In contrast to vessels mainly fulfilling hydraulic functions, fibres exist as the ground tissues in angiosperm xylem, which provide sufficient mechanical strength and serve as useful biomaterial for the pulp and wood industry. Vertically, a decline in fibre length with axial height has been reported in a few hardwood species [30,31], although it did not apply universally as no significant change of fibre length with axial height was observed in *Alnus rubra* [20]. In radial direction, fibre length tends to increase with cambial age in some species [23,27], while it decreases in other species [32]. Understanding how wood anatomical traits vary with cambial age and axial height would provide useful information on xylem hydraulics and the sapwood–heartwood transformation. Furthermore, our understanding on how pit geometry, such as the pit membrane diameter, might change radially or vertically remains surprisingly limited. Bordered pits contributed to 50% of the total hydraulic resistivity along the flow path for water transport in angiosperm xylem, which are small openings between secondary vessel walls facilitating water flow, both radially and axially [33,34]. Bordered pit resistance might be attributed to pit geometry, such as pit aperture diameter, pit membrane diameter, pit membrane thickness, etc.

When trees grow older with increasing cambial age, the xylem undergoes numerous changes that visibly differentiates sapwood from heartwood [35]. While the sapwood contains living cells, such as axial and radial parenchyma [36], heartwood is mainly composed of dead cells. In angiosperms, heartwood vessels are commonly infiltrated by tyloses, which are outgrowths with different morphological features entering through pit membrane pores from adjacent ray or axial parenchyma cells in a vessel wall [37–40]. Tyloses have multiple functions including limiting the spread of pathogens and wood decay, as well as compartmentalization post wounding [41,42]. This is especially common in temperate ring-porous tree species with only the outermost xylem being conductive and functional [43,44]. Nevertheless, the radial distribution of tyloses has not been fully investigated, which partially hinders our understanding of wood permeability. Besides, quantitative wood anatomical differences, as well as microscopic and ultrastructural wood anatomical patterns in the xylem radial direction, especially for vessel- and fibre-related features during sapwood and heartwood transformation, have been rarely demonstrated. In addition, some xylem traits are strongly

associated with wood utilization characteristics, such as wood density; therefore, investigating spatial wood anatomical patterns would further benefit high-value added timber utilization.

The main motivation of the present study was to analyse the effects of cambial age and axial height on the xylem anatomical traits in *Catalpa bungei* C.A. Mey, an economically important ring-porous tree species with high wood quality [45], hence improving our understanding of its xylem hydraulics and heartwood formation. Xylem structural features were investigated in the radial direction using scanning electron microscopy to further investigate the sapwood–heartwood transformation. Thirteen vessel- and fibre-related traits, as well as annual diameter growth, were measured radially at six different height levels of three trees, enabling us to understand structural changes during different developmental stages of the secondary xylem in both intra- and inter-tree levels. Moreover, biological and hydraulic implications of spatial patterns of wood anatomical traits were addressed in this study. We hypothesized significant effects of both the cambial age and axial height on studied wood anatomical traits, due to the driving force during the transformation between sapwood and heartwood, tree axial growth, and xylem water transport demand.

2. Materials and Methods

2.1. Study Site and Radial Strip Sampling

The experimental site is located in a valley in Shatuo village, Madian town, Luoyang City, China (111°26′–111°47′ E, 34°28′–34°37′ N), which has a temperate continental monsoon climate. The average annual temperature is 13.9 °C and average rainfall was 560 mm between the years 2013–2017. Three plantation-grown *C. bungei* trees of comparable age and size were randomly selected from plantation for this study (axial height: 14.9 m, 20.3 m, 15.8 m; diameter at breast height: 26.3 cm, 30.4 cm, 25.5 cm; tree age: 33 years, 44 years, 36 years, respectively). Disks with a thickness of 7–10 cm were harvested from six different height levels of the trunk in each tree (0 m, 1.3 m, 3.8 m, 6.3 m, 8.8 m, 11.3 m) from the base to the top in early 2018. In total, eighteen disks were obtained from the three trees. From each disk, a 50 mm thick pith-to-bark strip with a north orientation was collected, and all radial strips were frozen and stored in a freezer.

2.2. Wood Sampling and Light Microscopy Observation

A planer was used to provide a smooth surface of the radial strips and clarify tree rings, which were subsequently marked by pins. Subsequently, growth rings were counted and tree ring width was measured at 10× magnification using the software LINTAB 5 TM6-Tree Ring Station, Rinntech, Germany. In this species, sapwood and heartwood could easily be distinguished from their colour variation. Along the central direction of each radial strip, small wood samples (10 × 10 × 10 mm) were collected from the inner part of heartwood (Hi), centre of heartwood (Hc), outer part of the heartwood (Ho), centre of transition wood (T) and centre of the sapwood (S), and the growth ring locations of the wood samples were noted. In each sample, 1–6 growth rings were included. The annual diameter growth (AGR, cm yr⁻¹) for each sample was calculated as two times the sum of all growth ring widths spanned by the wood sample divided by the number of rings, i.e., $AGR = (2 \times \text{cumulative tree ring width}) / \text{total tree ring numbers}$, assuming that wood disks are cylindrical.

In total, 88 wood samples were collected and transversal and tangential sections with a thickness of 20 μm were cut from the samples with a sliding microtome (Leica SM 2010R, Leica Biosystems Inc., Buffalo Grove, IL, USA). Sections were stained with safranin (2%) for about one minute, dehydrated with an ethanol series (50%, 75%, 95%, 100%) for a few seconds, washed with xylene, and mounted with a drop of Canada balsam. Subsequently, sections were observed using a light microscope (Olympus BX 50, Olympus Corporation, Tokyo, Japan), and images were taken at 40×, 100×, 200× or 400× magnifications for analysis. Software ImageJ (National Institutes of Health, Bethesda, MD, USA) was applied for all image analysis and all the measurements were performed manually in the tangential direction from complete areas that included all tree rings in the sections. Details on the anatomical

parameters measured, as well as the magnifications used, are reported in Table 1. Specifically, tracheid was absent in this species. Vessel lumen diameter was calculated from the equivalent circle diameter, vessel element length was measured including the “tails”, and pit membrane diameter was calculated from the equivalent circle diameter based on measurements of the pit membrane surface area.

Table 1. Wood anatomical features measured in *C. bungei*. D_V , vessel lumen diameter; D_{MAX} , maximum vessel lumen diameter; L_V , vessel element length; V_D , vessel density; T_{FW} , fibre wall thickness; D_f , fibre lumen diameter; L_f , fibre length; t/b , fibre wall thickness/diameter ratio; D_{pm} , pit membrane diameter; AGR, annual diameter growth.

Variable	Unit	Wood Type	Magnification	<i>n</i>	Mean	SD
D_V	μm	earlywood	40×	100 vessels	180.7	28.2
		latewood	100×	100 vessels	21.6	3.9
D_{MAX}	μm	earlywood	40×	100 vessels	297.4	41.6
		latewood	100×	100 vessels	44.7	11.2
L_V	μm	both	40×	50 vessels	150.8	19.3
V_D	mm^{-2}	earlywood	40×	5 measurements	11.0	2.6
		latewood	40×	5 measurements	378.2	126.9
T_{FW}	μm	latewood	400×	50 fibres	1.5	0.4
D_f	μm	earlywood	200×	100 fibres	15.5	2.3
		latewood	400×	100 fibres	9.4	1.1
L_f	μm	both	40×	50 fibres	752.0	67.1
t/b	-	latewood	-		0.2	0.0
D_{pm}	μm	earlywood	400×	50 pits	8.2	0.6
AGR	mm yr^{-1}	both	10×	1–7 year rings	6.8	3.5

2.3. Scanning Electron Microscopy Observation

Five samples from the radial strip collected at breast height, i.e., at 1.3 m height, of tree No. 2 previously trimmed with the microtome were thereafter used for scanning electron microscope (SEM) observations, namely samples from Ho, Hc, Hi, T and S. These samples were split tangentially into small blocks ($10 \times 5 \times 1$ mm) and oven-dried at 60 °C for 24 h. The blocks were subsequently coated with gold at 15 mA in anion sputter coater (Hitachi E-1045, Hitachi, Ltd., Tokyo, Japan) for one minute, and observed with an SEM (Hitachi S4800, Hitachi, Ltd., Tokyo, Japan) under high vacuum (5 kV accelerate voltage, 8 mm or 14 mm working distance, se detector).

2.4. Statistical Analyses

To analyse the effects of cambial age and axial height on the wood anatomical traits of collected samples listed in Table 1, we fitted simple log-linear regression models using the natural logarithm of the traits as a response, and axial height, natural log-transformed cambial age and their interaction as predictor variables. As there were indications of tree-specific differences in the response variables, while the low number of three analysed trees did not warrant the inclusion of tree identity as a random effect, we accounted for tree effects by including an additive tree term in the fixed effects. Since many of the aggregate variables in Table 1 were measured as averages over a larger number of replications with a known standard error, we fitted the models for all aggregated variables with weighted least squares using the square inverse of the standard errors as inverse variance weights, thus down-weighting imprecise measurements. We also applied Pearson correlations to analyse correlations between all traits. All statistical analyses were performed with the statistical software R version 3.5.3 [46].

3. Results

3.1. Typical Xylem Features of Ring-Porosity

As a typical ring-porous tree species, tyloses of earlywood are rare (about 11%) in the sapwood of *C. bungei* but gradually increase in the transition zone (about 47%) and become frequently abundant (about 80%) in the heartwood (Figure 1). Warty layers with granule shapes are very rare in the pit chambers of sapwood (Figure 2A), but likewise become frequent in both the transition zone and heartwood (Figure 2C,E,F). Helical thickenings are common in vessel walls of latewood (Figure 2B), while absent in vessel walls of earlywood in this species (figure not shown). Pit membrane appears to be thicker in heartwood compared with the transition zone (Figure 2D,E). In sapwood and the transition zone, amorphous deposits were rare around cell walls in between pit chambers (Figure 2A,C) where microfibrils orientation is visible; in contrast, the deposits became abundant- around cells walls between pit chambers that microfibril arrangement is barely visible (Figure 2E,F). Tyloses are occluded to the S3 layers of vessel walls including pit apertures (Figure 2G–I), which contributed to an impermeable vessel network and nonconductive properties of vessels in heartwood.

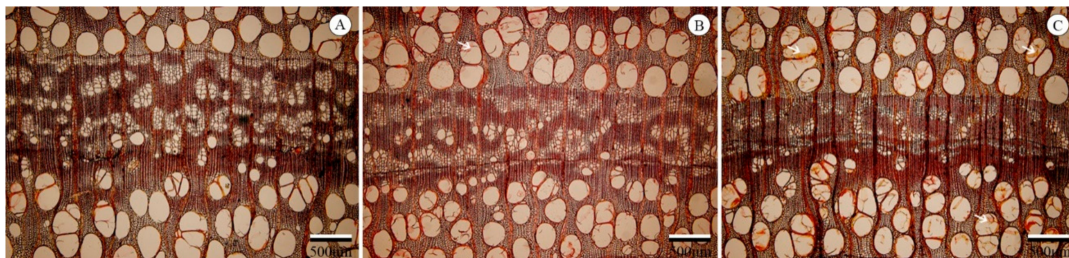


Figure 1. Transversal sections of wood blocks from sapwood (A), transition wood (B) and heartwood (C) in a radial strip at 6.3 m height in *C. bungei*. Tyloses were rarely distributed in sapwood, gradually reduced in the transition zone and common in the vessel lumen of heartwood. Arrows represent locations of tyloses.

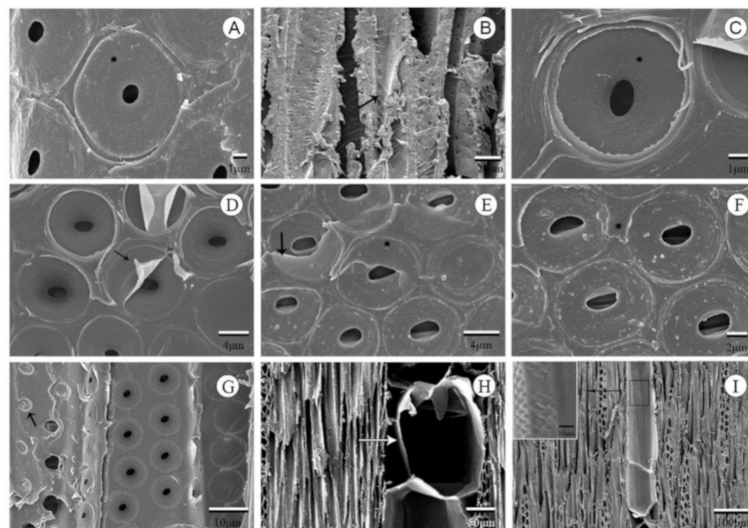


Figure 2. SEM photos of tangential surfaces of wood samples from sapwood (A,B), transition wood (C,D) and heartwood (E–I) of *C. bungei*. Warty layers and amorphous deposition were very rare in the pit chambers of sapwood (A), but became frequent in both the transition zone and heartwood (asterisks in C,E,F). Helical thickenings were common in vessel walls of latewood (B). Pit membrane appeared to be thicker in heartwood compared with the transition zone (D,E). Tyloses occluded pit apertures and vessel chambers (G–I). Arrows point to pit membranes in transition wood and heartwood (D,E), pit chambers occluded by amorphous deposition (G) and tyloses in the vessel chambers (H,I).

Another typical feature of ring-porous xylem was a distinct comparison between vessel sizes of earlywood and latewood (Figure 3). In *C. bungei*, vessel lumen diameters differed by roughly one order of magnitude between early- and latewood, averaging at about 180.69 μm in the former and 21.58 μm in the latter, with a much wider spread of values in the earlywood (note the logarithmic scale of Figure 3). Notably, the vessel lumen diameter distributions differed considerably between tree individuals, especially in the earlywood (Figure S1).

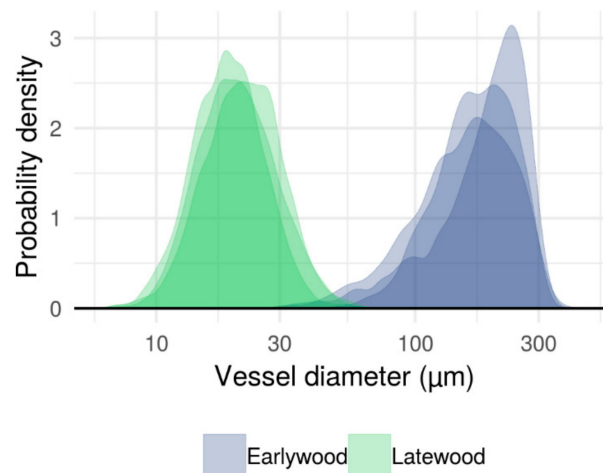


Figure 3. Empirical density plots of the observed vessel lumen diameter distributions in earlywood (blue) and latewood (green) separated by tree individuals (please note that the X-axis is displayed on a logarithmic scale). See Figure S1 for a breakdown by axial height and wood type.

3.2. Effects of Axial Height and Cambial Age on Xylem Traits

The effects sizes for the linear models used to analyse the combined effect of axial height, cambial age and their interaction on the analysed wood anatomical traits are shown in Figure 4. Additionally, partial residual plots of the main effects are given for a selection of vessel or fibre traits (Figures 5 and 6). Partial residual plots of the remaining traits are reported in Figure S2, and for the complete model output including unscaled parameter estimates, test statistics and significance levels refer to Table S1.

The patterns in average and maximum vessel lumen diameter were largely consistent, with older cambium producing significantly larger vessels in both early- and latewood (Figures 4 and 5, Figure S2, Table S1). In addition, vessel lumen diameter increased significantly with measured height in the latewood, but to a smaller extent with increasing cambial age, while vessel lumen diameter decreased insignificantly in earlywood (Figures 4 and 5, Table S1). In total, our models explained 56.4% of the variance in the earlywood and 51.3% of the variance in the latewood average vessel lumen diameter, but only 44.3% of the variance for earlywood and 16.4% of the variance for latewood maximum vessel lumen diameter due to relatively lower sample sizes.

Vessel element length significantly increased with cambial age, though a significant interaction with measured height counteracted this effect at upper positions ($R^2 = 0.373$, $p < 0.001$; Figures 4 and 5, Table S1). Pit membrane diameter responded significantly positively to cambial age as well, while it was relatively independent of the position along the vertical axis ($R^2 = 0.265$, $p > 0.05$; Figures 4 and 5, Table S1). Notably, vessel density increased significantly with axial height in the earlywood, while it declined significantly with axial height in the latewood (though the latter trend was counteracted by a significant positive interaction of both cambial age and axial height; cf. Figure 4 and Figure S2, Table S1). However, the latter trend might be due to a highly variable vessel density among samples (although about 500 vessels in earlywood and 1000 vessels in latewood were included for earlywood and latewood vessel density measurement, respectively), leading to a low amount of explained variance, especially in the latewood ($R^2 = 0.252$ for earlywood and 0.076 for latewood; see Table S1).

In contrast to the pronounced effects of radial and axial height on vessel lumen diameter, fibre lumen diameter was largely independent of these variables ($R^2 = 0.131$ for earlywood and 0.265 for latewood; Figures 4 and 6, Table S1). However, other fibre traits, i.e., fibre length and fibre wall thickness to diameter ratio, responded to the spatial position in the tree, with both fibre length and the fibre wall thickness to diameter ratio decreased significantly with axial height, the latter less strongly so in wood produced by older cambium ($R^2 = 0.563$ for L_f and 0.336 for t/b ; Figures 4 and 6, Table S1).

Interestingly, we observed an influence of spatial position on AGR, with significant negative effects from axial height and cambial age, and a significant negative interaction that together explained 26.0% of the variance in AGR (Figure 4, Table S1).

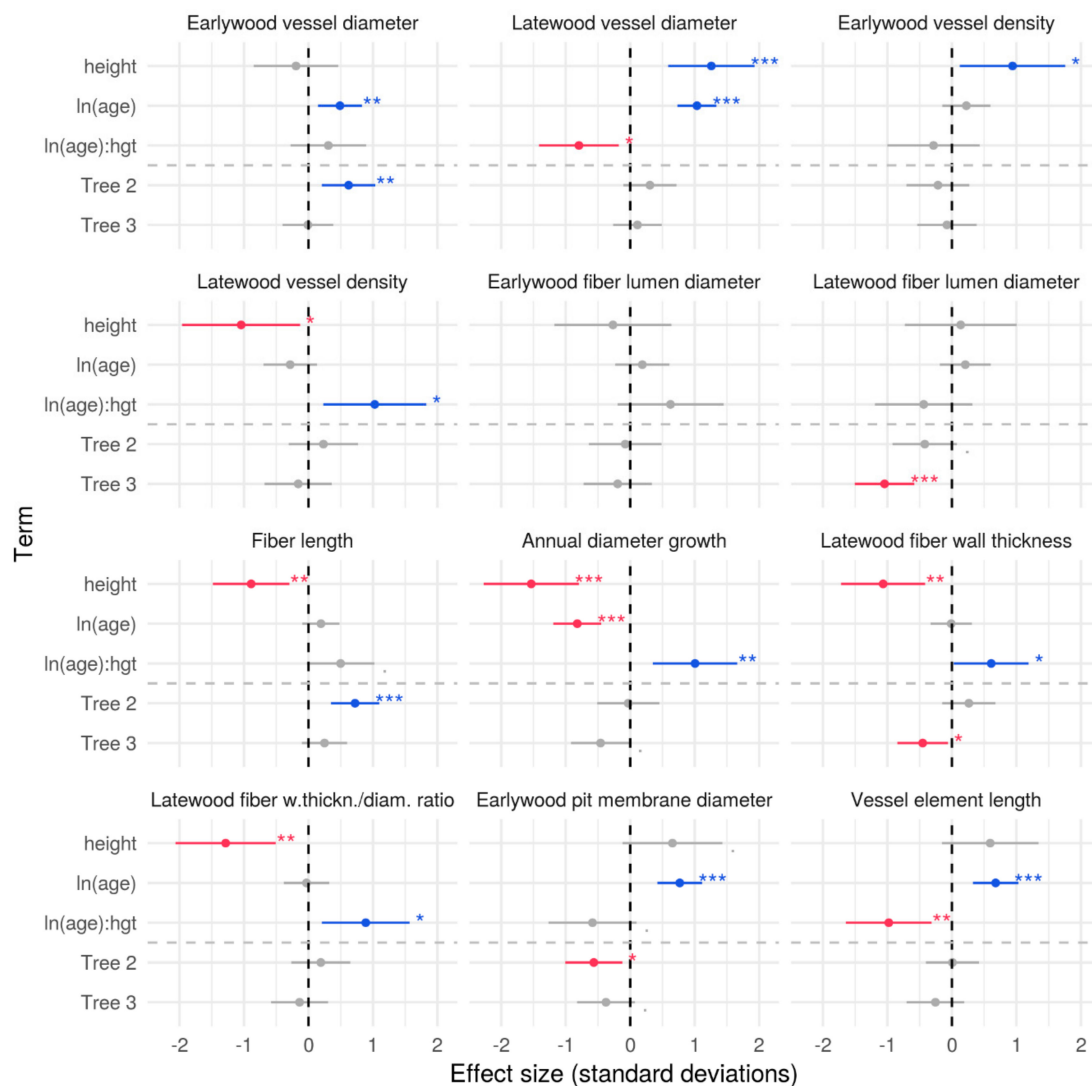


Figure 4. Effects sizes of the fitted linear models with their corresponding 95% confidence intervals. The change in standard deviations of the response when increasing the predictor by one standard deviation (for continuous predictor variables) or the difference between factor levels in standard deviations of the response (for tree effects) is shown. Blue: positive effects, red: negative effects, grey: insignificant effects. Stars show significance levels (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Note that Tree 1 does not appear in the table as it is included in the intercept term (not shown here as the scaled intercept is not relevant in terms of effect sizes). The black dashed vertical line is at zero and indicates no effect, and the horizontal dashed line is used to separate the effects from individual tree effects. Abbreviations: ln(age), natural logarithms of cambial age; hgt, axial height.

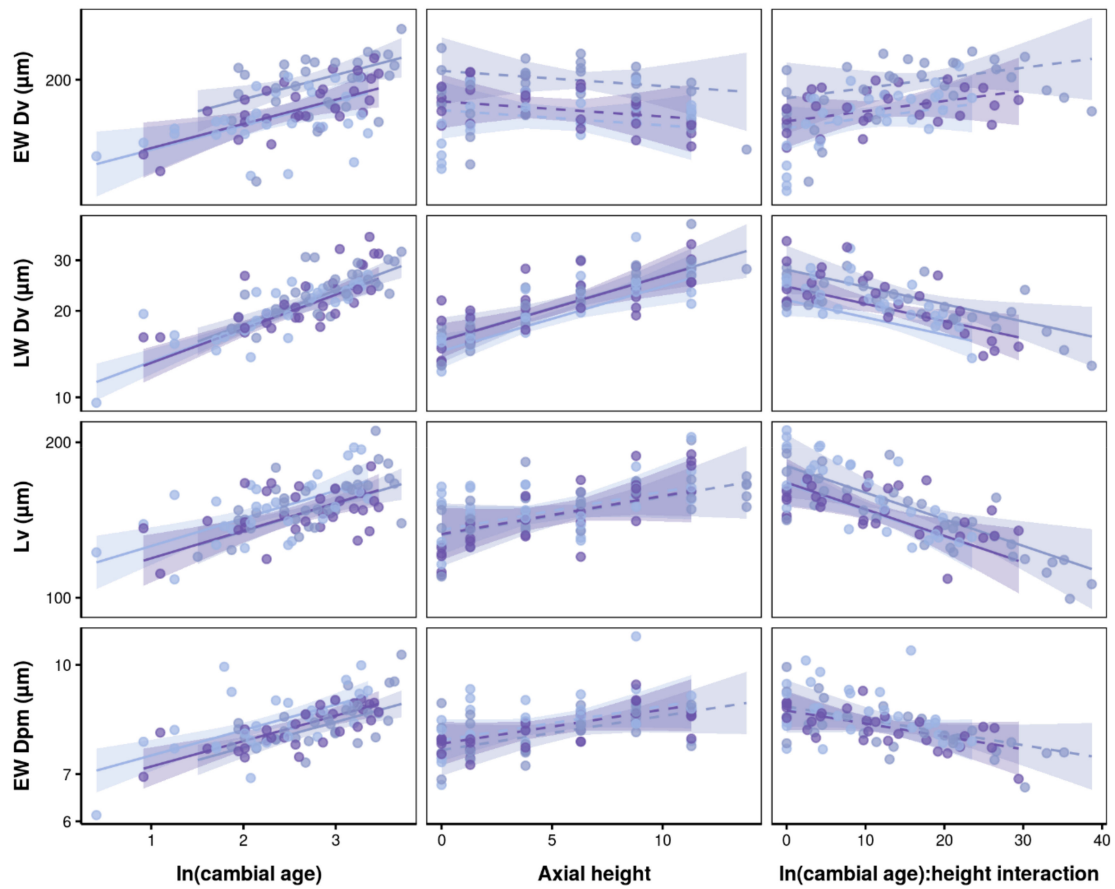


Figure 5. Partial residual plots for the models of vessel related traits. The predictions and 95% confidence intervals for each observed value of all three continuous predictor are shown while keeping the other predictors at the average value of the corresponding tree, and partial residuals corresponding to the theoretical values of the response that would be observed when the other predictors were at their average value. Colors represent different trees; significant relationships are illustrated by solid lines while insignificant relationships are illustrated by dashed lines. EW: earlywood, LW: latewood.

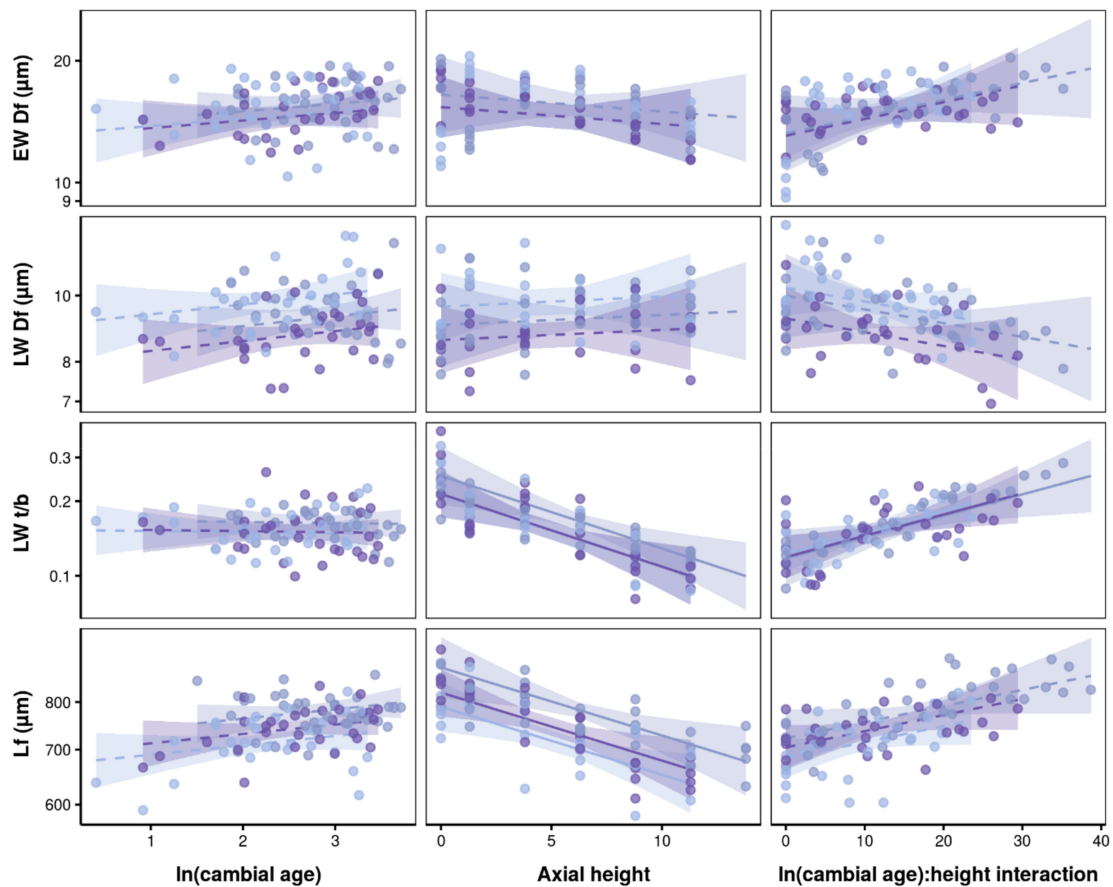


Figure 6. Partial residual plots for the models of fibre traits. The predictions and 95% confidence intervals for each observed value of all three continuous predictors are shown while keeping the other predictors at the average value of the corresponding tree, and partial residuals corresponding to the theoretical values of the response that would be observed when the other predictors were at their average values. Colors represent different trees; significant relationships are illustrated by solid lines while insignificant relationships are illustrated by dashed lines.

3.3. Interrelationships between Wood Anatomical Traits

A correlation heat map of all wood anatomical traits analysed in this study and the corresponding cambial age and vertical height in the tree indicates that the majority of the analysed traits tended to be negatively correlated to axial height and positively to the average cambial age of each wood segment (Figure 7). There was a correlation between earlywood vessel lumen diameter and earlywood maximum vessel lumen diameter, latewood vessel lumen diameter, earlywood fibre diameter and fibre length. Latewood fibre wall thickness was correlated with latewood fibre length and latewood fibre wall thickness to diameter ratio. However, the earlywood and latewood measurements of other traits were nearly unrelated to each other. In addition, only weak correlations between vessel density and both average and maximum vessel lumen diameter were found.

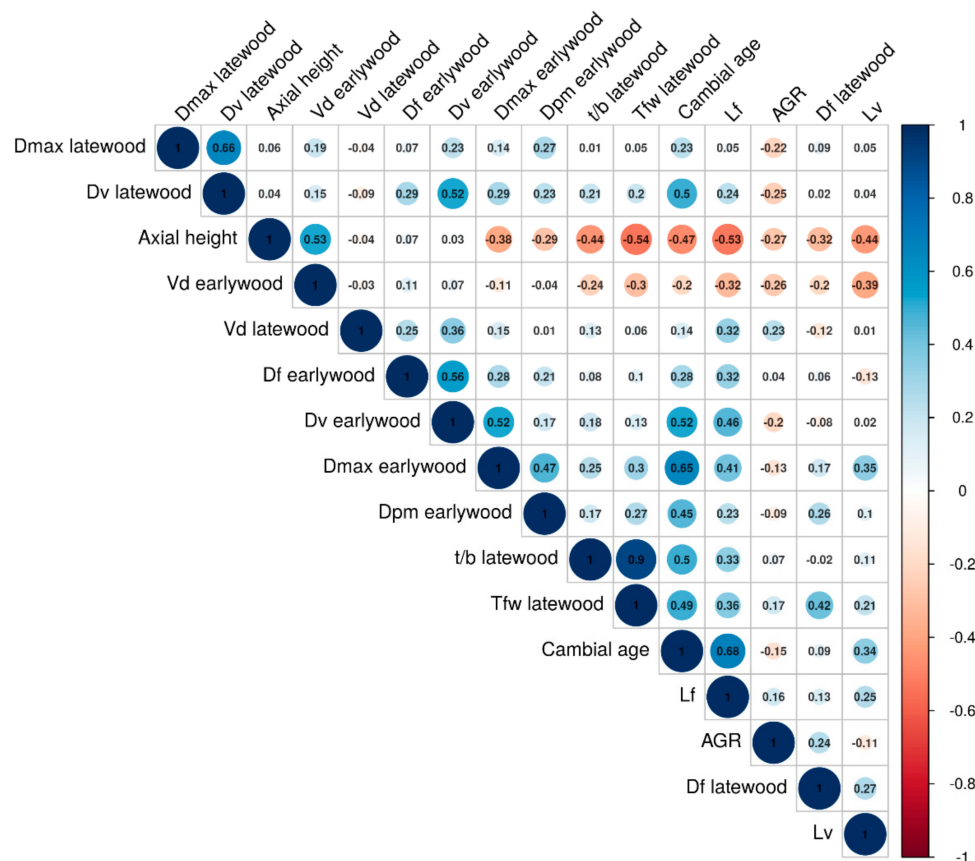


Figure 7. Correlation heat map of the analyzed variables (using the same transformations as in the fitted models). Numbers: Pearson product-moment correlation coefficient; blue: positive correlation; red: negative correlation. AGR, annual diameter growth; see Table 1 captions for remaining acronyms.

4. Discussion

4.1. Wood Anatomical Features of *C. bungei*

A typical feature of ring-porous tree species is the formation of large earlywood vessels at the beginning of the growing season and narrow latewood vessels later in the growing season [43]. Accordingly, the vessel lumen diameter distribution in *C. bungei* was bimodal, which is the same as poplar, a diffuse porous tree species showing consistently bimodal shapes [47,48]. In *C. bungei*, earlywood vessel lumen diameter distribution was right-skewed, demonstrating that large vessels with diameters ranging between 200–250 μm were highly frequent. In contrast, latewood vessel lumen diameter distribution was hump-shaped with average values around 20–25 μm , i.e., tentimes smaller than earlywood vessels.

While the large early-wood vessels commonly become embolized or occluded with tyloses by the end of the growing season in ring-porous tree species, the smaller and safer latewood vessels may remain functional for many years [43]. The occlusion of tyloses secreted from ray and axial parenchyma cells around vessels typically begins from the current or previous year growth ring [39] and functions as a defense system [42]. However, it might additionally be related to a response to environmental stimuli, such as drought- or frost-induced embolism formation, although no general consensus has been reached so far on which is the cause and which is the consequence [14,42,49–51]. Particularly, abundant tylose distribution in heartwood provided higher decay resistance and mechanical support [42], while on the other hand, it might reduce wood permeability and preservative penetration efficiency [42,52]. In our study, increasing amounts of tyloses from sapwood to heartwood in *C. bungei* was consistent with former reports [41]. Tyloses in *C. bungei* is the “normal” type [53] without lignification and

deposition, which contributes to sclerified tyloses and constrains water transfer [54,55]. Increasing the amount of tyloses from sapwood to heartwood could be a powerful defense system restraint to pathogen propagation [56]. Further work could be done to investigate the chemical components of tyloses in *C. bungei*, as well as its formation and expansion process, which is not well determined yet [42,52,57]. In addition, thicker pit membranes, abundant warty layers and amorphous deposits with increasing cambial age, and cell walls with helical thickening in *C. bungei*, tend to be more effective structures for maintaining moisture and thereafter resist microorganisms and embolism [58,59].

4.2. Vertical and Radial Patterns of Wood Anatomical Traits

Our finding that earlywood vessel lumen diameter decreased with axial height agreed with previous work, which showed a sharp decrease of vessel lumen diameter at certain axial height levels in multiple tree species [20,26,28,60]. However, in our study, this commonly observed relationship was not significant and was solely affected by the radial cambial age gradient, which seemed to be a more important driver in *C. bungei*. Decreased sizes of earlywood vessels and increased earlywood vessel density with axial height might be attributed to a declined auxin concentration distribution from young leaves along the flow path down to the roots and distal stems [61,62]. The length of flow path was frequently used in recent related studies as it describes the distance of auxin transport and hence the pressure drop over the flow resistance in sapwood. However, it changes significantly yearly as trees grow taller and is only available for the outermost growth ring when analysing its effect on wood anatomical data. Given that here we focus on patterns of wood anatomical traits in the whole life span of trees, axial height was used for analysis instead of flow path length. Since vessel lumen diameter is directly linked to vessel hydraulic diameter, the decline of vessel lumen diameter and thus hydraulic efficiency along the tree axis in earlywood also overcomes the increase in hydraulic resistance with flow path length. Meanwhile, our finding of significant effects on wood anatomical traits from different tree individuals suggested that tree identity should not be neglected.

Increased vessel lumen diameter with cambial age was in line with previous below- [63] and aboveground studies [12,28,64]. Increased early- and latewood vessel lumen diameter, as well as increased vessel element length with cambial age, improves water transport efficiency. The driver behind this commonly observed pattern of increasing conduit diameters with cambial age remain speculative, but it is most likely related to the radial concentration of auxin or other plant hormones affecting vascular differentiation. Fan et al. [22] found that after 40 years, vessel lumen diameter did not increase significantly anymore and transformed into mature wood, indicating that hormone concentration might remain constant at a given age and/or size. Although earlywood pit membrane diameter was independent of axial height in *C. bungei* in contrast to findings from conifers [65,66], we observed a strong cambial age effect on pit membrane diameter, which increased in diameter radially from heartwood to sapwood. This might be related to the increase in sapwood water demand when growing older, which requires larger pits to enhance hydraulic efficiency. A significant relationship between earlywood hydraulic vessel lumen diameter and pit membrane diameter in sapwood was also observed (data not shown), suggesting that vessel and pit structures coordinate to optimize water transport. Increased earlywood vessel density with axial height was in line with other studies [28], while a decreased pattern in latewood might be due to a significant interaction from cambial age, showing a large variance in latewood density data, as mentioned previously.

Relatively stable fibre lumen diameter values with cambial age and axial height suggested this mechanical trait was not affected by these two factors, in comparison with vessel-related and thus hydraulic traits. However, decreased fibre length and latewood fibre wall thickness to diameter ratio with axial height agrees with a former study [67], and might be associated with declined xylem water potential to the crown and thus the vertical drop in cell turgor pressure. This demonstrates that the cell turgor gradient affects cell elongations during cell differentiation, although the gradient might be rather small [68–71]. In general, a higher mechanical strength has been observed at the tree base in comparison to the tree top [72]. The elongation decline in most fibre-related traits along the axis, i.e.,

fibre length, fibre lumen diameter and fibre wall thickness to diameter ratio, is most likely adapted to the mechanical design of trees.

Negative effects of cambial age and axial height on annual diameter growth might be attributed to the developmental stages of studied trees, which are in later juvenile stages heading towards maturing stages. Weak correlations between wood anatomical traits agreed with previous work [73]. However, a strong relationship between vessel lumen diameter and vessel density was reported in other studies [74,75], which was not observed in this study. A possible explanation could be that wood anatomical data were collected from different sampling positions that were significantly affected by cambial age and axial height, as shown in this study, and hence may weaken the relationships.

5. Conclusions

An increasing amount of tyloses, warty layers and amorphous deposits were observed from the heartwood compared with sapwood of *C. bungei*, which might be biological traits to defend against fungi and keep moisture out during heartwood formation. The annual diameter growth of *C. bungei* decreased with both cambial age and axial height. Vessel lumen diameter differed by about one order of magnitude between earlywood and latewood, and vessel lumen diameter distribution patterns varied among tree individuals. Earlywood vessel lumen diameter decreased insignificantly with axial height and increased significantly with cambial age, while latewood vessel lumen diameter increased significantly with cambial age and axial height. Vessel density was significantly affected by axial height, and vessel element length and earlywood pit membrane diameter were positively influenced by cambial age. Fibre lumen diameter was independent of sampling position, while fibre wall thickness, fibre length and latewood fibre wall thickness to span ratio decreased with axial height. This study highlighted significant cambial age and axial height effects on xylem anatomical traits and confirmed earlier work that cautions interpreting wood anatomical structures observed from a random sample collected in the tree due to the significant within-tree variation as well as large wood anatomical variations among tree individuals. Moreover, cambial age and axial height might simultaneously affect wood formation, which may in turn influence xylem hydraulic function.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1999-4907/10/8/662/s1>, Figure S1: Empirical density plots of the observed vessel diameter distributions in earlywood (blue) and latewood (green), faceted by tree height (columns) and radial positions (rows) and separated by tree individuals (line types); Figure S2: Partial residual plots for the models of wood anatomical traits additional to Figures 6 and 7 in the main text; Table S1: Parameter estimates of all fitted models including the corresponding standard errors, 95% confidence intervals, t-statistics and P values for the test of the null hypothesis that the parameter value is equal to zero.

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References

1. Zheng, J.; Zhao, X.; Morris, H.; Jansen, S. Phylogeny best explains latitudinal patterns of xylem tissue fractions for woody angiosperm species across China. *Front. Plant Sci.* **2019**, *10*, 556. [[CrossRef](#)] [[PubMed](#)]

2. Tyree, M.T.; Zimmerman, M.H. *Xylem Structure and the Ascent of Sap*; Springer: Berlin, Germany, 2002; pp. 17–21.
3. Cai, J.; Hacke, U.; Zhang, S.; Tyree, M.T. What happens when stems are embolized in a centrifuge? Testing the cavitron theory. *Physiol. Plant.* **2010**, *140*, 311–320. [[CrossRef](#)] [[PubMed](#)]
4. Anfodillo, T.; Carraro, V.; Carrer, M.; Fior, C.; Rossi, S. Convergent tapering of xylem conduits in different woody species. *New Phytol.* **2006**, *169*, 279–290. [[CrossRef](#)] [[PubMed](#)]
5. Petit, G.; Anfodillo, T.; Mencuccini, M. Tapering of xylem conduits and hydraulic limitations in sycamore (*Acer pseudoplatanus*) trees. *New Phytol.* **2007**, *177*, 653–664. [[CrossRef](#)] [[PubMed](#)]
6. Zimmermann, M.; Tomlinson, P. Analysis of complex vascular systems in plants: Optical shuttle method. *Science* **1966**, *152*, 72–73. [[CrossRef](#)] [[PubMed](#)]
7. Kitin, P.; Fujii, T.; Abe, H.; Funada, R. Anatomy of the vessel network within and between tree rings of *Fraxinus lanuginosa*(Oleaceae). *Am. J. Bot.* **2004**, *91*, 779–788. [[CrossRef](#)] [[PubMed](#)]
8. Brodersen, C.R.; Lee, E.F.; Choat, B.; Jansen, S.; Phillips, R.J.; Shackel, K.A.; Mcelrone, A.J.; Matthews, M.A. Automated analysis of three-dimensional xylem networks using high-resolution computed tomography. *New Phytol.* **2011**, *191*, 1168–1179. [[CrossRef](#)]
9. Cai, J.; Tyree, M.T. Measuring vessel length in vascular plants: Can we divine the truth? History, theory, methods, and contrasting models. *Trees* **2014**, *28*, 643–655. [[CrossRef](#)]
10. Cai, J.; Tyree, M.T. The impact of vessel size on vulnerability curves: Data and models for within-species variability in saplings of aspen, *Populus tremuloides* Michx. *Plant Cell Environ.* **2010**, *33*, 1059–1069. [[CrossRef](#)]
11. Morris, H.; Gillingham, M.A.F.; Plavcová, L.; Gleason, S.M.; Olson, M.E.; Coomes, D.A.; Fichtler, E.; Klepsch, M.M.; Martínez-Cabrera, H.I.; McGlenn, D.J.; et al. Vessel diameter is related to amount and spatial arrangement of axial parenchyma in woody angiosperms. *Plant Cell Environ.* **2017**, *41*, 245–260. [[CrossRef](#)]
12. Leal, S.; Sousa, V.B.; Pereira, H. Radial variation of vessel size and distribution in cork oak wood (*Quercus suber* L.). *Wood Sci. Technol.* **2007**, *41*, 339–350. [[CrossRef](#)]
13. Rita, A.; Cherubini, P.; Leonardi, S.; Todaro, L.; Borghetti, M. Functional adjustments of xylem anatomy to climatic variability: Insights from long-term *Ilex aquifolium* tree-ring series. *Tree Physiol.* **2015**, *35*, 817. [[CrossRef](#)] [[PubMed](#)]
14. Cochard, H.; Tyree, T. Xylem dysfunction in *Quercus*: Vessel sizes, tyloses, cavitation and seasonal changes in embolism. *Tree Physiol.* **1990**, *6*, 393–407. [[CrossRef](#)] [[PubMed](#)]
15. Olson, M.E.; Rosell, J.A. Vessel diameter-stem diameter scaling across woody angiosperms and the ecological causes of xylem vessel diameter variation. *New Phytol.* **2013**, *197*, 1204–1213. [[CrossRef](#)] [[PubMed](#)]
16. Olson, M.E.; Anfodillo, T.; Rosell, J.A.; Petit, G.; Crivellaro, A.; Isnard, S.; León-Gómez, C.; Alvarado-Cárdenas, L.O.; Castorena, M. Universal hydraulics of the flowering plants: Vessel diameter scales with stem length across angiosperm lineages, habits and climates. *Ecol. Lett.* **2014**, *17*, 988–997. [[CrossRef](#)] [[PubMed](#)]
17. Rosell, J.A.; Olson, M.E.; Anfodillo, T. Scaling of xylem vessel diameter with plant size: Causes, predictions, and outstanding questions. *Curr. For. Rep.* **2017**, *3*, 46–59. [[CrossRef](#)]
18. Rodriguez-Zaccaro, F.D.; Valdovinos-Ayala, J.; Percolla, M.I.; Venturas, M.D.; Pratt, R.B.; Jacobsen, A.L. Wood structure and function change with maturity: Age of the vascular cambium is associated with xylem changes in current year growth. *Plant Cell Environ.* **2019**, *42*, 1816–1831. [[CrossRef](#)] [[PubMed](#)]
19. Dória, L.C.; Podadera, D.S.; Lima, R.S.; Lens, F. Axial sampling height outperforms site as predictor of wood trait variation. *IAWA J.* **2019**, *40*, 191–214. [[CrossRef](#)]
20. Gartner, B.L.; Lei, H.; Milota, M.R. Variation in the anatomy and specific gravity of wood within and between trees of red alder (*Alnus rubra* Bong.). *Wood Fibre Sci.* **1997**, *29*, 10–20.
21. Fan, Z.; Cao, K.; Shou-Qing, Z. Axial and radial changes in xylem anatomical characteristics in six evergreen broadleaved tree species in ailao mountain, yunnan. *Acta Phytoecol. Sin.* **2005**, *29*, 968–975.
22. Fan, Z.; Cao, K.; Becker, P. Axial and radial variations in xylem anatomy of angiosperm and conifer trees in Yunnan, China. *IAWA J.* **2009**, *30*, 1–13. [[CrossRef](#)]
23. Cardoso, S.; Sousa, V.B.; Quilho, T.; Pereira, H. Anatomical variation of teakwood from unmanaged mature plantations in East Timor. *J. Wood Sci.* **2015**, *61*, 326–333. [[CrossRef](#)]
24. Spicer, R.; Gartner, B.L. The effects of cambial age and position within the stem on specific conductivity in Douglas-fir (*Pseudotsuga menziesii*) sapwood. *Trees* **2001**, *15*, 222–229. [[CrossRef](#)]

25. Zobel, B.J.; van Buijtenen, J.P. *Wood Variation: Its Causes and Control*; Springer: Berlin, Germany, 1989; pp. 100–119.
26. James, S.A.; Meinzer, F.C.; Goldstein, G.; Woodruff, D.; Jones, T.; Restom, T.; Mejia, M. Axial and radial water transport and internal water storage in tropical forest canopy trees. *Oecologia* **2003**, *134*, 37–45. [[CrossRef](#)] [[PubMed](#)]
27. Jiang, X.; Yin, Y.; Urakami, H. Variation within tree of wood anatomical properties and basic density of I-214 poplar in Beijing area and their relationship modelling equations. *Sci. Silvae Sin.* **2003**, *39*, 115–121.
28. Zhao, X. Effects of cambial age and flow path-length on vessel characteristics in birch. *J. Res.* **2015**, *20*, 175–185. [[CrossRef](#)]
29. Domec, J.C.; Gartner, B.L. Age- and position-related changes in hydraulic versus mechanical dysfunction of xylem: Inferring the design criteria for Douglas-fir wood structure. *Tree Physiol.* **2002**, *22*, 91–104. [[CrossRef](#)] [[PubMed](#)]
30. Webb, C.D. Natural Variation in Specific Gravity, Fibre Length, and Interlocked Grain of Sweetgum (*Liquidambar styraciflua*) in the South Atlantic States. Ph.D. Thesis, North Carolina State University, Raleigh, NC, USA, 1964.
31. Saucier, J.R.; Thras, M.A. Specific gravity and fiber length variation within annual height increments increments of red maple. *For. Prod. J.* **1966**, *16*, 33–36.
32. Taylor, F.W. Fibre length variation within growth rings of certain angiosperms. *Wood Fibre Sci.* **1975**, *8*, 116–119.
33. Wheeler, J.K.; Sperry, J.S.; Hacke, U.G.; Hong, N. Inter-vessel pitting and cavitation in woody Rosaceae and other vesselled plants: A basis for a safety versus efficiency trade-off in xylem transport. *Plant Cell Environ.* **2005**, *28*, 800–812. [[CrossRef](#)]
34. Choat, B.; Cobb, A.R.; Jansen, S. Structure and function of bordered pits: New discoveries and impacts on whole-plant hydraulic function. *New Phytol.* **2008**, *177*, 608–626. [[CrossRef](#)] [[PubMed](#)]
35. Plomion, C.; Stokes, A. Update on wood formation in trees. *Plant Physiol.* **2015**, *127*, 1513–1523. [[CrossRef](#)]
36. Ziegler, H. Biological aspects of the Heartwood formation. *Holz Roh Werkst.* **1968**, *26*, 61–68. [[CrossRef](#)]
37. IAWA Committee. IAWA list of microscopic features for hardwood identification. *IAWA Bull.* **1989**, *10*, 219–332.
38. Bensen, K.J.M.; Kucera, L.J. Vessel occlusions in plants: Morphological, functional and evolutionary aspects. *IAWA Bull.* **1990**, *11*, 393–399. [[CrossRef](#)]
39. Saitoh, T.; Ohtani, J.; Fukazawa, K. The occurrence and morphology of tyloses and gums in the vessels of Japanese hardwoods. *IAWA J.* **1993**, *14*, 359–371. [[CrossRef](#)]
40. Jiang, X.; Zhang, L.; Zhou, Y. A study of tyloses and gums in the vessels of Chinese hardwood. *Sci. Silvae Sin.* **1995**, *31*, 155–159.
41. Evert, R.F. *Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Functions, and Development*, 3rd ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2006; pp. 297–298.
42. De Micco, V.; Balzano, A.; Wheeler, E.A.; Baas, P. Tyloses and gums: A review of structure, function and occurrence of vessel occlusions. *IAWA J.* **2016**, *37*, 196–205. [[CrossRef](#)]
43. Kitin, P.; Funada, R. Earlywood vessels in ring-porous trees become functional for water transport after bud burst and before the maturation of the current-year leaves. *IAWA J.* **2016**, *37*, 315–331. [[CrossRef](#)]
44. Jacobsen, A.L.; Valdovinos-ayala, J.; Pratt, R.B. Functional lifespans of xylem vessels: Development, hydraulic function, and post-function of vessels in several species of woody plants. *Am. J. Bot.* **2018**, *105*, 142–150. [[CrossRef](#)] [[PubMed](#)]
45. National Forestry and Grassland Administration. Available online: <http://www.forestry.gov.cn/main/4818/content-1045202.html> (accessed on 5 July 2019).
46. R Core Team. *R: A language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019.
47. Schreiber, S.G.; Hacke, U.G.; Hamann, A. Variation of xylem vessel diameters across a climate gradient: Insight from a reciprocal transplant experiment with a widespread boreal tree. *Funct. Ecol.* **2015**, *29*, 1392–1401. [[CrossRef](#)]
48. Hacke, U.G.; Spicer, R.; Schreiber, S.G.; Plavcová, L. An ecophysiological and developmental perspective on variation in vessel diameter. *Plant Cell Environ.* **2016**, *40*, 831–845. [[CrossRef](#)] [[PubMed](#)]

49. Pérez-de-lis, G.; Rozas, V.; Vázquez-ruiz, R.A.; García-gonzález, I. Do ring-porous oaks prioritize earlywood vessel efficiency over safety? Environmental effects on vessel lumen diameter and tyloses formation. *Agric. For. Meteorol.* **2018**, *248*, 205–214. [[CrossRef](#)]
50. Mishra, G.; Collings, D.A.; Altaner, C.M. Physiological changes during heartwood formation in young *Eucalyptus bosistoana* trees. *IAWA J.* **2018**, *39*, 1–13. [[CrossRef](#)]
51. Feng, Z.; Wang, J.; Rossler, R.; Kerp, H.; Wei, H.B. Complete tylosis formation in a latest Permian conifer stem. *Ann. Bot.* **2013**, *6*, 1075–1081. [[CrossRef](#)] [[PubMed](#)]
52. Chattaway, M.M. The development of tyloses and secretion of gum in heartwood formation. *Aust. J. Biol. Sci.* **1949**, *2*, 227–240. [[CrossRef](#)]
53. Wheeler, E.A. Inside Wood—A web resource for hardwood anatomy. *IAWA J.* **2011**, *32*, 199–211. [[CrossRef](#)]
54. Parke, J.L.; Oh, E.; Voelker, S.; Hansen, E.M.; Buckles, G.; Lachenbruch, B. *Phytophthora ramorum* colonizes tanoak xylem and is associated with reduced stem water transport. *Phytopathology* **2007**, *97*, 1558–1567. [[CrossRef](#)] [[PubMed](#)]
55. Collins, B.R.; Parke, J.L.; Lachenbruch, B.; Hansen, E.M. The effects of *Phytophthora ramorum* infection on the hydraulic conductivity and tylosis formation in tanoak sapwood. *Can. J. For. Res.* **2009**, *39*, 1766–1776. [[CrossRef](#)]
56. Khan, M.A.; Bera, M.; Spicer, R.A.; Spicer, T.E.V.; Bera, S. Evidence of simultaneous occurrence of tylosis formation and fungal interaction in a late cenozoic angiosperm from the eastern himalaya. *Rev. Palaeobot. Palynol.* **2018**, *259*, 171–184. [[CrossRef](#)]
57. McElrone, A.J.; Grant, J.A.; Kluepfel, D.A. The role of tyloses in crown hydraulic failure of mature walnut trees afflicted by apoplexy disorder. *Tree Physiol.* **2010**, *30*, 761–772. [[CrossRef](#)] [[PubMed](#)]
58. Kohonen, M.M. Engineered wettability in tree capillaries. *Langmuir* **2006**, *22*, 3148–3153. [[CrossRef](#)] [[PubMed](#)]
59. Kohonen, M.M.; Helland, A. On the function of wall sculpturing in xylem Conduits. *J. Bionics Eng.* **2009**, *6*, 324–329. [[CrossRef](#)]
60. Anfodillo, T.; Petit, G.; Crivellaro, A. Axial conduit widening in woody species: A still neglected anatomical pattern. *IAWA J.* **2013**, *34*, 352–364. [[CrossRef](#)]
61. Aloni, R.; Zimmermann, M.H. The control of vessel size and density along the plant axis: A new hypothesis. *Differentiation* **1983**, *24*, 203–208. [[CrossRef](#)]
62. Aloni, R. Ecophysiological implications of vascular differentiation and plant evolution. *Trees* **2014**, *29*, 1–15. [[CrossRef](#)]
63. Kirfel, K.; Leuschner, C.; Hertel, D.; Schuldt, B. Influence of root diameter and soil depth on the xylem anatomy of fine- to medium-sized roots of mature beech trees in the top- and subsoil. *Front. Plant Sci.* **2017**, *8*, 1194. [[CrossRef](#)] [[PubMed](#)]
64. Helin-Åska-Raczkowska, L.; Fabisiak, E. Radial variation of earlywood vessel lumen diameter as an indicator of the juvenile growth period in ash (*Fraxinus excelsior* L.). *Holz Roh Werkst.* **1999**, *57*, 283–286. [[CrossRef](#)]
65. Lazzarin, M.; Crivellaro, A.; Williams, C.B.; Dawson, T. Tracheid and pit anatomy vary in tandem in a tall *Sequoiadendron giganteum* tree. *IAWA J.* **2016**, *37*, 172–185. [[CrossRef](#)]
66. Losso, A.; Anfodillo, T.; Ganthaler, A.; Ko, W.; Markl, Y.; Nardini, A.; Oberhuber, W.; Purin, G.; Mayr, S. Robustness of xylem properties in conifers: Analyses of tracheid and pit dimensions along elevational transects. *Tree Physiol.* **2018**, *38*, 212–222. [[CrossRef](#)] [[PubMed](#)]
67. Sseremba, O.E.; Mugabi, P.; Banana, A.Y. Within-tree and tree-age variation of selected anatomical properties of the wood of Ugandan-Grown *Eucalyptus grandis*. *For. Prod. J.* **2016**, *66*, 433–442.
68. Lockhart, J.A. An analysis of irreversible plant cell elongation. *J. Theor. Biol.* **1965**, *8*, 264–275. [[CrossRef](#)]
69. Hsiao, T.C.; Acevedo, E.; Fereres, E.; Henderson, D.W. Water stress, growth, and osmotic adjustment. *Philos. Trans. R. Soc. Lond. B* **1976**, *273*, 479–500. [[CrossRef](#)]
70. Zhang, Y.; Zheng, Q.; Tyree, M.T. Factors controlling plasticity of leaf morphology in *Robinia pseudoacacia* L.I: Height-associated variation in leaf structure. *Ann. For. Sci.* **2012**, *69*, 29–37. [[CrossRef](#)]
71. Woodruff, D.R.; Bond, B.J.; Meinzer, F.C. Does turgor limit growth in tall trees? *Plant Cell Environ.* **2004**, *27*, 229–236. [[CrossRef](#)]
72. Vidaurre, G.B.; Vital, B.R.; de Cássia Oliveira, A.; da Silva Oliveira, J.T.; Moulin, J.C.; da Silva, J.G.M.; Soranso, D.R. Physical and mechanical properties of juvenile *Schizolobium amazonicum* wood. *Rev. Árvore* **2018**, *42*, e420101. [[CrossRef](#)]

73. Rungwattana, K.; Vienna, L.S.; Phumichai, T.; Rattanawong, R.; Hietz, P.; Vienna, L.S. Trait evolution in tropical rubber (*Hevea brasiliensis*) trees is related to dry season intensity. *Funct. Ecol.* **2018**, *32*, 2638–2651. [[CrossRef](#)]
74. Schuldt, B.; Leuschner, C.; Brock, N.; Horna, V. Changes in wood density, wood anatomy and hydraulic properties of the xylem along the root-to-shoot flow path in tropical rainforest trees. *Tree Physiol.* **2013**, *33*, 161–174. [[CrossRef](#)]
75. Pfautsch, S.; Harbusch, M.; Wesolowski, A.; Smith, R.; Macfarlane, C.; Tjoelker, M.G.; Reich, P.B.; Adams, M.A. Climate determines vascular traits in the ecologically diverse genus *Eucalyptus*. *Ecol. Lett.* **2016**, *19*, 240–248. [[CrossRef](#)]



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