RESEARCH ARTICLE

Functional Ecology | BRITISH ECOLOGICAL SOCIETY

High variation in hydraulic efficiency but not xylem safety between roots and branches in four temperate broad-leaved tree species

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Abstract

1. Xylem hydraulic safety and efficiency are key traits determining tree fitness in a warmer and drier world. While numerous plant hydraulic studies have focused on branches, our understanding of root hydraulic functioning remains limited, although roots control water uptake, influence stomatal regulation and have commonly been considered as the most vulnerable organ along the hydraulic pathway.

2. We investigated 11 traits related to xylem safety and efficiency along the hydraulic pathway in four temperate broad-leaved tree species.

3. Continuous vessel tapering from coarse roots to stems and branches caused considerable reduction in hydraulic efficiency. Wood density was always lowest in roots, but did not decline linearly along the flow path. In contrast, xylem embolism resistance (P_{50}) did not differ significantly between roots and branches, except for one species. The limited variation in xylem safety between organs did not adequately reflect the corresponding reductions in vessel diameter (by ~70%) and hydraulic efficiency (by ~85%). Although we did not observe any trade-off between xylem safety and specific conductivity, vessel diameter, vessel lumen fraction and wood density were related to embolism resistance, both across and partly within organs.

4. We conclude that coarse roots are not highly vulnerable to xylem embolism as commonly believed, indicating that hydraulic failure during soil drying might be restricted to fine roots.

KEYWORDS
embolism resistance, flow path, hydraulic architecture, hydraulic conductivity, vessel tapering, vulnerability curve, wood anatomy, wood density

1 | INTRODUCTION

Gravitation and frictional forces increasingly constrain water flow and the maintenance of intact water columns in the xylem of tall trees with increasing height, which causes a decline in xylem water potential from roots to leaves (Fulton et al., 2014; Woodruff & Meinzer, 2011). To compensate for these constraints, xylem conduits tend to taper along the hydraulic pathway from roots to leaves, as shown by a bulk of evidence from temperate tree species (Aloni, 1987; Anfodillo et al., 2013; Mencuccini et al., 2007; Tyree & Zimmermann, 2002; Zimmermann & Potter, 1982), while vessel frequency increases (Fan et al., 2009; Li et al., 2019; Lintunen & Kalliokoski, 2010). Although the continuous vessel tapering paradigm has not been confirmed for species from tropical environments (Kotowska et al., 2015; Schuldt et al., 2013), measurements conducted in temperate trees and in woody plants from arid environments indicate that the largest xylem vessels are located in roots (Domec et al., 2009; Lintunen & Kalliokoski, 2010; Martínez-Vilalta et al., 2002).

Yet, due to the generally assumed negative relationship between conduit diameter and embolism resistance within and across conifer and angiosperm species (Domec et al., 2010; Hajek et al., 2014; Maherali et al., 2006; Wheeler et al., 2005), several authors have argued that roots are the most vulnerable organ along the flow path in plants (Domec et al., 2004, 2010; Hacke et al., 2000; Maherali et al., 2006; Martínez-Vilalta et al., 2002; Sperry & Ikeda, 1997; Sperry & Saliendra, 1994). Actually, most former studies that have compared xylem embolism resistance between roots and stems in woody plants have supported this view (e.g. Alder et al., 1996; Domec et al., 2010; Kavanagh et al., 1999; Maherali et al., 2006; Pittermann et al., 2006; Pratt et al., 2007; Willson et al., 2008). However, methodological studies have revealed the existence of measurement artefacts during sample preparation, and called into question the ability of some standard techniques to properly estimate resistance to xylem embolism in long-vesseled specimens (Choat et al., 2010; Torres-Ruiz et al., 2014; Wheeler et al., 2013). The likelihood of roots to be dramatically more vulnerable to xylem embolism than stems and branches therefore requires re-examination. In fact, recent studies based on direct, non-invasive observations of embolism formation such as X-ray microtomography (micro-CT) and optical techniques of vulnerability assessment have found that roots are equally resistant, or even more resistant, to xylem embolism as stems (Losso et al., 2019; Rodriguez-Dominguez et al., 2018). Additional comparisons of hydraulic properties between roots and stems are thus needed to better document how resistance to drought is organized at the whole-plant level. Moreover, questions also remain regarding the relationship between embolism resistance and conduit size (Gleason et al., 2016). Thirty years ago, Sperry and Tyree (1988) argued that the mechanism of embolism formation might not be directly related to conduit size but to pit properties. In this regard, pit membrane thickness was recently identified as the key determinant of embolism resistance across a broad range of woody angiosperm species (Kaack et al., 2021; Li et al., 2016). However, the way this trait varies among trees and among organs within a single tree is largely unknown. Recent observations indicate that pit membrane thickness is comparable between large roots and branches in two *Acer* species, or even thicker in roots in the case of *Corylus avellana* (Kotowska et al., 2020; Wu et al., 2020). Overall, it remains unclear whether roots are less resistant to embolism than stems or branches, and how this matches the variability in wood anatomical features along the hydraulic pathway.

Furthermore, a growing number of studies could not support the anticipated relationship between hydraulic efficiency (capacity to transport water) and safety (capacity to resist embolism). This trade-off seems to be virtually absent both within (Hajek et al., 2014) and across tree species (Larter et al., 2017). In agreement herewith, a global analysis covering a large number of woody species observed only a weak safety–efficiency trade-off (Gleason et al., 2016). Hydraulic efficiency is mainly determined by conduit diameter and frequency, but none of these traits seem directly related to embolism resistance in both conifers (Delzon et al., 2010; Pittermann, 2010) and angiosperms (Lens et al., 2011; Sperry et al., 2006). Thus, although roots typically contain the largest vessels causing the highest hydraulic efficiency, this does not necessarily imply that they are also the most vulnerable organ along the flow path.

In the present study, we analysed wood structural properties along the hydraulic pathway from coarse roots to stem bases and further to sun-exposed branches in the crown top in four temperate deciduous tree species (*Acer pseudoplatanus* L., *Carpinus betulus* L., *Fagus sylvatica* L. and *Tilia cordata* Mill.). In addition, we determined the specific hydraulic conductivity and compared hydraulic efficiency and xylem safety between roots and branches. We hypothesized that (a) vessel diameter and xylem hydraulic efficiency decreases continuously along the hydraulic pathway from roots to branches to compensate for gravitational and frictional constraints with height, but (b) roots are not necessarily more vulnerable to embolism than branches due to a weak or no relationship between xylem efficiency and safety.

> **Highlight**
>
> Although vessel tapering resulted in a significant reduction in xylem hydraulic efficiency along the flow path in four temperate deciduous tree species, the resistance to xylem embolism did not vary.

2 | MATERIALS AND METHODS

2.1 | Study site and tree species

The study was conducted in a species-rich temperate old-growth deciduous forest located in Hainich National Park (HNP) in Central Germany. The Hainich mountain range in western Thuringia is covered by the largest non-fragmented deciduous forest area in Germany

(~160 km^2). The study site is located in the northeast of HNP and south of the village of Weberstedt (51°05′00″N, 10°30′27″E; 350 m a.s.l.). Permission for field work was granted by the HNP authorities. The climate is cool-temperate sub-oceanic to sub-continental with a mean annual precipitation of 590 mm and a mean annual temperature of 7.5°C (1973–2004, German Meteorological Office, Offenbach). The dominant soil type is Luvisol developed from loess covering Triassic limestone (Guckland et al., 2009). The studied stand is composed of up to 14 tree species per hectare, encompassing different families (*Fagaceae*, *Malvaceae*, *Betulaceae*, *Sapindaceae*) with varying strategies of leaf water status regulation and drought avoidance and tolerance (Köcher et al., 2009; Leuschner & Ellenberg, 2017). Mature tree individuals of the four mid- to late-successional short-vesseled broad-leaved species *Fagus sylvatica*, *Tilia cordata*, *Carpinus betulus* and *Acer pseudoplatanus* were sampled between 2012 and 2016 along a transect of approximately 200 m length and 30 m width (Table 1). Six trees per species with comparable diameter at breast height (DBH; 35–76 cm, depending on the species' dimensions in the stand) and tree height (25–34 m) were selected; all trees were co-dominant individuals with access to the upper sun canopy layer. The age of the sampled trees ranged between 80 and 120 years (Gebauer et al., 2008). The minimum distance between the sampled trees of a species was 10 m, in most cases 20–50 m; the individuals were treated as true replicates. A list of all xylem traits covered by the present study with corresponding acronyms and units is given in Table 2.

2.2 | Tree sampling

All branch samples were collected in autumn 2012 from the uppermost sun-exposed crown of five to six trees per species (*n* = 22). A mobile canopy lifter (model DL30, Denka-Lift AS) was used to access the sun canopy up to a height of 30 m. Per tree, at least three branch segments of approximately 50 cm length were selected for hydraulic and wood anatomical measurements (*n* = 94). After air-cutting, samples were immediately preserved in a sodium–silver chloride solution (16 μg/L Ag, 8 mg/L NaCl, Micropur katadyn©) filled in polyethylene tubes to prevent microbial activity, and stored at 4°C. All samples were processed within 2 weeks.

Coarse root samples were collected in January 2016 before the onset of frost; this winter was unusually mild. The roots were excavated in 5–25 cm soil depth in close vicinity to the target trees and traced back to the stem to verify its origin. To avoid tissue damage, no mechanical tension was applied to the root segments. They were

carefully excavated using light-weight tools (hand shovel, screwdriver, brush scrapers, etc.) only. Straight root segments of ~9 mm in diameter (range: 6–16 mm) and at least 150 cm length without major branching were selected for embolism resistance measurements (see below), harvesting one to four long root segments per tree and three to six trees per species (*n* = 22). Thus, branch and root samples had similar diameters. Additional coarse root segments (root length (mean ± *SE*) 44.2 ± 4.6 cm, 53.1 ± 5.1 cm, 64.5 ± 5.6 cm and 58.7 ± 5.3 cm in *Acer*, *Carpinus*, *Fagus* and *Tilia*, respectively) were sampled for axial xylem hydraulic conductivity measurements (*n* = 42). After cutting, root samples were cleaned from soil particles under running tap water, wrapped in paper towels soaked in a sodium–silver chloride solution (16 μg/L Ag, 8 mg/L NaCl, Micropur katadyn©) and stored in air-tight plastic bags at 4°C until processing. Branch segments, as well as all coarse root segments, were largely comparable within and among species with respect to age and diameter (for details, see below).

Stem wood cores were sampled in December 2015 from all six trees per species. Per tree, two cores were extracted next to each other at breast height from the outer stem wood (~10 cm length) using an increment borer (Ø 5.15 mm, Haglöf) to determine wood density and wood anatomical traits. The analysis was confined to the youngest 15 annual rings of the years 2001–2015.

2.3 | Wood density and wood anatomical analysis

Wood density (WD, g/cm^3), expressed as dry mass per fresh volume, was determined for all coarse root, stem wood and branch samples. In the branch and root samples, the basipetal end of ~5 cm length was analysed. After removal of pith and bark from the roots and branches, the fresh volume of all samples was determined through water displacement using Archimedes' principle. Subsequently, the samples were oven-dried at 105°C for 72 hr.

For wood anatomical analysis, stem wood cores and ~3-cm long branch and root segments from the basipetal end of the samples used for hydraulic measurements were stained with safranin (1% in 50% ethanol, Merck) and transverse sections cut using a sledge microtome (G.S.L.1, Schenkung Dapples). Subsequently, the complete cross-section was digitalized at ×100 magnification using a stereo-microscope equipped with an automatic stage (SteREOV20, Carl Zeiss MicroImaging GmbH, Software: AxioVision v4.8.2, Carl Zeiss MicroImaging GmbH). For image processing, Adobe Photoshop CS2 (Version 9.0, Adobe Systems Inc.) and the particle analysis function

TABLE 1 Successional status, drought tolerance according to Leuschner and Ellenberg (2017) as well as diameter at breast height (DBH, cm) and tree height (m) of the four investigated species

Species	Family	Success. stat.	Drought tol.	DBH (cm)	Height (m)
Acer pseudoplatanus	Sapindaceae	Mid	Mid/low	38.3–76.1	26.8–33.7
Carpinus betulus	Betulaceae	Mid/late	Mid	35.2–59.9	24.7–29.2
Tilia cordata	Malvacaeae	(Mid)/late	Mid	41.3–62.9	24.3–31.7
Fagus sylvatica	Fagaceae	Late	Low	40.8–61.0	28.2–33.1

Abbreviation	Unit	Definition
Wood properties		
WD	g/cm^3	Wood density
VD	n/mm^2	Vessel density
D	μm	Mean vessel diameter
D_h	μm	Hydraulically weighted vessel diameter
F		Vessel lumen fraction
S	mm^4	Vessel size to number ratio
Hydraulic properties		
P_{12}	MPa	Xylem pressure at 12% loss of hydraulic conductance
P_{50}	MPa	Xylem pressure at 50% loss of hydraulic conductance
P_{88}	MPa	Xylem pressure at 88% loss of hydraulic conductance
K_s	kg m^{-1}MPa^{-1} s^{-1}	Empirically determined specific conductivity
K_p	kg m^{-1} MPa^{-1} s^{-1}	Theoretically calculated potential conductivity

of ImageJ software (v1.44p, http://rsb.info.nih.gov/ij) were used to analyse the total xylem cross-section without pith and bark in all three tissue types, yielding on average $2{,}423 \pm 176$ analysed vessels in roots, $4{,}389 \pm 745$ in stem cores and $18{,}695 \pm 1{,}168$ in branches (means $\pm$ SE). We estimated vessel density (VD, n/mm^2), idealized vessel diameter (D, μm) after White (1991) as $D = ((32 \times (a \times b)^3)/(a^2 + b^2))^{\frac{1}{4}}$, hydraulically weighted vessel diameter (D_h, μm) according to Sperry et al. (1994) as $D_h = \Sigma D^5/\Sigma D^4$, and the vessel lumen fraction (F) and vessel size-to-number ratio (S, mm^4) according to Zanne et al. (2010); note that the vessel lumen fraction (F) is equal to the lumen-to-sapwood area ratio. Potential conductivity (K_p, kg m^{-1} MPa^{-1} s^{-1}) was calculated with the Hagen–Poiseuille equation as $K_p = ((\pi \times \rho \times \Sigma D^4)/(128 \eta \times A_{xylem}))$, where η is the viscosity ($1.002\ 10^{-9}$ MPa s) and ρ the density of water (998.2 kg/m^3), both at 20°C, and A_{xylem} (m^2) the analysed sapwood area.

2.4 | Xylem hydraulic efficiency and safety

Axial hydraulic conductivity (K_h, kg m MPa^{-1} s^{-1}) of roots and branches was measured with the Xyl'em apparatus and the XylWin 3.0 software (Bronkhorst, Montigny-les-Cormeilles, France) within 2 weeks after sample collection. Branch samples (mean basipetal age $\pm$ SE: 7.25 ± 0.32 years; mean diameter $\pm$ SE: 8.63 ± 0.09 mm; mean length $\pm$ SE: 31.79 ± 0.15 cm; $n = 94$) were recut at both ends (each 5–10 cm) and lateral scars were sealed with quick-drying adhesive (Loctite 431 and 7455, Henkel) to prevent lateral excision of water. Subsequently, the basipetal end of the segments was connected to the Xyl'em apparatus, flushed three times for 10 min at 120 kPa with filtered (0.2 μm; Maxi Culture Capsule, Pall Corp.) and degassed (at 30 mbar) demineralized water (10 mM KCl and 1 mM CaCl$_2$), and K_h determined at a pressure gradient of 6 kPa across the sample. Specific conductivity (K_s, kg m^{-1} MPa^{-1} s^{-1}) was calculated

by dividing K_h by the maximum basipetal (and not average) cross-sectional area (for methodological details, see Hajek et al., 2014; Schuldt et al., 2016). Considering the inverse flow direction in roots, root segments (mean basipetal age $\pm$ SE: 10.79 ± 0.73 years; mean diameter $\pm$ SE: 7.84 ± 0.45 mm; mean length $\pm$ SE: 55.34 ± 2.87 cm; $n = 43$) were connected with the acropetal end to the Xyl'em apparatus after cutting and sealing. Root K_s was calculated for the corresponding minimum cross-sectional area.

Xylem embolism resistance of coarse roots and branches was determined using the flow-centrifuge (Cavitron) technique (Cochard et al., 2005). Measurements on branches (mean basipetal age $\pm$ SE: 7.37 ± 0.35 years; mean diameter $\pm$ SE: 8.61 ± 0.11 mm; $n = 75$) were conducted at the University of Göttingen, Germany, with a standard (30 cm large) rotor attached to a commercially available centrifuge (Sorvall RC-5C, Thermo Fisher Scientific). Preliminary test assured that the maximum vessel length (L_{max}, cm) of the branch xylem was shorter than the rotor diameter in all diffuse-porous tree species of our sample ($L_{max} \pm$ SE: 9.4 ± 0.3 cm, 20.1 ± 0.7 cm, 19.3 ± 2.6 cm and 14.1 ± 1.6 in *Acer*, *Carpinus*, *Fagus* and *Tilia*, respectively). Long coarse root samples (mean basipetal age $\pm$ SE: 17.38 ± 1.20 years; mean diameter $\pm$ SE: 10.55 ± 0.56 mm; $n = 24$) were processed at the high-throughput phenotyping platform for hydraulic traits (Caviplace, University of Bordeaux, France) with a 100 cm large rotor (cf. Charrier et al., 2018; Lamarque et al., 2018). This large rotor allowed to overcome open-vessel artefact-related issues that were encountered during preliminary tests with the standard Cavitron and related to substantial maximum root vessel lengths ($L_{max} \pm SE$: 20.8 ± 3.6 cm, 51.8 ± 12.7 cm, 57.0 ± 1.0 cm and 42.8 ± 8.1 in *Acer*, *Carpinus*, *Fagus* and *Tilia*, respectively). Measurement preparation followed protocols detailed in Lobo et al. (2018) and Lamarque et al. (2018), with samples kept in water for ~1 hr for xylem relaxation before the start of measurements. The initial centrifuge

rotation speed was set at −0.8 MPa, and the xylem pressure levels were gradually lowered until at least 90% loss of hydraulic conductivity was reached. VCs were generated by plotting percentage loss of hydraulic conductivity (PLC) against xylem pressure. The pressure causing 50% loss of conductivity (P_{50}) was calculated after Pammenter and Van der Willigen (1998) as PLC = 100/$(1 + \exp(s/25 \times (P_i − P_{50})))$, where s (% MPa^{-1}) is the negative slope of the curve at the inflexion point and P_i the xylem pressure. The xylem pressures causing 12% (P_{12}, air entry point) and 88% (P_{88}) loss of conductivity were calculated as $P_{12} = 2/(s/25) + P_{50}$ and $P_{88} = −2/(s/25) + P_{50}$ (Domec & Gartner, 2001).

2.5 | Statistical analysis

All statistical analyses were performed with the R software, version 4.0.0 (R Development Core Team, Vienna, Austria). Data from different samples of the same tree were averaged prior to the analyses to avoid pseudo-replication (see Table S1 for the number of samples and trees). As the number of species did not reach the minimum number of random-effect level that is required when running a linear mixed model (Crawley, 2002; Gelman & Hill, 2006), the effects of tree organ (branch, stem, root), species and organ*species interactions on xylem anatomical and hydraulic traits were tested using two-factorial ANOVA models for unbalanced samples (SS Type III, *ANOVA()* function, package CAR). Model residuals were visually examined for normal distribution and homoscedasticity, and analyses were carried out on log-transformed data when necessary. One-way ANOVA models with Tukey contrats (*glht*() function, package MULTCOMP), a robust procedure for unbalanced designs and non-normality of data (Herberich et al., 2010), were further used to evaluate differences both among species and between organs within species. Heteroscedastic-consistent covariance estimation was implemented in the case of heterogeneous variances. Pairwise comparisons between hydraulic traits of roots and branches were analysed using Student's t test, Welch's t-test or Mann–Witney U-test, depending on the data structure. Pearson correlations (package CORMORANT; Link, 2020) were finally carried out to test for the interrelationships of all pairwise combinations of wood anatomical properties, WD, and hydraulic traits, using log10-transform data if needed.

3 | RESULTS

3.1 | Differences in xylem anatomical traits and hydraulic efficiency along the flow path

Plant organs (root, stem, branch) showed a highly significant influence on wood density (WD) and xylem anatomical traits (Figure 1; Table 3). In all species, WD was lower in coarse roots (0.27–0.41 g/cm^3) than in stem and branch wood (0.34–0.61 g/cm^3; Figure 1; Table S1), but WD did not decline linearly along the flow path. In *T. cordata* and in *C. betulus*, WD of branches was lower than in stems (0.34 vs. 0.46 g/cm^3 and

0.54 vs. 0.61 g/cm^3, respectively), which was not the case in the two other species. The hydraulically weighted vessel diameter (D_h) declined by 69%–71% along the hydraulic pathway from roots to branches (Figure 1; differences between organs were significant, except between coarse root and stem wood in *C. betulus*). All species showed significantly higher vessel densities (VD) in sun-canopy branches (164–380 n/mm^2) than in roots (44–72 n/mm^2) and stems (41–91 n/mm^2). The vessel size-to-number ratio (S) declined steeply from roots to branches, as did the vessel lumen fraction (F; Figure 1). F peaked in the roots and decreased towards the branches for *A. pseudoplatanus* and *F. sylvatica*, but, for *C. betulus* and *T. cordata*, it reached the minimum at the stem level. As a consequence of these anatomical adjustments, highest values in potential conductivity (K_p) were observed in the roots (56.4–93.3 kg m^{-1}MPa^{-1} s^{-1}) with a steep decrease to stem wood (15.4–19.7 kg m^{-1}MPa^{-1} s^{-1}) and branch wood (2.7–6.24 kg m^{-1} MPa^{-1} s^{-1}). Thus, stems and branches reached only 21%–36% and 4%–10% of the K_p values calculated for coarse roots, respectively. In accordance with K_p, higher specific conductivity (K_s) values were observed in roots (7.2–13.3 kg m^{-1} MPa^{-1} s^{-1}) than in branches (0.9–1.4 kg m^{-1} MPa^{-1} s^{-1}, Figure 1) for all species, thus causing declines by 81%–94% along the flow path (Figure 1). In contrast, species differences in K_p and K_s were mostly insignificant for a given organ.

3.2 | Differences in embolism resistance between roots and branches

Overall, branches and roots exhibited similar embolism resistance thresholds (non-significant organ effect in P_{12}, P_{50} and P_{88}; Table 3; Figure 2). In contrast, the three metrics of embolism resistance varied significantly among species (Table 3; Figure 2). Based on P_{50} and P_{88} values, the species embolism resistance ranking was the same for both roots and branches, with *Tilia* < *Fagus*/*Acer* < *Carpinus* (Figures 2 and 3). Pairwise comparisons conducted within species also indicated that branches and roots were equally resistant to xylem embolism in most cases (Figure 3), with differences sporadically emerging for *Carpinus* (P_{12}), *Fagus* (P_{88}) and *Tilia* (P_{50} and P_{88}).

3.3 | Interrelationships between wood density, wood anatomy and hydraulic functioning

A correlation analysis between pairs of log-transformed wood properties and hydraulic traits across species and all organs at the tree level could not confirm any trade-off between K_s and P_{50} ($p = 0.08$, $R = 0.32$; Figure 4). Likewise, no relationship between both efficiency-safety variables was observed within roots or branches ($p = 0.57$, $R = 0.21$ and $p = 0.11$, $R = 0.36$ for roots and branches, respectively; data not shown). For WD, however, we observed significant relationships with all anatomical variables except VD (Figure 4). WD was negatively related to D, F, K_p, K_s ($p < 0.001$) and, to a lesser degree, to D_h ($p < 0.01$) and S ($p < 0.05$). Furthermore, WD was strongly negatively related to P_{50} and P_{88} ($p < 0.001$; Figure 4). While
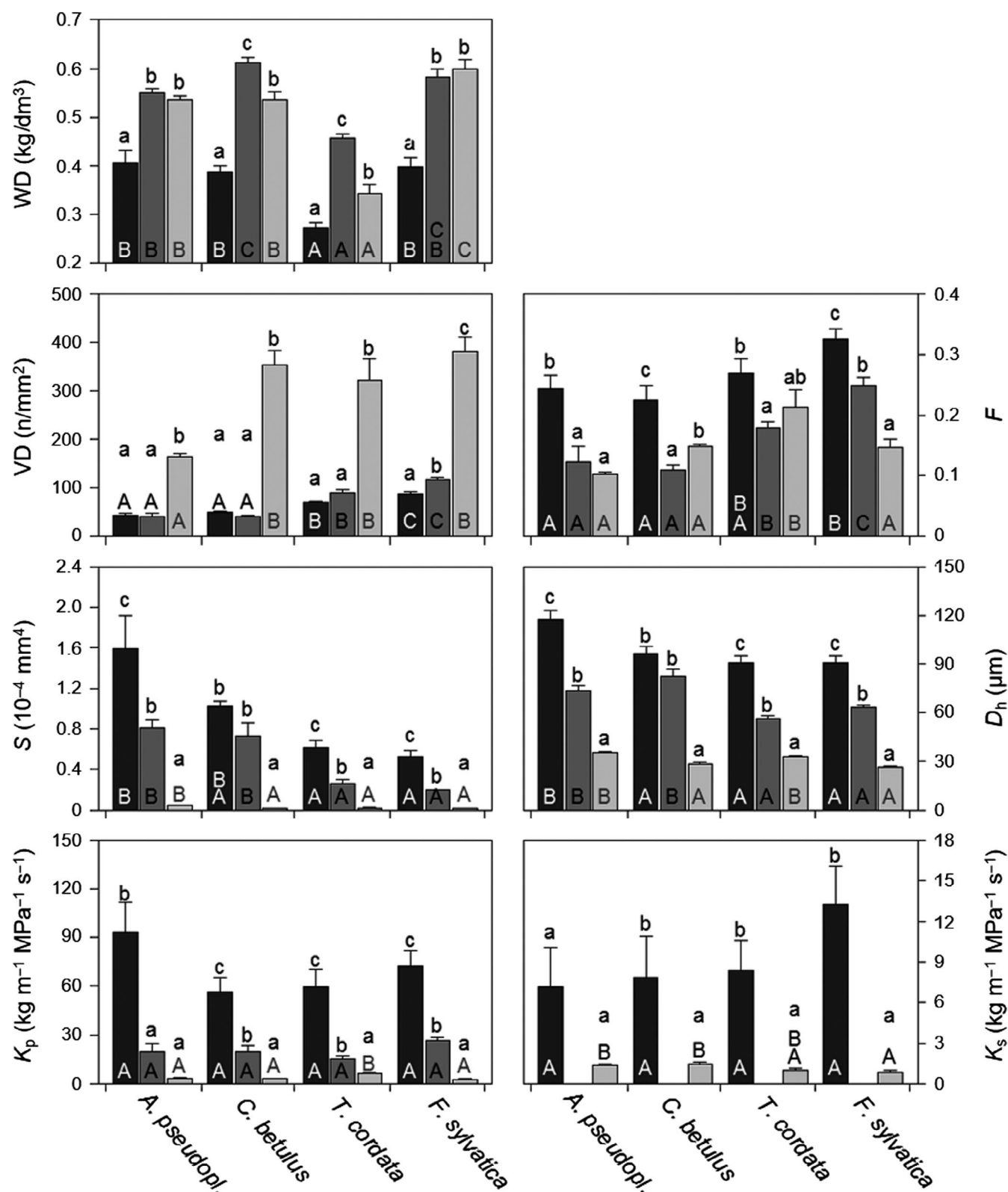
FIGURE 1 Means ± SE of seven wood traits from coarse roots (dark bars, left), stem cores (grey bars, centre) and branches (bright bars, right) arranged in blocs of the four investigated tree species. Different capital letters indicate significant differences ($p < 0.05$) between species within a given organ, and small letters between different organs of a given species ($p < 0.05$). Data for empirical specific conductivity (K_s, right bottom panel) of stem wood are not available. For definition of abbreviations, see Table 2

TABLE 3 Two-factorial analysis of variance for unbalanced designs (ANOVA SS Type III) on 10 structural or functional xylem traits. P_{12}, P_{50} and P_{88} of roots were obtained from sigmoidal VCs established on long root segments (100 cm). Bold F- and p-values indicate significant effects of species identity and/or organ type on the variance of wood traits (°: $p \leq 0.10$; *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$)

WD	SS	df	F	p	log (K_p)	SS	df	F	p
Spec	0.195	3	**47.87**	**2.37E−15*****	Spec	2.123	3	**5.30**	**2.66E−03****
Organ	0.073	2	**26.98**	**6.85E−09*****	Organ	31.344	2	**117.28**	**<2.2e−16*****
Spec:Organ	0.043	6	**5.27**	**2.37E−04*****	Spec:Organ	3.529	6	**4.40**	**9.69E−04*****
Residuals	0.075	55			Residuals	7.884	59		
log (VD)	SS	df	F	p	log (K_s)	SS	df	F	p
Spec	2.222	3	22.1727	**9.76E−10*****	Spec	0.987	3	0.70	5.61E−01
Organ	6.801	2	101.799	**<2.2e−16*****	Organ	3.181	1	**6.74**	**1.39E−02***
Spec:Organ	1.913	6	9.5437	**2.54E−07*****	Spec:Organ	3.344	3	2.36	8.87E−02°
Residuals	1.971	59			Residuals	16.058	34		
log (D_h)	SS	df	F	p	P_{12}	SS	df	F	p
Spec	0.290	3	**9.80**	**2.44E−05*****	Spec	2.127	3	**3.59**	**2.64E−02***
Organ	4.193	2	**212.45**	**<2.2e−16*****	Organ	0.021	1	0.11	7.47E−01
Spec:Organ	0.462	6	**7.80**	**3.36E−06****	Spec:Organ	1.744	3	2.95	5.08E−02°
Residuals	0.582	59			Residuals	5.329	27		
log (F)	SS	df	F	p	P_{50}	SS	df	F	p
Spec	1.213	3	**9.08**	**4.93E−05*****	Spec	3.651	3	**7.99**	**5.72E−04*****
Organ	2.659	2	**29.88**	**1.09E−09*****	Organ	0.160	1	1.05	3.15E−01
Spec:Organ	1.549	6	**5.80**	**8.49E−05*****	Spec:Organ	0.236	3	0.52	6.75E−01
Residuals	2.626	59			Residuals	4.114	27		
log (S)	SS	df	F	p	P_{88}	SS	df	F	p
Spec	5.830	3	**17.92**	**2.19E−08*****	Spec	6.583	3	**9.48**	**1.91E−04*****
Organ	36.670	2	**169.07**	**<2.2e−16*****	Organ	0.430	1	1.86	1.84E−01
Spec:Organ	2.980	6	**4.58**	**7.06E−04*****	Spec:Organ	0.355	3	0.51	6.78E−01
Residuals	6.400	59			Residuals	6.252	27		

K_s was strongly related to all wood anatomical traits ($p < 0.001$), P_{50} and P_{88} also depended positively on D, D_h and S ($p < 0.05$) and F and K_p ($p < 0.01$; Figure 4). Across organs, D and F were highly interrelated ($p < 0.001$; Figure 4). However, many of these significant relationships disappeared or were found to be highly variable when analysing the different organ types separately instead of the pooled dataset across all organs (cf. Figure 5). Accordingly, the observed variability for the majority of xylem traits measured was much lower between species than between organs (Table 3).

4 | DISCUSSION

4.1 | Alterations in xylem structure and hydraulic properties along the root-to-shoot flow pathway

Our results revealed a high intra-tree variability in the xylem hydraulic architecture of temperate broad-leaved tree species, corresponding to a steep decline in hydraulic efficiency from coarse roots to shoots, which was, however, not adequately reflected in xylem

safety. Acropetal conduit tapering, which is presumably caused by a combined gradient in auxin concentration (Aloni et al., 2006; Heyn, 1940), turgor pressure (Woodruff et al., 2004; Woodruff & Meinzer, 2011) and cambial age (Li et al., 2019; Rodriguez-Zaccaro et al., 2019), has been observed in many tree species (e.g. Aloni & Zimmermann, 1983; Anfodillo et al., 2013; Domec et al., 2009; Mencuccini et al., 2007; Petit et al., 2009). In correspondence, the four temperate species of this study showed continuous vessel tapering from the coarse roots through the stem wood to the terminal branches. However, this seems not to be a general pattern in angiosperms, since deviating vessel diameter patterns along the flow path have been reported for angiosperm trees species in tropical moist forests (e.g. Kotowska et al., 2015; Schuldt et al., 2013). Due to vessel tapering, water transport capacity in the conduits continuously declined from coarse roots to the stem wood and further to terminal sun-canopy branches in our sample, with fivefold to tenfold higher specific conductivities (K_s) in the root than the branch xylem. Although hydraulic efficiency is foremost determined by vessel diameter variation along the hydraulic pathway because conductivity increases with diameter raised to the fourth power according to the
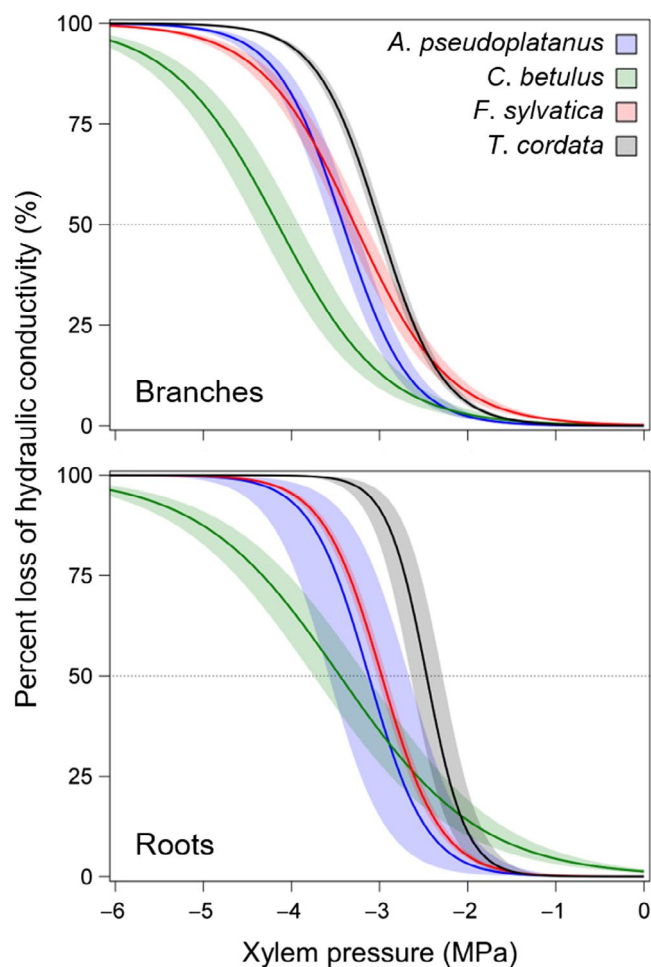
FIGURE 2 Averaged vulnerability curves for sun-canopy branches (upper panel) and coarse roots measured on long segments (100 cm, lower panel) of *Acer pseudoplatanus* (in blue), *Carpinus betulus* (in green), *Fagus sylvatica* (in red) and *Tilia cordata* (in black), with xylem pressure plotted against percentage loss of hydraulic conductivity (PLC). The shaded areas indicate the 95% confidence intervals for each curve

Hagen–Poiseuille law, the vessel lumen fraction was likewise closely associated with K_s in our species sample. The decline in hydraulic efficiency along the flow path might therefore have been even larger, if it were not partly offset by higher vessel numbers in branches causing greater vessel redundancy (Ewers et al., 2007).

4.2 | Variability in embolism resistance of branch- and coarse root xylem

Despite notable differences in wood anatomy between coarse roots and branches and a steep decline in hydraulic efficiency along the hydraulic pathway, xylem resistance to embolism remained remarkably constant along the flow path for all four species. The only significant difference in P_{50} was found in *T. cordata*, where roots had an 18% higher (less negative) P_{50}-value than branches. Thus, coarse roots were not significantly more vulnerable to xylem embolism than
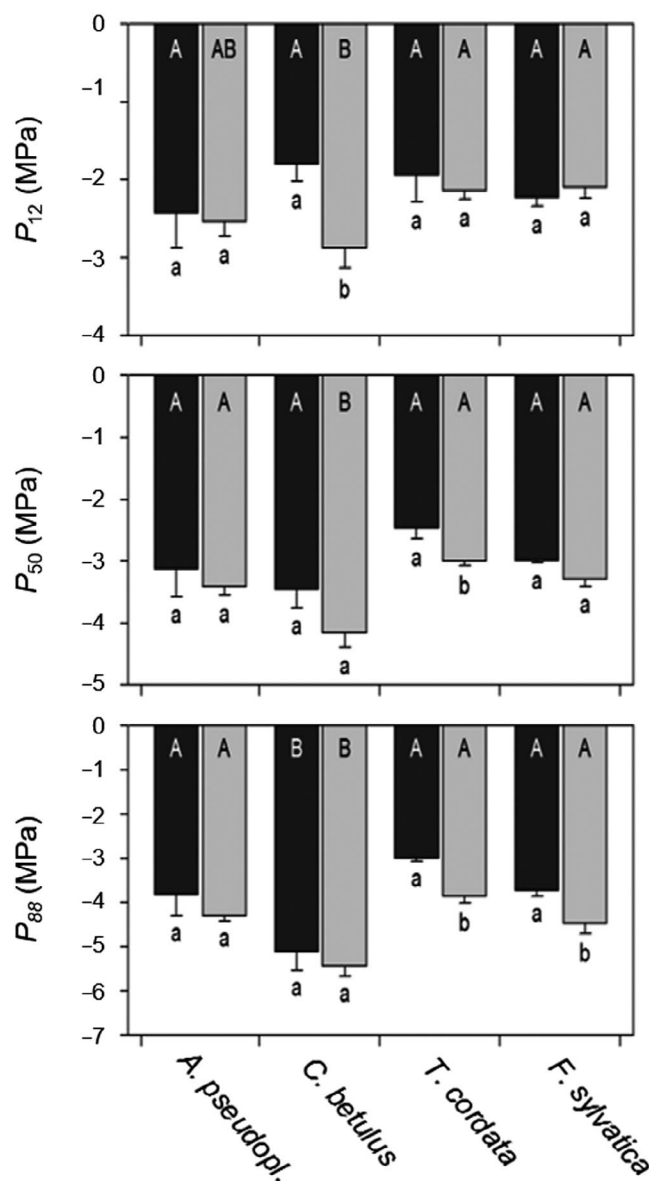

FIGURE 3 Means $\pm$ *SE* of three cardinal values of embolism resistance (P_{12}, P_{50}, P_{88}) of the four tree species for long coarse root segments (length 100 cm; dark bars) and sun-canopy branches (grey bars). Different capital letters indicate significant differences ($p < 0.05$) among the species for roots or branches. Small letters indicate significant differences between roots and branches for a given species ($p < 0.05$)

branches in three species. In line with this main result, a growing number of recent studies using different techniques have confirmed that roots are notably more embolism resistant than previously thought. These studies used the micro-CT technique and the optical method, both direct and non-invasive methods applied on intact plants (Losso et al., 2019; Peters et al., 2020; Rodriguez-Dominguez et al., 2018; Skelton et al., 2017), the rehydration kinetics technique (Creek et al., 2018), the single-vessel air-injection method (Wason et al., 2018) and the pneumatic method (Wu et al., 2020). Together with our study, the results from six independent methods are largely in contradiction with the majority of former comparative studies,
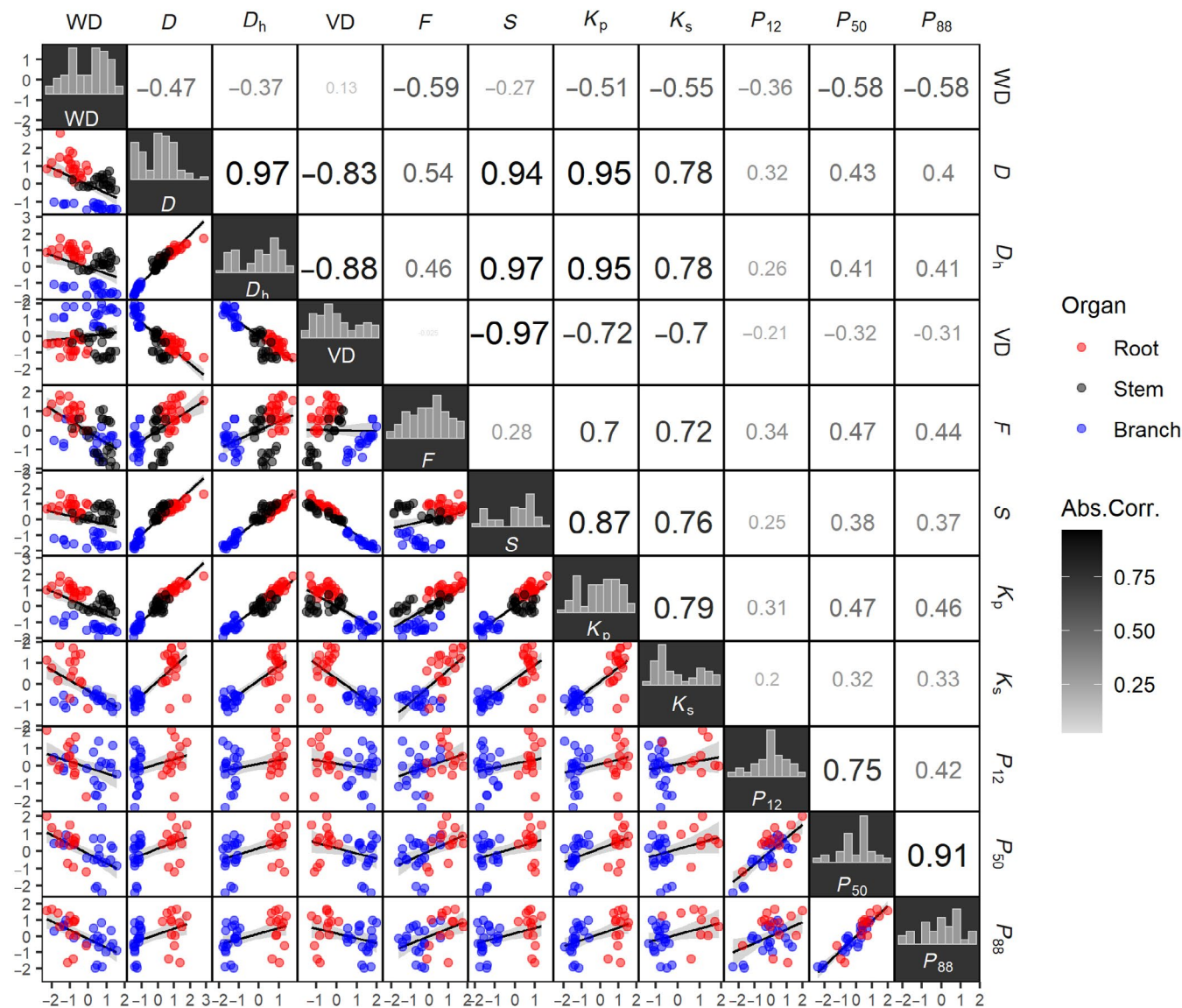
FIGURE 4 Pairwise Pearson correlation table (upper triangle), scatterplots (lower triangle) and density plots (diagonal panels) for pairs of traits across all three organs. Shown are averaged scaled and centred data per tree individual, including trend lines from linear regression with 95% confidence intervals. Colour and size indicate the strength of the correlation. Please note that P_{12}, P_{50} and P_{88} values as well as K_s were only available for roots and branches. Given variables were $\log^{10}$-transformed prior to the analysis (D_h, VD, F, S, K_p, K_s)

which found the root xylem to be the most vulnerable organ along the hydraulic pathway in a wide range of species from different environments (e.g. Alder et al., 1996; Domec et al., 2010; Kavanagh et al., 1999; Maherali et al., 2006; Pittermann et al., 2006; Pratt et al., 2007; Sperry & Ikeda, 1997; Willson et al., 2008). Most likely the majority of these mentioned studies have suffered from methodological limitations caused by the open-vessel artefact (Choat et al., 2016; Cochard et al., 2010, 2013; Jansen et al., 2015; López et al., 2019; Torres-Ruiz et al., 2014; but see Hacke et al., 2015; Jacobsen & Pratt, 2012; Pratt et al., 2015). Therefore, and according to these recent findings and the results obtained in the present study, the xylem of coarse roots (root diameter >2 mm) cannot be considered as 'hydraulic fuses' during soil drying as speculated by Jackson et al. (2000). It should rather be fine roots (<2 mm) or even

finest roots (<1 mm) that represent the level of replaceable units that would embolize during soil drying, thereby restricting hydraulic failure to redundant organs. During drought exposure, recent work indicates that the outside xylem hydraulic conductivity within the root cortical cells declines much earlier before any significant embolism is observed in coarse roots (Cuneo et al., 2016, 2020). Most likely, this can be attributed to cortical lacunae formation during soil drying (Passioura, 1988), which causes the loss of soil–root connectivity (Carminati & Javaux, 2020; North & Nobel, 1997) and dramatically increases root hydraulic resistance (Rodriguez-Dominguez & Brodribb, 2020). According to Passioura (1988), the root cortex, and not the inner xylem, will have a water potential much closer to that of the soil. It is therefore unlikely that the root xylem is exposed to very negative water potentials during moderate drought (e.g. Leuschner
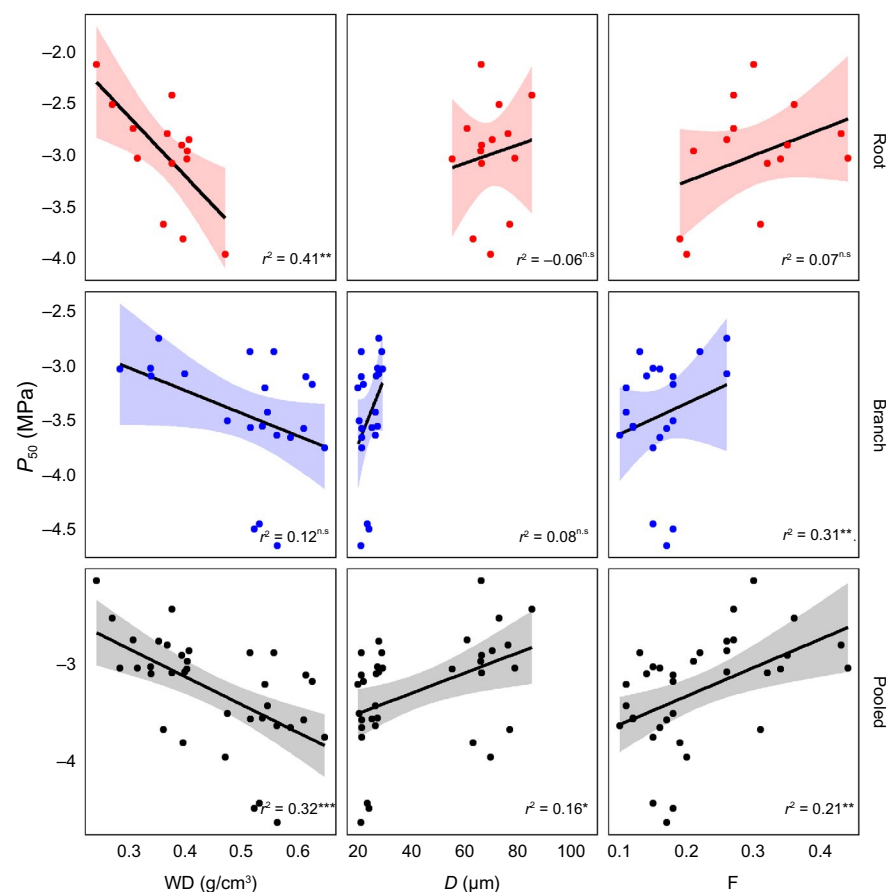
FIGURE 5 Wood density (WD, left), vessel diameter (*D*, middle) and the vessel lumen fraction (*F*, right) in relation to the xylem pressure causing 50% loss of hydraulic conductance (P_{50}) for coarse roots (top), branches (middle) and the pooled dataset (bottom). The shaded areas indicate the 95% confidence intervals for each linear regression analysis. Given are the mean values per tree individual, asterisks indicate the level of significance (*, $p < 0.05$; **, $p < 0.01$; ns, non-significant relationships) [Correction added on <18-January 2022>, after first online publication: Figure 5 corrected to include missing panels.]

et al., 2003). Under such conditions, hydraulic conductivity might therefore be restored rapidly after re-wetting (Bourbia et al., 2021). Even after severe drought, fine and finest roots will be more readily replaced as they have lower construction costs than coarse roots, and their production takes only weeks to months and not years.

4.3 | Missing trade-off between hydraulic efficiency and safety

Although several studies detected a significant negative relationship between conduit diameter and xylem safety across species (e.g. Christensen-Dalsgaard & Tyree, 2014; Domec et al., 2010; Fu et al., 2012; Maherali et al., 2006; Wheeler et al., 2005), others did not find evidence for any relationship at the intraspecific level (Hajek et al., 2016; Schuldt et al., 2016). In our study, P_{50} and P_{88} values showed an increase (shift to less negative potentials) with increasing vessel diameters (*D*, D_h), when considering the complete dataset including root and branch segments of all four species (Figure 4). However, this positive relationship disappeared when roots and branches were analysed separately. Thus, other factors than conduit diameter must be the principal determinants of hydraulic safety in these species, most likely pit membrane properties (e.g. Kaack et al., 2021; Lens et al., 2011; Li et al., 2016). In our study, the vessel lumen fraction was more closely related to embolism resistance than vessel diameter across organs and species ($p = 0.004$ vs. $p = 0.01$,

respectively). One might speculate that the vessel lumen fraction, which equals the lumen-to-sapwood area ratio, is a measure of the total vessel wall area and therefore related to the total number of pits per cross-section. According to Kaack et al. (2021), the number of pits per vessel affects xylem embolism resistance within a given range of pit membrane thickness (T_{PM}) values, approximately between 150 and 350 nm. This is exactly the range of T_{PM} values reported for our species sample (270, 315, 239 and 213 nm for *Acer*, *Carpinus*, *Fagus* and *Tilia*, respectively; Kaack et al., 2021; Schuldt et al., 2016), which might explain why we observed a close relationship between the vessel lumen fraction and embolism resistance. In contrast to this assumption, however, Hacke et al. (2017) proposed that vessel size might negatively relate to pit membrane thickness as a consequence of vessel widening. In contrast, and despite considerable differences in conduit size, the low intra-tree variability in embolism resistance found here supports the idea that the T_{PM} does not change considerably across organs. Indeed, pit membrane thickness was found to be surprisingly similar between different organs along the flow path in *A. pseudoplatanus* and *Acer campestre* (Kotowska et al., 2020; Wu et al., 2020). In agreement, empirically determined K_s, which besides K_p includes the resistivity caused by pit membranes, showed no relation to P_{50} and P_{88} in our study. In support of our second hypothesis, hydraulic safety and efficiency were thus independent of each other in the root-to-branch flow path of the four species, and the high intra-plant variability in hydraulic efficiency was not related to a similar variability in xylem safety.

4.4 | Influence of wood density on hydraulic functioning along the hydraulic pathway

Apart from the expected dependence of hydraulic efficiency on D, D_h and F, two main outputs result from the pairwise correlation analyses among anatomical and hydraulic parameters. On the one hand, the close negative relationship between WD and K_p and also K_s, mediated through the negative effect of WD on VD and F. On the other hand, the highly significant negative effect of WD on P_{50} and P_{88} across species and organs. In our species sample, denser wood decreased hydraulic efficiency and simultaneously increased hydraulic safety, although both K_s and P_{50} were unrelated to each other. Dense wood is supposed to be less vulnerable due to a smaller conduit lumen, higher wall thickness and reduced endangerment through cell wall implosion (Hacke et al., 2001; Jacobsen et al., 2005; Pratt et al., 2007). According to the strong negative relationship with F, WD seems to be both determined and restricted by the cumulative conduit volume in the xylem body. This dependence has been demonstrated for different taxa and regions (Jacobsen et al., 2007; Preston et al., 2006; Zanne et al., 2010). Yet, other studies on tropical trees did not find a close linkage between WD and vessel characteristics (e.g. Fan et al., 2012; Schuldt et al., 2013; Zhang & Cao, 2009), which may be interpreted as support for the assumption that WD is more dependent on fibre cell properties than on lumen structure (Martínez-Cabrera et al., 2009; Ziemińska et al., 2013). Exploring the importance of WD for xylem structure and functioning is particularly promising in our dataset, as it includes not only four functionally different tree species but also covers root, stem and branch xylem. In fact, separate analyses for structure–function interrelations for root, stem or branch xylem yielded weaker or missing relationships compared to correlation analyses covering the pooled data of all three compartments.

5 | CONCLUSIONS

This comparative study carried out on mature trees of four mid- to late-successional temperate broad-leaved species confirmed that differences in xylem anatomy and hydraulic functioning among organs can be as large, or even larger, than those observed among species. We also obtained evidence that coarse roots, despite being the most efficient organ to conduct water, are not inevitably more vulnerable to xylem embolism than branches. Instead, fine roots and the resistance at the soil–root interface, which greatly increases during soil drying, most likely act as 'hydraulic fuses' during drought periods, decoupling plant and soil water status. This opens new research avenues for a more mechanistic understanding of whole-plant responses to drought.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

B.S. designed the study, T.L. collected the field samples, L.J.L. and J.M.T.R. measured the vulnerability curves with the Cavi1000, which was developed and maintained by R.B. and S.D. and S.D. analysed the vulnerability curves. T.L. carried out the remaining hydraulic and wood anatomical observations and performed the statistical analyses. T.L., B.S. and C.L. wrote the first version of the manuscript, which was intensively discussed and revised by all authors.

DATA AVAILABILITY STATEMENT

Data deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.nzs7h44sz (Lübbe et al., 2021).

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