

Combined application of computer tomography and light microscopy for analysis of conductive xylem area in coarse roots of European beech and Norway spruce

Petia S. Nikolova · Helmut Blaschke ·
Rainer Matyssek · Hans Pretzsch · Thomas Seifert

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Abstract Axial water transport in trees is mainly determined by the gradient of negative water pressure and the structure of conductive xylem elements (i.e. conduits) connecting the fine roots with the foliage. There is still an essential lack of knowledge concerning the relationship between wood structure and hydraulic properties, especially of coarse roots. To this end, the study aimed (1) to work out a novel approach, based on the combination of computer tomography (CT) and light microscopy (LM), for determining the cumulative cross-sectional lumen area of conduits involved in the water transport of coarse roots in European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) and

(2) to demonstrate its adequacy in quantifying the functional relationship between sapwood anatomy and ascending water mass flow in the xylem. The cross-sectional sapwood area of coarse roots was assessed through CT. The cumulative cross-sectional lumen area of conduits in the sapwood (i.e. the lumen area of conductive conduits) was measured by LM in combination with interactive image analysis. The new approach was developed with coarse roots of both the tree species growing in a 60-year-old mixed forest in Bavaria, Germany. The combination of the two methods unveiled spruce to possess a distinct sapwood/heartwood boundary in small-diameter roots, whereas such roots of beech reflected a gradual transition zone; only large-diameter roots displayed a distinct boundary in beech. Additionally, the cumulative lumen area of conductive conduits was found to be approximately 12% of the total coarse root cross-sectional area in both the tree species. The new approach of measuring the conductive lumen area of coarse-root conduits yielded levels of specific sap flow (i.e. axial conductivity) that substantially differed from those derived from commonly applied methods, which were based on sap flow per unit of total cross-sectional root area or xylem cross-sectional area of individual roots. The combination of CT and LM will facilitate functional comparisons of woody roots differing in diameter and of tree species of different anatomical xylem structure.

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Petia S. Nikolova and Thomas Seifert contributed equally to this work.

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T. Seifert was formerly Chair of Forest Yield Science, Technische Universität München.

P. S. Nikolova · H. Blaschke · R. Matyssek
Ecophysiology of Plants, Technische Universität München,
Weihenstephan Center of Life and Food Sciences,
Am Hochanger 13, 85354 Freising, Germany

H. Pretzsch
Chair of Forest Yield Science, Technische Universität München,
Weihenstephan Center of Life and Food Sciences,
Am Hochanger 13, 85356 Freising, Germany

T. Seifert (✉)
Department of Forest and Wood Science,
Faculty of AgriSciences, Stellenbosch University,
Private Bag XI, 7602 Matieland, South Africa
e-mail: seifert@sun.ac.za

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Introduction

Acquisition of water by root systems and the axial water transport are essential components of the plant water flux

(Tyree 2003). Water transport is driven by the atmospheric evaporative demand across the canopy and transmitted via the hydraulic soil–plant–atmosphere continuum (SPAC) to the available water pool in the soil. Water uptake depends on factors such as hydraulic resistances in the soil–root interface and the water potential gradient across SPAC (Leuschner et al. 2004), as well as upon intrinsic anatomical and physiological root system characteristics (including aquaporins) that vary hydraulics during root ontogeny (Zeier and Schreiber 1998; Steudle 2001). Root hydraulic conductivity combines the components of conductivity for radial transport across the root, i.e. from the root surface to the xylem and conductivity for axial water transport to the base of the stem (Huang and Nobel 1994). The axial hydraulic conductivity increases with the number of conduits (i.e. vessels and/or tracheids) per unit of cross-sectional area and with their diameter to the fourth power according to the “Hagen–Poiseuille law” (cf. Tyree and Zimmermann 2002). The axial hydraulic conductivity based on the conductive cross-sectional xylem area yields the specific conductivity of a root, which is a measure for the efficiency of roots to conduct water to aboveground structures (Tyree et al. 1998; Tyree and Zimmermann 2002).

Despite major advances in the understanding of water uptake by plant roots (Steudle 2001; Coners 2001; Lindenmair et al. 2004; Leuschner et al. 2004) little is known about the hydraulic properties of woody roots in terms of their specific conductivity. High specific conductivity enables high xylem sap flow rates (Riedl 1937; Tyree and Zimmermann 2002) according to the demand by the foliage. The ability to acquire limiting resources from the soil more rapidly and efficiently than competitors determines the success in belowground competition between plants (Grime 1977; Nikolova 2007). This aspect is crucial for understanding competitiveness in mixed-species forest, in particular, if species of distinctly different root morphology, like angiosperm and coniferous trees, are involved.

Methodologies need to warrant comparisons of hydraulic characteristics between roots of contrasting anatomy. A basic technique is that of sap flow assessment in trunks through a heat-balance principle which yields whole-tree transpiration on a diurnal and seasonal basis (Senock and Ham 1993; Nadezhdina 1999; Čermak et al. 2004; Patzner 2004; Stöhr and Lösch 2004). This method has been adopted for flow assessment in woody roots (Lott et al. 1996; Coners 2001; Korn 2004) and for the investigation of the “hydraulic lift” phenomenon (Smith et al. 1999). When measured in shoot axes, the sap flow rate is usually expressed per unit of foliage area (Larcher, 2001), stem diameter or sapwood area (Čermak and Kučera 1987; Čermak et al. 2004). Below ground, sap flow rates have usually been presented per individual root (Lott et al. 1996; Smith et al. 1999; Lindenmair et al. 2004), related to the total cross-sectional

area of small-diameter roots (Korn 2004), or to the cross-sectional area of the xylem (Coners 2001; Lindenmair et al. 2004), or the integrated cross-sectional area of conduit lumina (Senock and Leuschner 1999). Studies focused on the conductive xylem area in coarse roots, analyzing root hydraulic conductivity, have been scarce, although they would allow for direct comparisons between tree individuals and tree organs (Čermak et al. 2004).

Given this deficit, the combination of the two methods, light microscopy (LM) and computer tomography (CT), is demonstrated in this study as a new approach for analyzing the cumulative cross-sectional lumen area of conduits in the sapwood (i.e. the conductive cumulative lumen area of conduits) of coarse roots (8–20 mm in diameter) in adult European beech and Norway spruce trees.

LM techniques were applied to assess root anatomical parameters related to the axial hydraulic conductivity of roots (i.e. conduit diameter, total number of conduits and the cumulative cross-sectional lumen area of conduits). Although these anatomical parameters in beech and spruce coarse roots of different diameter classes have been studied extensively (Riedl 1937; Schweingruber 2001; Korn 2004), the identification of non-conducting zones in the xylem cross-section has been neglected. In the present study, xylem density as well as moisture distribution in roots were determined by CT scanning. This X-ray based method commonly used in human medicine has been adopted also for non-destructive wood analysis (Lindgren 1991; Bhandarkar et al. 1999; zu Castell et al. 2005). The suitability of CT scanning for the detection of water content in woody tissues was proven in several studies (Fromm et al. 2001; Sandberg 2004; Vötter 2005; Longuetaud et al. 2006; Seifert et al. 2008), although analysis of tree roots has been neglected. The objectives of this study were, therefore, (1) to develop a new approach for assessing the cumulative lumen area of conduits involved in the axial water transport of beech and spruce coarse roots by combining LM and CT and (2) to demonstrate its adequacy in quantifying the functional relationship between sapwood anatomy and ascending water mass flow in the xylem.

Materials and methods

Site description

The study was performed at the experimental site “Kranzberger Forst” near Freising/Germany (*Fagus sylvatica*/*Picea abies* mixed forest; 48°25′08″N, 11°39′41″E, 490 m a.s.l.; Pretzsch et al. 1998). Spruce, planted in 1951, was the dominant species at the site, with beech established 7 years previously as groups of 60–100 individuals, according to the common silvicultural practices. Rooting

depth of beech and spruce trees was about 1 m in a Luvisol (FAO classification) that had developed from loess over tertiary sediments. Thus, trees have no access to the groundwater table. The climate on the study site is classified as temperate based on a 30-year record (1970–2000) of mean daily air temperature and annual precipitation (7.8°C and 785 mm, respectively; Nikolova 2007). For a more detailed stand description see Wipfler et al. (2005).

Sap flow measurements

Measurements were conducted during the assimilation period (June–October) in 2003. Sap mass flow was assessed on horizontal (surface) coarse roots (8–20 mm in diameter) of six beech and spruce trees each by means of miniaturized gauges (Sap Flow Meter T4.1, EMS, CZ; heat balance method with constant temperature difference of 4°C; Čermak et al. 2004). The maximum input power of the gauges was approximate 1.2 W in accordance with the heating voltage. Individual roots that originated directly from the root crown were excavated and the gauges were placed at positions within 0.5 m of the trunk. To ensure that effects of varying environmental conditions on the measurements were minimized, gauges were covered with aluminium foil, styrofoam plates and plastic boxes. The measuring device was equipped with two 6-channel data loggers recording data by 10-min intervals. Sap flow was calculated on a whole-day basis (1 day⁻¹). Two coarse roots (one with a small diameter of about 7 cm and one with a large diameter of about 17 cm) were selected per each tree species and their sap flow rates assessed across 50 days (day of year 149–199) for comparative sap flow analyses.

Light microscopy and digital image analysis

After completing the sap flow measurements the instrumented roots were cut and sections of 20-cm length were individually wrapped in wet paper and stored in plastic bags at 4°C for 2 days at maximum. This assured a minimum loss of water until the samples were processed. Transverse sectional cuts (thickness 20 µm) of representative segments (one-half to one-quarter of the total cross-sectional area A_t) were mounted in one-fifth glycerol/distilled water. Thereafter, transverse sections were stained separately with (1) phloroglucine–HCl reagent for colouring of lignified cell walls and (2) Lugol's I₂/KI solution for colouring of starch grains in living parenchyma cells, and then transferred with cover slips into a permanent mounting medium. Analyses were performed on the representative segment of each cut under light microscope (LM; Leitz Laborlux S) at 40× magnification. The LM was equipped with a video recording device system (PS30 CCD camera, progressive scan type) connected to an interface control software (DX30,

Kappa Image Base). Recorded images in 5–15 representative root sections were merged using image-processing software (Adobe Photoshop, vers. 8.01) and the areas of interest were converted in black–white image (grey level threshold technique). This procedure resulted in black lumina of conduits (i.e. vessels and/or tracheids) and white cell walls and white parenchyma cells. Resin channels in spruce were manually covered in white. Thickness of the entire cortical tissue outside the cambium (i.e. all non-xylem tissues) was assessed in all root segments of both the tree species, and such structures were removed from the image. The created bitmap (TIF-format) was processed with image software (Delta-T Scan, Cambridge, UK) to record the anatomical parameters: conduit lumen area (A), total number of conduits (N), total lumen area of conduits (A_l), and xylem cross-sectional area (A_s) of the representative segment. These parameters were then extrapolated to the whole xylem area in the individual coarse root samples. The inner diameter of the conduits (D) was calculated as the corresponding circular diameter from the accordant A .

Conduits with diameters smaller than 10–15 µm carry an insignificant amount of water, as pointed out by Tyree and Zimmermann (2002). That is why, in order to define the mean diameter of vessels and/or tracheids involved effectively in water sap flow (i.e. the effective diameter ED), only conduits with diameters larger than 15 µm were included in the ED calculation for each individual root analyzed by LM.

CT analysis of xylem density and moisture content

Water conducting cross-sectional area of the xylem (A_{sc}) was assessed by CT in individual beech and spruce roots. Like traditional X-ray radiographs, CT scanning is also based on the attenuation of X-rays by an object (according to Lambert–Beer's law in Eq. 1)

$$I_{out} = I_{in} e^{-\mu L}. \quad (1)$$

With I_{out} being the outgoing intensity, I_{in} the incoming intensity, e Euler's number, μ the material-specific attenuation coefficient and L the pathway length of radiation through the object.

Higher density and differences in atomic weight lead to a modification of the attenuation coefficient μ (Eq. 1). The difference of tomography compared to the existing X-ray methods is the scanning of objects from different radial positions. The resulting series of different projections is mathematically transformed and combined in a way that avoids 'shadowing' of single projections efficiently. The obtained tomogram is a picture of known size and coordinates of each pixel. The combination of multiple tomograms along the axial vector of the object yields a fully 3D representation. The different attenuations are measured

in ‘Hounsfield units’ (HU) and for visualization they are usually expressed by grey values for each pixel. The higher the attenuation (density) the brighter the grey value.

A “Siemens Somatom AR.HP” medical scanner was used. The predefined acceleration voltage was 130 kV with a dose of 200 mAs for 2 s. The scanner features a density resolution of 14 bits and a reconstruction matrix of 512×512 pixel in the region of interest (ROI). The reconstruction matrix is fixed and determines the spatial resolution according to the reselected ROI. Due to the small dimensions of the roots small ROIs were preset leading to average spatial resolutions of 0.37 mm per pixel with a standard deviation of 0.19 mm. Slice thickness was 1.0 mm with one picture every 5.0 mm along the root. A specific calibration of the CT scanner to the water content of the samples was not necessary. The constancy of density measurement was assured by regular maintenance based on test specimen of known density, so-called “water phantoms”.

The roots were scanned twice (1) immediately after cutting while the root was still fresh and (2) after oven drying (at 65°C to constant weight). Hence, water content and distribution within roots were assessed as the difference between density levels of dry and fresh scan. To avoid artefacts due to drying during the fresh scans, no scans at the cut root ends were used for further analysis. Only the central scans were analyzed. The roots showed a significant twist and distortion after drying compared to the fresh samples. Therefore, fresh and oven-dry scan was assigned according to the relative position along the root. AMIRA

3.0 (Mercury Computer Systems, Inc.) was used for the alignment. As the shrinkage of wood in the axial direction is known to be the smallest and the samples showed no significant reduction in total length upon drying, this matching method was adequate. In contrast, a significant radial shrinkage was observed in roots. This effect was corrected by expanding the pictures of the dried roots to the same diameter as their fresh counterparts. This shrinkage correction was done manually with Adobe Photoshop, vers. 6.0. Photoshop was used also for the subtraction of grey values (see earlier) so that the original DICOM format had to be rescaled to an 8-bit TIF format before by AMIRA.

Results

LM analyses of hydraulic anatomy

Coarse roots of spruce contained significantly less xylem area per unit of total root cross-section than such roots of beech (cf. A_s/A_t ratio; Table 1; Mann–Whitney U test; $P < 0.05$). In most coarse roots of spruce (9–19 mm in diameter), the xylem had a proportion of nearly 55 % of A_t , whereas in beech coarse roots (10–18 mm in diameter) A_s was 83% of A_t on average. A linear species-specific relationship existed between the cumulative lumen area of conduits (A_l) and A_t of analyzed roots (Fig. 1). A_l amounted to approximately 12% of A_t in beech and for 20% of A_t in spruce (see significantly different slope regression coefficients b in beech and spruce;

Table 1 Anatomical analysis of individual beech and spruce surface coarse roots

Root	Total root cross-sectional area A_t (mm ²)	Cross-sectional area of xylem A_s (mm ²)	Conducting cross-sectional area of xylem A_{sc} (mm ²)	Total area of conduit lumina A_l (mm ²)	Lumen area of effective conduits A_{lc} (mm ²)	A_s/A_t (%)	A_{sc}/A_s (%)	ED (μm)
Beech 1	13.72	10.52	10.52	2.38	2.38	77	1	25.5
Beech 2	84.99	70.62	70.62	10.04	10.04	83	1	27.0
Beech 3	138.60	116.98	99.83	16.01	13.60	84	0.85	55.0
Beech 4	183.58	151.51	135.11	24.61	21.94	83	0.89	36.0
Beech 5	71.14	60.79	60.79	16.57	16.57	85	1	31.0
Beech 6	98.38	82.88	82.88	15.54	15.54	84	1	30.3
Mean						83 (1)		34.1 (4.4)
Spruce 1	58.70	38.98	36.86	10.85	10.20	40	0.94	24.0
Spruce 2	32.53	16.88	14.00	3.59	2.98	52	0.83	26.3
Spruce 3	22.07	12.82	12.7	3.51	3.48	49	0.99	21.1
Spruce 4	35.63	17.62	5.25	2.86	0.86	49	0.30	19.7
Spruce 5	236.51	148.57	104.50	45.66	31.96	68	0.70	28.0
Spruce 6	158.29	102.02	63.55	26.90	16.68	64	0.62	27.0
Mean						54 (4)		24.4 (1.4)

All tissue enclosed by the cambium was considered as cross-sectional area of xylem (A_s), and the cross-sectional area of xylem which conducted water according to CT analysis was considered as A_{sc} . Cumulative area of vessel and/or tracheid lumina (A_l) as expressed per unit of cross-sectional xylem area, and lumen area of vessels and/or tracheids within the sapwood was considered as A_{lc} . For calculation of the weighted mean effective conduit diameter (ED) only diameters larger than 15 μm were considered; numbers in brackets as 1 SD of calculated means

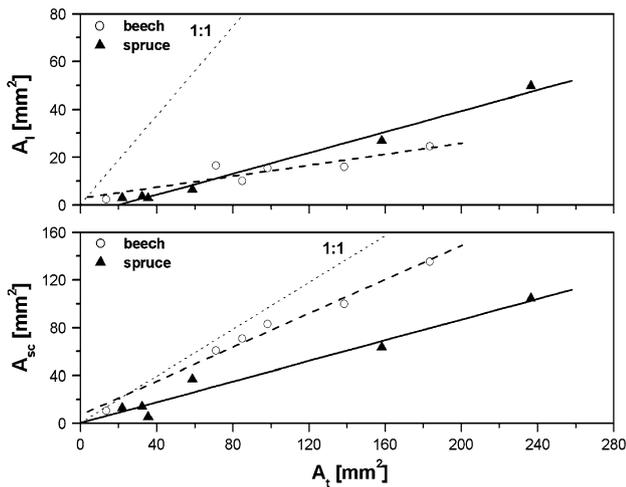


Fig. 1 Relationship between total cross-sectional root area (A_t , mm^2) in spruce and beech and the corresponding cumulative lumen area of vessels and/or tracheids (A_1 , mm^2) as determined by LM, and conducting cross-sectional area of xylem (A_{sc} , mm^2) as determined by CT. Dashed lines represent regression lines for beech, dotted lines denote the bisection line; regression statistics are shown in Table 2

Table 2 and Fig. 1). However, A_t in beech comprises of conduits with ED of $34 \mu\text{m}$, whereas in spruce ED was significantly lower (i.e. $24 \mu\text{m}$; Table 1; Mann–Whitney U test; $P < 0.05$). The space in the xylem between conduit lumina which is occupied by their cell walls and other types of cells (i.e. ray cells, wood fibre; cf. A_w on Fig. 2) amounted in beech roots to 63% of A_t ; hence, being significantly higher than in spruce roots (i.e. 40%; Mann–Whitney U test; $P < 0.05$). In contrast, within the total root cross-sectional area spruce possessed nearly two times more space in non-conducting structures than beech.

CT analyses

Only thick coarse roots in beech ($A_t > 100 \text{mm}^2$) showed regions of low X-ray absorption in the root centre, indicating

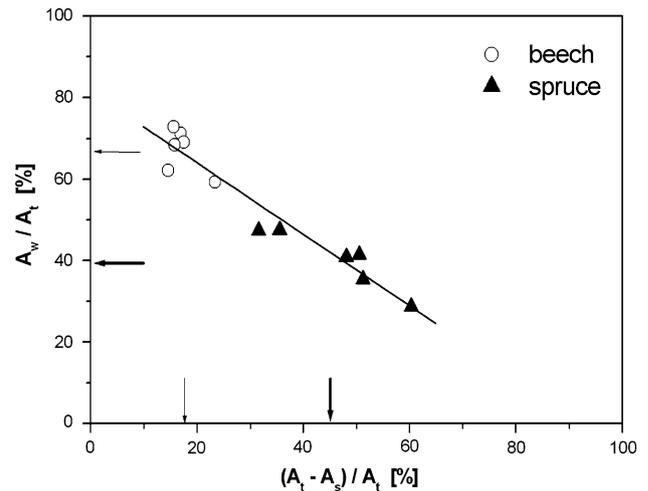


Fig. 2 : Relationship between the proportion of the area of cell walls and non-vessel cells (A_w) in the total cross-sectional root area (A_t) and the proportion of the entire tissue area outside the cambium ($A_t - A_s$) in A_t obeys the same regression ($y = 81.56 - 0.88x$; $R^2 = 0.93$; $P < 0.001$) in spruce and beech coarse roots. Means (cf. Table 1) are shown by arrows (thin, beech; thick, spruce)

heartwood formation. However, water content showed a gradual transition rather than a distinct sapwood–heartwood boundary between bark towards xylem (Fig. 3). Cross-sectional xylem area containing water in these roots (i.e. the root sapwood area A_{sc}) was 85–100 % of A_s (Table 1). In spruce, the heartwood boundary was more distinct (Fig. 3). Heartwood was present even in roots of small diameters (cf. A_{sc}/A_s ratio in Table 1). However, root sapwood area was linearly related to A_t in both the tree species (Fig. 1), showing significantly different slopes in the relationships of beech and spruce (cf. coefficient b ; Table 2; Fig. 1).

Combining LM and CT

Relationships shown on Fig. 1 allowed correction of the cumulative lumen area of conduits A_1 regarding for low or

Table 2 Regression statistics of the linear relationships ($y = a + bx$) shown in Figs. 1 and 3, with a and b being regression coefficients, R^2 the coefficient of determination and P the significance level of regression equations. Standard errors of a and b are shown in brackets

Species	Analysed relationships	a (mm^2)	b (mm^2)	n	R^2	P	
Figure 1	Beech	$A_1 - A_t$	2.87 (3.08)	0.12 (0.03)	6	0.81	0.014
		$A_{sc} - A_t$	6.87 (4.93)	0.71 (0.04)	6	0.90	<0.001
Figure 1	Spruce	$A_1 - A_t$	-4.38 (1.64)	0.21 (0.01)	6	0.98	<0.001
		$A_{sc} - A_t$	0.37 (5.11)	0.43 (0.04)	6	0.96	<0.001
Figure 3	Beech	$A_{lc} - A_t$	3.91(3.44)	0.10 (0.03)	6	0.71	0.036
	Spruce	$A_{lc} - A_t$	-0.92 (1.85)	0.13 (0.01)	6	0.94	0.001
	Beech and spruce	$A_{lc} - A_t$	0.72 (1.72)	0.12 (0.01)	12	0.87	<0.001

Bold letters indicate significant differences in the regression coefficients between both tree species ($P < 0.05$; t test)

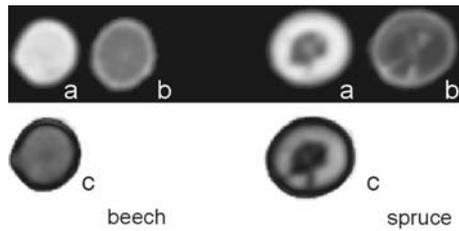


Fig. 3 Water content of roots of European beech (*left*) and Norway spruce (*right*) determined by CT scanning. High water content in the xylem is shown by high X-ray absorption and bright colours. In the upper part of the figure CT scans of the freshly cut (**a**) and oven dried (**b**) roots are displayed. The single picture in the lower part (**c**) represents the water content, calculated as the difference between oven-dried and fresh roots

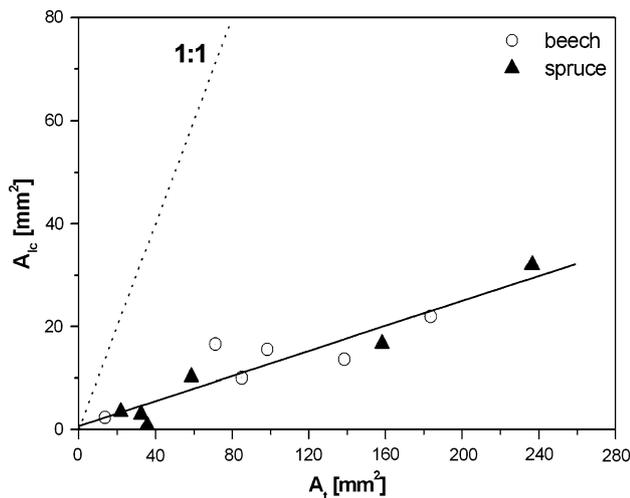


Fig. 4 Relationship between total cross-sectional root area (A_t , mm^2) in pooled data of spruce and beech roots and the corresponding conducting lumen area of effective conduits (A_{1c} , mm^2) as determined by combination of LM and CT; regression statistics is shown in Table 2

absent water content, yielding the respective lumen area effective in water transport (i.e. A_{1c} ; Table 1). The relationships between A_{1c} and A_t did not reveal significant differences between beech and spruce in regression coefficients a and b (Table 2). Hence, datasets of beech and spruce were pooled (Table 2; Fig. 4), indicating A_{1c} to be approximately 12% (cf. coefficient b ; Table 2) of the total cross-sectional area in both the tree species.

Root sap flow rates

When expressed per unit of total cross-sectional root area, sap flow rate (i.e. Q/A_t) assessed in roots with small diameter (i.e. beech 5 and spruce 1; Fig. 5) tended to be higher than in large-diameter roots (i.e. beech 4 and spruce 6; Fig. 5). Q/A_t ranging between 0.8 and $1.2 \text{ g mm}^{-2} \text{ day}^{-1}$ was inversely related in beech with root diameter. Spruce

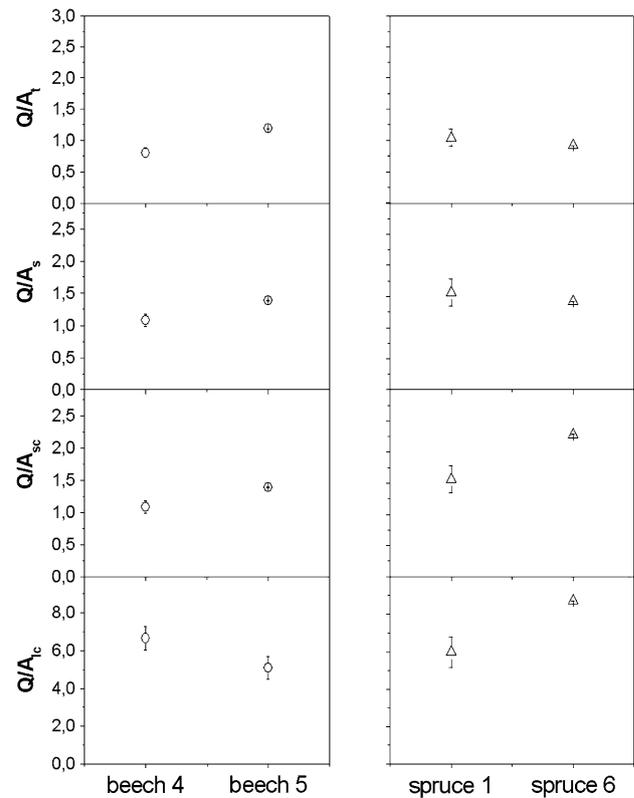


Fig. 5 Sap-flow rate Q ($\text{g mm}^{-2} \text{ day}^{-1}$) measured during 50 days in 2003 in individual beech and spruce coarse roots (description of root hydraulic anatomy is shown on Table 1). Sap-flow rate is expressed as means \pm 95% confidence interval either per unit of total cross-sectional root area (A_t), or cross-sectional xylem area (A_s), or conducting cross-sectional xylem area (A_{sc}), or lumen area of effective conduits (A_{1c})

roots showed intermediate Q/A_t relative to beech roots. When sap flow rate was expressed per unit of cross-sectional xylem area (i.e. Q/A_s), levels were significantly enhanced relative to Q/A_t by approximately 20% in beech roots, and about 53% in spruce roots. As a result, spruce roots reached Q/A_s typical for beech roots of small diameter (i.e. beech 5; Fig. 5).

In coarse roots with heartwood formation, Q/A_{sc} was higher than Q/A_t by about 40% in beech 4, and by 50 and 150% in spruce 1 and spruce 6, respectively. Lowest Q/A_{sc} was assessed at large root diameter in beech 4 (i.e. $1.1 \text{ g mm}^{-2} \text{ day}^{-1}$), whereas highest Q/A_{sc} resulted at large root diameter in spruce 6 (i.e. $2.3 \text{ g mm}^{-2} \text{ day}^{-1}$). Levels were highest when calculating Q/A_{1c} , i.e. as Q was related to the lumen area of effective conduits. In small roots, the increase relative to Q/A_t was 330% in beech 5 and 470% in spruce 1. Conversely, roots of high diameter displayed Q/A_{1c} increased to 740% in beech 4 and to 860% in spruce 6 relative to corresponding Q/A_t . When presented on an A_{1c} basis, lowest levels resulted at small root diameter in beech, and highest at large root diameter in spruce.

Discussion

The present study focussed on the development of a new approach for the assessment of cumulative lumen area of effective water transport vessels and/or tracheids (i.e. A_{lc}) in coarse roots of adult beech and spruce trees. The assessment is based on the combination of (1) LM to measure the cumulative lumen area of conduits and (2) CT scanning to determine the conducting cross-sectional xylem area (i.e. sapwood area A_{sc}). Both the methods implied the utilization of computer-based image analysis. Although several publications exist on anatomical traits of coarse roots in Norway spruce and European beech (Riedl 1937; Coners 2001; Korn 2004), none of these has covered the assessment of the conductive xylem area in roots.

Water transport capacity of individual woody roots may be expressed in several ways, depending on the basis which Q is related to. Often total root area A_t is used for normalization (Ong et al. 1998; in small diameter roots, Korn 2004). Doing so, our results indicated in beech and spruce average Q/A_t to be high in small-diameter roots, suggesting hydraulic conductivity (reflected by Q) to decrease with root age (i.e. diameter) in both the tree species. This finding appears to conflict with the current state of knowledge. Riedl (1937) and Tyree and Zimmermann (2002) report the diameter of vascular conduits (vessels and/or tracheids) to increase during root ontogeny. As a consequence, axial hydraulic conductivity of large (i.e. old) roots becomes higher (North and Nobel 1994). Our anatomical studies on woody roots confirm this tendency, given high effective conduit diameters (ED) in old rather than young (i.e. small) coarse roots (cf. Table 1). High ED related to high hydraulic conductivity (and Q) in the large roots, as indicated by the ratio of Q versus A_t . This discrepancy of the specific conductivity calculated from Q/A_t and the apparent results from wood anatomy indicates that the use of A_t as a basis of Q does not account for the internal hydraulic capacity of individual roots within the same tree species and may result in incorrect assessments of their specific sap flow rates.

The use of A_t as a basis of Q is disadvantageous, in addition, as non-xylem tissues are not accounted for. This deficit may cause misinterpretations when comparing Q across species. In spruce, 45% of A_t was non-xylem structures, whereas this proportion was 17% in beech (cf. Fig. 2). This finding explains the different Q/A_s in both the species, which was enhanced, contrasting with Q/A_t , in spruce by about 30% relative to beech (Fig. 5).

Coners (2001) reported for beech small roots (3–4 mm in diameter) Q/A_s to be similar to the levels found in our study in beech roots of 8–20 mm in diameter. However, the A_s cannot be used as a general basis for normalizing Q , since, as detected in the present study by CT scanning, spruce coarse roots have a distinct heartwood formation

already at diameters of 8 mm. Beech roots in contrast have a more gradual heartwood/sapwood transition (cf. Fig. 1) and only in large roots incipient heartwood formation was detected. The pattern of sapwood–heartwood transition (cf. Fig. 3) is consistent with the observations by Seifert et al. (2008) in stems of spruce and beech: stem wood of spruce had a distinct borderline and beech a more gradual transition. The higher proportion of heartwood in large coarse roots of spruce resulted in a distinct increase in Q/A_{sc} relative to the small coarse roots, whereas in beech the effect of heartwood formation was small.

The sapwood area A_{sc} delineates water containing, and thus potentially conductive areas from dry, definitely non-conducting areas. Though it, A_{sc} , is apparently not an adequate basis for normalizing Q when comparing woody roots of different anatomical characteristics, as the sapwood contains a substantial species specific proportion of cell walls and non-vessel tissues (i.e. xylem parenchyma and woody fibres; cf. Huber and Prütz 1938; Tyree and Ewers 1991; Korn 2004). The present study showed a higher proportion of cell walls and non-vessel tissues in beech compared to spruce (Fig. 2). These structures enhance the mechanical safety (Wagner et al. 1998) and the C storage capacity of beech roots (Riedl 1937; Gansert and Sprik 1998), but are not involved in axial water transport (Tyree and Zimmermann 2002). Therefore, using the LM technique in combination with CT analysis the cumulated lumen area of effective conduits A_{lc} was assessed. A_{lc} accounted for approximately 12% of A_t in both the tree species independent of the thickness of the coarse roots (Fig. 4). Calculated on an A_{lc} basis, Q in both the tree species increased by a factor of 4–8.5, being lowest in small coarse roots. These results correspond to the axial hydraulic conductivity of the individual roots indicated by ED and to the previous observations reported by North and Nobel (1994) and Tyree and Zimmermann (2002) that large roots have high hydraulic conductivity and high Q .

In this study, phloem area was not distinguished from xylem area during CT analysis. This leads to slightly overestimated sapwood proportions. A refinement of the image analysis procedure could solve this problem in the future. One needs to be aware, however, that the new approach cannot provide information about possible radial gradients of conductance within the xylem. Such gradients can be caused by embolism of older cells. In this study, water content was only used like a threshold to separate sapwood from heartwood in the CT scans. The different grey values depicting the water content within the sapwood were not taken into account. Future developments should therefore consider the specific water gradient in the sapwood as a possible indicator for conductance. Additionally, it would most probably need an analysis of the specific lumen diameters, frequency distributions and weighing the

potential sap flow according to Hagen–Poiseuille’s law. Additionally, a sound analysis of resistance factors which are not accounted for in Hagen–Poiseuille’s assumptions (e.g. pith resistance) would be necessary, as proposed, for example, by Haskins and Ford (1990). Further research is required here.

Conclusion

By combining LM and CT scanning additional information on cross-sectional conductive conduit area of coarse roots was retrieved. The proposed approach is able to characterize the axial hydraulic conductivity of coarse roots much more precisely than LM and CT alone and represents advancement in research on coarse root hydraulics. The new approach is advantageous in determining the relationship between sapwood anatomy and mass flow in the coarse root xylem, in particular, when comparing woody roots of different size and different xylem anatomy. This is a vital precondition for empirical analysis of growth and competition effects in mixed stands (Pretzsch and Schütze 2008) including tradeoff studies (Seifert and Müller-Starck 2008) and especially for modelling allocation with ecophysiological based process models (Rötzer et al. 2008).

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