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Review of embedding and non-embedding techniques for quantitative wood anatomy

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ABSTRACT

In recent decades, xylem anatomical traits have become increasingly important in dendrochronological research, as they offer the unique opportunity to assess eco-physiological drivers of tree growth at intra-annual resolution. However, standard protocols for generating such data are still missing, leading to methodological uncertainty, and complicating data exchange among laboratories. Here, we compare protocols for high-quality permanent slide preparation in dendroanatomy and address the effects of paraffin embedding vs. non-embedding approaches. Tests are conducted on both gymnosperm and angiosperm wood types of widely distributed European tree species, considering cell wall thickness (CWT), mean lumen area (MLA), and hydraulic diameter (Dh). Results indicate that non-embedding does not significantly alter the qualitative characteristics of permanent slides compared to embedded samples. Whereas the mean chronologies of MLA and Dh and their non-embedded counterparts share substantial high-frequency variance, the CWT chronologies reveal slightly larger discrepancies at inter-annual scale. However, methodological differences do not exceed 11.1 % for any parameter. While these results show high similarity between the two approaches, we recommend adopting the non-embedding procedure, since it saves resources and therefore allows to produce larger datasets. Regardless of the protocol used to build wood anatomical datasets, assembling large-scale networks of wood anatomical data could transform our understanding of forest responses to global changes.

1. Introduction

Microscopic analyses of xylem anatomical features provide a unique opportunity to study the response of forest growth to environmental changes at intra-annual resolution (Lopez-Saez et al., 2023; Piermattei et al., 2020). Methodological and technological advances in quantitative wood anatomy (QWA) progressively enabled detailed and resource-efficient analyses of xylem-specific components (Katzenmaier et al., 2023; Prendin et al., 2017; von Arx et al., 2016; von Arx and Carrer, 2014), namely the structural and conductive elements forming wood, such as fibers, vessels, and tracheids. As a result, recent tree-ring studies increasingly focused on the analyses of wood anatomical traits, indicating dendroanatomy to be a powerful new tool to study tree responses to environmental changes (Arnič et al., 2021; Carrer et al., 2016, 2018; von Arx et al., 2016). Among numerous wood anatomical traits, cell wall thickness (CWT, μ m), mean cell lumen area (MLA, μ m²), and hydraulic diameter (Dh, μ m) are most commonly used to address a variety of physiological, ecological and palaeoclimatological research questions (Castagneri et al., 2017; Fonti et al., 2009; Piermattei et al., 2020; von Arx et al., 2016; Ziaco et al., 2023).

Whereas QWA adds cell-specific information to traditional tree-ring proxies such as ring width (RW) or maximum latewood density (MXD), data development is quite time-consuming compared to conventional dendrochronological techniques. Standard protocols for generating wood anatomical data are still lacking, while methodological assessments (Björklund et al., 2019) routinely enable the construction of large-scale RW and MXD networks, providing a powerful tool for studying regional to continental forest responses to climate change

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(Briffa et al., 2001; Esper et al., 2002, 2012). In fact, tree-ring laboratories across the world currently use slightly different methodological approaches to prepare wood samples for the wide spectrum of QWA (Carrer et al., 2018; Seftigen et al., 2022), xylogenesis (Martinez del Castillo et al., 2018; Rossi et al., 2006), and other eco-physiological analyses (Schneider and Gärtner, 2013; Yeung et al., 2015). These methodological differences hamper the seamless comparison and exchange of data and material among laboratories and research groups and hinder the development of large-scale dendroanatomical networks that would likely add new insights to our understanding of large-scale forest changes.

One of the main methodological differences in sample preparation for QWA involves the procedure of sealing xylem tissue into an embedding medium, such as paraffin, versus boiling the sample in water or in baths of glycerine to soften the cellular walls before sectioning (Gärtner and Schweingruber, 2013; von Arx et al., 2016). While embedding is routinely used to prevent cell wall collapse during cutting by injecting liquid paraffin into cell lumina under vacuum pressure. especially in soft and living cells in the cambial zone (Rossi et al., 2006), non-embedding allows for a faster sample processing, simultaneously preventing the employment of harmful chemicals (i.e., xylol) intrinsic to the embedding procedure. Here, we compare these two protocols for high-quality permanent slide preparation in dendroanatomy, demonstrating the covariance of QWA data from embedding and non-embedding approaches. We use three major European tree species, including deciduous hardwoods and coniferous softwoods, and compare microsections obtained with and without paraffin embedding for any quantitative differences in CWT, Dh, and MLA.

2. Material and methods

Two coniferous and one deciduous tree species were selected for the dendroanatomical comparison: stone pine (Pinus cembra L. - PICE), European larch (Larix decidua L. - LADE), and European beech (Fagus sylvatica L. - FASY), growing in montane and subalpine belts between 1000 and 2200 m asl in the Italian Southern Alps (PICE, LADE) and Northern Apennines (FASY). One sample per tree, selected based on visual appearance to avoid damage, decay, and structural anomalies, was taken at breast height (1.3 m above ground) using a 10-mm-increment borer. Samples were split orthogonally to the longitudinal stem axis to produce two datasets of transverse microsections, one with and one without embedding. For the latter, core halves were separated into 4-5 cm long pieces and boiled in distilled water to soften these (5-10 min. for PICE and LADE; 20-25 min. for FASY). Ten µm thick transverse microsections were obtained using a rotary microtome (Leica, Heidelberg, Germany) and bleached in 10 % sodium hypochlorite for 10 min to decolour cell walls. After removing the bleach with water, microsections were stained in a 1:1 Safranin-Astra blue solution, dehydrated in successive ethanol baths (50 % and 100 % purity), permanently mounted onto glass microscope slides with Euparal (Carl Roth, Karlsruhe, Germany), and dried in an oven at 60°C for 48 hours. The remaining core halves were separated accordingly and embedded in paraffin using an automatic Slee MTP Tissue Processor (Slee, Nieder-Olm, Germany) and a Medite Tissue Embedding Center TES 99 (Medite, Burgdorf, Germany) before cutting 10 µm microsections. Digital images at a resolution of 2.2 pixels/µm were obtained from all microsections using a Hamamatsu NanoZoomer slide scanner (Hamamatsu Photonics, Shizuoka, Japan). For both the paraffin-embedded (hereinafter called PAR) and the non-embedded sections (NoPAR), images were taken exactly for the same areas, verified considering peculiarities in the xylem structure including resin ducts and parenchyma rays.

Images were processed using the ROXAS software (v4.0) to automatically identify individual tracheids in softwood and vessels in hardwood by using specific settings for both groups (von Arx and Carrer, 2014). Wood anatomical traits, namely conduit size (mean lumen area, MLA), cell wall thickness (CWT_{all}, the average between tangential and radial CWT), and hydraulic diameter (Dh), were measured on 15 rings in each section. While MLA was measured in all species, CWT was measured exclusively on LADE and PICE, whereas Dh was computed only for FASY (after Sperry et al., 1994). For all three parameters, arithmetic mean and standard deviation were calculated for each ring, thus building two chronologies per parameter. All tree-ring series were visually crossdated using RW measurements obtained from ROXAS to accurately compare the embedded and non-embedded slides.

To assess differences between measurements derived from embedded vs. non-embedded sections, we first measured the relative difference (|%|) between mean ring values of each anatomical trait as follows:

$$\Delta \overline{A} = - \left| rac{\overline{A}_{NoPAR} - \overline{A}_{PAR}}{\overline{A}_{PAR}}
ight|$$

where $\Delta \overline{A}$ is the absolute value of the ratio between the difference of the mean anatomical parameter measured on the non-embedded (\overline{A}_{NoPAR}) and embedded sections (\overline{A}_{PAR}) over the value for the embedded section. To quantify the average values of MLA, Dh, and CWT and assess their variability within each ring, we considered the relationship between absolute differences in the mean ($\Delta \overline{A}$) with annual means and standard deviations measured on the paraffin-embedded sections by using linear regressions. We used the absolute value since the aim was to quantify differences regardless of sign. High-frequency variability for each anatomical parameter was assessed by calculating the "Gleichläufigkeit" (glk, Eckstein and Bauch, 1969) of the mean and standard deviation series for MLA, Dh, and CWT using embedded and non-embedded samples. Standardized tracheidograms (Campelo et al., 2016; Olano et al., 2012; Vaganov, 1990; Ziaco, 2020), averaging 15 rings on each sample, were computed to evaluate intra-annual variability of MLA and CWT in LADE and PICE. The tracheidograms were computed by aligning cell radial files for each ring using RAPTOR (Peters et al., 2018) and then normalizing the number of cells to 20 (for LADE) and 50 (for PICE) using tgram (DeSoto et al., 2011). All statistical analyses were performed in the R environment (R Core Team, 2022).

3. Results and discussion

Visual assessment of the images of paraffin-embedded and nonembedded sections reveals no significant differences between the two approaches. Considering the minimal intrinsic differences due to the progressive cutting of a given sample, PAR and NoPAR sections are essentially indistinguishable from a qualitative perspective (Fig. 1). Mean and standard deviation chronologies show overall good interannual agreement in all parameters (Fig. 2, A1). The CWT chronologies, however, display larger high-frequency discrepancies, even though their $\Delta \overline{A}$ values are notably lower compared to MLA and Dh (Table 1). The largest $\Delta \overline{A}$ value (11.1 %) is recorded in MLA of LADE. For all species, $\Delta \overline{A}$ values are higher in MLA than in CWT or Dh, indicating a higher absolute measurement error for the larger anatomical features. High glk values among the PAR and NoPAR MLA chronologies ($glk_{FASY} = 0.64$, $glk_{LADE} = 1$, $glk_{PICE} = 0.86$, Table 1) on the other hand prove that interannual variability is less affected by this bias compared to the CWT measurements. Glk values calculated between the PAR and NoPAR CWT chronologies are comparably lower (glk_{LADE} = 0.5, glk_{PICE} = 0.57, Table 1). The visible differences between the CWT chronologies may be due to minor cutting imprecisions affecting the smaller CWT parameter more than the other features; the aforementioned progressive cutting issue, which inevitably causes the comparison of two sections with minimal differences between each other; and/or the tolerance of the instrument we used: even though microtomes are highly precise tools, they could still cause 0.5-1 µm errors in the cutting (cutting thickness range is usually 0.5-100 µm - Leica Biosystems, 2023), leading to slight differences in thickness among and within individual QWA samples.



Fig. 1. Visual comparison of microscopic sections (100x magnification) produced from the same sample of *Fagus sylvatica* (FASY), *Larix decidua* (LADE), and *Pinus cembra* (PICE) processed with both paraffin embedding (PAR) and without paraffin embedding (NoPAR) (scale bar = 1 mm).

However, the overall good agreement of the mean and standard deviation chronologies shows that both approaches are able to capture the intra-annual variability of the cellular parameters and describe their dispersion around the mean.

Differences in MLA between PAR and NoPAR are not affected by the average size of the vessel and tracheid lumina. There is neither a significant relationship between ring-specific $\Delta \overline{A}$ values and MLA in PAR sections for any species (Fig. 3a), nor between $\Delta \overline{A}$ and standard deviation (Fig. 3b). Similar results are observed for Dh and CWT, considering both the parameter-specific mean values and standard deviations

(Fig. 3c-f). Only CWT of LADE shows a significant (p < 0.05) negative relationship between $\Delta \overline{A}$ and standard deviation, which is in line with the low glk value (0.64) and suggests that differences between PAR and NoPAR decrease toward high-frequency (intra-annual) CWT variability. This might indicate that during periods of increased CWT variability fewer cells with particularly larger walls collapse, thereby mitigating damage (Hacke and Sperry, 2001) and reducing PAR *versus* NoPAR difference.

The tracheidograms of PAR and NoPAR sections show only minor differences reaching ${\sim}10$ % for MLA and ${\sim}5$ % for CWT, in LADE and



Fig. 2. Mean (blue) and standard deviation (green) chronologies of vessel area (a), tracheid lumen area (b, c), hydraulic diameter (d), and cell wall thickness (e, f) measured on paraffin-embedded (PAR) and non-paraffin-embedded (NoPAR) samples of *Fagus sylvatica* (FASY), *Larix decidua* (LADE) and *Pinus cembra* (PICE).

Table 1

Summary of measured anatomical traits on paraffin-embedded (PAR) and nonparaffin-embedded sections (NoPAR).

	PAR		NoPAR		Difference	Glk (PAR vs. NoPAR)	
	Mean	StDev	Mean	StDev	ΔĀ	Mean	StDev
Fagus sylv	atica						
MLA (µm ²)	982	789	974	817	7.7 % 5.3 %	0.64	0.79
DH (µm)	30.5	15.4	30.1	15.8		0.93	0.79
Larix decid	lua						
MLA (µm ²)	663	426	588	407	11.1 % 6.0 %	1	0.71
CWT (µm)	4.4	0.7	4.2	0.8		0.5	0.64
Pinus ceml	bra						
MLA (µm²)	672	454	660	443	7.7 %	0.86	0.57
CWT (um)	5.3	1	5.4	0.9	4.5 %	0.57	0.86

Mean: mean values. StDev: one standard deviation. Glk: gleichläufigkeit. $\Delta \bar{A}$: mean interannual ratio between the difference of the mean anatomical parameter measured on non-embedded (\bar{A} NoPAR) and embedded sections (\bar{A} PAR) over the value for the embedded section.

PICE (Fig. 4). While CWT differences $(\Delta \overline{A})$ are relatively stable throughout the ring, a sharp increase of >15 % in $\Delta \overline{A}$ values is recorded in the latewood sections. The increasing MLA difference between PAR and NoPAR tracheidograms in these last ring sections can either be attributed to an improved preservation of very small latewood cells after embedding or to a potential lack of paraffin infiltration in the thinnest

latewood cells, compromising the integrity of these while cutting (Fig. 4). Even though this effect must be considered when choosing one of the two approaches, it only affects a small portion of the ring and has no detectable impact on annually resolved MLA values. CWT measurements in the latewood appear to be completely unaffected by this tendency. Differences in late-season MLA chronologies (Fig. 4) may be less relevant, as the climate signals typically derived from these ring portions are usually weak, whereas CWT data from the same portions often retain highly significant climatic information (Carrer et al., 2018; Castagneri et al., 2017; Puchi et al., 2020).

Several factors might prevent a perfect match of the two approaches, including twisted fiber orientation, localized damages of woody tissue due to insufficient cutting precision, and arbitrary decisions of the sample section chosen for the analysis. Nevertheless, the small differences found in MLA, Dh, and CWT between the PAR and NoPAR are considered minor, since the recorded errors are towards the lower end of the range of 5-30 % typically introduced by inaccurate micro-slide preparation (von Arx et al., 2016). This conclusion is supported by the decision to exclude manual editing of images and filter application from this comparison, which are expected to further mitigate methodological differences. Moreover, it is important to note that the observed differences between the two approaches are probably accentuated by the exceptionally thin cutting thickness of $10\,\mu\text{m}$. By producing thicker slides, deviations in wood anatomical traits between embedding and non-embedding are likely to decrease. Lastly, necessary chemical treatments required to produce high-quality wood anatomical sections, such as bleaching with hypochlorite, might add some minor uncertainties on absolute QWA metrics through chemical reactions with the wood compounds, but are unproven and negligible due to the major benefit of minimizing other sources of error during the preparation (von Arx et al., 2016).



Fig. 3. Scatterplots showing the relationship between the average values of vessel/tracheid lumen area (a), hydraulic diameter (c), cell wall thickness (e), and their respective standard deviations (b, d, f) measured on paraffin-embedded sections (PAR) with the absolute difference between the mean values measured on PAR and NoPAR samples. Coloured lines are linear regressions (dashed lines indicate p > 0.05; solid lines p < 0.05).



Fig. 4. Standardized tracheidograms (solid bold lines) of lumen area and cell wall thickness from paraffin-embedded (PAR, dark blue lines) and non-paraffin-embedded (NoPAR, green lines) samples of LADE (a, b) and PICE (c, d). Thin curves represent ± 1 standard deviations. Dotted curves show mean differences between the tracheidograms.

4. Conclusion

We show that the non-embedding procedure does not significantly alter the quality of permanent slides prepared for QWA analysis. The lack of substantial biases between embedding and non-embedding approaches reinforces the reliability of diverse QWA protocols, data exchange, and the development of joint networks. Given the high similarity between the two approaches, we recommend following the non-embedding procedure since is less time-consuming and less expensive, while it also prevents the utilization of any harmful chemicals. Such approach, by saving more resources, allows to produce larger datasets and is therefore recommended for experienced applicants. However, attention might be required to certain tree species, such as Larix decidua, and anatomical features, such as particularly thin cell walls that are susceptible to tissue damage. In these cases, embedding is recommended, particularly for less experienced applicants. Deciduous species are generally less prone to tissue damage and can be processed and combined regardless of the preparation approach.

The establishment of large-scale, robust, and unbiased wood anatomical networks is essential for studying inter-regional forest growth responses to rapidly changing environmental conditions. We advise to continue working on the other steps of the QWA protocol to maximise the quality of the material used for anatomical measurements. Accurate sample collection, starting from the correct functioning of the tools (i.e., increment borers), boiling time, cutting precision, as well as specific ROXAS settings (i.e., protrusion removal), are crucial steps in the QWA protocol that should always be considered and performed in the best possible way to obtain high-quality thin sections for wood anatomical analyses.

Author contributions

E.Z., D.F., and P.R. conceived and planned the study. D.F., L.U., and H.Z.-Z. prepared the samples. D.F., P.R., and E.Z. performed the analyses. D.F., P.R., and E.Z. drafted the manuscript with inputs from J.E. and M.C. All authors provided critical discussion and agreed to the final version of manuscript.

CRediT authorship contribution statement

Emanuele Ziaco: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis,

Conceptualization. Jan Esper: Writing – review & editing, Supervision, Resources, Funding acquisition. Marco Carrer: Writing – review & editing, Supervision, Resources, Methodology, Investigation. Lucrezia Unterholzner: Writing – review & editing, Methodology. Heike Zimmer-Zachmann: Methodology. Davide Frigo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. Philipp Römer: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dendro.2024.126241.

References

- Arnič, D., Gričar, J., Jevšenak, J., Božič, G., von Arx, G., Prislan, P., 2021. Different wood anatomical and growth responses in European Beech (Fagus sylvatica L.) at three forest sites in Slovenia. Front. Plant Sci. 12, 669229 https://doi.org/10.3389/ fpls.2021.669229.
- von Arx, G., Carrer, M., 2014. ROXAS A new tool to build centuries-long tracheidlumen chronologies in conifers. Dendrochronologia 32 (3), 290–293. https://doi. org/10.1016/j.dendro.2013.12.001.
- von Arx, G., Crivellaro, A., Prendin, A.L., Čufar, K., Carrer, M., 2016. Quantitative wood anatomy—practical guidelines. Front. Plant Sci. 7 (781)), 1–13. https://doi.org/ 10.3389/fpls.2016.00781.
- Björklund, J., Arx, G., Nievergelt, D., Wilson, R., Van den Bulcke, J., Günther, B., Loader, N.J., Rydval, M., Fonti, P., Scharnweber, T., Andreu-Hayles, L., Büntgen, U., D'Arrigo, R., Davi, N., De Mil, T., Esper, J., Gärtner, H., Geary, J., Gunnarson, B.E., Frank, D., 2019. Scientific merits and analytical challenges of tree-ring densitometry. Rev. Geophys. 57 (4), 1224–1264. https://doi.org/10.1029/2019RG000642.
- Briffa, K.R., Osborn, T.J., Schweingruber, F.H., Harris, I.C., Jones, P.D., Shiyatov, S.G., Vaganov, E.A., 2001. Low-frequency temperature variations from a northern tree ring dendity network. J. Geophys. Res. 106 (D3), 2929–2941.
- Campelo, F., Nabais, C., Carvalho, A., Vieira, J., 2016. tracheideR—an R package to standardize tracheidograms. Dendrochronologia 37, 64–68. https://doi.org/ 10.1016/j.dendro.2015.12.006.
- Carrer, M., Brunetti, M., Castagneri, D., 2016. The imprint of extreme climate events in century-long time series of wood anatomical traits in high-elevation conifers. Front. Plant Sci. 7 (683)), 1–12. https://doi.org/10.3389/fpls.2016.00683.
- Carrer, M., Unterholzner, L., Castagneri, D., 2018. Wood anatomical traits highlight complex temperature influence on Pinus cembra at high elevation in the Eastern Alps. Int. J. Biometeorol. 62, 1745–1753. https://doi.org/10.1007/s00484-018-1577-4.
- Castagneri, D., Fonti, P., von Arx, G., Carrer, M., 2017. How does climate influence xylem morphogenesis over the growing season? Insights from long-term intra-ring anatomy in Picea abies. Ann. Bot. 119 (6), 1011–1020. https://doi.org/10.1093/aob/ mcw274.
- R. Core Team. (2022). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. (https://www.r-project. org/).

- DeSoto, L., De la Cruz, M., Fonti, P., 2011. Intra-annual patterns of tracheid size in the Mediterranean tree Juniperus thurifera as an indicator of seasonal water stress. Can. J. For. Res. 41 (6), 1280–1294. https://doi.org/10.1139/x11-045.
- Eckstein, D., Bauch, J., 1969. Beitrag zur Rationalisierung eines dendrochronologischen Verfahrens und zur Analyse seiner Aussagesicherheit. Forstwiss. Cent. 88 (1), 230–250. https://doi.org/10.1007/BF02741777.
- Esper, J., Cook, E.R., Schweingruber, F.H., 2002. Low-frequency signals in long tree-ring chronologies for reconstructing past temperature variability. Science 295 (5563), 2250–2253. https://doi.org/10.1126/science.1066208.
- Esper, J., Frank, D.C., Timonen, M., Zorita, E., Wilson, R.J.S., Luterbacher, J., Holzkämper, S., Fischer, N., Wagner, S., Nievergelt, D., Verstege, A., Büntgen, U., 2012. Orbital forcing of tree-ring data. Nat. Clim. Change 2 (12), 862–866. https:// doi.org/10.1038/nclimate1589.
- Fonti, P., Treydte, K., Osenstetter, S., Frank, D., Esper, J., 2009. Frequency-dependent signals in multi-centennial oak vessel data. Palaeogeogr. Palaeoclimatol. Palaeoecol. 275 (1–4), 92–99. https://doi.org/10.1016/j.palaeo.2009.02.021.
- Gärtner, H., & Schweingruber, F.H. (2013). Microscopic Preparation Techniques for Plant Stem Analysis. Remagen: Kessel Publishing House.
- Hacke, U.G., Sperry, J.S., 2001. Functional and ecological xylem anatomy. Perspect. Plant Ecol. Evol. Syst. 4 (2), 97–115. https://doi.org/10.1078/1433-8319-00017.
- Katzenmaier, M., Garnot, V.S.F., Björklund, J., Schneider, L., Wegner, J.D., von Arx, G., 2023. Towards ROXAS AI: deep learning for faster and more accurate conifer cell analysis. Dendrochronologia 81, 126126. https://doi.org/10.1016/j. dendro.2023.126126.
- Lopez-Saez, J., Corona, C., von Arx, G., Fonti, P., Slamova, L., Stoffel, M., 2023. Tree-ring anatomy of Pinus cembra trees opens new avenues for climate reconstructions in the European Alps. Sci. Total Environ. 855 (2023)), 158605 https://doi.org/10.1016/j. scitotenv.2022.158605.
- Martinez del Castillo, E., Prislan, P., Gričar, J., Gryc, V., Merela, M., Giagli, K., de Luis, M., Vavrčík, H., Čufar, K., 2018. Challenges for growth of beech and cooccurring conifers in a changing climate context. Dendrochronologia 52 (2018)), 1–10. https://doi.org/10.1016/j.dendro.2018.09.001.
- Olano, J.M., Eugenio, M., García-Cervigón, A.I., Folch, M., Rozas, V., 2012. Quantitative Tracheid anatomy reveals a complex environmental control of wood structure in continental Mediterranean climate. Int. J. Plant Sci. 173 (2), 137–149. https://doi. org/10.1086/663165.
- Peters, R.L., Balanzategui, D., Hurley, A.G., von Arx, G., Prendin, A.L., Cuny, H.E., Björklund, J., Frank, D.C., Fonti, P., 2018. RAPTOR: Row and position tracheid organizer in R. Dendrochronologia 47 (2018), 10–16. https://doi.org/10.1016/j. dendro.2017.10.003.
- Piermattei, A., von Arx, G., Avanzi, C., Fonti, P., Gärtner, H., Piotti, A., Urbinati, C., Vendramin, G.G., Büntgen, U., Crivellaro, A., 2020. Functional relationships of wood anatomical traits in Norway spruce. Front. Plant Sci. 11 (683), 1–14. https://doi.org/ 10.3389/fpls.2020.00683.
- Prendin, A.L., Petit, G., Carrer, M., Fonti, P., Björklund, J., von Arx, G., 2017. New research perspectives from a novel approach to quantify tracheid wall thickness. Tree Physiol. 37 (7), 976–983. https://doi.org/10.1093/treephys/tpx037.
- Puchi, P.F., Castagneri, D., Rossi, S., Carrer, M., 2020. Wood anatomical traits in black spruce reveal latent water constraints on the boreal forest. Glob. Change Biol. 26 (3), 1767–1777. https://doi.org/10.1111/gcb.14906.
- Rossi, S., Anfodillo, T., Menardi, R., 2006. Trephor: a new tool for sampling microcores from tree stems. IAWA J. 27 (1), 89–97. https://doi.org/10.1163/22941932-90000139.
- Schneider, L., Gärtner, H., 2013. The advantage of using a starch based non-Newtonian fluid to prepare micro sections. Dendrochronologia 31 (3), 175–178. https://doi. org/10.1016/j.dendro.2013.04.002.
- Seftigen, K., Fonti, M.V., Luckman, B., Rydval, M., Stridbeck, P., Von Arx, G., Wilson, R., Björklund, J., 2022. Prospects for dendroanatomy in paleoclimatology - a case study on Picea engelmannii from the Canadian Rockies. Climate 18 (5), 1151–1168. https://doi.org/10.5194/cp-18-1151-2022.
- Sperry, J.S., Nichols, K.L., Sullivan, J.E.M., Sonda, E.E., 1994. Xylem Embolism in Ring-Porous, Diffuse-Porous, and Coniferous Trees of Northern Utah and Interior Alaska. Ecology 75 (6), 1736–1752 https://www.jstor.org/stable/1939633.
- Vaganov, E.A. (1990). The Tracheidogram Method in Tree-ring Analysis and Its Application. In E.R. Cook & L.A. Kairiukstis (Eds.), *Methods of Dendrochronology* (pp. 63–76). Kluver, Dordrecht, The Netherlands.
- Yeung, E.C.T., Stasolla, C., Sumner, M.J., & Huang, B.Q. (2015). Plant Microtechniques and Protocols (E.C.T. Yeung, C. Stasolla, M.J. Sumner, & B.Q. Huang (eds.)). Springer International Publishing Switzerland 2015. https://doi.org/10.100 7/978-3-319-19944-3.
- Ziaco, E., 2020. A phenology-based approach to the analysis of conifers intra-annual xylem anatomy in water-limited environments. Dendrochronologia 59 (2020)), 125662. https://doi.org/10.1016/j.dendro.2019.125662.
- Ziaco, E., Liu, X., Biondi, F., 2023. Dendroanatomy of xylem hydraulics in two pine species: efficiency prevails on safety for basal area growth in drought-prone conditions. Dendrochronologia 81 (2023), 126116. https://doi.org/10.1016/j. dendro.2023.126116.