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SHORT ARTICLE ON PRELIMINARY RESEARCH

Simulation study to determine necessary sample sizes for image analysis-based quantitative wood anatomy of vessels of beech (*Fagus sylvatica*)

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ABSTRACT

Quantitative analysis of wood anatomy is a powerful dendroecological tool to provide new insights into environmental signals encoded in tree-rings. Nevertheless, studies with long time series or large sample sizes are not very common for diffuse-porous tree species due to the laborious sample preparation and image analysis. Finding a compromise between sample size and accuracy of sample estimators is therefore of crucial importance. In this simulation study, we present a subsampling method, which can make the quantitative analysis of wood anatomy of European beech more efficient. Based on our study material we demonstrate that for the parameters vessel size, vessel density, total vessel area and accumulated hydraulic conductivity the environmental signals are captured with high trueness (deviation $< 5\%$) when analysing only a 1 mm wide radial stripe along the increment core. Nevertheless, when information about vessel grouping indices is required, for high trueness of the results a wider radial stripe of ~ 2.2 mm needs to be analysed.

1. Introduction

Quantitative analysis of wood anatomy is a powerful tool for studying climate-growth relations of trees, and it provides valuable information about the xylem plasticity of tree species and their response to contrasting environments or extreme climatic events. Due to its laborious methodology, not many studies have been conducted on broadleaf tree species with diffuse porous wood structure so far (Fonti et al., 2010).

Different software tools like WinCELL (Regent Instruments, Québec, Canada), Image-Pro Plus (Media cybernetics, Silverspring, MA, USA) or ImageJ (Schneider et al., 2012) capable of measuring wood anatomical variables were developed or improved during recent years, facilitating an increasing number of studies that provide long chronologies of wood anatomical features (García-González and Fonti, 2008; Liang et al., 2013; Schuldt et al., 2013; Seo et al., 2014). A specialized tool for such analyses is ROXAS (Wegner et al., 2013; von Arx and Carrer, 2014), a semi-automatic image analysis software able to detect and measure conduits in micrographs of xylem cross-sections.

Fully automatic image analysis may not always be considered as being reliable or accurate enough, as the percentage of conduit detection in an image depends on e.g. the quality of the thin section, its thickness, the used stain, the used microscope for scanning or even on the performance of the computer system used for the analysis. Furthermore, available image analysis systems may not recognize a significant proportion of possible artifacts. Due to the laborious process of preparing and digitalizing thin sections of wood specimens, such deficits cannot totally be avoided. Hence, the automatically detected conduits need to be quality checked and manually corrected for misidentified or unrecognized vessels in order to increase accuracy of the results. The manual correction of non- or falsely recognized conduits is a time-consuming procedure. However, studies providing guidelines on how to reduce the amount of data and hence the time needed for quantitative wood anatomical analyses, while still capturing the environmental signals, are to our knowledge only available for conifers (Seo et al., 2014) and ring-porous tree species (García-González and Fonti, 2008). For diffuse-porous tree species, the manual correction of vessel detection is an extremely laborious task, due to the large number of conduits and high conduit density, small vessel sizes and large proportion of conduits being arranged in groups. Therefore, either the costs of the study are considerably increased or the analyst has to rely on less samples, in which case the environmental signals encoded in the conduits might not be preserved adequately. A compromise between those two extremes is to adjust the sample size, i.e. the size of the image used

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Table 1
List of the measured cell variables (CV) including their abbreviations used in the text, their definitions and units.

Notation	Variable	Unit
CD	Conduit density	No/mm^2
MCA	Mean conduit area	μm^2
RCTA	Total percentage of the vessel area within the xylem	[%]
Kh	Accumulated potential hydraulic conductivity as approximated by Poiseuille's law and adjusted to elliptical tubes (Nonweiler, 1975)	[kg*m*Mpa^{-1}* s^{-1}]
Vg	Vessel grouping index; mean number of vessels per group (counting a solitary vessel as 1, a pair of connected vessels as 2, etc.; Carlquist, 2001)	[no. vessel/group]
Vs	Vessel solitary fraction; ratio of solitary vessels to all vessels	[%]

for the analysis. However, reducing the sample size is a trade-off between the efficiency and the trueness of the results.

In this study, we try to determine the necessary sample size, respectively corridor size, i.e. the width of a radial stripe along a wood cross-section, to be analyzed in order to reduce the time needed for manual corrections, while still guaranteeing a specific level of accuracy. To achieve this, we analysed the properties of estimators for different wood anatomical variables in a simulation study. We investigated the difference between estimators for the vessel density, vessel area, total vessel area, accumulated potential hydraulic conductivity and vessel grouping indices (Table 1) of subsamples with different widths measured with ROXAS. For each simulated subsample we assessed the trueness and precision as measures of the accuracy of the estimators. In measurement theory, the term "*trueness*" refers to the closeness of agreement between the arithmetic mean of a large number of test results and the true or accepted reference value, whereas the term "*precision*" denotes the closeness of agreement between test results (ISO, https://www.iso.org). We present an approach, that helps to choose the necessary sample size i.e. the size of the image to be measured, so the user can adjust the time needed for measurements of wood anatomical features on broadleaves while still guaranteeing precise and unbiased sample statistics.

2. Materials and methods

2.1. Sampling method

Increment cores of European beech (*Fagus sylvatica* L.) were collected with a 5.15 mm Haglöf increment borer at 1.3 m stem height, perpendicular to the slope direction in order to avoid tension wood. The sample trees were randomly selected from beech stands in a valley in southwestern Germany, close to the city of Tuttlingen (see Diaconu et al., 2015 for more details on the study site). For the study, in total 32 tree-rings from 10 sample trees have been analyzed and a total amount of 22,358 vessels has been measured.

2.2. Preparation of thin sections

Thin sections with 15–20 μm thickness were prepared using the GSL 1 sledge microtome (Gärtner et al., 2014) and double-stained with a mixture of safranin and astrablue (0.4 g safranin, 0.25 g astrablue and 1 ml acetic acid in 100 ml distilled water) to increase contrasts for subsequent image analysis. In order to produce permanent samples the thin sections were dehydrated in 96% ethanol and mounted on microscope slides using Canada balsam as described by Gärtner and Schweingruber (2013). Finally, the thin sections were dried in the oven at 60 °C for 12–24 h.

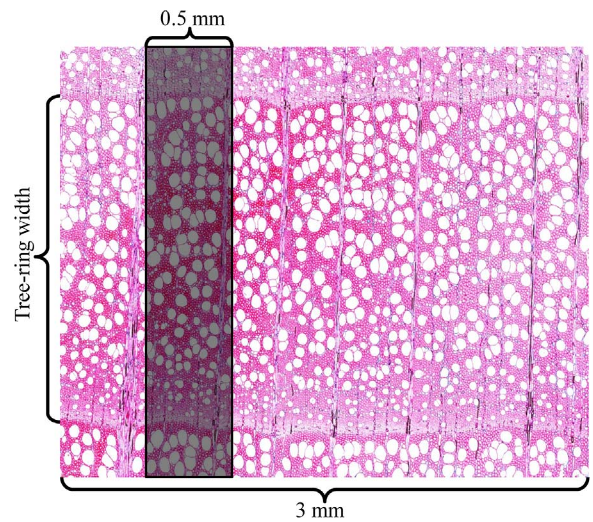

Fig. 1. Image of a thin-section of a European beech sample tree (cross-sectional plane); total width 3 mm, width of example corridor 0.5 mm (shaded area).

2.3. Image acquisition

Digital images of the thin sections were obtained using a Nikon Eclipse Ni-E upright motorized microscope with an automatic scanning-table and a colour camera with a resolution of 5 megapixels and 12-Bit colour depth (Nikon DS-Fi2). Micrographs were obtained by scanning the sample at a resolution of 0.49 μm/pixel. The individual images are automatically merged to form a large image using an implemented stitching function in the NIS-Elements software (Version 4.20.01, Nikon Corporation, 2014). The microscope images were rotated until radial parenchyma rays appeared parallel to the used corridor and the sides of the picture, cropped to individual tree-rings and subsequently analyzed in ROXAS in radial direction as presented in Fig. 1.

2.4. Finding the optimal sample size

In our simulation study "*sample size*" is determined by the tangential width of the cross-sectional corridor which is measured in radial direction. The length of the measurement corridor is defined by the tree-ring width. Depending on vessel density and tree-ring width the

number of vessels, i.e. sample units, at a given sample size can be largely different. The width of the analysed tree-rings was within a range of 0.4–4.0 mm. Tree-ring sections with medium and wide parenchyma rays (> 3-seriate) were excluded from the analysis. Considering these criteria and the possible presence of artifacts, for most of the samples a radial stripe of 3 mm in width along the sample was acceptable for the analysis. The raw data are presented in the Supplementary material 1 (Table S1) and the main descriptive statistics concerning the ring and anatomical parameters used in the analysis are presented in Table 2.

ROXAS tabulates in the output files x- and y-centre-coordinates of each automatically or manually detected conduit within a tree-ring.

Table 2
Descriptive statistics of the data used for the analysis (N: number of the measured tree-rings). Please refer to Table 1 for the full name of the measured traits.

Variable	N	Minimum	Mean	St. deviation	Maximum
MRW [mm]	32	0.46	1.88	1.07	4.00
MCA [μm^2]	32	1642	2108	367.98	2939
CD [no/mm^2]	32	87	135	25.96	178
RCTA [%]	32	21	28	4.36	37
Vg [no. vessel/group]	32	1	2	0.62	4
Vs [%]	32	10	33	11.21	49