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ORIGINAL

Variation of quantitative anatomical features due to carbonization and their impact on size classes for charcoal identification

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Abstract

Species identification of carbonized wood holds significance for various scientific disciplines, including botany, palaeontology, and archaeology. Identification also contributes to the preservation of endangered wood species and forests, and supports climate research. With regard to the identification of wood and wood products, all international research institutions adhere to the IAWA list of microscopic features for hardwood and softwood identification, established by the IAWA Committee in 1989.

Our comparative anatomical studies of 30 different species reveal significant dimensional losses of quantitative features during the charring process. Specifically, the findings indicate a shift in size classes, with varying percentages of loss in anatomical features from solid wood to charcoal for most of the taxa analyzed. Consequently, the size classes defined in databases for solid wood differentiation cannot be directly applied to charcoal identification. Furthermore, the present study employs statistical evaluations to illustrate the application of conventional size classes for the parameters: tangential diameter of vessel elements, intervessel pit diameter, ray height, and width. The implications of these findings for charcoal research are discussed in detail.

Introduction

The identification of charcoal is based on microscopic analysis of anatomical features. Qualitative features are generally well preserved after the charring process. (Prior and Alvin 1983, 1986; Rossen and Olson 1985; Prior and Gasson 1993; Ger-

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isch 2004; Kim and Hanna 2006; Kwon et al. 2009; Dias Leme et al. 2010; Nelle and Bankus 2012; Hubau et al. 2013; Goncalves et al. 2014; Goncalves and Scheel-Ybert 2016; Haag et al. 2020; Haag et al. 2023; Schmitz et al. 2020; Zemke et al. 2020). Only long-lasting charring temperatures of well over 800 °C (Dias Leme et al. 2010) can lead to fusion in the tissue and make it difficult to determine anatomical features. Such cases are uncommon, however, as both traditional and industrial charring do not normally involve such high temperatures. Basic charring studies by Dias Leme et al. (2010) have shown that processes at different temperatures (400 °C, 600 °C, 800 °C) influence the carbonization result on the cellular level, but do not influence or hinder the recognition of qualitative features.

Changes in dimensions occur due to the loss of mass during the charring process, typically resulting in higher losses in the radial and tangential directions (Bowyer et al. 2003; Braadbart and Poole 2008). This shrinkage can also lead to distortion of the anatomical structures and the formation of cracks in the charred tissue. In addition to the changes in the material that are visible to the eye, changes in the fine structures are also recognizable on a microscopic level. These changes have an impact on the size of quantitative features such as the tangential diameter of vessel elements, intervessel pit diameter, ray height and ray width. In this context, it is questionable whether the size changes deviate significantly from the values provided in international wood databases (such as InsideWood (2004-onwards) and Commercial Timbers (Richter and Dallwitz 2000-onwards), thereby limiting their application for the identification of charred wood species. Our preliminary investigations consistently indicate significant differences in shrinkage of quantitative anatomical features between different wood species, bringing into question the validity of a general approach. We assert that deriving an average shrinkage value for specific features (e.g., tangential vessel diameter, intervessel pit diameter, ray height, and ray width) from the data provided in wood databases is not universally applicable. Relying on these assumed initial values can result in erroneous identification outcomes.

We have carried out a statistical review of the size classes specified in the IAWA feature list for 30 wood species to assess the suitability of quantitative measurements for identifying charred wood species. Based on this analysis we have made recommendations for the application of size classes to features such as the tangential diameter of vessel elements, intervessel pit diameter, ray height, and ray width, accounting for the dimensional changes caused by the charring process.

Materials and methods

Wood collection, charcoal production and microscopy

For the present study, 30 wood species were selected from 22 different botanical families originating from different geographic regions (Table 1). Species were selected to capture high variation in anatomical trait combinations within the context of global species diversity. One sample of each species from the Thünen Institute's scientific wood collection was used for both the uncharred and charred analyses.

Table 1 Selected wood species for charring process

No.	Species	Family	Trade name
1	*Acacia dealbata* Link	Fabaceae-Mimosoideae	Akasia
2	*Acacia melanoxylon* R.Br.	Fabaceae-Mimosoideae	Akasia
3	*Acer pseudoplatanus* L.	Sapindaceae	Maple
4	*Afzelia africana* Pers.	Fabaceae	Doussié
5	*Alnus glutinosa* (L.) Gaertn.	Betulaceae	Common Alder
6	*Aspidosperma quebracho-blanco* Schltdl.	Apocynaceae	Quebracho-Blanco
7	*Aucoumea kleineana* Pierre	Burseraceae	Okoumé
8	*Autranella congolensis* (De Wild.) A.Chev.	Sapotaceae	Mukulungu
9	*Betula pubescens* Ehrh	Betulaceae	Birch
10	*Carpinus betulus* L.	Betulaceae	Common Hornbeam
11	*Cedrelinga cateniformis* (Ducke) Ducke	Fabaceae-Mimosoideae	Tornillo
12	*Coffea arabica* L.	Rubiaceae	Arabian Coffee
13	*Copaifera paupera* (Herzog) Dwyer	Fabaceae-Caesalpinioideae	Copaiba
14	*Dichrostachys cinerea* (L.) Wight & Arn	Fabaceae	Marabù
15	*Diospyros crassiflora* Hiern	Ebenaceae	Black Ebony
16	*Eucalyptus grandis* W. Hill ex Maiden	Myrtaceae	Eucalypt
17	*Fraxinus excelsior* L.	Oleaceae	Common Ash
18	*Ilex aquifolium* L.	Aquifoliaceae	English Holley
19	*Juglans nigra* L.	Juglandaceae	Black Walnut
20	*Milicia excelsa* (Welw.) C. C. Berg	Moraceae	Iroko, kambala
21	*Nauclea diderrichii* (De Wild.) Merr.	Rubiaceae	Bilinga, opepe
22	*Paulownia elongata* S. Y. Hu	Paulowniaceae	Kiri
23	*Pterocarpus angolensis* DC.	Fabaceae-Faboideae	Muninga
24	*Robinia pseudoacacia* L.	Fabaceae-Faboideae	False acacia
25	*Rubroshorea leprosula* (Miq.) P.S. Ashton & J. Heck.	Dipterocarpaceae	Light Red Meranti
26	*Senegalia chundra* (Roxb. Ex Rottler) Maslin	Fabaceae	Cutch
27	*Triplochiton scleroxylon* K. Schum.	Malvaceae	Wawa
28	*Ulmus minor* Mill.	Ulmaceae	Dutch Elm
29	*Vachellia nilotica (L.)* P.J.H.Hurter & Mabb.	Fabaceae	Babul Acacia
30	*Ziziphus mucronata* Willd.	Rhamnaceae	Buffalo thorn

To determine the quantitative changes in the charred material, light microscopy was first performed on the uncharred material as a reference. Microscopic sections were prepared in the three cutting directions (transverse/tangential/radial) and analysed with an OLYMPUS optical microscope using the "Cell^B for Live Science Microscopy" software. Quantitative and qualitative data were measured and documented. In addition to the qualitative examination of the structural features according to the IAWA feature list (IAWA Committee 1989), quantitative analyses were conducted measuring diameter of vessel elements ($n=100$), intervessel pit diameter ($n=50$), ray height ($n=100$) and ray width ($n=100$).

Samples of each species were then subjected to charring. One of the most widely used methods is carbonization in a muffle furnace at a temperature of around 400 °C (Prior and Gasson 1993; Gonçalves et al. 2012, 2014, 2016). Charring can also be performed using a gas burner, which was applied in the present study. Small wood cubes were wrapped airtight in aluminium foil and charred in a fireproof container over the gas burner at a temperature of about 400 °C for 5–10 min. The heating time varied depending on the specimen size (Zemke et al. 2020).

The charred samples were broken up in the aforementioned three anatomical directions (transverse/tangential/radial) and the surfaces cleaned to remove any accumulated dust. Microscopic analysis of charred wood can be performed using a range of high-resolution techniques, including reflected light microscopy (RLM); field emission scanning electron microscopy (FESEM); 3D-reflected light microscopy (3D-RLM) (Zemke et al. 2020). In the present study, the 3D-reflected light microscope VHX-series from KEYENCE was used, which works with an integrated polarization technology to provide high-resolution images of the anatomical structure. In addition, the microscope provides a software for measuring visible structures used to determine the tangential diameter of vessel elements ($n=100$), intervessel pit diameter ($n=50$), ray height ($n=100$) and ray width ($n=100$).

Statistical analysis

Based on the measurements obtained from both uncharred and charred wood samples, minimum, mean, and maximum values (MIN, MEAN, MAX) were calculated for several anatomical features of each wood species. These features include the tangential diameter of vessel elements, intervessel pit diameter, ray height, and ray width.

In order to choose the appropriate statistical analysis technique, data were checked for a normal distribution using the Kolmogorov-Smirnov test in Excel and the table of quantiles for the two-sided K-S adjustment test (University of Siegen). Data were then tested for significant differences at the significance level of $\alpha=5\%$. The results obtained were confirmed graphically using the quantile-quantile diagram.

We then tested if dimensions of features were significantly different between solid wood and charcoal for all wood species using the two-sample T-test with dependent samples (paired comparison test). Again, a significance level of $\alpha=5\%$ was applied. The effects of the charring process were evaluated for all features and the percentage shrinkage was calculated for each wood species. Finally, the mean dimensional loss for each feature was calculated from the individual results of all wood species.

Comparison of size classes between solid wood and charcoal

To compare the size classes of features between solid wood and charcoal, the calculated MIN and MAX values were used and assigned to the defined size classes (Table 2). The use of mean values was omitted because they represent only a single value rather than capturing the full range of dimensions of a feature for a wood species. Further size classes would be overlooked, although the presence of more than one class is entirely possible. The MIN and MAX values allow to define more than one size class.

The classification of the selected features for this study was based on the IAWA list of microscopic features (IAWA Committee 1989). The individual parameters:

Table 2 Overview of the size classes of anatomical features

	Size class [µm]			
	Class 1 **Minute**	**Class 2** **Small**	**Class 3** **Medium**	**Class 4** **Large**
Diameter of vessel elements*	≤ 50	50-100	100-200	≥200
Intervessel pit diameter*	≤ 4	4–7	7–10	≥ 10
Ray height – 1*	≤ 1000	≥ 1000		
Ray height – 2**	≤ 500	500-1000	≥1000	
Ray width	≤ 50	50-100	≥100	

* used according to IAWA-feature list (IAWA Committee 1989)
** used according to Commercial timbers (Richter and Dallwitz 2000 onwards)

tangential diameter of vessel elements, intervessel pit diameter and ray height were chosen for the comparison of feature dimensions. Since the IAWA feature list considers ray width only in terms of the number of cells, and not the exact dimensions in micrometres (µm), a “hypothetical” classification system for ray width was included in the present study that categorises the size classes based on the measured values (µm). To achieve higher resolution in differentiating ray height, the study incorporated the division of size classes into three subdivisions as per the classification system of the internationally recognized Delta-Intkey Commercial Timber Database (Richter and Dallwitz 2000 onwards), as opposed to the two size classes provided by the IAWA list.

Size class differences between solid wood and charcoal were evaluated and interpreted. The results were visualized by boxplot diagrams.

Results and discussion

Quantitative measurements were obtained for all 30 wood species with minimum, mean, and maximum values (MIN/MEAN/MAX) calculated for several anatomical features. Box-and-whisker plots were generated with Excel (Fig. 1) based on the measured dimensions of key features, including the diameter of vessel elements, intervessel pit diameter, ray height, and ray width. These plots illustrate the differences in feature dimensions among wood species following the charring process, with each species displaying a distinct range between its minimum and maximum values. The comparison of “percentage dimensional losses for individual species,” as shown in Table 3, reveals variations in shrinkage behaviour.

The Kolmogorov-Smirnov test and graphical quantile-quantile diagram indicate a normal distribution of the data. Differences between solid wood and charcoal were observed for all wood species with respect to the anatomical features studied: tangential diameter of vessel elements, intervessel pit diameter, ray height and ray width.

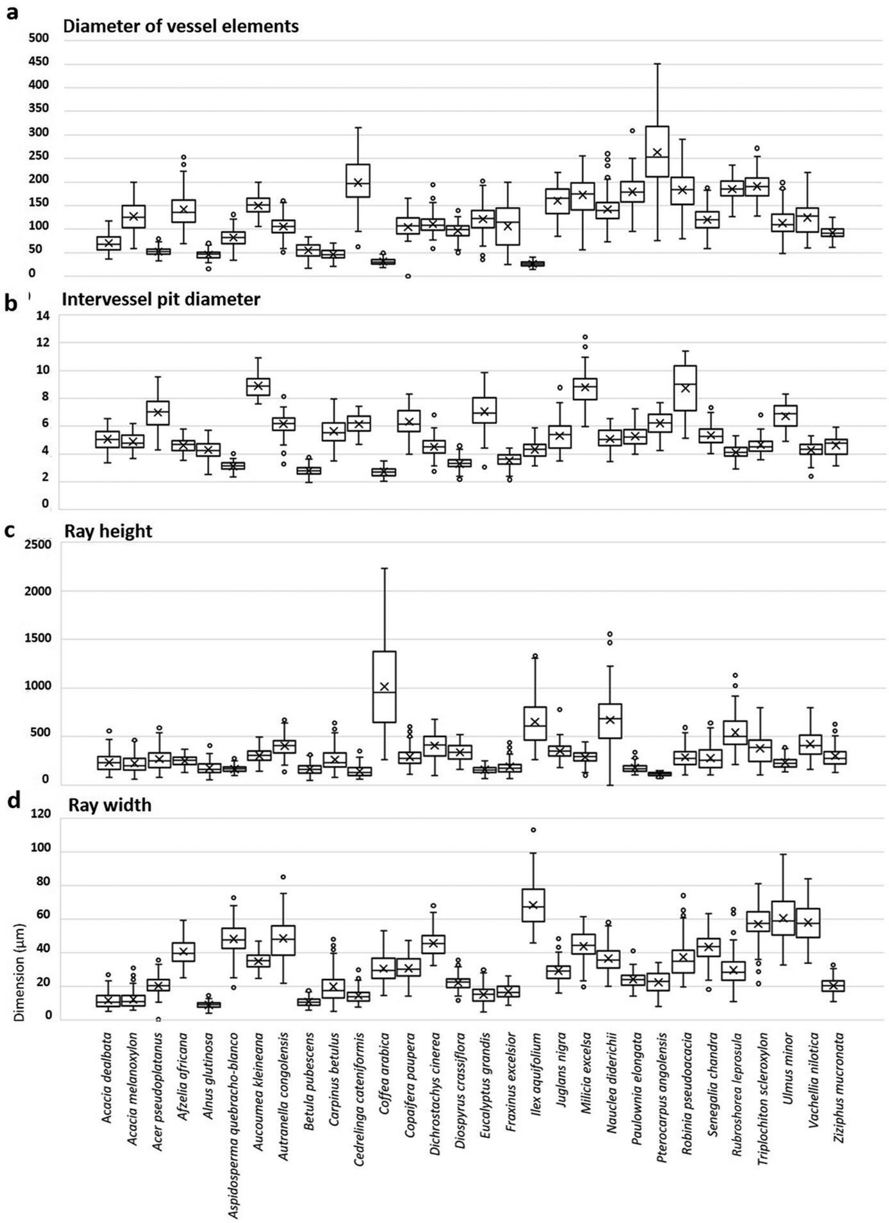

Fig. 1 Dimensions [µm] for diameter of vessel elements (**a**), intervessel pit diameter (**b**), ray height (**c**) and ray width (**d**) after the charring process for all 30 wood species

Shrinkage of quantitative features

The charring process induces varying dimensional changes across species, consistently resulting in a reduction in volume. Figure 2 presents a comparison of the

Table 3 Comparison of the percentage dimensional losses measured for all 30 wood species with a notation indicating where a change in size class has been observed

No.	Species	Vessel element diameter [%]	Intervessel pit diameter [%]	Ray height [%]	Ray width [%]
1	*Acacia dealbata* Link	40	$36^{\uparrow\downarrow}$	4	39
2	*Acacia melanoxylon* R.Br.	$21^{\downarrow}$	$38^{\uparrow\downarrow}$	4	29
3	*Acer pseudoplatanus* L.	9	$25^{\uparrow\downarrow}$	2	$48^{\downarrow}$
4	*Afzelia africana* Pers.	$37^{\uparrow}$	$33^{\uparrow\downarrow}$	15	0,2
5	*Alnus glutinosa* (L.) Gaertn.	12	$15^{\uparrow}$	17	37
6	*Aspidosperma quebracho-blanco* Schltdl.	22	26	21	14
7	*Aucoumea kleineana* Pierre	$19^{\downarrow}$	14	8 /$^{\downarrow}$	$5^{\downarrow}$
8	*Autranella congolensis* (De Wild.) A.Chev.	11	$33^{\uparrow\downarrow}$	$16^{\downarrow}/^{\downarrow}$	11
9	*Betula pubescens* Ehrh.	8	$31^{\downarrow}$	55 /$^{\downarrow}$	61
10	*Carpinus betulus* L.	12	$38^{\uparrow\downarrow}$	16	$24^{\downarrow}$
11	*Cedrelinga cateniformis* (Ducke) Ducke	$19^{\uparrow}$	4	26 /$^{\downarrow}$	21
12	*Coffea arabica* L.	29	$33^{\downarrow}$	3	25
13	*Copaifera paupera* (Herzog) Dwyer	$29^{\uparrow\downarrow}$	$29^{\uparrow\downarrow}$	13	$28^{\downarrow}$
14	*Dichrostachys cinerea* (L.) Wight & Arn.	$20^{\downarrow}$	$32^{\uparrow\downarrow}$	4	5
15	*Diospyros crassiflora* Hiern	18	29	6	1
16	*Eucalyptus grandis* W. Hill ex Maiden	19	$20^{\uparrow\downarrow}$	42	6
17	*Fraxinus excelsior* L.	$25^{\downarrow}$	4	25 /$^{\downarrow}$	26
18	*Ilex aquifolium* L.	5	$38^{\uparrow\downarrow}$	25	$25^{\uparrow}$
19	*Juglans nigra* L.	14	$53^{\uparrow\downarrow}$	14	$38^{\downarrow}$
20	*Milicia excelsa* (Welw.) C. C. Berg	22	$28^{\uparrow}$	23 /$^{\downarrow}$	18
21	*Nauclea diderrichii* (De Wild.) Merr.	39	$40^{\uparrow\downarrow}$	12	16
22	*Paulownia elongata* S. Y. Hu	$17^{\uparrow}$	$30^{\uparrow}$	14	20
23	*Pterocarpus angolensis* DC.	6	$24^{\downarrow}$	12	22
24	*Robinia pseudoacacia* L.	$2^{\uparrow}$	$2^{\uparrow}$	15	23
25	*Senegalia chundra* (Roxb. Ex Rottler) Maslin	$32^{\downarrow}$	13	22	29
26	*Rubroshorea leprosula* (Miq.) P.S. Ashton & J. Heck.	25	$44^{\uparrow\downarrow}$	33	47
27	*Triplochiton scleroxylon* K. Schum.	3	$39^{\uparrow\downarrow}$	18	$8^{\downarrow}$
28	*Ulmus minor* Mill.	$2^{\uparrow\downarrow}$	$2^{\downarrow}$	15 /$^{\downarrow}$	$23^{\downarrow}$
29	*Vachellia nilotica (L.)* P.J.H.Hurter & Mabb.	24	$42^{\uparrow\downarrow}$	$17^{\downarrow}/^{\downarrow}$	6
30	*Ziziphus mucronata* Willd.	6	$48^{\uparrow\downarrow}$	10	13

The arrows delineate the alterations in size classes associated with a subsequent to the charring process

$^{\uparrow}$ An additional size class has been detected.

$^{\downarrow}$ The feature has exhibited a loss of a size class.

structural features observed in transverse-sections of untreated and charred wood of *Acacia dealbata*, *Eucalyptus grandis*, and *Juglans nigra*. The images show a notable reduction in vessel diameter, particularly for the charred wood of *Acacia dealbata* and *Eucalyptus grandis*.

The mean values (uncharred and charred) for each wood species reveal a marked and statistically significant difference in size between the uncharred and charred wood groups for all examined features (significance level of $\alpha = 5\%$). The percentages of shrinkage differed among anatomical features (Fig. 3a). The average shrink-

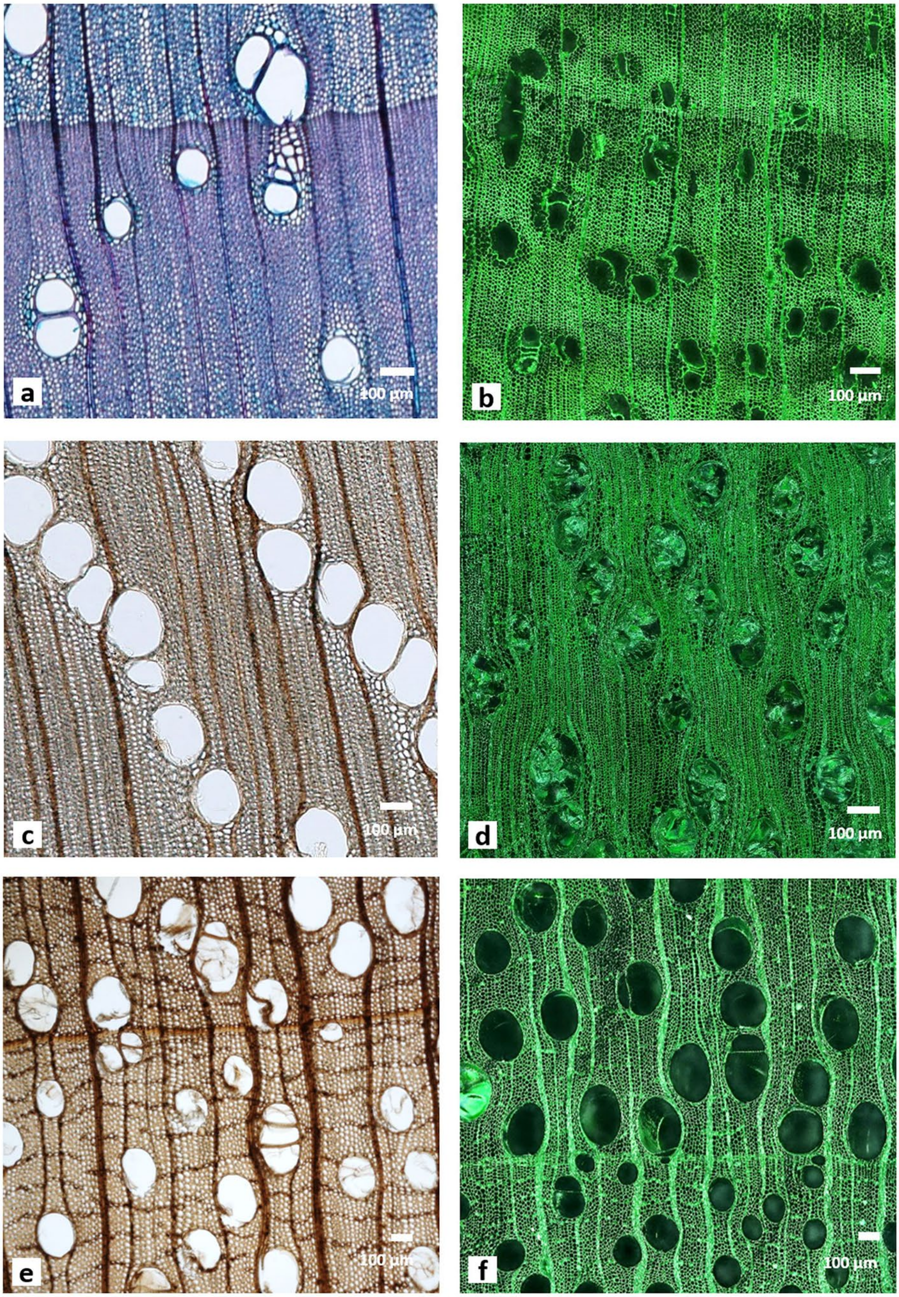

Fig. 2 Comparison of the transverse section of uncharred wood by light microscope (**a**, **c**, **e**), and charcoal by 3D- reflected light microscope (**b**, **d**, **f**) for *Acacia dealbata* (**a**-**b**), *Eucalyptus grandis* (**c**-**d**), and *Juglans nigra* (**e**-**f**)

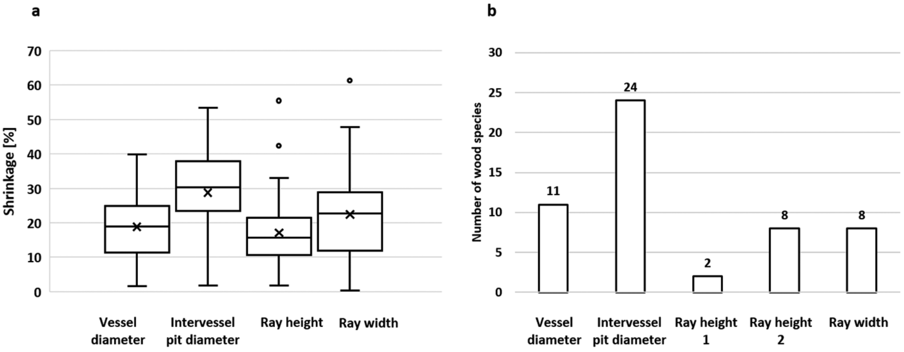

Fig. 3 Shrinkage of all wood species (**a**), and number of wood species with changed size class (**b**), for each anatomical feature

age for all 30 wood species is 19% for the tangential diameter of vessel elements, 29% for the intervessel pit diameter, 17% for the ray height, and 23% for ray width.

It is notable that the loss of dimension of the intervessel pit diameter is significantly higher compared to other features. As loss of dimension is the smallest among all the features examined, it can complicate the determination of measuring points and, in some cases, lead to inaccuracies. Additionally, the state of preservation after charring can vary. A fusion of the cell walls is often observed, which is why in anthracology the “pit aperture” and the “pit chamber” of charred intervessel pits are often measured individually. In the present study, we focussed on measuring the pit chamber to facilitate the comparison of measured values from uncharred wood samples with existing databases. Despite the “uneven” nature of the sample material, high-resolution images of intervessel pit areas were captured using the 3D-reflected light microscope, enabling successful measurements of the “pit chamber” for all wood species.

The data suggest notable differences in dimensional losses among the wood species studied. This becomes clear when considering the spectrum of MIN and MAX values of all species (given in MIN/MEAN/MAX [in %]– tangential diameter of vessel elements: 2/19/40; intervessel pit diameter: 2/29/53; ray height: 2/17/55; ray width: [0.2]/23/61). For example, *Robinia pseudoacacia* shows only 2% shrinkage in the tangential diameter of vessel elements, while *Acacia dealbata* exhibits a significantly higher dimensional loss of 40%. A similar trend is observed for the features of intervessel pit diameter, ray height, and ray width.

The results of the present study demonstrate that the degree of shrinkage varies considerably between different species. Consequently, new references should be considered, providing quantitative data on the diagnostic features. There were cases where the charring process had minor effects on the size of the structural features, for example *Robinia pseudoacacia*, with 2% shrinkage for the tangential diameter of vessel elements and the intervessel pits. Other species showed similar dimensional changes for the tangential diameter of vessel elements, such as *Triplochiton scleroxylon* and *Betula pubescens*, both 3%. Small losses were also observed for ray height and ray width with 2% for *Acer pseudoplatanus* and 1% for *Dichrostachys cinerea*.

In comparison, a number of wood species exhibited shrinkage of up to 60% for ray width (*Betula pubescens*). This species also had a loss of 55% for ray height. High shrinkage also occured for the tangential diameter of vessel elements and the intervessel pit diameter. Examples are *Acacia dealbata* with 40% loss for the tangential diameter of vessel elements and *Juglans nigra* with 53% loss for the intervessel pit diameter.

The present study, encompassing the examination of 30 wood species, represents only a fraction of the global species diversity despite the fact that species were selected from various family affiliations and geographical distributions. The findings of this study merely highlight trends that warrant further investigation.

The application of size classes of quantitative features for charcoal

The shrinking of anatomical features can affect the size classes of quantitative features. Often, the originally assigned size classes of wood are just above the threshold value of a documented size class, which means that the wood has to be classified in the class below due to shrinkage.

In addition to the percentage dimensional losses for all charred species, Table 3 indicates whether a shift in size class was observed. The analysis revealed two distinct outcomes: (↑) the detection of an additional size class, and (↓) the loss of a size class. The number of wood species exhibiting size class alterations varied across the measured anatomical features: tangential diameter of vessel elements 37% ($n=11$), intervessel pit diameter 80% ($n=24$), ray height (part 1) 7% ($n=2$), ray height (part 2) 27% ($n=8$), and ray width 27% ($n=8$) (Fig. 3b).

The composition of wood species exhibiting size class alterations differed for each anatomical feature, reflecting the differing losses of dimension for each species. This variation means that each wood species exhibits a unique number of traits that have undergone changes in size class. Additionally, the combination of traits with altered size classes also differs among species, and the range of altered size classes for a wood species spans from zero to four. While *Aspidosperma quebracho-blanco* and *Diospyros crassiflora* show no changes, *Ulmus minor* exhibits differences in all examined features compared to the original classification. The examples represent exceptions; most samples in our study exhibit two features with altered classifications.

The percentage of wood species with size class variations appears to depend on the number of size classes defined for a trait. Our observations indicate that an increase in the number of size classes correlates with a higher likelihood of changes detected in size class for a feature. To support this hypothesis, we expanded the original classification for ray height from two to three size classes. In this case, we utilized a finer classification for determining wood ray height based on the Commercial Timbers database (Richter and Dallwitz 2000 onwards), as opposed to the IAWA feature list (IAWA Committee 1989). This adjustment elevated the percentage of wood species with a changed size class assignment from 7 to 27%.

Therefore, employing a greater number of classes with more than two groups when determining quantitative features could be generally beneficial. The division into four classes, as already used in wood anatomy for the classification of the tangential diameter of vessel elements and intervessel pit diameter (small, medium, large, and very large), is considered highly useful by the authors. It would be advantageous to establish this “finer” subdivision for ray height as well.

Conclusion

The results of the present study show that all 30 examined wood species exhibit a different shrinkage percentage for the quantitative anatomical features. The parameters tangential diameter of vessel elements, intervessel pit diameter, ray height, and ray width were analysed. Subsequent examination of the size classes for the listed features between solid wood and charcoal revealed significant differences with shrinkage resulting in the loss of the original size class of a feature as observed in solid wood. This loss occurs because higher values in the spectrum are eliminated, causing the overall range of values to shift downwards. Size classes are therefore added when shrinkage results in the dimensions of a feature falling below the smallest size class of the solid wood reference. This effect must be taken into account when using quantitative features for wood identification. Since primarily qualitative features are used for the identification of charcoal, the significance of quantitative features has so far been of secondary importance considering the entire spectrum of anatomical features. Nevertheless, it would be an advantage for anthracological research to make use of the measured values of vessels, intervessel pits, and rays in order to be able to use them reliably with regard to size classification. In addition, quantitative features to qualitative features could be helpful in differentiating (sub-) tropical wood species and confirm an identification result at genus-/species level.

The 30 selected wood species in this study do not reflect the global diversity of all wood species. The aim of the present study was not to be exhaustive, but to address this complex topic and its associated challenges. The results make it clear that the assumption of an equal loss of dimension for all measured features will lead to erroneous results in the practice of wood species identification. Therefore, dimensional changes must be considered specifically for each species or genus. A “blanket” deduction of an assumed shrinkage is not appropriate for correct charcoal determination. As many wood anatomy laboratories build up references for charred wood, our ability to re-evaluate and adapt size classes will increase, in turn strengthening our capability for charcoal identification.

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Author contributions V.TH.Z. performed all microscopic experiments with the statistical evaluation of the data and wrote the main manuscript text with tables and figures. In the Selection of wood species and collection of samples were involved V.H. and V.TH.Z. The work was carried out under the supervision of G.K. All authors reviewed the manuscript.

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Declarations

Competing interests The authors declare no competing interests.

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