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ORIGINAL

Assessing the natural durability of xylarium specimens: mini-block testing and chemical fingerprinting for small-sized samples

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Abstract

The resistance of wood against fungal decay, in short the natural durability, is one of the main criteria for defining the potential use of a wood species. Wood collections, or xylaria, offer the unique opportunity to screen many specimens and species for the latter purpose yet sample size is often limited and standardized tests are often not possible neither desired given the historical and cultural value of these specimens. Two different methods to determine the natural durability are tested and presented here, more specifically the mini-block test and chemical fingerprinting by Direct Analysis in Real-Time Time-Of-Flight Mass Spectrometry (DART TOFMS). Fungal decay by *Trametes versicolor* was determined for 577 mini-blocks collected from xylarium specimens and 602 mini-blocks from commercial species, not belonging to the xylarium collection, were included as a benchmark. Mass loss percentages of the different species are similar to reported values, supporting the use of the mini-block test when standardized testing is hardly feasible. Furthermore, as expected there is also a significantly negative relationship between density and the mass loss percentages from the mini-block test (r-Spearman = −0.65***). Finally, partial least square-based prediction of recorded mass loss by using the DART TOFMS chemical fingerprints is promising (R^2-adjusted = 0.40***), yet the accuracy differs between species.

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Introduction

One of the main criteria for defining the potential use of a timber species is its resistance against wood destroying organisms (Plaschkies et al. 2014; Sundararaj et al. 2015). Although natural durability has been thoroughly studied and is well known for temperate species and a range of tropical timber species (see for example Cirad—Tropix 7: https://tropix.cirad.fr/), many species are understudied in this respect. Wood collections, or xylaria, can be considered as sleeping beauties concerning wood technological and biological output (Deklerck et al. 2019a) and, given the large number of (lesser-used) species and specimens, contain enormous potential. The Federal Xylarium of the Royal Museum for Central Africa (Tervuren, Belgium) holds the second largest wood collection in the world, and the largest for African species. Over 80,000 specimens covering more than 13,000 species have been collected in the last century, and the collection is still growing. The main challenge is the specimen's non-standardized, random format; ranging from branches, to disks and wood blocks. Not only does this complicate any standardized testing, these specimens also hold a historical and cultural value, and therefore, sampling of these specimens needs to be performed consciously. To determine the natural durability on xylarium specimens, it is not possible to obtain the standardized sample size from the methodology described in EN 113 (1996) and included in CEN/TS 15083-1 (2005). The Bravery (1979) mini-block test is therefore a valuable alternative. Several authors report on mini-block testing, however, mainly focusing on (un-/)treated Scots pine (*Pinus sylvestris* L.), radiata pine (*Pinus radiata* D. Don), spruce (*Picea abies* (L.) H. Karst.) or beech (*Fagus sylvatica* L.) samples (Carey 1988; Petrič et al. 1998; Sailer et al. 1998; Brown et al. 1991; Pohleven et al. 2000; Alfredsen et al. 2004; Verma et al. 2005; De Vetter et al. 2009; Pilgård et al. 2013). Van Acker et al. (2003) tested the preliminary screening ability of mini-blocks on both European and tropical species in comparison with EN 113. Carrillo et al. (2013) used mini-blocks to determine the resistance of 10 tree and shrub species from north-eastern Mexico against fungal decay caused by *Coniophora puteana* and *Trametes versicolor*. Palanti et al. (2012) used the mini-block and standard tests to determine treatment (copper complexes grafted to amino-functionalized silica gel) efficacy against fungal decay. Although most papers agree on the rapid screening ability of the mini-block test, little is known about using the mini-block test on a range of commercial and lesser-used tropical timber species, which tend to be more durable compared to their temperate counter parts.

The natural durability of a timber species is its resistance to biological decay due to, for example, the presence of natural components in the wood, which might exhibit toxicity towards biological organisms (Kutnik 2013; Sundararaj et al. 2015). Some of these natural components are toxic extractive compounds in the heartwood and are most important towards determining the natural durability (Scheffer and Cowling 1966; Bamber and Fukazawa 1985; Hillis 1987; Chang et al. 2000; Taylor et al. 2002; Sehlstedt-Persson and Karlsson 2010). It should be noted that next to toxic extractives, some extractives function as inhibitors of fungal growth (Hart and Hillis 1972). Lignin type and quantity, as well as wood

density, can also impact decay resistance (Eaton and Hale 1993; Gierlinger et al. 2003; Humar et al. 2008, Rana et al. 2010). Non-biocidal properties of heartwood extractives (for example antioxidant) might contribute to the natural durability as well (Schultz and Nicholas 2000). Finally, next to intrinsic biological durability, the resistance can be affected by the moisture dynamics of the material itself (Van Acker et al. 2014)

Extractives cover a large number of compounds of different classes (Hillis 1987); for example, alkaloids, phenolic compounds (e.g., flavonoids, tannins), terpenoids, quinones, etc. These compounds in the heartwood that determine the natural durability are secondary metabolites of low molecular weight (Chang et al. 2000; Sundararaj et al. 2015; Saha Tchinda et al. 2018). However, the amount of these extractives can differ within the tree, both vertically as well as radially leading to a difference in natural durability within the heartwood of the tree (Hillis 1987; Taylor et al. 2002; Wong et al. 2005; Sehlstedt-Persson and Karlsson 2010). All sapwood is considered not durable (durability class 5, EN 350 2016).

Fast prediction of the natural durability of a species or specimen has long been the aim. The colour of the wood, for example, has been shown to be related to the extractive content (Gierlinger et al. 2004). Fourier transform near-infrared (FT-NIR) spectrometry is one of the techniques explored for assessing natural durability. Reports on its use for larix (Gierlinger et al. 2003), red spruce sapwood (Jellison et al. 2002) and Scots pine (Flæte and Haartveit 2004) have shown its potential. These studies, however, have only focussed on assessing the natural durability of a single species. NIR has also been successfully used to determine the heartwood extractive contents of Scots pine (Rosenqvist and Karlsson 1999) and larch (Gierlinger et al. 2002), or to assess the chemical changes linked to biodegradation of spruce wood (Kelley et al. 2002; Schwanninger et al. 2004). Again, little information is available on rapid prediction of the natural durability of tropical timber species. Further, FT-NIR gives no indication of which extractives or compounds might be the main actors in determining the natural durability. As secondary metabolites are directly related to natural durability, mapping these metabolites by their chemical fingerprints and using the latter to predict the natural durability is highly interesting. Recently, a new technique has been proposed for rapid species identification in the timber trade (Cody et al. 2012; McClure et al. 2015; Deklerck et al. 2017). This technique is based on the comparison of chemical fingerprints (mass spectra, based on molecules below 900 daltons) using Direct Analysis in Real-Time Time-Of-Flight Mass Spectrometry (DART TOFMS, Cody and Laramée 2005). A single wood sliver is taken from a specimen, put in a heated helium gas stream, and within a couple of minutes the chemical fingerprint is created. The potential for using such chemical fingerprints to determine the natural durability was already mentioned by Finch et al. (2017) and will be explored here more in-depth.

The goal is to find out whether valid information on the natural durability of wood can be gained from xylarium samples. The objectives of this study are (1) to establish and validate a mini-block test suited for assessment of natural durability of xylarium specimens, (2) to establish the relationship between wood density and mass loss and (3) to determine whether chemical fingerprints can be used as a screening technique to predict the mass loss due to fungal decay.

Materials and methods

Mini-block test

In total, 577 heartwood mini-blocks (30 length × 10 width × 5 thickness mm) were collected from 237 xylarium specimens from 31 species (Supplementary Materials, Table 1). 602 mini-blocks from 10 species were collected from wood beams bought in trade stores (hereafter referred to as 'commercial specimens/species') (Supplementary Materials, Table 2). These commercial samples are not affected by possible ageing effects, as might be the case for the xylarium samples. Therefore, they allow us to assess the validity of the mini-block setup, which differs in block size and duration of exposure with the standard test (CEN/TS 15083-1). Additionally, because their natural durability is known, the commercial mini-block samples serve as a reliable reference for comparison for the xylarium samples. All mini-blocks were oven-dried (18–24 h at 103 °C) and weighed (m_1) prior to gamma irradiation and fungal testing. Subsequently, the mini-blocks were sterilized with gamma irradiation and placed on a fungal mat of *Trametes versicolor* (strain CTB 863A—as listed in the CEN/TS 15083-1 standard, Centre Technique du Bois et de l'Ameublement Allée de Boutaut—BP 227, F 23 028 Bordeaux cedex) grown on malt-agar (4% malt, 2% agar). To avoid direct contact with the agar, mini-blocks were positioned on a metal support in the Petri dishes (Supplementary Material, Figure 1). *Trametes versicolor* was chosen as it is a white rot fungus, primarily able to degrade hardwood species, and an obligatory test fungus in standard CEN/TS 15083-1. *Fagus sylvatica* L. was included as reference species for assessing the virulence of the fungus. For a first set of reference samples (319 in total), mass loss was determined after 8 weeks. The beech wood already had an average 23% mass loss, exceeding the minimum mass loss threshold of 20%, thus ensuring the virulence of the fungus (EN 113 1996; CEN/TS 15083-1). However, since beech is usually classified in durability class 5, corresponding to a mass loss of 30%, it was decided to prolong the mini-block test up to 12 weeks. More tropical species can be found in higher natural durability classes compared to temperate species (EN 350 2016). Since the xylarium samples are tropical, extending this period was expected to increase the likelihood of obtaining significant differences between species. After 12 weeks, mass loss was determined for the second set of commercial samples (283 total) and all xylarium samples (577 total). The mycelium was removed and all samples were weighed (m_2). Samples were then oven-dried (18–24 h at 103 °C) and weighed (m_3) once more. The corresponding mass loss (ML) was determined as:

$$\mathrm{ML}\ (\%) = \left(\left(m_1 - m_3\right)/m_1\right) \times 100 \tag{1}$$

The final moisture content (MC) was determined as:

$$\mathrm{MC}\ (\%) = \left(\left(m_2 - m_3\right)/m_3\right) \times 100 \tag{2}$$

The durability class was determined based on the median of the percentage mass loss and the classifications given in EN 350 (2016). These classifications

were compared to natural durability classes for the same species found in the literature: Houtvademecum (Klaassen 2018), Tropix 7 technical sheets (Cirad 2017) and the EN 350 standard (2016). In what follows numbers are used to indicate the natural durability class even though some sources use Latin numerals. Normality and homoscedasticity of mass loss per species and time period (8 weeks and 12 weeks) were assessed using the Shapiro–Wilk (1965) and Bartlett test (Snedecor and Cochran 1989), respectively. Mann–Whitney *U* tests (Mann and Whitney 1947) were performed to assess whether significant differences in natural durability are found between wood blocks exposed for 8 or 12 weeks and to determine whether there are significant differences in mass loss between commercial and xylarium species. To determine whether significant differences in mass loss between species changed from 8 to 12 weeks, the Kruskal–Wallis rank sum test in combination with Dunn's test of multiple comparisons using rank sums (*p* value adjustment via Benjamini–Hochberg method (Benjamini and Hochberg 1995)) from the dunn.test package (Dinno 2017) was used. These analyses were done in RStudio (RStudio Team 2016) and graphs were produced with the ggplot 2 package (Wickham and Chang 2016).

Wood density

The oven-dry wood density (kg/m^3) was determined for 233 xylarium specimens (31 species) before sampling for the mini-block test. The specimens were oven-dried (18–24 h at 103 °C) to determine the oven-dry weight and the volume was determined using the Archimedes principle (Maniatis et al. 2011), which allowed to relate mass loss and wood density.

DART TOFMS

Wood slivers were also taken from the same 233 xylarium specimens and analysed using a DART-SVP ion source (IonSense, Saugus, MA, USA) coupled to a JEOL AccuTOF 4G LC Mass Spectrometer (Jeol USA, Peabody, USA). The slivers were placed in a heated gas stream (7 s) containing electronically excited helium atoms produced by the DART ion source. This leads to the ionization and emissions of compounds from the wood, which then enter the mass analyser. Spectra were acquired in positive ion mode with DART ion source parameters settings (as indicated in Lancaster and Espinoza 2012; Espinoza et al. 2014; McClure et al. 2015): electrode 1 150 V; electrode 2 250 V. The mass spectrometer settings were: Orifice 1, 120 °C, 30 V; ring lens 5 V; Orifice 2 5 V; ion guide RF 600 V; ion guide bias voltage 33 V. The focus voltage was 10 V, quad voltage was 20 V, focus lens voltage was -120 V, push bias voltage was -0.43 V. Spectra were obtained over the mass range of *m/z* 100–1000 with a sampling interval of 0.25 ns and recording interval at 0.80 s. Accumulation time was 0.797 s and wait time 0.003 s. The text files of the mass-calibrated, centroided mass spectra were exported using TSS Unity (Shrader Software Solutions, Inc., Grosse Pointe Park, MI, USA) data reduction software and used for further analysis. The text files were transformed into a csv-excel format

using a 250 mmu binning and 1% threshold (see Deklerck et al. 2019b). Principal component analysis (PCA) was used for data exploration and preliminary relations between mass loss and ion abundance were determined. Only ions that were present in more than 75% of the specimens were included in the PCA. A first attempt at compound identification was also performed by cross-checking with the KNapSacK database (Afendi et al. 2012) in the Mass Mountaineer Mass Spectral Interpretation Tools software (RBC Software, Peabody, MA, USA). Partial least squares (PLS) regression (five components) with fivefold cross-validation from the pls package (Wehrens et al. 2018) in RStudio Team (2016) was performed to determine whether mass loss can be predicted via chemical fingerprinting. The graph indicating the difference between true mass loss value and prediction was constructed using the ggplot2 package (Wickham and Chang 2016).

Results and discussion

Mini-block test to determine the natural durability

The results and summary for the mini-block test on both xylarium (12 weeks) and commercial (8 and 12 weeks) samples can be found in Supplementary Materials Table 1 and 2, respectively. The beech reference mini-blocks exposed to fungal decay for 8 weeks showed 23% mass loss. After 12 weeks of degradation, the mass loss of the beech reference samples increased significantly to 33.3% ($p \leq 0.01$) (Fig. 1). *T. versicolor* is a white rot fungus capable of degrading carbohydrates and lignin at the same time and at a similar rate during all decay stages (Schmidt 2006). The hyphae produce degrading agents end excrete them into the slime layer that surrounds them. These degrading agents are thus only active in close proximity to the hyphae (Schmidt 2006). It, therefore, takes a while for the whole mini-block to be consumed, allowing degradation to gradually continue.

When comparing the mass loss for the commercial species after 8 and 12 weeks of degradation, it appears that all mass losses changed significantly, although most commercial species (European cherry, acajou d'Afrique, moabi, azobé, iroko, padouk d'Afrique and robinia) remain in the same natural durability class (Fig. 1). The additional 4 weeks of degradation did change the durability class of oak from class 1 to 3 ($p \leq 0.01$), sapeli from class 3 to 4 ($p \leq 0.01$) and iroko from class 2 to class 4 ($p \leq 0.01$). When comparing with the durability classes retrieved from the literature (Houtvademecum, Klaassen (2018)), online repositories (Tropix 7, Cirad 2017) as well as EN 350 (Supplementary Materials, Table 2), we see that 7 species end up in the same natural durability class (after 12 weeks). The biggest difference is for *Milicia excelsa*, which ends up in class 4 compared to class 1 or 2. The results of the Dunn's test (Table 1) showed that after 8 weeks there were nine species comparisons with no significant difference in mean (azobé—moabi, azobé—black locust, European beech—European cherry, European beech—acajou d'Afrique, European cherry—acajou d'Afrique, European cherry—sapeli, iroko—sapeli, moabi—European oak and moabi—black locus). After 12 weeks, however, there was a significant difference between moabi and European oak but no significant difference between

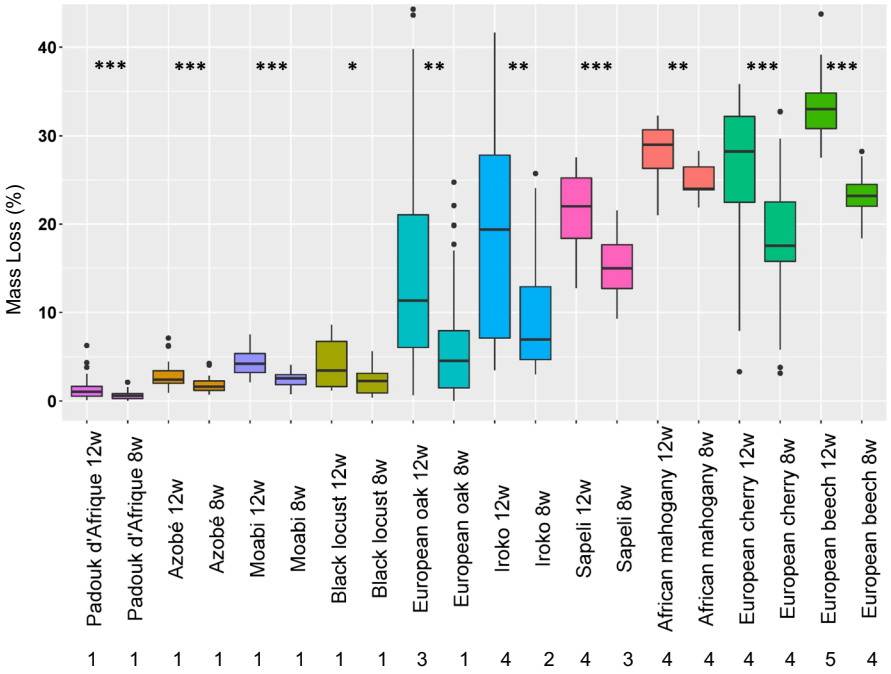

Fig. 1 Mass loss (%) for the commercial species, comparison between 8 and 12 weeks of fungal decay. Significance levels were determined via the Mann–Whitney *U* test or Welch's two-sample *t* test depending on normality (*ns* not significant, $*p \leq 0.5$; $**p \leq 0.01$; $***p \leq 0.001$). The numbers at the bottom of the figure indicate the natural durability class

azobé—padouk d'Afrique, padouk d'Afrique—black locust, European cherry—iroko, iroko—acajou d'Afrique, acajou d'Afrique—sapeli and sapeli—European oak, in addition to the nonsignificant differences after 8 weeks. Although the natural durability class for the commercial species only changed for three species (oak, sapeli and iroko), mass loss differences were significant between 8 and 12 weeks. This indicates the importance of time for determination of natural durability, as absolute mass loss values are compared to each other. In his initial mini-block test, Bravery (1979) suggested 6 weeks of fungal incubation. However, Bravery's aim was to develop a screening test for rapid evaluation of wood preservative fungicides on wood. The test was designed for wood species that are easily degradable when not treated with protection products, such as beech and Scots pine sapwood (Bravery 1979). A period of minimum 6 weeks should in that case suffice to reach a significant amount of mass loss when a product is not able to protect the wood (Bravery 1979).

The aim, however, was to apply the mini-block test to assess the natural durability of xylarium specimens. The most durable species (xylarium, class 1) are *Erythrophleum suaveolens* (tali), *Lophira alata* (azobé), *Millettia laurentii* (wengé), *Pterocarpus angolensis* (muninga), *soyauxi* (padouk d'Afrique) and

Table 1 Results of the Dunn's Test (above: 8 weeks, below: 12 weeks)

	Azobé	European beech	Cherry	Iroko	Acajou d'Afrique	Moabi	European oak	Padouk d'Afrique	Black locust
8 weeks									
European beech	*								
Cherry	*								
Iroko	*	*	*						
Acajou d'Afrique	*			*					
Moabi		*	*	*	*				
European oak	*	*	*	*	*				
Padouk d'Afrique	*	*	*	*	*	*	*		
Black locust		*	*	*	*		*	*	
Sapelli	*	*			*	*	*	*	*
12 weeks									
European beech	*								
Cherry	*								
Iroko	*	*							
Acajou d'Afrique	*								
Moabi		*	*	*	*				
European oak	*	*	*		*	*			
Padouk d'Afrique		*	*	*	*	*	*		
Black locust		*	*	*	*		*		
Sapelli	*	*				*		*	*

The coloured squares indicate that there is no significant difference in mean mass loss between the species. The star indicates there is a significant difference in mean mass loss between the species

tinctorius (padouk d'Afrique), while the least durable species (xylarium, class 5) are *Canarium schweinfurthii* (aiélé) and *Pycnanthus angolensis* (ilomba) (Fig. 2). Supplementary Materials Table 1 also lists the durability classes retrieved from the literature (Houtvademecum, Klaassen (2018)), online repositories (Tropix 7, Cirad 2017) as well as EN 350 (2016) (Fig. 2). Out of 31 species, 15 have a different classification compared to the literature classifications. 11 species have a lower durability and 4 have a better durability. Note the high within-species variability for *Milicia excelsa* (iroko), *Staudtia kamurensis* (niové) and *Zanthoxylum gilletii* (East African satin wood). The xylarium species applied are tropical species, which are, in general, more durable compared to temperate species (EN 350 2016). It was, therefore, opted to double the incubation period to ensure sufficient mass loss, allowing to determine significant differences in natural durability between species. The duration of the incubation period is important since the mass loss differences were significant between 8 and 12 weeks for the commercial species. However, when looking at differences in mean mass loss between the commercial species at 8 and between the commercial species at 12 weeks, we see that there are less significant differences at 12 weeks (Table 1). When comparing degradation after 12 weeks for xylarium specimens (Supplementary Materials,

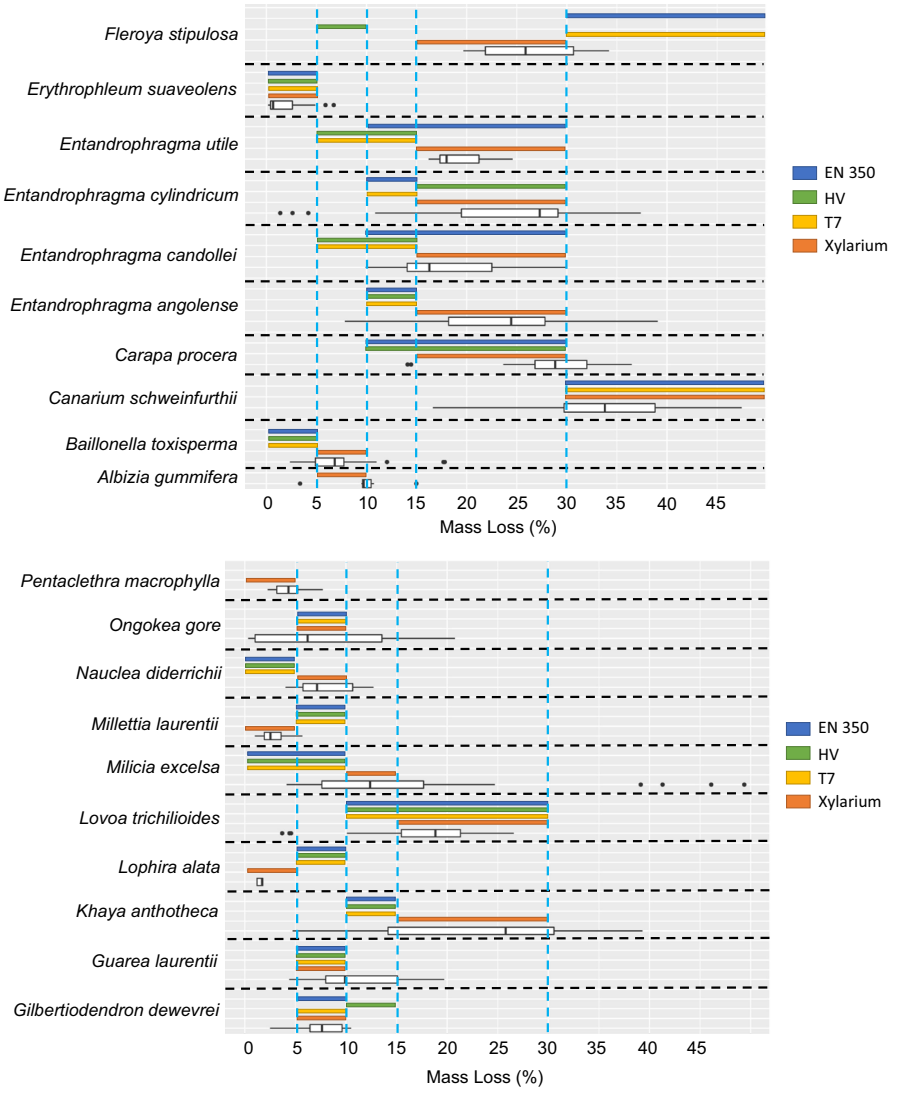

Fig. 2 Comparison of the mass loss from the xylarium species (boxplots based on mass loss from specimens) and the natural durability classes from the literature sources (HV = Houtvademecum, T7 = Tropix 7 technical sheets). The species are given in reverse alphabetical order per separate figure. Durability classes as defined in EN 350, based on mass loss (%): ≤ 5% = 1, > 5 to ≤ 10% = 2, > 10 to ≤ 15% = 3, > 15 to ≤ 30% = 4, > 30% = 5 (see blue dotted lines). For the xylarium species, the black bar on the boxplot indicates the median and as such the natural durability class. For the literature sources, when a species was in multiple classes, the full range possible is shown (for example, if a species is in class 3 and 4 it is indicated as ranging from > 10% mass loss to ≤ 30% mass loss)

Table 1) and commercial specimens originating from wood beams of the same species (Supplementary Materials, Table 2, 12 weeks) (Fig. 3), we see that *Baillonella toxisperma* (moabi) specimens from the xylarium showed stronger decay

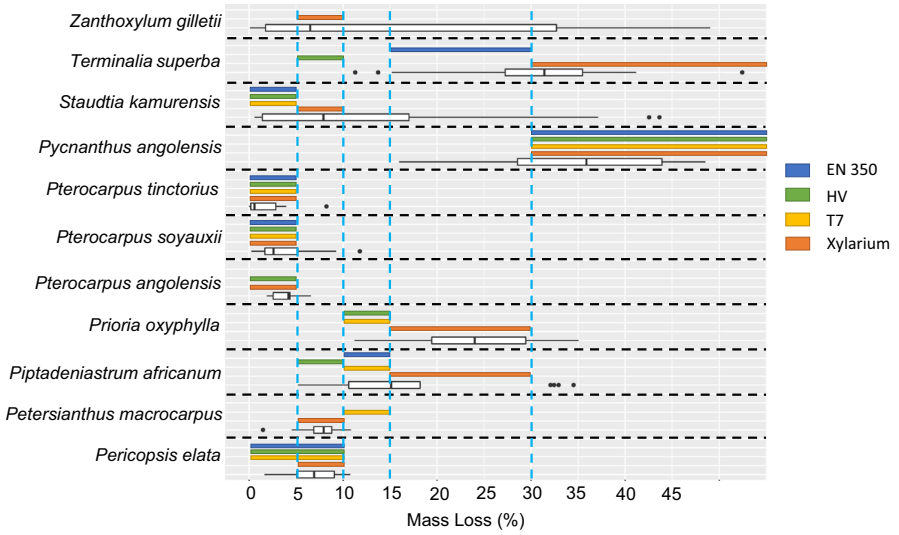

Fig. 2 (continued)

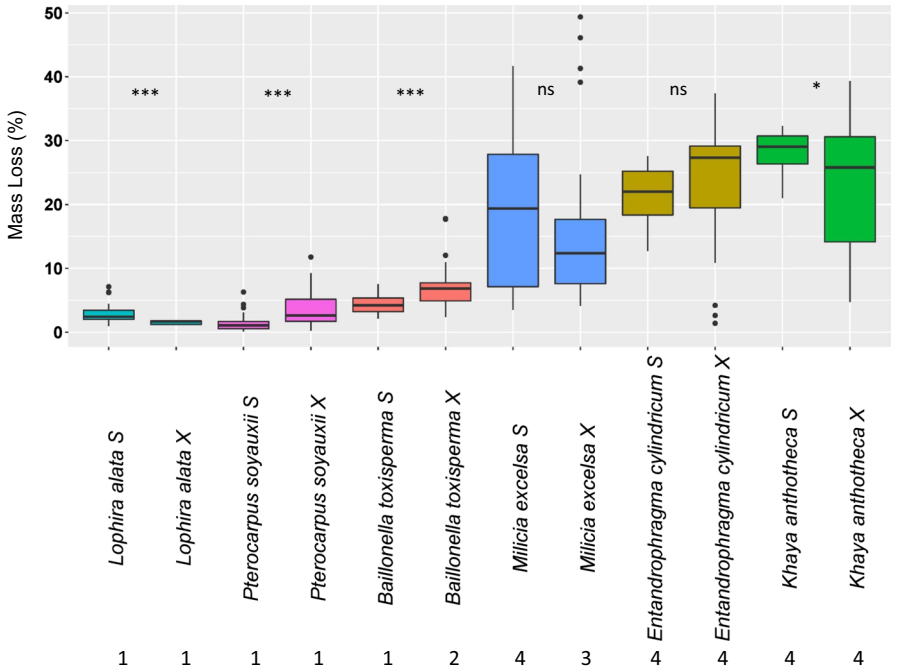

Fig. 3 Comparison of mass loss (%) after 12 weeks between the commercial specimens (C) and the xylarium specimens (X). Significance levels were determined via the Mann–Whitney *U* test (*ns* not significant, $*p \leq 0.5$; $**p \leq 0.01$; $***p \leq 0.001$). The natural durability class is shown at the bottom of the graph. The numbers at the bottom of the figure indicate the natural durability class

(2 vs. 1). Xylarium specimens from the species *Entandrophragma cylindricum* (sapeli), *Khaya anthotheca* (acajou d'Afrique) and *Pterocarpus soyauxii* (padouk d'Afrique) were in the same calculated durability class as the commercial ones (4, 4 and 1). However, there is a significant difference in mass loss (%) between the xylarium and commercial specimens for *Khaya anthotheca* and *Pterocarpus soyauxii*. The xylarium specimens from *Milicia excelsa* (iroko) appeared to have a higher natural durability compared to the commercial specimens (3 vs. 4). So in general, the natural durability classes of the xylarium specimens align well with those of the commercial specimens, however, almost half differ from those found in the literature. It should be noted that most xylarium specimens have been collected during the last 100 years and little is known about the treatment of these samples before archiving. As Scheffer and Cowling (1966) and Schultz and Nicholas (2000) indicate, younger heartwood (in terms of radial gradient) is usually more decay resistant compared to aged heartwood due to extractive degradation. As such, the extractive content of xylarium samples might have decreased. In addition, no information is available on the position of the xylarium samples, and commercial samples for that matter, in the tree, so it is difficult to assess the effect of within tree variation. Further, it is relevant to note that ranking the xylarium species from high to low natural durability still compares to EN 350 (2016). Nonetheless, the natural durability classes of the xylarium specimens in general align well with those of the commercial specimens. Only *Baillonella toxisperma* was classified as having a lower natural durability. This might indicate some loss of extractive components over time due to the age/position of the specimens in the tree or it might be due to any unknown pretreatments of the specimens.

Some species in the study presented here display high variability (Supplementary Materials, Table 1) and could belong to different durability classes based on the individual sample mass loss (for example; *Zanthoxylum gilletti*, *Staudtia kamurensis*, etc.). EN 350 (2016) states that when at least 40% of the mass loss values of samples for a species are distributed in each of the two possible durability classes, then the durability class should not be based on the median mass loss but should be expressed as falling in between durability class *X* and *Y*. Such high variability is indicated by the 'v' sign in the EN 350 norm (2016) and the range of individual values could also be reported by a fitted probability density function. *Zanthoxylum gilletti* shows the highest standard deviation and a large discrepancy between median mass loss (%) and average mass loss (%). One possible explanation is the accidental inclusion of sapwood samples in the test setup, as color difference between the heartwood and sapwood for this species is not straightforward. If we follow EN 350, *Zanthoxylum gilletti* would range between durability class 1 and 5. However, the 40% rule cannot be followed here as mass values are distributed over multiple classes, therefore, justifying the use of the median mass loss to determine natural durability classes. Iroko (*Milicia excelsa*) was classified as moderately durable based on the mini-block test (class III, range 1–5, xylarium specimens). Nzokou et al. (2005), however, classified iroko class 5, performing soil block and field tests with 16 weeks of fungal decay. Contrarily, padouk d'Afrique (*Pterocarpus soxyauxii*) and illomba (*Pycanthus angolensis*) had the same class as in Nzokou et al. (2005) (class 1 and class 5 respectively, xylarium specimens). Several species discussed here overlap

with the work of Kumi-Woode (1996), who determined the natural durability of a series of Ghanaian timber species also using *Trametes versicolor*. In the latter study, mean mass loss was considerably higher and the species appeared to be less resistant to decay.

Wood density and mass loss

The relationship between wood density and decay resistance has been studied intensively (Esenther 1977; Panshin and de Zeeuw 1980; Peralta et al. 2004; Bhat et al. 2005; Arango et al. 2006; Humar et al. 2008). Although the general trend is that the denser the wood the higher the natural durability, this is not always the case. For example, Arango et al. (2006) found a slightly positive correlation between mass loss and specific gravity, yet for softwoods. Figure 4 shows that there is indeed a significantly negative Spearman correlation between wood density and mass loss (r-Spearman = −0.65***). This is also shown by the negative linear relationship (R^2 adjusted = 0.39***). Noticeable exceptions to this trend are, for example, the specimens of *Zanthoxylum gilletti* with 50.8% average mass loss and 702 kg/m^3 wood density or 44.3% average mass loss mass loss and 664 kg/m^3 wood density (Supplementary Materials, Table 1). *Zanthoxylum gilletti* appears to be a difficult species to determine the natural durability class as it has the highest standard deviation on mass loss (%), even higher than the average itself (Supplementary Materials,

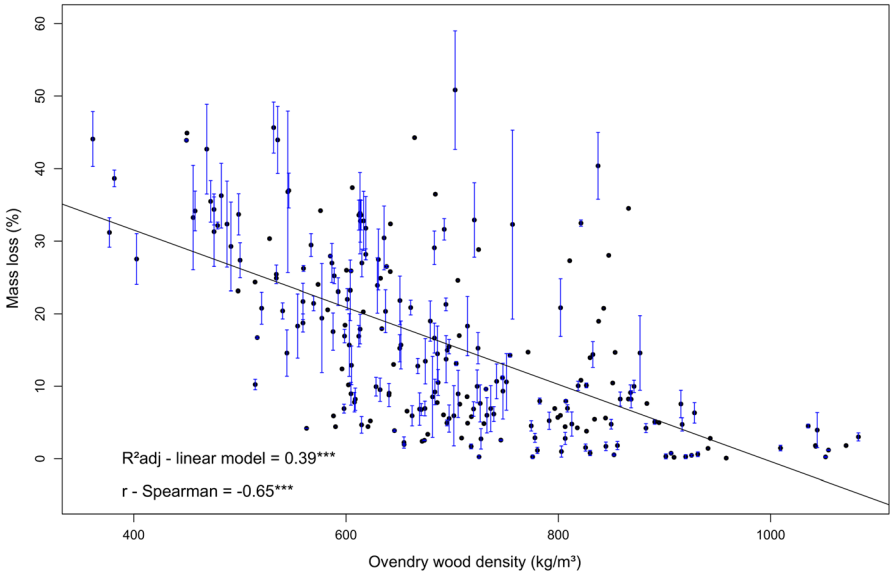

Fig. 4 Mass loss (%) versus oven-dry wood density (kg/m^3) for 233 Tervuren Xylarium specimens (31 species). The mass loss for the xylarium specimens is the average mass loss from the samples from that specimen. The standard deviation is given by the bars and the black line gives the linear relationship ($y = 52.80 - 0.05x$)

Table 1). High variation in the average mass loss of a single specimen is also possible (see Fig. 4, bars).

Chemical fingerprinting to predict mass loss

Previous research using FT-NIR spectra successfully predicted the natural durability of larch trees (Gierlinger et al. 2003). With DART TOFMS, a chemical fingerprint of a specimen based on its small molecules (<900 daltons) can be taken in seconds. One has to keep in mind that such a chemical fingerprint is from a single wood sliver, and variations might occur depending on the presence of extractives. Furthermore, DART TOFMS is not quantitative and only relative abundances of compounds were used in the analysis. The PCA with Spearman rank correlation for ions that are present in more than 75% of the specimens is shown in Fig. 5. It illustrates that certain ions are related to mass loss yet the explained variance of both components is rather low (22.6 and 9.5%). This is not surprising given the large number of compounds that might exist, and for some species, only a limited number has been identified or discovered (see Shinbo et al. 2006; Finch et al. 2017). Two compounds could be identified: 397.38 *m/z*, which is beta-sitosterol (linked to mass loss) and is found in, for example, *Terminalia superba*; and 273.11 *m/z*, which is angolensin (linked to resistance) and can be found in *Pterocarpus angolensis* (Supplementary Materials, Figure 2). The identification of the other compounds was less straightforward. It is surprising that a higher relative abundance of beta-sitosterol seems to be positively related to higher mass loss, as sitosterol was linked with some minor antifungal activity in the study by Mbambo et al. (2012). Angolensin is a flavonoid, and impact on natural durability has been shown in previous studies (Sundararaj et al. 2015; Wang et al. 2004). Small traces of pterocarpin were found as well in the spectra of the *Pterocarpus* species in this study. This compound and other pterocarpans

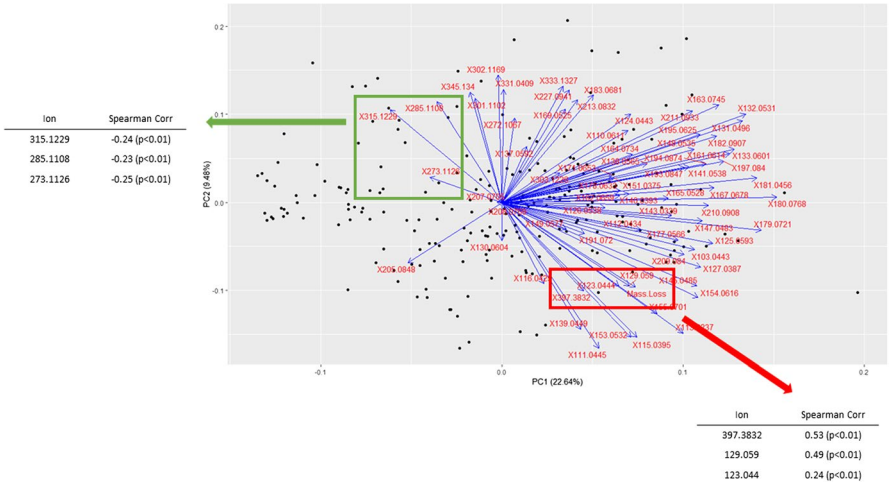

Fig. 5 Principal component analysis with the ions that are present in a minimum of 75% of the samples. The Spearman rank correlation with average mass loss is calculated for six ions

have shown anti-insect or anti-termite properties (Morimoto et al. 2006). The PCA (Fig. 5) illustrates that the compound with approximately 285.11 *m/z* ratio plays a potential role in the resistance against decay as well. There is a high relative abundance in *Pterocarpus* species and in *Millettia laurentii*, but compound identification is not univocal. Interesting to note is that for the samples of *Staudtia kamurensis* with a low average mass loss (1.8%, 1.4% and 0.8%), the relative abundance of this compound was high (100%, 100% and 43.5%), while the samples with high mass loss had very low relative abundances of this compound. It should be noted here that there might be other compounds with similar *m/z*-values for other species that cannot be discerned given the specifics of the pre-processing of the data (see Deklerck et al. 2019b). The identification was also restricted by the availability of information in the KNapSacK database, thus not every *m/z*-value can be linked to a chemical compound at this point. Future work should focus on single species analysis with increased sample size. This will allow for a more refined compound identification per species, instead of across all species together. Looking across species gives a broader scope, but certain interesting compounds might be missed because they are only present in one or some species depending on the dataset. An example of investigating compounds within a certain species for natural durability was given by Niamké et al. (2011), with a focus on *Tectona grandis* L. f. In the present study, only ions that were present in 75% of the specimens were included in the PCA analysis, thus eliminating potential species-specific molecules.

Figure 6 shows the results of a fivefold cross-validated PLS regression with five components and mass loss as response variable. There is a significant linear relationship between the true values of mass loss and the predicted ones

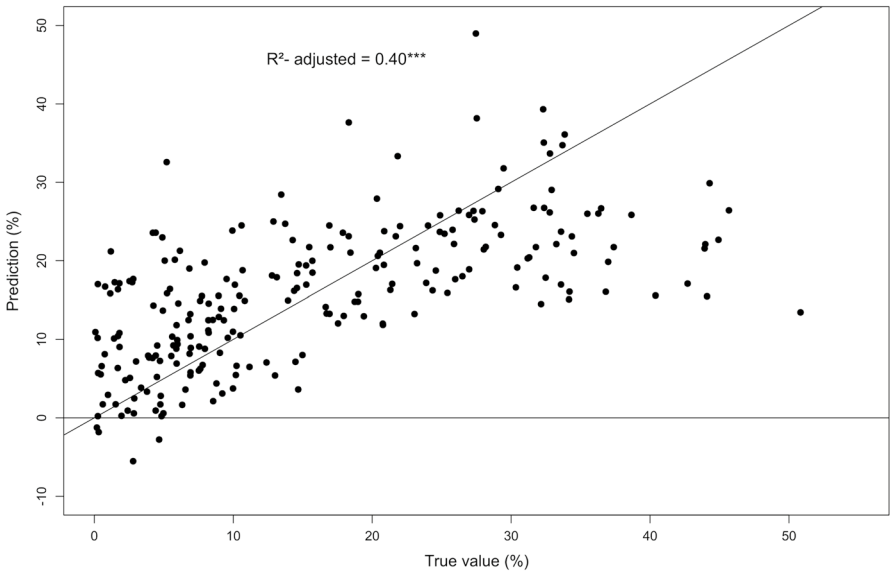

Fig. 6 PLS regression with individual specimen numbers showing the original mass loss (%) on the *x* axis and the predicted mass loss (%) on the *y* axis. The black line shows the 1:1 line

(R^2-adjusted = 0.40***). Overall, PLS regression in combination with these chemical fingerprints performs well to predict the mass loss of a specimen; keeping in mind that the model is for all species combined (Fig. 6). Looking at individual species, however, there are noticeable differences between the true mass loss and the prediction (Fig. 7). Even though the mass loss for *Entandrophragma utile* is substantial (class 4), the predictions of the mass loss are quite accurate. On the other hand, *Lophira alata* is in class 1, but the mass loss is overestimated for every specimen (with a minimum overestimation of 13.0%). For other species in class 1, the mass loss prediction is better (see the *Pterocarpus* species). However, the mass loss for *Pterocarpus soyauxii* is overpredicted for every sample. In addition, there are more significant differences between the mean difference (mean of true value mass loss (%) minus prediction mass loss (%)) and zero for the species in the highest natural durability class compared to the other classes. The prediction error rates of *Zanthoxylum gilletti* range from a 34.9% underprediction to 15.6% overprediction, again indicating the difficulties and variability of this species. Other examples of species with high variable predictions are *Entandrophragma cylindricum*, *Milicia excelsa*, *Ongokea gore*, *Piptadeniastrum africanum* and *Prioria oxyphylla*. PLS regression for every species individually with increased sample amount will lead to an improvement of the mass loss prediction (see Gierlinger et al. 2003, 2004). In addition, the chemical fingerprint is based on a single wood sliver from an individual.

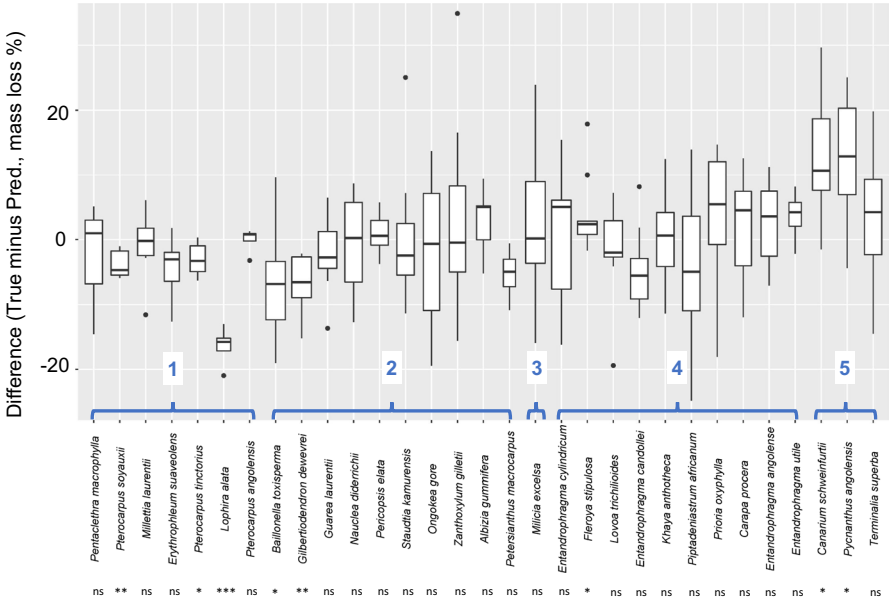

Fig. 7 Barplots per species showing the difference between the true mass loss value and the prediction through PLS regression. The species are ordered from highest natural durability (class 1) to lowest natural durability (class 5). The two way—Wilcoxon or *t* test was used (depending on whether the data had a normal distribution) to determine whether the mean of the difference in predictions was significantly different from zero. The significance levels are given to indicate whether the mean of the difference is significantly different from zero (*ns* not significant, $*p \leq 0.5$; $**p \leq 0.01$; $***p \leq 0.001$)

As mentioned before, there are substantial differences in extractive content throughout a tree (Hillis 1987; Taylor et al. 2002; Sehlstedt-Persson and Karlsson 2010), and the distribution can influence the natural durability (Taylor et al. 2002; Wong et al. 2005). DART TOFMS could also function as a quick screening technique for species with high variation in natural durability for one individual (for example, *Dicorynia guianensis* Amsh., see Amusant et al. (2004)). For several areas within the tree, wood slivers could be taken to construct chemical profiles and give an initial, quick indication of the natural durability.

Conclusion

The mini-block test can be used to determine the natural durability of xylarium samples. 12 weeks of incubation was deemed necessary to ensure sufficient mass loss based on the 30% mass loss needed for beech to be classified in durability class 5. It is, therefore, a serviceable method to obtain information on lesser-known wood species, abundantly available in xylaria. We advise to also include more durable species in the test setup to allow a reliable comparison with the results of the xylarium specimens. Furthermore, PLS regression in combination with chemical fingerprints, for all species combined, allows to estimate the mass loss of a specimen and is therefore a promising screening method to get an initial, fast indication of natural durability even if only a sliver of wood is available.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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