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Neglected role of fungal community composition in explaining variation in wood decay rates

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Abstract

Decomposition of wood is an important component of global carbon cycling. Most wood decomposition models are based on tree characteristics and environmental conditions; however, they do not include community dynamics of fungi which are the major wood decomposers. We examined the factors explaining variation in sapwood decay in oak tree stumps 2 and 5 years after cutting. Wood moisture content was significantly correlated with sapwood decay in younger stumps, whereas ITS-based composition and species richness of the fungal community were the best predictors for mass loss in the older stumps. Co-occurrence analysis showed that in freshly cut trees and in younger stumps fungal communities were non-randomly structured, whereas fungal communities in old stumps could not be separated from a randomly assembled community. These results indicate that the most important factors explaining variation in wood decay rates can change over time and that the strength of competitive interactions between fungi in decaying tree stumps may level off with increased wood decay. Our field analysis further suggests that ascomycetes may have a prominent role in wood decay, but their wood-degrading abilities need to be further tested under controlled conditions. The next challenging step will be to integrate fungal community assembly processes in wood decay models to improve carbon sequestration estimates of forests.

Key words: 454 pyrosequencing of ITS**,** assembly, fungal interactions, local scale, moisture content, saprotrophic fungi, sapwood, wood decomposition, *Quercus robur*.

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Introduction

Dead wood is an important component in the functioning of forest ecosystems. It plays a major role in nutrient cycling as a temporary storage stock of carbon and macronutrients which only become available again during decomposition (Cornelissen et al. 2012). Therefore, a better understanding of factors influencing the rate of wood decomposition can aid in estimating the carbon sequestration capacity of forests under climate change.

To date, most wood decay models are based on wood properties (physical and chemical characteristics of the tree species), moisture and temperature (Yin et al. 1999, Radtke et al. 2009, Zell et al. 2009) and are used to predict wood decay rates over large temporal and spatial scales. These models do not, however, account for the variation that is found at smaller temporal and spatial scales (Palviainen et al. 2010, Woodall 2010). Most carbon is lost during the first decade of wood decomposition, the period for which predictions by current models have the lowest accuracy (Fahey et al. 2005). This hampers the extrapolation of short-term, site-based measurements to larger temporal and spatial scales, thereby reducing the reliability of carbon sequestration estimates of forests. The gap between observed and predicted decay rates could be due to the fact that fungal community dynamics are not taken into account in current wood decay models.

In terrestrial ecosystems higher fungi are the main decomposers of the major wood polymers (cellulose, hemi-cellulose and lignin) (van der Wal et al. 2013). White rot fungi are the only organisms known to be able to completely decompose lignin, whereas brown rot fungi only modify lignin during decomposition of cellulose and hemi-cellulose. Soft rot occurs in wet wood, making wood soft by hydrolysis of part of the cellulose, but with little or no effect on lignin.

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Experiments have shown that the type of wood-rot and fungal identity can have a strong impact on wood decay rates (Boddy 2001).

When two or more fungal species are present in a woody resource, interactions between fungal species may occur that also affect the rate of decay. Freshly fallen wood may already contain established fungal species, or latently present fungal propagules which will be amongst the earliest colonizers (Boddy 2001, Parfitt et al. 2010). Furthermore, a number of wood-rot fungi have the ability to colonize living trees for instance by pathogenesis through the roots (Stokland et al. 2012) or vectoring by insects (Persson et. al. 2011). These parasitic and / or endophytic fungi may continue to live as decomposers in the fallen tree and hence, have a head start in the competition for available resources in the wood. Other early colonizers include opportunistic fungi and bacteria that grow on easily accessible (hemi-)cellulose and simple soluble substrates (Van der Wal et al. 2007). An already established fungal species may inhibit but also promote the colonization of successor species (Heilmann-Claussen & Boddy 2005). The positive or negative effects on later establishing fungi may depend on alternation of the chemical environment through the production of antibiotics (composition, amount) as well as physical modification of the wood (Niemelä et al. 1995). Pre-emptive competition through the consumption of easily degradable substrates as well as the occupation of space inside the wood also results in a limited availability of substrates for secondary colonizing fungi (Boddy 2001, Payne et al. 2000). Hence, the identity and interactions of species that colonize first, may affect colonization success of later arriving species. This effect is often referred to as a priority effect (Fukami et al. 2010).

In the next phase of wood decay, when two or more wood-decaying higher fungi have been able to colonize wood, competitive interactions continue. This can also affect decay rates e.g. fungi can invest more resources in the production of secondary metabolites than in growth

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and decomposition (Woodward & Boddy 2008). Thus, fungal species composition and interactions may have a strong impact on wood decay during all stages of decomposition.

The aim of this study was to assess the importance of fungal community composition to explain local variation in decay rates of naturally colonized woody resources. A few studies have indicated a possible relation between variation in wood decomposition rate and fungal community composition in naturally colonized logs. These studies used traditional methods to describe the fungal community such as isolation of mycelia by plating wood pieces on agar (Chapela et al. 1988, Boddy et al. 1989). Nowadays fungal communities can be described at much higher resolution using high-throughput DNA sequencing methods which are not biased by morphological or growth characteristics of the fungi. In this field study, we make use of a chronosequence of naturally decaying oak tree stumps in adjacent small-sized forest plots to minimize the variation in abiotic conditions.

Material and methods

Site description and field sampling

A chronosequence of decaying tree stumps of *Quercus robur* (English oak) was established in a forest stand on a sandy soil near Bergharen, The Netherlands (51°51'39 N, 5°40'15 E). The study stand consisted of *Q. robur* (about 70% of the vegetation) mixed with *Rubus fruticosus*, *Sorbus aucuparia*, *Betula pendula*, *Pteridium aquilinum* and *Amelanchier lamarckii*. At this location, three plots were chosen where oak trees had been cut in January 2007, November 2010 and March 2012, hereafter referred to as "old", "young" and "fresh" samples, respectively. Plots were situated next to each other and plot sizes were about 1 ha. At each tree harvest, all trees were cut within a plot. In April 2012, 20 randomly selected stumps (stumps with diameters of <15 cm

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were excluded), were sampled from the 2007 plot, 20 stumps from the 2010 plot, and 6 stumps were sampled from the 2012 plot to represent the starting point of decay. The average height of stumps in the 2007 and 2010 plots was 50 ± 10 cm, and the average height of stumps in the 2012 plot was 27 ± 7 cm. There was no significant relationship between stem height and sapwood or heartwood densities of stumps in the 2012 plots ($P > 0.4$), so we assumed that small differences in stem height of individual stumps were not affecting initial wood densities. The upper 5 cm of the stump was removed with a chain saw to avoid sampling mosses and fungal propagules present on the outer part, and a disc containing the next 3 cm was collected. Diameter of wood discs was 20 ± 2 cm in the 2007 plots, 21 ± 4 cm in the 2010 plots and 22 ± 3 cm in the 2012 plots. Discs were stored in plastic bags at -20°C until analyses.

Wood density and moisture content analyses

For each disc, a wedge-shaped piece (1/8 of the total disc) representing as much as possible all fungal decay patterns (e.g. interactions zones, type of wood decay) present in the whole disc was cut out and separated into sapwood, heartwood and, if still present, bark (Fig. 1). Volumes of each segment were calculated using Archimedes' volume displacement method. All samples were then oven dried at 70°C for three days and the density of each segment was calculated as dry weight per unit volume (g/cm^3). Moisture content (%) was calculated as ((mass wet wood - mass dry wood) / dry wood) * 100%.

Sample preparation

From each disc, sawdust samples were taken using an electric drill (bit diameter 8 mm). Sawdust from sapwood and heartwood were separately collected and the drill bit was sterilized between samples with ethanol. At least 15 drilled holes were made in both heartwood and sapwood. The

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131 resulting sawdust samples were pooled resulting in two samples per disc: one from heartwood
132 and one from sapwood. Samples were stored at -20°C until further analyses (Fig. 1).
133
134 DNA extraction, amplification and sequencing
135 Sapwood sawdust samples were frozen in liquid nitrogen and ground into a fine powder.
136 Heartwood samples were excluded for further analyses (see Results section below). DNA was
137 isolated from 0.15 g fresh weight of sapwood samples using the PowerSoil DNA Isolation kit
138 according to the manufacturer's instructions (MO BIO Laboratories, Inc.), with some
139 modifications: after adding solution C1 (causing cell lysis), samples were incubated at 60°C for
140 30 min., and after adding solution C6 (releasing DNA from spin filter), samples were incubated
141 at 30°C for 10 min. The nuclear rDNA internal transcribed spacer (ITS) region was amplified
142 using the fungal-specific primer pair fITS9 and ITS4 (Ihrmark et al. 2012). Adapter sequences
143 were added to the primers as recommended by Roche as well as 6-bp tags specific for each
144 sample. PCR reactions were performed in 25 µl reaction mixtures and contained 400 µM of each
145 dNTP, 1 U of FastStart Expand High Fidelity polymerase (Roche Applied Sciences, Indianapolis,
146 IN), 2.5 µl 10 x PCR buffer with $MgCl_2$, 10 µM of each of the two primers and 1 µl DNA (1-10
147 ng). The temperature cycling PCR conditions were: denaturation at 95°C for 5 min, followed by
148 30 cycles of 95°C for 30 s, 58°C for 30 s and 72°C for 1 min. The final extension step was 72°C
149 for 10 min. After confirming the presence of expected sizes of PCR products by agarose- gel
150 electrophoresis, PCR products from 4 reactions were pooled per sample and purified using a
151 QIAquick PCR Purification Kit (Qiagen). DNA in samples was quantified by a fluorescence-
152 based method (Pico Green assay) and the samples were sequenced (Macrogen Inc. Company,
153 South Korea) on a Roche 454 automated sequencer and GS FLX system using titanium chemistry
154 (454 Life Sciences, Branford, CT).

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156 Bioinformatics

157 Sequences and quality information were extracted from the Standard Flowgram Format (SFF)
158 files using the SFF converter tool in the Galaxy interface (Goecks et al. 2010). The 454 SFF files
159 are deposited in the European Nucleotide Archive
160 (http://www.ebi.ac.uk/ena/data/view/PRJEB4497). Sequences were analyzed using the Qiime
161 version 1.2.1 scripts (Caporaso et al. 2010), which were made available in the Galaxy interface.
162 Quality filtering of the sequences involved the removal of short sequences (< 200 bp), sequences
163 with low read quality, and sequences containing homopolymers or ambiguous characters
164 exceeding six nucleotides. The sequences were also checked for PCR chimeras using UCHIME
165 version 4.2.40 (Edgar et al. 2011). The sequences passing the quality control thresholds were
166 clustered into operational taxonomic units (OTUs) using USEARCH version 5.2.236 (Edgar,
167 2010) with a minimum sequence identity cutoff of 97%. Sequences within clusters of dominant
168 OTUs (accounting for ≥ 10% of all the sequences in each sample) were grouped based on %
169 identity scores in ClustalX v.2.1 and manually checked and blasted in the UNITE database
170 (Abarenkov et al., 2010) to confirm that sequences in each OTU resulted in the same taxonomic
171 identity. The average length of the ITS sequences passing the filtering step was 380 bp. For each
172 OTU, the most abundant sequence was selected as a representative for all sequences within an
173 OTU. Taxonomy was assigned to representative sequences by comparing them with known
174 reference sequences in the UNITE and GenBank (NCBI) database using the Blastn algorithm.
175 Sequences were, whenever possible, identified to the species (> 98% similarity) or genus (94-
176 97% similarity) level. The relative abundance of each OTU was calculated by dividing the
177 number of sequences per OTU by the total number of sequences per sample.

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Enzyme assays

Enzyme activities (laccase, manganese peroxidase, cellulase and hemicellulase) were assayed spectrophotometrically in the same extracts according to Van der Wal et al. (2007). Briefly, 8 ml of milliQ water was added to 1 g of sawdust and shaken for 1 h at room temperature, and then the slurry was pressed over a stainless steel filter (containing pores with a diameter of 2 mm). The supernatants were kept at -20°C until analysis of enzyme activities. Laccase activity was measured via oxidation of ABTS (2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)), and manganese peroxidase activity was measured via the oxidative coupling of DMAB (3-dimethylaminobenzoic acid) and MBTH (3-methyl-2-benzothiazolinone hydrazine hydrochloride) in the presence of Mn^{2+} and H_2O_2. The activity of endo-1,4-ß-glucanase as an indicator of cellulase was estimated using carboxymethyl cellulose linked with Remazol brilliant blue R (Azo-CMCellulose, Megazyme, Bray, Ireland), and the activity of endo-1,4-ß-xylanase (xylanase) as indicator of hemicellulase was estimated by using birchwood xylan linked with Remazol brilliant blue (Azo-xylan, Megazyme, Bray, Ireland) as a substrate.

Data analysis and statistics

The relation between the percentage of mass loss, wood moisture content, diameter, OTU richness, fungal diversity and fungal evenness was calculated by linear regression using SPPS (version 20.0.0). Statistical significance was assumed at $P < 0.05$.

Ordination analyses were performed in Canoco version 4.5. To identify if samples with the same “stump age” grouped together based on the composition of their fungal communities, a Detrended Correspondence Analysis (DCA) was performed. Canonical Correspondence Analysis (CCA) was used to test if the composition of the fungal community is related to the percentage of

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mass loss, tree diameter or wood moisture content, and to extracellular enzyme activities. Significance of canonical axes was assessed by the forward approach using Monte Carlo permutation tests under the reduced model. CCA was performed separately on the samples collected from the 2007 and 2010 plots.

Fungal diversity was calculated as the Shannon index $H' = -\Sigma(p_i \times \ln p_i)$, where p_i represents the relative abundance of species (OTU) i, and the Shannon's equitability (evenness) $E_H = H'/\ln S$, where S represents the total number of species (OTUs) present in the community.

Co-occurrence analysis of fungal communities were performed by a C-score analysis as well as a checkerboard index using default settings in EcoSim version 7.0 (Gotelli & Entsminger 2009). In a competitively structured community, both the observed C-score and checkerboard index are significantly larger than values derived from a community assembled in a random pattern. Samples with the same "stump age" were grouped and each group was analyzed separately. Species that occur in less than three samples (stumps) were removed from each matrix since they are unlikely to provide biologically meaningful information about co-occurrence.

Results

Wood densities

Heartwood density of old samples of oak stumps was almost the same as that of fresh samples (Fig. 2, Welch's test, $P = 0.16$). In contrast, the sapwood density of fresh samples was significantly higher than in young and old samples (Fig. 2, Welch's test, $P < 0.001$). Remarkably, sapwood densities between young and old samples did not significantly differ ($P = 0.57$). Since heartwood was apparently hardly degraded in 5 years, we focused our measurements and data analyses only on sapwood samples.

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226 Relation between sapwood decay, diameter and moisture content

227 For both young and old samples, there was no significant relationship between sapwood decay
228 and diameter of the tree stump (Appendix A, Fig. A1A & A1C). In young samples, we found a
229 significant positive relation between sapwood moisture content and wood decay (Appendix A,
230 Fig. A1B, $P = 0.02$) but we did not find this relation for old stumps (Appendix A, Fig. A1D, $P =$
231 0.45).

232

233 Fungal community composition and stump age

234 In total 425,766 quality sequences were obtained from 42 sapwood samples, and the mean
235 number of sequences per sample was 10,198. We identified in total 447 different OTUs
236 (Supplement 1) of which 262 were ascomycetes, 148 basidiomycetes, 7 zygomycetes, 3
237 glomeromycetes, 1 chytridiomycete and 26 could not be identified. In fresh samples 231 OTUs
238 were identified and the most abundant OTUs consisted of only 10 OTUs of which most had
239 sequence similarities with early successional fungal species such as sugar fungi (yeasts),
240 endophytes and plant parasites. Only one OTU had sequence similarities with a white rot fungus
241 (Appendix A, Fig. A2; Appendix B & C). In young samples, 270 OTUs were identified of which
242 26 OTUs comprised the most abundant ones. Eleven of these OTUs had the highest match with
243 saprotrophic fungi, of which ten were assigned to white rot fungi, two OTUs were assigned to
244 parasites, and two OTUs were assigned to fungi with both saprotrophic and parasitic abilities
245 (Fig. 3, Appendix B & D). In old samples, 243 OTUs were identified and the most abundant
246 OTUs consisted of 18 OTUs. Eight of these OTUs had the highest match with saprotrophic fungi,

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of which seven were assigned to white rot fungi. One OTU was assigned to a fungus with both saprotrophic and parasitic abilities (Fig. 4, Appendix B & E). In fresh, young and old sapwood samples 30%, 23% and 44% of the abundant OTUs could not be identified to the genus level. Most of the dominant OTUs were found in only one or two samples, showing that the abundant OTUs were not equally distributed among stumps. However, two OTUs were dominantly present in all stumps (fresh, young and old), i.e. *Mollisia sp*. and an unidentified *Helotiales* (Appendix B).

The fungal community composition of fresh samples grouped together on the left side in the DCA plot (Fig. 5). Young samples showed overlap in species composition both with fresh and old samples; old samples tended to group together on the right side of the DCA biplot. Therefore, the first DCA axis may represent a successional gradient of fungal communities. On the vertical axis young and old samples are much more spread out than fresh samples, and this can be interpreted as an increase in the variation in fungal community composition among young and old stumps.

Both the C-score and checkerboard pair analysis from the fresh and young samples showed that the fungal communities were non-randomly structured since both values were significantly higher compared to the mean values derived from the randomized communities. In old samples the observed community matrix could not be separated from the values derived from the randomized community (Appendix F).

Effect of fungal community composition on sapwood decay rates

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In old samples the number of OTUs (OTU richness) per sample was positively related with sapwood decay (Appendix A, Fig. A3, P = 0.04), which was not explained by a significant relationship between number of reads per sample and OTU richness (P>0.05). For both young and old samples, there was no relation between fungal diversity and / or fungal evenness with sapwood decay (P> 0.05).

Forward selection in CCA showed that fungal community composition was significantly correlated with sapwood decay in old samples (P = 0.02), explaining 10% of the total variance in species composition. Fig. 4 reveals that the most abundant OTUs in the samples that were hardly decomposed, consisted of *Phlebia radiata*, and in the samples that had strongly decomposed an two unidentified *Sordariales* and one unidentified *Heliotales* both belonging to the *Ascomycota* were most abundant. One OTU identified as *Trametes versicolor* was almost exclusively present in samples with similar mass loss (47 and 54%). In some samples showing different mass losses the same OTU, identified as *Mollisia sp.*, was among the dominant ones, but then always in combination with another fungal species. In young samples forward selection in CCA did not reveal a relationship between fungal community composition and wood decay (P> 0.89). In accordance, Fig. 3 shows that *Panellus stipticus* was abundant throughout several samples showing different mass losses, all in combination with an OTU identified as *Trametes versicolor*. Hence, in young samples, a similar composition of abundant fungal species in different stumps did not result in similar sapwood decay rates.

Other variables (moisture content and tree diameter) were not significantly related to fungal community composition (CCA, P > 0.17). Enzyme activities were also not significantly related to fungal community composition or wood decay (CCA, P > 0.2). Laccase activity was in general low in most samples (ranging from 0 to 0.43 nmol $g^{-1}h^{-1}$). Remarkably, the highest laccase activity was found in the least decayed old sample (5.61 nmol g^{-1} h^{-1}).

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Discussion

The variation in initial sapwood decay was significantly correlated with wood moisture content, whereas variation in later stages of sapwood decay was significantly related to the composition and OTU richness of the fungal community. This study is the first to indicate that the most important factors explaining variation in wood decay rates may change over time. Earlier studies focused only on the effect of abiotic factors and differences in traits of tree species (Mackensen et al. 2003, Brischke & Rapp 2008, Cornwell et al. 2009) In addition, other studies did not include a time series (Chapela et al. 1988, Boddy et al. 1989, Lindner et al. 2011), concentrated on decomposition stages instead of time of decomposition (Rajala et al. 2012) or used only fruiting body data and culture methods for detection of fungi (Chapela et al. 1988, Boddy et al. 1989). Despite the fact that early-successional fungal communities differed across individual young stumps, there was no indication that differences in community composition had an impact on initial sapwood decay. Instead, wood moisture content could partly (27%) explain variation in wood decay. However, the importance of moisture as explaining factor for longer term wood decay is unclear as there was no significant relationship with decay in older stumps.

As wood decomposition proceeded, we observed a significant relationship between the composition of the fungal community and mass loss. Potential mechanisms explaining this relation could be based on specific traits of fungal species, e.g. the presence or absence of a strong decay species (Lindner et al. 2011) or interactions between fungal species during community assembly processes (Fukami et al. 2010, Van der Wal et al. 2013). For instance, *Phlebia radiata* was the dominant fungus in samples that were hardly decomposed (Fig. 4). This fungus is known for its replacing ability at later stages in community development by lysing other

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315 wood-decaying fungi (Rayner & Todd 1979). This may indicate that fungi that colonized first
316 caused hardly any mass loss (i.e. consuming only the easily available carbon) and during
317 replacement by *P. radiata*, fungal hyphae instead of wood are consumed. Alternatively, during
318 competition fungi may invest more in defensive metabolites such as melanin than in growth and
319 decomposition. This idea is supported by the high laccase activity found in the least decayed
320 sample as laccases are thought to be involved in the formation of melanin (Baldrian 2006).
321 Most of the abundant OTUs were only found in one or two samples, showing that
322 dominant fungal species were unique on individual stumps. This reflects the stochastic nature of
323 fungal colonization (see discussion below). However, two OTUs, both assigned to the order
324 *Helotiales*, were dominantly present in fresh, young and old stumps. Members of the *Helotiales*
325 include saprotrophic-, parasitic- and endophytic fungi (Wang et al. 2006, Tedersoo et al. 2009).
326 Since these fungi were already present in freshly cut stumps, it may indicate that they first
327 displayed the endophytic lifestyle. This lifestyle will put these fungi in a good position to shift to
328 saprophytism after the tree died, given them a competitive advantage over other later-arriving
329 fungi for available resources in the wood (the so-called priority effect). In addition, their presence
330 may influence the success of later establishing fungi by altering the chemical environment
331 through the production of antibiotics or by physically modifying the wood (Niemelä et al. 1995,
332 Heilmann-Claussen & Boddy 2005). The effect of relative abundance of the *Helotiales* species on
333 wood decay did not show a clear pattern. The OTU assigned as *Mollisia sp.* was present in
334 quickly as well as in slowly decaying stumps, whereas the OTU assigned as *unidentified*
335 *Helotiales (4)* was present in stumps that lost already at least 40% of their mass in both young
336 and old stumps. Therefore, we cannot draw any conclusions about the effect of *Helotiales* fungi
337 on wood decay rate, but because of their abundance throughout all decay stages it is worthwhile
338 to include them in controlled wood decay experiments.

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A positive relation between OTU richness and mass loss was observed in old stumps (Fig. S3). We only found this relation based on richness, indicating that the presence of a certain species is more important for this relation than its abundance. Also Rajala et al. (2011) observed an increase of rRNA-based fungal richness with wood decay in naturally decaying spruce logs, but also the opposite has been found in wood that was artificially inoculated with different fungal species (Fukami et al. 2010). A positive relation between species richness and wood decay may point to additive or synergistic activities of fungal species (Fukasawa et al. 2011, Hättenschwiler et al. 2011). In wood, however, competitive interactions between wood-rot fungi are very common, can take place at all stages of wood decomposition and have a strong impact on fungal community composition (Boddy 2001). However, the fact that the fungal communities in old stumps could not be separated from a randomly assembled community in the co-occurrence analysis may indicate that competitive interactions are not the most dominant type of interaction in the old stumps (Appendix F). Indeed, in fresh and young samples, both the C-score and the checkerboard index showed that fungal communities were non-randomly structured, which could point to competitive species interactions (Gotelli & Entsminger 2009). Therefore, the strength of competitive interactions between fungi in decaying tree stumps may level off with increased wood decay. Alternatively, advanced decayed wood may cause an increase in the heterogeneity of wood polymer structures, creating a greater range of fungal microhabitats.

In wood that most quickly decomposed, 95% of the fungal community consisted of taxa belonging to the Ascomycota (Appendix G). It is generally assumed that basidiomycete fungi have greater enzyme diversity than ascomycetes and are thus causing more weight loss (Osono et al. 2003). Our results may imply that also ascomycete fungi can generate fast decomposition rates. Only one other study addressed the possible importance of ascomycete fungi in natural wood decay (Boddy et al. 1989). Another possibility is that these ascomycete fungi have replaced

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363 the basidiomycetes that first decayed the wood rapidly. Wood decay abilities of ascomycetes
364 need to be further investigated under controlled conditions.

365 Usually the differentiation between sapwood and heartwood is not made to determine the
366 decay rate. Here we observed a strong decomposition resistance of heartwood. This is probably
367 due to the presence of organic toxic compounds in oak heartwood, which inhibits microbial
368 growth (Schmidt 2006). In contrast, the sapwood was quickly decaying during the first year and
369 thereafter the average sapwood decay leveled off. Lumping the decay rate of heartwood and
370 sapwood may thus result in an underestimation of the decay rate of sapwood.

371 The relation between fungal community composition and wood mass loss was significant,
372 but only explained 10% of the total variance. This is very likely due to the huge differences of
373 fungal communities among individual tree stumps (Appendix B, Fig. 3 & 4). Large differences of
374 fungal communities across dead wood units have been reported before on spruce logs (Kubartová
375 et al. 2012). The large variation among tree stumps could be due to stochastic fungal colonization
376 processes of fungi via for instance dead tree roots, infection by wind- or insect-dispersed spores
377 or mycelial fragments (Persson et al. 2011) or, as indicated above, some fungi may be already
378 present in living trees (Parfitt et al. 2010). A further increase in variation among fungal
379 communities during later stages of decay can be expected due to subsequent community
380 assembly processes (Van der Wal et al. 2013).

381 In summary, current wood decay models do not explain the variation in wood mass loss
382 that is found on a local scale, consequently restricting the possibility to reliably estimate carbon
383 sequestration rates at larger temporal and spatial scales. Our results show that moisture content
384 may be a reliable predictor for wood mass loss during the first years of wood decay, but after this
385 period, the fungal community composition is contributing to explain the variance in wood mass

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386 loss. The identity of fungal species and their richness seem to affect decomposition. The effect of
387 ecologically relevant combinations of fungi on wood decay rates should be assessed under
388 controlled conditions to formulate rules that can be used to optimize current wood decay models.
389

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397

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Appendices

Appendix A: Three figures showing 1) relationships between sapwood decay, wood moisture content and stump diameter, 2) percentage of most abundant OTUs in freshly cut trees and 3) relationship between sapwood decay and OTU richness in old stumps.

Figure A1: Relation between sapwood decay in oak stumps (presented as % of wood mass loss), wood moisture content and tree stump diameter. A & B: measured in young samples (1.4 years since cutting), C & D: measured in old samples (5.3 years since cutting).

Figure A2: Percentage of most abundant operational taxonomic units (OTUs) in sapwood samples collected from freshly cut oak trees. (A): Ascomycota, (B): Basidiomycota. Numbers between brackets indicate OTU number (see Appendix B) of OTUs that could not be identified to the species level.

Figure A3: Relation between sapwood decay (presented as % of wood mass loss) and the number of OTUs (OTU richness) measured in old samples (5.3 years since cutting) taken from oak stumps.

Appendix B: Most abundant OTUs (representing ≥ 10% of the total number of sequences) in decaying oak sapwood. Ecological groups are defined as: p = parasite, wr = white-rot fungus, sr = soft-rot fungus, sa = fungus with saprotrophic abilities, sp = combining both saprotrophic and parasitic abilities, en = endophytic fungus. F = fresh, Y = young and O= old stumps, see Material and Methods section for description of sampling methods.

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Appendix C: Percentage of most abundant operational taxonomic units (OTUs) in samples collected from freshly cut oak stumps. (A): Ascomycota, (B): Basidiomycota. Light grey boxes represent OTUs that overlap with young and old oak stumps.

Appendix D: Percentage of most abundant operational taxonomic units (OTUs) in samples collected from young oak stumps. (A): Ascomycota, (B): Basidiomycota. Light grey boxes represent OTUs that overlap with fresh and old oak stumps; dark grey boxes represent OTUs that overlap with old oak stumps.

Appendix E: Percentage of most abundant operational taxonomic units (OTUs) in samples collected from old oak stumps. (A): Ascomycota, (B): Basidiomycota. Light grey boxes represent OTUs that overlap with fresh and young oak stumps; dark grey boxes represent OTUs that overlap with young oak stumps.

Appendix F: Co-occurrence analysis of sapwood-inhabiting fungal community data using C-score and checkerboard index analysis. * Only OTUs with 3 or more occurrences are included. † Estimated values from 5000 Monte Carlo randomizations. SES = Standardized effect size.

Appendix G: Number of sequence reads per phylum (A=Ascomycota, B = Basidiomycota, unk = unidentified sequence reads, G= Glomeromycota, Z= Zygomycota and C =Chytridiomycota), ratio between Basidiomycota and Ascomycota, H diversity, E eveness, the % Ascomycota and the % mass loss per sample. F=fresh, Y=young and O=old stumps.

Supplements

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562 **Supplement 1:** Representative ITS sequences of operational taxonomic units (OTUs) obtained
563 from decaying oak stumps.

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Figure legends

Figure 1: Sampling design for a wood disc collected from an oak tree stump. The wedge-shaped piece (S) was cut out and separated into heartwood, sapwood and bark and for every separate piece the moisture content and density was determined. Drill holes are made to extract sawdust for further lab analyses (see material and methods).

Figure 2: Density of heartwood (red dots, red number indicates the average of the heartwood samples) and sapwood (green dots, green number indicates the average of the sapwood samples) from freshly cut samples, young samples (1.4 years since cutting) and old samples (5.3 years since cutting) of oak stumps.

Figure 3: Percentage of most abundant operational taxonomic units (OTUs) in samples collected from young oak stumps (1.4 years since cutting). (A): Ascomycota, (B): Basidiomycota. Numbers between brackets indicate OTU number (see Appendix B) of OTUs that could not be identified to the species level. On the horizontal axis the percentage of sapwood mass loss per sample is presented. Bars with white dots represent fungal OTUs that overlap between young and old samples.

Figure 4: Percentage of most abundant operational taxonomic units (OTUs) in samples collected from old oak stumps (5.3 years since cutting). (A): Ascomycota, (B): Basidiomycota. Numbers between brackets indicate OTU number (see Appendix B) of OTUs that could not be identified to the species level. On the horizontal axis the percentage of wood mass loss per sample is presented. Bars with white dots represent fungal OTUs that overlap between young and old samples. * indicates that nearly all sapwood was decayed, only about 0.5 g of sapwood could be sampled to perform the DNA extraction.

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589 **Figure 5:** Detrended Correspondance Analysis (DCA) biplot showing the variation of the fungal
590 community composition in sapwood samples taken from oak stumps. Black dots: samples
591 collected from freshly cut trees, red dots: young samples (1.4 years since cutting), blue dots: old
592 samples (5.3 years since cutting). Samples closer together have more similar fungal communities.

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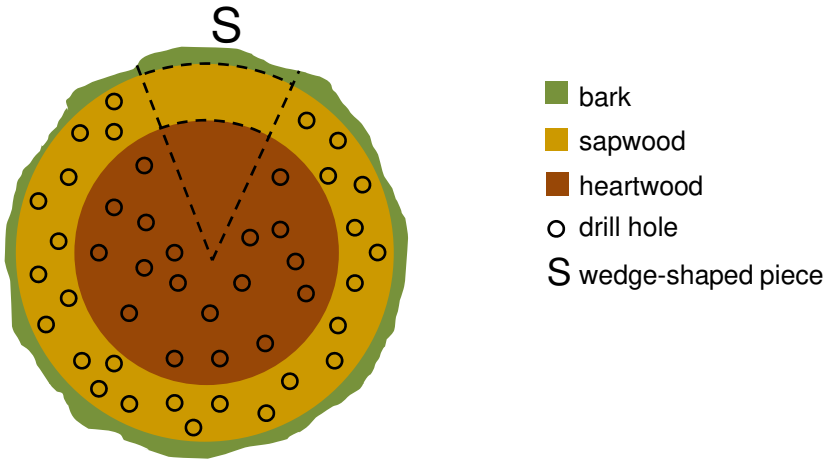
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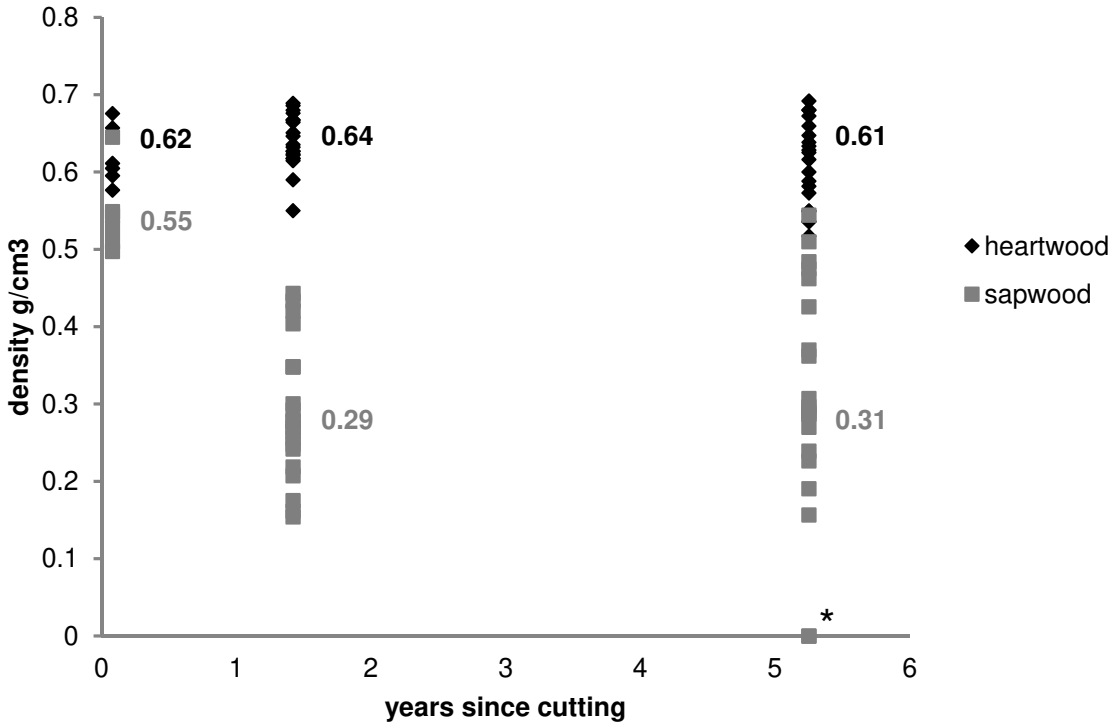
S wedge-shaped piece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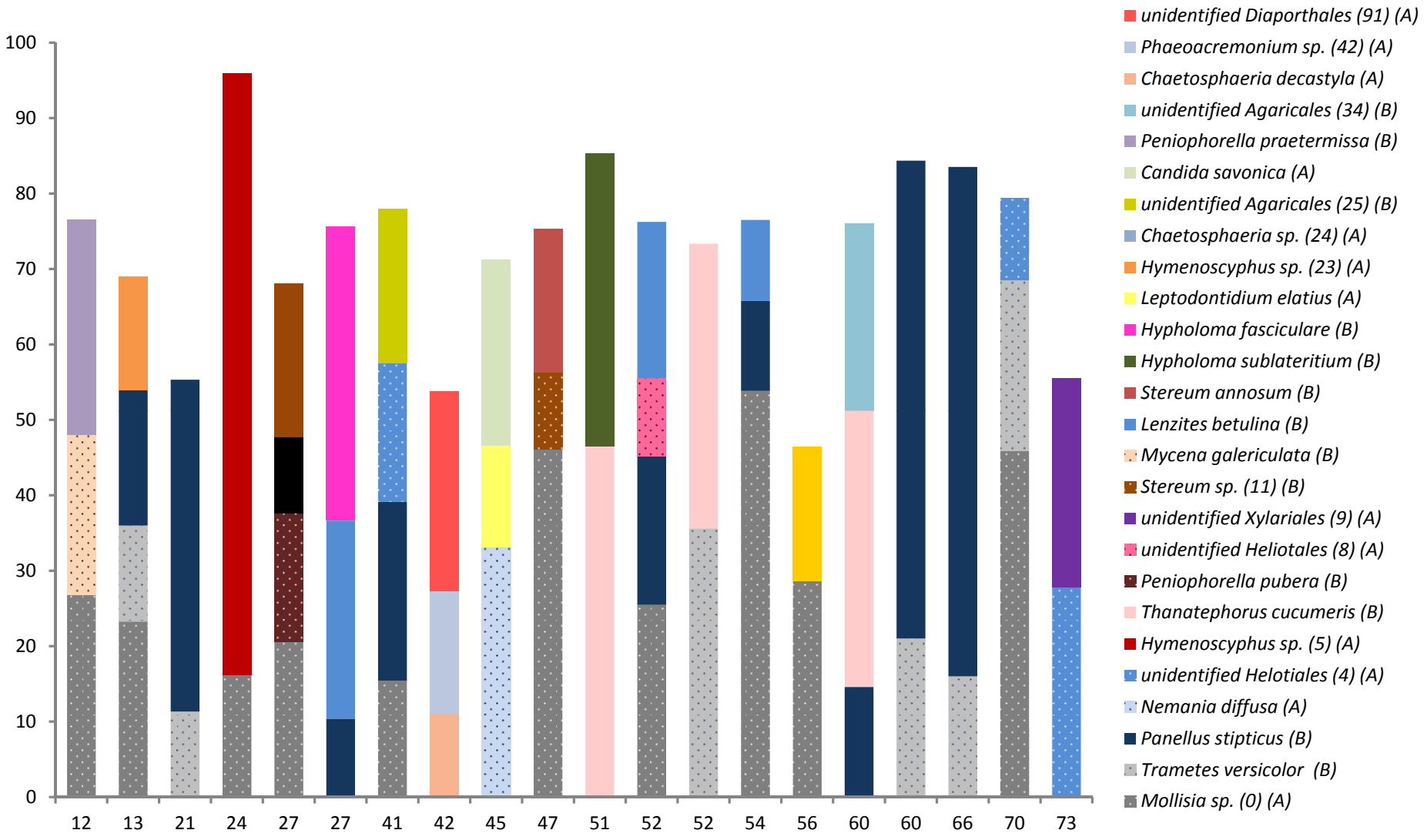
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unidentified Diaporthales (91) (A)
Phaeoacremonium sp. (42) (A)
Chaetosphaeria decastyla (A)
unidentified Agaricales (34) (B)
Peniophorella praetermissa (B)
Candida savonica (A)
unidentified Agaricales (25) (B)
Chaetosphaeria sp. (24) (A)
Hymenoscyphus sp. (23) (A)
Leptodontidium elatius (A)
Hypholoma fasciculare (B)
Hypholoma sublateritium (B)
Stereum annosum (B)
Lenzites betulina (B)
Mycena galericulata (B)
Stereum sp. (11) (B)
unidentified Xylariales (9) (A)
unidentified Heliotales (8) (A)
Peniophorella pubera (B)
Thanatephorus cucumeris (B)
Hymenoscyphus sp. (5) (A)
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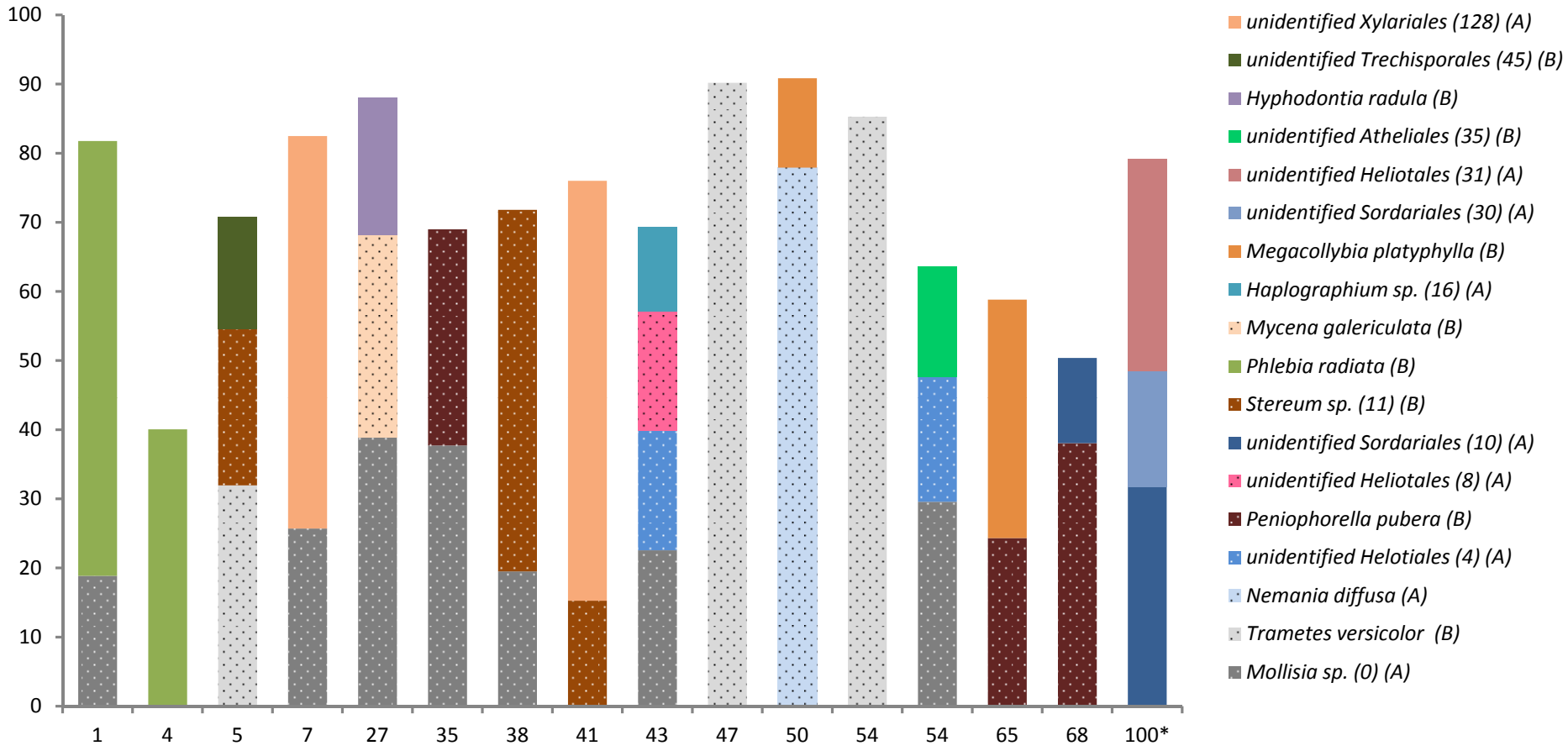
Panellus stipticus (B)
Trametes versicolor (B)
Mollisia sp. (0) (A)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Mycena galericulata (B)
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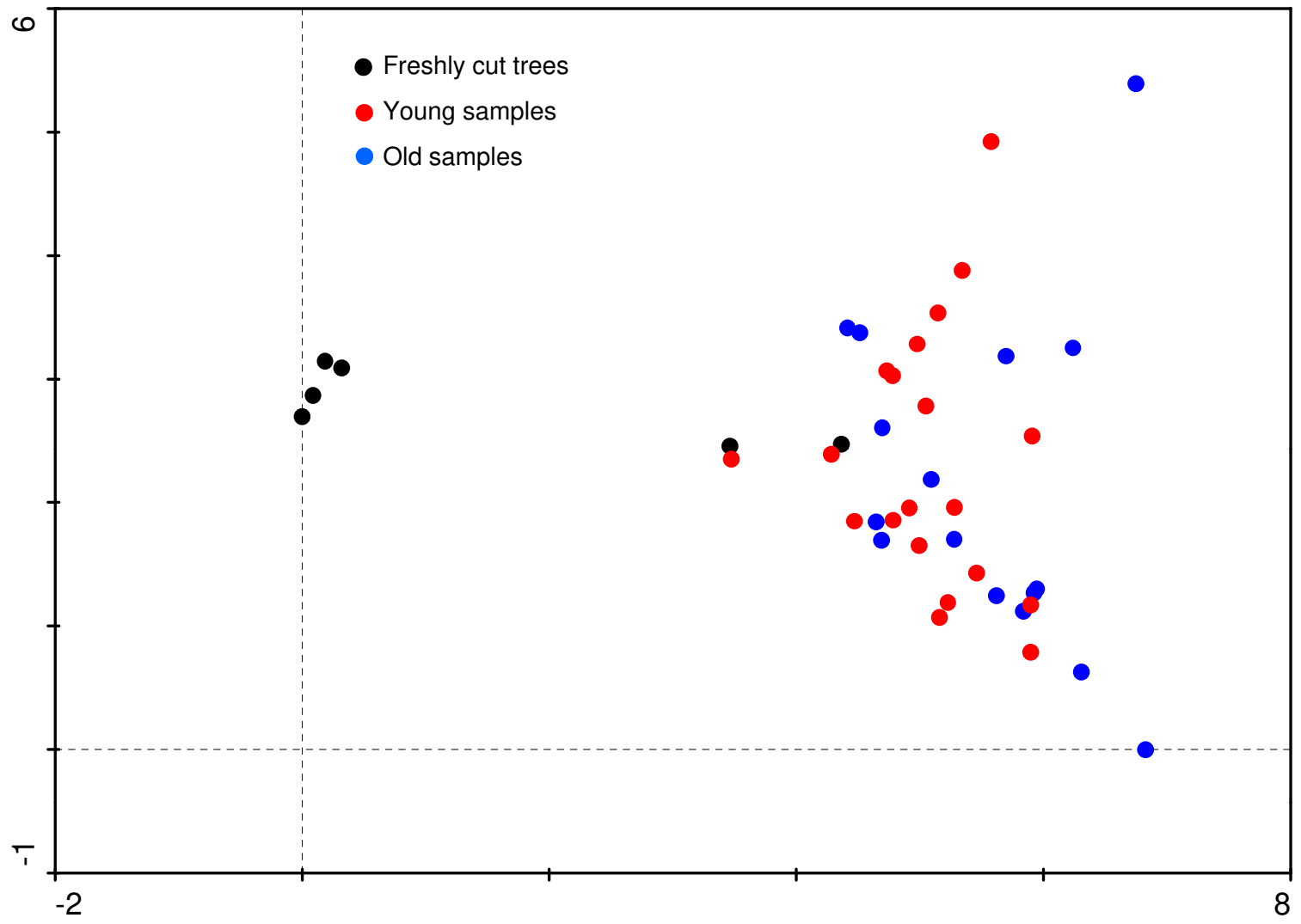
Freshly cut trees
Young samples
Old samples
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