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 that are the major wood decomposers. We examined the factors explaining variation in sapwood decay in oak tree stumps two and five 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 nonrandomly 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; Quercus robur; saprotrophic fungi; sapwood; wood decomposition.

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 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. 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 among 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-Clausen and 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). Preemptive 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 (Payne et al. 2000, Boddy 2001). 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 and decomposition (Woodward and 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 lamarkii*. 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 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 (mean ± SD), 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 (see Plate 1). 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 (one-eighth 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 mass per unit volume (g/cm³). Moisture content (%) was calculated as ([(wet wood mass − dry wood mass)/dry wood mass] × 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 resulting sawdust samples were pooled resulting in two samples per disc: one from heartwood and one from sapwood. Samples were stored at −20°C until further analyses (Fig. 1).

**DNA extraction, amplification, and sequencing**

Sapwood sawdust samples were frozen in liquid nitrogen and ground into a fine powder. Heartwood samples were excluded for further analyses (see Results). DNA was isolated from 0.15 g fresh mass of sapwood samples using the PowerSoil DNA Isolation kit according to the manufacturer’s instructions (MO BIO Laboratories, Carlsbad, California, USA), with some
modifications: after adding solution C1 (causing cell lysis), samples were incubated at 60°C for 30 min, and after adding solution C6 (releasing DNA from spin filter), samples were incubated at 30°C for 10 min. The nuclear rDNA internal transcribed spacer (ITS) region was amplified using the fungal-specific primer pair fITS9 and ITS4 (Ihrmark et al. 2012). Adapter sequences were added to the primers as recommended by Roche as well as 6-base-pair (bp) tags specific for each sample. Polymerase chain reactions (PCRs) were performed in 25-μL reaction mixtures and contained 400 μmol/L of each dNTP, 0.2 μL of FastStart Expand High Fidelity polymerase (Roche Applied Sciences, Indianapolis, Indiana, USA), 2.5 μL 10× PCR buffer with MgCl₂, 10 μmol/L of each of the two primers, and 1 μL DNA (1–10 ng). The temperature cycling PCR conditions were denaturation at 95°C for 5 min, followed by 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 for 10 min. After confirming the presence of expected sizes of PCR products by agarose-gel electrophoresis, PCR products from four reactions were pooled per sample and purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). DNA in samples was quantified by a fluorescence-based method (Pico Green assay) and the samples were sequenced (Macrogen Company, Seoul, South Korea) on a Roche 454 automated sequencer and GS FLX system using titanium chemistry (454 Life Sciences, Branford, Connecticut, USA).

Bioinformatics

Sequences and quality information were extracted from the Standard Flowgram Format (SFF) files using the SFF converter tool in the Galaxy interface (Goecks et al. 2010). The 454 SFF files are deposited in the European Nucleotide Archive (data available online). Sequences were analyzed using the Qiime version 1.2.1 scripts (Caporaso et al. 2010), which were made available in the Galaxy interface. Quality filtering of the sequences involved the removal of short sequences (<200 bp), sequences with low read quality, and sequences containing homopolymers or ambiguous characters exceeding six nucleotides. The sequences were also checked for PCR chimeras using UCHIME version 4.2.40 (Edgar et al. 2011). The sequences passing the quality control thresholds were clustered into operational taxonomic units (OTUs) using USEARCH version 5.2.236 (Edgar 2010) with a minimum sequence identity cutoff of 97%. Sequences within clusters of dominant OTUs (accounting for ≥10% of all the sequences in each sample) were grouped based on percentage of identity scores in ClustalX v.2.1 (Larkin et al. 2007) and manually checked and blasted in the UNITE database (Abarenkov et al. 2010) to confirm that sequences in each OTU resulted in the same taxonomic identity. The average length of the ITS sequences passing the filtering step was 380 bp. For each OTU, the most abundant sequence was selected as a representative for all sequences within an OTU. Taxonomy was assigned to representative sequences by comparing them with known reference sequences in the UNITE and GenBank (NCBI) database using the Blastn algorithm. Sequences were, whenever possible, identified to the species (>98% similarity) or genus (94–97% similarity) level. The relative abundance of each OTU was calculated by dividing the number of sequences per OTU by the total number of sequences per sample.

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 (Millipore, Amsterdam, The Netherlands) 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 hydrazide hydrochloride) in

4 http://www.ebi.ac.uk/ena/data/view/PRJEB4497
the presence of Mn²⁺ and H₂O₂. The activity of endo-
1,4-β-glucanase as an indicator of cellulase was esti-
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mated using carboxymethyl cellulose linked with Remazol
bright blue R (Azo-CMCellulose, Megazyme, Bray, Ireland), and the activity of endo-1,4-β-xylanase (xyla-
1,4-β-xylanase (xylanase) as an indicator of hemicellulase was estimated by
using birchwood xylan linked with Remazol brilliant blue (Azoxylan, 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 SPSS (version 20.0.0: IBM SPSS,
Chicago, Illinois, USA). Statistical significance was
assumed at *P* < 0.05. Ordination analyses were
performed in Canoco version 4.5 (ter Braak and
Smilauer 2006). To identify if samples with the same
“stump age” grouped together based on the composition
of their fungal communities, a detrended correspond-
ence analysis (DCA) was performed. Canonical corre-
spondence analysis (CCA) was used to test if the
composition of the fungal community is related to the
percentage of mass loss, tree diameter or wood moisture
content, and to extracellular enzyme activities. Signifi-
cance 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

\[ H' = -\sum (p_i \times \ln p_i) \]

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.

**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 five years, we focused our
measurements and data analyses only on sapwood
samples.

**Relation between sapwood decay, diameter, and moisture content**

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

**Fungal community composition and stump age**

In total, 425 766 quality sequences were obtained
from 42 sapwood samples, and the mean number of
sequences per sample was 10 198. We identified in total
447 different OTUs (Supplement) of which 262 were
ascomycetes, 148 basidiomycetes, 7 zygomycetes, 3
glomeromycetes, 1 chytridiomycete, and 26 could not
be identified. In fresh samples, 231 OTUs were
identified and the most abundant OTUs consisted of
only 10 OTUs of which most had sequence similarities
with early successional fungal species such as sugar
fungi (yeasts), endophytes and plant parasites. Only
one OTU had sequence similarities with a white rot
fungus (Appendix A: Fig. A1B; Appendixes B and C). In
young samples, 270 OTUs were identified of which 26
OTUs comprised the most abundant ones. Eleven of
these OTUs had the highest match with saprotrophic
fungi, of which 10 were assigned to white rot fungi, two

OTUs were assigned to parasites, and two OTUs were assigned to fungi with both saprotrophic and parasitic abilities (Fig. 3, Appendix B and D). In old samples, 243 OTUs were identified and the most abundant OTUs consisted of 18 OTUs. Eight of these OTUs had the highest match with saprotrophic fungi, 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). 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.

**Fig. 3.** Percentage of most abundant operational taxonomic units (OTUs) in samples collected from young oak stumps (1.4 years since cutting). OTUs are identified as Ascomycota (A) or Basidiomycota (B). Numbers in parentheses 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 hatching represent fungal OTUs that overlap between young and old samples.
Both the C-score and checkerboard pair analysis from the fresh and young samples showed that the fungal communities were nonrandomly 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**

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 *Heliotiales* both belonging to the *Ascomycota* were most abundant. One OTU identified

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**Fig. 4.** Percentage of most abundant OTUs in samples collected from old oak stumps (5.3 years since cutting). OTUs are identified as Ascomycota (A) or Basidiomycota (B). Numbers in parentheses 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 hatching represent fungal OTUs that overlap between young and old samples. In the bar identified as having 100% wood mass loss, nearly all sapwood was decayed; only about 0.5 g of sapwood could be sampled to perform the DNA extraction.
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$^{-1}$).

**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 and 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 wood-decaying fungi (Rayner and Todd 1979). This may indicate that fungi that colonized first caused hardly any mass loss (i.e., consuming only the easily available carbon) and during replacement by *P. radiata*, fungal hyphae instead of wood are consumed. Alternatively, during competition fungi may invest more in defensive
metabolites such as melanin than in growth and decomposition. This idea is supported by the high laccase activity found in the least decayed sample as laccases are thought to be involved in the formation of melanin (Baldrian 2006).

Most of the abundant OTUs were only found in one or two samples, showing that dominant fungal species were unique on individual stumps. This reflects the stochastic nature of fungal colonization (see discussion below). However, two OTUs, both assigned to the order Helotiales, were dominantly present in fresh, young and old stumps. Members of the Helotiales include saprotrophic, parasitic, and endophytic fungi (Wang et al. 2006, Tedersoo et al. 2009). Since these fungi were already present in freshly cut stumps, it may indicate that they first displayed the endophytic lifestyle. This lifestyle will put these fungi in a good position to shift to saprophytism after the tree died, given them a competitive advantage over other later-arriving fungi for available resources in the wood (the so-called priority effect). In addition, their presence may influence the success of later establishing fungi by altering the chemical environment through the production of antibiotics or by physically modifying the wood (Niemelä et al. 1995, Heilmann-Clausen and Boddy 2005). The effect of relative abundance of the Helotiales species on wood decay did not show a clear pattern. The OTU assigned as Mollisia sp. was present in quickly as well as in slowly decaying stumps, whereas the OTU assigned as unidentified Helotiales (4) was present in stumps that lost already at least 40% of their mass in both young and old stumps. Therefore, we cannot draw any conclusions about the effect of Helotiales fungi on wood decay rate, but because of their abundance throughout all decay stages it is worthwhile to include them in controlled wood decay experiments.

A positive relation between OTU richness and mass loss was observed in old stumps (Appendix A: Fig. A3). 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 nonrandomly structured, which could point to competitive species interactions (Gotelli and 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 heterogene-
ity 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 the basidiomycetes that first decayed the wood rapidly. Wood decay abilities of ascomycetes need to be further investigated under controlled conditions.

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

The relation between fungal community composition and wood mass loss was significant, but only explained 10% of the total variance. This is very likely due to the huge differences of fungal communities among individual tree stumps (Appendix B, Figs. 3 and 4). Large differences of fungal communities across dead wood units have been reported before on spruce logs (Kubartová et al. 2012). The large variation among tree stumps could be due to stochastic fungal colonization processes of fungi via for instance dead tree roots, infection by wind- or insect-dispersed spores or mycelial fragments (Persson et al. 2011) or, as indicated above, some fungi may be already present in living trees (Parfitt et al. 2010). A further increase in variation among fungal communities during later stages of decay can be expected due to subsequent community assembly processes (van der Wal et al. 2013). In summary, current wood decay models do not explain the variation in wood mass loss that is found on a local scale, consequently restricting the possibility to reliably estimate carbon sequestration rates at larger temporal and spatial scales. Our results show that moisture content may be a reliable predictor for wood mass loss during the first years of wood decay, but after this period, the fungal community composition is contributing to explain the variance in wood mass loss. The identity of fungal species and their richness seem to affect decomposition. The effect of ecologically relevant combinations of fungi on wood decay rates should be assessed under controlled conditions to formulate rules that can be used to optimize current wood decay models.

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LITERATURE CITED


**Supplemental Material**

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Appendices A–G and a Supplement are available online: [http://dx.doi.org/10.1890/14-0242.1.sm](http://dx.doi.org/10.1890/14-0242.1.sm)