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Linking molecular deadwood-inhabiting fungal diversity and community dynamics to ecosystem functions and processes in Central European forests

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Abstract Fungi play vital roles in the decomposition of deadwood due to their secretion of various enzymes that break down plant cell-wall complexes. The compositions of wood-inhabiting fungal (WIF) communities change over the course of the decomposition process as the remaining mass of wood decreases and both abiotic and biotic conditions of the wood significantly change. It is currently not resolved which substrate-related factors govern these changes in WIF communities and whether such changes influence the deadwood decomposition rate. Here we report a study on fungal richness and community structure in deadwood of Norway spruce and European beech in temperate forest ecosystems using 454 pyrosequencing. Our aims were to disentangle the factors that correspond to WIF community composition and to investigate the links between fungal richness, taxonomically-resolved fungal identity, and microbial-mediated ecosystem functions and processes by analyzing physico-chemical wood properties, lignin-modifying enzyme activities and wood decomposition rates. Unlike fungal richness, we found significant differences in community structure between deadwood of different tree species. The composition of WIF communities was related to the physico-chemical properties of the deadwood substrates. Decomposition rates and the activities of lignin-modifying enzymes were controlled by the succession of the fungal communities and competition scenarios rather than fungal OTU richness. Our results provide further insights into links between fungal community structure and microbial-mediated ecosystem functions and processes.

Keywords Ecosystem processes · Enzyme activity · Wood decay · Physico-chemical wood properties · Ascomycetes · Basidiomycetes · Pyrosequencing

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Introduction

Deadwood is one of the most important organic carbon pools in forest ecosystems (Floudas et al. 2012). Due to its lignin content of 15–40 %, deadwood is rather difficult to decompose and is therefore an important temporal store of carbon and macronutrients (Kopra and Fyles 2005; Krankina et al. 1999; Sarkanen and Ludwig 1971). Microorganisms, mainly fungi, play crucial roles in forest ecosystems. Their diversity and enzymatic activities constitute the basis for the food-webs in wood and litter (Pollierer et al. 2012; Stokland et al. 2012). Filamentous fungi of the phyla Basidiomycota and (to a lesser

extent) Ascomycota are particularly important in lignin mineralization. White-rot fungi secrete a set of extracellular oxidative enzymes such as laccase (Lac, EC 1.10.3.2), manganese peroxidase (MnP, EC 1.11.1.13) and/or manganese independent peroxidases (MiP) to oxidize the recalcitrant lignin polymer (Hofrichter et al. 2010). In contrast, brown-rot fungi do not secrete these enzymes. Instead, they oxidize lignin via a mechanism relying on hydroxyl radicals, which are generated via the Fenton reaction (Hatakka and Hammel 2011; Jellison et al. 1997; Martinez et al. 2005).

The decomposition processes driven by decomposer organisms are complex and influenced by the host tree species and environmental factors (Boddy 2001). Different fungal species have different capacities for wood decomposition (Valmaseda et al. 1990) and have further been reported to be dependently related to different tree species (Rajala et al. 2010). Moreover, the identity of the host tree determines several key abiotic substrate factors that influence the interactions between the resident microbiota along with their growth, reproduction, and metabolism (Gadd 2010; Kögel-Knabner 2002). These factors include the chemical properties and cell structure of the wood (Schwarze et al. 2000) as well as its pH and water content.

The existing ecological data on wood-inhabiting fungi (WIF) in Central European forests, including information on their diversity patterns, resource use and the determinants of their community structure, was primarily obtained via sporocarp surveys (Blaser et al. 2013; Heilmann-Clausen and Christensen 2003; Müller et al. 2007), which only record the composition of a portion of the actively reproducing fungal community at a specific point in time. However, the macroscopically observable fungal flora may not be fully representative of the fungal community that is present, especially if sampling is only conducted once or over a short period of time (Halme and Kotiaho 2012). In contrast, high-throughput sequencing allows for detailed analysis of community composition and may thus uncover a hitherto concealed fungal diversity residing in deadwood. This approach has been used to study WIF communities within and among Norway spruce (*Picea abies*) logs (Kubartova et al. 2012) and to test the link between fungal life history and population dynamics (Ovaskainen et al. 2013). As expected, these studies revealed much higher fungal species richness than previously published sporocarp surveys and suggested that highly abundant fruiting species may be only weakly representing the fungal community as a whole.

All of the European high-throughput sequencing based studies on WIF conducted to date focused on deadwood of either gymnosperm tree species, *Picea abies*, in a single biome, the boreal forest zone of Northern Europe (Kubartova et al. 2012; Ovaskainen et al. 2010, 2013) or two angiosperm tree species, *Fagus sylvatica* and *Quercus robur* in temperate forests (Hiscox et al. 2015; van der Wal et al. 2015). Therefore, our information on community dynamics of WIF in deadwood of different tree species in the same forest ecosystem at different locations is still limited. Our investigation is also the first to explore the fungal diversity in deadwood of European beech under natural condition using contemporary massively parallel sequencing techniques. In line with recent findings of Ottosson et al. (2015), we also expected deadwood of European beech and Norway spruce species to harbor a significant proportion of rare taxa, ones that are not presented through high sequence abundances. Links between fungal richness, taxonomically resolved community composition, and ecosystem processes are rarely studied, especially under natural conditions (van der Wal et al. 2015).

In this study, we aimed to: (i) using 454 pyrosequencing to compare the diversity and community structure of WIF in deadwood of two silviculturally important tree species found in Central European temperate forests - the coniferous *Picea abies* and the deciduous *Fagus sylvatica*, (ii) disentangling the ecological and environmental factors that correlate with WIF community structure, and (iii) using the resulting data to link WIF richness and taxonomically-resolved identity to microbial-mediated ecosystem functions (lignin-modifying enzyme activities) and processes (wood decomposition rates). We hypothesized that the different wood physico-chemical properties of the two tree species could lead to significant differences in fungal richness and community structure. The different physico-chemical parameters are linked to wood decay and how fungal decomposition of the wood alters their own environment. Specifically during wood decomposition (decreasing of wood density), C/N ratio decreases as a result of N accumulation and at the same time, lignin and wood moisture increase. The degrees how wood physico-chemical properties as well as macro-and micro-nutrient changes may depend greatly on the initial properties of each tree species.

In this study the lignin-modifying enzyme activities (indicator for lignin decomposition) and wood decomposition rates (indicator for decomposition process) were used as a proxy for microbial-mediated ecosystem functions and processes, respectively. Some studies have shown that species richness was found to positively link or promote stability of the ecosystem function in soil systems, however this is still unclear in the case of deadwood-inhabiting fungi under field conditions (van der Heijden et al. 1998; Coleman and Whitman 2005; Proulx et al. 2010; Eisenhauer et al. 2012). In a recent study, it was shown (under an artificial set-up) that fungal diversity was associated with wood decomposition rates in the intermediate decay stages, as determined by respiration rates (Valentin et al. 2014) . Since the loss of microbial biodiversity could alter ecosystem functioning and stability, we expected positive correlations between fungal richness and the activities of lignin-modifying enzymes and wood decomposition rates.

Material and methods

Experimental design, deadwood selection and sampling

The study was conducted on forest plots of the German Biodiversity Exploratories (Fischer et al. 2010) located in the UNESCO Biosphere Reserve "Schwäbische Alb" in southwestern Germany. The plot selection criteria were based on forest history and management regimes, dominant tree species and deadwood status (Fischer et al. 2010; Hessenmöller et al. 2011 and Luyssaert et al. 2011). All selected forest plots had, apparently, been subjected neither to clear-cutting procedures nor to a period of agricultural use in the past (Luyssaert et al. 2011). A minimum distance between the outer edges of each plot is 200 m and located at least 100 m from the nearest forest edge. Our survey took place on deadwood logs in 9 intensively investigated 1 ha plots, with three plots representing the following three forest management types, respectively: (i) natural beech forests (unmanaged for 100 years, natural regeneration, uneven-aged forest structure, with mature trees >100 years), (Hessenmöller et al. 2011), (ii) age-class managed beech forests dominated by *Fagus sylvatica* (natural regeneration, even-aged forest structure, 50–100 years) and (iii) age-class managed spruce forests dominated by *Picea abies* (planted forest, even-aged forest structure, 80 – 100 years) (Tables S1, S2). In April 2009, a set of 48 logs, equally representing the two tree species (*P. abies* and *F. sylvatica*) located on the forest floor were randomly selected and their properties (length, diameter, tree species, etc.) were characterized. Our selection assured that *Fagus* logs were present in *Picea*-dominated plots and vice versa. In June 2009, 3–7 wood samples were taken from each log (according to its size) using a cordless Makita BDF451 drill (Makita, Anja, Japan) equipped with a 2×42 cm wood auger as described in Hoppe et al. (2014, 2015) and Purahong et al. (2014a, b). Sporocarp data were available (Hoppe et al. 2014) and used as corroborative evidence for the presence of particular fungi that were detected as OTUs in the sequencing analysis.

Wood physico-chemical properties and lignin-modifying enzyme assays

The concentrations of C and N in wood samples were determined by total combustion using a Truspec elemental analyzer (Leco, St. Joseph, MI, USA). Klason lignin content was determined gravimetrically as the dry mass of solids remaining after sequential hydrolysis with sulfuric acid (72 % *w/w*); in a second step, acid soluble lignin was measured by UV-photometry in 4 % H_2SO_4 (Effland 1977; Liers et al. 2011). Total lignin was obtained by summing acid insoluble Klason lignin and acid soluble lignin (Raiskila et al. 2007). The wood samples' pH values and contents of nutrient ions and lignin-modifying enzymes were measured in aqueous extracts. The extractions were performed using 10 ml distilled water per 1 g dry mass of wood for 120 min on a rotary shaker (120 rpm). Macronutrients (Mg, K, Ca, Fe) and micronutrients (Cu, Mn, Zn, Ni) were determined using inductively coupled plasma (ICP) optical emission spectrometry (ICP-OES) and mass spectrometry (ICP-MS), according to the instrument manufacturers' specifications. Three oxidative extracellular oxidoreductases important for lignin degradation (laccase - Lac, manganese peroxidase – MnP, manganese-independent peroxidases - MiP) (Hatakka and Hammel 2011) were measured as described by Hahn et al. (2013). Nutrient ion and lignin-modifying enzyme analyses were conducted in triplicate and in duplicate, respectively, on the same subsamples.

Deadwood logs were assigned to four decay classes based on remaining mass (%) data by k-means cluster analysis as described in Hoppe et al. (2014) and Kahl et al. (2012). Decay rates were calculated based on a single exponential model (Harmon et al. 1986) using information on mass loss (density and volume loss) and time since death obtained by dendrochronological dating of the deadwood (further details are provided in suppl. information).

DNA isolation, PCR and pyrosequencing

Total community DNA was isolated from 1 g of each homogenized wood sample using a modified CTAB-protocol (Doyle and Doyle 1987) as described in Hoppe et al. (2014). All DNA extracts from the wood samples of each log were pooled into a composite extract prior to PCR. Fungal ITS rDNA amplicon libraries were produced as described in Wubet et al. (2012). Briefly we used fusion primers designed with pyrosequencing primer B, a barcode and the fungal specific primer ITS1-F (Gardes and Bruns 1993) as a forward primer and pyrosequencing primer A and the universal eukaryotic primer ITS4 (White et al. 1990) as a reverse primer to amplify the fungal nuclear ribosomal internal transcribed spacer (nrITS) rDNA. We used a set of 10 nt MID-barcodes provided by Roche Applied Science (Mannheim, Germany). Each composite DNA extract for the amplicon libraries was amplified separately by PCR in triplicate 50 μl reaction mixtures containing 25 μl 2x GoTaq Green Mastermix (Promega, Madison, WI, USA), 25 μM of each primer and approximately 20 ng template DNA. Amplification was performed using a touchdown PCR program with denaturation at 95 °C for 5 min followed by 10 cycles of denaturation at 94 °C for 30 s, annealing at 60–50 °C for 45 s (−1 °C per cycle), and extension at 72 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 50 °C for 45 s and 72 °C for 2 min, with a final 10 min extension step at 72 °C (Lentendu et al. 2014). The PCR products were separated on a 1.5 % agarose gel and equimolar volumes of the amplified products of the expected size from the three positive replicate amplicons per sample were homogenized. The pooled products were gel purified using a Qiagen Gel

Extraction Kit (Qiagen, Hilden, Germany). The purified DNA was quantified using a fluorescence spectrophotometer (Cary Eclipse, Agilent Technologies, Waldbronn, Germany). An equimolar mixture of each library was subjected to unidirectional pyrosequencing from the ITS4 end of the amplicons, using a 454 Titanium amplicon sequencing kit and a Genome Sequencer FLX 454 System (454 Life Sciences/ Roche Applied Science) at the UFZ Department of Soil Ecology.

Bioinformatic analysis

We performed multiple levels of sequence quality filtering. The fungal ITS sequences were extracted based on 100 % barcode similarity. Sequences were clipped of barcodes and trimmed to a minimum length of 300 nt to best cover the ITS2 part of the nrITS using MOTHUR (Schloss et al. 2009). Sequence reads with an average quality score of < 20, and homopolymers of> 8 bases were removed. Unique good quality sequences from the dataset were filtered and checked for chimeras using the uchime algorithm (Edgar et al. 2011) as implemented in MOTHUR. To avoid sampling size effects, the number of reads per sample was normalized for each data set by randomly subsampling to the lower number of reads per samples using the subsample script as implemented in MOTHUR. The sequence dataset was then clustered and assigned to OTUs using CD-HIT-EST of the CD-HIT package version 4.5.4 (Li and Godzik 2006) at a 97 % threshold of pairwise sequence similarity as in Wubet et al. (2012). We used MOTHUR to taxonomically assign representative sequences of the OTUs against the UNITE reference database (as downloaded in May 2013) using the default set-up (Abarenkov et al. 2010).

Statistical analysis

To link WIF richness and taxonomic identity to microbial-mediated enzyme activity and decomposition rates, we defined cumulative OTUs (cOTUs) as species synonyms by aggregating OTUs that were unequivocally given the same name by BLAST re-analysis against GenBank yielding at least 95 % (species level) and 90 % (genus level) maximum identity scores (Ovaskainen et al. 2013) for the same database species. The Chao1 and ACE diversity indices were calculated for all OTUs (including 1–3 tons) using the *estimate* function in the R package "vegan" (Oksanen 2013). All multivariate statistics were conducted on proportional abundance data using the WIF dataset excluding 1–3 tons. Analysis of similarities (ANOSIM) and nonmetric multidimensional scaling (NMDS) based on Bray-Curtis distances were conducted using PAST (Hammer et al. 2001) and the "vegan" package in R (Oksanen 2013), respectively, to compare the fungal community structure of *Fagus* and *Picea*. The influence of selected wood physico-chemical parameters, fungal family abundances and environmental factors on fungal community structure was investigated by fitting data on each factor to the NMDS ordinations of the fungal communities. The wood physico-chemical parameters considered in these analyses were decay class, concentrations of macronutrients (C, N, K, Ca, Mg, Fe) and micronutrients (Cu, Mn, Ni and Zn), relative wood moisture, wood density, remaining mass and pH. Goodness-of-fit statistics (R^2) for environmental variables fitted to the NMDS ordinations of fungal communities were calculated using the *envfit* function of "vegan", with *P* values being based on 999 permutations (Oksanen 2013). The *P* values were Bonferroni-corrected in all cases. We calculated non- parametric Kendall-Tau correlations (τ) (pairwise comparisons) to link the fungal taxonomic groups to lignin-modifying enzyme activities and decomposition rates using PAST. The differences in OTU richness (observed OTU and cOTU richness and estimated Chao1 and ACE richness) and wood physico-chemical properties among different decay classes were analyzed for differences among means ($P<0.05$) by performing one-way analysis of variance (ANOVA) incorporating Shapiro-Wilk's W test for normality and Levene's test to check for the equality of group variances. Fisher's Least Significant Difference (LSD) *post hoc* test was also performed.

Results

Wood physico-chemical properties in different decay classes

The C/N ratios of the deadwood decreased as it decayed, and were significantly higher in *Picea* logs ($P<0.0001$, ranging from 630±48.4 to 423±52.4) than in *Fagus* logs (365±14.9 to 194±15.6) (Fig. 1 and Table S3). This difference can be attributed to the significantly higher C concentrations in *Picea* deadwood compared to *Fagus* ($P<0.0001$), which ranged from 49.3 %±0.31 in decay class 1 to 51.4 %±0.94 in decay class 4, and also to the significantly higher N concentrations in *Fagus* logs ($P<0.0001$) compared to *Picea*. Nitrogen concentrations also increased ($P<0.0001$ for *Fagus* logs, $P<0.01$ for *Picea* logs) as wood decay progressed (Fig. 1). The mean total lignin concentrations in *Fagus* deadwood were lower than in that of *Picea* (28.9 and 36.0 %, $P<0.0008$) and the relative proportion of lignin in the wood increased significantly as it decayed. There were no differences in the mean relative wood moisture, mass loss and decay rate between the two tree species, but these parameters differed significantly between different decay classes within each tree species (Fig. 1). For example, relative wood moisture increased in parallel with decay, from 49.8 %±5.5 for decay class 1 to 155.2 %±9.1 for decay class 4 in *Fagus* ($P<0.0001$) and from 48.7 %±11.6 to 163.1 %±24.6 in *Picea* ($P<0.0001$). The pH of *Fagus*

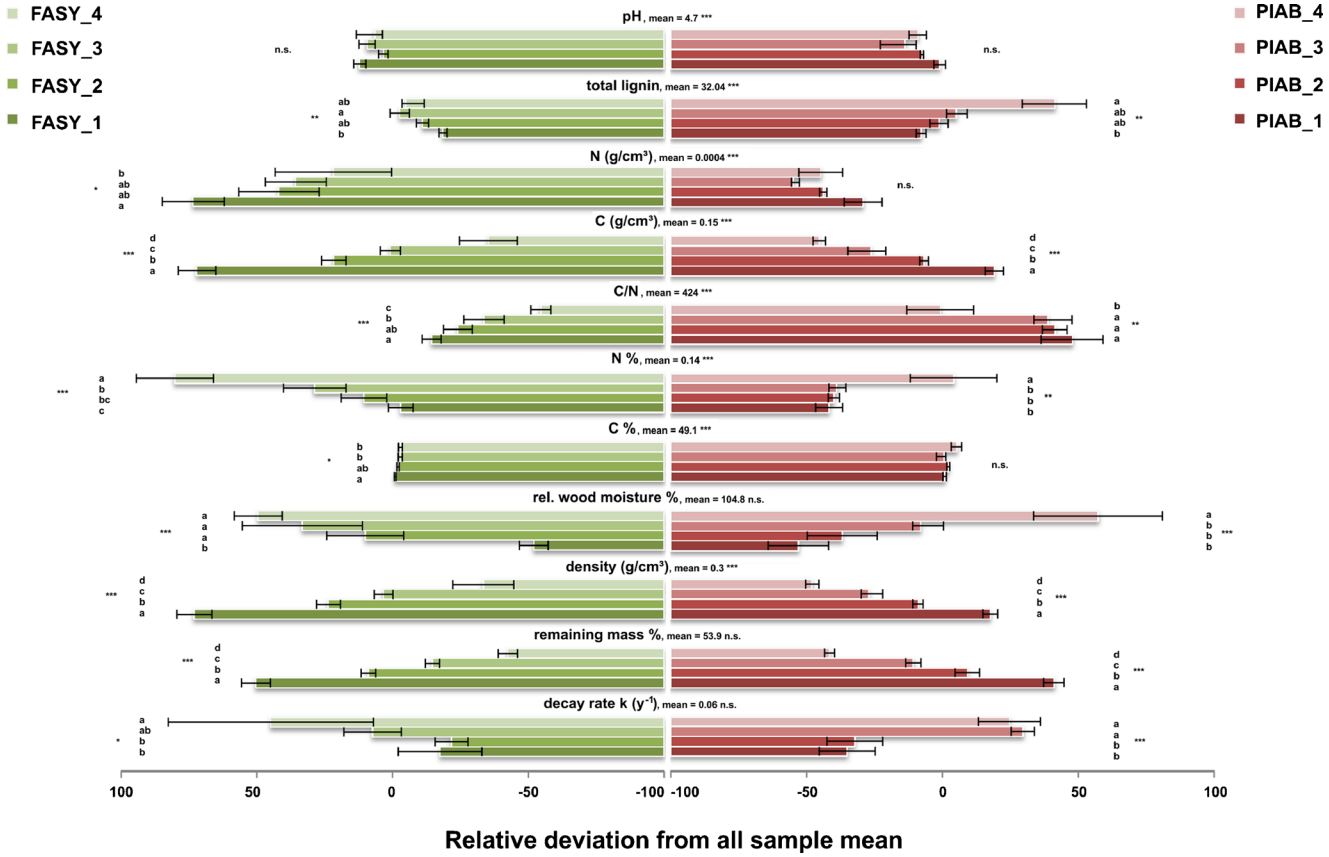

Fig. 1 Decay rates, mass losses, and wood physico-chemical properties of *Fagus* and *Picea* deadwood logs of different decay classes. The figure shows the relative deviation from the sample mean in each case. Mean values of all parameters for deadwood logs of both species are shown in the middle. The differences between the two deadwood species and also among different decay classes for individual deadwood species were analyzed by t-tests and one-way analysis of variance, incorporated in the form of Fisher's Least Significant Difference (ns=not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$). The four FASY (*Fagus sylvatica*) and PIAB (*Picea abies*) decay classes are shown in different color shades

deadwood was constant across decay classes and significantly higher than that of *Picea* deadwood, which declined from 4.6 ±0.1 for decay class 1 to 4.3±0.2 for decay class 4. The allocation of micronutrients and the activities of the lignin-modifying enzymes laccases (Lac), manganese peroxidase (MnP) and manganese independent peroxidases (MiP) are provided in supplementary Table S4.

Pyrosequencing and community sampling statistics

In total, 139,352 reads were obtained from 454 pyrosequencing of 48 deadwood samples. Sequences were initially quality checked, trimmed, normalized per sample and screened for potential chimeras (841 chimeras were removed). CD-HIT clustering of the remaining 86,935 sequences yielded 2,386 OTUs at a 97 % cutoff, of which 1,090 appeared as singletons and 305 as doubletons. Singletons, doubletons, and tripletons were removed from the final dataset, which was considered as having the "rare" taxa excluded. We performed a Mantel test on Bray-Curtis dissimilarities to assess the correlations between the whole matrix and a matrix excluding the rare taxa as just stated. This indicated that the removal of rare taxa from the community composition had no effect ($R=0.99$, $P=0.0001$). In total, 81,803 sequences clustering into 779 OTUs were retained for further statistical analysis after removing sequences that could not be taxonomically assigned to fungi. By applying a species-level sequence divergence threshold of 3 %, we were able to taxonomically assign 93.1 % of the filtered OTUs at the phylum level. Basidiomycota accounted for 338 of the filtered OTUs, Ascomycota for 375, and zygomycetes for 9. Chytridiomycota, Entomophthoromycota, and Glomeromycota were represented by 1 OTU each. Further, 614 (78.8 %), 552 (70.9 %) and 434 (55.7 %) of the filtered OTUs were classified at the order, family and genus levels, respectively. The remaining 6.9 % (54 OTUs) were grouped as unknown fungal OTUs.

Wood-inhabiting fungal richness and community structure

The total observed fungal OTU richness (excluding rare taxa) per sample ranged from 17 to 102 (25–159 including rare taxa) in logs of *Fagus sylvatica* and from 28 to 102 (38–151 including rare taxa) in *Picea abies* logs (Fig. S1). We did not

observe significant variation in mean OTU richness between the two tree species ($P = 0.32$) (Table S5). All four measures of fungal diversity/ richness (total observed, cumulative, Chao1, and ACE) correlated significantly and positively with decay class, whether it was quantified in terms of declining wood density ($P < 0.05$ *Fagus sylvatica*, $P < 0.001$ on *Picea abies*) or remaining wood mass (Fig. S2).

While the distribution of OTUs belonging to the Basidiomycota or Ascomycota was comparatively balanced in terms of their presence or absence, their relative abundances differed significantly. For example, the 201 Basidiomycota OTUs identified in *Fagus* samples contained 25,053 sequences (63.2 % of all sequences) whereas the 265 Ascomycota OTUs (Table S6) only contained 14,091 (35.5 %). There was an even more pronounced pattern in *Picea,* where the 217 identified Ascomycota OTUs contained only 5,614 (13.3 %) sequences whereas the Basidiomycota accounted for almost 83 % (34, 985) of all sequences clustered into 242 OTUs.

More specifically, 2,487 (6.35 %) sequences were assigned to *Annulohypoxylon cohaerens*, a common European beech saprotroph of the family *Xylariaceae*, which also represented the dominant family in the *Fagus* logs, accounting for 5,377 sequences in total (Figs. 2 and S3). Other important families in the *Fagus* fungal communities were the *Meruliaceae*, *Polyporaceae*, *Mycenaceae*, *Physalacriaceae* and *Marasmiaceae,* that together accounted for 42 % of all identified fungi (Fig. 2). OTUs assigned to the white-rot causing fungus *Resinicium bicolor*, which is listed as Agaricomycetes I.S. (*incertae sedis*) in the Index Fungorum (www.indexfungorum.org), were dominant in *Picea* deadwood, accounting for 25.3 % of all sequences. This fungus was detected in 83.3 % of all *Picea* logs (Fig. S3). *Bondarzewiaceae*, represented by *Heterobasidion sp.*, *Stereaceae*, *Fomitopsidaceae* and *Mycenaceae* also occurred frequently and accounted for 32.64 % of the identified sequences/ OTUs. Unidentified species of the *Helotiaceae* and an OTU identified as the potential soft-rot agent *Phialophora sp.* of the family *Herpotrichiellaceae* were the most abundant Ascoymcota in *Picea* but only accounted for 2 and 1.9 % respectively of all identified sequences.

We also examined the sharedness of WIF communities between tree species at species (cOTU) and genus level (Fig. S4ab). Among the 160 cOTUs with the same genus and species epithets, 74 (22 Ascomycota, 50 Basidiomycota, and 2 zygomycetes) were found in both, *Picea* and *Fagus* logs (Fig. S4a). Genus-level comparisons revealed that 35 genera were unique to *Fagus* and, 34 to *Picea*, while 58 genera were shared (Fig. S4b).

WIF dynamics on deadwood of different tree species

Different WIF dynamics were clearly observed in the *Fagus* and *Picea* logs (Figs. S5, S6). WIF communities in *Fagus* logs were highly dynamic with respect to wood decay, with no cOTU being dominant in all decay classes. The dominant fungal families and cOTUs in *Fagus* logs of decay classes 1–4 were *Xylariaceae* (mainly *Annulohypoxylon cohaerens*) and *Polyporaceae* (*Trametes versicolor*) in decay class 1, *Polyporaceae* (*Fomes fomentarius*) and *Meruliaceae* (*Phlebia livida*) in decay class 2, *Meruliaceae* (*Ceriporiopsis*

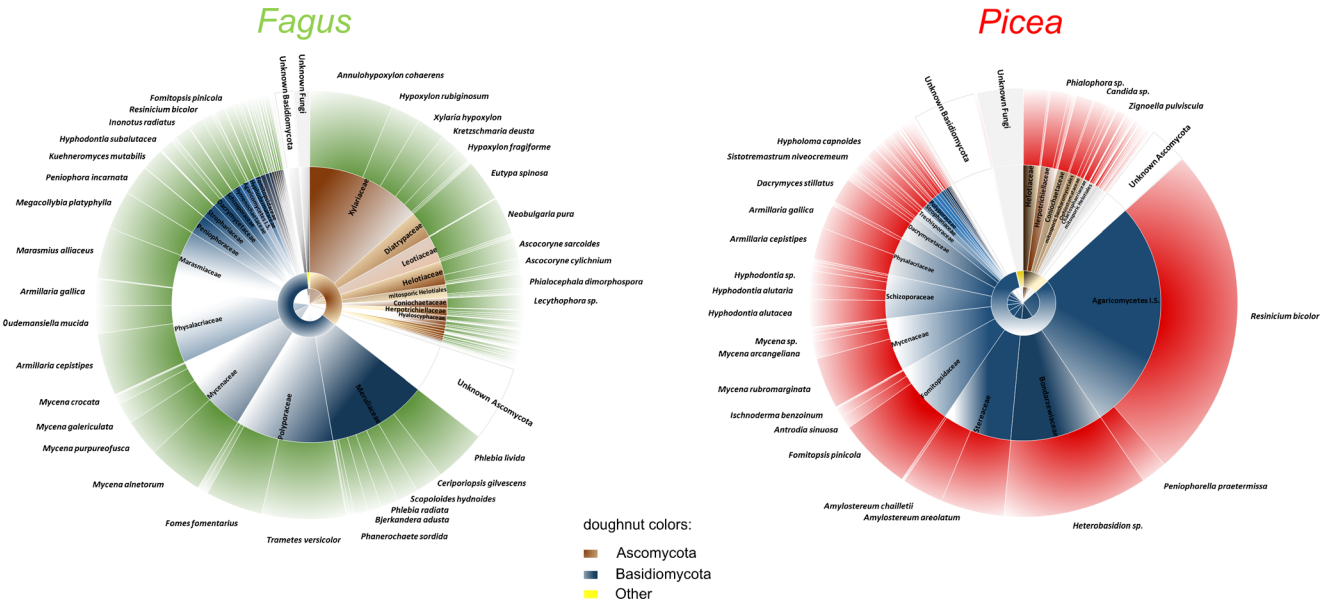

Fig. 2 Patterns of cOTUs on *Fagus* and *Picea* deadwood displayed as sunburst charts (beatexcel.com). The outer rings show the cumulative relative abundances of different fungal OTUs (cOTUs) at the species and genus levels with at least 95 and 90 % probability of correct identification, respectively, based on secondary BLAST re-analysis. The middle rings display the cumulative relative abundances at the family level. The inner rings display the cumulative relative abundances of different concordant fungal phyla. Only cOTUs that accounted for > 1 % of the total abundances and identified at least to genus are labeled

gilvescens), *Polyporaceae* (*Fomes fomentarius*) and *Marasmiaceae* (*Megacollybia platyphylla*) in decay class 3, and *Mycenaceae* (*Mycena alnetorum*), *Marasmiaceae* (*Megacollybia platyphylla, Marasmius alliaceus*) and *Polyporaceae* (*Trametes versicolor*) in decay class 4. In contrast, all of the *Picea* decay classes were dominated by a single cOTU assigned to Agaricomycetes I.S. (*Resinicium bicolor*); the mean abundances of this cOTU in decay classes 1, 2, 3, and 4 were 26.1, 36.6, 27.6, 16.5 %, respectively. Some fungal families and cOTUs were also co-dominant in different decay classes: *Stereaceae* (mainly *Amylostereum areolatum*) in decay class 1, *Fomitopsidaceae* (*Fomitopsis pinicola*) in decay class 3, and *Bondarzewiaceae* (*Heterobasidion* sp.) in decay classes 3 and 4. The WIF dynamics of both deadwood species are described at greater detail in the supporting information.

Factors correlating to fungal community structure in the two deadwood species

NMDS analysis clearly separated the WIF communities of the two tree species (Fig. 3ab; Table 1). Wood physico-chemical parameters correlated significantly with the fungal community structure (Fig. 3a). Factors that correlated significantly to the variation in the WIF community structure in both tree species were the decay class, relative wood moisture, pH, remaining mass, wood volume, wood density, C/N ratio and the concentrations of total lignin, C, N, Mg, Fe and Zn (P=0.024–0.0001). At the individual tree species level, WIF community structure correlated significantly with decay class, relative wood moisture, remaining mass, wood density and total lignin (Table 1). In addition, wood volume and C and N concentration were important in *Fagus* deadwood, while the concentrations of total lignin and Mg were contributing significantly in shaping the fungal community structure in *Picea* deadwood. Fungal families that correlated significantly with WIF community structure in *Fagus* and *Picea* logs are displayed in Fig. 3b.

Relationships between wood-inhabiting fungal richness, taxonomy and lignin-modifying enzyme activities

Correlations between WIF richness (in terms of total observed, cumulative, or estimated OTUs), WIF abundance (at both the family and cOTU levels), and the activities of lignin-modifying enzymes are presented in Table S7. There were no positive correlations between any actual richness of OTU/cOTU as well as the estimated richness of total OTU and the activities of lignin-modifying enzyme activities. Interestingly, however, there were some significant positive correlations between the abundances of certain fungal families and the measured activity of lignin-modifying enzymes. In *Fagus* logs, the abundance of the *Schizoporaceae* and *Xylariaceae* correlated positively with Lac and MiP activity, respectively (P<0.05). In *Picea* logs, the abundance of *Bondarzewiaceae* correlated positively with MiP and MnP activity (P=0.002 – 0.009). We hence found many significant correlations between fungal cOTU abundance and the activity of potential lignin-modifying enzymes (Table S7). In *Fagus* deadwood, the strongest significant positive correlations were found between *Mycena alnetorum* and Lac (τ= 0.43, P=0.003), *Hypoxylon rubiginosum* and MiP (τ= 0.42, P=0.004), and *Marasmius*

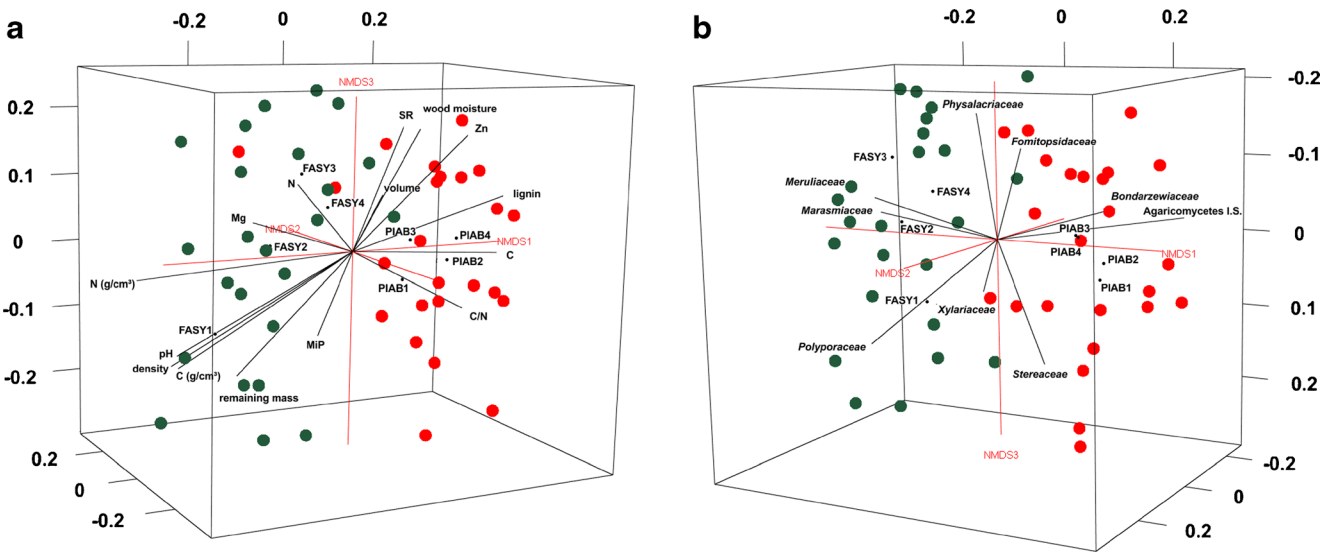

Fig. 3 3D-Nonmetric multidimensional scaling (NMDS) ordination of fungal community structure in *Fagus* (*green*) and *Picea* (*red*) deadwood using the *plot3d* and *ordigl* functions in R. The NMDS ordination (stress=0.16) was fitted to wood physico-chemical and anthropogenic factors (**a**) and also abundances of different fungal families (**b**) by using the *envfit* command in "vegan". ANOSIM revealed significant separation of fungal community structure according to tree species (R=0.60, P<0.001, 999 permutations). To better show the 3-dimensionality of the ordination, two movies in .mp4 format (BB Flashback Express Recorder, Blueberry Software, Birmingham, UK) are available in Suppl. files S1a and S1b

Table 1 Goodness-of-fit statistics (R^2) for parameters fitted to the nonmetric multidimensional scaling (NMDS) ordination of fungal community structure

Parameter	*Fagus vs Picea*		*Fagus*		*Picea*	
	R^2	*P*	R^2	*P*	R^2	*P*
Fungal OTU richness	0.1946	**0.024**	0.541	**0.003**	0.4758	**0.004**
Decay class	0.4461	**0.001**	0.4809	**0.005**	0.3634	**0.022**
Remaining mass	0.497	**0.001**	0.546	**0.003**	0.3413	**0.028**
Volume	0.2393	**0.008**	0.4975	**0.004**	0.1381	0.384
Density	0.6227	**0.001**	0.5472	**0.003**	0.3098	**0.047**
Relative wood moisture	0.3094	**0.002**	0.5331	**0.001**	0.4357	**0.01**
Total lignin	0.3788	**0.001**	0.1847	0.256	0.7645	**0.001**
pH	0.533	**0.001**	0.0853	0.603	0.3384	**0.039**
Decay rate	0.0728	0.378	0.2857	*0.079*	0.1189	0.499
Laccase (Lac)	0.0502	0.527	0.1092	0.487	0.0169	0.987
Manganese independent peroxidase (MiP)	0.1795	**0.021**	0.3451	**0.024**	0.0852	0.679
Manganese peroxidase (MnP)	0.0729	0.328	0.0815	0.76	0.1917	0.221
C/N	0.3765	**0.001**	0.2555	0.119	0.0933	0.588
C	0.2836	**0.001**	0.1083	0.523	0.6272	**0.001**
N	0.1941	**0.024**	0.2216	0.161	0.1828	0.23
C (g/cm^3)	0.6	**0.001**	0.5435	**0.003**	0.2658	0.094
N (g/cm^3)	0.6134	**0.001**	0.6111	**0.002**	0.1445	0.38
Mn	0.1507	0.057	0.0234	0.967	0.0497	0.802
Mg	0.3115	**0.002**	0.2087	0.196	0.3407	**0.027**
Ca	0.0736	0.316	0.151	0.332	0.2589	0.112
K	0.0684	0.362	0.2733	*0.099*	0.2357	0.127
Fe	0.1876	**0.016**	0.1352	0.418	0.2755	*0.065*
Cu	0.1143	0.145	0.1753	0.248	0.2928	*0.061*
Zn	0.3138	**0.001**	0.0863	0.613	0.0801	0.64
Ni	0.0718	0.366	0.153	0.357	0.1858	0.23

The significance estimates were based on 999 permutations. Significant factors (Bonferroni corrected $P<0.05$) are indicated in bold. Marginally significant variables (Bonferroni corrected $P<0.10$) are indicated in italics

alliaceus and MnP ($\tau = 0.31$, $P=0.036$). In *Picea* deadwood, the strongest significant positive correlations were found between *Armillaria gallica* and Lac ($\tau = 0.32$, $P=0.028$), *Heterobasidion sp*. and MiP ($\tau = 0.37$, $P=0.010$), and *Heterobasidion sp*. and MnP ($\tau = 0.45$, $P=0.002$). In addition, the abundances of certain families and cOTUs exhibited significant negative correlations with lignin-modifying enzyme activity (Table S7).

Relationships between wood-inhabiting fungal richness, taxonomic identity and wood decomposition rates

There were no significant correlations between total OTU richness (in terms of total observed, cumulative or estimated OTU richness) and wood decomposition rates (Table S8). However, the abundances of specific fungal taxonomic groups and cOTUs correlated significantly with wood decomposition rates. In *Fagus* deadwood, the abundance of the *Xylariaceae* family correlated significantly and negatively with decomposition rates ($\tau = -0.44$, $P=0.003$). The abundances of three individual species also correlated negatively with decomposition rates: *Hypoxylon fragiforme* and *Xylaria hypoxylon* (both *Xylariaceae*), and *Neobulgaria pura* (*Leotiaceae*). On the other hand, *Mycena purpureofusca, Phialocephala dimorphospora, Trametes versicolor* correlated positively with decomposition rates ($\tau = 0.31 - 0.41$, $P=0.034 - 0.005$; Table S8). In *Picea* deadwood, the abundances of the *Schizoporaceae* were positively correlated with decomposition rates ($\tau = 0.43$, $P=0.005$). Individual species whose abundances correlated positively with decomposition rates were *Botryobasidium botryosum, Hyphodontia alutacea, Hyphodontia alutaria* and *Mycena alnetorum* ($\tau = 0.32 - 0.45$, $P=0.036 - 0.003$; Table S8).

Discussion

This work builds on earlier studies that used high-throughput sequencing to investigate fungal community structures in deadwood (Kubartova et al. 2012; Ovaskainen et al. 2013) by providing the first comparison of two morphologically

different tree species often occurring in close proximity in temperate European forests. By assessing a very comprehensive dataset on physico-chemical wood properties, we were able to identify key factors that correlate with fungal community structure. Moreover, by linking fungal richness and community composition to enzyme activities and decomposition rates, we demonstrated that ecosystem processes are controlled by complex mechanisms such as assembly histories and competition scenarios.

Fungal diversity and community composition

We found no significant differences between the two deadwood species in terms of total or mean OTU richness. The mean fungal OTU richness tended to increase with increasing decay class. This is inconsistent with the findings of fructification pattern studies, which indicated that the abundance of fruiting bodies was highest at intermediate stages of decay (Heilmann-Clausen 2001; Hoppe et al. 2014). However, the fact that fungal OTU richness increased with losses of mass and density (i.e., as the decay class increased) is in agreement with the results of previous studies on spruce deadwood that used molecular techniques (Kubartova et al. 2012; Rajala et al. 2012). The discrepancy between these findings may be due to the fact that many fungi tend to reside as vegetative mycelia in deadwood and therefore do not develop fruiting bodies (Kubartova et al. 2012).

Only a small proportion of the sequences obtained from deadwood of *Picea abies* were assignable to Ascomycota, though the ratio of taxonomically assigned OTUs was balanced to Basidiomycota. This result was in line to recent findings on fungal diversity in Norway spruce (Ottosson et al. 2015) and reflects that large parts of biological diversity are only accessible via molecular based techniques (Hibbett et al. 2011)

WIF dynamics during decomposition processes and corresponding factors

Fungal community structure differed significantly between the two tree species as indicated by the NMDS analysis. The community structure was also more dependent on the species origin of deadwood than on the surrounding forest type. More specifically, fungal communities in *Fagus* logs in beech stands were more similar to fungal communities in *Fagus* logs in spruce forests than to fungal communities in *Picea* deadwood in beech forests. The same was true for *Picea* logs in spruce and beech stands. This further demonstrates that substrate type has a greater impact on WIF community structure than forest types. Nevertheless, the impact of tree species should be further tested in the future with more tree species, especially those tree species that have similar wood physico-chemical properties. Our results indicate that the physico-chemical properties of the wood (decay class, relative wood moisture, remaining mass, wood density, C/N ratio, total lignin) correspond significantly to community structure in both tree species. This is consistent with the report of Rajala et al. (2012) who investigated this aspect in 500 Norway spruce logs.

Deadwood of the two tree species also differed substantially with respect to the way in which their WIF communities changed as the wood decayed. In *Picea* logs, *Resinicium bicolor* was dominant in all decay classes; other fungi such as *Amylostereum areolatum*, *Heterobasidion sp.* and *Fomitopsis pinicola* were co-dominant in specific decay classes but much less abundant. Conversely, in *Fagus* logs, no single fungus was dominant in all decay classes. The dominant species changed from *Annulohypoxylon cohaerens* and *Trametes versicolor* in decay class 1 to *Fomes fomentarius* in decay classes 2 and 3 and then *Mycena alnetorum* and *Trametes versicolor* in decay class 4. It may be surprising that *Polyporaceae* were still dominant in the decay class 4 since species of this family require substrates with high energy contents to produce fruiting bodies and are therefore consequently rarely found in wood at later stages of the decomposition when using methods that focus on fruiting bodies (Bader et al. 1995; Lindblad 1998). This may be due to the presence of vegetative mycelia and/ or DNA residues in the wood (Kubartova et al. 2012), both of which would be captured by our methodology. However, the change in the dominant polypore species from *Fomes fomentarius* in decay class 3 to *Trametes versicolor* in decay class 4 indicates that this phenomenon may not be entirely related to DNA residues.

The fungal community dynamics in *Picea* deadwood observed in this study were distinctly different compared to those reported for boreal forest ecosystems in Fennoscandia (Kubartova et al. 2012; Ovaskainen et al. 2013; Rajala et al. 2011, 2012). First, the ascomycetes that were reported to be most dominant during the early stages of decay in boreal forests (Rajala et al. 2012) were largely absent in the Central European forests, where basidiomycetes were most abundant across all stages of decay. Secondly, different fungi were dominant at different stages of decay in the boreal environment (Kubartova et al. 2012; Ovaskainen et al. 2013; Rajala et al. 2012), whereas in our study *Resinicium bicolor* was most abundant in all decay classes. Third, species such as *Hyphodontia alutaria*, *Ascocoryne cylichnium*, *Heterobasidion parviporum* and *Fomitopsis pinicola* were classified as early colonizers in boreal forests (Kubartova et al. 2012) but were common in all decay classes in our study and even dominant in *Picea* logs of decay class 4. Finally, ectomycorrhizal (ECM) fungi that were already detected during the early stages of decay in previous studies and became strongly dominant during the final stages of decomposition in boreal forests (Rajala et al. 2012) were largely absent in our study. We did not observe any increase in the abundance of ECM species in the more mineralized wood of decay class 4. Only 4 ECM cOTUs were detected in a single *Picea* log in the

later stages of decay: *Lactarius* sp. (42 sequences), *Laccaria amethystina* (22 sequences) *Russula fellea* (9 sequences) and *Xerocomus pruinatus* (4 sequences). Together, these species accounted for only 0.18 % of all sequences detected in *Picea abies*. The relatively low abundance of ECM in *Picea* logs from temperate forests as compared to boreal forests could be due to differences in N-availability in the two forest ecosystems (Nasholm et al. 1998). We assume that ECM fungi in temperate forests preferentially acquire N from the soil, whereas in the N-limited boreal environment it is worthwhile for ECM fungi to acquire nitrogen by attacking deadwood (especially highly decayed deadwood) to avoid the competition with forest floor vegetation (Rajala et al. 2011).

The role of WIF in ecosystem functions and processes

Both the activities of lignin-modifying enzymes and the decomposition rate were related to the abundances of particular fungal families and cumulative OTUs. Laboratory scale studies demonstrated that members of different fungal taxa that were detected in this study (such as *Armillaria* sp., *Fomes fomentarius*, *Trametes versicolor*) can efficiently produce similar amounts of lignin-modifying enzymes (Baldrian 2006) and cause similarly high levels of mass loss (Valmaseda et al. 1990). The co-occurrence of these fungi in deadwood suggests that there is some functional redundancy within the studied WIF communities. Several authors have reported increased Lac and MnP activities due to two-species-interactions (Baldrian 2004; Freitag and Morrell 1992; Snajdr et al. 2011; White and Boddy 1992). Chi et al. (2007) found that some combinations of two fungi can accelerate the decay of wood due to increases in MnP production relative to that observed in equivalent cases featuring only a single fungal species. Whether enzymes are actually secreted due to fungus-fungus interactions or for other reasons may depend on the community structure and the state of degradation of the colonized wood. However, recent studies (Dickie et al. 2012; Dowson et al. 1988a, b; Fukami et al. 2010; Fukasawa et al. 2009) have demonstrated a high degree of interaction among co-existing fungal species, suggesting that WIF may invest more energy into competing with one-another than on producing wood-degrading enzymes under natural field conditions. Coates and Rayner (1985) also found that interaction reduced the rate of wood decay. Therefore, as demonstrated in this work, the high species richness of deadwood-resident fungal communities need not be associated with any increase in the production of wood degrading enzymes or wood decomposition rate. Nevertheless, we identified some fungal OTUs that are known to be active producers of lignin-modifying enzymes and strong wood decomposers that cause a white-rot with high mass loss (Table S9). *Trametes versicolor* is among the most important decomposers, occurring in all decay classes of *Fagus* deadwood. We further show that different wood types (deciduous vs. coniferous) directly relate to decomposer (fungal) community structure and dynamics. The decomposition rates on the two deadwood species were not significantly different. This may be due to functional redundancy in their WIF communities and demonstrates that the fungi in each community are adapted to their host tree species. Fungi in coniferous wood have to deal with larger amounts of extractives as well as more recalcitrant and condensed lignin than is encountered in *Fagus* wood (Blanchette 1991; Higuchi 2006). In respect to anatomy, coniferous woods have simpler structures than deciduous woods (Fengel and Wegener 1983). Such factors will affect fungal substrate preferences. Brown-rot fungi prefer coniferous wood while most white-rot fungi colonize both coniferous and deciduous wood (Hibbett and Donoghue 2001). The preference of brown-rot fungi for coniferous wood was apparent in our data: the three most abundant brown-rot fungi *Fomitopsis pinicola, Dacrymyces stillatus* and *Antrodia sinuosa* were found to be much more abundant in *Picea* than in *Fagus*. Interestingly, we found more sequences for white-rot fungi in *Picea* wood than we did for brown-rot fungi. The mean enzyme activities of MiP and MnP in *Picea* were only marginally lower than those observed for *Fagus*, further demonstrating the presence of white-rot species in the studied *Picea* deadwood. These observations are consistent with those of Olsson et al. (2011) and Rajala et al. (2012), who also found white-rot fungi to be more abundant than brown-rot fungi on *Picea abies*.

The relationships between fungal cOTU richness, family abundances and the activities of lignin-modifying enzymes and decomposition rates were very different in *Fagus* and *Picea* logs. The fungal communities in both deadwood species were composed of different cOTUs that were positively correlated and/ or expected to play roles with all lignin-modifying enzyme activities and wood decomposition rates. Interestingly, there were some fungi whose abundances did not correlate positively with lignin-modifying enzyme activity and/ or wood decomposition rates in this field study even though they secreted high titers of lignin-modifying enzymes and caused high mass losses under laboratory conditions. This could be due to the succession of the studied communities (i.e., priority effects) and the interspecific interactions among different fungal species, as discussed above. In addition, ITS is of variable copy number and may not directly correlate to biomass, which could distort the relationship between a fungus's 'abundance' in the dataset and the observed enzymatic activity.

Our results also revealed significantly negative correlations between the abundances of *Xylariaceae* species and decomposition rates on *Fagus* logs. Different members of the *Xylariaceae* were abundant in many *Fagus* logs of early to intermediate decay classes, and logs harboring these species often exhibited low rates of decomposition. This could be related to the ability of fungi of this family (which cannot produce MnP) to impede deadwood colonization by

secondary saprotrophic basidiomycetes (Fukasawa et al. 2009). *Xylariaceae* were shown to act very defensively against saprotrophic basidiomycetes on 2 % malt agar and were not displaced by saprotrophic basidiomycetes in twigs over an incubation period of 6 months (Fukasawa et al. 2009). *Xylariaceae* can form pseudosclerotial plates (PSPs) to delineate decay columns (demarcation lines) within the wood and protect them from attacks by competing saprotrophs. These decay columns can persist even after several years of decomposition (Fukasawa et al. 2009; Purahong and Hyde 2011). We observed similar recalcitrant dense matrices of melanized hyphae (PSPs) in *Fagus* logs that were highly dominated by *Xylariaceae* (Fig. S7). *Trametes versicolor* was also highly abundant in the *Fagus* deadwood at various stages of decay, and its abundance correlated negatively with that of *Xylariaceae* members. Interestingly, some logs in the early stages of decay that had been colonized by *Trametes versicolor* rather than *Xylariaceae* exhibited very high rates of wood decomposition. This demonstrates the importance of priority effects and interspecific interactions among different fungal species (Hiscox et al. 2015). *Trametes versicolor* has been shown to secrete large quantities of different lignin-modifying enzymes and yields high wood decomposition rates under laboratory condition (Valmaseda et al. 1990). We assume that the properties and species origin of deadwood affects both the dynamics of the fungal community and the interactions among different fungal species. Many fungal OTUs were present in both *Fagus* and *Picea* deadwood but the dominance patterns and temporal dynamics of the communities in each case differed substantially (Figs. S3, S4).

Conclusion

Fungal community structure was significantly different between deadwood of *Fagus sylvatica* and *Picea abies* occurring in close proximity in temperate forests of Germany. Wood physico-chemical properties are the main factors corresponding to the fungal communities in these deadwood species. Under the studied natural conditions, microbial-mediated ecosystem functions (i.e., the activities of lignin-modifying enzymes) and processes (wood decomposition rate) were controlled by successional assembly history, interspecific interactions and competition scenarios rather than total OTU/ -species richness (Dickie et al. 2012; Fukami et al. 2010; Hiscox et al. 2015).

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Data accessibility The raw sequence data are available from the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/Traces/study/) under experiment SRX589508. Corresponding MIDs and metadata are provided in Table S2.

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