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Habitat models of wood-inhabiting fungi along a decay gradient of Norway spruce logs

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ABSTRACT

Information on the habitat requirements of wood-inhabiting fungi is needed to understand the factors that affect their diversity. We applied culture-free DNA extraction and 454-pyrosequencing to study the mycobiota of decaying Norway spruce (*Picea abies*) logs in five unmanaged boreal forests. Fungal habitat preferences in respect of wood density gradient were then estimated with generalized additive mixed models. Fungal diversity and wood density were inversely related, i.e., OTU richness generally increased as the log became increasingly decomposed. White-rot fungi (e.g., *Phellinus nigrolimitatus*) and members of *Hyphodontia* did not show a clear response to the wood-density gradient, whereas abundance of *Phellinus viticola* and brown-rot fungi (e.g., *Fomitopsis pinicola*, *Antrodia serialis*, *Coniophora olivaceae*) peaked during intermediate decay and mycorrhizal fungi (e.g., *Piloderma*, *Tylospora*, *Russula*) increased in the later stages. This information on fungal habitat requirements facilitates the development of management practices that preserve fungal diversity in managed forests.

1. Introduction

Saproxylic fungi feed on dead wood and play a pivotal role in nutrient cycling of boreal forests, ensuring the productivity and function of these important ecosystems Rayner and Boddy 1988, Boddy et al., 2008, Stockland et al., 2012). Decomposing wood also provides a habitat for mycorrhizal fungi that engage in a symbiotic exchange of nutrients with their host-tree roots (Tedersoo et al., 2003; Smith and Read, 2008). Unfortunately, the amount of woody debris suitable for saproxylic species is decreasing due to forestry practices. Following the most recent assessment, 42% of the polypore species in Finland are now red-listed, i.e., classified as near threatened, endangered or regionally extinct (Kotiranta et al., 2010). Healthy and sustainable forestry relies on an underlying microbial diversity performing essential ecosystem services. The habitat preferences and environmental factors that affect saproxylic fungi are key to determining the fungal response patterns in ecosystem and forest-stand simulation models (Peltoniemi et al., 2013). However, much of our current knowledge of fungal habitats is based on the appearance of conspicuous fruit bodies of macrofungi rather than colonization and activity of mycelia. The recent development of cost-effective high-throughput sequencing techniques provides a window into this important but inconspicuous aspect of fungal ecology and offers a more comprehensive view into nutrient cycling in boreal forests.

The decomposition of woody tissue is a dynamic process tied to an ecological succession of the saproxylic fungal community (Lindblad, 1998; Rajala et al., 2012; Rayner and Boddy, 1988; Renvall, 1995; Stockland et al., 2012; Stockland and Siitonen, 2012). At the onset of the process, the woody substratum is dense with low moisture content and the decomposition rate is low (Mäkinen et al., 2006). The rate of decay gradually increases to a peak during the intermediate stages (Mäkinen et al., 2006) that are characterized by a high diversity of brown-rot (capable of decomposing cellulose and hemicellulose) and white-rot (capable of decomposing lignin) fungal fruit bodies (Bader et al., 1995; Renvall, 1995; Lindblad 1998). The decay rate gradually slows to a minimum (Mäkinen et al., 2006), fruit bodies of wood decomposers can no longer be detected (e.g., Renvall, 1995), and the remaining woody substratum contains only the most recalcitrant compounds (e.g., Rajala et al., 2012). However, recent studies based on direct molecular detection of *in situ* mycelia suggest that fungal richness

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increases towards the end of succession (Rajala et al., 2011; Kubartová et al., 2012; Rajala et al., 2012; Ovaskainen et al., 2013), the rate of decomposition does not slow down (Valentín et al., 2014), and saproxylic fungi are eventually replaced by mycorrhizal species (Rajala et al., 2011, 2012; Ovaskainen et al., 2013).

Polypore fungi are a well-known group of wood-inhabiting basidiomycetes. They form conspicuous fruit bodies and include white- and brown-rot fungi. Polypore fruiting preferences for the various decay stages have been characterized (Renvall, 1995; Junninen et al., 2006; Jönsson et al., 2008; Nordén et al., 2013) and incorporated into forest-stand simulation models (Peltoniemi et al., 2013). However, information modeled in these simulations is based solely on the occurrence of fruit bodies and little is known of mycelial activity. Recently, Ovaskainen et al. (2013) surveyed the fungi inhabiting Norway spruce logs with high-throughput sequencing of mycelia and a traditional fruit body inventory. These authors modeled the abundance of 30 fungal species as a function of knife-measured decay stages (i.e., phases 1–4) and observed a positive correlation between mycelium and fruit body occurrence for the majority of species, with some red-listed species being more abundant than implied by the occurrence of their fruit bodies. Unfortunately, the final stage of decay (i.e., phase 5) was excluded from their sampling although it is known to support specific functional groups and is expected to be the phase in which species diversity peaks.

In this study, we apply high-throughput sequencing of DNA recovered from dead wood across a decay gradient to infer a comprehensive and high-resolution profile of the saproxylic fungal community and its succession. We characterize decay stage in terms of wood density which allows habitat modeling with a continuous variable rather than the arbitrary classes applied earlier.

Our aim was to investigate the habitat preferences of the saproxylic fungal community as revealed by DNA sequences recovered from a wood decomposition gradient. Samples were taken from 535 Norway spruce logs comprising a chronosequence of recently-fallen to fully-decomposed logs in five unmanaged forest stands of southern Finland. We propose that: (1) fungal species richness increases with decreasing wood density; (2) the abundance of fungal species or other fungal groups can be modeled as a function of wood density; and (3) simulation models can improve our understanding of the habitat requirements of saproxylic fungi, and this information can be applied in forest management and conservation.

2. Material and methods

2.1. Study sites and wood sampling

Five unmanaged Norway spruce (*Picea abies*) dominated forests were selected from southern Finland: Sipoo (N60.46, E25.19); Lapinjärvi (N60.66, E26.12), Loppi (N60.79, E24.17); Petäjäjärvi (N61.91, E23.58); and Vesijako (N61.35, E25.11). A 75 × 75 m (0.56 ha) study site was established at each forest stand and 83–126 dead spruce logs per site were sampled as a representation of the decay continuum. Samples were drawn equally among decay phases regardless of their relative abundance at each site. Five cm thick discs were removed from the midpoint of each log, packed in plastic, and transported to the laboratory, where the samples were stored at −20 °C before sampling. In the laboratory, the bark was removed from the discs and they were drilled through the surface, sapwood and heartwood with a sterile drill-bit. The sawdust and shavings obtained from each sample were uniquely labeled and stored at −20 °C prior to DNA extraction. The remaining part of the disc was used to measure wood density by a water displacement method (Olesen, 1971): the sample dry mass (determined after 48 hr at 103 °C) was divided by its fresh volume determined by submergence in water. Rajala et al. (2012) provided a more detailed description of the study sites, sampling techniques and protocol for the determination of wood density.

2.2. Preparation of pooled DNA samples, amplification and 454-pyrosequencing

DNA was extracted from wood samples with the E.Z.N.A.™ SP Plant DNA Mini kit (Omega Bio-tec, Inc. USA) and purified with PEG precipitation as described in Rajala et al. (2010). DNA samples extracted in Rajala et al. (2011, 2012) were sorted according to wood density within each study site and five samples with similar wood density were pooled. Thus, each DNA sample analyzed in this study represented five logs (one sampling point per log) and the entire data set was based on 535 logs (pooled to create 107 samples subjected to 454-pyrosequencing). To test pooling and persistence of DNA in dead wood, we also subjected a small dataset of separate DNA and RNA samples from our previous study (Rajala et al., 2011) to the 454-pyrosequencing. Total RNA was extracted with the E.Z.N.A.™ SP Plant RNA Mini kit (Omega Bio-tec, Inc. USA), followed by DNase I digestion and cDNA synthesis as described in detail in Rajala et al. (2011).

Briefly, samples were amplified first in triplicate with ITS1f and ITS4 primers (Gardes and Bruns, 1993; White et al., 1990) under real-time amplification in RotorGene 6000 (Corbett Research, Australia) using Phusion polymerase (Thermo Scientific, USA). The concentration of amplicons was measured in RotorGene 6000, replicates were pooled, purified and concentrations were measured with the Qubit fluorometer (Invitrogen, USA). A second round of amplification was performed with 454-tagged A_ITS1f and B_ITS2 primers (White et al., 1990) in a S1000 thermal cycler (Bio-Rad, USA) using Herculase II Fusion polymerase (Agilent Technology, USA). Details of the amplification procedure can be found in the supplementary file (Supplementary_Sample Preparation).

The purification and sequencing of pooled amplicons was performed by the DNA sequencing and Genomics laboratory (Institute of Biotechnology, University of Helsinki, Finland) using the Genome Sequencer FLX Titanium XL + System (454 Life Sciences, Roche, Branford, CT, USA). The raw sequence data are available in the European Nucleotide Archive under accession number **PRJEB8282**.

2.3. Bioinformatic analysis

The raw sequence data consisting of 500,908 reads were processed by MOTHUR software package v.1.33 (Schloss et al., 2009) according to a standard operational procedure (Schloss et al., 2011) with slight modifications. First, barcodes were used to pin sequences to different samples, primer and barcode sequences were removed (pdiff = 0, bdiff = 0), and sequence quality was evaluated according to quality score. The following thresholds were used: minimum sequence length of 200 bp, maximum number of homopolymers 8, ambiguous bases 0 and average quality 25. Potentially chimeric sequences were identified and removed by UCHIME as implemented in MOTHUR (Edgar et al., 2011) referencing the UNITE/INSD database (UNITEv6_sh_dynamic; Kõljalg et al., 2013), and sequences that were part of larger segments were grouped (frag.clust). Data were normalized by sub-sampling 1141 sequences from each sample. Nine samples had fewer than 1141 sequences and were omitted. Pairwise distances were calculated for unique sequences (cutoff = 0.25, gapopen = −1), and sequences were clustered into operational taxonomic units (OTUs) at 97% similarity level using a nearest-neighbor algorithm. All singletons (OTUs with only one sequence) were removed, reducing the lowest number of sequences per sample to 1110. Accordingly, the data received a

second normalization treatment at 1110 sequences per sample. Number of OTUs was used to represent fungal species richness, whereas a phylotype approach was used to investigate taxonomic composition. In that approach, all sequences were classified as phylotypes using a naïve Bayesian classifier (WANG algorithm) with a bootstrap cutoff of 80% (Wang et al., 2007) and through comparison to known reference sequences in the UNITE/INSD database (UNITEv6_sh_dynamic; Kõljalg et al., 2013).

2.4. Statistical analysis

The relationship between fungal species richness and wood density was examined with the linear mixed-effect model (*lme*) function as applied in the software package *nlme* (Pinheiro et al., 2013) operating in R (R Development Core Team, 2013; Wood, 2006). The number of OTUs was examined in relation to wood-density factor, study site was treated as a random factor, and a random slope was applied to density within site.

Fungal response curves along a wood density gradient were estimated using the generalized additive mixed model (*gamm*) function in the package *mgcv* (Wood, 2013) of R. In the model, the abundance of each taxon or fungal group (i.e., number of sequences) was the dependent variable, smooth-termed wood density was an explanatory variable, and study site was a random effect. Log-linked Tweedie was selected as a distribution family, because the data contained many zero values, and an optimal variance function was selected for each model ($p = 1.3–1.7$) using the *gam.check* function.

3. Results

3.1. Fungal community in decaying spruce logs

The checked and normalized sequence dataset consisted of 108,780 reads assigned to 932 OTUs. The number of OTUs per sample (i.e., pooled from five separate logs) increased with decreasing wood density from around 50 in fresh and intact substrate to a maximum of 131 in loose, heavily-decayed wood (Fig. 1, $p = 0.008$).

All sequences were fungal and most belonged to Basidiomycota (76%). Ascomycetes comprised 14% of sequences, zygomycates 0.6%, Chytridiomycota 0.002%, with 9% of sequences being unidentified. Many of these sequences were attributed to the same taxon, and after the full list of phylotypes was compiled, 59% of them belonged to Basidiomycota, 34% to Ascomycota, 3% to zygomycetes, 0.002% to Chytridiomycota and 3.4% could not be matched to known sequences on reference databases (Table S1).

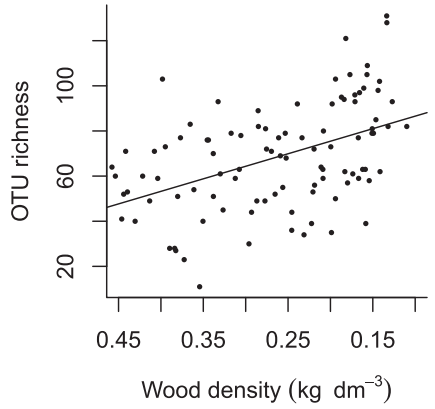

Fig. 1. Relationship between the number of fungal operational taxonomic units (OTUs) determined by ITS1 sequence similarity with a 97% threshold and density of decaying Norway spruce wood substrata. Number of sequences per sample was normalized to 1110, there were 98 samples and a total of 108,780 reads.

Nearly 49% of all fungal phylotypes (51% of qualified sequences) were identified to the species level. The most common sequences in the data set pertained to *Coniophora olivacea*, *Xeromphalina campanella* and *Phellinus viticola*. *Xeromphalina campanella* was the most frequently observed, being detected in 66% of samples, followed by *Tylospora fibrillosa* (65%) and *Botrybasidium subcoronatum* (63%) (Table 1, Table S2).

With respect to functional groups, white-rot fungi comprised 14% of sequences, brown-rot fungi 13% and mycorrhizal fungi 12%. Of the identified phylotypes, 6% were white-rot, 4% were brown-rot and 19% were mycorrhizal fungi (Table S1).

3.2. Relationship between fungi and wood density

Basidiomycetes were detected in all phases of decay, but their peak abundance was in low-density (i.e., highly decomposed) wood (Fig. 2A, significance of smooth-termed density for basidiomycetes $p = 0.002$). In contrast, ascomycetes displayed the opposite pattern of being more abundant in relatively fresh and intact wood of higher density (Fig. 2B, $p < 0.001$). Zygomycetes were infrequently observed, but were more common in low-density, heavily decayed wood (Fig. 2C, $p < 0.001$). Indeterminate fungi were found throughout the density gradient (Fig. 2D, $p = 0.437$), but the lack of information pertaining to these taxa precludes any taxonomic inferences from being drawn with respect to their habitat preferences or role in community succession and decomposition.

Abundance of white-rot fungi peaked slightly in moderately-decayed wood but response to the wood-density gradient was not significant (Fig. 3A, $p = 0.063$). Brown-rot fungi increased significantly in less-decayed wood and mycorrhizal fungi were most abundant in heavily-decayed wood (Fig. 3B–C; brown-rot $p < 0.001$ and mycorrhizal fungi $p < 0.001$).

Table 1
Frequency of fungal taxa presented in Figs. 4 and 5 as the number of pooled samples (i.e., 5 logs) detected in and significance of modeled smooth-termed density gradient.

Taxa	Frequency	*p*-value
Species		
Phellinus nigrolimitatus	54	0.697
Phellinus viticola	46	<0.001
Fomitopsis pinicola	28	<0.001
Antrodia serialis	47	<0.001
Coniophora olivacea	44	<0.001
Piloderma sphaerosporum	49	<0.001
Tylospora fibrillosa	65	<0.001
Russula emetica	23	<0.001
Xeromphalina campanella	66	0.012
Genera		
Hyphodontia sp.	51	0.064
Mycena sp.	75	0.541
Phialocephala sp.	57	0.010
Phialophora sp.	26	<0.001
Piloderma sp.	58	<0.001
Tylospora sp.	65	<0.001
Russula sp.	60	<0.001
Pseudotomentella sp.	40	0.006
Lactarius sp.	39	<0.001
Cortinarius sp.	36	<0.001
Meliniomyces sp.	25	<0.001
Tomentella sp.	31	<0.001
Thelephora sp.	18	<0.001
Amanita sp.	17	<0.001
Phialocephala sp.	57	0.010
Phialophora sp.	26	<0.001

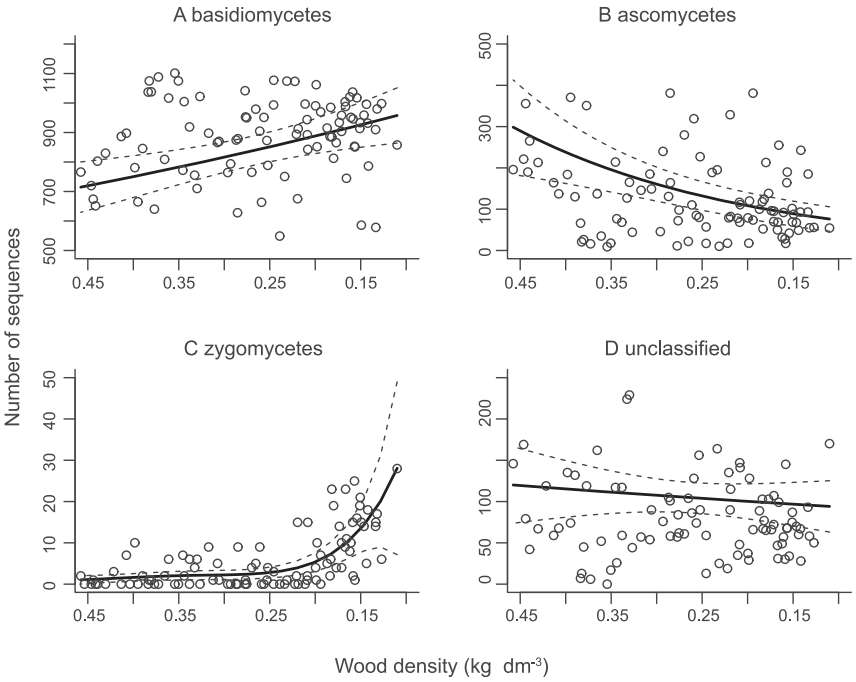

Fig. 2. Response of *fungal phylum* to decreasing wood density gradient. Estimated GAMM (generalized additive mixed model) response curves together with 95% confidence intervals. Original observations are shown by open symbols. Number of sequences per sample was normalized to 1110, there were 98 samples and a total of 108,780 reads.

In line with the general response of white-rot fungi, the abundance of *Phellinus nigrolimitatus* did not show a significant relationship to wood density and was observed throughout the decay gradient (Table 1, Fig. 4A). Similarly, *Hyphodontia* sp. and *Mycena* sp. did not show any significant covariation with density (Table 1), but the former was less abundant or absent from fresh/intact logs (Fig. 5A) and the latter was most abundant in slightly-decayed wood (Fig. 5B).

In contrast to other white-rot fungi, *Phellinus viticola* peaked in abundance during intermediate stages of decay (Table 1, Fig. 4B) and exhibited some specificity with respect to habitat. Similarly, the brown-rot fungi *Fomitopsis pinicola* and *Antrodia serialis* were most abundant in moderately-decayed wood although they could also be found in earlier stages (Fig. 4C, D). The basidiomycete brown-rot fungus *Coniophora olivaceae* (Fig. 4E), and ascomycete genera *Phillocephala* (Fig. 5C) and *Phialophora* (Fig. 5D) were typical of slightly decayed, high-density wood.

Most of the fungal species detected became more abundant as decomposition continued and wood density decreased, e.g., ectomycorrhizal fungi such as *Piloderma sphaerosporum* (Fig. 4F), *Tylospora fibrillosa* (Fig. 4G) and *Russula emetica* (Fig. 4H). These species, along with other members of *Piloderma* sp. (Fig. 5E), *Tylospora* sp. (Fig. 5F) and *Russula* sp. (Fig. 5G), as well as *Pseudotomentella* sp. (Fig. 5H), *Lactarius* sp. (Fig. 5I), *Cortinarius* sp. (Fig. 5J), *Meliniomyces* (Fig. 5K), *Tomentella* sp. (Fig. 5L), *Thelephora* sp. (Fig. 5M) and *Amanita* sp. (Fig. 5N) reached their peaked abundance in late-stages with low density used. *Xeromphalina campanella* was typical of heavily-decayed wood but was more frequently observed in other stages of the wood density gradient than mycorrhizal species (Fig. 4I).

A comprehensive account of the occurrence and abundance of all identified species and genera across the decay gradient is

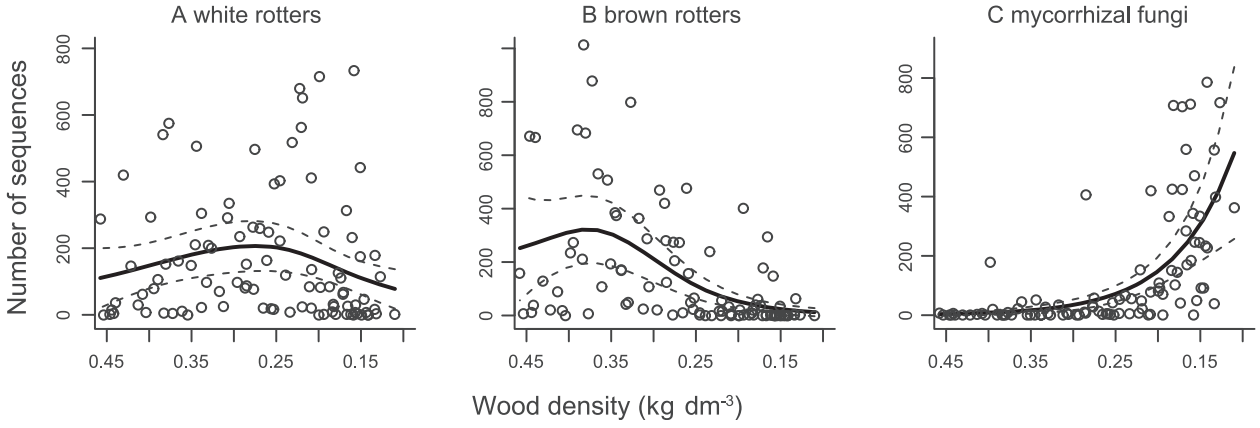

Fig. 3. Response of *functional groups* to decreasing wood density gradient. Estimated GAMM (generalized additive mixed model) response curves together with 95% confidence intervals. Number of sequences per sample was normalized to 1110, there were 98 samples and a total of 108,780 reads.

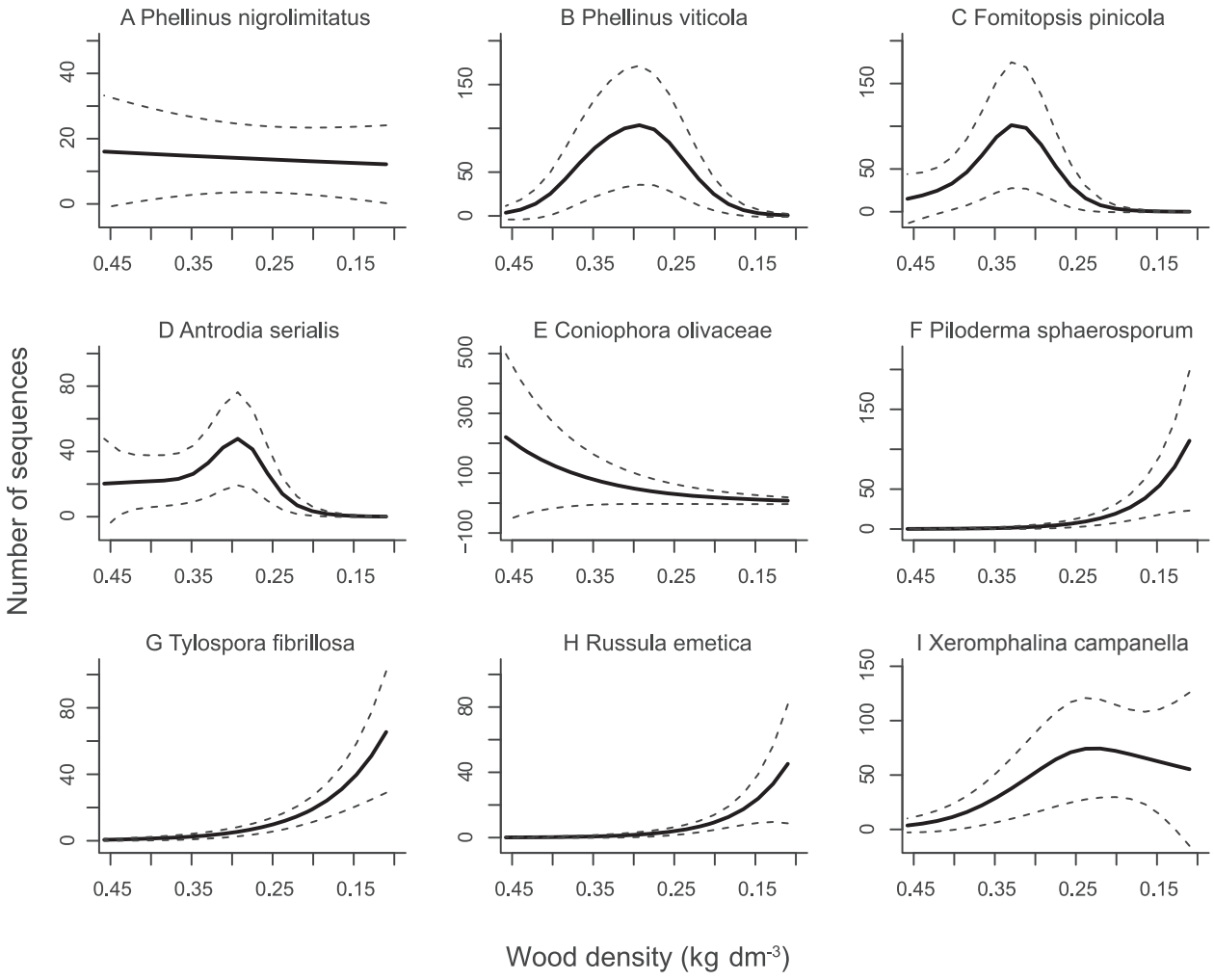

Fig. 4. Response of *fungal species* on decreasing wood density gradient. Estimated GAMM (generalized additive mixed model) response curves and 95% confidence intervals. Number of sequences per sample was normalized to 1110, there were 98 samples a total of 108,780 reads. Significance of smooth-termed density gradient for fungal genera and the number of observations are presented in Table 1. See Table S2 for a distribution of original observations along density gradient.

provided in Tables S2 and S3.

4. Discussion

Fungal species richness increased as the wood substratum decomposed to a lower density. The finding of the pooled samples is congruent with the observation of separately analyzed logs (Fig S1) and supports our earlier studies made with denaturing gradient gel electrophoresis (DGGE) (Rajala et al., 2011, 2012). Resolution of the 454-pyrosequencing was higher than DGGE, as it was able to detect more than 80 OTUs whereas DGGE revealed a maximum of 25 OTUs per drill-core sample from a single log (Rajala et al., 2012). A trend of increasing species richness also agrees with other studies investigating fungal communities in decomposing spruce logs with culture-free molecular techniques (Kubartová et al., 2012; Ovaskainen et al., 2013). In contrast, studies of polypore fruit bodies typically conclude that the number of fungal species peaks during the middle stages of decay (Bader et al., 1995; Renvall, 1995; Lindblad, 1998). However, it should be noted that culture-free molecular techniques are capable of detecting all taxa (including inconspicuous fungi) and statements based on polypore fruit bodies should be restricted to the patterns exhibited by the polypores rather than indicative of the entire fungal community. It should also be noted that species that form conspicuous fruit bodies may cease this activity in strongly decayed wood although viable mycelium remains (Ovaskainen et al., 2013). In theory, DNA may originate from inactive cells and in that sense RNA could be a better marker for the metabolically-active population, especially if the resolution of the technique (such as DGGE) is limited with respect to community richness (Rajala et al., 2011). However, the significance of any DNA persistence in high-throughput sequencing results seems marginal, as here DNA- and RNA-based fungal richness in dead wood was equal (Fig S2). At the end of decomposition, logs have already become part of the soil litter and exposed to a tremendous diversity of soil fungi. As such, it follows that as the log becomes increasingly decomposed and more integrated into the soil, the fungal richness increases and community composition gradually shifts from one specialized for wood decomposition to that which is resident in the underlying soil. Irrespective of fungal species richness, the decomposition rate is estimated to be highest during the intermediate stage of decay (Mäkinen et al., 2006) due to the abundance and activity of brown- and white-rot fungi. However, data concerning mass loss in heavily-decayed logs are scarce, making estimates of decomposition rate uncertain, and a recent study contradicted the notion of a decelerating rate in the later stages of decay (Valentín et al., 2014).

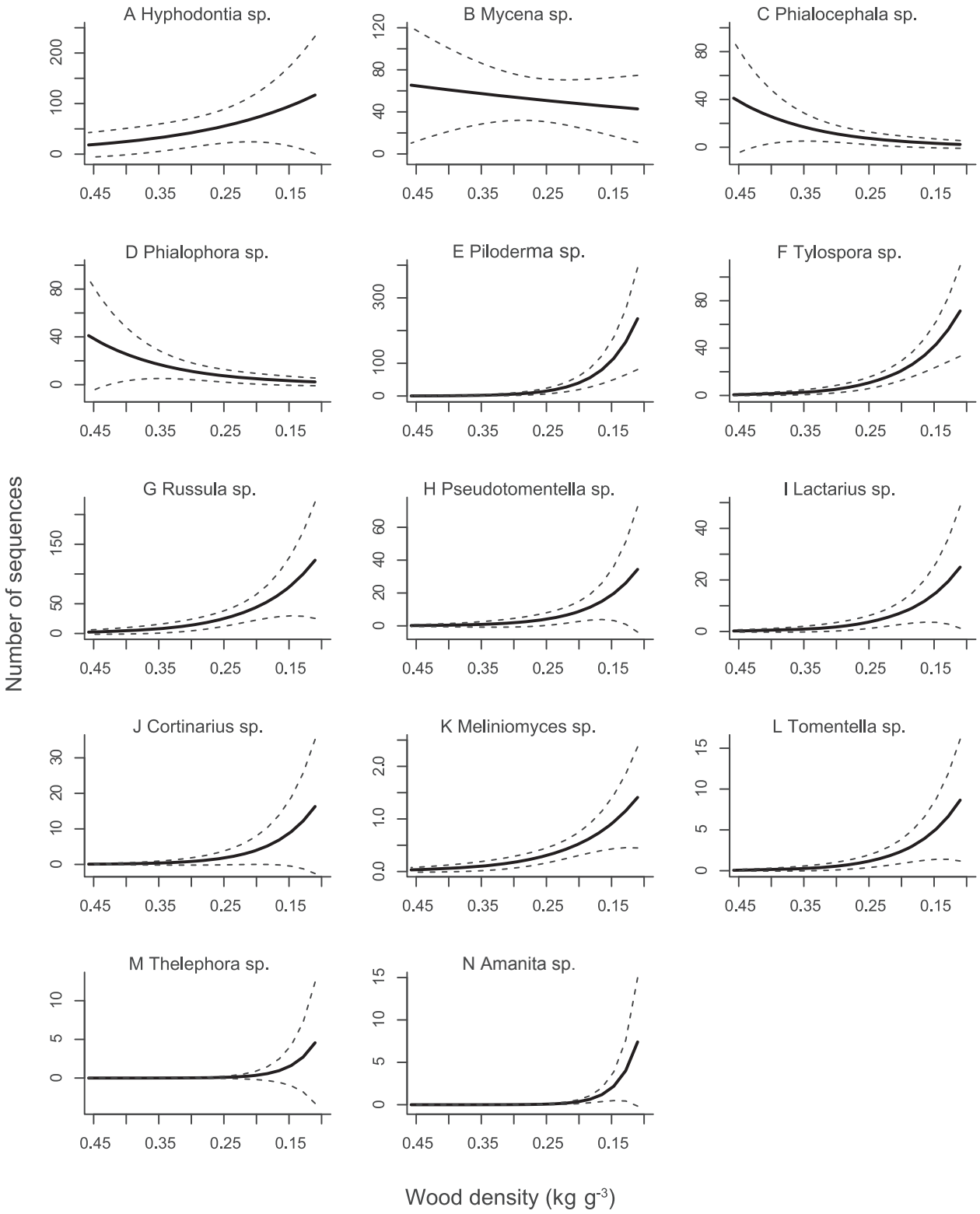

Fig. 5. Response of *fungal genera* to a decreasing wood density gradient. Estimated GAMM (generalized additive mixed model) response curves together with 95% confidence intervals. Number of sequences per sample was normalized to 1110, there were 98 samples and a total of 108,780 reads. Significance of smooth-termed density gradient for fungal genera and number of observations are presented in Table 1. See Table S3 for a distribution of original observations along density gradient.

We observed a succession of fungal species tied to decomposition of the woody matrix, and which correlates well with moisture content as well as structural and chemical composition (Rajala et al., 2011, 2012; Kubartová et al., 2012; Ovaskainen et al., 2013). To achieve reliable general habitat models, nearly 550 logs were sampled from five different sites in southern Finland. Without the large number of logs species occurrence data would have remained too small and only a few of the most common species could have

been modeled. However, within sites, five logs with similar densities were pooled to reduce sequencing costs. White-rot, brown-rot and mycorrhizal fungi showed different patterns of abundance change along the gradient of decreasing wood density, suggesting some niche segregation. This result agrees with our earlier work based on the sequencing of DGGE-OTUs (Rajala et al., 2011, 2012) but, in contrast to those studies, the dataset obtained with 454-pyrosequencing was large enough to inform precise models of species response to a changing quality of woody substratum. Quality was estimated as density, which correlates with other dead wood characteristics (Rajala et al., 2012). We accept that cause of tree death, type of stem breakage, local environment, species interactions and primary species may also influence fungal community structure and its dynamics (Renvall, 1995; Ovaskainen et al., 2010; Siitonen and Stockland, 2012; Stockland and Siitonen, 2012; Jonsson and Stockland, 2012; Ottosson et al., 2014), but these factors were not included because our primary aim was to develop habitat models that can be directly linked to forest stand simulation models that are applied widely in forest management planning.

Most white-rot fungi (e.g., *Phellinus nigrolimitatus* and *Hyphodontia* sp.) showed no significant relationship with wood density, suggesting these taxa to be rather generalist in their habitat requirements. Perennial, medium-sized fruit bodies of *P. nigrolimitatus* are typically observed during the late-intermediate stages of decay (Renvall, 1995; Lindblad, 1998; Jönsson et al., 2008), but we observed here that the mycelium of this species was already abundant in relatively fresh and intact wood. This result adds support to the claim that *P. nigrolimitatus* requires a considerable amount of time before fruiting (Ovaskainen et al., 2013). Some members of *Hyphodontia* might also require a similar growth-maturation period because we detected their mycelia in slightly-decayed wood when their fruit bodies are typically observed during the intermediate phase of decay (Renvall, 1995; Lindblad, 1998). An explanation for the strategy of delayed fruiting is not clear but may be related to a critical mass of mycelium required to support the production of fruit bodies, or external factors such as substratum quality and interactions with other species. In contrast to most other white-rot fungi, *Phellinus viticola* appeared to be specialized to a particular phase of decomposition, being detected and rapidly increasing in abundance in moderately-decayed wood. This species seems to have a rather narrow niche, with small and short-lived perennial fruit bodies that may be produced and supported with a relatively small mycelial mass (Renvall, 1995; Ovaskainen et al., 2013).

Brown-rot fungi were most abundant in slightly- or moderately-decayed wood, suggesting an important role in spruce decomposition and supporting results based on fruit-body inventories (Renvall, 1995). Thus, it seems that heavily-decayed wood is suboptimal habitat for brown-rot fungi and they quickly yield to more competitive species that are capable of decomposing lignin. *C. olivaceae* was the most common species in the data set throughout the density gradient and reached peak abundance in slightly decayed wood. This apparent generalist produces fruit bodies throughout decomposition (Lindblad, 1998) but less frequently in advanced stages (Renvall, 1995). Furthermore, Ovaskainen et al. (2013) observed a strong correlation between fruit bodies of *C. olivaceae* and extensive mycelial colonization. A similar observation was made for the common polypore *Fomitopsis pinicola*, and our results support earlier findings showing that this species tends to prefer moderately-decayed spruce logs but is also common on newly-fallen wood (Renvall, 1995; Lindblad, 1998; Jönsson et al., 2008; Ovaskainen et al., 2013). Saproxylic fungi such as *F. pinicola* can occur abundantly at the start of the decay process because this species can colonize living tissue, where it grows quickly, forms brown rot and produces large and long-lived fruit bodies. *Antrodia serialis,* another common brown-rot fungus, uses a different fruiting strategy as it forms annual and resupinate fruit bodies. We detected *A. serialis* in fresh, intact wood of high density and observed its peak abundance in the intermediate stage when it typically forms its fruiting bodies (Renvall, 1995; Lindblad, 1998; Jönsson et al., 2008).

Mycorrhizal fungi (e.g., *Cortinarius*, *Lactarius*, *Piloderma*, *Pseudotomentella*, *Russula*, *Tomentella* and *Tylospora*) clearly increased toward the end of the wood density gradient, suggesting that soil is their primary habitat. In boreal forest soil, ectomycorrhizal fungi compose one third of the microbial biomass (Högberg and Högberg 2002) where they exist in a symbiotic exchange of carbon and nutrients with their host trees (Högberg et al.,2010; Smith and Read, 2008). Mycorrhizal fungi gather nutrients efficiently from the soil and some species may also decompose complex and otherwise inaccessible organic compounds, such as woody substrata, via release of ligninolytic enzymes or the Fenton reaction (Chambers et al., 1999; Read and Perez-Moreno, 2003; Talbot et al., 2008; Bödeker et al., 2009). The extent to which mycorrhizal fungi are obligate symbionts or facultative saprotrophs is currently under debate (Talbot et al., 2008; Baldrian, 2009; Lindahl and Tunlid, 2014). Nevertheless, their colonization of decaying logs has been at least explained in terms of their search for nitrogen or other nutrient sources from the wood itself or the mycelia of other fungal decomposers (Lindahl et al., 1999; Buée et al., 2007). Mycorrhizal colonization may also be a prelude to the production of fruit bodies (Siitonen and Jonsson, 2012). Moreover, some mycorrhizal species detected in the wood may have been associated with small seedlings with their roots in decaying logs, but this study did not investigate the significance of such seedlings. More research is needed to fully understand the use of dead wood by mycorrhizal fungi.

5. Conclusions

We detected many more fungal phylotypes and inferred a greater diversity than described by previous studies relying on the observations of macroscopic fruit bodies or automated sequencing of different OTUs resolved by DGGE. In conflict with the prevailing concept of diminishing diversity in the late stages of decomposition, we detected a steady increase in diversity and a succession of brown-rot to white-rot to mycorrhizal-dominated communities as the decay process continued and wood density decreased. Although high-throughput sequencing is a powerful technique to identify most of the fungi inhabiting the woody substratum (Ovaskainen et al., 2013), we acknowledge the limitations related to the amount of samples, necessity for pooling, PCR biases and accuracy of species identification based on reference sequences (Stenlid et al., 2008; Lindahl et al., 2013). Nevertheless, the habitat models produced in this study generally agree with those based on fruit body inventories although they often proposed broader niches than previously defined due to the fact that mycelia may actively colonize and grow in the woody substrata without forming fruit bodies. Some forest simulation models already include wood decomposition parameters, which predict the amount and quality of dead wood substratum in different management scenarios (as demonstrated by Peltoniemi et al., 2013). The inclusion of the fungal species abundances in such simulation models allows the analysis of biodiversity indicator species in different management protocols and the development of sustainable forest management strategies.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.funeco.2015.08.007.

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