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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.

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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

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