Mycobiome analysis of asymptomatic and symptomatic Norway spruce trees naturally infected by the conifer pathogens *Heterobasidion* spp.

Running title: Mycobiome of Norway spruce

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Originality-Significance Statement

This study is the first to address the effect of root and butt rot disease on the composition of fungal communities associated with Norway spruce and the connection between health status of spruce trees and the composition of the resident mycobiota. Presented results showed the significant differences in structure of fungal communities inhabiting wood of symptomatic and asymptomatic spruce trees. Our study provides a new insight into the interaction of fungal plant pathogens with the resident plant microbiota.

Summary

Plant microbiome plays an important role in maintaining the host fitness as demonstrated by numerous studies. Despite a significant progress achieved in our understanding of the factors affecting the composition of microbial communities associated with trees, very little is known about the effect of plant pathogens on their structure. We analyzed the mycobiome of different parts of Norway spruce as well as their fungal communities on asymptomatic and symptomatic naturally infected trees. Using *Heterobasidion*-rotted and infected trees as a model, we investigated the primary impact of the disease on fungal communities associated with Norway spruce trees. Our results demonstrate that symptomatic and asymptomatic *Heterobasidion*-infected trees significantly differed in the structure of the fungal communities residing in their wood, but not in other anatomic regions. Each of the investigated tissues (wood, bark, needles and roots) harbored a unique fungal community. Symptomatic trees were more susceptible to co-infection by other wood-degrading fungi.

Introduction

All groups of land plants, ranging from mosses to angiosperms, live in close association with a diverse set of microorganisms. Both outer plant surfaces and inner parts of the plant body are

colonized by various taxa of bacteria, fungi, archaea, and protists, together comprising plant microbiota (Turner et al., 2013). It is widely accepted that plant microbiota influences host fitness (Vandenkoornhuyse et al., 2015). Certain plant-associated fungi and bacteria contribute to plant growth promotion and resistance against biotic and abiotic stresses (Hardoim et al., 2015). However, dormant pathogens and saprobes equally belong to plant microbiota (Porras-Alfaro and Bayman, 2011; Hardoim et al., 2015). Thus, the interactions between plants and the associated microorganisms can range from mutualism through commensalism to pathogenicity. The outcomes of specific interactions are influenced by a number of driving forces, including host and microbial genotypes, abiotic factors, and interactions within plant microbiome (Hardoim et al., 2015).

The application of metagenomics and metatranscriptomics boosted the studies on plant microbiome function and its role in plant health and stress tolerance (Lebeis, 2015). Nevertheless, the current information on factors driving the composition of plant microbiome and, particularly, forest trees is still very scarce. The available data suggest that microbial communities of the rhizosphere are mainly influenced by soil types, whereas host plant genotype has a limited effect on their composition (Weinert et al., 2011; Bulgarelli et al., 2012; Lundberg et al., 2012). In contrast, microbial communities of phyllosphere and endosphere are predominantly determined by host plant species (Redford et al., 2010; Bulgarelli et al., 2013).

Reports on abilities of microbial endophytes to improve host fitness and stress tolerance inspired the idea of using endophytic microorganisms as biocontrol and growth-promoting agents (Backman and Sikora, 2008; Mejia et al., 2008; Blumenstein et al., 2015; Pautasso et al., 2015). However, the research on the impact of microbiome on the plant disease resistance is still in its early stage. The effects of pathogens on microbiome community and *vice versa* observed in a few available studies differed among used experimental models (Hardoim et al., 2015), making it difficult to draw any general conclusions. Nevertheless, some pioneering studies do indicate that there might be a

correlation between the structure of microbiome communities and host plant resistance / susceptibility to pathogens (Ardanov et al., 2012; Martin et al., 2013).

Butt and root rot disease caused by fungi belonging to *Heterobasidion annosum* species complex has a great economic impact on forest industry in boreal zone (Asiegbu et al., 2005). The pathogen grows necrotrophically in the sapwood of living trees and saprotrophically in dead wood tissues. In Norway spruce, the disease develops slowly resulting in the formation of decay column within the tree trunk, but it rarely causes instant mortality of spruce trees (Asiegbu et al., 2005). Often, decay zone remains limited to heartwood, but occasionally pathogen can reach sapwood. Oliva et al (2013) have observed in field inoculations that heartwood of Norway spruce stumps were more susceptible to *H. parviporum* and *H. annosum* s.s. infection than pine. Current control strategies focus on prevention of fungal infection of tree stumps remaining after harvesting. No absolute protection and elimination of the fungus from already infected trees or stumps are available. Therefore, better understanding of the interactions between *Heterobasidion* fungi, their hosts and other components of host microbiome is needed for the development of novel, more efficient disease management strategies.

The aim of the presented study was to investigate the composition of fungal communities associated with different anatomical tissues of Norway spruce trees and to assess the impact of the root and butt rot caused by *Heterobasidion* sp. on the structure of these communities under field conditions. The pathogen establishment is likely to occur in an interaction with a resident microbiota of the infected tree. We hypothesized that there are significant differences in microbial communities of asymptomatic and symptomatic Norway spruce trees. As the extent of the potential effect exerted by *Heterobasidion* infection on spruce fungal communities was difficult to predict, we sampled not only tissues visibly closer to the decay zone (down stem, see Figure 1), but also included in our analysis more distant parts of spruce trees, namely root, bark and needles.

Results and discussion

MiSeq sequencing output

A total of 8 276 762 high quality sequences were generated across root, down stem, upper stem, bark and needle samples in the three sampling sites after sequence denoising and quality filtering. After filtering out unclassified sequences and sequences assigned to plant and animal domains, a core set of 7 673 670 sequences assigned to fungal domain was obtained. Due to technical problem of PCR amplification and sequencing, 16 out of 90 samples had lower number of reads (less than 7000) and were excluded from further analysis. The excluded sampled also had lower values of Good's coverage index than the remaining ones. The number of sequences in the remaining samples ranged from 194 915 to 22 278 with an average of 103 390 ± 44 630 (mean ± SD) sequences.

Occurrence of *Heterobasidion* in sampled trees

One of the unexpected findings of our study was that two OTUs assigned to the genus *Heterobasidion* were present not only in diseased trees showing symptoms of wood decay, but also in apparently healthytrees without decay symptoms (Fig. 1). Following this observation, the sampled trees were classified as "symptomatic (with decay)" and "asymptomatic (without decay), respectively.

Otu00011 was tentatively classified as *H. annosum*, whereas Otu00048 was assigned to *H. parviporum*. Both species occur naturally in Finland. *H. parviporum* predominantly infects Norway spruce trees, whereas *H. annosum* has broader host spectrum and infects both Scots pine and Norway spruce. Several explanations can be proposed for the presence of *Heterobasidion* spp. in asymptomatic trees. First, the entry of pathogen in these trees might have occurred relatively recently, i.e. at the time point of the sample collection the trees were at the initial stages of the disease development, with no detectable symptoms of wood decay. Alternatively, the lack of disease symptoms despite presence of *Heterobasidion* could be related to the genetic resistance

125 background of each individual tree, allowing asymptomatic trees to restrict the fungal growth and
126 invasion. It is known that spruce trees show natural variation in their susceptibility to
127 *Heterobasidion* infection, which could probably explain the absence of disease symptoms despite
128 the identification of the pathogen in the sampled trees. Finally, if two detected OTUs assigned to
129 *Heterobasidion* in fact represented two different species, it is possible that they differed in their
130 virulence. Our data show that Otu00048 was more abundant in asymptomatic trees, whereas
131 Otu00011 had higher abundance in symptomatic trees (Table S1). Taking into account the last
132 observation, we consider the cross-contamination during the sample processing rather unlikely
133 sources of *Heterobasidion*-specific reads in samples from asymptomatic trees. We however cannot
134 rule out completely the possibility that at least some of obtained reads were due to the presence of
135 *Heterobasidion* spores, which landed on the samples surface during harvesting. However, the spore
136 load is expected to be equal for all samples, whereas our data show clear differences in the
137 abundance of two OTUs assigned to the genus *Heterobasidion* among symptomatic and
138 asymptomatic trees.
139
140 ***Richness, diversity and evenness of mycobiome communities of Norway spruce***
141 Quality-filtered fungal sequences were clustered into 4375 OTUs (excluding singletons). The sub-
142 sampled set used to calculate richness, diversity and evenness contained 4315 OTUs. The highest
143 richness of fungal communities in asymptomatic and symptomatic trees were observed in needles
144 and in roots, respectively. The bark had the lowest numbers of OTUs in both symptomatic and
145 asymptomatic trees (Fig. 2A). There were no significant differences in the fungal species richness
146 among asymptomatic and symptomatic trees in any of the sampled tissues. Needles had the highest
147 community diversity in both symptomatic and asymptomatic trees. The lowest community diversity
148 was observed in roots and in down stem in asymptomatic and in symptomatic trees, respectively
149 (Fig. 2B). However, no statistically significant difference in fungal diversity was found in any of the

150 regions among symptomatic and asymptomatic trees. The highest evenness of fungal communities
151 was found in bark of symptomatic trees and in needles of asymptomatic trees, whereas the lowest
152 evenness was observed in roots of both groups (Fig. 2C). Evenness in needles of asymptomatic
153 trees was significantly higher than that of symptomatic trees.
154 The sampled tissues of the spruce trees shared 738 (16.9%) of the total 4375 OTUs. The proportion
155 of the OTUs unique to a certain tissue ranged from 1.6% (69 OTUs; bark) to 13.3% (584 OTUs;
156 roots) (Fig. 2D).
157 The PCoA based on the relative OTUs abundance explained 28.5% of the observed variation and
158 showed distinct clusters for each of the sampled tree tissues (Fig. 3), which were confirmed by
159 PERMANOVA ($p < 0.001$ in all possible pairs). The detailed taxonomic analysis of OTUs detected
160 in specific Norway spruce tissues is presented in Supporting Notes 1 and 2 and in Supporting
161 Figures S1-S6.
162
163 ***Impacts of health status on structure of fungal communities of Norway spruce***
164 We hypothesized that there are significant differences in microbial communities of asymptomatic
165 and symptomatic Norway spruce trees. This hypothesis was partially confirmed. There were no
166 significant differences among symptomatic and asymptomatic trees in the structure of fungal
167 communities inhabiting their needles, upper stem bark or roots. At the same time, the
168 PERMANOVA demonstrated that the structures of fungal communities associated with upper stem
169 and down stem of symptomatic and asymptomatic trees were significantly different (p=0.001 and
170 p=0.011, respectively) (Fig. S7). OTUs that significantly contributed to the shift in fungal
171 community structure among samples from symptomatic and asymptomatic trees are listed in Tables
172 S2 and S3. These results indicate that *Heterobasidion* infection has an effect on fungal communities
173 in the parts of the tree adjacent to the tissues colonized by the pathogen, but no significant effect on
174 more distant parts. However, it was demonstrated recently that *Heterobasidion* spp. infection

promotes mycorrhiza development in *Pinus pinea* (Zampieri et al., 2017), indicating that *Heterobasidion* infection might have more profound effects. Several explanations could be proposed for the lack of significant differences in the structure of fungal communities associated with asymptomatic and symptomatic spruce trees in our experiment. First, mycorrhizal fungi constituted only a small fraction in our dataset, and it might be due to our sampling strategy, as we sampled suberized roots and did not collect fine roots. The results might be different if fine roots colonized by ectomycorrhizal fungi were included in the analysis. Second, there might be differences among different tree species in a way they react to *Heterobasidion* infection. Pine trees in the experiment of (Zampieri et al., 2017) showed strong decline in vitality a few months after inoculation. At the same time, sampled spruce trees showed no symptoms of infection except for the heartwood decays, which could be observed only after tree felling. This is in line with the fact that *Heterobasidion* infection in Norway spruce develops slowly and infected trees show little or no symptoms. Thus, it is likely that changes in fungal communities in Norway spruce do not have a systemic effect and are restricted to tissues adjacent to *Heterobasidion* wood decay, at least at the early stages of infection.

OTUs with different relative abundance in asymptomatic and symptomatic trees

The abundance of certain OTUs differed between asymptomatic and symptomatic trees. Some saprotrophic (e.g., *Talaromyces* sp., *Trichoderma atroviridis*, *Penicillium* sp.) and wood-degrading species (e.g., *Inonotus* sp., *S. sanguinolentum*, *A. areolatum*) had higher abundance in symptomatic trees. However, many of them were detected in a limited number of trees. For example, OTU classified as *Inonotus* sp. was abundant only in 2 out of 9 symptomatic trees, and *S. sanguinolentum* and *A. areolatum* were abundant only in a single tree each. These observations might indicate that trees infected by *Heterobasidion* become more susceptible to co-infection with other wood-degrading fungi, but there might be additional factors that determine what particular species will

occupy an individual tree and, likely, the stage of the disease and its progression also have their effect.

At the same time, the observed difference in abundance of a number of OTUs among asymptomatic and symptomatic trees received statistical support in our analysis. Out of 50 OTUs with the highest abundance, 10 OTUs showed differences of abundance in at least one of the sampled spruce tissues (Tables 1 and S1). Nine of them were more abundant in asymptomatic trees. Only three of those could be assigned to a certain species, namely *Hypogymnia tubulosa*, *Scoliciosporum umbrinum* and *Phialocephala fortinii*. At the same time, Otu00002, classified as *Talaromyces* sp., was more abundant in symptomatic trees. *Talaromyces* is a large genus of saprotrophic fungi frequently isolated from various organic substrates, including plant litter. Some of them are potent producers of secondary metabolites, and *T. flavus* is widely used in the biological control of soil-borne plant pathogen. The corresponding OTU was abundant in all nine sampled symptomatic trees, but the biological significance of this finding merits further investigation.

Among OTUs that were more abundant in asymptomatic trees, two belonged to lichens and one – to the root endophyte *P. fortinii*. The higher abundance of *P. fortinii* in asymptomatic trees is noteworthy. Dark septate endophytes of *P. fortinii* species complex are nearly ubiquitously present in root of conifer trees and ericaceous plants (Grunig et al., 2008). Their biological role remains largely unknown, but it was demonstrated that secondary metabolites produced by members of this species complex have an inhibitory effect on plant pathogens (Tellenbach et al., 2013). Additionally, it was shown that a *Phialocephala* isolate protects Norway spruce seedlings from *H. parviporum* infection in *in vitro* experiments (Terhonen et al., 2016). In view of these observations, the protective ability of the endophytes of *P. fortinii* species complex against *Heterobasidion* infection in spruce trees deserves further investigation.

The higher abundance of two lichen species, *Hypogymnia tubulosa* and *Scoliciosporum umbrinum*, in samples from asymptomatic trees is remarkable, too. Lichens are well-known as producers of

biologically active secondary metabolites (Yilmaz et al., 2005). Further work will be required to elucidate whether identified lichen species have any antagonistic properties against root and butt rot pathogens.

To the best of our knowledge, only few studies have addressed the effect of plant pathogens on the composition of plant microbiota. This is also the first comprehensive report on mycobiome of different anatomic regions of Norway spruce documented in a single study. The results of our work provide additional evidence that disease progression causes significant changes in the structure of resident microbial communities. However, the situation is likely more complicated, as the effect of the pathogen is rather localized and it might not affect more distant parts of the tree. The observed higher abundance of the dark septate endophyte *P. fortinii* in asymptomatic trees justifies further studies on the role of *Phialocephala* species as natural antagonists to *Heterobasidion* spp. Our data demonstrate the suitability of *Heterobasidion* – spruce pathosystem to address the fundamental question of interactions between plant pathogens and the resident microbiota.

Experimental procedures

Study sites and sample collection

Three Norway spruce (*Picea abies* (L.) Karst.)-dominated forest sites in the municipality of Mäntsälä (Uusimaa region, Southern Finland) were chosen for sampling. The sites are located in privately-owned managed forest and are distributed in three selected plots: (1) plot 1 (60°44'51"N, 25°13'17"E), (2) plot 2 (60°45'11"N, 25°13'24"E, and (3) plot 3 (60°45'15" N, 25°15'34" E).

All three sampling plots are representative examples of managed spruce forest used for commercial timber production and growing at comparable conditions. Spruce stands at the selected sites were naturally regenerated and of the same age (approximately 55 years at the time of sampling). All three plots are located within an area with a relatively high incidence of *Heterobasidion* infection.

The elevation of the sites ranges from 87 to 95 m above sea level. Sample collections were conducted in May 2016.

Our sampling was performed simultaneously with the tree harvesting. The samples were collected immediately after tree felling. In this way, we could clearly distinguish asymptomatic and symptomatic trees based on presence or absence of wood decay column at stump height.

In each plot, six spruce trees were selected: three trees showing symptoms of *Heterobasidion*-induced wood decay (further referred to as symptomatic trees) (Fig. 1A), and three trees without decay symptoms (further referred to as asymptomatic trees) (Fig. 1B). Diameter of selected trees ranged from 40 to 64 cm. In total, samples of suberized roots, down stem (bark+sapwood+heartwood), upper stem sapwood (referred to as upper stem), upper stem bark (referred to as bark), and needles (Fig. 1) were taken from a total of nine asymptomatic and nine symptomatic trees.

DNA extraction, amplification of ITS2 region and sequencing

Spruce tissues samples were surface-sterilized with 70% ethanol prior to DNA extraction. Total DNA was extracted from grinded spruce tissues following a standard cetyl–trimethyl ammonium bromide (CTAB) method (Chang et al., 1993) with modifications described in (Terhonen et al., 2011). The concentrations and purity of the isolated DNA were measured using NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA).

PCR amplification of the fungal ITS2 region and sequencing were performed in the facilities of the Institute of Biotechnology (BI, University of Helsinki, Finland). The use of ITS2 region for metabarcoding of fungal communities was advocated in recent reports (Tedersoo et al., 2015; Tedersoo and Lindahl, 2016). Prior to sequencing, a nested PCR was performed. In the first PCR round, extracted DNA samples were used as templates, and the amplification was performed using the primers gITS7 and ITS4 (Ihrmark et al., 2012) containing partial TruSeq adapter sequences at

the 5´ends (ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC T and GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC T). The combination of primers gITS7 and ITS4 is widely used in metabarcoding of fungal communities. They have known limitations, as there are some mismatches between the sequences of the primers and the corresponding sequences of rRNA genes in many Tulasnellaceae, Archaeorhizomycetes and Microsporidia. A newly proposed pair of primers, which should have better performance, was published after the completion of the experimental part of our project (Taylor et al., 2016), and we were not able to assess its suitability. In the second round of PCR, full-length TruSeq P5 and Index containing P7 adapters were used as primers and the products of the first PCR were used as templates. The PCR products were purified and sequenced with Illumina MiSeq platform. Raw sequences were deposited at the European Bioinformatics Institute (EBI) under project accession number PRJEB21787 (http://www.ebi.ac.uk/ena/data/view/ PRJEB21787).

Pre-processing and analysis of ITS2 sequences

The raw ITS2 sequences were pre-processed at BI. The read quality was checked with FastQC (Andrews, 2010). Adapter and barcode sequences were removed using Cutadapt (Martin, 2011). The pre-processed data were analyzed using the mothur standard operation pipeline (SOP, v.1.37.6) (Schloss et al., 2011) with the modifications described earlier (Sun et al., 2016). Briefly, pair-end reads were converted to contigs with minimum overlap of 25bp. Sequences containing ambiguous bases (N) and homopolymers longer than eight nucleotides were removed. Processed sequences were pre-clustered with a distance of 2 nt/100 nt using a pseudosingle-linkage algorithm (Huse et al., 2010). Each sequence that passed quality filtering was truncated to a 230-bp length after primer and tag removal. All potential chimeric sequences were identified using the mothur-embedded UCHIME algorithm (Edgar et al., 2011) and removed. Unique sequences were pairwise aligned using the Needleman method (Needleman and Wunsch, 1970). The aligned distance matrices were

clustered into operational taxonomic units (OTUs) using the average neighbor algorithm and 97% sequence similarity. All global singletons (OTUs containing only one sequence across all samples) were removed, and the most abundant sequence in each OTU was selected to be the representative sequence. The sequences and OTUs were assigned to taxa using the mothur-formatted UNITE taxonomy reference database (UNITE+INSD, Version 7.2) (Koljalg et al., 2013) with an 80% bootstrap confidence threshold in mothur (Wang et al., 2007).

To correct the difference in sample size and ensure comparable estimators across samples, a subset of 22 500 sequences per sample (minimum number of sequences recovered among all samples) was randomly selected to calculate the diversity and to compare the community structure. The following parameters were calculated for all samples: observed and estimated fungal richness (Chao 1), diversity (Inverse Simpson's complement – 1-D), evenness (Simpson's equitability - ED) and Good's coverage (complement of the ratio between local singleton OTUs and the total sequence count).

Venn diagrams were constructed from the presence/absence transformed data (without singletons) with venn function from gplots (Warnes et al., 2016). Non-parametric Kruskall-Wallis tests with Hodges-Lehmann estimate were used to identify differences in diversity, species richness and evenness among the sampled tissues in symptomatic and asymptomatic trees. Principal coordinates analysis (PCoA) was used to visualize the fungal community structure with Bray-Curtis similarity using relative abundances of OTUs in PRIMER v.6 (Clarke and Gorley, 2006) with the add-on package of PERMANOVA + (Anderson et al., 2008). Prior to PCoA, the data were square root transformed to meet the analysis criteria. Subsequently, a PERMANOVA test was used to determine the significant difference in community structure between different regions in the tree.

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Conflict of interest statement

The authors declare no conflict of interest.

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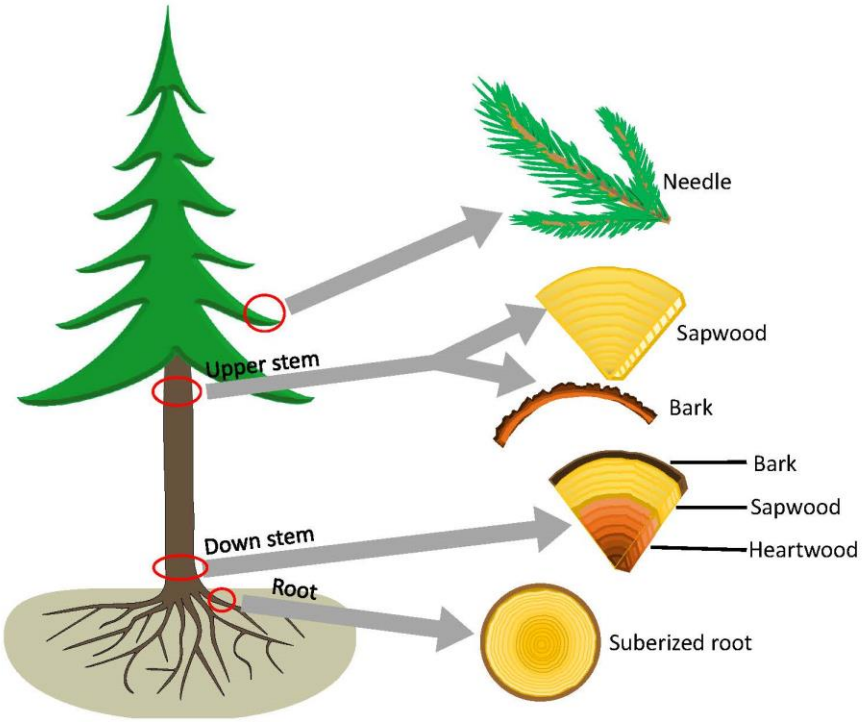
433 **Table and Figure legends**

434 **Table 1.** OTUs from the 50 most abundant OTUs in the combined dataset showing significant
435 differences in abundance among asymptomatic and symptomatic trees.



436

437 **Figure 1.** Representative pictures of sampled Norway spruce trees from the sampling site. (A) A
438 tree classified as symptomatic, with extensive wood decay caused by *Heterobasidion* sp. (B) An
439 asymptomatic tree without visible symptoms of wood decay. (C) Schematic diagram illustrating the
440 Norway spruce tissues sampled for the analysis of associated mycobiota.

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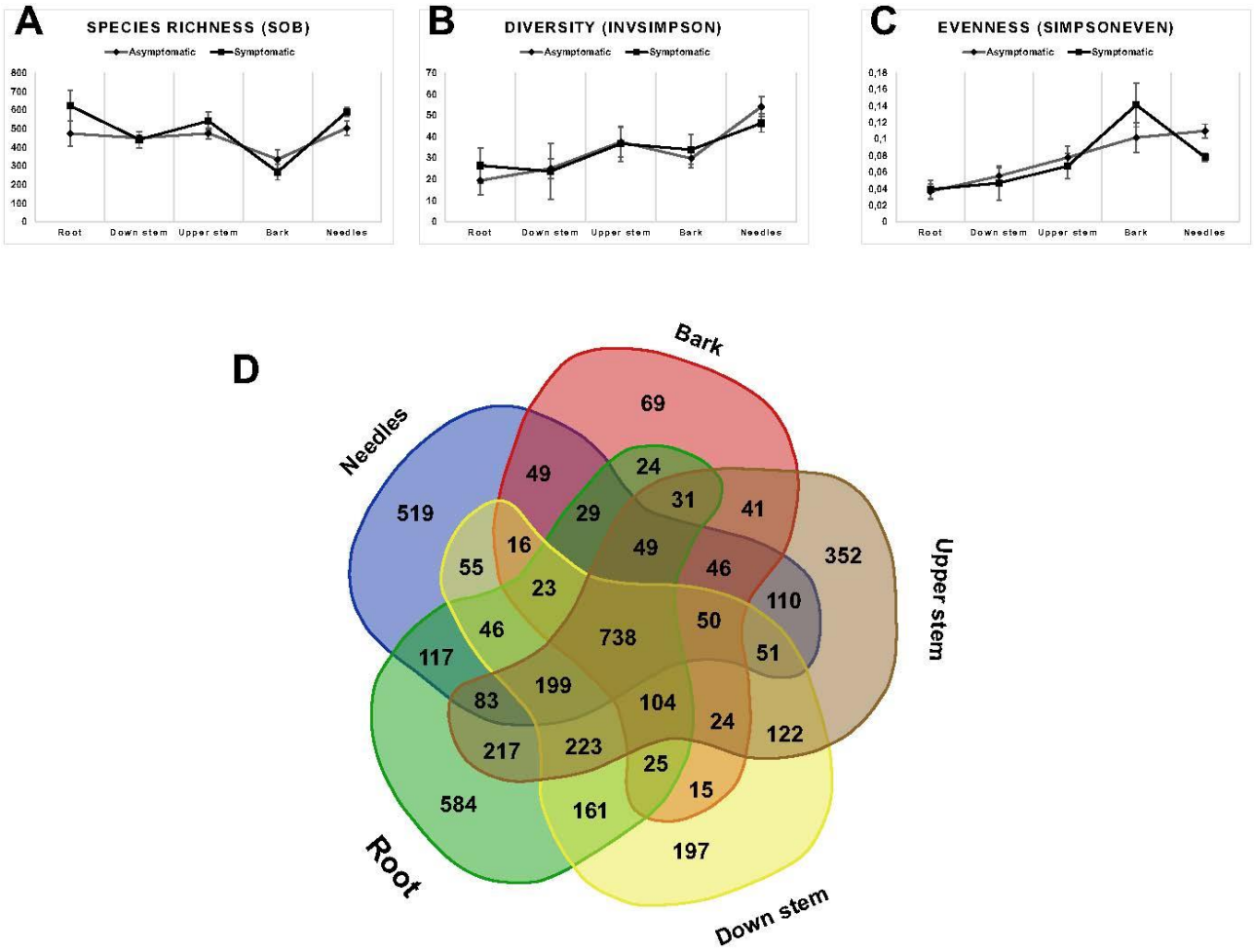

Figure 2. (A) Fungal richness (observed OTUs), (B) diversity and (C) evenness indices for ITS2 region libraries from different regions in symptomatic and asymptomatic trees. The mean values and standard errors are depicted on the graphs. (D) Unique and shared OTUs between different regions of the tree. The Venn diagram was constructed from the presence/absence transformed OTUs data (4375 OTUs without singletons).

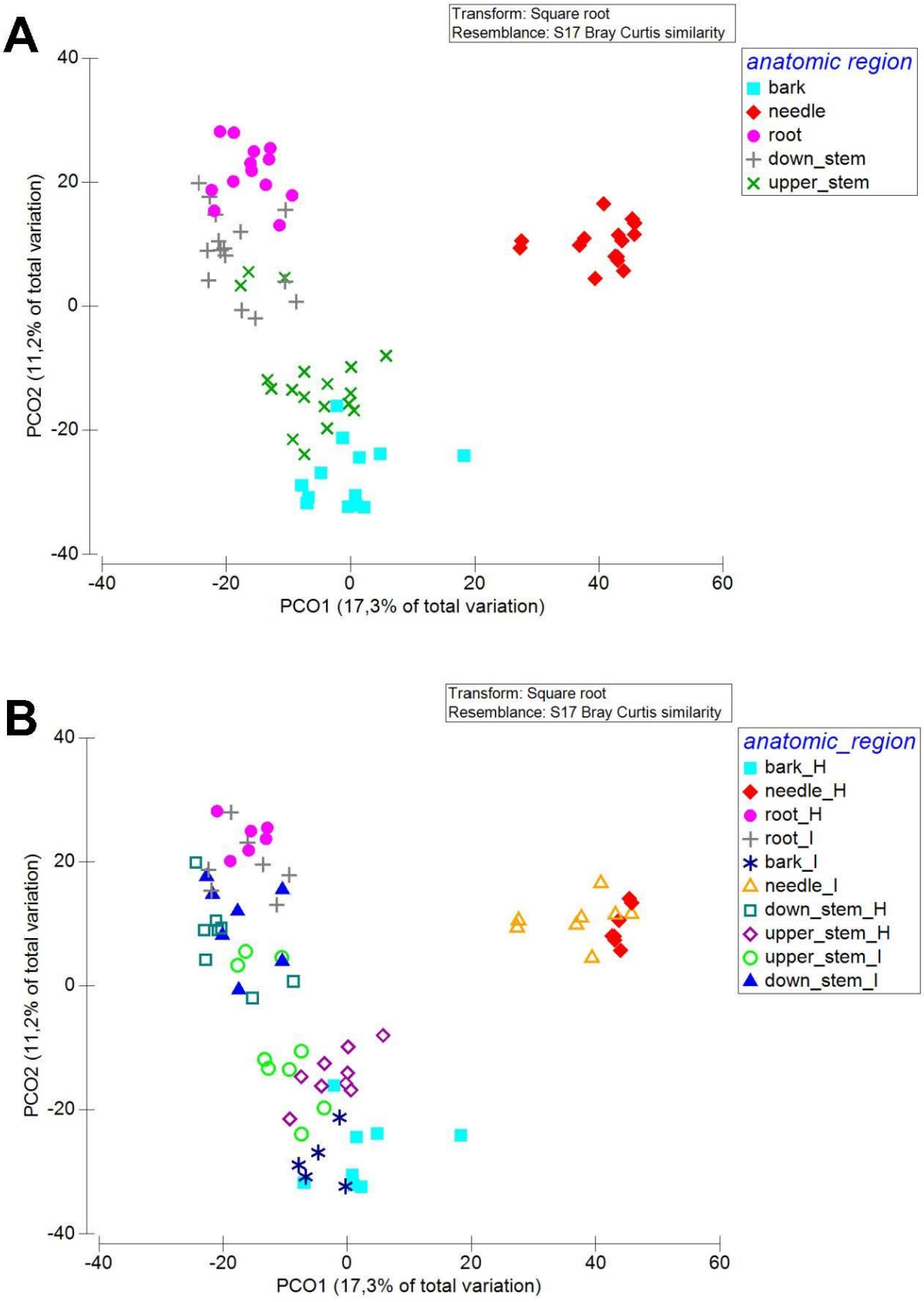

Figure 3. Principal coordinates analysis (PCoA) based on the relative abundance of fungal OTUs, showing the differences in fungal community structure in different anatomic regions of the studied

Norway spruce trees. (A) Origin of the samples (either from symptomatic or asymptomatic trees) is not indicated. (B) Samples from symptomatic (I) and asymptomatic (H) trees are indicated with different symbols.

Supporting Information

Supporting Note S1. Mycobiome composition and distribution among Norway spruce tissues.

Supporting Note S2. Dominant OTUs associated with Norway spruce tissues.

Supporting Table S1. Abundance (in %) of the top 50 fungal species in symptomatic and asymptomatic trees

Supporting Table S2. List of OTUs that significantly contributed to the shift in the structure of fungal communities of upper stem among asymptomatic and symptomatic trees.

Supporting Table S3. List of OTUs that significantly contributed to the shift in the structure of fungal communities of down stem among asymptomatic and symptomatic trees.

Supporting Figure S1. Abundance of fungal phyla (% of the total number of reads) in different tissues of the sampled spruce trees. Ascomycota is the most abundant group in all tissues, but their abundance is the highest in needles, whereas abundance of Basidiomycota increases in woody tissues. Abundance of the remaining groups in all sampled tissues was below 1% of total number of reads. Only phyla with the relative abundance >0.01% are shown on the diagrams.

476 **Supporting Figure S2.** Relative abundance of fungal phyla in different tissues of symptomatic (S)
477 and asymptomatic (A) spruce trees. Phyla with the relative abundance of >0.01% are depicted in
478 each diagram; phyla with the lower abundance are omitted.
479
480 **Supporting Figure S3.** Relative abundance of fungal classes in different tissues of the sampled
481 Norway spruce trees. Only classes with the relative abundance of >0.5% are depicted.
482
483 **Supporting Figure S4.** Relative abundance of fungal orders in various tissues of the sampled
484 Norway spruce trees. Only orders with the relative abundance of >1% are depicted.
485
486 **Supporting Figure S5.** Abundance of the 20 most abundant OTUs (% of total read counts). (A)
487 Abundance in the combined dataset. (B) Abundance in specific tree tissues.
488
489 **Supporting Figure S6.** Ten most abundant OTUs in each of the sampled tissue of asymptomatic
490 (A), (C), (E), (G), (I) and symptomatic (B), (D), (F), (H), (J) Norway spruce trees.
491
492 **Supporting Figure S7**. Principal coordinates analysis (PCoA) based on the relative abundance of
493 fungal OTUs, showing the differences in fungal community structure in upper stem and lower stem
494 of the studied Norway spruce trees.
495
496