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PLANT MICROBE INTERACTIONS

Tissue Microbiome of Norway Spruce Affected by *Heterobasidion*-Induced Wood Decay

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Abstract

Plants live in close association with microbial symbionts, which may affect the host fitness, productivity, and tolerance against biotic and abiotic stressors. The composition of plant microbial communities is influenced by many biotic and abiotic factors, but little is known about the effect of plant pathogens on the structure of these communities. In this study, we investigated the structure of bacterial communities associated with different tissues of asymptomatic and symptomatic (*Heterobasidion*-rotten) Norway spruce (*Picea abies* (L.) Karst.) trees. Our results demonstrated that each of the investigated anatomic tissues (root, bark, down stem, upper stem, and needles) harbored a unique bacterial assemblage. However, the health status of the host trees had little effect on the structure of bacterial communities, as the only significant differences among asymptomatic and symptomatic trees were found in the composition of the bacterial communities of needles. Proteobacteria was predominant in all anatomic regions with the highest abundance in needles (86.7%), whereas Actinobacteria showed an opposite trend, being more abundant in the woody tissues than in needles. Additionally, we performed profiling of terpenoid compounds present in spruce xylem and phloem. Total concentrations of monoterpenes and sesquiterpenes were considerably higher in asymptomatic trees. However, we found no significant correlations between terpenoid profiles of spruce trees and the composition of their bacterial communities. Our results provide an insight into the diversity of bacteria associated with Norway spruce tree tissues. At the same time, the health status and terpenoid content of host trees had a limited effect on the composition of bacterial communities in our survey.

Keywords Bacterial biota · Microbiome · Microbial community · Norway spruce · *Heterobasidion* · Host-microbe interactions · Terpenoids

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Introduction

All plants are colonized by heterogeneous communities of microorganisms, including bacteria, fungi, archaea, viruses, and protists [1]. Plant microbiota can interact with their hosts in a beneficial, harmful, or neutral way. Plant-associated microorganisms stimulate germination and growth, promote resistance or tolerance to biotic and abiotic stress factors, and influence plant fitness [2–4]. However, opportunistic pathogens and saprotrophs equally belong to plant microbiota [5, 6], which may have negative effect on host plants. The composition and structure of plant microbial communities is influenced by a number of factors, including host and microbial genotypes, environmental factors, and interactions within plant microbiome [6–8]. However, very little is known on factors driving the composition of microbiota associated with plants and, in particular, with forest trees. Bulgarelli et al. [9] noted that the microbial communities in the rhizosphere were mainly influenced by soil types, whereas in phyllosphere and endosphere, they were predominantly determined by host plant species. Other studies showed that rhizosphere microbiome was affected by plant development [10], whereas geographical location, host identity, site, and time determined phyllosphere microbiota [11, 12].

Due to the inherent abilities to improve host fitness in a beneficial way, microbial endophytes have great potential as biocontrol and growth-promoting agents [13, 14]. Nevertheless, the research on the microbiome impact on the plant disease resistance is still in its infancy. Similarly, the effects of pathogens on microbial communities have only been documented in a few available studies [6]; thus, it is very difficult to draw any general conclusions. However, there are some pioneering studies, which suggest a correlation between the structure of microbiota communities and host plant resistance or susceptibility to pathogens [15–18]. The root rot disease caused by the fungi *Heterobasidion annosum* (Fr.) Bref. species complex has a great economic impact on forest industry in boreal zone [19, 20]. The pathogen grows necrotrophically in the sapwood of living trees and saprotrophically in dead wood tissues. It develops very slowly and the host death can occur after several years [19]. Currently, the control strategies are focused on prevention of fungal infection of tree stumps after harvesting (e.g., biological and chemical stump treatments), but no methods for pathogen eradication from infected trees or stumps are available. Thus, studies on interactions among *Heterobasidion* spp., the host, and the resident microbiome are aimed at finding alternative novel ways to manage and protect this economically valuable natural timber resource.

Terpenoids represent the largest class of secondary metabolites in plants [21]. Conifer trees are rich in viscous oleoresin, stored in resin canals of xylem, phloem, bark, and needles. It is composed of mixture of solid diterpenes (C20) and essential oil formed of monoterpenes (C10) and sesquiterpenes (C15) [22]. Terpenoids have an important defense function, protecting conifer trees from pathogens and herbivores [23]. They are an important first line of defense to inhibit the initial growth of pathogens [18]. Key monoterpenes and diterpenes of Norway spruce (*Picea abies* (L.) Karst.) have shown antifungal properties, for instance, against *Heterobasidion* sp. [24]. Bullington et al. [18] reported that genetic resistance observed in natural populations of whitebark pine could be due to a combination of terpene concentration and endophytic microbial community residing in the needles. Several studies on the role of bacterial biota in the growth promotion and health of agricultural crops are available, but very little is known on its identity and composition on forest trees. Such knowledge on the identity of bacterial biota associated with forest trees is the first step in studies of growth promotion of tree microbiome that could be relevant for sustainable forest management. In this study, our aim was to investigate and compare the structure of bacterial communities associated with the Norway spruce tree tissues under field conditions, with or without symptoms of *Heterobasidion*-induced wood decay. Additionally, we assessed the correlation between the health status of the trees, the tree terpenoid profiles, and the structure of bacterial communities.

Materials and Methods

Study Sites and Sample Collection

Three Norway spruce-dominated forest sites in the municipality of Mäntsälä (Uusimaa region, Southern Finland) were chosen for samplings. The sites are located in privately owned managed forest and are distributed in three selected plots: plot 1 (60° 44′ 51″ N, 25° 13′ 17″ E), plot 2 (60° 45′ 11″ N, 25° 13′ 24″ E), and plot 3 (60° 45′ 15″ N, 25° 15′ 34″ E). Characteristics of all three plots are as previously described by Kovalchuk et al. [25]. Briefly, all the plots are growing under similar condition and represent typical examples of managed spruce forest used for commercial timber production. The spruce trees at the selected sites were naturally regenerated and are approximately 55 years of age at the time of sampling. The elevation of the sites ranges from 87 to 95 m above sea level. Sample collections were conducted in May 2016. In each forest site, one plot was chosen and six Norway spruce trees were selected: three trees showing symptoms of *Heterobasidion*-induced wood decay (visually observed immediately after tree felling during on-site logging, further referred to as symptomatic trees), and three trees without decay symptoms (also visually inspected after tree felling, further referred to as asymptomatic trees). Apart from the visual inspection of *Heterobasidion*-induced wood decay, our ITS-based high-throughput analysis of fungal communities of the

sampled trees confirmed the presence of *Heterobasidion* spp. in both symptomatic and asymptomatic trees [25]. Asymptomatic and symptomatic trees were intermixed in the selected sampling plots, and their distribution did not show a particular pattern. Diameter of selected trees ranged from 40 to 64 cm. In total, samples of suberized roots (containing periderm/secondary tissues), down stem at stump height (bark + sapwood + heartwood), upper stem (the part of the tree close to the crown/foliage), sapwood (referred to as upper stem, only xylem), upper stem bark (referred to as bark), and needles were taken from nine asymptomatic trees and nine symptomatic trees.

DNA Extraction, Amplification of 16S rDNA Region, and Sequencing

Surface of spruce tissue samples was sterilized with 70% ethanol before DNA extraction. Total DNA was extracted from homogenized and lyophilized plant tissues following a standard cetyltrimethylammonium bromide (CTAB) method [26] with modifications described in Terhonen et al. [27]. The concentrations and purity of DNA were measured using NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). PCR amplification of the bacterial 16S rDNA V3-V4 region and sequencing were performed at the Institute of Biotechnology (BI, University of Helsinki, Finland). The PCR products were purified and sequenced with Illumina MiSeq platform. The amplification primers were 341F and 785R [28] containing partial adapter sequences at the 5′ ends (ATCTACACTCTTTCCCTACACGACGCTCT TCCGATCT and GTGACTGGAGTTCA GACGTGTG CTCTTCCGATCT). Sequencing reads were produced paired-end sequences of approximately 250 nucleotides. Raw sequences were deposited at the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under project accession number SRP130315.

The raw 16S rDNA sequences were pre-processed at BI. The read quality was checked with FastQC v.0.11.2 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Adapter and barcode sequences were removed using Cutadapt v.1.15 [29]. The pre-processed data were analyzed and grouped into OTUs (operational taxonomic units) using the mothur standard operation pipeline (SOP, v.1.37.6) [30] with the modifications described earlier [31]. OTUs were assigned to taxonomic groups with mothur command classify.seqs (bootstrap cutoff set to 80) using SILVA database release 128 as a reference [32]. Venn diagrams were constructed using data before subsampling (without singletons) with InteractiVenn (http://www.interactivenn.net/) [33]. Sobs, invsimpson, and simpsoneven calculators were chosen to estimate community richness, diversity, and evenness, respectively (https://www.mothur.org/wiki/Calculators). One-way ANOVA tests were used to identify differences in community richness, diversity, and evenness among symptomatic and asymptomatic trees and abundance percentage differences. Tests of homogeneity of variances were done before one-way ANOVA tests. Principal coordinates analysis (PCoA) was used to visualize the bacterial community structure with Bray-Curtis similarity using relative abundances of OTUs in PRIMER v.6 [34] with the add-on package of PERMANOVA + [35]. 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. HOMOVA tests were done in mothur before PERMANOVA test.

Terpenoids Analysis

Terpenoids were analyzed from down stem (i.e., consisted of inner bark (phloem) and xylem (sapwood + heartwood)) of sampled trees using a modified method of Kainulainen et al. [36]. Samples (200 mg of inner bark and 300 mg of xylem) homogenized in liquid nitrogen were extracted in 2 ml of *n*-hexane at room temperature for 2 h and washed with 2 × 2 ml *n*-hexane. 1-Chloro octane was used as an internal standard. The extracts were analyzed using an Agilent 7890B gas chromatograph equipped with a mass selective detector (type 5977A). Separations were carried out on a 30-m HP-5 ms Ultra Inert (i.d. 0.25 mm; film thickness 0.25 μm, Agilent) column. Helium was used as carrier gas, and linear velocity was about 40 cm/s. The splitless (purge time off 1 min) sampling technique and 1 μl was injected. The column temperature was programmed from 50 to 115 °C at 5 °C/min, then to 280 °C at 15 °C/min and hold for 10 min. Mass numbers from *m*/*z* 33 to 350 were recorded. Compound identification and quantification was based on their mass spectra, retention time, and authentic standard compounds as described by Kainulainen et al. [36]. PCoA was used to visualize the structure of host trees terpenoid profiles.

Results

Information on the MiSeq Sequencing

A total of 13,353,946 high-quality sequences were generated across bark, down stem, needle, root, and upper stem samples in the three sampling sites after sequence de-noising and quality filtering. After filtering out unclassified sequences and sequences assigned to plant chloroplast, a core set of 647,055 sequences assigned to bacteria domain was obtained. Due to the technical problem of PCR amplification and sequencing, 10 samples with low read numbers were excluded and the remaining 80 samples were used for further analysis. The average number of sequences in the remaining samples is 7782 sequences.

Richness, Diversity, and Evenness of Bacterial Communities of Norway Spruce

Quality-filtered bacterial sequences were clustered into 3140 OTUs (excluding singletons). The sub-sampled set used to calculate richness, diversity, and evenness contained 2432 OTUs. Suberized root tissues had the highest richness of bacterial communities in both symptomatic and asymptomatic trees (Table 1). The highest community diversity and evenness was observed in upper stems and in roots in asymptomatic and symptomatic trees respectively. Needles had the lowest community richness, diversity, and evenness in both symptomatic and asymptomatic trees (Table 1). There were no statistically significant differences in the bacterial richness, diversity, and evenness among asymptomatic and symptomatic trees in any of the sampled tissues. The sampled tissues of the Norway spruce trees shared 371 (11.8%) of the total 3140 OTUs. The proportion of the OTUs unique to a certain tissue ranged from 0.5% (17 OTUs; upper stems) to 25.5% (800 OTUs; roots) (Fig. 1). Rarefaction curve showed that the numbers of OTUs were saturated in all samples, making them suitable for further community analysis (Supplementary Fig. S1).

Bacterial Community Composition and Distribution among Norway Spruce Tissues

Sequences assigned to bacteria domain were classified into 28 bacterial phyla. Overall, Proteobacteria was the most abundant group, accounting for 66.8% of all sequences, followed by Acidobacteria (12.3%) and Actinobacteria (11.9%). The abundance of bacterial phyla varied among different tree tissues and the top ten phyla of each tissue are presented in Fig. 2. Proteobacteria was predominant in all anatomic regions, but their relative abundance ranged from 49.9% in roots to 86.9% in needles. The abundance of Proteobacteria can be divided into three groups: the lowest in roots (49.9%); moderate in upper stem (58.5%), bark (62.4%), and down stem (70.9%); and the highest in needles (86.7%). Each group showed significant difference ($p < 0.05$ in all possible pair). The abundance of Actinobacteria showed an opposite trend compared with Proteobacteria. Their fraction was considerably larger in the woody tissues (up to 22.3% in roots) and much smaller (2.1%) in needles. The abundance of Acidobacteria in needles (7.5%) was significantly lower than that in bark (17.2%), down stem (11.3%), and upper stem (16.5%).

Ten classes of bacteria had a relative abundance of more than 1% (Supplementary Fig. S2A). Of which, three classes belonged to Proteobacteria including Alphaproteobacteria (48.7%), Gammaproteobacteria (15.1%), and Betaproteobacteria (2.5%); Actinobacteria (6.5%) and Thermoleophilia (3.4%) belonged to Actinobacteria; Acidobacteria (11.3%) and Acidimicrobiia (2.0%) belonged to Acidobacteria. The abundance of bacterial classes varied among the analyzed spruce tissues showing a distinct pattern. Alphaproteobacteria formed the largest fractions in bark, needle, root, and upper stem samples. The relative abundance of Alphaproteobacteria in needles (84.1%) was significantly higher than that in all other tissues ($p < 0.05$). Higher abundance ($p < 0.05$) was observed in bark (49.7%) than in root (33.9%). Gammaproteobacteria was the most abundant class in down stem (39.7%), where it was remarkably more abundant than in needles (0.6%) ($p < 0.05$). The relative abundance of Actinobacteria in roots (14.4%) was considerably higher than that in the other four tissues (from 1.9% in needle to 7.6% in upper stem) ($p < 0.05$). The abundance of Thermoleophilia in needles was significantly lower than that in the other four tissues ($p < 0.05$). Class Acidobacteria had significantly higher abundance in bark (16.1%) and upper stem (15.0%) than in needles (7.5%) and root (9.8%) ($p < 0.05$). Acidimicrobiia showed great differences in abundance among needles (only 0.04%) and the remaining tissues except for upper stem (bark 2.0%; down stem 2.6%; root 1.9%) ($p < 0.05$). The abundance of top ten bacterial phyla in different tree tissues of asymptomatic and symptomatic spruce was presented in Supplementary Fig. S2B. Furthermore, there were no significant differences in the abundance of any higher rank groups (phyla, classes, and orders) between symptomatic and asymptomatic tissues.

Table 1 Richness, diversity and evenness estimators of bacterial communities between asymptomatic (A) and symptomatic (S) trees

Samples	Sobs	invsimpson	simpsoneven
Bark A	167.11 ± 13.05	35.06 ± 4.94	0.20 ± 0.02
Bark S	149.75 ± 16.69	36.62 ± 6.55	0.23 ± 0.02
Down stem A	229 ± 16.99	41.75 ± 4.99	0.18 ± 0.01
Down stem S	174.14 ± 31.39	33.68 ± 10.05	0.17 ± 0.02
Needle A	110.50 ± 8.32	8.64 ± 0.64	0.08 ± 0.01
Needle S	141.88 ± 12.35	15.95 ± 2.18	0.11 ± 0.01
Root A	315.80 ± 29.76	51.22 ± 11.15	0.16 ± 0.03
Root S	382.86 ± 38.90	107.99 ± 27.18	0.27 ± 0.04
Upper stem A	284.83 ± 22.87	62.94 ± 11.89	0.21 ± 0.02
Upper stem S	291.00 ± 45.76	75.38 ± 27.56	0.24 ± 0.04

Bacterial Community Structure of Asymptomatic and Symptomatic Trees

The bacterial community structure showed significant difference ($p < 0.05$) among symptomatic and asymptomatic trees in needles, but not in other anatomic regions. The OTUs that significantly contributed to the shift in bacterial community structure in needles were listed in Table 2. The PCoA based on the relative OTU abundance explained 38.2% of the observed variation and showed distinct clusters for each of the

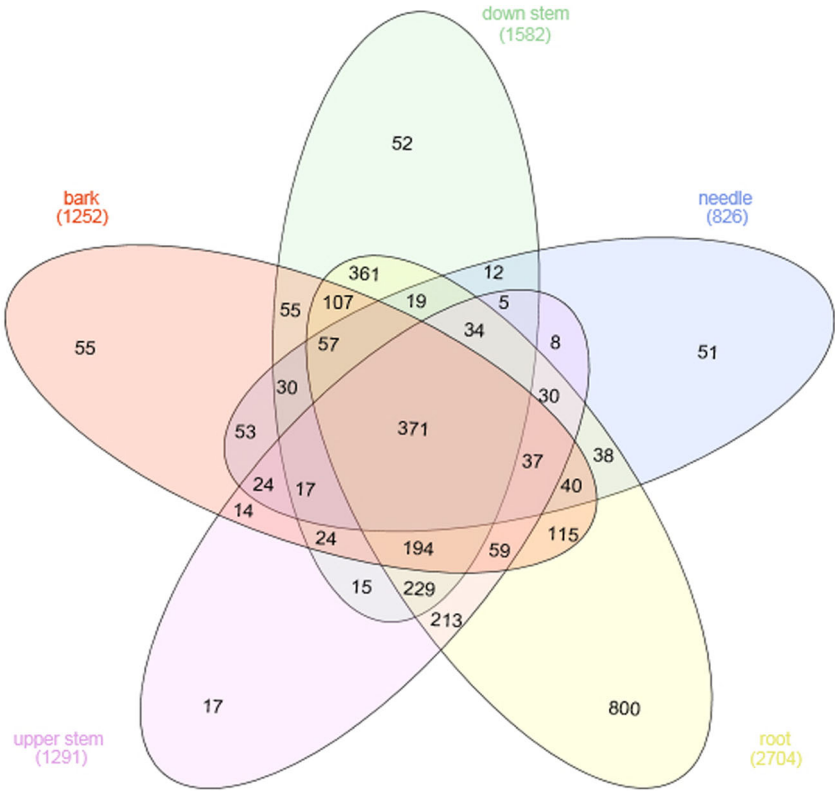

Fig. 1 Venn diagram showing common and unique OTUs in different tree tissues (3140 OTUs in total)

anatomic region (Fig. 3a, b). The subsequent PERMANOVA confirmed the significant differences in community structures among the five anatomic regions ($p < 0.05$ in all possible pairs), indicating that each anatomic region harbored unique bacterial community. There were no significant differences ($p > 0.05$) among bacterial community structure of three sites between symptomatic and asymptomatic trees (Supplementary Fig. S3). The combined dataset generated in our analysis contained 2432 bacterial OTUs (after subsampling). A total of 19 OTUs had an abundance exceeding 1%. *Endobacter* sp. (7.4%) and Rhizobiales (5.6%) were the most abundant. The ten most abundant OTUs varied among the anatomic regions, with four showing remarkably high abundance in needles (Fig. 4a). The most abundant OTUs in the individual tissue of asymptomatic and symptomatic trees were also identified. Among them, many could be classified to a genus level, others only down to a family or even to an order level. The top four OTUs detected in needles had a very high abundance with more than 58% in asymptomatic and 44% in symptomatic trees, which is in agreement with the low community evenness

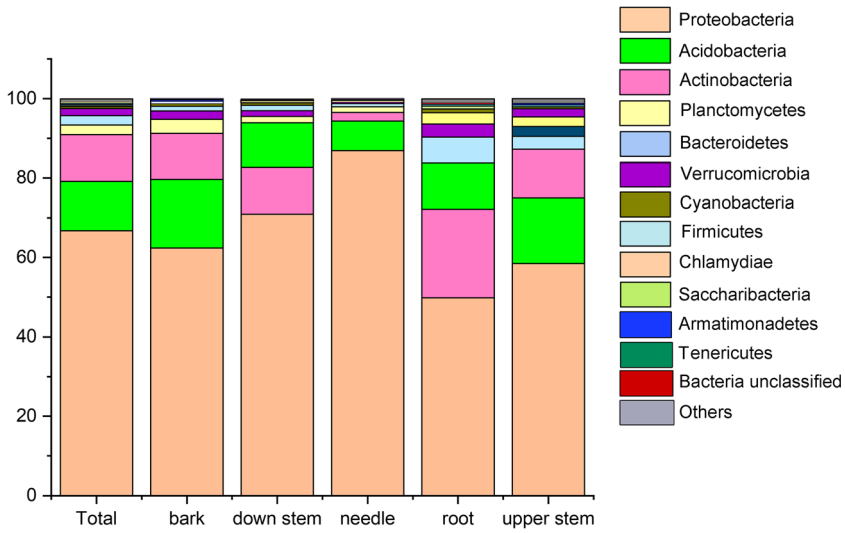

Fig. 2 Abundance of bacterial phyla (% of the total number of reads) in different anatomic regions of the sampled spruce trees. Proteobacteria is the most abundant group in all tissues, but their abundance is the highest in needles

Table 2 OTUs with significant differences in needles between asymptomatic (A) and symptomatic (S) trees

OTUs	Taxonomy	*p* value	Abundance pattern
Otu00001	*Endobacter* sp.	0.025974	A > S
Otu00024	*Acidiphilium* sp.	0.040959	S > A
Otu00029	*Burkholderia* sp.	0.004995	S > A
Otu00035	*Granulicella* sp.	0.016983	S > A
Otu00053	*Granulicella* sp.	0.008991	S > A
Otu00062	*Bryocella* sp.	0.034965	S > A
Otu00067	*Granulicella* sp.	0.029970	S > A
Otu00078	*Aquincola* sp.	0.022977	S > A
Otu00184	Comamonadaceae	0.035964	S > A
Otu00219	*Mucilaginibacter* sp.	0.028971	S > A
Otu00424	*Endobacter* sp.	0.007992	A > S
Otu00442	Armatimonadales	0.020979	S > A
Otu00638	*Hymenobacter* sp.	0.028971	A > S

values of needles in both asymptomatic and symptomatic trees (Table 1).

In spruce bark and upper stem, OTU00001 *Endobacter* sp. and OTU00002 Rhizobiales were the most abundant. OTU00007 *Endobacter* sp. constituted a large fraction of bacterial community in down stem. In needles, 13 OTUs significantly contributed to the community shift between asymptomatic and symptomatic trees (Table 1). Among them, only two OTUs (OTU00001, OTU00424) classified to *Endobacter* sp. and the OTU00638 assigned to *Hymenobacter* sp. had higher abundance in asymptomatic trees ($p < 0.05$). The remaining OTUs had higher abundance in symptomatic trees ($p < 0.05$). In root, OTU00017 classified as Bradyrhizobiaceae was the most abundant. The abundance of a number of OTUs differed between asymptomatic and symptomatic trees (Fig. 4b). Five OTUs out of the top 50 most abundant OTUs showed significant differences in abundance in the anatomic regions (Fig. 4b). The OTUs could not be assigned to a specific species, but to a genus or higher taxa. Of which, three were more abundant in asymptomatic trees including OTU00007 *Endobacter* sp., OTU00006 Rhizobiales, and OTU00019 Acetobacteraceae. Two OTUs were more abundant in symptomatic trees including OTU00018 Beijerinckiaceae and OTU00024 *Acidiphilium* sp.

Correlation between the Host Terpenoid Profiles and the Bacterial Community

Principal coordinates analysis showed that both monoterpenes and sesquiterpenes formed distinct clusters between asymptomatic and symptomatic trees, respectively. The PERMANOVA analysis indicated that the differences among asymptomatic and symptomatic trees were not statistically significant ($p = 0.064$ for phloem samples and $p = 0.594$ for xylem samples). However, the monoterpenes showed much higher variation than sesquiterpenes when the data were analyzed separately (86.0% vs. 58.2%) (Supplementary Fig. S4A, B). Interestingly, total concentrations of monoterpenes and sesquiterpenes were considerably higher in asymptomatic trees (Fig. 5). By using the terpene concentration as a variable factor, however, no significant correlations were observed between the terpene concentrations and the structure of bacterial communities in symptomatic and asymptomatic trees (data not shown).

Discussion

In the current study of bacterial biota, we opted for NGS-based technology as a robust method that captures most of the diversity of culturable and unculturable bacteria of Norway spruce. We investigated the structure of bacterial communities associated with different tissues of asymptomatic and symptomatic Norway spruce trees naturally infected by *Heterobasidion* under field conditions. Each of the tissues harbored a unique bacterial assemblage. We expected a profound impact of the pathogen on the bacterial biota of the different anatomic tissue regions. However, only the composition of bacterial communities of needles differed significantly between symptomatic and asymptomatic trees. There could be several reasons for this observation. In field sampling, there are several levels of consideration, longevity of the host, and the long timescale of the disease. This is particularly so for a disease that develops very slowly like *Heterobasidion* infection [19]. It is therefore possible that the significant effect of the disease documented on needles may indicate an early onset of metabolic changes on the host. Further studies using trees of diverse disease and resistant phenotypes could be necessary to clarify the composition of bacteria biota at late or dying stages of the tree.

In our study, 3140 bacterial OTUs were identified, which may be considered as representatives showing great species richness and diversity. These results do extend the knowledge on the diversity of the little studied bacterial communities inhabiting Norway spruce trees [37–39]. Only a few studies concerning bacterial biota in Norway spruce have been reported. One of the early reports indicated that bacteria can be isolated occasionally from reaction zones of Norway spruce, particularly in down stems where moisture was high [40]. Twenty years later, some slow-growing and difficult-to-detect bacteria in Norway spruce were considered to be the cause of failure of somatic embryo regeneration [37]. Previous works have also found several bacteria inhabiting Norway spruce trees, such as *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Arthrobacter*, and *Rhodococcus* [37–39, 41], but none on a large scale as performed in this study. We were able to find some species reported from Norway spruce previously like members of *Pseudomonas* and *Bacillus* [42], but their abundance was quite low (< 0.2%). Many of the OTUs analyzed

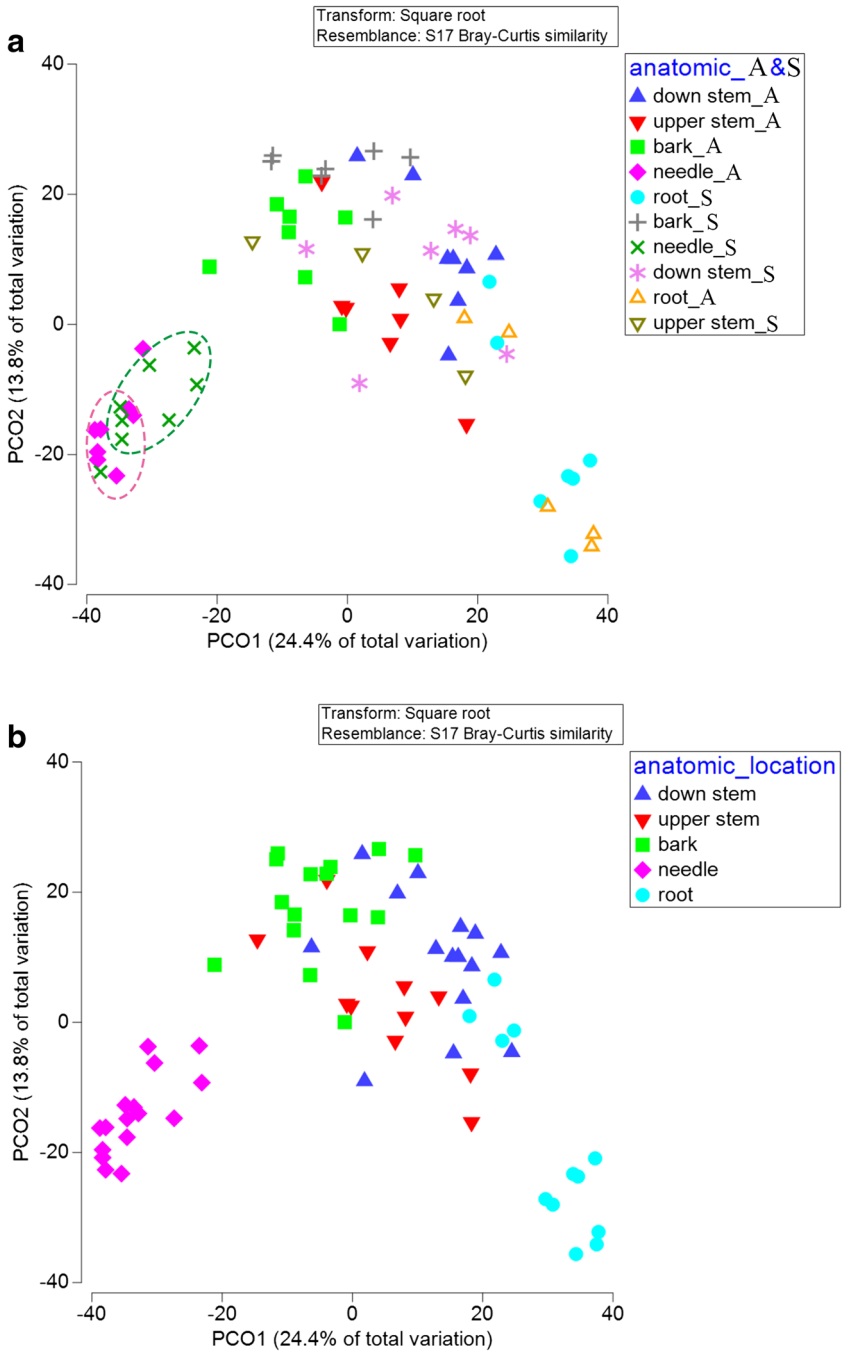

Fig. 3 Principal coordinates analysis (PCoA) based on the relative abundance of bacterial OTUs, showing the differences in bacterial community structure in different regions of the Norway spruce trees. **a** Samples from symptomatic (S) and asymptomatic (A) trees are indicated with different symbols. **b** Origin of the samples (either from symptomatic or asymptomatic trees) is not indicated

belonged to yet undescribed species since they had no reliable matches in public databases.

The bacterial communities of Norway spruce were classified into 28 phyla, mostly dominated by Proteobacteria followed by Acidobacteria and Actinobacteria, then Planctomycetes, Bacteroidetes, and Verrucomicrobia. Previous studies on many plants with cultivation-independent high-throughput sequencing analysis including the model plant *Arabidopsis* [43], crop plants [44, 45], and specialist plants like *Tamarix* trees [11] demonstrated that the plant bacterial biota was composed of only a few dominant phyla, mainly Proteobacteria, Actinobacteria, and Bacteroidetes, and to a lesser extent, Firmicutes [4]. Our results do extend the bacterial phyla range in plants and most particularly for little studied forest trees. The abundance of Proteobacteria, Actinobacteria, and Acidobacteria showed significant differences among anatomic tissues. Proteobacteria is a major phylum of Gram-negative bacteria. Their roles in Norway spruce remain to be clarified. Actinobacteria fraction was much larger in lignified woody tissues, mostly in roots, compared with needles. The phyla Verrucomicrobia and Planctomycetes are rarely identified as part of plant microbiota, and their presence within Norway spruce bacterial biota is somewhat unexpected.

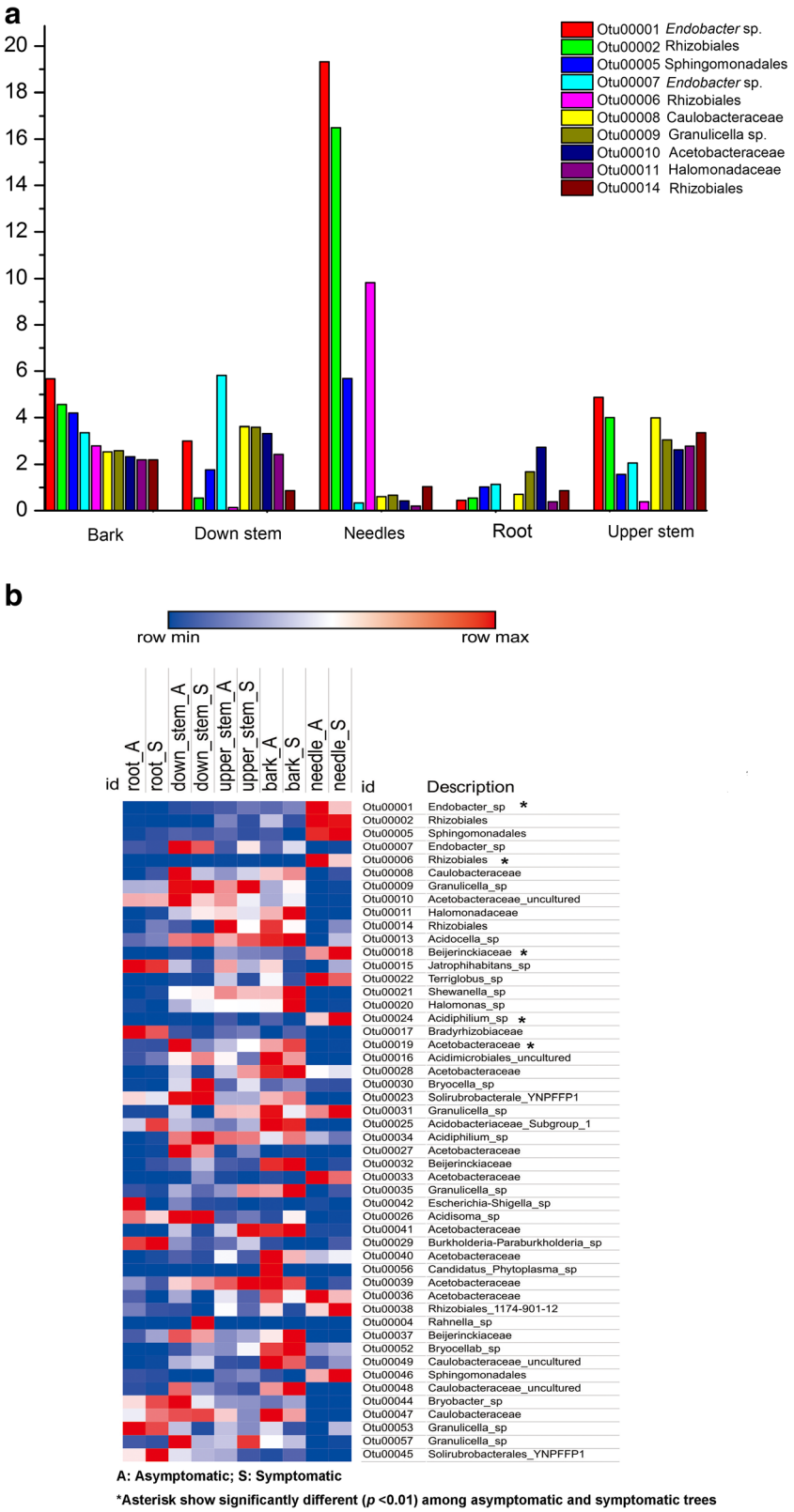

Fig. 4 Abundance of the most abundant OTUs (% of total read counts). **a** Top 10 OTU abundance in specific tree tissues. **b** The heatmap of 50 most abundant OTUs in each of the sampled tissue of asymptomatic (A) and symptomatic (S) Norway spruce trees

At class level, Alphaproteobacteria shared the largest fractions in bark, needle, root, and upper stem samples, whereas Gammaproteobacteria were the most abundant class in the down stem. Alphaproteobacteria is one of the most diverse groups of bacteria [46], which include members that can grow at very low nutrient level and is agriculturally important being

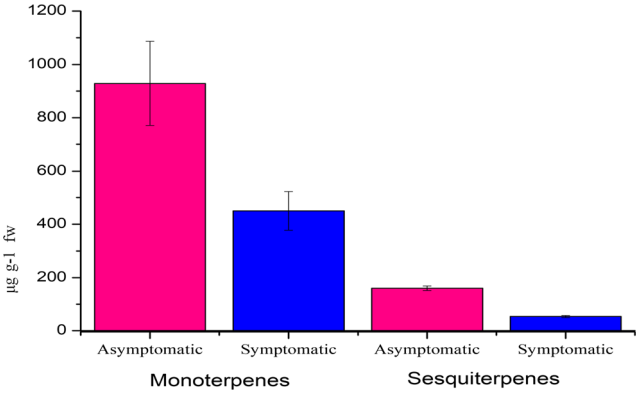

Fig. 5 Total amount of monoterpenes and sesquiterpenes of asymptomatic and symptomatic trees

capable of performing nitrogen fixation in symbiosis with plants [47]. It is the most abundant class in endophytic communities of *Pinus flexilis* James [48]. Gammaproteobacteria include many medically and scientifically important bacterial species, such as *Pseudomonas* spp., members having both pathogenic and growth-promoting properties [49]. Comparative microbiome analyses of healthy and diseased Gros Michel banana plants on *Fusarium* wilt-infested farms revealed significant shifts in the abundance of Gammaproteobacteria [50], showing that they could be indicator species of healthy banana plants. However, in our study, we found no statistically significant differences in the abundance of Gammaproteobacteria among symptomatic and asymptomatic trees. Therefore, it seems unlikely to be used as potential indicator of early *Heterobasidion* infection. Acidimicrobiia showed substantial differences in abundance among needle and bark, down stem, and root, indicating its more active roles in woody tissues.

Many of the bacterial OTUs identified in our survey belong to poorly characterized genera, some of which were formally described in the last decade [51–54], and very little is hitherto known about their ecological roles. Their identification as part of bacterial community associated with Norway spruce trees extends our knowledge about their ecological niche, but their functional significance is difficult to assess without further experimental work. In our survey, structures of bacterial communities differed significantly among all five sampled tissues. This is consistent with the previous studies that different plant tissues host different bacterial biota [4, 55, 56]. Apparently, bacterial communities responded to differences in anatomical tissues, providing further insight into ecological niche preferences of several of the identified bacterial taxa. Additionally, the microenvironment of tree tissues could contribute to alterations in bacterial community composition. It was obvious that the microenvironment of the tree tissues and niche preferences could have contributed to the alterations in bacterial community composition.

So far, only a few studies have addressed the effect of plant pathogens on the composition of plant microbiota and defensive chemicals, particularly for forest tree pathosystems. A recent study showed that Scots pine (*Pinus sylvestris* L.) root tips colonized by different ectomycorrhizal fungi may affect bacterial communities [57], whereas a possible contribution of fungal endophytic communities to pathogen resistance and terpenoid content was proposed in whitebark pine [18]. We hypothesized that asymptomatic and symptomatic Norway spruce tree tissues will be different in the composition of their bacterial communities. However, our results show only minor effect of tree health status on the composition of associated bacterial communities. The bacterial communities residing in needles differed significantly between asymptomatic and symptomatic trees. However, no significant differences were found for other examined tissues. This is in contrast to the pathogen effect on the fungal communities of Norway spruce, which was much more pronounced [25]. Changes in fungal communities were found to be restricted to tissues closest to *Heterobasidion* wood decay [25]. However, our data showed that bacterial communities of needles were characterized by the lowest species richness, diversity, and evenness and this might make these communities more sensitive to changes in the host tree health status. The trees with symptoms of decay may have reduced water transport as well as photosynthesis [58]. Oliva et al. [58] found that *Heterobasidion*-rotten trees have decreased periodic diameter increment than healthy trees. This was possibly due to photosynthesis product re-allocation. Consequently, a lot of energy and metabolites are channeled towards tree defense against infection thus leading to suppressed immunity. This invariably may have provided opportunity for latent and other bacteria biota to thrive in the needle part of symptomatic trees. Furthermore, although concentrations of monoterpenes and sesquiterpenes were considerably higher in asymptomatic trees, this was not statistically significant. However, our analysis also showed that three compounds, α-pinene ($p = 0.022$), tricyclene ($p = 0.025$), α-longipinene ($p = 0.028$), were present in significantly higher concentrations in phloem of asymptomatic trees than symptomatic trees (data not shown). Bullington et al. [18] also reported that resistant whitebark pine seedlings contained higher concentrations of total monoterpenes including (−)-α-pinene. According to Bullington et al. [18], it is also possible that fungal endophytes that reside within pine tissues could indirectly contribute to increase terpene levels by the induction of chemical defensive pathways in the tree.

As far as we know, only few studies [18] have addressed the effect of microenvironment of anatomic tissues and plant pathogens on the plant microbiota composition especially on conifer pathosystems. This is the first comprehensive study on bacterial biota on spruce tissues. We expect that the data generated in our project will form a useful resource for further studies on forest tree biology and forest pathology. Our results showed that root and butt rot induced by *Heterobasidion* sp.

have a limited effect on the composition of spruce bacterial communities, with the only significant changes observed in communities of needles. At the same time, our survey extends our knowledge about bacterial taxa associated with Norway spruce tissues under field conditions.

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