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Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter

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Summary

Understanding the ecology of coniferous forests is very important because these environments represent globally largest carbon sinks. Metatranscriptomics, microbial community and enzyme analyses were combined to describe the detailed role of microbial taxa in the functioning of the *Picea abies*-dominated coniferous forest soil in two contrasting seasons. These seasons were the summer, representing the peak of plant photosynthetic activity, and late winter, after an extended period with no photosynthate input. The results show that microbial communities were characterized by a high activity of fungi especially in litter where their contribution to microbial transcription was over 50%. Differences in abundance between summer and winter were recorded for 26–33% of bacterial genera and < 15% of fungal genera, but the transcript profiles of fungi, archaea and most bacterial phyla were significantly different among seasons. Further, the seasonal differences were larger in soil than in litter. Most importantly, fungal contribution to total microbial transcription in soil decreased from 33% in summer to 16% in winter. In particular, the activity of the abundant ectomycorrhizal fungi was reduced in winter, which indicates that plant photosynthetic production was likely one of the major drivers of changes in the functioning of microbial communities in this coniferous forest.

Introduction

Coniferous forest ecosystems represent significant global carbon sinks, especially in the boreal and temperate zones of the Northern Hemisphere. Consequently, understanding their ecology is essential for predicting and managing C-cycling processes and their impacts on climate change. We currently lack fundamental knowledge on how microorganisms function as key mediators of C-cycling processes in these ecosystems as well as the identification of the specific roles of individual taxa. Previously, litter and soil activity in forest ecosystems has been shown to be largely shaped by the activity of trees, which are the dominant primary producers. Trees affect the inflow and quality of complex organic compounds in the form of leaf and root litter, and provide root exudates (Prescott and Grayston, 2013). Additionally, trees contribute significantly to soil respiration (Högberg *et al*., 2010) and deposition of carbon (Clemmensen *et al*., 2013) either directly or indirectly through their fungal symbionts.

The concomitant production of aboveground litter and root exudates results in vertical stratification of forest soils. The accumulation of recalcitrant litter, as well as the absence or low abundance of roots on the soil surface, results in the formation of the litter horizon. This litter is characterized by a high activity of extracellular enzymes produced by fungal and bacterial decomposers, high heterotrophic respiration and high decomposition rates (Šnajdr *et al*., 2008; Baldrian *et al*., 2012). Ectomycorrhizal fungi (ECM; the plant root symbionts belonging to Dikarya) grows on the soil surface and are sustained at the cost of C that is allocated via plant roots to deeper soil and transported by their mycelia (Lindahl *et al*., 2007; Voříšková *et al*., 2014). In contrast, in deeper organic layers of coniferous forest soils, most of the C originates from rhizodeposition (Clemmensen *et al*., 2013), and the microbial community is generally richer in ECM fungi and bacteria (O'Brien *et al*., 2005; Lindahl *et al*., 2007; Baldrian *et al*., 2012).

Seasonally, temperate and boreal zone forests are characterized by the photosynthetic activity of trees during a vegetation period (with favourable temperature and light conditions) and during winter (with little light and temperatures below the freezing point) (Voříšková *et al*., 2014). The seasonality of photosynthetic production and its resulting carbon allocation can dramatically affect the availability of C to soil biota (Högberg *et al*., 2010; Kaiser *et al*., 2010), with belowground carbon allocation via plant roots limited to the vegetation period (Högberg *et al*., 2010). Previous observations from deciduous forest soils

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suggest that these changes in rhizodeposition, along with changing temperature and seasonal litter input, may be the most important factors affecting microbial community composition and activity (Kaiser *et al.*, 2010; Kuffner *et al.*, 2012; Voříšková *et al.*, 2014).

To understand microbial processes in forest ecosystems, it is essential to address the activity of both bacteria and fungi. Although bacterial biomass is quantitatively dominant, fungi have been shown to be more important in decomposition processes and link soil and plant interactions (Baldrian *et al.*, 2012; Štursová *et al.*, 2012). Given that the bulk of microbial community members have not yet been cultured, direct analysis of microbial processes is necessary to link community structure to specific functions and to provide insight into the contribution of individual microbial taxa to biogeochemical processes. The development of metatranscriptomic approaches and high-throughput sequencing offers the tools to address these questions. Metatranscriptomics has proven to be well suited for the identification of functional traits of various microbial taxa, especially in the oceans (Gilbert *et al.*, 2010; Shi *et al.*, 2011; Gifford *et al.*, 2013) where it has successfully been used to demonstrate seasonal changes in microbial activity (Gilbert *et al.*, 2010; Hewson *et al.*, 2014; Hollibaugh *et al.*, 2014). Compared with marine environments, the development of soil metatranscriptomics is much less advanced, mainly due to the difficulty of obtaining RNA in sufficient amounts and quality. These limitations, however, have recently been overcome (Urich *et al.*, 2008), and extracted RNA has been shown to identify the activity of various microbial taxa as well as specific genes (Baldrian *et al.*, 2012). Currently, however, there exist only few published metatranscriptomic studies on soils (Tveit *et al.*, 2013; 2014; Nacke *et al.*, 2014), and the understanding of soil functioning based on these studies is still fragmentary.

This work combines metatranscriptomics, microbial community analysis and enzyme activity measurements to investigate the influence of seasonality on forest top soil microbial activity. We investigate two seasons: the summer peak of plant photosynthetic activity and late winter with no photosynthate input in soil where we have previously observed high microbial activity, especially of fungi (Baldrian *et al.*, 2012). We also explore the extent of seasonal differences in ecosystem functioning and indicate whether these changes are due to differences in microbial community composition among seasons, differential transcription of the microbes, or both. Our overarching hypothesis is that observed seasonal differences in soil microbial activity are driven by changes in the availability of plant photosynthesis products and consequently that seasonality is soil horizon-specific. In winter, as decreases in the nutrient supply to root-symbiotic ECM fungi occur due to absent photosyntate allocation, we expect that ECM fungi abundance and activity will decrease. Moreover, because previous studies have shown that ECM fungi decrease the rates of organic matter decomposition (Gadgil and Gadgil, 1975; Ekblad *et al.*, 2013), it can be assumed that winter will be characterized by a relative increase of abundance and activity of decomposer microorganisms. Further, it was demonstrated that both fungi and selected bacteria are involved in decomposition in coniferous forests (Štursová *et al.*, 2012), and we will specifically investigate the relative contribution of the members of these two groups.

Results

Site and soil properties

Mean annual temperature in the year of sampling was 4.8°C and was the same for air on the soil surface and in litter and soil. The warmest month was August, with an air temperature of 13.4°C, litter temperature of 12.3°C and soil temperature of 12.2°C; the coldest month was February, with temperatures of –1.1°C, –0.4°C and –0.3°C in air, litter and soil, respectively (Fig. S1). The temperatures in litter and soil during the 14 days before sampling were 9.7°C in summer and –0.3 to –0.4°C in winter. Additionally, the summer sampling time was representative of the peak of the vegetative season, whereas the soil in winter was covered by 50 cm of snow for longer than 3 months (Fig. S1). Despite temperatures being slightly below 0°C in the winter, the water in litter and soil was never frozen.

Soils were characterized by high content of organic matter and low pH. The chemical properties of litter and soil differed dramatically, with the litter horizon containing significantly more organic matter, as well as nutrients (C, N and P) and exhibiting slightly but significantly higher pH and moisture content (Table 1). Litter also contained approximately twice as much bacterial biomass and approximately fourfold more fungal biomass than soil. The activity of all extracellular enzymes was higher in litter than in soil, with the exception of Mn-peroxidase and β-xylosidase. Seasonal differences within horizons were most apparent in the bacterial/fungal rDNA ratio, which was higher in winter than in summer in both horizons. In litter, the activity of the endocellulase and ergosterol content was higher in summer, whereas Mn-peroxidase activity was higher in winter. In soil, endocellulase activity was higher in summer, whereas endoxylanase activity was higher in winter (Table 1).

Composition and activity of the microbial community

Gene-targeted sequencing was performed to characterize the composition of the soil microbial community (16S rRNA gene amplicons, DNA) and activity (16S rRNA

Table 1. Characteristics of *Picea abies* forest litter and soil in summer and winter.

	Litter	Soil	Litter summer	Litter winter	Soil summer	Soil winter
Dry mass (%)	25.3 ± 2.2	**35.4 ± 1.8**	**31.8 ± 1.7**	18.9 ± 1.0	36.2 ± 2.9	34.7 ± 2.5
Organic matter (%)	**94.6 ± 0.7**	60.0 ± 3.6	94.1 ± 1.1	95.0 ± 1.0	64.0 ± 5.3	56.0 ± 4.8
pH	**3.4 ± 0.0**	3.2 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.1 ± 0.1	3.2 ± 0.1
C_{ox} (%)	**40.5 ± 0.6**	29.7 ± 0.9	40.9 ± 0.5	40.1 ± 1.0	30.0 ± 1.6	29.5 ± 1.0
N_{tot} (%)	**1.8 ± 0.0**	1.5 ± 0.1	1.8 ± 0.0	1.7 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
$P_{oxalate}$ (μg g^{-1})	**111 ± 6**	69 ± 3	110 ± 9	113 ± 9	72 ± 2	68 ± 5
Bacterial rDNA (10^8 copies g^{-1})	**6.7 ± 0.6**	3.9 ± 0.4	7.6 ± 0.7	5.8 ± 0.9	4.1 ± 0.7	3.6 ± 0.6
Fungal rDNA (10^6 copies g^{-1})	**35.5 ± 7.7**	8.1 ± 1.6	**60.6 ± 2.4**	10.5 ± 1.5	**12.2 ± 1.9**	4.0 ± 0.5
B/F ratio	34 ± 7	**63 ± 11**	13 ± 1	**56 ± 4**	33 ± 2	**92 ± 14**
Ergosterol (μg g^{-1})	**164 ± 7**	48 ± 3	**180 ± 10**	147 ± 6	50 ± 5	46 ± 4
Laccase (μmol min^{-1} g^{-1})	**2.9 ± 0.6**	0.5 ± 0.1	3.4 ± 0.9	2.4 ± 0.9	0.7 ± 0.1	0.4 ± 0.2
Mn-peroxidase (μmol min^{-1} g^{-1})	0.2 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	**0.4 ± 0.1**	0.6 ± 0.4	0.2 ± 0.1
Endocellulase (μmol min^{-1} g^{-1})	**13.5 ± 1.5**	7.4 ± 0.6	**16.7 ± 2.2**	10.4 ± 1.2	**9.0 ± 0.6**	5.9 ± 0.4
Endoxylanase (μmol min^{-1} g^{-1})	**13.6 ± 1.3**	4.5 ± 1.0	12.8 ± 2.0	14.4 ± 1.8	1.4 ± 0.4	**7.7 ± 0.4**
Exocellulase (μmol min^{-1} g^{-1})	**10.6 ± 2.0**	3.7 ± 0.6	11.8 ± 2.5	9.5 ± 3.1	4.1 ± 1.2	3.2 ± 0.5
β-glucosidase (μmol min^{-1} g^{-1})	**34.5 ± 4.0**	12.8 ± 1.2	34.6 ± 5.5	34.4 ± 6.5	12.4 ± 1.4	13.3 ± 1.9
β-xylosidase (μmol min^{-1} g^{-1})	6.9 ± 0.6	8.6 ± 0.8	6.9 ± 1.0	7.0 ± 0.8	8.0 ± 1.2	9.2 ± 1.2
β-galacturonidase (μmol min^{-1} g^{-1})	**1.7 ± 0.2**	0.5 ± 0.1	1.8 ± 0.2	1.6 ± 0.3	0.5 ± 0.1	0.4 ± 0.2
α-glucosidase (μmol min^{-1} g^{-1})	**4.0 ± 0.4**	1.7 ± 0.3	4.1 ± 0.8	3.9 ± 0.2	1.9 ± 0.4	1.5 ± 0.4
N-acetylglucosaminidase (μmol min^{-1} g^{-1})	**6.9 ± 0.4**	1.4 ± 0.3	6.6 ± 0.7	7.2 ± 0.3	1.6 ± 0.4	1.2 ± 0.4
Lipase (μmol min^{-1} g^{-1})	**257 ± 12**	159 ± 12	251 ± 20	263 ± 14	152 ± 16	165 ± 18
Phosphomonoesterase (μmol min^{-1} g^{-1})	**105 ± 4**	47 ± 4	103 ± 7	106 ± 4	48 ± 7	47 ± 4

The data represent the means and standard errors of six replicates for each horizon and season (horizon averages are based on data from both summer and winter). Significant differences ($P < 0.05$) among horizons and among seasons within each horizon are indicated in bold script.

amplicons, RNA). We detected a total of 27 164 bacterial operational taxonomic units (OTU) with best hits to 1005 genera. Soil biodiversity was observed to be between 4.54 and 5.21 (Shannon index) and evenness between 0.80 and 0.87. DNA and RNA communities from litter were significantly more diverse than those from soil ($P < 0.001$), and RNA-derived communities exhibited higher values of biodiversity (Shannon index) than those of DNA communities ($P < 0.02$; Table S1).

In both DNA- and RNA-derived communities, bacterial communities were dominated by *Proteobacteria*, followed by *Acidobacteria* and *Actinobacteria*. Although *Acidobacteria* were more abundant in soil, several bacterial taxa, including *Actinobacteria*, *Bacteroidetes*, *Betaproteobacteria* and *Verrucomicrobia*, were more abundant in litter. Bacterial OTUs were more horizon-specific than fungi, with 83% of the top 42 OTU showing preferential localization in one horizon. The most abundant bacterial genera in litter were *Pseudomonas* (7.5% of all sequences), *Beijerinckia* (7.2%) and *Acidiphila* (7.0%). The soil was especially rich in candidatus *Koribacter* (24%), *Beijerinckia* (6.3%) and *Rhodoplanes* (6.2%) (Table S2). Seasonal differences in abundance within each horizon were limited to a small number of taxa in the DNA community. Seasonal differences in rRNA content were more pronounced than in fungi, with 26% and 31% of genera showing seasonal differences in the litter and soil respectively (Table S2). Genome count estimates (based on the total number of associated rRNA amplicons), ribosome content (rRNA), ribosome production (mRNA reads of ribosomal proteins) and total activity (all mRNA reads) were used as proxies of bacterial community composition and activity. For example, although *Acidobacteria*-associated DNA was common, this bacterial taxon exhibited lower proportions of associated rRNA content and transcripts. In contrast, *Actinobacteria* were associated with a higher share of mRNA reads than of genomes or ribosomes, whereas *Verrucomicrobia* were abundant in DNA and RNA, but mRNA reads assigned to this phylum were rare (Fig. 1).

Amplicon sequencing of fungal internal transcribed spacer (ITS) sequences resulted in the identification of a total of 3942 fungal OTUs, with best hits to 424 genera. No significant differences were found in fungal OTU diversity between DNA and RNA samples, horizons and seasons. The species richness was 132–175, the biodiversity was 3.29–3.53 (Shannon index) and the evenness was 0.68–0.70 (Table S1). In both litter and soil, the fungal community was dominated by the Basidiomycota and Ascomycota, which represented 87–97% of sequences. The remaining sequences belonged primarily to the Mortierellomycotina (2% in litter, 12% in soil). The composition of fungal communities in litter and soil differed substantially, with 20 of 32 of the most abundant fungal OTU (63%) showing preferential localization in one horizon. The most abundant fungal genera in litter were *Mycena* (18%), *Tylospora* (16%) and *Cladophialophora* (9%), whereas in soil, *Russula* (17%), *Tylospora* (13%), *Mortierella* (12%) and *Piloderma* (11%) were the most abundant (Table S3). Seasonal differences in abundance within each horizon were limited to a small number of taxa in both DNA and RNA communities (Table S3). Moderate

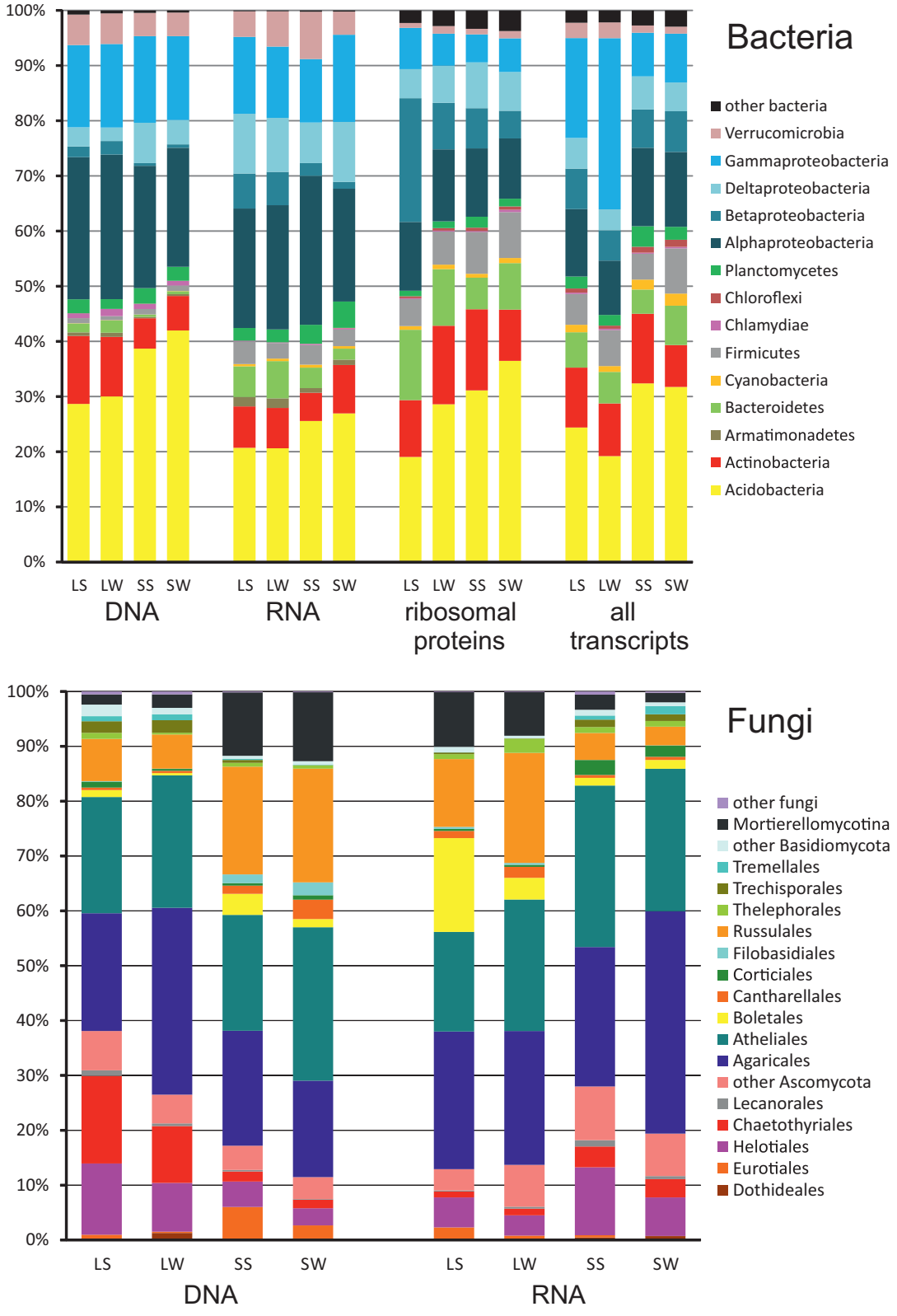

Fig. 1. Composition of total (DNA) and active (RNA) communities of *Picea abies* forest litter and soil in summer and winter, and expression of ribosomal proteins and all transcripts by bacteria. DNA and RNA represent the abundance of rDNA and rRNA-derived 16S and ITS2 sequences respectively. The data represent the means of six replicates for each horizon and season. Abbreviations: LS, litter summer; LW,litter winter; SS, soil summer; SW, soil winter.

seasonal differences were observable in the active RNA community (Fig. 1), such as a decrease of the Boletales and increase of the Russulales in soil during the winter. The DNA and RNA abundances frequently coincided, especially in soil: 30 of 39 dominant OTU in soil (77%) and 24 of 39 OTU in litter (62%) showed higher abundance in DNA and RNA in the same season.

The fungal community was significantly more variable than the bacterial community within the study area. The Bray–Curtis similarity values of community composition among the six study sites were similar in litter and soil, ranging from 0.48 to 0.60 in fungi and from 0.80 to 0.87 in bacteria. The difference in activity of bacterial and fungal genera between summer and winter were analysed by comparing the abundance ratios of RNA/DNA. Generally, for both bacteria and fungi, soil showed substantially higher differences in activity among seasons than did litter. Contrary to our hypothesis, ECM fungi did not show RNA enrichment in summer. Interestingly, all abundant soil *Actinobacteria* showed higher RNA/DNA ratios in winter (Fig. S2).

Microbial mRNA transcription in litter and soil

Metatranscriptomes were also obtained from the study site, providing broader insights for comparisons of expressed functions between horizons and seasons. Features identified as microbial (i.e., those assigned to either bacteria, fungi or archaea) represented a vast majority (83.4%) of annotated contigs. Of the other contigs, most had hits to the Streptophyta (4.2%), Arthropoda (3.7%), Nematoda (0.8%) and Chlorophyta (0.4%), whereas contigs identified as viral represented 0.2%. Overall, a total of 17 552 species were identified in assembled contigs. The contribution of microbial taxa to transcription differed widely between horizons. In litter, 69.9% of microbial transcripts were assigned to fungi, 28.9% to bacteria and 1.1% to archaea. However, in the soil, bacterial transcripts dominated at 74.1%, whereas fungal transcripts made up 24.6% and archaeal 1.3% of the total (Fig. 2A). The fraction of reads with functional classification associated with bacteria was high (72%) compared with those of archaea (26%) and fungi (only 19%).

The transcription profiles of selected microbial groups (Archaea, Fungi, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Firmicutes* and *Proteobacteria*) at level 3 of the Kyoto Encyclopedia of Genes and Genomes (KEGG) classification database were taxon-specific and differed significantly between all pairs of taxa ($P < 0.003$, analysis of similarities (ANOSIM) on Bray–Curtis distances with 9999 permutations). Fungal and archaeal transcription profiles also clearly separated from the bacterial transcription profiles (non-metric multidimensional scaling (NMDS); Fig. 2B). There were processes strongly dominated by bacteria (e.g., metabolism of terpenoids and polyketides), as well as those with a strong involvement of fungi, such as glycan biosynthesis and lipid metabolism (Fig. 2C). To evaluate phyla-specific processes (e.g., contribution of *Cyanobacteria* to photosynthesis), the total transcripts involved in a specific process (e.g., *Cyanobacteria*-associated photosynthesis transcripts) was compared with total transcripts associated with a specific phyla (e.g., all *Cyanobacteria*-associated transcripts). Broadly, specific taxa were observed to be highly enriched for group-specific processes (Table 2).

Comparing the transcripts between horizons, there was a significant difference between litter and soil in the relative abundance of observed functional assignments ($P = 0.0003$, ANOSIM on Bray–Curtis distances with 9999 permutations on KEGG level 3). Reads belonging to 132 out of 178 functional KEGG categories (74.1%) were observed to be significantly different in abundances between litter and soil (Table S4). The most highly expressed KEGG categories in litter relative to soil were ribosome (+45% compared with soil), fatty acid biosynthesis (+277%), starch and sucrose metabolism (+105%), protein processing in endoplasmic reticulum (+145%), RNA transport (+135%) and proteasome (+201%). In soil, significantly higher expression was recorded for oxidative phosphorylation (+57%), ATP-binding casette (ABC) transporters (+97%), glycolysis/gluconeogenesis (+29%), aminoacyl tRNA biosynthesis (+76%), purine metabolism (+67%) and pentose phosphate pathway (+67%) (Tables S5 and S6). Transcript abundances associated with all microbial phyla, with the exception of Archaea, were also significantly different between litter and soil ($P < 0.0011$, ANOSIM on Bray–Curtis distances with 9999 permutations). Together, the observed contrasting transcription abundance profiles between these two horizons suggest differences in their metabolism.

Seasonality of microbial transcription

In addition to the differences in transcription profiles among horizons, significant differences among transcription across seasons were also observed at this study site. Considering the taxonomic classification of reads, seasonality was much more pronounced in the soil, where the share of fungal transcripts was 33.4% in summer and only 15.7% in winter. Additionally, transcripts from *Actinobacteria*, *Planctomycetes* and *Proteobacteria* were significantly more frequent in summer, whereas those of the *Bacteroidetes* and *Chlorobi* were more than twofold higher in winter. In litter, only *Proteobacteria* and *Chlorobi* showed significantly different transcript abundances between summer and winter (Fig. 2A; Table S4).

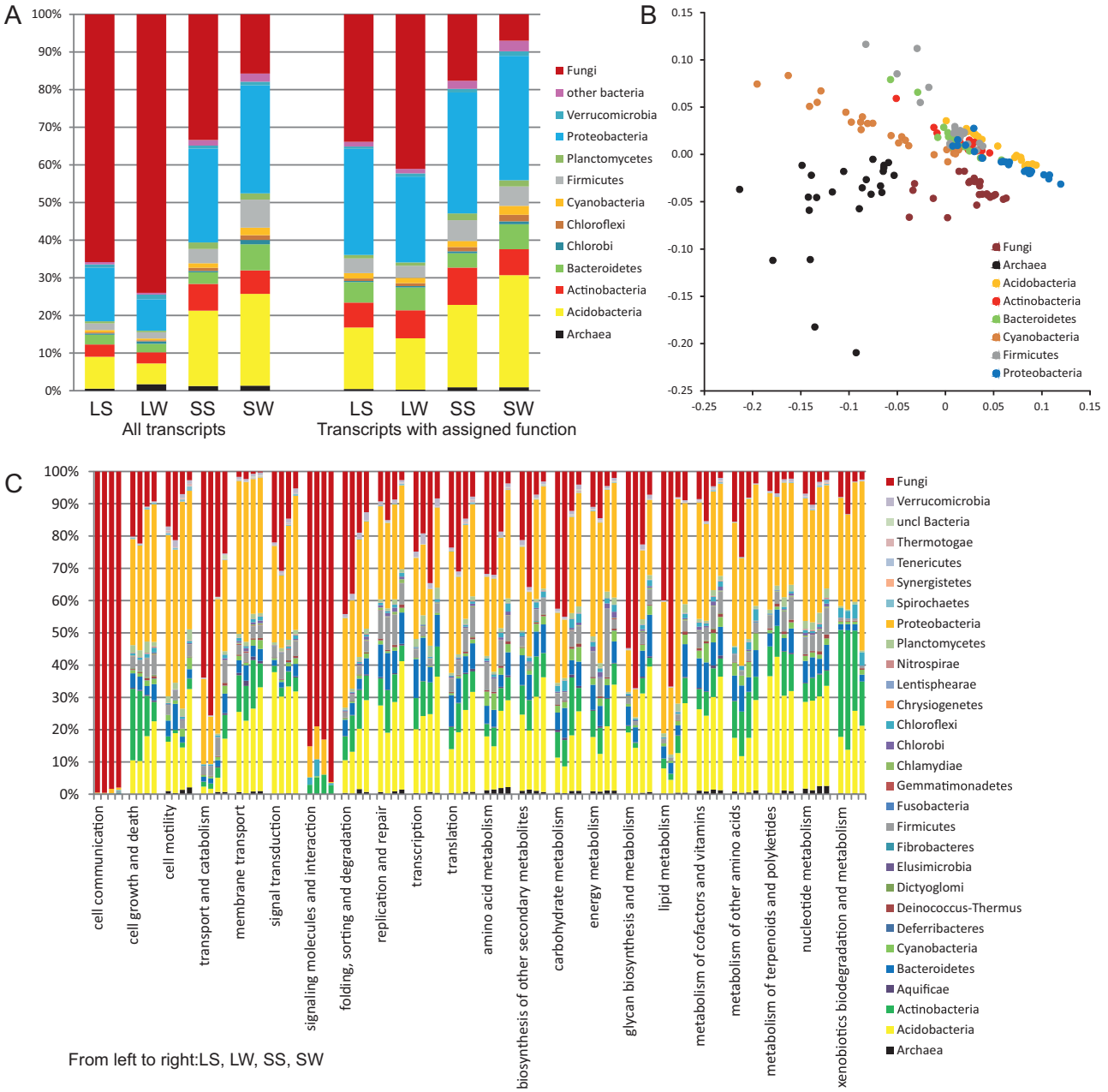

Fig. 2. Contribution of microbial taxa to the total transcription (A), the NMDS profiles of transcript categories grouped at KEGG3 level for selected microbial taxa (B) and transcription of specific functions (C) in the *Picea abies* forest litter and soil in summer and winter. In (A) and (C), data represent the means of six replicates for each horizon and season, In (B), data represent transcription profiles on KEGG3 level in individual samples. Abbreviations: LS, litter summer; LW, litter winter; SS, soil summer; SW, soil winter.

Functions associated with transcripts in litter and soil were also observed to be significantly different between summer and winter ($P < 0.005$, ANOSIM on Bray–Curtis distances with 9999 permutations, KEGG level 3). In litter, 26 functional KEGG categories (14.6%) were identified to be differentially expressed between seasons, including the two-component system (+60% in summer compared with winter), valine, leucine and isoleucine degradation (+66% in summer), phenylalanine metabolism (+293% in summer), RNA degradation (+65% in winter), proteasome (+88% in winter) and lysosome (+77% in winter). In soil, 24 categories (13.5%) showed differential expression between seasons, including valine, leucine and isoleucine degradation (+39% in summer), phenylalanine metabolism (+113% in summer) and proteasome (+92% in summer) (Tables S5 and S6). The seasonal differences in transcript functional profiles in soil were also significant within each single phylum of microbes except Archaea

Table 2. Selected processes in the *Picea abies* forest litter and soil where the contribution of microbial taxa to transcription (percentage of all reads assigned to each process) highly exceeds their contribution to general transcription.

Archaea	Arginine and proline metabolism (5.6%)
	N-glycan biosynthesis (3.2%)
	Chemotaxis (3.0%)
Fungi	Steroid biosynthesis (92%)
	Biosynthesis of various n-glycans (> 78%)
	Phenylalanine metabolism (70%)
	Fatty acid biosynthesis (62%)
	Starch and sucrose metabolism (52%)
	Amino sugar and nucleotide sugar metabolism (50%)
Acidobacteria	Synthesis of sesquiterpenes, triterpenes, flavonoids and carotenoids (> 60%)
	Fatty acid metabolism (43%)
Actinobacteria	Synthesis of type II polyketides (100%)
	Synthesis of macrolides (93%)
	Glycolysis/gluconeogenesis (29%)
Bacteroidetes	Biosynthesis of linoleic acid (62%)
	Metabolism of pyruvate, biotin and lipoic acid (> 19%)
	Prokaryotic C fixation (13%)
Cyanobacteria	Photosynthesis (80%)
	Photosynthetic C fixation (34%)
	Pentose phosphate pathway (9%)
Firmicutes	Methane metabolism (52%)
	Siderophore biosynthesis (23%)
	Cysteine and methionine metabolism (19%)
Proteobacteria	Steroid hormone biosynthesis (87%)
	Tetracycline biosynthesis (73%)
	Steroid degradation (69%)

($P < 0.018$, ANOSIM on Bray–Curtis distances with 9999 permutations, KEGG level 3), and in litter for all except *Bacteroidetes* and *Cyanobacteria*. The share of KEGG categories responding to seasonality was 2–10% in litter and 10–29% in soil. In *Acidobacteria*, *Proteobacteria* and Fungi, more than 20% of KEGG groups in soil showed significant seasonal trends.

We also identified representative assembled contigs from the transcriptome that were broadly present in our study area. This core metatranscriptome, defined as those contigs that represented > 0.001% of total transcription in each microbial domain (Archaea, Bacteria, Fungi) and that were observed in at least five samples from litter or from soil, had contrasting expressions in between horizons and seasons. The difference in relative transcription among seasons was at least 3-fold for 28–50% of the core contigs and at least 10-fold for 5–21% of the core contigs. More season-responding transcripts were found in soil than in litter for all domains (Fig. 3).

As root photosynthate allocation only occurs during the growing season, we expected that the abundance and activity of mycorrhizal fungi, depending on photosynthesis-derived carbon, will decrease between summer and winter. Based on the relative abundance of fungal ITS sequences belonging to mycorrhizal taxa in litter, we observed that this group was slightly but not significantly smaller in winter than in summer (46% and 51%), and the same was found for ITS transcripts (46% and 53%). In soil, the DNA and RNA abundance of mycorrhizal ITS sequences was not significantly different between seasons and was approximately 78–80%. Individual ECM, however, showed distinct seasonal patterns of abundance and activity that in some cases largely increased (*Russula*) and in other cases decreased (*Xerocomus*, *Amanita*) in winter in both horizons. Further, significant decrease in β-tubulin transcripts of Basidiomycota relative to those assigned to other fungi was observed from summer to winter; they represented 73.5 ± 3.6% in litter in summer, 57.1 ± 6.3% in winter,

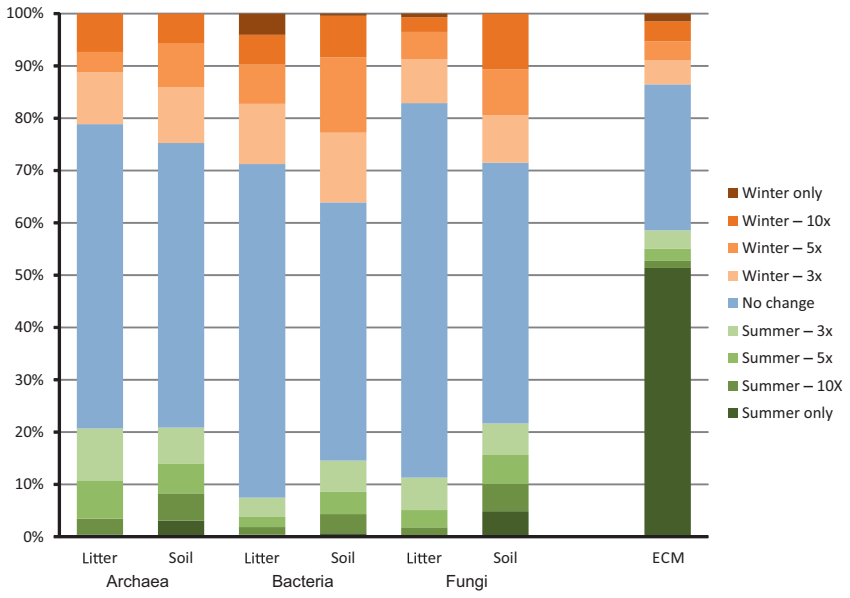

Fig. 3. The share of transcripts of Archaea, Bacteria and Fungi and of transcripts related to genes involved in the ectomycorrhizal symbiosis between summer and winter in the *Picea abies* forest litter and soil. The data represent the means of six replicates for each horizon. 3×, 5× and 10× indicate fold-change of transcript abundance among seasons.

83.4 ± 3.7% in soil in summer and 70.0 ± 2.5% in winter. The bulk of the remaining sequences belonged to the Ascomycota, with other fungi representing < 2.5% of transcripts. This may be due to the relative decrease in the transcription of the plant root-symbiotic ECM taxa because most of them belonged to the Basidiomycota.

To assess the seasonality of transcription of genes specific to mycorrhizal fungi, transcripts with high similarity to those induced during mycorrhizal symbiosis of *Laccaria laccata* with a plant host were identified. From a total of 19 969 of such putative ECM transcripts, 27.9% did not show difference in relative expression between seasons, whereas 51.4% showed expression exclusively in summer and another 7.2% showed a more than threefold increase in relative expression in summer. In contrast, only 1.5% and 12.0% of contigs were exclusively transcribed or increased in winter (Fig. 3) respectively.

Oxygen limitation has previously been reported as a major driver of soil activity in soils under snowpack (Robinson, 2001). In order to explore whether such anoxic conditions developed in our study area, we compared the relative summer and winter abundance of transcripts assigned to enzyme classes in anaerobic respiration, fermentation and fermentative pathways. These pathways included nitrate reduction, nitric and nitrous oxide reduction, sulphate reduction, homoacetogenesis, methanogenesis and synthesis of fermentation products. We observed no significant differences in transcripts associated with these functions between seasons, suggesting that seasonal oxygen limitation does not take place. Between soil and litter, however, we did observe higher abundance of anaerobic pathways in the soil compared with litter, suggesting that anaerobic niches may be present and influence microbial activity.

Discussion

Microbial transcription in forest litter and soil

Unlike many other soils, the soils of coniferous forests are unique in that they contain a high amount of fungal biomass in the soil, where root-symbiotic fungi proliferate because of their access to plant-produced C (Lindahl *et al.*, 2007; Clemmensen *et al.*, 2013). The litter of these soils also contains recalcitrant organic polymers that can be efficiently decomposed primarily by saprotrophic fungi (de Boer *et al.*, 2005; Šťursová *et al.*, 2012). In this study, we show that not only are these soils characterized by high diversity of both fungi and bacteria, consistent with previous results (Baldrian *et al.*, 2012), but also high metabolic diversity. The soil metatranscriptome identified over 4.6 million unique protein predictions in its assembled contigs. Assuming that a thousand expressed proteins are present in a single bacterial genome, this result suggests that thousands of microbial species are transcriptionally active at the same time.

Within these soils, a high proportion of assigned reads belonged to fungi, especially in the litter (Fig. 2). This result is much higher than previous estimates in both grassland and forest soils, where more than 90% sequences were assigned to bacteria and only 4% and 0.5% were fungal or archaeal respectively (Nacke *et al.*, 2014). The values observed here provide evidence to the importance of fungi in the functioning of the coniferous forest ecosystems (Fig. 2) and are consistent with other forest soil eukaryotic metatranscriptomes (Bailly *et al.*, 2007; Damon *et al.*, 2012; Takasaki *et al.*, 2013), where fungal reads were found to dominate among those of the eukaryota. In general, it is more challenging to reliably annotate fungal sequences relative to those of bacterial origin because of the lack of available reference genomes and performance of annotation tools (Meyer *et al.*, 2008). For example, it is challenging even to distinguish the genes of the Ascomycota and the Basidiomycota, the two most abundant fungal divisions. Despite these challenges, this study demonstrates that metatranscriptomics can provide insight into contrasting fungal and bacterial dynamics between soil horizons and seasons.

Forest topsoils have been previously demonstrated to exhibit vertical stratification of composition resulting from the different processes in litter and soil, as well as the stratification of organic matter in soil. This study confirms the differences in enzyme activities, microbial biomass and community composition previously observed with the decreasing content of bacterial and fungal biomass and increasing content of ECM with depth (Lindahl *et al.*, 2007; Baldrian *et al.*, 2012; Clemmensen *et al.*, 2013; Voříšková *et al.*, 2014). Metabolic potential has also been observed with metagenomics in the soil horizons of a *Picea abies* forest (Uroz *et al.*, 2013). This study extends these observations beyond microbial structure and gene potential to the functional level. We found that as many as 74% of functions were differentially expressed between horizons. In litter, this activity was dominated by fungi, whereas in soil, bacteria dominated. Increased synthesis of ribosomal proteins in litter suggests more active metabolism in this horizon, along with increased share of starch and sucrose metabolism (+105% compared with soil) suggesting higher production of decomposition-related enzymes. Differences in microbial transcription among soils from various depths has also been previously observed in Svalbard peat soils, but this environment is quite different and is also characterized by low fungal biomass and oxygen limitation (Tveit *et al.*, 2013; 2014), which our soils did not have.

Seasonal changes of microbial activity

Microbial communities in forest ecosystems were also demonstrated to change across seasons, likely due to seasonality of photosynthesis. In a deciduous temperate forest, the fungal community in litter exhibits profound seasonal changes: saprotrophic taxa reach their seasonal maxima on freshly fallen litter in autumn, whereas summer typically is characterized by the highest abundance of ectomycorrhizal taxa (Baldrian *et al*., 2013; Voříšková *et al*., 2014). In deeper soils, less change in fungal community composition is likely, but trends for minimum biomass in winter and peaks in summer are most likely due to rhizodeposition (Voříšková *et al*., 2014). Consistent with previous results (Baldrian *et al*., 2013), our soils did not exhibit differences in the fungal/bacterial biomass across seasons. Within the bacteria domain, only Actinobacteria exhibited seasonal changes in abundance in a forest soil while other groups did not (Kuffner *et al*., 2012). Broadly, community profiles of both soil DNA and RNA were largely consistent across seasons (Fig. 1). The seasonal changes in the activity of individual microbial species (RNA/DNA of OTUs) were much more pronounced in soil compared with litter (Fig. S2), with soil also exhibiting a higher share of functional categories with seasonal differences in expression (Figs 2 and 3). The increase of RNA degradation, proteasome and lysosome-related transcripts suggest reduced microbial biomass and activity in winter. Interestingly, we observed no reduction in the amount of bacterial biomass (16S) or that of fungal biomass (ergosterol) during the winter. Seasonal differences in soil dynamics could be the result of both changes in the abundance of microbial taxa as well as expression of transcribed functions. We also observed significant differences in genes expressed between seasons that exhibited very high sequence similarity ($E < 10^{-100}$) to the genome of *Solibacter usitatus*, supporting the idea that even individual microbial species change the repertoire of their transcribed genes among seasons.

Seasonal differences in the contribution of microbial groups to transcription were most markedly demonstrated in the soil relative to litter, where the share of fungal transcripts was 33.4% in summer but only 15.7% in winter. This corresponded to an observed increase in bacterial/fungal rRNA ratios in winter. No difference in total fungal biomass (ergosterol) was recorded between seasons, suggesting that possibly only the active part of fungal mycelial structures may be reduced during the winter. Because fungi are reported to be more abundant in the rhizosphere than in bulk soils (Turner *et al*., 2013), the seasonality of root processes that take place in soil and not in litter, such as the rhizodeposition of photosynthetically fixed C, may plausibly explain the decrease of fungal activity in winter where the system is dominated by ECM fungi. This result would be consistent with our observations that there is no significant difference in the relative amount of mycorrhizal fungi, but their activity in winter is reduced significantly. In the summer, we observed increases in the activity of genes associated with Ectomycorrhiza-specific transcripts (> 50% genes exclusively transcribed in that season; Fig. 3) as well as increased activity of Basidiomycota (which are mostly ECM), espsecially its housekeeping gene for β-tubulin. The higher ECM activity in summer was also accompanied by the increase of abundance of *Planctomycetes*, which were previously reported in association with functioning ECM hyphal networks (Lindahl *et al*., 2010), and higher activity of selected bacterial taxa that harbour mycorrhiza-helper bacteria such as *Burkholderia* spp., *Streptomyces* spp. or *Sphingomonas wittichii* (Churchland and Grayston, 2014).

The observed reduction of ECM activity in winter should theoretically decrease their inhibitory effects on decomposition of organic matter (Ekblad *et al*., 2013) and consequently lead to increased abundance of extracellular enzymes. Contrary to this expectation, enzymes in our soil did not show differences in activity between seasons. Reports from other deciduous forests are so far inconclusive, with some studies showing seasonality of enzymatic processes (Kaiser *et al*., 2010; Voříšková *et al*., 2014) and others observing no significance between seasons (Baldrian *et al*., 2013). Considering the temperature dependence of activity (Baldrian *et al*., 2013), the rates of enzymatic processes are likely to be higher in summer, supported by results from a recent study from another *Picea abies* forest where enzyme activity decreased with the reduction of C allocation by tree roots belowground (Štursová *et al*., 2014). Our results suggest that activity of enzymes involved in decomposition is promoted by the availability of simple C compounds, which is higher in summer. Our study has also excluded the possible oxygen limitation in unfrozen soil below deep snowpack in winter.

This paper provides the first comprehensive analysis of the seasonality of soil transcription that indicates contrasting dynamics in soil function both between litter and soils as well as between seasons. We find that the microbial community composition in soil of a coniferous forest, particularly on the DNA level, is quite stable, but that there are profound changes in microbial transcription across seasons. Most importantly, fungal contribution to total microbial transcription decreases in winter, especially in soil. Plant photosynthetic production seems to be the major driver of seasonality in the studied ecosystem because the activity of ECM fungi that are dependent on this process is highly affected. The results also indicate that the widely used DNA-based community surveys or metagenome analyses may represent less dynamic picture of studied ecosystems and to be thus inferior to the metatranscriptomic approaches in describing the

ecosystem functioning. Despite the potential of metatranscriptomics to reveal the dynamics of soil functioning, the results of this method should be in the future verified by complementary approaches, such as, for example, metaproteomics, metabolomics or direct isotopic labelling.

Experimental procedures

Study area, sample collection and characterization

The study area was located at high altitudes (1170–1200 m) of the Bohemian Forest mountain range (Central Europe; 49°02 N, 13°37 E) and was covered by an unmanaged Norway spruce (*Picea abies*) forest. The mean annual temperature was 5°C, and the mean annual precipitation was 1000 mm. The understory was either missing or composed of grasses (*Avenella*, *Calamagrostis*), bilberries (*Vaccinium*), mosses and ferns. The same study area was explored previously to identify the total and active microbial communities (Baldrian *et al*., 2012). Samples were collected on 24 July 2012 (summer, the peak of the vegetation period) and on 27 March 2013 (late winter, under a 50 cm snowpack after a long period of constant environmental conditions with uniform temperature and lack of daylight due to snow cover; Fig. S1). At six sites, located approximately 250 m from each other, eight soil cores (4.5 cm diameter) were collected from around the circumference of a 3 m diameter circle. Litter horizon (L, 2–4 cm) and organic soil horizon (S, 3–6 cm) materials were separately pooled within each site. After removal of roots, L material was cut into 0.5 cm pieces and mixed, whereas S material was passed through a 5 mm sterile mesh and mixed. A total of 24 samples were collected (6 sites × two seasons × 2 horizons). Soil and litter samples were immediately frozen in liquid nitrogen and stored on dry ice. Samples for nucleic acid extraction were stored at −80°C, samples for ergosterol quantification, chemical analysis and enzyme activity measurements were freeze-dried and stored at −45°C. Enzyme assays were performed in soil homogenates (Štursová and Baldrian, 2011).

Dry mass content was measured as a loss of mass during freeze-drying, organic matter content was measured after combustion at 650°C and pH was measured in distilled water (1:10). Soil C, N and extractable P content was measured in an external laboratory. Total ergosterol was extracted with 10% KOH in methanol and analysed by high-performance liquid chromatography (Šnajdr *et al*., 2008). Air temperature and soil temperatures at 2 cm and 5 cm depths were recorded hourly from 1 July 2012 until 30 June 2013 at all sampling sites.

Extraction and analysis of environmental RNA and DNA

For the metatranscriptome analysis, RNA was extracted using the RNA PowerSoil Total RNA Isolation Kit (MoBio Laboratories) combined with the OneStep PCR Inhibitor Removal Kit (ZymoResearch). Three aliquots (3 × 1 g of material) were extracted per sample. Triplicate RNA extracts were pooled, and RNA was purified using the RNA Clean and Concentrator Kit (ZymoResearch) on a column treated with DNase I (Fermentas) according to manufacturer's instructions. This product was checked for quality (RNA integrity number) and length distribution on an Agilent 2100 Bionalyser (Agilent Technologies). Approximately 1 μg of RNA was treated with an equimolar mixture of RiboZero rRNA Removal Kits Human-Mouse-Rat and Bacteria (Epicentre) to remove both prokaryotic and eukaryotic rRNA. rRNA removal was checked on an Agilent 2100 Bionalyser. A total of 50 ng of treated RNA served as the input for the ScriptSeq v2 RNA-Seq Library Preparation Kit (Epicentre), and the library size-distribution was re-checked on an Agilent 2100 Bionalyser (Agilent Technologies). Libraries were sequenced on an ILLUMINA HISEQ2000 at the Argonne National Laboratory, USA, to generate 150-base paired-end reads.

To analyse microbial community composition, 1 μg of total RNA was reverse transcribed using SUPERSCRIPT III REVERSE TRANSCRIPTASE (Life Technologies) using random hexamer primers. Total DNA was extracted in triplicate from all samples using a modified Miller method (Sagova-Mareckova *et al*., 2008) and cleaned with a Geneclean Turbo Kit (MP Biomedicals). Bacterial and fungal rDNAs were quantified by quantitative polymerase chain reaction (qPCR) using the 1108f and 1132r primers for bacteria (Wilmotte *et al*., 1993; Amann *et al*., 1995) and the FR1/FF390 primers for fungi (Prévost-Bouré *et al*., 2011).

For the microbial community analysis, PCR amplification of the fungal ITS2 region from DNA and cDNA was performed using barcoded gITS7 and ITS4 (Ihrmark *et al*., 2012) in three PCR reactions per sample. PCR reactions contained 2.5 μl of 10× buffer for DyNAzyme DNA Polymerase, 0.75 μl of BSA (20 mg ml^{-1}), 1 μl of each primer (0.01 mM), 0.5 μl of PCR Nucleotide Mix (10 mM each), 0.75 μl polymerase (2 U μl^{-1} DyNAZyme II DNA polymerase 1: 24 Pfu DNA polymerase) and 1 μl of template DNA or cDNA. Cycling conditions were 94°C for 5 min, 35 cycles of 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min. The V4 region of bacterial 16S rRNA was amplified using the barcoded primers 515F and 806R (Argonne National Laboratory) as described previously (Caporaso *et al*., 2012). Sequencing of fungal and bacterial amplicons was performed on ILLUMINA MISEQ.

Sequence data processing and analysis

The amplicon sequencing data were processed using the pipeline SEED 1.2.1 (Větrovský and Baldrian, 2013a). Briefly, pair-end reads were merged using FASTQ-join (Aronesty, 2013). Whole amplicons were processed for bacterial 16S, whereas the ITS2 region was extracted using ITS EXTRACTOR 1.0.8 (Nilsson *et al*., 2010) before processing. Chimeric sequences were detected using USEARCH 7.0.1090 (Edgar, 2010) and deleted, and sequences were clustered using UPARSE implemented within USEARCH (Edgar, 2013) at a 97% similarity level. Consensus Sequences were constructed for each cluster, and the closest hits at a genus or species level were identified using BLASTn against the Ribosomal Database Project (Cole *et al*., 2014) and Genbank databases (for bacteria) or UNITE (Koljalg *et al*., 2013) and GenBank for fungi. Sequences identified as nonbacterial or nonfungal were discarded. From 16S rRNA in DNA, bacterial genome count estimates were calculated based on the 16S copy numbers in

the closest available sequenced genome as described previously (Větrovský and Baldrian, 2013b). Sequence data have been deposited in the metagenomics Rapid Annotation using Subsystem Technology (MG RAST) public database (Meyer *et al*., 2008), data set number 4603354.3 for bacteria and 4603355.3for fungi). Shannon–Wiener Index, species richness and evenness were calculated for 1250 randomly chosen sequences per sample. The pipeline SEED 1.2.1 (Větrovský and Baldrian, 2013a) was used for data preprocessing and diversity calculations.

Metatranscriptome reads were quality trimmed by removing adapters with Trimmomatic (v 0.27) using ILLUMINA TRUSEQ2-PE adapters with a seed mismatch threshold, palindrome clip threshold, and simple clip threshold set at 2, 30, and 10 respectively (Bolger *et al*., 2014). Furthermore, sequencing reads were filtered by base call quality using the FASTX-TOOLKIT (http://hannonlab.cshl.edu/fastx_toolkit/index.html), specifically fastq_quality_filter, with the following parameters: –Q33 –q 30 –p 50. Resulting sequences were normalized using methods previously described in (Pell *et al*., 2012; Howe *et al*., 2014) and Khmer (v 0.7.1) and command normalise-by-median.py with the following parameters: –k 20 –C 20 –N 4 –x 50e9. Next, errors were trimmed by removing low abundance fragments of high coverage reads with Khmer and command filter-abund.py –V. The paired-end assembly of the remaining reads was performed with the VELVET ASSEMBLER (v 1.2.10, -exp_cov auto -cov_cutoff auto -scaffolding no; Zerbino and Birney, 2008) using odd k-mer lengths ranging from 33 to 63. Resulting assembled contigs were merged using CD-HIT v4.6 (Li and Godzik, 2006; Fu *et al*., 2012) and MINIMUS2 AMOS v3.1.0 (Sommer *et al*., 2007). Broadly, protocols for this metatranscriptome assembly can be found at https://khmer-protocols.readthedocs.org/en/latest/mrnaseq/index.html. Sequence data of all contig sequences have been deposited in the MG RAST data set number 4544233.3).

Contig annotation was performed in MG RAST with an E value threshold of 10^{-4} while also considering the representative hit option (i.e., single best annotation for each feature). For contigs where multiple KEGG categories were assigned (< 2%), all categories were counted as additional hits. Anoxia-induced reads were classified as those belonging to corresponding functions defined by Tveit and colleagues (2013). Transcripts of the putative mycorrhiza-related genes were identified as those contigs giving TBLASTX hits with E values of $< 10^{-50}$ to those genes of *Laccaria bicolor*, preferentially expressed in mycorrhizal symbiosis compared with free mycelial growth (Larsen *et al*., 2010; Kohler *et al*., 2015). Because reliable assignment of most fungal transcripts to divisions (such as Ascomycota or Basidiomycota) was not possible, the relative contribution of fungal divisions to transcription was quantified based on the abundance of transcripts of β-tubulin, a housekeeping gene whose sequences can be reliably assigned thanks to sufficient coverage in GenBank (Begerow *et al*., 2010).

For the metatranscriptomic data, individual sequence reads from each sample were mapped onto contigs using BOWTIE 2.2.1 (Langmead *et al*., 2009) with the default settings of: end-to-end alignment – sensitive. The mapping was used to calculate transcript abundance, and data were expressed as: per base coverage = read count × read length / contig length. Abundances were always reported as normalized values, i.e., shares of all transcripts in given sample, or, where indicated, shares of all transcripts of a selected microbial taxon. For the analysis of functional features, such as the KEGG categories (cf. Table S6), only those contigs belonging to archaea, bacteria and fungi and belonging to cellular processes, environmental information processing, genetic information processing and metabolism at the KEGG level 1 were considered.

Sequencing yielded 674×10^6 reads ($28 \times 10^6 \pm 3 \times 10^6$ reads per sample) that were assembled into 4 522 875 contigs over 200 bases, including 645 342 contigs over 500 bases and 98 246 over 1000 bases (mean length was 362 bases). The longest contig had a length of 33 888 bases. Protein prediction yielded a total of 4 662 356 predicted coding regions, of which 1 859 087 (39.9%) have been assigned an annotation. A total of 1 311 357 features (70.5% of annotated features, 28.1% of all features) were assigned to functional categories. In terms of reads, 327×10^6 reads (48.5%) mapped to contigs. Of the mapped reads, 24% had hits to contigs annotated with taxonomy, and 13.4% to contigs annotated with both taxonomy and function.

Statistical analysis

STATISTICA 7 (Statsoft, USA) or PAST 3.03 (http://folk.uio.no/ohammer/past/) were used for statistical analysis. Bray–Curtis distance was used as a metric of similarity between samples. Differences in soil variables were tested using ANOVA, and differences in relative abundances of individual features (transcripts or microbial taxa) were tested using the Mann–Whitney *U*-test, which assumes the measurements on a rank-order scale but does not assume normality of data. ANOSIM on Bray–Curtis distances was used for the analysis of differences among communities or transcript pools. Differences at $P < 0.05$ were considered to be statistically significant. NMDS on Bray–Curtis distances was used to visualize differences among the transcription profiles of microbial taxa.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Daily mean air and soil temperatures in the *Picea abies* forest and the view of the sampling sites at the time of sample collection.

Fig. S2. The ratio of sequences of the most abundant fungal and bacterial genera derived from RNA and DNA in the *Picea abies* forest litter and soil in summer and winter. Bacterial RNA/DNA ratio was calculated as a ratio of 16S rRNA (ribosomes) and bacterial genome counts estimated by 16S sequencing. Fungal RNA/DNA ratio represents the ratio of the ITS2 sequence abundance in PCR amplicons derived from RNA (transcripts leading to rRNA synthesis) and DNA (rDNA copies).

Table S1. Diversity of bacterial and fungal rDNA and rRNA sequences obtained from *Picea abies* forest litter and soil. The data were obtained for a sampling depth of 1250 sequences per sample and represent means and standard errors. Significant differences are indicated by different letters (Mann–Whitney *U*-test, $P < 0.05$).

Table S2. Overview of the abundance of bacterial taxa in a *Picea abies* forest litter and soil in summer and winter (mean abundance $> 0.5\%$) based on 16S amplicon sequencing. The data are means of data from six sites and represent estimated relative abundances of bacterial genome counts (for DNA) or rRNA molecules (for RNA). Statistically significant differences in abundance between horizons or between seasons within a horizon are indicated in bold script. Bacterial phyla/classes: Aci – Acidobacteria, Act – Actinobacteria, Alp – Alphaproteobacteria, Arm – Armatimonadetes, Bac – Bacteroidetes, Bet – Betaproteobacteria, Del – Deltaproteobacteria, Fir – Firmicutes, Gam – Gammaproteobacteria, Pla – Planctomycetes, Ver – Verrucomicrobia.

Table S3. Overview of the abundance of fungal taxa in a *Picea abies* forest litter and soil in summer and winter (mean abundance $> 0.5\%$) based on ITS amplicon sequencing. The data are means of data from six sites and represent relative abundances of ITS2 sequences derived from DNA and RNA respectively. Statistically significant differences in abundance between horizons or between seasons within a horizon are indicated in bold script. Fungal divisions/subdivisions: A – Ascomycota, B – Basidiomycota, M – Mortierellomycotina; ecology: ECM – ectomycorrhizal, ERM – ericoid mycorrhizal, SAP – nonmycorrhizal.

Table S4. Assignment to metatranscriptomic contigs and reads from *Picea abies* forest litter and soil to microbial taxa. The data represent means and standard errors of six replicates for each horizon and season. Differences among pairs of treatments were tested for statistical significance using Mann–Whitney *U*-test, differences at $P < 0.05$ are indicated in bold script. Relative contributions of fungi, archaea and bacteria are expressed as a share of all microbial reads, relative contributions of bacterial phyla as a share of all bacterial reads.

Table S5. Relative abundance KEGG all reads: Assignment of identified metatranscriptomic reads from the *Picea abies* litter and soil to KEGG classes. The data represent means of six replicated sites per horizon and season. Differences among pairs of treatments were tested for statistical significance using Mann–Whitney *U*-test, differences at $P < 0.05$ are indicated in bold script.

Table S6. KEGGs all samples: Assignment of identified metatranscriptomic reads from the *Picea abies* litter and soil to KEGG classes for all samples. Abbreviations: LS – litter summer, LW – litter winter, SS – soil summer, SW – soil winter, numbers indicate sampling sites.