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

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Abstract
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 *Pinus 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 photosynthetic 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.

Disciplines
Bioresource and Agricultural Engineering | Environmental Microbiology and Microbial Ecology

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

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Running title: Seasonal dynamics of a soil microbial community
Summary

Understanding the ecology of coniferous forests is very important because these environments represent significant global 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 in soil and litter were highly diverse both in composition and function and characterized by a high abundance of fungi. Differences in ecosystem functions between seasons consisted of a combination of moderate changes in microbial community composition and profound changes in taxon-specific microbial transcription profiles. Further, these differences were more significant 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 abundant ectomycorrhizal was reduced in winter. Overall, these results indicate that plant photosynthetic production was likely the major driver of changes in the functioning of microbial communities in this coniferous forest across seasons.

Introduction

Coniferous forests 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 have 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, as well 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 in 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 (Baldrian et al., 2012;
Šnajdr et al., 2008). In this region, ectomycorrhizal fungi (ECM; the plant root symbionts belonging to
the Dikarya) grows on the soil surface and is 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 (Baldrian et al., 2012; Lindahl et al., 2007; O'Brien et al., 2005).

Seasonally, temperate and boreal zone forests are characterised by the photosynthetic
activity of trees during a vegetative 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 mainly the vegetative season (Högberg et al., 2010).

Previous observations from deciduous forest soils 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. Though 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 (Gifford et al., 2013; Gilbert et al., 2010; Shi et al., 2011) 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 to 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 (Urish et al., 2008), where 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 a few published metatranscriptomic studies from soils (Nacke et al., 2014; Tveit et al., 2013; Tveit 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 and litter horizon on forest soil microbial activity. We investigate two seasons: the summer peak of plant photosynthetic activity and late winter with no photosynthate input in soil regions where we have previously observed high microbial activity, especially 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 abundance among seasons, differential transcription of the microbes, or both. Our over-arching 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 the 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 at 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 (Supplementary Fig. 1). The temperature in litter and soil during the 14 days before sampling was 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, while the soil in winter was covered by 50 cm of snow for longer than 3 months (Fig. 1). 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 (Fig. 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 (Fig. 1).

**Composition and activity of the microbial community**

Gene targeted sequencing was performed to characterize the composition of the soil microbial community (16S rRNA genes amplicons, DNA) and activity (16S rRNA amplicons, RNA). We detected a total of 27164 bacterial OTU with best hits to 1005 genera. Soil biodiversity was observed to be between 4.54-5.21 (Shannon Index) and evenness between 0.80-0.87. 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, Supplementary Table S1).

In both DNA- and RNA-derived communities, bacterial communities were dominated by Proteobacteria, followed by Acidobacteria and Actinobacteria. While 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 localisation 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%) (Supplementary 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 (Supplementary 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, while 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. 2).

Amplicon sequencing of fungal ITS genes 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 0.68-0.70 (Supplementary 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 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 (Supplementary Table S3). Seasonal differences in abundance within each horizon were limited to a small number of taxa in both DNA and RNA communities (Supplementary Table S3). Moderate seasonal differences were observable in the active RNA community (Fig. 2), 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, suggesting decreased activity during the summer season. Interestingly, all abundant soil Actinobacteria showed higher RNA/DNA ratios in winter (Supplementary Fig. 2).

Microbial 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 viruses represented 0.2%. Overall, a total of 17552 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 of 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. 3A). The fraction of reads with functional classification associated with bacteria was high (72%) compared to 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 KEGG classification database were taxon-specific and differed significantly between all pairs of taxa (P < 0.003, ANOSIM on Bray-Curtis distances with 9999 permutations). Fungal and archaeal transcription profiles also clearly separated from the bacterial transcription profiles (NMDS, Fig. 3B). 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. 3C).

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 to 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 1).

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 (Supplementary 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%), ABC transporters (+97%), glycolysis/gluconeogenesis (+29%), aminoacyl tRNA biosynthesis (+76%), purine metabolism (+67%) and pentose phosphate pathway (+67%) (Supplementary Tables S5, 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.
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. 3A, Supplementary Table S4).

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) (Supplementary Table S5, S6). The seasonal differences in transcript functional profiles in soil were also significant within each single phyla of microbes except archaea (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 threefold for 28-50% of the core contigs and at least tenfold for 5-21% of the core contigs. More season-responding transcripts were found in soil than in litter for all domains (Fig. 4).

As root photosynthate allocation only occurs during the growing season, we expected that the abundance and activity of mycorrhizal fungi depending root-derived carbon for C to 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 were not significantly different between seasons and was approximately 78-80%. Individual ectomycorrhizal fungi, however, showed distinct seasonal patterns of abundance and activity that in some cases largely increased (Russula) and in others decreased (Xerocomus, Amanita) in winter in both horizons. Further, significant decrease in β-tubulin transcripts of Basidiomycota relative to those associated with other fungi was observed from summer to winter; they represented 73.5 ± 3.6% in litter in summer, 57.1 ± 6.3% in winter, 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 seasonal 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 19969 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. 4), respectively.

Oxygen limitation has previously been reported as a major driver on 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 due to their access to plant-produced C (Clemmensen et al., 2013; Lindahl et al., 2007). The litter of these soils also contain recalcitrant organic polymers that can be efficiently decomposed primarily by saprotrophic fungi (de Boer et al., 2005; Štursová 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 strain, this result suggests 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. 3). 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 provides evidence to the importance of fungi in the functioning of the coniferous forest ecosystems (Fig. 1) and is 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, due to 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 ectomycorrhizal fungi with depth (Baldrian et al., 2012; Clemmensen et al., 2013; Lindahl et al., 2007; 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 fungal while in soil, bacteria dominated. Increased synthesis of ribosomal proteins in litter suggest more active metabolism in this horizon, along with increased
shares 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;
Tveit et al., 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. 2). The seasonal
changes in the activity of individual microbial species (RNA / DNA of OTUs) were much more
pronounced in soil compared to litter (Supplementary Fig. 2), with soil also exhibiting a higher share
of functional categories with seasonal differences in expression (Fig. 3, 4). 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, especially the functional profiles of genes with 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 was 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 in 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. 4)) as well as increased activity of Basidiomycota (which are mostly ECM), especially 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 abundances 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. In the future, the potential of metatranscriptomics to reveal the dynamics of soil functioning will hopefully be further increased when it is complemented by other methods, such as, for example, metaproteomics.

Experimental Procedures

Study area, sample collection and characterisation
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 24.7.2012 (summer, the peak of the vegetation period) and on 27.3.2013 (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, Supplementary Fig. 1). 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) material were separately pooled within each site. After removal of roots, L material was cut into 0.5 cm pieces and mixed while S material was passed through a 5-mm sterile mesh and mixed. A total of 24 samples were collected (6 sites × two seasons × two 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 HPLC (Šnajdr et al., 2008). Air temperature and soil temperatures at 2-cm and 5-cm depths were recorded hourly from July 1, 2012 until June 30, 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 & Concentrator Kit (ZymoResearch) on a column treated with DNase I (Fermentas) according to manufacturer’s instructions. This product was checked for quality (RIN 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 qPCR using the 1108f and 1132r primers for bacteria (Amann et al., 1995, Wilmotte et al., 1993) 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 10x 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, while 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 RDP (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 MG RAST public database ((Meyer et al., 2008), data set number 4603354.3 for bacteria and 4603355.3 for 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 pre-processing and diversity calculations.

Metatranscriptome reads were quality trimmed by removing adapters with Trimmomatic (v0.27) 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 normalised using methods previously described in (Howe et al., 2014, Pell et al., 2012) 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 (Fu et al., 2012, Li and Godzik 2006) 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 public database ((Meyer et al., 2008), http://metagenomics.anl.gov/, 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 et al., 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 to 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 x read length / contig length. Abundances were always reported as normalised 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. Supplementary 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 KEGG1 level were considered.

Sequencing yielded 674 x 10^6 reads (28 x 10^6 ± 3 x 10^6 reads per sample) that were assembled into 4522875 contigs over 200 bases, including 645342 contigs over 500 bases and 98246 over 1000 bases (mean length was 362 bases). The longest contig had a length of 33888 bases. Protein prediction yielded a total of 4662356 predicted coding regions, of which 1859087 (39.9%) have been assigned an annotation. A total of 1311357 features (70.5% of annotated features, 28.1% of all features) were assigned to functional categories. In terms of reads, 327 x 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. Nonmetric multidimensional scaling (NMDS) on Bray-Curtis distances was used to visualise differences among the transcription profiles of microbial taxa.

Acknowledgements

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There are no competing commercial interests in relation to the submitted work for any of the authors.

References


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

Fig. 2.: 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.

Fig. 3.: 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.

Fig. 4.: 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. 3x, 5x and 10x indicate fold-change of transcript abundance among seasons.
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July - soil temperature 9.7 °C

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

March - soil temperature -0.3 °C
A

- Fungi
- other bacteria
- Verrucomicrobia
- Proteobacteria
- Planctomycetes
- Firmicutes
- Cyanobacteria
- Chloroflexi
- Chlorobi
- Bacteroidetes
- Acnobacteria
- Acidobacteria
- Archaea

B

C

From left to right: LS, LW, SS, SW
Table 1.: 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.

<table>
<thead>
<tr>
<th>Archaea</th>
<th>arginine and proline metabolism (5.6%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n-glycan biosynthesis (3.2%)</td>
</tr>
<tr>
<td></td>
<td>chemotaxis (3.0%)</td>
</tr>
<tr>
<td>Fungi</td>
<td>steroid biosynthesis (92%)</td>
</tr>
<tr>
<td></td>
<td>biosynthesis of various n-glycans (&gt;78%)</td>
</tr>
<tr>
<td></td>
<td>phenylalanine metabolism (70%)</td>
</tr>
<tr>
<td></td>
<td>fatty acid biosynthesis (62%)</td>
</tr>
<tr>
<td></td>
<td>starch and sucrose metabolism (52%)</td>
</tr>
<tr>
<td></td>
<td>amino sugar and nucleotide sugar metabolism (50%)</td>
</tr>
<tr>
<td>Acidobacteria</td>
<td>synthesis of sesquiterpenes, triterpenes, flavonoids and carotenoids (&gt;60%)</td>
</tr>
<tr>
<td></td>
<td>fatty acid metabolism (43%)</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>synthesis of type II polyketides (100%)</td>
</tr>
<tr>
<td></td>
<td>synthesis of macrolides (93%)</td>
</tr>
<tr>
<td></td>
<td>glycolysis/gluconeogenesis (29%)</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>biosynthesis of linoleic acid (62%)</td>
</tr>
<tr>
<td></td>
<td>metabolism of pyruvate, biotin and lipoic acid (&gt;19%)</td>
</tr>
<tr>
<td></td>
<td>prokaryotic C fixation (13%)</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>photosynthesis (80%)</td>
</tr>
<tr>
<td></td>
<td>photosynthetic C fixation (34%)</td>
</tr>
<tr>
<td></td>
<td>pentose phosphate pathway (9%)</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>methane metabolism (52%)</td>
</tr>
<tr>
<td></td>
<td>siderophore biosynthesis (23%)</td>
</tr>
<tr>
<td></td>
<td>cysteine and methionine metabolism (19%)</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>steroid hormone biosynthesis (87%)</td>
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<td></td>
<td>tetracycline biosynthesis (73%)</td>
</tr>
<tr>
<td></td>
<td>steroid degradation (69%)</td>
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