Feed in summer, rest in winter: Microbial carbon utilization in forest topsoil

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**Abstract**

Evergreen coniferous forests contain high stocks of organic matter. Significant carbon transformations occur in these ecosystems, making them important for the global C cycle. Here, we show that organisms in topsoil encode a diverse set of carbohydrate active enzymes, including glycoside hydrolases and auxiliary enzymes. Microorganisms produce the bulk of these enzymes, with fungi strongly dominating transcription in litter and an equal contribution of bacteria and fungi in soil. Turnover of microbial biomass appears to be faster in the summer than in the winter, indicating fast growth and seasonal differences in gene transcription especially in soils. While the use of reserve compounds, such as starch or trehalose, is high in winter when the rhizodeposition of photosynthates is low, summer is characterized by high expression of ligninolytic, cellulolytic and xylanolytic enzymes produced mainly by fungi. Our results support the hypothesis that priming by photosynthate C may aid in the decomposition of recalcitrant biopolymers by fungi.

**Introduction**

Coniferous forests ecosystems in the boreal and temperate zones of the Northern Hemisphere represent significant global carbon pools and sinks (Scharlemann *et al.,* 2014). Consequently, understanding the processes of the carbon (C) cycle in these ecosystems is essential for modelling potential impacts of global climate change. Forest ecosystems are under strong influence of trees that mediate the influx of C into the ecosystem pool by producing both complex organic compounds in the form of leaf and root litter and dead wood, as well labile ones in the form of root exudates (Prescott and Grayston 2013). Trees thus directly affect and contribute to soil respiration and carbon deposition, both important microbial-mediated processes (Clemmensen *et al.,* 2013; Högberg *et al.,* 2010).

In the coniferous forest floor, litter and soil represent largely different compartments when considering C cycling processes. The litter layer is mostly derived from plant biopolymers and tree roots are scarce, resulting in the main C cycle process being the efficient decomposition of recalcitrant plant residues. In contrast, soil is a mixture of highly recalcitrant, humic materials and has a low abundance of plant biopolymers. The presence of roots makes simple, photosynthesis derived C compounds the most important C source that enters soil both directly and through root-associated ectomycorrhizal fungi (ECM) (Baldrian *et al.,* 2012; Clemmensen *et al.,* 2013). Fungi are abundant in both compartments, but litter is richer in saprotrophic taxa while Ectomycorrhizal (ECM) fungi largely dominate soil (Baldrian *et al.,* 2012; Lindahl *et al.,* 2007). The relative abundance of bacteria increases with depth, but the composition of their communities in litter and soil is similar.

Despite the differences among litter and soil, the C sources in both compartments are the same, though with differing relative proportions. With the exception of photosynthesis-derived C compounds, all other C sources are biopolymers composing dead plant debris (cellulose, hemicelluloses, lignin) and living and dead bacterial and fungal biomass (peptidoglycan, chitin, and other polysaccharides). The turnover of these compounds is essential for microbial activity (resulting in CO2 respiration) and biomass growth and remodeling. All of these processes can be tracked by the analysis of enzymes that mediate them – the carbohydrate active enzymes (CAZymes). Specifically, glycoside hydrolases (GH) and selected auxiliary carbohydrate-active enzymes (AA) are associated with the decomposition of polysaccharides and lignin (Lombard *et al.,* 2014). The classification of GH and AA proteins or genes into families that contain structurally similar proteins, makes it possible, to some extent, to assign catalytic functions to sequences (Lombard *et al.,* 2014). CAZymes have previously been studied in soil transcriptomes, though only by approaches targeting individual enzymes (Baldrian *et al.,* 2012; Kellner and Vandenbol 2010) or exclusively eukaryotic transcripts at low resolution (Damon *et al.,* 2012). Proteomic studies of these enzymes have also been performed in beech litter (Schneider *et al.,* 2012) but suffered from the limited resolution of soil protein characterization.

Much of our present appreciation of the C cycle in forest soils is originates from culture-based studies and genomics. The traditional view is that saprotrophic cord-forming basidiomycota are best suited and thus most involved in the decomposition of biopolymers (de Boer *et al.,* 2005), and however, it is increasingly apparent that several other groups of organisms are involved as well. For example, saprotrophic ascomycetous “microfungi” possess a wide array of carbohydrate-active enzymes even though they lack ligninolytic peroxidases (Eichlerová *et al.,* 2015). These organisms may be interacting with root-symbiotic ECM fungi that aid in decomposition (Lindahl and Tunlid 2015), with some taxa even producing ligninolytic peroxidases (Bödeker *et al.,* 2014). In general, ECM genomes are less rich in carbohydrate-active enzymes, especially GHs and AAs (Kohler *et al.,* 2015). It is possible that genes-associated with N acquisition rather than C utilization may be related to decomposition activity. It has previously been observed that ECM decomposition is often dependent on “priming,” or the availability of simple C compounds (Lindahl and Tunlid 2015). Additionally, bacteria may interact with decomposition communities as GHs, including cellulases and hemicellulases, are identified as widespread in bacterial genomes (Berlemont and Martiny 2015) along with genes associated with cellulose decomposition being a common trait in soil and litter bacteria (López-Mondéjar *et al.,* 2016). While previous studies of litter proteoms have indicated the dominance of fungal decomposition enzymes over bacterial ones (Schneider *et al.,* 2012), the results of stable isotope probing experiments indicate that fungi and bacteria are both involved significantly in cellulose and hemicelluloses utilization in forest soils (Eichorst and Kuske 2012; Leung *et al.,* 2016; Rime *et al.,* 2016; Štursová *et al.,* 2012). In the utilization of dead fungal biomass, a quantitatively highly important pool of C in forest topsoil, bacteria were even indicated to dominate over fungi (Brabcová *et al.,* 2016). The relative importance of fungi, bacteria, and other groups of organisms in the soil C cycle processes, however, remains largely undisclosed.

drivers ofa. C cycling in soil is expected to largely respond to seasonal climatic conditions in temperate and boreal zones. The high photosynthetic activity of trees during the summer period (with favourable temperature and light conditions) is minimal in winter, when it is reduced by light limitations and temperatures below the freezing point. Consequently, carbon allocation belowground varies dramatically and directly impacts soil biota (Högberg *et al.,* 2010; Kaiser *et al.,* 2010). In our previous study, we have demonstrated that the presence of microbial communities in the coniferous *Picea abies* forest topsoil are similar among seasons, but their activities differ dramatically (Žifčáková *et al.,* 2016). The pool of transcripts differs among seasons, especially in the soil where fungal transcripts were observed to significantly decrease (by 50%) in winter, with ECM-associated activity being particularly reduced (Žifčáková *et al.,* 2016). The consequences of seasonality on the C cycle are not known, but it can be hypothesized that the reduced input of photosynthate C in winter can be replaced by more intensive utilization of recalcitrant C compounds, especially polysaccharides, in winter by saprotrophic bacteria and fungi. These organisms should also benefit from the reduced competition with the starving ECM fungi. The reduction of saprotrophic activity by ECM, called the “Gadgill effect” was frequently addressed but its existence and extent are still under debate (Fernandez and Kennedy 2016).

In this study, we leverage the power of metagenomics to characterize the community composition and the functional potential of individual taxa combined with metatranscriptomic datasets to predict C-cycling GH- and AA-associated activities with sufficient reliability and resolution using a comprehensive database of available fungal genomes. We hypothesize that the absence of simple C compounds in winter results in the increase of importance of other C sources, namely plant polysaccharides (cellulose, pectin and hemicellulose) and fungal biomass from decomposing ECM mycelia. The reduction of fungal activity in winter in general and of ESM in particular that was observed previously (Žifčáková *et al.,* 2016) is expected to increase the share of bacteria and saprotrophic fungi in decomposition. Here, we identify which sources of C are used, who is associated with their metabolism, how C cycling varies with changing labile C abundance between summer and winter.

**Materials and Methods**

**Sampling area and sample collection**

The study sites were located in the highest altitudes of the Bohemian Forest National Park, Czech Republic (49° 2′ 38″ N, 13° 37′ 2″ E), 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. This study used the samples of DNA and RNA collected in July 2012 and March 2013 previously described in the study of (Žifčáková *et al.,* 2016). Briefly, samples were taken from the litter layer (L) and the organic horizon of soil (S). Material from each of six study sites were pooled separately for each site and horizon. 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). Samples were immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

**Extraction and analysis of environmental RNA and DNA**

RNA and DNA extraction and the metatranscriptome sequencing and assembly were described previously by (Žifčáková *et al.,* 2016). Briefly, RNA was extracted using the RNA PowerSoil Total RNA Isolation Kit (MoBio Laboratories) combined with the OneStep PCR Inhibitor Removal Kit (ZymoResearch) from three 1-g aliquots per sample, pooled, and RNA purified using the RNA Clean & Concentrator Kit (ZymoResearch) on a column treated with DNase I (Fermentas). Approximately 1 μg of RNA was treated with an equimolar mixture of RiboZero rRNA Removal Kits Human-Mouse-Rat and Bacteria (Epicentre) and a total of 50 ng of treated RNA served as the input for the ScriptSeq v2 RNA-Seq Library Preparation Kit (Epicentre) that was used for library construction. 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). DNA samples were fragmented to reach the mean fragment lengths around 400 bp and libraries were prepared using TruSeq PCR Free Kit (Illumina). Metatranscriptome and metagenome libraries were sequenced on an Illumina HiSeq2000 to generate 150-base paired-end reads.

Metagenome reads were processed in the same way as originally described for the metatranscriptome (Žifčáková *et al.,* 2016). The 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 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).

Assembly of metagenomic reads was performed in the same way as described for the metatranscriptome and sequence data of all contig sequences have been deposited in the MG RAST public database (Meyer *et al.,* 2008) under the dataset number 4627252.3; metatranscriptome contigs are available as 4544233.3. Metagenome sequencing yielded 567 x 106 reads (24 x 106 ± 2 x 106 reads per sample) that were assembled into 9,380,309 contigs over 200 bases, including 1,882,204 contigs over 500 bases, 569,720 over 1000 bases and 6,665 over 10,000 bases (mean length was 454 bases). The longest contig had a length of 179,090 bases. Protein prediction yielded a total of 9,178,489 predicted coding regions, of which 4,355,554 (47.5%) have been assigned an annotation by MG RAST.

**Annotation of the metagenome and metatranscriptome**

Contig annotation was first 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) and taxonomic information was retrieved for each identified contig. Because MG RAST is not suitable for annotation of fungal proteins, predicted proteins were subsequently annotated by finding the best protein match in an in-house database containing protein predictions from all publicly available fungal genomes available at the time of analysis (155 genomes). For all hits that received closer hit to the fungal predicted protein database (FPPD) then using MG RAST, taxonomic information was retrieved from the FPPD.

Glycoside hydrolases (GH) and auxiliary enzymes (AA) were identified among the metagenome and metatranscriptome contigs using the CAZy pipeline (Lombard *et al.,* 2014), which combines Blast and HMM tools with the manual curation of CAZy database (http://www.CAZy.org). Protein models are compared with the sequence and profile libraries created from catalytic and non-catalytic modules of the CAZy database. Based on the major reported activities of GH and AA families according to the CAZy database, families were grouped according to function (Table 1).

To assess the abundance in the metagenome and the relative rate of expression in the metatranscriptome, individual sequence reads from each sample were mapped onto contigs identified as GH or AA using bowtie 2.2.1 (Langmead *et al.,* 2009) with the default settings of: end to end alignment –sensitive. To calculate gene or transcript abundance, 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.

**Statistical analysis**

R (R Core Team 2014) and PAST 3.03 (http://folk.uio.no/ohammer/past/) were used for statistical analysis. Differences in relative abundances of gene or transcript abundances were tested using the Mann-Whitney U test, which assumes the measurements on a rank-order scale but does not assume normality of data. One-way or two-way PERMANOVA on Bray-Curtis distances with 99999 permutations was used to analyze differences among communities or transcript pools. Two-dimensional non-metric multidimensional scaling (NMDS) ordination analysis on Bray-Curtis distances was performed in R with package vegan (Oksanen *et al.,* 2016; R Core Team 2014). In all cases, differences at P < 0.05 were considered to be statistically significant.

**Results**

**Gene pool of auxiliary enzymes and glycoside hydrolases**

Among genes predicted in soil metagenomes, 5.5 % of genes were annotated as Carbohydrate Active Enzymes - CAZymes. In total, 91,195 glycoside hydrolases from 108 families and 7,709 auxiliary enzymes from 11 families were identified among the protein predictions of the *P. abies* topsoil metagenome. GH13 (amylase) was the most diverse family with 16,412 contigs followed by GH3 (-glucosidase) and GH15 (trehalase), another 29 GH families and the family AA3 were identified in >1,000 contigs (Supplementary Table 1). Among contigs, 67 % of GH and 46% of contigs AA were of assigned to bacteria and 27% and 24% to fungi.

Considering the frequency of occurrence, 66.2% of glycoside hydrolase reads were assigned to bacteria (Proteobacteria 20.0%, Acidobacteria 20.9%, Actinobacteria 13.4%, Bacteroidetes 4.9%), 24.9% to fungi (Ascomycota 14.1%, Basidiomycota 6.1%). Of auxilliary enzyme reads 61.8% were assigned to bacteria (Proteobacteria 43.2%, Acidobacteria 6.3%, Actinobacteria 7.6%), 37.5% to fungi (Ascomycota 26.1%, Basidiomycota 9.1%). Reads assigned to other organisms were rare (Fig. 1). Most reads mapped to GH13 (-glucosidase), GH3 (-glucosidase), GH23 (chitinase), GH15 (trehalase), AA3 (ligninolytic oxidase), GH2 (-glucosidase), GH18 (chitinase), GH5 (cellulase), and GH1 (-glucosidase) in total representing approximately one half of all reads (Fig. 2). The gene pool differed significantly between litter and soil, but not between seasons (Fig. 3). Among X individual gene families, abundance was significantly different among horizons for 72 gene families, among season for 9 gene families in litter and 2 gene families in soils indicating that the community composition is horizon-specific but similar in both seasons (Supplementary Table 1).

**Transcription of auxilliary enzymes and glycoside hydrolases**

Of 4.5 millions contigs of the *P. abies* topsoil metatranscriptome, 42,872 (0.83%) were identified as glycoside hydrolases from 105 families and 5,111 (0.11%) as auxiliary enzymes from 12 families. GH13 (amylase) was the most diverse family with 4,574 contigs followed by GH5 (2,011 contigs, cellulase), GH3 (1,707 contigs, -glucosidase) and AA3 (1,683 contigs, ligninolytic oxidase), and additional six GH families and the family AA1 were identified in >1,000 contigs (Supplementary Table 1). Glycoside hydrolases and auxiliary enzymes were more frequently of fungal origin (43% GH and 71% AA) then of bacterial origin (42% and 22%).

The transcription of glycoside hydrolases represented between 0.26% and 0.34% of the total transcription in both litter and soil. Auxiliary enzymes were more transcribed in litter (0.07-0.08% of total transcription) than in soil (0.03-0.04%; Fig. 1). Most GH and AA transcripts were produced by fungi that accounted for 51.6% of GH reads (27.6% Basidiomycota, 19.3% Ascomycota) and as much as 81.5% of AA reads (44.7% Ascomycota, 35.1% Basidiomycota). Bacteria were responsible for 34.7% of GH transcription (Acidobacteria 13.7%, Proteobacteria 7.6%) and 13.1% of AA transcription. GH were also frequently transcribed by animals (3.7%) and plants (2.6%) and AA by plants (3.3%; Fig. 1).

Most metatranscriptome reads mapped to the -glucosidases of the GH13 family, to the cellulolytic enzymes in GH5, GH7, and AA9, ligninolytic oxidases AA3, to the endoglucanase/endogalactanase GH16 and to the chitinase GH18. Although the most transcribed GH family was also the one most abundant in the metagenome, the gene and transcript CAZyme gene profiles were not statistically similar? (Fig. 2).

It was apparent that different groups of organisms transcribe different enzyme sets (Fig. 3). In all taxa, -glucosidases and cellulases belonged to the most transcribed enzymes, however, for dikaryotic fungi, cellulases and ligninolytic enzymes were the most transcribed groups. -Glucosidases and chitinases were also often among the most produced enzymes while xylanases were missing in archaea and rare in Proteobacteria (Supplementary Table 2). The contribution of individual groups to the production of enzymes was highly variable with a high contribution of fungi to the production of the dominant plant cell wall-degrading enzymes – cellulases, ligninases (up to 90% of fungal transcripts in both groups), and xylanases (up to 70%); all other enzyme groups are produced by a wide range of taxa (Fig. 2, 4).

The transcript pool differed significantly between litter and soil: 86 gene families showed significantly different expression rate between horizons (Supplementary Table 1). This partly reflects the differences in the metagenome and thus of the community composition but also the gene pools produced by different groups of microorganisms differed significantly among horizons (Fig. 3). Among the functional groups of enzymes, enzymes targeting cellulose were the most transcribed followed by those acting on lignin, fungal cell wall, and starch and glycogen indicating the importance of plant and fungal biomass as nutrient sources. Soil samples showed higher production of enzymes targeting reserve compounds (starch/glycogen and trehalose) and peptidoglycan while the enzymes degading cellulose and lignin and pectin were more transcribed in litter (Fig. 4).

**Seasonality of transcription**

The transcript pool differed significantly between summer and winter in both horizons but was more pronounced in soil, where it affected 60 gene families compared to 18 gene families in litter (Fig. 3, Supplementary Table 1). At the level of functional groups of enzymes, differences in their metagenome content among seasons was negligible as well as the variation in the taxonomic groups that produced them (Supplementary Figure 1). Most functional groups of enzymes showed seasonal differences in transcription and the shift in the contribution of taxa to their production (Fig. 4), especially in soil. Winter samples were associated with an increase in the use of reserve compounds (glycogen/starch and trehalose) while the share of enzymes targeting recalcitrant plant biomass (cellulose, and lignin) decreased. Significantly higher production of enzymes targeting fungal and bacterial cell wall components such as chitin, peptidoglycan, and selected glucans was observed in summer compared to winter indicating higher turnover and growth rates in the warm season (Fig. 4). For all enzyme groups, the share of enzymes produced by fungi in soil decreased in winter while the contribution of bacteria increased. For example, fungi produced 62% of cellulases in soil in summer but only 29% in winter (Fig. 4).

Of 2,836 CAZyme-associated transcripts that appeared in at least five litter samples, 219 (7.7%) were significantly increased in summer and 103 (3.6%) in winter. In soil, of 2,119 transcripts, 287 (13.5%) were increased in summer and 215 (10.1%) in winter confirming the more pronounced seasonality in soil. It was especially the enzymes targeting cellulose, lignin and microbial cell walls that were significantly more transcribed in soil in summer while enzymes targeting starch, glycogen and trehalose were often more frequently transcribed in winter (Fig. 5).

**Discussion**

**Gene pool of carbohydrate-active enzymes and its expression**

The size and composition of the gene pool encoded in the environmental metagenome is often regarded to indicate the functional properties of a specific ecosystem or habitat (Fierer *et al.,* 2012) despite the fact that the links between genome content and expression were found to be weak in individual bacteria and fungi (Eichlerová *et al.,* 2015; López-Mondéjar *et al.,* 2016). This study clearly demonstrates that there is no clear relationship between gene abundance in the metagenome and its expression. One of the reasons is the low representation of eukaryotic genes that is generally reported from metagenomic studies (Pold *et al.,* 2016; Fierer *et al.,* 2012). This is caused by the high abundance of noncoding DNA in eukaryotic sequences, the occurrence of introns as well as by the fact that fungal and other eukaryotic genomes are so far underrepresented in genomic databases (Kollmar *et al.,* 2015). Although our metagenome and metatranscriptome datasets are not exhaustive, they confirm that the share of bacterial reads in the metagenome is higher than in the metatranscriptome, while the opposite is true for fungal genes and transcripts. However, correlations between gene and transcript abundances were also weak if exclusively bacterial genes were considered (Fig. 2), which indicates that the genomic potential is a poor predictor of expression.

Both the metagenome and metatranscriptome differed significantly in their composition between litter and soil (Fig. 3). This is consistent with the fact that the composition of bacterial and fungal communities differs among these horizons in the studied ecosystem (Baldrian *et al.,* 2012) as well as elsewhere (Lindahl and Tunlid 2015; López-Mondéjar *et al.,* 2015), reflecting the properties of these habitats (Baldrian 2017). The differences were more pronounced in the metatranscriptome. Litter was enriched in transcripts associated with cellulases and ligninases (Fig. 4), indicating the importance of decomposition of recalcitrant plant biopolymers. Soil showed an increased share of enzymes acting of α-glucosidases and especially trehalases, the storage compounds of ECM fungi (Nehls *et al.,* 2010) that represents a major part of fungal community in soil (Baldrian *et al.,* 2012). The share of AA and GH reads in the total metagenome (5%) was approximately double compared to 1.6-2.1% in the metagenome of a mixed temperate forest (Pold *et al.,* 2016) and the share of their transcription (0.36%) was comparable to the share of CAZymes in the metatranscriptome of a maple forest (Hesse *et al.,* 2015), indicating comparable importance.

**Involvement of soil organisms in C transformation**

The vast majority of glycoside hydrolases and auxiliary enzymes (83-92% and 93-95%, respectively) were produced by microorganisms, i.e. fungi, bacteria and archaea, which was slightly more than their share on the overall transcription (83%; (Žifčáková *et al.,* 2016)). Of these, the share of archaea was negligible. Fungi accounted for the higher share of AA production then bacteria and this was also the case of GH production in litter; in soil, GH were produced equally by fungi and bacteria in summer, but bacteria largely dominated GH production in soil in winter (Fig. 1). The share of fungi is substantially higher than in metatranscriptomic studies from a maple forest or in peatlands, where bacterial CAZy transcripts were 2.6 to 5-fold more abundant then eukaryotic ones (Hesse *et al.,* 2015; Ivanova *et al.,* 2016). This indicates high importance of fungi in coniferous forests, although the comparison may be biased by the fact that previous studies annotated short reads and used a limited reference database for fungi so that relevant transcripts might have been overlooked. The observed dominance of fungal-associated CAZymes is consistent with previous results obtained by proteomic analysis of decomposing beech litter, where fungal transcripts also dominated the enzyme pool (Schneider *et al.,* 2012). Compared to this study, we have observed a much higher share of transcripts from Basidiomycota which is also consistent with their higher abundance in the coniferous forest soil (Žifčáková *et al.,* 2016). The share of bacterial transcription of GH and AA, however, was substantial, especially in soil, and bacteria produce a significant share of enzymes that allow them to access C in cellulose or hemicelluloses (Eichorst and Kuske 2012; López-Mondéjar *et al.,* 2016; Štursová *et al.,* 2012). Acidobacteria, Proteobacteria, Bacteroidetes and Actinobacteria were the most important bacterial producers of CAZymes (Fig. 1). These groups are known to be abundant in acidic forest topsoils (Žifčáková *et al.,* 2016) and have also been found to be dominant in CAZy production in another acidic environment – the boreal peatland (Ivanova *et al.,* 2016). The culturing and analysis of Acidobacteria, Proteobacteria, Bacteroidetes from coniferous forests has also previously confirmed the production of a wide range of extracellular enzymes by the individual members of these phyla, especially the Acidobacteria and Bacteroidetes (Lladó *et al.,* 2016).

Litter and soil transcription is associated with contrasting groups of organisms, reflecting carbon availability in these systems (Fig. 3). Although bacteria were associated with all GH and AA transcripts, the majority of these enzymes are associated with labile substrates such as starch, cellobiose or other oligosaccharides (Fig. 4). Importantly, bacteria were also important producers of chitinolytic enzymes, confirming their important role in the turnover of dead fungal mycelia (Brabcová *et al.,* 2016). The decomposition of lignin, cellulose, and xylan was, on the other hand, dominated by fungi that appear to be better adapted to decompose recalcitrant plant-derived biomass components (van der Wal *et al.,* 2013). GH13 genes, putative α-glucosidases/α-amylases, were the most abundant present (in metagenomes) and transcribed gene of bacteria (in metatranscriptomes). These genes, commonly present in bacterial genomes (Berlemont and Martiny 2015), were also found abundant in another forest soils (Pold *et al.,* 2016) and to be highly transcribed in peatlands and lignocellulose-degrading consortia in bioreactors (Ivanova *et al.,* 2016; Jimenez *et al.,* 2015). Additional enzymes that were associated with plant biomass decomposition for fungi were identified in dominant transcripts of both Ascomycota and Basidiomyota related to cellulolytic enzymes, namely the GH7 cellulases and the AA9 lytic polysaccharide monooxygenases (Suppl. Tab. 2).

**Seasonality of carbon utilization**

Seasonality of community composition was observed in the topsoil of temperate deciduous forests for both bacteria and fungi (López-Mondéjar *et al.,* 2015; Voříšková *et al.,* 2014). The seasonal changes occurred in soil and especially in litter that is produced each autumn and rapidly degraded, resulting in litter-decomposing bacteria and fungi undergo successional changes (Tláskal *et al.,* 2016; Voříšková and Baldrian 2013). The seasonality of coniferous topsoil is different because the litterfall period is not distinct and litter decomposes much more slowly (Haňáčková *et al.,* 2015) so that communities in litter remain similar in summer and winter, with similar conditions in soil (Žifčáková *et al.,* 2016). In this study, we observed this result as summer and winter metagenomes were not significantly different (Fig. 3). However, while community composition remains stable, the ratio of fungi to bacteria and fungal biomass are significantly higher in summer than in winter (Žifčáková *et al.,* 2016). Our findings also support a higher ), as the the expression of chitin and lysozyme are higher in summer then in winter.

Consistent with the assumption that the nutrient sources in litter (mainly plant biomass) remain the same across the year while soil experiences the seasonal input of photosynthates, the seasonality of transcription is substantially higher in soil, which applies both for total transcription (Žifčáková *et al.,* 2016) and expression of CAZymes. The production of GH and AA by Ascomycota and Basidiomycota increased in winter is consistent with the decrease of expression of genes related to ECM symbiosis (Žifčáková *et al.,* 2016), indicating lower activity of the ECM fungi, mainly Basidiomycetes. Alongside, the relative contribution to CAZyme expression increased for bacteria and Ascomycota. This observation may either indicate the relief of inhibition of nonmycorrhizal microorganisms due to the Gadgill effect (Fernandez and Kennedy 2016) or simply the decrease of activity of ECM fungi. The observation of the increase in activity of enzymes associated with mobilizing storage compounds – starch, glycogen and trehalose in winter soil indicates that this is likely a time of nutrient limitation and biomass may need to be maintained at the costs of metabolic reserves. The use of trehalose and mannitol as energy reserves by ECM during winter starvation has been previously observed (Druebert *et al.,* 2009; Nehls 2008).

Contrary to expectations, the microbial communities in soil did not switch from the utilization of simple C compounds in summer to complex carbohydrates in winter. Instead, the expression of enzymes degrading lignin, cellulose and xylan was increased in the summer. Although it was shown that summer microbial community is more adapted to utilization of labile C sources such as root exudates (Dennis *et al.,* 2010; Jones *et al.,* 2009), experiments indicate that labile substrates may prime decomposition of recalcitrant SOM (Kuzyakov 2010). Simple C has been demonstrated to fuel the decomposition of organic matter by ECM fungi in their search for organic N (Lindahl and Tunlid 2015) and is consistent with the hypothesis that there is a priming effect of rhizodeposited C on OM decomposition (Talbot *et al.,* 2008). Our results further support that C priming may be essential for the decomposition of complex biopolymers by both Basidiomycete and Ascomycete fungi. The existence of this C priming effect is further supported by the results of (Štursová *et al.,* 2014), who showed substantial decrease of decomposition following the termination of rhizodeposition as a result of large-scale defoliation of trees by bark beetles.

**Conclusions**

Organisms in coniferous litter and soil possess a diverse set of enzymes that participate in decomposition of complex C compounds. Microorganisms are the most important producers of these enzymes, especially glycoside hydrolases and auxiliary enzymes, with fungi strongly dominating transcription in litter and equal contributions of bacteria and fungi in soil. In contrast to deciduous forest soils, microbial community composition remains stable across the year but gene transcription shows seasonality. Our results indicate that microbial biomass turnover is faster in summer than in winter, and seasonality of gene transcription is especially high in soil. While the use of reserve compounds such as starch or trehalose is high in winter, summer is characterized by high expression of ligninolytic, cellulolytic, and xylanolytic enzymes. This supports the hypothesis that priming by photosynthesis-derived C is necessary to support fungal decomposition of recalcitrant biopolymers. This paper also shows that metagenomics is not suitable for the analysis of ecosystem functioning since there is no correlation between gene and transcript abundance.

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**Figure legends**

Figure 1.: Contribution of microbial and other taxa to the transcription and gene pool of auxiliary enzymes (A) and glycoside hydrolases (B) in the *Picea abies* topsoil and the share of AA and GH reads in the total metatranscriptome (C). Abbreviations: LS – litter summer, LW – litter winter, SS – soil summer, SW – soil winter

Figure 2.: Share of the read counts of glycoside hydrolases and auxilliary enzymes on total transcription and total metagenome in the *Picea abies* topsoil. Read abundances are in parts per million. Colors indicate taxonomic affiliation of transcripts and genes, functional groups of enzymes are colours-coded.

Figure 3.: Nonmetric multidimensional scaling of the (A) transcript counts and (B) gene pools of glycoside hydrolases and auxiliary enzymes in the *Picea abies* topsoil by horizons and seasons and the composition of the pool of GH and AA transcribed by topsoil organisms in litter and soil (C). Abbreviations: LS – litter summer, LW – litter winter, SS – soil summer, SW – soil winter.

Figure 4.: Transcript abundances of GH and AA by functional groups in the litter and soil of the *Picea abies* forest by seasons. Numbers indicate the share of reads in the total metatranscriptome in ppm. Significant differences in read abundances among seasons are indicated by different letters.

Figure 5.: Transcripts of auxiliary enzymes and glycoside hydrolases from the *Picea abies* forest topsoil with significant difference of transcription among seasons.

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