Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers

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Abstract

Organic matter decomposition in the globally widespread coniferous forests has an important role in the carbon cycle, and cellulose decomposition is especially important in this respect because cellulose is the most abundant polysaccharide in plant litter. Cellulose decomposition was 10 times faster in the fungi-dominated litter of Picea abies forest than in the bacteria-dominated soil. In the soil, the added 13C-labelled cellulose was the main source of microbial respiration and was preferentially accumulated in the fungal biomass and cellulose induced fungal proliferation. In contrast, in the litter, bacterial biomass showed higher labelling after 13C-cellulose addition and bacterial biomass increased. While 80% of the total community was represented by 104–106 bacterial and 33–59 fungal operational taxonomic units (OTUs), 80% of the cellulolytic communities of bacteria and fungi were only composed of 8–18 highly abundant OTUs. Both the total and 13C-labelled communities differed substantially between the litter and soil. Cellulolytic bacteria in the acidic topsoil included Betaproteobacteria, Bacteroidetes and Acidobacteria, whereas these typically found in neutral soils were absent. Most fungal cellulose decomposers belonged to Ascomycota; cellulolytic Basidiomycota were mainly represented by the yeasts Trichosporon and Cryptococcus. Several bacteria and fungi demonstrated here to derive their carbon from cellulose were previously not recognized as cellulolytic.

Introduction

Soils contain one of the largest pools of organic carbon compounds on the Earth, and soil processes thus play a major part in the global C cycle. This is especially true for the forest soils that cover large areas of the northern hemisphere, where dead plant biomass is not removed during harvests but accumulates on the soil surface. The understanding of organic matter decomposition in forest ecosystems is thus essential for any predictions of carbon balance now and in the future. Dead plant biomass accumulating on the forest floor is mainly composed of plant cell wall polymers – cellulose, hemicelluloses and lignin. Cellulose is the most abundant biopolymer and typically constitutes 20–30% of the plant litter mass (Berg & Laskowski, 2006).

Decomposition of cellulose was the subject of intensive research for decades, and our current understanding shows that soil microorganisms have a dominant role in this process (Lynd et al., 2002; Baldrian & Valašková, 2008). Previous studies using cultured microbial isolates and enzyme activity measurements formed our current view of the involvement of individual microbial taxa in cellulose decomposition. It is currently assumed that while cellulolytic capabilities are restricted to certain groups of bacteria (Lynd et al., 2002), these capabilities are common to various fungi and that the latter dominate cellulose decomposition in soils (Kjoller & Struwe, 2002; de Boer et al., 2005).

Although the above assumptions might be essentially true, the current knowledge has to be revisited for several reasons. First of all, the current views are largely based on data obtained with easily culturable species that have been preferentially studied. This is reflected by the fact that over 80% of fungal cellulolytic enzymes characterized were isolated from wood-inhabiting species (Baldrian &
Valášková, 2008), although the genes encoding these enzymes are also common in soil fungi (Edwards et al., 2008; Kellner et al., 2010). Similarly, some recent reports have demonstrated the ability to degrade cellulose in bacterial taxa that were not previously recognized for their cellulytic capabilities (Ward et al., 2009; Pankratov et al., 2011).

Novel molecular approaches, such as stable isotope probing (SIP) and next-generation sequencing, make it possible to analyse substrate utilization by microorganisms at sufficient resolution. The first published studies on cellulose decomposition using 13C-SIP focused on bacteria in agricultural soils (Haichar et al., 2007; Schellenberger et al., 2010; Lee et al., 2011) and fungi in hardwood forests (Bastias et al., 2009). None of these studies, however, covered both fungi and bacteria, and none used sufficient sequencing effort. Although more than 85% of cellulose is decomposed in the forest litter layer, only the rest being available to soil microorganisms (Snajdr et al., 2011b), microbial cellulolytic community in litter has not yet been the subject of targeted analysis. Compositions of bacterial and especially fungal communities have been demonstrated to differ significantly between litter and deeper soil and also the relative abundances of fungi and bacteria change with soil depth (Lindahl et al., 2007; Baldrian et al., 2012), but it is unclear how these differences are reflected in the relative contribution of fungi and bacteria, and their individual taxa, to the decomposition of organic matter including cellulose.

The aim of this work was to characterize the composition of the microbial community that is actively decomposing cellulose in topsoil – litter and soil organic horizon – of Picea abies forest by examining both bacteria and fungi. In addition, the genes encoding fungal cellobiohydrolase (exocellulase) gene cbhI sequences were also analysed. Its gene product catalyses the rate-limiting step in the decomposition of cellulose (Baldrian & Valášková, 2008), and its activity in fungal cultures correlates with litter mass loss (Volfíšková et al., 2011). The fact that cbhI gene is found in Ascomycota, Basidiomycota and Mucoromycotina (Edwards et al., 2008; Weber et al., 2011) makes it a suitable tracer of fungi involved in enzymatic cellulose hydrolysis. The relatively large nutrient patches present in litter of the size up to the whole needles/leaves can be better exploited by fungi as larger, filamentous organisms that can more easily colonize it (de Boer et al., 2005). In soil, the low nutrient content might be insufficient to support large mycelia of saprotrophic fungi and the relative content of bacteria, capable of utilizing the dilute nutrients, is higher (Baldrian et al., 2012). It is thus likely that the relative importance of fungi and bacteria in the decomposition of biopolymers, including cellulose, might differ between litter and soil.

Materials and methods

Study site and sample collection

The study area was located in the highest altitudes (1200 m) of the Bohemian Forest mountain range (Central Europe; 48°59.01 N, 13°35.05 E). The area is nearly completely covered with P. abies forest, the mean annual temperature is 5.5 °C and the annual precipitation 1000 mm (Kopáček et al., 2002). Topsoil was collected in February 2009 at four 100 m² sites, each located within 250 m from each other. Litter samples (L) and soil organic horizon (S) material were separately pooled. After the removal of roots, the litter was cut into 0.5 cm pieces, the S material was passed through a 5-mm sterile mesh and both were kept at 4 °C until the construction of microcosms. Dry mass, organic matter and cellulose content and pH were determined as previously described (Snajdr et al., 2011b; Baldrian et al., 2012).

SIP microcosms

Microcosms were established to permit destructive harvesting in three replicate microcosms for each combination of material (litter or soil) and time point (0, 8, 15 and 22 days). The soil and litter were preincubated for 36 h at the target incubation temperature prior to microcosm set-up. Each microcosm contained 5.0 g wet mass of either the L or the S material in a sterile 160-mL serum bottle, and 100 mg of 13C-labelled Zea mays cellulose (97 atom% 13C; IsoLife, Wageningen, the Netherlands) was mixed with the whole volume of each sample. Microcosms were sealed with Teflon stoppers and aluminium crimp caps to contain the headspace for later 13CO₂ sampling. Microcosms were incubated in the dark at 11 °C, the mean summer temperature in the study area and destructively harvested after 0, 8, 15 and 22 days. Microcosm materials were frozen immediately at −80 °C for DNA extraction.

Concentration and carbon isotopic composition of CO₂

One millilitre of the headspace gas was collected at microcosm initiation and after 8, 15 and 22 days of incubation using an N₂-purged syringe and was stored at room temperature in 12-mL serum bottles. The carbon dioxide in the stored bottles was analysed for δ13C within 7 days on a Trace Gas system interfaced to an IsoPrime mass spectrometer (GV Instruments, Manchester, UK) at the Alaska Stable Isotope Facility (University of Alaska Fairbanks, Fairbanks, AK) as previously described (Leigh et al., 2007).

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DNA extraction and $^{13}$C-DNA separation

DNA was extracted from 0.5 g aliquots of microcosm material using the Fast DNA Spin Kit for Soil (MP Biomedicals, Solon, OH). DNA yield and purity were checked on ND1000 (NanoDrop, Wilmington, DE). Labelled DNA was separated by isopycnic centrifugation using a caesium trifluoroacetate solution (CsTFA; GE Healthcare, Piscataway, NJ) with a starting buoyant density (BD) of 1.60 g mL$^{-1}$ as previously described (Leigh et al., 2007). The CsTFA solution was combined with 3 μg DNA and subjected to ultracentrifugation for 48 h at 141 400 g using 5.1-mL tubes in an NVT 100 rotor and L-100XP Optima Ultracentrifuge (Beckman Coulter, Brea, CA). Each centrifugation run included duplicates of a blank control (no DNA) for BD determination, two duplicates of microcosm samples and corresponding time zero samples. Gradients were fractionated into 250 μL fractions, and the BD of each fraction was determined gravimetrically using fractions from the blank gradients. The labelled $^{13}$C-DNA and unlabelled $^{12}$C-DNA were separated and formed two distinct peaks as determined by normalized quantitative PCR (qPCR) data plots with $^{13}$C-DNA banding at BD around 1.60 g mL$^{-1}$ and unlabelled $^{12}$C-DNA at around 1.57 g mL$^{-1}$. Sample DNA was precipitated from fractions with 1 mL of 2-propanol overnight at $-20^\circ$C and then centrifuged at 10 000 g. The pellets were cleaned twice with 0.5 mL 2-propanol, vacuum dried and re-suspended in EB elution buffer (Qiagen, Valencia, CA).

The distribution of $^{13}$C-DNA in the fractions was determined with qPCR using 1108f and 1132r universal primers for bacteria (Wilmotte et al., 1993; Amann et al., 1995) and ITS1/qITS2$^*$ universal primers for fungi (White et al., 1990; Sˇnajdr et al., 2011a). For each fraction, qPCR was performed in duplicate reactions as previously described (Baldrian et al., 2012). The labelled and unlabelled fractions were determined by plotting the normalized DNA concentration vs. the fraction number. Fractions representing the $^{13}$C-DNA and the $^{12}$C-DNA were pooled together to form representative samples for each microcosm. qPCR was also used to determine the content of fungal and bacterial rDNA sequences in DNA and for the calculation of fungal/bacterial rDNA copy ratio (F/B DNA ratio).

Community fingerprinting

Terminal restriction fragment length polymorphism (T-RFLP) analyses were performed on all $^{12}$C-DNA and $^{13}$C-DNA samples to examine the changes in community composition with time. For bacterial DNA analysis, samples were amplified using primers 27F and 1392R targeting eubacterial 16S rRNA genes (Johnson, 1994). For fungal DNA analysis, primers ITS1f and ITS4 targeting fungal ITS regions were used (White et al., 1990; Gardes & Bruns, 1993). Primers 27F and ITS1f were fluorescently labelled with 5-hexachlorofluorescein (HEX) on the 5′ end. All PCRs were prepared with PPP mix (Top-Bio, Prague, Czech Republic) at 12.5 μL per reaction and with 0.5 μL of forward and reverse primer (10 μM) and 2–50 ng DNA template in a total reaction volume of 25 μL as previously described (Leigh et al., 2007; Gryndler et al., 2010). Bacterial PCR products were digested for 60 min with 10 U HhaI and fungal PCR products digested with 5 U NlaIII were analysed on an ABI PRISM 3100 genetic analyser (Applied Biosystems, Foster City, CA). T-RFLPs were analysed using GeneMarker 1.85 software (SoftGenetics, State College, PA).

Tag-encoded amplicon pyrosequencing and sequence analysis

Amplicon pyrosequencing was performed with L and S samples collected on days 0, 8 and 15 and followed the previously described protocol (Baldrian et al., 2012). Samples from day 22 were not analysed owing to the fact that with the increasing incubation period, the danger of nonspecific $^{13}$C-labelling of microbial cross-feeders may increase and affect the identification of cellulose degraders. The eubacterial primers eub530F/eubi1000AR [modified from Dow et al. (2008)] were used to amplify the V4-V6 region of bacterial 16S rRNA gene; the fungi-specific primers ITS1/ITS4 (White et al., 1990) were used to amplify the ITS1, 5.8S rDNA and ITS2 regions of fungal rDNA; and the primers cbhIF and cbhIR (Edwards et al., 2008) were used to amplify a partial sequence of fungal cellobiohydrolase I. PCR amplifications were performed in two steps, and the products were purified as previously described (Baldrian et al., 2012). The mixture was subjected to sequencing on a GS Junior 454-pyrosequencer (Roche, Basel, Switzerland).

The pyrosequencing yielded in total 25 968 reads of sufficient quality and length. Pyrosequencing noise reduction was performed using the DENOISING 0.851 (Reeder & Knight, 2010), and chimeric sequences were deleted after detection using UCHIME (Edgar, 2010). In subsequent analyses, sequences with > 300 bases were used. Fungal sequences were clustered using CD-HIT (Li & Godzik, 2006) at 97% similarity (O’Brien et al., 2005) to yield operational taxonomic units (OTUs). Consensus sequences were constructed for each OTU in Seaview4.2.10 (http://phil.univ-lyon1.fr/software/seaview.html). PlutoF pipeline (Tedersoo et al., 2010) was used to generate best species hits. In the bacterial analysis, sequences were clustered at 97% similarity, and the Ribosomal Database
Project (Cole et al., 2009) was used to generate best hits. Clusters of cbhI sequences were constructed at 96% similarity. For identification, cbhI sequences were retrieved from GenBank and also obtained by the analysis of isolates or cultured strains from the studied ecosystem by cloning/sequencing (GenBank accession numbers HQ896433-HQ896468 and JF343545-JF343547). All cbhI sequences were aligned after intron removal in MAFFT (http://mafft.cbrc.jp/alignment/server/index.html), and a phylogenetic tree was constructed using PhyML online (Dereeper et al., 2008) with the GTR substitution model.

**Statistical analyses**

One-way ANOVA followed by Tukey’s post hoc test was used to test for statistical differences among treatments in STATISTICA 9.0 (Statsoft, Tulsa, OK). Differences at P < 0.05 were regarded as statistically significant. The abundance of fungal and bacterial OTUs was analysed by Canonical Correspondence Analysis in CANOCO 4.55 (Biometris, Wageningen, the Netherlands). Treatments were used as environmental variables, and the relative abundances of the 50 most abundant genera or cbhI clusters in the entire dataset were used as species variables. The Monte Carlo test (499 permutations) was exploited to evaluate the significance of the results (P < 0.05).

**Results**

**Microbial response to the addition of 13C-cellulose to forest litter and soil**

The topsoil of the P. abies forest consisted of a 1–4-cm-thick litter layer and a 2–4-cm-thick organic horizon. Both were acidic with pH 4.6 and 3.6, organic matter content of 93% and 57%, and cellulose content of 36 and 11 mg g⁻¹ dry mass, respectively. 13C-cellulose addition resulted in the final cellulose content of 61 mg g⁻¹ dry mass in the L and 45 mg g⁻¹ dry mass in the S microcosms. Microbial activity in microcosms was reflected by a gradual increase in CO₂ owing to respiration that was approximately 10× faster in the L microcosms (Fig. 1a). The added 13C-cellulose contributed to respiration, especially in the S microcosms, where the relative content of 13CO₂ reached 61%, compared to 31% in the L microcosms (Fig. 1b).

In the beginning of the experiment, L microcosms contained 4.3 ± 1.3 × 10⁸ bacterial and 4.6 ± 1.1 × 10⁸ fungal rDNA copies per g dry mass, several times more than the S microcosms (1.7 ± 1.3 × 10⁷ and 0.10 ± 0.09 × 10⁸, respectively). The L and S microcosms differed significantly in the F/B DNA ratio, which was 1.1 in L and 0.06 in S. During the experiment, the fungal DNA content in L microcosms remained unchanged, while the abundance of bacteria increased significantly after day 15. In contrast, the bacterial abundance in the S microcosms remained unchanged, while the fungal rDNA copy numbers increased by a factor of 45 (Fig. 2a). A substantial part of the microbial community derived their biomass from 13C cellulose. In the S microcosms, as much as 84 ± 2% of total fungal DNA was in the 13C-fraction (compared to 17 ± 5% of bacterial DNA), whereas in the L microcosms, bacteria were more abundant in the 13C-fraction (32 ± 3%) than the fungi (20 ± 1%) (Fig. 2b). Preferential accumulation of 13C in the fungal biomass of the S microcosms also resulted in the increase of F/B ratio in the 13C-DNA from 0.3 to 6.8 (Fig. 2c).

T-RFLP analyses showed a clear distinction of patterns between samples of unlabelled 12C-DNA and labelled 13C-DNA communities of bacteria and fungi in both
microcosms. The 13C-DNA communities generally contained fewer terminal restriction fragments. Sampling at different times, 8, 15 and 22 days after microcosm set-up, did not show high variation in the patterns of terminal restriction fragments and thus indicated stable community composition over time (Supporting Information, Fig. S1).

Identification of cellulose-decomposing bacteria and fungi in litter and soil

Pyrosequencing yielded a total of 12,111 bacterial, 7,075 fungal and 6,782 cbhI sequences. A total of 1,164 bacterial OTUs, 413 fungal OTUs and 297 fungal cbhI clusters were detected, with 204, 78 and 119 present in the respective 13C-DNA communities. In the canonical correspondence analysis of the 50 most abundant bacterial genera, first two canonical axes explained 55% and 17% of species abundance variability, and the Monte Carlo test indicated statistical significance of the first canonical axis as well as of the sum of all four axes. The 12C-DNA and 13C-DNA samples were separated along the first axis, while the second axis separated the L and S samples (Fig. 3a). Among OTUs with abundances over 0.5% in the whole dataset, 22% were strongly associated with L (at least 10× higher relative abundance), 25% were strongly associated with the S and 53% showed comparable abundance in both types of microcosms. The total unlabelled bacterial 12C-DNA communities were relatively diverse: 80% of the whole community was set-up by 106 ± 23 OTUs in the L and 104 ± 8 in the S. The 10 ± 3 and 18 ± 14 dominant OTUs represented 80% of total 13C-DNA communities in the L and S microcosms, respectively. The abundance of the dominant OTU was 2–3% in 12C-DNA but 10–17% in the 13C-DNA.

In the unlabelled 12C-DNA community from the L microcosms (days 8 and 15), 98% of the sequences belonged to five bacterial phyla: the Proteobacteria (44%), Bacteroidetes (20%), Acidobacteria (16%), Actinobacteria (12%) and Verrucomicrobia (6%). In the S microcosms, Acidobacteria were the most abundant (49%), followed by Proteobacteria (30%), Actinobacteria (11%) and Verrucomicrobia (4%). In the 13C-DNA communities of both the L and S microcosms, Betaproteobacteria and Bacteroidetes were the most enriched phyla (Fig. 3c).

The identification of the 50 most abundant bacterial OTUs is summarized in Table S1. When OTU abundance data were grouped by genera, the Gp1 Acidobacterium, Mucilaginibacter, Rhodanobacter, Herminiimonas and Bradyrhizobium were the most abundant in the L microcosms, whereas Gp1, Gp2 and Gp3 Acidobacteria and Actinoallomurus were the most abundant in the S microcosms (Table 1). Herminiimonas and Mucilaginibacter were the most abundant genera in the labelled 13C-DNA communities in both litter and soil. High enrichment in the 13C-DNA was also apparent in Pedobacter, Streptacidiphilus (both L and S), Cytophaga, Asticcacaulis (L),

![Fig. 2.](attachment:fig2.png)

(a) Fungal and bacterial biomass in 13C-cellulose-supplemented microcosms with litter and soil from Picea abies forest: (a) Fungal and bacterial rDNA content in soil, (b) The relative amount of the labelled 13C-enriched DNA fraction, (c) Fungal-to-bacterial DNA ratio in the 12C-DNA and 13C-DNA. The data represent the means and standard errors of three replicates.
Other fungi showed comparable abundance in both. The $^{12}$C-DNA with the L and 33% with the S microcosms and 25% in the first canonical axes and all four axes. Both the $^{12}$C-DNA and the Monte Carlo test indicated statistical significance explained 30% and 27% of species abundance variability, most abundant fungal genera, the first two canonical axes (Table 1).

Alkanindiges and Collimonas (in the S microcosms; Table 1).

In the canonical correspondence analysis of the 50 most abundant fungal genera, the first two canonical axes explained 30% and 27% of species abundance variability, and the Monte Carlo test indicated statistical significance in the first canonical axes and all four axes. Both the $^{12}$C-DNA and $^{13}$C-DNA samples and L and S microcosm samples were clearly separated (Fig. 3b). Among OTUs with abundances $>0.5\%$, 42% were strongly associated with the L and 33% with the S microcosms and 25% showed comparable abundance in both. The $^{12}$C-DNA communities were more diverse than the labelled ones, and 80% of the whole community was set-up by 33 ± 1 OTUs in the $^{12}$C-DNA from the L microcosms and 59 ± 2 in the S microcosms. These values were only 8 ± 6 and 9 ± 8 in the $^{13}$C-DNA communities. The abundance of the dominant OTU was 6–12% in $^{12}$C-DNA but as much as 25% in the $^{13}$C-DNA.

The unlabelled $^{12}$C-DNA communities differed among L and S microcosms. Although Ascomycota and Basidiomycota were dominant in both, Ascomycota were more abundant in the S microcosms (88% compared to 56% in L) and Basidiomycota were more abundant in the L microcosms (39% compared to 5% in S). In addition to the ascomycetous orders Dothideales and Helotiales, which

![Fig. 3. Bacterial and fungal community composition in $^{13}$C-cellulose-supplemented microcosms with litter and soil from Picea abies forest based on the amplicon pyrosequencing of bacterial 16S rRNA gene and fungal ITS. Canonical correspondence analysis of the relative abundance of the members of top 50 bacterial (a) and fungal (b) genera. Localizations of individual DNA samples are indicated as circles, and environmental variables are indicated as arrows. Genus names are provided for the genera with abundance >1% in any of the samples. Phylogenetic assignment of bacterial (c) and fungal (d) sequences in (d), sequences belonging to the Ascomycota and the Basidiomycota are indicated by thin red and blue lines, respectively. The data represent means from values obtained for days 8 and 15.](image-url)
were highly abundant in both types of microcosms, the L samples were also rich in the members of the *Aetheliales*, *Russulales* and *Agaricales* orders. The 13C-DNA community of the L microcosms contained fewer *Basidiomycota* (17%) than the 12C-DNA. The members of the *Dothideales* order were frequent in the labelled fraction, but also the abundance of the *Leotiomycetes*, *Tremellales* and *Chaetothyriales* increased. The 13C-DNA of the S samples was dominated by the *Ascomycota* (94%), with the *Dothideales*, *Leotiomycetes* and *Helotiales* being most abundant (Fig. 3d).

The identification of the most abundant fungal OTUs is summarized in Table S2. The *Amphinema*, *Russula*, *Oxidiadendron* and *Hygropybe* were the most abundant in the L microcosms, whereas *Cadophora*, *Geomyces*, *Lepidotodontium*, *Aquitapetum* and *Diplotomma* were the most abundant in the S microcosms (Table 1). High enrichment in the 13C-DNA was found for the members of the following genera: *Lepidotodontium* in both the L and S, *Chaetosphaeria* and *Hypocreia* in the L, and *Cryptococcus* and *Umbelopsis* in the S microcosms (Table 2).

To predict cbhI clusters, individual samples with similar diversities in L and S and the 13C-DNA and 13C-DNA were analysed at a sampling depth of 400 sequences. Of the 80–150 cbhI clusters predicted as Chao1 estimates, between 30 and 80 clusters were recovered. Among clusters with abundances > 0.5%, 55% were strongly associated with the L and 30% with the S microcosms. Only 15% showed comparable abundance in both. This was confirmed by the canonical correspondence analysis on the 50 most abundant cbhI clusters, which separated clusters from the L and S microcosms along the first canonical axis. These clusters were then separated from 12C-DNA and 13C-DNA along the second axis. The Monte Carlo test indicated statistical significance of the first canonical axis and the sum of all four axes. The cbhI gene DNA pools showed similar diversity in labelled and unlabelled communities; 80% of whole communities were set-up by 11–23 clusters, and the abundance of dominant clusters was between 8% and 22%.

We were able to identify the taxonomic affiliation of producers for 21 cbhI clusters (Fig. S2), and the environmental cbhI sequences showed > 97% similarity with sequences of known fungi. Most of the identified sequences belonged to the members of the order *Helotiales*. Other sequences belonged to the ascomycetous orders *Dothideales*, *Magnaportheales*, *Capnodiales*, *Hypocreales* and *Leotiomycetes*, and two clusters belonged to basidiomycetous genus *Myccena* (*Agaricales*). Clusters 4 and 6, with > 99% cbhI similarity to *Cadophora* and

### Table 1. The most abundant bacterial genera in the 12C-DNA and 13C-DNA communities of the *Picea abies* forest litter and soil microcosms based on the abundance of DNA sequences on days 8 and 15 after 13C-cellulose addition

<table>
<thead>
<tr>
<th>Genus (Ac)</th>
<th>12C - Litter</th>
<th>13C - Litter</th>
<th>13C/12C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gp1 Acidobacterium</td>
<td>8.2</td>
<td>36.4</td>
<td>4.5</td>
</tr>
<tr>
<td>Muclaginibacter (B)</td>
<td>5.0</td>
<td>21.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Rhodanobacter (P)</td>
<td>3.4</td>
<td>9.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Herminimonas (P)</td>
<td>2.2</td>
<td>3.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Bradyrhizobium (P)</td>
<td>2.0</td>
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<td>1.7</td>
</tr>
<tr>
<td>Burkholderia (P)</td>
<td>1.7</td>
<td>2.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Salinicola (P)</td>
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<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Actinosapta (At)</td>
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<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Arthrobacter (At)</td>
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<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Pedobacter (At)</td>
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**12C - Soil**

<table>
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<th>13C - Litter</th>
<th>13C/12C</th>
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</thead>
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<tr>
<td>Gp1 Acidobacterium (Ac)</td>
<td>17.3</td>
<td>29.9</td>
<td>1.7</td>
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<tr>
<td>Gp2 Acidobacterium (Ac)</td>
<td>10.5</td>
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<td>2.3</td>
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<tr>
<td>Gp3 Acidobacterium (At)</td>
<td>2.2</td>
<td>6.3</td>
<td>2.9</td>
</tr>
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<td>Salinicola (P)</td>
<td>1.6</td>
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</tr>
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<td>Bradyrhizobium (P)</td>
<td>0.8</td>
<td>1.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Steroidobacter (P)</td>
<td>0.6</td>
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<td>1.8</td>
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<td>Skermanella (P)</td>
<td>0.6</td>
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<td>1.7</td>
</tr>
<tr>
<td>Conexibacter (At)</td>
<td>0.5</td>
<td>0.9</td>
<td>1.8</td>
</tr>
<tr>
<td>Herminimonas (P)</td>
<td>0.4</td>
<td>0.3</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Bacterial phyla: Ac, Acidobacteria; At, Actinobacteria; B, Bacteroidetes and P, Proteobacteria.

*Ratio of abundance in the 13C and 12C community.

Genera with high abundance in the 13C-control (day 0).
Table 2. The most abundant fungal genera in the $^{12}$C-DNA and $^{13}$C-DNA communities of the Picea abies forest litter and soil microcosms based on the abundance of DNA sequences on days 8 and 15 after $^{13}$C-cellulose addition

<table>
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<tr>
<th>Genus</th>
<th>Abundance (%)</th>
<th>Genus</th>
<th>Abundance (%)</th>
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<tbody>
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<td>$^{12}$C - Litter</td>
<td></td>
<td>$^{13}$C - Litter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphinema (B)</td>
<td>26.3</td>
<td>Oidiodendron† (A)</td>
<td>32.5</td>
<td>5.6</td>
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<td>Russula (B)</td>
<td>12.6</td>
<td>Trichosporon (B)</td>
<td>27.9</td>
<td>92.0</td>
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<tr>
<td>Oidiodendron (A)</td>
<td>5.9</td>
<td>Chaetosphaeria (A)</td>
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<td>40.2</td>
</tr>
<tr>
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<td>Geomyces* (A)</td>
<td>4.6</td>
<td>13C only</td>
</tr>
<tr>
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<td>Hypocrea (A)</td>
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<td>40.7</td>
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<tr>
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<td>Mortierella* (M)</td>
<td>3.2</td>
<td>1.1</td>
</tr>
<tr>
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<td>Leptodontium (A)</td>
<td>2.6</td>
<td>2.1</td>
</tr>
<tr>
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<td>Hygrocybe (B)</td>
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<td>0.2</td>
</tr>
<tr>
<td>Trechispora (B)</td>
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<td>Amphinema (B)</td>
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</tr>
<tr>
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<td>Cadophora (A)</td>
<td>0.6</td>
<td>0.3</td>
</tr>
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</table>

$^{12}$C - Soil

<table>
<thead>
<tr>
<th>Genus</th>
<th>Abundance (%)</th>
<th>Genus</th>
<th>Abundance (%)</th>
<th>$^{13}$C/$^{12}$C*</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>Aquapoterium (A)</td>
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<td>Cryptococcus (B)</td>
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<td>Umbelopsis (M)</td>
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</tr>
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<td>0.3</td>
</tr>
<tr>
<td>Mortierella (M)</td>
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<td>Mortierella* (M)</td>
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<td>0.5</td>
</tr>
<tr>
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<td>Rhizoscyphus (A)</td>
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<td>Articulospora (A)</td>
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<td>1.3</td>
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<td>Cryptococcus (B)</td>
<td>2.1</td>
<td>Cadophora (A)</td>
<td>1.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Fungal divisions: A, Ascomycota; B, Basidiomycota; C, Chytridiomycota and M, Mucoromycotina.

*Ratio of abundance in the $^{13}$C and $^{12}$C community.
†Genera with high abundance in the $^{13}$C-control (day 0).

Ceuthospora, were among the most abundant clusters in $^{13}$C-DNA from the S microcosms, each representing 5% of all sequences. Phylogenetic analysis allowed coarse taxonomic placement of 29–65% of the cbhI sequences into either the Ascomycota or Basidiomycota. In both the L microcosm and S microcosm, cbhI genes of ascomycetous origin were dominant, representing 48% of sequences in $^{12}$C-DNA from L, 28% in $^{13}$C-DNA from L, 65% in $^{12}$C-DNA from S and 45% in $^{13}$C-DNA from S, compared to only 8.6% assigned to the Basidiomycota in the $^{12}$C-DNA and < 1% in all other DNA samples.

Discussion

Although both fungi and bacteria in forest topsoil substantially accumulated cellulose-derived carbon, their relative contributions to cellulose degradation in litter and soil differed. In soil, where polysaccharides were scarce and bacteria-dominated, cellulose addition was mainly incorporated into fungal biomass, which resulted in their proliferation. In contrast, cellulose added to litter rich in fungal biomass was mainly accumulated by bacteria. This might reflect the fact that cellulose represents a preferred substrate for bacteria, whereas fungi are able to utilize a wider range of litter constituents.

Bacterial growth was previously shown to be promoted when fungi decomposed lignocellulose (Romani et al., 2006; Snajd et al., 2011a), supposedly supporting bacteria living as mycoparasites or cheaters (de Boer et al., 2005). Because the degradation products of cellulose (cellobiose and especially glucose) are suitable substrates for most fungi and bacteria, the ‘cheater’ community theoretically might comprise most microorganisms present. If cheater microorganisms receive enough $^{13}$C, they should appear in the $^{13}$C-DNA. The microorganisms feeding on the $^{13}$C-rich biomass of cellulose decomposers should appear as labelled later in the experiment. Our results show that there is a limited subgroup of the total community accumulating the $^{13}$C-label. It comprises a few highly abundant OTUs whose composition is stable over time, which indicates that cheating and cross-labelling were not quantitatively important.

Cellulolytic abilities are only limited to certain, although phylogenetically diverse, bacterial taxa (Lynd et al., 2002). In neutral to moderately acidic agricultural soils, cellulolytic bacteria were recorded in the genera Arthrobacter, Cellulomonas, Kitasatospora, Micromonospora, Oerskovia, Streptomyces (Actinobacteria), Burkholderia, Dyella, Fulvimonas, Mesorhizobium, Methylbacterium, Sphingomonas, Variorax (Proteobacteria), Bacillus,
Acidobacteria culturing of isolates showed that some Gp1 and Gp3 are able to utilize cellulose. Both the genome sequencing and the virtual absence of basidiomycetous decomposers in the heavy-fraction 13C-DNA in forest topsoil was also rich in Acidobacteria (Baldrian & Valašková, 2008; Baldrian et al., 2011). Of these fungi, Geomyces, Umbelopsis and Hypocrea were identified in 13C-DNA in this study. Additionally, the fungi associated with P. abies needles in various stages of decomposition were shown to be able to produce cellulases. In this study, members of the Helotiales and Dothideales orders were found in the 13C-DNA (Zifčáková et al., 2011). Oidiodendron and Geomyces belonged to the most abundant genera in the heavy-fraction 13C-DNA in forest topsoil. Because these genera were also abundant in the 13C-DNA from control samples collected before cellulose addition, it is unclear whether the incorporated 13C is from the added cellulose. This is likely, however, because several isolates of both genera were isolated from litter-degraded cellulose in culture (Rice et al., 2006; Zifčáková et al., 2011).

This study shows that the involvement of the Basidiomycota in cellulose decomposition was low because of the virtual absence of basidiomycetous cord-formers, although these did occur in this ecosystem being represented mainly by the genera Marasmius and Mycena (Zifčáková et al., 2011; Baldrian et al., 2012). The basidiomycetous yeasts of the genera Trichosporon and Cryptococcus were the most abundant basidiomycete cellulose decomposers in the L and S microcosms, respectively. While Trichosporon was previously reported to degrade cellulose (Dennis, 1972), Cryptococcus neoformans lacks exocellulase in its genome (Baldrian & Valašková, 2008), and members of the genus reportedly did not degrade cellulose (Dennis, 1972). Recent studies, however, reported endocellulase production by Cryptococcus S-2 (Thongekkaew et al., 2008) and Cryptococcus was shown to accumulate 13C from cellulose added to soil (Bastias et al., 2009). These findings, along with our current results, are of high importance, considering that the genus Cryptococcus represents as much as one-third of the total fungal community in some soils (Buée et al., 2009).

Cellulolytic gene complement of ectomycorrhizal fungi is usually limited when compared to saprotrophs, and some species completely lack cellulase genes (Baldrian, 2009). During the screening of soil fungi for cbhI genes in this study, we identified the presence of two cbhI sequences in Russula emetica and Russula paludosa and showed that the cbhI cluster 41 is highly similar to the R. emetica sequence. Russula emetica was an abundant species in L microcosms but showed only low abundance in the 13C-DNA. In a previous study, members of the Russulales have been found to be inactive during the organic matter decomposition period (Baldrian et al., 2012). Thus, their involvement in decomposition is questionable.

This study also showed that fungal communities differ substantially between soil and litter: as much as 76% of the quantitative dominance of Acidobacteria in acidic soils, their contribution to carbon cycling in this environment may be significant (Lauber et al., 2009; Jones et al., 2011).

Among fungi, cellulose decomposition abilities are relatively common, especially among the saprotrophic Ascomycota and Basidiomycota and many saprotrophic species were recently reported to contain the cbhI gene (Weber et al., 2011; Baldrian et al., 2012). In addition to cord-forming basidiomycetes, many fast-growing nonbasidiomycetous fungi that inhabit litter are also able to efficiently degrade cellulose (Deacon et al., 2006; Baldrian & Valašková, 2008; Baldrian et al., 2011).
dominant fungal OTUs and 47% of bacterial ones were preferentially associated with a particular component of the forest floor. The same applies also to cellulose-utilizing fungi, showing that cellulose transformation in litter and soil is performed by largely different communities that are suited for environments with different organic matter content and quality.

The analysis of the cbhI genes of fungal isolates obtained in this study confirmed the previous findings of Edwards et al. (2008) that most fungal species contain more than one cbhI gene (typically 2–3) and that these are very often highly dissimilar (Fig. S2). It also confirmed that Ascomycota were particularly important for cellulose hydrolysis, and that the fungal members of the major orders involved in cellulose decomposition contain the cbhI gene. Unlike in the bacterial and fungal communities, the cbhI gene pools in 13C-DNA and 13C-DNA showed similar diversity. Moreover, all but one of the cbhI clusters with an abundance of > 1% were present in the 13C-DNA, which showed that the possession of a cbhI gene is a reliable indicator of cellulolytic ability.

Contrary to the theoretical expectations, bacteria incorporated relatively more cellulose-derived carbon than fungi in litter, where cellulose decomposition was fast, than in the soil. Cellulolytic microorganisms represent only a minor part of the total community and differ between litter and soil. Several microbial taxa that were previously not recognized as cellulolytic were now reported. In the case of fungi, cellulose decomposers belonged mainly to Ascomycota and Basidiomycota (primarily yeasts) rather than cord-forming saprotrophs with known cellulolytic abilities. This shows that further research targeting decomposer microorganisms in different habitats is needed to gain sufficient understanding of this important process.

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References


Cellulose decomposition in forest litter and soil


Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Relative abundances of individual fragments in the T-RFLP profiles of bacterial 16S rRNA gene amplicons digested with HhaI and fungal ITS1/ITS2 amplicons digested with NlaIII.

**Fig. S2.** Phylogenetic relationships among reference *cbhI* gene sequences and sequences obtained in this study.

**Table S1.** Identification (based on RDP), best NCBI-BLASTN hit and relative abundances of 50 most abundant bacterial OTUs; 12L = 12C-litter horizon, 12H = 12C-organic horizon, 13L = 13C-labeled litter, 13H = 13C-labeled organic horizon.

**Table S2.** Identification, best NCBI-BLASTN hit and relative abundances of 50 most abundant fungal OTUs; 12L = 12C-litter horizon, 12H = 12C-organic horizon, 13L = 13C-labeled litter, 13H = 13C-labeled organic horizon.

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