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ORIGINAL ARTICLE

Fungal community on decomposing leaf litter undergoes rapid successional changes

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Fungi are considered the primary decomposers of dead plant biomass in terrestrial ecosystems. However, current knowledge regarding the successive changes in fungal communities during litter decomposition is limited. Here we explored the development of the fungal community over 24 months of litter decomposition in a temperate forest with dominant *Quercus petraea* using 454-pyrosequencing of the fungal internal transcribed spacer (ITS) region and cellobiohydrolase I (*cbhI*) genes, which encode exocellulases, to specifically address cellulose decomposers. To quantify the involvement of phyllosphere fungi in litter decomposition, the fungal communities in live leaves and leaves immediately before abscission were also analysed. The results showed rapid succession of fungi with dramatic changes in the composition of the fungal community. Furthermore, most of the abundant taxa only temporarily dominated in the substrate. Fungal diversity was lowest at leaf senescence, increased until month 4 and did not significantly change during subsequent decomposition. Highly diverse community of phyllosphere fungi inhabits live oak leaves 2 months before abscission, and these phyllosphere taxa comprise a significant share of the fungal community during early decomposition up to the fourth month. Sequences assigned to the *Ascomycota* showed highest relative abundances in live leaves and during the early stages of decomposition. In contrast, the relative abundance of sequences assigned to the *Basidiomycota* phylum, particularly basidiomycetous yeasts, increased with time. Although cellulose was available in the litter during all stages of decomposition, the community of cellulolytic fungi changed substantially over time. The results indicate that litter decomposition is a highly complex process mediated by various fungal taxa.
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Introduction

Plant litter represents a major source of organic carbon in forest soils. Its decomposition is a complex process that involves mineralisation and transformation of organic matter. Decomposition of plant litter is a key step in nutrient recycling (Berg *et al.*, 2001). As most of the plant biomass-derived carbon in the temperate and boreal forests is mineralised in the litter layer, an understanding of this process and the microorganisms involved is essential for the identification of factors that affect global carbon fluxes.

Fungi are considered to be the key players in litter decomposition because of their ability to produce a wide range of extracellular enzymes, which allows them to efficiently attack the recalcitrant lignocellulose matrix that other organisms are unable to decompose (Kjoller and Struwe, 1982; de Boer *et al.*, 2005). Biochemical decomposition of leaf litter is a sequential process that initially involves the loss of the less recalcitrant components (for example, oligosaccharides, organic acids, hemicellulose and cellulose) followed by the degradation of the remaining highly recalcitrant compounds (for example, lignin or suberin). Litter quality changes during the course of its transformation and so does the activity of litter-associated microorganisms (Dilly *et al.*, 2001). These changes are accompanied by a succession of microbial litter decomposers that reflect the varied catabolic capabilities that are sequentially required to complete the process of litter decomposition (Frankland, 1998; Osono *et al.*, 2006).

The ability of fungi to decompose leaf litter has been investigated many times under laboratory conditions (for example, Osono, 2007; Baldrian *et al.*, 2011). Furthermore, many studies have combined litterbag techniques with cultivation-based methods followed by the isolation and identification of fungal decomposers (Koide *et al.*, 2005; Osono, 2005; Zhang *et al.*, 2008; Osono *et al.*,

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2009). Using these methods, fungi involved in the decomposition of litter have been divided into early, intermediate and late decomposers (Frankland, 1998; Tang *et al.*, 2005). This observation was supported by a recent study performed by Šnajdr *et al.* (2011), as these three phases were distinguished during oak litter decomposition based on the differences in the activity of extracellular enzymes and the rates of decomposition of the individual litter constituents. In most previous studies, fungi from the *Ascomycota* phylum were found to dominate during the initial stages of litter decay along with a few basidiomycetous fungi. The abundance of fungi from the *Ascomycota* phylum decreases during the process of degradation as they are gradually replaced by fungi from the *Basidiomycota* phylum, especially the saprotrophic cord formers, during the later stages of decomposition (Frankland, 1998; Osono, 2007).

Plant organic matter transformation leads to the disappearance of easily utilisable compounds and to the formation of recalcitrant ones. As a consequence, the chemical and spatial heterogeneity of the substrate changes with time. This process can theoretically result in the formation of novel niches and a potential increase in fungal diversity or to the creation of more uniform environment with a potential decrease in diversity. Both scenarios have been reported from litter or wood (Melillo *et al.*, 1989; Dickie *et al.*, 2012) but the actual development of fungal community on decaying litter is so far unknown. Culture-dependent approaches are typically selective because only a small fraction of microbial taxa grow under the conditions used for strain isolation (Amann *et al.*, 1995). Molecular methods, such as community fingerprinting or direct sequencing of cloned PCR sequences that have recently been applied to litter (Aneja *et al.*, 2006; Kubartova *et al.*, 2009) suffered from limited resolution. Therefore, next-generation sequencing approaches currently represent the only technique that can be used to sufficiently describe the development of fungal community composition during succession.

The degradation of plant leaves is not limited to the litter layer on the forest floor. Indeed, the decomposition process begins as soon as the leaf is formed (Stone, 1987). Phyllosphere fungi that are established in the interior or on the surfaces of live leaves have the advantage of gaining access to readily available nutrients in live leaves and later, after senescence, to the dead leave biomass. Recently, 454-pyrosequencing was used to assess fungal diversity in live oak leaves and demonstrated the presence of a diverse fungal community (Jumpponen and Jones, 2009a, b). It is highly probable that at least some of these fungi participate in litter decomposition. There is some evidence that certain phyllosphere fungi are able to transform various components of litter because they produce the extracellular enzymes that are involved in decomposition in pure culture and their ability to decompose litter material has been described (Korkama-Rajala *et al.*, 2008; Žifčáková *et al.*, 2011). Although potential leaf endophytes have been isolated from litter in various stages of decomposition (Osono, 2002; Koide *et al.*, 2005), their importance in the community of litter-associated fungi is currently unknown.

The aim of this study was to characterise the development of the fungal community composition over 24 months following litterfall in a temperate forest dominated by *Quercus petraea*. As some litter components, including cellulose, remain present in a considerable quantity during the entire 24-month period (Šnajdr *et al.*, 2011), the fungi capable of cellulose decomposition may be present during all phases of decomposition. To address this possibility, the composition of the gene pool of the *cbhI* exocellulase gene, which is an enzyme that catalyses the rate-limiting step in the decomposition of cellulose (Baldrian and Valášková, 2008; Edwards *et al.*, 2008), was monitored at various stages of litter decomposition. To evaluate the role of phyllosphere fungi in litter decomposition, fungal communities associated with live *Q. petraea* leaves and senescent leaves immediately before abscission were also analysed. The results of this study are discussed in light of previously published data derived from the same experiment where litter decomposition (mass loss), fungal and bacterial biomass content based on ergosterol and phospholipid fatty acid analysis, and the activity of the extracellular enzymes in the litterbags were explored by Šnajdr *et al.* (2011).

Materials and methods

Study site and sample collection

The study site was an oak (*Q. petraea*) forest in the Xaverovský Háj Natural Reserve, near Prague, Czech Republic (50°5′38"N, 14°36′48"E). The site was previously explored with respect to the activity of decomposition-related extracellular enzymes in the forest topsoil (Šnajdr *et al.,* 2008) and during the successive transformation of *Q. petraea* litter (Šnajdr *et al.*, 2011). In this study site, the saprotrophic fungi were characterised (Valášková *et al.*, 2007; Baldrian *et al.*, 2011). The soil was acidic cambisol with a litter thickness of 0.5–1.5 cm, a pH of 4.3, a C content of 46.2% and an N content of 1.76%. The mean annual temperature at the soil surface was 9.3 °C (winter mean 1.3 °C, summer mean 16.6 °C; Baldrian *et al.*, in press).

The litterbag experiment was run as described previously (Šnajdr *et al.*, 2011). Litter material (*Q. petraea* leaves, tree age 100–120 years) for litterbag construction was collected immediately after abscission and allowed to air dry at 20 °C. Litterbags containing 5 g of air-dried leaves (10 × 20 cm, 1 mm nylon mesh size) were placed on the top of the litter horizon at the study site at the end of the litterfall

season. To prevent extensive desiccation, litterbags were overlaid with freshly fallen oak leaves. Litterbags were removed after 2, 4, 8, 12 and 24 months of incubation, three litterbags were collected at each sampling time for DNA extraction. For the analysis of the phyllosphere fungal community composition, live *Q. petraea* leaves were collected 2 months before abscission (August) by hand-picking (month −2), and senescent leaves were collected during the litterfall period by gently shaking oak twigs and collecting falling leaves before their contact with the soil (month 0). Collected material was transferred to the laboratory and processed immediately. Leaves or litter were cut into 0.25 cm^2 pieces and used immediately for DNA extraction. The same material was also used for chemical analyses, measurement of enzyme activities and quantification of microbial biomass as described in Šnajdr *et al.* (2011).

*454-Pyrosequencing of fungal internal transcribed spacer (ITS) and cellobiohydrolase I (*cbhI*) genes*

The total genomic DNA was extracted from 300 mg of material using the Powersoil Kit (MoBio, Carlsbad, CA, USA). The primers ITS1/ITS4 (White *et al.*, 1990) were used to amplify the ITS1 region, the 5.8S ribosomal DNA and the ITS2 region of the fungal ribosomal DNA. The primers *cbhI*F and *cbhI*R (Edwards *et al.*, 2008) were used to amplify a partial sequence of the fungal *cbhI* gene. These primers amplify *cbhI* genes belonging to the GH7 family of fungi from the *Basidiomycota*, *Ascomycota* and *Mucoromycotina* unless the template contains intron in the primer sequence (Štursová *et al.*, 2012).

A two-step PCR amplification using composite primers containing multiplex identifiers was performed to obtain amplicon libraries for 454-pyrosequencing following a previously described method (Baldrian *et al.*, 2012). PCR amplicons were quantified using the Quant-iT PicoGreen Kit (Invitrogen, Grand Island, NY, USA). An equimolar mix of PCR products was prepared for each primer pair, and the pooled products were sequenced on a GS FLX Titanium platform (Roche, Basel, Switzerland). Fungal ITS sequences were analysed from all sampling times, and the *cbhI* gene diversity was analysed in the samples collected at −2, 0, 4 and 12 months.

Bioinformatic analysis

The pyrosequencing data were processed as described previously (Baldrian *et al.*, 2012). Pyrosequencing noise reduction was performed using the Denoiser 0.851 (Reeder and Knight, 2010) and chimeric sequences were detected using UCHIME (Edgar, 2010) and deleted. Fungal sequences were shortened to 380 bases and clustered using cd-hit (Li and Godzik, 2006) at a 97% similarity level (O'Brien *et al.*, 2005) to obtain the operational taxonomical units (OTUs). Consensus sequences were constructed for each cluster, and the closest hits were identified using the PlutoF pipeline (Tedersoo *et al.*, 2010). For the *cbhI* gene, the sequences were truncated to 300 bp and clustered at a 96% similarity level (Baldrian *et al.*, 2012) to obtain the OTUs. Consensus sequences were constructed, and the introns were removed. Data sets containing the *cbhI* sequences representing the OTUs and sequences retrieved from GenBank were aligned using SeaView 4 (http://pbil.univ-lyon1.fr/software/seaview.html) with MUSCLE (http://www.drive5.com/muscle/). Maximum likelihood phylogenetic trees were computed with the GTR substitutions model using GARLI (http://www.molecularevolution.org/sofware/phylogenetics/garli/garli_create_job). The OTUs of *cbhI* genes that clustered with sequences of known fungal taxa from the GenBank with bootstrap support >70% were taxonomically assigned to fungal phyla.

Sequence data have been deposited in the MG-RAST public database (http://metagenomics.anl.gov/, data set numbers 4497081.3 for fungal ITS region and 4497080.3 for *cbhI* genes).

Diversity and statistical analyses

Owing to the fact that the sampling depth achieved in this study did not allow to make realistic estimates of total diversity and since next-generation sequencing derived data were demonstrated to be affected by artefacts (Tedersoo *et al.*, 2010), the only measure of diversity of OTUs used was the amount of the most abundant OTUs that represented 80% of all sequences. This metric in our opinion fairly represents the diversity of the quantitatively important part of the fungal or *cbhI* community. To avoid possible effects of variable sampling depth, these estimates were calculated for a data set containing 700 randomly chosen ITS sequences or 495 *cbhI* sequences from each litterbag. The sequences were clustered again as described above. The OTU richness and Chao1 were calculated using EstimateS 8.00 (http://viceroy.eeb.uconn.edu/estimates).

One-way analysis of variance with the Fisher's least significant difference *post hoc* test was used to analyse the significant differences in relative abundance of individual OTUs or fungal taxa among sampling times. Principal component analysis was performed with the relative abundance data of the 50 most abundant fungal genera. PC1 and PC2 loads were subjected to analysis of variance with the Fisher's least significant difference *post hoc* test. Differences with a $P<0.05$ were regarded as statistically significant.

Results

Fungal communities associated with oak leaves

In total, 23 760 sequences of the fungal ITS region with >380 bp were used for analysis after denoising and removal of the chimeric sequences and

sequences not belonging to fungi (<0.6%). These sequences clustered into 1874 OTUs (including 1193 singletons) at a 97% similarity level. Although 80% of all sequences at month −2 were represented by 23 dominant OTUs, at month 0 70–90% of sequences belonged to the single most abundant OTU (assigned to *Mycosphaerella punctiformis*). Within a relatively short time (month 4), the diversity peaked with 30 OTUs representing 80% of the total fungal community at month 8 and then levelled off (Figure 1).

A total of 387 fungal genera were identified as the closest hits of individual OTUs. *Mycosphaerella*, *Naevala*, *Troposporella* and *Trichosporon* were the most abundant fungi in the amplicon pool. The 50 most abundant fungal OTUs with their closest identified hits and abundances are listed in Supplementary Table 1. In all, 40 of the 50 most abundant fungal OTUs and 27 of the top 33 genera demonstrated significant changes in abundance over time (Supplementary Table 1, Figure 2). Altogether, the ascomycetous OTU 0 (closest hit: *Mycosphaerella punctiformis*) and OTU 1 (*Naevala minutissima*) were the most abundant fungi. OTU 0 was predominant during the early stages of succession (month −2 and 0) but disappeared almost completely during later stages. OTU 1 and OTU 4 (*Athelia*) were highly abundant during the initial stages of litter decomposition (months 2 and 4). However, OTU 3 and OTU 11 (both *Troposporella fumosa*) dominated the later stages of decomposition (months 8 and 12) but were almost absent in other samples. The latest stages of litter decomposition were dominated by OTU 2 (*Trichosporon porosum*) and OTU 5 (*Trichosporon miniliiforme*) (Supplementary Table 1).

The majority of fungal sequences were assigned to the *Ascomycota* (71%) and *Basidiomycota* (26%) phylum. *Glomeromycota* were represented by 1.8% of all sequences, and fungi from *Mucoromycotina* comprised 0.76% of all sequences. Fungi from the *Ascomycota* phylum dominated in the live and senescent leaves, which is in contrast to month 24 when fungi from the *Basidiomycota* phylum represented 60% of the amplicons. *Glomeromycota* and *Mucoromycotina* sequences were rare until month 4 and then rapidly increased until month 8 and 24 when they represented 8.6% and 2.2% of the amplicon pool, respectively (Figure 3). The most abundant fungal orders were *Capnodiales* (22% of sequences), *Helotiales* (20%) and *Tremellales* (12%). Members of the *Helotiales* order were present during

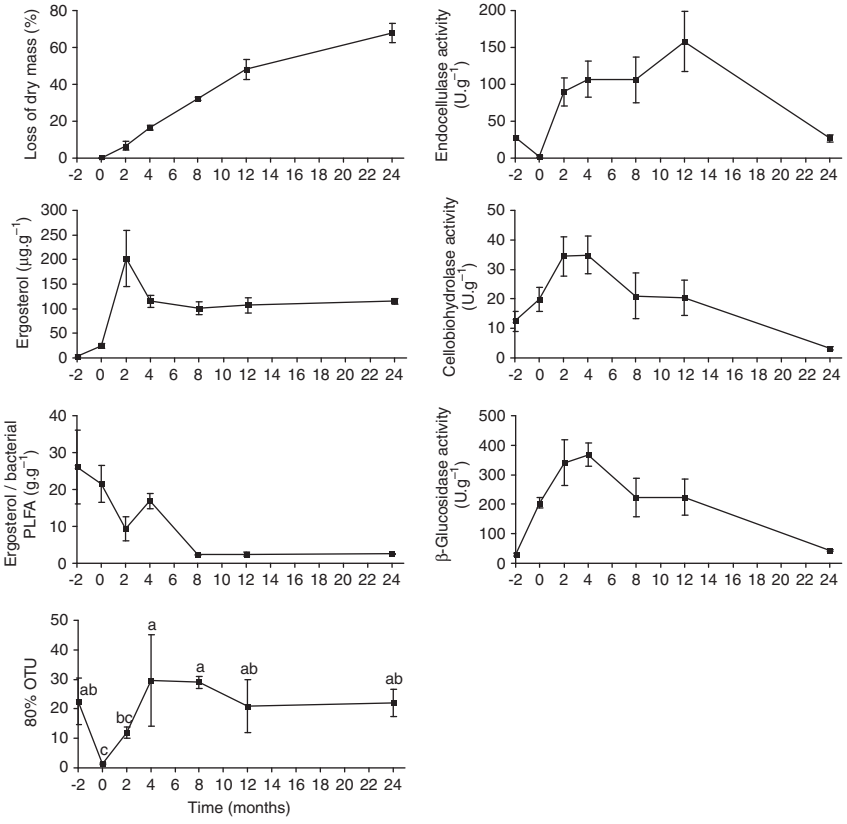

Figure 1 Loss of dry mass, activity of extracellular enzymes, development of fungal and bacterial biomass and estimates of fungal diversity in *Q. petraea* live leaves and leaves at different stages of decomposition. Fungal biomass is expressed as ergosterol content. The ratio of fungal and bacterial biomass is based on the ratio of ergosterol content and the content of bacteria-specific phospholipid fatty acids. Data on leaf chemistry and microbial biomass are derived from Šnajdr *et al.* (2011). 80% OTU represents the number of the most abundant OTUs, which represent 80% of all sequences. The data are shown as the means and s.e. from three litterbags.

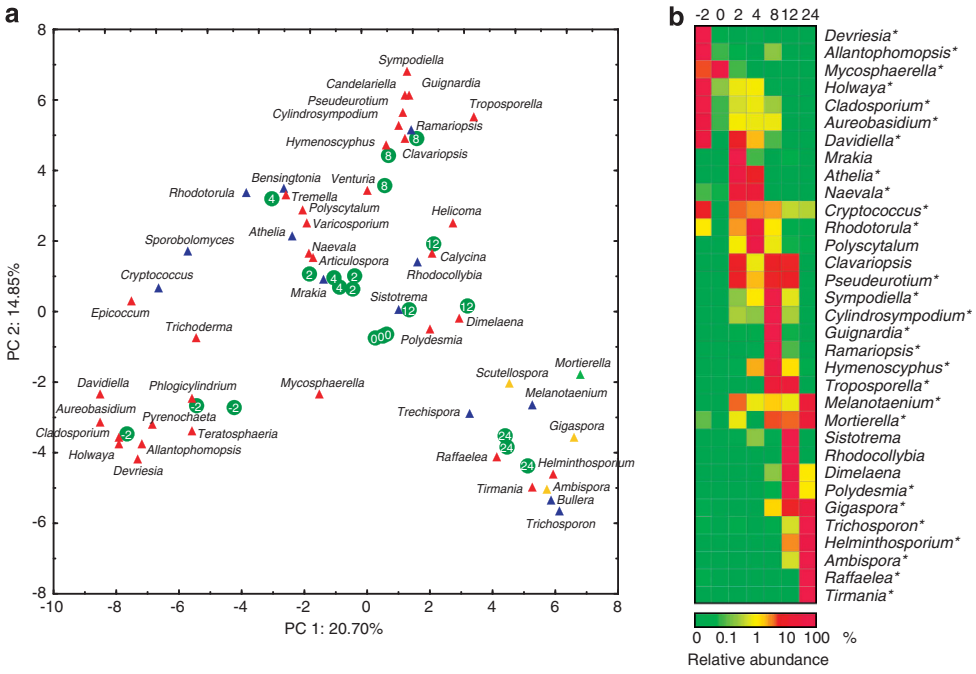

Figure 2 (**a**) Principal component analysis of the relative abundance of the 50 most abundant fungal genera in *Q. petraea* live leaves and leaves at different stages of decomposition. *Ascomycota*—red, *Basidiomycota*—blue, *Glomeromycota*—yellow, *Mucoromycotina*—green. Green circles with numbers indicate the positions of individual samples (litterbags) with ages in months. (**b**) Time course of the relative abundance of dominant fungal genera in *Q. petraea* live leaves and leaves at different stages of decomposition. Mean abundances are shown for each time point. A statistically significant effect of time on abundance is indicated by an asterisk ($P<0.05$, analysis of variance (ANOVA) followed by Fisher's *post hoc* test).

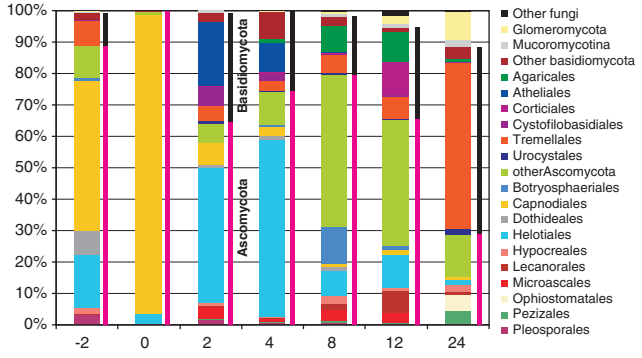

Figure 3 Phylogenetic assignment of fungal sequences from *Q. petraea* live leaves and leaves at different stages of decomposition. The data are represented as the mean values from three litterbags.

all phases of succession and peaked at month 2 and 4 together with fungi from the *Atheliales* family. Fungi belonging to the ascomycetous order *Capnodiales* dominated among amplicons from the live and senescent leaves, whereas the basidiomycetous order *Tremellales* predominated at later stages (Figure 3).

Each sampling time was characterised by a specific fungal community, which was different from the community in the previous or the next stage. When the abundance of the top 50 fungal genera was analysed by principal component analysis, the first two canonical axes explained 20.70% and 14.85% of the total variability (Figure 2). The analysis of variance of the PC1 and PC2 loadings demonstrated that community changes over time were significant between any two dates except between months 2 and 4 ($P<0.015$). This is supported by the fact that most of the fungi that were highly abundant at a certain time only dominated for a short period. Among the 28 genera that represented >3% of the sequences at any particular sampling time, 20 did not exceed 1% at any other time (Figure 2).

The amplicons from live *Q. petraea* leaves were dominated by the *Ascomycota* (88.5%) fungi, and *Capnodiales*, *Helotiales*, *Dothideales* and *Pleosporales* were the major orders. The *Basidiomycota* phylum (10.6%) was mainly represented by *Tremellales* (Figure 3). The fungal community on senescent leaves was dominated by the same OTUs that were dominant on live leaves, and the fungi that were highly abundant on live leaves comprised approximately 50% of all fungi until month 4 (Figure 4).

Cellulose-decomposing fungi associated with oak leaves

The gene *cbhI* encoding for cellobiohydrolase was used as a marker for the cellulolytic members of the fungal community. In total, 3351 denoised, non-

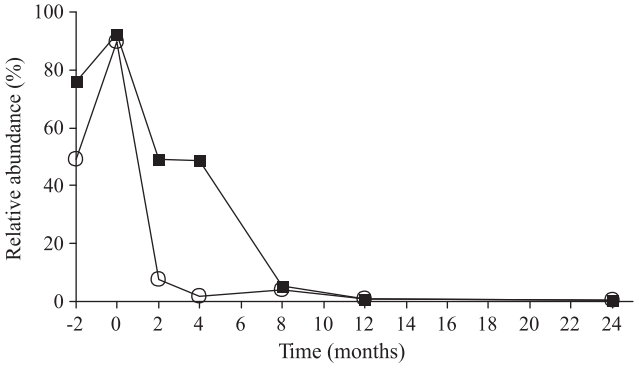

Figure 4 The persistence of fungal OTUs recorded in live *Q. petraea* leaves (endophytes) during subsequent decomposition of litter. Data represent the sum of the relative abundances of dominant endophytes with abundances >5% in live leaves (open circles) and abundant endophytes (>1%; black squares).

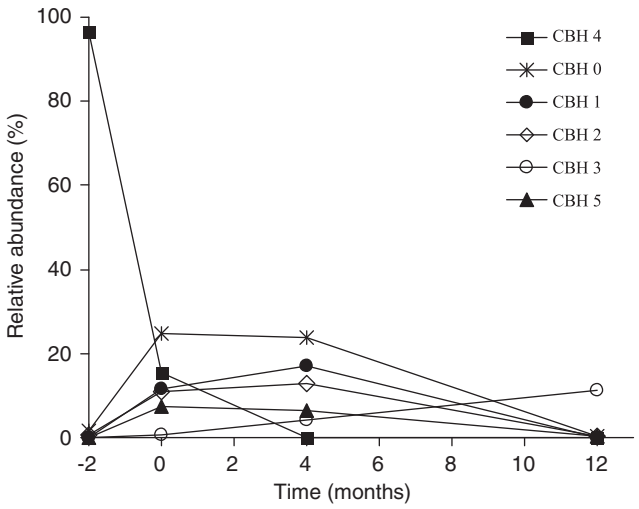

Figure 5 Time course of the relative abundance of dominant cellulolytic fungi represented by the *cbhI* gene OTUs in *Q. petraea* live leaves and leaves at different stages of decomposition. The data are shown as the mean values from three litterbags.

chimeric sequences of *cbhI* were analysed. The sequences clustered into 235 OTUs (including 107 singletons). The diversity of the cellulolytic fungal community increased between months 4 and 12: 80% of the *cbhI* gene sequences were represented by only 7 ± 1 and 8 ± 1 dominant OTUs during months 0 and 4 while it was 26 ± 2 at month 12.

The most abundant OTUs were OTU 4 and OTU 0, which represented 28% and 12.5% of all sequences, respectively. OTU 4 dominated during month -2 where it represented over 90% of all sequences. Among the other abundant OTUs, OTU 0, OTU 1 and OTU 2 predominated during months 0 and 4 but were almost absent at month 12. In contrast, OTU 3 was the most abundant at month 12 (Figure 5). This observation indicates that, despite the fact that cellulose was available during the entire decomposition process, specific cellulolytic fungi were present during different stages of decomposition. The abundance of one-half of the 30 most abundant OTUs significantly changed over time (Supplementary Table 2).

Phylogenetic analysis of the *cbhI* gene sequences showed that 38% of all sequences clustered with sequences of known fungal taxa with >70% bootstrap support (Supplementary Figure 1). Of these sequences, the majority belonged to the *Basidiomycota* (29%) phylum, which was represented by two OTUs, and the other 9% belonged to seven ascomycetous OTUs. Sequence similarities >97% allowed us to determine the taxonomic affiliation of OTU 11 to the ectomycorrhizal basidiomycete *Russula paludosa* and OTU 46 to the ascomycete *Aureobasidium pullulans*.

Discussion

During the decomposition of *Q. petraea* leaves used in this study, approximately 70% of the total mass was lost within 24 months (Figure 1; Šnajdr *et al.*, 2011). The C/N ratio decreased from 49 to 22 within 12 months and remained constant later. Fungal biomass increased rapidly from low values in the live and senescent leaves to a maximum at month 2 and remained lower but constant until the end of the experiment. The activity of cellulolytic enzymes was detected in live and senescent leaves, which indicates that decomposition started before leaf abscission (Figure 1; Šnajdr *et al.*, 2011). Three distinct decomposition phases have been distinguished that are characterised by the sequential mass loss of extractables and hemicelluloses, cellulose, and lignin (Supplementary Figure 2; Šnajdr *et al.*, 2011). This seems to be consistent with the culture-based observations that divided fungi into early, intermediate and late decomposers (Frankland, 1998; Osono and Takeda, 2001; Tang *et al.*, 2005; Osono, 2007). The culture-dependent studies, however, tend to underestimate the total diversity of fungi and are biased towards rapidly growing species (Hering, 1967; Frankland, 1998). Thus, they do not provide reliable information about fungal communities associated with leaves/litter during its degradation. Despite several limitations (see for example Amend *et al.*, 2010), next-generation sequencing seems to be better suitable to explore the fungal community because it can deliver information at higher quantitative resolution and is not biased towards easily culturable and fast growing taxa.

The live oak leaves used in this study harboured a relatively rich and even fungal community with its diversity comparable to previous reports from *Quercus macrocarpa* (Jumpponen and Jones, 2009a, b). The low biomass of the fungi on live leaves is possibly a consequence of the action of the

protective mechanisms of the plant. After leaf senescence, rapid proliferation of opportunistic *Mycosphaerella* spp. resulted in a sevenfold increase in fungal biomass but a rapid decrease in diversity. The rapid increase in fungal diversity after the litterfall was caused by the invasion of new colonisers and was detected during month 2 (Figure 1). Fungal diversity continued to increase until month 4, which indicates the arrival of new species on the substrate. However, the abundance of the most common fungal genera did not change significantly (Figure 2).

Fungi from the *Ascomycota* phylum prevailed in the live and senescent leaves on the trees (88.5% and 99.5% of amplicons, respectively). These data are in accordance with previous culture-based studies on various trees (Osono, 2002; Santamaría and Bayman, 2005) and the pyrosequencing analyses of live *Q. macrocarpa* leaves (Jumpponen and Jones, 2009a, b). However, the most common genera recorded from a *Q. macrocarpa* phyllosphere in North America were quite distinct on the genus level. Of the major genera, *Microsphaeropsis*, *Alternaria*, *Epicoccum, Aureobasidium*, *Phoma* and *Erysiphe* were detected, and only *Aureobasidium* and *Epicoccum* were recovered from live leaves in this study with a >1% frequency, which demonstrates that either the tree species, geographic distance or different environmental conditions affect the composition of phyllosphere mycoflora.

Some fungi associated with living tree leaves are also found in association with decomposing leaf litter (Koide *et al*., 2005; Osono, 2006). The fact that some live leaf-associated fungi are able to produce extracellular enzymes or decompose sterile senescent leaves (Korkama-Rajala *et al*., 2008; Žifčáková *et al*., 2011) led to the hypothesis that certain taxa may change from endophytism to a saprotrophic strategy. In addition, molecular evidence indicates that fungi cultured from live leaves and decaying litter may indeed belong to the same taxa (Promputtha *et al*., 2007). This study shows that phyllosphere fungi are still quantitatively important during the subsequent stages of decomposition, at least until month 4 (Figures 2 and 4). Establishment in live, nonsenescent leaves created an opportunity for these fungi to readily exploit leaf-derived nutrients during decomposition after leaf senescence. Fungi belonging to the genera *Holwaya*, *Cladosporium*, *Aureobasidium*, *Davidiella* and *Cryptococcus* were predominant in living oak leaves, in senescent leaves nearly disappeared and their abundance rose again in early phases of litter decomposition. The genera *Aureobasidium* and *Cladosporium* contain well-known phyllosphere fungi that have been repeatedly isolated from various trees (Sadaka and Ponge, 2003; Slavikova *et al*., 2007; Unterseher and Schnittler, 2009), and their persistence until early decomposition has been reported (Sadaka and Ponge, 2003). OTUs belonging to the *Mycosphaerella* genus, which comprises pathogenic and saprotrophic species (Suto, 1999), were the most dominant in live leaves and made up a significant portion of the population at month 0 where they represented 90% of sequences, which indicates that they are both endophytes and efficient early saprotrophs.

The first year of our experiment was characterised by a relatively rapid loss of litter mass, a decrease in the C/N ratio and the cellulose content, and a relatively high activity of cellulolytic enzymes, which causes faster decomposition of cellulose (Supplementary Figure 2). These conditions were associated with the continuous dominance of fungi from the *Ascomycota* phylum, which are generally known to selectively decompose cellulose over lignin. Dominance of ascomycetous fungi in the early stages of beech litter decomposition was currently also demonstrated using metaproteomic approach (Schneider *et al*., 2012). Similar results were obtained when 8-week-old *Fagus sylvatica* litter was analysed, except that it also contained a significant proportion of fungi from the *Mucoromycotina* phylum (Aneja *et al*., 2006). Despite the fact that the *Mucoromycotina* fungi are often considered to be opportunistic microorganisms that are associated with nutrient-rich substrates, their abundance in *Q. petraea* litter was low until month 8. Among the fungal genera that were dominant during month 2, *Naevala*, *Cryptococcus* and *Mycosphaerella* were detectable in the live leaves, and the basidiomycetous genera *Athelia* and *Mrakia* appeared anew during month 2 and immediately became dominant, which demonstrates their ability to rapidly proliferate on fresh litter. *Naevala*, *Athelia* and *Cryptococcus* fungi maintained their prevalence until month 4, whereas *Mrakia* fungi nearly disappeared and were replaced by *Polyscytalum* and *Rhodotorula* fungi. Month 8 was characterised by an entirely different fungal community, which was most likely caused by a depletion of the majority of the readily available organic compounds and was associated with a sharp decrease in the phyllosphere fungi. Fungi belonging to the *Glomeromycota* and the *Mucoromycotina* phyla were detectable on litter beginning at month 8, and their abundances gradually increased until the end of the experiment. The fungal genera *Troposporella*, *Guignardia*, *Ramariopsis*, *Sympodiella* and *Cryptococcus* prevailed at month 8. *Troposporella* fungi have been recorded in seasonally flooded soil ecosystems (Carrino-Kyker and Swanson, 2008), where they were most likely involved in the decomposition of allochthonous carbon input. *Troposporella* remained frequent until month 12, whereas basidiomycetous *Sistotrema* and *Rhodocollybia* and lichenised ascomycetous *Dimelaena* fungi appeared for the first time at this stage. *Rhodocollybia* is a typical saprotroph (Valášková *et al*., 2007), and the polyphyletic genus *Sistotrema* contains both ectomycorrhizal and decomposer fungal species (Di Marino *et al*., 2008; Boberg *et al*., 2011).

During the second year, the rate of litter mass loss was relatively slow and the activity of cellulolytic enzymes decreased, which indicated that the easily accessible polysaccharides were depleted. Also, the substrate was richer in the recalcitrant lignin and nitrogen and characteristic with the increased activity of ligninolytic enzymes (Supplementary Figure 2). Fungi from the *Basidiomycota* phylum distinctively dominated over fungi from the *Ascomycota* phylum at month 24. In previous studies, basidiomycetous species, particularly the saprotrophic cord formers, have often been demonstrated to be late litter decomposers (Osono, 2007; Duong *et al.*, 2008) because of their capability to synthesise enzymes required for the degradation of complex polymers (Baldrian, 2008). Interestingly, basidiomycetous cord formers were not among the most frequent taxa observed in our study. Instead, the basidiomycetous yeast genus *Trichosporon* comprised 50% of all sequences at month 24. This is consistent with a recent report that identified this species as the second most abundant cellulose decomposer in litter using stable isotope probing (Štursová *et al.*, 2012). Although the cellulose content of the litter at this stage is relatively low (Šnajdr *et al.*, 2011), it may be still sufficient to support the growth of yeasts and may not be sufficient to support the growth of the large mycelia of basidiomycetous cord formers. There is only a small overlap of the fungal community in litter and in the uppermost (organic) soil horizon at the site of study (unpublished data) with >50% of fungi in soil being ectomycorrhizal. Among the genera reported in this study, *Mortierella*, *Cryptococcus*, *Trichosporon*, *Ambispora* and *Naevala* are also frequent in soil, which can serve as a reservoir for their spread.

This study demonstrated that fungal succession during litter decomposition is much faster than so far expected from the culture-based studies (Figure 2). The fast appearance–disappearance of fungal taxa seems to contrast with the reported persistence of DNA from inactive fungi in decaying wood (Rajala *et al.*, 2011) and to support the rapid turnover of early/intermediate/late saprotrophs (Lindahl and Finlay, 2006). The successional changes are likely governed not only by the relatively slow changes of the polysaccharide, lignin and nitrogen content in litter but possibly by other factors including more subtle changes in litter chemistry and interspecific fungal interactions.

Cellulose is the major polysaccharide in plant litter, and cellulose-degrading enzymes are thus an obvious target for the study of the decomposing microorganisms. Among these, cellobiohydrolases (exocellulases), which catalyses the rate-limiting step of cellulose decomposition (Baldrian and Valášková, 2008), and the *cbhI* gene represent suitable markers for the study of cellulolytic fungi (Edwards *et al.*, 2008; Weber *et al.*, 2011; Baldrian *et al.*, 2012). Here, we show that several exocellulase-producing fungi are present in the live leaves of *Q. petraea* although the community is dominated by a single species (Figure 5). This OTU of *cbhI* genes was assigned to fungi from the *Basidiomycota* phylum, which is surprising if we consider the dominance of fungi from the *Ascomycota* phylum at this stage. The diversity of cellulolytic fungi is high in senescent leaves with 58 observed and >200 predicted OTUs. As the leaves are still attached to the trees, these fungi must have colonised the substrate before its contact with soil. Later in decomposition, estimates of *cbhI* richness were approximately 200 in number, which indicates that there are approximately 100 cellulolytic fungal species when multiple copies of the gene per fungal genome are considered (Edwards *et al.*, 2008; Weber *et al.*, 2011). The overall fungal diversity did not correlate with the diversity of fungi harbouring the *cbhI* gene, which indicates that the proportion of cellulolytic fungi changes during decomposition; no clear link was observed between the diversity of the *cbhI* genes and the activity of cellobiohydrolase. Although cellulose represents a substrate that is present in the litter during the entire decomposition process, the community of cellulolytic fungi also showed successive changes similar to those of the total fungal community with dominant OTUs appearing and disappearing (Figure 5). This may indicate that the individual cellulolytic fungi have specific additional nutritional requirements or competitive abilities. Interestingly, the sequences dominating in the *cbhI* gene pool at month 12 belonged to the ectomycorrhizal genus *Russula*. Our results are consistent with previous observations that identified cellulases and class II peroxidases in these fungi (Bodeker *et al.*, 2009; Štursová *et al.*, 2012) that may combine the mycorrhizal and saprotrophic lifestyle to some extent.

This study demonstrates that the composition of the fungal community changes with changing litter quality much faster than previously thought. Furthermore, similar changes during the decomposition process are observed among cellulolytic fungi, which indicates that succession is not only driven by the availability of the major nutrient sources but also by other factors, perhaps other nutritional requirements or the competitive abilities of individual taxa. The initial steps of decomposition where fungi dominate the decomposer community are characterised by a high involvement of fungi that occur on the live leaves of the tree. However, further research on both the structural and functional aspects of fungal community composition, for example, use of the metatranscriptomic or metaproteomic approaches, are needed to better understand the functional role of individual fungal taxa during decomposition.

Conflict of Interest

The authors declare no conflict of interest.

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