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FUNGAL MICROBIOLOGY

Small-scale Diversity and Succession of Fungi in the Detritusphere of Rye Residues

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Abstract Transport of litter carbon in the detritusphere might determine fungal abundance and diversity at the small scale. Rye residues were applied to the surface of soil cores with two different water contents and incubated at 10°C for 2 and 12 weeks. Fungal community structure was analysed by constructing clone libraries of 18S rDNA and subsequent sequencing. Litter addition induced fungal succession in the adjacent soil and decreased detectable fungal diversity mainly due to the huge supply of substrates. Ergosterol content and *N*-acetyl-glucosaminidase activity indicated fungal growth after 2 weeks. Simultaneously, the structure of the fungal community changed, with *Mortierellaceae* proliferating during the initial phase of litter decomposition. Ergosterol measurements were unable to detect this early fungal growth because *Mortierellaceae* do not produce ergosterol. In the late phase during decomposition of polymeric substrates, like cellulose and chitin, the fungal community was dominated by *Trichocladium asperum*. Water content influenced community composition only during the first 2 weeks due to its influence on transport processes in the detritusphere and on competition between fungal species. Our results underline the importance of species identification in understanding decomposition processes in soil.

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Introduction

Fungi form a diverse group of organisms with an estimated 1.5 million species, of which most are unknown [13]. Soil is an important fungal habitat because many fungi spend at least some part of their life cycles in soils [2]. Until recently, however, methodological limitations hampered studying this great diversity. Isolation and culture approaches, for example, favour fast-growing fungi, whereas it is not possible to grow many other species in culture [2]. Molecular techniques offer the opportunity to detect rare, not-cultivable species and, thereby, to identify novel taxa of the soil fungal community [18, 27]; this yields further insight into the ecology of soil fungi.

Fungi have many important functions in soils. For example, saprotrophic fungi are involved in the decomposition of soil organic matter and recycling of nutrients, whereas mycorrhizal fungi improve plant growth by providing additional nutrients [25]. Despite these important roles, little is known about what governs fungal diversity in soils and its spatiotemporal distribution [23, 35]. Substrate supply and distribution are among the factors influencing fungal diversity. For example, Waldrop et al. [36] observed a unimodal relation between substrate supply and fungal diversity, but none between plant diversity and fungal diversity. Differences in substrate quality induced a shift in the fungal community between

soil horizons of a boreal forest [23]. In that study, saprotrophic fungi like *Mycena* and *Marasmius* spp. were mainly associated with relatively fresh litter on the soil surface, whereas mycorrhizal fungi, like *Cortinarius* and *Piloderma* spp., dominated the more decomposed litter and humus horizons. The influence of substrate quality was also observed by Wallenstein et al. [37], who detected a shift in the abundance of *Ascomycota* and *Zygomycota* between tussock and shrub soils of the arctic tundra. Likewise, substrate characteristics change during litter decomposition, inducing shifts in the community of fungal decomposers [16]. Generally, succession of fungi on litter starts with phyllosphere fungi, which contribute only slightly to decomposition of soluble compounds [28]. They are followed by saprotrophs utilising soluble compounds and, finally, by fungi, which degrade more complex substrates like cellulose and lignin [8, 16]. Beside changes in substrate quality, water availability at constant water content increases during litter decomposition [4]. This in turn affects fungal succession with xerotolerant fungi growing during the initial stage and species less tolerant to water stress during later stages. Most of the studies on fungal succession on decomposing litter were related to fungal communities attached to the litter. However, it is known that fungi are actively involved in the bidirectional transport of litter C and soil mineral N [9], and that soil moisture significantly affects fungal community structure [24].

The present study therefore aims to identify fungal succession in soil adjacent to decomposing litter by construction of clone libraries based on the 18S rDNA. In the detritusphere, soluble litter C is transported into the adjacent soil, which induces gradients of increased microbial activity and C turnover in the soil as far as 4 mm from the litter layer [10, 20]. In previous studies, we found this process to differ at temporal as well as spatial scales, depending on the soil water content and the transport mechanisms of the soluble litter C [29, 30]. We hypothesised that soil fungi in the detritusphere show similar successional stages as litter-born fungi. We further expected that fungal succession starts after a lag phase of 2 weeks, when biomass markers indicated fungal growth [30]. Finally, we hypothesised that soil moisture affects fungal succession during litter decomposition.

Methods

Soil and Plant Residues

Soil was sampled from the long-term field experiment in Rotthalmünster (Germany, 48° 21' N, 13° 12' E) near the Danube River in September 2002. Samples were taken from the clay–loamy topsoil of a Stagnic Luvisol (WRB) [pH ($CaCl_2$) 5.5, total C content 12.6 g kg^{-1}, total N content 1.6 g kg^{-1}]. Wheat has been cultivated at the site in monoculture for the last 36 years with NPK fertilisation (171 kg N ha^{-1}). After sampling, the soil was sieved (<2 mm) and stored at −20°C to minimise disturbance by soil faunal activity during the experiments. For the incubation, rye residues with a C/N ratio of 40 were chosen. Rye residues were stored air-dried until the start of the experiment.

Experimental Design

The experiment consisted of four treatments with two different matric potentials and without or with litter addition: (1) −316 hPa without litter (control), (2) −316 hPa with litter addition, (3) −63 hPa without litter (control), and (4) −63 hPa with litter addition. For the sake of simplicity, soils at −316 and −63 hPa will be considered below as dry and wet, respectively.

For a detailed description of microcosm preparation, see Poll et al. [30]. Briefly, soil cores of 1.5 cm height (density=1.2 g cm^{-3}) were placed into air-tight glass containers (microcosm, 750 mL). Each soil core of litter treatments was covered by 0.3 g of rye residues. The microcosms were incubated at 10°C. After 3, 7, 14, 28, 56, and 84 days, microcosms of each treatment were sampled destructively. Based on previous results of ergosterol contents and N-acetyl-glucosaminidase activity, which indicated a strong increase in fungal biomass between days 14 and 28 (Table 1) [30], we selected samples taken after 14 and 84 days for the following analyses. We expected these two sampling dates to cover two different stages of fungal succession. Controls without litter addition were not analysed since ergosterol content and *N*-acetyl-glucosaminidase activity previously indicated a stable fungal biomass in control soils [30]. During the experiment, loss of water was minimal.

Sample Preparation

After incubation, the litter was removed from the soil cores, which were immediately frozen at −20°C. Subsequently, four soil cores of each treatment were sliced on a cryostat microtome (HM 500 M, MICROM International GmbH, Walldorf, Germany) in 0–1-, 1–2-, and 2–3-mm slices in sequence from the top of the core to obtain a good representation of the fungal community within the first 3 mm of the detritusphere. The respective slices of two of the four sampled soil cores were pooled to obtain a sufficient amount of soil for analyses. This yielded six (three depths × two pooled soil cores) replicates for each treatment.

Table 1 Microbial biomass, ergosterol content and *N*-acetyl-glucosaminidase activity in three layers of increasing distance to the litter layer and at two different water contents 14 and 84 days after litter addition

		Microbial biomass (μg g^{-1})		Ergosterol (μg g^{-1})		N-acetyl-glucosaminidase (nmol $g^{-1}h^{-1}$)	
		14 days	84 days	14 days	84 days	14 days	84 days
−63 hPa	0–1 mm	401.7 (46.7)	639.7 (34.1)	0.63 (0.07)	1.40 (0.28)	193.0 (20.4)	778.4 (98.9)
	1–2 mm	259.1 (5.0)	309.0 (55.1)	0.50 (0.12)	1.05 (0.06)	123.6 (12.4)	400.0 (24.9)
	2–3 mm	241.0 (20.5)	336.3 (11.7)	0.46 (0.01)	1.15 (0.03)	141.2 (2.3)	260.8 (40.1)
−316 hPa	0–1 mm	479.8 (52.1)	555.6 (43.4)	0.43 (0.06)	2.18 (0.27)	174.2 (34.9)	751.4 (137.6)
	1–2 mm	274.3 (14.1)	579.9 (41.7)	0.39 (0.05)	1.17 (0.14)	128.7 (5.9)	423.3 (86.2)
	2–3 mm	237.5 (33.9)	381.6 (32.2)	0.49 (0.10)	0.94 (0.06)	125.4 (14.2)	313.7 (39.4)

Values in parentheses are standard deviations of three replicates. All data are taken from Poll et al. [30]

DNA Extraction and Polymerase Chain Reaction Amplification

Fourteen and 84 days after litter addition, DNA was extracted from the three layers. To characterise the initial fungal communities in soil and litter, two subsamples of each were included into the phylogenetic analyses. Total community DNA was extracted from 0.3 g soil using the FastDNA Spin Kit for soil (BIO101, MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. The quantity of the extracted DNA was checked with a BioPhotometer (Eppendorf AG, Hamburg, Germany).

The primer set NS23 and NS24 was used to amplify a 600–800-bp fragment of the fungal small-subunit rDNA [11]. Each 25-μL polymerase chain reaction (PCR) contained 1× PCR standard buffer ($MgCl_2$), 200 μM of each dNTP, 1.25 mM of each primer, 1 U of *Taq* polymerase (Eppendorf AG), and approximately 10 ng of soil DNA. The following PCR conditions were applied: initial denaturation at 94°C 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 49°C for 45 s, and elongation at 72°C for 1 min. Cycling was completed by a final elongation step at 72°C for 7 min. Following PCR, products were purified using the QIAquick Purification Kit (Qiagen GmbH, Hilden, Germany).

Cloning and Sequencing

The PCR products were cloned using *Escherichia coli* and the pCRII-TOPO TA Cloning kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. From each of the 28 clone libraries, 36 positive colonies were randomly sampled and added to PCR mixtures containing the primers M13 F and R (Invitrogen). The following PCR conditions were applied: initial denaturation at 94°C for 10 min, followed by 25 cycles of 94°C for 30 s, 55°C for 45 s, 72°C for 1 min, and a final step at 72°C for 7 min. After PCR, products were purified using the QIAquick Purification Kit (Qiagen GmbH) and sent to GATC Biotech AG (Konstanz, Germany) for sequencing. All sequences have been submitted to GenBank (EF628508–EF629260, EF629262, EF629263, EF629265–EF629267, EF629269–EF629284, EF629286–EF629307, EF629309, and EF629317–EF629320).

Sequence Analyses and Identification

All sequences derived from one clone library were aligned and the number of operational taxonomic units (OTUs) in each clone library was determined by the following phylogenetic method. We applied MAFFT to align the sequences using the *fft-nsi* option [21] and a fast neighbour-joining algorithm using the Kimura-2-parameter substitution model as implemented in Paup* [33].

Each sequence of all samples was compared to published sequences in GenBank using BLAST [1]. Sequences with similarity above 95% were assigned to the published sequences. If more than one published sequence was detected, the sequence with the highest similarity score was assigned. The identified sequences and the number of OTUs for each clone library were cross checked to reveal misidentification of sequences with low quality. Sequences with low similarity (<95%) or significant similarity to different phylogenetic groups at both ends of the sequences were removed as candidates for chimeric PCR products. Sequences with affiliation to a phylogenetic group, but with less than 95% identity to a published sequence, were treated as new OTUs of the respective group. The taxonomic classification was based on the system published by Hibbett et al. [14].

Data Analyses

The numbers of observed OTUs were tested for significant differences by one-way and two-way ANOVA. Sample-based rarefraction curves were calculated using

EstimateS [3]. Therefore, we pooled the six replicates (2 replicates*3 depths) of each treatment and sampling date to obtain a better coverage of total diversity. For calculating ACE, a non-parametric estimator of total species (OTU) richness [17], we pooled sequences of all soil samples because we did not expect any fungal species to completely disappear during the experiment. According to Tiunov and Scheu [34], a discriminant function analysis preceded by multidimensional scaling was performed to test for shifts in the fungal community (STATISTICA 6.0, Statsoft, Tulsa, OK, USA).

Results

A total number of 812 sequences were obtained, of which 123 were most probably chimeric (15.2%) and 10 represented soil animals. The remaining 679 sequences belonged to three fungal phyla and one subphylum, with *Ascomycota*, *Mucoromycotina*, *Basidiomycota*, and *Chytridiomycota* representing 417, 216, 37, and 9 sequences, respectively. The relative abundance of fungal groups at phylum level shifted during the experiment (Fig. 1). The initial fungal community was dominated by ascomycetes, with only small differences between soil and litter, whereas, 14 days after litter addition, more than 65% of the fungi were *Mucoromycotina*. Twelve weeks after litter addition, more than 90% of the sequences were obtained from ascomycetes. Water content induced only small differences.

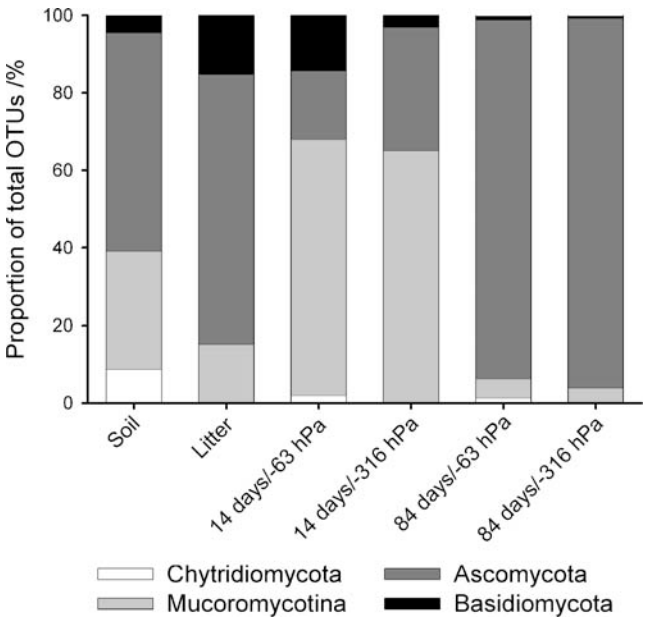

Figure 1 Relative abundance of fungal phyla based on 18S rDNA analysis. Values are means of two replicates for soil and litter and of six replicates for all other samples

Among the 679 fungal sequences, 51 different OTUs were identified (Table 2). The sequences assigned to the *Mortierellales* were difficult to distinguish due to low sequence divergence and, therefore, these sequences were pooled together for data analyses. This reduced the number of different OTUs to 44. Although the initial fungal communities in soil and litter were similar at the phylum level, OTU richness was much higher in soil than in litter (Fig. 2). OTU richness was significantly affected by soil water content ($F_{1,20}$=9.29) and time ($F_{1,20}$=28.45), with the highest diversity found in wet soil 14 days after litter addition. These differences in fungal diversity were confirmed by sample-based rarefraction curves, which showed the highest diversity for the same treatment (significant at approximately 95% confidence interval, Fig. 3). Since rarefraction curves did not reach saturation, the true OTU richness probably lies above the observed values. Calculating the non-parametric richness estimator ACE, the total OTU richness in experimental soils was estimated to be at least 50.9 OTUs.

Fungal communities were compared by discriminant analysis based on relative abundance of OTUs yielding two axes, which added significantly to the model and explained nearly 84% of the total variation (Fig. 4). Overall, differences in OTU richness correlated to a shift in fungal community composition during the experiment. The clone libraries of the initial soil and litter were clearly separated from libraries of incubated samples. Litter addition induced fungal succession, with samples taken after a 14-day incubation differing significantly from samples taken after 84 days. Wet and dry soils were divided only 14 days after litter addition, although not significantly. After 12 weeks, no differences were observed. The correlation between identified species and discriminant axes provides information about which species contributed to the separation of groups. The most important species were *Cladosporium cladosporioides*, *Trichocladium asperum*, *Waitea circinata*, *Amylomyces rouxii*, *Claviceps pupurea*, *Spizellomycete* sp. *JEL371*, and *Motierella* spp. (Table 3). The most abundant fungi revealed different temporal patterns (Fig. 5). *Mucoraceae* and *Clavicipitaceae*, as well as *Spizellomycete* sp. *JEL371* were strongly present in the initial soil community, but were rare in soils after litter addition. The same was true for the dominant litter fungi *Mucoraceae*, *Mycosphaerellaceae* (*C. cladosporioides*), and *Ceratobasidiaceae* (*W. circinata*). Two weeks after litter addition, *Mortierellaceae* showed a strong increase, whereas abundance fell to the initial level after 84 days. A similar pattern was found for *Thamnidiaceae* and the genus *Fusarium*. Twelve weeks after litter addition, the fungal community was dominated by *T. asperum*, which represented approximately 79% and 87% of the identified sequences in the wet and dry soil, respectively. Other fungi, which showed their highest abundance 84 days after litter

Table 2 Fungal taxa which were most similar to sequences obtained from soil and litter clone libraries

Phylum, order, family, BLAST match	Accession	Ecology	Frequency
Chytridiomycota			
Spizellomycetales			
Spizellomycetaceae			
Spizellomycete sp. JEL371	DQ536490	S/P	1.33
Mucoromycotina			
Mortierellales			
Mortierellaceae			
JAB SMS 01_G09	AY646704	S[a]	1.33
Mortierella alpina	AJ271630	S	2.65
Mortierella chlamydospora	AF157143	S	3.39
Mortierella parvispora	AY129549	S	0.29
Mortierella verticillata	AF157145	S	8.54
S_Canopy_300_02_11	AY382451	S[a]	4.42
soil clone RSC-CHU-46	AJ506020	S[a]	3.09
New OTU of Mortierellaceae	EF628739	S[a]	0.88
Mucorales			
Mucoraceae			
Amylomyces rouxii	AY054697	S	4.86
Mucor plumbeus	AF548078	S	0.15
Rhizomucor variabilis	AF113435	S	0.88
Thamnidiaceae			
Backusella ctenidia	AF157122	S	1.33
Ascomycota			
anamorphic Hypocreales			
Fusarium culmorum	AF548073	PP	0.29
Fusarium oxysporum	AB110910	PP	3.53
New OTU of Hypochreales	EF628810	?	0.74
Coniochaetales			
Coniochaetaceae			
Lecythophora lignicola	AJ496246	S/W	1.33
Eurotiales			
Trichocomacaceae			
Penicillium brevicompactum	AF548085	S	0.44
Penicillium chrysogenum	AF411201	S	0.44
Helotiales			
Bulgariaceae			
Bulgaria inquinans	DQ471008	W	0.15
Hypocreales			
Clavicipitaceae			
Claviceps purpurea	AB160993	PP	0.59
Cordyceps sinensis	AB067700	IP	0.74
Paecilomyces carneus	AB258369	IP[a]/K[a]	0.15
Paecilomyces marquandii	AB114223	K	0.15
New OTU of Clavipitaceae	EF628795	E[a]	0.88
mitosporic Ascomycota			
Pezizomycotina clone Sey062	AY605205	?	0.88
Phoma herbarum	AY337712	PP/N	0.74
Trichocladium asperum	AY706336	S	39.62
Pezizales			
Pezizaceae			
Peziza echinispora	AF006309	W	2.21

Table 2 (continued)

Phylum, order, family, BLAST match	Accession	Ecology	Frequency
Pyronemataceae			
Inermisia terrestris	Z30241	C	0.29
Pleosporales			
Pleosporaceae			
Alternaria sp. CBS 174.52	DQ678016	S/N	0.15
Pleosporales sp. GFL014	DQ085396	S/N	0.15
Pyrenophora trichostoma	U43459	PP	0.44
Saccharomycetales			
Saccharomycetaceae			
Candida sp. MA6	DQ438181	S	0.15
Zygowilliopsis californica	Y12108	S	0.15
Sordariales			
Phyllachoraceae			
Plectosphaerella cucumerina	AF176951	NP	0.15
Thelebolales			
Thelebolaceae			
Thelebolus stercoreus	AY942194	C	0.74
Thelebolus stercoreus	AY942192	C	1.47
incertae sedis			
Mycosphaerellaceae			
Cladosporium cladosporioides	AF548071	S	4.86
Basidiomycota			
Agaricales			
Bolbitiaceae			
Conocybe lactea	DQ437683	S	0.44
Tricholomataceae			
Hemimycena gracilis	DQ440644	L	0.29
Marasmius sp. MCA1611	AY916724	L[a]	0.44
Xeromphalina campanella	DQ465344	L	0.44
New OTU of Tricholomataceae	EF628704	?	0.59
Cantharellales			
Ceratobasidiaceae			
Waitea circinata	D85647	M	1.62
Filobasidiales			
Bullera sp. VY-120	AB110692	S[a]	0.15
Cryptococcus clone RSC-CHU-60	AJ506031	S[a]	0.44
Cryptococcus terreus	AB032649	S	0.29
Polyporales			
Albatrellaceae			
Albatrellus flettii	AF518569	S/M	0.15
Phanerochaetaceae			
Lopharia mirabilis	AY293141	W	0.15
incertae sedis			
New OTU of Agaricomycetes	EF628876	?	0.44

Frequency gives the proportion of each clone within the total number of 679 clones.

C coprophilic, *E* endophytic, *IP* insect pathogenic, *K* keratinophilic, *L* litter decaying, *M* mycorrhizal, *N* necrotic, *NP* nematophagous, *PP* plant pathogenic, *S* saprobic, *W* wood decaying

[a] Probable ecology based on knowledge of closely related species of the same genus

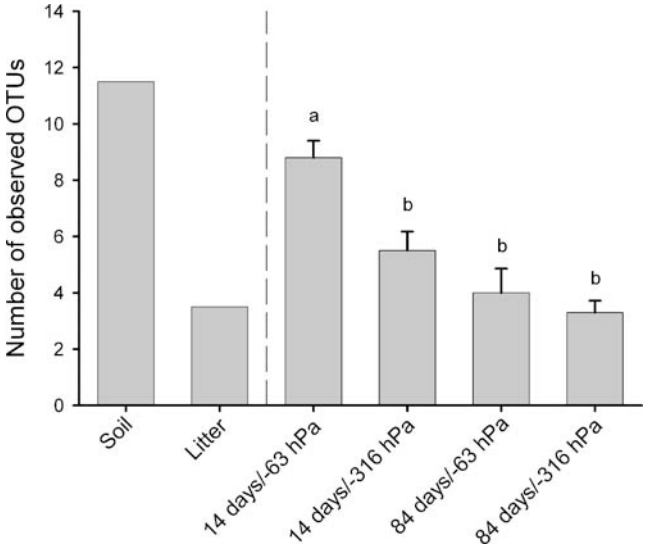

Figure 2 Observed OTU richness of fungal communities. *Error bars* indicate standard error of six replicates. *Different letters* indicate statistically different groups (Tukey-B, $P<0.05$)

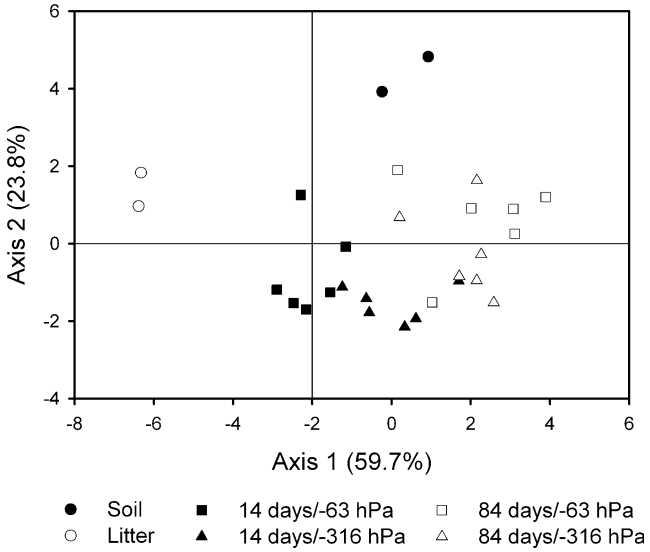

Figure 4 Discriminant analysis showing the separation of fungal communities due to time of incubation and soil water content. *Axis 1* was significant at $P<0.001$, *axis 2* at $P<0.01$

addition, were associated with the families *Pezizaceae* and *Coniochaetaceae*.

The ecology of most fungi is given in Table 2. The greatest functional diversity was found in the initial soil fungal community (Fig. 6). During the experiment, saprotrophic fungi became more dominant, representing more than 85% of the sequences after 84 days. Additionally, a greater proportion of probably wood-decaying fungi was found at the end of the experiment.

Discussion

Cloning and sequencing of the fungal 18S rDNA was suitable to detect fungal succession within the detritusphere of rye residues. We obtained a good coverage of approximately 85% of the estimated total fungal richness and probably detected the most abundant fungi. The estimated richness of approximately 50.9 OTUs in our soil is relatively low compared to other studies. For example, Jumpponen and Johnson [19] estimated the total species richness of a grassland soil to be 127 to 175, and Lindahl et al. [23] found 100 different genotypes among

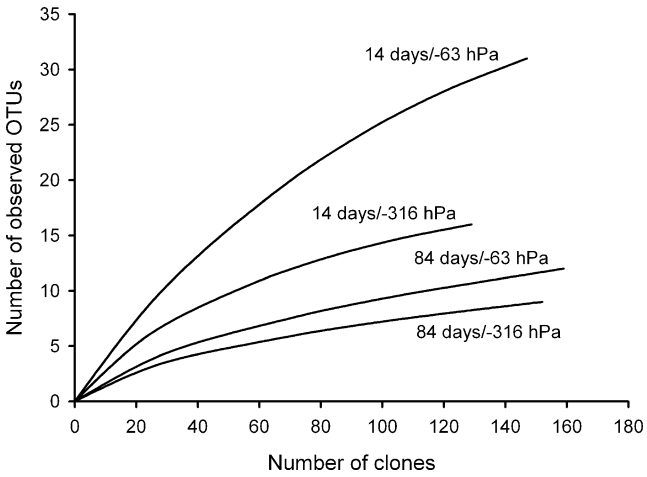

Figure 3 Sample-based rarefraction curves of observed fungal diversity. Replicates of each treatment are pooled together

Table 3 Linear correlation (r values) between frequency of fungal clones (proportion of the total number of clones) and discriminant axes

Clone	Axis 1	Axis 2
Cladosporium cladosporioides	−0.715***	0.213
Trichocladium asperum	0.702***	0.151
Waitea circinata	−0.601**	0.181
Alternaria sp. CBS 174.52	−0.486**	0.107
Lecythophora lignicola	0.382*	−0.128
Claviceps purpurea	0.158	0.674***
Amylomyces rouxii	−0.309	0.641***
Spizellomycete sp. JEL371	0.041	0.622***
Mortierella spp.	−0.323	−0.611***
Clavicipitaceae sp	−0.038	0.560**
Candida sp. MA6	0.071	0.534**
Cordyceps sinensis	0.071	0.534**
Inermisia terrestris	0.174	0.491**
Paecilomyces marquandii	−0.018	0.434*
Bulgaria inquinans	−0.018	0.434*
Bullera sp. VY-120	−0.018	0.434*
Backusella ctenidia	−0.200	−0.413*

*$P<0.05$; **$P<0.01$; ***$P<0.001$

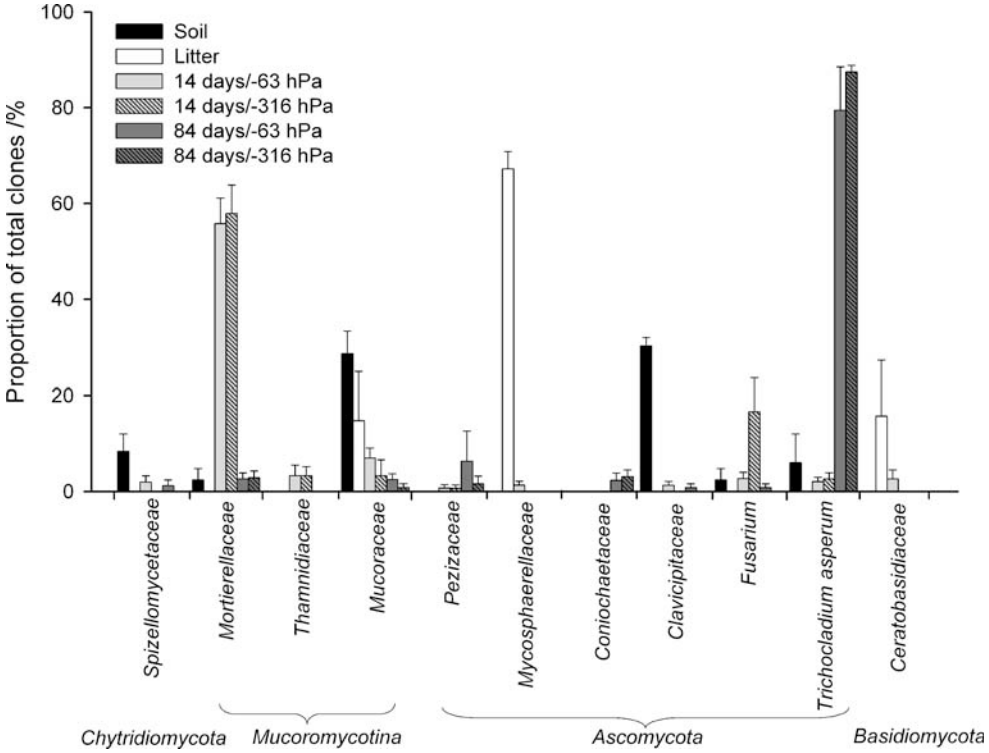

Figure 5 Relative abundance of most abundant fungal groups (>2.5% in at least one treatment). Fungal groups showing no response to time of incubation were excluded. *Error bars* indicate standard error of six replicates

248 sequenced clones of a forest soil. This discrepancy in fungal diversity might be because we used an arable soil, which represents a highly disturbed ecosystem. Wu et al. [39], for example, observed that disturbance by land management practices significantly reduced fungal diversity. This is in accordance with a low fungal biomass in soils with a high cultivation intensity [32].

We previously found a succession of *r* and *K* strategists in soil adjacent to decomposing litter, which we explained by the availability of substrates of different quality and quantity during the initial and later stages of litter decomposition [30]. Since fungi are a heterogeneous group with a huge range of physiological capabilities [5], we expected a succession of soil fungi comparable to the succession of fungi attached to decomposing litter. Overall, we detected an increasing abundance of saprotrophic fungi in the soil during the experiment. This agrees with the finding that, due to their capabilities to degrade organic polymers, saprotrophic fungi predominate in soil habitats where litter is the primary C source [15]. In contrast, mycorrhizal fungi predominate in habitats with root exudates as the primary C source, although they might be able to degrade polymers as well.

The change in relative abundance of OTUs throughout the experiment further supports the hypothesis of fungal succession in soil during litter decomposition. The initial litter was dominated by *Mucoraceae*, *W. circinata*, and *C. cladosporioides*. The latter is a typical fungus of the phylloplane of living and senescent leaves [5]. Due to its low competitive abilities compared to soil microorganisms [31], *C. cladosporioides* did not become established in the soil. The initial soil community was dominated by *Mucoromycotina* and *Ascomycota*. Litter addition induced

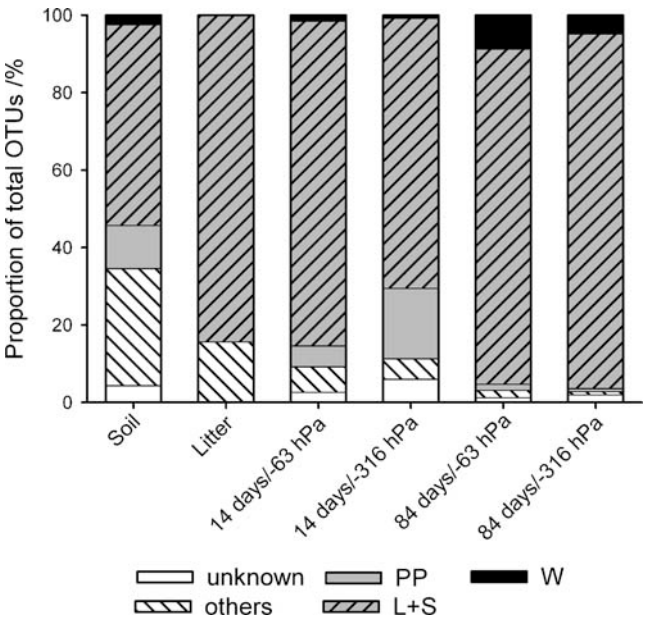

Figure 6 Relative abundance of ecological groups of fungi based on assignment in Table 2. Values are means of two replicates for soil and litter and of six replicates for all other samples. *Others*, coprophilic+endophytic+insect pathogenic+keratinophilic+mycorrhizal+necrotic+nematophagous; *PP*, plant pathogenic; *L*, litter decaying; *S*, saprobic; *W*, wood decaying

a shift towards *Mucoromycotina*, which was mainly related to the high abundance of *Mortierellaceae*, even though *Backusella ctendia* and *Fusarium* spp. benefited from litter addition as well. Members of *Mortierellaceae* are known to be fast-growing fungi capable of rapidly exploring new substrates and mainly utilising simple soluble substrates [22]. Due to these properties, they belong to the pioneer colonisers of freshly fallen litter [5] and typically dominate the utilisation of freshly added substrates in arable soils even many years after abandonment [35].

The increase in fast-growing fungi at day 14 contrasts our hypothesis of a delayed succession of soil fungi after litter addition. Previous results showed an increase of the fungal biomarkers ergosterol and *N*-acetyl-glucosaminidase activity between sampling days 14 and 28 [30]. Ergosterol is the predominant fungal sterol and is often used as an indicator for fungal biomass [6], which might be correct for higher fungi of the *Ascomycota* and *Basidiomycota*. However, *Chytridiomycota* do not produce ergosterol [26], whereas the *Zygomycota* are intermediate, with some fungi producing ergosterol and some not. Since fungi belonging to the genus *Mortierella* produce desmosterol and cholesterol as main sterols [38], an increase in their biomass, as in our study, will not be detected by measuring the ergosterol content. *N*-acetyl-glucosaminidase is involved in the degradation of chitin, which, in soil, is mainly derived from dead fungal hyphae [7]. Therefore, more dead hyphae after the death of pioneer colonisers might explain the delayed increase in *N*-acetyl-glucosaminidase activity compared to the growth of *Mortierella* species.

Twelve weeks after litter addition, the fungal community was dominated by ascomycetes, which are generally able to degrade more complex substrates like cellulose [5]. These substrates were decomposed during the late phase of the incubation [30], thereby inducing a shift from *Mucoromycotina* to *Ascomycota*. At a lower taxonomic level, *T. asperum* strongly dominated the fungal community, reflecting its high potential to degrade cellulose and chitin [7]. Throughout the incubation, basidiomycetes were only of minor importance. Generally, basidiomycetes are important during the final stages of litter decomposition, mainly in forest ecosystems, because they degrade lignin [8]. The arable soil in our study, however, might have been less favourable for basidiomcytes, and the incubation time might have been too short for the establishment of a basidiomycetes-dominated fungal community.

The shift in dominating soil fungi was accompanied by a change in observed fungal diversity. The detected diversity of soil fungal communities declined after litter addition. This might support results of Waldrop et al. [36], who found a unimodal relationship between substrate supply and fungal diversity. At low resource availability, fungal diversity increases as substrate supply rises because more species meet their minimum requirements. However, as substrates become increasingly abundant, processes like competitive exclusion may reduce fungal diversity. In our experiment, adding litter provided a huge amount of substrates, which might have favoured certain highly competitive species. Consequently, fungal diversity was reduced in terms of evenness, and only the most abundant fungi were detectable, whereas other fungi were below the detection limit.

Fungal activity is known to be limited only at very low matric potentials [12]. In our experiment, we adjusted soil cores to moderate matric potentials of −63 and −316 hPa, and hence, great shifts in the fungal community due to water stress were unlikely. However, even at moderate soil moisture, differences in water potential may affect competition between fungal species [5] and, therefore, we expected soil moisture to affect fungal succession. Soil water content induced only small differences at the phylum level. At a finer taxonomic resolution, however, water content had an influence at day 14. Such an impact was also shown by McLean and Huhta [24], who found a higher species richness in soils of fluctuating vs uniform soil moisture. Another reason for the effect of water content, besides differences in the competitiveness, might be the transport of soluble C. Our previous studies revealed that soluble litter compounds were transported mainly during the first 2 weeks [30] and that this was significantly influenced by the water content [29]. Here, different concentrations of litter C in the soil solution might have induced the activity of different soil fungi. After exhaustion of soluble litter compounds, transport processes and, therefore, soil moisture might have become less important to soil fungi, and fungal succession was probably driven by degradative capabilities of fungi.

Conclusion

Our results revealed a decrease in detectable fungal diversity and a shift in relative abundance of dominant OTUs already 2 weeks after addition of rye residues. The temporal pattern of litter decomposition—with utilisation of soluble substrates during the initial phase and subsequent degradation of polymeric substances—defined fungal succession in soil that was comparable to that in litter. After 2 weeks of incubation, pioneer colonisers like *Motierellaceae* were most competitive because they grow rapidly on simple substrates. At the end of the incubation, those fungi capable of degrading polymeric substrates dominated, with *T. asperum* being most prominent. Water content might have influenced competitiveness of fungal species, as well as substrate transport mainly during the initial stage of litter decomposition; this yielded different community structures 2 weeks after litter

addition. Finally, our study underlined the importance of species identification, since both ergosterol content and *N*-acetyl-glucosaminidase activity as indicators for fungal biomass were not able to detect fungal decomposers during the initial phase.

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Authors contribution: C. Poll was responsible for the experimental design and work, conducted the final statistic analyses, and wrote the manuscript; T. Brune conducted the construction of the clone libraries, the sequencing of 18S, and sequence quality control; D. Begerow was responsible for the design of the molecular experiment and data analysis of sequences and supervised the molecular work of T. Brune; E. Kandeler was responsible for the overall experimental design and concept and supervised the Ph.D. of C. Poll. All authors read and approved the final manuscript.

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