Microb Ecol (2009) 58:98–107
DOI 10.1007/s00248-008-9458-8

ENVIRONMENTAL MICROBIOLOGY

Diversity and Decomposing Ability of Saprophytic Fungi from Temperate Forest Litter

Ariana Kubartová · Jacques Ranger · Jacques Berthelin · Thierry Beguiristain

Received: 21 May 2008 / Accepted: 30 September 2008 / Published online: 4 November 2008

Abstract This study was designed to examine saprophytic fungi diversity under different tree species situated in the same ecological context. Further, the link between the diversity and decomposition rate of two broadleaved, two coniferous and two mixed broadleaved-coniferous litter types was targeted. Litter material was decomposed in litter bags for 4 and 24 months to target both early and late stages of the decomposition. Fungal diversity of L and F layers were also investigated as a parallel to the litter bag method. Temperature gradient gel electrophoresis fingerprinting was used to assess fungal diversity in the samples. Mass loss values and organic and nutrient composition of the litter were also measured. The results showed that the species richness was not strongly affected by the change of the tree species. Nevertheless, the community compositions differed within tree species and decomposition stages. The most important shift was found in the mixed litters from the litter bag treatment for both variables. Both mixed litters displayed the highest species richness (13.3 species both) and the most different community composition as compared to pure litters (6.3–10.7 species) after 24 months. The mass loss after 24 months was similar or greater in the mixed litter (70.5% beech–spruce, 76.2% oak–Douglas-fir litter) than in both original pure litter types. This was probably due to higher niche variability and to the synergistic effect of nutrient transfer between litter types. Concerning pure litter, mass loss values were the highest in oak and beech litter (72.8% and 69.8%) compared to spruce and D. fir (59.4% and 66.5%, respectively). That was probably caused by a more favourable microclimate and litter composition in broadleaved than in coniferous plantations. These variables also seemed to be more important to pure litter decomposition rates than were fungal species richness or community structure.

A. Kubartová (✉)
Department of Forest Mycology and Pathology, SLU,
P.O. Box 7026, 750 07 Uppsala, Sweden
e-mail: Ariana.Kubartova@mykopat.slu.se

J. Ranger
Unité Biogéochimie des Ecosystèmes Forestiers,
INRA, Centre de Nancy,
Champenoux 54 280, France

J. Berthelin · T. Beguiristain
LIMOS, CNRS UHP UMR 7137, Université Henri Poincaré,
P.O. Box 239, Vandoeuvre-les-Nancy 54 506, France

Introduction

It is widely viewed that changes in forest above-ground diversity and structure indirectly affect the soil microbial community and its functions. Plant litter decomposition is a key process in nutrient recycling and humus formation in forest ecosystems [4, 6, 38]. Saprophytic fungi play an important role in decomposition because they can attack the lignocellulose matrix in litter that other organisms are not able to assimilate [1, 11]. The change in the litter quality during decomposition induces a succession of microbial communities: the r-strategists (opportunists) dominate during the early stages and are replaced later by K-strategists (persisters) due to the growth of limiting substrate concentrations [10]. The process typically begins with litter colonisation by bacteria, Ascomycetes, and imperfect fungi (Deuteromycetes), which consume the less recalcitrant components. The cellulose present in non-lignified tissues is then attacked by some of these organisms. Subsequently, the remaining lignified litter is colonised mainly by brown-rot and white-rot Basidiomycetes that degrade it further.

The remaining highly recalcitrant compounds become a part of the soil organic matter.

An increase of tree species diversity in forests should lead to an increase in microbial biomass, diversity and activity due to greater niche variability (e.g. diversity of carbon sources in more variable litters and root exudates, greater primary production, variable microclimate, presence of dead wood or spatial and age variability of trees) [43]. Deciduous litter should be more favourable for microbial decomposers than the coniferous one, and its greater quality may have a positive influence on the diversity and decomposition processes. Several recent studies focused on decomposition rate, nutrient dynamics and/or decomposer activity of both pure and mixed plant litters, as reviewed by Gartner and Cardon [16]. Similarly, certain studies assessed microbial diversity within forest ecosystems using molecular or phospholipid fatty acid analyses, as reviewed by Leckie [25]. However, studies investigating decomposition rates in situ together with the fungal species richness and community composition are still scanty.

Greater microbial species richness is generally expected to increase the average rates of related ecological processes due to greater enzyme diversity and niche complementarity [12]. Nevertheless, the decomposing ability of each species varies depending on environmental conditions and also on interactions with other fungi. Many fungi should be functionally redundant and the potential capacity for inter-specific competition can be large. In such a case, the effect of diversity could be weak caused due to saturation at low species richness and/or enhanced competition [7, 21, 44, 47].

The main purpose of this study was to assess in situ fungal colonisation of forest tree litter and to investigate the role of the fungal diversity on the decomposition rate. The targeted tasks were: (1) the effect of tree species on fungal species richness and community structure during litter decay, (2) their dependence on stage of decomposition and (3) the coincidence of litter mass loss rates with fungal species richness and diversity.

Methods

Site Description and In Situ Experiments

Experiments were conducted in the Breuil forest experimental site, Burgundy, France. This site represents an opportunity to specifically evaluate the influence of tree species which were composed of control semi-native forest and mono-specific even-aged plantation stands in a homogeneous area. Hence, the impact of vegetation cover results only from different stand structure, litter quality and/or changed microclimate. The Breuil site is located in the Morvan Mountains, France at an altitude of 640 m, latitude 47°18′10″ and longitude 4°4′44″. Mean annual temperature is 9°C, with 1,280 mm precipitation and evapotranspiration of 640 mm. The previous forest was an old coppice with standards, dominated by *Fagus sylvatica* L. and *Quercus petraea* Smith, associated with several other dominant species such as *Betula verrucosa* Ehrh. and *Corylus avelana* L. It evolved slowly towards a high forest structure, not having been harvested for more than 50 years. This native forest was partially cut down in 1976 and replaced by various monocultures planted in 1,000 m^2 stands each. Five different stands were selected for the litter bag experiment: four young plantations, namely European beech (*F. sylvatica* L.), sessile oak (*Q. petraea* Smith.), Norway spruce (*Picea abies* Karst.) and Douglas-fir (D. fir; *Pseudotsuga menziesii* Franco.) and the native forest as a reference stand.

Leaf and needle litters of the four plantations were decomposed in 5×5-cm litter bags of mesh size 5 μm in the plantation where they originated. Two leaf-needle litter mixtures were decomposed on the border of the adjoining involved stands using the same bags. These included spruce–beech mixture and Douglas-fir–oak mixture, both 50% of each litter type. The distance between two replicates was 0.5 m. The bags were placed in the stands in late November 2002. They were collected after 4 months (March 2003) and 24 months (November 2004) of the incubation. Five replicates were sampled at each tree species, and three of them were later used for molecular analyses. Mass loss of the decomposed litter was calculated for the five replicates as the difference between initial and final weight of litter dried at 65°C for 24 h.

Forest floor sampling was carried out in late June 2004 in the four plantations and in the native forest. Three replicates of L and F layers were sampled in each stand. They were at a distance of 10 cm from each other. All the samples were immediately frozen (−80°C) for storage in the lab. Organic and nutrient composition of the falling litter and nutrient composition of the forest floor were analysed.

Molecular Analyses

The structure of fungal communities was assessed by temperature gradient gel electrophoresis (TGGE), which has been shown to be a sensitive and robust technique for investigating soil microbial communities [3, 29]. Total genomic DNA was isolated from the samples using the Dneasy plant mini kit (Qiagen) following the manufacturer's protocol without any modification. Fungal communities were analysed using ITS1F and ITS2 primer pairs to amplify by polymerase chain reaction (PCR) the 280-bp fragment of the fungal ITS rDNA [15, 48]. A 40-bp GC-clamp was attached to the 5′ end of the primer ITS2 to avoid complete separation of DNA strands during the denaturing electrophoresis. The reaction medium consisted of 5 μl of PCR buffer (Sigma, 100 mM Tris-HCl, pH 8.3,

500 mM KCl, 15 mM $MgCl_2$), 1 μl of dNTP (10 mM), 1 μl of each primer (20 μM), 0,5 μl of Taq-polymerase (5 units/μl, Sigma), 1 μl of GC-rich solution (Sigma), 2.5 μl of BSA (3%) and 2 μl of genomic DNA in a final volume of 50 μl. The amplification regime consisted of an initial cycle of denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s and extension at 72°C for 1 min 15 s. The amplification concluded with a final elongation step at 72°C for 8 min. The PCR reactions were performed using an iCycler Thermal cycler (Bio-rad). PCR products were tested on 1.5% (*w*/*v*) agarose gels followed by ethidium bromide (0.5 mg/l, Bio-rad) staining. TGGE was performed with a Dcode Universal Mutation Detection system (Bio-rad). Polyacrylamide gels (8% acrylamide (*w*/*v*), 8 M urea, 1.25× TAE and 0.2% glycerol (*v*/*v*), 300 μl of ammonium persulphate (10%; *w*/*v*) and 30 μl of TEMED) were used. Amplified DNA samples with the same volume of loading buffer (10 μl each) were separated by electrophoresis in 1.25× TAE at a constant voltage (145 V) at a temperature gradient from 50°C to 55°C with a temperature increment of 1°/h. Gels were stained with silver nitrate after the electrophoresis.

Statistics

A matrix of species distribution was calculated based on image analyses using the Quantity One software (Bio-Rad) with a band position tolerance of 2 mm, giving a maximum of 50 detectable species. A presence–absence matrix was created. Species richness, i.e. species number per sample, was calculated (referred SR below). Analysis of variance tests of SR and mass loss were counted using the Statistica software (StatSoft). Multivariable analyses of the litter bags and forest floor fungal community composition were done using CANOCO (Microcomputer Power). Principal component analysis was used first, corresponding to the successive dimensions of maximum variance of the scatter of samples. Redundancy analysis (RDA) than ordinated fungal communities and tree stands as environmental variables, such that the relative position of the communities reflect their similarity and/or dissimilarity. The relative significance of the fungal species vectors was indicated by their length and direction from the axes origin. Monte Carlo permutation tests calculated the significance of the environmental factors [41].

Results

Decomposition Rate and Litter Properties

The values of cumulative mass loss in the litter bags are given in Fig. 1. The major part of the litter material was decomposed in both mixed litter experiments after 4 months of incubation. Concerning the pure litter material, the mass loss decreased from oak, Douglas-fir, spruce and beech. The effect of litter type was significant. Beech and coniferous mass loss values significantly differed from oak and both mixed litter values. The tree species order changed after 24 months of incubation, when most of the material was decomposed in the oak–Douglas-fir mixed litter. The classification was then: oak > beech–spruce mixed litter > beech > Douglas-fir and spruce. The litter-type effect was not significant in this case; only spruce significantly differed from oak and mixed litters.

The litter types differed in their mineral and organic composition (Table 1). Litter from beech, oak and native forest had a higher nitrogen content than spruce and Douglas-fir. This was both in the falling litter and forest floor case. The broadleaved trees also had a lower C/N ratio and a higher potassium and calcium concentration in falling litter. The native forest had the highest concentration of N, P and Ca from all tree species and the second highest of K in the forest floor. Beech litter was the richest on soluble compounds, while Douglas-fir was the poorest. However, it had the highest concentrations of both cellulose and hemicelluloses. The lowest concentrations were found in oak and beech, respectively. Beech showed the lowest amount of lignin, and oak the highest. Oak had also a high lignin/N ratio, followed by spruce, and it also had a high ash content.

Fungal Diversity in Litter Decomposed in Litter Bags

All of the 50 potentially detectable fungal species were identified from the incubated samples. The SR (number of species per sample) ranged from five to 20. The average SR per sample within all tree stands was 11.8 after 4 months of incubation and 10.6 for the 24 months sampling. Hence, it

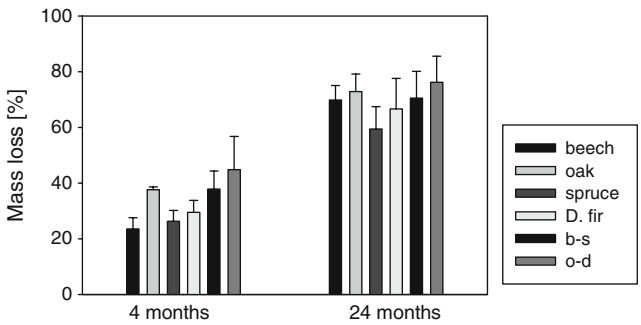

Figure 1 Mass loss of the litters decomposed in the litter bags, sampled after 4 and 24 months of decomposition (*b–s* beech–spruce mixed litter, *o–d* oak–Douglas-fir mixed litter). *4 months* beech and coniferous litter values significantly differed from oak and both mixed litter values; *24 months* only spruce differed significantly from oak and mixed litters

Table 1 Properties of the studied substrates

	Soluble	Hemicellul.	Cellulose	Lignin	Lignin/n	Ash	N	C/N	P	K	Ca	Mg
A												
Native f.	32.2	16.7	23.5	24.1	1.5	3.5						
Beech	44.2	17.6	18.0	16.2	1.2	4.1						
Oak	30.2	13.3	22.1	29.9	2.2	4.5						
Spruce	37.1	16.5	19.4	23.6	1.8	3.5						
Douglas	19.6	19.0	42.5	17.1	1.4	1.9						
B												
Native f.							23.2	22.3	1.03	9.51	2.28	0.84
Beech							25.2	21.0	1.00	8.24	2.33	0.50
Oak							23.9	21.3	1.16	8.52	2.75	1.20
Spruce							15.1	34.4	1.11	7.34	1.44	0.71
Douglas							18.0	29.1	0.99	6.77	2.26	1.08
C												
Native f.							16.1	32.1	0.81	1.19	2.72	0.61
Beech							13.9	37.3	0.63	1.22	2.46	0.56
Oak							13.6	38.1	0.70	1.12	2.64	0.69
Spruce							12.9	36.9	0.67	1.12	1.27	0.55
Douglas							12.6	44.6	0.63	1.2	1.98	0.58

A organic composition of the litters decomposed in the litter bags and in the native forest (%), *B* concentrations of nutrients in the litters decomposed in the litter bags and in the native forest (g/kg), *C* concentrations of nutrients in the forest floor (g/kg)

did not significantly differ between samplings. The beech stand showed the highest and significantly different SR in the first sampling, followed by conifers, mixed litter samples and oak. Mixed litter samples presented the highest SR in the second sampling, followed by spruce, oak, beech and Douglas-fir. The Douglas-fir value was significantly different from all the others and the beech value significantly differed from both mixed litters. Comparing the two sampling dates, a higher SR was found in oak and mixed litters in the later sampling as compared to the earlier, but lower in the others (Fig. 2a). The effect of tree species on SR was found to be significant in both samplings.

Each tree species was characterised by a specific fungal species pattern in the litter bag samples. In RDA, 49.3% and 47.7% of the variability of the community composition was explained by the forest tree species effect in the first and the second sampling, respectively. The stage of decomposition accounted for 11.5% of the fungi species shift between earlier and later samplings within all tree species combined. Random tests confirmed the significant influence of both forest tree species and decomposition stage. In the RDA plot of the first sampling (Fig. 3a), beech was clearly discriminated from other tree species on the x-axis by the presence of certain characteristic species (e.g. 19, 25, 30, 47, 48; for identity and Blast match, see Table 2). The spruce stand was the most distant on the y-axis and also had a different species composition (1, 14, 32). The mixed litter samples were positioned in the central part of the graph. No typical species was associated with them in

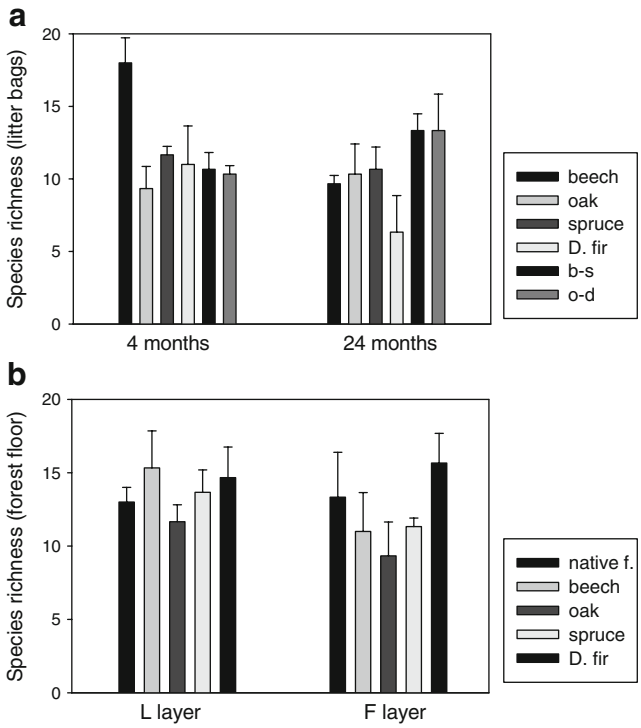

Figure 2 Fungal species richness (SR), i.e. average number of species per sample, detected: **a** in litter decomposed in litter bags, sampled after 4 and 24 months of in situ decomposition (*b–s* beech–spruce mixed litter, *o–d* oak–Douglas mixed litter); beech significantly differed in *4 months*, Douglas significantly differed from all the others and beech differed from both mixed litters in *24 months*; **b** in L and F layer of forest floor; in *L*, only beech and oak values differed significantly, in *F*, Douglas was the only significantly different stand

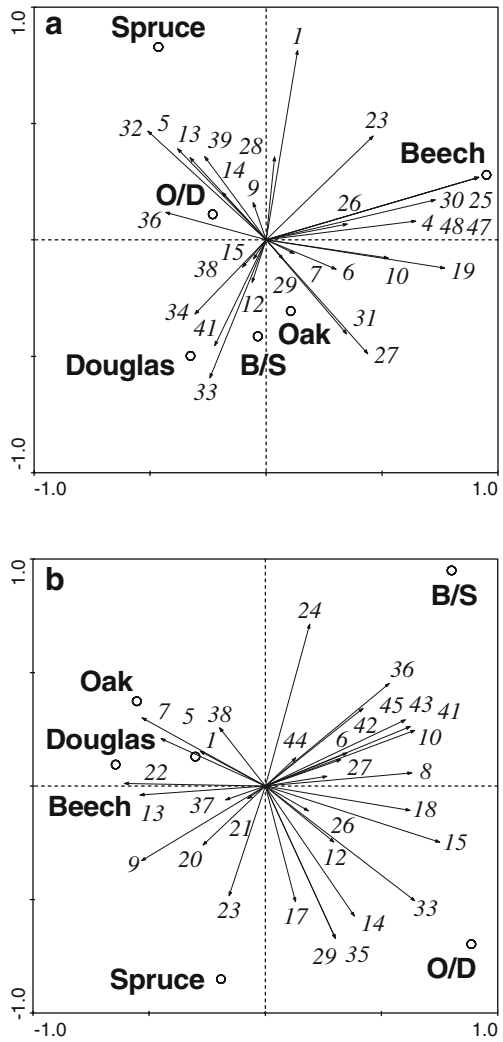

Figure 3 RDA plot showing fungal community composition in litters decomposed in litter bags, after **a** 4 months, **b** 24 months of the decomposition in two broadleaves, two coniferous plantations and in two mixed litters (*B*/*S* beech, spruce; *O*/*D* oak, Douglas), decomposed on the border of adjoining plantations. *Length of arrow* indicates the relative importance of the fungal species, while the *angle between arrows* indicates the degree to which they are correlated

the earlier sampling. Nevertheless, they were highly separated from pure litter types in the second sampling, tending to occupy the right part of the RDA plot (Fig. 3b). However, they were well discriminated on the y-axis from each other. Many species were present only in these mixed litter samples in the later sampling (e.g. species 10, 24, 41, 43 in beech–spruce litter, species 14, 29, 33 in oak–D. fir litter). The species occurring in mixed litter samples were not always present in either of the original pure litter samples and conversely. Spruce showed the most distinct community structure of the pure litter samples, similar to the first sampling. It was positioned separately along the y-axis but kept the same position as other pure litter types along the x-axis. These pure litter types, i.e. beech, oak and Douglas-fir, were clustered together, indicating that relatively similar fungal communities were involved in their decomposition processes compared to mixed litter. The beech stand showed the most pronounced shift in community composition between the earlier and later sampling dates. The shift of the mixed litter communities was also significant. Oak and spruce communities changed less, and Douglas-fir community remained almost identical.

Thirty-three of the 50 species were identified in both earlier and later samplings. However, there was a clear abundance difference in the case of the majority of species between the sampling dates. Species 7, 9, 12, 28, 31 were identified mostly in the early stages of colonisation, species 17, 22, 37, 42, 44 were positively correlated with the later stages. Five of the most common species were detected in all tree litter types (6, 7, 9, 13, 28). Five other species were detected only in one sample. Two of more abundant species were specific to only one tree stand (47, 48, beech, 4 months).

Fungal Diversity in Forest Floor Layers

Forty-five different fungal taxa were detected in the L and F forest floor layers together. Eight to 18 species were identified per sample. The average SR was 13.7 species per sample in the L layer and 12.1 species in the F layer indicating no significant difference. The beech stand expressed the highest SR in the L layer, followed by conifers, the native forest and oak, but only beech and oak values differed significantly from the others. In the F layer, Douglas-fir was the species richest and the only significantly different stand, followed by the native forest, spruce, beech and lastly, oak again (Fig. 2b). Comparing the layers together, Douglas-fir and the native forest had a higher SR in the F layer than in the L layer, but for the other tree species, the SR was higher in the L layer. The tree species effect on SR was not significant in the L layer, but it was in the F layer.

When testing the effect of tree species on community composition by RDA, it explained 43.6% of L layer variability and 46.1% of F layer variability. The L or F layer effect (i.e. decomposition stage) explained 10.7% of the variability within all five tree species together. Random tests showed the tree species effect in both the L and F layers and the L or F layer effect to be significant to fungal species distribution. In the RDA plot of L layer diversity (Fig. 4a), the fungal communities associated with the native forest and spruce were discriminated from the others along the x-axis, characterised e.g. by species 15, 20, 28. Beech and oak stands occupied a similar position on the x-axis (common species e.g. 6, 10, 41). However, beech was discriminated on the y-axis, while oak was positioned in the central part of the plot. The Douglas-fir position was distant

Table 2 List of sequenced species. N. indicates the number used in the text and figures, Identity is the most particular species, together with Blast database match and percentage of similarity, A-Ascomycetes, B-Basidiomycetes, Frequency implies in how many samples the species was detected

N	Identity		Blast match	Percentage	Freq.
6	Leaf litter ascomycete its 408 isolate	A	AF502889	96	43
7	*Megacollybia platyphylla*	B	AF498289	100	48
10	Unpublished species		–	–	28
12	*Epacris microphylla* root associated f.		AY268197	98	23
14	Uncultured f. clone B1c from forest		AY324159	98	21
17	Leaf litter ascomycete strain its 356	A	AF502859	97	21
19	*Trametes versicolor*	B	AY673076	93	12
21	*Gerronema strombodes*	B	U66433	92	11
23	*Phlebia albida*	B	AY219368	94	21
25	*Dactylaria* sp. P24	A	AY265332	88	18
26	*Phanerochaete sordida*	B	AY219383	96	16
28	Fungal endophyte WMS13	A	AY063309	94	28
30	*Cryphonectria* sp. CMW 11302	A	AY214324	98	13
33	*Epacrid* root endophyte E4–5–5	A	AF148952	91	13
35	*Menispora tortuosa*	A	AF178558	92	12
37	*Coniosporium apollinis*	A	AJ244271	90	16
39	*Helotiales* sp. sd2aN4b(A)	A	AY465452	95	8
41	Ascomycete sp. olrim349	A	AY354279	95	20
42	*Woollsia* root associated f. XVIII	A	AY230788	90	14
46	*Chaetosphaeria pulviscula*	A	AF178544	94	12

from the other tree species on both axes, being the closest to oak. The native forest and spruce had a similar position on the first axis even in the F layer RDA graph (Fig. 4b, common species e.g. 14, 27, 34) as did oak and beech (species 26, 36). Douglas-fir was discriminated from other tree species again. The native forest displayed a high SR value but had many species in common with the plantations. The most pronounced community shift between the L and F layers were found in Douglas-fir. The communities changed in other tree species as well, but least in oak and beech stands. Certain species were associated with L layer, independently on tree stand (e.g. 6, 10, 37, 44, 49); others were more abundant in F layer (13, 24, 25, 26, 30).

Forty of 45 species occurred in both forest floor layers, often showing preferences to one or the other. Sixteen species were present in all tree stands in at least in one of the layers. Only one species was identified only once. All the samples both from litter bags and forest floor layers were also analysed together (Fig. 5). Canonical axes expressed 16.2% of the whole dataset variability. Samples from the 24 months sampling were positioned between the L and F layers on both axes, clustered in the upper left part of the RDA plot. Four months sampling, representing the early stages of decomposition, was clearly separated from the others on the x-axis. The F layer, representing the later stages, was well discriminated on the y-axis. This corresponded well to expected decomposition stages of the samples (4 months > L layer > 24 months > F layer).

Discussion

Decomposition Rate

The differences in decomposition rate among forest tree species are associated not only with different organic composition of litters [36] and the heterogeneous distribution of carbon and nitrogen resources [34] but also with diverse SR and community composition and/or different microclimates of the stands [6]. Early decomposition stages, in this study 4 months, are expected to be strongly related to microclimate and litter chemistry of water soluble nutrients and structural carbohydrates. Later decomposition stages (24 months) are more influenced by lignin concentrations in the litter material [4]. Oak, as the warmest and moistest stand [24] with the nutrient richest litter, displayed the highest mass loss from pure litter samples both after 4 and 24 months of decomposition, despite high lignin concentrations and high lignin/N values. In the beech stand, the decomposition was the slowest after 4 months of decomposition, despite the highest N amount. It might be, among others, caused by a high concentration of soluble compounds which included inhibiting phenolic substances [46]. However, it displayed the second highest mass loss in the 24 months sample, coinciding with low lignin concentrations and lignin/N values. Conifers had less favourable litter compositions and microclimatic conditions and displayed lower mass loss values than broadleaved stands in

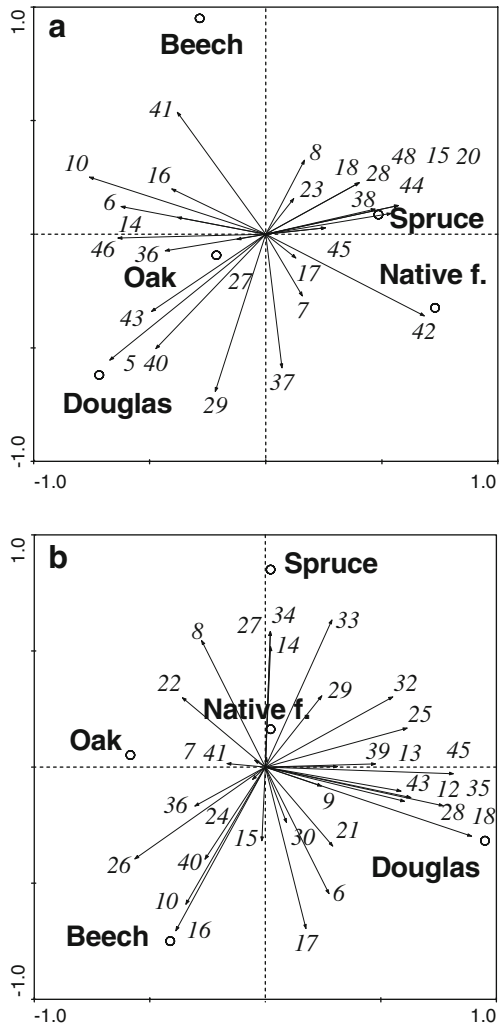

Figure 4 Fungal community composition in L and F layer of the forest floor, in the four plantations and in the native forest as the control stand **a** L layer, **b** F layer

the 24 months. Douglas-fir needles decomposed more rapidly than the spruce litter being richer in N and having less soluble compounds and lignin concentrations. Differences in microbial activity within forest ecosystems were also documented e.g. by Emmerling et al. [13], Fisk et al. [14], Ohtonen and Vare [31].

From numerous previous experiments using mixed litter material, it is clear that decomposition patterns are not always predictable from simple-species dynamics [16, 23]. Litter mixture is expected to support a greater number of microhabitats and chemical diversity and can also influence overall decomposition rate and microbial activity through the transfer of nutrients and secondary chemicals. Nutrients released from rapidly decaying, higher quality litter can stimulate decay in adjacent, more recalcitrant litter or conversely, decay can be slowed by the release of inhibitory compounds such as phenolics and tannins [16, 21]. The change in decomposition rate after mixing broadleaved litter with coniferous litter was documented previously [16, 39, 46]. Also, in the present study, the mixed litter showed different dynamics than corresponding pure litter types.

Fungal Diversity

The study supported the hypothesis of tree species influence on decomposing fungi communities. In fact, a majority of species should originate in the native forest being suppressed or favoured by the conditions in the young plantations. In the 4-month litter bag sampling in March, fungi could be inhibited by unfavourable conditions during winter due to low temperatures. Conversely, later sampling in November could have high fungal activity due to favourable moisture and temperature conditions and to an input of nutrients leached from newly fallen litter. Fungal community in the litter bags was expected to develop under partly different conditions, mainly modified moisture and exclusion of soil fauna, compared to surrounding litter layers [38]. The forest floor was sampled at the end of June 2004, thus, during a rather warm and humid period and would be expected to support high fungal activity.

Lejon et al. [27] also described significant discrimination of fungal communities within the top 5–10 cm of the soil profile in the same study site, using the ARISA technique. Lindahl et al. [28] documented a clear shift in fungal community composition between the L and F horizon in a Scots-pine boreal forest. Hyphal length was previously found to be greater in the L layer than in the F and H layers [5, 33]. Other studies documented the differences in microbial community composition in diverse forest ecosystems [17, 20, 26, 37, 40, 42, 49, 50], seasonal changes in community composition [30] and succession of fungi during decomposition [32], reviewed by Osono and Takeda [35] and Virzo De Santo et al. [45]. However, the studies

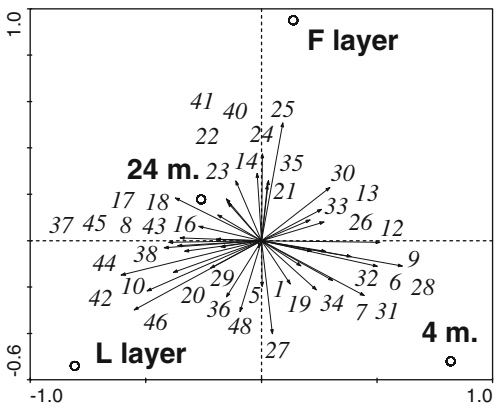

Figure 5 Fungal community compositions of all samples analysed together in order to show the resemblance or divergence between the datasets (L and F layers of the forest floor, *4 m.* 4 months sampling of litters decomposed in the litter bags, *24 m*. 24 months sampling)

are not easily compared due to the use of different sampling scales and techniques in various climatic conditions.

Links Between Biodiversity and Decomposition Rate

Fungal diversity and decomposition rate were not necessarily correlated (Figs. 1, 2). The nutrient status of the litter and its organic properties together with biotic interactions and actual activity of species according to environmental conditions [2] seemed to be more important for mass loss values than either species richness or community composition. A certain positive diversity effect was found in the 24-month samples for species-rich, well-decomposed mixed litters. It seemed that higher niche variability and resource diversity allowed more fungal species to coexist in the mixed litter without increasing the competition and inhibiting their activity. Rather, negative relations were also found, e.g. in the oak stand in the 4 months sampling, where low SR was detected together with relatively fast decay. Conversely, the beech litter with significantly higher SR decomposed slowly, since enhanced competition in the rich community might have slowed down the decay process. After 24 months, litter bags from Douglas-fir, relatively poor in fungal species, showed higher mass loss than spruce with rather higher SR. Certain species present mainly in later stages (e.g. 17, 37, 38, 42, 44) were positively correlated to higher decomposition rate (tested by RDA, all litter types together, data not shown), whereas other species (e.g. 7, 12, 27, 28, 31) were correlated negatively.

The tree species studied seemed to retain sufficient fungal diversity to compensate for species suppressed by the change of the dominant tree. There was no clear diversity decrease observed to influence the decomposition rate negatively by elimination of key species. Community composition seemed to have only minor effects on decomposition rate probably due to a high degree of functional redundancy of decomposing fungi [8]. Favourable microhabitats might be preferentially colonised, but less favourable are also exploited as total abundance and competition rise largely independently of the number of species involved [12]. Unexploited gaps after a species loss can, thus, be eventually closed by the remaining species [22]. Setala and McLean [44] reported that functional efficiency of fungal communities increased with the number of taxa only at the species-poor end of the gradient in a study of gradients of SR in mixtures of saprophytic fungi from pine–spruce forest. Similarly, no decrease in functional diversity of microorganisms in plant debris did result in decline of decomposition rate [9], and the experimental reduction of the microbial communities had no direct effects on soil functions in arable soils [19]. Conversely, short-term decomposition in pasture soil decreased with decreasing biodiversity after fumigation [18]. According to the present study, Wilkinson et al. [50] documented decomposer community composition in pine and spruce forests to be mainly determined by climatic conditions and litter quality whereas differences in community composition had no apparent functional consequences for litter decomposition.

Conclusions

The study showed that saprophytic fungal communities under the studied tree species differed in species composition. Succession of species according to decomposition stage was observed as well. The mixed litter samples were found to sustain fungal diversity, including the most distant communities composed of characteristic species in the later decomposition stages. Decomposition rate was also increased, mainly when compared to the corresponding coniferous pure litter. The species richness was not found to be positively correlated to the decomposition rate. Higher decomposition rate values in the species-rich mixed litter might rather be explained by higher niche and resource variability. On the contrary, enhanced competition in the species-richest communities might possibly slow the decay (e.g. beech litter in 4 months).

Acknowledgment The authors thank the technician team of the INRA Biogéochimie des Ecosystèmes Forestiers Unit, in charge of collecting the database of the site, and J. Moukoumi for preparation and treatment of the samples. The authors thank also the GIP-Ecofor for providing subsidies for monitoring the Breuil site experiment, the Marie-Curie association, the Czech Science Foundation and the Swedish Institute. We are also grateful to M. Ruth for the language corrections.

References

1. Adl S (2003) The ecology of soil decomposition. CABI, Wallingford
2. Aerts R (1997) Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos 79:439–449
3. Anderson IC, Cairney JWG (2004) Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Env Microbiol 6:769–779
4. Berg B (2000) Litter decomposition and organic matter turnover in northern forest soils. For Ecol Manag 133:13–22
5. Berg B, Kniese JP, Verhoef HA (1998) Dynamics and stratification of bacteria and fungi in the organic layers of Scots pine forest soil. Biol Fertil Soils 26:313–322
6. Berg B, McClaugherty C (2003) Plant litter—decomposition, humus formation, carbon sequestration. Springer, Berlin
7. Cox P, Wilkinson SP, Anderson JM (2001) Effects of fungal inocula on the decomposition of lignin and structural polysaccharides in Pinus sylvestris litter. Biol Fertil Soils 33:246–251
8. Deacon LJ, Janie Pryce-Miller E, Frankland JC, Bainbridge BW, Moore PD, Robinson CH (2006) Diversity and function of decomposer fungi from a grassland soil. Soil Biol Biochem 38:7–20

9. Degens BP (1998) Decreases in microbial functional diversity do not result in corresponding changes in decomposition under different moisture conditions. Soil Biol Biochem 30:1989–2000
10. Dilly O, Bartsch S, Rosenbrock P, Buscot F, Munch JC (2001) Shifts in physiological capabilities of the microbiota during the decomposition of leaf litter in a black alder (Alnus glutinosa (Gaertn.) L.) forest. Soil Biol Biochem 33:921–930
11. Dix NJ, Webster JW (1995) Fungal ecology. Capman and Hall, London
12. Ekschmitt K, Klein A, Pieper B, Wolters V (2001) Biodiversity and functioning of ecological communities-why is diversity important in some cases and unimportant in others? J Plant Nutrit Soil Sci 164:239–246
13. Emmerling C, Schloter M, Hartmann A (2002) Functional diversity of soil microorganisms-a review of recent research activities in Germany. J Plant Nutrit Soil Sci 165:408–420
14. Fisk MC, Ruether KF, Yavitt JB (2003) Microbial activity and functional composition among northern peatland ecosystems. Soil Biol Biochem 35:591–602
15. Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for Basidiomycetes: application to the identification of mycorrhizae and rusts. Molec Ecol 2:113–118
16. Gartner TB, Cardon ZG (2004) Decomposition dynamics in mixed-species leaf litter. Oikos 104:230–246
17. Grayston SJ, Prescott CE (2005) Microbial communities in forest floors under four tree species in coastal British Columbia. Soil Biol Biochem 37:1157–1167
18. Griffiths BS, Ritz K, Bardgett RD, Cook R, Christensen S, Ekelund F, Sorensen SJ, Baath E, Bloem J, de Ruiter P, Dolfing J, Nicolardot B (2000) Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship. Oikos 90:279–294
19. Griffiths BS, Ritz K, Wheatley R, Kuan HL, Boag B, Christensen S, Ekelund F, Sorensen SJ, Muller S, Bloem J (2001) An examination of the biodiversity-ecosystem function relationship in arable soil microbial communities. Soil Biol Biochem 33:1713–1722
20. Hackl E, Zechmeister-Boltenstern S, Bodrossy L, Sessitsch A (2004) Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. Appl Envir Microbiol 70:5057–5065
21. Hättenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. Annu Rew Ecol Evol Syst 36:191–218
22. Jones TH, Bradford MA (2001) Assesing the functional implications of soil biodiversity in ecosystems. Ecol Res 16:845–858
23. King RF, Dromph KM, Bardgett RD (2002) Changes in species evenness of litter have no effect on decomposition processes. Soil Biol Biochem 34:1959–1963
24. Kulhankova A, Beguiristain T, Ranger J, Moukoumi J, Berthelin J (2006) Spatial and temporal diversity of wood decomposer communities in different forest stands, determinated by ITS rDNA targeted TGGE. Ann For Sci 63:547–556
25. Leckie SE (2005) Methods of microbial community profiling and their application to forest soils. For Ecol Manag 220:88–106
26. Leckie SE, Prescott CE, Grayston SJ, Neufeld JD, Mohn W (2004) Characterization of humus microbial communities in adjacent forest types that differ in nitrogen availability. Microb Ecol 48:29–40
27. Lejon DP, Chaussod R, Ranger J, Ranjard L (2005) Microbial community structure and density under different tree species in an acid forest soil (Morvan, France). Microb Ecol 50:614–625
28. Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J, Finlay RD (2007) Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol 173:611–620
29. Muyzer G (1999) DGGE/TGGE a method for identifying genes from natural ecosystems. Curr Opin Microbiol 2:317–322
30. Myers RT, Zak DR, White DC, Peacock A (2001) Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Sci Soc Amer J 65:359–367
31. Ohtonen R, Vare H (1998) Vegetation Composition Determines Microbial Activities in a Boreal Forest Soil. Microb Ecol 36:328–335
32. Osono T (2002) Phyllosphere fungi on leaf litter of Fagus crenata: occurrence, colonization, and succession. Can J Bot 80:460–469
33. Osono T, Ono Y, Takeda H (2003) Fungal ingrowth on forest floor and decomposing needle litter of Chamaecyparis obtusa in relation to resource availability and moisture condition. Soil Biol Biochem 35:1423–1431
34. Osono T, Takeda H (2001a) Effects of organic chemical quality and mineral nitrogen addition on lignin and holocellulose decomposition of beech leaf litter by Xylaria sp. Eur J Soil Biol 37:17–23
35. Osono T, Takeda H (2001b) Organic chemical and nutrient dynamics in decomposing beech leaf litter in relation to fungal ingrowth and succession during 3-year decomposition processes in. a cool temperate deciduous forest in Japan. Ecol Res 16:649–670
36. Osono T, Takeda H (2002) Comparison of litter decomposing ability among diverse fungi in a cool temperate deciduous forest in Japan. Mycologia 94:421–427
37. Pennanen T, Liski J, Baath E, Kitunen V, Uotila J, Westman CJ, Fritze H (1999) Structure of the microbial communities in coniferous forest soils in relation to site fertility and stand development stage. Microb Ecol 38:168–179
38. Prescott CE (2005) Do rates of litter decomposition tell us anything we really need to know? For Ecol Manag 220:66–74
39. Prescott CE, Zabek LM, Stanley CL, Kabzems R (2000) Decomposition of breadleaf and needle litter in forests of British Columbia: influence of litter type, forest type and litter mixtures. Can J For Res 30:1742–1750
40. Priha O, Grayston SJ, Hiukka R, Pennanen T, Smolander A (2001) Microbial community structure and characteristics of the organic matter in soils under Pinus sylvestris, Picea abies and Betula pendula at two forest sites. Biol Fertil Soils 33:17–24
41. Ramette A (2007) Multivariate analyses in microbial ecology. FEMS Microbiol Ecol 62:142–160
42. Saetre P, Baath E (2000) Spatial variation and patterns of soil microbial community structure in a mixed spruce-birch stand. Soil Biol Biochem 32:909–917
43. Setala H (2002) Sensitivity of ecosystem functioning to changes in trophic structure, functional group composition and species diversity in bellowground food webs. Ecol Res 17:207–215
44. Setala H, McLean MA (2004) Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. Oecologia 139:98–107
45. Virzo De Santo A, Rutigliano FA, Berg B, Fioretto A, Puppi G, Alfani A (2002) Fungal mycelium and decomposition of needle litter in three contrasting coniferous forests. Acta Oecologica 23:247–259
46. Wardle DA, Nilsson MC, Zackrisson O, Gallet C (2003) Determinants of litter mixing effects in a Swedish boreal forest. Soil Biol Biochem 35:827–835

47. Wardle DA, van der Putten WH (2003) Biodiversity, ecosystem functioning and above-ground-bellow-ground linkages. In: Loreau M (ed) Biodiversity and ecosystem functioning. Oxford University Press, Oxford, pp 155–168
48. White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In: Innis N (ed) PCR - Protocols and applications - A laboratory manual. Academic, New York, pp 315–322
49. White C, Tardif JC, Adkins A, Staniforth R (2005) Functional diversity of microbial communities in the mixed boreal plain forest of central Canada. Soil Biol Biochem 37:1359–1372
50. Wilkinson SC, Anderson JM, Scardelis SP, Tisiafouli M, Taylor A, Wolters V (2002) PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. Soil Biol Biochem 34:189–200