Supporting Information

Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition

Witoon Purahong, Tesfaye Wubet, Guillaume Lentendu, Michael Schloter, Marek J. Pecyna, Danuta Kapturska, Martin Hofrichter, Dirk Krüger, François Buscot

Appendix S1. PCR conditions

Samples were amplified for pyrosequencing using a forward and reverse fusion primer. For bacteria, the forward primer was constructed with (5'-3') the Roche A linker (CCATCTCATCCCTGCGTGTCTCCGACTCAG), an 8-10bp barcode, and the BAC 341F primer ([CCTACG GGAGGCAGCAG, Muyzer & Smalla, 1998; Muyzer et al, 1995]). The reverse fusion primer was constructed with (5'-3') a biotin molecule, the Roche B linker (CCTATCCCCTG TGTGCCTTGGCAGTCTCAG), and the BAC 907R primer ([CCGTCAATTCMTTTGAGTTT, Muyzer & Smalla, 1998; Muyzer et al, 1995]). We sequenced the samples from the forward primer. For fungi, the forward primer was constructed with (5'-3') the Roche A linker, an 8-10bp barcode, and the ITS1F primer ([CTTGGTCATTTAGAGGAAGTAA, Gardes & Bruns, 1993]). The reverse fusion primer was constructed with (5'-3') the Roche B linker, and the ITS4 primer ([TCCTCCGCTTATTGATATGC, White et al, 1990]). We sequenced the samples from the ITS4 (reverse primer). Amplifications were performed in 25 µl reactions with Qiagen HotStar Taq master mix (Qiagen Inc, Valencia, California), 1 µl of each 5 µM primer, and 1 µl of template. Reactions were performed on ABI Veriti thermocyclers (Applied Biosytems, Carlsbad, California) under the following thermal profile: 95 $^{\circ}$C for 5 min, then 35 cycles of 94 $^{\circ}$C for 30 sec, 54 $^{\circ}$C for 40 sec, 72 $^{\circ}$C for 1 min, followed by one cycle of 72 $^{\circ}$C for 10 min and 4 $^{\circ}$C hold.

Appendix S2. Decomposition of European beech leaf litter

Information related to the decomposition of beech leaf litter used in this study, i.e. variations in microbial biomass, enzyme activity patterns (five hydrolytic and three oxidative enzymes) and nutrient dynamics, has been published elsewhere (Purahong et al, 2014a, b). Briefly, after 473 days, almost 40 % of the total leaf litter mass was lost (Purahong et al, 2014a), and the decomposition rate was consistent with other litterbag studies carried out at the same location and using the similar leaf litter (Jacob et al, 2009, 2010). Substantial amounts of lignin were decomposed between days 284 to 473 (~20 % – 40 %), demonstrating that leaf litter in our litterbag experiment had reached the later (second phase) decomposition stage mentioned in Berg (2000). C:N and Lignin:N ratios also leveled out at 284 days. The microbial biomass measured with phospholipid fatty acid (PLFA) clearly showed that fungi dominated during the entire decomposition processes (fungal:bacterial ratios ranged from 5.74 at 89 days to 1.36 at 473 days) (Purahong et al, 2014b). Enzyme activities in leaf litter sampled at different times were observed to fluctuate. Interestingly, the two lignin-degrading enzymes (laccase and manganese peroxidase activity (MnP)) that were measured, exhibited patterns linked to lignin decomposition rate in the unmanaged beech forests under investigation (Purahong et al, 2014a). While oxidative activities in the leaf litter were dominated by laccase over the whole sampling time, from 284 days on there was a strong increase of MnP that has a higher redox potential than laccase, (Lundell et al, 2010) coupled with a significantly decreased lignin content (Purahong et al, 2014a). We hypothesized that there should be a community shift perhaps involving newly arriving microbes by 284 – 473 days (Purahong et al, 2014a). The dynamics of most macronutrients (C, N, P, K, Ca, Mg) were studied. Nitrogen immobilization was clearly observed from the first sampling date (89 days) to 362 days (Purahong et al, 2014a).

Appendix S3. Bacterial community dynamics

The initial leaf litter (day 0) was highly dominated by two common bacterial OTUs: OTU 2 (closest hit: Actinobacteria, *Frigoribacterium* sp., 8.9 %) and OTU 4 (closest hit: Proteobacteria, *Sphingomonas* sp., 8.4 %) (Figure 1 a, Figure 2). These two were also the most dominant OTUs in the leaf litter at 89 and 180 days with relative abundances ranging from 5.2 % – 12.8 %, but their respective abundances gradually decreased to between 1.4 % and 3.5 % at 473 days. Six bacterial OTUs (closest hit: *Pseudomonas* sp.1, *Rhizobiales* OTU 27, *Massilia* sp.2, *Novosphingobium* sp., *Janthinobacterium* sp., *Massilia* sp.1; relative abundances 3 % - 4 %) co-dominated freshly fallen leaf litter. The abundance of the majority of dominant bacterial OTUs in freshly fallen leaf litter decreased or became absent by the next sampling dates (Figure 1 a, Figure 2). At 89 and 180 days, the

bacterial communities were also co-dominated by ten further OTUs (closest hit: *Pedobacter* sp., *Novosphingobium* sp., *Massilia* sp.1, *Rhizobium* sp.1, *Kineosporia* sp., *Nitrobacter* sp., *Flavobacterium johnsoniae*, *Flavobacterium* sp., *Caulobacter* sp. and *Chitinophaga* sp. (Figure 1 a, Figure 2). The two bacterial OTUs from the early stages of decomposition (*Nitrobacter* sp. and *Kineosporia* sp.) maintained their high levels of abundance at all sampling dates until the later stages of decomposition at the end of the experiment (180 – 473 days). The abundance of *Flavobacterium johnsoniae* and *Flavobacterium* in leaf litter had declined markedly by 284 and 362 days and became co-dominant at 473 days. *Streptomyces* and *Bradyrhizobium* appeared and co-dominated the later decompositional stage of leaf litter (284 – 473 days) (Figure 1 a, Figure 2). Interestingly, almost all of them were already present in 0 day leaf litter, mostly at low levels of abundance, except for *Pseudomonas* and *Rhizobium,* which were already very abundant at this early stage. This result can be partly explained by some studies which have shown that *Rhizobium* and *Pseudomonas* could be transported from root to leaf or to the top of the plant and live there as bacterial endophytes (Bodenhausen et al., 2013). Such endophytic *Rhizobium* and *Pseudomonas* may serve as inoculum and start to fix nitrogen shortly after leaf fall. One might argue that some of these genera (*Frankia*, *Rhizobium* and *Bradyrhizobium*) commonly found as symbionts in roots of trees and shrubs (e.g. *Alnus, Robinia, Genista*) or herbaceous legumes (e.g. *Lupinus, Vicia, Lathyrus*) were detected in leaf litter because the roots of their host plant might have entered the litterbags during the experiment. However, we can rule out this hypothesis in our experiment as we did not find any roots in the litterbags and all small plants that grew near by the litterbags were removed.

Appendix S4. Fungal community dynamics

The initial leaf litter (day 0) was highly dominated by an aquatic hyphomycetes (Ingoldian fungi; closest hit: *Gyoerffyella* sp.1) that alone contributed 71.9 % of the total sequences at this stage (Figures 1 b, Figure 3). *Mycosphaerella* sp. was also frequently detected in freshly fallen leaf litter where it accounted for 10.7 %. Its abundance had dropped sharply by the next sampling time (89 days) and almost disappeared after 180 days (Figure 1 b, Figure 3). *Ceratobasidium* sp. was the most frequently detected basidiomycete fungus in freshly fallen leaf litter, but it only contributed to 1.6 % of total sequences (Figure 1 b). *Gyoerffyella* sp.1 was still dominant or frequently detected but its abundance declined markedly to 31.7 % and 7.4 % at 89 and 180 days, respectively, and had almost disappeared from the leaf litter at 284 – 473 days (Figure 1 b, Figure 3). The abundance of six fungal OTUs increased greatly from the freshly fallen leaf stage to co-dominate the leaf litter at 89 – 180 days (closest hit: *Ceratobasidium* sp., 10.9 % – 20.6 %; Xylariales OTU 3, 9.4 % – 19.9 %; *Cylindrosympodium* sp.1, 6.5 %; Leotiomycetes OTU 12, 5.7 %; Ceratobasidiaceae OTU 79, 3.9 % – 5.0 %;

Apodus sp., 6.4 %). These six OTUs also co-dominated until 362 or 473 days (Figure 1 b, Figure 3). Some Basidiomycota significantly increased in their abundance (*Mycena* spp., cumulative relative abundance = 4.3 % – 15.6 %) or were newly detected (*Clitocybe phaeophthalma* 11.4 % – 15.5 %; Agaricomycetes OTU 7, 6.8 %; *Lepiota clypeolaria* 5.6 %) and co-dominated leaf litter at 284 – 473 days (Figure 1 b, Figure 3).).

Appendix S5. Factors significantly corresponding with microbial community successions

Our work has identified factors that correspond with successions within the microbial community (see below). It is worth noting that, in the investigated litter, the abiotic factors correlating with both bacterial and fungal community successions were identical, and that the directions of the correlations were also similar. In particular, leaf litter quality parameters, microbial macronutrients, micronutrients and pH were significant factors. Leaf litter quality e.g. C:N ratio is an important factor influencing microbial communities as it relates both to the nutrient status of the leaf litter and to its stage of decomposition (Purahong et al, 2014a, 2015). Macronutrients such as C, N, K, Mg and Ca are required in large amounts for microbial growth and reproduction (Prescott et al, 1999). Specifically, C and N are important elements in all macromolecules including carbohydrates, proteins, lipids, and nucleic acids (Aggarwal, 2007). K and Mg are needed for the activity of enzymes as cofactors (Aggarwal, 2007). Ca is cofactor for certain enzymes and is also a constituent of bacterial endospores. Micronutrients such as Co, Cu and Mn have important roles as structural components of enzymes involved in catalysis of reactions (Aggarwal, 2007). Co is biologically important for microorganisms as it is involved in diverse enzymatic reactions (Antony et al, 2010): it forms an essential component of non corrin-cobalt-containing enzymes (methionine aminopeptidase, prolidase, nitrile hydratase, glucose isomerase, methylmalonyl-CoA carboxytransferase, aldehyde decarbonylase, lysine-2,3-aminomutase, and bromoperoxidase) (Kobayashi and Shimizu, 1999). In this ecosystem, decomposing leaf litter is dominated by laccase - the so-called ‘blue copper proteins’ or ‘blue copper oxidases’, which are proteins containing four catalytic copper atoms (Kunamneni et al, 2007). Thus, we can deduce that Cu must be important for all microbes that produce laccase. In fact, Cu is required by most living organisms as it is the component of many metalloenzymes and proteins involved in electron transport, redox, and other important reactions, and as a cofactor in many proteins (Mendez de Souza et al, 2005). Mn is an essential micronutrient required for the growth, metabolism, reproduction and survival of microorganisms (Sujith & Bharathi, 2011). It is important for scavenging trace metals and breaking down complex organic matter such as lignin as it has roles in general as well as carbohydrate metabolic pathways (Crowley et al, 2000). Four metalloenzymes including manganese superoxide dismutase, mangani-catalase, arginase, and O-phosphatases are containing Mn

(Christianson, 1997; Shi, 2004). The Mn^{2+} containing O-phosphatases are known to regulate many important processes for microbial growth and reproduction such as spore formation, the assimilation of carbon and nitrogen, vegetative growth, the development of fruiting bodies, and cell segregation (Shi, 2004; Sujith & Bharathi, 2011). Mn is also required for microbial protection against toxic metals, UV light, viruses and oxidative stress (Christianson, 1997; Spiro et al, 2010; Sujith & Bharathi, 2011).

Goodness-of-fit statistics (R^2) of environmental variables fitted to the nonmetric multidimensional scaling (NMDS) ordination of bacterial and fungal communities

Variables	**Bacterial community**		**Fungal community**	
	R^2	*P* value	R^2	*P* value
Total C	0.6363	**0.001**	0.627	**0.002**
Total N	0.9088	**0.001**	0.8374	**0.001**
C: N	0.8849	**0.001**	0.8716	**0.001**
pH	0.4898	**0.008**	0.3425	**0.045**
Mg	0.6197	**0.002**	0.4171	**0.021**
K	0.742	**0.001**	0.7953	**0.001**
Ca	0.7983	**0.001**	0.7107	**0.002**
P	0.1218	0.377	0.1387	0.300
Mn	0.4271	**0.013**	0.3684	**0.036**
Fe	0.1275	0.375	0.0458	0.744
Cu	0.4599	**0.017**	0.4478	**0.012**
Co	0.5371	**0.003**	0.3042	**0.05**
V	0.207	0.175	0.1168	0.417
Water content	0.3094	**0.048**	0.3201	**0.05**
Lignin	0.3236	**0.049**	0.2064	0.176
L: N	0.8569	**0.001**	0.8297	**0.001**

Fig. S1 Individual rarefaction analysis for each sample. TP = time point: TP0 = 0 day, TP1 = 89 days, TP2 = 180 days, TP3 = 284 days, TP4 = 362 days, TP5 = 473 days.

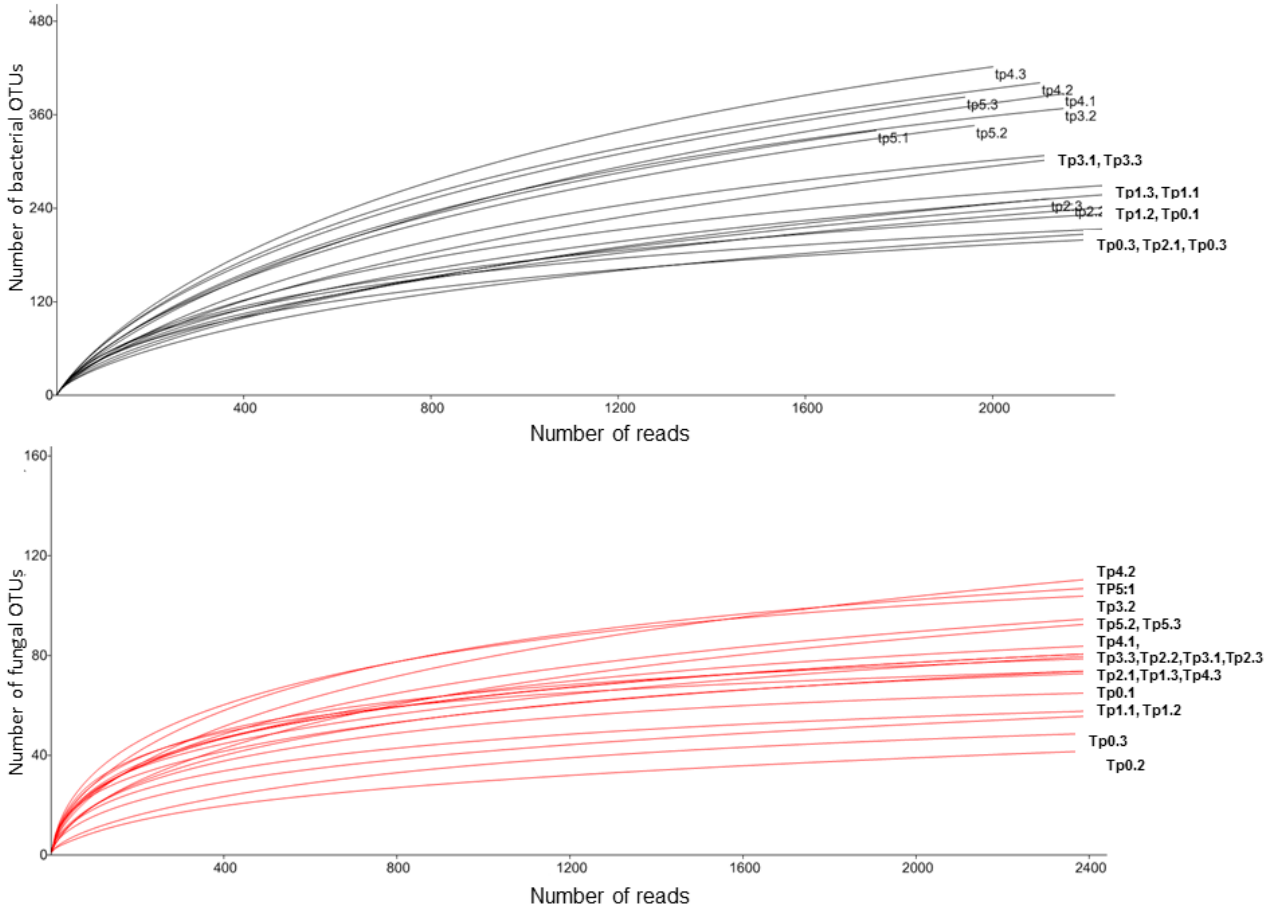


Fig. S2 Heat-map of relative abundances of dominant bacterial (a) and fungal (b) OTUs which account for at least 1 % of the relative abundance at one or more sampling times (mean and standard error (SE), n = 3). The heatmap was overlaid with overall values. TP = time point: TP0 = 0 day, TP1 = 89 days, TP2 = 180 days, TP3 = 284 days, TP4 = 362 days, TP5 = 473 days.

Bacterial OTU	Mean TP0	SE TP0	Mean TP1	SE TP1	Mean TP2	SE TP2	Mean TP3	SE TP3	Mean TP4	SE TP4	Mean TP5	SE TP5
Nitrobacter sp.	0.76	0.22	3.80	0.62	9.70	1.33	12.25	0.88	13.32	2.45	9.22	0.50
Frigoribacterium sp.	8.88	0.39	8.25	1.14	12.83	2.30	4.97	1.08	3.69	0.72	3.45	0.57
Kineosporia sp.	1.70	0.50	3.01	0.29	10.05	2.07	17.37	1.36	5.55	0.98	4.53	0.23
Sphingomonas sp.	8.38	1.39	5.22	0.76	8.65	1.26	4.02	0.83	2.49	0.49	1.39	0.24
Rhizobium sp.1	2.26	0.23	3.92	0.69	3.88	1.27	2.19	0.44	1.84	0.14	1.80	0.10
Flavobacterium sp.1	0.52	0.09	4.77	0.95	3.94	0.79	0.67	0.04	1.04	0.38	3.37	1.32
Flavobacterium johnsoniae	0.55	0.16	4.05	1.24	3.19	1.24	0.42	0.18	0.39	0.11	4.41	1.52
Pedobacter sp.	2.18	0.41	5.41	1.68	3.42	1.18	0.23	0.07	0.20	0.10	0.38	0.19
Novosphingobium sp.	2.95	0.08	3.63	0.51	2.36	0.41	0.79	0.28	0.44	0.18	0.37	0.08
Caulobacter sp.	0.42	0.03	2.39	0.53	2.80	0.28	1.88	0.36	1.16	0.13	1.92	0.22
Bradyrhizobium sp.	0.07	0.05	0.16	0.10	0.12	0.04	2.20	0.33	3.50	0.91	4.61	0.27
Massilia sp.1	2.60	0.13	3.80	0.81	2.20	0.37	0.36	0.14	0.34	0.17	0.16	0.09
Actinoplanes sp.	0.25	0.14	1.33	0.71	0.92	0.29	2.11	0.43	2.17	0.36	1.88	0.23
Massilia sp.2	3.58	0.77	2.27	0.64	0.81	0.06	0.51	0.12	0.44	0.15	0.17	0.09
Pseudomonas sp.1	4.26	0.69	1.16	0.51	1.31	0.93	0.12	0.07	0.34	0.11	0.14	0.08
Chitinophaga sp.	0.21	0.09	1.31	0.21	2.87	1.42	1.47	0.30	0.96	0.48	0.66	0.15
Variovorax paradoxus	1.59	0.21	0.97	0.20	0.90	0.11	0.73	0.14	0.92	0.01	0.67	0.16
Flavobacterium sp2	0.33	0.11	2.33	0.70	0.85	0.05	0.56	0.16	0.36	0.13	1.38	0.64
Streptomyces sp.1	0.00	0.00	0.00	0.00	0.05	0.03	2.51	1.21	1.09	0.10	1.79	0.32
Devosia sp.1	0.64	0.18	1.40	0.13	0.73	0.16	0.59	0.13	0.56	0.11	0.87	0.19
Umezawaea tangerina	0.02	0.02	0.13	0.09	0.29	0.06	1.07	0.33	1.86	0.45	1.56	0.21
Janthinobacterium sp.	2.64	0.42	1.10	0.47	0.40	0.04	0.12	0.06	0.17	0.01	0.03	0.03
Polaromonas sp.	0.89	0.29	1.28	0.33	0.83	0.22	0.34	0.20	0.67	0.13	0.35	0.05
Devosia sp.2	0.40	0.21	1.33	0.44	1.05	0.13	0.50	0.10	0.68	0.12	0.40	0.03
Rhizobium sp.2	0.45	0.05	0.72	0.14	0.55	0.05	0.62	0.08	1.00	0.19	0.89	0.09
Rhizobiales OTU27	3.77	0.16	0.01	0.01	0.00	0.00	0.08	0.08	0.00	0.00	0.00	0.00
Luteibacter rhizovicinus	0.01	0.01	0.01	0.01	0.03	0.02	0.98	0.24	1.00	0.49	1.90	0.47
Rhizobacter sp.	1.64	0.47	0.32	0.05	0.67	0.31	0.15	0.09	0.21	0.09	0.46	0.03
Micromonospora sp.	0.04	0.04	0.04	0.03	0.15	0.02	0.82	0.27	1.28	0.68	1.25	0.35
Dyadobacter sp.	1.25	0.26	1.00	0.14	0.68	0.13	0.09	0.05	0.11	0.03	0.03	0.02
Xylophilus sp.	2.10	0.43	0.40	0.09	0.11	0.08	0.05	0.03	0.22	0.06	0.19	0.06
Rhizomicrobium sp.	0.01	0.01	0.00	0.00	0.08	0.03	0.96	0.25	1.02	0.10	1.18	0.03
Methylobacterium adhaesivum	2.19	0.19	0.27	0.07	0.11	0.02	0.12	0.08	0.03	0.02	0.02	0.02
Hymenobacter sp.	2.47	0.20	0.03	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Streptomyces sp.2	0.00	0.00	0.01	0.01	0.00	0.00	0.76	0.22	0.85	0.29	1.06	0.16
Duganella sp.	1.05	0.50	0.44	0.13	0.35	0.07	0.05	0.03	0.05	0.05	0.04	0.04
Flavobacterium sp.3	1.81	0.86	0.12	0.05	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Pedobacter roseus	1.44	0.17	0.32	0.04	0.11	0.05	0.00	0.00	0.02	0.02	0.02	0.02
Kineococcus sp.	1.07	0.23	0.18	0.09	0.17	0.10	0.00	0.00	0.00	0.00	0.00	0.00
Friedmanniella sp.	1.07	0.12	0.12	0.05	0.01	0.01	0.05	0.03	0.02	0.02	0.06	0.06
Pseudomonas sp.2	0.00	0.00	1.12	1.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Acidiphilium sp.	1.07	0.32	0.04	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

a

Fig. S2 (continue)

Fungal OTU	Mean TP0	SE TP0	Mean TP1	SE TP1	Mean TP2	SE TP2	Mean TP3	SE TP3	Mean TP4	SE TP4	Mean TP5	SE TP5
Apodus sp.	0.00	0.00	0.23	0.16	6.40	3.61	13.90	5.72	16.98	2.12	38.09	13.47
Gyoerffyella sp.1	71.91	5.42	31.72	6.47	7.40	2.04	0.00	0.00	0.06	0.04	0.03	0.01
Xylariales otu3	0.36	0.28	9.35	1.66	19.87	0.57	9.00	1.25	1.64	0.52	2.23	0.39
Ceratobasidium sp.	1.61	0.55	20.60	3.93	10.87	4.74	4.21	1.30	15.51	7.72	8.15	3.78
Cylindrosympodium sp.1	0.10	0.05	1.12	0.41	6.49	1.19	10.00	1.24	1.31	0.48	0.85	0.08
Cercophora sp.	0.00	0.00	0.00	0.00	1.27	1.09	0.95	0.78	4.85	3.79	1.47	1.37
Agaricomycetes otu7	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.54	6.85	6.85	0.00	0.00
Cylindrosympodium sp.2	0.11	0.09	0.52	0.32	3.61	0.34	9.23	1.24	2.42	0.65	1.72	0.18
Efibulobasidium albescens	0.08	0.04	4.13	2.79	1.94	1.07	0.00	0.00	0.38	0.38	0.00	0.00
Clitocybe phaeophthalma	0.00	0.00	0.00	0.00	0.00	0.00	11.38	10.52	15.53	7.98	0.07	0.04
Mycosphaerella sp.	10.72	1.69	1.81	0.63	0.62	0.04	0.00	0.00	0.08	0.05	0.00	0.00
Leotiomycetes otu12	0.04	0.02	1.59	1.08	5.73	1.17	4.98	0.97	1.72	0.20	2.50	0.45
Flagellospora sp.	2.10	0.62	3.24	0.68	1.27	0.49	0.67	0.10	0.26	0.07	0.22	0.04
Mycena terena	0.00	0.00	0.32	0.32	0.68	0.35	1.05	0.54	3.42	2.02	6.35	5.16
Podospora sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.54	0.54	4.46	2.82
Hydnodontaceae otu16	0.00	0.00	0.19	0.19	1.57	1.57	0.00	0.00	0.03	0.03	0.00	0.00
Tricholomataceae otu17	0.00	0.00	3.63	3.30	0.97	0.77	0.04	0.04	0.01	0.01	0.00	0.00
Leptodiscella sp.	0.00	0.00	0.10	0.08	1.70	0.52	2.48	0.39	0.26	0.12	0.32	0.18
Lepiota clypeolaria	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	5.60	4.12
Cylindrotrichum gorii	0.00	0.00	0.11	0.09	1.01	0.56	1.51	0.30	0.07	0.05	0.07	0.01
Apiognomonia sp.	1.53	0.94	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aureobasidium sp.	0.03	0.01	1.23	0.23	1.90	0.38	2.62	1.12	0.47	0.22	0.65	0.13
Hydropisphaera sp.	0.00	0.00	0.19	0.17	2.54	1.34	0.92	0.54	0.07	0.03	0.03	0.03
Cryptosporiopsis sp.	1.25	0.28	1.08	0.71	0.22	0.03	0.15	0.05	0.22	0.01	0.17	0.08
Mycena sp.1	0.00	0.00	0.00	0.00	0.05	0.04	1.08	0.53	0.40	0.03	2.94	0.41
Phialea strobilina	0.38	0.06	1.98	0.32	0.96	0.36	0.14	0.08	0.23	0.11	0.26	0.10
Xylariales otu30	0.00	0.00	0.00	0.00	0.70	0.29	1.09	0.28	0.72	0.55	0.04	0.00
Helotiales otu31	0.67	0.18	2.67	0.40	2.26	0.55	1.20	0.28	0.34	0.12	0.25	0.02
Chalara holubovae	0.00	0.00	0.03	0.03	0.52	0.14	1.23	0.93	0.22	0.10	0.77	0.60
Phaeohelotium monticola	0.00	0.00	0.03	0.03	0.26	0.24	0.25	0.25	2.57	1.77	0.08	0.08
Podospora glutinans	0.00	0.00	0.00	0.00	1.30	0.46	0.52	0.20	0.95	0.48	0.15	0.09
Venturiaceae otu35	0.00	0.00	0.08	0.06	0.53	0.11	1.72	0.66	0.14	0.08	0.14	0.01
Helotiales otu40	0.19	0.07	1.27	0.42	0.66	0.39	0.00	0.00	0.01	0.01	0.00	0.00
Clitocybe metachroa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.46	0.77
Herpotrichia sp.	0.00	0.00	0.11	0.06	0.37	0.18	0.40	0.33	1.07	0.46	0.19	0.11
Mycena sp.3	0.00	0.00	0.00	0.00	0.89	0.81	2.18	0.53	2.59	1.24	3.23	1.44
Ceratobasidiaceae otu79	0.01	0.01	5.00	1.78	3.91	2.85	1.70	0.99	3.39	3.03	1.09	0.50
Ascomycota otu144	0.00	0.00	0.14	0.04	1.11	0.46	0.86	0.28	0.39	0.10	0.44	0.10

b

Fig. S3 Observed bacterial and fungal OTU richness (a and c) and Shannon diversity (b and d) (mean ± SE, n = 3) in leaf litter across different sampling dates.

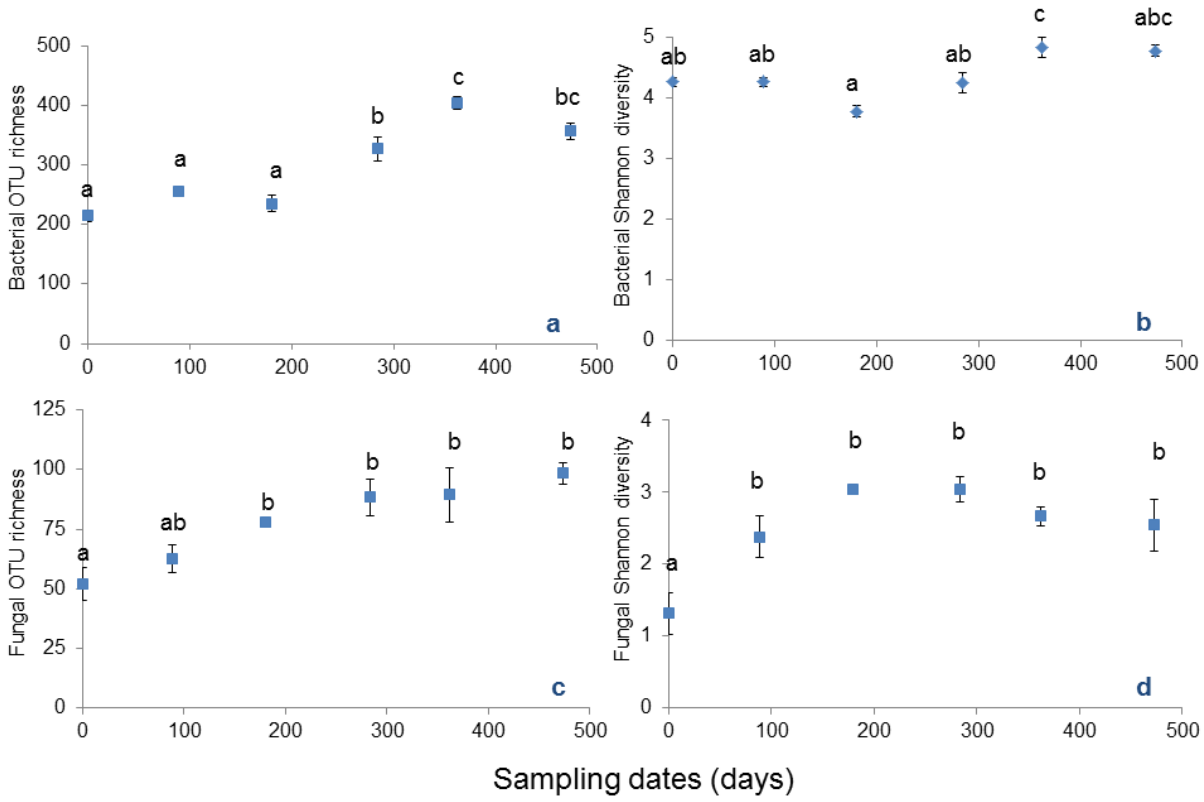


Fig. S4 Spearman's rank correlations between specific bacterial (a) and fungal (b) OTUs (account for at least 1 % of the relative abundance at one or more sampling times) and leaf litter quality parameters and physicochemical properties. The heatmap was overlaid with overall values.

Bacterial OTU	C	N	C/N	Mg	K	Ca	Mn	Cu	Co	pH	Water	Lignin
Nitrobacter sp.	**-0.64**	**0.84**	**-0.73**	**0.54**	**-0.57**	**0.83**	0.30	**0.53**	**0.76**	0.10	0.03	0.11
Frigoribacterium sp.	**0.78**	**-0.74**	**0.78**	-0.17	**0.69**	**-0.64**	-0.11	**-0.50**	**-0.68**	-0.19	-0.37	**0.49**
Kineosporia sp.	**-0.53**	**0.68**	**-0.56**	**0.53**	**-0.49**	**0.68**	0.23	0.39	0.45	0.02	-0.36	0.46
Sphingomonas sp.	**0.79**	**-0.64**	**0.77**	0.06	**0.74**	**-0.48**	0.15	-0.45	**-0.52**	-0.40	-0.43	**0.61**
Rhizobium sp.1	**0.54**	**-0.48**	**0.55**	-0.17	**0.53**	**-0.47**	-0.17	-0.19	**-0.59**	0.20	-0.22	0.27
Flavobacterium sp.1	-0.14	-0.06	-0.04	**-0.55**	-0.25	-0.15	**-0.75**	0.29	-0.25	**0.67**	0.26	-0.20
Flavobacterium johnsoniae	-0.04	-0.33	0.15	**-0.57**	-0.18	-0.26	**-0.82**	0.10	-0.42	**0.72**	**0.48**	-0.12
Pedobacter sp.	**0.68**	**-0.72**	**0.66**	-0.44	**0.60**	**-0.75**	-0.33	-0.32	**-0.78**	0.01	-0.20	0.17
Novosphingobium sp.	**0.83**	**-0.71**	**0.75**	-0.39	**0.81**	**-0.76**	-0.14	-0.38	**-0.70**	-0.09	-0.30	0.20
Caulobacter sp.	-0.11	0.09	-0.04	-0.20	-0.21	-0.01	**-0.51**	0.21	-0.33	**0.60**	0.05	0.26
Bradyrhizobium sp.	**-0.84**	**0.71**	**-0.79**	0.10	**-0.81**	**0.64**	-0.04	0.34	**0.62**	0.34	0.40	-0.36
Massilia sp.1	**0.82**	**-0.70**	**0.74**	-0.35	**0.80**	**-0.73**	-0.08	-0.30	**-0.78**	-0.15	-0.25	0.23
Actinoplanes sp.	**-0.65**	**0.64**	**-0.65**	-0.01	**-0.50**	**0.48**	0.02	0.24	**0.54**	0.34	0.09	-0.24
Massilia sp.2	**0.87**	**-0.72**	**0.77**	-0.27	**0.88**	**-0.72**	0.01	-0.46	**-0.66**	-0.25	-0.25	0.24
Pseudomonas sp.1	**0.73**	**-0.74**	**0.67**	-0.23	**0.63**	**-0.60**	0.01	-0.37	**-0.51**	-0.27	-0.18	0.04
Chitinophaga sp.	-0.24	0.35	-0.35	0.17	-0.16	0.37	-0.01	0.47	0.11	0.20	0.09	0.30
Variovorax paradoxus	**0.63**	**-0.48**	**0.61**	-0.09	**0.51**	-0.40	0.21	-0.09	**-0.48**	-0.27	-0.16	-0.04
Flavobacterium sp2	-0.07	-0.15	-0.01	**-0.58**	-0.04	-0.33	**-0.64**	0.11	-0.40	**0.57**	-0.01	-0.12
Streptomyces sp.1	**-0.84**	**0.77**	**-0.82**	0.28	**-0.74**	**0.63**	0.06	0.23	**0.63**	0.15	-0.21	-0.05
Devosia sp.1	0.24	-0.26	0.28	**-0.63**	0.11	**-0.49**	**-0.52**	-0.14	**-0.53**	**0.58**	0.13	-0.17
Umezawaea tangerina	**-0.80**	**0.78**	**-0.82**	0.22	**-0.74**	**0.64**	0.03	0.25	**0.75**	0.22	0.09	-0.26
Janthinobacterium sp.	**0.91**	**-0.72**	**0.83**	-0.15	**0.84**	**-0.63**	0.05	-0.32	**-0.59**	-0.29	-0.22	0.18
Polaromonas sp.	**0.50**	-0.41	**0.47**	-0.28	**0.52**	-0.42	-0.06	-0.27	-0.47	0.09	-0.18	-0.02
Devosia sp.2	0.28	-0.10	0.22	-0.14	0.08	-0.07	-0.21	0.37	-0.34	0.41	-0.06	0.13
Rhizobium sp.2	**-0.60**	0.42	**-0.53**	-0.21	**-0.56**	0.38	-0.17	0.32	**0.50**	**0.48**	0.45	**-0.60**
Rhizobiales OTU27	**0.61**	**-0.61**	**0.61**	-0.08	**0.73**	**-0.60**	0.29	**-0.66**	-0.33	**-0.56**	-0.18	0.11
Luteibacter rhizovicinus	**-0.84**	**0.80**	**-0.84**	0.29	**-0.85**	**0.65**	0.05	0.43	**0.58**	0.07	**0.56**	-0.22
Rhizobacter sp.	0.26	**-0.70**	0.44	-0.16	0.17	-0.41	-0.24	**-0.54**	-0.41	-0.17	-0.04	0.09
Micromonospora sp.	**-0.79**	**0.79**	**-0.77**	0.20	**-0.79**	**0.62**	-0.05	0.27	**0.68**	0.18	0.13	-0.18
Dyadobacter sp.	**0.88**	**-0.75**	**0.86**	-0.38	**0.77**	**-0.69**	-0.16	-0.41	**-0.64**	-0.13	-0.16	0.20
Xylophilus sp.	**0.57**	**-0.59**	**0.54**	-0.42	0.45	**-0.62**	-0.09	-0.46	-0.36	-0.11	-0.10	-0.34
Rhizomicrobium sp.	**-0.92**	**0.77**	**-0.85**	0.43	**-0.90**	**0.81**	0.12	**0.47**	**0.70**	0.11	0.39	-0.15
Methylobacterium adhaesiv	**0.85**	**-0.75**	**0.82**	-0.17	**0.82**	**-0.71**	0.08	**-0.49**	**-0.50**	-0.36	-0.19	0.18
Hymenobacter sp.	**0.77**	**-0.72**	**0.73**	-0.24	**0.75**	**-0.72**	0.07	-0.42	**-0.47**	-0.37	-0.15	-0.08
Streptomyces sp.2	**-0.83**	**0.72**	**-0.79**	0.20	**-0.75**	**0.62**	0.15	0.39	**0.65**	0.17	0.25	-0.42
Duganella sp.	**0.82**	**-0.79**	**0.84**	-0.23	**0.79**	**-0.72**	-0.11	-0.46	**-0.72**	-0.24	-0.21	0.27
Flavobacterium sp.3	**0.85**	**-0.81**	**0.84**	-0.37	**0.75**	**-0.81**	-0.03	**-0.54**	**-0.63**	-0.29	-0.13	0.03
Pedobacter roseus	**0.73**	**-0.84**	**0.77**	-0.35	**0.79**	**-0.70**	-0.11	-0.40	**-0.57**	-0.24	-0.16	-0.05
Kineococcus sp.	**0.81**	**-0.82**	**0.83**	-0.12	**0.69**	**-0.66**	0.01	-0.47	**-0.66**	-0.38	-0.19	0.29
Friedmanniella sp.	**0.51**	**-0.51**	**0.51**	-0.29	**0.66**	**-0.64**	0.10	**-0.51**	**-0.48**	-0.43	-0.19	0.00
Pseudomonas sp.2	0.16	-0.21	0.21	-0.40	0.26	-0.21	-0.26	-0.16	-0.21	0.35	0.17	-0.07
Acidiphilium sp.	**0.77**	**-0.70**	**0.72**	-0.25	**0.74**	**-0.72**	0.07	-0.40	**-0.50**	-0.38	-0.14	-0.08

a

Fig. S4 (continue)

Fungal OTU	C	N	C/N	Mg	K	Ca	Mn	Cu	Co	pH	Water
Apodus sp.	**-0.88**	**0.75**	**-0.83**	0.28	**-0.85**	**0.77**	-0.12	0.44	**0.71**	0.33	**0.47**
Gyoerffyella sp.1	**0.82**	**-0.86**	**0.83**	-0.42	**0.78**	**-0.79**	-0.20	**-0.52**	**-0.65**	-0.20	-0.26
Xylariales otu3	-0.03	0.13	-0.07	0.08	-0.11	0.15	-0.18	0.42	-0.21	0.35	-0.11
Ceratobasidium sp.	0.10	0.14	-0.10	**-0.48**	0.02	-0.27	-0.25	0.27	-0.20	0.18	-0.01
Cylindrosympodium sp.1	-0.38	**0.55**	-0.46	0.42	-0.32	**0.53**	0.14	0.33	0.35	0.08	0.01
Cercophora sp.	**-0.50**	**0.57**	-0.40	0.43	**-0.57**	**0.75**	0.14	**0.57**	**0.55**	0.18	0.27
Agaricomycetes otu7	-0.18	0.42	-0.29	0.47	-0.14	0.34	**0.51**	0.13	0.38	-0.11	-0.42
Cylindrosympodium sp.2	**-0.52**	**0.68**	**-0.57**	**0.51**	**-0.50**	**0.66**	0.25	0.34	**0.48**	-0.05	0.00
Efibulobasidium albescens	0.39	-0.45	0.44	-0.12	0.34	-0.18	-0.17	-0.08	**-0.50**	0.23	-0.14
Clitocybe phaeophthalma	**-0.66**	**0.58**	**-0.76**	0.30	-0.47	**0.49**	0.43	0.24	0.47	-0.17	0.17
Mycosphaerella sp.	**0.87**	**-0.84**	**0.87**	-0.33	**0.86**	**-0.70**	-0.06	**-0.47**	**-0.60**	-0.24	-0.27
Leotiomycetes otu12	**-0.48**	0.40	-0.41	0.37	-0.37	**0.56**	0.03	0.30	0.22	0.19	0.17
Flagellospora sp.	**0.80**	**-0.67**	**0.70**	-0.20	**0.72**	**-0.65**	0.07	-0.21	**-0.69**	-0.17	-0.39
Mycena terena	**-0.78**	**0.69**	**-0.75**	0.20	**-0.78**	**0.66**	-0.01	0.39	**0.55**	0.23	**0.54**
Podospora sp.	**-0.70**	0.28	**-0.51**	-0.27	**-0.65**	0.23	-0.45	0.15	0.29	0.25	**0.62**
Hydnodontaceae otu16	-0.02	-0.14	0.04	0.10	0.07	0.24	-0.05	0.02	0.12	0.39	0.28
Tricholomataceae otu17	0.30	-0.23	0.26	-0.33	0.29	-0.30	-0.19	0.05	**-0.52**	0.27	-0.20
Leptodiscella sp.	**-0.48**	**0.55**	-0.43	**0.50**	-0.44	**0.62**	0.13	0.29	0.33	0.10	-0.06
Lepiota clypeolaria	**-0.61**	0.31	-0.42	-0.16	-0.46	0.21	-0.13	0.13	0.25	0.04	0.28
Cylindrotrichum gorii	-0.40	0.45	-0.41	0.40	-0.30	**0.49**	0.04	0.20	0.27	0.07	0.02
Apiognomonia sp.	**0.64**	**-0.71**	**0.71**	-0.20	**0.73**	**-0.55**	0.10	**-0.68**	-0.38	-0.44	-0.14
Aureobasidium sp.	-0.18	0.28	-0.25	0.23	-0.17	0.30	0.00	0.43	-0.03	0.25	-0.01
Hydropisphaera sp.	-0.14	0.40	-0.24	0.42	-0.20	0.33	0.23	0.38	0.04	-0.09	-0.46
Cryptosporiopsis sp.	**0.52**	**-0.47**	0.44	-0.23	**0.56**	-0.43	0.03	-0.41	**-0.49**	-0.23	0.01
Mycena sp.1	**-0.89**	**0.72**	**-0.82**	0.21	**-0.77**	**0.60**	-0.11	0.24	**0.68**	0.19	0.35
Phialea strobilina	**0.49**	**-0.59**	**0.49**	-0.41	0.35	**-0.51**	-0.31	-0.08	**-0.52**	0.32	0.05
Xylariales otu30	**-0.49**	**0.74**	**-0.56**	**0.63**	**-0.47**	**0.77**	0.37	0.43	**0.59**	-0.10	-0.04
Helotiales otu31	**0.51**	-0.31	0.41	-0.16	0.41	-0.35	-0.05	0.08	**-0.50**	0.05	-0.26
Chalara holubovae	**-0.63**	**0.49**	**-0.49**	0.40	**-0.54**	**0.65**	0.03	0.23	0.37	0.18	0.08
Phaeohelotium monticola	-0.16	0.36	-0.21	0.28	-0.25	0.36	0.06	0.38	0.32	0.17	-0.23
Podospora glutinans	**-0.57**	**0.57**	**-0.58**	**0.54**	**-0.49**	**0.75**	0.27	0.39	0.47	-0.10	0.14
Venturiaceae otu35	-0.33	0.46	-0.34	0.46	-0.36	0.45	0.14	0.24	0.31	0.13	-0.10
Helotiales otu40	**0.71**	**-0.69**	**0.70**	-0.40	**0.61**	**-0.61**	-0.25	-0.20	**-0.73**	0.09	-0.20
Clitocybe metachroa	**-0.64**	0.13	-0.39	-0.30	**-0.65**	0.08	**-0.64**	-0.05	0.13	0.42	**0.59**
Herpotrichia sp.	**-0.54**	**0.56**	**-0.59**	0.15	-0.34	**0.51**	0.08	0.28	0.36	0.19	0.11
Mycena sp.3	**-0.79**	**0.76**	**-0.73**	0.29	**-0.74**	**0.68**	0.06	0.43	**0.65**	0.01	0.22
Ceratobasidiaceae otu79	-0.14	0.33	-0.27	-0.28	-0.26	-0.02	-0.36	0.27	-0.05	0.38	0.21
Ascomycota otu144	**-0.59**	**0.54**	**-0.49**	0.43	**-0.51**	**0.64**	0.00	0.29	0.39	0.25	0.03

b

Fig. S5 Mean manganese peroxidase activities (mU/g litter dry mass) in beech leaf litter at different sampling times (n = 3): Tp1 = 89, Tp2= 180, Tp3= 284, Tp4 = 362, Tp5 = 473 days (**reproduced** from Purahong et al., 2014).

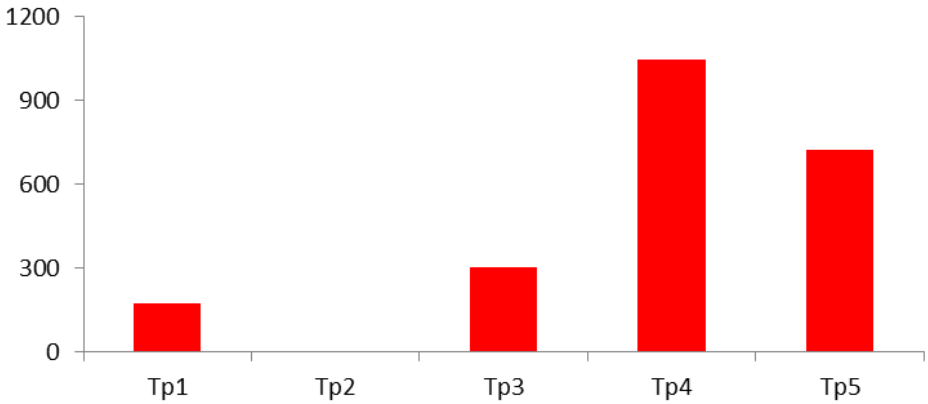
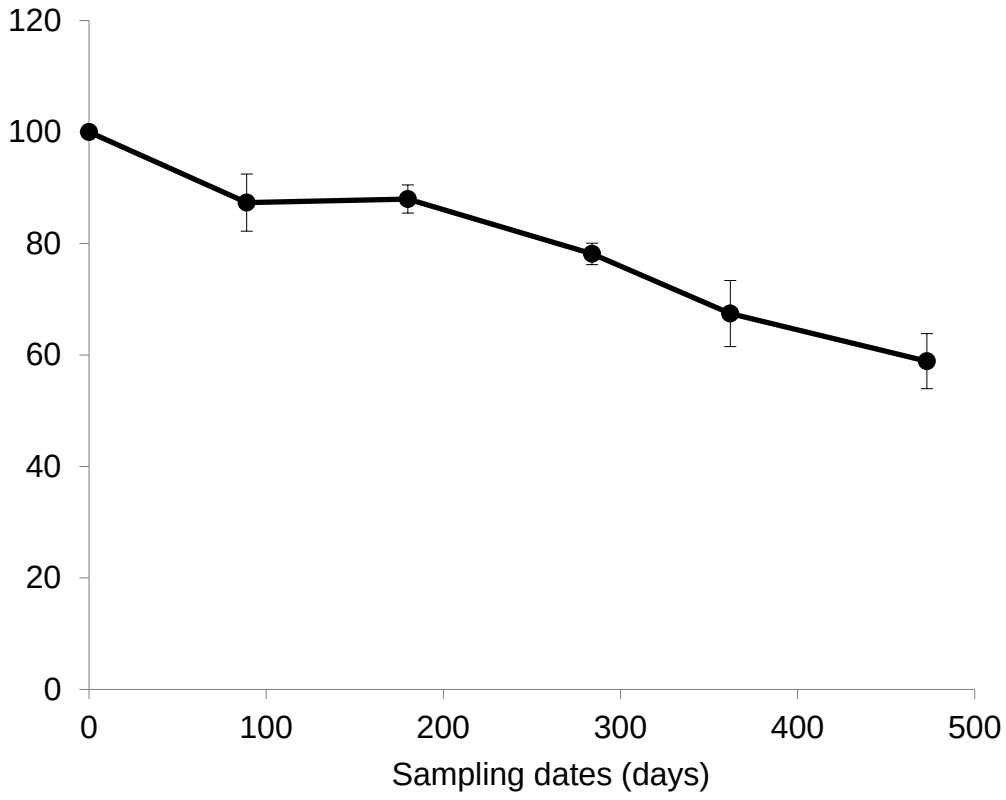


Fig. S6 Mean amount of total lignin remaining during decomposition (mean ± SE, n = 3) (reproduced from Purahong et al., 2014).



References

Aggarwal S. (2007). General Microbiology: Principles of Microbial Nutrition and Growth. Department of Microbiology Institute of Home Economics, New Delhi.

Antony R, Sujith PP, Fernandes S O, Verma P, Khedekar VD, Loka Bharathi PA. (2010). Cobalt Immobilization by Manganese Oxidizing Bacteria from the Indian Ridge System. Curr Microbiol, doi: 10.1007 /s00284-010-97 84-1.

Berg B. (2000). Litter decomposition and organic matter turnover in northern forest soils. Forest Ecol Manage 133:13–22.

Christianson DW. (1997). Structural chemistry and biology of manganese metalloenzymes. Prog Biophys Mol Biol 67:217–252.

Crowley JD, Traynor DA, Weatherburn DC. (2000). Enzymes and proteins containing manganese:an overview. Met Ions Biol Syst 37:209–278.

Gardes M, Bruns TD. (1993). ITS primers with enhanced specificity for basidiomycetes-application for the identification of mycorrhizae and rusts. Mol Ecol 2:113-118.

Jacob M, Weland N, Platner C, Schaefer M, Leuschner C, Thomas FM 2009. Nutrient release from decomposing leaf litter of temperate deciduous forest trees along a gradient of increasing tree species diversity. Soil Biol Biochem 41: 2122–2130.

Jacob M, Viedenz K, Polle A, Thomas F 2010. Leaf litter decomposition in temperate deciduous forest stands with a decreasing fraction of beech (*Fagus sylvatica*). Oecologia 164: 1083–1094.

Kobayashi M, Shimizu S (1999) Cobalt proteins. Eur J Biochem 261:1-9.

Kunamneni A, Ballesteros A, Plou FJ, Alcalde M. 2007. Fungal laccase–a versatile enzyme for biotechnological applications. In: Mendez-Vilas A, editor. Communicating current research and educational topics and trends in applied microbiology. Badajoz: Formatex Research Center, pp. 223–245.

Mendez de Souza P, Marinho, PH, Lima, MA, Elesbão do Nascimento, A., Maria de Campos Takaki, G, (2005) Copper influence on polyphosphate metabolism of Cunninghamella elegans. Braz. J. Microbiol. 36: 315-320.

Muyzer G, Smalla K, 1998. Application of denaturing gradient gel electrophore-sis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie Van Leeuwenhoek 73, 127–141.

Muyzer G, Teske A, Wirsen C, Jannasch H, 1995. Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal ventsamples by denaturing gradient gel electrophoresis of 16S rDNA fragments. Arch Microbiol 164: 165–172.

Prescott LM, Harley JP, Klein DA 1999. Microbiology, 4 edn. McGraw-Hill Higher Education

Purahong W, Kapturska D, Pecyna MJ, Schloter M, Buscot F, Hofrichter M, Krüger D 2014a. Influence of different forest system management practices on leaf litter decomposition rates, nutrient dynamics and the activity of ligninolytic enzymes: a case study from Central European forests. PLoS ONE 9(4): e93700. doi:10.1371/journal.pone.0093700.

Purahong W, Schloter M, Pecyna J M, Kapturska D, Däumlich V, Mital S, Buscot F, Hofrichter M, Gutknecht LM J, Krüger D 2014b. Uncoupling of microbial community structure and function in decomposing litter across beech forest ecosystems in Central Europe. Scientific reports, 4:7014. doi: 10.1038/srep07014.

Shi L 2004. Manganese-dependent protein o-phosphatases in prokaryotes and their biologicalfunctions. Front Biosci 9: 1382–1397.

Sujith PP and Loka Bharathi PA 2011. Manganese oxidation by bacteria: Biogeochemical aspects. In Molecular Biomineralis: Progress in Molecular and Sub-cellular Biology (ed. Muller, W. E. G.), Springer, vol. 52, pp. 49 - 76.

White TJ, Bruns TD, Lee S, Taylor J. (1990). Amplification and direct sequencing of fungal ribosomal DNA genes for phylogenies. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds). PCR Protocols: A Guide to Methods and Applications. Academic Press: San Diego, pp 315-322.