

Controls of litter chemistry over early lignin decomposition in beech litter ¹

Lukas Kohl¹, Wolfgang Wanek¹, Katharina Keiblinger^{2,3}, Sonja Leitner^{1,3}, Maria Mooshammer¹, Ieda Hämmerle¹, Lucia Fuchslueger¹, Jörg Schneckner¹, Thomas Schneider^{4,5}, Sandra Moll⁷, Markus Gorfer^{7,8}, Joseph Strauss^{7,8}, Katharina Riedel^{4,6}, Leo Eberl^{4,5}, Sophie Zechmeister-Boltenstern^{2,3}, Andreas Richter¹,

1 Department of Chemical Ecology and Ecosystem Research, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

2 Federal Research and Training Centre for Forests, Natural Hazards and Landscape, Department of Soil Biology, Seckendorff-Gudent-Weg 8, A-1131 Vienna, Austria

3 Current address: Institute for Soil Science, University of Natural Resources and Life Sciences, Peter Jordan-Straße 82, A-1180, Vienna, Austria

4 Institute of Plant Biology, University of Zurich, Winterthurerstrasse 190, CH-8057, Zurich, Switzerland

5 Current address: Institute of Plant Biology, University of Zurich, Zollikerstrasse 107, CH-8008, Zurich, Switzerland

6 Current address: Institute of Microbiology, Ernst-Moritz-Arndt University of Greifswald, Friedrich-Ludwig-Jahn-Strasse 15, D-17487 Greifswald, Germany

7 Fungal Genetics and Genomics Unit, Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Konrad-Lorenz-Strasse 24, A-3430 Tulln, Austria

8 AIT Austrian Institute of Technology GmbH, Bioresources Unit, Konrad-Lorenz-Strasse 24, A-3430 Tulln, Austria

*** E-mail: Corresponding author@institute.edu**

Lignin is a major component of plant litter and is considered highly resistant to decomposition. Polymeric carbohydrates, in contrast, are more easily accessible carbon sources. We studied the decomposition rates of these two compound classes, to which extent they are controlled by litter C:N:P stoichiometry, and whether this control changes over time. Therefore, we conducted a 15-months mesocosm experiment under controlled climatic conditions, comparing beech litter of different N and P contents, which was sterilized and re-inoculated with a litter/topsoil mixture from one of the sites to ensure identical microbial communities at the start of the experiment. Lignin and carbohydrate decomposition rates were calculated for 2 periods (0-6 months and 6-15 months) by pyrolysis-GC/MS.

Positive correlations of carbohydrate decomposition rates with litter N content were found during the entire experiment. Lignin decomposition rates during the initial period were highly variable and negatively correlated to litter P content and positively correlated to the microbial P demand ($C:P_{\text{litter}}/C:P_{\text{microbial}}$). During the later stage, both lignin and carbohydrate decomposition loss were positively correlated to N contents and respiration. Initial lignin decomposition rates were highest in litter with low fungi:bacteria ratios, which occurred in N and P poor litter.

Our results showed that a substantial amount of lignin can be degraded during early decomposition. In the present study, early lignin decomposition was coupled to low N and P availability, and the establishment of K-strategist microorganisms. However, early lignin decomposition rates did not depend on fungi, which are commonly assumed to mediate lignin decomposition, or stoichiometric conditions that favor fungal growth.

2 Plant litter is quantitatively dominated by macromolecular compounds. In foliar litter, lignin and carbohy-
3 drate polymers together make up 40-60% of litter dry mass [1], while leachable substances ("DOM") account
4 for only 1.5-6% [2]. The breakdown of these high molecular weight compounds into smaller molecules acces-
5 sible to microbes is mediated by extracellular enzymes and is therefore considered to be the rate limiting for
6 decomposition processes [3]

7 Common models of litter decomposition [4-7] assume that organic compounds in litter form up to three
8 independent pools of increasing recalcitrance, i.e. (1) soluble compounds, (2) cellulose and hemi-celluloses,
9 and (3) lignin and waxes (cutin and suberin). Soluble compounds are most accessible to microbes and
10 are usually consumed first, followed by regular polymers, such as cellulose. Lignin is not degraded until
11 accumulated to a certain, critical level when it inhibits the degradation of less recalcitrant compounds. Most
12 studies quantified these pools by gravimetric determination of the amount of cellulose, hemi-celluloses and
13 lignins after sequential extractions with selective solvents. These methods were repeatedly criticized for being
14 unspecific for lignin determination [8]. When analyzed with alternative methods (NMR, CuO-oxidation,
15 Pyrolysis-GC/MS), extracted lignin fractions were shown to contain also many other substances [9].

16 Recent studies based on more specific methods to determine litter lignin contents question the intrinsic
17 recalcitrance of lignin. Isotope labeling experiments with soils and litter/soil mixtures, undertaken both
18 in-situ and under controlled conditions, revealed mean residence times of lignin in soils in the range of 10-
19 50 years, much less then expected and shorter than that of bulk soil organic matter [10-12]. While the
20 ability to completely degrade lignin was traditionally attributed exclusively to Basidiomycetes, it has been
21 demonstrated for several bacterial taxa over the last years [13].

22 For leaf litter, lignin decomposition even at early stages of litter decay and lignin decomposition rates
23 that decreased during decomposition were recently reported by Klotzbücher and colleagues [14]. Based on
24 these results, they proposed a new concept for lignin degradation in which fastest lignin degradation occurs
25 during early litter decomposition when the availability of labile carbon sources is high. Lignin decomposition
26 during late decomposition, in contrast, is limited by the availability of easily assimilated C and therefore
27 slows down. Additionally, the decomposition of lignin may also be dependent on the nutrient content of the
28 litter and thus the nutritional status of the microbial community. During radical polymerization, significant
29 amounts of cellulose and protein are incorporated into lignin structures [15]. In isolated lignin fractions
30 from fresh beech litter, N contents twice as high as in bulk litter were found [16]. It was therefore argued
31 that, while yielding little C and energy, lignin decomposition makes protein accessible to decomposers that is

occluded in plant cell walls, and that lignin decomposition is therefore not driven by C but by the N demand of the microbial community ("Nitrogen mining theory", [17]).

In favor of the N mining theory, fertilization experiments indicated N exerts an important control on lignin degradation: N addition increased mass loss rates in low-lignin litter while slowing down decomposition in lignin-rich litter [18] and decreased the activity of lignolytic enzymes in forest soils [3]. Moreover, cellulose triggered a stronger priming effect in fertilized than in unfertilized soils indicating that the mineralization of recalcitrant carbon may be controlled by an interaction of N and labile C availability [19].

Addition of N has a different effect on litter decomposition than varying N levels in the litter [20]. This is due to the fact that leaf litter N is stored in protein and lignin structures and not directly available to microorganisms, while fertilizer N is added in the form of readily available inorganic N (ammonium, nitrate or urea). N-fertilization experiments can thus simulate increased N-deposition rates but not the effect of litter N on decomposition processes.

Our study therefore aimed at analyzing the effect of variations in beech litter nutrient (N and P) content and stoichiometry (C:N and N:P ratios) on lignin and carbohydrate decomposition rates. Towards this end, we followed the breakdown of lignin and polymeric carbohydrates by pyrolysis-GC/MS (pyr-GC/MS) during a mesocosm experiment under constant environmental conditions over a period of 15 month. In order to exclude effects resulting from different initial microbial communities, we sterilized beech litter samples from 4 different locations in Austria and re-inoculated them prior to the experiment with an litter/top-soil inoculum from one of the sites. Additionally, we analyzed the micorbial community in a subset of the samples using a metaproteomic approach.

With the experiment, we addressed the following questions:

(Q1) Is lignin decomposition delayed until late decomposition stages or are significant amounts of lignin already degraded during early litter decomposition, and is the timing of lignin decomposition dependent on litter stoichiometry? We hypothesized, that ligin decomposition is initially slower in litter with a narrow C:N ratio (higher availability of assimilable nitrogen), than in litter with a high C:N ratio.

(Q2) Are high lignin degradation rates related to a higher fungal activity? We hypothesized that wider C:N and C:P ratios favor lignin degradation by fungi while narrow C:N and C:P ratios favor carbohydrate degradation by bacteria.

Initial litter chemistry

Initial litter chemistry of the four sites (Achenkirch, AK, Klausenleopoldsdorf, KL, Ossiach, OS, Schottenwald, SW), measured 14 days after incubation, is presented in supplemental table 1. C:N ratios varied between 41:1 and 58:1 and C:P ratios between 700:1 and 1300:1, while N:P ratios ranged between 15:1 and 30:1. No significant changes occurred during litter incubation except a slight decrease of the C:N ratio (41.8:1 to 37.4:1) found in the most active litter type (SW) after 15 months. Fe concentrations were more than twice as high for OS (approx. 450 ppm) than for other litter types (approx. 200 ppm). Litter Mn also was highly variable between litter types, ranging between 170 and 2130 ppm. Changes of micro-nutrient concentrations during litter incubation were significant, but in all cases <15% of the initial concentration. In initial litter, lignin accounted for 28.9-31.2% and carbohydrates for 25.9-29.2% of the total peak area of all pyrolysis products.

Mass loss, respiration and soluble organic carbon

Litter mass loss was not significant after 2 weeks and 3 months, and significant for 2 litter from two sites after 6 months. After 15 months, litter mass loss was significant for all collection sites, and ranged between 5 and 12 % of the initial dry mass, and was strongly correlated to litter N content ($R=0.794$, $p<0.001$). Detailed results were reported by [21].

Highest respiration rates were measured at the first measurement after 14 days incubation (150-350 $\mu\text{g CO}_2\text{-C d}^{-1} \text{ g}^{-1} \text{ litter-C}$), which dropped to 75 to 100 $\mu\text{g CO}_2\text{-C d}^{-1} \text{ g}^{-1} \text{ litter-C}$ after 3 months. After 6 and 15 months, respiration rates for AK and OS further decreased, while SW and KL showed a second maximum in respiration after 6 months (fig 1). Accumulated respiration was strongly correlated to litter mass loss after 15 months ($r=0.738$, $p<0.001$, $n=20$).

Soluble organic carbon concentrations decreased between the first three harvests (14 days to 6 months), and strongly increased to 15 months (from 0.1 to 0.7 $\text{mg C g}^{-1} \text{ d.w.}$ to 1.5 to 4 $\text{mg C g}^{-1} \text{ d.w.}$ after 15 months, fig. 1). After 14 days and 3 months, the highest soluble organic C concentrations were found in SW litter followed by AK. Soluble organic C concentrations were weakly correlated with litter N content after 14 days ($r=0.69$, $p<0.001$, $n=20$) and after 3 months ($r = 0.65$, $p<0.01$, $n=20$), but strictly correlated after 6 months ($r=0.85$, $p<0.001$, $n=20$) and 15 months ($r=0.90$, $p<0.001$, $n=20$).

Potential enzyme activities

Within each time point, all potential extracellular enzyme activities were correlated with litter N and actual respiration rates (all $R > 0.8$, $p < 0.001$, $n = 20$). Cellulase activity increased from the first harvest onwards to 15 months, with a small depression after 6 months (Fig. 1), phenoloxidase and peroxidase activities reached their maximum between 3 and 6 months (fig. 1). For all enzymes and at all time points, SW showed the highest and AK the lowest activities. Differences between these two sites were more pronounced in cellulase activity (SW 10x higher than AK) than in oxidative enzymes (SW 4x higher). Conversely, the phenoloxidase/cellulase ratio was highest for AK and lowest for SW at all time points and decreased during litter decomposition (fig. 1).

Microbial biomass abundance and community composition

Microbial biomass contents ranged from 0.5 to 6 mg C g⁻¹ d.w., 0.05 to 0.55 mg N g⁻¹ d.w. and 0.05 to 0.35 mg P g⁻¹ litter d.w (fig. 2). After an initial increase in microbial biomass, in KL and OS microbial biomass remained constant after 3 months while AK and SW showed further accumulation of microbial biomass which reaches a maximum of microbial C and N contents after 6 months (AK also for P). Microbial C:N ratios ranged between 6:1 and 18:1, C:P ratios between 8:1 and 35:1, and N:P ratios between 0.5:1 and 3.5:1 (fig. 2).

Microbial biomass was stoichiometrically homeostatic during the first 6 months (no or negative correlations between microbial C:N:P and litter C:N:P, see also [21]), but after 15 months (microbial C:N:P ratios were significantly and positively correlated to resource stoichiometry: $R = 0.53-0.64$, all $p < 0.002$). The homeostatic regulation coefficients [22] were $H_{C:P} = 1.68$, $H_{C:N} = 2.01$, and $H_{N:P} = 2.29$ after 15 month incubation. Microbial C:N ratios after 3 and 6 months were within a tightly constrained range, 14.5:1 to 18.2:1 after 3 months and 6.9:1 to 9.0:1 after 6 months, but significantly different between the two sampling events. In contrast, microbial C:P and N:P ratios were less constrained, with the highest variance between litter from different sites after 3 months of incubation (fig. 2).

Metaproteome analysis yielded between 451 and 1113 (average 639) assigned spectra per sample (one replicate per collection site after 14 days, 3, 6, and 15 months). For community profiling only spectra assigned to bacteria or fungi were used. Fungal proteins were dominant in all litter types at all stages, but most prominent in SW and least pronounced in AK. Fungi:bacteria (F:B) protein abundance ratios were highest after 14 days (5 to 12) and decreased during litter decomposition (1.7 to 3 after 15 months, see fig. 4). The large initial differences in F:B ratios between litter from different sites decreased during decomposition.

118 In addition, F:B ratios were measured on a DNA basis (qPCR) the results showing a similar pattern but
119 with a much larger fungal DNA dominance (F:B ratios between 10-180). F:B ratios were highly correlated
120 between protein- and log-transformed DNA-based estimates ($r=0.785$, $p<0.001$, $n=20$).

121 Fungal communities were dominated by Ascomycota, with smaller contributions by Basidiomycota (<5%
122 of fungal protein). Among the fungal classes found, Sordariomycetes and Eurotiomycetes were most abun-
123 dant with further contributions of Dothideomycetes, Leothiomycetes and Saccharomycetes (fig. 3). Bacteria
124 were dominated by Proteobacteria (mainly γ , declining, and α - and β -Proteobacteria, increasing with litter
125 decomposition) with minor contribution of Actinomycetes and Bacterioidetes (both increasing) and Ther-
126 motogae (decreasing, fig. 3).

127 Pyrolysis-GC/MS and Lignin content

128 In total 128 pyrolysis products were detected, quantified, identified and assigned to their substances of origin
129 (suppl. tab. 2 -4). We found only minor changes in the relative concentration of litter pyrolysis products
130 during decomposition, and differences between sites were small but well preserved during decomposition.
131 However, the high precision and reproducibility of pyrolysis GC/MS analysis of litter allowed tracing small
132 changes in lignin and carbohydrate abundance during decomposition. Lignin-derived compounds made up
133 between 29 and 31 % relative peak area (TIC) in initial litter, and increased by up to 3 %. The increase
134 occurred almost exclusively during the first 6 months. Carbohydrate-derived pyrolysis products accounted
135 for 26 to 29 % in initial litter and decreased by up to 2.6 % within 15 months of incubation. The initial
136 (pyrolysis-based) lignin:carbohydrate indices (LCI) were highly similar between litter from different collection
137 sites, ranging between 0.517 and 0.533 (Fig. 4). During decomposition, the LCI increased by up to 9 % of
138 the initial value. The highest increase was found in SW litter, while LCI slightly decreased in AK litter.
139 All significant changes in LCI occurred within the first 6 months (fig. 5). As differences in lignin and
140 carbohydrate contents between 0-3 and 3-6 months were not significant, we analyzed differences for two time
141 intervals, i.e. between 0-6 months and 6-15 months.

142 During the first 6 months, between one and 6 % of the initial lignin pool and between 4 and 17% of the
143 initial carbohydrate pool were degraded (Fig. 6). Lignin decomposition was highest in AK and KL litter,
144 while microbial communities of KL, OS and SW litter decomposed carbohydrates faster. Lignin preference
145 values (% lignin decomposed : %carbohydrates decomposed) were lowest in SW and highest in AK litter
146 (Figure 5). In AK litter, lignin macromolecules were 50 % more likely to be decomposed than carbohydrates,
147 while in SW litter carbohydrates were 10 times more likely to be decomposed (fig. 6). Between 6 and 15

148 months, no further accumulation of lignin occurred. Lignin and carbohydrates were both degraded at the
149 same rate and their relative concentrations remained constant between 6 and 15 months (fig. 6).

150 **Correlations between lignin and carbohydrate decomposition and litter chem-** 151 **istry, microbial community and decomposition processes**

152 Relationships between lignin and carbohydrate degradation, litter chemistry, microbial biomass and decom-
153 position processes were tested after 6 and 15 months (tables 5 and 6) including data presented by [21]
154 and [23]. After 6 months, we found that the ratio of lignin/cellulose degradation was positively correlated
155 with the ratio of phenoloxidase : cellulase ($R=0.599$, $p=0.005$, $n=20$) and peroxidase : cellulase ($R=0.734$
156 $p<0.001$, $n=20$). Carbohydrate decomposition was positively correlated with litter N content, and nega-
157 tively with litter C:N ratios and litter-microbial C:N imbalances. In contrast, lignin decomposition was
158 negatively correlated to litter P, but positively with litter C:P and N:P ratios, and litter-microbial C:P and
159 N:P imbalances (fig. 7). After 15 months, the ratio of lignin : carbohydrate decomposition was no longer
160 related to stoichiometry or elemental composition any more. Most interestingly, lignin and carbohydrate
161 decomposition exhibited the same controls, being positively correlated to soluble organic C, litter N and
162 litter P (table 6) between 6 and 15 months. Mass loss and accumulated respiration were positively correlated
163 to lignin and carbohydrate decomposition (table 6), a pattern that we did not find for lignin decomposition
164 in the early decomposition phase (table 5). Protein abundance F:B ratios were negatively correlated to the
165 ratios of lignin : cellulose decomposition and to LCI change during the first 6 months, pointing to bacterial
166 engagement in lignin decomposition. In contrast, both lignin and carbohydrate decomposition rates, were
167 positively correlated with F:B ratios after 15 months, pointing to fungal dominance of both lignin and car-
168 bohydrate decompostion. No correlation between F:B ratio and the ratio of lignin : cellulose decomposition
169 was found in this later period (fig. 4).

170 To asses the interaction between litter chemistry, microbial community and degradation processes, we
171 conducted a correspondence analysis (CA) of the metaprotomen data (relative protein abundances, fig. 8).
172 The results indicate that incubation time (i.e. succession) is the dominant factor controlling the microbial
173 community, with samples collected at the first (14 days) and the last (15 month) sampling grouping closely
174 together, while litter quality (i.e. elemental stoichiometry of litter collected at different sites) had a higher
175 impact after 3 and 6 months. The first factor (CA 1), which explained 35.7 % of the total variance, separates
176 litter sampled after 15 months (positive values) from litter sampled earlier (negative values). Consequently,
177 CA 1 was also positively correlated to incubation time and negatively to litter C content (i.e. decreas-

ing C:N ratios during decomposition). A number of bacterial taxa (Actinobacteri, Bacteroidetes, α - and β -proteobacteria), and two fungal classes (Leotiomycetes and Tremellomycetes) were positively correlated to CA1 i.e. increased in abundance towards 15 months, while Cyanobacteria, ϵ -proteobacteria and Saccharomycetes were negatively correlated. CA 2, which explained 26.0 % variance, separated litter sampled within the first 6 months. Dothideomycetes and Sordariomycetes were positively and γ -proteobacteria negatively correlated to this factor, which also correlated to the F:B protein abundance ratio. Litter collected 14 days after inoculation have the highest scores on CA 2, while sites with active lignin degradation within the first 6 months (AK, KL) have the most negative scores. The axis was furthermore correlated to the microbial biomass P content and C:P and N:P imbalances (and free NH_4^+ , not shown). For samples analyzed after 6 months, where direct comparison to lignin degradation rates was possible, significant correlations to CA 2 were found for lignin : carbohydrate degradation ($r=-0.97$, $p=0.028$), % Lignin loss : % Carbon loss ($r=-0.96$, $p=0.040$) and LCI increase ($r=0.973$, $p=0.027$), even though the number of independent samples was very low ($n=4$). Differences in CA2 strongly decreased after 15 months, suggesting that the differences in the microbial community found within the first 6 months were diminished with succession of the decomposer community. Litter N and P contents were not correlated to either factor, although differences in resource quality evidently affected community composition after 3 and 6 months, as can be seen in the differences in the microbial communities as observed in CA 2. Correlation of CA factors with litter stoichiometry, and microbial stoichiometry, and the abundance of the analyzed taxa are provided in supplemental table 7.

Discussion

The experimental approach chosen allowed us to single out the effects of litter quality on the microbial decomposer community and decomposition processes, while excluding effects of fauna, climate and different initial microbial communities. By exploiting intra-specific differences in beech litter stoichiometry, we were able to minimize differences in the chemical composition of initial litter (e.g. similar lignin and cellulose content, table 1), while exploring the effect of litter nutrient contents on lignin and carbohydrate decomposition. Therefore, we can attribute different rates of carbohydrate and lignin decomposition to the intrinsic qualities of litter collected at different sites, i.e. elemental and stoichiometric composition. Analytical pyrolysis allowed specific determination of litter lignin contents (simultaneously with carbohydrates), avoiding the limitations of common methods for lignin quantification as AUR [8].

Contradicting traditional concepts of litter decomposition, our results demonstrate that variable amounts

of lignin were degraded during the first 6 months of incubation. During this early stage, lignin decomposition rates depended on litter quality (P) and ranged from non-significant to degradation rates similar to bulk carbon mineralization rates (i.e. no discrimination against lignin). Our study therefore provided evidence that early lignin decomposition rates are by far underestimated, as recently proposed by Klotzbücher and coworkers [14], based on a complementary analytic approach. Unlike them, we found no decrease but constant or increasing lignin decomposition rates during litter decomposition over 15 months.

Our results provide strong evidence that litter C:N:P stoichiometry and litter element concentrations exerted a major control on the extent of lignin decomposition during the initial decomposition phase. Carbohydrate decomposition was positively correlated with litter N contents and negatively to litter C:N ratios, as were the majority of decomposition processes (mass loss, respiration, potential extracellular enzymatic activities). In contrast, lignin decomposition rates were positively correlated with litter C:P ratios and negatively with dissolved and total litter P. The relationship was strongest when lignin decomposition rates were compared to litter-microbe C:P imbalances, i.e. the greater the imbalance between resource and consumer C:P (greater P limitation), the lower the lignin decomposition rates. Additionally, we found a marked change in the controls of lignin decomposition during this period. While carbohydrate and lignin decomposition were differently controlled by litter chemistry (N versus P) during the first 6 months, these litter components were decomposed at similar rates thereafter and decomposition rates were only related to litter N availability.

Cultivation studies showed that lignin decomposition by fungi is triggered by N starvation, and that lignin does not provide sufficient energy to maintain the decomposer's metabolism without the use of other organic C i.e. energy sources [24]. Moreover, lignin decomposition was found in wild-type *A. thaliana* litter containing abundant cellulose as a C source, but not in a low-cellulose mutant during a 12-month incubation experiment in a boreal forest [20]. In the N- and P-(co-)limited situation commonly encountered during early litter decomposition, we may speculate that lignin is degraded to access additional nutrients (mainly N) or to use a C surplus by decomposing a less C efficient but nutrient enriched substrate (nutrient mining hypothesis). However, a stimulation of lignin decomposition by low P availability or microbial P limitation, as indicated by the strong negative correlations to P pools that we found, has not been reported yet. Though lignified materials have been reported to be N-rich and decomposition of these materials may therefore enhance N supply to microbial communities, lignins are not expected to contain quantitative important amounts of P.

In order to decompose litter lignin and carbohydrates, microbial decomposers rely on the production and excretion of hydrolytic and oxidative extracellular enzymes. While the absolute amounts, in which these enzymes are produced, were largely controlled by N availability, the ratio in which they were produced was

strongly related to differences in the ratio of cellulose:lignin decomposition. Talbot and coworkers [20] recently¹¹ suggested that lignin decomposition comprises a strategy of slow-growing microbes to evade competition through colonizing more lignin-rich and nutrient-poor substrates. Indeed we found lignin decomposition in low quality litter (low N and P) with microbial communities that were subject to large imbalances in C:N and C:P between resource and consumer, pointing to N or P limitation. Low P availability may limit fast growth of microbial populations and select for slow-growing lignin-degrading microbes during early decomposition and provide K-strategists (slow growing on recalcitrant carbon) an advantage over r strategists (fast growing on labile carbon). Indeed we found that lignin decomposition was highest in AK litter, where resource C:P and N:P were highest, i.e. low P supply may have limited microbial growth generally or the establishment of r strategists in particular.

Differences in initial lignin contents were marginal (29-31 % relative peak area), and lignin degradation rates of sites with high lignin contents were not higher than that of sites with low lignin contents. Therefore, in early lignin decomposition was not triggered by critical lignin contents (i.e. lignin limiting the use of other C sources) as is suggested by traditional litter decomposition models. Neither did low lignin decomposition rates result from a lack of metal cofactors of oxidative lignin decay (i.e. Mn or Fe), which were suggested to be rate limiting for late lignin decomposition [1]. While Mn and Fe concentrations indeed strongly varied between litter collected at different sites, concentrations were lowest in the litter with highest lignin decomposition rates (AK, see Table 1). Moreover, soluble organic C ("DOC") was suggested to limiting lignin decomposition since the process of lignin decomposition does not generate sufficient energy to power the metabolism of its decomposers [14]. However, soluble organic C did not control lignin decomposition in this experiment since we found highest (initial) concentrations in the two litter types that showed the highest and the lowest lignin decomposition rates (SW and AK). In contrast, while the initial content of soluble C is not related to litter stoichiometry or respiration, we found a strong correlation of soluble C, litter N after 6 and 15 months. This rather supports a perspective in which soluble C is rapidly used by litter decomposers, while its production is limited by the production of extracellular enzymes and therefore by N availability, which in our experiment also correlated with overall C mineralization rates.

While the mode of negative P regulation on lignin decomposition remains unknown, we found corresponding differences in the composition of the microbial decomposer communities on litter with fast and slow lignin decomposition. Unlike predicted by ecological stoichiometry theory, not bacteria but fungi were more successful in colonizing high N and P litter during initial decomposition. Fungi colonized litter faster than bacteria and therefore dominated early litter decomposition, however the F:B ratios decreased over

269 the entire incubation period pointing to increasing population sizes of bacteria with time. Interestingly, low¹²
270 F:B communities (AK) were more active in decomposing lignin than those being dominated by fungi. This
271 does not necessarily indicate that bacteria play the key role in lignin decomposition, though bacteria were
272 also reported to produce oxidative enzymes that can decompose lignified materials in litter [13]. However,
273 decreases in F:B ratios may be superimposed on the increase of smaller subpopulations of e.g. fungi that
274 are key mediators of lignin decomposition, or alternatively general increases in the size of microbial com-
275 munities with declining fungi/bacteria ratios may as well mask stable fungal populations when bacterial
276 abundance increases. Furthermore, we are well aware of the short-coming of metaproteomic approaches that
277 under-represent important soil phyla such as uncultivated and unsequenced groups (e.g. Lecanoromycetes).

278 The onset of lignin degradation in litter from all sites between 15 months was accompanied by increased
279 abundance of fungal and bacterial taxa associated with late litter decomposition: Complete lignin degradation
280 is most commonly accounted to Basidiomycetes [1], which however accounted for less than 5 % of fungal
281 protein in all analyzed samples. Among bacteria, lignin degradation was reported for Actinomycetes, α -
282 , and γ -Proteobacteria [13]. The change in controls over lignin degradation after 6 months (i.e. lignin
283 degradation dependent on litter stoichiometry) was accompanied by increased abundances of Actinomycetes
284 and α -Proteobacteria while γ -Proteobacteria abundance was correlated to lignin degradation activity after 6
285 months of incubation. In contrast, several Ascomycetes classes (Sordariomycetes, Saccheromycetes), which
286 were highly abundant in early decomposition stages and are associated with the rapid degradation of labile C
287 substrates, decreased in their relative abundance. However, since the metaproteomic approach did not find
288 relevant amounts of oxidative extracellular enzymes we so far cannot dissect the contributions of bacteria
289 and fungi to the lignin decomposition process.

290 Conclusions

291 Our results contradict the traditional concept that lignin decomposition is slow during early litter decom-
292 position. While traditional litter decomposition models propose that lignin decomposition mainly occurs
293 during late decomposition stages, we found that variable but in some cases substantial amounts of lignin
294 were decomposed during the first 6 months. We can therefore conclude, that low nutrient levels (mainly
295 P) led to an earlier onset of lignin degradation (Q1). On the other hand, the results of our experiment
296 contradicted our second hypothesis (Q2): We found that regardless of their wider C:N and C:P ratios, fungal
297 communities were more dominant in litter with high litter N and P contents, especially within the first 6

months of decomposition. Regardless of low F:B ratios, P-poor, but bacteria-rich communities were most actively degrading lignin during early decomposition. In contrast, late lignin decomposition rates were highest in N-rich litter with high F:B ratios, indicating a fundamental change in the controls of lignin decomposition between early and later decomposition stages.

Material and methods

Litter decomposition experiment

Beech litter was collected at four different sites in Austria (Achenkirch (AK), Klausenleopoldsdorf (KL), Ossiach (OS), and Schottenwald (SW); referred to as litter types) in October 2008. Litter was cut to pieces of approximately 0.25cm², homogenized, sterilized twice by γ -radiation (35 kGy, 7 days between irradiations) and inoculated (1.5% w/w) with a mixture of litter and soil to assure that all litter types share the same initial microbial community. From each type, four samples of litter were taken immediately after inoculation, dried and stored at room temperature. Batches of 60g litter (fresh weight) were incubated at 15 °C and 60% relative water content in mesocosms for 15 months. For each litter type 5 replicates were removed and analyzed after 14, 97, 181 and 475 days. A detailed description of the litter decomposition experiment was published by [25].

Bulk litter, extractable, and microbial biomass nutrient content

To calculate litter mass loss, litter dry mass content was measurement in 5 g litter (fresh weight) after 48 h at 80 °C. Dried litter was ball-milled for further chemical analysis. Litter C and N content was determined using an elemental analyzer (Leco CN2000, Leco Corp., St. Joseph, MI, USA). Litter phosphorus content was measured with ICP-AES (Vista-Pro, Varian, Darmstadt, Germany) after acid digestion [26]). To determine dissolved organic C, dissolved N and P, 1.8 g litter (fresh weight) were extracted with 50 ml 0.5 M K₂SO₄. Samples were shaken on a reciprocal shaker with the extractant for 30 minutes, filtered through ash-free cellulose filters and frozen at -20 °C until analysis. To quantify microbial biomass C, N and P, further samples were additionally extracted under the same conditions after chloroform fumigation for 24 h [27]. Microbial biomass was determined as the difference between fumigated and non-fumigated extractions . C and N concentration in extracts were determined with a TOC/TN analyzer (TOC-VCPH and TNM, Shimadzu), P was determined photometrically as inorganic P after persulfate digestion [28].

Substrate to consumer stoichiometric imbalances $C:X_{imbal}$ were calculated as

14

$$C : X_{imbal} = \frac{C : X_{litter}}{C : X_{microbial}} \quad (1)$$

where X stand for the element N or P.

Microbial Respiration

Respiration was monitored weekly during the entire incubation in mesocosms removed after 6 month and on the last incubation day for all mesocosms using an infrared gas analyzer (IRGA, EGM4 with SRC1, PPSystems, USA). CO₂ concentration was measured over 70 seconds and increase per second was calculated based on initial dry mass. Accumulated respiration after 6 month was calculated assuming linear transition between measurements, accumulated respiration after 15 month was estimated from respiration rates after 181 and 475 days.

Potential enzyme activities

Potential activities of β -1,4-cellobiosidase (“cellulase”), phenoloxidase and peroxidase were measured immediately after sampling. 1 g of litter (fresh weight) was suspended in sodium acetate buffer (pH 5.5) and ultrasonicated. To determine cellulase activity, 200 μ l suspension were mixed with 25 nmol 4-methylumbelliferyl- β -D-cellobioside (dissolved in 50 μ l of the same buffer) in black microtiter plates and incubated for 140 min in the dark. The amount of methylumbelliferyl (MUF) set free in by the enzymatic reaction was measured fluorimetrically (Tecan Infinite M200, excitation at 365 nm, detection at 450 nm). To measure phenoloxidase and peroxidase activity litter suspension was mixed 1:1 with a solution of L-3,4-dihydroxyphenylalanine (DOPA) to a final concentration of 10 mM. Samples were incubated in microtiter plates for 20h to determine phenoloxidase activity. For peroxidase activity, 1 nmol of H_2O_2 was added before incubation. Absorption at 450 nm was measured before and after incubation. All enzyme activities were measured in three analytical replicates. The assay is described in detail in [29].

Pyrolysis-GC/MS

Pyrolysis-GC/MS was performed with a Pyroprobe 5250 pyrolysis system (CDS Analytical) coupled to a Thermo Trace gas chromatograph and a DSQ II MS detector (both Thermo Scientific) equipped with a carbowax column (Supelcowax 10, Sigma-Aldrich). Between 2-300 μ g of dried and finely ground litter (MM2000

ball mill, Retsch) was heated to 600 °C for 10 seconds in a helium atmosphere. GC oven temperature was constant at 50 °C for 2 minutes, followed by an increase of 7 °C/min to a final temperature of 260 °C, which was held for 15 minutes. The MS detector was set for electron ionization at 70 eV in the scanning mode (m/z 20 to 300).

Peaks were assignment was based on NIST 05 MS library after comparison with measured reference materials. 128 peaks were identified and selected for integration either because of their abundance or diagnostic value. This included 28 lignin and 45 carbohydrate derived substances. The pyrolysis products used are stated in tables 2 -4 For each peak between one and four dominant and specific mass fragments were selected, integrated and converted to TIC peak areas by multiplication with a MS response coefficient [30,31]. Peak areas are stated as % of the sum of all integrated peaks.

A pyrolysis-based lignin to carbohydrate index (*LCI*) was calculated to derive a ratio between these two substance classes without influences of changes in the abundance of other compounds .

$$LCI = \frac{Lignin}{Lignin + Carbohydrates} \quad (2)$$

Accounting for carbon loss, we estimate % lignin and cellulose degraded during decomposition according to equation 3, where %_{init} and %_{act} stand for initial and actual %TIC area of lignin or cellulose pyrolysis products, *C_{init}* for the initial amount of C and *R_{acc}* for the accumulated CO₂-C respired by a mesocosm.

$$\%_{loss} = 100 \cdot \frac{\%_{init} - \%_{act}}{\%_{init}} \cdot \frac{(1 - R_{acc})}{C_{init}} \quad (3)$$

Metaproteome analysis and quantitative PCR

From each harvest (14, 97, 181, and 475 days), one replicate per litter type was stored at -80°C for metaproteome analysis. 3 g of each sample were grounded in liquid nitrogen and extracted with Tris/KOH buffer (pH 7.0) containing 1% SDS. Samples were sonicated for 2 min, boiled for 20 min and shaken at 4 °C for 1 h. Extracts were centrifuged twice to remove debris and concentrated by vacuum-centrifugation. An aliquot of the sample was applied to a 1D-SDS-PAGE and subjected to in-gel tryptic digestion. The resulting peptide mixtures were analyzed on a hybrid LTQ-Orbitrap MS (Thermo Fisher Scientific) as described earlier [32]. Protein database search against the UniRef 100 database, which also comprised the translated metagenome of the microbial community of a Mennesota farm silage soil [33] and known contaminants, was performed using the MASCOT Search Engine. A detailed description of the extraction procedure and search crite-

ria was published by [34]. If more than one protein was identified based on the same set of spectra these¹⁶
 proteins were grouped together resulting in one protein cluster. The obtained protein/protein cluster hits
 were assigned to phylogenetic and functional groups and assignments were validated by the PROPHANE
 workflow (<http://prophane.svn.sourceforge.net/viewvc/prophane/trunk/;> [35]). Higher protein abundance
 is represented by a higher number of MS/MS spectra acquired from peptides of the respective protein. Thus,
 protein abundances were calculated based on the normalised spectral abundance factor (NSAF) [36,37]. This
 number allows relative comparison of protein abundances over different samples [38]. Protein abundances
 was aggregated at class level for fungi and proteobacteria and at phylum level for other bacterial taxa. These
 abundances were subjected to a canonical correspondance analysis without constraints. Vectorial fittings of
 stoichiometrical ratios (litter, microbial biomass and imbalance) were calculated and plotted when $p < 0.05$.
 Quantitative PCR was used to determine fungal and bacterial abundance as described recently [39]. F:B
 ratios were calculated as the ratio between estimated amounts of bacterial and fungal DNA found.

Statistical analysis

All statistical analyses were performed with the software and statistical computing environment R [40].
 If not mentioned otherwise, results were considered significant when $p < 0.05$. Due to frequent variance
 inhomogeneities Welch ANOVA and paired Welch's t-tests with Bonferroni corrected p limits were used. All
 correlations mentioned refer to Pearson correlations. A correspondence analysis (CA) and vectorial fittings
 were calculated using the R package "vegan" [41].

Acknowledgments

This study formed part of the national research network MICDIF (Linking microbial diversity and functions
 across scales and ecosystems, S-10007-B01, -B06 and -B07) by the Austrian Research Fund (FWF). Katharina
 Keiblinger is a recipient of a DOC-IFORTE fellowship of the Austrian Academy of Sciences. Vital support
 regarding Pyr-GC/MS measurements was given by Clemens Schwarzingger, Andreas Blöchl and Birgit Wild.

References

1. Berg, B & McClaugherty C (2008) Plant Litter. Decomposition, Humus Formation, Carbon Sequestration. Berlin: Springer.

- 401 2. Don A, Kalbitz K (2005) Amounts and degradability of dissolved organic carbon from foliar litter at
402 different decomposition stages. *Soil Biology and Biochemistry* 37: 2171–2179.
- 403 3. Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology*
404 *and Biochemistry* 42: 391–404.
- 405 4. Berg B, Staaf H (1980) Decomposition rate and chemical changes of Scots pine needle litter. II.
406 Influence of chemical composition. *Ecological Bulletins* : 373–390.
- 407 5. Coûteaux MM, Bottner P, Berg B (1995) Litter decomposition, climate and litter quality. *Trends in*
408 *ecology & evolution* 10: 63–66.
- 409 6. Moorhead DL, Sinsabaugh RL (2006) A theoretical model of litter decay and microbial interaction.
410 *Ecological Monographs* 76: 151–174.
- 411 7. Adair EC, Parton WJ, Del Grosso SJ, Silver WL, Harmon ME, et al. (2008) Simple three-pool model
412 accurately describes patterns of long-term litter decomposition in diverse climates. *Global Change*
413 *Biology* : 2636–2660.
- 414 8. Hatfield RD, Romualdo SF (2005) Can Lignin Be Accurately Measured? *Crop Science* 45: 832–839.
- 415 9. Preston CM, Trofymow JA, Sayer BG, Niu J (1997) ^{13}C nuclear magnetic resonance spectroscopy with
416 cross-polarization and magic-angle spinning investigation of the proximate analysis fractions used to
417 assess litter quality in decomposition studies. *Canadian Journal of Botany* 75: 1601–1613.
- 418 10. Amelung W, Brodowski S, Sandhage-Hofmann A, Bol R (2008) Combining Biomarker with Stable
419 Isotope Analyses for Assessing the Transformation and Turnover of Soil Organic Matter. *Advances in*
420 *Agronomy* 100: 155–250.
- 421 11. Thevenot M, Dignac MF, Rumpel C (2010) Fate of lignins in soils: A review. *Soil Biology and*
422 *Biochemistry* 42: 1200–1211.
- 423 12. Bol R, Poirier N, Balesdent J, Gleixner G (2009) Molecular turnover time of soil organic matter in
424 particle - size fractions of an arable soil. *Rapid Communications in Mass Spectrometry* 23: 2551–2558.
- 425 13. Bugg TD, Ahmad M, Hardiman EM, Singh R (2011) The emerging role for bacteria in lignin degra-
426 dation and bio-product formation. *Current opinion in biotechnology* 22: 394–400.

- 427 14. Klotzbücher T, Kaiser K, Guggenberger G, Gatzek C, Kalbitz K (2011) A new conceptual model for
428 the fate of lignin in decomposing plant litter. *America* 92: 1052–1062.
- 429 15. Achyuthan KE, Achyuthan AM, Adams PD, Dirk SM, Harper JC, et al. (2010) Supramolecular
430 self-assembled chaos: polyphenolic lignin's barrier to cost-effective lignocellulosic biofuels. *Molecules*
431 (Basel, Switzerland) 15: 8641–88.
- 432 16. Dyckmans J, Flessa H, Brinkmann K, Mai C, Polle A (2002) Carbon and nitrogen dynamics in acid de-
433 tergent fibre lignins of beech (*Fagus sylvatica* L.) during the growth phase. *Plant, Cell & Environment*
434 25: 469–478.
- 435 17. Craine JM, Morrow C, Fierer N (2007) Microbial nitrogen limitation increases decomposition. *Ecology*
436 88: 2105–13.
- 437 18. Knorr M, Frey S, Curtis P (2005) Nitrogen addition and litter decomposition : A meta-analysis.
438 *Ecology* 86: 3252–3257.
- 439 19. Fontaine S, Henault C, Aamor a, Bdioui N, Bloor J, et al. (2011) Fungi mediate long term sequestration
440 of carbon and nitrogen in soil through their priming effect. *Soil Biology and Biochemistry* 43: 86–96.
- 441 20. Talbot JM, Yelle DJ, Nowick J, Treseder KK (2011) Litter decay rates are determined by lignin
442 chemistry. *Biogeochemistry* : 1–17–17.
- 443 21. Mooshammer M, Wanek W, Schneckner J, Wild B, Leitner S, et al. (2011) Stoichiometric controls of
444 nitrogen and phosphorus cycling in decomposing beech leaf litter. *Ecology* in press.
- 445 22. Sterner RW, Elser JJ (2002) *Ecological Stoichiometry*.
- 446 23. Leitner S, Wanek W, Wild B, Haemmerle I, Kohl L, et al. (2011) Linking resource quality to decom-
447 position processes: Influence of litter chemistry and stoichiometry on glucan depolymerization during
448 decomposition of beech (*Fagus sylvatica* L.) litter. *Soil Biology and Biochemistry* in review.
- 449 24. Janshekar H, Fiechter A (1988) Cultivation of *Phanerochaete chrysosporium* and production of lignin
450 peroxidases in submerged stirred tank reactors. *Journal of Biotechnology* 8: 97–112.
- 451 25. Wanek W, Mooshammer M, Blöchl A, Hanreich A, Keiblinger K, et al. (2010) Determination of gross
452 rates of amino acid production and immobilization in decomposing leaf litter by a novel N-15 isotope
453 pool dilution technique. *Soil Biology and Biochemistry* 42: 1293–1302.

- 454 26. Kolmer J, Spaulding E, Robinson H (1951) Approved Laboratory Techniques. New York: Appleton
455 Century Crafts.
- 456 27. Brooks P, Kragt J, Powlson D, Jenkinson D (1985) Chloroform fumigation and the release of soil
457 nitrogen: the effects of fumigation time and temperature. *Soil Biology & Biochemistry* 17: 831–835.
- 458 28. Schinner F, Öhlinger R, Kandeler E, Margesin R (1996) *Methods in Soil Biology*. Berlin: Springer
459 Verlag, pp. 389 pp.
- 460 29. Kaiser C, Koranda M, Kitzler B, Fuchslueger L, Schnecker J, et al. (2010) Belowground carbon alloca-
461 tion by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community
462 composition in a beech forest soil. *New Phytologist* 187: 843–858.
- 463 30. Schellekens J, Buurman P, Pontevedra-Pombal X (2009) Selecting parameters for the environmental
464 interpretation of peat molecular chemistry - A pyrolysis-GC/MS study. *Organic Geochemistry* 40:
465 678–691.
- 466 31. Kuder T, Krueger MA (1998) Preservation of biomolecules in sub-fossil plants from raised peat bogs -
467 a potential paleoenvironmental proxy. *Organic Geochemistry* 29: 1355–1368.
- 468 32. Schneider T, Gerrits B, Gassmann R, Schmid E, Gessner MO, et al. (2010) Proteome analysis of fungal
469 and bacterial involvement in leaf litter decomposition. *Proteomics* 10: 1819–30.
- 470 33. Tringe SG, von Mering C, Kobayashi A, Salamov Aa, Chen K, et al. (2005) Comparative metagenomics
471 of microbial communities. *Science (New York, NY)* 308: 554–7.
- 472 34. Keiblinger KM, Schneider T, Roschitzki B, Schmid E, Eberl L, et al. (2011) Effects of stoichiometry and
473 temperature perturbations on beech litter decomposition, enzyme activities and protein expression.
474 *Biogeosciences Discussions* 8: 11827–11861.
- 475 35. Schneider T, Schmid E, de Castro JaV, Cardinale M, Eberl L, et al. (2011) Structure and function of
476 the symbiosis partners of the lung lichen (*Lobaria pulmonaria* L. Hoffm.) analyzed by metaproteomics.
477 *Proteomics* : 2752–2756.
- 478 36. Florens L, Carozza MJ, Swanson SK, Fournier M, Coleman MK, et al. (2006) Analyzing chromatin
479 remodeling complexes using shotgun proteomics and normalized spectral abundance factors. *Methods*
480 (San Diego, Calif) 40: 303–11.

- 481 37. Zybaylov B, Mosley AL, Sardiū ME, Coleman MK, Florens L, et al. (2006) Statistical analysis of
482 membrane proteome expression changes in *Saccharomyces cerevisiae*. *Journal of proteome research* 5:
483 2339–47.
- 484 38. Bantscheff M, Schirle M, Sweetman G, Rick J, Kuster B (2007) Quantitative mass spectrometry in
485 proteomics: a critical review. *Analytical and bioanalytical chemistry* 389: 1017–31.
- 486 39. Inselsbacher E, Hinko-Najera Umana N, Stange FC, Gorfer M, Schüller E, et al. (2010) Short-term
487 competition between crop plants and soil microbes for inorganic N fertilizer. *Soil Biology and Bio-*
488 *chemistry* 42: 360–372.
- 489 40. R Development Core Team (2008). R: A Language and Environment for Statistical Computing. URL
490 <http://www.r-project.org>.
- 491 41. Oksanen J, Blanchet FG, Kindt R, Legendre P, O'Hara R, et al. (2011). *vegan: Community Ecology*
492 *Package*. R packge version 1.17-9. URL <http://cran.r-project.org/package=vegan>.

493 Figure Legends

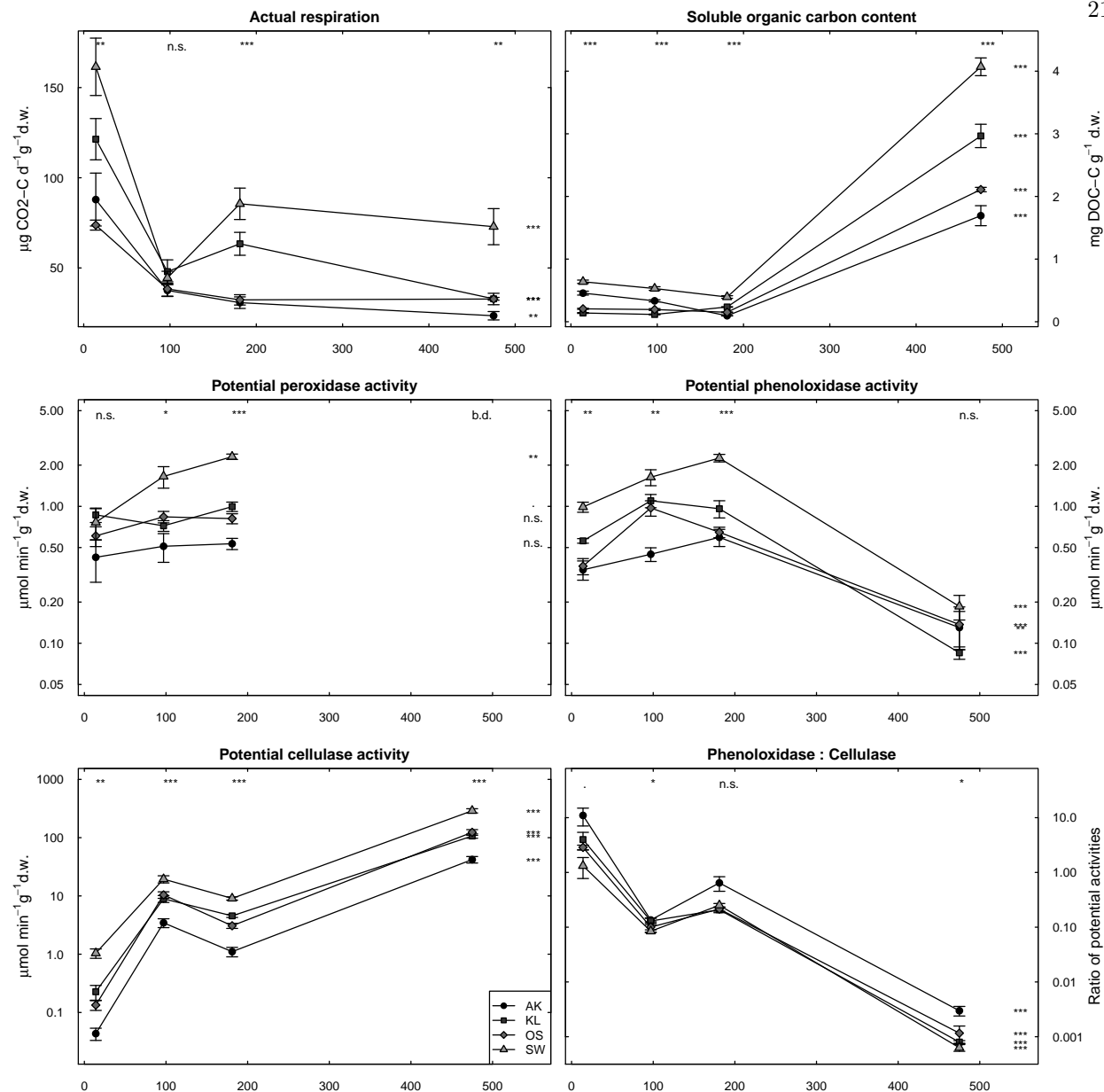


Figure 1. Respiration rates, concentration of soluble organic C and potential extracellular enzyme activities in decomposing beech leaf litter from a mesocosm experiment. Beech litter was collected in: triangles, Schottenwald (SW); diamonds, Ossiach (OS); squares, Klaus-nleopoldsdorf (KL); circles, Achenkirch, AK. Error bars indicate standard errors (n=5). Significant differences between litter types are presented by asterisks above the symbols, significant differences between time points by asterisks to the right of the curves. *, P<0.05, **, P<0.01, ***, P<0.001, b.d. - below detection limit.

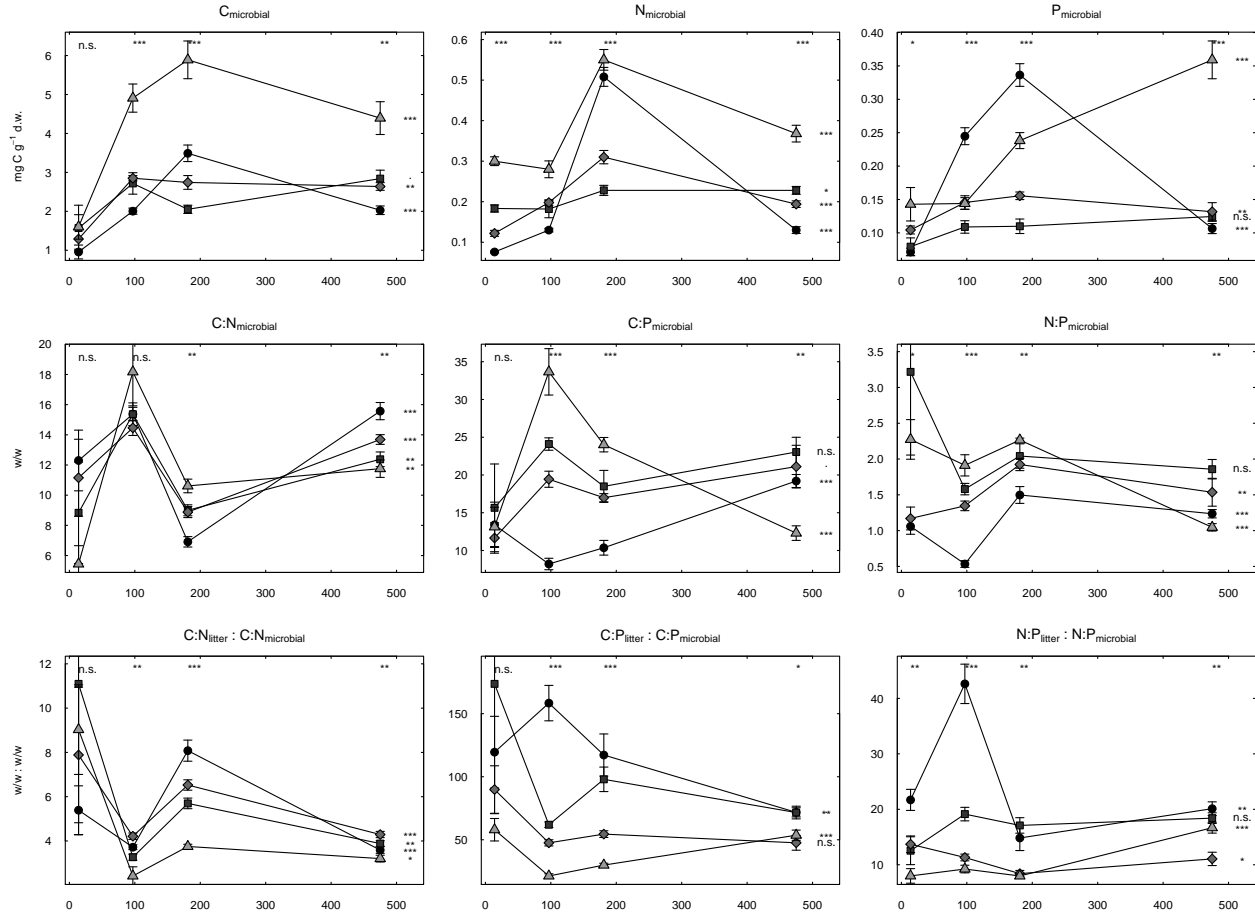


Figure 2. Microbial biomass C, N and P, microbial C:N:P stoichiometry and resource:consumer stoichiometric imbalance in these elements in decomposing beech leaf litter from a mesocosm experiment. Beech litter was collected in: triangles, Schottenwald (SW); diamonds, Ossiach (OS); squares, Klausenleopoldsdorf (KL); circles, Achenkirch, AK. Error bars indicate standard errors (n=5). Significant differences between litter types are presented by asterisks above the symbols, significant differences between time points by asterisks to the right of the curves. *, P<0.05, **, P<0.01, ***, P<0.001.

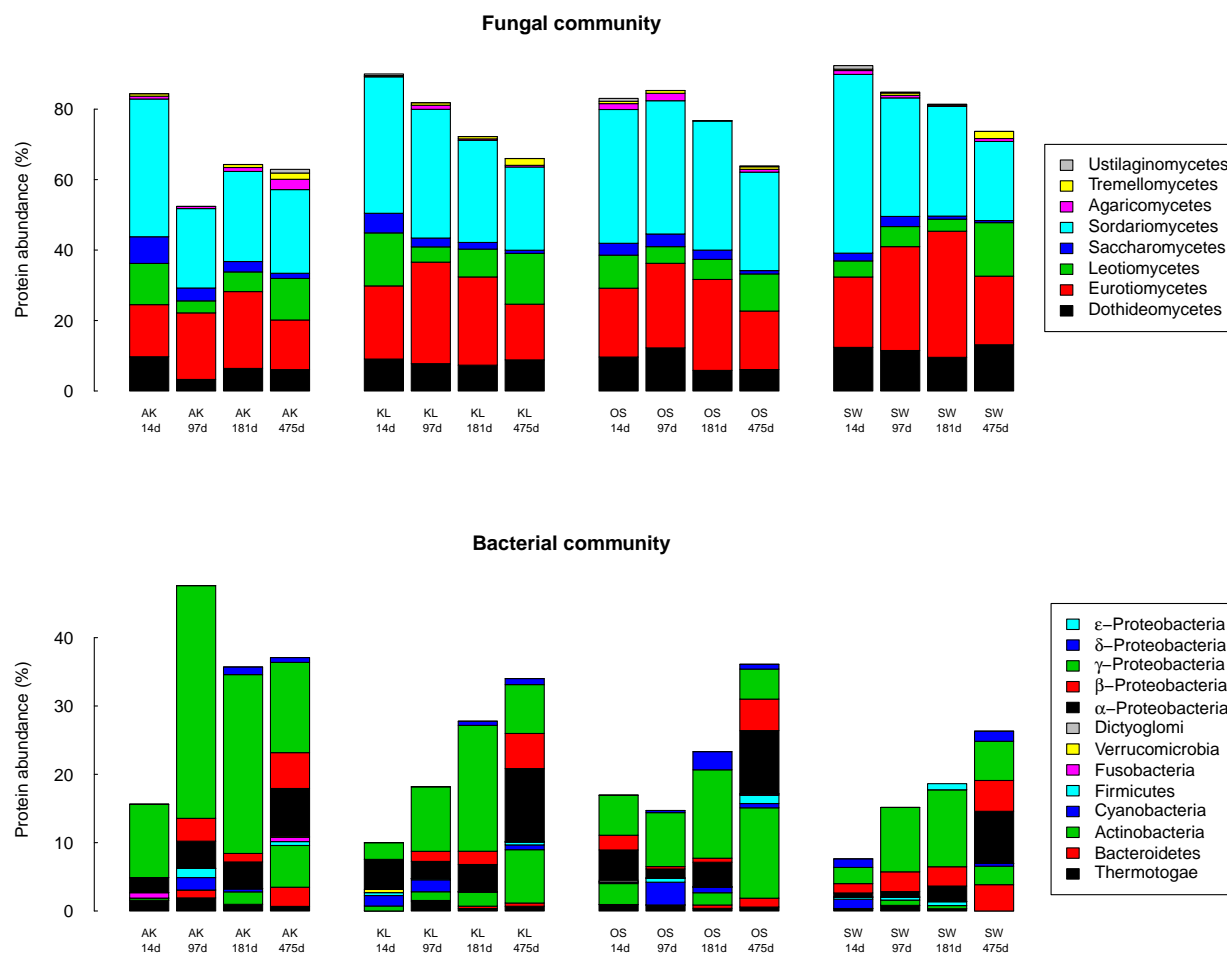


Figure 3. Protein abundance of fungal and bacterial taxa. Litter was collected in Achenkirch (AK); Klausenleopoldsdorf (KL); Ossiach (OS); Schottenwald (SW). Samples were analyzed after sterilization, re-innoculation and incubation for 14, 97, 181, or 475 days.

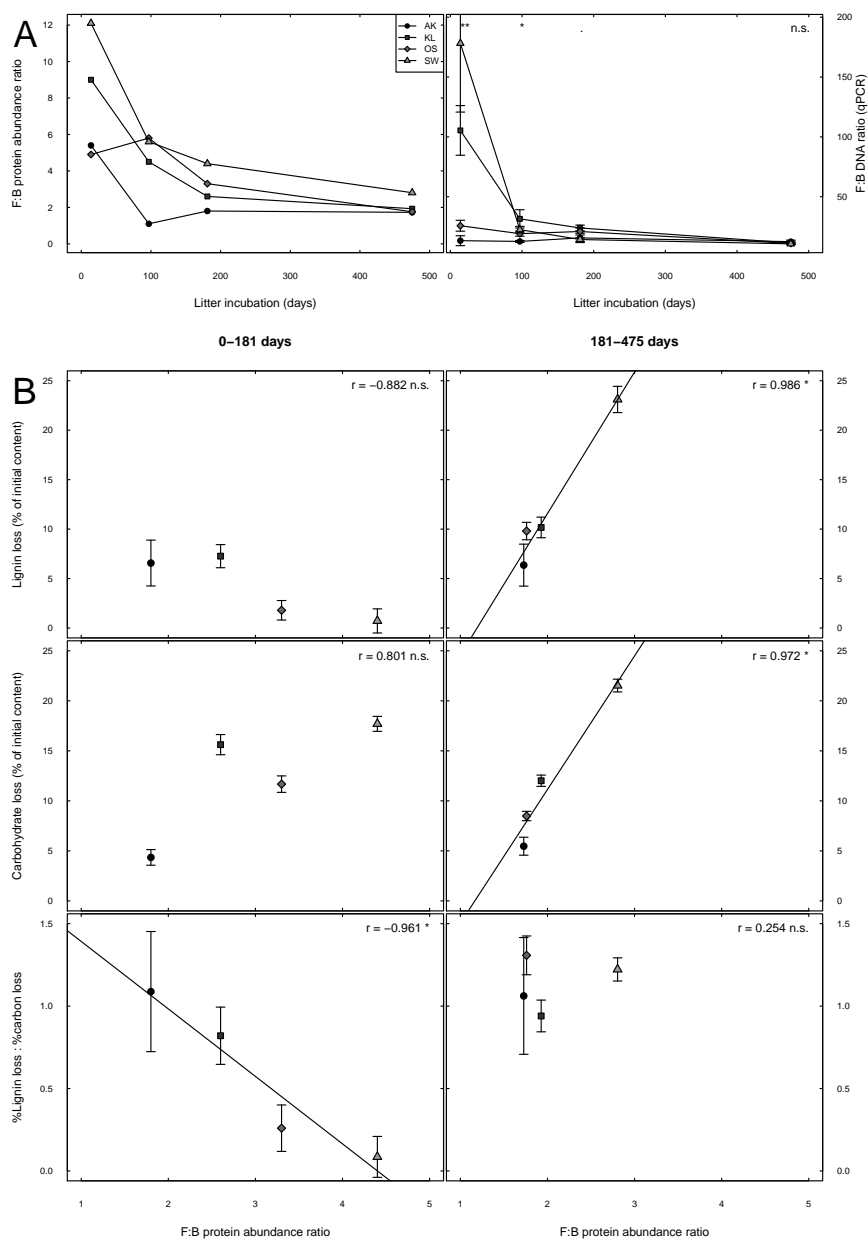


Figure 4. Fungi:Bacteria (F:B) ratios and their correlations with LCI change: A: F:B protein abundance (left) and DNA (right) ratio. B: Correlations between F:B preprotein abundance ratios and lignin loss (top), carbohydrate loss (mid) and lignin loss : carbon loss (bottom) for 0-6 months (left) and 6-15 months (right, errorbars indicate standard errors, n=4-5). Beech litter was collected in: triangles, Schottenwald (SW); diamonds, Ossiach (OS); squares, Klausenleopoldsdorf (KL); circles, Achenkirch, AK. Error bars indicate standard errors (n=5). Significant differences between litter types are presented by asterisks above the symbols, significant differences between time points by asterisks to the right of the curves. *, P<0.05, **, P<0.01, ***, P<0.001.

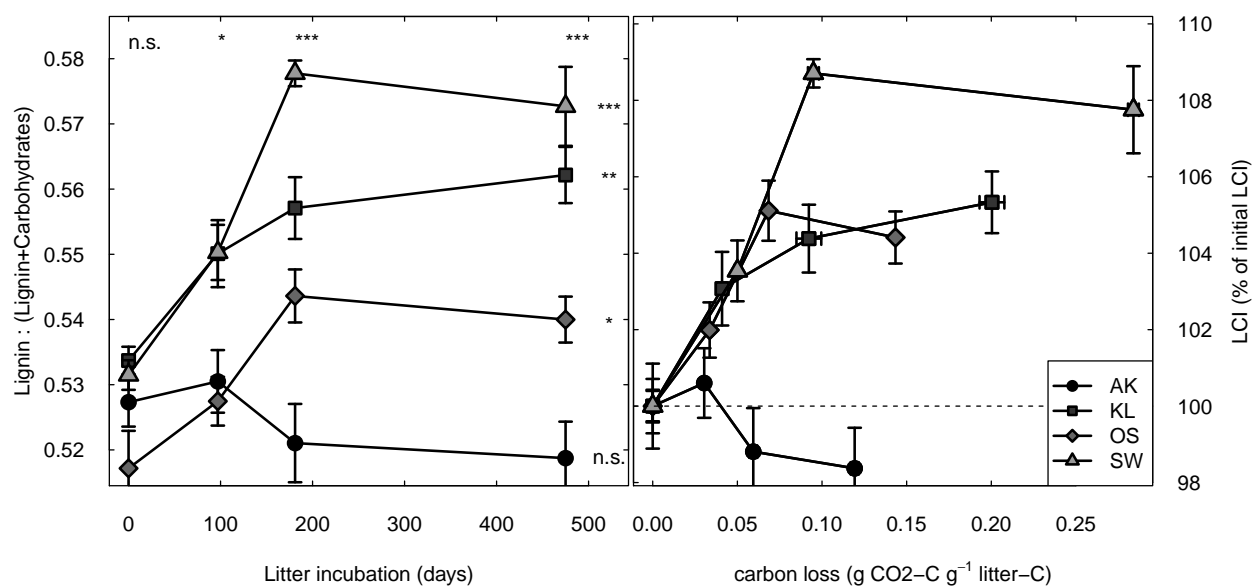


Figure 5. Development of lignin to carbohydrate index (lignin : (lignin+carbohydrates), LCI) during time of beech litter decomposition (left) or plotted against cumulative C loss (right). Errorbars indicate standard errors (n=4-5). The dashed line indicates a constant ratio between lignin and carbohydrates (i.e. no preferential decomposition of carbohydrates). Beech litter was collected in: triangles, Schottenwald (SW); diamonds, Ossiach (OS); squares, Klausenleopoldsdorf (KL); circles, Achenkirch, AK. Error bars indicate standard errors (n=5). Significant differences between litter types are presented by asterisks above the symbols, significant differences between time points by asterisks to the right of the curves. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

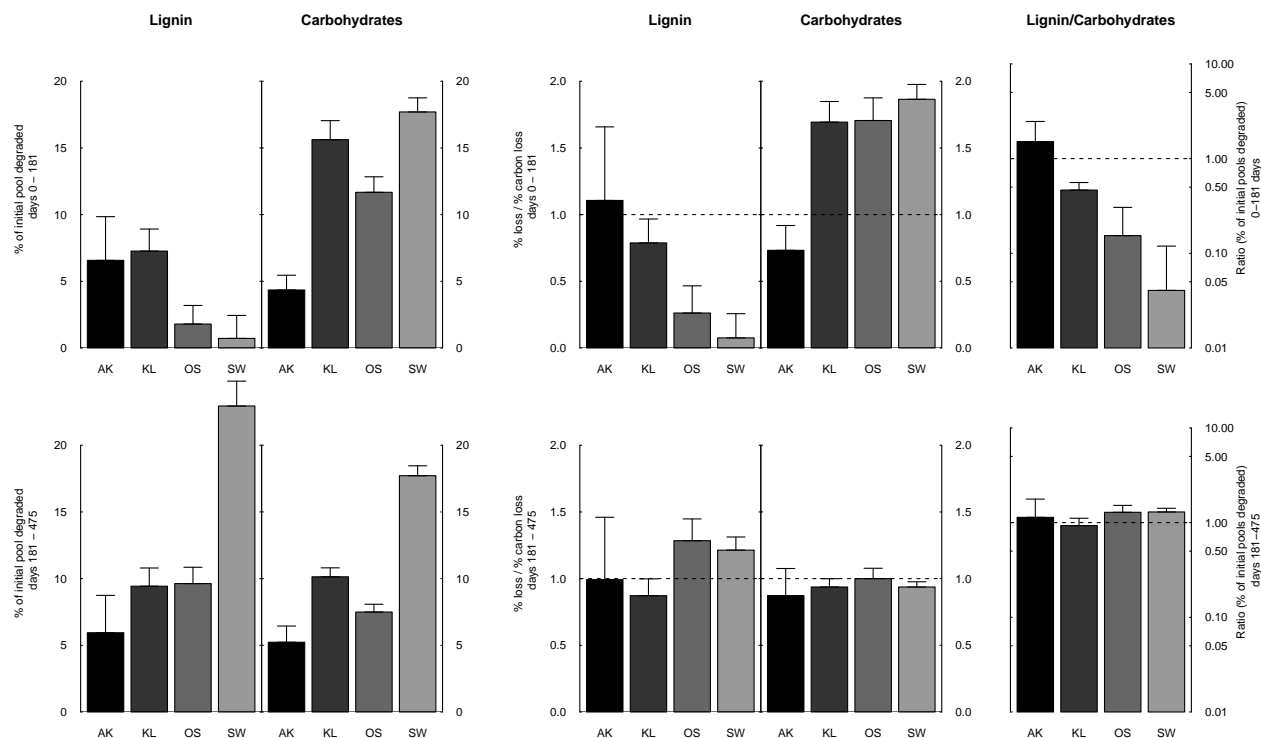


Figure 6. Carbon loss corrected amounts of lignin and carbohydrates degraded in beech litter collected in Achenkirch (AK), Klausenleopoldsdorf (KL), Ossiach (OS) and Schottenwald (SW). Carbon loss was calculated based on accumulated respiration for each mesocosm. Error bars indicate standard errors (n=4-5). The dashed line marks no discrimination during decomposition between lignin, carbohydrates and bulk carbon

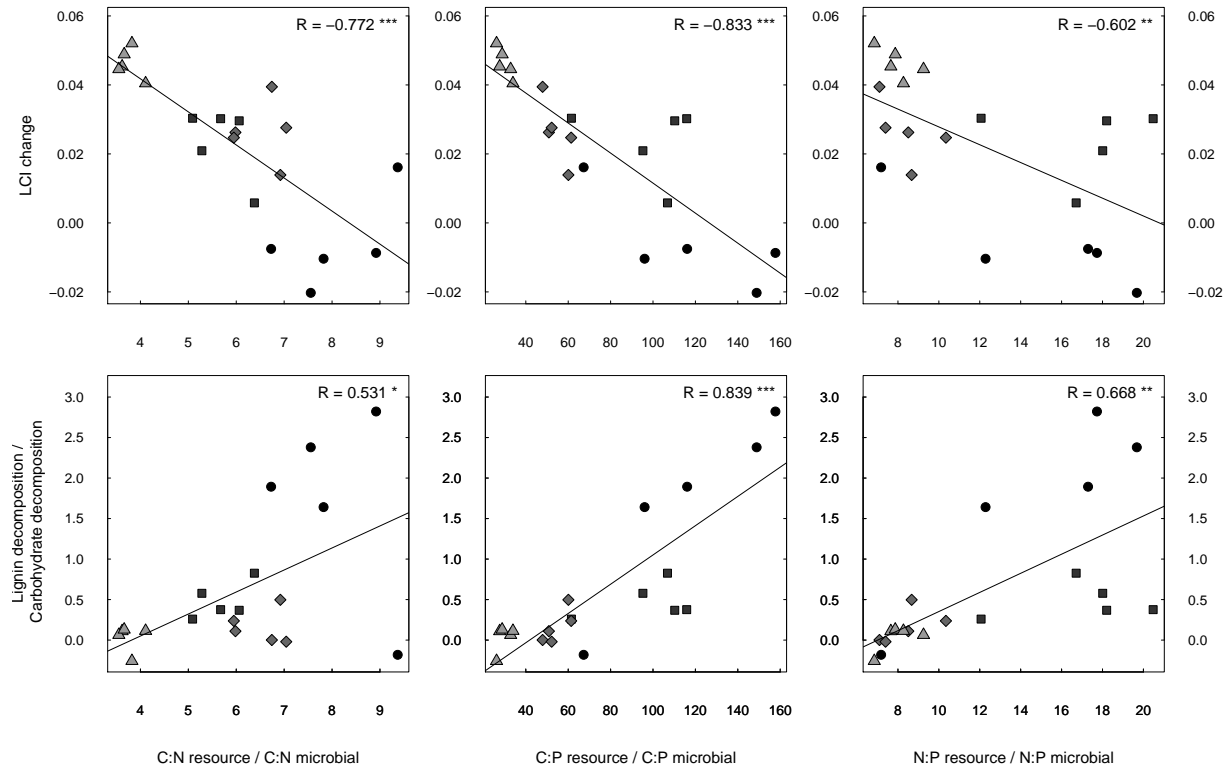


Figure 7. Correlation between the LCI change or the ratio of lignin : carbohydrate decomposition during the first 6 months of litter decomposition correlate to litter : microbe stoichiometric imbalances. and change and Correlations between lignin accumulation during the first 6 month of litter incubation and stoichiometric resource:consumer imbalances. LCI is calculates as of lignin/(lignin+Carbohydrates). Beech litter was collected in: triangles, Schottenwald (SW); diamonds, Ossiach (OS); squares, Klausenleopoldsdorf (KL); circles, Achenkirch, AK. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

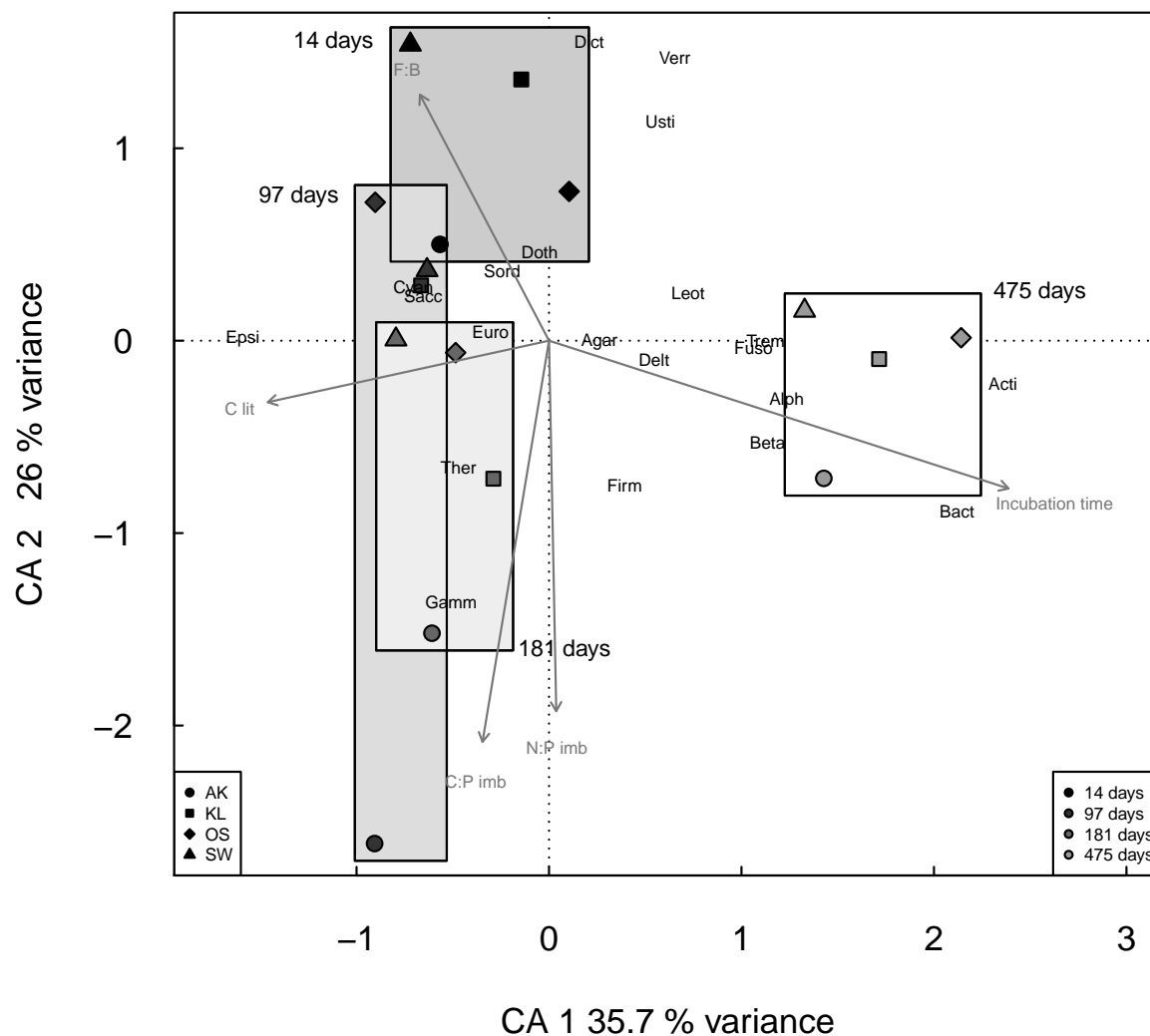


Figure 8. Microbial community composition. The first two components of a correspondence analysis (CA) of protein abundances found. Rectangles indicate samples of identical incubation time. Peptides were aggregated at class level (fungi and proteobacteria) or phylum level (other bacterial phyla): Dothideomycetes (Doth); Eurotiomycetes (Euro); Leotiomyces (Leot); Saccharomycetes (Sacc); Sordariomycetes (Sord); Agaricomycetes (Agar); Tremellomycetes (Trem); Ustilaginomycetes (Usti); Thermotogae (Ther); Bacteroidetes (Bact); Actinobacteria (Acti); Cyanobacteria (Cyan); Firmicutes (Firm); Fusobacteria (Fuso); Verrucomicrobia (Verr); Dictyoglomi (Dict); Alphaproteobacteria (Alph); Betaproteobacteria (Beta); Gammaproteobacteria (Gamm); Deltaproteobacteria (Delt); Epsilonproteobacteria (Epsi). Taxa factor loadings were printed x2 for better readability. Correlations between CA 1, CA 2, and litter chemistry, microbial stoichiometry, and protein abundance of microbial taxa are stated in supplemental table 7. Arrows represent vectorial fittings of these variables calculated independently from the CA, plotted only if $p < 0.05$: Litter C content (C lit); $C:X_{\text{Microbial}}/C:X_{\text{Litter}}$ (C:P imb, C:N imb).

Table 1. Element concentrations, elemental stoichiometry and cellulose and lignin concentrations in beech litter measured after 14 days incubation. Standard errors are given in brackets (n=5). C extr represents for soluble organic carbon. Beech litter was collected in AK, Achenkirch, KL, Klausenleopoldsdorf, OS, Ossiach, and SW, Schottenwald.

	AK	(SE)	KL	(SE)	OS	(SE)	SW	(SE)	p value
C (% d.w.)	50.86	(0.39)	49.41	(0.53)	48.15	(0.39)	48.90	(0.34)	0.002
C extr (mg g ⁻¹)	0.46	(0.03)	0.14	(0.01)	0.21	(0.01)	0.64	(0.03)	<0.001
N (% d.w.)	0.878	(0.012)	0.938	(0.012)	0.806	(0.013)	1.172	(0.016)	<0.001
P (% d.w.)	0.040	(0.000)	0.030	(0.000)	0.052	(0.002)	0.070	(0.000)	<0.001
C:N (w/w)	57.86	(0.57)	52.60	(0.49)	59.97	(0.72)	41.78	(0.76)	<0.001
C:P (w/w)	1282	(21)	1548	(25)	905	(15)	699	(9)	<0.001
N:P (w/w)	22.17	(0.47)	29.45	(0.60)	15.10	(0.29)	16.75	(0.39)	<0.001
K (mg g ⁻¹)	0.26	(0.00)	0.54	(0.00)	0.21	(0.00)	0.55	(0.00)	<0.001
Ca (mg g ⁻¹)	1.33	(0.01)	1.26	(0.01)	1.63	(0.01)	1.23	(0.01)	<0.001
Mg (mg g ⁻¹)	0.27	(0.00)	0.14	(0.00)	0.20	(0.00)	0.15	(0.00)	<0.001
Fe (ppm)	210	(2)	208	(4)	453	(12)	192	(4)	<0.001
Mn (ppm)	172	(2)	1430	(10)	776	(9)	2137	(51)	<0.001
Zn (ppm)	30.8	(0.4)	33.0	(0.3)	36.0	(1.0)	42.4	(0.7)	<0.001
Lignin	28.9	(28.9)	29.9	(29.9)	31.2	(31.2)	30.5	(30.5)	<0.001
Carbohydrates	25.9	(25.9)	26.1	(26.1)	29.2	(29.2)	26.9	(26.9)	<0.001

Table 2. Lignin derived and other phenolic pyrolysis products

Name	RT	MW	integrated fragments	Origin	Class
Guaiacol	18.87	124	109+124	Lignin	Guaiacyl
Methylguaiacol	20.32	138	123+138	Lignin	Guaiacyl
Ethylguaiacol	21.40	152	137+152	Lignin	Guaiacyl
Propenylguaiacol	23.29	164	149+164	Lignin	Guaiacyl
Vinylguaiacol	23.69	150	135+150	Lignin	Guaiacyl
Propenylguaiacol	24.48	164	149+164	Lignin	Guaiacyl
Syringol	24.58	154	139+154	Lignin	Syringyl
Propenylguaiacol	25.66	164	149+164	Lignin	Guaiacyl
Methylsyringol	25.67	168	153+168	Lignin	Syringyl
Ethylsyringol	26.39	182	167+182	Lignin	Syringyl
Propenylsyringol	27.97	194	179+194	Lignin	Syringyl
Vinylsyringol	28.37	180	165+180	Lignin	Syringyl
Guaiacolaldehyde	28.40	152	109+152	Lignin	Guaiacyl
Propylguaiacol	28.72	166	137+166	Lignin	Guaiacyl
Oxo-hydroxy-ethylguaiacol	28.77	182	182	Lignin	Guaiacyl
Propenylsyringol	28.91	194	179+194	Lignin	Syringyl
Oxo-ethylguaiacol	29.20	166	151+166	Lignin	Guaiacyl
Oxo-propylguaiacol	29.36	180	137+180	Lignin	Guaiacyl
Propenylsyringol	30.16	194	194+179	Lignin	Syringyl
Syringolaldehyde	32.68	182	139+182	Lignin	Syringyl
Oxo-hydroxy-ethylsyringol	32.80	212	212	Lignin	Syringyl
Guaiacolacetic acid	32.88	182	137+182	Lignin	Guaiacyl
Propylsyringol	33.15	196	181+196	Lignin	Syringyl
Oxo-propylsyringol	33.32	210	167+210	Lignin	Syringyl
Oxopropenylguaiacol	35.30	178	135+178	Lignin	Guaiacyl
Hydroxypropenylguaiacol	37.10	180	137+180	Lignin	Guaiacyl
Syringolacetic acid	38.78	212	212	Lignin	Syringyl
Oxo-propenylsyringol	43.06	208	165+208	Lignin	Syringyl
Phenol	21.02	94	65+66+94	Phenolic	
4-Methylphenol	22.11	108	107+108	Phenolic	
3-Methylphenol	22.22	108	107+108	Phenolic	
Ethylphenol	23.38	122	107+122	Phenolic	
Propenylphenol	26.93	134	133+134	Phenolic	
Propenylphenol	27.76	134	133+134	Phenolic	
Propylphenol	31.11	136	151+166	Phenolic	
Butylphenol	31.86	150	107+150	Phenolic	
4-Hydroxybenzaldehyde	32.70	122	121+122	Phenolic	
Hydroquinone	33.40	110	81+110	Phenolic	

Table 3. Carbohydrate derived pyrolysis products

Name	RT	MW	integrated fragments	Origin	Class
Acetaldehyde	2.06	44	29+44	Carbohydrates	
Furan	2.35	68	39+68	Carbohydrates	Furan
Methylfuran	2.74	82	81+82	Carbohydrates	Furan
Methylfuran	2.91	82	81+82	Carbohydrates	Furan
Dimethylfuran	3.43	96	95+96	Carbohydrates	Furan
Dimethylfuran	3.66	96	95+96	Carbohydrates	Furan
Vinylfuran	5.01	94	65+94	Carbohydrates	Furan
Unknown furan	6.36	108	107+108	Carbohydrates	Furan
Cyclopentanone	6.99	105	84+105	Carbohydrates	Cyclopentenone
Methylfuran	7.62	82	53+82+83	Carbohydrates	Furan
2-Oxopropanoic acid, methylester	7.92	102	43+102	Carbohydrates	
1-Hydroxypropanone	9.24	74	43	Carbohydrates	
2-Cyclopenten-1-one	10.26	82	53+54+52	Carbohydrates	Cyclopentenone
2-Methyl-2-cyclopenten-1-one	10.51	96	53+96	Carbohydrates	Cyclopentenone
1-Hydroxy-2-propanone	10.69	88	57+88	Carbohydrates	Cyclopentenone
Unknown	11.38	unk	65+66+94	Carbohydrates	
3-Furaldehyd	11.57	96	95+96	Carbohydrates	Furan
2(5H)Furanon	11.69	98	55+98	Carbohydrates	Furan
Propanoic acid, methylester	12.10	102	43+102	Carbohydrates	
2-Furaldehyd	12.22	96	95+96	Carbohydrates	Furan
Acetylfuran	12.99	110	95+110	Carbohydrates	Cyclopentenone
3-Methyl-cyclopentanone	13.31	96	67+96	Carbohydrates	Cyclopentenone
Dimethylcyclopentenone	13.69	110	67+95+110	Carbohydrates	Cyclopentenone
5-Methyl-2-furancarboxaldehyde	14.23	110	109+110	Carbohydrates	Furan
2-Cyclopenten-1,4-dione	14.44	96	54+68+96	Carbohydrates	Cyclopentenone
Butyrolactone	15.22	86	56+86	Carbohydrates	
Unknown	15.56			Carbohydrates	
Furanmethanol	15.61	98	98	Carbohydrates	Cyclopentenone
5-Methyl-2(5H)-furanone	16.06	98	55+98	Carbohydrates	Furan
Unknown	16.17	unk	110	Carbohydrates	
1,2-Cylopentandione	17.51	98	55+98	Carbohydrates	Cyclopentenone
Unknown	17.67	unk	42+70	Carbohydrates	
2-Hydroxy-3-methyl-2-cyclopenten-1-one	18.14	98	98	Carbohydrates	Cyclopentenone
3-Methy-11,2-cyclopentanedione	18.42	112	69+112	Carbohydrates	Cyclopentenone
Unknown	19.06		58+86+114	Carbohydrates	
Unknown	19.35		98+126	Carbohydrates	
Unknown	21.77		116	Carbohydrates	
Unknown	22.33		44	Carbohydrates	
Unknown	26.18		57+69	Carbohydrates	
5-Hydroxymethylfuran-1-carboxaldehyde	27.51	126	97+126	Carbohydrates	Furan
Unknown	31.67		73+135	Carbohydrates	
Laevoglucosan	40.44	172	60+73	Carbohydrates	

Table 4. Other pyrolysis products

Name	RT	MW	integrated framents	Origin	Class
25:0 Alkan	27.74	352	57+71	aliphatic	Alkan
25:1 Alken	28.34	350	57+69	aliphatic	Alken
27:0 Alkan	30.04	380	57+67	aliphatic	Alkan
27:1 Alken	30.63	378	57+65	aliphatic	Alken
29:0 Alkan	32.20	408	57+63	aliphatic	Alkan
29:1 Alken	32.82	406	57+61	aliphatic	Alken
Myristic acid (14:0)	2.35	68	39+68	Lipid	Fatty Acid
Palmitic acid (16:0)	2.74	82	81+82	Lipid	Fatty Acid
Stearuc acid (18:0)	2.91	82	81+82	Lipid	Fatty Acid
N-methyl-pyrrol	6.15	81	80+81	Protein	Pyrrol
Pyridine	6.90	95	52+79+95	Protein	Pyridine
Methylpyridine	7.50	93	66+92+93	Protein	Pyridine
Methylpyridine	7.54	93	66+92+93	Protein	Pyridine
methylpyridine	9.02	93	66+93	Protein	Pyridine
Pyrrol	13.11	67	39+41+67	Protein	Pyrrol
Methylpyrrol	13.81	81	80+81	Protein	Pyrrol
Methylpyrrol	14.10	81	80+81	Protein	Pyrrol
3-Hydroxypyridine	26.52	95	67+95	Protein	Pyridine
Indole	26.85	117	89+117	Protein	Indole
Methylindole	27.42	131	130+131	Protein	Indole
Toluene	4.54	92	91+92		Aromatic
Xylene	5.94	106	91+105+106		Aromatic
Xylene	6.09	106	91+105+106		Aromatic
Xylene	6.20	106	91+105+106		Aromatic
Xylene	6.99	105?	84+105?		Aromatic
Methoxytoluene	11.78	122	121+122		Aromatic
Indene	12.64	116	115+116		Aromatic
Benzaldehyde	13.35	106	77+106		Aromatic
Dihydrobenzofuran	26.19	120	91+119+120		Aromatic
Limonene	7.22	136	93		Terpene
Phytol	20.00	276	95+123	Chlorophyll	Terpene
Unknown aliphatic	22.82		58+71		aliphatic
Aceton	2.46	58	43		
2-Propenal	2.60	56	55+56		
Methanol	2.88	32	29+31+32		
3-Buten-2-one	3.39	70	55+70		
2,3-Butandione	3.67	86	69+86		
3-Penten-2-one	3.89	86	69+86		
2-Butanal	4.56	70	69+70		
2,3-Pentadione	4.77	100	57+100		
Hexanal	5.16	82	56+72+82		
1-Penten-3-one	11.28	84	55+84		
Hexan-2,4-dion	23.92	114	56+84+114		
unknown	15.98		119+134		
Unknown	20.85		81		
Unknown	20.86		82+95		
Unknown	22.43		98+128		
Unknown	27.76		138		

Table 5. Results of correlation analysis (R) between lignin and carbohydrate decomposition and other decomposition processes (mass loss, respiration), extracellular enzyme activities, litter chemistry, and litter and microbial biomass C:N:P stoichiometry. Significant ($p < 0.05$) correlations are presented in bold. Data taken from [21, 23]. Changes in litter chemistry (lignin and carbohydrate decomposition) were calculated between 0 and 181 days, other data were measured after 181 days. L acc - lignin accumulation, Ch acc - Carbohydrate accumulation, LCI - LCI difference, L dec - lignin decomposition rate, C dec - carbohydrate decomposition, rate, L resp - lignin loss / carbon loss, C resp - carbohydrate loss / carbon loss, L/C dec - lignin loss / carbohydrate loss, Per/Cell - Potential peroxidase activity / potential cellulase activity, Phen/Cell - Potential phenol activity / potential cellulase activity.

	L acc	Ch acc	LCI diff	L dec	C dec	L resp	C resp	L/C dec	Per/Cell	Phen/Cell
Mass loss	0.291	-0.15	0.245	-0.328	0.106	-0.201	0.125	-0.081	0.048	0.053
Actual respiration	0.333	-0.723	0.606	-0.082	0.771	-0.195	0.594	-0.368	-0.268	-0.362
Accumulated Respiration	0.494	-0.704	0.688	-0.132	0.856	-0.332	0.557	-0.525	-0.506	-0.534
Cellulase activity	0.657	-0.76	0.803	-0.431	0.801	-0.497	0.664	-0.589	-0.436	-0.539
Protease activity	0.186	-0.296	0.264	-0.132	0.274	-0.157	0.301	-0.27	-0.26	-0.18
Chitinase activity	0.409	-0.749	0.663	-0.17	0.795	-0.312	0.677	-0.559	-0.49	-0.607
Phosphatase activity	0.549	-0.813	0.776	-0.302	0.851	-0.407	0.702	-0.556	-0.418	-0.522
Phenoxidase activity	0.632	-0.669	0.737	-0.415	0.719	-0.449	0.552	-0.484	-0.305	-0.356
Peroxidase activity	0.599	-0.588	0.677	-0.412	0.639	-0.438	0.47	-0.435	-0.173	-0.302
N mineralization	0.466	-0.664	0.65	-0.167	0.739	-0.299	0.527	-0.387	-0.282	-0.367
Nitrification	0.587	-0.707	0.732	-0.38	0.74	-0.432	0.621	-0.499	-0.369	-0.45
P mineralization	0.665	-0.55	0.684	-0.544	0.596	-0.576	0.414	-0.478	-0.212	-0.255
C litter	-0.545	0.506	-0.578	0.604	-0.368	0.643	-0.618	0.698	0.525	0.581
extractable C	0.609	-0.766	0.782	-0.37	0.814	-0.446	0.658	-0.54	-0.392	-0.484
N litter	0.354	-0.517	0.503	-0.14	0.587	-0.187	0.366	-0.203	-0.119	-0.159
P litter	0.682	-0.222	0.517	-0.747	0.175	-0.68	0.188	-0.491	-0.073	-0.16
C:N litter	-0.405	0.586	-0.57	0.175	-0.654	0.234	-0.44	0.273	0.195	0.242
C:P litter	-0.636	0.174	-0.453	0.754	-0.082	0.649	-0.176	0.418	0.049	0.08
N:P litter	-0.512	-0.029	-0.264	0.714	0.147	0.577	-0.02	0.316	-0.032	-0.019
C:N mic	0.666	-0.758	0.799	-0.43	0.798	-0.515	0.678	-0.609	-0.584	-0.596
C:P mic	0.692	-0.787	0.834	-0.476	0.814	-0.562	0.726	-0.672	-0.564	-0.648
N:P mic	0.582	-0.729	0.74	-0.415	0.729	-0.508	0.715	-0.67	-0.545	-0.671
C:N imbalance	-0.56	0.81	-0.772	0.288	-0.859	0.391	-0.71	0.531	0.564	0.56
C:P imbalance	-0.817	0.663	-0.833	0.757	-0.61	0.799	-0.668	0.839	0.575	0.67
N:P imbalance	-0.724	0.351	-0.602	0.81	-0.253	0.764	-0.397	0.668	0.301	0.41
Fungi/bacteria(qPCR)	0.002	-0.122	0.079	-0.024	0.087	-0.066	0.135	-0.072	0.199	-0.033
Fungi/bacteria (metaproteome)	0.998	-0.854	0.958	-0.882	0.801	-0.961	0.824	-0.873	-0.679	-0.676

Table 6. Results of correlation analysis (R) between lignin and carbohydrate decomposition and other decomposition processes (mass loss, respiration), extracellular enzyme activities, litter chemistry, and litter and microbial biomass C:N:P stoichiometry. Significant ($p < 0.05$) correlations are presented in bold. Data taken from [21, 23]. Changes in litter chemistry (lignin and carbohydrate decomposition) were calculated between 181 and 475 days, other data were measured after 475 days. L acc - lignin accumulation, Ch acc - Carbohydrate accumulation, LCI - LCI difference, L dec - lignin decomposition rate, C dec - carbohydrate decomposition, rate, L resp - lignin loss / carbon loss, C resp - carbohydrate loss / carbon loss, L/C dec - lignin loss / carbohydrate loss, Per/Cell - potential peroxidase activity / potential cellulase activity, Phen/Cell - potential phenol activity / potential cellulase activity.

	L acc	Ch acc	LCI diff	L dec	C dec	L resp	C resp	L/C dec	Per/Cell	Phen/Cell
Massloss	0.246	0.156	0.068	0.582	0.708	0.005	0.279	-0.137	-0.444	0.403
Actual respiration	-0.011	0.244	-0.212	0.86	0.856	0.122	0.192	-0.044	-0.403	0.29
Accumulated Respiration	0.283	0.354	-0.009	0.852	0.968	0.015	0.298	-0.177	-0.608	0.486
Cellulase activity	0.073	0.218	-0.137	0.848	0.881	0.148	0.295	-0.081	-0.575	0.414
Protease activity	0.004	0.054	-0.086	0.448	0.455	0.16	0.316	-0.11	-0.456	0.381
Phosphatase activity	0.256	0.31	0.069	0.298	0.373	-0.102	-0.014	-0.115	-0.152	0.017
Chitinase activity	0.163	0.339	-0.086	0.643	0.671	0.167	0.253	-0.029	-0.58	0.395
Phenoloxidase activity	0.319	-0.389	0.436	-0.248	-0.003	-0.221	0.505	-0.443	-0.483	0.692
Peroxidase activity	-0.277	0.379	-0.385	0.173	-0.049	0.16	-0.51	0.382	0.546	-0.708
N mineralization	0.246	0.337	0.078	0.009	0.062	-0.191	-0.113	-0.167	0.062	0.089
Nitrification	-0.027	0.567	-0.32	0.63	0.567	0.09	-0.148	0.114	-0.105	-0.023
P mineralization	-0.017	0.202	-0.138	0.507	0.508	-0.136	-0.063	-0.128	0.043	-0.027
C litter	0.123	-0.065	0.177	-0.325	-0.264	-0.204	-0.289	0.024	0.501	-0.348
extractable C	0.231	0.435	-0.086	0.828	0.89	0.074	0.218	-0.109	-0.538	0.409
N litter	0.21	0.356	-0.065	0.816	0.896	-0.004	0.172	-0.12	-0.431	0.349
P litter	-0.117	-0.037	-0.182	0.764	0.762	0.161	0.318	-0.075	-0.464	0.325
C:N litter	-0.272	-0.365	0.016	-0.794	-0.901	0.027	-0.207	0.155	0.49	-0.404
C:P litter	0.329	0.122	0.315	-0.645	-0.541	-0.276	-0.218	-0.067	0.283	-0.162
N:P litter	0.471	0.289	0.328	-0.336	-0.179	-0.293	-0.113	-0.148	0.048	0.034
C:N mic	-0.184	-0.408	0.093	-0.658	-0.703	-0.032	-0.318	0.25	0.57	-0.513
C:P mic	0.237	-0.06	0.312	-0.609	-0.505	-0.192	-0.072	-0.063	0.233	-0.223
N:P mic	0.336	0.127	0.29	-0.373	-0.247	-0.18	0.048	-0.157	-0.002	-0.009
C:N imbalance	-0.145	-0.014	-0.076	-0.354	-0.447	0.061	0.043	-0.05	0.027	0.02
C:P imbalance	0.021	0.246	-0.074	-0.137	-0.2	-0.02	-0.241	0.095	0.16	-0.032
N:P imbalance	0.025	0.231	-0.085	0.04	-0.007	0.003	-0.268	0.172	0.16	-0.08
Fungi/bacteria(qPCR)	-0.03	-0.008	0.017	-0.236	-0.254	-0.089	-0.115	-0.003	0.161	-0.219
Fungi/bacteria (metaproteome)	0.158	0.57	-0.369	0.986	0.972	0.254	0.484	-0.274	-0.601	0.55

Table 7. Correlations coefficients between correspondance analysis factors CA 1 and 2, litter and microbial stoichiometry and protein abundance of microbial taxa. Significant ($p < 0.05$) correlations are presented in bold.

	CA1	CA2
Incubation time	0.872	-0.281
Respiration	-0.158	0.601
NH4 conc.	0.084	0.032
NO3 conc.	0.53	-0.009
PO4 conc	0.161	0.051
C litter	-0.787	-0.172
N litter	-0.174	0.268
P litter	-0.149	0.308
C:N litter	-0.06	-0.272
C:P litter	0.077	-0.334
N:P litter	0.112	-0.223
C micr.	-0.159	-0.04
N micr.	-0.22	-0.14
P micr.	-0.11	-0.59
C:N micr.	0.104	0.04
C:P micr.	-0.022	0.485
N:P micr.	-0.174	0.472
C:N imbalance	-0.225	-0.228
C:P imbalance	-0.118	-0.714
N:P imbalance	0.013	-0.659
F:B prot.	-0.417	0.795
Dothideomycetes	-0.078	0.745
Eurotiomycetes	-0.578	0.083
Leotiomycetes	0.731	0.253
Saccharomycetes	-0.501	0.18
Sordariomycetes	-0.511	0.762
Agaricomycetes	0.167	-0.004
Tremellomycetes	0.723	0
Ustilaginomycetes	0.188	0.37
Thermotogae	-0.336	-0.469
Bacteroidetes	0.638	-0.267
Actinobacteria	0.896	-0.085
Cyanobacteria	-0.319	0.122
Firmicutes	0.183	-0.35
Fusobacteria	0.227	-0.009
Verrucomicrobia	0.114	0.256
Dictyoglomi	0.027	0.2
Alphaproteobacteria	0.924	-0.232
Betaproteobacteria	0.766	-0.358
Gammaproteobacteria	-0.348	-0.929
Deltaproteobacteria	0.229	-0.043
Epsilonproteobacteria	-0.205	0.002