

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

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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 estimated 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, lignin decomposition rates were positively correlated to N contents, respiration, and carbohydrate decomposition. 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 ac-
5 cessible to microbes is mediated by extracellular enzymes and considered rate limiting for decomposition
6 processes [3]

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

17 Recent studies based on more specific methods to determine litter lignin contents question the assumed
18 intrinsic recalcitrance of lignin. Experiments using isotope labeling used to calculate mean residence times
19 for lignin in soils and litter/soil mixtures in both laboratory and in-situ incubation reported lignin residence
20 times no longer than that of other carbon compounds or bulk soil organic matter [10,11]. Also, the capability
21 to degrade lignin was demonstrated for several bacterial taxa in addition to fungi [12].

22 For leaf litter, lignin depletion even at early stages of decomposition and lignin decomposition rates that
23 decreased during decomposition were recently reported by KlotzbÄijcher and colleagues [13]. Based on these
24 results, they proposed a new concept for lignin degradation in which fastest lignin degradation occurs during
25 early litter decomposition when the availability of labile carbon is high. Lignin decomposition during late
26 decomposition, in contrast, is limited by the availability of easily assimilated C and therefore slows down.
27 Additionally, the decomposition of lignin may also be dependent on the nutrient content of the litter and
28 thus the status of the microbial community. During radical polymerization, significant amounts of cellulose
29 and protein are incorporated into lignin structures [14]. In isolated lignin fractions from fresh beech litter,
30 N contents twice as high as in bulk litter were found [15]. It was therefore argued that, while yielding little
31 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", [16]).

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 [17] 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 easily accessible C and N availability [18].

Addition of N has a different effect on litter decomposition than varying N levels in the litter [19]. 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 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.

We addressed the following questions in our study:

(1) Is lignin decomposition delayed until late decomposition stages or are significant amounts of lignin already degraded during early litter decomposition, and if the timing of lignin decomposition depended on litter stoichiometry? We hypothesized, that lignin 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.

(2) 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 (14 days after incubation) is presented in table 1. C:N ratios between 41:1 and 58:1 and C:P ratios between 700:1 and 1300:1 were found, 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 month. 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 extractable organic carbon

Litter mass loss was not significant after 2 weeks and 3 months, and significant for 2 litter types after 6 months. After 15 months, litter mass loss was significant for all litter types, 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 [20].

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 days (fig 1). Accumulated respiration was correlated to litter mass loss ($r=0.738$, $p<0.001$, $n=20$).

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 concentration was found in SW litter followed by AK. Soluble organic C concentrations were weakly correlated with litter N content after 14 days ($r=0.69$, $p<0.001$) and after 3 months ($r = 0.65$, $p<0.01$), but were strictly correlated after 6 months ($r=0.85$, $p<0.001$) and 15 months ($r=0.90$, $p<0.001$).

Potential enzyme activities

Potential extracellular enzyme activities were correlated with litter N, respiration and other decomposition processes (all $R > 0.8$, $p < 0.001$). Cellulase activity increased from first harvest onwards to 15 months, with a small depression after 6 months (Fig. 1), phenoloxidase and peroxidase activities reached their maximum after between 3 and 6 months (fig. 1). For all enzymes and at all time points, SW showed the highest and AK the lowest activity. Differences between these two sites were more pronounced in cellulase activity (SW 10x higher than AK) than in oxidative enzymes (4x higher). Conversely, the phenoloxidase/cellulase ratio was highest for AK and lowest for SW at all time points and decreased during litter decomposition. This indicates that microbial communities in AK litter invested more energy and nitrogen into degrading lignin and less into degrading carbohydrates than in litter from other sites. (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 5.5 mg N g⁻¹ d.w. and 0.05 to 3.5 mg P g⁻¹ litter d.w (fig. 2). In KL and OS microbial biomass buildup reached a plateau after 3 months, AK and SW showed further microbial biomass growth reaching a maximum of microbial C and N contents after 6 months (AK also for P). Microbial C:N ratios ranged between 6 and 18, C:P ratios between 8 and 35, and N:P ratios between 0.5 and 3.5 (fig. 2).

Litter microbial biomass was stoichiometrically homeostatic during the first 6 months (no or marginally negative correlations between microbial C:N:P and litter C:N:P, see also [20]), but after 15 months (microbial C:N:P ratios were significantly correlated to resource stoichiometry: $R = 0.53-0.64$, all $p < 0.002$), when the homeostatic regulation coefficients [21] $H_{C:P} = 1.68$, $H_{C:N} = 2.01$, and $H_{N:P} = 2.29$ were found. Microbial C:N ratios were tightly constrained after 3 months (14.5 to 18.2) and 6 months (6.9 to 9.0), but significantly different between the two time points. Microbial C:P and N:P ratios were less constrained, with the highest variance between litter from different sites after 3 months incubation (fig. 2).

Fungi/bacteria ratios derived from metaproteomics data of the litter (one replicate per litter type and harvest) were highest after 14 days (5 to 12) and decreased during litter decomposition (1.7 to 3 after 15 months). The large differences in fungi/bacteria ratios between litter types decreased during decomposition. Fungal proteins were dominant in all litter types at all stages, but most prominent in SW and least pronounced in AK. The fungi/bacteria ratios were negatively correlated to the ratios of lignin/cellulose decomposition and to LCI change during the first 6 months. In contrast, lignin decomposition rates were positively correlated with fungi/bacteria ratios after 15 months but not to the ratios of lignin/cellulose decomposition (fig. 3). In

115 addition, fungi: bacteria ratios were measured on a DNA basis (qPCR) the results showing a similar pattern
116 between litter types and harvests but with a much larger fungal DNA dominance (ratios between 10-180).
117 Fungi/bacteria ratios were highly correlated between protein- and DNA-based estimates ($r=0.801$, $p<0.001$,
118 with log-transformed qPCR ratios).

119 **Pyrolysis-GC/MS and Lignin content**

120 In total 128 pyrolysis products were detected, quantified, identified and assigned to their origin (2 -4). We
121 found only minor changes in the relative concentration of litter pyrolysis products during decomposition, and
122 differences between sites were small but well preserved during decomposition. However, the high precision and
123 reproducibility of pyrolysis GC/MS analysis of litter allowed tracing small changes in lignin and carbohydrate
124 abundance during decomposition. Lignin-derived compounds made up between 29 and 31 % relative peak
125 area (TIC) in initial litter, and increased by up to 3 % over the first 6 months. Carbohydrate-derived
126 pyrolysis products accounted for 26 to 29 % in initial litter and decreased by up to 2.6 % during litter
127 decomposition. The pyrolysis-based LCI index showed a small range between 0.517 and 0.533 initially (Fig.
128 4). During decomposition, LCI increased by up to 9 % of the initial value, with SW showing the highest
129 increase while in AK LCI decreased. The changes in LCI almost completely occurred over the first 6 months,
130 with insignificant changes thereafter (fig. 4).

131 During the first 6 months of litter decomposition, between one and 6 % of the initial lignin pool and
132 between 4 and 17% of the initial carbohydrate pool were degraded (Fig. 5). Lignin decomposition was highest
133 in AK and KL litter, while KL, OS and SW decomposed carbohydrates fastest. Lignin preference values
134 (% lignin decomposed/%carbohydrates decomposed) were lowest in SW and highest in AK litter (Figure 5).
135 In AK litter, lignin macromolecules were 50 % more likely to be decomposed than carbohydrates, while in
136 SW litter carbohydrates were 10 times more likely to be decomposed (fig. 5). Between 6 and 15 months, no
137 further accumulation of lignin occurred, lignin and carbohydrates were both degraded at the same rates and
138 their relative concentrations remained constant (fig. 5).

139 **Correlations between lignin and carbohydrate decomposition and litter chem-** 140 **istry, microbial community and decomposition processes**

141 Relationships between lignin and carbohydrate degradation, litter chemistry, microbial biomass and decom-
142 position processes were tested after 6 and 15 months (tables 5 and 6) including data presented by [20] and [22].
143 After 6 months, we found that the ratio of lignin/cellulose degradation was positively correlated with the

ratio of phenoloxidase/cellulase ($R=0.599$, $p=0.005$) and peroxidase/cellulase ($R=0.734$ $p<0.001$, table 5)⁸. Carbohydrate decomposition was positively correlated with litter N content, and negatively with litter C:N ratios and litter-microbial C:N imbalances. In contrast, lignin decomposition was negatively correlated to litter P, but positively with litter C:P and N:P ratios, and litter-microbial C:P and N:P imbalances (fig. 6). After 15 months, the ratio of lignin/carbohydrate decomposition was not related to stoichiometry or elemental composition any more. Most interestingly, lignin and carbohydrate decomposition exhibited the same controls, being positively correlated to soluble organic C, litter N and litter P (table 6). Mass loss and accumulated respiration were positively correlated to lignin and carbohydrate decomposition (table 6), a pattern that we did not find for lignin decomposition in the early decomposition phase (table 5).

Discussion

Our experimental approach allowed us to single out the effects of litter quality on the microbial decomposer community as well as decomposition processes, while excluding effects of fauna, climate and the initial microbial community. 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.

Contradicting the traditional concepts of litter decomposition, our results demonstrate that relevant but 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). We can therefore confirm that early lignin decomposition rates are by far underestimated, as recently proposed by [13], based on a complementary analytic approach. Unlike them, we found no decreases but constant or increasing lignin decomposition rates during litter decomposition over 15 months. 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.

Differences in initial lignin contents were marginal (29-31 % relative peak area), and lignin contents of sites with high initial lignin decomposition rates were not higher than that of sites with low rates. Therefore,

173 differences in early lignin decomposition did not result from high or low lignin contents as is suggested by⁹
174 traditional litter decomposition models. Low lignin decomposition rates were also not caused by a lack of Mn
175 or Fe, the metals being important cofactors of oxidative lignin decay, which were suggested to be rate limiting
176 during late lignin decomposition [1]. While Mn and Fe concentrations strongly varied between litter collected
177 at different sites, Mn and Fe concentrations were lowest in the litter with highest lignin decomposition
178 rates (AK, see Table 1). Low contents of these elements would explain decreased but not enhanced lignin
179 decomposition. Moreover, soluble organic C was suggested to be limiting for lignin decomposition since the
180 process of lignin decomposition does not generate enough energy for survival of lignin decomposers [13].
181 Soluble organic C apparently did not control lignin decomposition since we found highest concentrations in
182 the two litter types that showed the highest and the lowest lignin decomposition rates.

183 We found strong evidence that litter C:N:P stoichiometry and litter element concentrations exerted a
184 major control on the extent of lignin decomposition during the initial decomposition phase. Carbohydrate
185 decomposition was positively correlated with litter N contents and negatively to litter C:N ratios, as were
186 the majority of decomposition processes (mass loss, respiration, potential extracellular enzymatic activities).
187 In contrast, lignin decomposition rates were positively correlated with litter C:P ratios and negatively with
188 dissolved and total litter P. The relationship was strongest when lignin decomposition rates were compared
189 to litter-microbe C:P imbalances, i.e. the greater the imbalance between resource and consumer C:P became
190 (greater P limitation) the lower lignin decomposition rates became.

191 Cultivation studies showed that lignin decomposition by fungi is triggered by nitrogen starvation, and
192 that lignin does not provide sufficient energy to maintain the decomposer's metabolism without the use of
193 other organic C i.e. energy sources [23]. Moreover, lignin decomposition was found in wild-type *A. thaliana*
194 litter containing abundant cellulose as a C source, but not in a low-cellulose mutant during a 12-month
195 incubation experiment in a boreal forest [19]. In the N- and P-(co-)limited situation commonly encountered
196 during early litter decomposition, we may speculate that lignin is degraded to access additional nutrients
197 (mainly N) or to use a C surplus by decomposing a less C efficient but nutrient enriched substrate (nutrient
198 mining hypothesis). However, a stimulation of lignin decomposition by low P availability or microbial P
199 limitation, as indicated by the strong negative correlations to P pools that we found, has not been reported
200 yet. Though lignified materials have been reported to be N-rich and decomposition of these materials
201 may therefore enhance N supply to microbial communities, lignins are not expected to contain quantitative
202 important amounts of P.

203 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. [19] 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 and P limitation or high N and P uns efficiency of these communities. 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 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.

While the mode of negative P regulation on lignin decomposition remains unknown, we found 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 high P litter during initial decomposition. Fungi colonized litter faster than bacteria and therefore dominated early litter decomposition, however the fungi: bacteria ratios decreased over the entire incubation period pointing to increasing population sizes of bacteria with time. Fungi-rich communities more efficiently used high litter N to produce extracellular enzymes that degrade carbohydrates immediately after inoculation (fungi: bacteria ratios were correlated to litter N 14 days after inoculation) and high litter P to build up microbial biomass on a longer time scale (fungi: bacteria ratios were correlated to litter P after 6 months). Interestingly, bacteria-rich communities (AK) were more active in decomposing lignin than those being dominated by fungi. This does not necessarily indicate that bacteria play the key role in lignin decomposition, though bacteria were also reported to produce oxidative enzymes that can decompose lignified materials in litter [12]. However, decreases in fungi/bacteria ratios may be superimposed on the increase of smaller subpopulations of e.g. fungi that are key mediators of lignin decomposition, or alternatively general increases in the size of microbial communities with declining fungi/bacteria ratios may as well mask stable fungal populations when bacterial abundance increases. The fungal communities were dominated by Ascomycetes (Dothideomycetes, Eurotiomycetes, Leotiomyces and Sordariomycetes), with smaller contributions by Saccharomycetes and Basidiomycetes (Agaricomycetes and Tremellomycetes). It is particularly the latter, Basidiomycetes, that catalyze the cellulolytic and lignolytic decomposition of dead

235 plant material, however they comprised less than 5% of the fungal protein ensemble. The bacterial com¹¹
236 munity in contrast was dominated by Proteobacteria (mainly γ , declining, and α - and β -Proteobacteria,
237 increasing with litter decomposition), Actinobacteria and Bacteroidetes both of which strongly increased
238 with time. Actinobacteria are also known as important decomposers of plant detritus, with the potential
239 to excrete oxidative enzymes and being oligotrophic, and Bacteroidetes also excrete a broad range of hy-
240 drolytic enzymes targeting cellulose and other polymers. Since the metaproteomic approach did not find
241 oxidative extracellular enzymes we so far cannot dissect the contributions of bacteria and fungi to the lignin
242 decomposition process.

243 While the microbial communities were strictly homeostatic during the first 6 months, substrate stoi-
244 chiometry had a minor, but significant influence on microbial stoichiometry after 15 months. Together, these
245 changes indicate that the microbial communities were able to compensate for differences in substrate quality
246 by adjusting their C-, N- and P-use efficiency (Mooshammer et al. 2011) which was coupled to differences
247 in substrate preference (lignin/carbohydrate) and occurred at the expense of microbial community growth
248 and overall decomposition speed. However, stoichiometric compensation of the microbial communities was
249 limited after 6-15 months which points to larger stoichiometric differences between the microbial populations
250 dominating the later stage decomposition processes.

251 Conclusions

252 Our results contradict the traditional concept that lignin decomposition is slow during early litter decom-
253 position. While traditional litter decomposition models propose that lignin decomposition mainly occurs
254 during late decomposition stages, we found that variable but in some cases substantial amounts of lignin
255 were decomposed during the first 6 months. The extent to which lignin was decomposed was controlled by
256 litter P during the first 6 months, but by litter N thereafter as was carbohydrate decomposition. Our results
257 further question that recalcitrance is intrinsic to lignin as a chemical compound, but suggests that lignin
258 decomposition also depends on litter chemistry and environmental conditions, which both affect microbial
259 community structure including the abundance of fungal and bacterial groups that are key to decomposition
260 of plant debris by excretion of hydrolytic and oxidative extracellular enzymes.

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 [24].

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 [25]). 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 [26]. 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 inorganig P after persulfate digestion [27].

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

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

where X stand for the element N or P.

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-cellubiosidase (“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 [28].

Metaproteome analysis and quantitative PCR

For metaproteome analysis, 3 g of each sample were ground 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 mixtures were analyzed on a hybrid LTQ-Orbitrap MS (Thermo Fisher Scientific). Protein database search against the UniRef library, the translated metagenome of the microbial community of a Minnesota farm silage soil [29] and known contaminants was performed using the MASCOT Search Engine. A detailed description of the extraction procedure and search criteria was published by [30]. Additionally, fungi/bacteria

ratios were determined with quantitative PCR as described recently [31].

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 µg 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 [32,33]. 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.

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

Statistical analysis

All statistical analyses were performed with the software and statistical computing environment R [34]. 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

339 Acknowledgments

340 References

- 341 1. Berg, B & McClaugherty C (2008) Plant Litter. Decomposition, Humus Formation, Carbon Seques-
342 tration. Berlin: Springer.
- 343 2. Don A, Kalbitz K (2005) Amounts and degradability of dissolved organic carbon from foliar litter at
344 different decomposition stages. *Soil Biology and Biochemistry* 37: 2171–2179.
- 345 3. Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology*
346 *and Biochemistry* 42: 391–404.
- 347 4. Berg B, Staaf H (1980) Decomposition rate and chemical changes of Scots pine needle litter. II.
348 Influence of chemical composition. *Ecological Bulletins* : 373–390.
- 349 5. Coûteaux MM, Bottner P, Berg B (1995) Litter decomposition, climate and liter quality. *Trends in*
350 *ecology & evolution* 10: 63–66.
- 351 6. Moorhead DL, Sinsabaugh RL (2006) A theoretical model of litter decay and microbial interaction.
352 *Ecological Monographs* 76: 151–174.
- 353 7. Adair EC, Parton WJ, Del Grosso SJ, Silver WL, Harmon ME, et al. (2008) Simple three-pool model
354 accurately describes patterns of long-term litter decomposition in diverse climates. *Global Change*
355 *Biology* : 2636–2660.
- 356 8. Hatfield RD, Romualdo SF (2005) Can Lignin Be Accurately Measured? *Crop Science* 45: 832–839.
- 357 9. Preston CM, Trofymow JA, Sayer BG, Niu J (1997) ¹³C nuclear magnetic resonance spectroscopy
358 with cross-polarization and magicâ€šangle spinning investigation of the proximate analysis fractions
359 used to assess litter quality in decomposition studies. *Canadian Journal of Botany* 75: 1601–1613.
- 360 10. Thevenot M, Dignac MF, Rumpel C (2010) Fate of lignins in soils: A review. *Soil Biology and*
361 *Biochemistry* 42: 1200–1211.

- 362 11. Bol R, Poirier N, Balesdent J, Gleixner G (2009) Molecular turnover time of soil organic matter 16
363 particle - size fractions of an arable soil. *Rapid Communications in Mass Spectrometry* 23: 2551–2558.
- 364 12. Bugg TD, Ahmad M, Hardiman EM, Singh R (2011) The emerging role for bacteria in lignin degra-
365 dation and bio-product formation. *Current opinion in biotechnology* 22: 394–400.
- 366 13. Klotzbücher T, Kaiser K, Guggenberger G, Gatzek C, Kalbitz K (2011) A new conceptual model for
367 the fate of lignin in decomposing plant litter. *America* 92: 1052–1062.
- 368 14. Achyuthan KE, Achyuthan AM, Adams PD, Dirk SM, Harper JC, et al. (2010) Supramolecular
369 self-assembled chaos: polyphenolic lignin’s barrier to cost-effective lignocellulosic biofuels. *Molecules*
370 (Basel, Switzerland) 15: 8641–88.
- 371 15. Dyckmans J, Flessa H, Brinkmann K, Mai C, Polle A (2002) Carbon and nitrogen dynamics in acid de-
372 tergent fibre lignins of beech (*Fagus sylvatica* L.) during the growth phase. *Plant, Cell & Environment*
373 25: 469–478.
- 374 16. Craine JM, Morrow C, Fierer N (2007) Microbial nitrogen limitation increases decomposition. *Ecology*
375 88: 2105–13.
- 376 17. Knorr M, Frey S, Curtis P (2005) Nitrogen addition and litter decomposition : A meta-analysis.
377 *Ecology* 86: 3252–3257.
- 378 18. Fontaine S, Henault C, Aamor a, Bdioui N, Bloor J, et al. (2011) Fungi mediate long term sequestration
379 of carbon and nitrogen in soil through their priming effect. *Soil Biology and Biochemistry* 43: 86–96.
- 380 19. Talbot JM, Treseder KK (2011) Interactions between lignin, cellulose, and nitrogen drive litter chem-
381 istry - decay relationships. *Ecology in press*.
- 382 20. Mooshammer M, Wanek W, Schneckner J, Wild B, Leitner S, et al. (2011) Stoichiometric controls of
383 nitrogen and phosphorus cycling in decomposing beech leaf litter. *Ecology in press*.
- 384 21. Sterner RW, Elser JJ (2002) *Ecological stoichiometry: the biology of elements from molecules to the*
385 *biosphere*. Princeton University Press, 439 pp.
- 386 22. Leitner S, Wanek W, Wild B, Haemmerle I, Kohl L, et al. (2011) Linking resource quality to decom-
387 position processes: Influence iof litter chemistry and stoichiometry on glucan depolymerization during
388 decomposition of beech (*Fagus silvatica* L.) litter. *Soil Biology and Biochemistry in review*.

- 389 23. Janshekar H, Fiechter A (1988) Cultivation of *Phanerochaete chrysosporium* and production of lignin¹⁷
390 peroxidases in submerged stirred tank reactors. *Journal of Biotechnology* 8: 97–112.
- 391 24. Wanek W, Mooshammer M, Blöchl A, Hanreich A, Keiblinger K, et al. (2010) Determination of gross
392 rates of amino acid production and immobilization in decomposing leaf litter by a novel N-15 isotope
393 pool dilution technique. *Soil Biology and Biochemistry* 42: 1293–1302.
- 394 25. Kolmer J, Spaulding E, Robinson H (1951) *Approved Laboratory Techniques*. New York: Appleton
395 Century Crafts.
- 396 26. Brooks P, Kragt J, Powlson D, Jenkinson D (1985) Chloroform fumigation and the release of soil
397 nitrogen e the effects of fumigation time and temperature. *Soil Biology & Biochemistry* 17: 831–835.
- 398 27. Schinner F, Öhlinger R, Kandeler E, Margesin R (1996) *Methods in Soil Biology*. Berlin: Springer
399 Verlag, pp. 389 pp.
- 400 28. Kaiser C, Koranda M, Kitzler B, Fuchslueger L, Schnecker J, et al. (2010) Belowground carbon alloca-
401 tion by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community
402 composition in a beech forest soil. *New Phytologist* 187: 843–858.
- 403 29. Tringe SG, von Mering C, Kobayashi A, Salamov Aa, Chen K, et al. (2005) Comparative metagenomics
404 of microbial communities. *Science (New York, NY)* 308: 554–7.
- 405 30. Schneider T, Gerrits B, Gassmann R, Schmid E, Gessner MO, et al. (2010) Proteome analysis of fungal
406 and bacterial involvement in leaf litter decomposition. *Proteomics* 10: 1819–30.
- 407 31. Inselsbacher E, Hinko-Najera Umana N, Stange FC, Gorfer M, Schüller E, et al. (2010) Short-term
408 competition between crop plants and soil microbes for inorganic N fertilizer. *Soil Biology and Bio-*
409 *chemistry* 42: 360–372.
- 410 32. Schellekens J, Buurman P, Pontevedra-Pombal X (2009) Selecting parameters for the environmental
411 interpretation of peat molecular chemistry - A pyrolysis-GC/MS study. *Organic Geochemistry* 40:
412 678–691.
- 413 33. Kuder T, Kruege MA (1998) Preservation of biomolecules in sub-fossil plants from raised peat bogs -
414 a potential paleoenvironmental proxy. *Organic Geochemistry* 29: 1355–1368.

- 415 34. R Development Core Team (2008). R: A Language and Environment for Statistical Computing. URL
416 <http://www.r-project.org>.

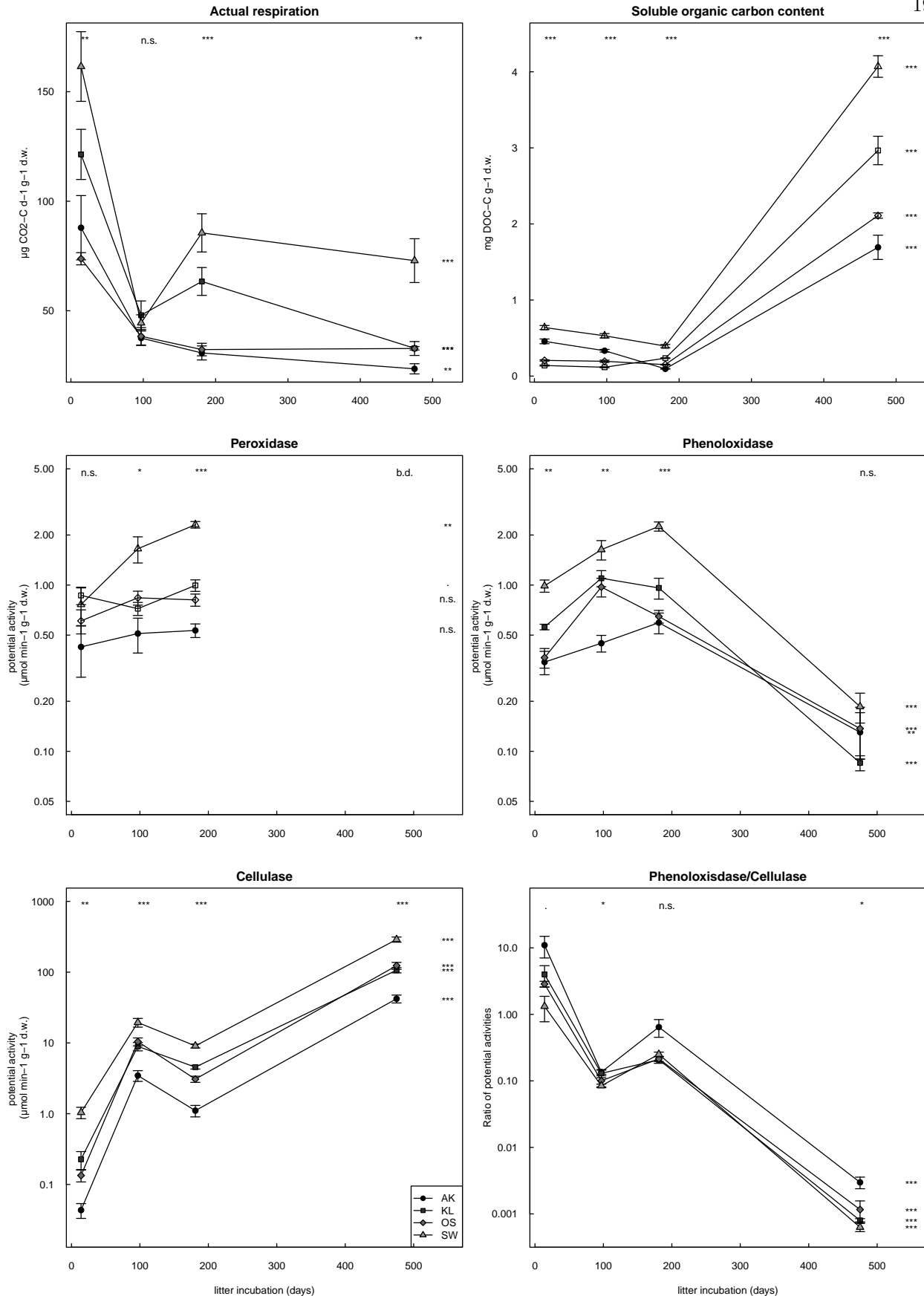


Figure 1. Respiration rates, concentration of soluble organic C and potential extracellular enzyme activities in decomposing beech leaf litter from a mesocosm experiment. Beech litter was collected in: triangles, Schottenwald (SW); diamonds, Ossiach (OS); squares, Klaus-leopoldsdorf (KL); circles, Achenkirch, AK. Error bars indicate standard errors ($n=5$). Significant differences between litter

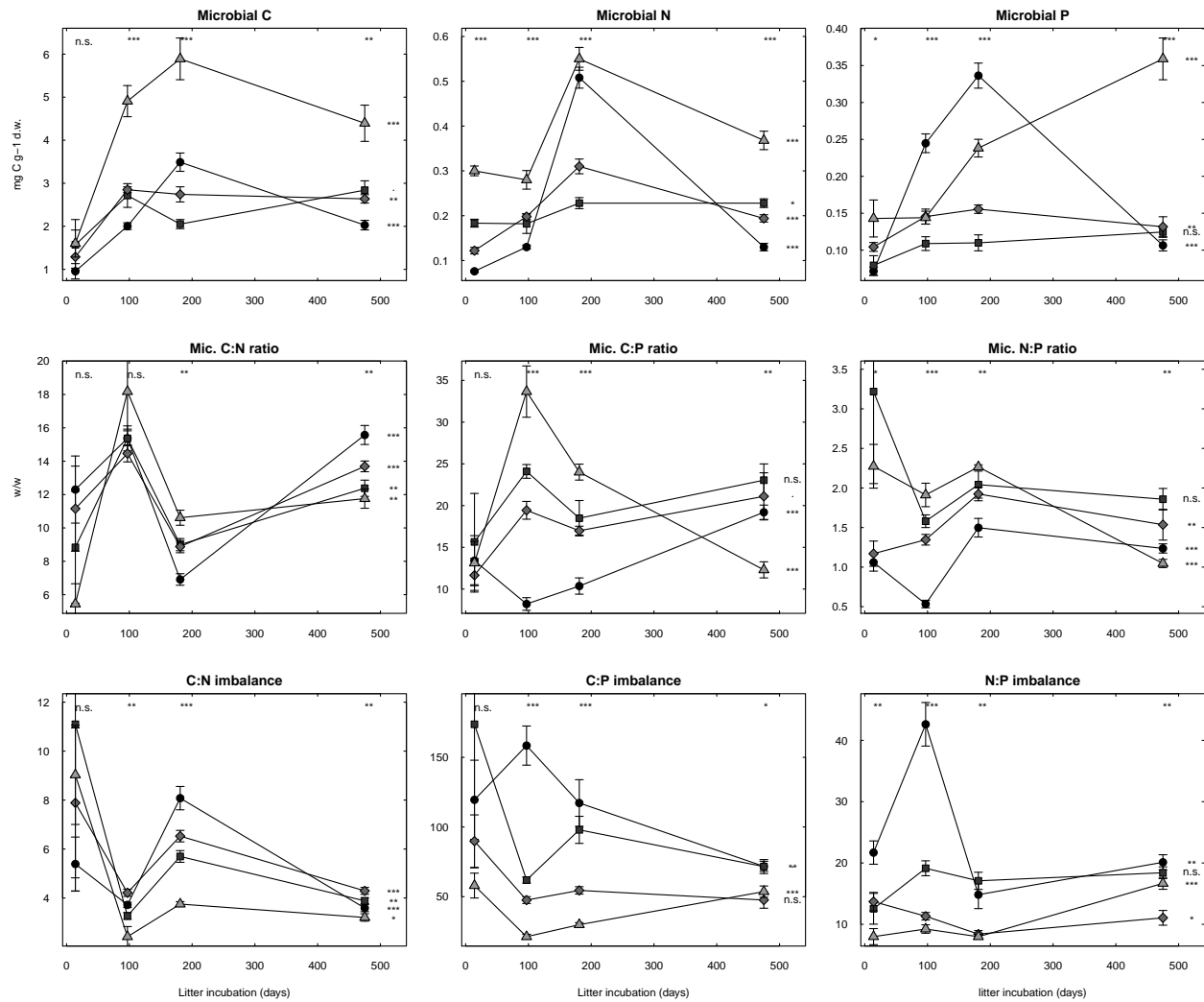


Figure 2. Microbial biomass C, N and P, microbial C:N:P stoichiometry and resource/consumer stoichiometric imbalance in these elements in decomposing beech leaf litter from a mesocosm experiment. Beech litter was collected in: triangles, Schottenwald (SW); diamonds, Ossiach (OS); squares, Klausenleopoldsdorf (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.

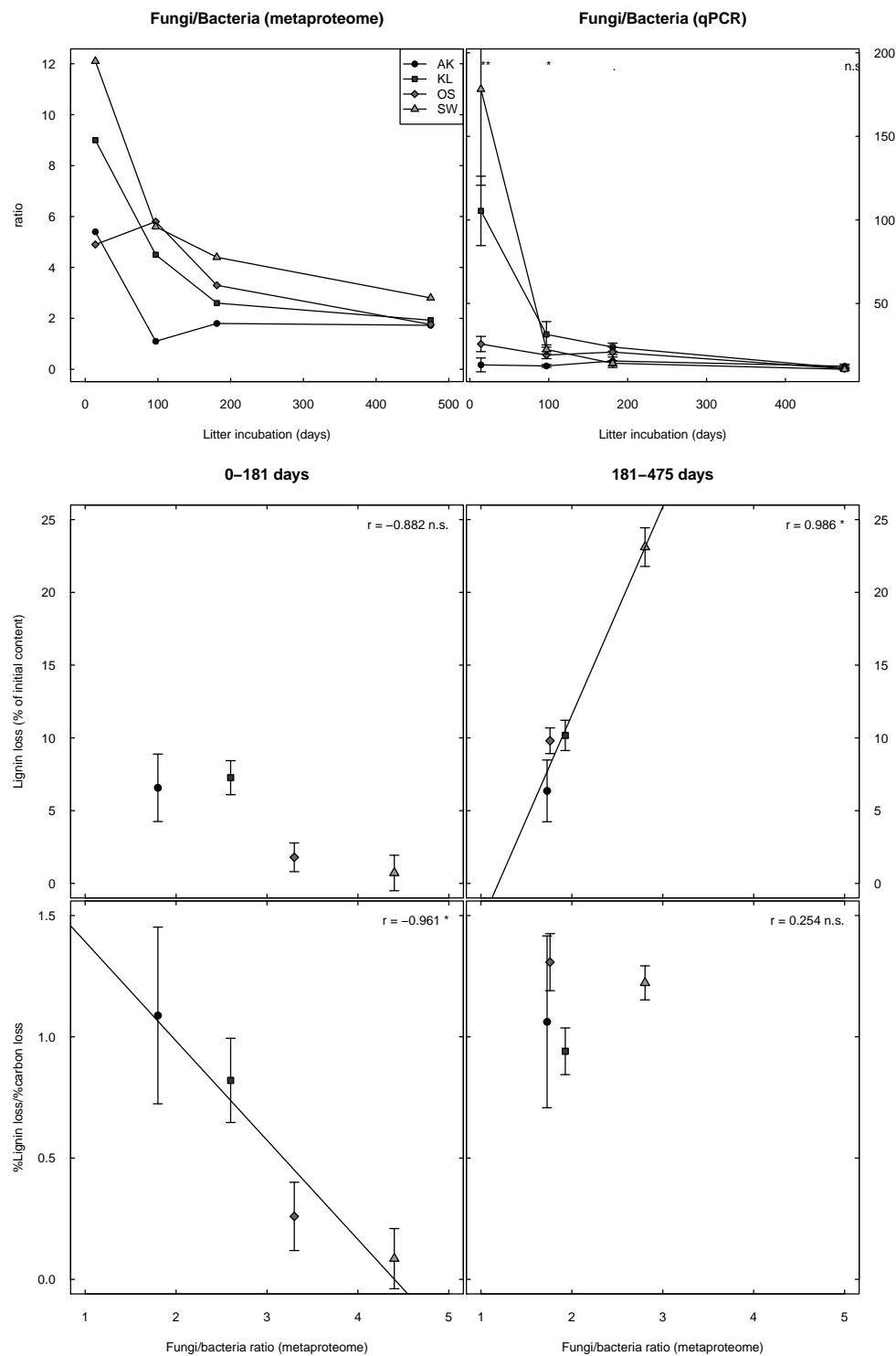


Figure 3. Fungi/Bacteria ratios and their correlations with LCI change: Top: Fungi/bacteria ratios derived from metaproteome (left) and qPCR (right) analysis. Bottom: The correlations between metaproteomic fungi/bacteria ratios with Lignin decomposition rates (mid) and lignin loss / carbon loss (bottom) for 0-6 months (left) and 6-15 months (right). Error bars 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. *, $P<0.05$, **, $P<0.01$, ***, $P<0.001$.

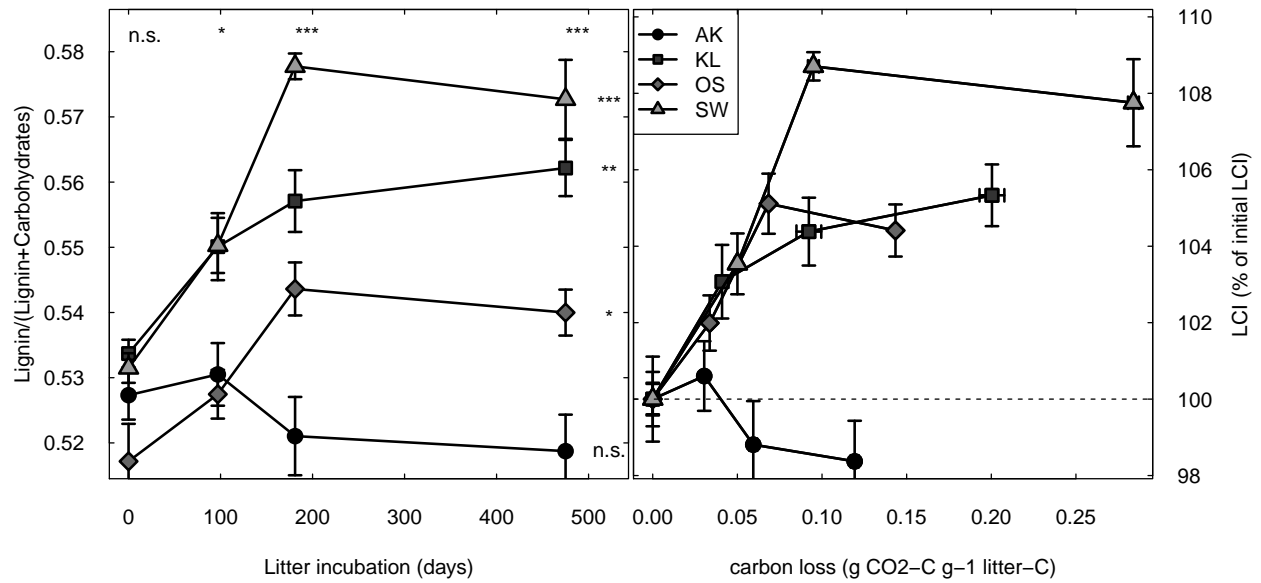


Figure 4. Development of the LCI (lignin/(lignin+carbohydrates)) during time of beech litter decomposition (A) or plotted against cumulative C loss (B). Errorbars indicate standard errors (n=4-5). The dashed line indicates a constant ratio between lignin and carbohydrates (i.e. no preferential decomposition 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. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

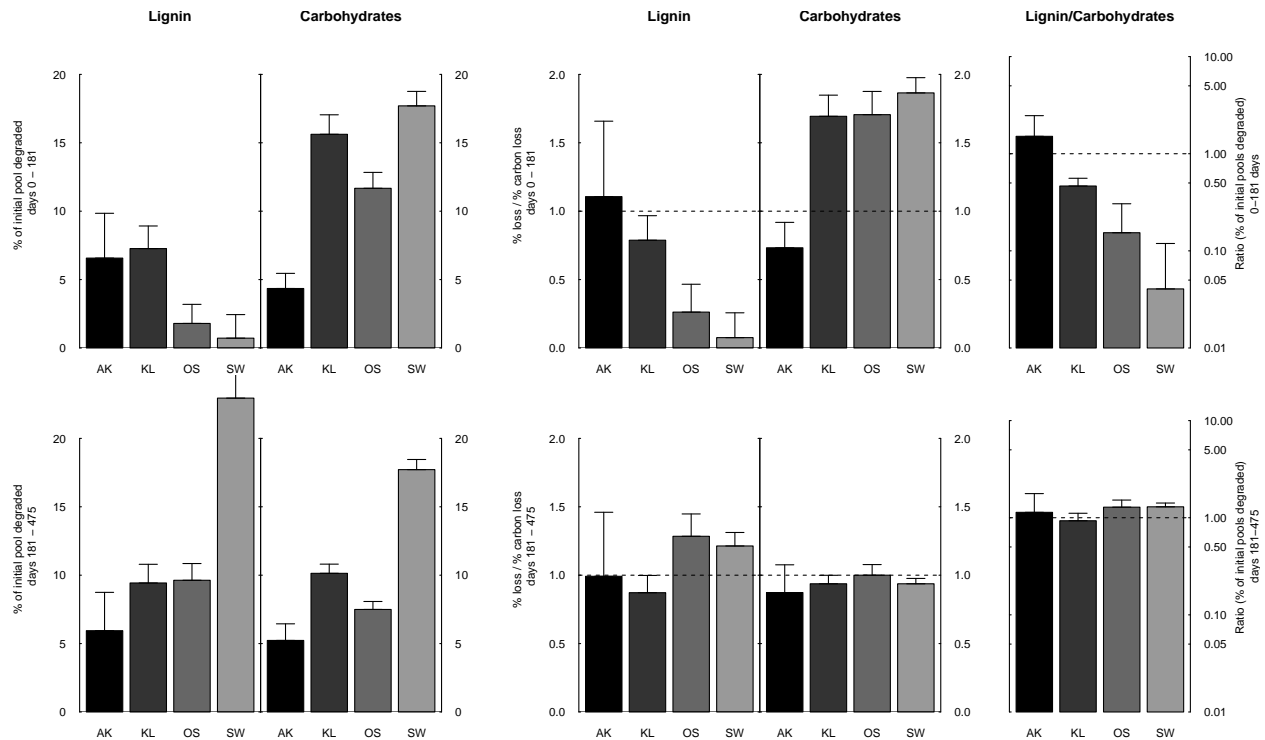


Figure 5. Carbon loss corrected amounts of lignin and carbohydrates degraded in beech litter collected in Achenkirch (AK), Klausenleopoldsdorf (KL), Ossiach (OS) and Schottenwald (SW). Carbon loss was calculated based on accumulated respiration for each mesocosm. Error bars indicate standard errors (n=4-5).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

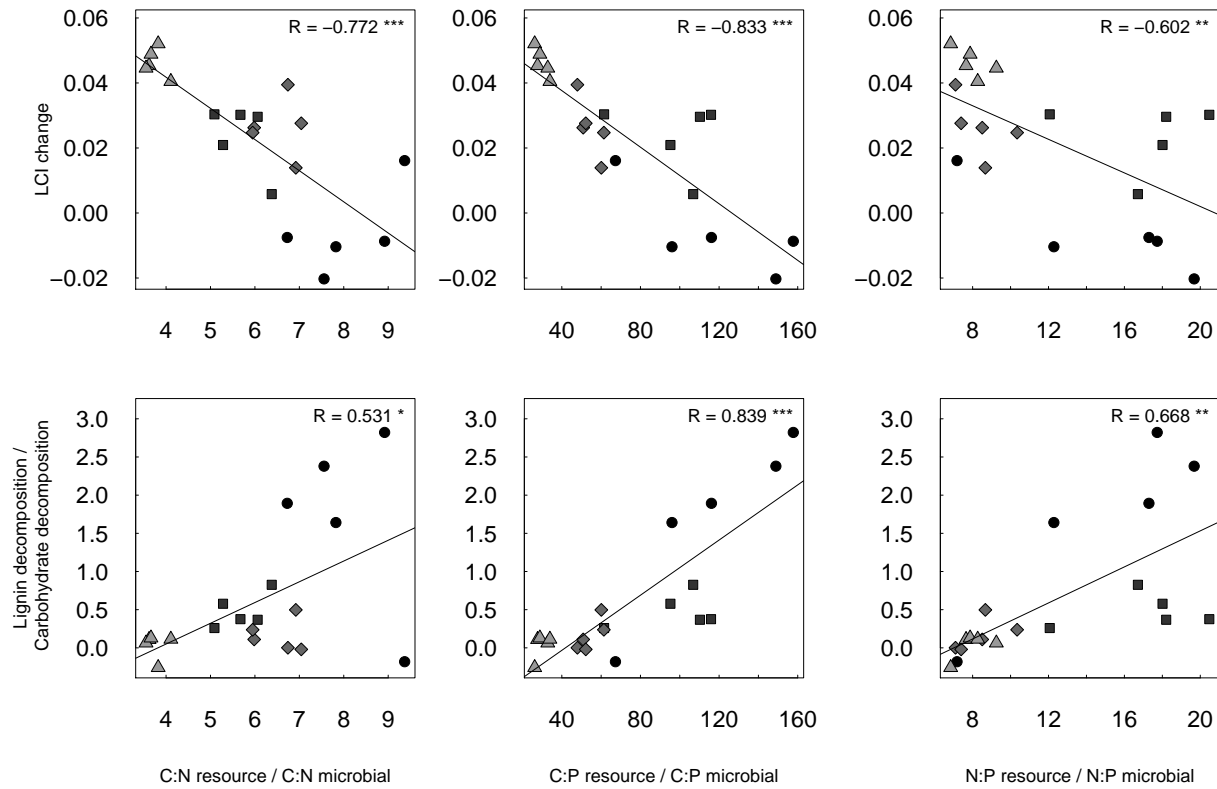


Figure 6. Correlation between the LCI change or the ratio of lignin/carbohydrate decomposition during the first 6 months of litter decomposition correlate to litter/microbe stoichiometric imbalances. and change and Correlations between lignin accumulation during the first 6 month of litter incubation 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. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

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 quantified

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 [20, 22]. 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	0.0534
Actual respiration	0.333	-0.723	0.606	-0.0822	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
Phenoloxidase 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	-0.0728	-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	-0.0823	0.649	-0.176	0.418	0.049	0.0805
N:P litter	-0.512	-0.0287	-0.264	0.714	0.147	0.577	-0.0202	0.316	-0.0316	-0.0192
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)	0.00234	-0.122	0.0794	-0.0242	0.0874	-0.0664	0.135	-0.072	0.199	-0.0333
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 [20, 22]. 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, L/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 - Potetial peroxidase activity / potential cellulase activity, Phen/Cell - Potetial phenolo 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	0.00521	0.279	-0.137	-0.444	0.403
Actual respiration	-0.0114	0.244	-0.212	0.86	0.856	0.122	0.192	-0.0444	-0.403	0.29
Accumulated Respiration	0.283	0.354	-0.00931	0.852	0.968	0.0149	0.298	-0.177	-0.608	0.486
Cellulase activity	0.0733	0.218	-0.137	0.848	0.881	0.148	0.295	-0.0811	-0.575	0.414
Protease activity	0.00361	0.0538	-0.086	0.448	0.455	0.16	0.316	-0.11	-0.456	0.381
Phosphatase activity	0.256	0.31	0.0689	0.298	0.373	-0.102	-0.0136	-0.115	-0.152	0.0167
Chitinase activity	0.163	0.339	-0.0858	0.643	0.671	0.167	0.253	-0.0289	-0.58	0.395
Phenoloxidase activity	0.319	-0.389	0.436	-0.248	-0.0034	-0.221	0.505	-0.443	-0.483	0.692
Peroxidase activity	-0.277	0.379	-0.385	0.173	-0.0488	0.16	-0.51	0.382	0.546	-0.708
N mineralization	0.246	0.337	0.0777	0.00915	0.0616	-0.191	-0.113	-0.167	0.0624	0.0892
Nitrification	-0.0272	0.567	-0.32	0.63	0.567	0.0904	-0.148	0.114	-0.105	-0.0234
P mineralization	-0.0165	0.202	-0.138	0.507	0.508	-0.136	-0.0626	-0.128	0.0433	-0.0273
C litter	0.123	-0.0651	0.177	-0.325	-0.264	-0.204	-0.289	0.0236	0.501	-0.348
extractable C	0.231	0.435	-0.0861	0.828	0.89	0.074	0.218	-0.109	-0.538	0.409
N litter	0.21	0.356	-0.0654	0.816	0.896	-0.00431	0.172	-0.12	-0.431	0.349
P litter	-0.117	-0.037	-0.182	0.764	0.762	0.161	0.318	-0.0746	-0.464	0.325
C:N litter	-0.272	-0.365	0.0158	-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	-0.0672	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	0.0338
C:N mic	-0.184	-0.408	0.0928	-0.658	-0.703	-0.0319	-0.318	0.25	0.57	-0.513
C:P mic	0.237	-0.06	0.312	-0.609	-0.505	-0.192	-0.0716	-0.063	0.233	-0.223
N:P mic	0.336	0.127	0.29	-0.373	-0.247	-0.18	0.0482	-0.157	-0.00191	-0.00931
C:N imbalance	-0.145	-0.014	-0.0759	-0.354	-0.447	0.0611	0.0435	-0.0495	0.0273	0.0196
C:P imbalance	0.0215	0.246	-0.0739	-0.137	-0.2	-0.02	-0.241	0.0948	0.16	-0.0317
N:P imbalance	0.0248	0.231	-0.085	0.0398	-0.00715	0.00271	-0.268	0.172	0.16	-0.0803
Fungi/bacteria(qPCR)	-0.03	-0.00782	0.0166	-0.236	-0.254	-0.0887	-0.115	-0.00256	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