

# Controls of litter chemistry over early lignin decomposition in beech litter<sup>1</sup>

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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 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 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 is not degraded until  
11 accumulated to a certain, critical level when it inhibits the degradation of less recalcitrant compounds [4-7].  
12 These pools are usually quantified by gravimetric determination of the amount of cellulose, hemi-celluloses  
13 and lignins after sequential extractions with selective solvents. These methods were repeatedly criticized  
14 for being unspecific for lignin determination [8]. When analyzed with alternative methods (NMR, CuO-  
15 oxidation, Pyrolysis-GC/MS), extracted lignin fractions were shown to contain also many other substances  
16 [9].

17 Recent studies based on more specific methods to determine litter lignin contents question the assumed  
18 intrinsic recalcitrance of lignin. Isotope labeling experiments with soils and litter/soil mixtures, undertaken  
19 both in-situ and under controlled conditions, revealed mean residence times of lignin in soils are in the range  
20 of 10-50 years, much less than expected and shorter than that of bulk soil organic matter or many other  
21 carbon compounds [10-12]. Also, the capability to degrade lignin was demonstrated for several bacterial  
22 taxa in addition to fungi [13].

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

32 yielding little C and energy, lignin decomposition makes protein accessible to decomposers that is occluded<sup>4</sup>  
33 in plant cell walls, and that lignin decomposition is therefore not driven by C but by the N demand of the  
34 microbial community ("Nitrogen mining theory", [17]).

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

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

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

52 We addressed the following questions in our study:

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

57 (2) Are high lignin degradation rates related to a higher fungal activity? We hypothesized that wider  
58 C:N and C:P ratios favor lignin degradation by fungi while narrow C:N and C:P ratios favor carbohydrate  
59 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 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 soluble 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 [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 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 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<sup>-1</sup> d.w., 0.05 to 0.55 mg N g<sup>-1</sup> d.w. and 0.05 to 0.35 mg P g<sup>-1</sup> 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 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 correlated to resource stoichiometry:  $R = 0.53-0.64$ , all  $p < 0.002$ ), when the homeostatic regulation coefficients [22]  $H_{C:P} = 1.68$ ,  $H_{C:N} = 2.01$ , and  $H_{N:P} = 2.29$  were found. Microbial C:N ratios after 3 and 6 month were within a tightly constrained ranges (14.5:1 to 18.2:1 and 6.9:1 to 9.0:1, respectively), 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 of incubation (fig. 2).

Fungi:bacteria (F:B) 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, see fig. 3). The large differences in fungi:bacteria ratios between litter types decreased during decomposition. Fungal proteins abundance was 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 addition, fungi: bacteria ratios were measured on a DNA basis (qPCR) the results showing a similar pattern between litter types and harvests but with a much larger fungal DNA dominance (ratios between 10-180). Fungi:bacteria ratios were highly correlated between protein- and log-transformed DNA-based estimates ( $r=0.785$ ,  $p<0.001$ ).

## Pyrolysis-GC/MS and Lignin content

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

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

## Correlations between lignin and carbohydrate decomposition and litter chemistry, microbial community and decomposition processes

Relationships between lignin and carbohydrate degradation, litter chemistry, microbial biomass and decomposition processes were tested after 6 and 15 months (tables 5 and 6) including data presented by [21] and [23]. 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)<sup>8</sup>. 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 [14], 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,



175 differences in early lignin decomposition did not result from high or low lignin contents as is suggested by<sup>9</sup>  
176 traditional litter decomposition models. Low lignin decomposition rates were also not caused by a lack of Mn  
177 or Fe, the metals being important cofactors of oxidative lignin decay, which were suggested to be rate limiting  
178 during late lignin decomposition [1]. While Mn and Fe concentrations strongly varied between litter collected  
179 at different sites, Mn and Fe concentrations were lowest in the litter with highest lignin decomposition  
180 rates (AK, see Table 1). Low contents of these elements would explain decreased but not enhanced lignin  
181 decomposition. Moreover, soluble organic C was suggested to be limiting for lignin decomposition since the  
182 process of lignin decomposition does not generate enough energy for survival of lignin decomposers [14].  
183 Soluble organic C apparently did not control lignin decomposition since we found highest concentrations in  
184 the two litter types that showed the highest and the lowest lignin decomposition rates.

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

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

205 In order to decompose litter lignin and carbohydrates, microbial decomposers rely on the production and

206 excretion of hydrolytic and oxidative extracellular enzymes. While the absolute amounts, in which these<sup>10</sup>  
207 enzymes are produced, were largely controlled by N availability, the ratio in which they were produced  
208 was strongly related to differences in the ratio of cellulose/lignin decomposition. [20] suggested that lignin  
209 decomposition comprises a strategy of slow-growing microbes to evade competition through colonizing more  
210 lignin-rich and nutrient-poor substrates. Indeed we found lignin decomposition in low quality litter (low N  
211 and P) with microbial communities that were subject to large imbalances in C:N and C:P between resource  
212 and consumer, pointing to N and P limitation or high N and P uns efficiency of these communities. Low  
213 P availability may limit fast growth of microbial populations and select for slow-growing lignin-degrading  
214 microbes during early decomposition and provide K-strategists (slow growing on recalcitrant carbon) an  
215 advantage over r strategists (fast growing on labile carbon). Indeed we found that lignin decomposition was  
216 highest in litter, where resource C:P and N:P were highest, i.e. low P supply may have limited microbial  
217 growth generally or the establishment of r strategists in particular.

218 While the mode of negative P regulation on lignin decomposition remains unknown, we found differences  
219 in the composition of the microbial decomposer communities on litter with fast and slow lignin decomposition.  
220 Unlike predicted by ecological stoichiometry theory, not bacteria but fungi were more successful in colonizing  
221 high N and high P litter during initial decomposition. Fungi colonized litter faster than bacteria and therefore  
222 dominated early litter decomposition, however the fungi: bacteria ratios decreased over the entire incubation  
223 period pointing to increasing population sizes of bacteria with time. Fungi-rich communities more efficiently  
224 used high litter N to produce extracellular enzymes that degrade carbohydrates immediately after inoculation  
225 (fungi: bacteria ratios were correlated to litter N 14 days after inoculation) and high litter P to build up  
226 microbial biomass on a longer time scale (fungi: bacteria ratios were correlated to litter P after 6 months).  
227 Interestingly, low F:B communities (AK) were more active in decomposing lignin than those being dominated  
228 by fungi. This does not necessarily indicate that bacteria play the key role in lignin decomposition, though  
229 bacteria were also reported to produce oxidative enzymes that can decompose lignified materials in litter [13].  
230 However, decreases in fungi:bacteria ratios may be superimposed on the increase of smaller subpopulations  
231 of e.g. fungi that are key mediators of lignin decomposition, or alternatively general increases in the size  
232 of microbial communities with declining fungi/bacteria ratios may as well mask stable fungal populations  
233 when bacterial abundance increases. The fungal communities were dominated by Ascomycetes, with smaller  
234 contributions by Basidiomycetes. It is particularly the latter, Basidiomycetes, that catalyze the cellulolytic  
235 and lignolytic decomposition of dead plant material, however they comprised less than 5% of the fungal protein  
236 ensemble. The bacterial community in contrast was dominated by Proteobacteria (mainly  $\gamma$ , declining, and  $\alpha$ -

and  $\beta$ -Proteobacteria, increasing with litter decomposition), Actinobacteria and Bacteroidetes both of which strongly increased with time. Actinobacteria are also known as important decomposers of plant detritus, with the potential to excrete oxidative enzymes and being oligotrophic, and Bacteroidetes also excrete a broad range of hydrolytic enzymes targeting cellulose and other polymers. Since the metaproteomic approach did not find oxidative extracellular enzymes we so far cannot dissect the contributions of bacteria and fungi to the lignin decomposition process.

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

## Conclusions

Our results contradict the traditional concept that lignin decomposition is slow during early litter decomposition. While traditional litter decomposition models propose that lignin decomposition mainly occurs during late decomposition stages, we found that variable but in some cases substantial amounts of lignin were decomposed during the first 6 months. The extent to which lignin was decomposed was controlled by litter P during the first 6 months, but by litter N thereafter as was carbohydrate decomposition. Our results further question that recalcitrance is intrinsic to lignin as a chemical compound, but suggests that lignin decomposition also depends on litter chemistry and environmental conditions, which both affect microbial community structure including the abundance of fungal and bacterial groups that are key to decomposition 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 0.25cm<sup>2</sup>, homogenized, sterilized twice by  $\gamma$ -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 K<sub>2</sub>SO<sub>4</sub>. 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 inorganig P after persulfate digestion [28].

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). CO<sub>2</sub> 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  $\beta$ -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  $\mu$ l suspension were mixed with 25 nmol 4-methylumbelliferyl- $\beta$ -D-cellobioside (dissolved in 50  $\mu$ 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  $\mu$ 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 ma-

terials. 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 CO<sub>2</sub>-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.

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 Minnesota farm silage soil [33] and known contaminants, was performed using the MASCOT Search Engine. A detailed description of the extraction procedure and search criteria was published by [2]. If more than one protein was identified based on the same set of spectra these 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/>; [34]).

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) [35, 36]. This number allows relative comparison of protein abundances over different samples [37].

Quantitative PCR was used to determine fungal and bacterial abundance as described recently [38]. 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 [39]. 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.

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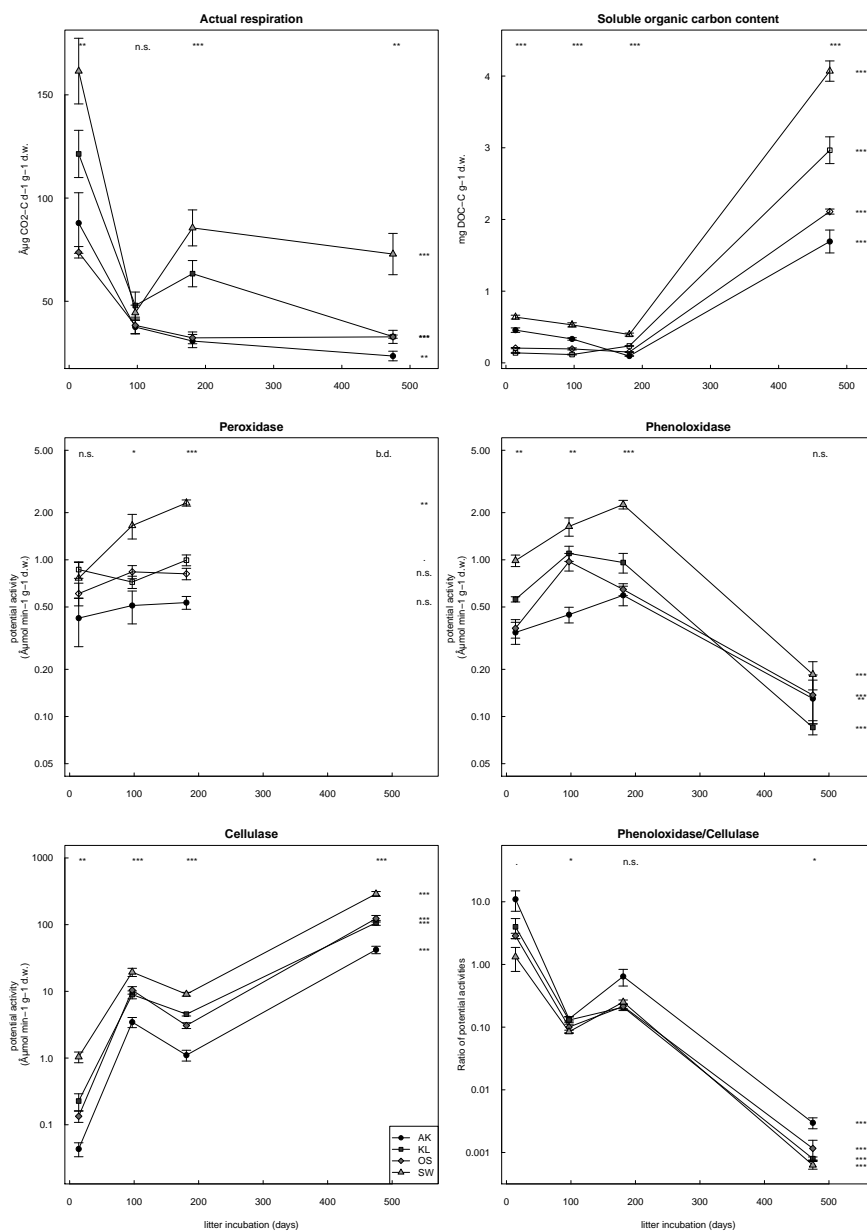
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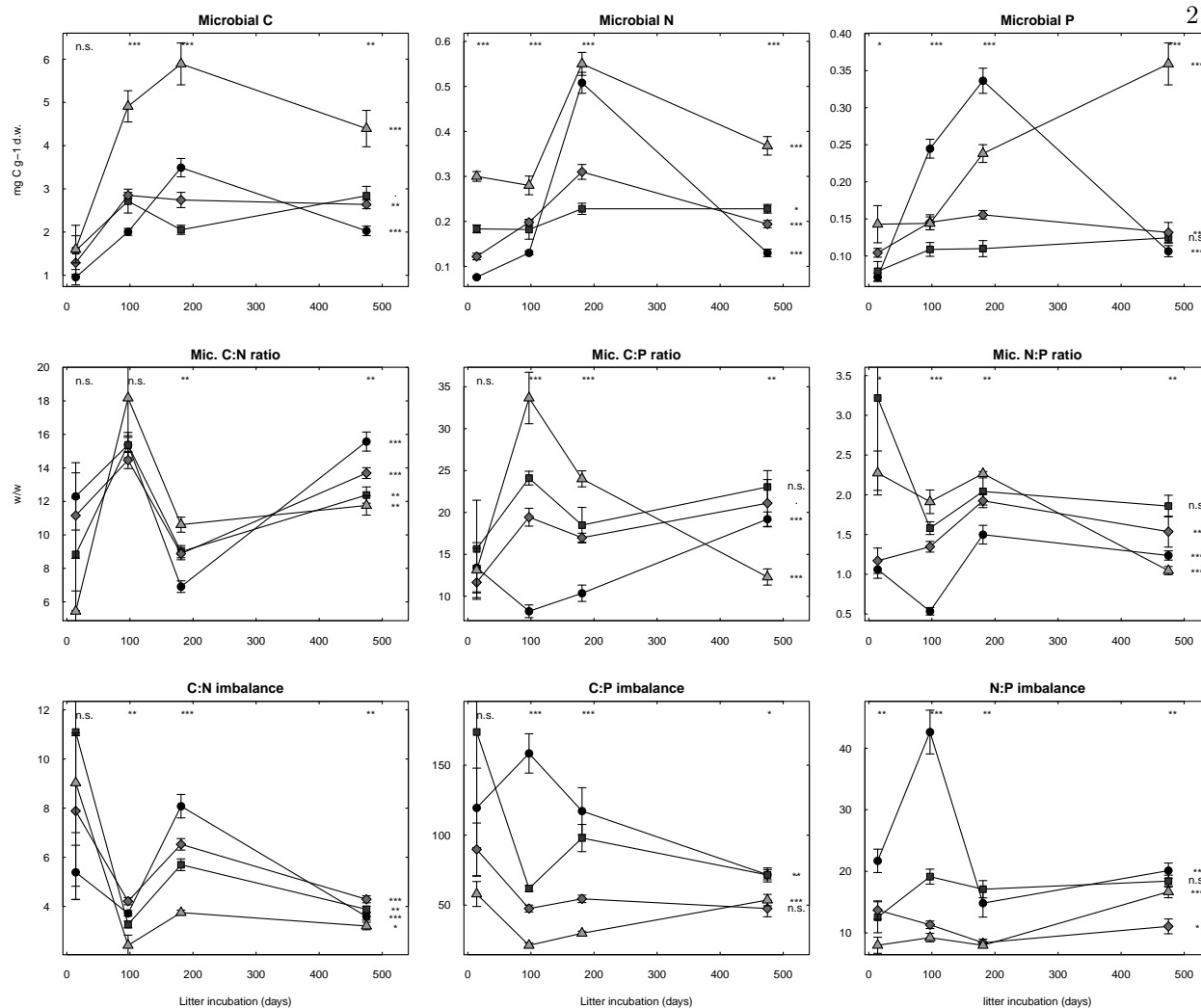
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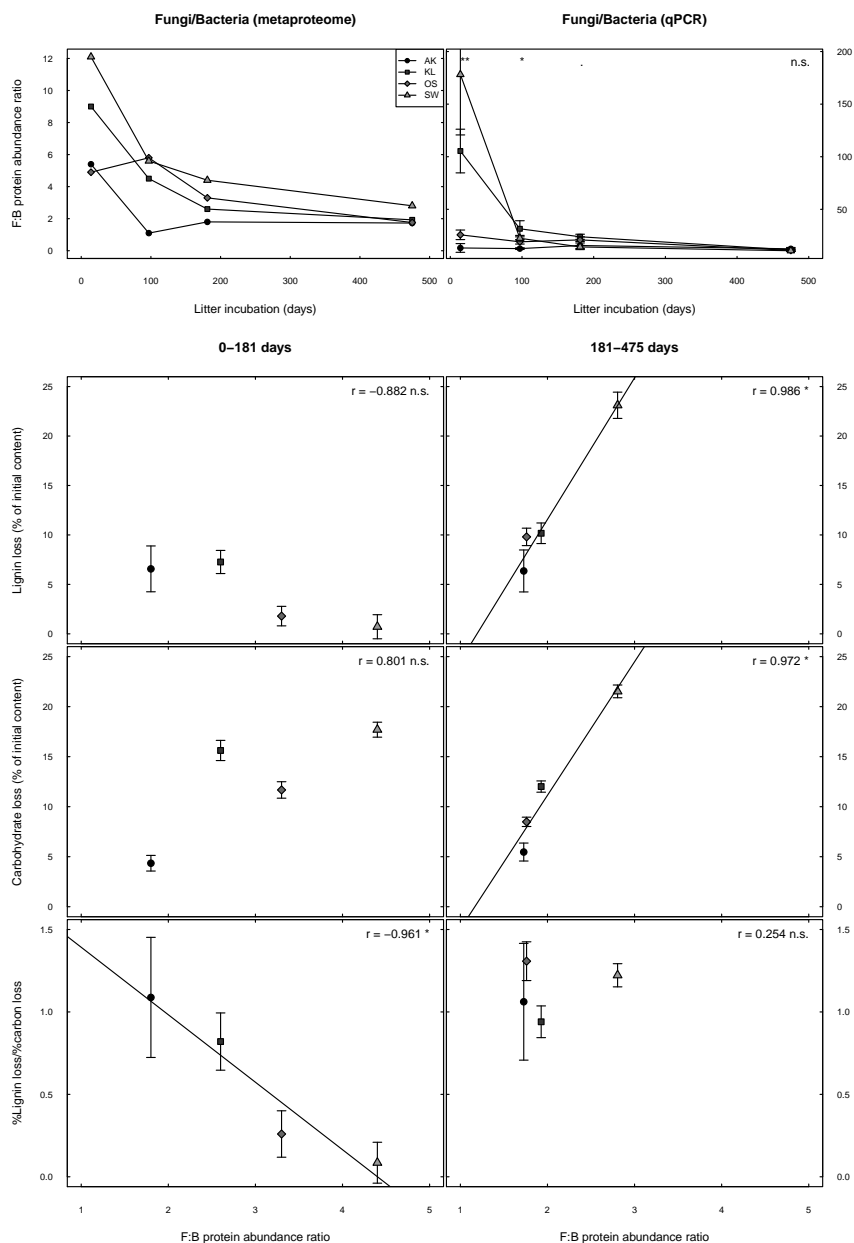




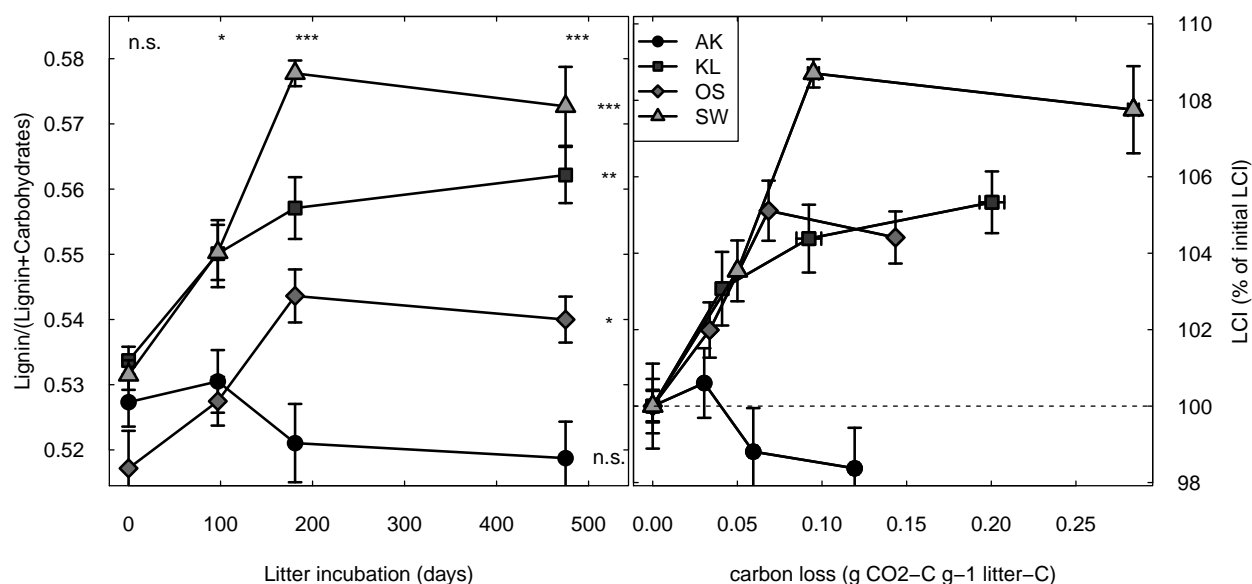
**Figure 1. Respiration rates, concentration of soluble organic C and potential extracellular enzyme activities** in decomposing beech leaf litter from a mesocosm experiment. Beech litter was collected in: triangles, Schottenwald (SW); diamonds, Ossiach (OS); squares, Klaus-leopoldsdorf (KL); circles,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



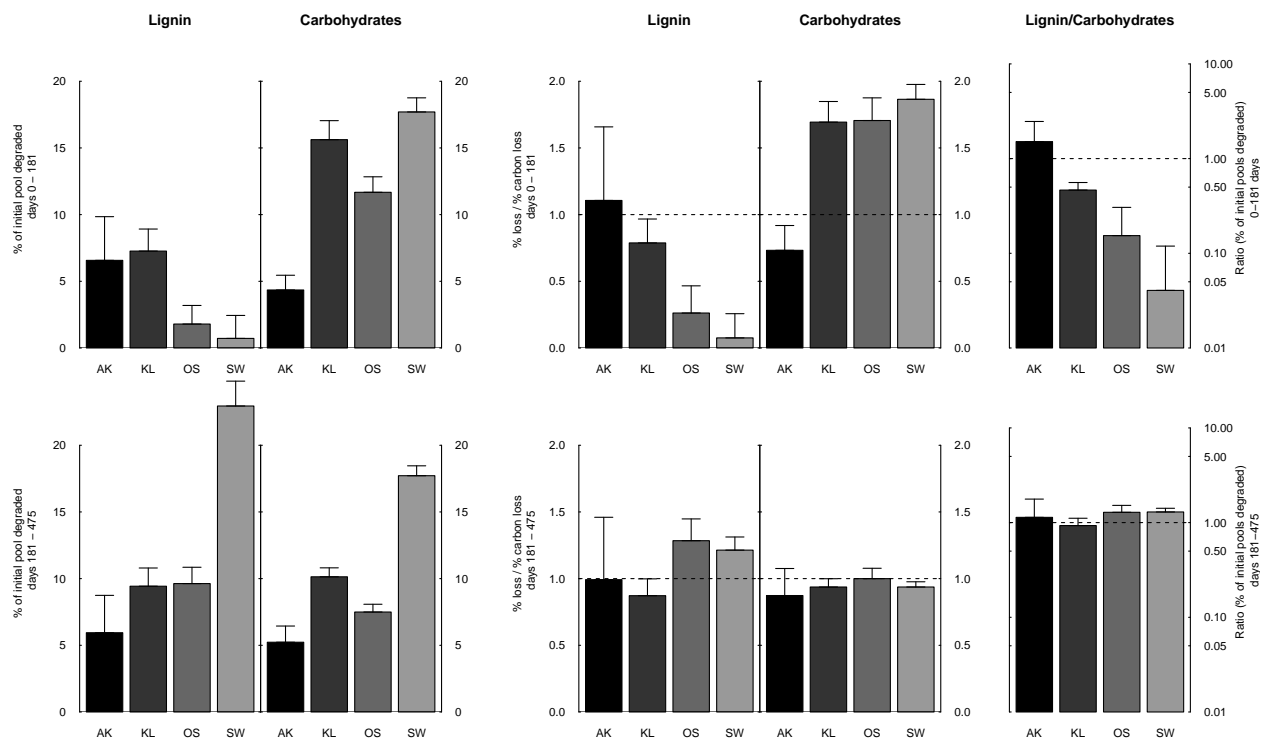
**Figure 2. Microbial biomass C, N and P, microbial C:N:P stoichiometry and resource/consumer stoichiometric imbalance in these elements in decomposing beech leaf litter from a mesocosm experiment.** Beech litter was collected in: triangles, Schottenwald (SW); diamonds, Ossiach (OS); squares, Klausenleopoldsdorf (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. Fungi:Bacteria (F:B) ratios and their correlations with LCI change:** Top: F:B protein abundance (left) and DNA (right) ratio. Bottom: Correlations between F:B pretein abundance ratios and Lignin loss (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. \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ .

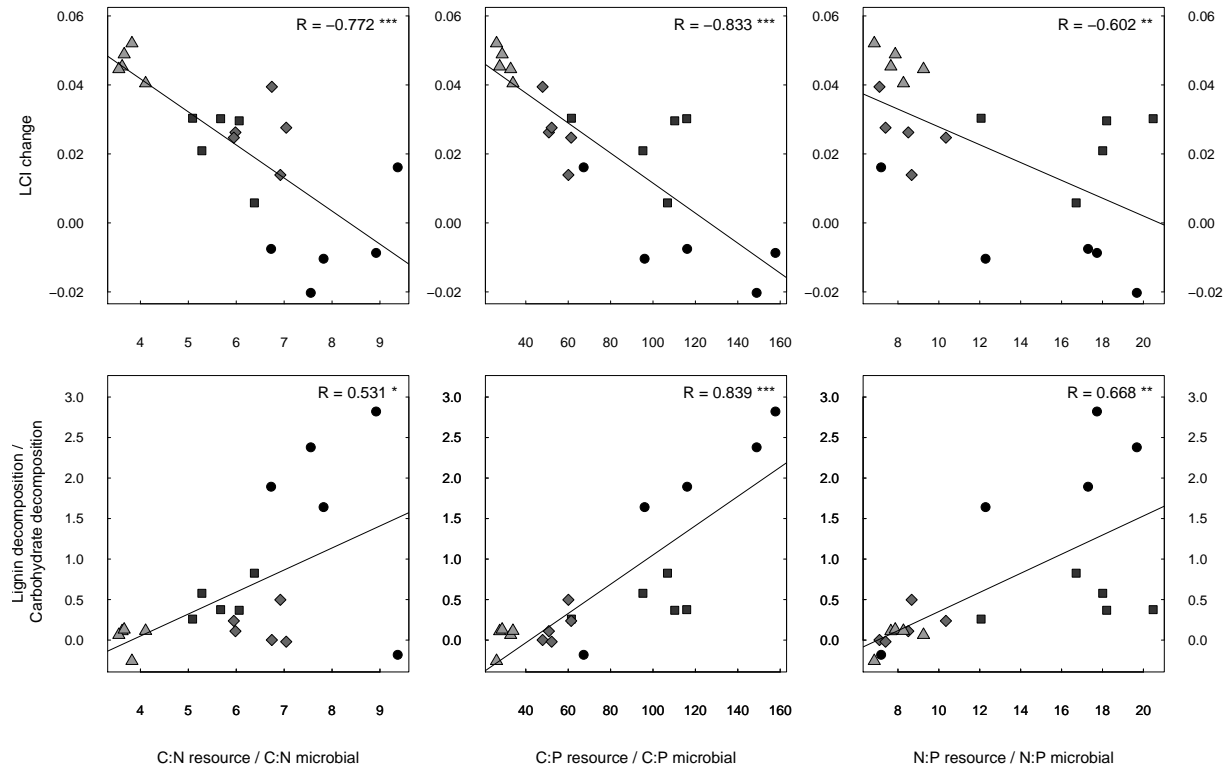


**Figure 4. Development of the LCI (lignin/(lignin+carbohydrates)) during time of beech litter decomposition (A) or plotted against cumulative C loss (B).** Errorbars indicate standard errors (n=4-5). The dashed line indicates a constant ratio between lignin and carbohydrates (i.e. no preferential decomposition 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 <sup>-1</sup> ) | 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 <sup>-1</sup> )      | 0.26  | (0.00)  | 0.54  | (0.00)  | 0.21  | (0.00)  | 0.55  | (0.00)  | <0.001  |
| Ca (mg g <sup>-1</sup> )     | 1.33  | (0.01)  | 1.26  | (0.01)  | 1.63  | (0.01)  | 1.23  | (0.01)  | <0.001  |
| Mg (mg g <sup>-1</sup> )     | 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 [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 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     |
|-------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| 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         | <b>-0.723</b> | <b>0.606</b>  | -0.0822       | <b>0.771</b>  | -0.195        | <b>0.594</b>  | -0.368        | -0.268        | -0.362        |
| Accumulated Respiration       | <b>0.494</b>  | <b>-0.704</b> | <b>0.688</b>  | -0.132        | <b>0.856</b>  | -0.332        | <b>0.557</b>  | <b>-0.525</b> | <b>-0.506</b> | <b>-0.534</b> |
| Cellulase activity            | <b>0.657</b>  | <b>-0.76</b>  | <b>0.803</b>  | -0.431        | <b>0.801</b>  | <b>-0.497</b> | <b>0.664</b>  | <b>-0.589</b> | -0.436        | <b>-0.539</b> |
| 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         | <b>-0.749</b> | <b>0.663</b>  | -0.17         | <b>0.795</b>  | -0.312        | <b>0.677</b>  | <b>-0.559</b> | <b>-0.49</b>  | <b>-0.607</b> |
| Phosphatase activity          | <b>0.549</b>  | <b>-0.813</b> | <b>0.776</b>  | -0.302        | <b>0.851</b>  | -0.407        | <b>0.702</b>  | <b>-0.556</b> | -0.418        | <b>-0.522</b> |
| Phenoxidase activity          | <b>0.632</b>  | <b>-0.669</b> | <b>0.737</b>  | -0.415        | <b>0.719</b>  | <b>-0.449</b> | <b>0.552</b>  | <b>-0.484</b> | -0.305        | -0.356        |
| Peroxidase activity           | <b>0.599</b>  | <b>-0.588</b> | <b>0.677</b>  | -0.412        | <b>0.639</b>  | -0.438        | <b>0.47</b>   | -0.435        | -0.173        | -0.302        |
| N mineralization              | <b>0.466</b>  | <b>-0.664</b> | <b>0.65</b>   | -0.167        | <b>0.739</b>  | -0.299        | <b>0.527</b>  | -0.387        | -0.282        | -0.367        |
| Nitrification                 | <b>0.587</b>  | <b>-0.707</b> | <b>0.732</b>  | -0.38         | <b>0.74</b>   | -0.432        | <b>0.621</b>  | <b>-0.499</b> | -0.369        | -0.45         |
| P mineralization              | <b>0.665</b>  | <b>-0.55</b>  | <b>0.684</b>  | <b>-0.544</b> | <b>0.596</b>  | <b>-0.576</b> | 0.414         | <b>-0.478</b> | -0.212        | -0.255        |
| C litter                      | <b>-0.545</b> | <b>0.506</b>  | <b>-0.578</b> | <b>0.604</b>  | -0.368        | <b>0.643</b>  | <b>-0.618</b> | <b>0.698</b>  | <b>0.525</b>  | <b>0.581</b>  |
| extractable C                 | <b>0.609</b>  | <b>-0.766</b> | <b>0.782</b>  | -0.37         | <b>0.814</b>  | <b>-0.446</b> | <b>0.658</b>  | <b>-0.54</b>  | -0.392        | <b>-0.484</b> |
| N litter                      | 0.354         | <b>-0.517</b> | <b>0.503</b>  | -0.14         | <b>0.587</b>  | -0.187        | 0.366         | -0.203        | -0.119        | -0.159        |
| P litter                      | <b>0.682</b>  | -0.222        | <b>0.517</b>  | <b>-0.747</b> | 0.175         | <b>-0.68</b>  | 0.188         | <b>-0.491</b> | -0.0728       | -0.16         |
| C:N litter                    | -0.405        | <b>0.586</b>  | <b>-0.57</b>  | 0.175         | <b>-0.654</b> | 0.234         | -0.44         | 0.273         | 0.195         | 0.242         |
| C:P litter                    | <b>-0.636</b> | 0.174         | <b>-0.453</b> | <b>0.754</b>  | -0.0823       | <b>0.649</b>  | -0.176        | 0.418         | 0.049         | 0.0805        |
| N:P litter                    | <b>-0.512</b> | -0.0287       | -0.264        | <b>0.714</b>  | 0.147         | <b>0.577</b>  | -0.0202       | 0.316         | -0.0316       | -0.0192       |
| C:N mic                       | <b>0.666</b>  | <b>-0.758</b> | <b>0.799</b>  | -0.43         | <b>0.798</b>  | <b>-0.515</b> | <b>0.678</b>  | <b>-0.609</b> | <b>-0.584</b> | <b>-0.596</b> |
| C:P mic                       | <b>0.692</b>  | <b>-0.787</b> | <b>0.834</b>  | <b>-0.476</b> | <b>0.814</b>  | <b>-0.562</b> | <b>0.726</b>  | <b>-0.672</b> | <b>-0.564</b> | <b>-0.648</b> |
| N:P mic                       | <b>0.582</b>  | <b>-0.729</b> | <b>0.74</b>   | -0.415        | <b>0.729</b>  | <b>-0.508</b> | <b>0.715</b>  | <b>-0.67</b>  | <b>-0.545</b> | <b>-0.671</b> |
| C:N imbalance                 | <b>-0.56</b>  | <b>0.81</b>   | <b>-0.772</b> | 0.288         | <b>-0.859</b> | 0.391         | <b>-0.71</b>  | <b>0.531</b>  | <b>0.564</b>  | <b>0.56</b>   |
| C:P imbalance                 | <b>-0.817</b> | <b>0.663</b>  | <b>-0.833</b> | <b>0.757</b>  | <b>-0.61</b>  | <b>0.799</b>  | <b>-0.668</b> | <b>0.839</b>  | <b>0.575</b>  | <b>0.67</b>   |
| N:P imbalance                 | <b>-0.724</b> | 0.351         | <b>-0.602</b> | <b>0.81</b>   | -0.253        | <b>0.764</b>  | -0.397        | <b>0.668</b>  | 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) | <b>0.998</b>  | -0.854        | <b>0.958</b>  | -0.882        | 0.801         | <b>-0.961</b> | 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 - 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    | <b>0.582</b>  | <b>0.708</b>  | 0.00521  | 0.279        | -0.137   | -0.444        | 0.403         |
| Actual respiration            | -0.0114      | 0.244        | -0.212   | <b>0.86</b>   | <b>0.856</b>  | 0.122    | 0.192        | -0.0444  | -0.403        | 0.29          |
| Accumulated Respiration       | 0.283        | 0.354        | -0.00931 | <b>0.852</b>  | <b>0.968</b>  | 0.0149   | 0.298        | -0.177   | <b>-0.608</b> | <b>0.486</b>  |
| Cellulase activity            | 0.0733       | 0.218        | -0.137   | <b>0.848</b>  | <b>0.881</b>  | 0.148    | 0.295        | -0.0811  | <b>-0.575</b> | 0.414         |
| Protease activity             | 0.00361      | 0.0538       | -0.086   | 0.448         | 0.455         | 0.16     | 0.316        | -0.11    | <b>-0.456</b> | 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  | <b>0.643</b>  | <b>0.671</b>  | 0.167    | 0.253        | -0.0289  | <b>-0.58</b>  | 0.395         |
| Phenoloxidase activity        | 0.319        | -0.389       | 0.436    | -0.248        | -0.0034       | -0.221   | <b>0.505</b> | -0.443   | <b>-0.483</b> | <b>0.692</b>  |
| Peroxidase activity           | -0.277       | 0.379        | -0.385   | 0.173         | -0.0488       | 0.16     | <b>-0.51</b> | 0.382    | <b>0.546</b>  | <b>-0.708</b> |
| 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      | <b>0.567</b> | -0.32    | <b>0.63</b>   | <b>0.567</b>  | 0.0904   | -0.148       | 0.114    | -0.105        | -0.0234       |
| P mineralization              | -0.0165      | 0.202        | -0.138   | <b>0.507</b>  | <b>0.508</b>  | -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   | <b>0.501</b>  | -0.348        |
| extractable C                 | 0.231        | 0.435        | -0.0861  | <b>0.828</b>  | <b>0.89</b>   | 0.074    | 0.218        | -0.109   | <b>-0.538</b> | 0.409         |
| N litter                      | 0.21         | 0.356        | -0.0654  | <b>0.816</b>  | <b>0.896</b>  | -0.00431 | 0.172        | -0.12    | -0.431        | 0.349         |
| P litter                      | -0.117       | -0.037       | -0.182   | <b>0.764</b>  | <b>0.762</b>  | 0.161    | 0.318        | -0.0746  | <b>-0.464</b> | 0.325         |
| C:N litter                    | -0.272       | -0.365       | 0.0158   | <b>-0.794</b> | <b>-0.901</b> | 0.027    | -0.207       | 0.155    | <b>0.49</b>   | -0.404        |
| C:P litter                    | 0.329        | 0.122        | 0.315    | <b>-0.645</b> | <b>-0.541</b> | -0.276   | -0.218       | -0.0672  | 0.283         | -0.162        |
| N:P litter                    | <b>0.471</b> | 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   | <b>-0.658</b> | <b>-0.703</b> | -0.0319  | -0.318       | 0.25     | <b>0.57</b>   | <b>-0.513</b> |
| C:P mic                       | 0.237        | -0.06        | 0.312    | <b>-0.609</b> | <b>-0.505</b> | -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   | <b>0.986</b>  | <b>0.972</b>  | 0.254    | 0.484        | -0.274   | -0.601        | 0.55          |