

# Nutrient content controls onset of lignin decomposition in Beech litter

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## Abstract

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.

# Introduction

Plant litter is quantitatively dominated by macromolecular compounds. In foliar litter, lignin and carbohydrate polymers like cellulose together make up 40-60% of litter dry mass [1], while leachable substances account for only 1.5-6% [2]. The breakdown of high molecular weight compounds into smaller molecules that are accessible to microbes is mediated by extracellular enzymes and is considered to be rate limiting for decomposition [3].

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

Recent studies based on more specific methods to determine litter lignin contents question the intrinsic recalcitrance of lignin. Isotope labeling experiments with soils and litter/soil mixtures, undertaken both in-situ and under controlled conditions, revealed mean residence times of lignin in soils in the range of 10-50 years, much less than expected and shorter than that of bulk soil organic matter [11–13]. While the ability to completely degrade lignin was traditionally attributed exclusively to Basidiomycetes, it has been demonstrated for several bacterial taxa over the last years [14].

For leaf litter, lignin decomposition even at early stages of litter decay and lignin decomposition rates that decreased during decomposition were recently reported by Klotzbücher and colleagues [15]. They proposed that lignin decomposition is limited by labile C sources and that therefore fastest lignin degradation occurs during early litter decomposition.

Additionally, the decomposition of lignin may also be dependent on the nutrient content of the litter and thus the nutritional status of the microbial community. During radical polymerization, significant amounts of cellulose and protein are incorporated into lignin structures [16]. In isolated lignin fractions from fresh beech litter, N contents twice as high as in bulk litter were found [17]. It was therefore argued that, while

yielding little C and energy, lignin decomposition makes protein accessible to decomposers that is occluded in plant cell walls, and that lignin decomposition is therefore not driven by C but by the N demand of the microbial community ("Nitrogen mining theory", [18]). 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 [19] and decreased the activity of lignolytic enzymes in forest soils [3]. Incubation experiments with soil-litter mixtures showed that N fertilization led to a decrease in the mineralization of complex soil carbon, while no such effect was found after P fertilization [18]. This is explained because soil P is protected by inorganic mechanisms rather than incorporation into humic substances, however, no data is available whether this is also the case in decomposing plant litter.

It was recently been shown that addition of N has a different effect on litter decomposition than varying N levels in litter [20]. This is due to the fact that leaf litter N is stored in protein and lignin structures and not directly available to microorganisms, while fertilizer N is added in the form of readily available inorganic N (ammonium, nitrate or urea). A similar effect has to be expected for P. Fertilization experiments can thus simulate increased nutrient deposition but not the effect of litter nutrient contents on decomposition processes.

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

With the experiment, we addressed the following questions:

(Q1) Is lignin decomposition delayed until late decomposition stages or are significant amounts of lignin already degraded during early litter decomposition, and does the timing of lignin decomposition depend 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.

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

degradation by bacteria.

## Results

### Initial litter chemistry

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

### Mass loss, respiration and soluble organic carbon

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

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

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

14 days ( $r=0.69$ ,  $p<0.001$ ,  $n=20$ ) and after 3 months ( $r = 0.65$ ,  $p<0.01$ ,  $n=20$ ), but strictly correlated after 6 months ( $r=0.85$ ,  $p<0.001$ ,  $n=20$ ) and 15 months ( $r=0.90$ ,  $p<0.001$ ,  $n=20$ ).

### Potential enzyme activities

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

### Microbial biomass abundance and community composition

Microbial biomass contents ranged from 0.5 to 6 mg C g<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). After an initial increase in microbial biomass, in KL and OS microbial biomass remained constant after 3 months while AK and SW showed further accumulation of microbial biomass which reaches a maximum of microbial C and N contents after 6 months (AK also for P). Microbial C:N ratios ranged between 6:1 and 18:1, C:P ratios between 8:1 and 35:1, and N:P ratios between 0.5:1 and 3.5:1 (fig. 2). The differences between microbial and litter stoichiometry led to an accumulation of substantial amounts of P (up to 80% of the total litter P in AK after 6 months). In AK and KL insoluble P was rapidly incorporated into the microbial biomass, while soluble P contents increased (ie. insoluble P was mobilized). In contrast, microbial P contents pools increased more slowly in sites that did degraded little or no lignin (OS and SW), and microbial P was taken up from a shrinking soluble P pool, ie. not from insoluble P (fig 1).

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

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<sup>1</sup>prepare final figure

months and 6.9:1 to 9.0:1 after 6 months, but significantly different between the two sampling events. In contrast, microbial C:P and N:P ratios were less constrained, with the highest variance between litter from different sites after 3 months of incubation (fig. 2).

Metaproteome analysis yielded between 451 and 1113 (average 639) assigned spectra per sample (one replicate per collection site after 14 days, 3, 6, and 15 months). For community profiling only spectra assigned to bacteria or fungi were used. Fungal proteins were dominant in all litter types at all stages, but most prominent in SW and least pronounced in AK. Fungi:bacteria (F:B) protein abundance ratios were highest after 14 days (5 to 12) and decreased during litter decomposition (1.7 to 3 after 15 months, see fig. 4). The large initial differences in F:B ratios between litter from different sites decreased during decomposition. In addition, F:B ratios were measured on a DNA basis (qPCR) the results showing a similar pattern but with a much larger fungal DNA dominance (F:B ratios between 10-180). F:B ratios were highly correlated between protein- and log-transformed DNA-based estimates ( $r=0.785$ ,  $p<0.001$ ,  $n=20$ ).

Fungal communities were dominated by Ascomycota, with smaller contributions by Basidiomycota (<5% of fungal protein). Among the fungal classes found, Sordariomycetes and Eurotiomycetes were most abundant with further contributions of Dothideomycetes, Leothiomycetes and Saccharomycetes (fig. 3). Bacteria were dominated by Proteobacteria (mainly  $\gamma$ , declining, and  $\alpha$ - and  $\beta$ -Proteobacteria, increasing with litter decomposition) with minor contribution of Actinomycetes and Bacterioidetes (both increasing) and Thermotogae (decreasing, fig. 3).

## Pyrolysis-GC/MS and Lignin content

In total 128 pyrolysis products were detected, quantified, identified and assigned to their substances of origin (suppl. tab. ??-??). 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 %. The increase occurred almost exclusively during the first 6 months. Carbohydrate-derived pyrolysis products accounted for 26 to 29 % in initial litter and decreased by up to 2.6 % within 15 months of incubation. The initial (pyrolysis-based) lignin:carbohydrate indices (LCI) were highly similar between litter from different collection sites, ranging between 0.517 and 0.533 (Fig. 4). During decomposition, the LCI increased by up to 9 % of the initial value. The highest increase was found in SW litter, while LCI slightly decreased in AK litter.

All significant changes in LCI occurred within the first 6 months (fig. 5). As differences in lignin and carbohydrate contents between 0-3 and 3-6 months were not significant, we analyzed differences for two time intervals, i.e. between 0-6 months and 6-15 months.

During the first 6 months, between one and 6 % of the initial lignin pool and between 4 and 17% of the initial carbohydrate pool were degraded (Fig. 6). Lignin decomposition was highest in AK and KL litter, while microbial communities of KL, OS and SW litter decomposed carbohydrates faster. 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. 6). Between 6 and 15 months, no further accumulation of lignin occurred. Lignin and carbohydrates were both degraded at the same rate and their relative concentrations remained constant between 6 and 15 months (fig. 6).

### **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 2 and 3) 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 ( $R=0.599$ ,  $p=0.005$ ,  $n=20$ ) and peroxidase : cellulase ( $R=0.734$ ,  $p<0.001$ ,  $n=20$ ). 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. 7). The best correlation of lignin : carbohydrates degradation rates and LCI were found with both  $C:P_{litter} : C:P_{microbial}$  and  $C:N_{litter} : C:N_{microbial}$ . However, the two stoichiometric ratios were also intercorrelated, as were microbial  $C:N_{microbial}$  and  $C:P_{microbial}$ , probably due to more narrow C:N and C:P ratios of bacterial than fungal biomass. Between 6 and 15 months, the ratio of lignin : carbohydrate decomposition was no longer related to stoichiometry or elemental composition any more. During this later period, lignin and carbohydrate decomposition exhibited the same controls, being positively correlated to soluble organic C, litter N and litter P (table 3) between 6 and 15 months. Mass loss and accumulated respiration were positively correlated to lignin and carbohydrate decomposition (table 3), a pattern that we did not find for lignin decomposition in the early decomposition phase (table 2). Protein abundance F:B



ratios were negatively correlated to the ratios of lignin : cellulose decomposition and to LCI change during the first 6 months, pointing to bacterial engagement in lignin decomposition. In contrast, both lignin and carbohydrate decomposition rates, were positively correlated with F:B ratios after 15 months, pointing to fungal dominance of both lignin and carbohydrate decomposition. No correlation between F:B ratio and the ratio of lignin : cellulose decomposition was found in this later period (fig. 4).

To assess the interaction between litter chemistry, microbial community and degradation processes, we conducted a correspondence analysis (CA) of the metaproteome data (relative protein abundances, fig. 8). The results indicate that incubation time (i.e. succession) is the dominant factor controlling the microbial community, with samples collected at the first (14 days) and the last (15 month) sampling grouping closely together, while litter quality (i.e. elemental stoichiometry of litter collected at different sites) had a higher impact after 3 and 6 months. The first factor (CA 1), which explained 35.7 % of the total variance, separates litter sampled after 15 months (positive values) from litter sampled earlier (negative values). Consequently, CA 1 was also positively correlated to incubation time and negatively to litter C content (i.e. decreasing C:N ratios during decomposition). A number of bacterial taxa (Actinobacteria, Bacteroidetes,  $\alpha$ - and  $\beta$ -proteobacteria), and two fungal classes (Leotiomyces and Tremellomyces) were positively correlated to CA1 i.e. increased in abundance towards 15 months, while Cyanobacteria,  $\epsilon$ -proteobacteria and Saccharomycetes were negatively correlated. CA 2, which explained 26.0 % variance, separated litter sampled within the first 6 months. Dothideomycetes and Sordariomycetes were positively and  $\gamma$ -proteobacteria negatively correlated to this factor, which also correlated to the F:B protein abundance ratio. Litter collected 14 days after inoculation have the highest scores on CA 2, while sites with active lignin degradation within the first 6 months (AK, KL) have the most negative scores. The axis was furthermore correlated to the microbial biomass P content and C:P and N:P imbalances (and free  $\text{NH}_4^+$ , not shown). For samples analyzed after 6 months, where direct comparison to lignin degradation rates was possible, significant correlations to CA 2 were found for lignin : carbohydrate degradation ( $r=-0.97$ ,  $p=0.028$ ), % Lignin loss : % Carbon loss ( $r=-0.96$ ,  $p=0.040$ ) and LCI increase ( $r=0.973$ ,  $p=0.027$ ), even though the number of independent samples was very low ( $n=4$ ). Differences in CA2 strongly decreased after 15 months, suggesting that the differences in the microbial community found within the first 6 months were diminished with succession of the decomposer community. Litter N and P contents were not correlated to either factor, although differences in resource quality evidently affected community composition after 3 and 6 months, as can be seen in the differences in the microbial communities as observed in CA 2. Correlation of CA factors with litter stoichiometry, and microbial stoichiometry, and the abundance of the analyzed taxa are provided in supplemental table 4.

## Discussion

We used beech litter collected at different sites to study the effect of litter quality on decomposition. Differences in litter quality led to different mass loss rates and the development of different decomposer communities from the same inoculate. Lignin decomposition was highly variable between litter of different quality within the first 6 month; lignin decomposition rates ranged from no detectable degradation (SW litter) to decomposition at bulk carbon loss rates (ie. no lignin accumulation, AK litter). Our results therefore provide further evidence that the use of extractive methods to measure lignin contents led to an underestimation of early lignin degradation rates, as recently suggested [15], and that substantial amounts of lignin can be degraded during early decomposition. In contrast, between 6 and 15 months, lignin was degraded at the same rate as bulk carbon in all litter, regardless of litter quality. During this time, the different lignin contents acquired within the first six months remained in place, but lignin contents no further increased.

Our study was designed to investigate the effect of litter N and P contents on decomposition, and we chose our collection sites to provide litter with different N and P contents. We found positive correlations between litter N and bulk decomposition parameters like carbon mineralization rates and extracellular enzymatic activities, indicating litter decomposition was limited by litter N. No such correlation was found for litter P contents. However, AK litter, which had low contents in both N and P, had a lower C mineralization rate than KL and OS, which were had lower contents of N or P, respectively, than AK, suggesting a co-limitation of both elements. The use of litter of a single species from different sites minimized differences in other litter traits, and eg. initial carbohydrate and lignin contents of all samples fell in a narrow range for litter from all sites. However, litter N and P contents are also proxies for other litter traits not directly measurable (e.g. leaf morphology), which resulted from the plant's response to nutrient availability (e.g. for low P adaptation see [24]). N and P were also demonstrated to be correlated to a wide area of leaf traits in plants (ref<sup>2</sup>), and such leaf traits were successfully used to predict litter decomposability in the past [25].

The composition of the microbial community changed with both by time (i.e. succession) and collection site (i.e. litter quality). While all samples measured were dominated by fungi, fungi:bacteria ratios decreased over time and were higher in nutrient-rich litter than in nutrient-poor litter (SW ■ AK). Our results contradicted the often-cited predictions that higher N and P contents would favor bacterial over fungal growth because bacterial biomass has lower C:N and C:P ratios than fungal biomass [26]. In contrast, we found fungi : bacteria ratios were higher in nutrient-rich litter. Similar observations were reported by GÃijsewell and

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<sup>2</sup>good ref for leaf economy spectrum needed!

Gessner 2009, who suggested that bacteria compensate N deficiency by heterotrophic N fixation, and therefore colonize low-N litter more successfully. However, microbial decomposers excrete important amounts of N as extracellular enzymes, what further raise their N demand; a factor not represented in the biomass C:N ratios. The higher abundance of bacteria on low nutrient litter would also be explained if fungi produced more extracellular enzymes per biomass than bacteria, therefore have creating a more narrow C:N demand than bacteria even though their biomass has a wider C:N ratio. In result, bacteria-rich decomposers with more narrow C:N and C:P biomass developed on low-nutrient sites, with more narrow C:N and C:P ratios, further increasing difference between microbial and litter stoichiometry. To consider both litter and microbial stoichiometry, we used  $C:X_{litter} : C:X_{consumer}$  ratios as integrated measure for nutrient availability to nutrient demand.

Litter quality controlled the composition of the microbial communities, and thereby the difference between litter and microbial stoichiometry, both of which were correlated to the extend of early lignin decomposition. Litter which accumulated more lignin had microbial communities with high fungi:bacteria ratios and lower differences between litter and microbial stoichiometry. Our results therefore indicate that lignin degradation is associated with bacteria-rich communities, a low availability of nutrients to decomposers, or both.

Traditionally, the capability to completely degrade lignin was exclusively attributed to Basidiomycota fungi [1]. However, Basidiomycota made up less than 5% of fungal protein. Over the last years, the capability to completely degrade lignin was demonstrated for several bacterial taxa (eg. actinomycetes,  $\alpha$ -, and  $\gamma$ -proteobacteria [14]). Of these three taxa, we found one ( $\gamma$ -proteobacteria) correlated to lignolytic activities after 3 and 6 months. The other two taxa (actinomycetes and  $\alpha$ -proteobacteria) were enriched after 15 months in all litter types, when lignin decomposition was found in litter from all sites (ie. independent from litter quality). However, since our metaproteomic analysis only sporadically detected lignolytic enzymes, we can not attribute lignolytic activities to specific taxa at this time. Nevertheless, our data indicates that corresponding trends for lignin degradation and fungal : bacterial protein abundance both along succession and between litter from different sites after 6 months (higher lignolytic activity at low fungi:bacteria ratios). Overall, lignin decomposition was not coupled to fungal abundance, hinting that bacterial contributions to lignin decomposition might be greater than traditionally assumed. Also, the activity of  $\gamma$ -proteobacteria deserves special attention in further studies on early litter decomposition.

Lignin degradation in the first 6 months was positively  $C:P_{litter} : C:P_{microbial}$  and  $C:N_{litter} : C:N_{microbial}$ , ie. more lignin was degraded when the difference between litter and microbial stoichiometry was higher. Dif-

ferentiating between the effects of these two stoichiometric ratios was difficult, since both were intercorrelated. Lignin decomposition rates were also negatively correlated to litter P, but not N contents and positively correlated to microbial biomass P contents, while carbohydrate decomposition was positively correlated to litter N. This would indicate a differential control of lignin (inhibited by P) and carbohydrate (stimulated by N) decomposition. Also, actively lignin degrading litter (AK and KL) net-mobilized insoluble litter P during this time, while in slowly lignin degrading litter (SW and OS) an accumulation of microbial P led to a decrease in soluble P. This suggests that lignin degradation increased the mobilisation of P from insoluble litter biomass into the rapidly recycled P fractions (biomass and dissolved), and would explain higher lignin degradation rates in litter with high microbial P demand. However, such a nutrient mobilization by lignin degradation is commonly assumed for N, but not for P [18]: Lignin (like humic compounds) does not provide sufficient C and energy to sustain the decomposers' metabolism, but occludes important amounts of protein during polymerization [16], which is available only after lignin degradation. Therefore, lignin degradation was proposed to constitute a strategy of N sequestration (N mining theory, [?, 18]).

The degradation of lignin and carbohydrate polymers depends on the excretion of different extracellular enzymes. Their production is N intensive, therefore a trade-off exists between the production of cellulolytic and lignolytic enzymes [3]. Lignin decomposition was also suggested to allow decomposers direct competition by the early colonization of lignin rich sites [27]. We found higher activities of both cellulolytic and lignolytic enzymes in N-rich litter, but their ratio was well correlated to lignin : carbohydrate decomposition. Lignin degradation yields less C and energy than carbohydrates degradation, but might provide additional N. When  $C:X_{substrate} : C:X_{consumer}$  is low, as it was the case for lignin degrading litter, additional carbon can not be used by decomposers to build up biomass. Therefore, the observed increase in lignolytic activities might result from a microbial strategy to optimize N allocation between cellulolytic and lignolytic enzyme systems when additional C can not be utilized by the decomposer due to a lack of nutrients.

In summary, litter quality had profound influence on the litter decomposition process, including community decomposition and lignin accumulation. Lignin decomposition within the first six months was highly variable between litter of the same species but different in nutrient contents, and ranged from no lignin degradation to lignin degradation at bulk litter C loss rates. It was associated with nutrient-rich microbial biomass on low-nutrient litter, i.e. a high difference between litter and decomposer stoichiometry, low fungi:bacteria ratios and an elevated abundance of  $\gamma$ -proteobacteria peptides. Differences in lignin contents acquired within the first six months remained in place during later decomposition, although - regardless of litter quality - lignin was not further accumulated between 6 and 15 months.

## Material and methods

### Litter decomposition experiment

Beech litter was collected at four different sites in Austria (Achenkirch (AK), Klausenleopoldsdorf (KL), Ossiach (OS), and Schottenwald (SW); referred to as litter types) in October 2008. Litter was cut to pieces of approximately 0.25cm<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 [28].

### 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 [29]). 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 [30]. 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, Schimadzu), P was determined photometrically as inorganig P after persulfate digestion [31].

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.

## Microbial Respiration

Respiration was monitored weekly during the entire incubation in mesocosms removed after 6 month and on the last incubation day for all mesocosms using an infrared gas analyzer (IRGA, EGM4 with SRC1, PPSystems, USA). 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<sub>2</sub>O<sub>2</sub> 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 [32].

## 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 supplementary tables nn-nn<sup>3</sup> 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 [33,34]. 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 the % lignin and cellulose degraded during decomposition ( $L_{\%loss}$  and  $Ch_{\%loss}$ ) according to equation 3, where  $X_{init}$  and  $X_{act}$  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.

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

Furthermore, we calculated the % lignin or carbohydrates lost per % carbon respired ( $L_{resp}$  and  $Ch_{resp}$ )

$$X_{resp} = \frac{X_{\%loss}}{100 \cdot R_{acc}} \quad (4)$$

## Metaproteome analysis and quantitative PCR

From each harvest (14, 97, 181, and 475 days), one replicate per litter type was stored at -80°C for metaproteome analysis. 3 g of each sample were grounded in liquid nitrogen and extracted with Tris/KOH buffer (pH 7.0) containing 1% SDS. Samples were sonicated for 2 min, boiled for 20 min and shaken at 4 °C for 1 h. Extracts were centrifuged twice to remove debris and concentrated by vacuum-centrifugation. An aliquot of the sample was applied to a 1D-SDS-PAGE and subjected to in-gel tryptic digestion. The resulting peptide mixtures were analyzed on a hybrid LTQ-Orbitrap MS (Thermo Fisher Scientific) as described earlier [35]. Protein database search against the UniRef 100 database, which also comprised the translated metagenome of the microbial community of a Mennesota farm silage soil [36] and known contaminants, was performed using the MASCOT Search Engine. A detailed description of the extraction procedure and search crite-

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<sup>3</sup>check numbers!

ria was published by [37]. 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/>; [38]). 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) [39,40]. This number allows relative comparison of protein abundances over different samples [41]. Protein abundances was aggregated at class level for fungi and proteobacteria and at phylum level for other bacterial taxa. These abundances were subjected to a canonical correspondance analysis without constraints. Vectorial fittings of stoichiometrical ratios (litter, microbial biomass and imbalance) were calculated and plotted when  $p < 0.05$ .

Quantitative PCR was used to determine fungal and bacterial abundance as described recently [42]. 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 [43]. If not mentioned otherwise, results were considered significant when  $p < 0.05$ . Due to frequent variance inhomogeneities Welch ANOVA and paired Welch's t-tests with Bonferroni corrected p limits were used. All correlations mentioned refer to Pearson correlations. A correspondence analysis (CA) and vectorial fittings were calculated using the R package "vegan" [44].

## Acknowledgments

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## Figure Legends

**Figure 1. Respiration rates, concentration of soluble organic C and potential extracellular enzyme activities** in decomposing beech leaf litter from a mesocosm experiment. Beech litter was collected in: triangles, Schottenwald (SW); diamonds, Ossiach (OS); squares, 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. \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ , b.d. - below detection limit.

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

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

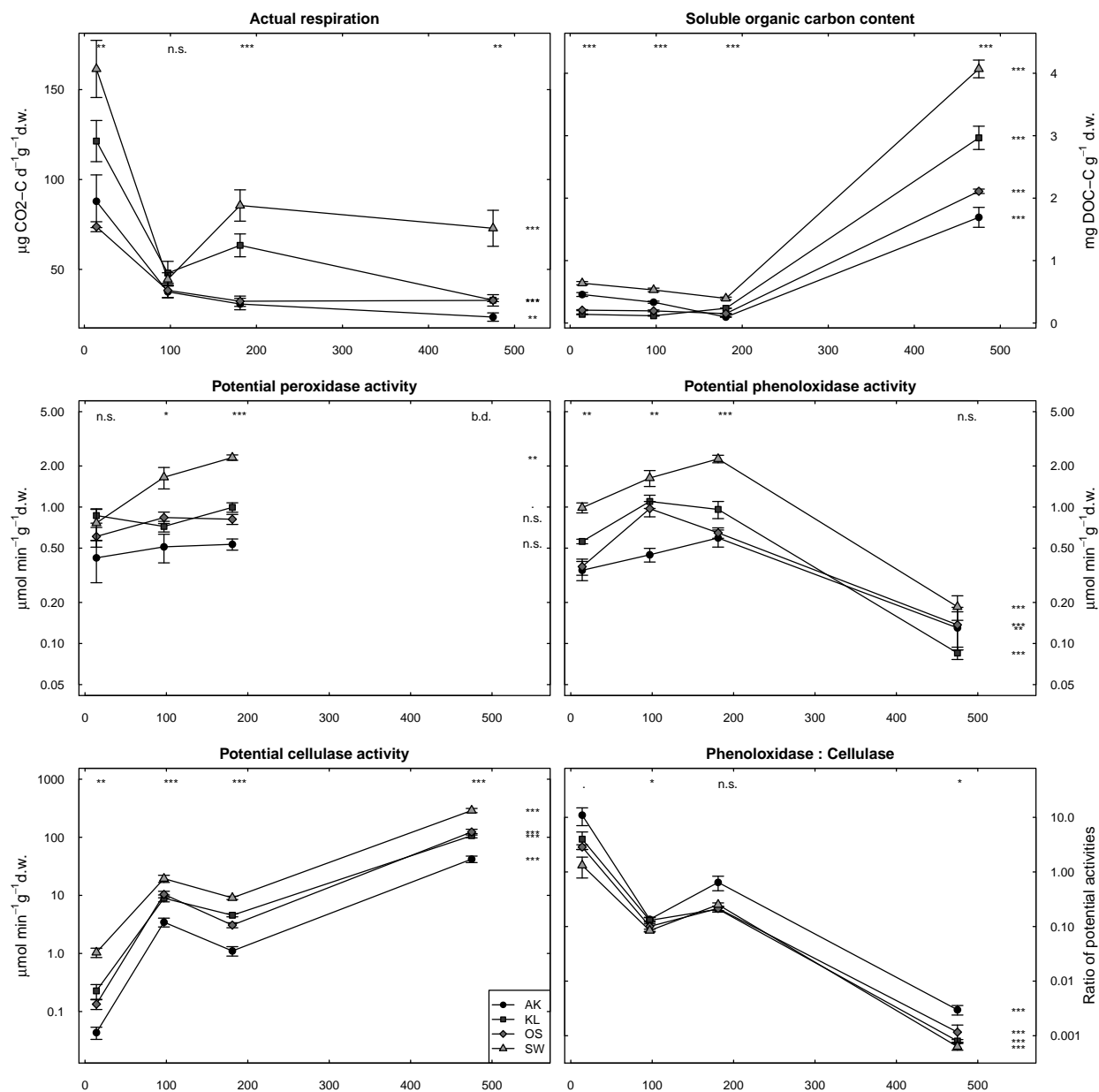
**Figure 4. Fungi:Bacteria (F:B) ratios and their correlations with LCI change:** A: F:B protein abundance (left) and DNA (right) ratio. B: Correlations between F:B preprotein abundance ratios and lignin loss (top), carbohydrate loss (mid) and lignin loss : carbon loss (bottom) for 0-6 months (left)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

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

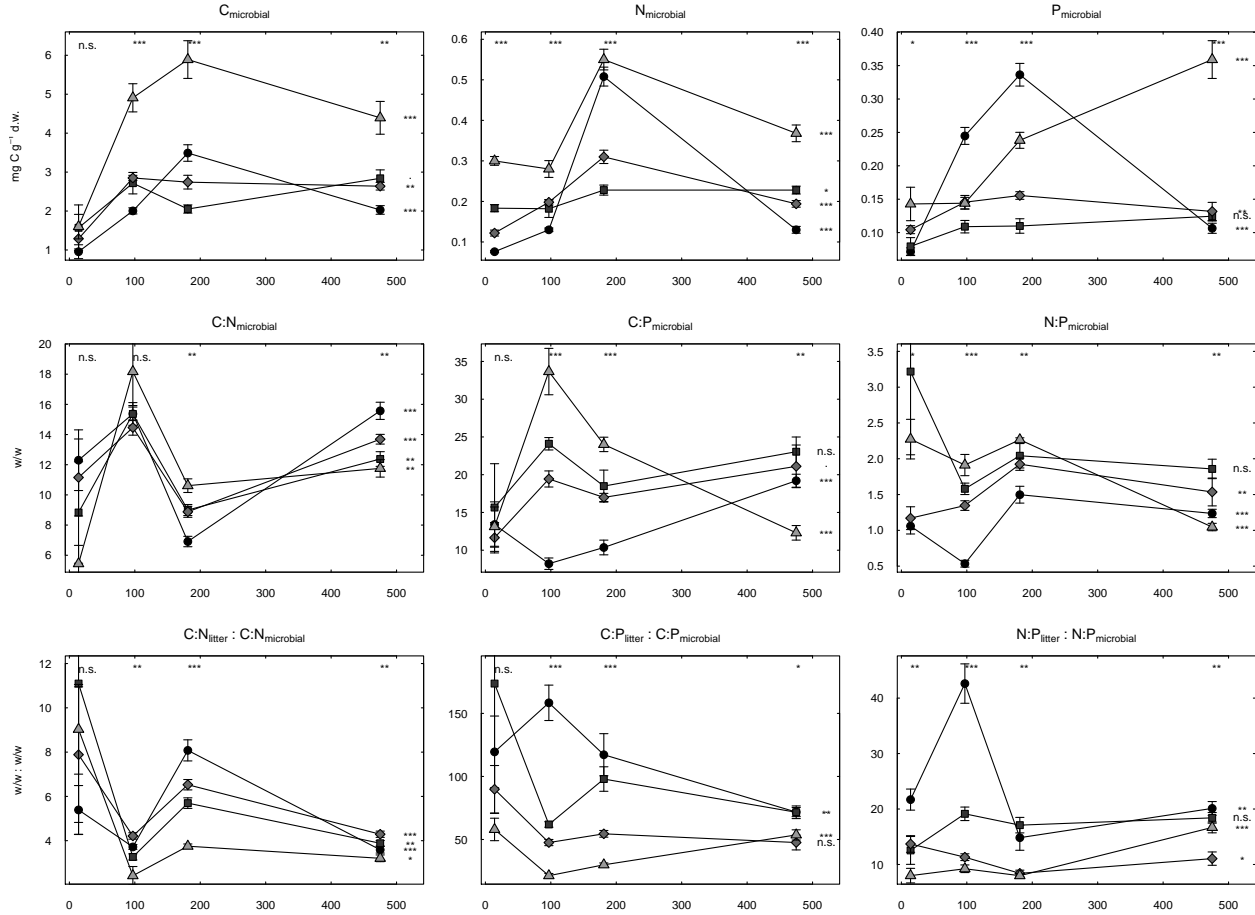
**Figure 6. Carbon loss corrected amounts of lignin and carbohydrates** degraded in beech litter collected in Achenkirch (AK), Klausenleopoldsdorf (KL), Ossiach (OS) and Schottenwald (SW). Carbon loss was calculated based on accumulated respiration for each mesocosm. Error bars indicate standard errors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

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

**Figure 8. Microbial community composition.** The first two components of a correspondance analysis (CA) of protein abundances found. Rectangles indicate samples of identical incubation time. Peptides were aggregated at class level (fungi and proteobacteria) or phylum level (other bacterial phyla): Dothideomycetes (Doth); Eurotiomycetes (Euro); Leotiomyces (Leot); Saccharomycetes (Sacc); Sordariomycetes (Sord); Agaricomycetes (Agar); Tremellomycetes (Trem); Ustilaginomycetes (Usti); Thermotogae (Ther); Bacteroidetes (Bact); Actinobacteria (Acti); Cyanobacteria (Cyan); Firmicutes (Firm); Fusobacteria (Fuso); Verrucomicrobia (Verr); Dictyoglomi (Dict); Alphaproteobacteria (Alph); Betaproteobacteria (Beta); Gammaproteobacteria (Gamm); Deltaproteobacteria (Delt); Epsilonproteobacteria (Epsi). Taxa factor loadings were printed x2 for better readability. Correlations between CA 1, CA 2, and litter chemistry, microbial stoichiometry, and protein abundance of microbial taxa are stated in supplemental table 4. Arrows represent vectorial fittings of these variables calculated independently from the CA, plotted only if p<0.05: Litter C content (C lit); C:X<sub>Microbial</sub>/C:X<sub>Litter</sub> (C:P imb, C:N imb).

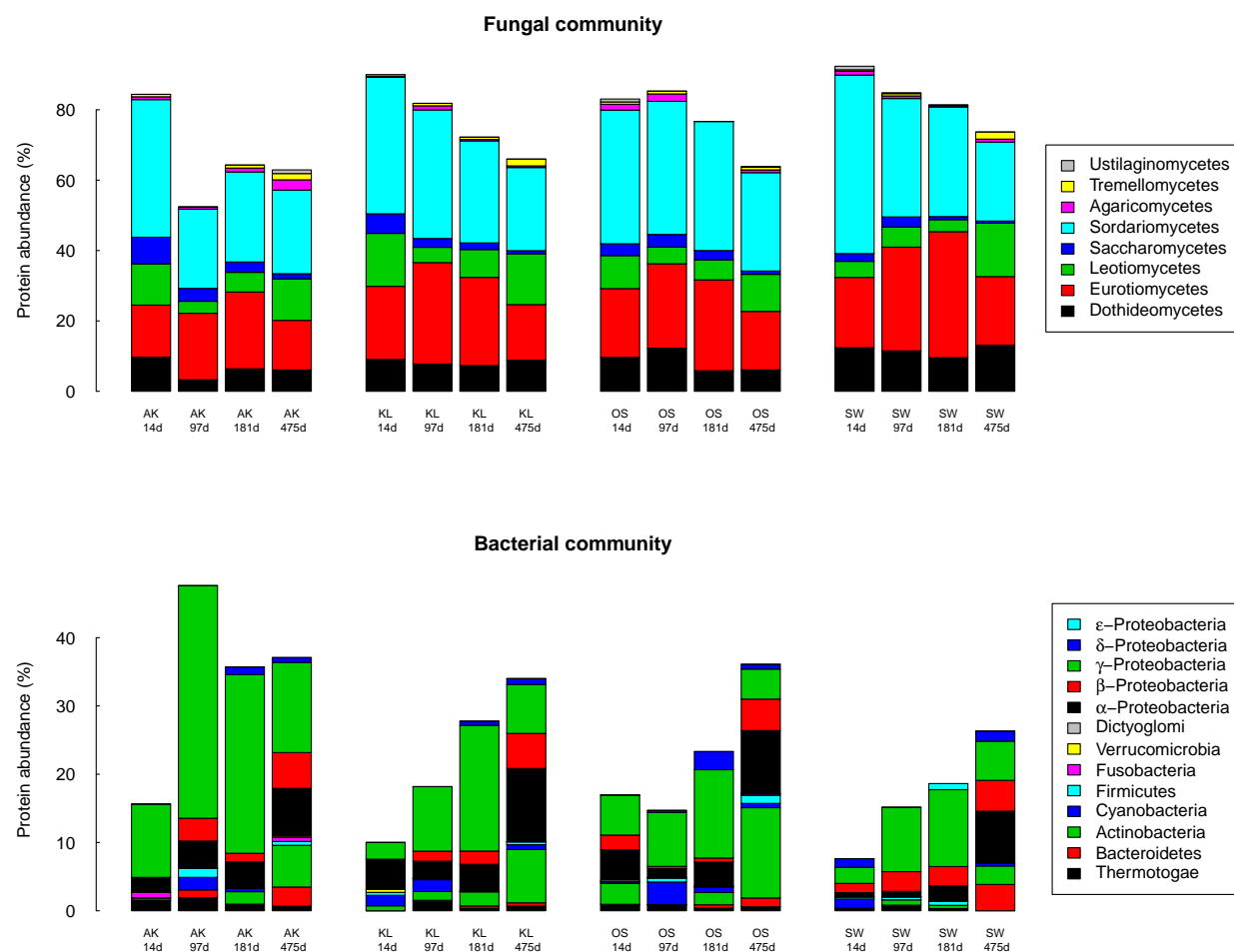


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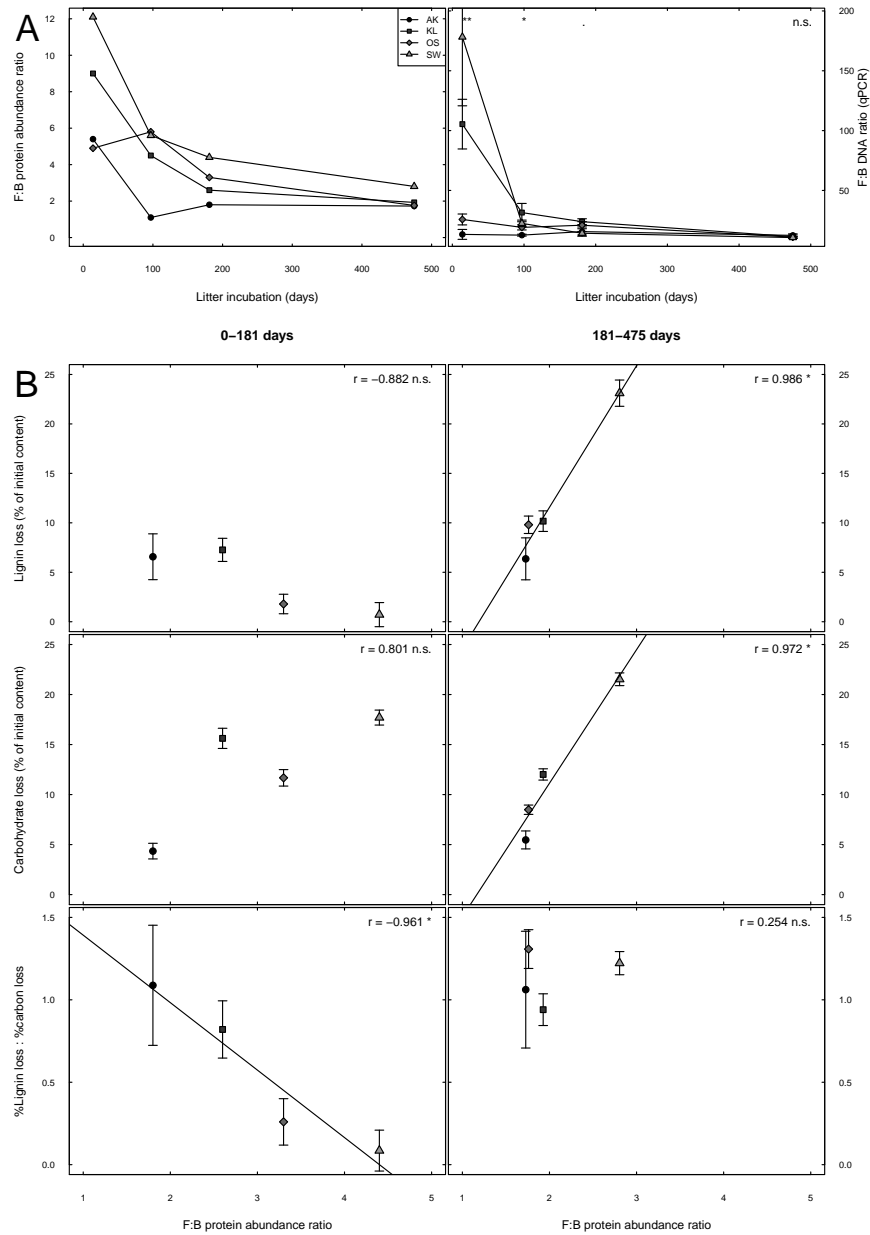


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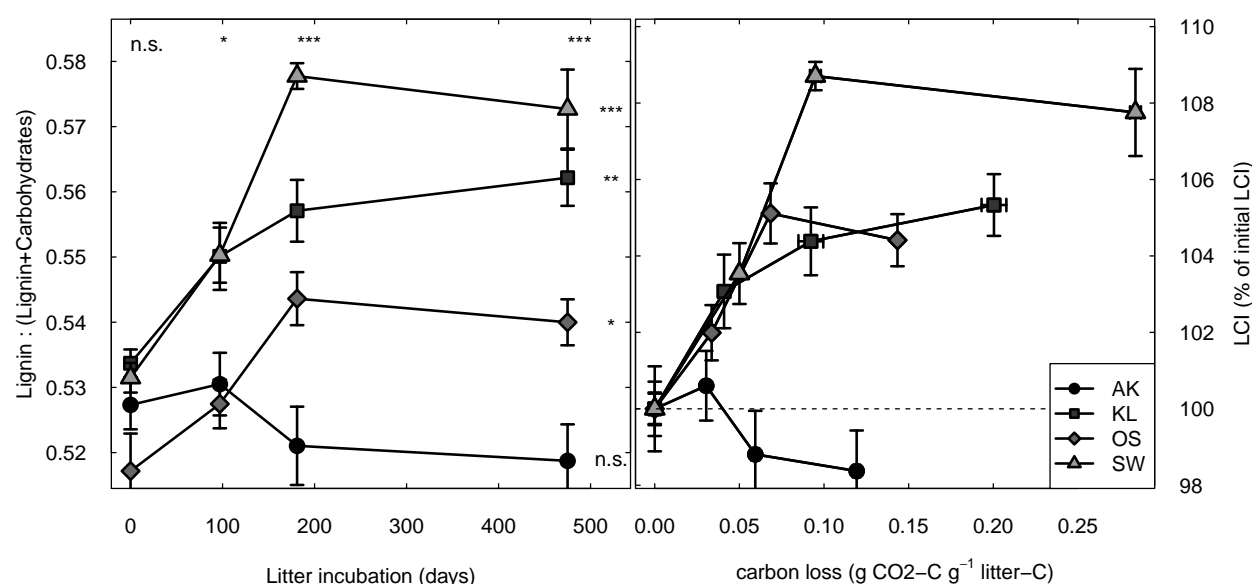




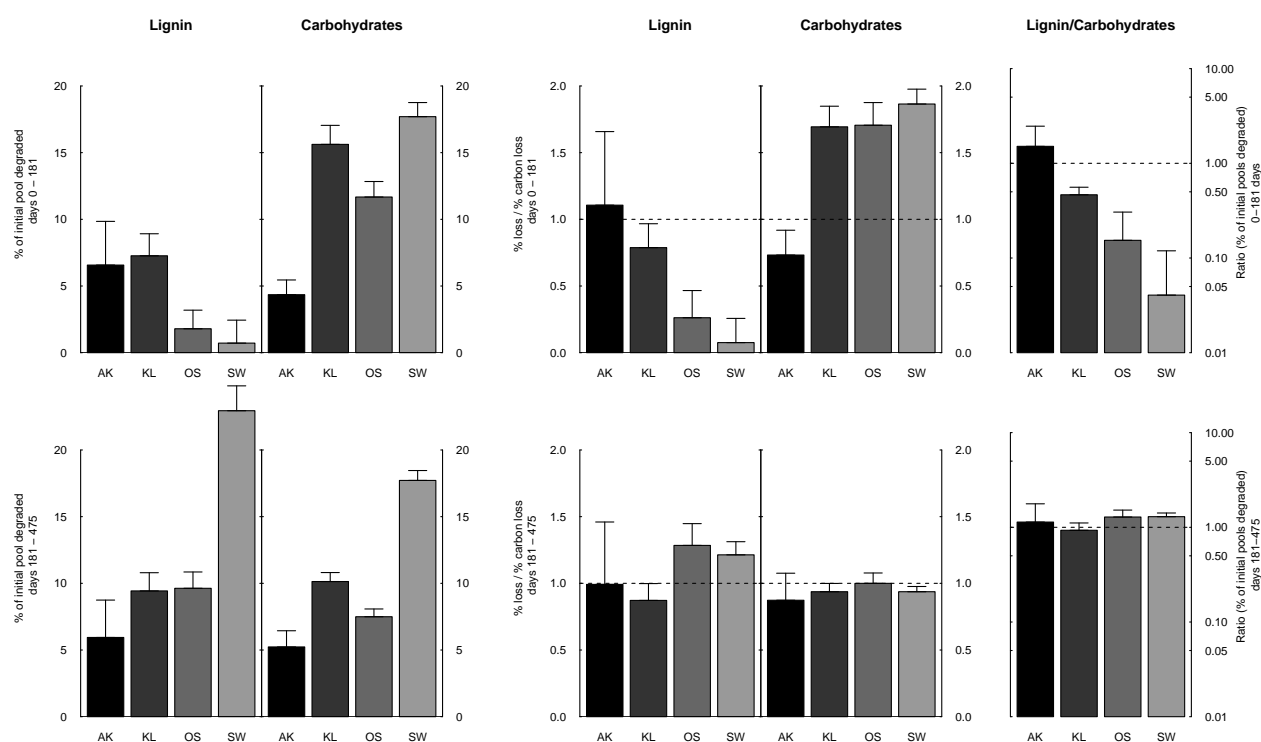
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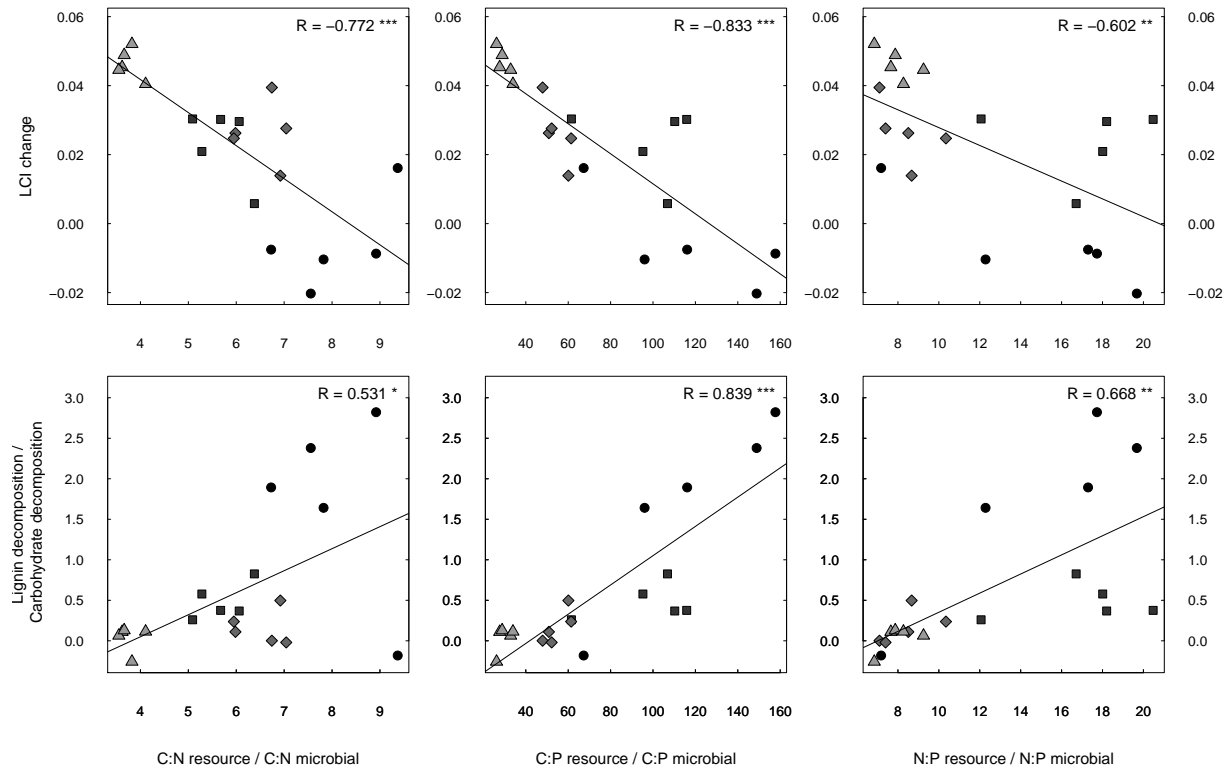
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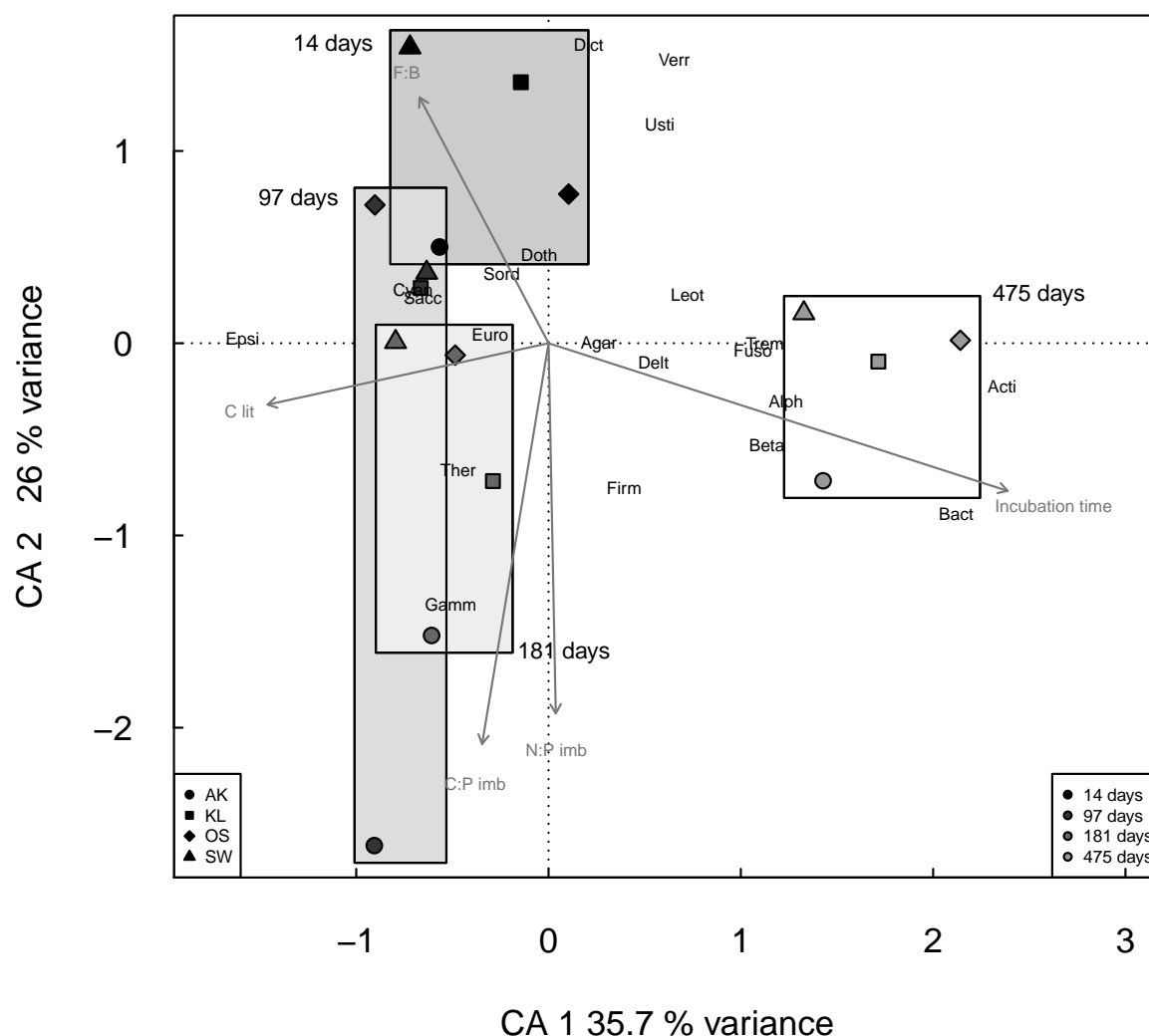
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## Tables

**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.** 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.  $\Delta_L$  - lignin accumulation,  $\Delta_{Ch}$  - Carbohydrate accumulation,  $\Delta_{LCI}$  - LCI difference,  $L\%_{loss}$  - lignin decomposition,  $Ch\%_{loss}$  - carbohydrate decomposition,  $L_{resp}$  - lignin loss : carbon loss,  $Ch_{resp}$  - carbohydrate loss : carbon loss,  $L : Ch\%_{loss}$  - lignin loss : carbohydrate loss, Per:Cell - Potetial peroxidase activity : potential cellulase activity, Phen:Cell - Potetial phenolo activity : potential cellulase activity.

	$\Delta_L$	$\Delta_{Ch}$	$\Delta_{LCI}$	$L\%_{loss}$	$Ch\%_{loss}$	$L_{resp}$	$Ch_{resp}$	$L : Ch\%_{loss}$	Per:Cell	Phen:Cell
Massloss	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
Phosphatase 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>
Chitinase 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>
Phenoloxidase 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>0.692</b>	-0.262	<b>0.543</b>	<b>-0.756</b>	0.204	<b>-0.689</b>	0.232	<b>-0.501</b>	-0.0902	-0.173
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>	0.74	-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 3.** 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.  $\Delta_L$  - lignin accumulation,  $\Delta_{Ch}$  - Carbohydrate accumulation,  $\Delta_{LCI}$  - LCI difference,  $L\%_{loss}$  - lignin decomposition,  $Ch\%_{loss}$  - carbohydrate decomposition,  $L_{resp}$  - lignin loss : carbon loss,  $Ch_{resp}$  - carbohydrate loss : carbon loss,  $L : Ch\%_{loss}$  - lignin loss : carbohydrate loss, Per:Cell - Potetial peroxidase activity : potential cellulase activity, Phen:Cell - Potetial phenolo activity : potential cellulase activity.

	$\Delta_L$	$\Delta_{Ch}$	$\Delta_{LCI}$	$L\%_{loss}$	$Ch\%_{loss}$	$L_{resp}$	$Ch_{resp}$	$L : Ch\%_{loss}$	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	-0.209	-0.0272	-0.266	<b>0.775</b>	<b>0.721</b>	0.228	0.243	0.0168	-0.359	0.234
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

**Table 4.** Correlations coefficients between correspondance analysis factors CA 1 and 2, litter and microbial stoichiometry and protein abundance of microbial taxa. Significant ( $p < 0.05$ ) correlations are presented in bold.

	CA1	CA2
Incubation time	<b>0.872</b>	-0.281
Respiration	-0.158	<b>0.601</b>
NH4 conc.	0.0838	0.0317
NO3 conc.	<b>0.53</b>	-0.00945
PO4 conc	0.161	0.0506
C litter	<b>-0.787</b>	-0.172
N litter	-0.174	0.268
P litter	-0.162	0.367
C:N litter	-0.0597	-0.272
C:P litter	0.0771	-0.334
N:P litter	0.112	-0.223
C micr.	-0.159	-0.0404
N micr.	-0.22	-0.14
P micr.	-0.11	<b>-0.59</b>
C:N micr.	0.104	0.0403
C:P micr.	-0.0223	0.485
N:P micr.	-0.174	0.472
C:N imbalance	-0.225	-0.228
C:P imbalance	-0.118	<b>-0.714</b>
N:P imbalance	0.0129	<b>-0.659</b>
F:B prot.	-0.417	<b>0.795</b>
Dothideomycetes	-0.0779	<b>0.745</b>
Eurotiomycetes	<b>-0.578</b>	0.0834
Leotiomycetes	<b>0.731</b>	0.253
Saccharomycetes	<b>-0.501</b>	0.18
Sordariomycetes	<b>-0.511</b>	<b>0.762</b>
Agaricomycetes	0.167	-0.00414
Tremellomycetes	<b>0.723</b>	-0.000103
Ustilaginomycetes	0.188	0.37
Thermotogae	-0.336	-0.469
Bacteroidetes	<b>0.638</b>	-0.267
Actinobacteria	<b>0.896</b>	-0.0846
Cyanobacteria	-0.319	0.122
Firmicutes	0.183	-0.35
Fusobacteria	0.227	-0.00858
Verrucomicrobia	0.114	0.256
Dictyoglomi	0.027	0.2
Alphaproteobacteria	<b>0.924</b>	-0.232
Betaproteobacteria	<b>0.766</b>	-0.358
Gammaproteobacteria	-0.348	<b>-0.929</b>
Deltaproteobacteria	0.229	-0.0427
Epsilonproteobacteria	-0.205	0.00168