Litter nutrient contents controls extend of lignin decomposition via decomposer community composition in Beech litter

Lukas Kohl[1], Wolfgang Wanek[1], Katharina Keiblinger[2,3], Sonja Leitner[1,3], Maria Mooshammer[1], Ieda Hämmerle[1], Lucia Fuchslueger[1], Jörg Schnecker[1], Thomas Schneider[4,5] Sandra Moll[7] Markus Gorfer[7,8] Joseph Strauss[7,8] Katharina Riedel[4,6] Leo Eberl[4,5] Sophie Zechmeister-Boltenstern[2,3], Andreas Richter[1],

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

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

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

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

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

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

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

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

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

Abstract

Lignin is a major component of plant litter and is considered highly resistant to decomposition. Carbohydrates, in contrast, are more easily degraded. We studied the decomposition rates of these two compound classes, and to which extent they are controlled by litter C:N:P stoichiometry.

Herein we report results from a 15-months mesocosm experiment under controlled climatic conditions with beech litter of different N and P contents. Litter was sterilized and re-inoculated prior to the experiment to minimize differences in the initial microbial community, but study the effect of N and P contents on identical initial decomposer communities. Lignin and carbohydrate decomposition rates were determined by pyrolysis-GC/MS for 2 periods (0-6 months and 6-15 months), the composition of the microbial community was monitored via metaproteome analysis.

Different in litter nutrient contents led to the establishment of different decomposer communities. Fungi were dominant on all litter, but fungi:bacteria ratios were highest on high-nutrient litter, leading to a negative correlation between litter and microbial stoichiometry and high differences between litter and microbial C:N and C:P ratios on nutrient poor litter.

Rates of lignin decomposition were highly variable during the first six months, ranging from insignificant amounts decomposed to decomposition at bulk C mineralization rates. Between 6 and 15 months, lignin was degraded at bulk mass loss rates independent of the litter nutrient contents, however, different lignin contents aquired within the initial 6 months remained in place. Early lignin degradation rates were highest in litter with low fungi:bacteria ratios, and were correlated to differences between litter and microbial stoichiometry ($C:P_{litter:microbial}$ and $C:P_{litter:microbial}$). Lignin degrading communities were enriched in γ-proteobacteria after 6 months incubation.

Our results indicate that - contradicting common models - significant amounts of lignin were be degraded during early decomposition in low nutrient litter. We demonstrate that litter quality profoundly affects the lignin decomposition via the composition of the decomposer community. Even though bacterial biomass is enriched in N and P, communities low nutrient litter were enriched in bacteria. This led to higher differences between litter and microbial stoichiomety, a possible control over lignin degradation during early decomposition.

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 [?], while leachable substances account for only 1.5-6% [?]. The breakdown of high molecular weight compounds into smaller molecules that are accessible to microbes is mediated by extracellular enzymes and is considered to rate limiting for decomposition [?].

Common models of litter decomposition [?, ?, ?, ?] assume that organic compounds in litter form up to three independent pools of increasing recalcitrance, i.e. (1) soluble compounds, (2) cellulose and hemicelluloses, 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 [?]. When analyzed with alternative methods (NMR, CuO-oxidation, Pyrolysis-GC/MS), extracted lignin fractions were shown to contain also many other substances [?], which led to an overestimation of lignin accumulation during early decomposition [?].

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 then expected and shorter than that of bulk soil organic matter [?, ?, ?]. While the ability to completely degrade lignin was traditionally attributed exclusively to Basidomycetes, it has been demonstrated for several bacterial taxa over the last years [?].

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 [?]. 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 [?]. In isolated lignin fractions from fresh beech litter, N contents twice as high as in bulk litter were found [?]. 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", [**?**]). 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 [**?**] and decreased the activity of lignolytic enzymes in forest soils [**?**]. 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 [**?**]. 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 [**?**]. 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 asses the activity of bacterial and fungal taxa.

With the experiment, we addressed the following questions:

(1) 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 ligin decomposition is initially slower in litter with a narrow C:N ratio (higher availability of assimilable nitrogen), than in litter with a high C:N ratio.

(2) Are high lignin degradation rates related to a higher fungal activity? We hypothesized that wider C:N and C:P ratios favor lignin degradation by fungi while narrow C:N and C:P ratios favor 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 **??**. 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. We found no significant changes of litter stoichiomrtry during the incubation except of the C:N ratio in the fastest degrading litter (SW), which slight decreased after 15 months (41.8:1 to 37.4:1) . Lignin and carbohydrate contents before incubation were in a similar range in all litter, lignin accounted for 28.9-31.2% and carbohydrates for 25.9-29.2% of the total peak area of all pyrolysis products. Micronutrient contents stronger varied: Fe concentrations were more than twice as high in OS (approx. 450 ppm) than for other litter (approx. 200 ppm), Mn contents were highly variable and ranged between 170 and 2130 ppm. All changes of micro-nutrient concentrations during incubation were <15% of the initial concentration.

Mass loss, respiration and soluble organic C, N and P

Highest respiration rates were measured at the first measurement after 14 days incubation (150-350 µg CO_2-C d^{-1} g^{-1} litter-C), which dropped to 75 to 100 µg CO_2-C d^{-1} g^{-1} 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 **??**).

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 [**?**]. 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 mg C g^{-1} d.w. to 1.5 to 4 mg C g^{-1} d.w. after 15 months, fig. **??**). 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 were initially only 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), indicating an increasing independence on litter N contents. Soluble N (not shown) was tighly correlated to soluble organic C (R=0.992, p <0.001, n=80) with Corg:N ratios falling from initially 30:1-40:1 to 20:1-30:1. Soluble P contents ranged between 70 and 400 µg g^{-1} d.w., and were highly correlated to litter P contents (R=0,830, p <0.001, n=80). Within the initial 6 month of incubation, soluble P decreased in high-P litter and increased in low-P litter (fig. **??**).

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. **??**), phenoloxidase and peroxidase activities reached their maximum between 3 and 6 months (fig. **??**). 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. **??**).

Microbial biomass abundance and community composition

Microbial biomass contents ranged from 0.5 to 6 mg C g^{-1} d.w., 0.05 to 0.55 mg N g^{-1} d.w. and 0.05 to 0.35 mg P g^{-1} litter d.w (fig. **??**). 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. **??**). The differences between microbial and litter stoichiometry led to an accumulation of substancial amounts of P (up to 80% of the total litter P in AK after 6 months). In AK and KL litter biomass P includes initially insoluble P that was mobilized (ie. the sum of soluble + biomass P contents increased), while in OS and SW litter and microbial P was taken up from a shrinking soluble P pool (fig. **??**).

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 [**?**]), 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 [**?**] 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 tighly constrained range, 14.5:1 to 18.2:1 after 3 months and 6.9:1 to 9.0:1 after 6 months, but significantly different between the two sampling events. In contrast, microbial C:P and N:P ratios were less constrained, with the highest variance between litter from different sites after 3 months of incubation (fig. **??**).

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 high-nutrient SW and least pronounced in low-nutrient AK litter. 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. **??**). 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. **??**). Bacteria were dominated by Proteobacteria (mainly γ, declining, and α- and β-Proteobacteria, increasing with litter decomposition) with minor contribution of Actinomycetes and Bacterioidetes (both increasing) and Thermotogae (decreasing, fig. **??**).

Pyrolysis-GC/MS and Lignin content

In total 128 pyrolysis products were detected, quantified, identified and assigned to their substances of origin (suppl. tab. 1). 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 occured 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. **??**). 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. **??**). 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. **??**). 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. **??**).

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

To asses possible controls over lignin degradation, we tested relationships between lignin and carbohydrate degradation, litter chemistry, microbial biomass and decomposition processes after 6 and 15 months for correlations (tables **??** and **??**) including data presented previously [**?**, **?**]. After the initial 6 months, when differences in ligin accumulation were highest, 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). In contrast, lignin decomposition was negatively correlated to litter P, but positively with litter C:P and N:P ratios. The best correlation of lignin : carbohydrates degradation rates and LCI were found with both C:$P_{litter:microbial}$ and C:$N_{litter:microbial}$ (fig. **??**), however, these two ratios were also intercorrelated (R=0.641, p=0.002, n=20). In contrast, carbohydrate decomposition was positively correlated with litter N content, and negatively with litter C:N ratios and C:$N_{litter:microbial}$.

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 decom-

position exhibited the same controls, being positively correlated to soluble organic C, litter N and litter P (table **??**) between 6 and 15 months. Mass loss and accumulated respiration were positively correlated to lignin and carbohydrate decomposition (table **??**), a pattern that we did not find for lignin decomposition in the early decomposition phase (table **??**). 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. **??**).

To asses the interaction between litter chemistry, microbial community and degradation processes, we conducted a correspondence analysis (CA) of the metaproteome data (relative protein abundances, fig. **??**). 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 (Actinobacteri, Bacteroidetes, α- and β-proteobacteria), and two fungal classes (Leotiomycetes and Tremellomycetes) were positively correlated to CA1 i.e. increased in abundance towards 15 months, while Cyanobacteria, ϵ-proteobacteria and Saccharomycetes were negatively correlated. CA 2, which explained 26.0 % variance, separated litter sampled within the first 6 months. Dothideomycetes and Sordariomycetes were positively and γ-proteobacteria negatively correlated to this factor, which also correlated to the F:B protein abundance ratio. Litter collected 14 days after inoculation have the highest scores on CA 2, while sites with active lignin degradation within the first 6 months (AK, KL) have the most negative scores. The axis was furthermore correlated to the microbial biomass P content and C:$P_{litter:microbial}$ and N:$P_{litter:microbial}$. 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 **??**.

Discussion

Different in litter qualities led to different mass loss rates and the development of of different decomposer communities from the same inoculate. Lignin decomposition was highly variable between litter of different quality within the first 6 month; it's decomposition ranged from non-detectable (SW litter) to decomposition at bulk carbon loss rates (ie. no lignin accumulation, AK litter). This provides further evidence that the use of extractive methods to measure lignin contents led to an underestimation of early lignin degradation rates, as recently suggested [**?**], and that substancial 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 aquired within the first six months remained in place, but lignin contents no further increased.

We chose our collection sites to provide litter with different N and P contents since we focused on the effect of these nutrients on decomposition. We found positive correlations between litter N and bulk decomposition parametres 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 [**?**]). N and P were also demostrated to be correlated to a wide area of leaf traits in plants (ie. [**?**]), and such leaf traits were successfully used to predict litter decomposeability in the past [**?**].

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 con-

tradicted 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 [**?**]. In contrast, we found fungi : bacteria ratios were higher in nutrient-rich litter. Similar observations were reported by Güsewell and 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, which 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 $C:X_{litter:microbial}$ ratios as integrated measure for nutrient availability to nutrient demand.

Litter quality controlled the composition of the microbial communities, and microbial biomass stoichiometry, which influenced the stoichiometric offset between resource (litter) and consumer (microbial biomass) that decomposers had to overcome. Both community composition, and $C:X_{litter:microbial}$ and were correlated to the rate of early lignin decomposition; Litter with high fungi:bacteria ratios and lower differences between litter and microbial stoichiometry accumulated more lignin. Our results therefore indicate that lignin degradation is associated with bacteria-rich degrader communities, a low availability of nutrients to decomposers, or both. In contrast, we can exclude that lignin decomposition was triggered by critical lignin contents or inhibited by insufficient Mn (highest lignolytic activity in litter with lowest contents of lignin and Mn), as suggested for late lignin decomposition [**?**].

Traditionally, the capability to completely degrade lignin was exclusively attributed to Basidomycota fungi [**?**]. However, fungi:bacteria ratios were lower in lignin degrading litter and Basidomycota produced less than 5% of fungal protein. Over the last years, lignin degradation was also demonstrated for several bacterial taxa (eg. actinomycetes, α-, and γ-proteobacteria [**?**]). Of these three taxa, we found one (γ-proteobacteria) correlated to lignolytic activities after 3 and 6 months. The other two taxa (actinomycetes and α-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, the metaproteomic analysis only sporadically detected lignolytic enyzmes, 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).

Lignin degradation in the first 6 months was best predicted by $C:P_{litter:microbial}$ and $C:N_{litter:microbial}$, ie. more lignin was degraded when the difference between litter and microbial stoichiometry was higher. $C:P_{litter:microbial}$ and $C:N_{litter:microbial}$ were intercorrelated, so we can not differentiate the effects of N and P. However, lignin decomposition rates were 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 indicating a differential control of lignin (stimulated by P demand) and carbohydrate (stimulated by N availability) decomposition. Litter which rapidly degraded lignin (AK and KL) net-mobilized insoluble P into rapidly cycled P forms (soluble and microbial), while in slowly lignin degrading litter (SW and OS) microbial P originated only from soluble P (fig. **??**B). This indicates that lignin degradation increased the mobilization of P from insoluble litter biomass , and explains higher lignin degradation rates in litter with high microbial P demand. Such a nutrient mobilization by lignin degradation is assumed for N [**?**]: Lignin (like humic compounds) occludes important amounts of protein during polymerization [**?**, **?**], which is available only after lignin degradation. Therefore, the degradation of complex carbon sources was proposed to constitute a strategy of N sequestration (N mining theory, [**?**, **?**]). However, ambivalent results are reported for whether P is protected in lignin and humic compounds, and whether P demand triggers the decomposition of complex carbon compounds [**?**, **?**].

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 [**?**]. Lignin decomposition was also suggested to allow decomposers direct competition by the early colonization of lignin rich sites [**?**]. 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 provides additional N. When $C:X_{substrate:consumer}$ 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 exercised a profound control on the litter decomposition process, including community composition and lignin accumulation. Lignin decomposition within the first six months was highly variable between litter of of the same species but different in nutrient contents, and ranged from no

lignin degradation to lignin degradation at bulk litter C loss rates. Lignin decomposition was not coupled to fungi-rich decomposer communities, indicating important bacterial contributions to lignin decomposition. Early lignin decomposing litter was characterized by nutrient-rich microbial biomass on low-nutrient litter, ie. a high difference between litter and decomposer stoichiometry, low fungi:bacteria ratios and an elevated abundance of γ-proteobacteria peptides. Different lignin contents acquired during early decomposition remained in place, potentially affecting late decomposition and humification.

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^2, homogenized, sterilized twice by γ-radiation (35 kGy, 7 days between irradiations) and inoculated (1.5% w/w) with a mixture of litter and soil to assure that all litter types share the same initial microbial community. From each type, four samples of litter were taken immediately after inoculation, dried and stored at room temperature. Batches of 60g litter (fresh weight) were incubated at 15 °C and 60% relative water content in mesocosms for 15 months. For each litter type 5 replicates were removed and analyzed after 14, 97, 181 and 475 days. A detailed description of the litter decomposition experiment was published by [**?**].

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 [**?**]). To determine dissolved organic C, dissolved N and P, 1.8 g litter (fresh weight) were extracted with 50 ml 0.5 M K_2SO_4. 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 [**?**]. 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 [**?**].

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

$$C : X_{s:c} = \frac{C : X_{litter}}{C : X_{microbial}} \tag{1}$$

where X stand for the element N or P.

Microbial Respiration

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

Potential enzyme activities

Potential activities of β-1,4-cellobiosidase ("cellulase"), phenoloxidase and peroxidase were measured immediately after sampling. 1 g of litter (fresh weight) was suspended in sodium acetate buffer (pH 5.5) and ultrasonicated. To determine cellulase activity, 200 µl suspension were mixed with 25 nmol 4-methylumbelliferyl-β-D-cellobioside (dissolved in 50 µl 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 flourimetrically (Tecan Infinite M200, exitation 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-dihydroxyphenylalanin (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. Absortion 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 [**?**].

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 colomn (Supelcowax 10, Sigma-Aldrich). Between 2-300 µg of dried and finely ground litter (MM2000 ball mill, Retsch) was heated to 600 °C for 10 seconds in a helium atmosphere. GC oven temperature was constant at 50 °C for 2 minutes, followed by an increase of 7 °C/min to a final temperature of 260 °C, which was held for 15 minutes. The MS detector was set for electron ionization at 70 eV in the scanning mode (m/z 20 to 300).

Peaks were assignment was based on NIST 05 MS library after comparison with measured reference materials. 128 peaks were identified and selected for integration either because of their abundance or diagnostic value. This included 28 lignin and 45 carbohydrate derived substances. The pyrolysis products used are stated in supplementary tables nn-nn[1] 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 [**?**, **?**]. 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 .

$$LCI = \frac{Lignin}{Lignin + Carbohydrates} \tag{2}$$

Accounting for carbon loss, we estimate the % lignin and cellulose degraded during decomposition (L_{degr} and Ch_{degr}) according to equation **??**, 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_2-C respired by a mesocosm.

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

Furthermore, we calculated the % of initial lignin or carbohydrates degraded per % of initial carbon respired (L:C_{degr} and Ch:C_{degr}):

$$X_{resp} = \frac{X_{\%loss}}{100} \cdot \frac{C_{init}}{R_{acc}} \tag{4}$$

[1] check numbers!

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 [**?**]. Protein database search against the UniRef 100 database, which also comprised the translated metagenome of the microbial community of a Mennesota farm silage soil [**?**] and known contaminants, was performed using the MASCOT Search Engine. A detailed description of the extraction procedure and search criteria was published by [**?**]. 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/; [**?**]). 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) [**?**, **?**]. This number allows relative comparison of protein abundances over different samples [**?**]. Protein abundances was aggregated at class level for fungi and protebacteria and at phylum level for other bacterial taxa. These abundances were subjected to a canonical correspondance analysis without constrains. 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 [**?**]. 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 [**?**]. 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" [**?**].

Acknowledgments

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KEY: Zybailov2006

ANNOTATION: From Duplicate 1 (Statistical analysis of membrane proteome expression changes in Saccharomyces cerevisiae. - Zybailov, Boris; Mosley, Amber L; Sardiu, Mihaela E; Coleman, Michael K; Florens, Laurence; Washburn, Michael P)

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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. Mobilization of litter P (A) Insoluble litter P is mobilized into recycled P pools (dissolved and microbial biomass P) in lignin degrading litter (AK and KL), while the increase in biomass P on non lignin-degrading litter (OS and SW) origininates from soluble P. (B) correlation between mobilization of P and lignin accumulation, 0-6 months incubation. Beech litter was collected in: triangles, Schottenwald (SW); diamonds, Ossiach (OS); squares, Klausenleopoldsdorf (KL); circles, Achenkirch, AK. Error bars indicate standard errors (n=5).

Figure 4. 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 5. 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 preotein 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.

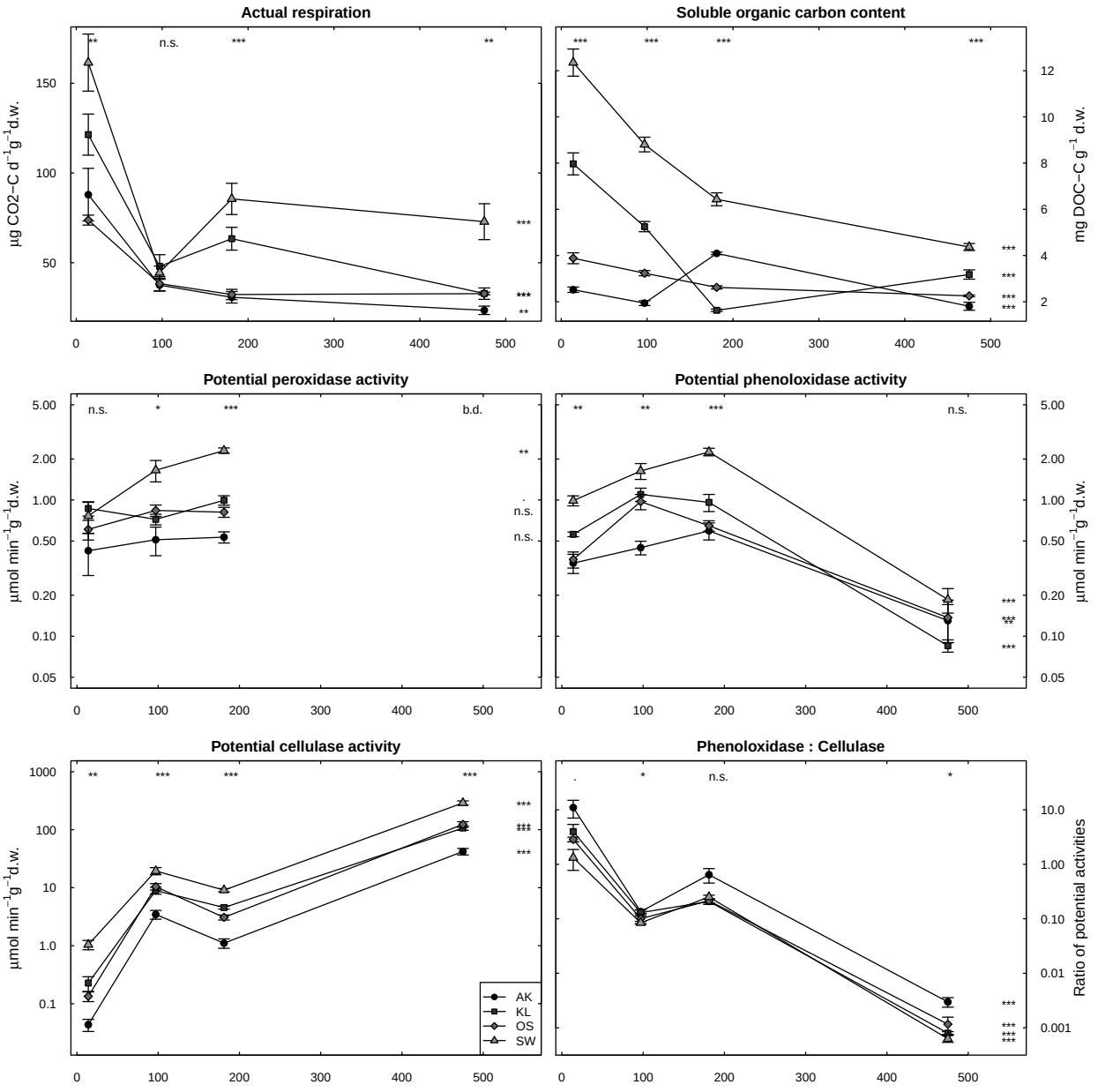
Figure 6. Develoment of lignin to carbohydrate index (lignin : (lignin+carbohydrates), LCI) during time of beech litter decomposition (left) or plotted against cumulative C loss (right). Errorbars indicate standard errors (n=4-5). The dashed line indicates a constant ratio between lignin and carbohydrates (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. *, $P<0.05$, **, $P<0.01$, ***, $P<0.001$.

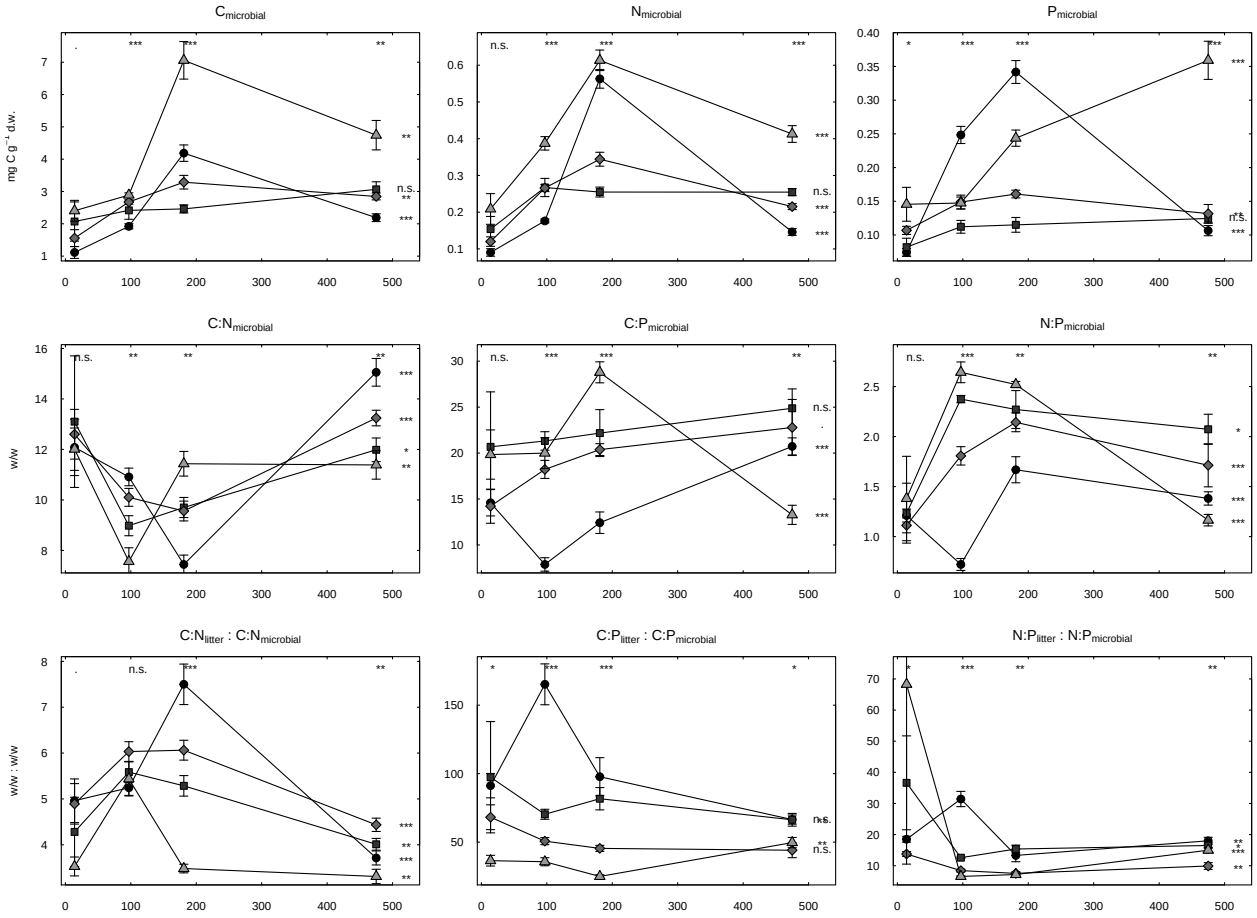
Figure 7. Carbon loss corrected amounts of lignin and carbohydrates degraded in beech litter collected in Achenkirch (AK), Klausenleopoldsdorf (KL), Ossiach (OS) and Schottenwald (SW). Carbon loss was calculated based on accumulated respiration for each mesocosm. Error bars indicate standard errors (n=4-5). The dashed line marks no discrimation during decomposition between lignin, carbohydrates and bulk carbon

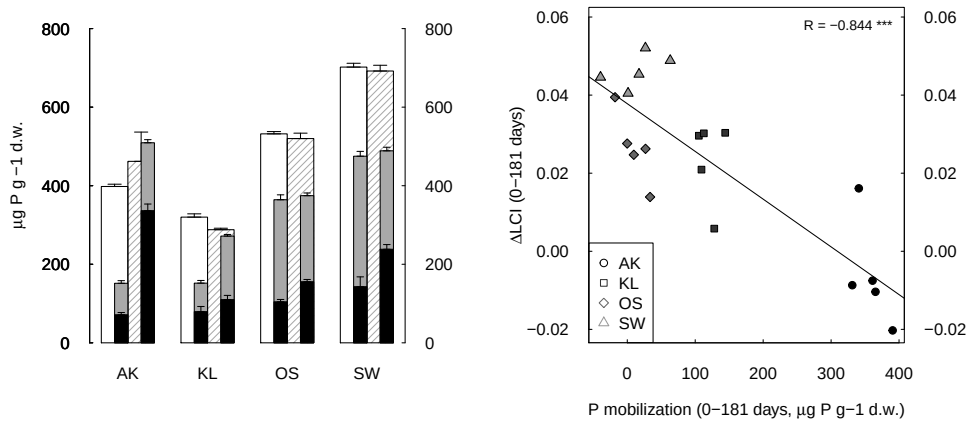
Figure 8. Correlation between the LCI change or the ratio of lignin : carbohydrate decomposition ratio during the first 6 months of litter decomposition correlate to litter : microbe stoichiometric imbalances. and change and Correlations between lignin accumulation during the first 6 month of litter 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. *, $P<0.05$, **, $P<0.01$, ***, $P<0.001$.

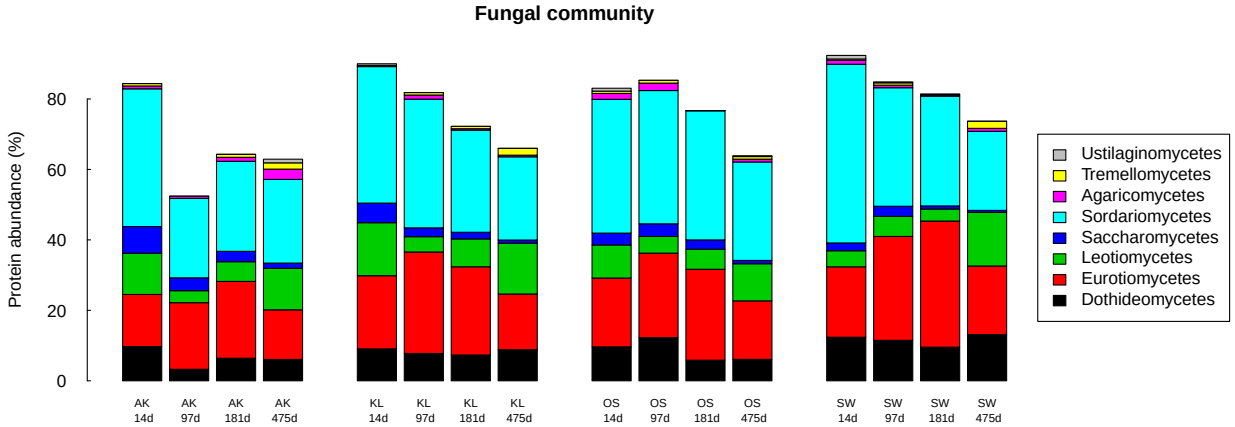
Figure 9. Microbial commuity composition. The first two components of a correspondance analysis (CA) of protein abundances found. Rectangles indicate samples of identical incubation time. Peptides were aggregated at class level (fungi and proteobacteria) or phylum level (other bacterial phyla): Dothideomycetes (Doth); Eurotiomycetes (Euro); Leotiomycetes (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 **??**. Arrows represent vectorial fittings of these variables calculated independently from the CA, plotted only if $p<0.05$: Litter C content (C lit); $C{:}X_{Microbial}/C{:}X_{Litter}$ (C:P imb, C:N imb).

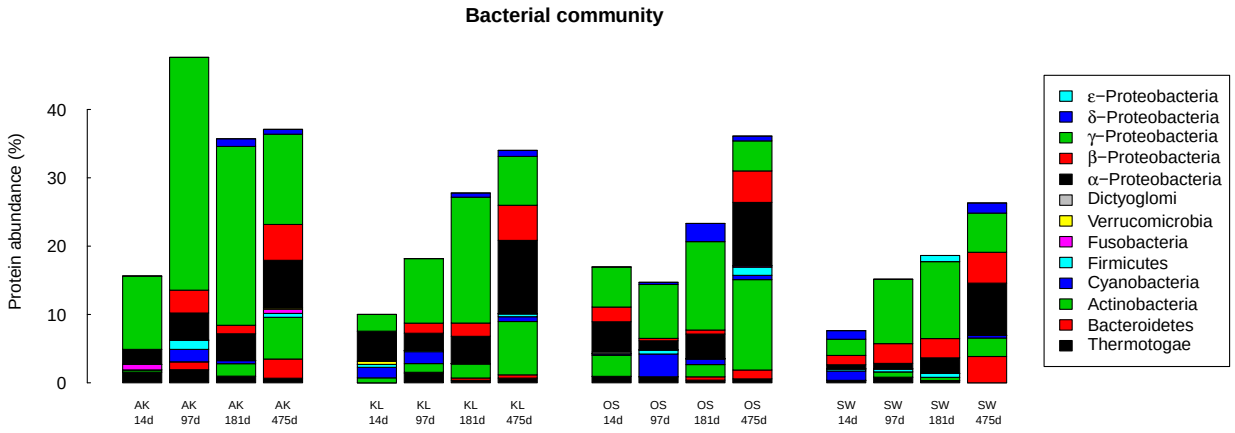
Tables

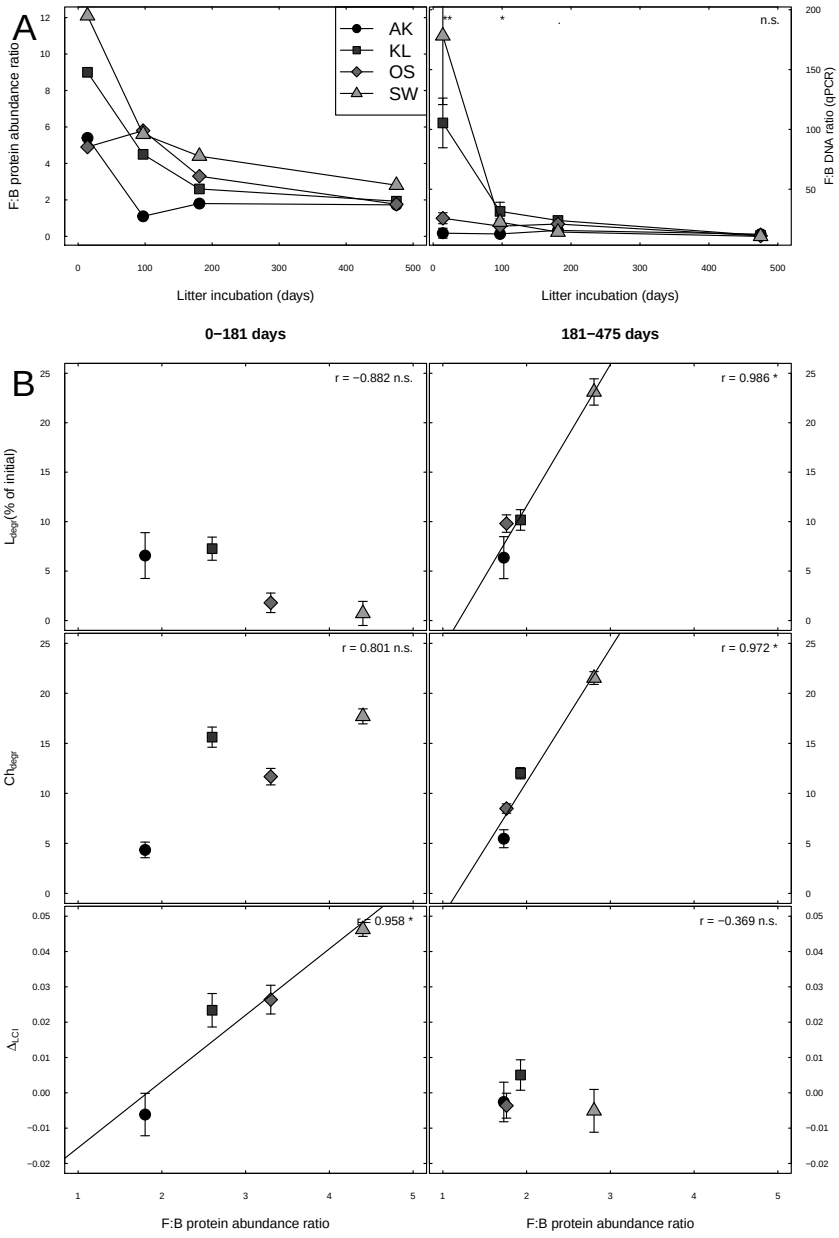

Actual respiration
µg CO2−C d−1g−1d.w.
Soluble organic carbon content
mg DOC−C g−1 d.w.
Potential peroxidase activity
µmol min−1g−1d.w.
Potential phenoloxidase activity
µmol min−1g−1d.w.
Potential cellulase activity
µmol min−1g−1d.w.
Phenoloxidase : Cellulase
Ratio of potential activities
AK
KL
OS
SW

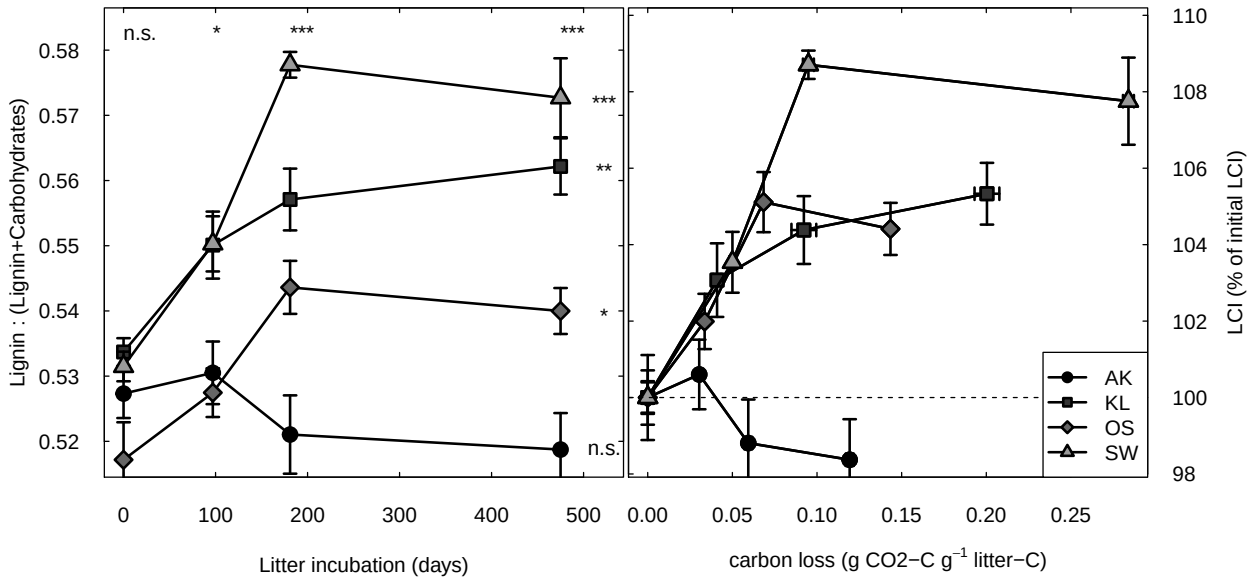

C_microbial
N_microbial
P_microbial
C:N_microbial
C:P_microbial
N:P_microbial
C:N_litter : C:N_microbial
C:P_litter : C:P_microbial
N:P_litter : N:P_microbial
mg C g⁻¹ d.w.
w/w
w/w : w/w
n.s.

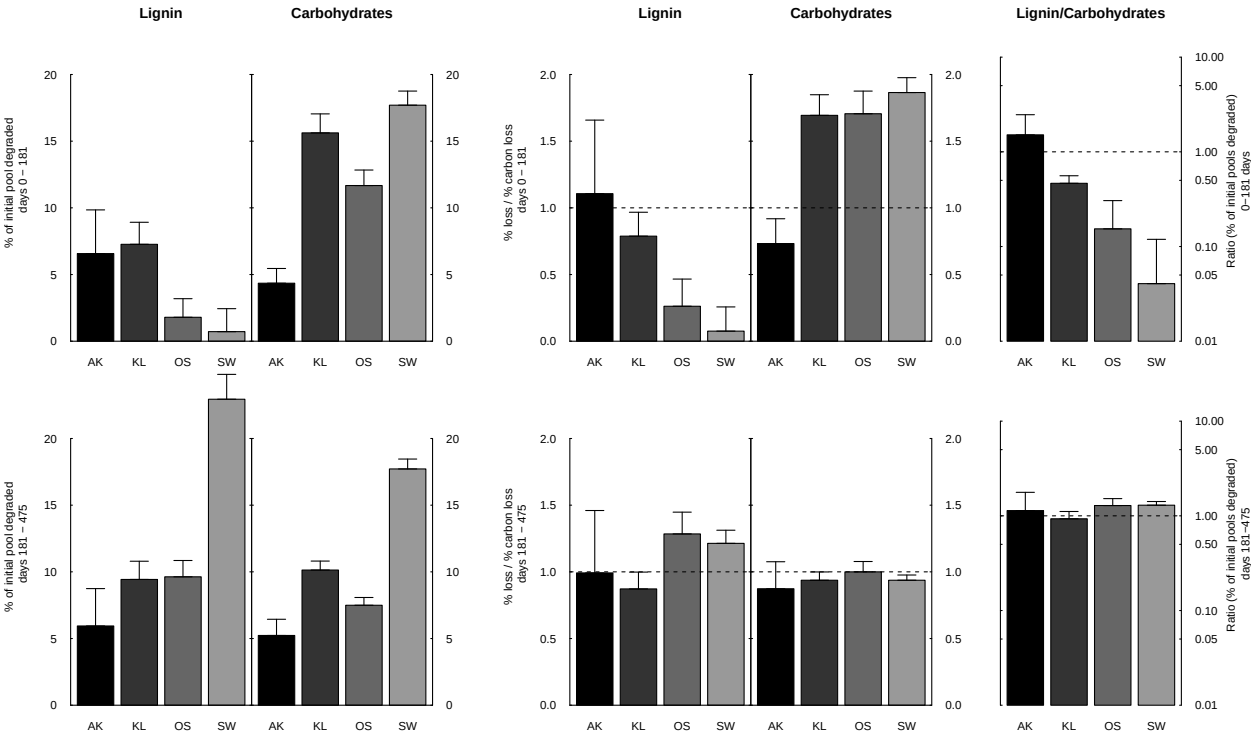

µg P g −1 d.w.
AK
KL
OS
SW
ΔLCl (0−181 days)
R = −0.844 ***
P mobilization (0−181 days, µg P g−1 d.w.)

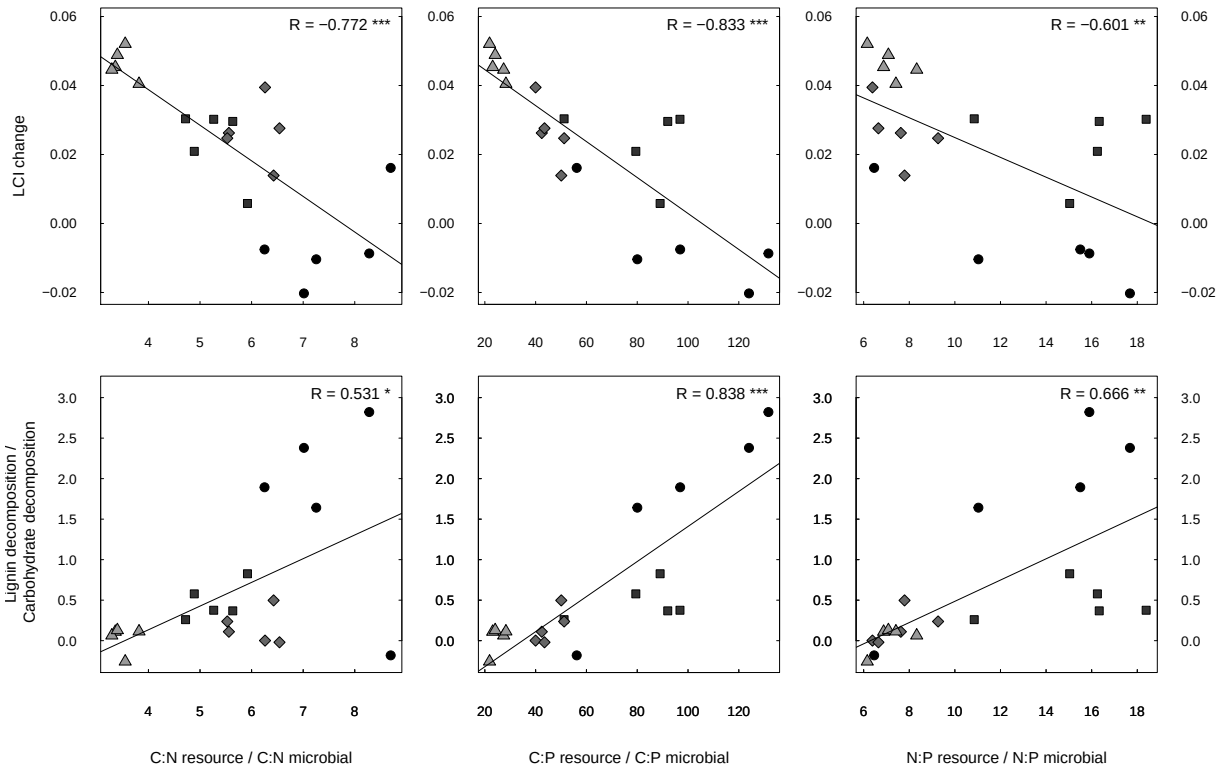

Fungal community
Protein abundance (%)
0
20
40
60
80
AK 14d
AK 97d
AK 181d
AK 475d
KL 14d
KL 97d
KL 181d
KL 475d
OS 14d
OS 97d
OS 181d
OS 475d
SW 14d
SW 97d
SW 181d
SW 475d
Ustilaginomycetes
Tremellomycetes
Agaricomycetes
Sordariomycetes
Saccharomycetes
Leotiomycetes
Eurotiomycetes
Dothideomycetes

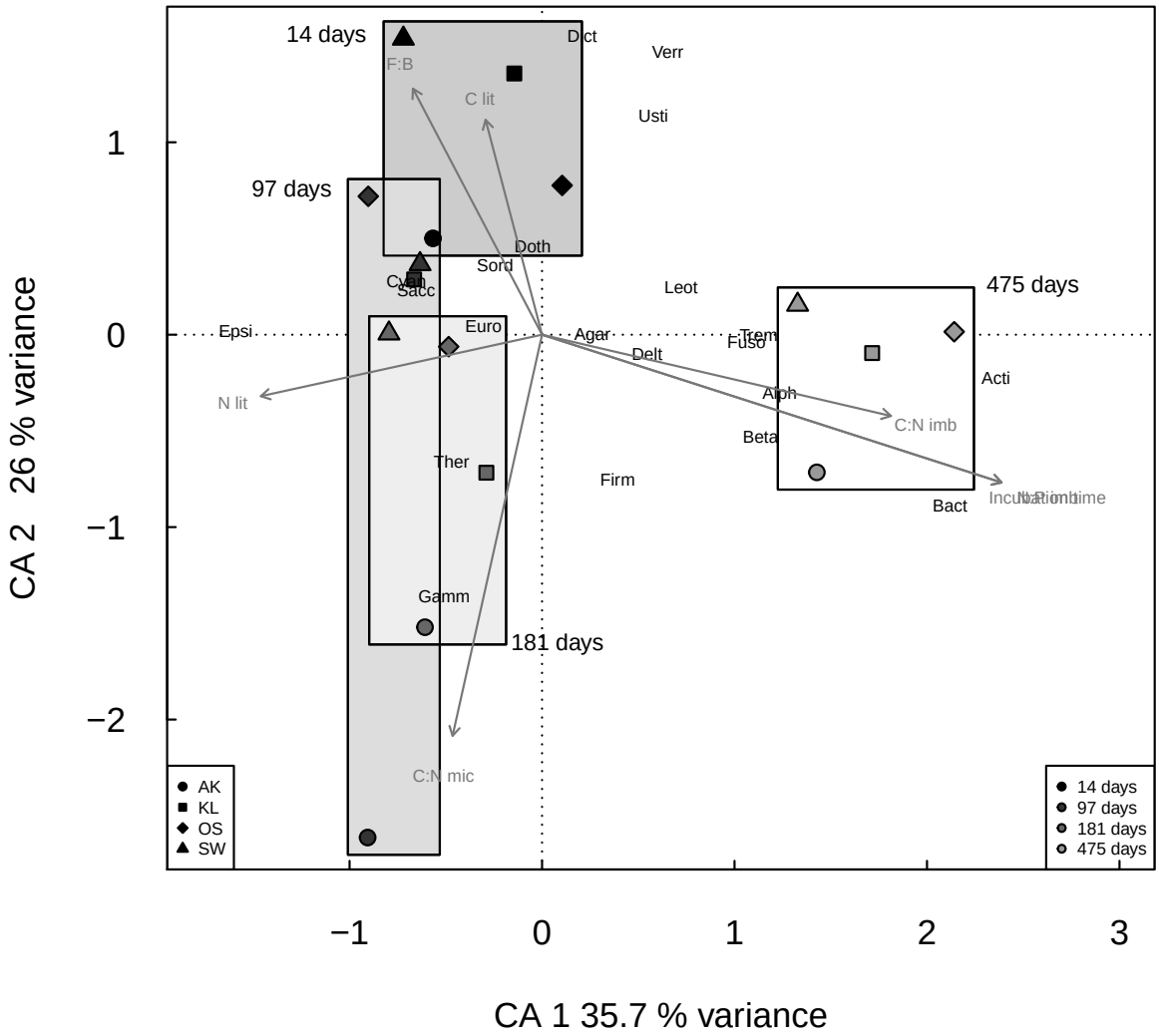

Bacterial community
Protein abundance (%)
0
10
20
30
40
AK 14d
AK 97d
AK 181d
AK 475d
KL 14d
KL 97d
KL 181d
KL 475d
OS 14d
OS 97d
OS 181d
OS 475d
SW 14d
SW 97d
SW 181d
SW 475d
ε−Proteobacteria
δ−Proteobacteria
γ−Proteobacteria
β−Proteobacteria
α−Proteobacteria
Dictyoglomi
Verrucomicrobia
Fusobacteria
Firmicutes
Cyanobacteria
Actinobacteria
Bacteroidetes
Thermotogae


A
AK
KL
OS
SW
F:B protein abundance ratio
F:B DNA ratio (qPCR)
**
*
.
n.s.
Litter incubation (days)
Litter incubation (days)
0−181 days
181−475 days
B
r = −0.882 n.s.
r = 0.986 *
r = 0.801 n.s.
r = 0.972 *
r = 0.958 *
r = −0.369 n.s.
L_degr (% of initial)
Ch_degr
Δ_LCI
F:B protein abundance ratio
F:B protein abundance ratio


n.s.
*
***
***
***
**
*
n.s.
Lignin : (Lignin+Carbohydrates)
0.58
0.57
0.56
0.55
0.54
0.53
0.52
0
100
200
300
400
500
Litter incubation (days)
0.00
0.05
0.10
0.15
0.20
0.25
carbon loss (g CO2−C g−1 litter−C)
110
108
106
104
102
100
98
LCI (% of initial LCI)
AK
KL
OS
SW


Lignin
Carbohydrates
Lignin
Carbohydrates
Lignin/Carbohydrates
% of initial pool degraded days 0 – 181
% loss / % carbon loss days 0 – 181
Ratio (% of initial pools degraded) 0–181 days
% of initial pool degraded days 181 – 475
% loss / % carbon loss days 181 – 475
Ratio (% of initial pools degraded) days 181–475
AK
KL
OS
SW


R = −0.772 ***
R = −0.833 ***
R = −0.601 **
R = 0.531 *
R = 0.838 ***
R = 0.666 **
LCI change
Lignin decomposition / Carbohydrate decomposition
C:N resource / C:N microbial
C:P resource / C:P microbial
N:P resource / N:P microbial


14 days
97 days
181 days
475 days
Dct
Verr
F:B
C lit
Usti
Doth
Sord
Cytr
Sacc
Leot
Epsi
Euro
Agar
Trem
Fuso
Delt
Acti
N lit
Alph
C:N imb
Beta
Ther
Firm
Bact
Gamm
C:N mic
AK
KL
OS
SW
14 days
97 days
181 days
475 days
CA 2 26 % variance
CA 1 35.7 % variance
1
0
−1
−2
−1
0
1
2
3

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

Table 2. 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 [**?**, **?**]. Changes in litter chemistry (lignin and carbohydrate decomposition) were calculated between 0 and 181 days, other data were measured after 181 days. Δ_L, Δ_{Ch} - differences in lignin/carbydrate contents (pyr-GC/MS), Δ_{LCI} - LCI (lignin : (lignin + carbohydrates)) difference (pyr-GC/MS), L_{degr}, Ch_{degr} - % of initial lignin/carbohydrate loss, L/C_{degr}, Ch/C_{degr} - % lignin/carbohydrates loss : % carbon respired, $L : Ch_{degr}$ - lignin loss : carbohydrate loss, Per:Cell - Potetial peroxidase activity : potential cellulase activity, Phen:Cell - Potetial phenoloxidase activity : potential cellulase activity.

	Δ_L	Δ_{Ch}	Δ_{LCIC}	L_{degr}	Ch_{degr}	$L : C_{degr}$	$Ch : C_{degr}$	$L : Ch_{degr}$	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	**0.569**	**-0.453**	**0.565**	**-0.543**	0.392	**-0.503**	**0.453**	**-0.458**	-0.294	-0.346
Accumulated Respiration	-0.149	-0.32	0.114	0.4	**0.446**	0.208	0.258	-0.0982	-0.251	-0.347
Cellulase activity	**0.657**	**-0.76**	**0.803**	-0.431	**0.801**	**-0.497**	**0.664**	**-0.589**	-0.436	**-0.539**
Protease activity	0.186	-0.296	0.264	-0.132	0.274	-0.157	0.301	-0.27	-0.26	-0.18
Phosphatase activity	0.409	**-0.749**	**0.663**	-0.17	**0.795**	-0.312	**0.677**	**-0.559**	**-0.49**	**-0.607**
Chitinase activity	**0.549**	**-0.813**	**0.776**	-0.302	**0.851**	-0.407	**0.702**	**-0.556**	-0.418	**-0.522**
Phenoloxidase activity	**0.632**	**-0.669**	**0.737**	-0.415	**0.719**	**-0.449**	**0.552**	**-0.484**	-0.305	-0.356
Peroxidase activity	**0.599**	**-0.588**	**0.677**	-0.412	**0.639**	-0.438	**0.47**	-0.435	-0.173	-0.302
N mineralization	**0.466**	**-0.664**	**0.65**	-0.167	**0.739**	-0.299	**0.527**	-0.387	-0.282	-0.367
Nitrification	**0.587**	**-0.707**	**0.732**	-0.38	**0.74**	-0.432	**0.621**	**-0.499**	-0.369	-0.45
P mineralization	**0.665**	**-0.55**	**0.684**	**-0.544**	**0.596**	**-0.576**	0.414	**-0.478**	-0.212	-0.255
C litter	**-0.545**	**0.506**	**-0.578**	**0.604**	-0.368	**0.643**	**-0.618**	**0.698**	**0.525**	**0.581**
extractable C	0.318	-0.015	0.197	-0.268	0.0745	-0.166	-0.118	0.0531	0.203	0.203
N litter	0.354	**-0.517**	**0.503**	-0.14	**0.587**	-0.187	0.366	-0.203	-0.119	-0.159
P litter	**0.692**	-0.262	**0.543**	**-0.756**	0.204	**-0.689**	0.232	**-0.501**	-0.0902	-0.173
C:N litter	-0.405	**0.586**	**-0.57**	0.175	**-0.654**	0.234	-0.44	0.273	0.195	0.242
C:P litter	**-0.636**	0.174	**-0.453**	**0.754**	-0.0823	**0.649**	-0.176	0.418	0.049	0.0805
N:P litter	**-0.512**	-0.0287	-0.264	**0.714**	0.147	**0.577**	-0.0202	0.316	-0.0316	-0.0192
C:N mic	**0.664**	**-0.758**	**0.799**	-0.428	**0.798**	**-0.514**	**0.678**	**-0.609**	**-0.583**	**-0.595**
C:P mic	**0.691**	**-0.786**	**0.833**	**-0.475**	**0.813**	**-0.561**	**0.725**	**-0.671**	**-0.564**	**-0.647**
N:P mic	**0.582**	**-0.729**	**0.74**	-0.416	**0.728**	**-0.508**	**0.715**	**-0.67**	**-0.545**	**-0.672**
C:N imbalance	**-0.559**	**0.81**	**-0.772**	0.287	**-0.86**	0.39	**-0.71**	**0.531**	**0.563**	**0.56**
C:P imbalance	**-0.816**	**0.663**	**-0.833**	**0.756**	**-0.61**	**0.798**	**-0.668**	**0.838**	**0.576**	**0.67**
N:P imbalance	**-0.724**	0.349	**-0.601**	**0.811**	-0.251	**0.764**	-0.395	**0.666**	0.3	0.408
Fungi/bacteria(qPCR)	0.00234	-0.122	0.0794	-0.0242	0.0874	-0.0664	0.135	-0.072	0.199	-0.0333
Fungi/bacteria (metaproteome)	**0.998**	-0.854	**0.958**	-0.882	0.801	**-0.961**	0.824	-0.873	-0.679	-0.676

Table 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 [**?**, **?**]. Changes in litter chemistry (lignin and carbohydrate decomposition) were calculated between 181 and 475 days, other data were measured after 475 days. Δ_L, Δ_{Ch} - differences in lignin/carbydrate contents (pyr-GC/MS), Δ_{LCI} - LCI (lignin : (lignin + carbohydrates)) difference (pyr-GC/MS), L_{degr}, Ch_{degr} - % of initial lignin/carbohydrate loss, L/C_{degr}, Ch/C_{degr} - % lignin/carbohydrates loss : % carbon respired, $L : Ch_{degr}$ - lignin loss : carbohydrate loss, Per:Cell - Potetial peroxidase activity : potential cellulase activity, Phen:Cell - Potetial phenoloxidase activity : potential cellulase activity.

	Δ_L	Δ_{Ch}	Δ_{LCIC}	L_{degr}	Ch_{degr}	$L : C_{degr}$	$Ch : C_{degr}$	$L : Ch_{degr}$	Per:Cell	Phen:Cell
Massloss	0.246	0.156	0.068	**0.582**	**0.708**	0.00521	0.279	-0.137	-0.444	0.403
Actual respiration	-0.171	0.0554	-0.273	**0.826**	**0.773**	0.238	0.216	0.0432	-0.365	0.229
Accumulated Respiration	**0.611**	0.402	0.356	0.00736	0.229	-0.274	0.119	-0.283	-0.334	0.344
Cellulase activity	0.0733	0.218	-0.137	**0.848**	**0.881**	0.148	0.295	-0.0811	**-0.575**	0.414
Protease activity	0.00361	0.0538	-0.086	0.448	0.455	0.16	0.316	-0.11	**-0.456**	0.381
Phosphatase activity	0.256	0.31	0.0689	0.298	0.373	-0.102	-0.0136	-0.115	-0.152	0.0167
Chitinase activity	0.163	0.339	-0.0858	**0.643**	**0.671**	0.167	0.253	-0.0289	**-0.58**	0.395
Phenoloxidase activity	0.319	-0.389	0.436	-0.248	-0.0034	-0.221	**0.505**	-0.443	**-0.483**	**0.692**
Peroxidase activity	-0.277	0.379	-0.385	0.173	-0.0488	0.16	**-0.51**	0.382	**0.546**	**-0.708**
N mineralization	0.246	0.337	0.0777	0.00915	0.0616	-0.191	-0.113	-0.167	0.0624	0.0892
Nitrification	-0.0272	**0.567**	-0.32	**0.63**	**0.567**	0.0904	-0.148	0.114	-0.105	-0.0234
P mineralization	-0.0165	0.202	-0.138	**0.507**	**0.508**	-0.136	-0.0626	-0.128	0.0433	-0.0273
C litter	0.123	-0.0651	0.177	-0.325	-0.264	-0.204	-0.289	0.0236	**0.501**	-0.348
extractable C	0.368	**0.552**	0.0304	0.416	**0.493**	-0.0431	0.12	-0.155	**-0.473**	0.423
N litter	0.21	0.356	-0.0654	**0.816**	**0.896**	-0.00431	0.172	-0.12	-0.431	0.349
P litter	-0.209	-0.0272	-0.266	**0.775**	**0.721**	0.228	0.243	0.0168	-0.359	0.234
C:N litter	-0.272	-0.365	0.0158	**-0.794**	**-0.901**	0.027	-0.207	0.155	**0.49**	-0.404
C:P litter	0.329	0.122	0.315	**-0.645**	**-0.541**	-0.276	-0.218	-0.0672	0.283	-0.162
N:P litter	**0.471**	0.289	0.328	-0.336	-0.179	-0.293	-0.113	-0.148	0.048	0.0338
C:N mic	-0.185	-0.409	0.0927	**-0.657**	**-0.703**	-0.0296	-0.315	0.251	**0.569**	**-0.512**
C:P mic	0.237	-0.0601	0.312	**-0.609**	**-0.505**	-0.191	-0.0713	-0.0629	0.233	-0.223
N:P mic	0.338	0.125	0.293	-0.378	-0.25	-0.185	0.0482	-0.16	0.000192	-0.00981
C:N imbalance	-0.146	-0.0154	-0.0749	-0.358	-0.451	0.0585	0.0407	-0.0496	0.031	0.0163
C:P imbalance	0.0217	0.247	-0.0737	-0.138	-0.2	-0.0205	-0.241	0.0946	0.16	-0.032
N:P imbalance	0.0233	0.233	-0.0878	0.0459	-0.00203	0.00691	-0.268	0.175	0.157	-0.0784
Fungi/bacteria(qPCR)	-0.03	-0.00782	0.0166	-0.236	-0.254	-0.0887	-0.115	-0.00256	0.161	-0.219
Fungi : bacteria (metaproteome)	0.158	0.57	-0.369	**0.986**	**0.972**	0.254	0.484	-0.274	-0.601	0.55

Table 4. Correlations coeffitients 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
Incubatation time	**0.872**	-0.281
Respiration	-0.158	**0.601**
NH4 conc.	0.0368	0.029
NO3 conc.	**0.584**	-0.0564
PO4 conc	0.0905	0.321
C litter	**-0.787**	-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.	-0.107	-0.0562
N micr.	-0.288	-0.141
P micr.	-0.132	**-0.59**
C:N micr.	0.417	0.212
C:P micr.	0.0775	**0.589**
N:P micr.	-0.296	0.446
C:N imbalance	**-0.508**	-0.34
C:P imbalance	-0.157	**-0.773**
N:P imbalance	0.119	**-0.6**
F:B prot.	-0.417	**0.795**
Dothideomycetes	-0.0779	**0.745**
Eurotiomycetes	**-0.578**	0.0834
Leotiomycetes	**0.731**	0.253
Saccharomycetes	**-0.501**	0.18
Sordariomycetes	**-0.511**	**0.762**
Agaricomycetes	0.167	-0.00414
Tremellomycetes	**0.723**	-0.000103
Ustilaginomycetes	0.188	0.37
Thermotogae	-0.336	-0.469
Bacteroidetes	**0.638**	-0.267
Actinobacteria	**0.896**	-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	**0.924**	-0.232
Betaproteobacteria	**0.766**	-0.358
Gammaproteobacteria	-0.348	**-0.929**
Deltaproteobacteria	0.229	-0.0427
Epsilonproteobacteria	-0.205	0.00168