Controls of litter chemistry over early lignin decomposition in beech litter

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Abstract

Lignin is considered the most recalcitrant component of plant litter, accumulated during early decomposition and degraded only during late decomposition stages when its concentration limits litter decomposition rates. A recent study based on the more specific (CuO-oxidation) to determine lignin contents reports challenges this concept, reporting highest lignin decomposition rates during early litter decomposition. Until now, no further studies exploring early lignin decomposition were published, and its potential controls remain unknown.

We follow early litter decomposition with analytical pyrolysis in a climate-chamber decomposition experiment, focusing on resource control over early lignin decomposition and microbial carbon substrate preferences. Beech litter with different C:N:P stoichiometry but innoculated with identical initial microbial communities was incubated at constant climatic conditions to identify the control of litter chemistry on the developing microbial community and its decomposition activity. We use analytical pyrolysis to quantify lignin and carbohydrate break-down.

During the first 6 month fundamental differences in lignin degrading activities were found between sites. Lignin discrimination in litter decomposition ranges between only marginal amounts of lignin being decomposed and lignin decomposition at the same rate average

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litter, leading to different niveaus of lignin accumulation. Between 6 and 15 month, no lignin discrimination was found, but different lignin contents aquired earlier reminded.

Neither nitrogen nor labile carbon availability could be identified as a control over lignin decomposition. However, amounts of lignin decomposed were correlated to the buildup of resource to decomposer C:N and C:P imbalances.

Keywords: litter decomposition, lignin, analytical pyrolysis, Pyr-GC/MS

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1. Introduction

Plant litter biomass is dominated by macromolecular compounds. Together, lignin, carbohydrate and protein polymers make up xx% of litter dry mass, while leach-able substances in litter account for only xx %.

Litter decomposition models [lit] follow the concept that macromolecules in litter form three independent carbon pools of increasing recalcitrance attributed to (1) soluble compounds, (2) cellulose and hemi-celluloses and (3) lignin. During decomposition, soluble compounds are most accessible to microbes and consumed first, followed by carbohydrates (i.e. cellulose). Lignin can be decomposed only by specialists and is not degraded until accumulated to a certain, critical level when it inhibits the degradation of less recalcitrant compounds (Berg and Staaf, 1980; Coûteaux et al., 1995; Moorhead and Sinsabaugh, 2006).[2] Most common methods to quantify these carbon pools gravimatrically determine cellulose, hemi-celluloses and lignin contents after sequential extractions with selective solvents. These methods ("Klason"- and "ADF"-lignin) were repeatedly criticize as unspecific for lignin determination (**?**). When analyzed with alternative methods (NMR, CuO-oxidation, Pyrolysis-GC/MS), extracted lignin fractions contain many other than the proclaimed substances. (i.e. Preston et al. (1997) [3].

Recent studies based on specific methods to determine litter lignin content (CuO - oxidation, Pyrolysis-GC/MS, NMR) question the assumed intrinsic recalcitrance of lignin. Ex-

[2] more lit.

[3] [lit CuO], lit[Pyr]

periments using isotope labeling used to calculate mean residence times for lignin in soils and litter/soil mixtures in both laboratory and outdoor incubation reported lignin residence times no longer than that of other carbon compounds or bulk SOM (Thevenot et al., 2010; Bol et al., 2009)[4].

For leaf litter, lignin depletion during early decomposition and decreasing lignin decomposition rates were recently by Klotzbücher et al. (2011). Based on their results, the authors proposed a new concept for lignin degradation in which fastest lignin degradation occurs during early litter decomposition. Lignin decomposition during late decomposition is limited by (dissolved organic) carbon availability, a pulsed input of labile carbon (during litterfall or experimental manipulations like drying and rewetting) causes higher lignin degradation rates for a limited time period .

Klotzbücher et al. (2011) do not elaborate the of stoechiometric constrains on lignin decomposition. Nitrogen fertilization experiments on litter and soils indicate a that litter N contents are important controls of lignin degradation: N addition increases mass loss rates in low-lignin litter while slowing down decomposition in lignin-rich litter (Knorr et al., 2005). High nitrogen levels were reported to inhibit lignolytic enzyme in forest soils(Sinsabaugh, 2010). Cellulose triggered higher priming effect in fertilized than in unfertilized soils indicating that the mineralization of recalcitrant C is controlled by an interaction of labile C and N availability (Fontaine et al., 2011).

Decomposer behavior in fertilization eperiments might be different from the behavior in litter with different nutrient levels, as leaf litter N is stored in protein and lignin structures and not directly availeable to microorganisms. To our knowledge, no other experiment has yet compared effects of intra-specific variance in litter nutrient contents on decomposition processes. N-fertilization experiments can simulate increased N-deposition rates. To simulate variations litter C:N ratios, our approach is preferable, because potential variations in litter N content occur in complex substrates. N location and accessibility is different from the low molecular weight N species used in fertilization experiments.

[4]more lit?

In this study we analyze samples from climate-chamber incubated beech litter varying in N and P content with pyrolysis-GC/MS (pyr-GC/MS). The experiment was designed to study the effect of resource stroichiometry on microbial decomposition, exclude decomposing fauna and keep climatic conditions constant.

We hypothesize that

(1) Microorganisms have to allocate N in the production of different enzymes. When environmental conditions are constant, microbial substrate preference is determined by litter chemistry.

(2) While (non-lignified) carbohydrates are easier degraded than lignin and the resulting sugar monomers yield more energy, lignin degradation improves to accessibility of nitrogen ("lignin mining", Craine et al. (2007)). More lignin is decomposed when nitrogen availability is low, and high nitrogen availability inhibits lignin degradation.

(3) Lignin degradation is inhibited when labile carbon availability is low and decomposition is energy limited (as proposed by Klotzbücher et al. (2011)).

(4) From a decomposers perspective, lignin is less effective than carbohydrate mining and slows microbial litter decomposition.

2. Material and methods

2.1. Litter decomposition experiment

A detailed description of our litter decomposition experiment was published in Wanek et al. (2010). Briefly, beech litter was collected at four different sites in Austria (Achenkirch (AK), Klausenleopoldsdorf(KL), Ossiach(OS), and Schottenwald(SW); refered to as litter types) in October 2008. Litter was cut to pieces of approximately 0.25cm^2, homogenized, sterilized twice by gamma[5] 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 after inoculation and stored dried at room temperature. Samples of 60g litter (fresh weight) were incubated

[5]greek gamma here

at 15 °C and 60% water content in mesocosms for a duration between 2 weeks to 15 month. For each litter type 5 replicas were removed and analyzed after 14, 97, 181 and 475 days.

Litter chemistry as analyzed 14 days after incubation is listed in table 1. C:N ratios between 1:41 and 1:58 and C:P ratios between 1:700 and 1:1300 were found, N:P ratios ranged between 1:15 and 1:30. No significant changes occurred during litter incubation except a slight decrease of the C:N ratio (1:41.8 to 1:37.4) found in the most active litter type (SW) after 15 month. Fe content 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.

2.2. 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 were 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 Henschler (1988)).

To determine soluble C, N, and P contents, 1.8g litter (fresh weight) were extracted with 50 ml 0.5M K_2SO_4. Samples were shaken on a reciprocal shaker with the extractant for 30 minutes, filtered with ash-free filters and frozen at -20 °C until analysis. To quantify microbial biomass C, N and P pools, sample were extracted under the same conditions after chloroform fumigation. 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), Phosphorous was determined photometrically.[6]

Substrate to consumer stoichiometric imbalances $X{:}Y_{inbal}$ were calculated as

$$X : Y_{inbal} = \frac{X : Y_{litter}}{X : Y_{microbial}} \tag{1}$$

[6] lit!!

where X and Y stand for one of the elements C, N, or P.

2.3. 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. Measurements of ambient air were performed before and after each measurement to assess possible leaks or base-line drifts IRGA. 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.

2.4. Enzyme activities

Measurements of potential exo-enzyme activities for cellulases, peroxidases and phenoloxidase were described by Leitner et al. (2011). Activities were determined with a series of micro-plate assays based on the hydrolysis of 4-methyl-β-D-cellobioside (cellulase) and L-3,4-dihydroxyphenylalanin (oxidative enzymes). Products of enzyme catalyzed reactions were detected photometrically (oxidative enzymes) or flourometrically (cellulase) (Marx, 2001; Sinsabaugh, 1999; Kaiser et al., 2010).

2.5. 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).

Litter analyzed was sampled immediately after inoculation and after 3, 6, and 15 month incubation. 2-300 µg dried and finely ball-milled litter were heated to 600°C for 10 seconds in 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 cycling between m/z 20 and 300.[7]

Peaks were assignment was based on NiSt 05 MS library after comparison with reference material measured. 128 peaks were identified and selected for integration due to their hight abundance or diagnostic value, including 28 lignin and 45 carbohydrate derrived substances. For each peak between one and four dominant mass fragments selected for high abundance and specificity were integrated and converted to TIC peak areas by a multiplication with a MS response coefficient (Schellekens et al., 2009; **?**). For principal component analysis, unconverted areas were used. Peak areas are stated as % of the sum of all integrated peaks of a sample.

Relative peak areas are different from weight%, but allow tracing of accumulation/depletion of substance classes during decomposition (Schellekens et al., 2009).

We use the terms "accumulation" and depletion to refer to changes in litter composition and "degradation" to refer to the amount of lignin and carbohydrates decomposed.

A lignin to carbohydrates index was calculated to measure the 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 % lignin and cellulose degraded during decomposition according to equation 3, where TIC_{init} and TIC_{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.

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

We provide % of initial lignin and carbohydrate pools decomposed, % decomposed per % litter carbon mineralized and the ratio between the twodecomposition rates.

[7] maybe cite other paper for method?

2.6. Statistical analysis

All statistical analyses were performed with the software and statistical computing environment R using the package "vegan" (Oksanen et al., 2011). If not mentioned otherwise, results were considered significant when p <0.05. Due to the frequent of variance inhomogenities Welch anova and paired Welch's t-tests with Bonferroni corrected p limits were used. Principal component analysis was performed using vegan function "rda" scaling variables. All correlations refer to Pearson correlations. We calculated correlations between depletion and degradation rates found in this study with litter chemistry parametres and process data reported by Mooshammer et al. (2011) and Leitner et al. (2011).

3. Results

3.1. Mass loss, respiration and extractable organic carbon

Litter mass loss was not significant after 2 weeks and 3 month, significant for 2 litter types after 6 month. After 15 month, litter mass loss was significant for all litter types, and strongly correlated to litter N content (R=0.794, p=***). Detailed results were reported by (Mooshammer et al., 2011). After 15 month, between 5 and 12% of the initial dry mass was lost. This is less than reported in litter decomposition studies on other species, but in a similar range as recently reported for beech litter from an in-situ litterbag-study (Kalbitz et al., 2006) .

Highest respiration rates were measured after 14 days incubation (150-350 µg CO_2-C d-1 g-1 litter-C), dropped to rates between between 75 and 100 µg CO_2-C d-1 g-1 litter-C after 97 days. After 181 and 375 days, respiration rates for AK and OS further decreased, while SW and KL show a second maximal respiration after 181 days.

Soluble organic carbon content decreased between the first three harvests (14 to 181 days), to strongly increase after 475 days (fig. **??** 0.1 to 0.7 mg C g^{-1} d.w. were found after 14, 97 and 181 days, and increased to amounts between 1.5 and 4 mg/g after 375 days. After 14 and 97 days, the highest C content was found in SW litter followed by AK (see fig. 1. DOC content was loosely correlated to litter N content after 14 (R=0.69, p<0.001)

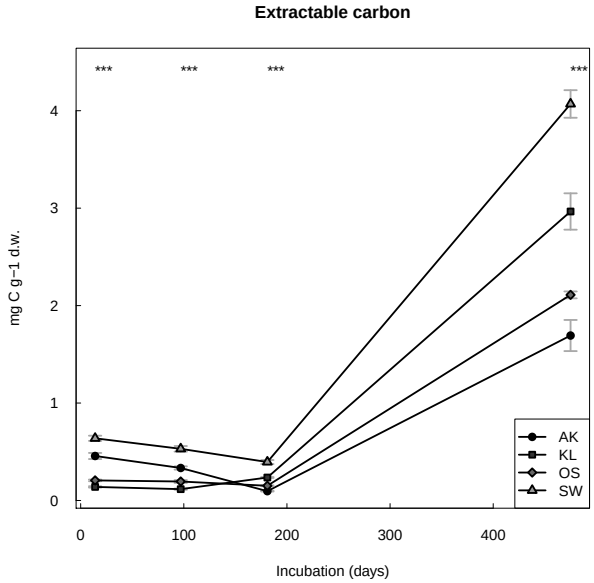

Figure 1: Extractable organic carbon. Error bars indicate standard errors (n=5).

and 97 days (R = 0.65, p =<0.01), they were strictly correlated after 181 days (R = 0.85, p=<0.001) and 375 days (R=0.90, p=<0.001).

3.2. Microbial Community

3.2.1. Microbial biomass abundance and stoichiometry

Microbial biomass contents ranged from 0.5 to 6 mg C, 0.05 to 5.5mg N and 0.05 to 3.5 mg P per g litter (d.w.). In KL and OS biomass buildup reaches a plateau after 3 month, AK and SW show further growth reaching a maximum of microbial C and N contents after 6 month (AK also for P). Microbial C:N ratios measured range between 1:6 and 1:18, C:P ratios between 1:8 abd 1:35, and N:P ratios between 1:0.5 and 1:3.5. Microbial C:N ratios (Fig. 2).

Litter microbial biomass is homeostatic during the first 6 month (no or marginally negative correlation between microbial stoichiometry and litter stoichiometry) (Mooshammer et al., 2011), but not after 15 month, when all three ratios show correlations (R 0.53 - 0.64, $p < 0.016$). Litter C:N ratios are tighly constrained after 3 (1:14.5 - 1:18.2) and 6 month

(1:6.9 - 1:9.0), but significantly different between the two time points. C:P and N:P ratios are less constrained, with the highest variance between litter from different sites after 3 month incubation (Fig. 2).

3.2.2. Potential enzyme activities

Absolute potential enzyme activities were correlated to litter N, respiration and other other decomposition processes (all R >0.8, p <0.001). For all enzymes and at all time points, SW showed the highest and AK the lowest activity. Cellulase was below detecten limit after 14 days, oxidatitve enzymes after 15 month. Cellulases activity is highest after 3 month and decreases between 97 and 181 days. Peroxidase and Peroxidase activities reach their maximum after 181 (fig. 3). After between 6 and 15 month, cellulase activity strongly increased. After 475 days, the activity of oxidative enzymes was below the detection limit [data not shown]

The ratio between the potential activities of cellulases and oxidative enzymes was lowest for AK at all time points. Microbial communities in AK litter invest more energy and nitrogen into degrading lignin and less into degrading carbohydrates than other litter types. (fig. 3)

3.3. Pyrolysis-GC/MS and Lignin content

3.4. Changes in litter chemistry

Litter pyrolysis products and different sites are reported in detail elsewhere (Kohl, in preparation). We found only minor changes during pyrograms during decomposition, differences between sites were were small but well preserved during decomposition. The high similarity allowed tracind small changes in lignin and carbohydrate abundance during decomposition.

When measured by pyr-GC/MS, lignin derrived compounds make up between 29 and 31 %TIC in the initial litter, with an increase of up to 3 %TIC over the first 3 month. Carbohydrate derrived pyrolysis products account for 26 to 29 %TIC in initial litter and decrease by up to 2.6 % during litter decomposition. Carbohydrate depletion and lignin

accumulation were coorelated (R = 0.47, p <0.01) in all samples measured. The initial (pyrolysis-) LCI index (applied to excludes influences of changes in the abundance of other pyrolysis products) ranges between 0.517 and 0.533. During decomposition, it increases by up to 8.7% of the initial value, with SW showing the highest and KL the lowest increase. This increase almost completely occurs over the first 6 month, with insignificant changes in both directions between 6 and 15 month incubation. Figure **??**[8] shows changes in the relative abundance of in pyrolysis products versus incubation time and accumulated respiration. Lignin to carbohydrate ratios in a similar range (increasing from 0.565 to 0.588 over 24 month) were reported for in situ oak litter decomposition by **?** using thermochemolysis. [9]

To check wether the sum of lignin and carbohydrate peaks represents trends of the majority of derrived from these substances, for each peak in each sample, we substracted the mean relative peak area of the respective peak in initial litter of the same litter type and applied a principal component analysis to the results. (fig. 4). The first two principal components represent 45.2% of the total variance. Initial litter samples cluster cluster in the bottom right corner of the graph with positive loadings on PCA 1 and negative loadings on PCA2. Decomposed samples are shifted versus fresh litter along different axis: While decomposed SW samples are in the bottom left quadrant of the samples, shifted along PCA 1 toward more negative values and indifferent along PCA2, decomposed AK samples are shifted along PCA2 towards more positive values and do not shift along PCA1. KL and OS show intermediate decomposition trends. Their decomposed samples are placed in the top left corner, combining both decomposition trends.

Pyrolysis products that are positioned in the bottom-right quadrant are depleted in all litter types, while products in the top left quadrant are accumulated in all litter types. Substances in the bottom left quadrant are depleted in AK and accumulated in SW, substances in the top-right quadrant show the opposite trend.

[8] check fig.

[9] I converted the L:C ratio stated by Snajdr to L/(L+C). This demonstrates a surprising coherence between quite different analytical methods, different peaks analyzed.

21 of 28 lignin markers cluster with the most negative loadings on both PCA1 and PCA2. The trends described herein therefore are based on on a consistent set of independently quantified lignin markers (fig.4. Carbohydrate products show diverging trends, with both accumulated and depleted compounds, representing the polymorphy of this compounds class in plant litter.

3.4.1. Amounts of Lignin and Carbohydrates degraded

During the first 6 month of litter decomposition, between one and 6% of the initial lignin pool and between 4 and 17% of the initial carbohydrate pool were degraded. Lignin decomposition was highest in AK and KL litter, while KL and SW decomposed the highest part of their carbohydrate pools. Lignin discimination (compared to carbohydrates) was highest in SW and lowest in AK litter. In AK litter, lignin molecules were 50% more likely to be decomposed than carbohydrates, while in SW litter carbohydrates were 10 times more likely to be decomposed (fig. 7).

Between 6 and 15 month, no further discrimination occurs, lignin and carbohydrate are degraded at the same rates and their content in pyrograms remains constant (fig. 5).

3.5. Correlation between litter chemistry, lignin decomposition, other processes

Table 2 provides linear regressions found between lignin and carbohydrate degradation, litter chemistry, microbial biomass and decomposition processes after 6 month incubation including data presented by Mooshammer et al. (2011) and Leitner et al. (2011). We found The lignin to cellulose degradation ratio was correlated to phenoloxidase to cellulase and peroxidase to cellulase enzymatic activity ratios (R=0.729 and R=0.863, p=?). Lignin accumulation and carbohydrate depletion were found to increase with enzymatic activities measured (including lignolytic enzymes) N, and P gross depolymerization rates but not with glucan depolymerization.

While carbohydrate degradation and depletion was correlated litter N content, C:N ratio and C:N imbalances. lignin degradation and accumulation were correlated to litter P, litter C:P and N:P ratios, C:P and N:P imbalances and extractable organic C and PO_4. High

lignin accumulation and carbohydrate depletion were also connected to wide C:N, C:P and N:P ratios.

Correlations litter Mn, Zn and Mg contents, lignin and carbohydrate decomposition found can be explained by intercorrelations of these elements with litter N and P contents.

4. Discussion

4.1. Early lignin decomposition

Our results demonstrates the relevance of lignin degradation during the the first 6 month of beech litter decomposition. Lignin decomposition rates depend on litter quality and vary between only marginal amounts to bulk carbon mineralization rates during the first 6 month of incubation and uniformly reach average carbon decomposition rates between 6 an 15 month. Lignin discrimination in carbon mineralization ranges from no discrimination to lignin being 10 times less likely to be degraded. We can therefore confirm that early lignin decomposition rates are by far underestimated (Klotzbücher et al., 2011) applying a complementary anlytical approach. Unliky their results, we found no significant decreases in lignin contents during early decomposition. Also, lignin degradation rates were constant or increased during decomposition.

4.2. Temporal changes in lignin decomposition

During the first 6 month, lignin accumulation strongly varies between beech litter from different sites. As the original microbial community was destroyed and replaced with a commun innoculum for all sites, differences found result from differences in litter chemistry. Between 6 and 15 month lignin contents remained constant in all litter types, indicating that lignin is not degraded slower than other litter compounds, but differences in lignin contents aquired during the first 6 month remain.

The absolute amount of lignin and the ratio in which lignin and carbohydrates are degraded during the first 6 month was ranges from marginal amounts (SW: not significant different from 0) to lignin degraded at same rate as bulk litter. Between 6 and 15 month,

lignin degradation rates reach bulk litter carbon mineralization rates in all litter types, differences in lignin contents that developed during the frist 6 month are preserved at least until 15 month of incubation.

Klotzbücher et al. (2011) suggests a change in decoposition dynamics after 100 to 200 days of incubation, after which lignin decomposition rates due to lack of labile carbon. They also report a correlation between respiration rates and extractable carbon after this change. We can confirm the correlation between extractable carbon and respiration after 181 days, see both processes adapt to litter N content. Our data agrees that respiration is limited by labile carbon availability, but the production of labile carbon itself seems controled by litter N content. As the process of degrading macromolecular is conducted by extracellular enzymes and is more N intensive that the mineralization of labile carbon, depolymerization is the point in the decomposition process, where a N limitation would be most likely to become effective.

4.3. Resource controls on carbon chemistry

Recent literature suggests the availability of labile carbon (Klotzbücher et al., 2011) or redox-active micronutrients like Mn and Fe [10] or low N availability (Craine et al., 2007) as key controls for (late) lignin degradation.

Mn and Fe are important co-factors of oxidative enzymes involved into lignin decomposition. Several authors suggest Mn availability limits lignin degradation and is rate limiting in late litter decomposition stages. Mn and Fe contents strongly vary between the different litter types used. An experiment studying aquatic decomposition of the same litter suggested Mn contents controll decomposition processes like enzyme activties. We can exclude micronutrient availability as a limiting factor for lignin decomposition in our experiment, because Mn and Fe contents are lowest in the litter with the highest lignin decomposition (AK, table 1). Low contents of these Elements would explain inhibited, not enhance lignin decomposition.

[10] Lit.!

Most decomposition processes (enzyme activities and depolymerization rates) were correlated to litter nitrogen content and C:N ratio. We find lignin decomposition rates is negatively correlated to these processes, but not to litter N contents. Also, no little lignin decomposition occurs OS litter, which has a N content just as low as AK litter. The correlation found between LCI change and litter N and C:N seems based on an enhanced carbohydrate degradation, but no inhibition in lignin decomposition.

Availability of extractable carbon was highest in AK and SW litter, two sites which have the highest and the lowest lignin decomposition trends. Therefore, extracable carbon can be excluded as a control over lignin decomposition.

Most interestingly, lignin degradation activities are tightly correlated to litter/microbe C:P imbalances. These imbalances are not exclusively based in litter chemistry, but are also controled by microbial comunity. C:P imbalance reaches a maximum after 3 month whil C:N imbalances have a maximum after 6 month. Lignin decomposition found (especially changes in LCI) correlate to C:N imbalance. This indicantes that either elevated N demand triggers lignin decomposition or lignin decomposition allows additional N to be incorporated into the microbial community. However, the correlation to C:P imbalances are stricter ($R=0.83$, $p < 0.001$) and C:P imbalances develop earlier (after 3 month).

High C:N and C:P imbalances indicate C surpluss. Following the hypothesis that lignin decomposition is carbon limited, the carbon surplus available to microorganism might be invested into lignin degradation.

5. Conclusions

6. Acknowledgements

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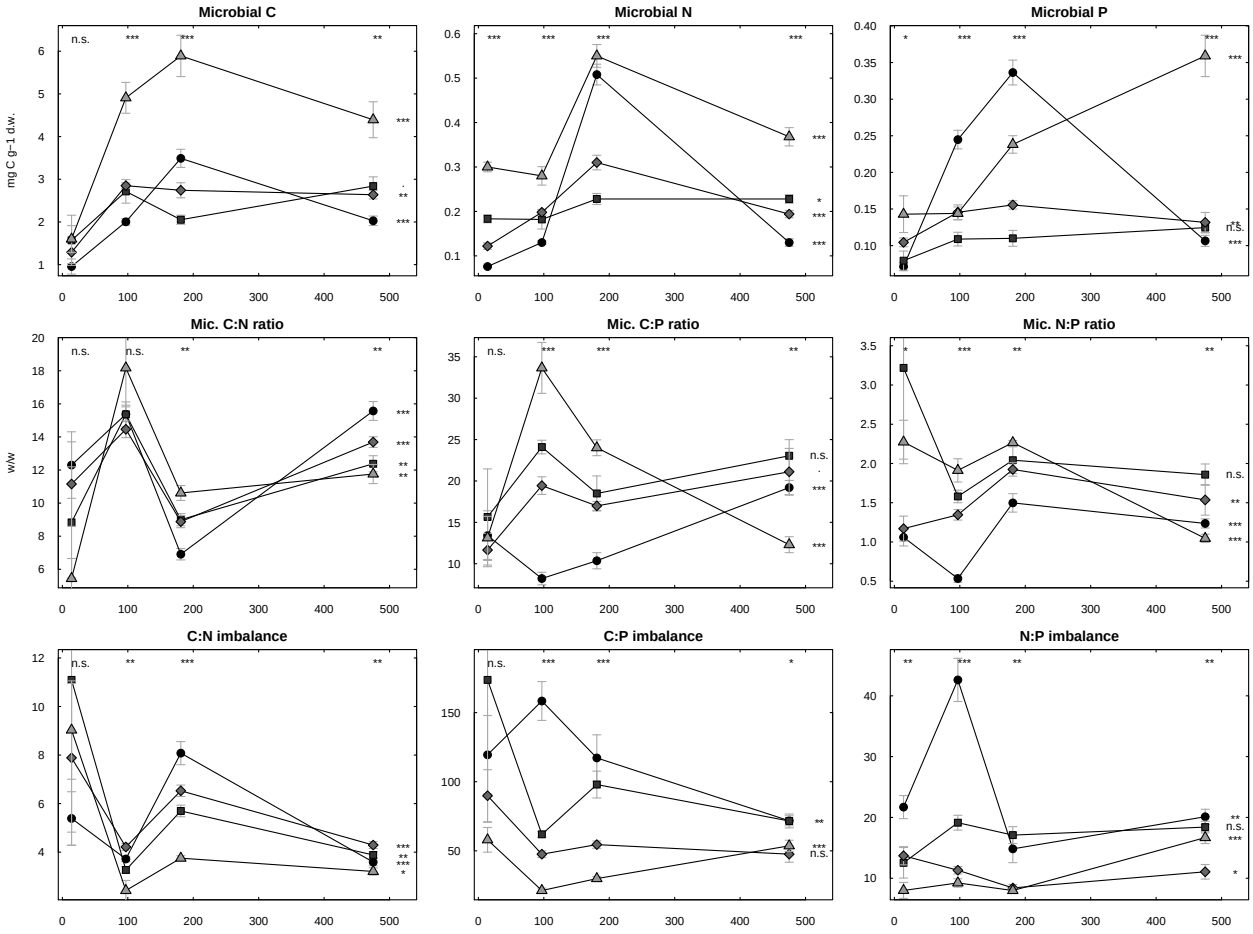

Figure 2: Microbial biomassm, microbial stoichiometry and resource:consumer stoichiometric imbalance. Error bars indicate standard errors (n=5).

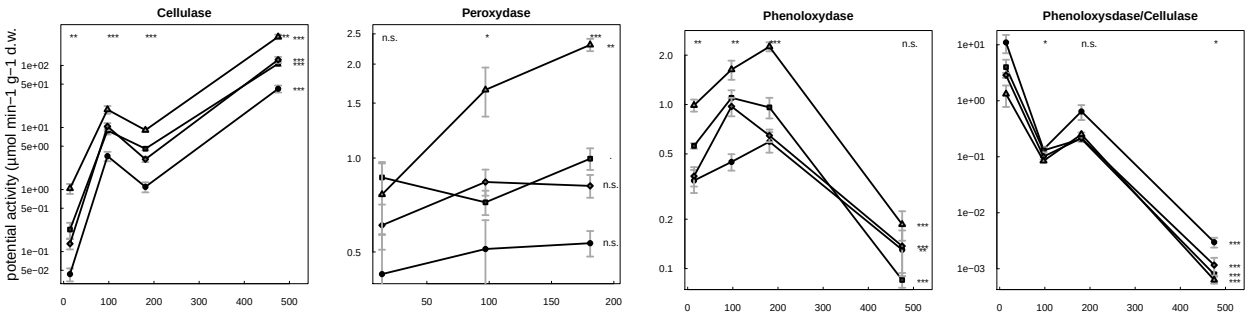

Figure 3: Potential eco-enzyme activities

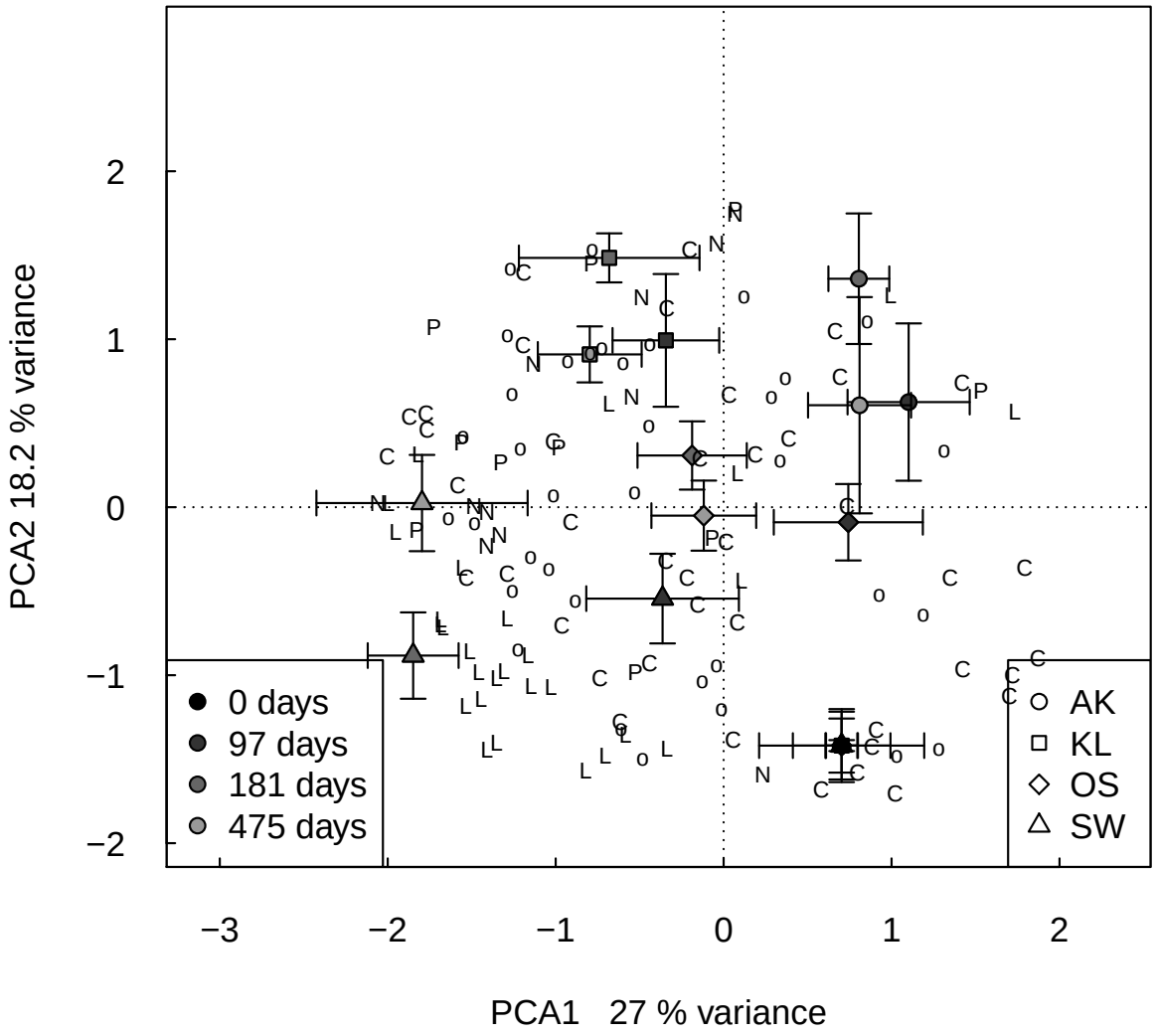

Figure 4: Principal component analysis of the relative peak areas of 128 pyrolysis products, corrected by their abundance before incubation. Symbols indicate samples measured (means per litter type and time point), error bars indicate +/- 1 SE (n=4-5). Letters indicate pyrolysis products (L - lignin, P - other phenolic compounds, C - carbohydrates, N - nitrogen containing compounds, open circles: nonspecific and unidentified peaks). Factor loadings of pyrolysis products were multiplied by 2.5 for better readability.

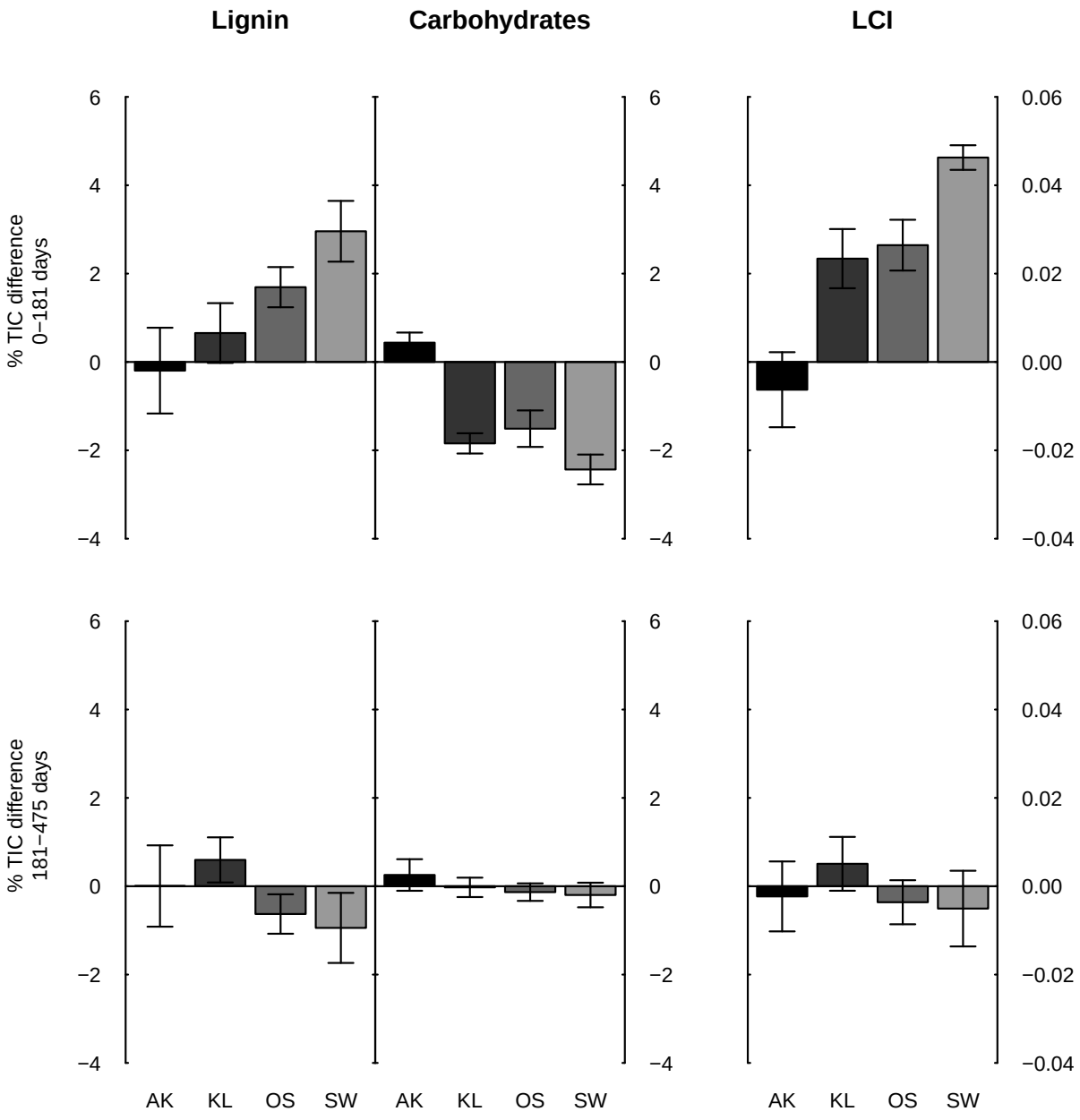

Figure 5: Accumulation and depletion of lignin and carbohydrates

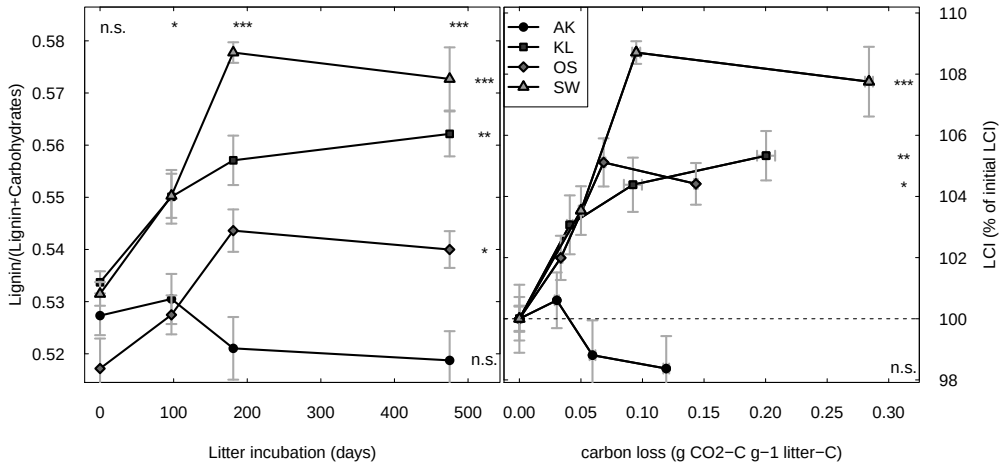

Figure 6: Develoment of the LCI (lignin/(lignin+carbohydrates)). Errorbars indicate standard errors (n=4-5). The dashed line indicates a constant ratio between lignin and carbohydrates (i.e. no preferential decomposition of carbohydrates.)

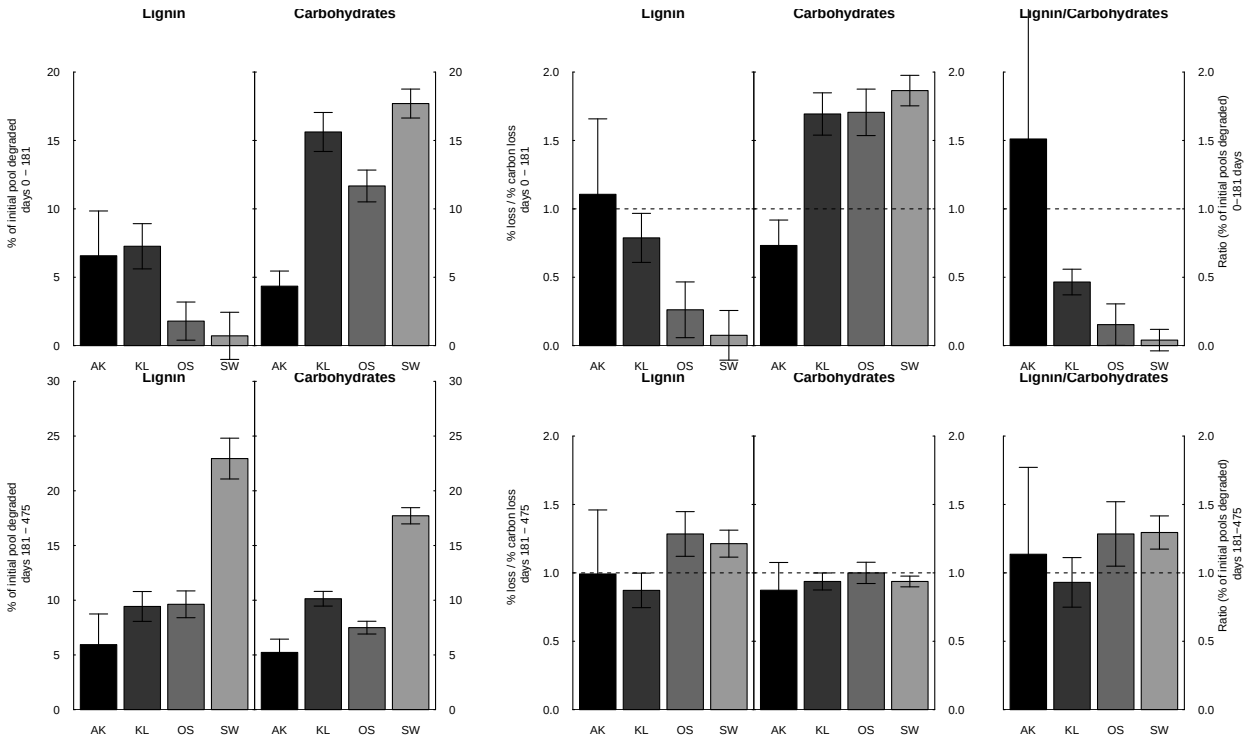

Figure 7: Carbon loss corrected amounts of lignin and carbohydrates degraded. Carbon loss was calculated based on accumulated respiration. Error bars indicate standard errors (n=4-5). The dashed line marks no discrination between lignin, carbohydrates and bulk carbon loss.

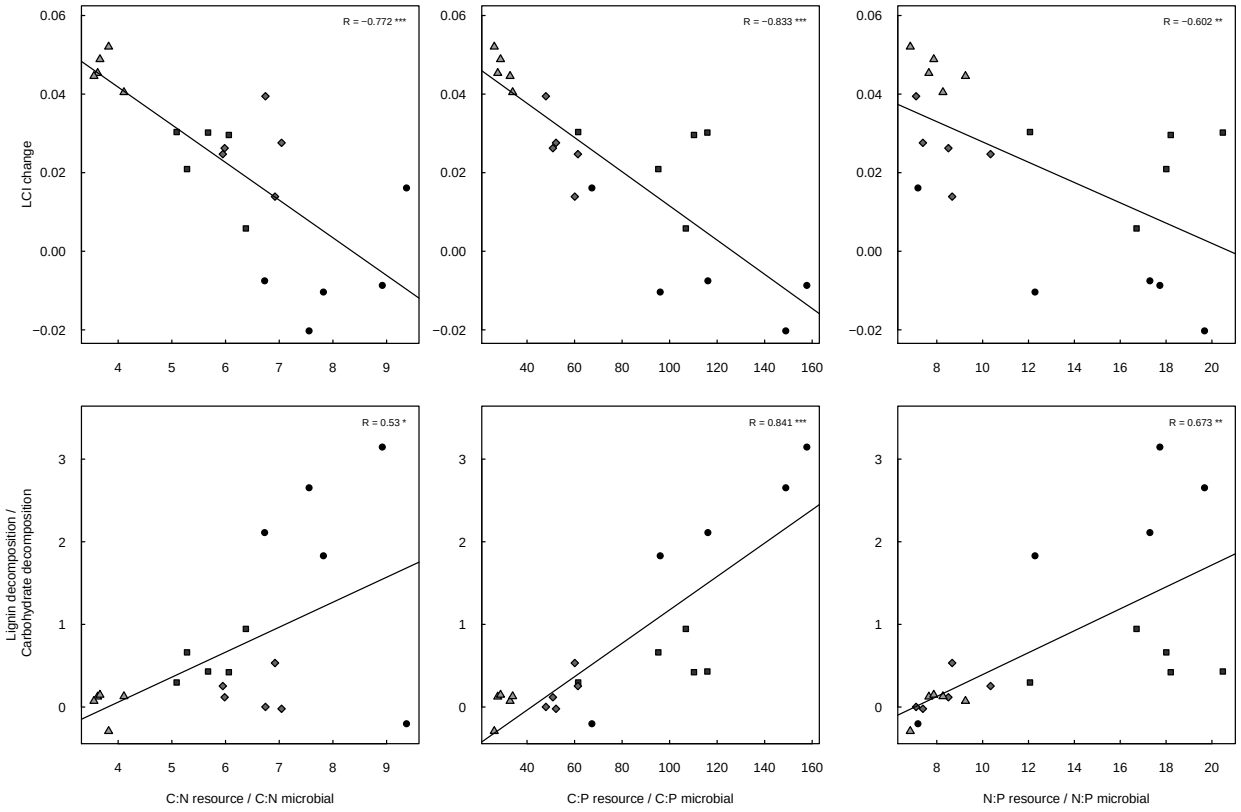

Figure 8: Correlations between Lignin accumulation during the first 6 month of litter incubation and stoichiometric resource:consumer imbalances

Table 1: Litter stoichiometry and mineral elemental contents measured after 14 days incubation. Standard errors are stated in brackets (n=5). C extr stands for extractable carbon.

	AK	(SE)	KL	(SE)	OS	(SE)	SW	(SE)
C (% d.w.)	50.86	(0.39)	49.41	(0.53)	48.15	(0.39)	48.90	(0.34)
C extr (% d.w.)	45.66	(3.13)	13.92	(0.70)	20.61	(0.86)	63.81	(2.67)
N (% d.w.)	0.878	(0.012)	0.938	(0.012)	0.806	(0.013)	1.172	(0.016)
P (% d.w.)	0.040	(0.000)	0.030	(0.000)	0.052	(0.002)	0.070	(0.000)
C:N (w/w)	57.86	(0.57)	52.60	(0.49)	59.97	(0.72)	41.78	(0.76)
C:P (w/w)	1282	(21)	1548	(25)	905	(15)	699	(9)
N:P (w/w)	22.17	(0.47)	29.45	(0.60)	15.10	(0.29)	16.75	(0.39)
K (mg g-1)	0.26	(0.00)	0.54	(0.00)	0.21	(0.00)	0.55	(0.00)
Ca (mg g-1)	1.33	(0.01)	1.26	(0.01)	1.63	(0.01)	1.23	(0.01)
Mg (mg g-1)	0.27	(0.00)	0.14	(0.00)	0.20	(0.00)	0.15	(0.00)
Fe (ppm)	210	(2)	208	(4)	453	(12)	192	(4)
Mn (ppm)	172	(2)	1430	(10)	776	(9)	2137	(51)
Zn (ppm)	30.8	(0.4)	33.0	(0.3)	36.0	(1.0)	42.4	(0.7)

Table 2: Correlation (R) between Lignin and Carbohydrate degration with litter chemistry, microbial community and decomposition processes. Significant ($p<0.05$) correlations are printed bold. Data taken from Mooshammer et al. (2011); Leitner et al. (2011); **?**

	L acc	Ch acc	LCI diff	L dec	C dec	L resp	C resp	L/C dec	Per/C	Phen/C
Massloss	0.291	-0.15	0.245	-0.339	0.0964	-0.211	0.0965	-0.0818	0.0534	0.048
Respiration	0.333	**-0.723**	**0.606**	-0.0747	**0.732**	-0.19	**0.507**	-0.364	-0.362	-0.268
Cellulase activity	**0.657**	**-0.76**	**0.803**	-0.424	**0.789**	**-0.493**	**0.611**	**-0.588**	**-0.539**	-0.436
Phenoloxidase activity	**0.632**	**-0.669**	**0.737**	-0.412	**0.708**	**-0.448**	**0.503**	**-0.484**	-0.356	-0.305
Peroxidase activity	**0.599**	**-0.588**	**0.677**	-0.41	**0.618**	-0.439	0.412	-0.435	-0.302	-0.173
Phenoloxidase/Cellulase	-0.359	**0.642**	**-0.556**	0.178	**-0.66**	0.331	**-0.698**	**0.729**	**0.875**	**1**
Peroxidase/Cellulase	**-0.538**	**0.707**	**-0.709**	0.377	**-0.713**	**0.554**	**-0.731**	**0.863**	**1**	**0.875**
Protein depolymerization	**0.454**	-0.332	**0.455**	-0.253	0.413	-0.302	0.14	-0.289	-0.166	-0.0549
Amino acid immobilization	0.321	-0.0878	0.247	-0.271	0.11	-0.192	-0.0604	-0.0592	0.0838	0.188
N mineralization	**0.466**	**-0.664**	**0.65**	-0.159	**0.703**	-0.295	0.45	-0.384	-0.367	-0.282
NH4 immobilization	**0.507**	**-0.681**	**0.68**	-0.207	**0.716**	-0.342	**0.477**	-0.42	-0.362	-0.259
Nitrification	**0.587**	**-0.707**	**0.732**	-0.377	**0.721**	-0.431	**0.565**	**-0.497**	-0.45	-0.369
NO3 immobilization	**0.596**	**-0.658**	**0.708**	-0.381	**0.688**	-0.432	**0.519**	**-0.5**	-0.452	-0.374
P mineralization	**0.665**	**-0.55**	**0.684**	**-0.544**	**0.59**	**-0.58**	0.387	**-0.479**	-0.255	-0.212
P immobilization	0.198	0.00338	0.135	-0.139	0.0411	-0.155	-0.189	-0.0669	0.0137	0.0365
Glucan depolymerization	0.206	-0.257	0.275	-0.19	0.221	-0.247	0.225	-0.249	-0.257	-0.129
Glucose consumption	0.197	-0.341	0.319	-0.111	0.324	-0.202	0.244	-0.225	-0.212	-0.0394
NH4	**0.637**	**-0.647**	**0.732**	-0.397	**0.699**	**-0.446**	**0.456**	**-0.479**	-0.393	-0.316
NO3	0.128	**-0.504**	0.359	0.0818	**0.511**	0.00814	0.375	-0.27	-0.382	-0.291
PO4	**0.623**	-0.421	**0.572**	**-0.684**	0.367	**-0.615**	**0.515**	**-0.455**	-0.198	-0.171
C litter	**-0.545**	**0.506**	**-0.578**	**0.589**	**-0.45**	**0.631**	**-0.704**	**0.702**	**0.581**	**0.525**
N litter	0.354	**-0.517**	**0.503**	-0.14	**0.546**	-0.189	0.286	-0.201	-0.159	-0.119
P litter	**0.682**	-0.222	**0.517**	**-0.75**	0.204	**-0.686**	0.211	**-0.496**	-0.16	-0.0728
C:N litter	-0.405	**0.586**	**-0.57**	0.173	**-0.616**	0.234	-0.36	0.271	0.242	0.195
C:P litter	**-0.636**	0.174	**-0.453**	**0.758**	-0.136	**0.655**	-0.234	0.425	0.0805	0.049
N:P litter	**-0.512**	-0.0287	-0.264	**0.718**	0.079	**0.583**	-0.107	0.324	-0.0192	-0.0316
K litter	0.391	**-0.502**	**0.532**	-0.148	**0.558**	-0.299	0.268	**-0.453**	**-0.455**	-0.277
Ca litter	0.0212	0.0452	-0.0432	-0.214	-0.119	-0.142	0.245	-0.138	-0.0295	-0.13
Mg litter	**-0.578**	**0.747**	**-0.764**	0.346	**-0.782**	**0.548**	**-0.659**	**0.814**	**0.794**	**0.594**
Fe litter	0.0933	-0.117	0.0924	-0.235	0.0469	-0.217	0.393	-0.258	-0.187	-0.235
Mn litter	**0.72**	**-0.66**	**0.8**	**-0.535**	**0.692**	**-0.661**	**0.509**	**-0.76**	**-0.612**	-0.417
Zn litter	**0.668**	-0.428	**0.638**	**-0.577**	**0.451**	**-0.622**	0.328	**-0.616**	**-0.47**	-0.327
Cmic	**0.554**	-0.259	**0.46**	**-0.496**	0.281	-0.405	0.129	-0.221	-0.0407	-0.0604
Nmic	0.257	0.162	0.0575	-0.34	-0.167	-0.183	-0.254	0.0925	0.298	0.269
Pmic	-0.162	**0.592**	-0.422	-0.0163	**-0.6**	0.176	**-0.647**	**0.511**	**0.674**	**0.567**
C:N mic	**0.666**	**-0.758**	**0.799**	-0.423	**0.807**	**-0.511**	**0.657**	**-0.609**	**-0.596**	**-0.584**
C:P mic	**0.692**	**-0.787**	**0.834**	**-0.468**	**0.818**	**-0.557**	**0.694**	**-0.671**	**-0.648**	**-0.564**
N:P mic	**0.582**	**-0.729**	**0.74**	-0.406	**0.733**	**-0.502**	**0.685**	**-0.669**	**-0.671**	**-0.545**
extractable C	**0.609**	**-0.766**	**0.782**	-0.364	**0.793**	-0.443	**0.593**	**-0.538**	**-0.484**	-0.392
C:N imbalance	**-0.56**	**0.81**	**-0.772**	0.28	**-0.851**	0.386	**-0.662**	**0.53**	**0.56**	**0.564**
C:P imbalance	**-0.817**	**0.663**	**-0.833**	**0.748**	**-0.653**	**0.794**	**-0.691**	**0.841**	**0.67**	**0.575**
N:P imbalance	**-0.724**	0.351	**-0.602**	**0.807**	-0.313	**0.763**	**-0.455**	**0.673**	0.41	0.301

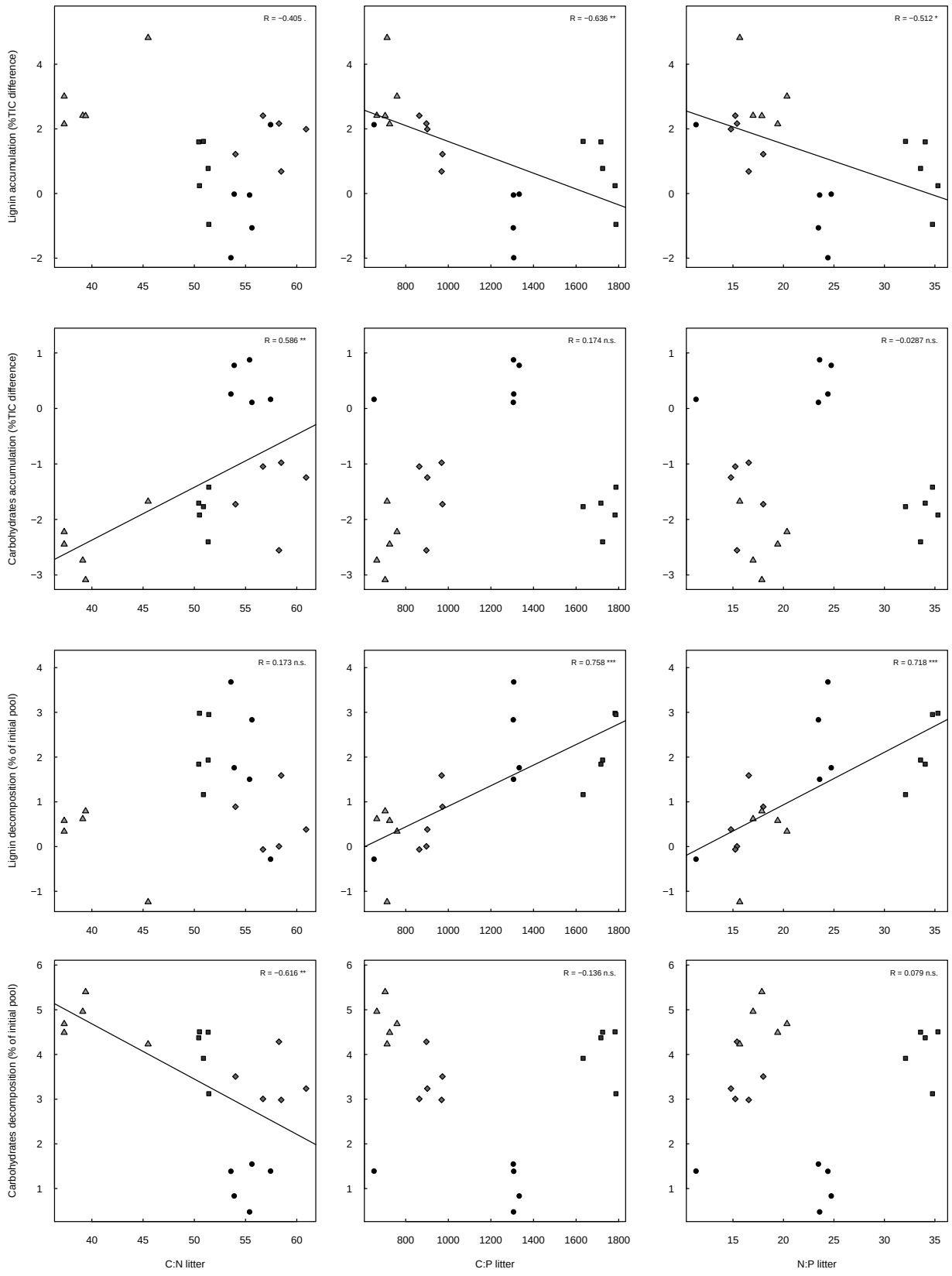

Figure 9: Correlations between Lignin and Carbohydrates accumulation and decomposition during the first 6 month of litter incubation and litter C:N:P ratios

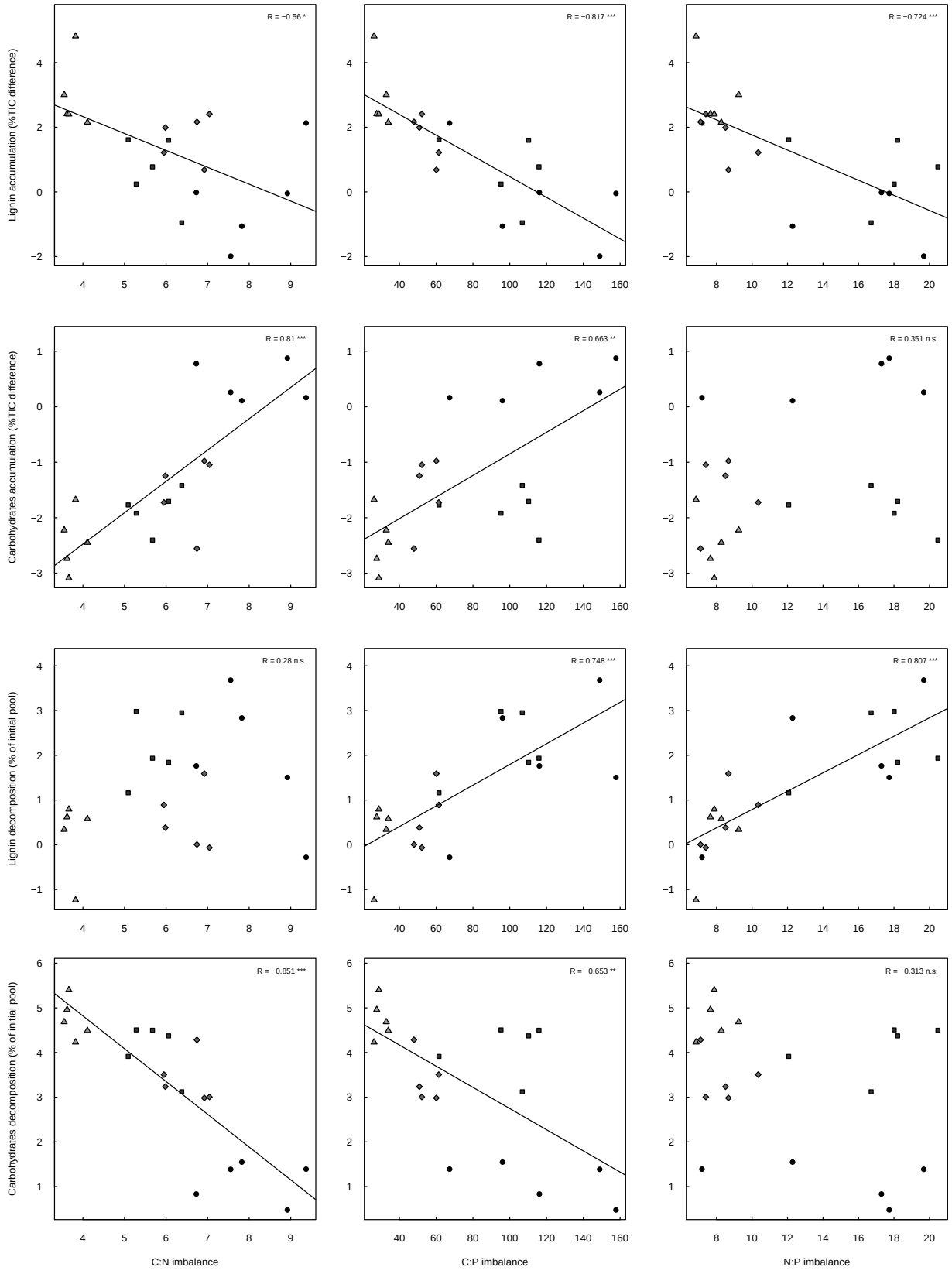

Figure 10: Correlations between Lignin and Carbohydrates accumulation and decomposition during the first 6 month of litter incubation and stoichiometric resource:consumer imbalances