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

Lukas Kohl[a], Wolfgang Wanek[a], Katharina Keiblinger[b,1], Sonja Leitner[a,1], Maria Mooshammer[a], Ieda Hämmerle[a], Lucia Fuchslueger[a], Jörg Schnecker[a], Sophie Zechmeister-Boltenstern[b,1], Andreas Richter[a]

[a]*Department of Chemical Ecology and Ecosystem Research, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria*
[b]*Federal Research and Training Centre for Forests, Natural Hazards and Landscape, Department of Soil Biology, Seckendorff-Gudent-Weg 8, A-1131 Vienna, Austria*

**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.

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 litter, leading to different niveaus of lignin accumulation. Between 6 and 15 month, no lignin

[1]Current adress: Institute for Soil Science, University of Natural Resources and Life Sciences, Peter Jordan-Straße 82, A-1190, Vienna, Austria

discrimination was found, but different lignin contents aquired earlier reminded.

[results]

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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 were repeatedly criticize as unspecific for lignin determination (Hatfield and Romualdo, 2005). 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. Experiments 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;

[2] more lit.

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

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 stoecheometric 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.

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

[4]more lit?

fauna and keep climatic conditions constant.

We hypothesize that

(1) 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.

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

(3) Lignin decomposition yields less energy than carbohydrate decomposition, therefore carbon loss and decomposition process rates are lower in litter that decomposes more lignin.

## 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 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

[5]greek gamma here

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 (Schinner, 1996). 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

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

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

[6] lit!!

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-$\beta$-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

[7] maybe cite other paper for method?

a MS response coefficient (Schellekens et al., 2009; Kuder and Kruge, 1998). 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.

### *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 (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. **??**. DOC content was loosely correlated to litter N content after 14 (R=0.69, p<0.001) 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 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. 1).

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, all $p < 0.002$, $H_{C:N}=2.01$, $H_{C:P}=1.68$, $H_{N:P}=2.29$). Microbial 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. 1).

*3.2.1. 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. 2). 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. 2)

*3.3. Pyrolysis-GC/MS and Lignin content*

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]

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. 4).

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. **??**).

### *3.4. Correlation between litter chemistry, lignin decomposition, other processes*

Table 3 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

[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.

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.

## 4. Discussion

Our experimental approach allows to single out litter quality and it's influence on the development of the microbial decomposer community as the sole source of the differences in decomposition processes found, while excluding fauna, climate and the initial microbial community as controlling factors. By exploiting intra-specific differences in beech litter stoichiometry, we were able to minimize differences in the composition of initial lignin and carbohydrate contents while exploring the effect of different litter nutrient contents. Therefore, we can attribute different levels of carbohydrate-over-lignin preference encountered to the intrinsic qualities of different litter types used.

Contradicting traditional concepts of litter decomposition (**?**)Berg1982), our results demonstrate that relevant amounts of lignin are degraded during the the first 6 month of litter decomposition. Lignin decomposition rates found during this early stage depend on litter quality and ranges from non-significant amounts decomposed to degradation at bulk carbon mineralization rates (i.e. no discrimination against lignin). We can therefore confirm that early lignin decomposition rates are by far underestimated, as proposed by Klotzbücher et al. (2011), with complementary analytic approach. Contrasting their results, we found no significant decreases in lignin contents and constant or increasing lignin degradation rates during early decomposition. Additionally, we found a change in controls over lignin discrimination after this initial period. While the preference of carbohydrate over lignin decomposition was

controlled by litter chemistry over the first 6 month, all components of litter were degraded at similar rates thereafter.

Several control mechanisms of litter chemistry over (late) lignin decomposition were proposed over the last years. In the traditional model for chemical changes during litter decomposition **?** suggest lignin is decomposed only if its high content in litter limits the decomposition of other substrates. We found differences in initial lignin contents below 10%, and lignin contents of sites with high initial lignin decomposition rates were not higher than that of sites with low rates.

Mineral elements are co-factors of oxidative enzymes involved into lignin decomposition, and Mn availability was suggested to limit mass loss rates during lignin-rich late decomposition stages [10]. While Mn and Fe contents strongly vary between litter collected at different sites, we can exclude their 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, see tab. 1). Low contents of these Elements would explain inhibited, not enhance lignin decomposition.

Relative decomposition rates of Lignin were strictly correlated to C:P imbalances and a number of P pools analyzed. Correlation was highest when lignin decomposition was compared to resource:consumer C:P ratios. C:N imbalances were also found correlated to carbohydrate-over-lignin discrimination, but linked stronger to carbohydrate decomposition stimulation than lignin decomposition inhibition.

Strong evidence already exists that labile carbon and nitrogen availability control (late) lignin decomposition. Beech litter lignin was shown to occlude significant amounts of nitrogen during it's polymerization [11]. The 'nitrogen mining hypothesis' (Craine et al., 2007) suggests that microbial degradation of recalcitrant carbon compounds is directed to access nitrogen, not carbon, and several experiments found lignin decomposition inhibited by N fertilizationcit.. Nitrogen starvation was also shown to trigger lignin decomposition in cul-

[10] cit.

[11] citation!

tivation studies. However, it is unclear to which extend results gathered from the addition of readily available nitrogen can be applied to different levels of leaf protein content. On the other hand, lignin decomposition was suggested to be inhibited when little labile carbon is available. Cultivation experiments suggest, that lignin does not provide sufficient energy to maintain the decomposer's metabolism without the input of other carbon sources [12]. Klotzbücher et al. (2011) found highest lignin decomposition rates immediately after the manipulation of litter (i.e. draught-rewetting during experimental setup), but no increase in lignin decomposition activities from fresh to 3-year old litter used. In our experiment, amounts of extractable carbon were highest in AK and SW litter, two sites which have the highest and the lowest lignin decomposition trends. Therefore, extractable carbon can be excluded as a control over lignin decomposition.

A stimulation of lignin decomposition by a high P imbalance or a delay of lignin decomposition under high P availability, as indicated by the high correlation to P pools found, was not reported yet. We obseved a N- and P- co-limited situation during early litter decomposition, in which lignin is degraded either to access additional nutrients or as a mean to use a C surplus in a N and P limited situation.

Between 6 and 15 month lignin contents remained constant in all litter types. This indicates that lignin is not degraded slower than other litter compounds, but differences in lignin contents acquired during the first 6 month remain. The controls which lead to differences in the extend of lignin discrimination over the first six month are no longer predominant between 6 and 15 month.

This change in decomposition dynamics corresponds to change from early to late decomposition (Berg, B. & McClaugherty, 2008), although mass loss rates found after 6 month (6-10%) are by far lower than those proposed.

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 decompo-

[12] citation

sition 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.

Klotzbücher et al. (2011) suggests a change in decomposition dynamics after 100 to 200 days of incubation, after which lignin decomposition rates decrease 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 controlled 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, de-polymerization is the point in the decomposition process, where a N limitation would be most likely to become effective.

## 5. Conclusions

Our results further question the concept that lignin decomposition is inhibited until late decomposition. Our results support the hypothesis that the decomposition of recalcitrant compounds depends more on ambient factors than on intrinsic chemical properties.

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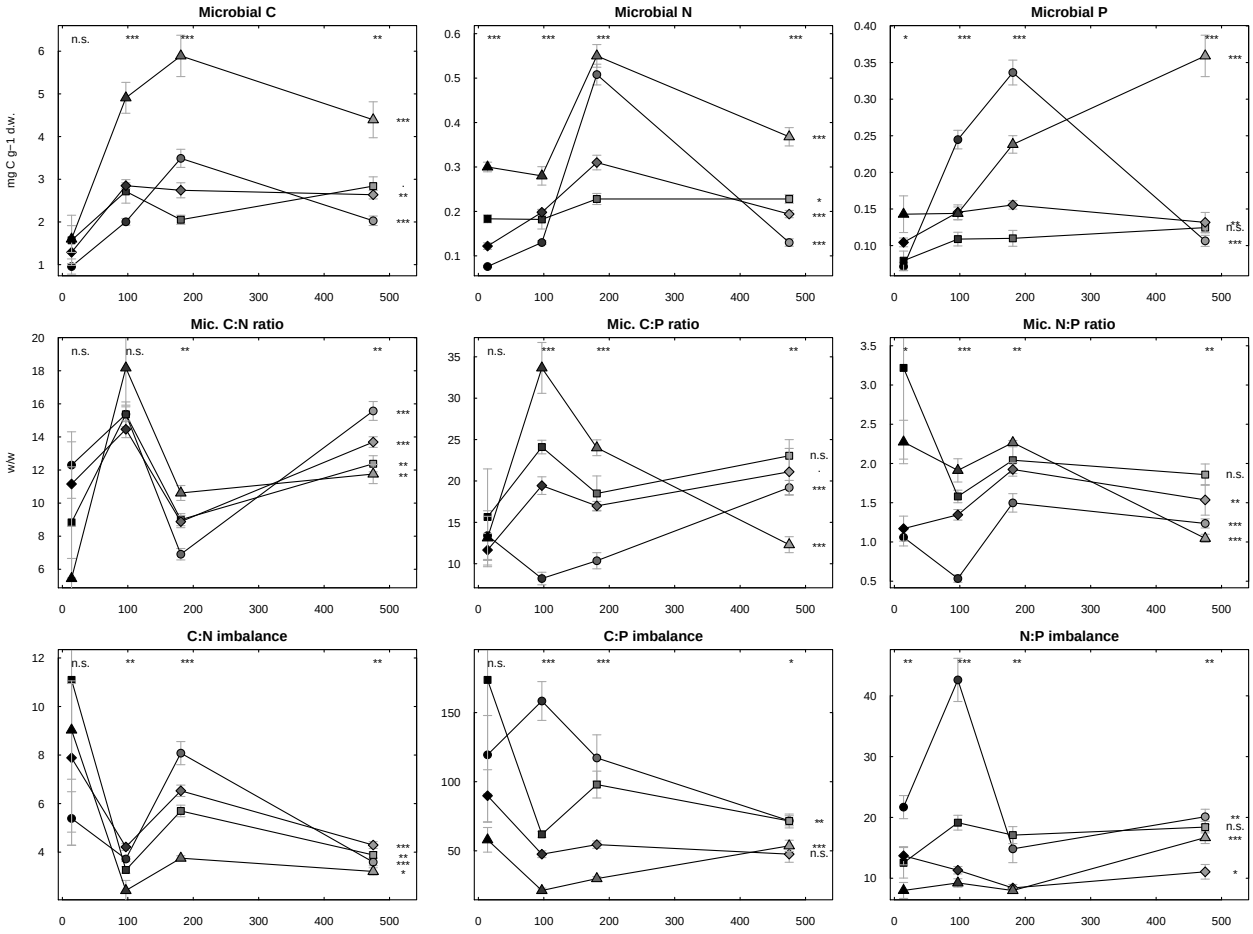


Figure 1: Microbial biomassm, microbial stoichiometry and resource:consumer stoichiometric imbalance. Error bars indicate standard errors (n=5).

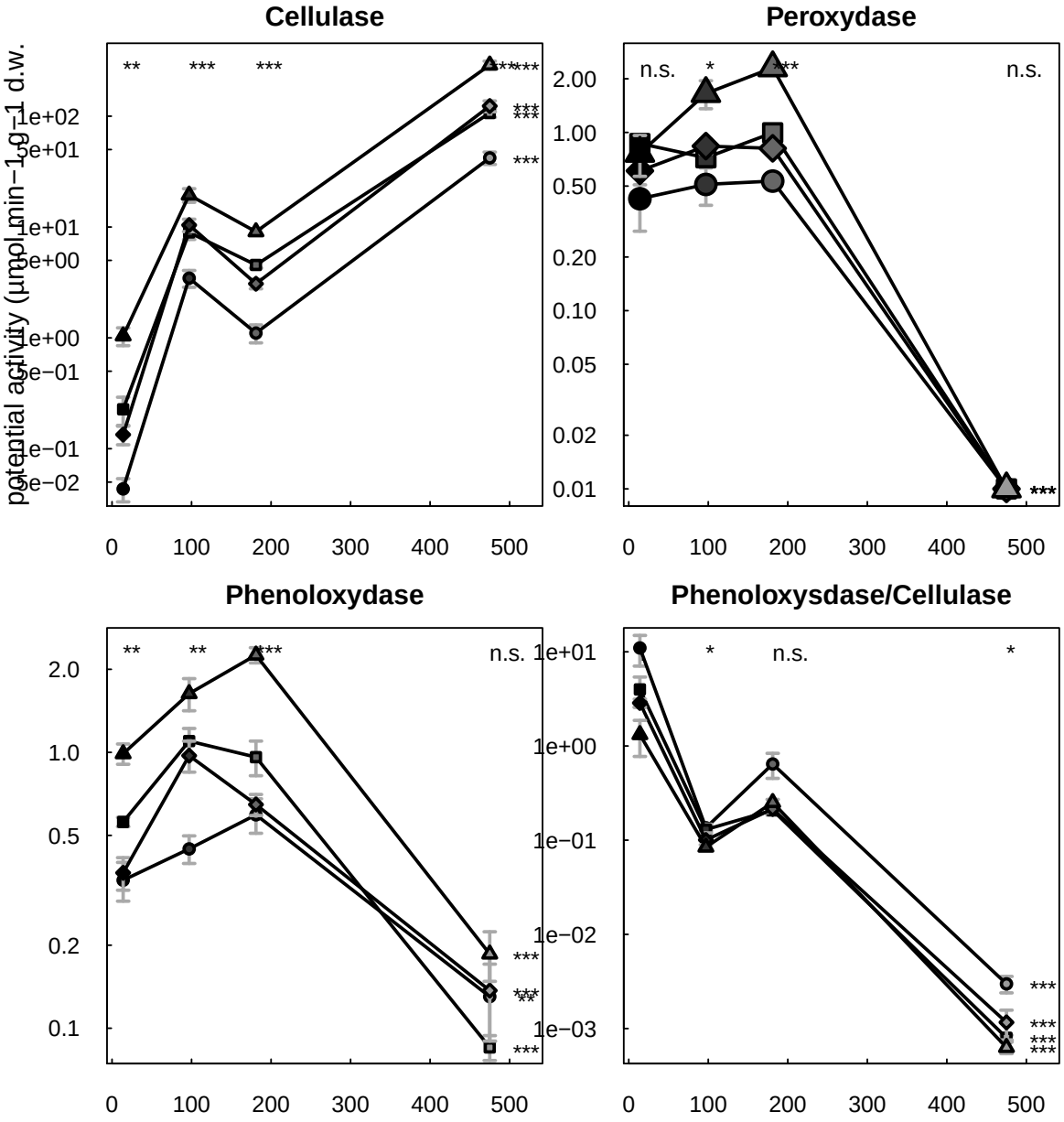


Figure 2: Potential eco-enzyme activities

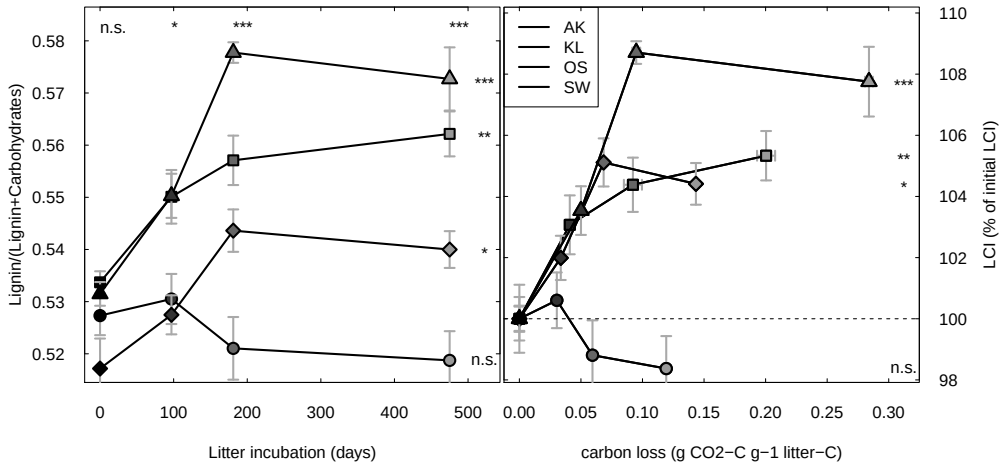


Figure 3: Develoment of the LCI (lignin/(lignin+carbohydrates)). Errorbars indicate standard errors (n=4-5). The dashed line indicates a constant ratio between lignin and carbohydrates (i.e. no preferential decomposition of carbohydrates. )

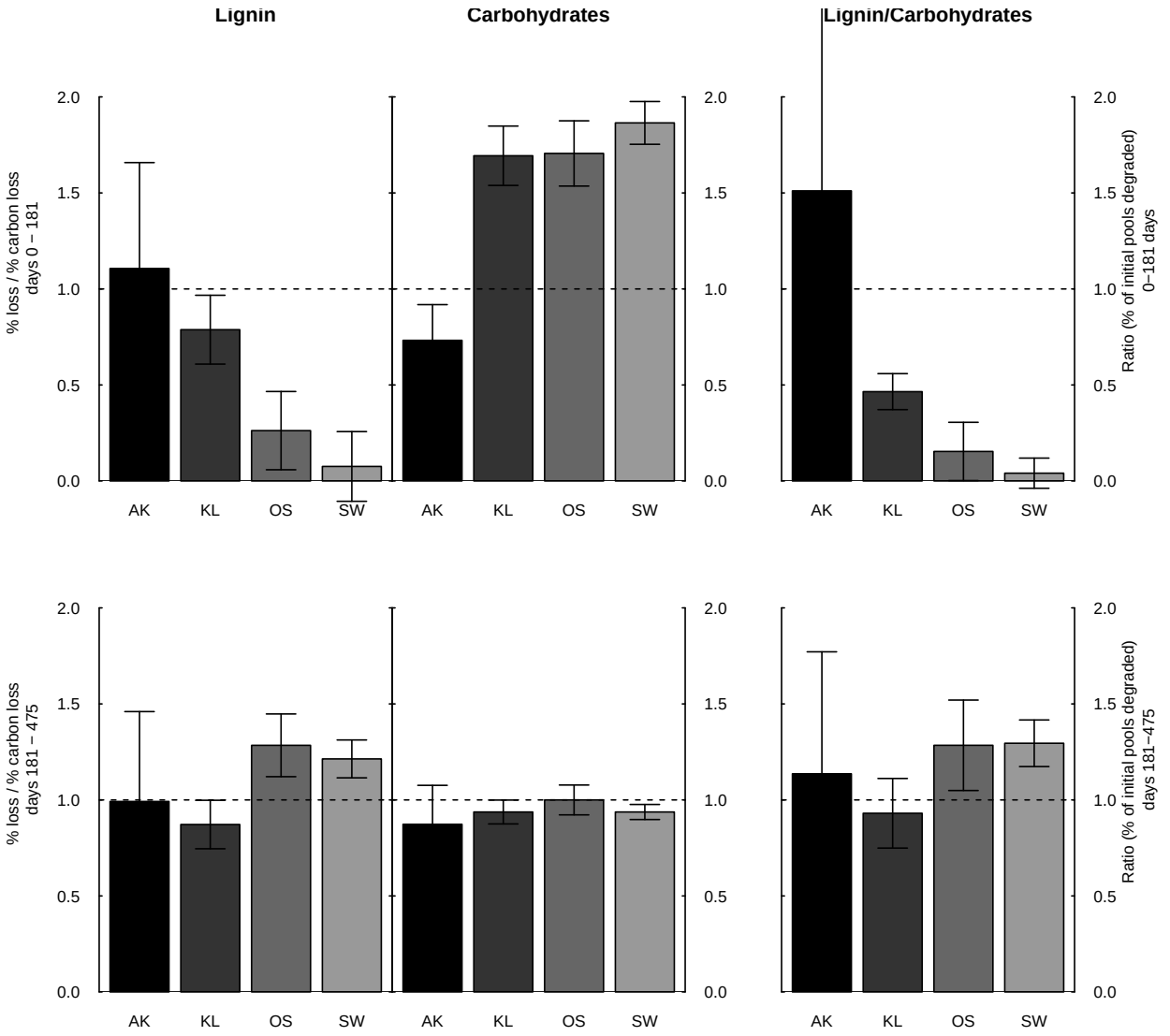


Figure 4: Carbon loss corrected amounts of lignin and carbohydrates degraded. Carbon loss was calculated based on accumulated respiration. Error bars indicate standard errors (n=4-5). The dashed line marks no discrination between lignin, carbohydrates and bulk carbon loss.

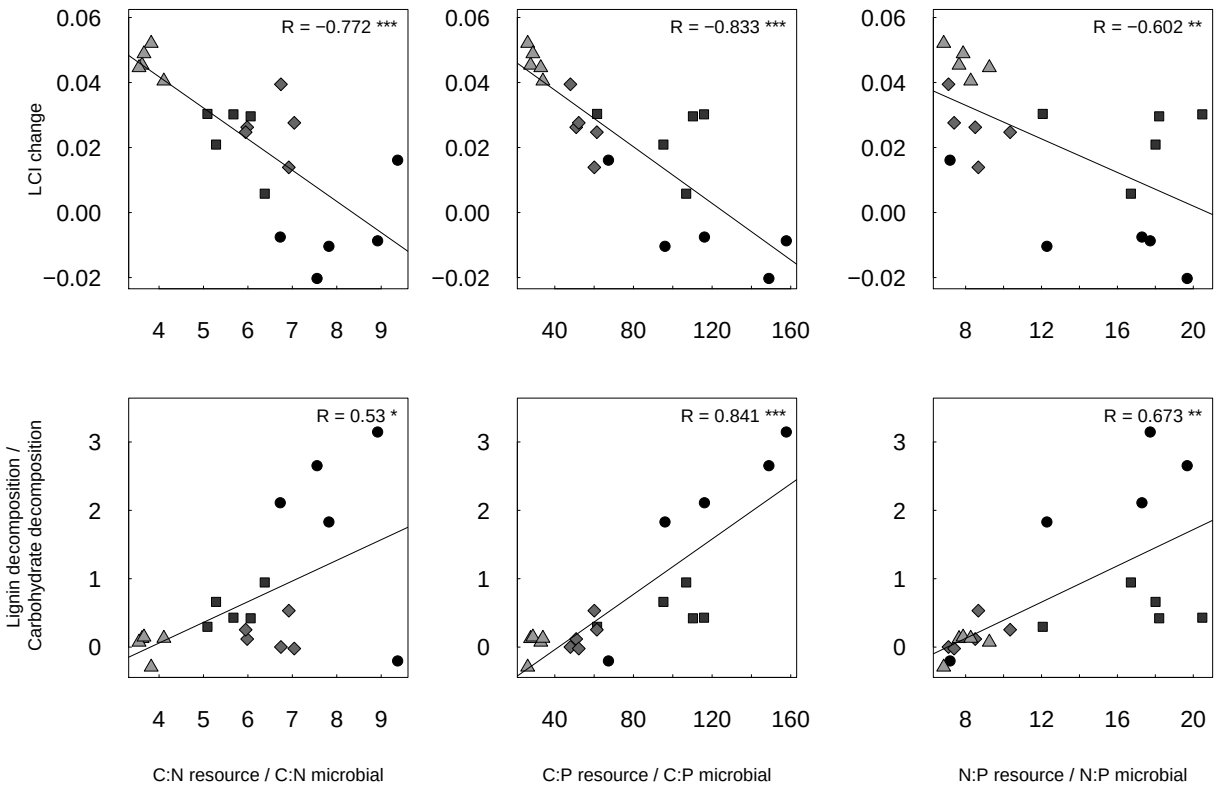


Figure 5: Correlations between Lignin accumulation during the first 6 month of litter incubation and stoichiometric resource:consumer imbalances

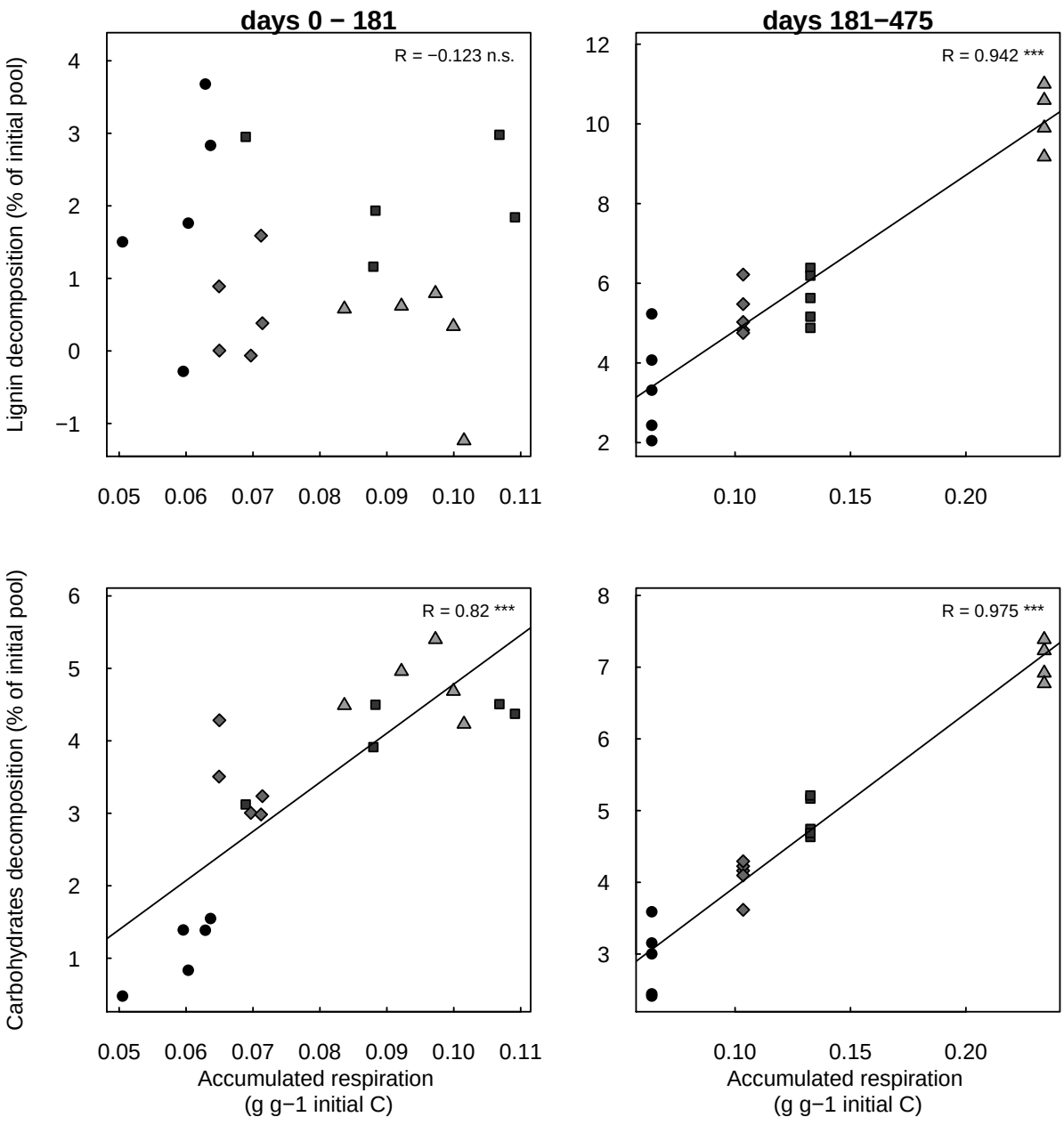


Figure 6: caption

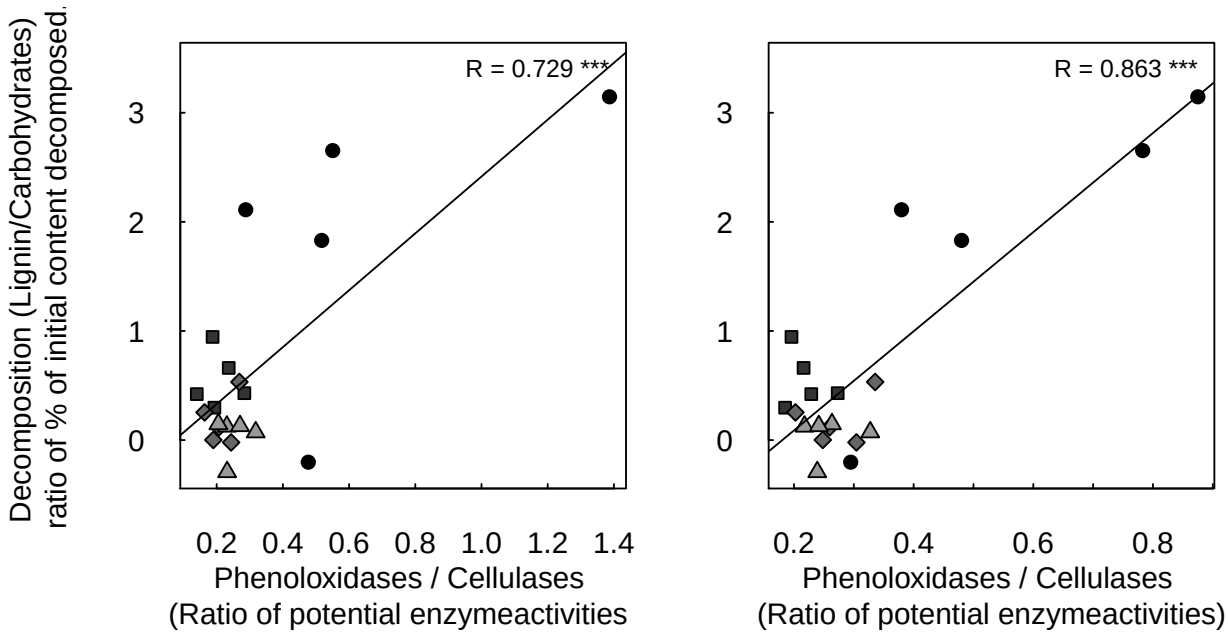


Figure 7: caption

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) | 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) | 0.002 |
| 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 |

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/Cell | Phen/Cell |
|---|---|---|---|---|---|---|---|---|---|---|
| Massloss | 0.291 | -0.15 | 0.245 | -0.339 | 0.0964 | -0.211 | 0.0965 | -0.0818 | 0.048 | 0.0534 |
| Actual respiration | 0.333 | **-0.723** | **0.606** | -0.0747 | **0.732** | -0.19 | **0.507** | -0.364 | -0.268 | -0.362 |
| Accumulated Respiration | **0.494** | **-0.704** | **0.688** | -0.123 | **0.82** | -0.327 | **0.483** | **-0.522** | **-0.506** | **-0.534** |
| Cellulase activity | **0.657** | **-0.76** | **0.803** | -0.424 | **0.789** | **-0.493** | **0.611** | **-0.588** | -0.436 | **-0.539** |
| Protease activity | 0.186 | -0.296 | 0.264 | -0.123 | 0.295 | -0.148 | 0.314 | -0.272 | -0.26 | -0.18 |
| Phosphatase activity | 0.409 | **-0.749** | **0.663** | -0.157 | **0.766** | -0.301 | **0.604** | **-0.555** | **-0.49** | **-0.607** |
| Phenoloxidase activity | **0.632** | **-0.669** | **0.737** | -0.412 | **0.708** | **-0.448** | **0.503** | **-0.484** | -0.305 | -0.356 |
| Peroxidase activity | **0.599** | **-0.588** | **0.677** | -0.41 | **0.618** | -0.439 | 0.412 | -0.435 | -0.173 | -0.302 |
| N mineralization | **0.466** | **-0.664** | **0.65** | -0.159 | **0.703** | -0.295 | 0.45 | -0.384 | -0.282 | -0.367 |
| Nitrification | **0.587** | **-0.707** | **0.732** | -0.377 | **0.721** | -0.431 | **0.565** | **-0.497** | -0.369 | -0.45 |
| P mineralization | **0.665** | **-0.55** | **0.684** | **-0.544** | **0.59** | **-0.58** | 0.387 | **-0.479** | -0.212 | -0.255 |
| C litter | **-0.545** | **0.506** | **-0.578** | **0.589** | **-0.45** | **0.631** | **-0.704** | **0.702** | **0.525** | **0.581** |
| extractable C | **0.609** | **-0.766** | **0.782** | -0.364 | **0.793** | -0.443 | **0.593** | **-0.538** | -0.392 | **-0.484** |
| N litter | 0.354 | **-0.517** | **0.503** | -0.14 | **0.546** | -0.189 | 0.286 | -0.201 | -0.119 | -0.159 |
| P litter | **0.682** | -0.222 | **0.517** | **-0.75** | 0.204 | **-0.686** | 0.211 | **-0.496** | -0.0728 | -0.16 |
| C:N litter | -0.405 | **0.586** | **-0.57** | 0.173 | **-0.616** | 0.234 | -0.36 | 0.271 | 0.195 | 0.242 |
| C:P litter | **-0.636** | 0.174 | **-0.453** | **0.758** | -0.136 | **0.655** | -0.234 | 0.425 | 0.049 | 0.0805 |
| N:P litter | **-0.512** | -0.0287 | -0.264 | **0.718** | 0.079 | **0.583** | -0.107 | 0.324 | -0.0316 | -0.0192 |
| C:N mic | **0.666** | **-0.758** | **0.799** | -0.423 | **0.807** | **-0.511** | **0.657** | **-0.609** | **-0.584** | **-0.596** |
| C:P mic | **0.692** | **-0.787** | **0.834** | **-0.468** | **0.818** | **-0.557** | **0.694** | **-0.671** | **-0.564** | **-0.648** |
| N:P mic | **0.582** | **-0.729** | **0.74** | -0.406 | **0.733** | **-0.502** | **0.685** | **-0.669** | **-0.545** | **-0.671** |
| C:N imbalance | **-0.56** | **0.81** | **-0.772** | 0.28 | **-0.851** | 0.386 | **-0.662** | **0.53** | **0.564** | **0.56** |
| C:P imbalance | **-0.817** | **0.663** | **-0.833** | **0.748** | **-0.653** | **0.794** | **-0.691** | **0.841** | **0.575** | **0.67** |
| N:P imbalance | **-0.724** | 0.351 | **-0.602** | **0.807** | -0.313 | **0.763** | **-0.455** | **0.673** | 0.301 | 0.41 |

Table 3: –>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/Cell | Phen/Cell |
|---|---|---|---|---|---|---|---|---|---|---|
| Massloss | -0.0455 | -0.264 | 0.0665 | **0.608** | **0.685** | **0.49** | 0.436 | -0.144 | -0.444 | 0.403 |
| Actual respiration | -0.374 | -0.22 | -0.213 | **0.882** | **0.84** | **0.84** | **0.771** | -0.293 | -0.403 | 0.29 |
| Accumulated Respiration | -0.165 | -0.29 | -0.0113 | **0.91** | **0.965** | **0.752** | **0.765** | -0.409 | **-0.608** | **0.486** |
| Cellulase activity | -0.317 | -0.307 | -0.137 | **0.891** | **0.887** | **0.82** | **0.885** | -0.442 | **-0.575** | 0.414 |
| Protease activity | -0.229 | -0.271 | -0.086 | **0.493** | **0.495** | **0.463** | **0.664** | **-0.475** | **-0.456** | 0.381 |
| Phosphatase activity | 0.0425 | -0.0182 | 0.0685 | 0.368 | 0.405 | 0.285 | **0.489** | -0.408 | -0.152 | 0.0167 |
| Phenoloxidase activity | 0.34 | -0.436 | 0.435 | -0.218 | 0.00927 | -0.346 | -0.134 | -0.184 | **-0.483** | **0.692** |
| Peroxidase activity | -0.274 | 0.452 | -0.385 | 0.148 | -0.0609 | 0.269 | 0.0763 | 0.17 | **0.546** | **-0.708** |
| N mineralization | 0.175 | 0.195 | 0.0757 | 0.0241 | 0.0543 | -0.0988 | -0.189 | 0.0091 | 0.0624 | 0.0892 |
| Nitrification | -0.289 | 0.23 | -0.321 | **0.631** | **0.54** | **0.559** | 0.352 | 0.0392 | -0.105 | -0.0234 |
| P mineralization | -0.164 | 0.0616 | -0.137 | **0.497** | **0.458** | **0.505** | 0.367 | -0.0317 | 0.0433 | -0.0273 |
| C litter | 0.33 | 0.231 | 0.176 | -0.392 | -0.346 | -0.413 | **-0.713** | **0.639** | **0.501** | -0.348 |
| extractable C | -0.205 | -0.188 | -0.0882 | **0.88** | **0.894** | **0.727** | **0.717** | -0.366 | **-0.538** | 0.409 |
| N litter | -0.17 | -0.166 | -0.0672 | **0.838** | **0.861** | **0.702** | **0.567** | -0.153 | -0.431 | 0.349 |
| P litter | -0.4 | -0.369 | -0.181 | **0.782** | **0.756** | **0.806** | **0.885** | -0.399 | **-0.464** | 0.325 |
| C:N litter | 0.124 | 0.196 | 0.018 | **-0.827** | **-0.874** | **-0.664** | **-0.564** | 0.194 | **0.49** | -0.404 |
| C:P litter | **0.508** | 0.277 | 0.313 | **-0.637** | **-0.538** | **-0.746** | **-0.793** | 0.292 | 0.283 | -0.162 |
| N:P litter | **0.477** | 0.189 | 0.325 | -0.307 | -0.179 | **-0.496** | **-0.56** | 0.171 | 0.048 | 0.0338 |
| C:N mic | 0.216 | 0.186 | 0.095 | **-0.728** | **-0.741** | **-0.582** | **-0.661** | **0.557** | **0.57** | **-0.513** |
| C:P mic | 0.395 | 0.0762 | 0.312 | **-0.565** | **-0.466** | **-0.584** | -0.426 | -0.0723 | 0.233 | -0.223 |
| N:P mic | 0.333 | 0.0142 | 0.288 | -0.298 | -0.189 | -0.389 | -0.199 | -0.287 | -0.00191 | -0.00931 |
| C:N imbalance | -0.0522 | 0.084 | -0.0756 | -0.322 | -0.37 | -0.273 | -0.0657 | -0.317 | 0.0273 | 0.0196 |
| C:P imbalance | 0.0913 | 0.335 | -0.0757 | -0.189 | -0.234 | -0.264 | **-0.552** | **0.493** | 0.16 | -0.0317 |
| N:P imbalance | 0.0576 | 0.293 | -0.0865 | -0.027 | -0.0722 | -0.0929 | **-0.464** | **0.615** | 0.16 | -0.0803 |

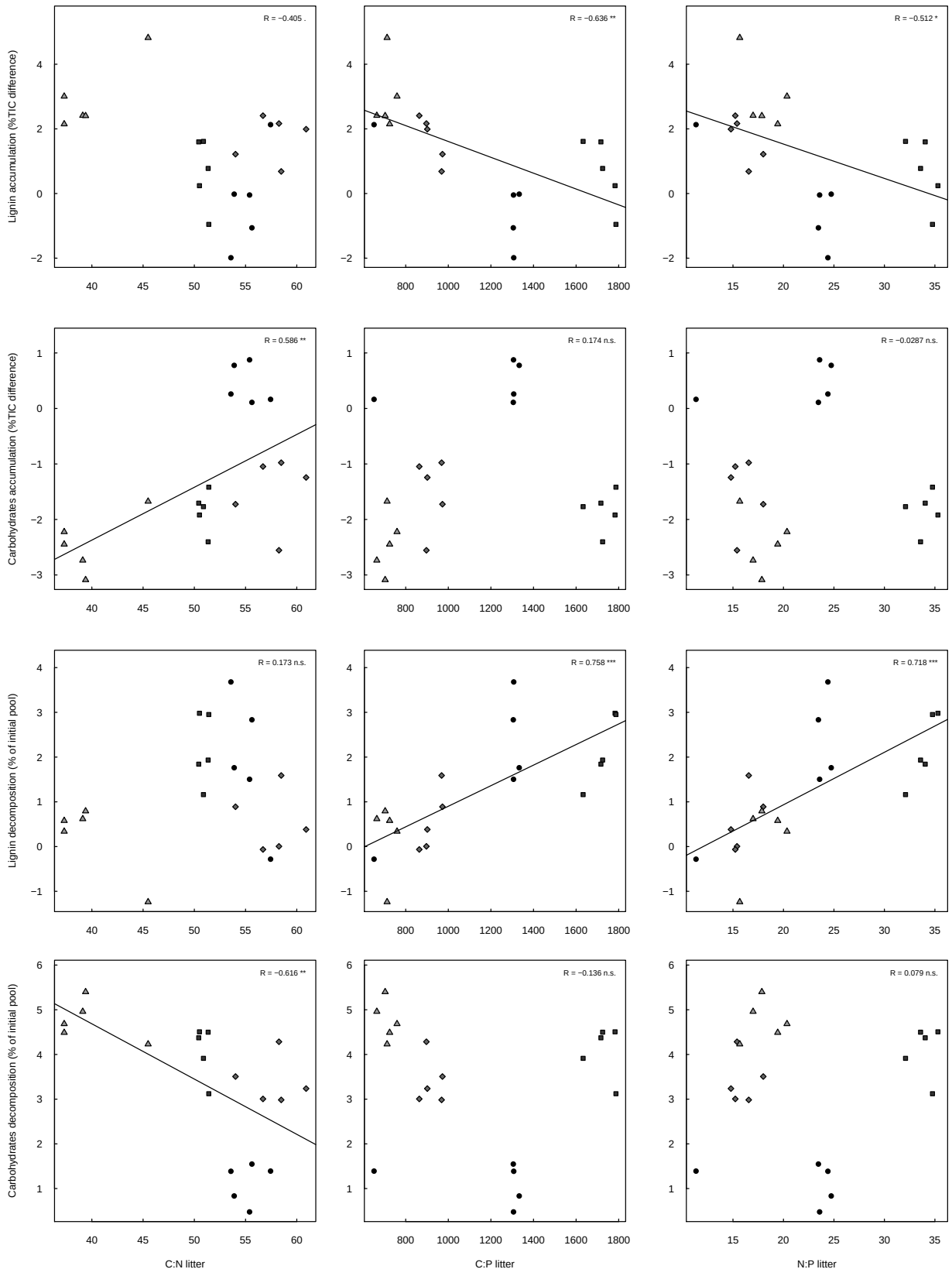




Figure 8: Correlations between Lignin and Carbohydrates accumulation and decomposition during the first 6 month of litter incubation and litter C:N:P ratios

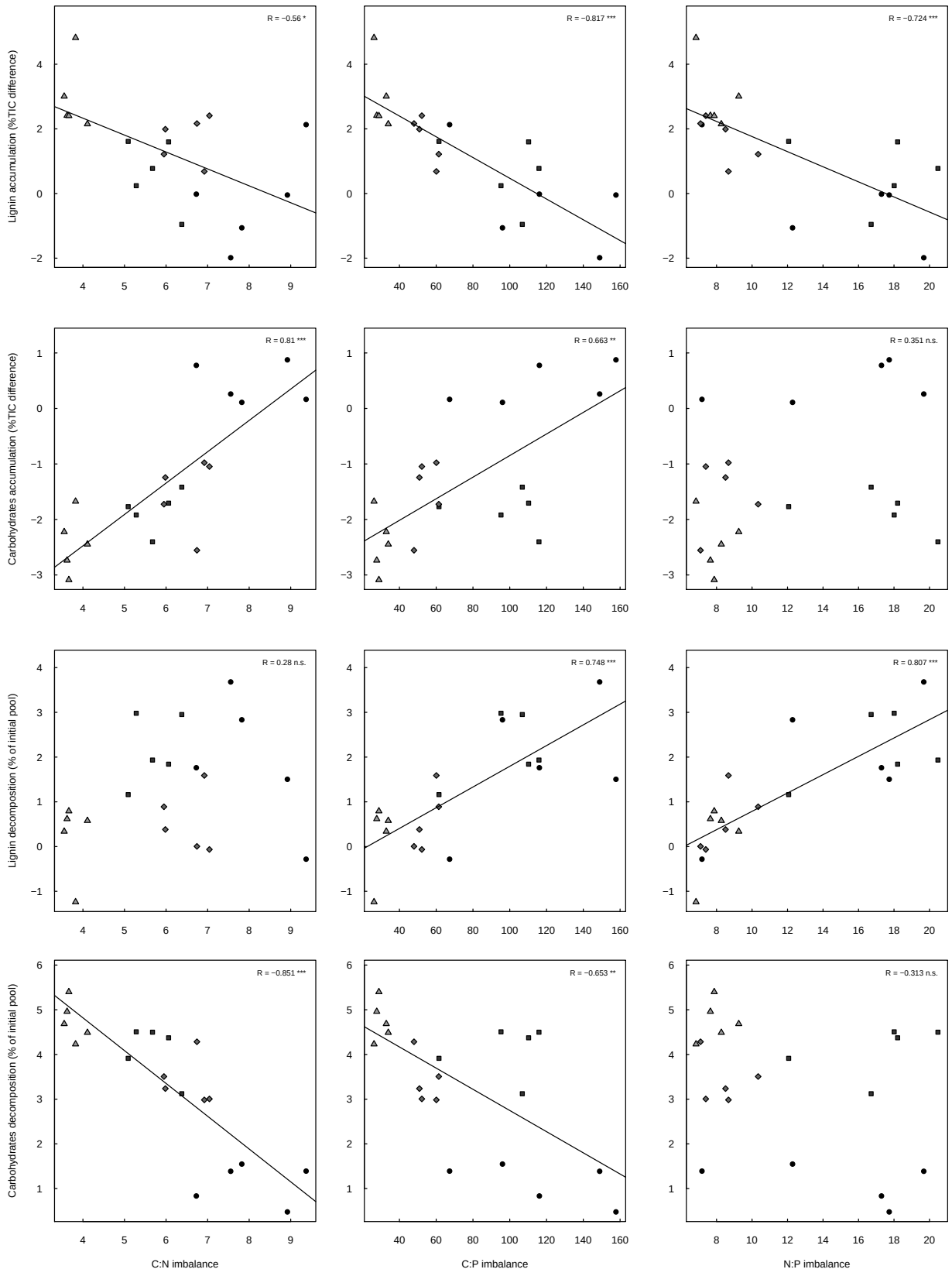




Figure 9: Correlations between Lignin and Carbohydrates accumulation and decomposition during the first 6 month of litter incubation and stoichiometric resource:consumer imbalances