

Controls of litter chemistry over early lignin decomposition in beech litter ¹

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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 methods 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 lignin and carbohydrate decomposition during early litter decay with analytical pyrolysis in a climate-chamber decomposition experiment, focusing on resource control over microbial carbon substrate preferences. Beech litter with different C:N:P stoichiometry but identical initial microbial communities was incubated 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 from insignificant amounts of lignin decomposed to lignin decomposition at the same rate bulk 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.

[results]

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

4 Litter decomposition models [lit] follow the concept that macromolecules in litter form three indepen-
 5 dent carbon pools of increasing recalcitrance attributed to (1) soluble compounds, (2) cellulose and hemi-
 6 celluloses and (3) lignin. During decomposition, soluble compounds are most accessible to microbes and
 7 consumed first, followed by carbohydrates (i.e. cellulose). Lignin can be decomposed only by specialists
 8 and is not degraded until accumulated to a certain, critical level when it inhibits the degradation of less
 9 recalcitrant compounds [1–3].¹ Most common methods to quantify these carbon pools gravimetrically deter-
 10 mine cellulose, hemi-celluloses and lignin contents after sequential extractions with selective solvents. These
 11 methods were repeatedly criticize as unspecific for lignin determination [4]. When analyzed with alternative
 12 methods (NMR, CuO-oxidation, Pyrolysis-GC/MS), extracted lignin fractions contain many other than the
 13 proclaimed substances. (i.e. [5] ².

14 Recent studies based on specific methods to determine litter lignin content (CuO - oxidation, Pyrolysis-
 15 GC/MS, NMR) question the assumed intrinsic recalcitrance of lignin. Experiments using isotope labeling
 16 used to calculate mean residence times for lignin in soils and litter/soil mixtures in both laboratory and
 17 outdoor incubation reported lignin residence times no longer than that of other carbon compounds or bulk
 18 SOM [6, 7]³.

19 For leaf litter, lignin depletion during early decomposition and decreasing lignin decomposition rates
 20 were recently by [8]. Based on their results, the authors proposed a new concept for lignin degradation in
 21 which fastest lignin degradation occurs during early litter decomposition. Lignin decomposition during late
 22 decomposition is limited by (dissolved organic) carbon availability, a pulsed input of labile carbon (during
 23 litterfall or experimental manipulations like drying and rewetting) causes higher lignin degradation rates for
 24 a limited time period. The authors also suggest, that lignin decomposition is hampered in late decomposition
 25 stage, when labile (soluble) carbon source are limited.

26 [8] do not elaborate the of stoecheometric constrains on lignin decomposition. In isolated lignin fractions
 27 from fresh beech litter, N contents twice as high as bulk litter were found ⁴. It was argued that, while yielding
 28 little C and energy, lignin decomposition makes occluded cell wall protein accessable to decomposers, and

¹more lit.

²[lit CuO], lit[Pyr]

³more lit?

⁴cit

lignin decomposition is driven not by carbon but by nitrogen demand ("Nitrogen mining theory", [9]).

Nitrogen fertilization experiments on litter and soils indicate 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 [10]. High nitrogen levels were reported to inhibit lignolytic enzyme in forest soils [11]. 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 [12].

N fertilization has different effects on litter decomposition than different nutrient levels in litter, as leaf litter N is stored in protein and lignin structures and not directly available to microorganisms. N-fertilization experiments can simulate increased N-deposition rates. To simulate variations in litter C:N ratios, our approach is preferable, because potential variations in litter N content occur in complex substrates.

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 stoichiometry on microbial decomposition, exclude decomposing fauna and keep climatic conditions constant.

We test several proposed mechanisms, by which lignin degradation is affected by litter chemistry:

(1) High lignin contents inhibit the degradation of other carbon sources, and trigger lignin decomposition [1].

(2) Lignin degradation is inhibited, when the availability of cofactors for oxidative enzymes (mainly Mn) is low⁵.

(3) Lignin degradation is directed to degrade N, therefore less lignin is decomposed in litter with narrow C:N ratios where more lignin is available [9].

-(4) The availability of dissolved carbon limits lignin decomposition, and lignin decomposition is inhibited when DOC content becomes rate limiting (and therefore correlated to) carbon respiration rates [8].

Results

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 [13]. After 15 month, between 5 and 12% of the initial

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57 dry mass was lost. This is less than reported in litter decomposition studies on other species, but in a similar⁴
58 range as recently reported for beech litter from an in-situ litterbag-study [14] .

59 Highest respiration rates were measured after 14 days incubation (150-350 $\mu\text{g CO}_2\text{-C d}^{-1}\text{ g}^{-1}\text{ litter-C}$),
60 dropped to rates between between 75 and 100 $\mu\text{g CO}_2\text{-C d}^{-1}\text{ g}^{-1}\text{ litter-C}$ after 97 days. After 181 and
61 375 days, respiration rates for AK and OS further decreased, while SW and KL show a second maximal
62 respiration after 181 days.

63 Soluble organic carbon content decreased between the first three harvests (14 to 181 days), to strongly
64 increase after 475 days (0.1 to 0.7 $\text{mg C g}^{-1}\text{ d.w.}$ were found after 14, 97 and 181 days, and increased to
65 amounts between 1.5 and 4 mg/g after 375 days. After 14 and 97 days, the highest C content was found
66 in SW litter followed by AK (see fig. ??). DOC content was loosely correlated to litter N content after 14
67 ($R=0.69$, $p<0.001$) and 97 days ($R = 0.65$, $p = <0.01$), they were strictly correlated after 181 days ($R =$
68 0.85 , $p=<0.001$) and 375 days ($R=0.90$, $p=<0.001$).

69 Microbial biomass abundance and stoichiometry

70 Microbial biomass contents ranged from 0.5 to 6 mg C , 0.05 to 5.5 mg N and 0.05 to 3.5 mg P per g litter
71 (d.w.). In KL and OS biomass buildup reaches a plateau after 3 month, AK and SW show further growth
72 reaching a maximum of microbial C and N contents after 6 month (AK also for P). Microbial C:N ratios
73 measured range between 1:6 and 1:18, C:P ratios between 1:8 and 1:35, and N:P ratios between 1:0.5 and
74 1:3.5. Microbial C:N ratios (Fig. 1).

75 Litter microbial biomass is homeostatic during the first 6 month (no or marginally negative correlation
76 between microbial stoichiometry and litter stoichiometry) [13], but not after 15 month, when all three ratios
77 show correlations (R 0.53 - 0.64, all $p < 0.002$, $H_{\text{C:N}}=2.01$, $H_{\text{C:P}}=1.68$, $H_{\text{N:P}}=2.29$). Microbial C:N ratios
78 are tightly constrained after 3 (1:14.5 - 1:18.2) and 6 month (1:6.9 - 1:9.0), but significantly different between
79 the two time points. C:P and N:P ratios are less constrained, with the highest variance between litter from
80 different sites after 3 month incubation (Fig. 1).

81 Potential enzyme activities

82 Absolute potential enzyme activities were correlated to litter N, respiration and other other decomposition
83 processes (all $R > 0.8$, $p < 0.001$). For all enzymes and at all time points, SW showed the highest and AK
84 the lowest activity. Cellulase was below detecten limit after 14 days, oxidativte enzymes after 15 month.
85 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)

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 small but well preserved during decomposition. The high similarity allowed tracing small changes in lignin and carbohydrate abundance during decomposition.

When measured by pyr-GC/MS, lignin derived 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 derived 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 correlated ($R = 0.47$, $p < 0.01$) 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 ??⁶ 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 [15] using thermochemolysis.⁷

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 discrimination (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

⁶check fig.

⁷I 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.

same rates and their content in pyrograms remains constant (fig. ??).

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 [13] and [16]. 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.

Discussion

Our experimental approach allows to single out litter quality and its influence on the microbial decomposer community as the only source of the differences in decomposition processes found, while we can 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 litter while exploring the effect of different litter nutrient contents on lignin and carbohydrate decomposition. 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, 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 [8], 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

142 over lignin decomposition was controlled by litter chemistry over the first 6 month, all components of litter⁷
143 were degraded at similar rates thereafter.

144 In the search of the control over this early lignin decomposition, we can discard hypothesis (1) and (2):
145 Differences in initial lignin contents were below 10%, and lignin contents of sites with high initial lignin
146 decomposition rates were not higher than that of sites with low rates. Mn and Fe contents strongly vary
147 between litter collected at different sites, but both Mn and Fe contents are lowest in the litter with the
148 highest lignin decomposition (AK, see tab. 1). Low contents of these Elements would explain inhibited, not
149 enhance lignin decomposition. Hypothesis (3) can be excluded because we found highest amounts of soluble
150 carbon in litter from two different sites who show the highest and the lowest lignin degradation.

151 We did find strong evidence that C:N:P stoichiometric ratios wield key control over the extend of lignin
152 accumulation during this first decomposition stage. While carbohydrate decomposition was correlated to
153 litter N contents (as were a majority of decomposition processes found, from respiration to enzymatic activ-
154 ities), relative decomposition rates of Lignin were strictly correlated to C:P imbalances and a number of P
155 pools analyzed. Correlation was highest when lignin decomposition was compared to resource:consumer C:P
156 ratios.

157 Strong evidence exists that labile carbon and nitrogen availability control (late) lignin decomposition.
158 Cultivation studies show that lignin decomposition in fungi is triggered by nitrogen starvation, and that
159 lignin does not provide sufficient energy to maintain the decomposer's metabolism without the use of other
160 carbon sources ⁸. Lignin decomposition was found in wild-type *A. thaliana* litter, but not in a low-cellulose
161 mutant during 12 month incubation in a boreal forest [17]. However, a stimulation of lignin decomposition
162 by a high P imbalance or a delay of lignin decomposition under high P availability, as indicated by the high
163 correlation to P pools we found, was not reported yet. In the N- and P- co-limited situation during early
164 litter decomposition, in which lignin is degraded either to access additional nutrients or as a mean to use a
165 C surplus in a N/P co-limited situation.

166 [17] also suggests that lignin decomposition might be interpreted a k-strategy used by microbes to be able
167 to colonize more lignin-rich and nutrient-poor substrates. Low nutrient availability might favor this strategy,
168 as the high P- demands of a fast growing microbial community can not be met under such conditions.

169 While we found different levels of lignin degradation during the first 6 month, lignin contents remained
170 constant in all litter types between 6 and 15 month. This indicates that lignin is not degraded slower than
171 other litter compounds, but differences in lignin contents acquired during the first 6 month remain in place.

⁸citation

172 The controls which lead to differences in the extend of lignin discrimination over the first six month are n⁸
173 longer predominant between 6 and 15 month.

174 This change in decomposition dynamics corresponds to change in soluble carbon. While during the first
175 3 month, extractable carbon contents were not or to a lesser extend correlated to litter N, soluble carbon
176 is strictly correlated to litter N and actual respiration after 6 and 15 month⁹. [8] suggests a change in
177 decomposition dynamics after 100 to 200 days of incubation, after which lignin decomposition rates decrease
178 due to lack of labile carbon. They also report a correlation between respiration rates and extractable
179 carbon after this change. The authors interprets this correlation as carbon limited respiration, and suggest
180 that lignin decomposition is inhibited under such a limitation. We can confirm the correlation between
181 extractable carbon and respiration after 181 days, but not the inhibition of lignin decomposition. Also,
182 we found that both respiration and the production of soluble carbon are controled by litter N content.
183 The process of degrading macromolecular compounds into soluble molecules is conducted by extracellular
184 enzymes and is therefore N intensive that the mineralization of labile carbon, de-polymerization is the point
185 in the decomposition process where a N limitation would be most likely to become effective.

186 Another notable change occurs in the homeostasis of the microbial community. While is was strictly
187 homeostatic during the first 6 month, substrate stoichiometry had a minor, but significant influence on mi-
188 crobial stoichiometry after 15 month. Together, those changes indicate that the microbial community is able
189 to compensate for differences in substrate quality (on the expense of community growth and overall decom-
190 position speed) and can select preferred compounds during the first 6 month. However, this compensation
191 is limited and imbalances can not be upheld at the same intensity after the first 200 days.

192 Our results further question the concept that lignin decomposition is inhibited until late decomposition.
193 While traditional litter decomposition concepts locate lignin decomposition only during late decomposition,
194 we find substancial amounts of lignin decomposed over the first six month. The extend, to which lignin
195 is decomposed, was controlled by litter chemistry over the first 6 month. However, we did not find lignin
196 decomposition rates controlled by litter quality thereafter. Soluble carbon contents were not restrictive to
197 lignin decomposition.

198 While carbohydrate decomposition was stimulated by high N contents, early lignin decomposition rates
199 were highly correlated C:P ratio and resource:consumer imbalances. High lignin contents accumulated during
200 this stages remained in place during later decomposition. For further studies, this raises the question, to which
201 extend late decomposition is influenced by this early, stoichiometry-controlled accumulation of recalcitrant

⁹stats

compounds.

Material and methods

Litter decomposition experiment

A detailed description of our litter decomposition experiment was published in [18]. Briefly, 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 0.25cm², homogenized, sterilized twice by gamma¹⁰ 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 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.

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 [19]).

To determine soluble C, N, and P contents, 1.8g litter (fresh weight) were extracted with 50 ml 0.5M K₂SO₄. 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

¹⁰greek gamma here

229 extracted under the same conditions after chloroform fumigation. Microbial biomass was determined as the
230 difference between fumigated and non-fumigated extractions [20]. C and N concentration in extracts were
231 determined with a TOC/TN analyzer (TOC-VCPH and TNM, Shimadzu), Phosphorous was determined
232 photometrically.¹¹

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

$$X : Y_{inbal} = \frac{X : Y_{litter}}{X : Y_{microbial}} \quad (1)$$

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

235 Microbial Respiration

236 Respiration was monitored weekly during the entire incubation in mesocosms removed after 6 month and
237 on the last incubation day for all mesocosms using an infrared gas analyzer (IRGA, EGM4 with SRC1,
238 PPSystems, USA). CO₂ concentration was measured over 70 seconds and increase per second was calculated
239 based on initial dry mass. Measurements of ambient air were performed before and after each measurement
240 to assess possible leaks or base-line drifts IRGA. Accumulated respiration after 6 month was calculated
241 assuming linear transition between measurements, accumulated respiration after 15 month was estimated
242 from respiration rates after 181 and 475 days.

243 Enzyme activities

244 Measurements of potential exo-enzyme activities for cellulases, peroxidases and phenoloxidase were described
245 by [16]. Activities were determined with a series of micro-plate assays based on the hydrolysis of 4-methyl-
246 β -D-cellobioside (cellulase) and L-3,4-dihydroxyphenylalanin (oxidative enzymes). Products of enzyme cat-
247 alyzed reactions were detected photometrically (oxidative enzymes) or flourometrically (cellulase) [21–23].

248 Pyrolysis-GC/MS

249 Pyrolysis-GC/MS was performed with a Pyroprobe 5250 pyrolysis system (CDS Analytical) coupled to a
250 Thermo Trace gas chromatograph and a DSQ II MS detector (both Thermo Scientific) equipped with a
251 carbowax column (Supelcowax 10, Sigma-Aldrich).

¹¹lit!!

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 300.¹²

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 high 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 [24, 25]. 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 [24].

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

$$LCI = \frac{Lignin}{Lignin + Carbohydrates} \quad (2)$$

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 CO₂-C respired by a mesocosm.

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

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

¹²maybe cite other paper for method?

All statistical analyses were performed with the software and statistical computing environment R using the package “vegan” [26]. 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 [13] and [16].

Acknowledgments

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339 Figure Legends

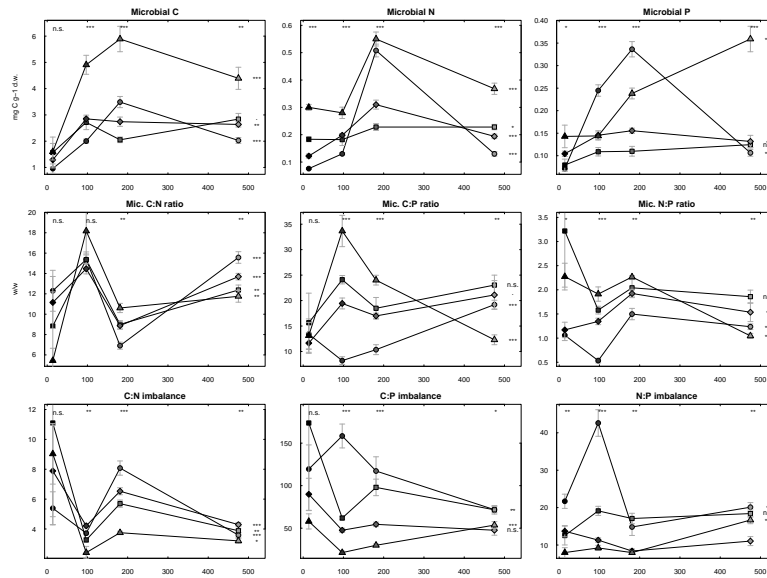


Figure 1. Microbial biomass, microbial stoichiometry and resource:consumer stoichiometric imbalance. Error bars indicate standard errors (n=5).

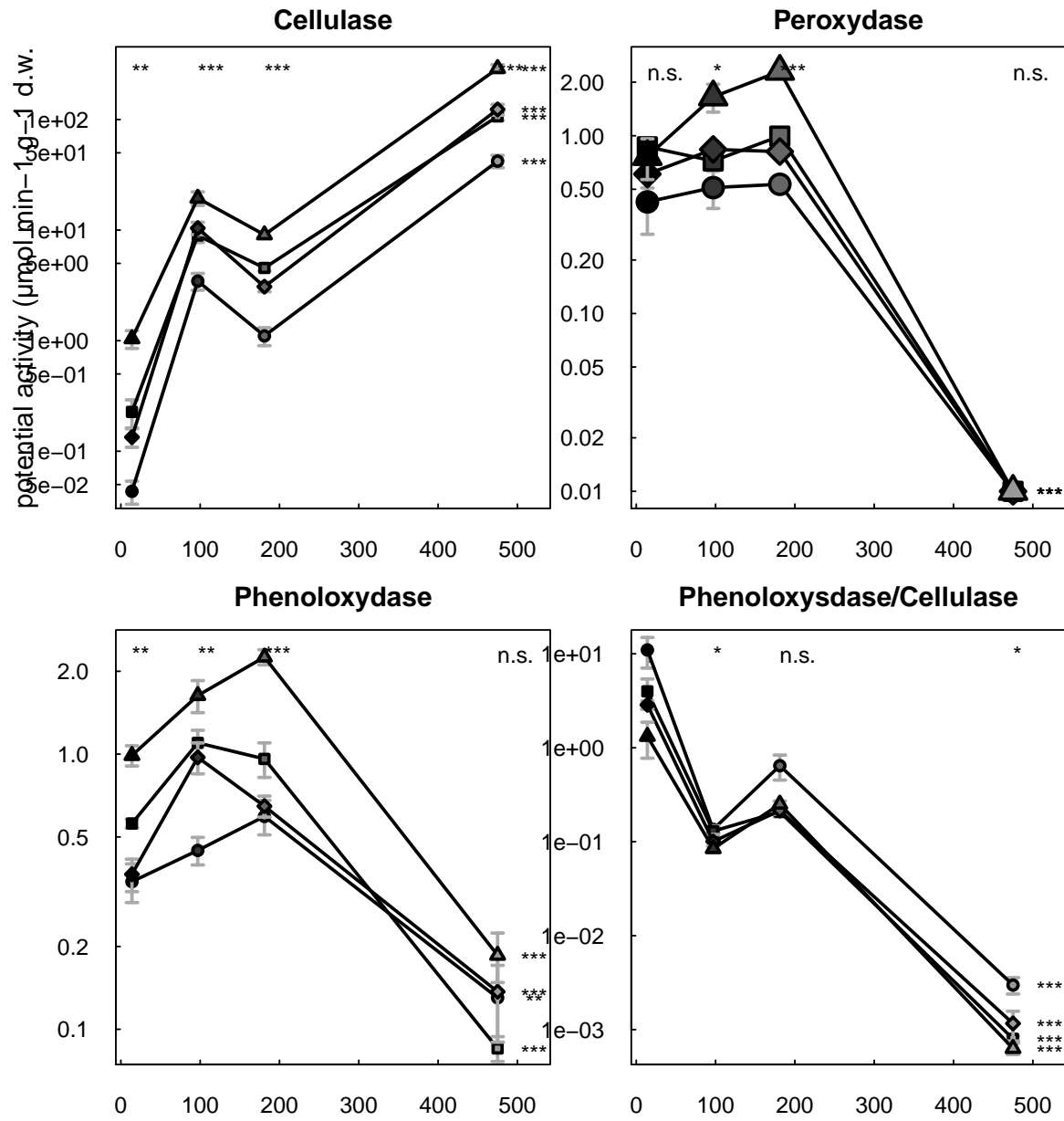


Figure 2. Potential eco-enzyme activities

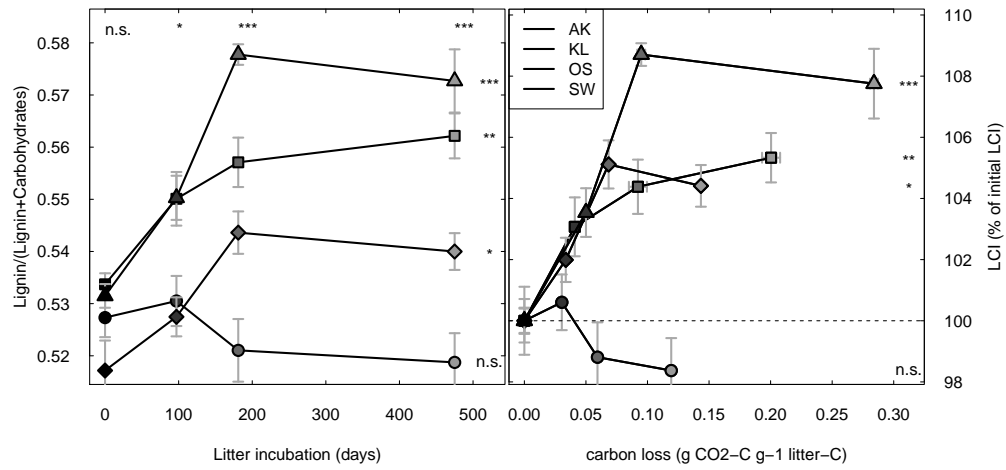


Figure 3. Development of the LCI (lignin/(lignin+carbohydrates)). Errorbars indicate standard errors (n=4-5). The dashed line indicates a constant ratio between lignin and carbohydrates (i.e. no preferential decomposition of carbohydrates.)

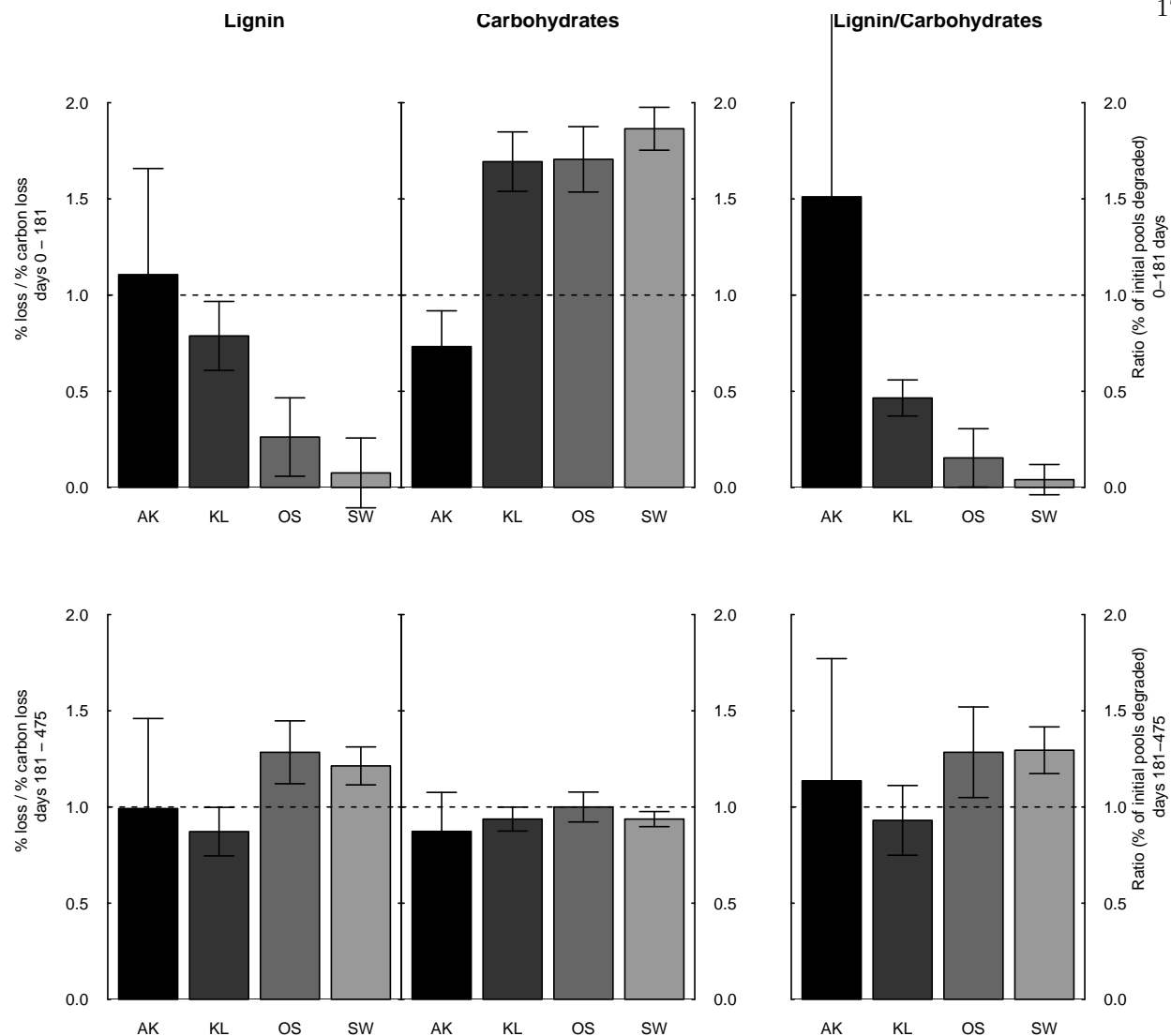


Figure 4. Carbon loss corrected amounts of lignin and carbohydrates degraded. Carbon loss was calculated based on accumulated respiration. Error bars indicate standard errors (n=4-5). The dashed line marks no discrimination between lignin, carbohydrates and bulk carbon loss.

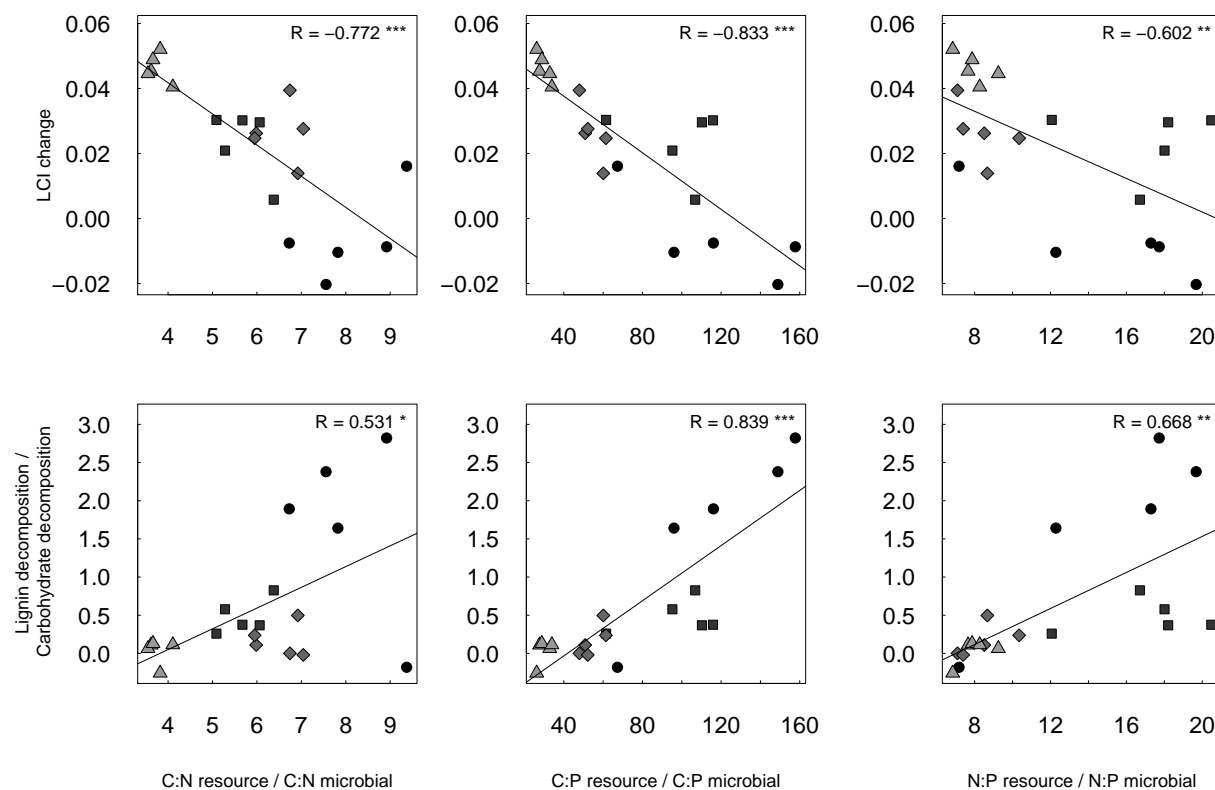


Figure 5. Correlations between Lignin accumulation during the first 6 month of litter incubation and stoichiometric resource:consumer imbalances

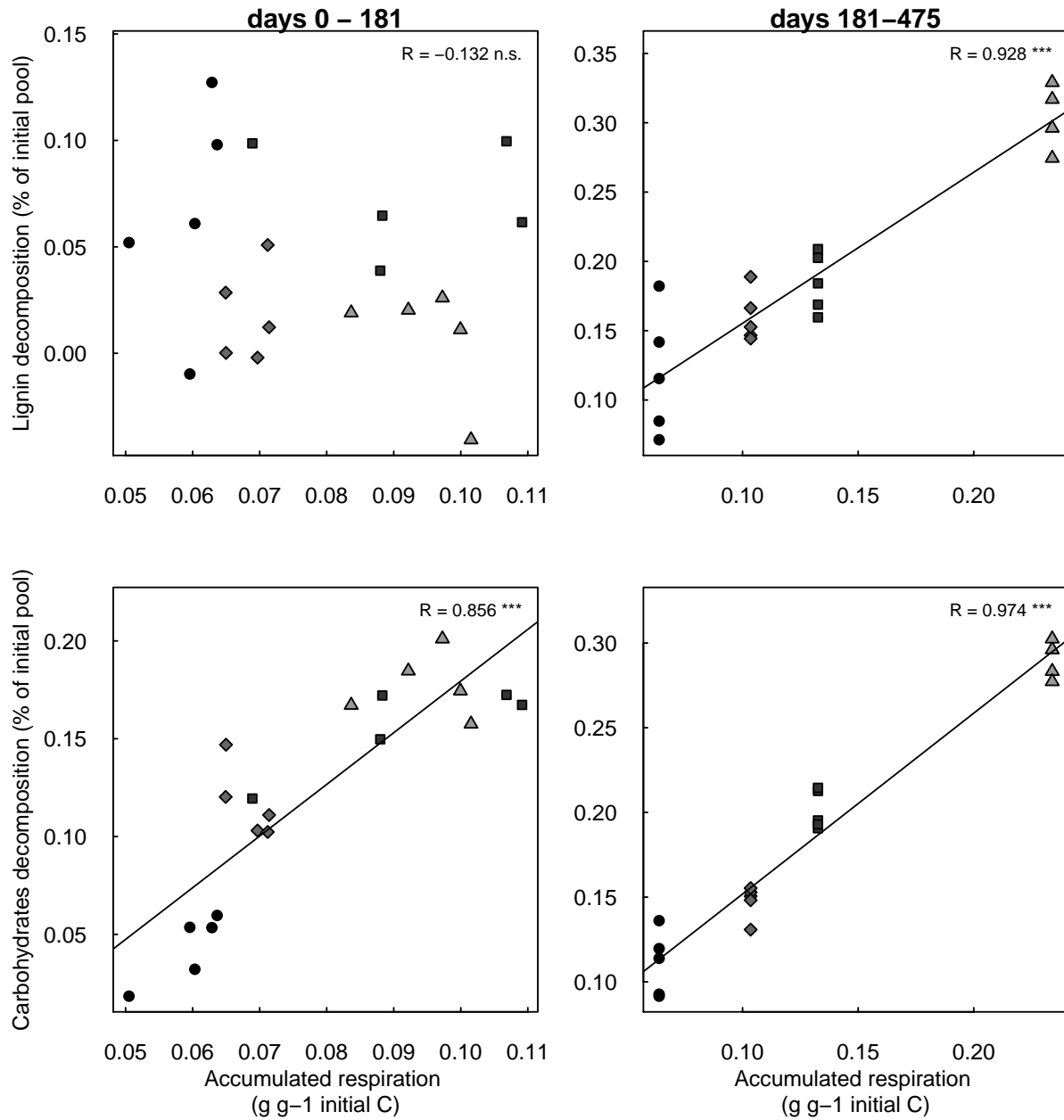


Figure 6. caption

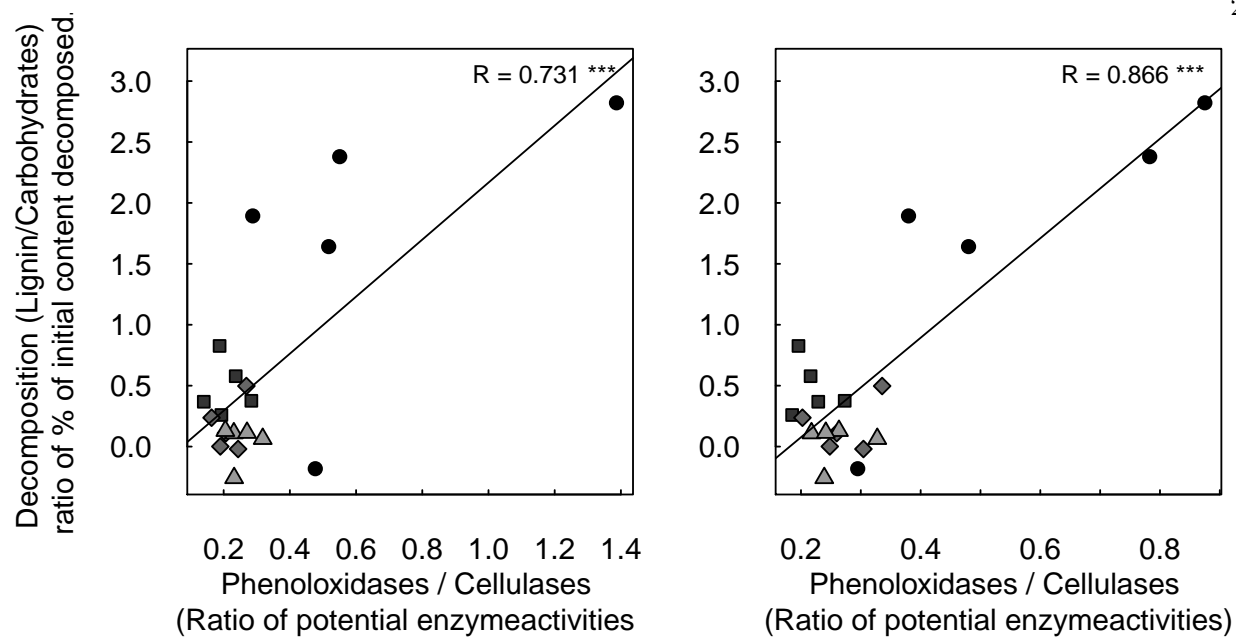


Figure 7. caption

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 degradation with litter chemistry, microbial community and decomposition processes. Significant ($p < 0.05$) correlations are printed bold. Data taken from [13, 16]. Differences in litter chemistry were calculated between 0 and 181 days, process rates were measured after 181 days.

	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	-0.328	0.106	-0.201	0.125	-0.081	0.048	0.0534
Actual respiration	0.333	-0.723	0.606	-0.0822	0.771	-0.195	0.594	-0.368	-0.268	-0.362
Accumulated Respiration	0.494	-0.704	0.688	-0.132	0.856	-0.332	0.557	-0.525	-0.506	-0.534
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
Chitinase activity	0.409	-0.749	0.663	-0.17	0.795	-0.312	0.677	-0.559	-0.49	-0.607
Phosphatase activity	0.549	-0.813	0.776	-0.302	0.851	-0.407	0.702	-0.556	-0.418	-0.522
Phenoxidase 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	0.609	-0.766	0.782	-0.37	0.814	-0.446	0.658	-0.54	-0.392	-0.484
N litter	0.354	-0.517	0.503	-0.14	0.587	-0.187	0.366	-0.203	-0.119	-0.159
P litter	0.682	-0.222	0.517	-0.747	0.175	-0.68	0.188	-0.491	-0.0728	-0.16
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	0.666	-0.758	0.799	-0.43	0.798	-0.515	0.678	-0.609	-0.584	-0.596
C:P mic	0.692	-0.787	0.834	-0.476	0.814	-0.562	0.726	-0.672	-0.564	-0.648
N:P mic	0.582	-0.729	0.74	-0.415	0.729	-0.508	0.715	-0.67	-0.545	-0.671
C:N imbalance	-0.56	0.81	-0.772	0.288	-0.859	0.391	-0.71	0.531	0.564	0.56
C:P imbalance	-0.817	0.663	-0.833	0.757	-0.61	0.799	-0.668	0.839	0.575	0.67
N:P imbalance	-0.724	0.351	-0.602	0.81	-0.253	0.764	-0.397	0.668	0.301	0.41

Table 3. Correlation (R) between Lignin and Carbohydrate degradation with litter chemistry, microbial community and decomposition processes. Significant ($p < 0.05$) correlations are printed bold. Data taken from [13, 16]. Differences in litter chemistry were calculated between 181 and 475 days, process rates were measured after 475 days.

	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	0.623	0.71	0.505	0.496	-0.118	-0.444	0.403
Actual respiration	-0.374	-0.22	-0.213	0.86	0.83	0.837	0.809	0.0279	-0.403	0.29
Accumulated Respiration	-0.165	-0.29	-0.0113	0.909	0.981	0.753	0.825	-0.119	-0.608	0.486
Cellulase activity	-0.317	-0.307	-0.137	0.861	0.863	0.805	0.91	-0.00551	-0.575	0.414
Protease activity	-0.229	-0.271	-0.086	0.455	0.447	0.434	0.645	-0.0269	-0.456	0.381
Phosphatase activity	0.0425	-0.0182	0.0685	0.334	0.39	0.259	0.487	-0.0904	-0.152	0.0167
Chitinase activity	-0.221	-0.228	-0.0874	0.695	0.7	0.578	0.78	0.0348	-0.58	0.395
Phenoloxidase activity	0.34	-0.436	0.435	-0.196	0.0177	-0.338	-0.121	-0.456	-0.483	0.692
Peroxidase activity	-0.274	0.452	-0.385	0.126	-0.067	0.261	0.0631	0.397	0.546	-0.708
N mineralization	0.175	0.195	0.0757	0.0631	0.111	-0.0805	-0.142	-0.145	0.0624	0.0892
Nitrification	-0.289	0.23	-0.321	0.645	0.573	0.574	0.407	0.164	-0.105	-0.0234
P mineralization	-0.164	0.0616	-0.137	0.475	0.461	0.516	0.402	-0.0877	0.0433	-0.0273
C litter	0.33	0.231	0.176	-0.329	-0.269	-0.358	-0.654	-0.0539	0.501	-0.348
extractable C	-0.205	-0.188	-0.0882	0.884	0.912	0.727	0.774	-0.0383	-0.538	0.409
N litter	-0.17	-0.166	-0.0672	0.854	0.896	0.722	0.644	-0.0751	-0.431	0.349
P litter	-0.4	-0.369	-0.181	0.727	0.701	0.786	0.883	-0.00155	-0.464	0.325
C:N litter	0.124	0.196	0.018	-0.846	-0.912	-0.683	-0.643	0.113	0.49	-0.404
C:P litter	0.508	0.277	0.313	-0.572	-0.463	-0.721	-0.765	-0.144	0.283	-0.162
N:P litter	0.477	0.189	0.325	-0.233	-0.0883	-0.466	-0.5	-0.205	0.048	0.0338
C:N mic	0.216	0.186	0.095	-0.723	-0.745	-0.568	-0.693	0.136	0.57	-0.513
C:P mic	0.395	0.0762	0.312	-0.559	-0.453	-0.599	-0.45	-0.122	0.233	-0.223
N:P mic	0.333	0.0142	0.288	-0.288	-0.169	-0.409	-0.207	-0.174	-0.00191	-0.00931
C:N imbalance	-0.0522	0.084	-0.0756	-0.348	-0.412	-0.311	-0.132	0.00942	0.0273	0.0196
C:P imbalance	0.0913	0.335	-0.0757	-0.114	-0.16	-0.218	-0.499	0.0773	0.16	-0.0317
N:P imbalance	0.0576	0.293	-0.0865	0.0497	0.0088	-0.0352	-0.392	0.128	0.16	-0.0803

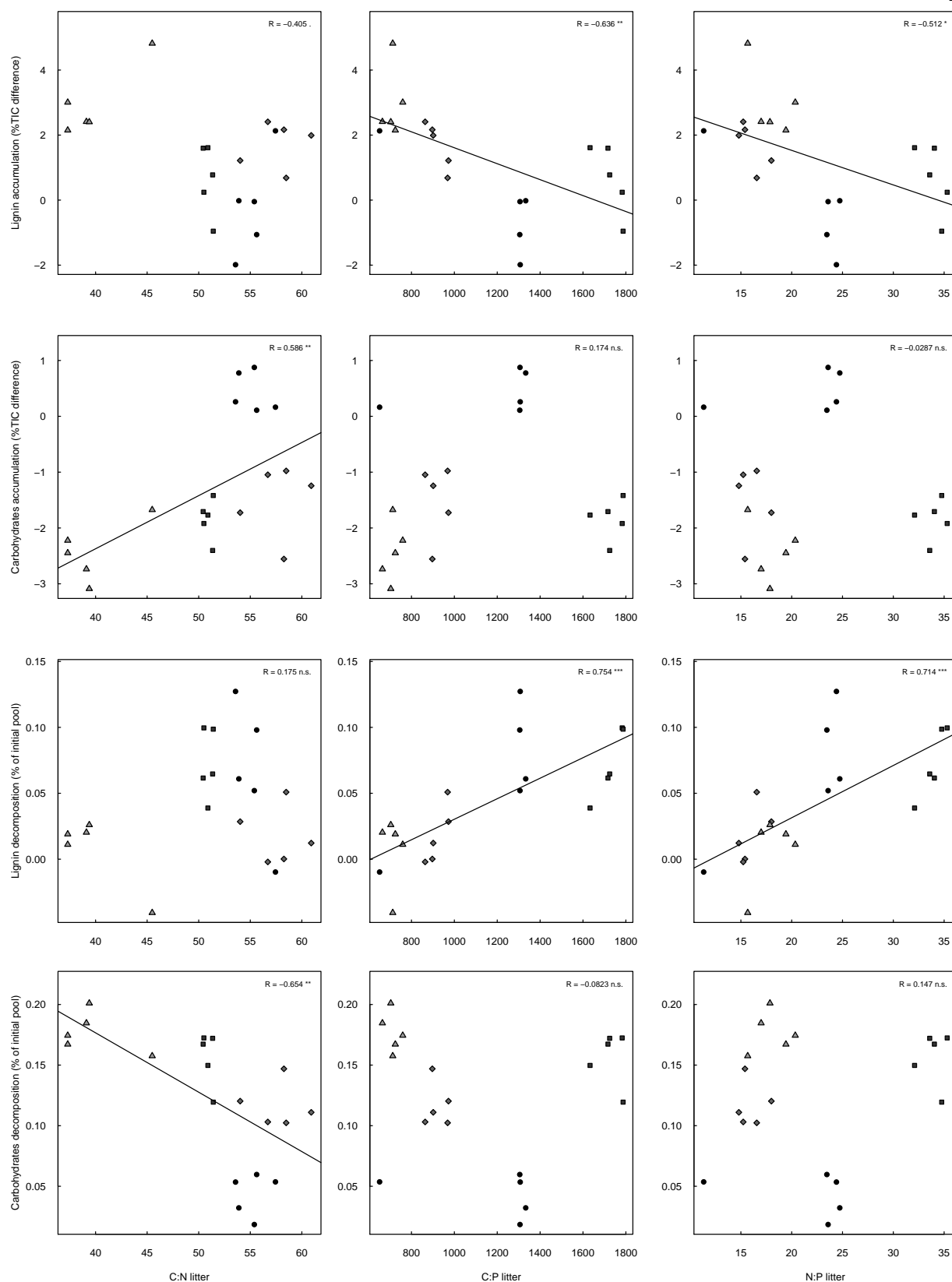


Figure 8. Correlations between Lignin and Carbohydrates accumulation and decomposition during the first 6 months of litter incubation and litter C:N:P ratios

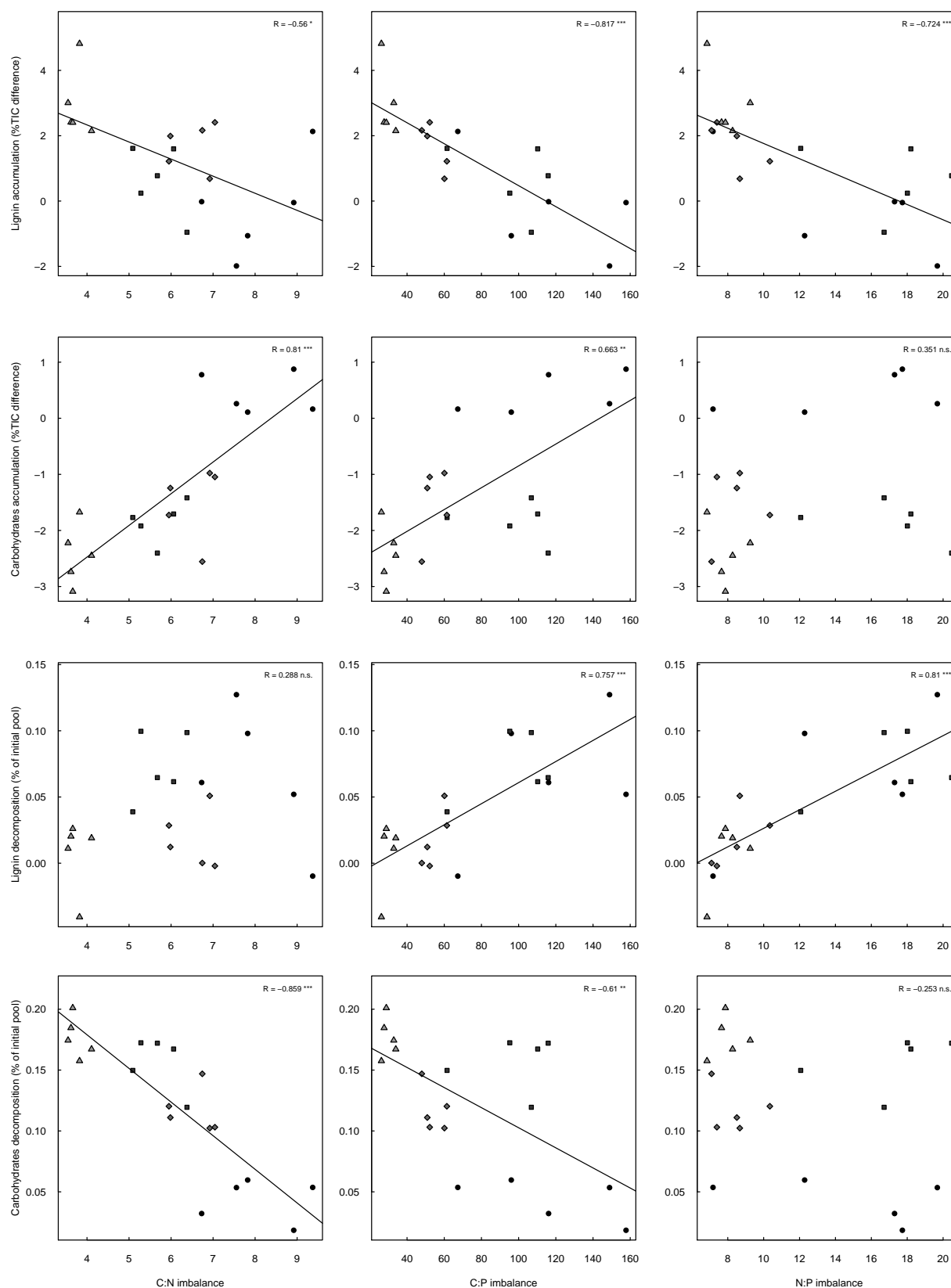


Figure 9. Correlations between Lignin and Carbohydrates accumulation and decomposition during the first 6 months of litter incubation and stoichiometric resource:consumer imbalances

Table 4. Correlations between C25/27/29 alkanes and alkenes, 14:0, 16:0 and 18:0 fatty acids and phytol. Differences between 0 and 181 days.

	alkanacc	alkenacc	faacc	phytolacc	alkandeg	alkendeg	fadeg	phytoldeg	alkanresp	alkenresp	faresp	phytolresp
Massloss	0.17	0.49	-0.0933	0.226	-0.15	-0.348	-0.00722	-0.462	-0.189	-0.227	-0.0146	-0.291
Actual respiration	0.752	0.428	0.701	0.792	-0.357	0.163	-0.656	-0.867	0.119	0.349	-0.767	-0.863
Accumulated Respiration	0.703	0.0679	0.703	0.781	-0.221	0.545	-0.507	-0.714	0.431	0.673	-0.792	-0.87
Cellulase activity	0.665	0.548	0.673	0.894	-0.154	0.112	-0.531	-0.905	0.308	0.332	-0.719	-0.903
Protease activity	0.027	0.0541	0.139	0.304	0.245	0.178	0.09	-0.172	0.344	0.3	-0.0935	-0.21
Phosphatase activity	0.673	0.169	0.756	0.778	-0.229	0.405	-0.574	-0.691	0.231	0.574	-0.75	-0.774
Chitinase activity	0.744	0.519	0.748	0.916	-0.234	0.157	-0.617	-0.931	0.27	0.382	-0.78	-0.93
Peroxidase activity	0.526	0.601	0.574	0.838	-0.157	0.0178	-0.482	-0.911	0.286	0.238	-0.653	-0.87
N mineralization	0.535	0.614	0.478	0.79	-0.0898	-0.0474	-0.625	-0.917	0.282	0.155	-0.551	-0.843
Nitrification	0.724	0.453	0.662	0.828	-0.327	0.132	-0.426	-0.941	0.197	0.298	-0.74	-0.923
P mineralization	0.654	0.487	0.551	0.836	-0.223	0.121	-0.43	-0.912	0.19	0.319	-0.615	-0.876
C litter	-0.0337	0.695	0.368	-0.284	-0.052	-0.174	-0.317	-0.762	0.353	0.0259	-0.437	-0.712
extractable C	0.715	-0.192	0.688	-0.369	-0.244	-0.0544	-0.0979	0.0362	-0.155	0.0115	0.0999	0.0999
N litter	0.688	0.65	0.502	0.7	-0.376	0.17	-0.562	-0.944	0.263	0.374	-0.741	-0.937
P litter	0.728	0.728	0.076	0.317	0.133	-0.494	-0.107	-0.429	0.0454	0.0547	-0.645	-0.829
C:N litter	0.054	-0.734	-0.562	-0.759	0.38	0.0768	0.598	0.943	0.197	-0.113	0.0955	-0.294
C:P litter	0.305	0.054	0.0372	-0.219	-0.216	0.57	-0.0572	0.273	-0.185	0.418	-0.0817	0.135
N:P litter	0.535	-0.561	0.24	0.0377	-0.348	0.584	-0.263	-0.0351	-0.156	0.49	-0.324	-0.139
C:N mic	0.557	0.398	0.62	0.826	0.00763	0.226	-0.345	-0.704	0.439	0.48	-0.609	-0.782
C:P mic	0.557	0.397	0.647	0.864	-0.00386	0.246	-0.38	-0.762	0.469	0.469	-0.624	-0.821
N:P mic	0.486	0.28	0.613	0.773	0.0068	0.288	-0.358	-0.661	0.396	0.487	-0.554	-0.717
C:N imbalance	-0.695	-0.486	-0.713	-0.91	0.167	-0.193	0.507	0.87	-0.367	-0.42	0.738	0.915
C:P imbalance	-0.289	-0.576	-0.46	-0.684	-0.196	0.0083	0.213	0.566	-0.164	0.375	0.738	0.874
N:P imbalance	0.0124	-0.566	-0.155	-0.356	-0.31	0.303	-0.00124	0.271	-0.354	0.128	0.0428	0.216

Table 5. Correlations between C25/27/29 alkanes and alkenes, 14:0, 16:0 and 18:0 fatty acids and phytol. Differences between 181 and 475 days.

	alkenacc	alkenacc	faacc	phytolacc	alkandeg	alkdeg	fadeg	phytoldeg	alkanresp	alkenresp	faresp	phytolresp
Massloss	-0.634	-0.289	0.0683	0.709	0.448	0.557	0.344	-0.478	0.152	0.525	-0.535	-0.731
Actual respiration	-0.471	-0.301	0.432	0.676	0.313	0.636	0.0176	-0.253	0.157	0.464	-0.326	-0.584
Accumulated Respiration	-0.795	-0.35	0.324	0.835	0.53	0.764	0.242	-0.402	0.241	0.77	-0.63	-0.829
Cellulase activity	-0.596	-0.244	0.662	0.784	0.356	0.613	-0.0828	-0.288	0.317	0.5	-0.274	-0.547
Protease activity	-0.38	-0.00818	0.659	0.439	0.12	0.196	-0.394	-0.0506	0.264	0.194	-0.011	-0.0894
Phosphatase activity	-0.451	0.0332	0.461	0.323	0.25	0.194	-0.224	-0.000321	0.28	0.255	-0.178	-0.192
Chitinase activity	-0.633	-0.104	0.589	0.611	0.3	0.44	-0.189	-0.186	0.244	0.536	-0.353	-0.4
Peroxidase activity	-0.0256	0.134	-0.345	-0.134	-0.0858	-0.13	0.379	0.137	-0.184	0.0737	-0.0468	-0.0142
N mineralization	0.0627	-0.106	0.343	0.127	0.028	0.0833	-0.401	-0.172	0.119	-0.106	0.0258	0.0264
Nitrification	-0.0379	0.0275	-0.156	0.236	-0.0981	0.0926	0.259	-0.395	-0.397	0.283	-0.569	-0.421
P mineralization	-0.496	-0.451	0.253	0.663	0.417	0.628	0.112	-0.424	0.179	0.535	-0.57	-0.657
C litter	0.0444	-0.336	0.206	0.473	0.333	0.543	0.0642	-0.249	0.235	0.211	-0.182	-0.447
N litter	-0.733	-0.294	0.398	-0.0325	0.223	0.145	0.685	-0.412	0.0415	0.0415	-0.212	-0.3
extractable C	-0.76	-0.181	0.116	0.876	0.452	0.701	0.14	-0.5	0.174	0.735	-0.666	-0.809
C:N litter	-0.354	-0.548	0.0977	0.575	0.254	0.448	-0.246	-0.0722	0.345	0.17	0.0983	-0.261
C:P litter	0.0939	0.0836	-0.682	-0.818	-0.614	-0.843	-0.448	0.494	-0.248	-0.804	0.701	0.922
N:P litter	-0.241	-0.0878	-0.627	-0.422	-0.0492	-0.249	0.48	-0.191	-0.251	0.0934	-0.341	-0.0041
C:N mic	0.549	0.241	-0.502	-0.0957	0.187	0.0732	0.654	-0.000689	-0.202	0.445	-0.659	-0.377
C:P mic	0.206	0.413	-0.218	-0.611	-0.18	-0.547	0.04	0.245	0.0205	-0.687	0.569	0.55
N:P mic	-0.0201	0.326	-0.054	-0.503	-0.241	-0.297	-0.0471	0.26	-0.217	-0.175	0.0327	0.355
C:N imbalance	0.429	0.393	0.363	-0.443	-0.175	-0.515	-0.028	0.156	-0.253	0.13	-0.243	0.124
C:P imbalance	-0.0373	-0.357	-0.544	0.0137	-0.586	-0.515	-0.584	0.403	-0.335	-0.297	0.27	0.611
N:P imbalance	-0.2	-0.486	-0.619	0.199	0.13	0.208	0.535	-0.327	-0.143	0.262	-0.427	-0.324
					0.37	0.391	0.687	-0.468	0.045	0.324	-0.457	-0.522

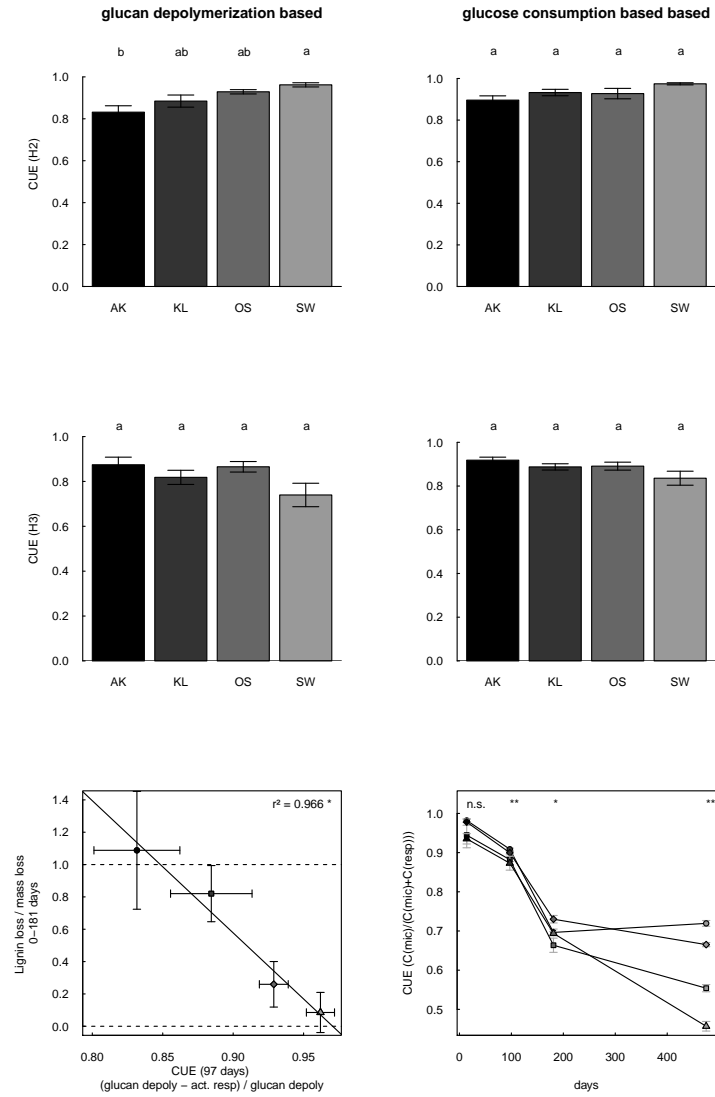


Figure 10. caption

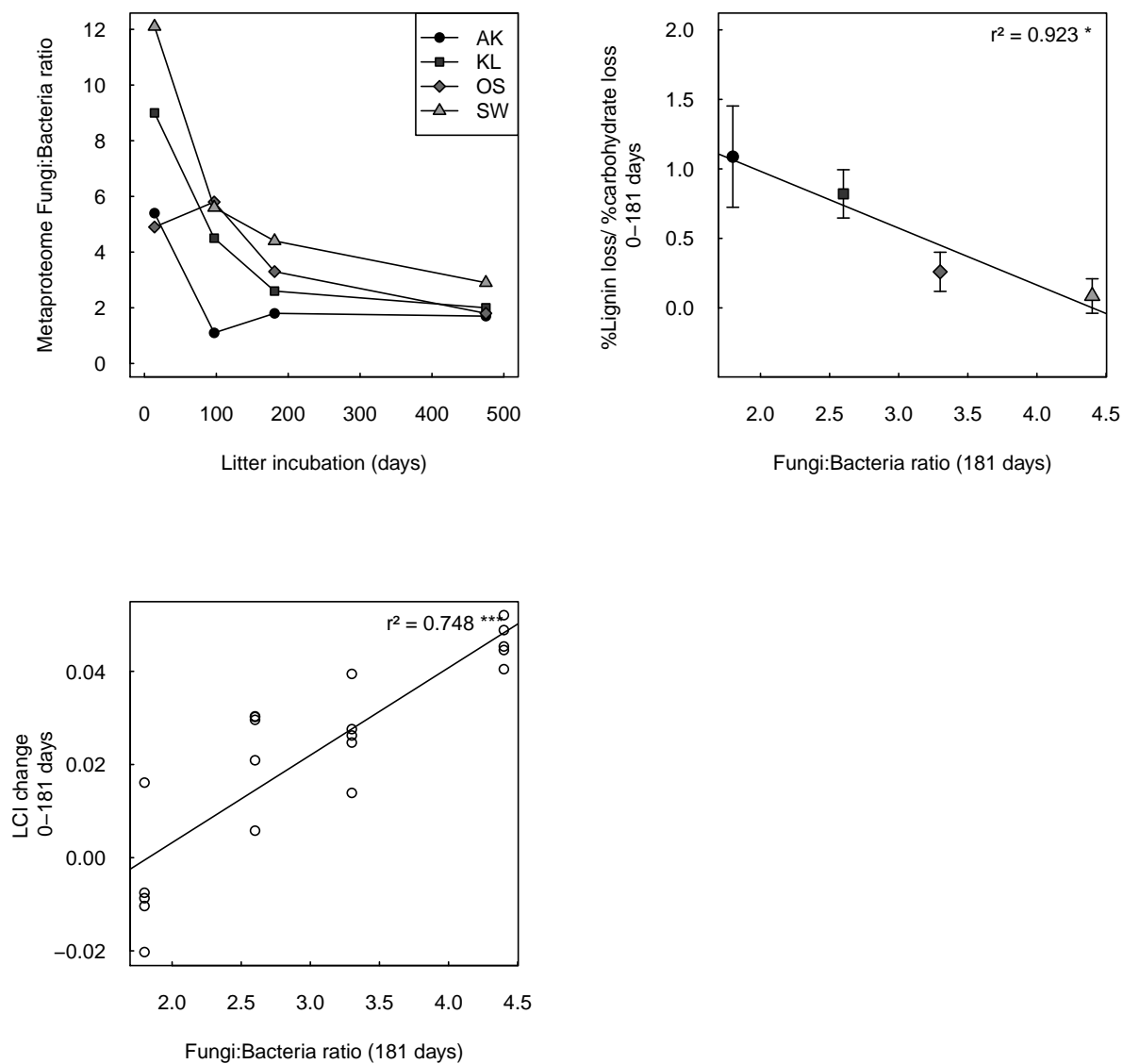


Figure 11. Bold the first sentence. Rest of figure 2 caption. Caption should be left justified, as specified by the options to the caption package.