universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

"High molecular weight compounds in beech litter decomposition"

Verfasser

Lukas Kohl

angestrebter akademischer Grad

Magister der Naturwissenschaften (Mag. rer. nat.)

Wien, 2011

Studienkennzahl lt. Studienblatt: A444

Studienrichtung lt. Studienblatt: Ökologie

Betreuer: Ao. Univ.-Prof. Mag. Dr. Andreas Richter

Contents

Introduction

This work is composed of three major parts that serve different perposes and are aimed at different readers:

General introduction gives a broad introduction to litter decomposition and explains its importance in biogeochemical carbon and nutrient cycles. Plant polymers present in beech litter briefly presented. Furthermore, models and concepts used to describe litter decomposition and stoichiometrical approaches are briefly reviewed. This chapter aims to give non-experts an compact overview over the background of this work. Finally, the experiment, of which this work forms part, is presented.

Methodological comments aims at future users of analytical pyrolysis shares experiences with this technique. It explaines the ratio behind choices taken in the implemention of our pyrolysis-GC/MS methods, points out alternatives, pitfalls encountered and possible further developments. It is not written not so much for professional analytical chemists as for biologists and other amateurs working with the method.

[**name**] Is a manuscript written to be submitted to Soil Biology and Biochemistry, and aims to become a state-of-the-art original research article in a peer-reviewed international journal.

1

General Introduction

1.1 Litter decomposition and the global carbon cycle

Rising atmospheric CO_2 concentrations and global climate changes caused by them (Treut et al., 2007) lead to an increased interest in natural carbon cycles and their anthropogenic modifications. Since pre-industrial times, annual means of atmospheric CO_2 concentration increased from 280 to 379 ppm (v/v) (Treut et al., 2007). The source of approximately 80% of the increase was be pined down to fossil fuel usage by comparing atmospheric CO_2 concentration to its ^{13} C signature (fossil fuel C is depleted in ^{13} C) or corresponding decrease in atmospheric O_2 concentrations(Treut et al., 2007). Furthermore, land use change and cement production are accounted for additional CO_2 emissions. Between 2000 and 2005, mean annual CO_2 emissions from fossil fuel burning and cement production accounts for 7.2 $\pm$ 0.3 Gt CO_2-C. Additionally, land use change causes the annual emissions 1.6 $\pm$ 1.1 Gt CO_2-C. Together with other greenhouse gases, elevated CO_2 concentrations are expected to raise earth mean surface temperature by nn degree by 20nn (IPCC?).

Anthropogenic CO_2 emissions are tightly interconnected with natural carbon cycles. Only 45% of the emissions are found in the athmosphere, 30% of the emmited CO_2 is absorbed in oceans and 25% in terrestrial ecosystems . Oceanic CO_2 absorption is based on export of particular and dissolved organic carbon and dissolved inorganic carbon (HCO_3^- , CO_3^{2-}) to intermediate and deep water layers. Land sinks take up carbon into larger vegetation- and soil C pools, i.e. due to a nothward shift of climatic limits for vegetation and CO_2 and N fertilization. However, a large part (-2.6 Gt a^{-1}) of this terrestrial carbon sinks is unaccounted for (Denman et al., 2007, p. 515).

Finding this “missing sink” and modeling feedback mechanisms of CO_2 emissions challenged scientists to strife for deepening their understanding of large scale biotic carbon transformation processes and storage. Globally, land plants assimilates 120 Gt C annually (gross primary production). This is almost one

sixth of the global atmospheric CO_2 pool (750 Gt a-1) and more than 15 times more than anthropogenic C emissions. Auto-trophic (plant) respiration consumes one half of the assimilated carbon, the other half is introduced into decomposition process as plant litter. Animal biomass and herbivore form only a neglect-able part of the total biomass [lit.], but can wield key controls on vegetation and its succession.

Ecosystem carbon balances are determined by the difference between carbon assimilation (photosynthesis) and respiration. While controls on photosynthesis rates are well understood, knowledge about decomposition processes is by far more limited. This is due to the fact that organisms capable of photosynthesis generally are green, seesile and grow aboveground, are therefore easy to find and study, while a large part of heterotrophic respiration is conducted by soil microbial communities of microscopic scale that dwell belowground, are hard to identify, and live in a chemically complex environment. Due to the complex nature of soils, studying chemical transformation processes and chemical controls over microbial communities and physiology is easier in aquatic than in terrestrian habitates (for example, differences between nutrient contents and bioavailable nutrient amounts are smaller in aquatic environments, facilitating studies of nutrient control on microbial communities). However, research interest in terrestrial decomposition processes, especially litter decomposition, which sees the highest biomass turnover, is enormous, with more than one peer-reviewed research article per day published on litter decomposition between 2005 and 2009 (Prescott, 2010).

Global litterfall summs up to for approximately 60 Gt C a^{-1}. Frequently between 30 and 70% of this mass are lost in the first year and further 20 to 30 % within another 5 to 10 years (Chapin et al., 2002, p.157).

Temperate forests are highly productive, average net primary production is estimated for 1550 g m^{-2} a^{-1} (1/3 of which is allocated into belowground biomass). They cover 1.7 * 10^7 km^2 (1/15th of earth land surface) and account for 8.1 Gt a-1 NPP (1/8th of total terrestrial NPP) (Chapin et al., 2002, p?). European beech (*Fagus silvaticus* L.) is the dominant forest forming tree species in potential vegetation in large parts of western and central Europe.

1.1.1 Decomposition processes

Decomposition processes are based on chemical, physical and biological degradation of dead organic matter. Thereby, three types of processes can be distinguished:

Leaching selectively dissolves litter components and transfers them into deeper soil layers. There, these components can either be degraded by soil organisms, adsorbed to minerals or exported to ground waters.

Fragmentation physically breaks down large pieces of litter into smaller units, which provide a bigger surface that can be colonized by microbes and can easier be taken up by soil fauna.

Chemical alteration occurs in both biotic and abiotic processes (Chapin et al., 2002). In the current work, we focus exclusively on the chemical transformation that occurs in beech litter.

1.1.2 Modeling litter decomposition

The most simple models discribe litter decomposition as a exponential loss over decomposition time, which can be described by mathematically described as

$L_t = L_0 e^{-kt}$

or

$ln(L_t/L_0) = -kt$

Where L_0 and L_t for the initial amount of litter and the amount of litter left after the time t has passed. The constant k describes the decomposition rate of a particular material Chapin et al. (2002).

1.1.3 Ecological stoichiometry

Carl Sprengel proposed in 1828 that crops rely on nutrients in a given ratio, and that growth is limited by the nutrient that is least frequent compared to this given ratio. While since then plants - and other organisms - were shown to be capable of a certain plasticity in their nutrient requirement, there is a trade-off between adaption to nutrient availability and competitive fitness. [1]

But organisms do not only rely on elements in a certain ratio, they are also bound (within an adaptive range) to keep them in specific range within their internal mileu. An homeostatic organism keeps this internal mileu constant independent of their ambiental conditions, while in on that is not homeostatic, the internal milieu changes with the elemental ratio in their substrate (Sterner and Elser, 2002).

By 1958, marine biologist Albert C. Redford pusblished results from measurements of the elementel composition of marine biomass featuring a constant ratio between carbon, nitrogen, and phosphorous (C:N:P = 106:16:1 (n/n)) in both living and dead biomass. The high constancy of this ratios is based on controls over CO_2 assimilation by N and P availability and controls of the biogeochemical cycling of nutrients (i.e. export by sedimentation) by biological systems (Cleveland and Liptzin, 2007).

Several attempts to find similar ratios in terrestrial ecosystem mostly failed due to the complex nature of terrestrial soils and difficulties to determine actual bio-availability of nutrients. [2]

Plants are able to assimilate carbon from atmospheric CO_2, but have to sequestrate other elements from soils. Furthermore, a significant part of nitrogen and other nutrients is removed from senescenced

[1] citation

[2] paper "redfield ratio for soils" here!

leaves before abscission. Therefore, plant detritus is enriched in carbon and depleted in nitrogen when compared to soils or decomposer organisms. C:N ratios (w/w) found in fresh beech litter are between 1:40 and 1:50 (Mooshammer et al., 2011), while soil C:N ratios are in the order of 1:20 [3]. During litter decomposition, the part of carbon mineralized is higher therefore than the part of nitrogen. Microbial decomposer communities found on early decomposition litter have biomass C:N ratios between 1:6 and 1:18, indicating microbes live in a environment characterized by a carbon surpluss and a lack of nitrogen. Litter decomposition rates were found to correlate with detritus C:N and C:P rateson a global scale (**?**).

1.2 Chemical constituents of initial beech litter

The dry biomass of freshly fallen plant litter is chemically dominated by polymeric compounds. Nitrogen is present almost exclusively in for of protein (Wanek et al., 2010), among carbohydrates, cellulose (the β - 1-4 glycosidic polymer of glucose) is most common (10-50 % of litter dry mass). Other carbohydrates - refered to as hemicelluloses - together make up between 30 and 40 % of litter dry mass. A wide variety of carbohydrate monomeres and glycosidic bindings occur in are leaf litter. Lignin, forms 15-40% biomass, is an aromatic polymer fromed through the radicalic polymerisation of several different phenylpropanoid monomers. (Berg, B. & McClaugherty, 2008, pp. 54f). The polymerization process of lignin can incorporates protein and carbohydrates into lignin polymers, thereby occluding them to decomposing enzymes and lowering their bioavailability. Nitrogen content of beech lignin was found twice as high as in bulk litter (**?**). Furthermore, cutin waxes (ester-bound long chain aliphaic compounds with aromatic components) and tannins are present.

Only a small fraction of foliar plant litter (approximately 25% for decidous litter, and less in conifer litter) is soluble in water (Berg, B. & McClaugherty, 2008). Therefore, decomposing microorganisms rely on the excretion of extracellular enzymes to break down plant biomass into soluble fragments Klotzbücher et al. (2011). Hydrolases break down protein and carbohydrates to amino acids and sugars, while the degradation of lignin is facilitated by oxidoreductases (**?**).

1.2.1 Micronutrients

Litter micronutrient content varies and depends in Transition metals, especially manganese and iron, are important co-factors of oxidoreductases. The aerobic degradation of complex aromatic compounds is facilitated by reactive oxigen species generated by such enzymes. Therefore, a lack of their cofactors can limit the degradation of complex material (especially phenolic compounds like lignin) in the decomposition processes of litter and other complex organic material like soil organic matter or dissolved organic

[3] citation

matter. [4]

1.3 Changes of litter carbon chemistry during decomposition

1.3.1 The traditional model developed by B. Berg

Traditional models of chemical changes during litter decomposition describe three phases of litter decomposition. In the early phase, which can expand until 40% of dry mass are lost, availability of labile carbon sources like soluble compounds and non-lignified carbohydrates is high. In this phase, mass loss rate were usually found to be nitrogen limited and - more generally - enhanced by high levels of nitrogen, phosphorous and dissolved carbon. In the late phase, lignin content inhibits further decomposition and mass loss rates are repressed by lignin and nitrogen contents, but enhances by high manganese contents. During this phase, lignin contents reach a constant value. Finally, at the end of decomposition, mass loss of near-humus litter reaches a limit value, and remaining biomass becomes incorporated into soils (fig. 1.1).

Berg, B. & McClaugherty (2008)).

1.3.2 Microbial nitrogen mining hypothesis

The "nitrogen mining hypothesis" is based on the concept that the breakdown of recalcitrant carbon allows soil microbiota to access recalcitrant nitrogen, while yielding little to no energy. This explains why nitrogen starvation triggers the excretion of enzymes degrading phenolic compounds (Craine et al., 2007; Moorhead and Sinsabaugh, 2006). In plant litter, nitrogen is often occluded within lignin molecules, which are degraded by similar enzymes. Isolated lignin fractions from fresh beech leaves were shown to contain twice as much nitrogen as bulk material (Dyckmans et al., 2002). Craine et al. (2007) incubated mixtures from 50 different soils and plant litter from 50 species, adding mineral nitrogen and phosphorous. They find that nitrogen, but not phosphorous addition lowers the amount of recalcitrant carbon decomposed. However, their quantification of labile and recalcitrant carbon respired are based exclusively of mathematical modeling, compare linear and exponential decline of respiration rates.

1.3.3 Carbon limitation of lignin decomposition

Recently, Klotzbücher et al. (2011) suggested that the degradation of lignin depends on the availability of labile carbon sources. In a climate chamber experiment, the authors incubated samples previously decomposed in-sitio for up to three years, recording microbial respiration, lignin content (based on CuO-

[4] references needed!!

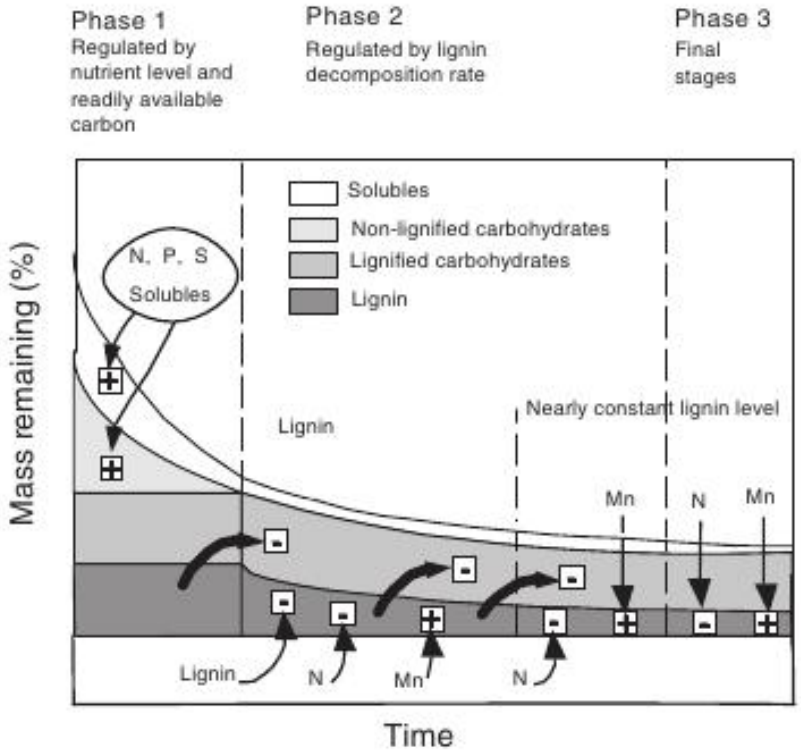

Fig. 6.1 Model for chemical changes and rate-regulating factors during decomposition (modified from Berg and Matzner 1997). The decomposition of water-soluble substances and unshielded cellulose/hemicellulose is stimulated by high levels of the major nutrients (early stage – *phase 1*). When all unshielded holocellulose is decomposed, only lignin-encrusted holocellulose and lignin remain. The early phase has been observed to last up to ca. 40% mass loss (case B in Table 6.1), with a very high mass-loss rate, or leaching. It may also be close to nonexistent, as in case C in Table 6.1. In the late stage (*phase 2*), the degradation of lignin controls the litter decomposition rate. Nitrogen hampers the degradation of lignin, and higher N concentrations suppress decomposition, whereas Mn appears to have a stimulating effect on lignin degradation. Finally, in the humus-near stage (*phase 3*), the lignin level is nearly constant, often at values of 50–55%, the litter decomposition rate is close to zero, and the accumulated mass loss also reaches its limit value

Figure 1.1: Litter decomposition model (taken from Berg, B. & McClaugherty (2008))

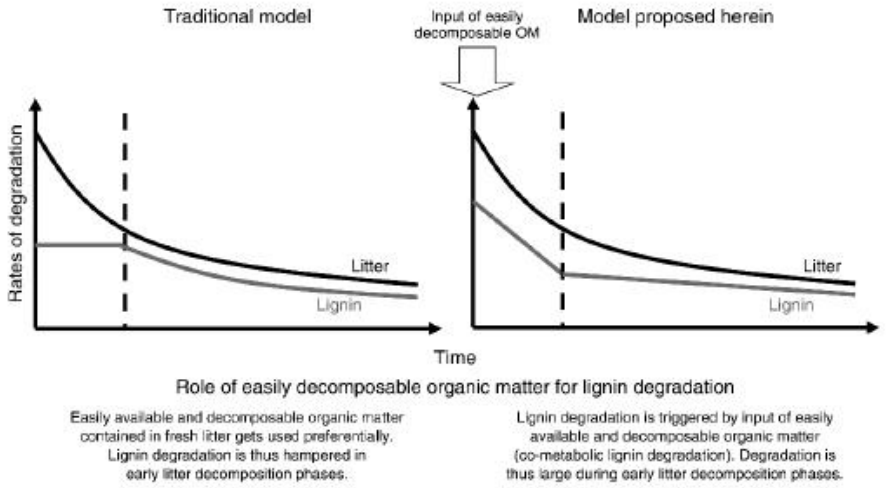

FIG. 5. Revised conceptual model for the fate of lignin during litter decomposition, based on data presented herein. The traditional model was proposed in Berg and Staaf (1980) and based on data from the decomposition of Scots pine needles. In our model, lignin will be degraded if easily degradable OM is available.

Figure 1.2: Lignin decomposition model (taken from Klotzbücher et al. (2011))

oxidation), and the production of soluble carbon (DOC). Regardless of the previous field incubation time, samples showed the highest lignin degradation rates at the beginning of the laboratory incubation, and a decrease of lignin contents over the first 200 days. Soluble carbon production was correlated to respiration rates thereafter. Therefore, the authors conclude that soluble carbon limits carbon mineralization rates in litter after an initial pool of labile carbon (which can result from experimental manipulation (i.e. draught-rewetting) at the experimental setup) are degraded. They also suggest, that lignin is not decomposed under such carbon-limited conditions. As lignin decomposition rates do not increase with the previous field incubation time, they contradict the longstanding hypothesis that lignin is not degraded during early litter decomposition, until a critical lignin content is reached, but propose a concept of early lignin decomposition, where lignin degradation rates are highest during early litter decomposition, when plenty of labile carbon is available (fig. 1.2).

1.3.4 N control over priming effects

The priming effect is defined as the amount of recalcitrant carbon that is mineralized when a source of labile carbon is added to the system (e.g. a soil). It is determined by adding a labeled substrate (e.g. 13C cellulose) and determining the difference in respiration and the amount of respired label. Fontaine et al. (2011) conducted an experiment adding cellulose, nitrogen or both and calculating the priming effect of high-N and low-N treatments.

1.3.5 Modeling litter decomposition

1.4 Analytical Pyrolysis

Pyrolysis is the decomposition of complex organic compounds under elevated temperatures in the absence of oxygen (Moldoveanu, 1998). In the case of analytical pyrolysis, samples are typically heated to temperatures above 500°C in an helium atmosphere. At this temperature, high molecular weight compounds break down to smaller, voluntile compounds. Most frequently, the helium atmosphere with the pyrolysis products is injected into a GC/MS-system. Pyrolysis products are separated on a GC column, and identified and quantified on a MS detector. Analytical pyrolysis was first applied by G. Williams in 1860 to proof that caoutchouc is a polymer of isoprene units. Currently, analytical pyrolysis is used to identify artificial polymers and plasticizers added in their production, natural polymers from plant material to soil and dissolved organic matter, and whenever only minimal sample amounts are available (forensics, art history, ..).

Three types of pyrolytic systems are frequently used:

(1) microfurnance type pyromates have an oven that is constantly heated to the pyrolysis temperature, into which the sample is droped,

(2) resistively heated systems use a metal filament (mostly platin) to electrically heat the pyrolysis chamber, and

(3) curie-point systems inductively heat a metal cup containing the sample to it's material specific curie-point, at which ferromagnetism is lost and the cup therefore is not further heated (Sobeih et al., 2008).

Detected pyrolysis products can be related to their polymer origin. While this is a straightforward interpretation for artificial polymers and natural homopolymers, the analysis of pyrograms from complex organic polymers can be challanging to the point where pyrolysis is used as simple fingerprinting method, allowing not qualitative interpretation but provide insight into difference between humic substances found in different samples. Nevertheless, analytical pyrolysis usually provides a good overview over the monomers present in complex organic material.

1.4.1 Research questions and experimental approach

The current work studies the influence of litter quality on the decomposition of high molecular weight substances.

2

Methodological comments

2.1 Column choice and temperature programm

This work uses a Carbowax column (Supelcowax 10) for seperation. The column was chosen for better peak seperation after comparing several measuremts on this column with a RTX 35 (Restec) column. A good part of the published Pyrolysis-GC/MS studies use simple HP-5/SP-5 or similar standard GC columns.

However, during analysis, limitations of the column became evident, especially the limited temperature range (maximum temperature 280°C) of the column. Due to this and probably long retention of polar substances on the column, we were not able to detect several interesting compounds: long chained (C18+) n-alkyl-alcohols, ω - hydroxy - n-alkyl-fatty acids, and $\alpha - \omega$ - n-alkyl-dicarboxylic acids (all common in cuticulary waxes). Our detection of n-alkanes and alkenes was limited to C27 compounds (C29 compounds could be detected, but strong discrimination against them was suspected). Among the carbohydrate products, only traces of leavoglycosan and no other dehydroxysugars. Laevoglycosan is usually among the major decomposion products of cellulose, and among anhydrosugars, products originating from different sugar monomeres can be differenciated, especially between pentoses and hexoses. Among the lignin products, pyrolysis products with functional groups in the side chain and syringol derivatives in general were discriminated against.

The GC temperature program was designed to freeze - trap pyrolysis products at the beginning of the column. Therefore a low initial temperature was chosen (50 °C). The maximum temperature of the column according to the producer is 280 °C. Again, reaching a higher temperature would be of advantage, because larger molecules (which have high diagnostic value) would be detected.

2.2 Internal standards and absolute quantification

Quantifying pyrolysis products can be a challange itself: Beside the high number of complex products to be quantified, comercial availability of these substances is limited. Due to the low sample amounts (100-500 µg) exact balancing of the sample is difficult, especially as pyrolysis vials are usually not optimized for balancing of to avoid sample losses. For the GSG Pyromat instrumentation, recovery rates strongly varied between samples, suposedly due to gas leakage in the Pyr-GC interface. Generally, reproductivity of recovery rates and balancing is not sufficiently high enough to relate absolute peak areas to sample inweight for quantitative analysis.

Other chromatographic applications commonly exclude this "injection bias" by the use of an internal standard. Until now, this is not common in pyr-GC/MS analysis. Two recent publication add an internal standard to the sample: Steinbeiss et al. (2006) uses p-methoxyphenone, Bocchini et al. (1997) tests several substances and conclude that xx is most suited as an internal standard for lignin determination. In both approaches the internal standard is not chemically modified during pyrolysis but evaporated ("themal desorption") and results in a single peak in the pyrogram. Internal standard amounts found can account for losses of pyrolysis products. It does not account for losses during the pyrolysis process itself, i.e. incomplete pyrolysis of the sample is not throughoutly heated to the intented temperature. Adding the internal standard to the sample in a known ratio is also difficult: usually the internal standard is applied by pipetting a small amount of a solution onto the sample (1-5 µL). Larger volumes do no fit into the pyrolysis vials and often provoce leakage of the solution from the bottom-open vials.

A substancial part of the products formed by the pyrolysis of natural organic polymers are not or not exactly identified, commercial availability of pyroylsis products is limited. Also, if their thermal stability is insufficient, these substances can not be induced to the chromatic system by thermal desorption in the pyrolysis unit. Due to this problems and the high number of compounds produced, no publication quantifying single pyrolysis was published yet.

Quantifying substances of origin of pyrolysis products is even harder than quantifying the products themselves. For plant material, the most 1important classes of compounds analyzed - carbohydrates and lignin - are present in different forms in plant litter. However, especially for Carbohydrates can not be distinguished by pyr-GC/MS, but it has to be assumed that during pyrolysis they do not produce the same product in the same ratios. Lignin components different among plant families, reference material for angiosperm is scare. Chemical alternations in lignin structures are unavoidable during preparation.

Due to the reasons above, commonly, analytical pyrolysis studies do not aim for an absolute quantification of pyrolysis products or their substances of origin.

2.3 Peak assignment

Peak assignment is the crucial step in the analysis of pyr-GC/MS data. Usually not the whole dataset, but a small number of representative files are screened.

For the current litter analysis, one replicate of initial litter and litter after 15 month incubation (from two different litter types) were analyzed. However, it was known from previous studies that litter types were highly similar in their composition. For more heterogenous samples, at least one replicate for each treatment should be analyzed.

The following steps were applied:

A List all peaks over a certain area treshhold was compiled. This is done by (1) automatic integration with the Xcalibur Qual Browser and (2) manual screening of print-outs of the chromtogram. Initial air contamination peaks are excluded. These are usually between 0.8 and and 2 minutes GC runtime, have characteristic molecule (M+) ions at m/z 28 (N2) 32 (O2) and 44 (CO2) and are often by far the highest peaks in the pyrogram.

An attempt to identify peaks with a relative peak area over a critical treshhold (i.e. 0.1 % total peak area).

When one substance class is detected, missing pyrolysis products from the same substance of origin are looked for, usually using their most abundant MS fragments.

Finally, critical diagnostic peaks can be found when looked for (specific ion traces)

For plant material, **?** present the most relevant data for the identification of pyrolysis products. They confirm the identity of over 100 pyrolysis products by standard addition. Recently, several studies supervised by Peter Buurman (Buurman and Roscoe, 2010; Schellekens et al., 2009; **?**) feature (1) up-to-date lists of peaks found and (2) good examples for information to be extracted from large datasets based on 100+ peaks in soil organic matter fractions.

2.4 Peak classification

Lignin pyrolysis products are 2- and 6- methoxylated and dimethoxylated phenols with alkyl groups of up to three carbon atoms in position 4. The peak list for lignin markers presented in this work is extensive and reliable. Other peaks of potential lignin origin include non-methoxylated phenoles with similar side chains. However, while lignin is expected to be accounted as source of a large part of free phenol produced during pyrolysis, it can also be a product of protein, carbohydrate and non-lignin phenolic compounds.

Carbohydrates products are derrivatives of furan and cyclopentenone with methyl-, oxomethyl and hydroxymethyl sidechains. Furan and Cyclopentenonederrivatives often show different trends. Some

authors attribute cyclopentenones to lipids in soils. Additionally, carbohydrates produce a large number of smaller molecules, including short chained aldehydes and carboxylic acids. In the current work, an important part of carbohydrate peaks could not be identified by their MS spetrum, but were assigned based on the measurement of reference carbohydrates (cellulose, glucose, xylan).

Protein is decomposed to pyridin and pyrrol and their methylated derrivatives during pyrolysis. Additionally, indole and methylindole were found, which are characteristic decomposition products of tryptophan. In literature, a number of small aromatic compounds (i.e. toluene) are described as pyrolysis products of individual amino acids.

Lipids

Bibliography

C. Berg, B. & McClaugherty. *Plant Litter. Decomposition, Humus Formation, Carbon Sequestration.* Springer, Berlin, 2008.

P Bocchini, G.C Galletti, S Camarero, and A.T Martinez. Absolute quantitation of lignin pyrolysis products using an internal standard. *Journal of Chromatography A*, 773 (1-2):227–232, June 1997. ISSN 00219673. doi: 10.1016/S0021-9673(97)00114-3. URL http://linkinghub.elsevier.com/retrieve/pii/S0021967397001143.

P. Buurman and R. Roscoe. Different chemical composition of free light, occluded light and extractable SOM fractions in soils of Cerrado and tilled and untilled fields, Minas Gerais, Brazil: a pyrolysis-GC/MS study. *European Journal of Soil Science*, 62(April): no–no, December 2010. ISSN 13510754. doi: 10.1111/j.1365-2389.2010.01327.x. URL https://univpn.univie.ac.at/+CSCO+c0756767633A2F2F6A6A6A2E7661747261676E706261617270672E70627A++/c
http://apps.isiknowledge.com/full_record.do?product=UA&search_mode=GeneralSearch&qid=1&SID=Y1lNPe9
http://doi.wiley.com/10.1111/j.1365-2389.2010.01327.x.

F. Stuart Chapin, Pamela A. Matson, and Harold A. Mooney. *Principles of Terrestrial Ecosystem Ecology.* Springer Science and Buisness Media, Inc., New York, 2002.

Cory C. Cleveland and Daniel Liptzin. C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry*, 85(3):235–252, July 2007. ISSN 0168-2563. doi: 10.1007/s10533-007-9132-0. URL http://www.springerlink.com/index/10.1007/s10533-007-9132-0.

Joseph M Craine, Carl Morrow, and Noah Fierer. Microbial nitrogen limitation increases decomposition. *Ecology*, 88(8):2105–13, August 2007. ISSN 0012-9658. URL http://www.ncbi.nlm.nih.gov/pubmed/17824441.

K.L. Denman, G. Brasseur, A. Chidthaisong, P. Ciais, P.M. Cox, R.E. Dickinson, D. Hauglustaine, C. Heinze, E. Holland, D. Jacob, U. Lohmann, S Ramachandran, P.L. da Silva Dias, S.C. Wofsy, and X. Zhang. Couplings between changes in the climate system and biogeochemistry. In S. Solomon,

D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor, and H.L. Miller, editors, *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, chapter 7, pages 499–587. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2007. URL http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Couplings+Between+Changes+in+the+Clir

J Dyckmans, H Flessa, K Brinkmann, C Mai, and A Polle. Carbon and nitrogen dynamics in acid detergent fibre lignins of beech (Fagus sylvatica L.) during the growth phase. *Plant, Cell & Environment*, 25(4):469–478, 2002. URL http://onlinelibrary.wiley.com/doi/10.1046/j.1365-3040.2002.00826.x/full.

S. Fontaine, C. Henault, a. Aamor, N. Bdioui, J.M.G. Bloor, V. Maire, B. Mary, S. Revaillot, and P.a. Maron. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. *Soil Biology and Biochemistry*, 43(1): 86–96, January 2011. ISSN 00380717. doi: 10.1016/j.soilbio.2010.09.017. URL http://linkinghub.elsevier.com/retrieve/pii/S0038071710003500.

Thimo Klotzbücher, Klaus Kaiser, Georg Guggenberger, Christiane Gatzek, and Karsten Kalbitz. A new conceptual model for the fate of lignin in decomposing plant litter. *America*, 92(5):1052–1062, 2011.

Serban C. Moldoveanu. Analytical Pyrolysis of Natural Organic Polymers, 1998.

Daryl L. Moorhead and Robert L. Sinsabaugh. A theoretical model of litter decay and microbial interaction. *Ecological Monographs*, 76(2):151–174, 2006. URL http://www.esajournals.org/doi/pdf/10.1890/0012-9615(2006)076[0151:ATMOLD]2.0.CO;2.

Maria Mooshammer, , and the Crew. Marias paper. *ecosystems*, 2011.

Cindy E. Prescott. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry*, 101(1-3):133–149, April 2010. ISSN 0168-2563. doi: 10.1007/s10533-010-9439-0. URL http://www.springerlink.com/index/10.1007/s10533-010-9439-0.

Judith Schellekens, Peter Buurman, and Xabier Pontevedra-Pombal. Selecting parameters for the environmental interpretation of peat molecular chemistry - A pyrolysis-GC/MS study. *Organic Geochemistry*, 40(6):678–691, June 2009. ISSN 01466380. doi: 10.1016/j.orggeochem.2009.03.006. URL http://linkinghub.elsevier.com/retrieve/pii/S0146638009000680.

Karina L Sobeih, Mark Baron, and Jose Gonzalez-Rodriguez. Recent trends and developments in

pyrolysis-gas chromatography. *Journal of chromatography. A*, 1186(1-2):51–66, April 2008. ISSN 0021-9673. doi: 10.1016/j.chroma.2007.10.017. URL http://www.ncbi.nlm.nih.gov/pubmed/17980891.

Sibylle Steinbeiss, Christian M. Schmidt, Klaus Heide, and Gerd Gleixner. δ13C values of pyrolysis products from cellulose and lignin represent the isotope content of their precursors. *Journal of Analytical and Applied Pyrolysis*, 75(1):19–26, January 2006. ISSN 01652370. doi: 10.1016/j.jaap.2005.03.009. URL http://linkinghub.elsevier.com/retrieve/pii/S016523700500080X.

Robert W. Sterner and James J. Elser. *Ecological Stoichiometry*. Princeton University Press, Princeton, 2002.

Le Treut, R Somerville, U Cubasch, Y Ding, C Mauritzen, A Mokssit, T Peterson, M Prather, D Qin, M Manning, Z Chen, M Marquis, K B Averyt, M Tignor, and United Kingdom. Historical Overview of Climate Change Science. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor, and H.L. Miller, editors, *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, chapter 1, pages 93–127. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2007.

Wolfgang Wanek, Maria Mooshammer, Andreas Blöchl, Angelika Hanreich, Katharina Keiblinger, Sophie Zechmeister-Boltenstern, and Andreas Richter. Determination of gross rates of amino acid production and immobilization in decomposing leaf litter by a novel N-15 isotope pool dilution technique. *Soil Biology and Biochemistry*, 42(8):1293–1302, 2010. ISSN 00380717. doi: 10.1016/j.soilbio.2010.04.001. URL http://linkinghub.elsevier.com/retrieve/pii/S0038071710001331.

3

Manuscript

Controls of litter chemistry over early lignin decomposition in beech litter

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

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

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

3 Current address: Institute for Soil Science, University of Natural Resources and Life Sciences, Peter Jordan-Straße 82, A-1190, Vienna, Austria

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

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

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

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

Abstract

Lignin is considered the most recalcitrant component of plant litter, which is accumulated during early decomposition and is degraded only in late decomposition stages when its concentration limits litter decomposition rates. A recent study 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.

Depending on litter stoichiometry, significant amounts of lignin were decomposed during the first 6 month of incubation. Lignin degradation rates ranged from in litter decomposition ranges from marginal amounts to lignin decomposition at average carbon mineralization rates (i.e. no discrimination against lignin), leading to different of lignin accumulated at the end of this period. Between 6 and 15 month, no lignin content remained constant, but different lignin contents acquired earlier reminded in place.

Lignin degradation rates were correlated positively to stoichiometric resource/consumer ratios (i.e. correlated with high microbial nutrient demand) and best correlated to C:P ratios. Lignin decomposition was also correlated to low bacteria/fungi ratios.

Introduction

Plant litter biomass is dominated by macromolecular compounds. In deciduous foliar litter, lignin and carbohydrate polymers together make up 40-60% of litter dry mass [1], while leachable substances ("DOM") account for only 1.5-6% [2].

Litter decomposition models generally follow the concept that organic compounds in litter form up to three independent pools of increasing recalcitrance (i.e. soluble compounds, cellulose and hemi-celluloses, and lignin). During decomposition, soluble compounds are most accessible to microbes and are consumed first, followed by carbohydrates (i.e. cellulose). Lignin can be decomposed only by specialized fungi and is not degraded until accumulated to a certain, critical level when it inhibits the degradation of less recalcitrant compounds [3–6]. The pools are usually quantified by gravimatric determination of cellulose, hemi-celluloses and lignin after sequential extractions with selective solvents. These methods were repeatedly criticized as unspecific for lignin determination [7]. When analyzed with alternative methods (NMR, CuO-oxidation, Pyrolysis-GC/MS), extracted lignin fractions contain many other than the proclaimed substances (e.g. [8]). [1]

Recent studies based on more specific methods to determine litter lignin content 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 soil organic matter [9, 10]. It was also shown that several bacterial taxa are able to degrade lignin[2].

For leaf litter, lignin depletion during early decomposition and lignin decomposition rates decreasing with litter age were recently reported [11]. Based on this results, the authors proposed a new concept for lignin degradation in which fastest lignin degradation occurs during early litter decomposition when labile carbon availability is high. Lignin decomposition during late decomposition is limited by (dissolved organic) carbon availability. However, they do do not elaborate stoichiometric constraints of lignin decomposition[3]. During radical polymerization, significant amounts of cellulose and protein are incorporated into lignin structures [12]. In isolated lignin fractions from fresh beech litter, N contents twice as high as in bulk litter were found [13]. It was therefore argued that, while yielding little C and energy, lignin decomposition makes occluded cell wall protein accessible to decomposers, and lignin decompostion is therefore not driven by C but by N demand ("Nitrogen mining theory") [14].

[1] [lit CuO], lit[Pyr]
[2] ref.
[3] ev. nutrient contents instead of stoichiometry?

Nitrogen fertilization experiments with litter and soils indicated that litter N contents are important controls of lignin degradation: N addition increased mass loss rates in low-lignin litter while slowing down decomposition in lignin-rich litter [15]. High nitrogen levels were reported to inhibit lignolytic enzymes in forest soils [16]. Moreover, cellulose triggered a stronger 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 [17].

Addition of N has very different effects on litter decomposition than varying N levels in litter. This is due to the fact that leaf litter N is stored in protein and lignin structures and not directly available to microorganisms, while fertilizer N is added in the form of readily available inorganic N (NH_4^+, NO_3^-). N-fertilization experiments can simulate increased N-deposition rates but not the effect of litter N on decomposition processes [18].

In this study we analyze samples of beech litter varying in N and P content with pyrolysis-GC/MS (pyr-GC/MS) after up to 15 month climate chamber incubation. We sterilized and re-innoculated the litter prior to incubation to study the effect of resource stoichiometry on microbial decomposition, but exclude effects resulting from different initial microbial communities. We address the following questions:

(1) Is lignin decomposition delayed until late decomposition stages or are significant amounts of lignin degraded during early litter decomposition?

(2) Are lignin and carbohydrate degradation rates controlled[4] by litter N, P, and soluble C content? Do this controls change during decomposition?

(3) Do high lignin degradation rates correspond to higher fungal activity? Do wider C:N and C:P ratios favor lignin degradation by fungi while more narrow C:N and C:P ratios favor carbohydrate degradation by bacteria?

Results

Mass loss, respiration and extractable organic carbon

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 [19]. 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

[4] correlated?

correlated to litter N content (R=0.794, p=***). Detailed results were reported by [20].

Highest respiration rates were measured at the first measurement after 14 days incubation (150-350 µg CO_2-C d-1 g-1 litter-C), dropped to rates between between 75 and 100 µg CO_2-C d-1 g-1 litter-C after 97 days. After 181 and 475 days, respiration rates for AK and OS further decreased, while SW and KL show a second maximal respiration after 181 days. Accumulated respiration

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 475 days. After 14 and 97 days, the highest C content was found in SW litter followed by AK (data not shown). 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 475 days ($r = 0.90$, $p < 0.001$).

Microbial biomass abundance and community

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 distinct 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. 3).

Litter microbial biomass is homeostatic during the first 6 month (no or marginally negative correlation between microbial stoichiometry and litter stoichiometry) [20], 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. 3).

Metaproteome fungi:bateria ratios were highest (1:5 - 1:12) after 14 days and decrease during decomposition (1:1.7 - 1:3 after 475 days). Differences between litter from different sites decrease during decomposition. Fungi are most dominant in SW, bacteria in AK. The fungi to bacteria ratio is negatively correlated to lignin decomposition during the first 6 month 11.

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). Cellulases activity is highest after 3 month and decreases between 97 and 181 days, phenoloxidase and peroxidase activities reach their maximum after 181 days (fig. **??**). For all enzymes and at all time points, SW showed the highest and AK the lowest activity. Differences between these two sites were more pronounced in cellulase activity (SW 10x higher than AK) than oxidative enzymes (4x higher). The phenoloxidase/cellulases and peroxidase/cellulase ratio was lowest for AK and highest for SW at all time points and continously decreased during decomposition. This indicates that microbial communities in AK litter invest more energy and nitrogen into degrading lignin and less into degrading carbohydrates than other litter types. (fig. **??**)

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 **??**[5] 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 [21] using thermochemolysis. [6]

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

[5] check fig.

[6] 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.

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. **??**).

Correlations between lignin and carbohydrate decomposition and litter chemistry, microbial community and decomposition 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 [20] and [22]. 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 allowed us to single out the effects of litter quality on the microbial decomposer community as well as decomposition processes, while excluding effects of fauna, climate and the initial microbial community. By exploiting intra-specific differences in beech litter stoichiometry, we were able to minimize differences in the chem ical composition of initial litter (e.g. lignin and cellulose content), while exploring the effect of litter nutrient contents on lignin and carbohydrate decomposition. Therefore, we can attribute different rates of carbohydrate and lignin decomposition to the intrinsic qualities of different litter types.

Contradicting the traditional concepts of litter decomposition, our results demonstrate that relevant amounts of lignin are degraded during the the first 6 months of incubation. During this early stage, lignin

decomposition rates depended on litter quality and ranged from non-significant amounts decomposed to degradation rates similar to bulk carbon mineralization rates (i.e. no discrimination against lignin). We can therefore confirm that early lignin decomposition rates are by far underestimated, as recently proposed Klotzbücher et. al. [11], based on a complementary analytic approach. Unlike them, we found no decreases in lignin contents and constant or increasing lignin degradation rates during early decomposition. Additionally, we found a change in the controls of lignin discrimination after this initial period. While the preference for carbohydrate to lignin decomposition was controlled by litter chemistry over the first 6 month, all components of litter were degraded at similar rates thereafter.

Differences in initial lignin contents were marginal (below 29-31 %TIC), and lignin contents of sites with high initial lignin decomposition rates were not higher than that of sites with low rates. Therefore, early lignin accumulation did not result from high lignin contents as is suggested by traditional decomposition models. Also, these differences are not caused by a lack of Mn or Fe, which were sugested as rate limiting for late lignin decomposition [1]. While Mn and Fe contents strongly varied between litter collected at different sites, but both 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. Also, soluble carbon was not limiting lignin decomposition since we found it's highest amounts in litter from the two different sites who show the highest and the lowest lignin degradation.

We found strong evidences that C:N:P stoichiometry exerts a key control over the extent of lignin decomposition during initial decomposition. Carbohydrate decomposition was correlated positively to litter N contents and negatively to litter C:N ratios, as were a majority of decomposition processes (mass loss, respiration, potential extracellular enzymatic activities). In contrast, relative decomposition rates of Lignin were positively correlated with litter C:P ratios and negatively with disolved and total P. Correlation was highest when lignin decomposition rates were compared to resource/consumer C:P ratios.

Cultivation studies showed that lignin decomposition in fungi is triggered by nitrogen starvation, and that lignin does not provide sufficient energy to maintain the decomposer's metabolism without the use of another carbon sources[7]. Lignin decomposition was found in wild-type *A. thaliana* litter , but not in a low-cellulose mutant a during 12 month incubation in a boreal forest [18]. In the N- and P- co-limited situation during early litter decomposition, in which lignin is degraded to access additional nutrients or to use a C surplus by decomposing an less C efficient but nutrient enriched substrate. However, a stimulation of lignin decomposition by a high P imbalance or a delay of lignin decomposition under high P availability,

[7] citation

as indicated by the high correlation to P pools we found, was not reported yet.

For the decomposition of litter lignin and carbohydrates, microbial decomposers rely on the production and excretion of hydrolytic and oxidative extracellular enzymes. While the absolute amounts, in which these enzymes where produced, were controlled by N availability, the ratio in which they were produced correlated to differences in cellulose and lignin degradation [8]. Talbot et. al. [18] suggested that lignin decomposition is a micorbial strategy to evade competition by colonizing more lignin-rich and nutrient-poor substrates. Indeed we find lignin decomposition in low quality litter with a high difference between resource and difference, which are high in stress (N/P deficit), but low in competition. Low P availability further limits the fast growth rates during early decomposition and gives k-strategists (slow growing on recalcitrant carbon) an advantage over r strategists (fast growing on labile carbon) of of a fast growing microbial community can not be met. Indeed we found that lignin degradation is higher in litter, where the C:P and N:P are highest, i.e. high P demand limits growth.[9]

While the mode of this regulation is remains unknown, we find a corresponding shift in the microbial community composition. Unlike predicted by stoichiometric theory, not bacteria but fungi were more successful in using high litter N and P during initial decomposition. Fungi colonized litter faster than bacteria and therefore dominated early litter decomposition, but the bacteria/fungi ratio decreased over the entire incubation period. This apparent competitive advantage of fungi at high nutrient levels lead to negative correlations between litter and microbial stoichiometry during the first 6 month. Fungi-rich communities more efficiently used additional N input to produce extracellular enzymes that degrade carbohydrates immediately after inoculation (B/F is correlated to litter N 14 days after inoculation) and additional P to build up microbial biomass on a longer time scale(B/F is correlated to litter P after 6 month). Interestingly, it were the bacteria-dominated communities were more active in decomposing lignin than those dominated by fungi. This does not necessarily indicate that bacteria play a key role in lignin decomposition. However, a bacteria-rich decomposer consortium has elevated N and P, but less C demand, conditions that favor lignin over carbohydrate decomposition. Another notable change occured in the homeostasis of the microbial community. While the microbial community was strictly homeostatic during the first 6 month, substrate stoichiometry had a minor, but significant influence on microbial stoichiometry after 15 month. Together, those changes indicate that while the microbial communities is able to compensate for differences in substrate quality (on the expense of community growth and overall decomposition speed) and to select preferred compounds during the first 6 month. However, this compensation is limited and imbalances can

[8] cor stat
[9] naja..

not be upheld at the same intensity after the first 200 days. [10]

While we found different levels of lignin degradation during the first 6 month, lignin contents remained constant in litter from all sites between 6 and 15 month. This indicates that lignin was decomposed at the same rate as other litter compounds, but differences in lignin contents acquired during the first 6 month remain in place. 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. Lignin loss in this decomposition stage was positively correlated to litter N content, as were carbohydrate loss, litter mass loss and respiration. The higher fungi/bacteria ratio continuously decreases during decomposition, as fungi, which grew fast in N and P rich litter become out-competed by a bacteria-dominated lignin degrading community, with little differences in F/B rates between litter types at this later stage.

The change in decomposition dynamics corresponds to change in DOM production. While during the first 3 month, DOM contents were not (or to a lesser extend) correlated to litter N, and actual respiration after 6 and 15 month[11]. KltzbÄijcher et. al. [11] suggest 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. The authors interpret this correlation as carbon limitation to respiration, and suggest that lignin decomposition is inhibited under such a limitation. We found a similar correlation between extractable carbon and respiration after 181 days, but not the inhibition of lignin decomposition. Also, both respiration and DOM production are correlated litter N content at this stage. We therefore suggest that litter N content is the key control over both DOM production and respiration, since the process of degrading macromolecular compounds into soluble molecules is conducted by extracellular enzymes and is therefore N intensive.

Conclusions

Our results contradict the traditional concept that lignin decomposition is inhibited until late decomposition. While traditional litter decomposition concepts propose that lignin decomposition in late decomposition stages, we find substantial amounts of lignin decomposed during the first six month. The extend to which lignin is decomposed was controlled by litter chemistry over the first 6 month, but not thereafter. While carbohydrate decomposition was stimulated by high N contents, early lignin decomposition rates were highly correlated C:P ratio and resource:consumer imbalances. Our results also further questions, that recalcitrance

[10] naja..
[11] stats

is intrinsic to lignin as a chemical compound, but suggests that the accumulation of lignin also depends on ecological conditions, under which decomposition processes take place. High lignin contents accumulated during this stages remained in place during later decomposition. For further studies, this raises the question, to which extend late decomposition is influenced by this early, stoichiometry-controlled accumulation of recalcitrant compounds.

Material and methods

Litter decomposition experiment

A detailed description of our litter decomposition experiment was published in [23]. 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[12] 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

[12] greek gamma here

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 [24]).

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 [25]. C and N concentration in extracts were determined with a TOC/TN analyzer (TOC-VCPH and TNM, Schimadzu), Phosphorous was determined photometrically.[13]

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.

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.

Enzyme activities

Measurements of potential exo-enzyme activities for cellulases, peroxidases and phenoloxidase were described by [22]. 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) [26–28].

[13] lit!!

Metaproteome analysis

[..]

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.[14]

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 [29, 30]. 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 [29].

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

[14] maybe cite other paper for method?

products, C_{init} for the initial amount of C and R_{acc} for the accumulated CO_2-C respired by a mesocosm.[14]

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

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

Statistical analysis

All statistical analyses were performed with the software and statistical computing environment R using the package "vegan" [?,31]. 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 [20] and [22].

Acknowledgments

References

1. Berg, B & McClaugherty C (2008) Plant Litter. Decomposition, Humus Formation, Carbon Sequestration. Berlin: Springer.
2. Don A, Kalbitz K (2005) Amounts and degradability of dissolved organic carbon from foliar litter at different decomposition stages. Soil Biology and Biochemistry 37: 2171–2179.
3. Berg B, Staaf H (1980) Decomposition rate and chemical changes of Scots pine needle litter. II. Influence of chemical composition. Ecological Bulletins : 373–390.
4. Coûteaux MM, Bottner P, Berg B (1995) Litter decomposition, climate and liter quality. Trends in ecology & evolution (Personal edition) 10: 63–66.
5. Moorhead DL, Sinsabaugh RL (2006) A theoretical model of litter decay and microbial interaction. Ecological Monographs 76: 151–174.

6. Adair EC, Parton WJ, Del Grosso SJ, Silver WL, Harmon ME, et al. (2008) Simple three-pool model accurately describes patterns of long-term litter decomposition in diverse climates. Global Change Biology : 2636–2660.

7. Hatfield RD, Romualdo SF (2005) Can Lignin Be Accurately Measured? Crop Science 45: 832–839.

8. Preston CM, Trofymow JA, Sayer BG, Niu J (1997) 13C nuclear magnetic resonance spectroscopy with cross-polarization and magicâĂŞangle spinning investigation of the proximate analysis fractions used to assess litter quality in decomposition studies. Canadian Journal of Botany 75: 1601–1613.

9. Thevenot M, Dignac MF, Rumpel C (2010) Fate of lignins in soils: A review. Soil Biology and Biochemistry 42: 1200–1211.

10. Bol R, Poirier N, Balesdent J, Gleixner G (2009) Molecular turnover time of soil organic matter in particle - size fractions of an arable soil. Rapid Communications in Mass Spectrometry 23: 2551–2558.

11. Klotzbücher T, Kaiser K, Guggenberger G, Gatzek C, Kalbitz K (2011) A new conceptual model for the fate of lignin in decomposing plant litter. America 92: 1052–1062.

12. Achyuthan KE, Achyuthan AM, Adams PD, Dirk SM, Harper JC, et al. (2010) Supramolecular self-assembled chaos: polyphenolic lignin's barrier to cost-effective lignocellulosic biofuels. Molecules (Basel, Switzerland) 15: 8641–88.

13. Dyckmans J, Flessa H, Brinkmann K, Mai C, Polle A (2002) Carbon and nitrogen dynamics in acid detergent fibre lignins of beech (Fagus sylvatica L.) during the growth phase. Plant, Cell & Environment 25: 469–478.

14. Craine JM, Morrow C, Fierer N (2007) Microbial nitrogen limitation increases decomposition. Ecology 88: 2105–13.

15. Knorr M, Frey S, Curtis P (2005) Nitrogen addition and litter decomposition : A meta-analysis. Ecology 86: 3252–3257.

16. Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry 42: 391–404.

17. Fontaine S, Henault C, Aamor a, Bdioui N, Bloor J, et al. (2011) Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biology and Biochemistry 43: 86–96.

18. Talbot JM, Treseder KK Interactions between lignin, cellulose, and nitrogem drive litter chemistry decay relationships. Ecology (in press) .

19. Kalbitz K, Kaiser K, Bargholz J, Dardenne P (2006) Lignin degradation controls the production of dissolved organic matter in decomposing foliar litter. European Journal of Soil Science 57: 504–516.

20. Mooshammer M, , the Crew (2011) Marias paper. ecosystems .

21. Snajdr J, Cajthaml T, Valášková V, Merhautová V, Petránková M, et al. (2011) Transformation of Quercus petraea litter: successive changes in litter chemistry are reflected in differential enzyme activity and changes in the microbial community composition. FEMS microbiology ecology 75: 291–303.

22. Leitner S, Wanek W, Wild B, Haemmerle I, Kohl L, et al. (2011) Linking resource quality to decomposition processes: Influence iof litter chemistry and stoichiometry on glucan depolymerization during decomposition of beech (Fagus silvatica L.) litter. Soil Biology and Biochemistry .

23. Wanek W, Mooshammer M, Blöchl A, Hanreich A, Keiblinger K, et al. (2010) Determination of gross rates of amino acid production and immobilization in decomposing leaf litter by a novel N-15 isotope pool dilution technique. Soil Biology and Biochemistry 42: 1293–1302.

24. Henschler G (1988) Analysen im biologischen Material. Weinheim: VCH Verlagsgesellschaft mbH.

25. Schinner (1996) chloroform fumigation method (dummy) .

26. Marx M (2001) A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biology and Biochemistry 33: 1633–1640.

27. Sinsabaugh RL (1999) Characterizing soil microbial communities. Standard Soil Methods for Long-Term Ecological Research 2: 318–348.

28. Kaiser C, Koranda M, Kitzler B, Fuchslueger L, Schnecker J, et al. (2010) Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. New Phytologist 187: 843–858.

29. Schellekens J, Buurman P, Pontevedra-Pombal X (2009) Selecting parameters for the environmental interpretation of peat molecular chemistry - A pyrolysis-GC/MS study. Organic Geochemistry 40: 678–691.

30. Kuder T, Kruge MA (1998) Preservation of biomolecules in sub-fossil plants from raised peat bogs: a potential paleoenvironmental proxy. Organic Geochemistry 29: 1355–1368.

31. Oksanen J, Blanchet FG, Kindt R, Legendre P, O'Hara R, et al. (2011). vegan: Community Ecology Package. R packge version 1.17-9. URL http://cran.r-project.org/package=vegan.

Figure Legends

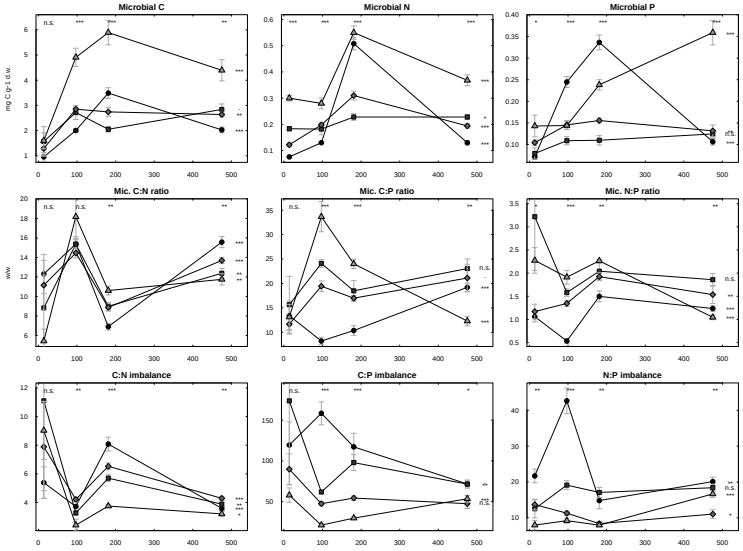

Figure 1. Microbial biomassm, microbial stoichiometry and resource:consumer stoichiometric imbalance. Error bars indicate standard errors (n=5).

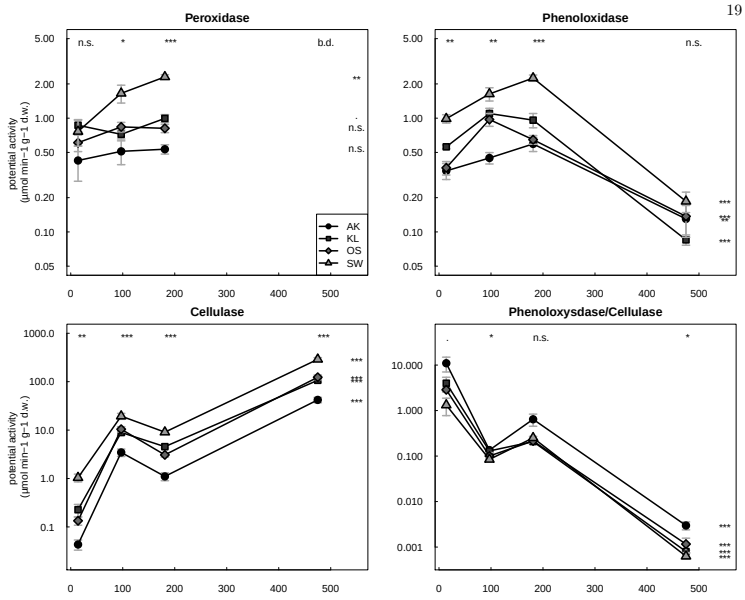

Figure 2. Potential eco-enzyme activities [caption]

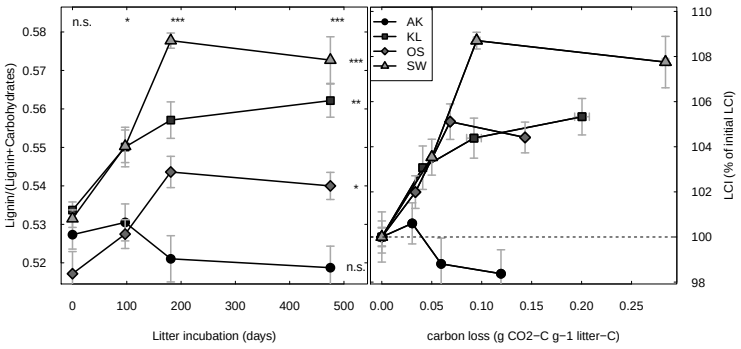

Figure 3. Develoment of the LCI (lignin/(lignin+carbohydrates)). Errorbars indicate standard errors (n=4-5). The dashed line indicates a constant ratio between lignin and carbohydrates (i.e. no preferential decomposition of carbohydrates.

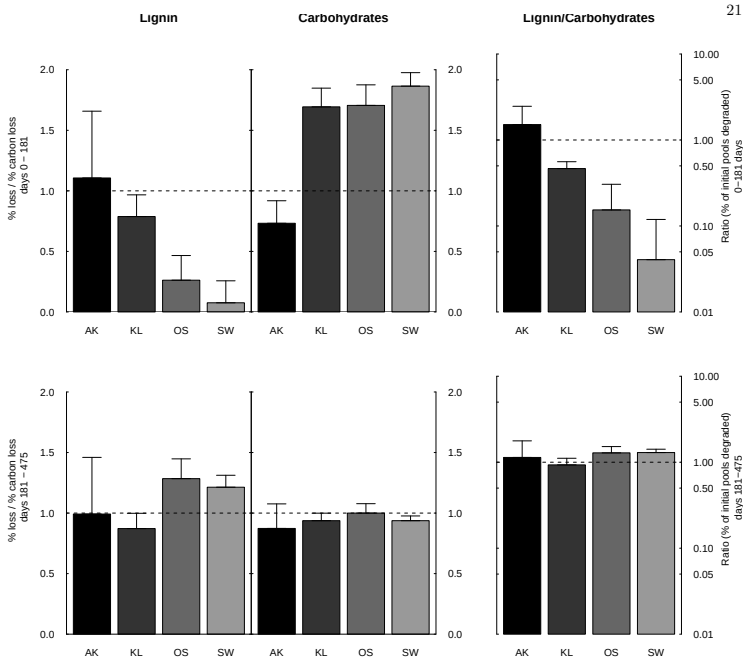

Figure 4. Carbon loss corrected amounts of lignin and carbohydrates degraded. Carbon loss was calculated based on accumulated respiration. Error bars indicate standard errors (n=4-5). The dashed line marks no discrination between lignin, carbohydrates and bulk carbon loss.

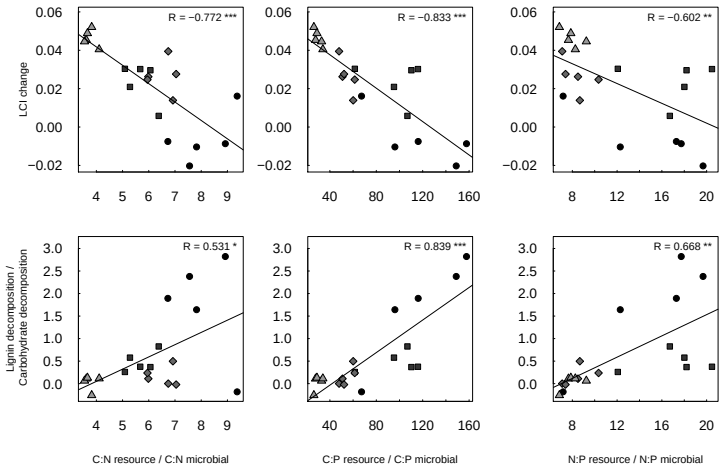

Figure 5. Correlations between Lignin accumulation during the first 6 month of litter incubation and stoichiometric resource:consumer imbalances

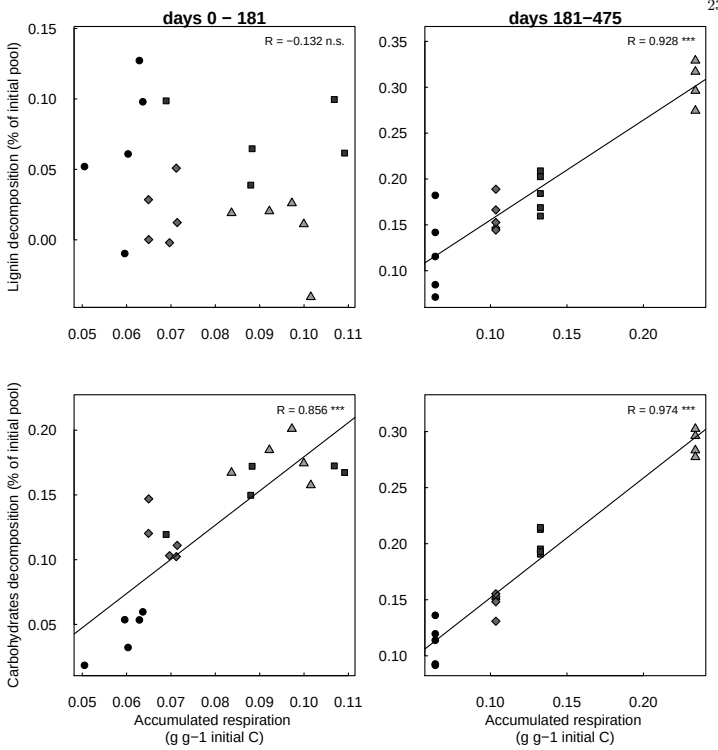

Figure 6. caption

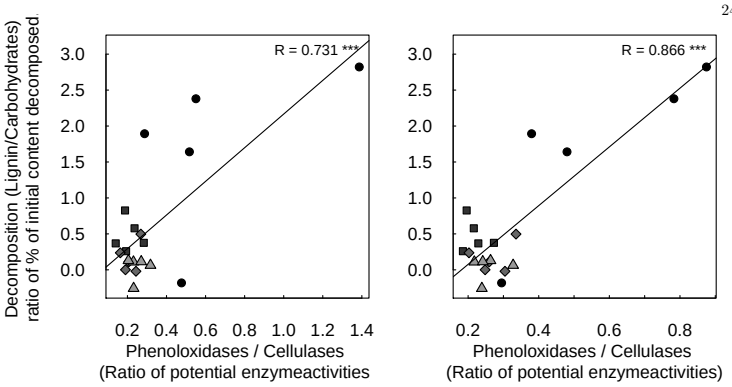

Figure 7. caption

Tables

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 [20, 22]. Differences in litter chemistry were calculaten 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**
Phenoloxidase activity	**0.632**	**-0.669**	**0.737**	-0.415	**0.719**	**-0.449**	**0.552**	**-0.484**	-0.305	-0.356
Peroxidase activity	**0.599**	**-0.588**	**0.677**	-0.412	**0.639**	-0.438	**0.47**	-0.435	-0.173	-0.302
N mineralization	**0.466**	**-0.664**	**0.65**	-0.167	**0.739**	-0.299	**0.527**	-0.387	-0.282	-0.367
Nitrification	**0.587**	**-0.707**	**0.732**	-0.38	**0.74**	-0.432	**0.621**	**-0.499**	-0.369	-0.45
P mineralization	**0.665**	**-0.55**	**0.684**	**-0.544**	**0.596**	**-0.576**	0.414	**-0.478**	-0.212	-0.255
C litter	**-0.545**	**0.506**	**-0.578**	**0.604**	-0.368	**0.643**	**-0.618**	**0.698**	**0.525**	**0.581**
extractable C	**0.609**	**-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 degration with litter chemistry, microbial community and decomposition processes. Significant ($p<0.05$) correlations are printed bold. Data taken from [20, 22]. Differences in litter chemistry were calculaten 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

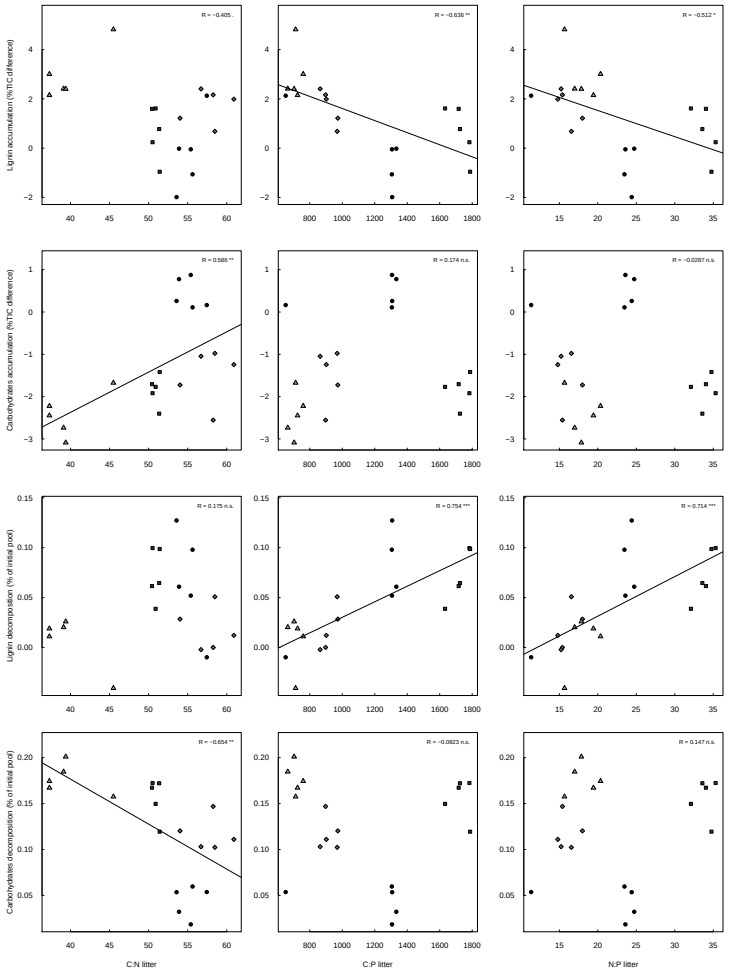

Figure 8. Correlations between Lignin and Carbohydrates accumulation and decomposition during the first 6 month of litter incubation and litter C:N:P ratios

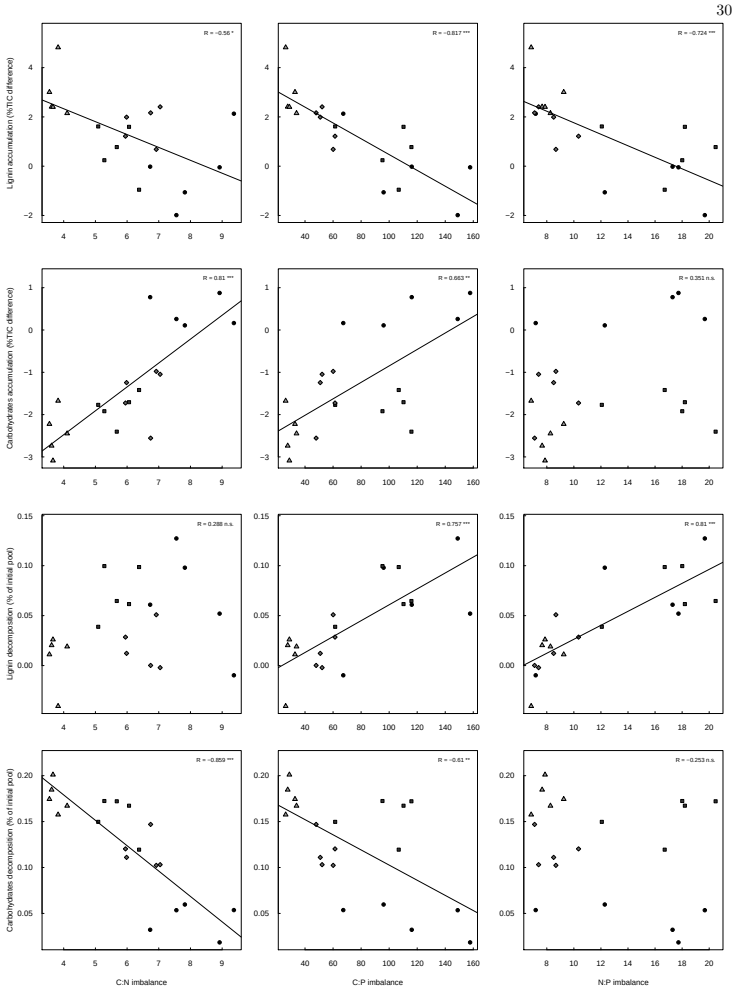

Figure 9. Correlations between Lignin and Carbohydrates accumulation and decomposition during the first 6 month of litter incubation and stoichiometric resource:consumer imbalances

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	**-0.707**	**-0.863**
Accumulated Respiration	**0.703**	0.0679	**0.703**	**0.781**	-0.221	**0.545**	**-0.507**	**-0.714**	0.431	**0.673**	**-0.702**	**-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**
Phenoloxidase activity	**0.626**	**0.601**	**0.574**	**0.838**	-0.157	0.0178	**-0.482**	**-0.911**	0.286	0.238	**-0.653**	**-0.87**
Peroxidase activity	**0.535**	**0.614**	**0.478**	**0.79**	-0.0898	-0.0474	-0.426	**-0.917**	0.282	0.155	**-0.581**	**-0.843**
N mineralization	**0.724**	0.453	**0.662**	**0.828**	-0.327	0.132	**-0.625**	**-0.941**	0.197	0.298	**-0.74**	**-0.923**
Nitrification	**0.654**	**0.487**	**0.551**	**0.836**	-0.223	0.121	-0.43	**-0.912**	0.19	0.319	**-0.615**	**-0.876**
P mineralization	0.438	**0.695**	0.368	**0.852**	-0.052	-0.174	-0.317	**-0.762**	0.353	0.0259	-0.437	**-0.712**
C litter	-0.0337	-0.192	-0.284	-0.369	-0.244	-0.0544	-0.0979	0.0362	-0.303	-0.155	0.0115	0.0999
extractable C	**0.715**	**0.406**	**0.688**	**0.906**	-0.225	0.17	**-0.562**	**-0.944**	0.263	0.374	**-0.741**	**-0.937**
N litter	**0.688**	**0.65**	**0.502**	**0.7**	-0.376	-0.13	**-0.579**	**-0.92**	0.0454	0.0547	**-0.645**	**-0.829**
P litter	0.0781	**0.728**	0.076	0.317	0.133	**-0.494**	-0.107	-0.429	0.197	-0.334	-0.0955	-0.294
C:N litter	**-0.728**	**-0.636**	**-0.562**	**-0.759**	0.38	0.0768	**0.598**	**0.943**	-0.0723	-0.113	**0.689**	**0.87**
C:P litter	0.054	**-0.734**	0.0372	-0.219	-0.216	**0.57**	-0.0572	0.273	-0.185	0.418	-0.0817	0.135
N:P litter	0.305	**-0.561**	0.24	0.0377	-0.348	**0.584**	-0.263	-0.0351	-0.156	**0.49**	-0.324	-0.159
C:N mic	**0.535**	0.398	**0.62**	**0.826**	0.00763	0.226	-0.345	**-0.704**	**0.48**	0.439	**-0.609**	**-0.782**
C:P mic	**0.557**	0.397	**0.647**	**0.864**	-0.00386	0.246	-0.38	**-0.762**	**0.458**	**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	0.0683	0.213	**0.566**	**-0.487**	-0.164	0.375	**0.574**
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.

	alkanacc	alkenacc	faacc	phytolacc	alkandeg	alkendeg	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	**0.638**	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	**0.562**	**0.784**	0.386	**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
Phenoloxidase activity	-0.0256	0.134	-0.345	-0.134	-0.0858	-0.13	0.379	0.157	-0.184	0.0737	-0.0468	-0.0142
Peroxidase activity	0.0627	-0.106	0.343	0.127	0.028	0.0833	-0.401	-0.172	0.119	-0.106	0.0258	0.0264
N mineralization	-0.0379	0.0275	-0.156	0.236	-0.0981	0.0926	0.259	-0.395	-0.397	0.283	**-0.569**	-0.421
Nitrification	**-0.496**	-0.451	0.253	**0.663**	0.417	**0.628**	0.112	-0.424	0.179	**0.535**	**-0.57**	**-0.657**
P mineralization	-0.303	**-0.466**	0.206	**0.473**	0.333	**0.543**	0.0642	-0.249	0.235	0.211	-0.182	-0.447
C litter	0.0444	-0.336	**-0.825**	-0.0325	0.223	0.145	**0.685**	-0.412	-0.0238	0.0415	-0.212	-0.3
extractable C	**-0.733**	-0.294	0.398	**0.876**	0.452	**0.701**	0.14	**-0.5**	0.174	**0.735**	**-0.666**	**-0.809**
N litter	**-0.76**	**-0.548**	0.116	**0.836**	**0.618**	**0.802**	0.415	**-0.52**	0.28	**0.764**	**-0.652**	**-0.909**
P litter	-0.354	-0.181	**0.597**	**0.575**	0.254	0.448	-0.246	-0.0722	0.345	0.17	0.0983	-0.261
C:N litter	**0.784**	**0.503**	-0.0977	**-0.818**	**-0.614**	**-0.843**	-0.448	**0.494**	-0.248	**-0.804**	**0.701**	**0.922**
C:P litter	0.0939	0.0836	**-0.682**	-0.422	-0.0492	-0.249	**0.48**	-0.0006689	-0.281	0.0934	-0.341	-0.0041
N:P litter	-0.241	-0.0878	**-0.627**	-0.0957	0.187	0.0732	**0.654**	-0.191	-0.202	0.445	**-0.659**	-0.377
C:N mic	**0.549**	0.241	**-0.502**	**-0.611**	-0.18	**-0.547**	0.04	0.245	0.0205	**-0.687**	**0.589**	**0.55**
C:P mic	0.206	0.413	-0.218	**-0.503**	-0.241	**-0.508**	-0.0471	0.26	-0.217	-0.175	0.0327	0.355
N:P mic	-0.0201	0.326	-0.054	-0.273	-0.175	-0.297	-0.028	0.156	-0.253	0.13	-0.243	0.124
C:N imbalance	0.429	0.393	0.363	-0.443	**-0.586**	**-0.515**	**-0.584**	0.403	-0.335	-0.297	0.27	**0.611**
C:P imbalance	-0.0373	-0.357	**-0.544**	0.0137	0.13	0.208	**0.535**	-0.327	-0.143	0.262	-0.427	-0.324
N:P imbalance	-0.2	**-0.486**	**-0.619**	0.199	0.37	0.391	**0.687**	**-0.468**	0.045	0.324	**-0.457**	**-0.522**

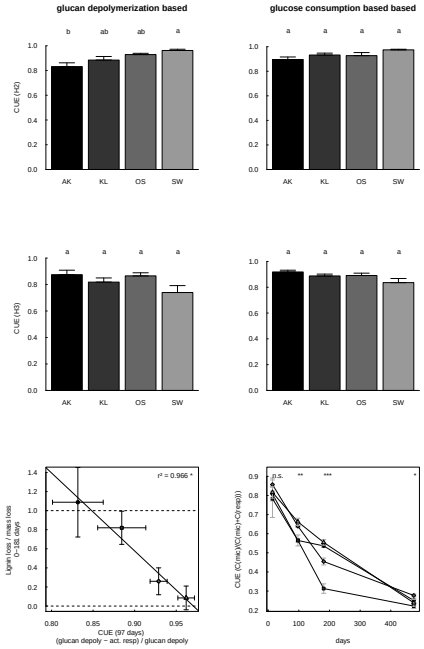

Figure 10. caption

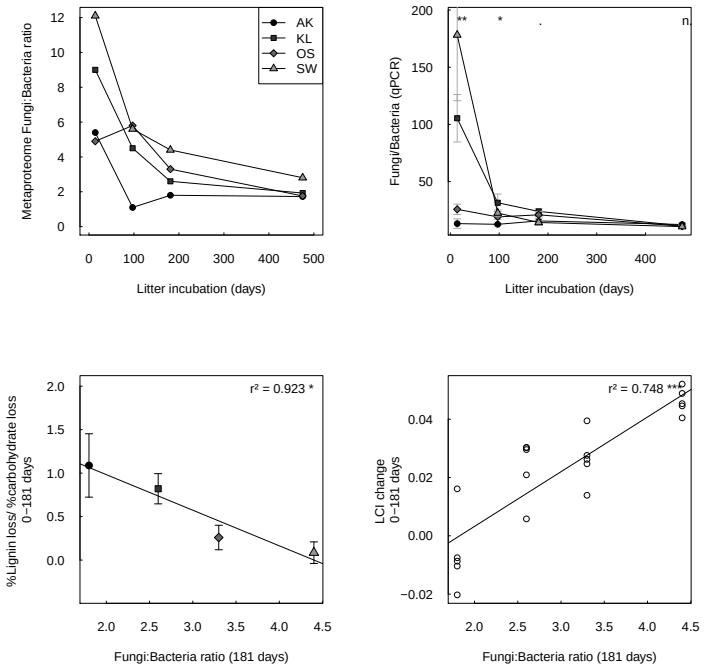

Figure 11. Bold the first sentence. Rest of figure 2 caption. Caption should be left justified, as specified by the options to the caption package.