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"High molecular weight compounds in beech litter decomposition"

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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 to describe decomposition processes 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 athmospheric 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 athmospheric CO_2 concentration to its ^{13} C signature (fossil fuel C is depleted in ^{13} C) or correnspondingdecrease in athmospheric 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 emmissions 1.6 $\pm$ 1.1 Gt CO_2-C. Together with other greenhouse gases, elevated CO_2 concentrations are prognosted 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 prognosting feedback mechanisms of CO_2 emissions challenged scientists to strife for depthening 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 antropogenic C emissions. Autotrophic (plant) respiration consumes one half of the assimilated carbon, the other half is introduced into decomposition process as plant litter. Animal biomass and herbivory form only a neglectable 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 tree species in potential western and central europe. The distribution area is shown in figure nn.

cultivation

Temperate forests 8.1 Gt a-1 Boreal forests 2.6 Gt a-1 Mediterranean shrublands 1.4 Gt a-1 Tropical savannas and grasslands 14.9 Temperate grasslands 5.6 Desserts 3.5 Arctic tundra 0.5 Crops 4.1 sum 62.6 Gt

1.1.1 Ecological stoichiometry

Carl Sprengel proposed in 1828 that crops rely on nutrints 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 capeable of a certain plasticity in their nutrient requirement, there is a tradeoff between addaption 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 mileu 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 terrestrian ecosystem mostely failed due to the complex nature of terrestrian soils and difficulties to determine actual bioavailability of nutrients. [2]

Plants are able to assimilate carbon from atmosphaeric CO_2, but have to sequestrate other elements from soils. Furthermore, a significant part of nitrogen and other nutrients is removed from senecenced 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]. Therefore, during litter decomposition, the part of carbon mineralized is higher 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 surplass and a lack of nitrogen. Litter decomposition rates were found to correlate with detritus C:N and C:P rates (**?**).

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

[1] citation
[2] paper "redfield ratio for soils" here!
[3] citation

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 orgnic matter or dissolved organic 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)).

[4] references needed!!

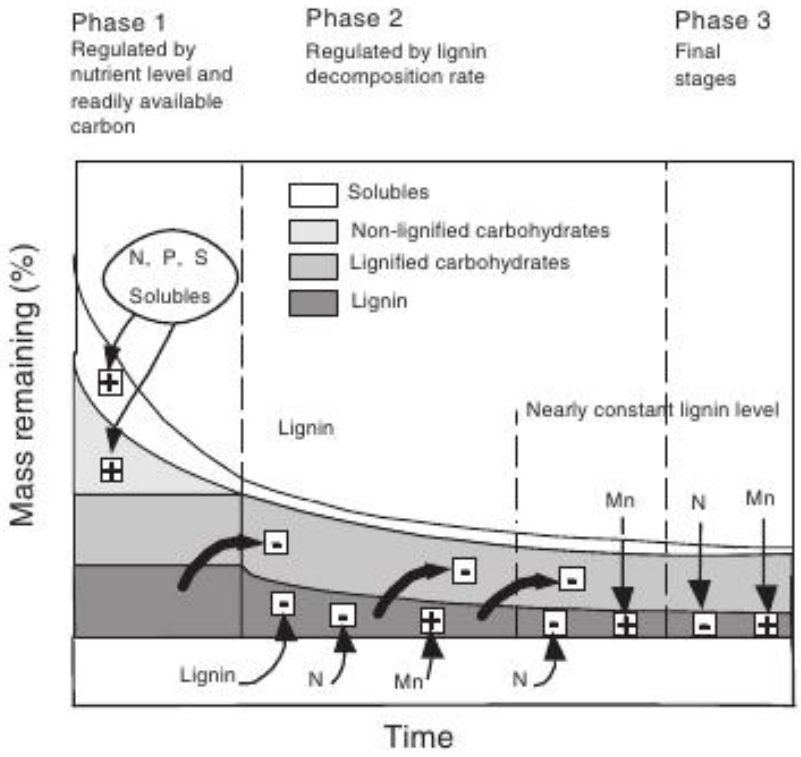

Fig. 6.1 Model for chemical changes and rate-regulating factors during decomposition (modified from Berg and Matzner 1997). The decomposition of water-soluble substances and unshielded cellulose/hemicellulose is stimulated by high levels of the major nutrients (early stage – *phase 1*). When all unshielded 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))

1.3.2 Microbial nitrogen mining hypothesis

The "nitrogen mining hypothesis" is based on the idea, that the breakdown of recalcitrant carbon - while yielding little to no energy - allows soil microbia to access recalcitrant nitrogen. This explains why nitrogen starvation triggers the excretion of enzymes degrading phenolic compounds (Craine et al., 2007). In plant litter, nitrogen is often occluded within lignin molecules, which are degraded by similar enyzmes.

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.

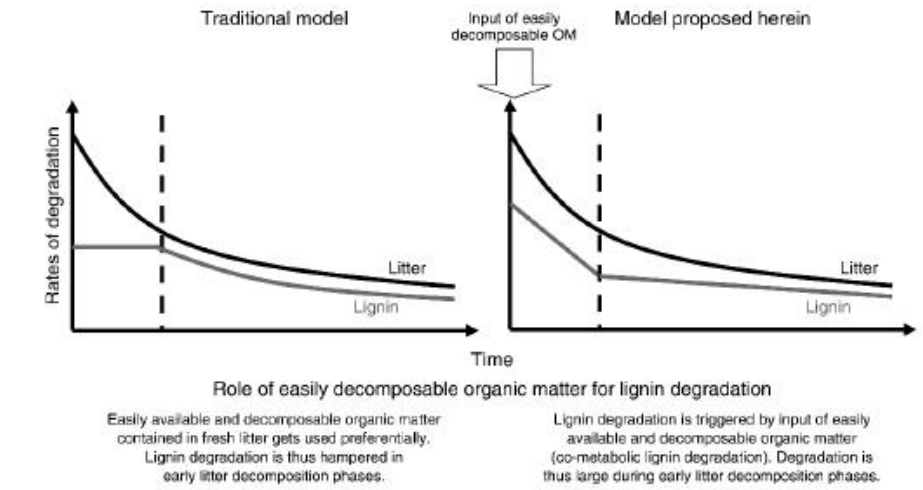

FIG. 5. Revised conceptual model for the fate of lignin during litter decomposition, based on data presented herein. The traditional model was proposed in Berg and Staaf (1980) and based on data from the decomposition of Scots pine needles. In our model, lignin will be degraded if easily degradable OM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

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

1.3.4 Analytical methods to characterize high molecular weight compounds

Carbohydrates The characterization of sugar monomers present can be achieved by HPLC or GC analysis after hydrolysis (i.e. Snajdr et al. (2011)). Specific starch analysis is usually conducted by enzymatic hydrolysis with amylases, described by Leitner et al. (2011). Cellulose and hemicelluloses are often analyzed by selective extraction and gravimetry. This method is describe in more detail with lignin analysis.

Lignin Several methods have been developed to determine lignin, but [they are all problematic] (Hatfield and Romualdo, 2005).

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 preparaation.

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, **?** presents the most relevant data for the identification of pyrolysis products. The 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

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3

Manuscript 1

Controls of litter chemistry over early lignin decomposition in beech litter

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Abstract

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

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

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

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

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

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

1. Introduction

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

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

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

[2] more lit.

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

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

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

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

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

[4]more lit?

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

We hypothesize that

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

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

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

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

2. Material and methods

2.1. Litter decomposition experiment

A detailed description of our litter decomposition experiment was published in Wanek et al. (2010). Briefly, beech litter was collected at four different sites in Austria (Achenkirch (AK), Klausenleopoldsdorf(KL), Ossiach(OS), and Schottenwald(SW); refered to as litter types) in October 2008. Litter was cut to pieces of approximately 0.25cm^2, homogenized, sterilized twice by gamma[5] radiation (35 kGy, 7 days between irradiations) and inoculated (1.5% w/w) with a mixture of litter and soil to assure that all litter types share the same initial microbial community. From each type, four samples of litter were taken after inoculation and stored dried at room temperature. Samples of 60g litter (fresh weight) were incubated

[5]greek gamma here

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

Litter chemistry as analyzed 14 days after incubation is listed in table 2. C:N ratios between 1:41 and 1:58 and C:P ratios between 1:700 and 1:1300 were found, N:P ratios ranged between 1:15 and 1:30. No significant changes occurred during litter incubation except a slight decrease of the C:N ratio (1:41.8 to 1:37.4) found in the most active litter type (SW) after 15 month. Fe content were more than twice as high for OS (approx. 450 ppm) than for other litter types (approx. 200 ppm). Litter Mn also was highly variable between litter types, ranging between 170 and 2130 ppm. Changes of micro-nutrient concentrations during litter incubation were significant, but in all cases <15% of the initial concentration.

2.2. Bulk litter, extractable, and microbial biomass nutrient content

To calculate litter mass loss, litter dry mass content was measurement in 5 g litter (fresh weight) after 48 h at 80 °C. Dried litter was ball-milled for further chemical analysis. Litter C and N content were determined using an elemental analyzer (Leco CN2000, Leco Corp., St. Joseph, MI, USA). Litter phosphorus content was measured with ICP-AES (Vista-Pro, Varian, Darmstadt, Germany) after acid digestion Henschler (1988)).

To determine soluble C, N, and P contents, 1.8g litter (fresh weight) were extracted with 50 ml 0.5M K_2SO_4. Samples were shaken on a reciprocal shaker with the extractant for 30 minutes, filtered with ash-free filters and frozen at -20 °C until analysis. To quantify microbial biomass C, N and P pools, sample were extracted under the same conditions after chloroform fumigation. Microbial biomass was determined as the difference between fumigated and non-fumigated extractions (**?**). C and N concentration in extracts were determined with a TOC/TN analyzer (TOC-VCPH and TNM, Schimadzu), Phosphorous was determined photometrically.[6]

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

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

[6] lit!!

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

2.3. Microbial Respiration

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

2.4. Enzyme activities

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

2.5. Pyrolysis-GC/MS

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

Litter analyzed was sampled immediately after inoculation and after 3, 6, and 15 month incubation. 2-300 µg dried and finely ball-milled litter were heated to 600°C for 10 seconds in helium atmosphere. GC oven temperature was constant at 50 °C for 2 minutes, followed by an increase of 7°C/min to a final temperature of 260 °C, which was held for 15 minutes.

The MS detector was set for electron ionization at 70 EV cycling between m/z 20 and 300.[7]

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

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

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

A lignin to carbohydrates index was calculated to measure the ratio between these two substance classes without influences of changes in the abundance of other compounds

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

Accounting for carbon loss, we estimate % lignin and cellulose degraded during decomposition according to equation 3, where TIC_{init} and TIC_{act} stand for initial and actual %TIC area of lignin or cellulose pyrolysis products, C_{init} for the initial amount of C and R_{acc} for the accumulated CO_2-C respired by a mesocosm.

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

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

[7] maybe cite other paper for method?

2.6. Statistical analysis

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

3. Results

3.1. Mass loss, respiration and extractable organic carbon

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

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

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

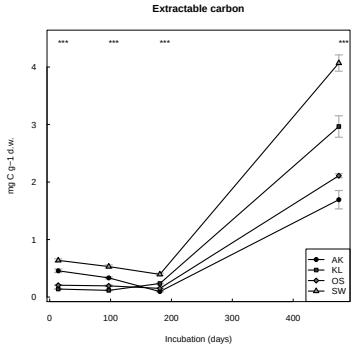

Figure 1: Extractable organic carbon. Error bars indicate standard errors (n=5).

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

3.2. Microbial Community

3.2.1. Microbial biomass abundance and stoichiometry

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

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

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

3.2.2. Potential enzyme activities

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

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

3.3. Pyrolysis-GC/MS and Lignin content

3.4. Changes in litter chemistry

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

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

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

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

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

[8] check fig.

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

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

3.4.1. Amounts of Lignin and Carbohydrates degraded

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

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

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

Table 3 provides linear regressions found between lignin and carbohydrate degradation, litter chemistry, microbial biomass and decomposition processes after 6 month incubation including data presented by Mooshammer et al. (2011) and Leitner et al. (2011). We found The lignin to cellulose degradation ratio was correlated to phenoloxidase to cellulase and 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 inbalances. lignin degradation and accumulation were correlated to litter P, litter C:P and N:P ratios, C:P and N:P inbalances and extractable organic C and PO_4. High lignin

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

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

4. Discussion

4.1. Early lignin decomposition

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

4.2. Temporal changes in lignin decomposition

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

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

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

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

4.3. Resource controls on carbon chemistry

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

Mn and Fe are important co-factors of oxidative enzymes involved into lignin decomposition. Several authors suggest Mn availability limits lignin degradation and is rate limiting in late litter decomposition stages. Mn and Fe contents strongly vary between the different litter types used. An experiment studying aquatic decomposition of the same litter suggested Mn contents controll decomposition processes like enzyme activties. We can exclude micronutrient availability as a limiting factor for lignin decomposition in our experiment, because Mn and Fe contents are lowest in the litter with the highest lignin decomposition (AK, table 2). Low contents of these Elements would explain inhibited, not enhance lignin decomposition.

[10] Lit.!

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

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

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

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

5. Conclusions

6. Acknowledgements

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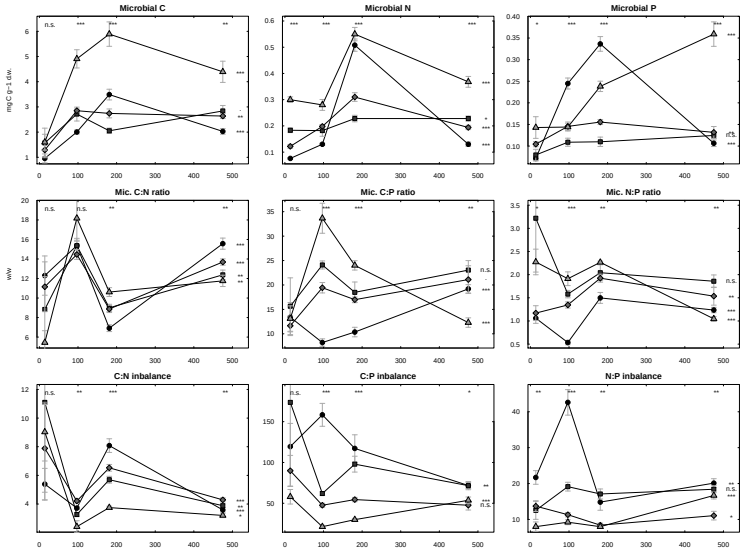

Figure 2: Microbial biomassm, microbial stoichiometry and resource:consumer stoichiometric inbalance. Error bars indicate standard errors (n=5).

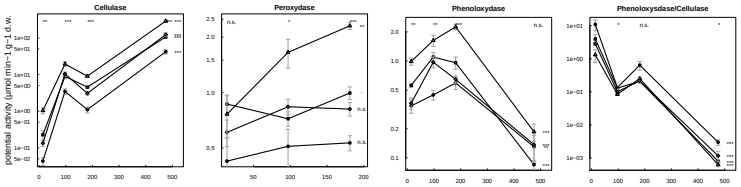

Figure 3: Potential eco-enzyme activities

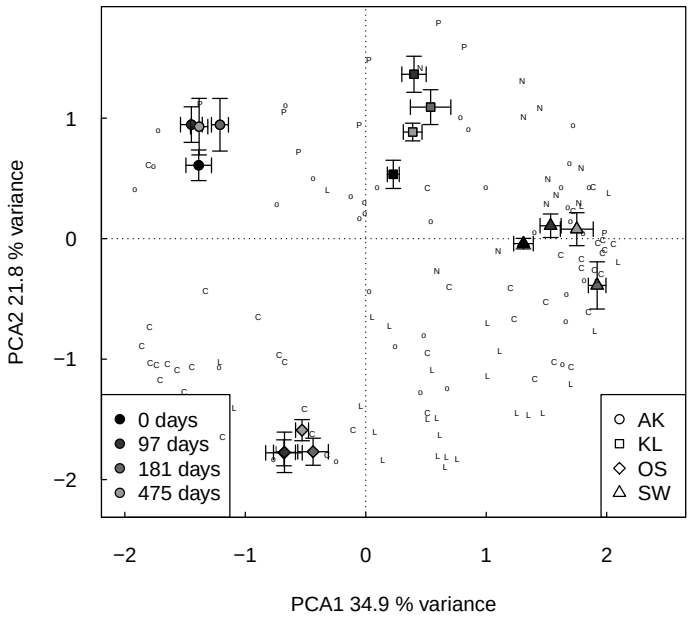

Figure 4: Principal component analysis of the relative peak areas of 128 pyrolysis products, corrected by their abundance before incubation. Symbols indicate samples measured (means per litter type and time point), error bars indicate +/- 1 SE (n=4-5). Letters indicate pyrolysis products (L - lignin,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

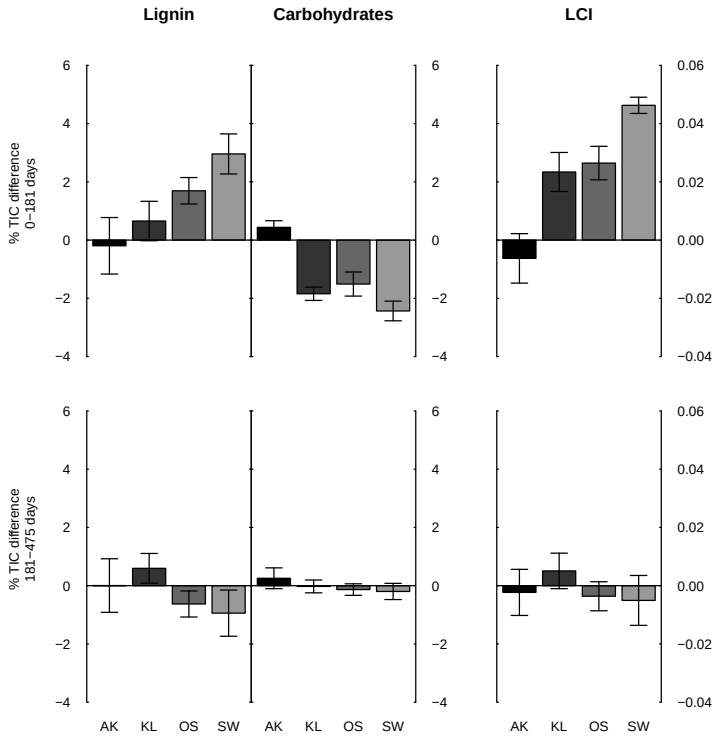

Figure 5: Accumulation and depletion of lignin and carbohydrates

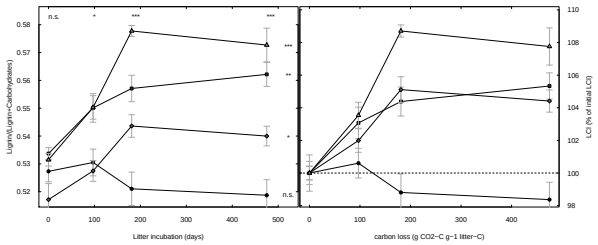

Figure 6: Develoment of the LCI (lignin/(lignin+carbohydrates)). Errorbars indicate standard errors (n=4-5). The dashed line indicates a constant ratio between lignin and carbohydrates (i.e. no preferential decomposition of carbohydrates.)

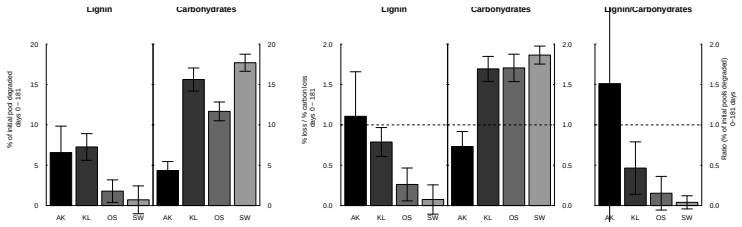

Figure 7: Carbon loss corrected amounts of lignin and carbohydrates degraded. Carbon loss was calculated based on accumulated respiration. Error bars indicate standard errors (n=4-5). The dashed line marks no discrination between lignin, carbohydrates and bulk carbon loss.

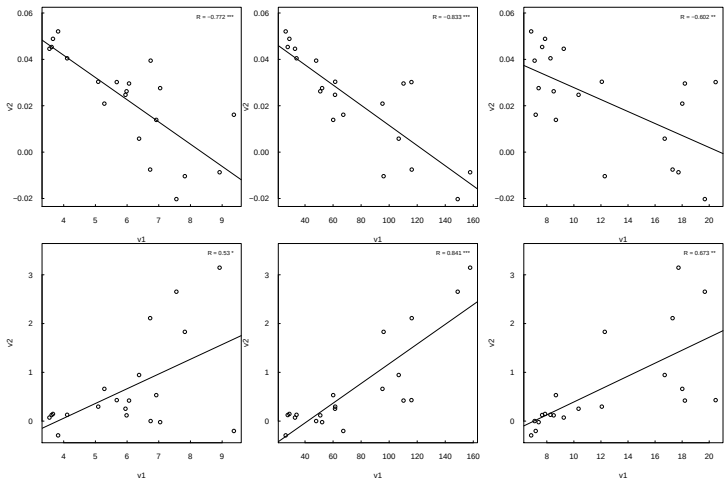

Figure 8: Correlations between Lignin accumulation during the first 6 month of litter incubation and stoichiometric resource:consumer inbalances

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

	C	(% d.w.)	C extr	(% d.w.)	N	(% d.w.)	P	(% d.w.)	C:N	(w/w)	C:P	(w/w)	N:P	(w/w)
AK	50.86	(0.39)	45.66	(3.13)	0.878	(0.012)	0.040	(0.000)	57.86	(0.57)	1282	(21)	22.17	(0.47)
KL	49.41	(0.53)	13.92	(0.70)	0.938	(0.012)	0.030	(0.000)	52.60	(0.49)	1548	(25)	29.45	(0.60)
OS	48.15	(0.39)	20.61	(0.86)	0.806	(0.013)	0.052	(0.002)	59.97	(0.72)	905	(15)	15.10	(0.29)
SW	48.90	(0.34)	63.81	(2.67)	1.172	(0.016)	0.070	(0.000)	41.78	(0.76)	699	(9)	16.75	(0.39)

Table 2: Mineral element content measured 14 days after incubarion. Standard errors are stated in brackets (n=5). All contents are given per g dry weight.

	K	mg g-1	Ca	mg g-1	Mg	mg g-1	Fe	ppm	Mn	ppm	Zn	ppm
AK	0.26	(0.00)	1.33	(0.01)	0.272	(0.002)	210	(1.985)	0.272	(0.002)	30.80	(0.37)
KL	0.54	(0.00)	1.26	(0.01)	0.142	(0.002)	208	(3.813)	0.142	(0.002)	33.00	(0.32)
OS	0.21	(0.00)	1.63	(0.01)	0.198	(0.002)	453	(11.625)	0.198	(0.002)	36.00	(1.05)
SW	0.55	(0.00)	1.23	(0.01)	0.150	(0.000)	192	(4.352)	0.150	(0.000)	42.40	(0.75)

Table 3: Correlation (R) between Lignin and Carbohydrate degration with litter chemistry, microbial community and decomposition processes. Significant ($p<0.05$) correlations are printed bold. Data taken from Mooshammer et al. (2011); Leitner et al. (2011); **?**

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

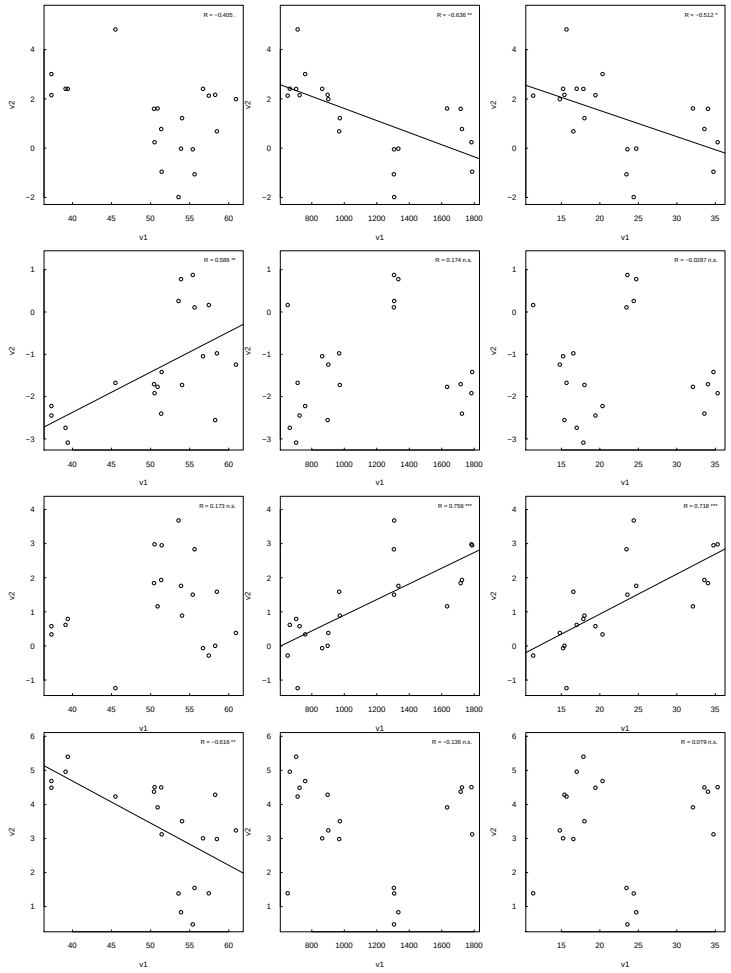

Figure 9: Correlations between Lignin and Carbohydrates accumulation and decomposition during the first 6 month of litter incubation and litter C:N:P ratios

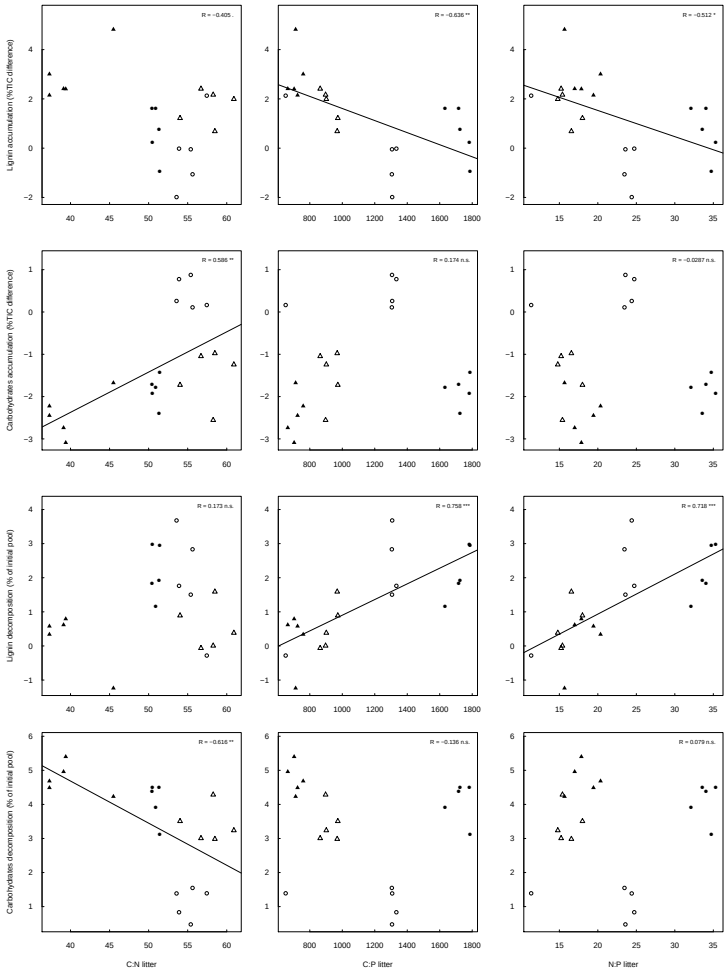

Figure 10: Correlations between Lignin and Carbohydrates accumulation and decomposition during the first 6 month of litter incubation and stoichiometric resource:consumer inbalances

4

Manuscript 2

Following beech litter decomposition with analytical pyrolysis: Inter-site variance and decomposition trends

Abstract

Litter decomposition studies are key to understanding global carbon fluxes and soil formation. Analytical pyrolysis has the potential to provide insight into carbon transformations during decomposition. Most interestingly, this potential was not exploited yet. We report data of beech litter pyrolysis products of litter optained from different sites in Austria and changes in pyrolysis products during litter decomposition

Keywords:

1. Introduction

Understanding plant litter decomposition processes is key to understanding the global carbon cycle, nutrient recycling and soil formation[1]. However, little is known about changes in the chemistry of high molecular weight substances during decomposition. The insufficient specifity of traditional methods to determine plant fibres[2] led to misinterpretation of decomposition dynamics. Analytical pyrolysis can provide and fast a cheap method to investigate chemical transformations of plant polymers during decomposition. However, while the use of analytical pyrolysis to quantify lignin and cellulose contents becomes more frequent, the full potential of an high definition interpretation of pyrograms is rarely used.

Analytical pyrolysis is frequently applied to characterize natural organic polymers in soil organic matter [and dissolved organic matter?]. Plant derived compounds make up an important part of pyrolysis products found. The relative abundances of such markers were shown to hold key information on past climates and decomposition conditions [3, 4, 5].

Only a handful of Pyr-GC/MS studies tracing changing pyrolysis markers during litter decomposition were published, none of them includes indepth. The only published work

directly studying the decay of plant leaf litter is [6]. [**?**] presents basic thermochemolysis data. Straw was incubated with n. Several studies monitor the decomposition of woody material with Pyr GC/MS [lit.] or litter/soils mixtures[lit.]. None of the studies mentioned above analysis decomposition trends of individual pyrolysis products.

While assignment to major compounds classes (like ligin or carbohydrate) based on pyrolysis reaction mechnisms is common, little is known on whether the composition of individual pyrolysis products within this groups yields information.

This study compares pyrograms of beech litter from different site after up to 15 month of climate chamber decomposition. Beech litter were reported for exceptionally slow decomposition rates (12% mass losso in the first year) in nature [**?**] and showed similar decomposition rates our climate chamber experiment. Therefore, we do not focus on the accumulation of recalcitrant compounds, but focus on how individual pyrolysis markers accumulate or deplete relative to their compounds of origin which might provide insight into changes in polymer condensation patterns.

2. Material and Methods

Beech litter from 4 different sites in Austria was collected in October 2008, cut to pieces <0.5 cm, homogenized, sterilized and inoculated with a common inoculate for all sites. Litter was incubated in a climate chamber at 15 ℃ and was kept at 60 % moisture [7]. Total mass loss after 15 month was between 7 and 12 %. Accumulated respiration after 6 month accounts for nn.to nn% of the litter carbon pool, after 15 month between n and n.

Pyrolysis-GC/MS was performed on a Pyroprobe 5250 pyrolysis system (CDS Analytical) coupled to a Thermo Trace gas chromatograph an a DSQ II MS detector (both Thermo Scientific) equipped with a carbowax colomn (Supelcowax 10, Sigma-Aldrich). 2-300 µg dried and finely ball-milled litter were heated to 600°C for 10 seconds in helium atmosphere. The temperature of the valve oven and the transfer line to the GC injection port were set to 250°C,a 10x split injection was applied with the injector heated to 240°C. Carrier gas flow was set to 1ml min^{-1}. 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 transfer line was heated to 270 °C. The MS detector was set for electron ionization at 70 EV, the ion source was heated to 270°C. Detection was set to cycle between m/z 20 and 300 with a cycle time of 0.3 seconds.

Peaks were assignment was based on NiSt 05 MS library and comparison with reference material measured. 128 peaks were selected for integration due to their hiht abundance or diagnostic value. For each peak between one and four mass fragments selected for high abundance and specificity were integrated (i.e. [4]). Peak areas are stated as % of the sum of all integrated peaks of a sample.

Pyrolysis products were assigned to their substances of origin by comparison to reference material, structural similarity and in accordance with literature ([8, 4, 9][more lit!]). We confirmed the identity of two products rarely reported for plant material (Phytol and 3-Hydroxypyridine) by the addition of reference material to the sample and comparison to MS libraries. Both substances were bought from Sigma-Aldrich (St. Louis, MO, USA).

$$\%lost = \frac{mean()actual\%)}{1 - \frac{carbonrespired}{initialcarboncontent}} - \%meaninitialcontent \quad (1)$$

2.1. Litter mass loss and respiration

2.2. Statistical analysis

All statistical analyses were performed with the software and statistical computing environment R using the R package "vegan" [10]. If not mentioned otherwise, results were considered significant, when $p<0.05$. All correlations refer to Pearson correlations. All data presented was tested for significant differences between harvests and litter types. Normal distribution assumed but could not be tested due to the small number of cases per treatment (n=4-5). A substantial part of variables had heterogeneous variances when tested ážČith Levene's test. Therefore, (one-way) Welch anova was used to calculate significant differences between harvests within each litter type and litter types within each harvest (alpha=0.05). For post-hoc group assignment, paired Welch's t-tests with Bonferroni corrected p limits

were used. Principal component analysis was performed using vegan function "rda" scaling variables.

3. Results

3.1. Mass loss and respiration

Accumulated mass loss during the first 181 days ranged between

3.2. Pyrolysis products

A total of 128 peaks quantified including 28 phenolic, 11 nitrogen containing compounds and 42 carbohydrate derived compounds. Inter-site differences dominate both initial and incubated litter: In 127 peaks significant differences between litter from different sites was found during one time point, in 95 differences were significant in all 4 harvests. In 113 peaks significant differences between time points were found, but only 49 peaks had significant changes during incubation in more than two litter types.

3.3. Lignin and other phenolic compounds

Of 38 phenolic pyrolysis products were identified, 15 of which had guaiacol-, 13 syringol-, and 10 non-methoxylated ring systems.

Guaiacol/Syringol (G/S) ratios were tightly (all R=0.68-0.94, $p<0.001$) correlated between the more abundant side chains (-H, $-CH_3$, $-CH_2CH_3$, $-CH{=}CH_2$, $-CH_3CH{=}CH_2$). This correlation is weaker or non-significant for less abundant side chains (-CHO, $-CH_2CH_2CHO$) due to higher variances in their detection.

Part of non-methoxylated phenols pyrolysis products (side chains $-CH_2CH_3$, $-CH_2CH_2CH_3$ $-CH_3CH{=}CH_2$)show clear structural similarity to pyrolysis products of lignin, suggesting that they are pyrolysis products of p-hydroxypropanylphenol based lignin. Other side chains (-H, $-CH_3$, -CHO) are less specific and are reported for other sources, although in small amounts. Furthermore, some of the phenolic compounds (i.e. hydroxyquinone) are not lignin derived.

In both syringol and gaiacol the ratio between the abundances of pyrolysis products is the same.

Guaiacol to Syringol ratios were constant over decomposition time. The ratios are are different for G/S compounds with different side chains, propably due to changing MS resposes of the compounds

Most important difference between sites were observed in the ratio between Guaiacol

The pattern of abundances of different side chains was the same for guaiacol and syringol derived compounds. Fig 4 shows that pairwise ratios between 5 different lignin side chains are highly significant in guaiacol and syringol lignin based pyrolysis products. However, there are some site-specific differences in these patterns: the abundance of -H and -CH_3 side chains relative to other side chains is different between the litter types, but consistent between guaiacol and syringol derivatives. de chains with oxygen based functional groups were present, but in abundances much lower than the before mentioned.

[3, 4] use the Guaiacol+Syringol to C_3-Gaiacol+Syringol ratio as an indicator for ?aerobic/anaerobic degradation.

3.4. N compounds

In total, 11 nitrogen containing compounds were identified, including two indole three pyridine and 5 pyrrol derrivatives. 8 of them were stricly correlated to litter N content (all R>0.8, p<0.001).Two groups could be identified amoung them: indol and methylindole were highly correlated to each other (R=0.98, p<0.05?0.001?), as were 6 pyridin and pyrrol derivatives (all R >0.9, p<0.05?0.001?). Correlations between peaks of two groups were still high, but lower than within the groups (R = 0.77-0.87, p<0.05?0.001?). Both groups show a continuous increase during litter decomposition: after 15 month pyridine and pyrrol derrivatives increase by 8-20% of their initial content, indol and methylindol by 20-30% (fig. 8).

Two pyrolysis products show different trends: The abundance of 3-Hydroxypyrrol strongly (30-50%) decreases during first six month, then follows general N trend. N-methyl-pyrrol shows an increase between 5 and 35% (%TIC) during the first 6 month and remains stable thereafter (fig. 8).

3.5. Carbohydrates

In total, 42 carbohydrate derived pyrolysis products were identified either by their structure or by comparison to reference material Unlike lignin or N compounds, carbohydrate pyrolysis products do not follow a common trend, but - reflecting the differences within this group. About a third of the peaks could not be identified but were assigned to carbohydrates because they are present reference pyrograms of cabrohydrates, but not in other compounds.

Methylated cyclopentenone derivatives show an increase over decomposition, while derivative with keto- or hydroxy- side chains show decreases over decomposition.

Furanmethanol shows an especially high degradation rate, loosing 15 to 30 % of it's initial contribution to the total peak area over 15 month.

3.6. Other compounds

3.6.1. Phytol

We found two compounds with terpenoid structure: Phytol is a chlorphyll derived C_{20} alcohol mentioned as a pyrolysis product only by [**?**]. We confirmed the compounds identity by comparison with comercially available material. Phytol is strongly depleted during decomposition in low-N sites (up to 50% of its relative peak area in OS and AK), but not in high-N sites (SW). (fig **??**)

3.6.2. Fatty acids

Three saturated fatty acids were found in litter pyrograms (14:0, 16:0, 18:0). Fatty acids were reported as underrepresented in pyrgrams due to their decarboxylation during pyrolysis [lit], so only the most abundant fatty acids are expected to be found. Additionally, free fatty acids are hard to seperate on our chromatographic system . The three fatty acids were also most abundant in THM analysis of this litter material (data not shown).

For fatty acid analysis 2 samples were excluded as contaminations were encountered.

Initial content of fatty acids showed differences of up to 30 % between sites. Fot the three fatty acids found, between 15 and 50 % were degraded over 15 month, indication that both accumulation and depletion occurs in certain sites.

3.6.3. Long chained alkanes and alkenes

We found C25-C29 alkanes and alkenes in our pyrograms. The two had different decomposition trends: While

4. Discussion

4.1. Inter-site variance during litter decomposition

We found that differences in litter pyrolysis products persist at least during early litter decomposition. Differences in Lignin side chains are characteristic for

4.2. Lignin side chains

Until now, studies following lignin degradation with Pyr-GC/MS were focusing on changes between the frequency of lignin pyrolysis products, only the ratio between guaiacol/syringol/phenol based ring system was investigated. We found substantial differences in the frequency of different lignin side chains between litter collected at different sites. We found that these differences remained nearly constant during during 15 month of litter decomposition.

4.3. Pyridine/Pyrrol to Indole ratio

The ration between indene and pyridine/pyrrol ring pyroylsis products drops during decomposition by about 20%. Initial differences between sites (OS is higher than the rest) remain. Increase in tryptophan content among amino acids in bacterial protein?

4.4. Lipophilic compounds

From our dataset, we see that (1) alcenes are accumulated

5. Conclusions

6. Acknowledgements

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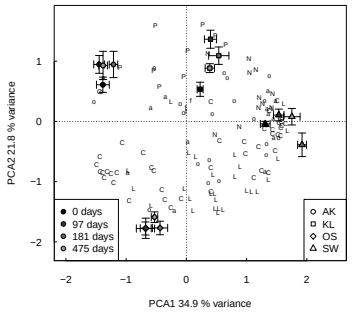


Figure 1: PCA based on 128 peaks quantified in 74 samples. Error bars indicate standard errors (n=4-5). Letters indicate pyrolysis products: C - carbohydrates, L - lignin, P - other phenolic compounds, N - N containing compounds, a - long chained aliphatic compounds (fatty acids, n- alkanes, n-alkenes, phytol

Table 1: Lignin derrived and other phenolic pyrolysis products

	Name	RT	MW	integrated framents	Origin	Class
1	Guaiacol	18.87	124	109+124	L	g
2	Methylguaiacol	20.32	138	123+138	L	g
3	Ethylguaiacol	21.40	152	137+152	L	g
4	Propenylguaiacol	23.29	164	149+164	L	g
5	Vinylguaiacol	23.69	150	135+150	L	g
6	Propenylguaiacol	24.48	164	149+164	L	g
7	Syringol	24.58	154	139+154	L	sy
8	Propenylguaiacol	25.66	164	149+164	L	g
9	Methylsyringol	25.67	168	153+168	L	sy
10	Ethylsysringol	26.39	182	167+182	L	sy
11	Propenylsyringol	27.97	194	179+194	L	sy
12	Vinylsyringol	28.37	180	165+180	L	sy
13	Guaiacolaldehyde	28.40	152	109+152	L	g
14	Propylguaiacol	28.72	166	137+166	L	g
15	Oxo-hydroxy-etylguaiacol	28.77	182	182	L	g
16	Propenylsyringol	28.91	194	179+194	L	sy
17	Oxo-ethylguaiacol	29.20	166	151+166	L	g
18	Oxo-propylguaiacol	29.36	180	137+180	L	g
19	Propenylsyringol	30.16	194	194+179	L	sy
20	Syringolaldehyde	32.68	182	139+182	L	sy
21	Oxo-hydroxy-ethylsyringol	32.80	212	212	L	sy
22	Guaiacolacetic acid	32.88	182	137+182	L	g
23	Propylsyringol	33.15	196	181+196	L	sy
24	Oxo-propylsyringol	33.32	210	167+210	L	sy
25	Oxopropenylguaiacol	35.30	178	135+178	L	g
26	Hydroxypropenylguaiacol	37.10	137+180	180	L	g
27	Syringolacetic acid	38.78	212	212	L	sy
28	Oxo-propenylsyringol	43.06	208	165+208	L	sy
29	Phenol	21.02	94	65+66+94	Ph	ph
30	4-Methylphenol	22.11	108	107+108	Ph	ph
31	3-Methylphenol	22.22	108	107+108	Ph	ph
32	Ethylphenol	23.38	122	107+122	Ph	ph
33	Propenylphenol	26.93	134	133+134	Ph	ph
34	Propenylphenol	27.76	134	133+134	Ph	ph
35	Propylphenol	31.11	136	151+166	Ph	ph
36	Butylphenol?	31.86	150	107+150	Ph	ph
37	4-Hydroxybenzaldehyde	32.70	122	121+122	Ph	ph
38	Hydroquinone	33.40	110	81+110	Ph	ph

Table 2: Carbohydrate derrived pyrolysis products

	Name	RT	MW	integrated frament	Origin	Class
1	Acetaldehyde	2.06	44	29+44	C	cp
2	Furan	2.35	68	39+68	C	f
3	Methylfuran	2.74	82	81+82	C	f
4	Methylfuran	2.91	82	81+82	C	f
5	Dimethylfuran	3.43	96	95+96	C	f
6	Dimethylfuran	3.66	96	95+96	C	f
7	Vinylfuran	5.01	94	65+94	C	f
8	Unknown furan	6.36	108	107+108	C	f
9	Cyclopentanone	6.99	105?	84+105?	C	cp
10	Methylfuran	7.62	82	53+82+83	C	f
11	2-Oxopropanoic acid, methylester	7.92	102	43+102	C	s
12	1-Hydroxypropanone	9.24	74	43	C	s
13	2-Cyclopenten-1-one	10.26	82	53+54+52	C	cp
14	2-Methyl-2-cyclopenten-1-one	10.51	96	53+96	C	cp
15	1-Hydroxy-2-propanone	10.69	88	57+88	C	cp
16	Unknown	11.38	unk	65+66+94	C	cp
17	3-Furaldehyd	11.57	96	95+96	C	f
18	2(5H)Furanon	11.69	98	55+98	C	f
19	Propanoic acid, methylester	12.10	102	43+102	C	s
20	2-Furaldehyd	12.22	96	95+96	C	f
21	Acetylfuran	12.99	110	95+110	C	cp
22	3-Methyl-cyclopentanone	13.31		67+96	C	cp
23	Dimethylcyclopentenone	13.69	110	67+95+110	C	cp
24	5-Methyl-2-furancarboxaldehyde	14.23	110	109+110	C	f
25	2-Cyclopenten-1,4-dione	14.44	96	54+68+96	C	cp
26	Butyrolactone	15.22	86	56+86	C	cp
27	Unknown	15.56	?	?	C	cp
28	Furanmethanol	15.61	98	98	C	cp
29	5-Methyl-2(5H)-furanone	16.06	98	55+98	C	f
30	Unknown	16.17	unk	110	C	cp
31	1,2-Cylopentandione	17.51	98	55+98	C	cp
32	Unknown	17.67	unk	42+70	C	cp
33	2-Hydroxy-3-methyl-2-cyclopenten-1-one	18.14	98	98	C	cp
34	3-Methy-l1,2-cyclopentanedione	18.42	112	69+112	C	cp
35	Unknown	19.06	unk	58+86+114	C	unk
36	Unknown	19.35	unk	98+126	C	unk
37	Unknown	21.77	unk	116	C	unk
38	Unknown	22.33	unk	44	C	unk
39	Unknown	26.18	unk	57+69	C	unk
40	5-Hydroxymethylfuran1–carboxaldehyde	27.51	126	97+126	C	f
41	Unknown	31.67	unk	73+135	C	unk
42	Laevoglucosan	40.44	172	60+73	C	f

Table 3: Other pyrolysis products quantified

	Name	RT	MW	integrated frements	Origin	Class
1	25:0				Cut	cut0
2	25:1				Cut	cut1
3	27:0				Cut	cut0
4	27:1				Cut	cut1
5	29:0				Cut	cut0
6	29:1				Cut	cut1
7	Myristic acid (14:0)	2.35	68	39+68	lip	fa
8	Palmitic acid (16:0)	2.74	82	81+82	lip	fa
9	Stearuc acid (18:0)	2.91	82	81+82	lip	fa
10	N-methyl-pyrrol	6.15	81	80+81	N	N-me-pyr
11	Pyridine	6.90	95	52+79+95	N	p
12	Methylpyridine	7.50	93	66+92+93	N	p
13	Methylpyridine	7.54	93	66+92+93	N	p
14	methylpyridine	9.02	93	66+93	N	p
15	Pyrrol	13.11	67	39+41+67	N	p
16	Methylpyrrol	13.81	81	80+81	N	p
17	Methylpyrrol	14.10	81	80+81	N	p
18	3-Hydroxypyridine	26.52	95	67+95	N	pyridol
19	Indole	26.85	117	89+117	N	ind
20	Methylindole	27.42	131	130+131	N	ind
21	Aceton	2.46	58	43	non	short
22	2-Propenal	2.60	56	55+56	non	short
23	Methanol	2.88	32	29+31+32	non	short
24	3-Buten-2-one	3.39	70	55+70	non	short
25	2,3-Butandione	3.67	86	69+86	non	short
26	3-Penten-2-one	3.89	86	69+86	non	short
27	Toluene	4.54	92	91+92	non	ar
28	2-Butanal	4.56	70	69+70	non	short
29	2,3-Pentadione	4.77	100	57+100	non	short
30	Hexanal	5.16	82	56+72+82	non	short
31	Xylene	5.94	106	91+105+106	non	ar
32	Xylene	6.09	106	91+105+106	non	ar
33	Xylene	6.20	106	91+105+106	non	ar
34	Xylene	6.99	105?	84+105?	non	ar
35	Limonene	7.22	?	93	non	ter
36	1-Penten-3-one	11.28	84	55+84	non	short
37	Methoxytoluene	11.78	122	121+122	non	ar
38	Indene	12.64	116	115+116	non	ar
39	Benzaldehyde	13.35	106	77+106	non	ar
40	1-Methyl-4-methoxybenzene	15.98	?	?	non	ar
41	Phytol	20.00	?	95+123	phytol	phytol
42	Unknown	20.85	unk	?	unk	unk
43	Unknown	20.86	unk	?	unk	unk
44	Unknown	22.43	unk	98+128	unk	unk
45	Unknown aliphatic	22.82	unk	58+71	unk	al
46	Hexan2,4dion	23.92	114	56+84+114	unk	o
47	Dihydrobenzofuran	26.19	120	91+119+120	unk	bf
48	Unknown	27.76	unk	138	unk	unk

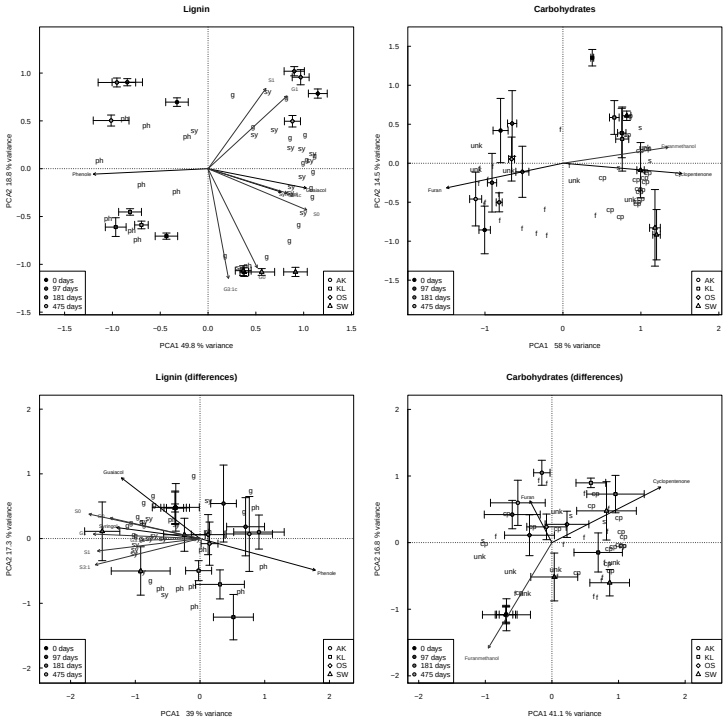
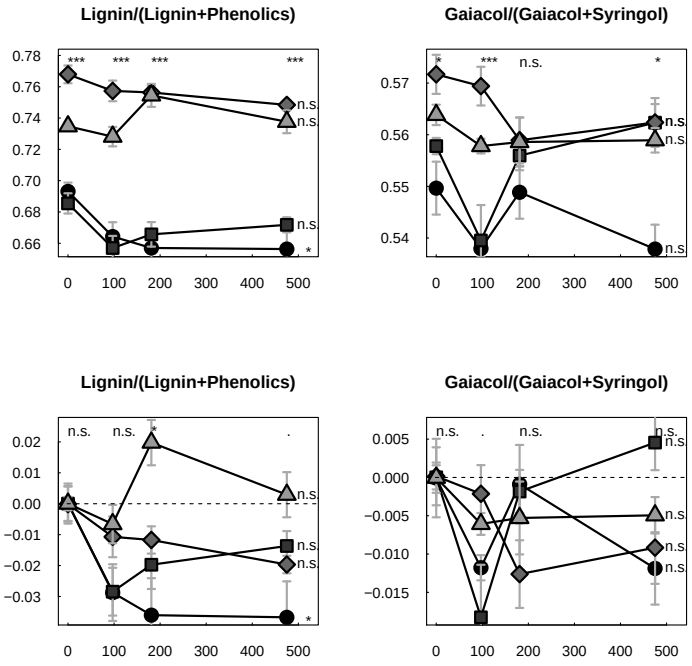


Figure 2: The upper left graphs shows a PCA of relative contributions of lignin and phenol pyrolysis products to the sum of these products, the lower left graph shows the difference of this relative contribution to initial contributions. The both graphs on the right hand show the same relations for carbohydrate derrived pyrolysis products. Sample means (n=4-5) and standard errors are stated as indicated in the plot legend. Letters indicate pyrolysis products (g - guaiacol lignin, sy - syringol lignin, ph - phenolic compounds, cp - cyclopentenone-type and f - furan type carbohydrade markers). Black arrows indicate fits for %TIC sum of compound classes (guaiacol and syringol lignin, other phenolic compounds, furan- and cyclopentenone-type carbohydrate markers), grey arrows indicate selected individual markers (G1/S0 - guaiacol/syringol, G/S1 - methylguaiacol/-syringol, G3:1/S3:1 - Propenylguaiacol/syringol.) The lower graph shows a PCA based on differences to initial peak abundance.


Lignin/(Lignin+Phenolics)
Gaiacol/(Gaiacol+Syringol)
Lignin/(Lignin+Phenolics)
Gaiacol/(Gaiacol+Syringol)
0.78
0.76
0.74
0.72
0.70
0.68
0.66
0.57
0.56
0.55
0.54
0.02
0.01
0.00
−0.01
−0.02
−0.03
0.005
0.000
−0.005
−0.010
−0.015
0
100
200
300
400
500
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n.s.
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Figure 3:

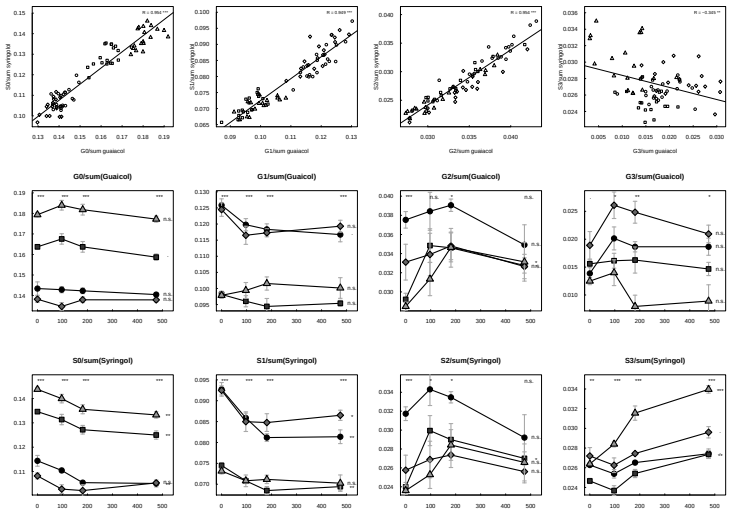

Figure 4: Lignin side chains occur in the same ratios for both guaiacol and syringol lignin, but differences in the content of -H and -CH_3 side chains were found.

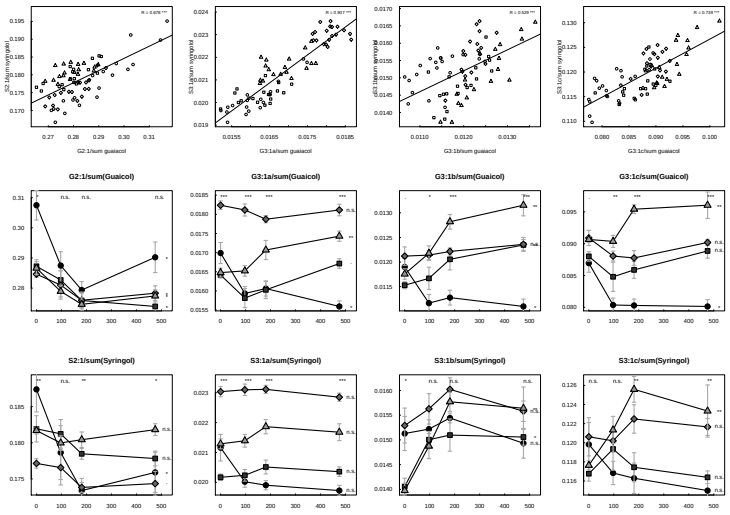

Figure 5: Lignin side chains occur in the same ratios for both guaiacol and syringol lignin, but differences in the content of -H and -CH_3 side chains were found.

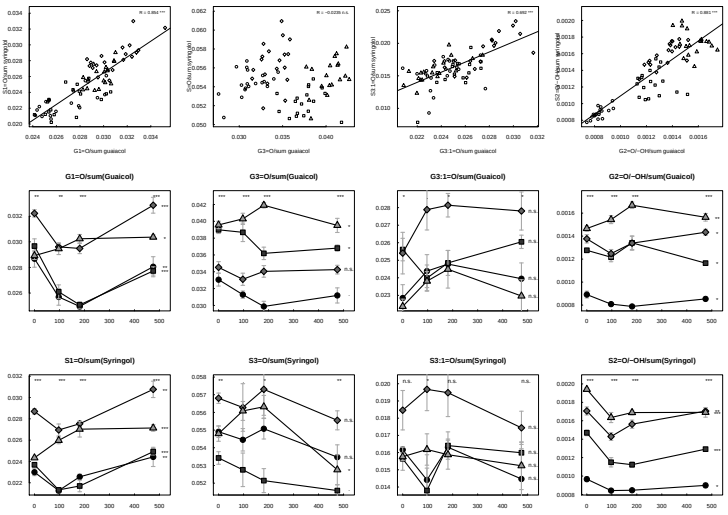

Figure 6: Lignin side chains occur in the same ratios for both guaiacol and syringol lignin, but differences in the content of -H and -CH_3 side chains were found.

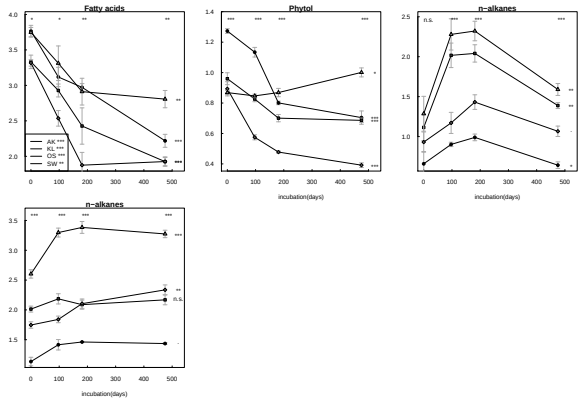

Figure 7: Trends for lipophilic compounds found in isolated lignin. Two samples were excluded due to contaminations.

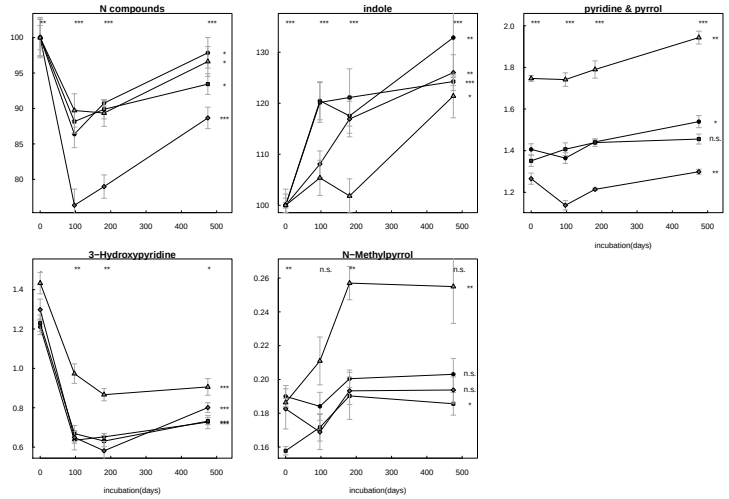

Figure 8: Trends for nitrogen compounds found in isolated lignin. Two samples were excluded due to contaminations.