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VORWORT

An dieser Stelle möchte ich ganz herzlich allen danken, die mich während meines Studiums und meiner Diplomarbeit begleitet und unterstützt haben.

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1. INTRODUCTION

A major scientific challenge of the 21st century lies in understanding the interactions between ecological, evolutionary and socio-economic factors, and their effect on biodiversity and ecosystem functioning across scales. MICDIF – "Linking microbial diversity and functions across scales and ecosystems" – a National Research Network funded by the Austrian Research Council (http://micdif.net/, FWF- S1007, Andreas Richter) was performed with the aim to advance the understanding of the functional role of microbial diversity in ecosystems and its importance for ecosystem functioning in connected terrestrial and aquatic ecosystems. In detail, MICDIF research topics focussed on the alteration of microbially-driven ecosystem functions by ecological stoichiometry (i.e. elemental composition of resources) and environmental conditions.

The MICDIF individual project 07 (Wolfgang Wanek) dealt with microbial processes and functional microbial community structure. Its goal was to investigate the influence of substrate stoichiometry and environmental conditions on transformations of carbon, nitrogen and phosphorus when litter is decomposed in terrestrial as well as aquatic ecosystems. Different analytical methods were used to analyse how gross transformation processes are affected by elemental stoichiometry, microbial community structure and environmental modulation of metabolism by focussing on gross rates of organic matter depolymerisation and of carbon, nitrogen and phosphorus mobilization/immobilization.

The major aim of this diploma thesis within MICDIF was to explore potential differences in carbon quality between similar litter types (*Fagus sylvatica* L.) that differed in elemental stoichiometry (C:N:P), and how these may influence litter decomposition. Therefore, beech litter was collected from different sites and was analyzed for intraspecific differences in carbon quality and litter chemistry. We expected that litter from the same plant species but different sites will differ in elemental composition but not (markedly) in carbon quality. Fur-

thermore, decomposition patterns of plant litter components, e.g. lignins and fatty acids were investigated and possible controls are discussed.

2. PLANT LITTER DECOMPOSITION

Plant litter decomposition represents a major carbon transformation process, and due to the complexity of this process, only a definition in general terms is possible. Decomposition can be described as the process by which a fraction of organic material is broken down into simpler forms of matter while the residue is transformed into more stable compounds based on the interaction of physical, chemical and biological processes. Physical mechanisms include fragmentation by wet-dry, shrink-swell, freeze-melt events and even by wind, animals and other plants, as well as leaching and transport in water. Moreover, depolymerization, oxidation and condensation reactions, as chemical and biological mechanisms, together with ingestion and digestion by fauna and extracellular enzymatic processes account for essential biological decomposition processes. The significance of the litter decomposition process can be seen in concomitant CO_2 production, humus formation, long-term carbon storage and the regeneration of organically bound nutrients (Berg & McClaugherty 2008).

2.1. THE IMPORTANCE OF PLANT LITTER DECOMPOSITION FOR ECOLOGICAL PROCESSES

At the biosphere-level the significance of litter decomposition basically derives from two reasons. First, a relationship between the release of so-called greenhouse gases (e.g. CO_2, CH_4 and nitrogen-based gases) and plant litter decomposition rate is assumed. Factors causing an increase in the decomposition rate may also enhance the release carbon-based greenhouse gases into the atmosphere. Second, large amounts of carbon are stored in soils as humus and

other related stable organic compounds. The understanding of the processes of litter decomposition, humus formation and carbon sequestration, and moreover of the factors which govern these processes is important in order to predict global atmospheric carbon budgets.

Furthermore, litter decomposition should also be examined at the ecosystem level. There exists a clear relationship between litter decomposition and nutrient recycling. Dynamics of the decay of organic matter in soils influence the availability of nutrients through mineralization of organically bound nutrients. Humus formation also has an impact on soil cation exchange capacity (CEC) which is related to clay and humus content. As humus itself can be regarded as a reservoir of nutrients that are released slowly during mineralization, organic matter can positively affect the nutrient holding capacity via CEC and increase the nutrient reservoir of soils. However, it should also be kept in mind that soil nutrient availability can be temporally reduced during initial stages of litter decay due to immobilisation and thereby transient sequestration of nutrients from the general nutrient cycle.

In addition, other soil processes and decomposition are interrelated. Decomposition, humus formation and weathering are processes which are linked to carbon and nutrient storage as well as to controlled nutrient uptake of plants and microorganisms. For instance, humus and organic acids formed during litter decomposition may enhance weathering of minerals in soils, and thereby increase the supply of nutrients to plants. Moreover, soil pH is affected by decomposition processes. An increase in pH takes place when cations taken up by plants from deeper soil layers are released due to litter leaching and litter decomposition. In contrast release of CO_2 and the formation of carbonic acids and humic acids cause a decrease in soil pH.

The diversity and stability of ecological communities are also affected by decomposition processes given that detrital food webs process carbon and energy, thereby releasing nutrients and organic intermediates that can be used by other organisms. Another aspect concerns the changes in litter quality during decomposition and formation of humus viewed from

the perspective of microbial ecology. Different groups of microflora can only use carbon (energy) in specific organic forms which may greatly change in abundance during litter decomposition. This means that due to plant litter decomposition, the diversity of microbial populations is impacted by changes in litter composition and the release of intermediate degradation products which serve as sources for energy and nutrients for other microbial subpopulations (Berg & McClaugherty 2008).

2.2. FACTORS INFLUENCING PLANT LITTER DECOMPOSITION

It is well known that a complex and interacting set of factors influences mass loss, humus formation, nutrient dynamics and patterns of plant litter decay. Already in 1929, Tenney and Waksman (in (Berg & McClaugherty 2008)) postulated four distinct factors that have an impact on decomposition rates of soil organic matter (SOM). These factors were as follows:

a. the substrate's chemical composition
b. an adequate supply of nitrogen for decomposer organisms
c. the diversity of decomposer microorganisms
d. the environmental conditions, such as aeration, moisture, pH and temperature

In general, these factors are still valid nowadays (Berg & McClaugherty 2008). Zhang et al (2008) described the factors that regulate litter decomposition rates in more detail. These factors are climatic factors, e.g. mean annual temperature (MAT), mean annual precipitation (MAP) and annual actual evapotranspiration (AET), which vary with geographic variables, such as latitude and altitude, litter quality, in particular total nutrient content, nitrogen content, C:N ratio, lignin content and L:N (lignin:nitrogen) ratio, as well as vegetation and litter type . Moreover, they showed that nutrients, such as N, P, K, Ca, Mg, have a positive effect on decomposition rates, whereas the rates decreased with increasing C:N and L:N ratios (Zhang et al 2008). Litter chemistry can greatly vary between plant species and tissue type, and is affected by climate, site, and nutrient availability (Berg & McClaugherty 2008), thereby having

a major control on litter decomposition. It was shown that nitrogen has adverse effects on litter decomposition. High-quality (i.e. low-lignin) litter is decomposed faster when nitrogen is highly available compared to high-lignin litter. Additionally, nitrogen can increase the mineralization of old soil carbon and thereby decrease the stabilization of soil organic matter (Prescott 2010).

Litter pH is also an important factor for two reasons. First, it directly affects decomposer microorganisms, and second, it influences the solubility and availability of phosphorus and some micronutrients indirectly. Moreover, the chemical composition of newly shed litter affects the composition of the microbial community as well as the pattern and course of the decomposition process. Other important factors are edaphic factors such as soil texture, soil nutrient availability and soil chemistry, e.g. as determined by parent rock material. Vegetation on nutrient-poor materials such as granite forms a poor litter substrate, whereas limestone is able to positively affect the nutrient content of litter. Nutrient mobility and hydrology are determined by soil texture (Berg & McClaugherty 2008). The effect of increased CO_2 concentrations on litter decomposition was studied e.g. by Rouifed et al. (2010). Though several studies indicated that increased CO_2 concentrations result in wider C:N ratio of litter, and therefore have a negative impact on decomposition and nutrient release rates in litter, their study showed that elevated CO_2 had a much weaker effects on litter decomposition than litter species composition (Rouifed et al. 2010).

2.3. LITTER DECOMPOSITION VIEWED AS A PROCESS

The plant litter decomposition process itself can be regarded as a complex set of physical, chemical and biological processes that are influenced by a great variety of factors and their interactions. Decomposition, often defined as litter mass loss, is generally described as the sum of CO_2 released and organic matter and nutrients leached during a time interval. Leaching is defined as the loss of nutrients and incompletely decayed organic matter through disso-

lution and export by water. The different processes change in dominance during litter decomposition. In general, the litter decomposition sequence starts with newly shed litter. Its chemical composition affects the composition of the microbial community as well as the course and pattern of the decomposition process. During litter decomposition a variety of changes take place, such as mass loss, chemical changes of the remaining litter, fast or slow decay of different chemical components, occurrence of newly synthesized organic compounds and imported substances, in particular nutrients. Plant litter consists of a varying amount of different organic compounds dependent on plant part (leaves, stems, roots, bark) and species. These compounds can be grouped by their molecular size, solubility and primary element composition. Some compounds, namely sugars, low-molecular weight phenolics and nutrients can be lost in the early stages of decomposition due to dissolution, leaching and the activity of opportunistic microorganisms. The decay of high-molecular weight molecules such as cellulose, hemicellulose and lignin represents a slower process.

Net accumulation of newly formed compounds can be seen as the result of condensation reactions of phenolics and lignin degradation products and the import of nutrients. Figure 1 shows the main flows of carbon and general pathways for litter transformation to humus and inorganic carbon. At the beginning of decomposition, microorganisms form CO_2 and soluble compounds, and newly formed water-soluble components (so-called dissolved organic matter, DOM) leach out. Only the remaining more stable compounds are eventually transformed into humus. The decomposition process generally proceeds in a sequential manner with different classes of organic compounds dominating the different phases as litter decays. For Scots pine needle litter, a three-phase model for decomposition of litter to humus was proposed by Berg and Staaf (1980) (Fig. 2A). In this model, which focuses on the effects of lignin, nitrogen and climate, the decomposition process is divided into three functionally defined stages as follows:

a. early stage

b. late stage

c. humus-near stage or limit-value stage

The early stage is characterized by a high leaching rate of water-soluble compounds, enhanced production of CO_2 (under aerobic conditions) or of organic acids (under anaerobic or oxygen-limited conditions) by microorganisms, and rapid degradation of soluble and low-molecular weight compounds. Four groups of soluble organic matter can be distinguished, namely sugars (mono- and oligosaccharides), phenolics (e.g. partly hydrolyzed tannins), hydrocarbons and glycerides. Moreover, un-lignified cellulose and hemicellulose are degraded. This stage is mainly affected by climate. Further, high concentrations of the nutrients nitrogen, phosphorus and sulphur positively affect microbial decomposition of litter.

In the late stage, degradation of hemicellulose, cellulose and lignin takes place. Here the influence of climate approaches almost zero, but the effects of nitrogen and lignin increase. The effects of nitrogen and lignin are discussed later. It is well known that lignin degradation is normally slower than the decay of other litter components such as cellulose, which may be due to the complex irregular structure of lignins. As the decomposition process proceeds, lignin accumulates and becomes the factor determining litter decomposition rate.

During the humus-near stage, the decomposition is reaching a limit value and in some cases decomposition rates approach zero. Limit values for litter decomposition represent the proportion in decomposing litter stabilized and potentially regarded as SOM. The limit value for litter mass loss is estimated using an asymptotic function where decomposition rate approaches zero and decomposing litter reaches the 'limit-value stage' or 'humus-near stage'. Decomposition rate in the humus-near stage is mostly controlled by nitrogen and lignin content.

In the modified model (Fig. 2B) only two phases are proposed, where the late stage does not occur or is nearly non-existent. Furthermore, the modified model considers the role of manganese during lignin degradation and the significance of lignin decay in earlier stages.

In both models, nitrogen and lignin concentrations increase from the onset of decomposition. Furthermore, is should be kept in mind that all processes may occur throughout the decomposition even if some processes are dominant in a particular stage. The decomposition of lignified substances already takes place during the initial phase influenced to some extent by climate and by manganese. As in the established model, decomposition approaches a limit value in the humus-near stage (Berg & McClaugherty 2008).

Figure 1 Main carbon flows and general transformation pathways during litter decomposition (Berg et al. 2008, p.12)

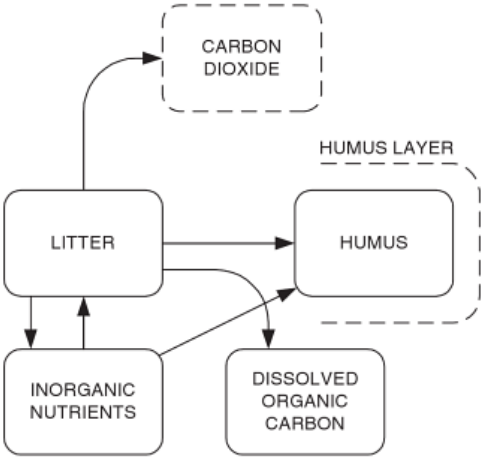
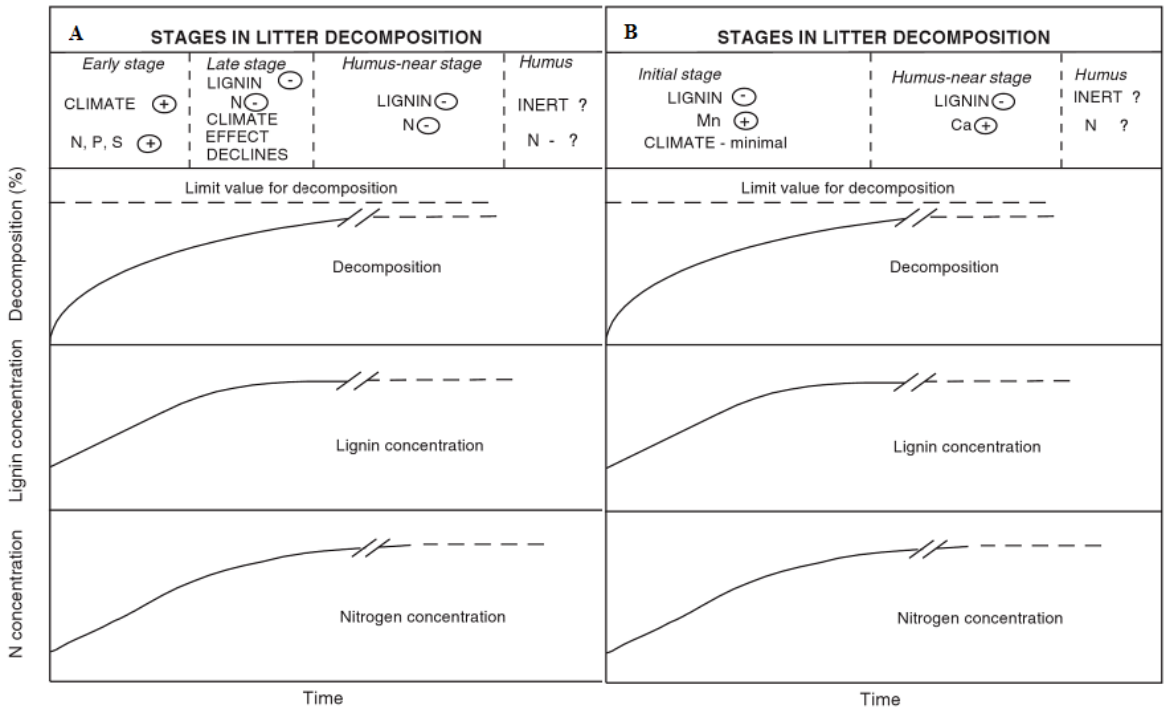


Figure 2 A Established model for litter decomposition and its controls by climate, lignin and nitrogen concentrations (Berg et al. 2008, p.13). **2 B** Modified model for litter decomposition and its controls (Berg et al. 2008, p.14)



2.4. EFFECTS OF NITROGEN AND MANGANESE ON LIGNIN DEGRADATION

A general phenomenon in decomposing litter is the increase in lignin and nitrogen as the decomposition process proceeds. Nitrogen is a determining factor in lignin decay and humus formation. It was shown that concentrations of N above a certain level cause a decrease in lignin decomposition. There are two main mechanisms that may explain the influence of N on lignin; one is of biological nature whereas the other is based upon chemical mechanisms. The biological mechanism is based on the repression of the formation of lignolytic enzymes in organisms when amounts of available N are high. The chemical mechanism is based on condensation reactions of nitrogen and lignin, which result in recalcitrant compounds (e.g. humins) that cannot be easily degraded by microorganisms. These compounds may be able to limit the access of microorganisms to degradable hemicellulose and cellulose. In addition,

manganese is an important element for the formation and activity of lignolytic enzymes, such as manganese peroxidase, laccase, and lignin peroxidase (Berg & McClaugherty 2008).

2.5. SOIL FAUNA AND MICROFLORA

Soil fauna plays a significant role in litter decay via fragmentation, gut processing, translocation of litter material (bioturbation) and enhancement of soil structure (Couteaux et al 1995). The prominent decomposer organisms in boreal and temperate forest soils are fungi and bacteria. A variety of microorganisms are able to degrade cellulose and hemicellulose through the production of extracellular hydrolytic enzymes. In contrast, lignin degradation is thought to be mainly performed by fungi (white- and brown-rot type) and less so by aerobic bacteria (Berg & McClaugherty 2008).

3. THERMALLY ASSISTED HYDROLYSIS AND METHYLATION

The term pyrolysis originates from the Greek “pyros” (= fire) and “lyso” (= decompose). In simple terms it describes the chemical decomposition of organic compounds by heat. In this reaction, high-molecular weight compounds are thermally degraded to lower-molecular weight products, part of which are volatile. Pyrolysis can be linked to different analytic methods, such as gas chromatography coupled to mass spectrometry. This combination represents an appropriate analytical tool for identification and structural characterisation of high-molecular weight substances.

Furthermore, it is possible to perform pyrolysis using an alkylation reagent. This analysis method is known as “thermally assisted hydrolysis and methylation” (THM). Thermally assisted hydrolysis and methylation (THM), also named thermochemolysis, is a type of analytical pyrolysis. This method is used to analyse a wide range of complex and intractable materials (Shadkami & Helleur 2010), such as synthetic and natural materials including resins,

waxes, lipids, carbohydrates, wood products, soil, sediments, proteins (Challinor 2001), polymers and materials in art and conservation (Shadkami & Helleur 2010). "Pyrolytic methylation" reactions are chemical degradation processes that occur when high-molecular weight molecules are heated in an oxygen-free environment in the presence of alkylation reagents, such as tetraalkylammonium hydroxide (TAAH) (Challinor 2001).

The use of heat in this reaction has two main effects. First, it drives the reaction between the analyte molecule and the alkylation reagent, which results in alkylated products. Second heat participates in the base-catalyzed bond cleavage of esters and ethers. Furthermore, heat induces thermal bond cleavage of the analyte molecule, but to a lower extent. The THM reaction results in the formation of smaller molecular weight, alkylated, and therefore less polar volatile pyrolysis products (Shadkami & Helleur 2010).

3.1. COMPARISON OF ANALYTICAL PYROLYSIS (PY) AND THM

In analytical pyrolysis, thermal fragmentation of complex molecules under oxygen-free conditions is performed at temperatures ranging from 400 °C to 700 °C. This form of analysis is commonly used for non-volatile or indissoluble organic compounds. It is applicable to direct analysis of solid compounds by techniques such as GC and GC/MS. The disadvantages of analytical pyrolysis are the resulting underivatized, polar products, which have a poor chromatographic behaviour, the need of high energy input, complex fragmentation patterns and the multitude of pyrolysis products.

In contrast, for THM a lower temperature is required, hence less thermal fragmentation occurs. Moreover, THM has a greater selectivity in ether and ester bond cleavage and the resulting respective alkyl derivatives of the compounds containing acidic functional groups show enhanced chromatographic resolution. Analytical pyrolysis as well as THM are used for analysing the chemical alteration of polymeric or complex materials and mixtures. Based on the pyrolysis and THM profiles, chemical analysis can be performed resulting in structural

characterisation, chemical profiling and determination of the analyte's origin (Shadkami & Helleur 2010).

3.2. ALKYLATION REAGENTS

Nowadays a variety of alkylation reagents are used. They have specific behaviours and characteristics, and are therefore used for different purposes. They all possess the ability to derivatize acidic functional groups. Moreover, they are persistent and chemically reactive over a temperature range of 300 °C to 600 °C. Some reagents selectively cleave ether and ester bonds. The products resulting from the reaction of an analyte molecule and the alkylation reagent are derivatized compounds, which are more volatile and less polar, and therefore exhibit enhanced chromatographic behaviour.

Tetramethylammonium hydroxide (TMAH, pK_b 4.2) is the most common reagent used for alkylation. It is a relatively strong base and a THM reagent. Other alkylation reagents used and their applications are:

- Tetraethylammonium acetate (TEAAC) for derivatisation of free fatty acids,
- Tetramethylammonium acetate (TMAAC) and phenyltrimethylammonium hydroxide (TMPAH) for quantitative analyses of organic acids in atmospheric particles,
- m-(trifluoromethyl)-Phenyltrimethylammonium hydroxide (TFTMAH) for methylation of alcohols including fatty acid alcohols, hydroxyl acids, terpenes and glycerols (Shadkami & Helleur 2010).

3.3. REACTION MECHANISM

Kossa et al. (1979) proposed the following mechanism for a thermochemolysis reaction of a compound containing an acidic functional group and the alkylation reagent tetramethylammonium hydroxide (Shadkami & Helleur 2010):

$$R-OH\,(aq) + (CH_3)_4N^+OH^-\,(aq) \xrightarrow{-H_2O} (CH_3)_4N^+ + R-O^-\,(aq) \xrightarrow{heat} R-OCH_3 + N(CH_3)_3\,(g)$$

Challinor (2001) predicted an additional hydrolysis step and described the mechanism as follows:

(1) $OH^- + A-B \longrightarrow A^- + B-OH$

(2) $A^- + R_4N^+OH^- \longrightarrow R_4N^+A^- + OH^-$

$B-OH + R_4N^+OH^- \longrightarrow R_4N^+OB^- + H_2O$

(3) $R_4N^+A^- \longrightarrow A-R + R_3N$

$R_4N^+OB^- \longrightarrow B-OR + R_3N$

Reaction (1) represents a hydrolysis step, in which A – B forms the hydrolysable analyte molecule. This step is valid for ester and ether bond cleavages by OH^- groups originating from the alkylation reagent. The formation of a TAA salt is shown in reaction (2). Thermal dissociation of the TAA salt under pyrolysis conditions results in the respective alkyl derivatives, shown in reaction (3).

In general, the THM reaction mechanism consists of a high-temperature hydrolysis reaction linked with the hydrolytic cleavage of ether and ester bonds induced by e.g. TAAH. In succession, TAA salts are formed and pyrolytically transformed to alkyl derivatives (Challinor 2001). The mechanisms proposed by Kossa et al. (1979) and Challinor (2001) are the most accepted ones; both bring into focus the bond cleavage of ester and ether bonds. But some important aspects concerning thermochemolysis should not be disregarded. It should be considered, that other bond cleavages can occur, such as thermal bond cleavage for example for interflavonoid bonds in tannins, C-C bonds in polycyclic aromatic hydrocarbons and amide bonds in aromatic polyesters. It has been supposed that these reactions are concurrent. Moreover, it is known that during analytical pyrolysis free radical degradation mechanisms

occur. The possibility of occurrence of such mechanisms in THM under high-temperature conditions should not be neglected (Shadkami & Helleur 2010).

Another aspect of importance concerns methanol as solvent for the TMAH reagent. Under TMH conditions, it is possible that methylated products not only result from hydrolysis by TMAH, but also to some extent from methanolysis. In conclusion, the formation of products during THM cannot be explained only by hydrolysis and alkylation reactions; other bond cleavage mechanisms such as homolytic cleavage of C-C bonds must be present to explain pyrolysis and concurrency of pyrolytic processes (Challinor 2001).

4. EXPERIMENTAL SETUP, MATERIALS AND METHODS

For this study samples of beech (*Fagus sylvatica L.*) litter were collected at four different sites in Austria, namely Achenkirch (AK), Schottenwald (SW), Klausen-Leopoldsdorf (KL) and Ossiach (OS). They all differ in elemental and stoichiometric quality, thus in C:N:P ratios. For litter biochemical (carbon quality) analysis thermally assisted hydrolysis and methylation linked to pyrolysis-gas chromatography/mass spectrometry (THM Py-GC/MS) was used.

4.1. MESOCOSM EXPERIMENT AND SAMPLE PRE-TREATMENT

To obtain similar conditions for all samples, the collected litter was first dried at 40 °C for 48 hours. Then the litter was chopped into small pieces (between 1 mm and 1 cm) and gamma-ray sterilized. The sterilized litter was inoculated with a suspension of a O-horizon:litter mixture (1:1 (w:w)) from Klausen-Leopoldsdorf to ensure that initial microbial community composition was similar for all samples. Finally, 60 g of each inoculated litter sample were placed into mesocosms constructed from PVC tubes (Wanek et al 2010). The litter samples were incubated in a climate chamber at a temperature of 15°C and collected after several periods of time. The first harvest took place after (two weeks) three months of incubation, the second

after six months and the last one after a period of 15 months. For each litter type and harvest five replicate mesocosms were sampled with the exception of the initial samples; for those only four replicates were kept. A small amount of the sample was homogenized in a ball mill and transferred into Eppendorf reaction tubes for storage.

4.2. METHOD

For TMH analysis, aliquots of 100 to 200 µg of litter were weighed into quartz pyrolysis tubes covered with glass wool and 2 µL of methylation reagent (25 % methanolic solution of tetramethylammonium hydroxide) was added. Prior to thermochemolysis a drying step was performed at 60 °C for 5 minutes to evaporate the surplus of methanol. The THM reaction and pyrolysis were performed on a CDS Analytical Pyroprobe 5250 Autosampler. The Pyroprobe was coupled to a Thermo Scientific Trace GC Ultra Gas Chromatograph with Trace DSQ II Mass Spectrometer. Pyrolysis was carried out at 600 °C for 10 seconds. Valve oven and interface were constantly set at a temperature of 250 °C. A Supelcowax 10 carbowax column (30 m × 0,25 mm × 0,25 µm, Sigma Aldrich) was used for GC separation of TMH products. The following temperature conditions were applied to separate the pyrolysates: the GC oven temperature was programmed from 50 °C as initial temperature (held for 2 minutes) to a final temperature of 260 °C (held for 15 minutes) at a heating rate of 7 °C min^{-1}. The settings for the mass spectrometer were: ionization energy of 70 eV and a mass range between m/z 20 and 300.

4.3. IDENTIFICATION AND STATISTICAL ANALYSIS

The components were identified by interpretation of their GC chromatograms and comparison of their EI mass spectra with NIST Mass Spectral Database. Furthermore, the identified pyrolysis products were compared with data reported in literature. For quantitative analysis, the peak areas were integrated. Peak integration was performed in the total ion chromatogram

(TIC) and in the selected ion chromatogram (SIM) at specific m/z for each peak. The calculated TIC (CTIC) data and the relative peak areas were essential for further statistical analysis. The relative peak areas were calculated by dividing the peak area for each compound by that of the sum of all compounds to allow comparison of peak areas of all samples. The identified compounds were classified into a number of source groups according to type and possible origin. These source groups were lignins (syringol and guaiacol subunits), carbohydrates, aromatic compounds, fatty acids, nitrogen-containing compounds and short chain compounds. For statistical analysis the program Statgraphics Plus 5.0 (Statistical Graphics Inc., Rockville, MD, USA) was used. The data were analysed with Principal Component Analysis (PCA) and One-Way and Multifactor ANOVA (Analysis of Variance).

5. RESULTS

MICDIF context data (provided by Ieda Nunes Cornelio Hämmerle, IP1; Katharina Keiblinger, IP6; and Sonja Leitner and Maria Mooshammer, IP7) are presented in Table 1, Table 2 and Figure 3, and are used to allow a better interpretation of this study's experimental results. Only data on lignins, fatty acids and aromatic compounds will be elaborated in this diploma thesis due to the low abundance of carbohydrates and nitrogen-containing compounds in THM traces (Nierop 2001, Shadkami & Helleur 2010). Short-chain compounds are not discussed since are cleavage products of unknown origin.

Table 1 Litter mass loss and initial contents of cellulose and lignin in litter collected in Achenkirch (AK), Klausen-Leopoldsdorf (KL), Ossiach (OS) and Schottenwald (SW). All data in percent of dry weight (% d.w.).

	AK	KL	OS	SW
Mass loss (% d.w.)	6,61	8,34	5,84	10,78
Lignin content (% d.w.)	20,39	16,64	15,27	14,91
Cellulose content (% d.w.)	20,70	19,39	17,98	18,74

Notes: Values are means of five replicates (mass loss) or four samples (initial contents of lignin and cellulose). Mass loss data correspond to values measured after 15 month of litter incubation.

Litter mass loss ranged from 6 to 11% with highest mass loss observed in Schottenwald and lowest in Ossiach. The highest lignin as well as cellulose content was found in litter collected in Achenkirch (Table 1). Mass loss was negatively related to lignin content and litter C:N (data not shown). Highest nitrogen concentrations and therefore lowest C:N ratios were reported for Klausen-Leopoldsdorf and Schottenwald. Furthermore, remarkable differences between litter types were found in C:P ratios, Fe and Mn concentrations (Table 2).

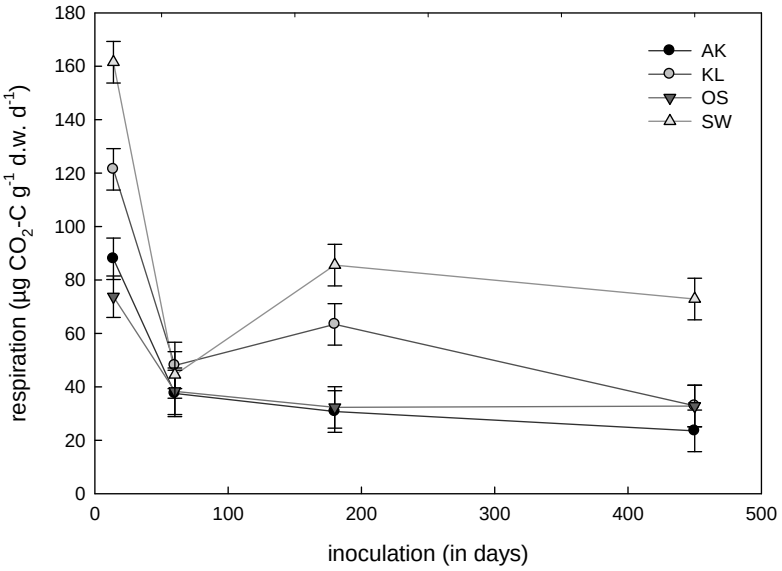
Table 2 Litter chemistry and elemental stoichiometry of all litter types and harvests. Data in percent of dry weight.

	AK				KL			
	H1	H2	H3	H4	H1	H2	H3	H4
C:N	57,8	56,9	55,1	55,5	52,5	49,7	50,9	47,8
C:P	1281	1258	1095	1364	1546	1484	1727	1610
K	0,26	0,24	0,32	0,24	0,54	0,55	0,54	0,54
Ca	1,33	1,33	1,38	1,38	1,26	1,30	1,29	1,33
Mg	0,27	0,28	0,26	0,28	0,14	0,15	0,15	0,16
Fe	209	210	210	183	207	226	221	200
Mn	172	166	165	164	1429	1489	1524	1448
Zn	30,6	33,4	36,4	33,5	33,0	38,6	39,4	37,7

	OS				SW			
	H1	H2	H3	H4	H1	H2	H3	H4
C:N	59,9	60,6	57,6	58,5	41,7	40,3	39,5	37,3
C:P	904	913	918	940	698	694	711	643
K	0,21	0,20	0,24	0,20	0,55	0,55	0,55	0,58
Ca	1,63	1,67	1,67	1,72	1,23	1,32	1,31	1,40
Mg	0,20	0,20	0,20	0,21	0,15	0,16	0,17	0,18
Fe	452	501	445	406	192	214	203	197
Mn	775	767	922	759	2136	2271	2279	2332
Zn	35,7	40,0	37,4	37,6	42,2	49,2	45,4	48,2

Notes: Values are means of five replicates; data presented are for harvests after two weeks (H1), two months (H2), six months (H3) and 15 months (H4) of litter incubation.

Figure 3 Time course of microbial respiration during beech litter decomposition. Analyses were performed after two weeks, and two, six and 15 months of litter incubation. Data represent mean values ± standard error (SE, n=5)



A major part of the occurring TMAH-pyrolysis products could be identified (in total 102 compounds) which are shown in Table 3 below.

Table 3 Identified TMAH-pyrolysis compounds grouped into syringol (S) and guaiacol units (G) of lignins (LIG), carbohydrates (C), fatty acids (FA), aromatics (A), nitrogen-containing components (N) and short chain compounds (SCC).

RT	name	formula	MW	category	FA	m/z	m/z
2,42	Acetic acid, methyl ester	C3H6O2	74	SCC		43	74
3,19	2-Propenoic acid, methyl ester	C4H6O2	86	SCC		55	85
3,72	Carbonic acid, dimethyl ester	C3H6O3	90	SCC		45	59
3,99	Acetaldehyde, methoxy-	C3H6O2	74	SCC		45	29
5,34	2-Propanone, 1-methoxy-	C4H8O2	88	SCC		45	43
5,44	2-Butenoic acid, methyl ester, (E)-	C5H8O2	100	SCC		69	85
6,00	N-Methylpyrrole	C5H7N	81	N		80	81
6,07	Benzene, 1,4-dimethyl-	C8H10	106	A		91	106

6,33	Propanoic acid, 2-methoxy-, methyl ester	C5H10O3	118	SCC		59	31
6,67	Furan, 2-methoxy-	C5H6O2	98	C		83	98
6,76	3-Pentenoic acid, methyl ester	C6H10O2	114	SCC		55	114
6,90	unidentified N compound		117	N		58	42
7,06	Acetic acid, methoxy-, methyl ester	C4H8O3	104	SCC		45	74
7,70	5-Hexenoic acid, methyl ester	C7H12O2	128	SCC		74	43
7,79	Furan, 2-(methoxymethyl)-	C6H8O2	112	C		81	112
8,20	Styrene	C8H8	104	A		104	103
8,78	Heptanoic acid, methyl ester	C8H16O2	144	SCC		74	87
9,33	Propanoic acid, 2-hydroxy-, methyl ester	C4H8O3	104	SCC		45	43
9,81	Benzene, methoxy-	C7H8O	108	A		108	78
9,98	Benzenemethanamine, N,N-dimethyl-	C9H13N	135	N		58	91
10,11	2-Cyclopenten-1-one	C5H6O	82	C		82	39
10,35	2-Cyclopenten-1-one, 2-methyl-	C6H8O	96	C		67	96
10,72	Octanoic acid, methyl ester	C9H18O2	158	FA	8:0	74	87
11,09	Benzene, 1-methoxy-2-methyl-	C8H10O	122	A		107	122
11,40	3-Furaldehyde	C5H4O2	96	C		95	96
11,63	Benzene, 1-methoxy-4-methyl-	C8H10O	122	A		121	122
11,72	Benzene, 1-methoxy-3-methyl-	C8H10O	122	A		122	91
12,07	Furfural	C5H4O2	96	C		96	95
12,40	2-Methoxy-4-methyl-3,4-dihydro-2H-pyran	C7H12O2	128	C		58	97
12,62	Nonanoic acid, methyl ester	C10H20O2	172	FA	9:0	74	87
12,82	2,5-Dimethylanisole	C9H12O	136	A		121	136
13,26	Benzene, 1-ethyl-4-methoxy-	C9H12O	136	A		121	136
14,07	Methyl 2-furoate	C6H6O3	126	C		95	126
14,19	Pyridine, 3-methoxy-	C6H7NO	109	N		109	66
14,34	Butanedioic acid, dimethyl ester	C6H10O4	146	SCC		115	55
14,45	Decanoic acid, methyl ester	C11H22O2	186	FA	10:0	74	87
14,78	2-Hexenoic acid, 3-methyl-, methyl ester	C8H14O2	142	SCC		111	142
14,90	Benzoic acid, methyl ester	C8H8O2	136	A		105	77
15,40	2,3-Dimethoxytoluene	C9H12O2	152	A		152	137
15,83	Benzene, 1-ethenyl-4-methoxy-	C9H10O	134	A		134	119
16,55	Benzene, 1,2-dimethoxy-	C8H10O2	138	A		138	95
16,82	Benzene, 1,4-dimethoxy-	C8H10O2	138	A		123	138
16,97	Benzene, 1,3-dimethoxy-	C8H10O2	138	A		138	109
17,12	Benzaldehyde, 4-(1-methylethyl)-	C10H12O	148	A		133	148
17,36	Fructofuranose, 2,6-anhydro-1,3,4-tri-O-methyl-, β-d-	C9H16O5	204	C		45	101
17,74	2,5-Dimethoxytoluene	C9H12O2	152	A		137	152

17,87	3,4-Dimethoxytoluene	C9H12O2	152	A		137	152
18,27	Benzene, 1-methoxy-4-(1-propenyl)-	C10H12O	148	A		147	148
18,92	Phenol, 3,5-dimethoxy-	C8H10O3	154	G/LIG		152	125
19,00	Benzene, 4-ethyl-1,2-dimethoxy-	C10H14O2	166	G/LIG		151	166
20,20	1,2,3-Trimethoxybenzene	C9H12O3	168	S/LIG		168	153
20,40	N-Methylindole	C9H9N	131	N		130	131
20,51	3(2H)-Furanone, 4-methoxy-2,5-dimethyl-	C7H10O3	142	C		142	71
20,96	Benzene, 1,2-dimethoxy-4-(2-propenyl)-	C11H14O2	178	G/LIG		178	91
21,02	Tetradecanoic acid, methyl ester	C15H30O2	242	FA	14:0	74	87
21,14	Benzaldehyde, 4-methoxy-	C8H8O2	136	A		135	136
21,47	Benzene, 1,2,3-trimethoxy-5-methyl-	C10H14O3	182	S/LIG		182	167
21,64	Benzoic acid, 3,4,5-trimethoxy-	C10H12O5	212	S/LIG		197	212
21,96	1,2,4-Trimethoxybenzene	C9H12O3	168	C		153	168
22,15	Benzoic acid, 4-methoxy-, methyl ester	C9H10O3	166	A		135	166
22,23	2,4,5-Trimethoxybenzaldehyde	C10H12O4	196	S/LIG		181	196
22,49	Pentadecanoic acid, methyl ester	C16H32O2	256	FA	15:0	74	87
22,73	Nonanedioic acid, dimethyl ester	C11H20O4	216	FA	9:0	152	74
22,87	Benzene, 1,3,5-trimethoxy-	C9H12O3	168	A		168	139
23,21	2,4,6-Trimethoxytoluene	C10H14O3	182	A		182	151
23,36	Benzeneacetic acid, 4-methoxy-, methyl ester	C10H12O3	180	G/LIG		121	180
23,91	Hexadecanoic acid, methyl ester	C17H34O2	270	FA	16:0	74	87
24,07	Acetophenone, 2,4-dimethoxy-, 3-methyl	C11H14O3	194	G/LIG		194	179
24,53	Benzenepropanoic acid, 4-methoxy-, methyl ester	C11H14O3	194	G/LIG		121	194
24,71	Asarone	C12H16O3	208	S/LIG		193	208
25,20	1,2,3,4-Tetramethoxybenzene	C10H14O4	198	A		198	183
25,26	Heptadecanoic acid, methyl ester	C18H36O2	284	FA	17:0	74	87
26,06	Benzaldehyde, 3,4-dimethoxy-	C9H10O3	166	G/LIG		166	165
26,12	Isoelemicin	C12H16O3	208	S/LIG		193	208
26,24	Benzoic acid, 2,6-dimethoxy-, methyl ester	C10H12O4	196	A		165	163
26,56	Octadecanoic acid, methyl ester	C19H38O2	298	FA	18:0	74	87
26,96	Benzoic acid, 3,4-dimethoxy-, methyl ester	C10H12O4	196	G/LIG		165	196
26,81	9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	296	FA	18:1	55	69
26,89	11-Octadecenoic acid, methyl ester	C19H36O2	296	FA	18:1	55	69
26,99	1,2-Dimethoxy-4-(2-methoxy-1-propenyl)benzene	C12H16O3	208	G/LIG		208	193
27,12	3,4-Dimethoxyacetophenone	C10H12O3	180	G/LIG		165	180
27,37	1,2-Dimethoxy-4-(2-methoxyethenyl)benzene	C11H14O3	194	G/LIG		194	179
27,41	Benzeneacetic acid, 3,4-dimethoxy-, methyl ester	C11H14O4	210	G/LIG		151	210
27,68	3,4,5-Trimethoxybenzaldehyde	C10H12O4	196	S/LIG		196	181

27,84	1-(3,4-Dimethoxyphenyl)-1-propanone	C11H14O3	194	G/LIG		165	194
28,30	2-Propenoic acid, 3-(4-methoxyphenyl)-, methyl ester, (E)-	C11H12O3	192	A		161	192
28,43	Benzoic acid, 3,4,5-trimethoxy-, methyl ester	C11H14O5	226	S/LIG		226	211
28,51	1,2-Dimethoxy-4-(3-methoxy-1-propenyl)benzene	C12H16O3	208	G/LIG		208	146
28,59	3,4,5-Trimethoxyacetophenone	C11H14O4	210	S/LIG		195	210
28,93	1,2-Dimethoxy-4-(1,3-dimethoxy-1-propenyl)-benzene	C13H18O4	238	G/LIG		238	223
29,03	Eicosanoic acid, methyl ester	C21H42O2	326	FA	20:0	74	87
29,88	unidentified N compound			N		196	211
30,20	Heneicosanoic acid, methyl ester	C22H44O2	340	FA	21:0	74	87
30,47	Benzoic acid, 3,4,5-trimethoxy-, methyl ester	C11H14O5	226	S/LIG		226	195
30,63	(3,4-Dimethoxyphenyl)-acatic acid	C11H16O3	196	G/LIG		151	196
30,85	1,2-Dimethoxy-4-(1,3-dimethoxy-1-propenyl)benzene	C13H18O4	238	G/LIG		207	238
30,91	1,2-Dimethoxy-4-(2,3-dimethoxy-1-propenyl)benzene	C13H18O4	238	G/LIG		238	207
31,33	Docosanoic acid, methyl ester	C23H46O2	254	FA	22:0	74	87
32,05	2-Propenoic acid, 3-(3,4-dimethoxyphenyl)-, methyl ester	C12H14O4	222	G/LIG		191	222
33,68	Tetracosanoic acid, methyl ester	C25H50O2	382	FA	24:0	74	87
34,08	2-Propenoic acid, 3-(3,4,5-trimethoxyphenyl)-, methyl ester	C13H16O5	252	G/LIG		252	237
36,92	Triacontanoic acid, methyl ester	C31H62O2	466	FA	30:0	74	87

The compounds were grouped according to their possible origin and chemical similarity. All other compounds were subsumed in a remaining group, termed short chain compounds, which may be unspecific cleavage products of many different compounds during pyrolysis. The assignment was carried out by comparison with literature, e.g. del Rio et al. (2007), Nierop (2001), McKinney et al. (1996), Fabbri et al. (1996), Fabbri and Helleur (1999) and Asperger et al. (2000).

According to Fabbri et al. (1996), some of the described compounds are related to humic acids and humin substances. These are aromatic compounds: e.g. benzoic acid methyl ester, 2,3-dimethoxytoluene, 1,2/1,3/1,4-dimethoxybenzene, 4-methoxybenzoic acid methyl ester and 3-(4-methoxyphenyl)-2-propenoic acid methyl ester; fatty acids: methyl esters of pentadecanoic acid, hexadecanoic acid, octadecanoic acid and 9-octadecenoic acid; N-methylindole as nitrogen-containing compound and a short chain compound, namely butanedioic acid methyl ester.

Furthermore, some cutan and cutin derived TMAH pyrolysis products could be identified. As reported by Nierop (2001), cutin THM pyrolysis products are methyl esters of alkanoic acids, hydroxyalkanoic acids, α,ω-alkanoic acids and alkanols (Nierop 2001). Cutan derived products are fatty acid methyl esters ranging from C_{15} to C_{31} as well as aromatic products which amount to approximately eight percent of the total weight of the polymer (McKinney et al 1996). Some examples of products that may result from cutin and cutan are 1,3,5-trimethoxybenzene, 2,4,6-trimethoxytoluene, 2,6-dimethoxybenzoic acid methyl ester and long-chain fatty acid methyl esters.

Principal Component Analyses (PCA) were performed to obtain a small number of linear combinations of different variables which account for most of the variability in the data. The first PCA of the major source groups, i.e. lignins, fatty acids, carbohydrates, aromatic compounds, nitrogen-containing compounds and short chain compounds is shown in Figure 4. The summary of the analysis is presented in Table 4. Two principal components could be extracted which together account for 81% of the variability in the original data. Lignins, carbohydrates and aromatic compounds were positively related and these were negatively correlated with short chain compounds and nitrogen-containing compounds (PC1). Fatty acids were independent of the other source groups (PC2).

Figure 4 Principal Component Analysis of source groups

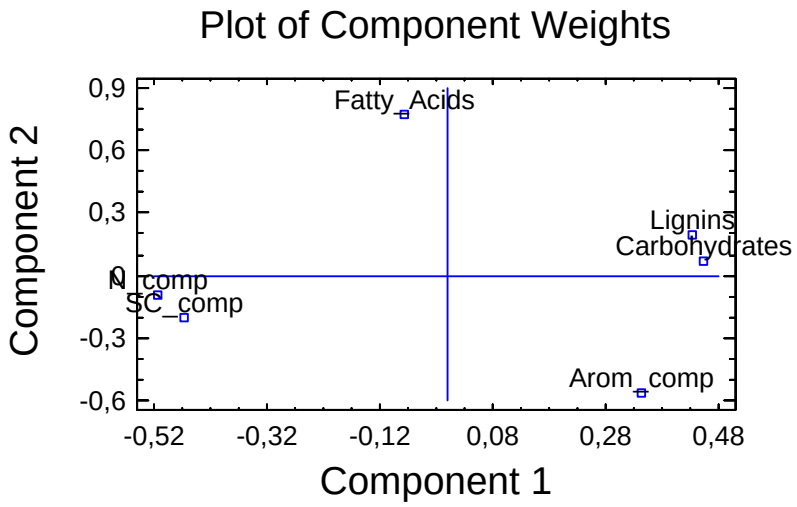


Table 4 Principal Component Analysis summary of source groups

	Principal component 1	Principal component 2
Percent of variance	56	25
Component weights		
Aromatic compounds	0,344	-0,561
Carbohydrates	0,454	0,066
Short chain compounds	-0,512	-0,092
Fatty acids	-0,465	-0,196
Lignins	-0,077	0,771

To investigate the similarities or dissimilarities of lignins and aromatics in more detail, a second PCA was performed (shown in Figs. 5 and 6). Here three principal components were extracted which together accounted for about 59% of the variance. We did not find a clear grouping between aromatics and/or lignins, on either PC axis, pointing to close relatedness of compounds classified as clearly lignin-derived or phenolics/aromatic compounds. Aromatics were most strongly represented on PC1 axis being positively correlated to many lignin markers, the same holding true for PC3 (where all significant markers were negatively related to

PC axis). Only on PC2 some aromatic compounds and some lignin biomarkers were positively, some negatively related.

Figure 5 PCA of lignins and aromatic compounds; PC1 vs. PC2

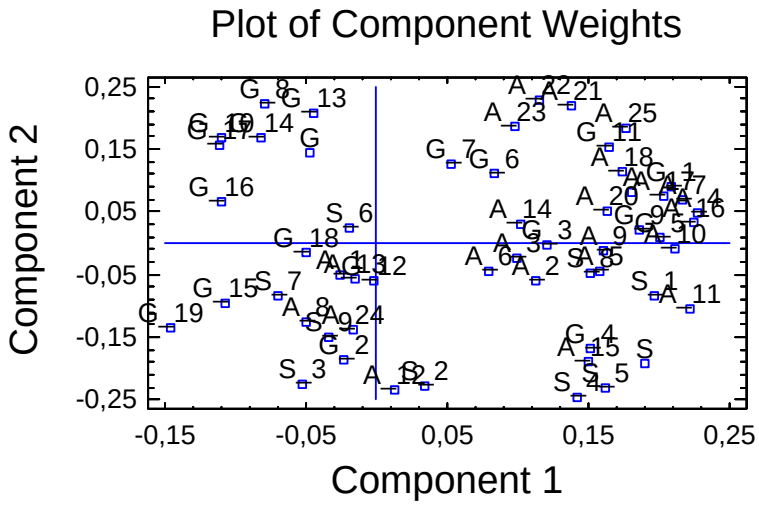


Figure 6 PCA of lignins and aromatic compounds; PC1 vs. PC3

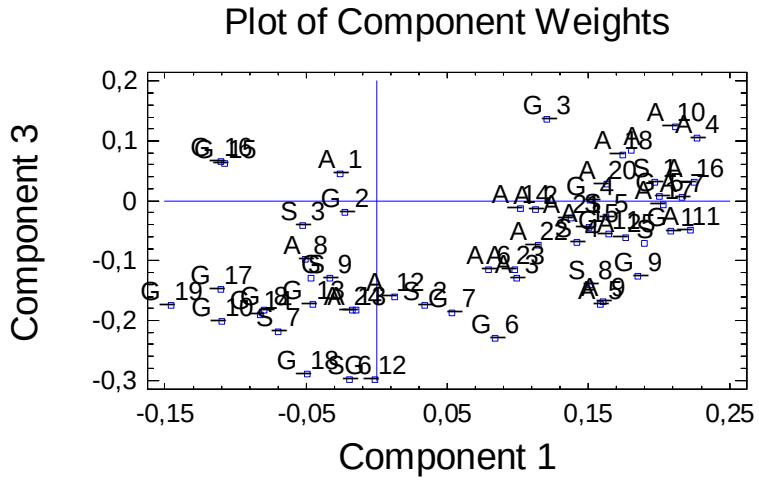


Table 5 PCA summary of lignins and aromatic compounds

		PC1	PC2	PC3
Percent of variance		25	18	16
Component weights				
Benzene, 1,4-dimethyl-	A	0.181	0.081	0.084
Styrene	A_1	-0.026	-0.051	0.046
Benzene, methoxy-	A_2	0.113	-0.059	-0.015
Benzene, 1-methoxy-2-methyl-	A_3	0.099	-0.025	-0.129
Benzene, 1-methoxy-4-methyl-	A_4	0.227	0.049	0.105
Benzene, 1-methoxy-3-methyl-	A_5	0.158	-0.044	-0.173
2,5-Dimethylanisole (od. 2,4/2,3/2,6)	A_6	0.079	-0.046	-0.115
Benzene, 1-ethyl-4-methoxy-	A_7	0.217	0.068	0.005
Benzoic acid, methyl ester	A_8	-0.050	-0.125	-0.098
2,3-Dimethoxytoluene	A_9	0.160	-0.012	-0.169
Benzene, 1-ethenyl-4-methoxy-	A_10	0.212	-0.010	0.123
Benzene, 1,2-dimethoxy-	A_11	0.223	-0.106	-0.049
Benzene, 1,4-dimethoxy-	A_12	0.013	-0.233	-0.160
Benzene, 1,3-dimethoxy-	A_13	-0.015	-0.057	-0.182
Benzaldehyde, 4-(1-methylethyl)-	A_14	0.102	0.030	-0.013
2,5-Dimethoxytoluene	A_15	0.150	-0.190	-0.045
3,4-Dimethoxytoluene	A_16	0.225	0.032	0.030
Benzene, 1-methoxy-4-(1-propenyl)-	A_17	0.203	0.076	-0.006
Benzaldehyde, 4-methoxy-	A_18	0.175	0.114	0.077
Benzoic acid, 4-methoxy-, methyl ester	A_20	0.163	0.050	0.027
Benzene, 1,3,5-trimethoxy-	A_21	0.138	0.218	-0.029
2,4,6-Trimethoxytoluene	A_22	0.115	0.229	-0.075
1,2,3,4-Tetramethoxybenzene	A_23	0.098	0.185	-0.115
Benzoic acid, 2,6-dimethoxy-, methyl ester	A_24	-0.017	-0.137	-0.182
2-Propenoic acid, 3-(4-methoxyphenyl)-, methyl ester, (E)-	A_25	0.176	0.183	-0.061
Phenol, 3,5-dimethoxy-	G	-0.047	0.144	-0.130

Benzene, 4-ethyl-1,2-dimethoxy-	G_1	0.209	0.091	-0.051
Benzene, 1,2-dimethoxy-4-(2-propenyl)-	G_2	-0.023	-0.185	-0.020
Benzeneacetic acid, 4-methoxy-, methyl ester	G_3	0.121	-0.003	0.137
Acetophenone, 2,4-dimethoxy-, 3-methyl	G_4	0.151	-0.167	0.001
Benzenepropanoic acid, 4-methoxy-, methyl ester	G_5	0.200	0.009	0.007
Benzaldehyde, 3,4-dimethoxy-	G_6	0.084	0.111	-0.230
Benzoic acid, 3,4-dimethoxy-, methyl ester	G_7	0.053	0.125	-0.188
1,2-Dimethoxy-4-(2-methoxy-1-propenyl)benzene	G_8	-0.080	0.223	-0.183
3,4-Dimethoxyacetophenone	G_9	0.185	0.022	-0.126
1,2-Dimethoxy-4-(2-methoxyethenyl)benzene	G_10	-0.109	0.169	-0.202
Benzeneacetic acid, 3,4-dimethoxy-, methyl ester	G_11	0.164	0.154	-0.055
1-(3,4-Dimethoxyphenyl)-1-propanone	G_12	-0.001	-0.059	-0.299
1,2-Dimethoxy-4-(3-methoxy-1-propenyl)benzene	G_13	-0.045	0.207	-0.174
1,2-Dimethoxy-4-(1,3-dimethoxy-1-propenyl)-benzene	G_14	-0.082	0.168	-0.191
(3,4-Dimethoxyphenyl)-acatic acid	G_15	-0.108	-0.096	0.062
1,2-Dimethoxy-4-(1,3-dimethoxy-1-propenyl)benzene	G_16	-0.110	0.065	0.066
1,2-Dimethoxy-4-(2,3-dimethoxy-1-propenyl)benzene	G_17	-0.111	0.156	-0.148
2-Propenoic acid, 3-(3,4-dimethoxyphenyl)-, methyl ester	G_18	-0.049	-0.016	-0.289
2-Propenoic acid, 3-(3,4,5-trimethoxyphenyl)-, methyl ester	G_19	-0.146	-0.135	-0.176
1,2,3-Trimethoxybenzene	S	0.190	-0.191	-0.071
Benzene, 1,2,3-trimethoxy-5-methyl-	S_1	0.197	-0.083	0.031
Benzoic acid, 3,4,5-trimethoxy-	S_2	0.034	-0.229	-0.175
2,4,5-Trimethoxybenzaldehyde	S_3	-0.053	-0.225	-0.041
Asarone	S_4	0.142	-0.247	-0.070
Isoelemicin	S_5	0.162	-0.232	-0.027
3,4,5-Trimethoxybenzaldehyde	S_6	-0.019	0.023	-0.299
Benzoic acid, 3,4,5-trimethoxy-, methyl ester	S_7	-0.070	-0.084	-0.219
3,4,5-Trimethoxyacetophenone	S_8	0.151	-0.049	-0.140
Benzoic acid, 3,4,5-trimethoxy-, methyl ester	S_9	-0.033	-0.149	-0.130

Third a PCA of fatty acids was calculated (Figure 7, Table 6). About 66% of the variability were explained by the two principal components extracted. Fatty acids were assorted into two groups, namely short chain fatty acids ($\leq C_7$, FA-FA7) and long chain fatty acids ($\geq C_{11}$, FA11-15) on the one hand, and FA8-10 i.e. octadecanoic acid methyl ester, 9-octadecenoic acid methyl ester and 10-octadecenoic acid methyl ester, on the other hand on PC1. The first group clearly separated on PC2, i.e. short chain fatty acids were negatively correlated to long chain fatty acids.

Figure 7 PCA of fatty acids

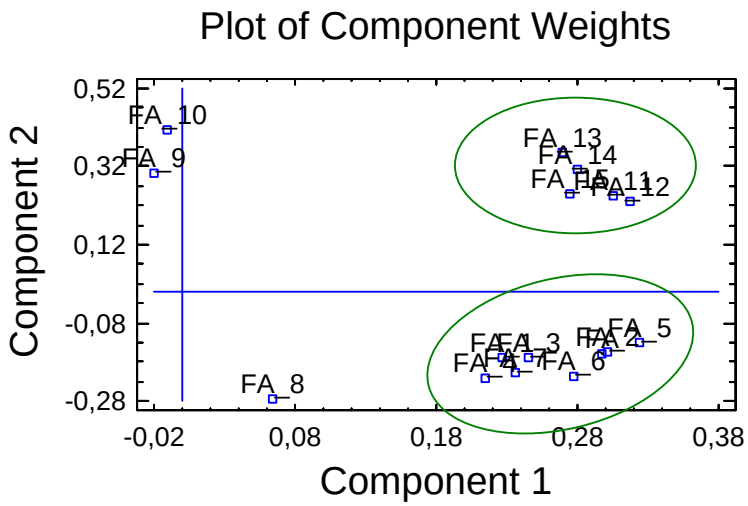
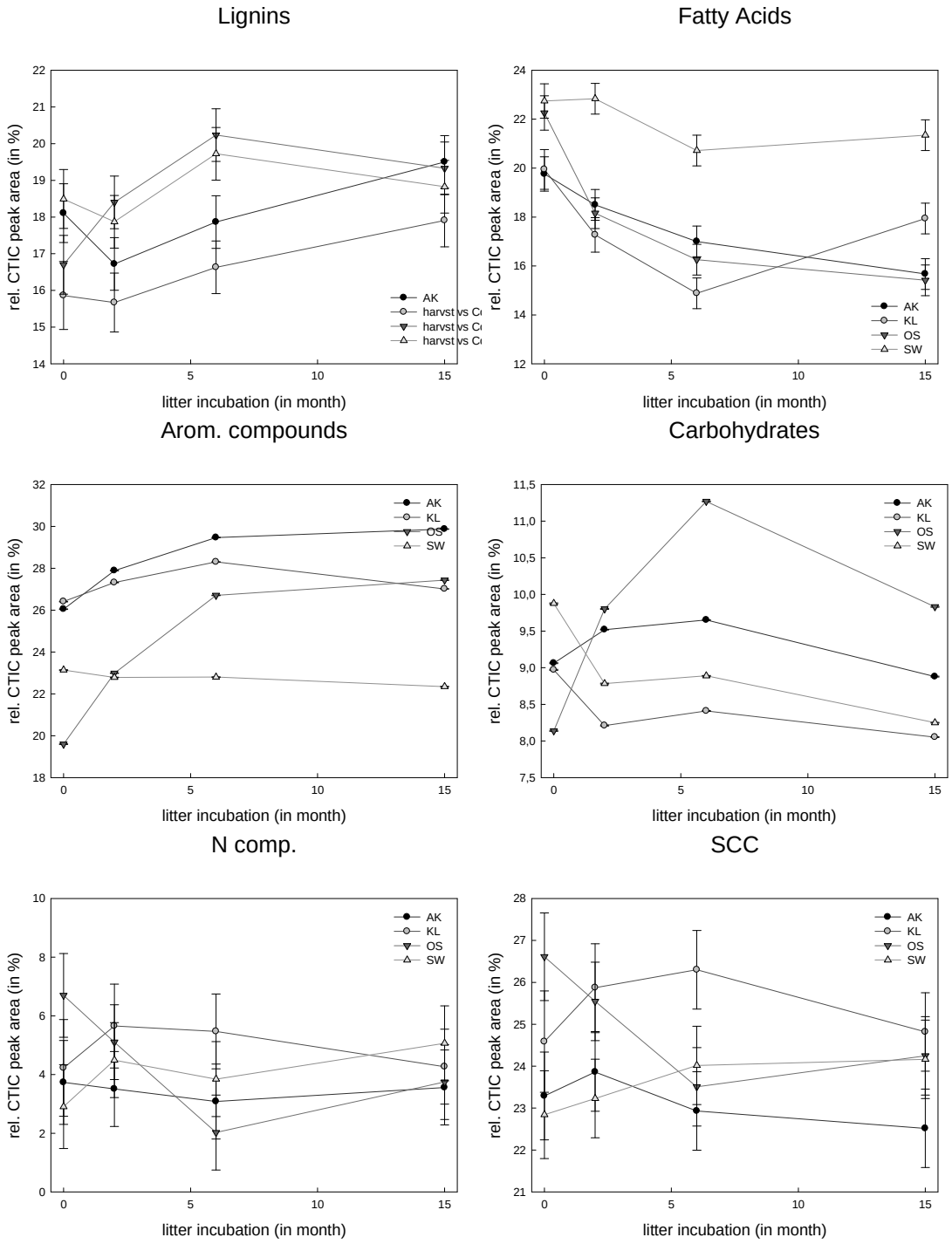


Table 6 PCA summary of fatty acids

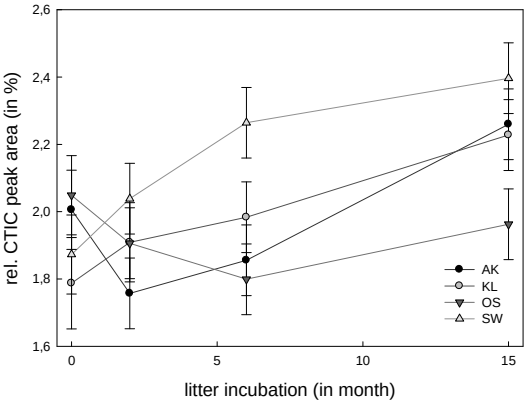
		PC1	PC2
Percent of variance		49	17
Component weights			
Octanoic acid, methyl ester	FA	0,297	-0,158
Nonanoic acid, methyl ester	FA_1	0,226	-0,168
Decanoic acid, methyl ester	FA_2	0,301	-0,155
Tetradecanoic acid, methyl ester	FA_3	0,245	-0,169
Pentadecanoic acid, methyl ester	FA_4	0,214	-0,220
Nonanedioic acid, dimethyl ester	FA_5	0,323	-0,128
Hexadecanoic acid, methyl ester	FA_6	0,277	-0,216
Heptadecanoic acid, methyl ester	FA_7	0,236	-0,205
Octadecanoic acid, methyl ester	FA_8	0,064	-0,274
9-Octadecenoic acid (Z)-, methyl ester	FA_9	-0,019	0,303
11-Octadecenoic acid, methyl ester	FA_10	-0,011	0,413
Eicosanoic acid, methyl ester	FA_11	0,304	0,246
Heneicosanoic acid, methyl ester	FA_12	0,317	0,226
Docosanoic acid, methyl ester	FA_13	0,269	0,354
Tetracosanoic acid, methyl ester	FA_14	0,279	0,312
Triacontanoic acid, methyl ester	FA_15	0,274	0,251

A multifactor ANOVA was performed to determine which factor (site-litter type, harvest) had a significant effect on the decomposition patterns of compounds classes grouped by origin. Time courses of the main litter components during decomposition are shown in Figure 8. The results of the Multifactor ANOVA are summarized in Table 7.

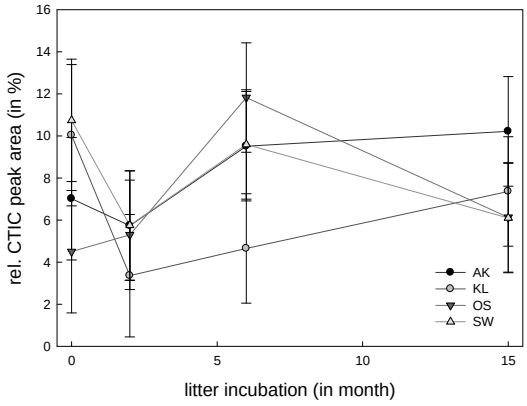
Figure 8 Courses of main litter components, and L:C, L:N and N:C ratios during litter decomposition. Values are means ± SE (n=5).



L:C


2,6
2,4
2,2
2,0
1,8
1,6
rel. CTIC peak area (in %)
0
5
10
15
litter incubation (in month)
AK
KL
OS
SW

L:N


16
14
12
10
8
6
4
2
0
rel. CTIC peak area (in %)
0
5
10
15
litter incubation (in month)
AK
KL
OS
SW

N:C

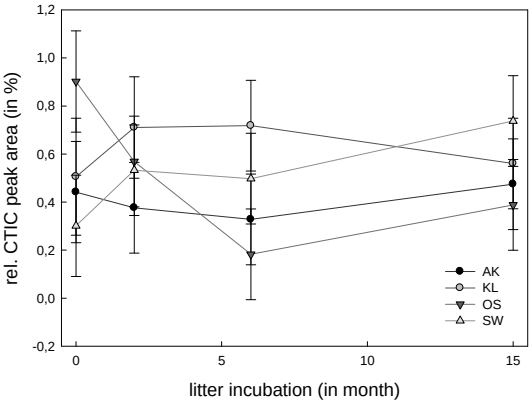

1,2
1,0
0,8
0,6
0,4
0,2
0,0
-0,2
rel. CTIC peak area (in %)
0
5
10
15
litter incubation (in month)
AK
KL
OS
SW

Table 7 Two-way ANOVA to test the effects of litter type and harvest on main components (lignin, fatty acids, carbohydrates, aromatics, N containing compounds, short chain compounds), L:C, L:N and N:C ratios and the principal components 1 and 2 (PC1, PC2) for main litter components.

Df	3		3		9		
fraction	**harvest effect**		**site effect**		**interaction**		**relative amount**
	F	P	F	P	F	P	**in %**
LIG	5,67	0,0018**	7,00	0,0004***	1,22	0,3008	15 - 21
G	3,26	0,0279*	6,38	0,0008***	1,38	0,2195	8 - 14
S	10,17	0,0000***	19,56	<0,0001***	3,30	0,0026**	5 - 9
FA	27,57	<0,0001***	40,89	<0,0001***	3,74	0,0009***	14 - 24
A	6,14	0,0011*	22,74	<0,0001***	2,39	0,0224*	18-32
C	1,56	0,2082	3,96	0,0122*	1,39	0,0660	8-11
SCC	0,37	0,7775	4,79	0,0048**	1,00	0,4489	22 - 28
N	0,50	0,6854	0,78	0,5095	0,83	0,5900	1 - 8
L:C	6,95	0,0004***	3,04	0,0361*	1,80	0,0878	
L:N	1,54	0,2134	0,39	0,7606	0,83	0,5886	
N:C	0,33	0,8008	0,78	0,5079	1,05	0,4153	
PC1	1,52	0,2197	2,17	0,1018	1,36	0,2290	
PC2	18,17	<0,0001***	49,53	<0,0001***	2,83	0,0079**	

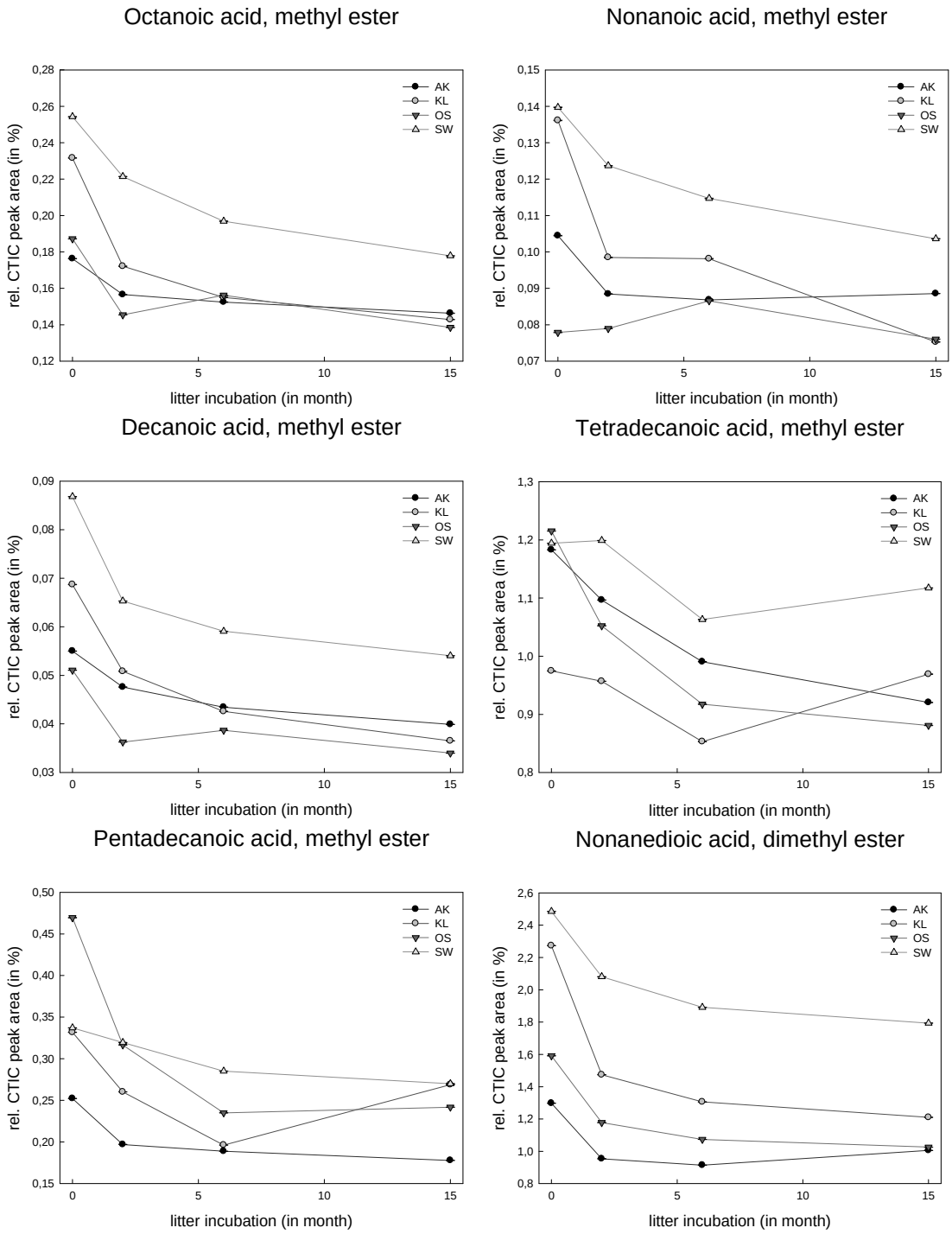
Notes: Analysis includes data from all litter types; initial litter and harvests after 3, 6 and 15 months. Data are F-ratios and P-values (95% confidence level). Significance levels: *** P < 0,001; ** P < 0,01; * P < 0,05; no symbol P > 0,05

The highest relative amounts were accounted for by aromatic compounds (18-32%), followed by short chain compounds (22-28%), fatty acids (14-24%) and lignins (15-21%). Carbohydrates (8-11%) and nitrogen-containing compounds (1-8%) made up only a small percentage

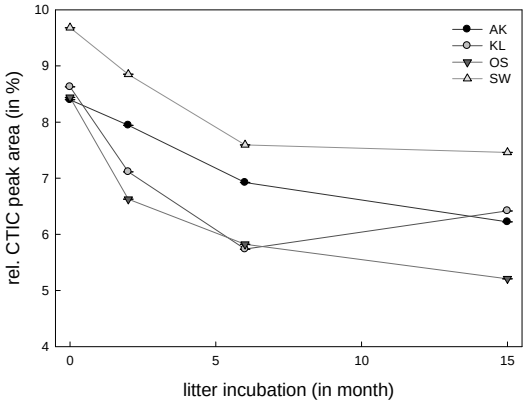
of CTIC. Significant effects of harvest and site were obtained for lignins, fatty acids, aromatics and the L:C ratio. For carbohydrates and short chain compounds only a site effect is observed, while N, L:N mand N:C were not affected by both factors. Moreover, PC1 scores (LIG, C, A; N, SCC) were not affected by both while PC2 scores were highly significantly affected by harvest and site.

From two months of incubation onwards a slight increase in lignins became evident. Although lignins showed significant site and harvest effects, they behaved similar in all litter types as the interaction P-value was > 0,05. Fatty acids decreased at the beginning of decomposition, particularly in litter from Achenkirch and Klausen-Leopoldsdorf. The only exception was litter from Schottenwald which had the highest fatty acid concentrations and remained nearly constant during decomposition. For aromatic compounds an increase over the 15-month period was found for all litter types except for Schottenwald. The relative amount of aromatics in litter from Schottenwald did not change to a great extent. After six months the concentration of aromatics remained nearly constant in all litter types. The L:C ratios increased for all litter types, most notably after six months of decomposition.

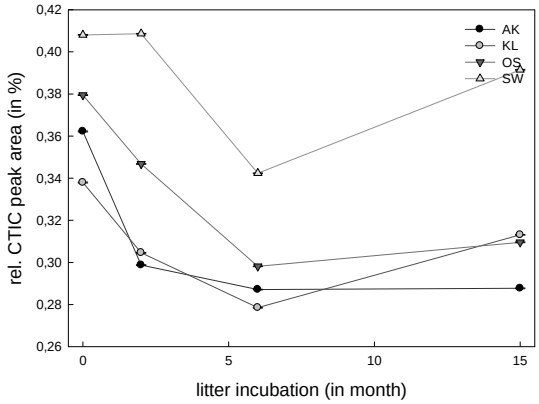
Figure 9 Courses of fatty acids during decomposition; values are means ± SE



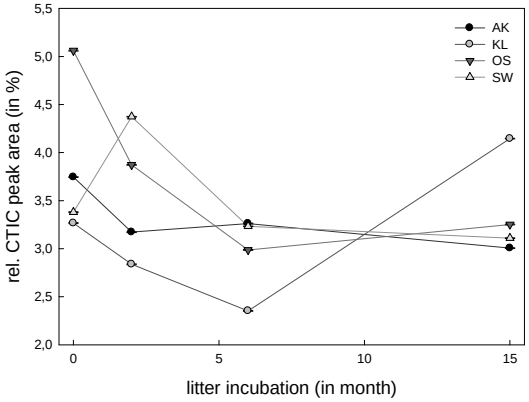
Hexadecanoic acid, methyl ester



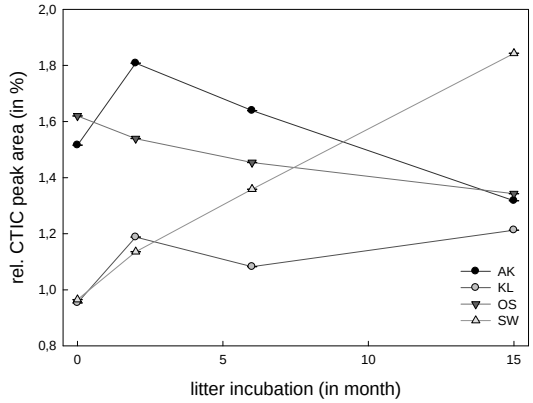
Heptadecanoic acid, methyl ester



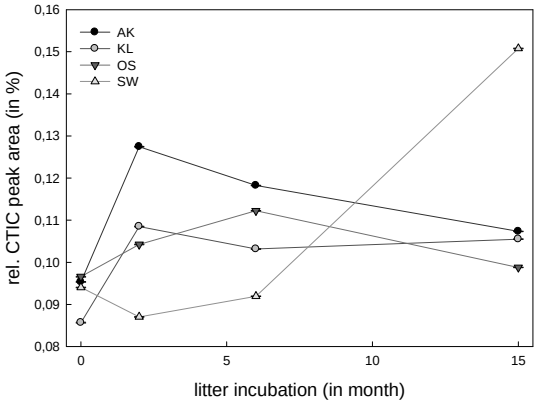
Octadecanoic acid, methyl ester



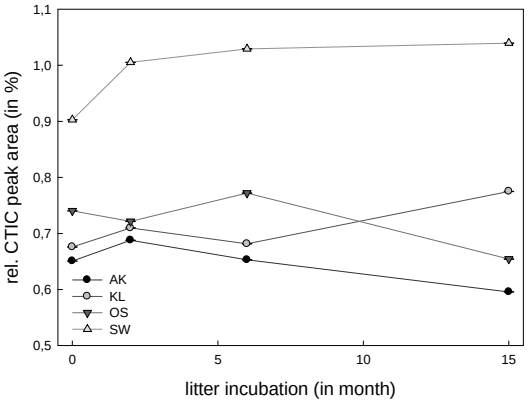
9-Octadecenoic acid (Z)-, methyl ester

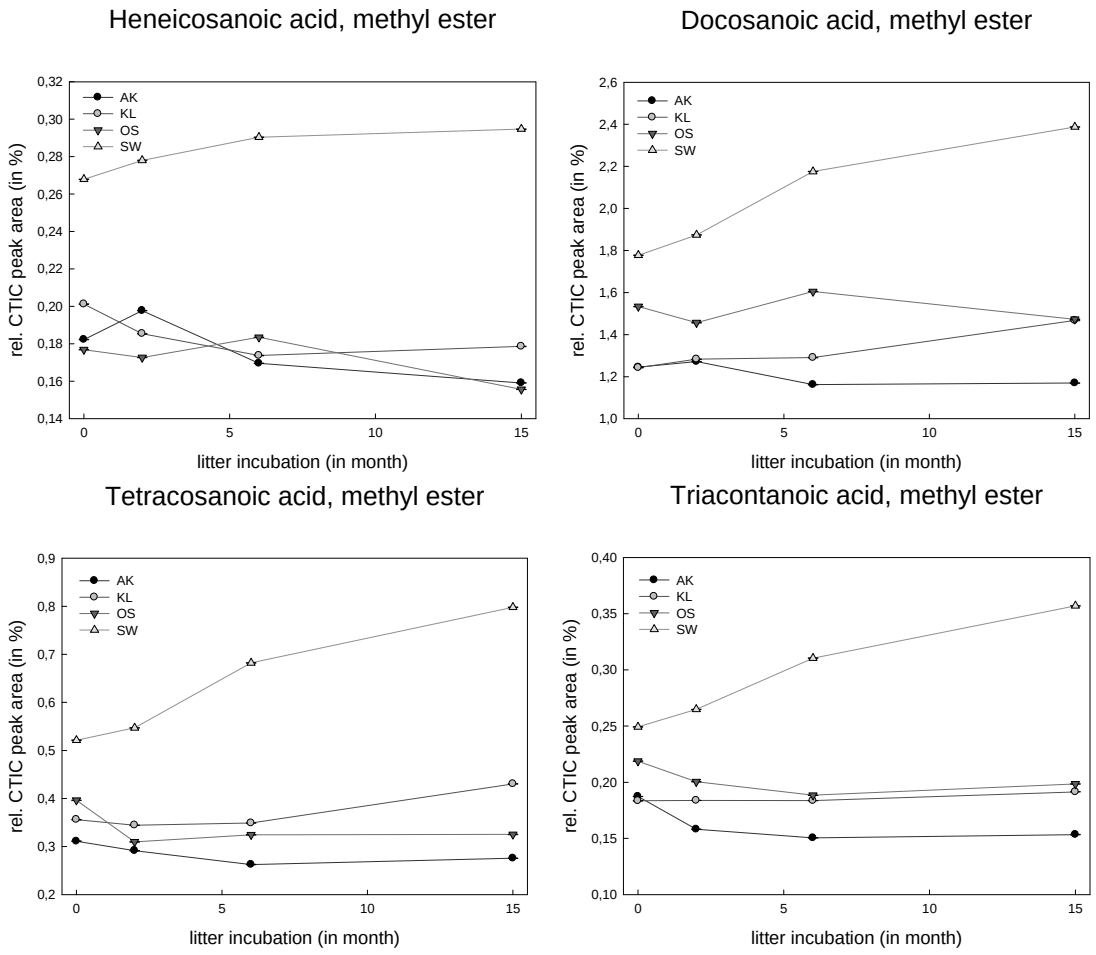


11-Octadecenoic acid, methyl ester



Eicosanoic acid, methyl ester





Moreover, time courses of single fatty acids were analyzed and are presented in Figure 9, as well as results of Multifactor ANOVA in Table 8.

Table 8 Results of two-way ANOVA to test the effect of litter type and harvest on fatty acids during beech litter decomposition.

Df	3		3		9		
fraction	**harvest effect**		**site effect**		**interaction**		**relative amount**
	F	P	F	P	F	P	**in %**
Octanoic acid, methyl ester	36,54	0,0000***	41,83	0,0000***	2,46	0,0190*	0,14 – 0,26
Nonanoic acid, methyl ester	10,90	0,0000***	25,72	0,0000***	2,49	0,0175*	0,08 – 0,14
Decanoic acid, methyl ester	63,58	0,0000***	83,62	0,0000***	3,15	0,0037**	0,03 – 0,09
Tetradecanoic acid, methyl ester	22,96	0,0000***	83,62	0,0000***	3,20	0,0033**	0,08 – 1,2
Pentadecanoic acid, methyl ester	54,89	0,0000***	53,83	0,0000***	8,64	0,0000***	0,2 – 0,5
Nonanedioic acid, dimethyl ester	93,31	0,0000***	243,71	0,0000***	5,08	0,0000***	0,9 – 2,5
Hexadecanoic acid, methyl ester	74,67	0,0000***	40,87	0,0000***	2,21	0,0338*	5 – 10
Heptadecanoic acid, methyl ester	9,82	0,0000***	17,01	0,0000***	0,80	0,6200	0,28 – 0,41
Octadecanoic acid, methyl ester	3,26	0,0277*	1,82	0,1534	2,56	0,0148*	2,3 – 5,2
9-Octadecenoic acid (Z)-, methyl ester	5,23	0,0029**	40,84	0,0000***	17,43	0,0000***	0,9 – 1,9
11-Octadecenoic acid, methyl ester	6,31	0,0009***	1,90	0,1402	6,40	0,0000***	0,08 – 0,15
Eicosanoic acid, methyl ester	1,18	0,3236	89,04	0,0000***	2,65	0,0121*	0,6 – 1,0
Heneicosanoic acid, methyl ester	0,82	0,4896	85,19	0,0000***	1,52	0,1616	0,16 – 0,3
Docosanoic acid, methyl ester	2,53	0,0662	55,55	0,0000***	2,34	0,0253*	1,2 – 2,4
Tetracosanoic acid, methyl ester	3,02	0,0368*	56,76	0,0000***	2,83	0,0079**	0,3 – 0,8
Triacontanoic acid, methyl ester	1,98	0,1266	65,25	0,0000***	3,85	0,0007***	0,15 – 0,35

Notes: Analysis includes data from all litter types; initial litter and harvests after 3, 6 and 15 month. Data are F-ratio and P-value (95% confidence level). Significance levels: *** P < 0,001; ** P < 0,01; * P < 0,05; no symbol P > 0,05

In total, fatty acids accounted for 14 to 24% of CTIC. Highest relative amounts were found for hexadecanoic acid methyl ester (C16:0, class I), octadecanoic acid methyl ester (C18:0, class III) and docosanoic acid methyl ester (C22:0, class II), which belong to the different

fatty acid classes as identified by PCA of FA. Significant effects by harvest and site were observed for almost all fatty acids.

Short-chain fatty acids (class I) decreased during litter decomposition, especially during the first two months. The relative amount of long-chain fatty acids (class II) remained almost constant during the incubation period. Only in litter from Schottenwald long-chain fatty acids slightly increased. Of the class III fatty acids, octadecanoic acid methyl ester resembles short-chain fatty acid esters. In the first six months a decrease occurred in abundance; then an increase or stable concentration was noticed. The course of 9-octadecenoic acid methyl ester (C18:1Δ9) differed between litter types. Litter from Schottenwald showed a continuous increase, whereas a decrease was observed in litter from Ossiach. Litter samples from Achenkirch and Klausen-Leopoldsdorf showed similar trends, with initial increases in the contents and after two months the fatty acid concentrations decreasing. 11-Octadecenoic acid methyl ester (C18:1Δ11) increased continuously in litter collected from Schottenwald. The other litter types showed an increase during the first two months while later on they decreased or showed a nearly consistent content of fatty acids.

6. DISCUSSION AND CONCLUSIONS

As mentioned before carbohydrates and nitrogen-containing compounds show a poor pyrolytic recovery and yield upon THM and were most probably underrepresented in the TMH products. This is a known phenomenon as aliphatic compounds are more abundant then sugar-derived substances due to structural differences and the different abilities of analytical pyrolysis and thermochemolysis in releasing GC amenable substances (Nierop 2001). Moreover, competitive pyrolytic reactions may be caused by insufficient contact between the sample and the methylation reagent (Asperger et al 2001), thus producing non-derivatized products. The discussion is therefore focused on lignins, aromatic compounds and fatty acids.

The studies by Jacob et al. (2010), Rouifed et al. (2010) and Klotzbücher et al. (2011) showed that beech produces comparatively recalcitrant litter which therefore is slowly degraded. This results conforms to the ones established in the MICDIF network with mass loss ranging between 6 and 11% after 15 months. It should also not be disregarded, that decomposition might be retarded by a lack of leaching and mechanical breakdown by larger animals and thereby diminished hydrological losses and lower available surface area which is available for microbial attack (Heim & Frey 2004). Another notable factor that influences litter decomposition is litter chemistry. Zhang et al (2008) reported that litter with high lignin concentrations and a low amount of nitrogen is generally decomposed slowly (Zhang et al 2008).

For lignin degradation no significant intraspecific differences could be observed although the litter types differed in their litter chemistry (i.e. lignin and cellulose content, C:N:P ratios and nutrient concentrations such as Mn^{2+}). Thus, in this case neither carbon quality nor nutrient concentrations had a significant effect on lignin decay. This is in clear contrast to current understanding that lignin decay is negatively related to N concentration and positively to Mn^{2+} (Prescott 2010). However, the lignin decomposition patterns found in this study conformed to the model proposed by Berg and Staaf (1980). This model states that lignin degradation is hampered in the early stages of decay due to easily available and decomposable organic matter which is preferentially used by microorganisms. Moreover, this model is supported by the litter respiration data and the increase in L:C ratios. In the first two weeks of litter incubation litter respiration was high, implying high amounts of available carbon being present as carbon source for microorganisms. The L:C ratios increased during decomposition showing that cellulose underwent faster decay than lignins. Some lignin compounds could be identified as typical lignin degradation products, e.g. 3,4-dimethoxybenzaldehyde and 3,4,5-trimethoxybenzaldehyde (Vane 2003). Both accumulated during litter decomposition, mainly during later stages of decomposition. Therefore, lignin must be degraded to some extent even

though not to an appreciable extent until later stages. This fact further supports the model by Berg and Staaf (1980).

Aromatic compounds accumulated during litter decay. Most aromatic compounds were highly correlated with lignin markers, no outlier group being found. These data therefore suggest that the aromatic compounds detected by TMH were related to lignin decay, or may be regarded as lignin biomarkers in this study. In addition, cutin releases aromatic humus precursors during decomposition but was found not as resistant to decay as it was firstly assumed (Prescott 2010). As some aromatic humus-like substances were found to accumulate during litter decomposition in this study, they may indicate cutin degradation. Some examples of aromatic compounds which may derive from cutin are benzoic acid methyl ester, 2,3-dimethoxytoluene, 1,2/1,3/1,4-dimethoxybenzene, 4-methoxybenzoic acid methyl ester and 3-(4-methoxyphenyl)-2-propenoic acid methyl ester.

The estimation of the origin of fatty acids found in this study was difficult, because they may be released by from plant lipids and waxes as well as from microbial lipids deriving from fungi and bacteria (Asperger et al 2001). Strait-chain fatty acids are common in plants as well as in microorganisms and thus are widely distributed (Zelles 1999). Plant waxes contain a wide range of high molecular components, e.g. waxy esters, fatty acids, fatty alcohols, sterols and hydrocarbons (Asperger et al 2001). Long-chain alkanoic acids (C_{20}-C_{32}) are characteristic for plant waxes whereas short-chain fatty acids (C_{14}-C_{18}) are used as biomarkers for microorganisms in soils (Otto & Simpson 2005). In particular, some fatty acids, e.g. C15:0, C17:0 and C20:0 are common in bacteria and hence used as unspecific bacterial biomarkers (Zechmeister-Boltenstern et al 2011, Zhang et al 2007). 11-Octadecenoic acid (C18:1Δ11) is characteristic for eubacteria (Zelles 1999). Only one fungi-specific fatty acid was found, namely 9-octadecenoic acid methyl ester (C18:1Δ9) according to Zechmeister-Boltenstern et al (2011) and Zhang et al (2007).

The MICDIF data showed an increase in microbial biomass, thus an increase of microbial fatty acids can also be expected. For common bacterial fatty acids, ranging from C_{15} to C_{20}, no increase was evident during the early period of decomposition. Only after six months of incubation, a slight increase of straight-chain alkanoic acids, such as C14:0, C15:0, C17:0, C18:0 and C20 was seen. The eubacterial fatty acid C18:1Δ11 showed an increase during the first two months in litter from Achenkirch, Klausen-Leopoldsdorf and Ossiach however in litter collected from Schottenwald, C18:1Δ11 accumulated not until the later stages of litter decomposition. The fungal fatty acid accumulated during the first two months in Achenkirch and Klausen-Leopoldsdorf and as decomposition proceeded, the amount decreased. For litter from Schottenwald a consistent accumulation of fungal fatty acids was found while in Ossiach a continuous decrease was observed. These results agree with the general model of litter degradation by microorganism reported in Swift et al (1979). The main decomposers of primary litter resources are fungi due to the fact, that the most widespread distribution of cellulases and lignin-degrading enzymes is found within this group of microorganisms. Bacteria are better adapted to degradation of labile organic matter and of secondary resources (Swift et al 1979). The results established in this study for lignin degradation and patterns of fatty acids are in agreement with this theory for microbial decay, where fungi are dominant in the early stages of decomposition in Schottenwald (high Mn concentration) forcing cellulose and to some extent lignin degradation and bacteria do not occur until decomposition proceeds. In comparison, bacteria were the dominating microorganisms in litter from Ossiach (low Mn concentration) thus lignins accumulated at the beginning of decomposition and lignin degradation began at later stages.

Furthermore, nonanedioic acid dimethyl ester is known to inhibit the growth of gram-positive and gram-negative bacteria (Broberg et al 2007). In consequence of the relatively high concentrations in initial litter this fatty acid may suppress bacterial growth and establishment during the early stages of decay and thus enable fungi to be the major decomposer

microorganisms. As the litter decomposition process proceeded, the concentration of nonanedioic acid dimethyl ester decreased, allowing bacteria to colonise the decaying litter. This can be seen in increasing bacterial fatty acid methyl esters after six months of incubation.

Long-chain fatty acids (> C_{20}) which mainly originate from plant waxes accumulated or remained nearly constant over the incubation period. From this follows that plant waxes, in particular wax fatty acids were recalcitrant and nearly resistant to microbial decay under the conditions applied in this study.

In conclusion, this study show that not only element stoichiometry but also C chemistry varied intra-specifically in beech litter. Litter mass loss was more closely related to litter N content (or litter C:N ratio) than to lignin content or L:C ratios. Litter elemental composition affected the decomposition of major groups of macromolecules (i.e. fatty acids, aromatic compounds; but not lignins and carbohydrates) comprising beech litter in a complex way. Due to insufficient applicability of thermochemolysis to carbohydrates and nitrogen-containing compounds, other methods such as analytical pyrolysis should be used to further investigate the impact of litter chemistry on litter decomposition and the composition of microbial communities.

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8. SUMMARY

This study on beech (*Fagus sylvatica L.*) litter decomposition was performed within the framework of the MICDIF (-"Linking microbial diversity and functions across scales and ecosystems") FWF-project. The major aim was the investigation of potential intraspecific differences in beech litter decomposition due to differences in carbon quality and litter chemistry (i.e. lignin and cellulose content, C:N:P ratios and essential elements such as Ca, K, Mg, Fe, Mn and Zn). Therefore a mesocosm experiment was carried out and beech litter collected at four different sites in Austria (Achenkirch, Klausen-Leopoldsdorf, Schottenwald and Ossiach) was incubated in a climate chamber at 15 °C for 15 months. Initial litter as well as litter samples collected after three, six and fifteen month of incubation were analysed. Litter carbon quality was analyzed by thermally assisted hydrolysis and methylation (THM) linked to pyrolysis – gas chromatography/mass spectrometry. A methanolic solution of tetramethylammonium hydroxide (TMAH) was used as alkylation reagent.

Generally, beech litter decomposition proceeded slowly; mass loss after 15 months ranged between 6 and 11%. Lignin degradation followed mainly the model for lignin decay established by Berg and Staaf (1980). Thereby lignin degradation is hampered in the early stages of decay due to easily available and decomposable organic matter, e.g. glucans such as sugars and cellulose, which are preferentially used as carbon and energy source by microorganisms. Fatty acids derived from plant waxes were not degraded during the incubation period. Moreover the analysis of fatty acid methyl esters showed significant intraspecific differences in microbial community structure during the decomposition process. Differences in microbial community structure may be linked to differences in litter chemistry and degradation patterns. On the basis of litter from Schottenwald and Ossiach this effect could be seen. Litter from Schottenwald (high N and Mn concentrations) was firstly colonized by fungi which forced cellulose and to some extent also lignin degradation. Later on, the litter was also

colonized by bacteria. In comparison, bacteria were the dominant microorganisms in litter from Ossiach (low N and Mn concentrations) during the first period, when lignin accumulated. As litter decomposition proceeded, fungal biomass increased resulting in enhanced lignin degradation. Under the given conditions, fungi therefore were the main decomposer organisms of primary resources such as cellulose and lignin and bacteria were better adapted to the degradation of secondary resources produced by fungi.

The THM method used in this study was more amenable to fatty acids, aromatic compound and lignins then to carbohydrates and nitrogen-containing compounds. Thus it would be reasonable to analyse the effects of litter chemistry on litter decomposition and microbial community structure with other methods such as analytical pyrolysis GC/MS as well.

9. ZUSAMMENFASSUNG

Im Zuge des MICDIF („Linking microbial diversity and functions across scales and ecosystems") Projektes wurde diese Studie zum Abbau von Buchenstreu durchgeführt. Das Hauptziel war, potentielle intraspezifische Unterschiede während des Abbaus von Buchenstreu aufgrund unterschiedlicher chemischer Streuzusammensetzung aufzudecken. Unterschiede in der Streuzusammensetzung betrafen dabei den Lignin- und Cellulosegehalt, die C:N:P Quotienten und essentielle Nährelemente wie Ca, K, Mg, Fe, Mn und Zn.

Dazu wurden Mesokosmos-Experimente durchgeführt. Hierzu wurde Buchenstreu unterschiedlicher Standorte (Achenkirch, Klausen-Leopoldsdorf, Ossiach und Schottenwald) gesammelt und für 15 Monate unter gleichen Bedingungen in einer Klimakammer (Temperatur: 15°C) inkubiert. Nach Inkubationszeiten von drei, sechs und fünfzehn Monaten wurden Proben entnommen und gemeinsam mit der ursprünglichen Steu untersucht. Die Analysenmethode war eine Pyrolyse-GC/MS mit vorangehender sogenannter „thermally assisted hydrolysis and methylation (THM)", d.h. einer thermisch unterstützten Hydrolysierungs- und Methylierungsreaktion. Als Methylierungsreagenz wurde eine methanolische Lösung von Tetramethylammoniumhydroxid eingesetzt.

Unter Einbeziehung von bereits erfassten MICDIF Daten konnten folgende Ergebnisse beschrieben werden. Im Allgemeinen verlief der Abbau von Buchenstreu eher langsam; der Massenverlust lag nach 15 Monaten nur zwischen sechs und elf Prozent. Der Lignin-Abbau verlief hauptsächlich nach den Mustern, die für den Abbau von Lignin von Berg und Staaf im Jahre 1980 beschrieben wurden. Diesem Modell zufolge wird der Lignin-Abbau in den frühen Phasen des Streuabbaus durch leicht verfügbare und zersetzbare organische Substanz, wie zum Beispiel Zucker und Cellulose, behindert, da diese von den Mikroorganismen bevorzugt werden. Fettsäuren, die aus pflanzlichen Wachsen stammen, konnten während der Inkubationszeit und unter den gegebenen Bedingungen nicht abgebaut werden. Die weitere Analyse

der Fettsäuremethylester ergab signifikante Unterschiede in mikrobieller Gemeinschaftsstruktur während des Streuabbaus. Diese Unterschiede konnten anhand zweier Streutypen (Schottenwald und Ossiach) gut gezeigt werden. Die Streuproben aus Schottenwald (gekennzeichnet durch hohe Stickstoff- und Mangankonzentrationen) wurden primär von Pilzen besiedelt, welche Cellulose und in geringerem Ausmaß auch Lignin abbauten. Erst nach einiger Zeit erfolgte die Besiedelung auch durch Bakterien. Im Vergleich dazu, wurden in den Proben aus Ossiach (gekennzeichnet durch niedrige Stickstoff- und Mangankonzentrationen) zu Beginn des Abbaus hauptsächlich Bakterien vorgefunden. Dies hatte zur Folge, dass Lignin angereichert wurde. Im Verlauf des Abbauprozesses nahm die pilzliche Biomasse zu und Lignin wurde verstärkt abgebaut. Unter den gegebenen Versuchsbedingungen waren Pilze die wichtigsten Zersetzer von primären Streukomponenten wie Cellulose und Lignin, wohingegen Bakterien sich als die geeigneten Zersetzer für Sekundärsubstrate erwiesen.

THM eignet sich besonders gut für die Analyse von Fettsäuren, Ligninen und Aromaten, ist aber für Kohlenhydrate und Stickstoffverbindungen eher unzureichend. Daher wäre es sinnvoll, den Einfluss der chemischen Streuzusammensetzung auf den Streuabbau und die mikrobielle Gemeinschaftsstruktur und Aktivität auch mit anderen analytischen Pyrolyse-Methoden zu untersuchen.

10. LEBENSLAUF

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Zusätzliche Kenntnisse und Fertigkeiten

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