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DIPLOMARBEIT

Titel der Diplomarbeit

Influence of litter chemistry and stoichiometry on glucan depolymerisation during decomposition of beech (*Fagus sylvatica*) litter

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General introduction

1. Decomposition and stoichiometry

1.1. Decomposition of organic material

Decomposition of organic matter is a key process of the global C-cycle. It is responsible for the mineralization of organic matter to carbon dioxide (CO_2) and for the mineralization of nutrients and thus also a key component of global N and P cycles. The major substrate for decomposition and the primary source of C and nutrients for decomposer organisms is plant litter, demonstrating the pivotal role of litter decomposition in most terrestrial ecosystems.

Decomposition is a very complex and complicated interplay of various processes and stages. It includes a variety of mechanisms that can be grouped into physical (fragmentation, leaching), chemical (oxidation, condensation) and biological ones (ingestion, digestion, extracellular enzymatic activity) (Chapin *et al.*, 2002), and can be roughly divided into three major stages (Berg & McClaugherty, 2008). In a first early stage, soluble sugars, amino acids, small aromatic compounds and cations are lost through leaching or metabolised. Secondly, beginning with the synthesis of extracellular enzymes (Sinsabaugh *et al.*, 1991), degradation of macromolecules like cellulose, hemicelluloses, starch and proteins takes place. Finally, more recalcitrant compounds like lignin are attacked. It has been stated, that organic material, especially if it is rich in recalcitrant compounds, is not completely mineralized but only to a 'threshold' level that is marked by a high concentration of lignin and other recalcitrant compounds (Berg & Meentemeyer, 2002). The residues of decomposition undergo a variety of transformation and condensation processes and are stabilized by association with soil minerals, finally forming the more or less stable fraction of soil organic matter that we know as "humus".

1.2. Biological decomposition

Depending on the ecosystem, a large proportion of dead organic matter can be degraded biologically. Soil animals are considered responsible for fragmentation of large particles, creating new surfaces for microbial colonization, and for mixing of the decomposing litter into the soil

(Chapin *et al.*, 2002). Further breakdown and chemical alteration of dead organic matter is ascribed to the activity of soil microorganisms (Chapin *et al.*, 2002). There are three crucial controls on biological decomposition (Chapin *et al.*, 2002): (i) environmental factors like aeration, moisture, temperature and soil pH, (ii) chemical composition of the litter and (iii) the soil microbial community. The relevance of these factors changes as does the course of decay (Couteaux *et al.*, 1995; Berg & McClaugherty, 2008). In the beginning, when easily degradable substances like cellulose and hemicelluloses are still available, climate and litter nutrient content especially of macronutrients like carbon (C), nitrogen (N) and phosphorus (P) play a fundamental role. In later, more humus-near stages, lignin content becomes important. Interestingly, the effect of N has been reported to be inverted during decomposition: in the beginning, a high N content usually accelerates decay because it is essential for microbial metabolism, whereas it can lead to a deceleration at later stages of decomposition (Berg & Matzner, 1997; Berg & Meentemeyer, 2002), depending on the type of litter and environmental conditions during decomposition (De Santo *et al.*, 2009). This effect sometimes arises because N can form stable condensation products with lignin and phenolic compounds that are very recalcitrant and resist microbial attack. In the early stage of decomposition, nutrients may be transported into the litter, which leads to an initial net accumulation, and immobilized by the microbial community before net release of nutrients occurs.

1.3. The concept of stoichiometry

In ecology, the concept of stoichiometry is an important tool for connecting processes mediated by organisms with nutrient supply. The basic assumption of stoichiometry is that certain biological entities on various scales like biomolecules, cells, multicellular organisms or even whole ecosystems possess a characteristic composition of the biologically most important elements (Sterner & Elser, 2002). In 1958, A. C. Redfield discovered that the elemental composition of marine organic matter is astonishingly stable with a C:N:P ratio of 106:16:1 (Redfield, 1958). A similar connection of these elements with a characteristic elemental ratio was later discovered for various terrestrial and aquatic organismic groups like terrestrial higher plants (McGroddy *et al.*, 2004) and marine algae, vertebrate and invertebrate animals as well as soil microorganisms (Makino *et al.*, 2003; Cleveland & Liptzin, 2007). Most stoichiometric studies are based on the

three elements C, N and P. Carbon is the fundament of all life on earth, all biomolecules contain C. Of all non-transition elements in the periodic table, C has the highest binding energy (Williams, 1997) and is therefore favored due to its ability to store energy. Additionally, C can form single, double and triple bonds with itself and other elements and therefore provides a great architectural flexibility (Williams, 1997). Nitrogen can assume a diversity of chemically stable redox states (NH_4^+, NH_3, N_2, N_2O, NO, NO_2^-, NO_3^-), which makes it biochemically important (Williams, 1997; Sterner & Elser, 2002). The amino group ($-NH_2$) is an integral part of the peptide bond found in every protein, be it enzymes with catalytic activity or structural polypeptides with stabilizing function. Finally, P is found wherever genetic information is stored or transcribed (DNA, RNA), as a vehicle for biochemical energy (ATP) and in biomembranes (phospholipids) (Westheimer, 1992).

For decomposition, stoichiometry can be a helpful tool because it links the involved processes, i.e. deconstruction and transformation of dead organic material, mineralization and immobilization of nutrients and processes linked to microbial metabolism like growth and respiration, directly to the chemical composition of the decomposing material (Guesewell & Gessner, 2009) and the microbial community. Stoichiometry can be expressed not only as molar C:N:P ratios of microbial biomass or plant litter, but also as ratios of activities of exoenzymes that are involved in microbial nutrient acquisition, namely cellulases (C acquisition), proteases (N acquisition) and phosphatases (P acquisition) (Sinsabaugh *et al.*, 2008; Sinsabaugh *et al.*, 2009), and as ratios of microbial processes such as C, N or P mineralization (Achat *et al.*, 2010).

2. Carbon sources during litter decomposition

2.1. Glucans in plant litter: Cellulose

2.1.1. Structure of cellulose

During decomposition, glucans play an important role because they are major sources of C. The most abundant glucans in plant litter are cellulose and starch. Cellulose has been denoted the most abundant biopolymer on earth (Perez *et al.*, 2002). It can primarily be found in plant cell

walls of terrestrial plants and marine algae (Brett & Waldron, 1996; Taiz & Zeiger, 2002), acting as major structural component and accounting for about one-third of the total plant biomass (Somerville, 2006). Cellulose is a homopolymer composed of D-glucose subunits that are linked by β-1,4 glycosidic bonds (Perez *et al.*, 2002; Taiz & Zeiger, 2002). One single cellulose molecule can contain between 500 up to 15.000 glucose monomers (Brett, 2000), forming long chains also known as cellulose fibrils. These cellulose fibrils interact through hydrogen bonds and van der Waals forces, forming densely packed crystalline regions (Nishiyama *et al.*, 2002; Nishiyama *et al.*, 2003) that are interrupted by amorphous regions which are more susceptible to enzymatic attack (Beguin & Aubert, 1994).

2.1.2. Cellulose biodegradation

One of the main obstacles for the biodegradation of cellulose is its insolubility (Perez *et al.*, 2002). Microbes cannot take up these large macromolecules and therefore degradation has to take place either exocellularly in association with the outer cell envelope layer, or extracellularly in soil solution. Bacteria and fungi produce an array of hydrolytic and oxidative enzymes that act in synergy to completely degrade cellulose and the accompanying hemicellulose and lignin (Perez *et al.*, 2002; Berg & McClaugherty, 2008).

Cellulose is degraded by a group of hydrolytic enzymes referred to as ‘cellulases’ (Perez *et al.*, 2002). Currently, there have been discovered more than 100 different families of glycosyl hydrolases which are organized into 14 classes as listed at the CAZy server (The carbohydrate-active enzymes database, www.cazy.org). In contrast to other hydrolytic enzymes, cellulases are able to hydrolyze the β-1,4 glycosidic bonds between glucosyl residues by an acid catalysis, requiring a proton donor and a nucleophile or base. There are two main different strategies of enzymatic cellulose degradation: 1) aerobic breakdown by extracellular enzymes, and 2) anaerobic digestion by cellulosomes.

1) Aerobic bacteria and fungi like *Trichoderma reesei* and *Phanerochaete chrysosporium* degrade cellulose extracellularly in three steps mediated by a cluster of different extracellular enzymes (Figure 1) (Perez *et al.*, 2002; Berg & McClaugherty, 2008). First, endoglucanases (endo-1,4-β-glucanases) hydrolyze internal bonds, preferably in amorphous regions of cellulose,

producing new terminal ends of cellulose chains. Secondly, exoglucanases (exo-1,4-β-glucanases or cellobiohydrolases) act on existing or endoglucanase-derived cellulose chain ends, breaking up crystalline and amorphous cellulose, releasing cellobiose molecules. Finally, β-glucosidases break down soluble cellobiose molecules releasing two glucose molecules. Nearly all of these extracellular cellulolytic enzymes contain two sites that mediate binding to the substrate (Durrant *et al.*, 1991; Ramalingam *et al.*, 1992): the active site of the catalytic domain and a non-catalytic carbohydrate binding module that is essential for effective degradation of non-soluble crystalline cellulose (Lee & Brown, 1997). An overview of this breakdown mechanism is given in Figure 1.

2) Some anaerobic microorganisms like *Clostridium thermocellum* possess a multi-enzyme complex called the cellulosome that attaches both to the microbial cell envelope and to the substrate (Schwarz, 2001). In this complex, the cellulolytic enzymes are tightly bound to each other via a non-catalytic peptide called the scaffolding protein which also mediates the binding of the cellulosome to the substrate and the microbial cell envelope (Boisset *et al.*, 1999). The close neighborhood between cell and substrate minimizes diffusion losses of hydrolytic products and increases the effectiveness of this cellulolytic strategy. Nevertheless, only 5-10% of total cellulose degradation occurs anaerobically (Schwarz, 2001).

2.2. Glucans in plant litter II: Starch

2.2.1. Structure of starch

A large quantity of glucose in plant cells is found in the polymeric form of starch, an osmotically inactive storage homopolysaccharide that is synthesized during photosynthesis (Taiz & Zeiger, 2002). It consists of two different molecules: the linear amylose whose glucose units are linked by α-1,4-glucosidic bonds, and the branched amylopectin that has additional α-1,6-glucosidic bonds. Starch is stored mainly in form of semi-crystalline starch granules in chloroplasts or plastids (Smith, 2001; Taiz & Zeiger, 2002) and can be found in large quantities in twigs of trees near the buds at the end of the growing season, and in various kinds of fruits, seeds and rhizomes, but can be produced by most plant cells.

2.2.2. Starch biodegradation

Starch is degraded by an array of hydrolytic enzymes capable of cleaving α-1,4-glycosidic bonds that are referred to as amylases (Smith *et al.*, 2005). The first enzymes to attack the starch granules are the α-amylases (1,4-α-D-glucan glucanohydrolases, glycogenases), a group capable of hydrolyzing α-1,4-glucosidic bonds at random locations along the starch chain releasing maltose and maltotriose from amylose, and maltose, glucose and "limit dextrin[1]" from amylopectin. Because α-amylases can act anywhere on the substrate, they are faster than β-amylases (1,4-α-D-glucan malthohydrolases, glycogenases, saccharogen amylases), which can only attack the α-1,4 linkages from the non-reducing ends of starch molecules producing maltose units. γ-amylases (glucan 1,4-α-glucosidases, amyloglucosidases, glucoamylases) hydrolyze the last α-1,4-glycosidic linkage at the non-reducing end of amylose and amylopectin yielding glucose. In addition, γ-amylases are capable of cleaving α-1,6-glycosidic linkages. Amylases are produced by animals (e.g. human saliva, pancreas), plants (leaves, germinating seeds) and microorganisms.

2.3. Hemicelluloses and lignin

Cellulose micro fibrils are covered with hemicelluloses and lignin forming the matrix of the plant cell wall (Perez *et al.*, 2002). Hemicelluloses are heteropolymers consisting of different sugars, mainly D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, D-galacturonic and D-glucuronic acid (Taiz & Zeiger, 2002). The sugars and sugar acids are connected by β-1,4 and β-1,3 glycosidic bonds, forming shorter chains than cellulose with numerous lateral branches. Due to their arborescent form, hemicelluloses do not aggregate like cellulose, but have a more amorphous structure that makes them easier to degrade (Perez *et al.*, 2002). In contrast to starch, cellulose and hemicelluloses, which are all polysaccharides, lignin is a highly condensed heteropolymer consisting of aromatic phenylpropane units (Taiz & Zeiger, 2002). The basic building blocks are three phenyl propionic alcohols, coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (p-hydroxyphenyl propanol) and sinapyl alcohol (syringyl propanol), which are

[1] Dextrins are mixtures of short polymers of D-glucose units linked by α-1,4- or α-1,6-glycosidic bonds that are released during starch degradation. Limit dextrins are short chained branched products of amylopectin cleavage by α- or β-amylases which cannot hydrolyse the α-1,6- bonds at branching points.

linked together during lignin biosynthesis by the generation of the free radicals of these three alcohols. Because of its heterogeneous, highly condensed structure, lignin is very difficult to degrade (Berg & McClaugherty, 2008). There are two major enzyme groups involved in lignin decomposition (Kirk, 1984): (i) peroxidases, also known as ligninases or lignin peroxidases, and (ii) phenoloxidases, also known as polyphenol oxidases or laccases. (i) Using H_2O_2 as oxidant, peroxidases oxidize aromatic nuclei by removing one electron, forming unstable cation radicals that then undergo a variety of non-enzymatic reactions producing multiple intermediate degradation products (Kirk & Farrell, 1987). A special group of peroxidases are Manganese(Mn)-peroxidases that in addition to H_2O_2 also require Mn (Perez & Jeffries, 1992). These enzymes oxidate Mn(II) to Mn(III), which in turn oxidizes the organic substrate. Peroxidases are used by soil microorganisms because they are able to degrade molecules without a precisely repeated structure. (ii) Phenoloxidases catalyze the one-electron oxidation of phenols to phenoxy radicals, leading to C_α-oxidation, demethoxylation and aryl-C_α cleavage (Kirk & Farrell, 1987). Similar to peroxidases, the radical-mediated lignin degradation by phenoloxidases also produces a variety of phenolic condensation products.

Lignin, cellulose and hemicelluloses in plant litter are often referred to as “lignocellulose” because they are tightly associated with each other and therefore must be degraded in a coordinated way (Perez *et al.*, 2002; Berg & McClaugherty, 2008). They are complex high molecular weight heteropolymers, which are considered highly recalcitrant substrates for microbial breakdown during biodegradation, and their degradation demands multi-component enzyme systems that are produced not by single organisms but by an interacting interdependent decomposer community (Sinsabaugh *et al.*, 1991; Dilly *et al.*, 2001).

3. A method to study glucan decomposition

3.1. Isotope pool dilution

The isotope pool dilution (IPD) assay is a common method to determine the gross transformation rates of the inorganic macronutrients N and P in soil, i.e. N mineralization, nitrification, immobilization of NO_3^- and NH_4^+ and P_i transformation (Di *et al.*, 2000; Murphy *et al.*,

2003). Nevertheless, it has not yet been used to measure gross rates of depolymerization of structural and storage polysaccharides. We developed a new pool dilution assay by using ^{13}C labelled glucose to assess depolymerization of cellulose and starch.

Soil transformation processes are often defined as input and output fluxes that influence pools of available nutrients. For example, N mineralization provides an input into the inorganic NH_4^+ pool by breaking down larger organic compounds that contain N, whereas microbial or plant uptake or immobilization of NH_4^+ represents its output. Similarly, nitrification releases NO_3^- into the soil whereas NO_3^- uptake removes it from the available nutrient pool. A simple measurement of the changes of the NH_4^+ or NO_3^- pool size will only reflect net transformation rates, but will not allow quantification of the involved gross processes. Therefore, in 1954 Kirkham and Bartholomew published a method that uses tracer experiments to measure the gross rates of soil nutrient mineralization and immobilization (Kirkham & Bartholomew, 1954). In the IPD technique, a mineral nutrient pool (e.g. NH_4^+ or NO_3^-) is labelled with a tracer, for example with a highly enriched $^{15}NH_4^+$ or $^{15}NO_3^-$ salt, and the dilution of the tracer is then measured. The dilution occurs because the input process continues to release unlabelled nutrient into the labelled pool, while the output process takes out both labelled and unlabelled nutrient, therefore reducing the amount of tracer in the pool. The involved processes are defined as the fluxes F_{input} and F_{output} and the following equations (1) and (2) can be used to determine them (Di *et al.*, 2000):

(1) $$F_{input} = \frac{(Q_1 - Q_2) * \ln(\frac{A_1}{A_2})}{(t_2 - t_1) * \ln(\frac{Q_1}{Q_2})}$$

(2) $$F_{output} = F_{input} - \frac{Q_2 - Q_1}{t_2 - t_1}$$

where Q_1 and Q_2 are the amount of tracer plus tracee (e.g. labelled and unlabelled NH_4^+ in the soil NH_4^+ pool), and A_1 and A_2 are the tracer excess abundances if stable isotopes are used, or specific activities if radioactive tracers are used. All variables are measured at two time points, t_1 and t_2, after the labelling of the mineral nutrient pool. The tracer excess abundance is the amount of heavy isotope in atom% corrected for the level of natural abundance of heavy isotope

that is always present at background level. To get correct estimates, the following assumptions need to be fulfilled: (i) the influx and efflux rates must be constant over the period for which they are calculated, (ii) the amount of added tracer must kept as small as possible to prevent disturbance of the target processes, (iii) the tracer has to be distributed homogeneously in the target pool, and (iv) backflux of immobilized tracer (e.g., re-mineralization of assimilated $^{15}N\text{-}NH_4^+$) must not occur.

3.2. Adaption of the isotope pool dilution method to study glucan decomposition

To assess glucan decomposition (Figure 3), we transformed the abovementioned equations (1) and (2) for calculating the influx into and efflux from the soluble glucose pool to compute gross glucan depolymerization (GGD, equation 3) and gross glucose immobilization (GGI, equation 4, both given in µg C g^{-1} d.w. d^{-1})

(3) $$GGD = \frac{C_{t2} - C_{t1}}{t_2 - t_1} * 60 * 24 * \frac{\ln(APE_{t1}/APE_{t2})}{\ln(C_{t2}/C_{t1})}$$

(4) $$GGI = \frac{C_{t1} - C_{t2}}{t_2 - t_1} * 60 * 24 * \left(1 + \frac{\ln\left(APE_{t2}/APE_{t1}\right)}{\ln\left(C_{t2}/C_{t1}\right)}\right)$$

(5) $$APE = \text{atom\%}^{13}C_{sample} - \text{atom\%}^{13}C_{background}$$

(6) $$\delta^{13}C = \left(\frac{R_{sample}}{R_{V-PDB}} - 1\right) * 1000$$

(7) $$\text{atom\%} = \frac{R_{V-PDB} * (\delta^{13}C_{sample}/1000 + 1)}{1 + R_{V-PDB} * (\delta^{13}C_{sample}/1000 + 1)} * 100$$

where C_{t1} and C_{t2} are the amount of carbon (µg Glc-C g^{-1} d.w.) in the glucose, t_1 and t_2 are the times of stopping the pool dilution assay (30 and 120 minutes) and APE_{t1} and APE_{t2} are the values of ^{13}C atom percent excess (%) of glucose as shown in equation (5), giving the amount of ^{13}C (atom%) in the labelled sample corrected for the background natural ^{13}C abundance (about 1.08 atom%). The amount of ^{13}C is usually expressed as δ notation (‰, Equation 6) where R_{sample} and $R_{V\text{-}PDB}$ are the ratios of ^{13}C:^{12}C of the sample and the international standard V-PDB (Vienna

Pee Dee Belemnite), respectively, with $R_{V\text{-}PDB}$ being 0.0112372. Equation (7) was used to calculate atom% from the measured $\delta^{13}C$ values of the sample.

4. Figures

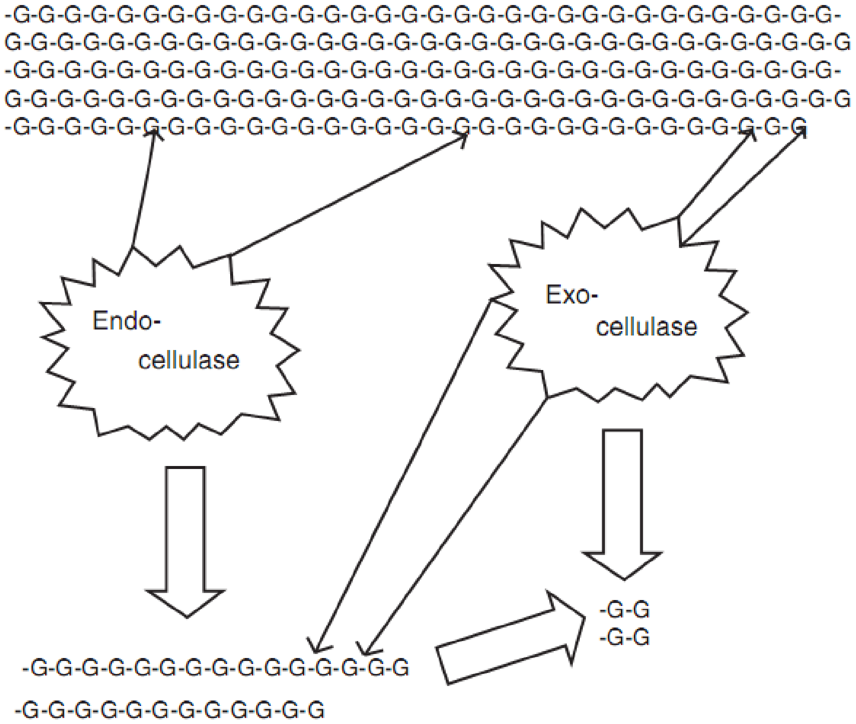

Figure 1: Part of the cellulose fibre is attacked by an endocellulase (endo-1,4-β-glucanase) breaking the chains and splitting off oligosaccharides in a random manner, including soluble shorter chains with a few glucose units. An exocellulase (exo-1,4-β-glucanase) splits off cellobiose units from the non-reducing end of the carbohydrate chains. *G* glucose unit. From Berg & McClaugherty (2008)

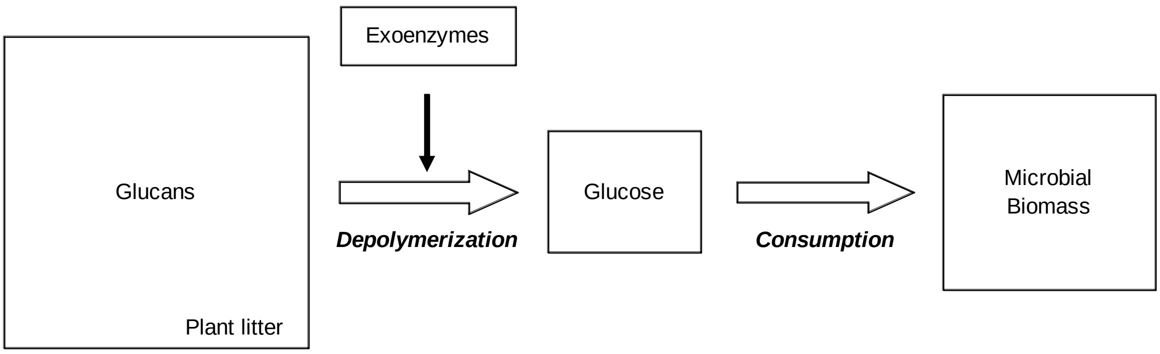

Figure 2: Conceptual model of the main processes involved in glucan degradation. Squares represent pools, white arrows represent mass fluxes and black arrows represent controls on fluxes. The size of the squares and fluxes is schematic and does not correlate with actual quantities.

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Manuscript

Influence of litter chemistry and stoichiometry on glucan depolymerisation during decomposition of beech (*Fagus sylvatica* L.) litter

Abstract

The influence of litter stoichiometry and chemical composition on glucan decomposition was investigated by a laboratory decomposition experiment. Leaf litter of beech (*Fagus sylvatica* L.) differing in litter stoichiometry and chemistry was sterilized, re-inoculated with a beech forest soil suspension (organic horizon) to obtain an identical initial microbial community, and incubated in mesocosms for six months at 15°C. Samples were taken two weeks and three and six months after inoculation and litter C:N:P ratios, starch, cellulose and lignin content, cellulase, peroxidase and phenoloxidase activities and respiration were measured. In addition gross rates of glucan depolymerisation into glucose and subsequent glucose consumption by the microbial community were estimated by a newly developed isotope pool dilution technique. Gross rates of glucan depolymerisation and glucose consumption were highly correlated, indicating that both processes are co-regulated and intrinsically linked by the microbial demand for C and energy and the consequential resource allocation to enzymes that depolymerize glucans. Initially glucan depolymerisation rates were correlated with starch content, indicating that starch was the primary source for glucose in early stages of decomposition. Subsequently, the correlation with starch diminished and glucan depolymerisation rates were correlated with cellulase activities three and six months after inoculation, suggesting that starch depolymerisation was no longer predominant and that cellulose was the primary substrate for glucan depolymerisation at this stage of decomposition. Litter stoichiometry did not affect glucan depolymerization or glucose consumption rates early in decomposition. At later stages of decomposition, however, we found significantly negative relationships between glucan depolymerisation and litter C:N and lignin:N ratio and a positive relationship between glucan depolymerisation and litter N content. We found no correlation between respiration and glucose consumption rates, indicating that glucose was not the primary substrate for respiration. Litter C:N and C:P ratios were negatively related to cellulase,

peroxidase and phenoloxidase activities three and six months after incubation, further corroborating the importance of N and P for glucan depolymerization. Taken together our study was able to demonstrate, for the first time, the importance of litter nutrient content and chemical composition for the process of glucan depolymerisation during litter decomposition.

Introduction

Plant litter is the primary input of C into the soil, with up to 90% of global terrestrial plant production entering the soil dead organic matter pool (Cebrian, 1999), and constitutes the trophic base for detritus food webs, which in turn represent a major driver of global C and nutrient cycles (Chapin *et al.*, 2002). Many studies have therefore attempted to elucidate the processes and controls of litter decomposition and soil formation (Prescott, 2010).

Plant litter compounds can be divided into two major classes regarding their degradability (Berg & McClaugherty, 2008): (i) recalcitrant substances like lignin and aliphatic substances (e.g., suberin, cutin) that are considered highly resistant to decomposition and the primary source of stable soil organic matter (SOM) (Berg & Meentemeyer, 2002) that is degraded slowly in time spans from decades to millennia (Prescott, 2010), and (ii) more labile compounds such as polysaccharides (e.g., cellulose, starch) that are more easily degraded within weeks to years and constitute an important short term C source for the microbial community. The relative amounts of easily degradable versus recalcitrant compounds have previously been reported to substantially influence the rate of litter decomposition (Berg & Agren, 1984; Aber *et al.*, 1990; Couteaux *et al.*, 1995; Fioretto *et al.*, 2005; Austin & Ballare, 2010).

A major step of litter decomposition is accomplished by extracellular enzymes (Sinsabaugh, 1994; Sinsabaugh & Moorhead, 1994) which are produced and excreted by microbes to depolymerize macromolecules into smaller, soluble substrates that can be taken up by microorganisms. The production of extracellular enzymes is regulated by environmental nutrient availability and reflects the demand of the microbial community for nutrients and energy (Sinsabaugh *et al.*, 2009). Because of the structural complexity of plant litter, a set of different extracellular enzymes is required for its breakdown. It is assumed that one single microorganism is not able to synthesize all enzymes necessary for litter decomposition, but that several groups of microorganisms produce different enzymes that work together in a synergistic way (Perez *et al.*, 2002; Berg & McClaugherty, 2008). Therefore, the composition of the saprotrophic microbial community and its enzymatic apparatus is thought to be fundamentally influenced by the chemical composition of litter (Cox *et al.*, 2001; Lucas *et al.*, 2007). A change in enzymatic activity in turn

influences the relative quantities of chemical compounds in the remaining litter and the release of nutrients from litter (Sinsabaugh *et al.*, 2002; Sinsabaugh *et al.*, 2005).

In addition to chemical composition of the litter, its elemental stoichiometry (i.e. C:N, C:P and N:P ratios) constitutes an important control of decomposition. The concept of stoichiometry has been widely used to link processes involved in litter decomposition (i.e. depolymerisation of macromolecules, mineralization and immobilization of nutrients) to the quality of the substrate and the composition of the microbial decomposer community (Guesewell & Gessner, 2009; Achat *et al.*, 2010; Keiblinger *et al.*, 2010; Manzoni *et al.*, 2010). Many dominant saprotrophic microorganisms are heterotrophs (Tezuka, 1990), which are considered homeostatic within a relatively narrow range of biomass C:N, C:P and N:P ratios (Sterner & Elser, 2002). Observed changes in microbial stoichiometry are usually ascribed to shifts in the decomposer community structure (Sterner & Elser, 2002; Makino *et al.*, 2003).

A major fraction of litter C occurs in form of polysaccharides like cellulose and starch, which can make up about half of the plant biomass. Both substances are homopolymers that are composed entirely of D-glucose subunits. Cellulose is the major structural component of the plant cell wall (Brett & Waldron, 1996), which can be found in nearly all plant tissues, and has therefore been denoted the most abundant biopolymer on earth (Perez *et al.*, 2002). Starch is an osmotically inactive storage molecule that is synthesized during photosynthesis and stored in form of starch granules in chloroplasts. The enzymatic depolymerisation of glucans by extracellular enzymes is considered to be the rate-limiting step in glucan decomposition (Perez *et al.*, 2002). Up to now, decomposition rates of cellulose and starch in plant litter have only been examined by observation of long-term changes in the respective pool size compared to absolute litter mass loss, disregarding actual decomposition rates. We therefore developed a new method based on the isotope pool dilution (IPD) technique (Kirkham & Bartholomew, 1954; Di *et al.*, 2000), using ^{13}C labelled glucose as a tracer to estimate gross rates of glucose production during decomposition, which we consider to derive mainly from enzymatic glucan depolymerisation.

The goal of the present study was to elucidate the controls of litter chemistry and stoichiometry on glucan decomposition. We hypothesized, (i) that glucan depolymerisation is controlled by litter stoichiometry, i.e., rates of glucan depolymerisation to be negatively correlated

with litter C:N ratio due to increased resource allocation to C-acquiring enzymes such as cellulases and amylases with increasing litter N availability, and (ii) that starch, as the more labile compound, will be degraded in early decomposition stages, while decomposition of lignocellulose will start later during decomposition.

To examine the controls of litter chemistry and stoichiometry on glucan depolymerisation, we conducted a short-term laboratory incubation experiment under controlled conditions using beech (*Fagus sylvatica* L.) litter of varying elemental stoichiometry (C:N:P) and chemical composition that had been sterilized and re-inoculated with a beech forest soil inoculum (organic horizon) to obtain an equal initial microbial community for each litter type and incubated for six months in mesocosms in a climate chamber (Wanek *et al.*, 2010).

Material and methods

Litter decomposition experiment

A litter decomposition experiment was carried out over six months to explore the influence of litter quality on glucan decomposition. Undecomposed beech litter (*Fagus sylvatica*, L.) with different litter chemistry and elemental stoichiometry (referred to as 'litter type') was collected in October 2008 from four sampling sites in Austria: Klausenleopoldsdorf (K), Achenkirch (A), Ossiach (O) and Schottenwald (S). Site characteristics were described by Wanek et al. (2010). The leaves did not show any visible signs of decomposition or fungal colonization. They were dried at 40°C for 48h, shred to pieces between 1 - 20 mm and sterilized twice with gamma rays of 35 kGy with one week between irradiation events. Then all litter types were inoculated with a suspension of an O-horizon:litter mixture (1:1 (w:w)) collected from Klausenleopoldsdorf in December 2007 to obtain an identical initial microbial community structure on all four litter types. Thereafter, we prepared mesocosms (n = 5) containing 60 g of litter fresh weight for all four litter types (K, A, O and S) and three harvest time points (two weeks, three and six months after inoculation), making up 60 mesocosms in total. The mesocosms had been prepared from PVC plastic tubing of 10 cm length and 12.5 cm diameter and had both ends covered with a removable plastic grid. Additionally, the top opening was sealed loosely with laboratory film to prevent desiccation. They were placed on humid sponge cloth in a climate chamber at 15°C and watered weekly with autoclaved tap water to keep the water content stable at 60% fresh weight. Additional mesocosms were prepared for each harvest and litter type to determine the size of the soluble glucose pool one week before the actual harvests. The litter from each mesocosm was homogenized before analysis.

Isotope pool dilution assay

To determine rates of gross glucan depolymerisation and gross glucose immobilisation, a new isotope pool dilution (IPD) assay was developed. IPD is a common method to determine the gross transformation rates of N, P and S in soil (Di *et al.*, 2000) by labeling a target pool and subsequently measuring the dilution of the label and the change in pool size. We adapted this

method using ^{13}C glucose (99 atom% $^{13}C_6$ D-glucose, Isotec Inc.) as a tracer. For the assay, 1.5 g of litter fresh weight were filled into 50 ml HDPE centrifuge tubes in duplicates. To start the assay, a maximum of 50% of the free glucose pool in the sample was added in form of ^{13}C-labelled glucose dissolved in high purity water (MilliQ, >18.2 MOhm, Millipore) (5 ml label with concentrations ranging from 0.01 to 5 mg ^{13}C-Glc l^{-1}). The vials were shaken vigorously to distribute the label homogeneously. The amount of liquid added via the tracer solution was adjusted to form a thin water film on the leaf particles that assured a homogeneous tracer distribution without causing anoxic conditions. After shaking the vials, they were re-opened, sealed loosely with cotton wool to enable gas exchange and then incubated at 15°C for 30 and 120 minutes, respectively. To stop the pool dilution assay, samples were extracted with 30 ml of MilliQ water at room temperature on a laboratory shaker for 15 min and centrifuged for 5 minutes at 10.000 rpm. The supernatant was then decanted into 30 ml syringes that had a plug of cotton wool on the bottom to prevent blockage of the luer taper and subsequently filtered over a carbohydrate-free glass microfiber filter (GF/C, Whatman) inside a filter device (Swinnex, Millipore). The procedure of filtration was considered to be sufficient for stopping the assay because cellulases bind firmly onto cellulose fibres and are removed by filtration, preventing ongoing degradation (data not shown).

Isolation of glucose from litter

Immediately after filtration, the solution was applied to coupled cation and anion exchange cartridges (OnGuard II H, volume 1 cc, H^+ form, on top of OnGuard II A, volume 1 cc, bicarbonate form; both from Dionex) which had been soaked by flushing with 10 ml of MilliQ water for two hours prior to sample application. After the sample solution had passed through the ion exchange cartridges, they were flushed with 5 ml of MilliQ water to obtain the solution that was still in the cartridges. The flow-through containing the sample solution plus the flushing water was collected and transferred into 250 ml vacuum proof round bottom flasks, frozen at -20°C and freeze-dried for 24 h. The residue was dissolved in 3 ml of MilliQ water, transferred into 20 ml HDPE vials, frozen again and freeze-dried over night. The dried extract was dissolved in 0.5-1 ml MilliQ water and stored frozen until analysis.

Isotopic analysis

The amount and $\delta^{13}C$ value of glucose in the samples was measured via compound specific isotope analysis on a high performance liquid chromatography-isotope ratio mass spectrometer (HPLC-IRMS) system as described by Wild et al. (2010). The HPLC system consisted of an ICS-3000 pump, an AS50 autosampler with a 25 µl injection loop and an Ultimate 3000 column compartment (all provided by Dionex). The separation column was a HyperREZ XP Carbohydrate Ca^{2+} 8 µm column (Thermo Fisher Scientific, USA), run at 85°C with 0.5 ml min^{-1} MilliQ water as eluent. The HPLC was connected to the IRMS via a Finnigan LC IsoLink Interface (Thermo Fischer Scientific, USA), where the glucose was oxidized to CO_2 in by acid persulfate digestion inside an oxidation reactor at 99.9°C. As oxidant, a 0.5 M solution of sodium persulfate (sodium peroxodisulfate purum p.a., ≥99%, Fluka, Sigma-Aldrich), and 1.7 M phosphoric acid (orthophosphoric acid puriss. p.a., crystallized, ≥99%, Fluka, Sigma-Aldrich) were used. Both reagents were prepared freshly every week and degassed in an ultrasonic bath by applying vacuum with a membrane pump. Flow rates of both reactant pumps were set to 50 µl min^{-1} and the system was left for at least one hour of equilibration.

Coming from the HPLC, the mobile phase entered the IsoLink interface via a six-port valve. Oxidant and acid were mixed (1:1) and added to the sample stream inside the oxidation reactor where the glucose was oxidized to CO_2. In a separation unit, the CO_2 was transferred over membranes to a counter flow of helium as carrier gas. Afterwards, the gas stream was dried over Nafion tubes. Before entering the IRMS, excess oxygen was removed as described by Hettman et al. (2007) inside a reduction reactor to improve both filament lifetime and reproducibility of the analysis. Subsequently, the sample stream entered a Finnigan Delta V Advantage Mass Spectrometer (Thermo Fisher Scientific, USA) by an open split for stable isotope analysis.

Spiking

The limit of isotope quantification for glucose on the HPLC-IsoLink-IRMS system in use lies at about 20 mg Glc l^{-1}. Samples with glucose concentrations below this limit had to be measured through spiking of the samples. A standard stock solution with a concentration of 10 g l^{-1} D-glucose (Merck, Vienna, Austria) in MilliQ water was prepared and a working solution was

prepared freshly every time by diluting the stock solution 1:10. Then, 10 µl of the working solution were pipetted into a 250 µl glass insert for GC vials and 90 µl sample were added. The concentration of the standard in the spiked sample now constituted 100 mg Glc l^{-1}. The $\delta^{13}C$ of the spiked sample was then measured on the HPLC-IRMS system. Additionally, the glucose concentration of samples was determined on a high performance liquid chromatography-pulsed amperometric detection system (HPLC-PAD), which has a lower detection limit than the HPLC-IRMS system (0.024 mg l^{-1}). The HPLC-PAD system consisted of an ICS3000 SP-1 Pump, an AS50 Autosampler with a 10 µl injection loop and an ICS3000 DC-2 Detector/Chromatography Module (all provided by Dionex, Vienna, Austria). As separation column a CarboPac PA20 (3 x 150 mm Analytical Column with a CarboPac PA20, 3 x 30 mm Guard Column, Dionex) was run with 0.5 ml min^{-1} 20 mM NaOH as eluent. The $\delta^{13}C$ value of the glucose in the sample could then be determined using an isotopic mixing model (2):

$$\delta_{sample} = \frac{c_{all} * \delta_{all} - c_{spike} * \delta_{spike}}{c_{sample}} \quad (2)$$

$$c_{all} = c_{sample} + c_{spike} \quad (3)$$

where c_{sample} is the concentration of glucose in the sample as measured by the HPLC-PAD system. For calibration, the glucose stock solution was used in concentrations between 0.1 and 50 mg l^{-1}. c_{spike} is the concentration of the glucose standard, which was 100 mg l^{-1}, and c_{all} is the calculated concentration of the spiked sample as shown in equation (3). δ_{spike} and δ_{all} were measured on the HPLC-IRMS system. As standards for calibration on the HPLC-IRMS, the glucose stock solution was used in concentration of 100 and 150 mg l^{-1} and injected at least eight times each, four times at the beginning and four times at the end of the measurement, with additional injections of the 100 mg l^{-1} standard every 15 samples. For δ_{spike}, the mean value of the 100 mg l^{-1} standard was taken. Equation (2) was then used to calculate the δ_{sample}.

Potential enzyme activity

Data for extracellular enzyme activities were provided by Ieda Nunez-Hämmerle. The potential activities of β-1,4-cellobiosidase (‘cellulase’), peroxidase and phenoloxidase were

estimated using standard microplate fluorimetric (Marx *et al.*, 2001) and photometric (Sinsabaugh *et al.*, 1999) assays with small modifications as described by Kaiser et al. (2010). Aliquots of fresh leaf litter (2 g) were suspended in 100 ml sodium acetate buffer (100 mM, pH 5.5) and homogenized with an ultrasonicator for two minutes at low intensity. For cellulase activity, 50 µl of substrate (4-methylumbelliferyl-β-D-cellobioside, 0.5 mM in 100 mM sodium acetate buffer) were incubated with 200 µl of leaf litter suspensions in triplicates. For calibration, a quenched standard with 200 µl of any litter-in-buffer solution and 50 µl of the pure MUF-substrate-in-buffer in concentrations between 0 and 1750 pmol were used. As controls, 200 µl of sodium acetate buffer and 50 µl of MUF-substrate-in-buffer were used in triplicates. The microplates were incubated at room temperature for 140 min. The assay was stopped by adding 10 µl 1 M NaOH to each well (samples, controls and standards). Fluorescence was measured with a fluorescence spectrophotometer (Tecan Infinite M200 fluorimeter, Werfen, Austria) at 450 nm emission and 365 nm extinction. Peroxidase and phenoloxidase activity were measured photometrically using L-3,4-dihydroxphenylalanin (L-DOPA, 20 mM) as substrate. Equal amounts of substrate and litter-in-buffer solution of the samples (see above) were mixed, shaken for 10 min and centrifuged. Then aliquots were pipetted into microtiter plates (six analytical replicates). Additionally, 10 µl of a 0.3% H_2O_2 solution were added to half of the wells for peroxidase measurement. Absorption was measured at 450 nm at the starting point and after 20 h. Enzyme activity was calculated from the difference in absorption between the two time points divided by the molar extinction coefficient, which had been previously determined.

Litter and microbial stoichiometry, respiration and litter chemistry

Litter and microbial elemental contents (C, N, P) and litter respiration were measured by K. Keiblinger and S. Zechmeister-Boltenstern at the Federal Forest Office, Vienna (BFW). Ratios of litter and microbial C:N, C:P and N:P as well as litter Cellulose:N and lignin:N were calculated on a mass basis. $C:N_{imbalance}$ as a measure of the imbalance between microbial and litter stoichiometry was calculated as follows:

(4) $$C:N_{imbalance} = \frac{C:N_{litter}}{C:N_{microbes}}$$

Starch content was determined following the method described in Göttlicher et al. (2006). Aliquots of finely ground plant material (20 mg, dried at 60°C) were repeatedly extracted with ethanolic solutions (1 ml of 50% ethanol at room temperature, 80% ethanol at 60°C and 96% ethanol at 60°C) to remove soluble carbohydrates. The remaining pellet was dried *in vacuo* and incubated at 85°C for 30 min with a solution of heat-stable α-amylase (Sigma-Aldrich, Vienna, Austria) from *Bacillus licheniformis* (500 U ml^{-1} MilliQ water) to hydrolyze starch. After cooling and centrifugation, 100 µl of the supernatant were further incubated for 30 min at 55°C with amyloglucosidase (Roche Diagnostics, Vienna, Austria) from *Aspergillus niger* (10 U in 0.5 ml 20 mM sodium acetate buffer, pH 4.6). The assay was stopped and the enzymes were precipitated by mixing vigorously with 0.5 ml chloroform. Glucose released from starch hydrolysis was then quantified by high performance liquid chromatography with pulsed amperometric detection (HPLC-PAD) on an anion-exchange column (CarboPac PA20, 3 x 150 mm Analytical Column, with CarboPac PA20, 3 x 30 mm Guard Column) on an ICS3000 system. The HPLC system consisted of an ICS3000 SP-1 Pump, an AS50 Autosampler with a 10 µl injection loop and an ICS3000 DC-2 Detector/Chromatography Module (all provided by Dionex, Austria). As eluent 20 mM NaOH was used with a flow of 0.5 ml min^{-1}.

Cellulose and lignin were determined following a modification of the CTAB/sulfuric acid method (Rowland & Roberts, 1994) as published in the manual “Use of isotope and radiation methods in soil and water management and crop nutrition” by the International Atomic Energy Agency (http://www-pub.iaea.org/MTCD/publications/PDF/TCS-14.pdf). Aliquots of finely ground plant litter (0.5 g, dried at 60°C) were weighed into F57 ANKOM filter bags for fibre analysis (ANKOM technology, Macedon, NY, USA) of known dry weight and heat sealed. In a first step hemicelluloses, protein and starch were removed by extracting the plant material for 1.5 hrs in a boiling sulfuric acid (0.5 M) with cetyltrimethylammonium bromide (CTAB, 20 g l^{-1}) solution (100 ml CTAB/sulfuric acid per bag). The bags were washed under running demineralized water until no more foam was left, then with boiling MilliQ water until the pH was neutral, and then five times with acetone. Then the bags were dried at 60°C over night and re-weighed. The residue in the bags now consisted of acid detergent fibre (ADF) containing α-cellulose, lignin and ash. In a second step following the acid detergent extraction, α-cellulose was removed by an acid hydrolysis with 72% sulfuric acid (20 ml per sample) for 3 hrs at room temperature. The bags

were washed again as described after the CTAB/sulfuric acid treatment, dried at 60°C over night, re-weighed and the cellulose content was determined by difference and calculated as % initial sample dry weight. Finally, the filter bags containing the remaining fraction (lignin and ash) were ashed at 515°C for 3 hrs in porcelain cups, re-weighed and the amount of lignin (% dry weight) was calculated by subtraction of the ash content from the remaining mass after cellulose removal.

Statistical analyses

Significant differences ($p < 0.05$) between litter types and harvests were analyzed by two-way ANOVA followed by Fisher's LSD post-hoc test. Kolmogorov-Smirnov-test was performed to test for normal distribution and Levene's test to test for homogeneity of variance. If necessary, outliers were excluded and/or the data were log-transformed to obtain normal distribution. Relations between gross rates and litter stoichiometry and chemistry as well as enzymatic activity were examined through simple Pearson correlations on untransformed data. Statistical analyses were performed with Statgraphics 5.0 (Statistical Graphics Inc.) and Statistica 7.1 (StatSoft Inc.).

Results

Litter stoichiometry

Stoichiometry of the initial litter used in this experiment has already been described elsewhere (Wanek *et al.*, 2010). Differences in $C:N_{lit}$, $C:P_{lit}$ and $N:P_{lit}$ were significant for the four litter types (Table 1) and those differences persisted at all three harvests. After two weeks, litter collected from Schottenwald had the lowest $C:N_{lit}$ (42), litter from Klausenleopoldsdorf was intermediate with a $C:N_{lit}$ of 53, while litter collected from Achenkirch and Ossiach had high $C:N_{lit}$ ratios (58 and 60, respectively). $C:N_{lit}$ of litter from Achenkirch and Ossiach slightly decreased over time (55 and 58 at harvest three for Achenkirch and Ossiach, respectively), but did not change significantly between harvests in the litter from Schottenwald and Klausenleopoldsdorf. $C:P_{lit}$ was initially low in Schottenwald litter (699), intermediate in Ossiach litter (905), and high in Achenkirch litter (1280) and Klausenleopoldsdorf litter (1550), and was constant over time with exception for litter from Klausenleopoldsdorf that showed a slight increase of $C:P_{lit}$ (1730 thereafter 6 months). $N:P_{lit}$ was initially low in litter collected from Ossiach (15) and Schottenwald (17) and high in litter from Achenkirch (22) and Klausenleopoldsdorf (30), and changed only in the Klausenleopoldsdorf litter, where it slightly increased (34 at harvest three).

Litter chemistry

Litter chemistry was significantly affected by harvest and litter type (Table 1). As expected, starch content (Figure 1) decreased fast in the first three months from initial concentrations ranging between 0.11 and 0.29 % d.w. to amounts between 0.06 and 0.18 % d.w. at harvest two, but did not further decrease between harvests two and three. The cellulose concentration of the litter (Figure 1) stayed constant throughout the experiment with a slight but insignificant increase from initial values ranging between 18 and 21 % d.w. up to amounts between 20 and 26 % d.w. after three months, presumably due to reduction of absolute litter dry weight, until finally reaching values between 18 and 24 % d.w. at the end of the experiment. Lignin concentrations (Figure 1) did not change significantly in litter collected from Ossiach and Schottenwald throughout the experiment, ranging between 13 and 16 % d.w., while in litter from Klausenleopoldsdorf and

Achenkirch lignin concentrations increased between harvest one (15 and 20 % d.w., respectively) and two (29 and 34 % d.w., respectively) due to loss of absolute litter dry mass, and then only slightly decreased reaching final values around 26 % d.w. after six months of degradation. The lignin:N ratios of litter from Schottenwald and Ossiach did not change significantly over time, with average lignin:N ratios of 11.9 and 18.4, respectively, while the lignin:N ratios for litter from Klausenleopoldsdorf and Achenkirch peaked at harvest two, with average values of 24.2 and 29.9, respectively.

Gross rates of glucan depolymerisation and glucose consumption

The gross rates of glucan depolymerisation and glucose consumption (Figure 2) varied significantly between harvests and litter types (Table 1). Glucan depolymerisation showed a different time pattern for each of the four litter types. After two weeks, litter from Ossiach had the highest rates of all four litter types (1390 µg Glc-C g^{-1} d.w. d^{-1}), then decreased to similar levels as litter from Klausenleopoldsdorf and Schottenwald after three months, and finally reached its lowest value after six months (268 µg Glc-C g^{-1} d.w. d^{-1}). The depolymerisation rates of litter from Klausenleopoldsdorf varied between 366 and 562 µg Glc-C g^{-1} d.w. d^{-1} but did not change significantly between the harvests. The Achenkirch litter, which had the lowest rates of all four litter types in the beginning, showed a slight increase in glucan depolymerisation reaching levels similar to those of the other litter types at harvest three, where differences between rates were no longer significant, ranging between 281 and 366 µg Glc-C g^{-1} d.w. d^{-1}. Depolymerisation in Schottenwald litter was relatively low in the beginning, then peaked at harvest two after three months, where Schottenwald litter had the highest rates of all four litter types at this time point (963 µg Glc-C g^{-1} d.w. d^{-1}), and then decreased to levels similar to the beginning.

Gross rates of glucose consumption (Figure 2) also showed significant differences between harvests and litter types (Table 1). Three of the four litter types (Ossiach, Schottenwald and Klausenleopoldsdorf) showed a decrease in glucose consumption over time with exception for litter from Achenkirch, which had generally very low rates at all time points that did not change over time. After two weeks of decomposition, the glucose consumption rates of litter from Klausenleopoldsdorf, Ossiach and Schottenwald were at their highest levels (ranging from 1690

to 2580 µg Glc-C g^{-1} d.w. d^{-1}), being approximately 10-fold higher than the rates of the Achenkirch litter (237 µg Glc-C g^{-1} d.w. d^{-1}). After three months all consumption rates had declined until reaching very low levels at the end of the incubation after six months, varying only slightly between the four litter types (between 331 and 574 µg Glc-C g^{-1} d.w. d^{-1}).

Glucose concentration and mean residence time

Glucose concentrations (Figure 1) in litter from Klausenleopoldsdorf, Ossiach and Schottenwald were highest at the harvest after two weeks (160 to 354 µg C g^{-1} d.w.), then decreased significantly and did not further change (ranging between 9 and 56 µg C g^{-1} d.w.). Litter from Achenkirch exhibited very low glucose concentrations from the beginning, which did not change throughout the experiment (13 to 17 µg C g^{-1} d.w). Mean residence times (MRT) for glucose (Figure 2), calculated by dividing the pool size by influx or efflux rates, were highest at harvest one (0.23 to 0.82 d) and low at harvests two (0.11 to 0.16 d) and three (0.08 to 0.16 d), showing the accelerating turnover of the glucose pool with declining glucose availability.

Correlations between glucan depolymerisation, glucose consumption, litter stoichiometry and litter chemistry

Linear regressions were performed to explore the influence of litter chemistry and stoichiometry on glucan depolymerisation and glucose consumption rates. Correlation coefficients are shown in Table 2 for harvest one and Table 3 for harvests two and three combined. We decided to split the dataset examining harvest one (n = 20) separately from the combined data of harvests two and three (n = 40), because the correlations of glucan depolymerisation and glucose consumption with litter stoichiometry and chemistry were significantly different between early stages (harvest 1) and later stages (harvests two and three). Our data suggests that initially starch was the main glucan being degraded, which was indicated by a correlation between litter starch content and glucan depolymerisation at harvest one and a rapid decrease of starch content in the first three months of our experiment. However, we did not find any correlation, neither at harvest two nor at harvest three, between glucan depolymerisation and starch content. Instead,

glucan depolymerisation was highly correlated with cellulase activity, indicating that cellulose was the primary substrate for glucan depolymerisation at later stages of decomposition.

After two weeks of decomposition litter stoichiometry was not correlated with glucan depolymerisation and glucose consumption rates. For the combined data of harvests two and three, however, we found significant relationships of $C:N_{lit}$ (Figure 3) with glucan depolymerisation ($r = -0.44$, $p < 0.01$) and glucose consumption ($r = -0.47$, $p < 0.01$). $C:P_{lit}$ and $N:P_{lit}$ was not correlated with depolymerisation and consumption rates. At harvest one, $C:N_{lit}$ was negatively correlated with glucose mean residence time ($r = -0.86$, $p < 0.001$). At harvests two and three we found significant positive relationships of MRT Glc with $C:P_{lit}$ ($r = 0.52$, $p = 0.001$) and $N:P_{lit}$ ($r = 0.62$, $p < 0.001$).

The pattern of correlations between litter chemistry and glucan depolymerisation also changed over time: after two weeks of decomposition, glucan depolymerisation was positively correlated with starch content ($r = 0.63$, $p < 0.05$), and negatively with cellulose ($r = -0.65$, $p = 0.01$) and lignin ($r = -0.55$, $p < 0.05$) content. After three months, however, glucan depolymerisation was not correlated with starch, cellulose or lignin concentration, but we found significant negative relationships of glucan depolymerisation with litter Cellulose:N ($r = -0.40$, $p < 0.05$) and lignin:N ($r = -0.38$, $p < 0.05$) ratios. Glucose concentration was not correlated with glucan depolymerisation and glucose consumption rates at harvest one, but we found significant relationships of glucose concentration with glucan depolymerisation ($r = 0.73$, $p < 0.001$) and glucose consumption ($r = 0.90$, $p < 0.001$) after three and six months (Figure 4). MRT Glc was negatively correlated with starch content ($r = -0.75$, $p < 0.01$) and Cellulose:N ($r = -0.71$, $p < 0.01$) at harvest one, but we did not find any correlations between litter chemistry and MRT Glc after three and six months.

Cellulases (Figure 5) were very low at harvest one and showed no correlation with glucan depolymerisation at this time point. After three and six months, however, we found very close relationships of glucan depolymerisation with cellulases (Figure 6, $r = 0.80$, $p < 0.001$) as well as with lignolytic peroxidases ($r = 0.33$, $p < 0.05$) and phenoloxidases ($r = 0.43$, $p < 0.05$). Glucose consumption rates were also related to activities of cellulases ($r = 0.81$, $p < 0.001$), peroxidases ($r = 0.36$, $p < 0.05$) and phenoloxidases ($r = 0.47$, $p < 0.01$) after three and six months. Cellulose

and lignin degrading enzymes were generally closely related with each other (cellulases and peroxidases $r = 0.58$, $p < 0.001$; cellulases and phenoloxidases $r = 0.63$, $p < 0.001$; peroxidases and phenoloxidases $r = 0.90$, $p < 0.001$) at harvests two and three. MRT Glc was related to cellulases ($r = 0.64$, $p = 0.01$), peroxidases ($r = 0.64$, $p = 0.01$) and phenoloxidases ($r = 0.72$, $p < 0.01$) at harvest one, but there were no such correlations at harvests two and three.

Glucose consumption was very closely related to glucan depolymerisation (Figure 7) at harvest one ($r = 0.76$, $p < 0.001$) and harvests two and three ($r = 0.94$, $p < 0.001$). However, we found no correlation whatsoever between glucose consumption and respiration.

Discussion

Glucans make up a large proportion of plant litter (accounting for up to 50% of plant biomass) (Perez *et al.*, 2002) and represent one of the dominant substrates for soil microorganisms during earlier stages of litter decomposition. Although being of high molecular weight, glucans are relatively easy to degrade compared to more recalcitrant C-sources like lignin (Perez *et al.*, 2002; Berg & McClaugherty, 2008). Nevertheless, the depolymerisation of glucans is mediated by extracellular enzymes (Perez *et al.*, 2002) and therefore requires considerable amounts of energy and nutrients (i.e., N for enzyme production) (Schimel & Weintraub, 2003). The aim of our study was to elucidate the influence of litter quality and stoichiometry on rates of glucan depolymerisation and glucose consumption during early-stage litter decomposition.

Our data suggest that the influence of litter stoichiometry on glucan depolymerisation rates changed over the course of our experiment. Two weeks after inoculation of the litter we found no relationships between glucan depolymerisation and $C:N_{lit}$, $C:P_{lit}$, $N:P_{lit}$, or N and P content of the litter. This suggests that litter N or P content did not significantly influence starch degradation initially. At later stages of decomposition, however, glucan depolymerisation was negatively correlated to $C:N_{lit}$ (Figure 3), which indicates that glucans are preferably decomposed at low $C:N_{lit}$ ratios. We also found a positive correlation of glucan depolymerisation with N_{lit} (Table 3) but no correlation with C_{lit} at this time point, which suggests that the influence of $C:N_{lit}$ on glucan depolymerisation was linked to the N content of the litter. $C:N_{lit}$ was negatively correlated with N_{lit} but not with C_{lit}, indicating that changes in $C:N_{lit}$ occurred due to changes in litter N content. Furthermore, we found negative relationships between cellulase activity and $C:N_{lit}$ and $C:P_{lit}$ at all time points, indicating that low $C:N_{lit}$ and $C:P_{lit}$ ratios resulted in increased production of cellulolytic enzymes. It has been stated that the ratio of activities of C- to nutrient-acquiring enzymes is relatively constant across scales and ecosystems with the mean C:N:P ratio of extracellular enzyme activities being approximately 1:1:1 (Sinsabaugh *et al.*, 2009). This relationship is ascribed to a limitation in the capacity of microbial communities to alter relationships among extracellular enzyme C, N and P acquisition activities, because those acquisition activities are linked to the equilibrium between microbial growth efficiency and the elemental compositions of substrate and microbial biomass (Sinsabaugh *et al.*, 2009). Considering this premise and

assuming that the decrease in $C:N_{lit}$ that we observed in our experiment was caused by an increase in N_{lit}, which in turn suggests an increase in N-availability of the substrate, it is tempting to speculate that the decrease in $C:N_{lit}$ of the litter of our experiment lead to a higher resource-allocation to C-delivering enzymes like cellulases and amylases and a consequential increase in glucan depolymerisation on litter with low C:N ratios.

In addition, we found a significant relationship between glucan depolymerisation and $C:N_{imbalance}$, the ratio of $C:N_{lit}$ to $C:N_{mic}$, which reflects the relation between resource and demand availability. A high $C:N_{imbalance}$ implies that $C:N_{lit}$ is high compared to $C:N_{mic}$, thus microbes experience a rather N-limited situation due to excess C. Otherwise, if $C:N_{lit}$ was very close to $C:N_{mic}$, $C:N_{imbalance}$ would be low and microbes might be rather C-limited. The negative relationship between glucan depolymerisation and $C:N_{imbalance}$ implies that glucans, which are rich in C and only contain low amounts of associated N in form of proteins in plant cell walls (Brett & Waldron, 1996) or starch granules, are depolymerized to a greater extent if the microbial community is sufficiently supplied with N.

In addition to litter stoichiometry, its chemistry also appeared to play an important role in the regulation of glucan depolymerisation and glucose consumption. Two weeks after inoculation, glucan depolymerisation rates were highest and positively correlated with starch content, suggesting that starch was the primary substrate for glucan depolymerisation at this early stage of decomposition. This assumption was supported by the lack of cellulolytic enzyme activity at this time point and the decrease of starch concentration in the litter between harvests one and two. Furthermore, glucan depolymerisation was correlated with mass loss at harvest one, suggesting it to be a quantitatively considerable decomposition process at this stage. After three months, the correlation between glucan depolymerisation rates and starch content diminished, which is in line with the often reported rapid degradation of starch in the first weeks of litter decomposition (Berg & McClaugherty, 2008). Amylase activity was not measured in our experiment, so we cannot directly link glucan depolymerisation to amylase activity. However, amylase activity in decaying litter has previously been observed to decrease after the first weeks of decomposition (Fioretto *et al.*, 2005; Papa *et al.*, 2008). In addition, we found a good linear relationship between cellulase activity and glucan depolymerisation for the combined data of harvests two and three (Figure 6).

We therefore assume that the glucose production we measured at harvests two and three derived mostly from cellulose degradation. To determine possible differences in the controls of starch and cellulose degradation, we split the dataset and examined harvest one (mainly starch degradation) separately from harvests two and three combined (mainly cellulose degradation). At harvests two and three gross glucan depolymerisation rates had declined and now showed a highly significant relationship to potential cellulase activity (Figure 6), suggesting cellulose being the main substrate for glucan depolymerisation at this time point. Nevertheless, we found no correlation with cellulose content at harvests two and three; we therefore assume that cellulose depolymerisation was enzyme not substrate limited at this stage. This hypothesis is supported by the observation that cellulose content hardly changed over time. As cellulose is present in such high amounts in plant litter (Perez *et al.*, 2002; Osono & Takeda, 2005; Papa *et al.*, 2008; Pauly & Keegstra, 2008; Preston *et al.*, 2009), a cellulose-limited situation would only be likely to occur at more advanced stages of degradation.

Examining the relationships between enzymatic activities, we found close relationships between cellulases and oxidative enzymes (peroxidases and phenoloxidases) after three and six months of decomposition, probably indicating synergistic action between cellulose and lignin degrading enzymes. This was confirmed by the correlation between glucan depolymerisation and the lignin degrading enzymes peroxidase and phenoloxidase. As cellulose fibres are tightly associated with lignin molecules (Perez *et al.*, 2002), a 'coordinated' degradation of the lignocellulose complex seems plausible and has been previously reported (Cooke & Whipps, 1993; Perez *et al.*, 2002; Romani *et al.*, 2006; Berg & McClaugherty, 2008).

Gross glucose consumption was highly correlated with glucan depolymerisation, although both processes are thought to have different direct controls: glucan depolymerisation may be driven by the activities of extracellular enzymes secreted into the soil solution whereas glucose is taken up directly by soil microorganisms. However, both processes are intrinsically linked by the demand of microbes for glucose and the consequential resource allocation to enzymes that degrade glucans and thus liberate glucose (Sinsabaugh *et al.*, 2009). Our results suggest that extracellular glucan depolymerisation and microbial glucose consumption are tightly co-regulated and that glucan depolymerisation is a 'fine tuned' process meeting the momentary microbial

demand for glucose. As the production of extracellular enzymes is very nutrient- and energy-demanding and therefore expensive for microorganisms, it is very likely that cellulolytic enzymes are only produced to an extent necessary to satisfy the microbial demand for C (Schimel & Weintraub, 2003; Sinsabaugh *et al.*, 2008). In support of this assumption we found a negative correlation between glucose consumption and $C:N_{lit}$ (Figure 3) and a positive correlation between glucose consumption and N_{lit} (Table 3) for the combined data of harvests two and three. These relationships suggest that a higher N content of the substrate leads to a better N-supply of the microbial community and a subsequent higher demand for C. Furthermore, we found significant correlations between glucose consumption and glucose concentration of the litter (Figure 4) after three and six months, indicating a high requirement of glucose by the microbial community. As glucose is an easily accessible carrier of C and energy, it should be readily taken up by microbes. Nevertheless, we did not find any correlation between glucose consumption and respiration throughout the experiment, suggesting glucose not to be a direct substrate for microbial respiration. A correlation between glucose consumption and respiration would only be expected, if all (or a constant proportion) of the consumed carbon source (e.g., glucose) was actually respired. However, it is likely that a variable part of the C acquired by glucose consumption is assimilated and used for buildup of new microbial biomass. The proportion of glucose that is assimilated would strongly depend on the availability of nutrients, most notably N.

In summary our results suggest that plant litter stoichiometry and chemistry constitute important controls of litter glucose production rates and subsequent glucose consumption by microorganisms during litter decomposition. Glucan depolymerisation was clearly controlled by the availability of suitable substrates, which changed over the course of experiment: at initial stages of decomposition starch was the primary substrate for depolymerisation, while at later stages of decomposition, cellulose was the main substrate. Interestingly, potential cellulase activities were highly correlated with glucan depolymerization, indicating that at these stages of litter decomposition, the depolymerization rates were controlled by enzyme availability not by the accessibility of the cellulose. We were further able to demonstrate that the N content and C:N ratio of the litter strongly effected glucan depolymerisation at later stages of decomposition, suggesting increased resource allocation to C-requiring enzymes with increasing N availability of the substrate.

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Figures and tables:

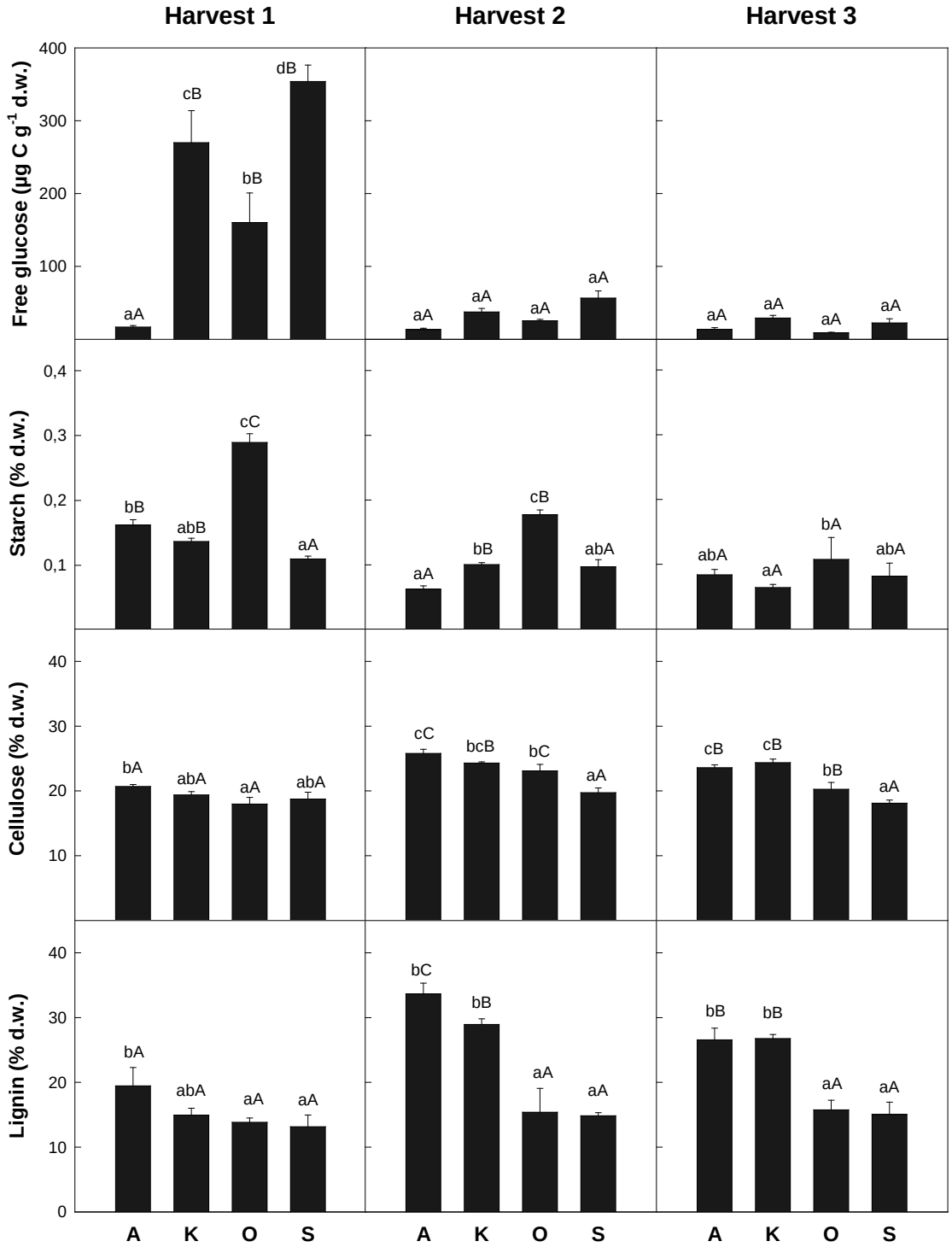

Figure 1: Concentrations of free glucose, starch, cellulose and lignin in the litter during litter decomposition (A, Achenkirch; K, Klausenleopoldsdorf; O, Ossiach; S, Schottenwald). Harvests 1, 2 and 3 refer to sampling time points after 2 weeks and 3 and 6 months, respectively, for glucose, and 0, 3 and 6 months for starch, cellulose and lignin. Given are means of 5 mesocosms ± SE with exception for the first sampling time point (0 months) of starch, cellulose and lignin (n = 4). Lower case letters indicate significant differences between litter types, upper case letters indicate significant differences between harvests (two-way ANOVA followed by Fisher's LSD, p = 0.05).

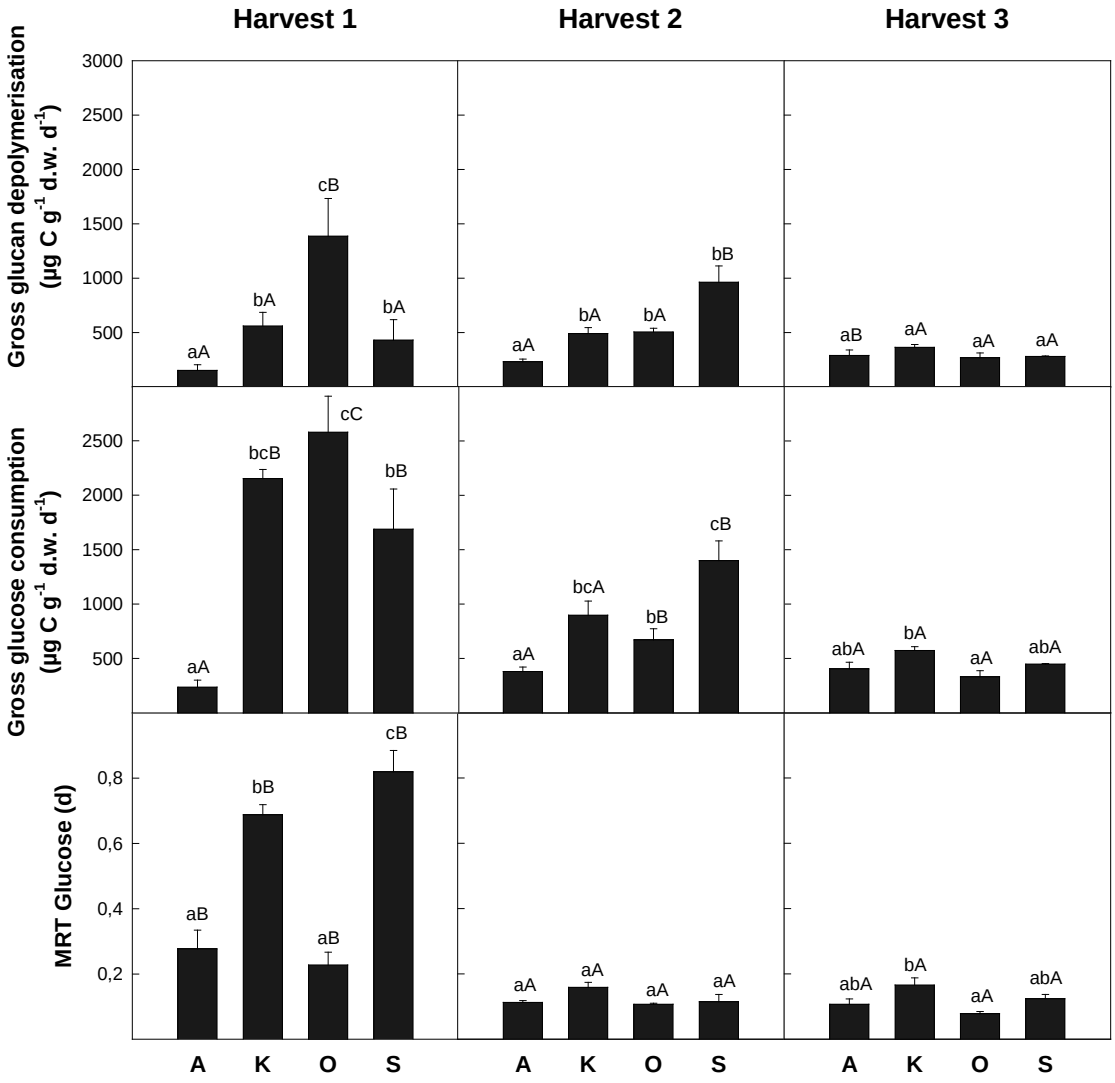

Figure 2: Gross rates of glucan depolymerisation and glucose immobilisation and mean residence time (MRT) of glucose at the three sampling time points (2 weeks, 3 and 6 months after inoculation of the litter) of the litter decomposition experiment (A, Achenkirch; K, Klausenleopoldsdorf; O, Ossiach; S, Schottenwald). Given are means of 5 mesocosms ± SE. Lower case letters indicate significant differences between litter types, upper case letters indicate significant differences between harvests (two-way ANOVA followed by Fisher's LSD, p = 0.05).

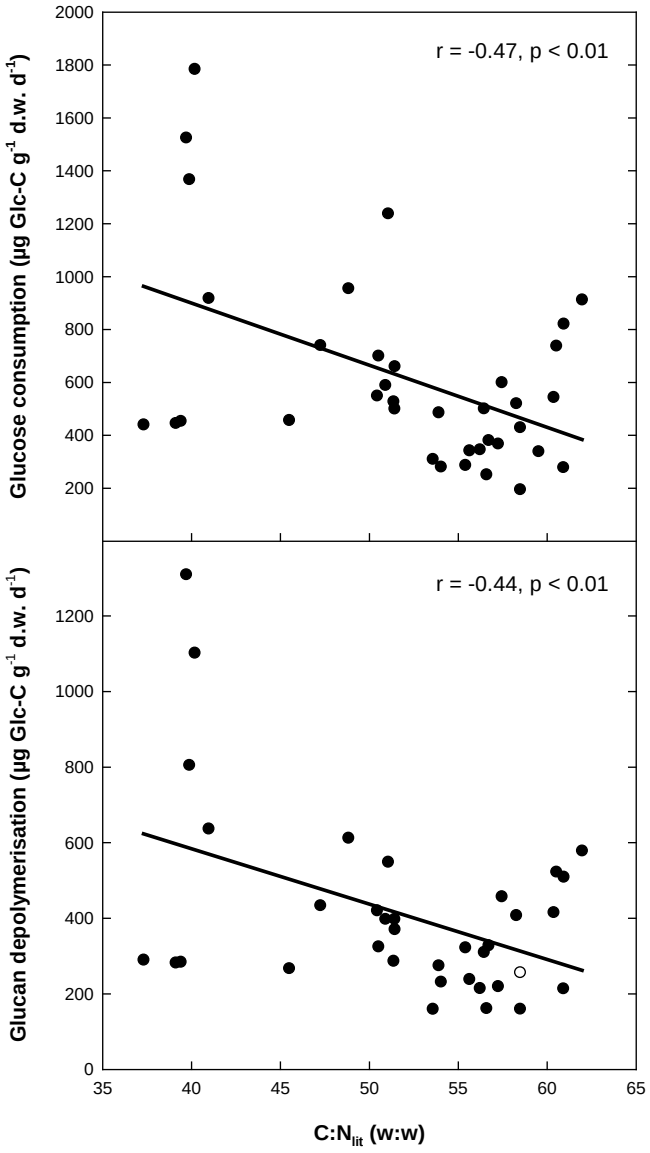

Figure 3: Influence of $C:N_{lit}$ on rates of glucan depolymerisation and glucose consumption. Shown are combined data of four litter types (Achenkirch, Klausenleopoldsdorf, Ossiach and Schottenwald) and two time points (three and six months after inoculation of the litter) (n = 40). Letters indicate correlation coefficients (r) and p-values.

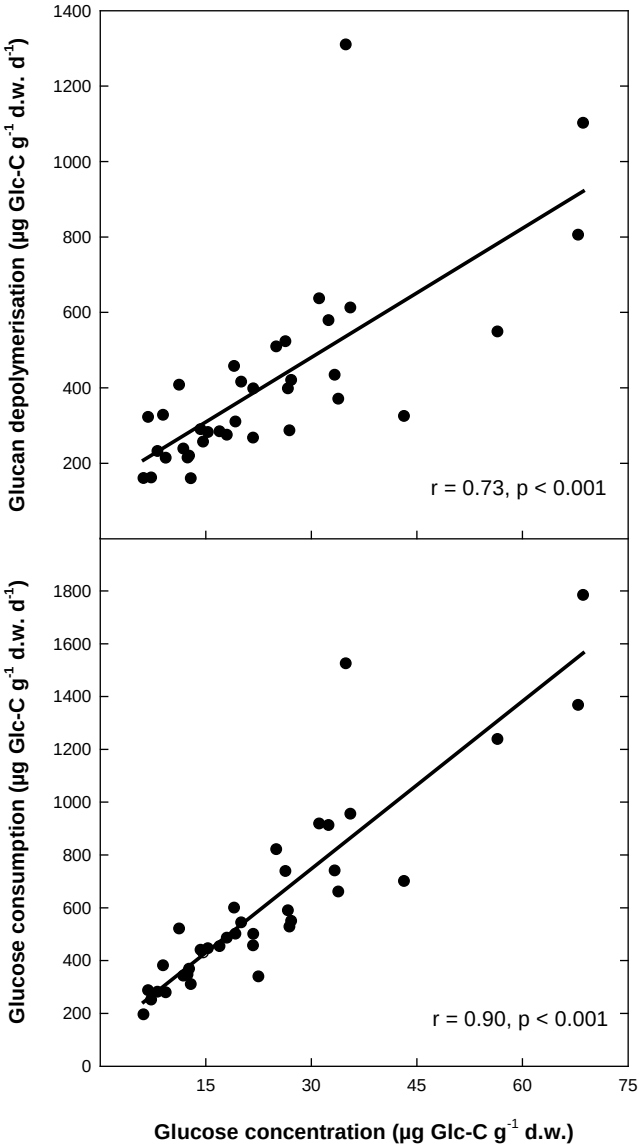

Figure 4: Linear regression of glucan depolymerisation and glucose consumption with litter glucose concentration. Shown are combined data of four litter types (Achenkirch, Klausenleopoldsdorf, Ossiach and Schottenwald) and two time points (three and six months after inoculation of the litter) (n = 40). Letters indicate correlation coefficients (r) and p-values.

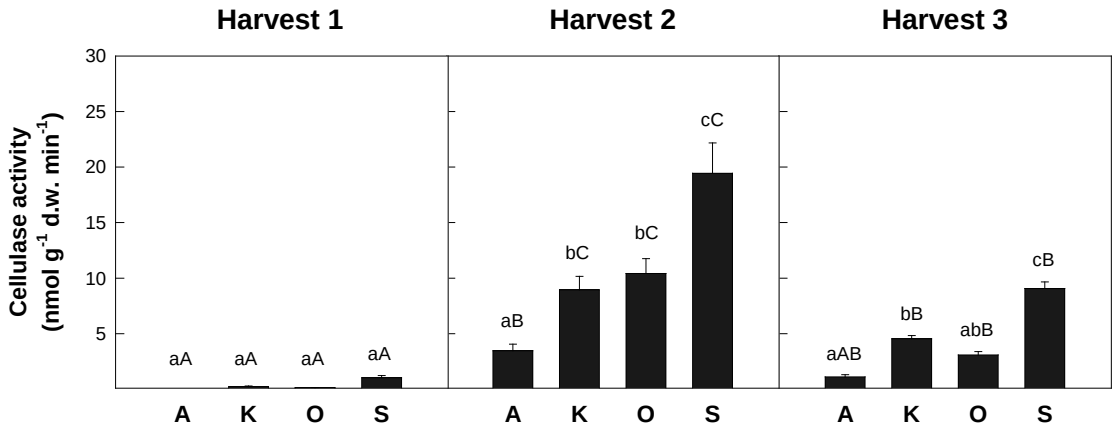

Figure 5: Potential activities of cellulases after 2 weeks and 3 and 6 months after inoculation of the litter (A, Achenkirch; K, Klausenleopoldsdorf; O, Ossiach; S, Schottenwald). Given are means of 5 mesocosms ± SE. Lower case letters indicate significant differences between litter types, upper case letters indicate significant differences between harvests (two-way ANOVA followed by Fisher's LSD, p = 0.05).

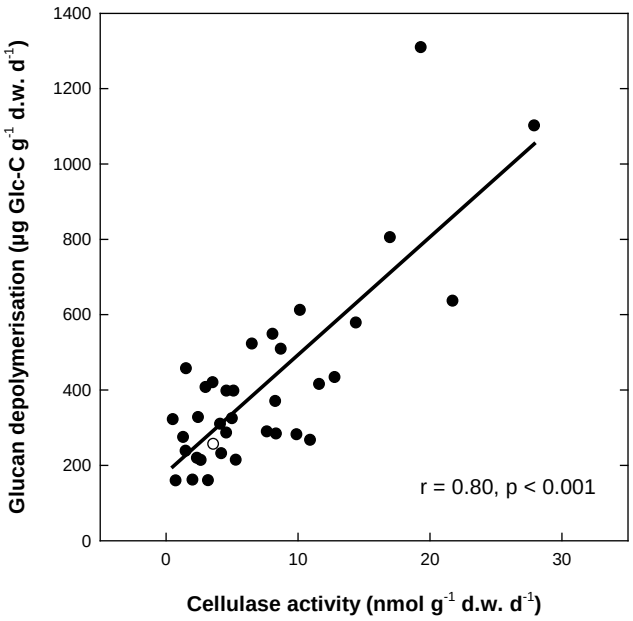

Figure 6: Linear regression of gross glucan depolymerisation and potential cellulase activity. Shown are combined data of four litter types (Achenkirch, Klausenleopoldsdorf, Ossiach and Schottenwald) and two time points (three and six months after inoculation of the litter) (n = 40). Letters indicate correlation coefficients (r) and p-values.

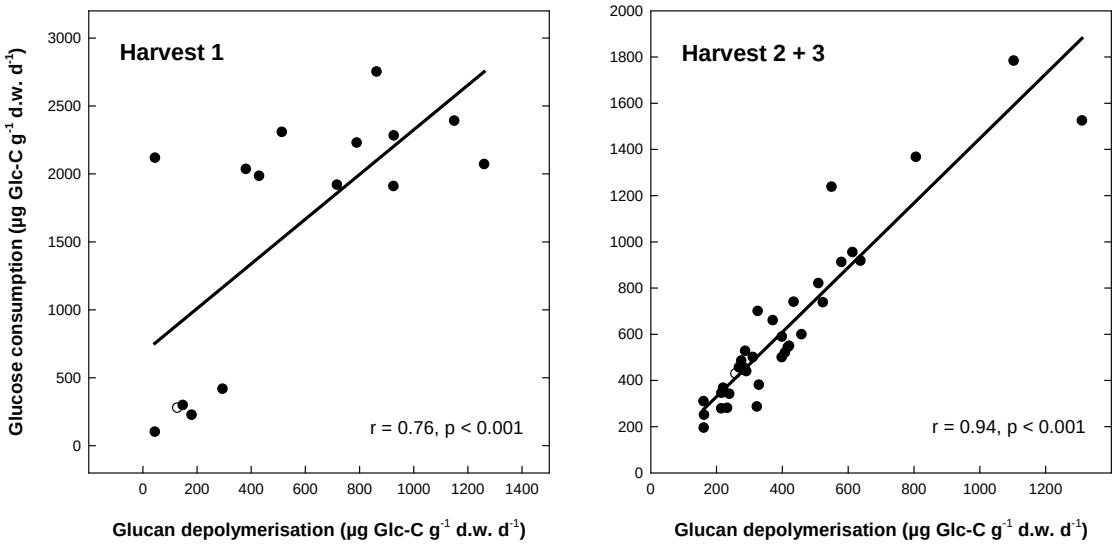

Figure 7: Linear regressions of glucose consumption and glucan depolymerisation after two weeks (harvest one, n = 20) and after three and six months (harvests two and three combined, n = 40). Shown are data of four litter types (Achenkirch, Klausenleopoldsdorf, Ossiach and Schottenwald). Letters indicate correlation coefficients (r) and p-values.

	Harvest		Litter type		Harvest x Litter type	
	F	p	F	p	F	p
$C:N_{lit}$	8.45	0.0007	369.99	<0.0001	1.58	0.1749
$C:P_{lit}$	1.25	0.2958	251.45	<0.0001	3.10	0.0120
$N:P_{lit}$	3.37	0.0425	184.66	<0.0001	1.82	0.1152
Glucose concentration	112.70	<0.0001	26.90	<0.0001	16.43	<0.0001
MRT Glucose	230.31	<0.0001	48.01	<0.0001	28.01	<0.0001
Starch concentration	51.08	<0.0001	35.94	<0.0001	7.61	<0.0001
Cellulose concentration	29.20	<0.0001	24.84	<0.0001	3.41	0.0079
Lignin concentration	20.18	<0.0001	37.11	<0.0001	4.56	0.0012
Gross glucan depolymerisation	4.21	0.0216	10.35	<0.0001	6.47	0.0001
Gross glucose consumption	22.22	<0.0001	22.86	<0.0001	10.01	<0.0001
Cellulase activity	108.53	<0.0001	36.88	<0.0001	10.19	<0.0001
Peroxidase activity	8.44	0.0007	32.08	<0.0001	1.51	0.1962
Phenoloxidase activity	31.71	<0.0001	54.31	<0.0001	2.55	0.0317

Table 1: Significance of effects of litter type and harvest and their interactions on C:N, C:P and N:P of the litter, glucose, starch, cellulose and lignin concentrations, mean residence time (MRT) of glucose, gross rates of glucan depolymerization and glucose consumption, and cellulase, peroxidase and phenoloxidase activities assessed by two-way analysis of variance (ANOVA). Data given are F-ratio and p-value (95% confidence level) and include values from three harvests (two weeks, three and six months) and four litter types (Achenkirch, Klausenleopoldsdorf, Ossiach and Schottenwald). Units for rates are µg C g^{-1} d.w. d^{-1}; units for glucose concentrations are µg Glc-C g^{-1} d.w. and for MRT glucose are d; units for starch, cellulose and lignin concentrations are % d.w. and for enzyme activities are nmol g^{-1} d.w. min^{-1}.

	C_{lit}	N_{lit}	P_{lit}	$C:N_{lit}$	$C:P_{lit}$	$N:P_{lit}$	C_{mic}	N_{mic}	$C:N_{mic}$	$C:N_{imbalance}$	Glc conc.	MRT Glc	Starch conc.	Cellulose conc.	Lignin conc.	Cellulose:N	Lignin:N	Depoly	Glc cons.	Cellulase	Peroxid.	Phenolox.	Respiration	Mass loss
C_{lit}		0,006	-0,374	0,119	0,426	0,381	0,078	-0,288	0,443	**-0,528**	-0,343	0,104	-0,319	0,478	**0,513**	0,286	0,428	**-0,643**	**-0,689**	-0,204	-0,169	-0,220	0,061	**-0,447**
		0,980	0,104	0,618	0,061	0,097	0,766	0,219	0,075	0,029	0,139	0,714	0,228	0,061	0,042	0,283	0,099	0,005	0,001	0,389	0,476	0,352	0,800	0,048
N_{lit}	0,006		**0,594**	**-0,988**	-0,415	-0,059	0,377	**0,843**	-0,384	0,006	**0,642**	**0,815**	**-0,751**	0,043	-0,282	**-0,825**	**-0,598**	-0,453	-0,088	**0,839**	0,245	**0,880**	**0,776**	0,016
	0,980		0,006	0,000	0,069	0,804	0,136	0,000	0,129	0,981	0,002	0,000	0,001	0,876	0,290	0,000	0,014	0,068	0,721	0,000	0,299	0,000	0,000	0,948
P_{lit}	-0,374	**0,594**		**-0,584**	**-0,975**	**-0,828**	0,200	**0,565**	-0,351	0,015	**0,459**	0,027	-0,037	-0,305	-0,355	**-0,667**	**-0,499**	0,157	0,216	**0,733**	0,059	**0,608**	0,395	-0,011
	0,104	0,006		0,007	0,000	0,000	0,442	0,009	0,167	0,956	0,042	0,925	0,893	0,250	0,177	0,005	0,049	0,547	0,374	0,000	0,805	0,004	0,085	0,965
$C:N_{lit}$	0,119	**-0,988**	**-0,584**		0,403	0,036	-0,355	**-0,881**	0,452	-0,104	**-0,684**	**-0,864**	**0,739**	0,005	0,343	**0,845**	**0,650**	0,381	-0,003	**-0,840**	-0,297	**-0,906**	**-0,785**	-0,097
	0,618	0,000	0,007		0,078	0,881	0,162	0,000	0,069	0,690	0,001	0,000	0,001	0,985	0,194	0,000	0,006	0,131	0,990	0,000	0,203	0,000	0,000	0,684
$C:P_{lit}$	0,426	-0,415	**-0,975**	0,403		**0,928**	-0,152	-0,407	0,260	0,023	-0,351	0,202	-0,159	0,327	0,339	**0,528**	0,416	-0,271	-0,234	**-0,601**	0,043	**-0,458**	-0,236	0,043
	0,061	0,069	0,000	0,078		0,000	0,562	0,075	0,313	0,931	0,129	0,471	0,556	0,217	0,199	0,036	0,109	0,292	0,334	0,005	0,858	0,042	0,316	0,856
$N:P_{lit}$	0,381	-0,059	**-0,828**	0,036	**0,928**		0,008	-0,078	0,080	0,084	-0,090	0,470	-0,452	0,333	0,210	0,235	0,179	-0,419	-0,217	-0,318	0,179	-0,130	0,058	0,106
	0,097	0,804	0,000	0,881	0,000		0,977	0,743	0,760	0,750	0,707	0,077	0,079	0,207	0,435	0,381	0,506	0,095	0,372	0,172	0,451	0,586	0,809	0,658
C_{mic}	0,078	0,377	0,200	-0,355	-0,152	0,008		0,291	**0,511**	**-0,656**	0,123	0,345	0,045	-0,066	-0,452	-0,305	-0,481	-0,296	0,154	0,427	0,277	0,217	0,431	-0,310
	0,766	0,136	0,442	0,162	0,562	0,977		0,258	0,036	0,004	0,639	0,226	0,874	0,816	0,091	0,269	0,070	0,285	0,555	0,088	0,282	0,403	0,084	0,227
N_{mic}	-0,288	**0,843**	**0,565**	**-0,881**	-0,407	-0,078	0,291		**-0,595**	0,316	**0,903**	**0,832**	**-0,590**	-0,265	-0,452	**-0,893**	**-0,701**	-0,100	0,182	**0,687**	0,168	**0,932**	**0,758**	0,260
	0,219	0,000	0,009	0,000	0,075	0,743	0,258		0,012	0,217	0,000	0,000	0,016	0,321	0,079	0,000	0,002	0,703	0,456	0,001	0,479	0,000	0,000	0,269
$C:N_{mic}$	0,443	-0,384	-0,351	0,452	0,260	0,080	**0,511**	**-0,595**		**-0,814**	**-0,692**	-0,356	**0,579**	0,141	-0,047	**0,526**	0,150	-0,353	-0,414	-0,419	-0,346	**-0,576**	-0,198	-0,407
	0,075	0,129	0,167	0,069	0,313	0,760	0,036	0,012		0,000	0,002	0,212	0,024	0,616	0,869	0,044	0,594	0,197	0,098	0,094	0,174	0,015	0,447	0,105
$C:N_{imbalance}$	**-0,528**	0,006	0,015	-0,104	0,023	0,084	**-0,656**	0,316	**-0,814**		**0,580**	0,187	-0,336	-0,190	-0,096	-0,264	-0,160	**0,555**	0,433	0,023	0,135	0,235	-0,049	**0,648**
	0,029	0,981	0,956	0,690	0,931	0,750	0,004	0,217	0,000		0,015	0,522	0,221	0,497	0,735	0,341	0,569	0,032	0,082	0,930	0,607	0,365	0,852	0,005
Glc conc.	-0,343	**0,642**	**0,459**	**-0,684**	-0,351	-0,090	0,123	**0,903**	**-0,692**	**0,580**		**0,717**	-0,474	-0,313	-0,452	**-0,823**	**-0,666**	0,036	0,159	0,381	-0,085	**0,840**	**0,560**	0,319
	0,139	0,002	0,042	0,001	0,129	0,707	0,639	0,000	0,002	0,015		0,003	0,064	0,238	0,079	0,000	0,005	0,892	0,516	0,098	0,723	0,000	0,010	0,171
MRT Glc	0,104	**0,815**	0,027	**-0,864**	0,202	0,470	0,345	**0,832**	-0,356	0,187	**0,717**		**-0,747**	-0,054	-0,173	**-0,705**	-0,447	-0,221	0,208	**0,643**	0,637	**0,721**	**0,790**	0,091
	0,714	0,000	0,925	0,000	0,471	0,077	0,226	0,000	0,212	0,522	0,003		0,003	0,862	0,573	0,007	0,126	0,429	0,456	0,010	0,011	0,002	0,000	0,746
Starch conc.	-0,319	**-0,751**	-0,037	**0,739**	-0,159	-0,452	0,045	**-0,590**	**0,579**	-0,336	-0,474	**-0,747**		-0,257	-0,063	**0,517**	0,237	**0,626**	0,168	**-0,537**	-0,387	**-0,670**	**-0,661**	-0,079
	0,228	0,001	0,893	0,001	0,556	0,079	0,874	0,016	0,024	0,221	0,064	0,003		0,337	0,816	0,041	0,376	0,017	0,535	0,032	0,139	0,004	0,005	0,772
Cellulose conc.	0,478	0,043	-0,305	0,005	0,327	0,333	-0,066	-0,265	0,141	-0,190	-0,313	-0,054	-0,257		0,482	**0,523**	0,366	**-0,649**	**-0,510**	-0,223	-0,297	-0,129	-0,104	-0,171
	0,061	0,876	0,250	0,985	0,217	0,207	0,816	0,321	0,616	0,497	0,238	0,862	0,337		0,059	0,038	0,163	0,012	0,044	0,406	0,263	0,634	0,701	0,526
Lignin conc.	**0,513**	-0,282	-0,355	0,343	0,339	0,210	-0,452	-0,452	-0,047	-0,096	-0,452	-0,173	-0,063	0,482		**0,509**	**0,931**	**-0,547**	**-0,533**	-0,425	-0,364	-0,323	-0,443	**-0,539**
	0,042	0,290	0,177	0,194	0,199	0,435	0,091	0,079	0,869	0,735	0,079	0,573	0,816	0,059		0,044	0,000	0,043	0,034	0,101	0,166	0,223	0,086	0,031
Cellulose:N	0,286	**-0,825**	**-0,667**	**0,845**	**0,528**	0,235	-0,305	**-0,893**	**0,526**	-0,264	**-0,823**	**-0,705**	**0,517**	**0,523**	**0,509**		**0,718**	-0,061	-0,465	**-0,895**	-0,604	**-0,853**	**-0,681**	-0,213
	0,283	0,000	0,005	0,000	0,036	0,381	0,269	0,000	0,044	0,341	0,000	0,007	0,041	0,038	0,044		0,002	0,837	0,070	0,000	0,013	0,000	0,004	0,427
Lignin:N	0,428	**-0,598**	**-0,499**	**0,650**	0,416	0,179	-0,481	**-0,701**	0,150	-0,160	**-0,666**	-0,447	0,237	0,366	**0,931**	**0,718**		-0,350	**-0,526**	**-0,670**	-0,508	**-0,615**	**-0,657**	**-0,524**
	0,099	0,014	0,049	0,006	0,109	0,506	0,070	0,002	0,594	0,569	0,005	0,126	0,376	0,163	0,000	0,002		0,220	0,036	0,004	0,044	0,011	0,006	0,037
Depoly	**-0,643**	-0,453	0,157	0,381	-0,271	-0,419	-0,296	-0,100	-0,353	**0,555**	0,036	-0,221	**0,626**	**-0,649**	**-0,547**	-0,061	-0,350		**0,764**	-0,148	-0,018	-0,193	-0,228	**0,549**
	0,005	0,068	0,547	0,131	0,292	0,095	0,285	0,703	0,197	0,032	0,892	0,429	0,017	0,012	0,043	0,837	0,220		0,000	0,570	0,944	0,458	0,378	0,022
Glc cons.	**-0,689**	-0,088	0,216	-0,003	-0,234	-0,217	0,154	0,182	-0,414	0,433	0,159	0,208	0,168	**-0,510**	**-0,533**	-0,465	**-0,526**	**0,764**		0,241	0,472	0,010	-0,034	0,403
	0,001	0,721	0,374	0,990	0,334	0,372	0,555	0,456	0,098	0,082	0,516	0,456	0,535	0,044	0,034	0,070	0,036	0,000		0,320	0,041	0,967	0,891	0,087
Cellulase	-0,204	**0,839**	**0,733**	**-0,840**	**-0,601**	-0,318	0,427	**0,687**	-0,419	0,023	0,381	**0,643**	**-0,537**	-0,223	-0,425	**-0,895**	**-0,670**	-0,148	0,241		0,433	**0,695**	**0,669**	-0,040
	0,389	0,000	0,000	0,000	0,005	0,172	0,088	0,001	0,094	0,930	0,098	0,010	0,032	0,406	0,101	0,000	0,004	0,570	0,320		0,057	0,001	0,001	0,867
Peroxid.	-0,169	0,245	0,059	-0,297	0,043	0,179	0,277	0,168	-0,346	0,135	-0,085	**0,637**	-0,387	-0,297	-0,364	**-0,604**	**-0,508**	-0,018	**0,472**	0,433		0,170	0,273	0,086
	0,476	0,299	0,805	0,203	0,858	0,451	0,282	0,479	0,174	0,607	0,723	0,011	0,139	0,263	0,166	0,013	0,044	0,944	0,041	0,057		0,475	0,244	0,718
Phenolox.	-0,220	**0,880**	**0,608**	**-0,906**	**-0,458**	-0,130	0,217	**0,932**	**-0,576**	0,235	**0,840**	**0,721**	**-0,670**	-0,129	-0,323	**-0,853**	**-0,615**	-0,193	0,010	**0,695**	0,170		**0,763**	0,124
	0,352	0,000	0,004	0,000	0,042	0,586	0,403	0,000	0,015	0,365	0,000	0,002	0,004	0,634	0,223	0,000	0,011	0,458	0,967	0,001	0,475		0,000	0,603
Respiration	0,061	**0,776**	0,395	**-0,785**	-0,236	0,058	0,431	**0,758**	-0,198	-0,049	**0,560**	**0,790**	**-0,661**	-0,104	-0,443	**-0,681**	**-0,657**	-0,228	-0,034	**0,669**	0,273	**0,763**		0,019
	0,800	0,000	0,085	0,000	0,316	0,809	0,084	0,000	0,447	0,852	0,010	0,000	0,005	0,701	0,086	0,004	0,006	0,378	0,891	0,001	0,244	0,000		0,935
Mass loss	**-0,447**	0,016	-0,011	-0,097	0,043	0,106	-0,310	0,260	-0,407	**0,648**	0,319	0,091	-0,079	-0,171	**-0,539**	-0,213	**-0,524**	**0,549**	0,403	-0,040	0,086	0,124	0,019	
	0,048	0,948	0,965	0,684	0,856	0,658	0,227	0,269	0,105	0,005	0,171	0,746	0,772	0,526	0,031	0,427	0,037	0,022	0,087	0,867	0,718	0,603	0,935	

Table 2: Correlation analysis for harvest 1 between litter chemistry and stoichiometry, gross rates of glucan depolymerisation and glucose consumption and extracellular enzyme activities in litter mesocosms. C_{lit}, N_{lit}, P_{lit}, $C:N_{lit}$, $C:P_{lit}$, $N:P_{lit}$, Glc conc., Starch conc., Cell. conc. Lignin conc., Cellulose:N and Lignin:N represent the respective elemental and compound concentrations and ratios of beech litter, C_{mic}, N_{mic} and $C:N_{mic}$ the elemental content and ratio of the microbial biomass, $C:N_{imbalance}$ the ratio of $C:N_{litter}/C:N_{mic}$. Abbreviations for gross processes are Depoly, gross glucan depolymerisation; Glc cons., gross glucose consumption, Cellulase, Peroxid., Phenolox., the respective potential activities of cellulase, peroxidase and phenoloxidase; Respiration, microbial respiration in the mesocosms. Numbers represent correlation coefficients (upper lines) and p-values (lower lines). Correlation coefficients in bold denote significant relationships between variables ($p < 0.05$).

	C_{lit}	N_{lit}	P_{lit}	$C:N_{lit}$	$C:P_{lit}$	$N:P_{lit}$	C_{mic}	N_{mic}	$C:N_{mic}$	$C:N_{imbalance}$	Glc conc.	MRT Glc	Starch conc.	Cellulose conc.	Lignin conc.	Cellulose:N	Lignin:N	Depoly	Glc cons.	Cellulase	Peroxid.	Phenolox.	Respiration	Mass loss
C_{lit}		0,159 0,327	**-0,361** 0,022	-0,090 0,581	**0,458** 0,003	**0,490** 0,001	-0,044 0,788	0,189 0,242	-0,210 0,194	0,223 0,166	-0,124 0,445	0,276 0,104	**-0,433** 0,012	**0,376** 0,020	**0,557** 0,000	0,084 0,615	**0,443** 0,005	-0,212 0,214	-0,156 0,355	-0,252 0,116	-0,097 0,553	-0,114 0,485	0,121 0,482	0,304 0,057
N_{lit}	0,159 0,327		**0,487** 0,001	**-0,991** 0,000	-0,311 0,051	0,056 0,730	**0,715** 0,000	**0,417** 0,007	0,190 0,240	**-0,467** 0,002	**0,488** 0,001	0,197 0,249	-0,286 0,106	**-0,534** 0,001	-0,290 0,078	**-0,891** 0,000	**-0,526** 0,001	**0,420** 0,011	**0,448** 0,005	**0,539** 0,000	**0,778** 0,000	**0,775** 0,000	**0,648** 0,000	0,200 0,216
P_{lit}	**-0,361** 0,022	**0,487** 0,001		**-0,459** 0,003	**-0,963** 0,000	**-0,820** 0,000	**0,757** 0,000	**0,510** 0,001	0,157 0,333	-0,221 0,170	0,216 0,180	**-0,398** 0,016	0,190 0,289	**-0,720** 0,000	**-0,679** 0,000	**-0,629** 0,000	**-0,693** 0,000	**0,394** 0,017	0,282 0,091	**0,518** 0,001	**0,610** 0,000	**0,576** 0,000	0,189 0,270	0,165 0,309
$C:N_{lit}$	-0,090 0,581	**-0,991** 0,000	**-0,459** 0,003		0,280 0,080	-0,093 0,567	**-0,695** 0,000	**-0,380** 0,016	-0,203 0,208	**0,483** 0,002	**-0,524** 0,001	-0,229 0,179	0,284 0,110	**0,537** 0,001	0,299 0,068	**0,896** 0,000	**0,537** 0,001	**-0,435** 0,008	**-0,471** 0,003	**-0,553** 0,000	**-0,764** 0,000	**-0,768** 0,000	**-0,636** 0,000	-0,157 0,333
$C:P_{lit}$	**0,458** 0,003	-0,311 0,051	**-0,963** 0,000	0,280 0,080		**0,928** 0,000	**-0,671** 0,000	**-0,432** 0,005	-0,182 0,260	0,199 0,218	-0,098 0,547	**0,517** 0,001	-0,296 0,094	**0,676** 0,000	**0,686** 0,000	**0,492** 0,002	**0,646** 0,000	-0,306 0,070	-0,185 0,273	**-0,446** 0,004	**-0,495** 0,001	**-0,447** 0,004	-0,035 0,840	-0,116 0,476
$N:P_{lit}$	**0,490** 0,001	0,056 0,730	**-0,820** 0,000	-0,093 0,567	**0,928** 0,000		**-0,431** 0,005	-0,304 0,056	-0,114 0,484	0,020 0,902	0,107 0,511	**0,618** 0,000	**-0,394** 0,023	**0,495** 0,002	**0,586** 0,000	0,166 0,321	**0,453** 0,004	-0,143 0,406	-0,005 0,975	-0,241 0,134	-0,213 0,188	-0,157 0,332	0,212 0,215	-0,062 0,704
C_{mic}	-0,044 0,788	**0,715** 0,000	**0,757** 0,000	**-0,695** 0,000	**-0,671** 0,000	**-0,431** 0,005		**0,698** 0,000	0,151 0,354	-0,307 0,054	0,221 0,170	-0,049 0,776	0,009 0,962	**-0,724** 0,000	**-0,605** 0,000	**-0,805** 0,000	**-0,712** 0,000	**0,344** 0,040	**0,338** 0,041	**0,540** 0,000	**0,824** 0,000	**0,773** 0,000	**0,436** 0,008	0,213 0,187
N_{mic}	0,189 0,242	**0,417** 0,007	**0,510** 0,001	**-0,380** 0,016	**-0,432** 0,005	-0,304 0,056	**0,698** 0,000		**-0,564** 0,000	**0,410** 0,009	-0,179 0,269	-0,181 0,291	-0,184 0,305	**-0,534** 0,001	**-0,371** 0,022	**-0,529** 0,001	**-0,434** 0,007	-0,178 0,299	-0,225 0,180	-0,105 0,518	**0,447** 0,004	**0,404** 0,010	0,320 0,057	**0,333** 0,036
$C:N_{mic}$	-0,210 0,194	0,190 0,240	0,157 0,333	-0,203 0,208	-0,182 0,260	-0,114 0,484	0,151 0,354	**-0,564** 0,000		**-0,873** 0,000	**0,467** 0,002	0,134 0,437	0,233 0,192	0,017 0,920	-0,071 0,674	-0,103 0,540	-0,097 0,563	**0,618** 0,000	**0,685** 0,000	**0,678** 0,000	0,218 0,178	0,223 0,166	-0,064 0,710	-0,197 0,223
$C:N_{imbalance}$	0,223 0,166	**-0,467** 0,002	-0,221 0,170	**0,483** 0,002	0,199 0,218	0,020 0,902	-0,307 0,054	**0,410** 0,009	**-0,873** 0,000		**-0,518** 0,001	-0,252 0,138	-0,153 0,395	0,162 0,330	0,164 0,325	**0,360** 0,027	0,263 0,111	**-0,495** 0,002	**-0,584** 0,000	**-0,712** 0,000	**-0,442** 0,004	**-0,482** 0,002	-0,306 0,069	0,110 0,499
Glc conc.	-0,124 0,445	**0,488** 0,001	0,216 0,180	**-0,524** 0,001	-0,098 0,547	0,107 0,511	0,221 0,170	-0,179 0,269	**0,467** 0,002	**-0,518** 0,001		**0,560** 0,000	0,074 0,683	-0,076 0,652	-0,143 0,391	**-0,364** 0,025	-0,283 0,085	**0,727** 0,000	**0,896** 0,000	**0,653** 0,000	0,273 0,089	**0,368** 0,019	0,157 0,360	-0,218 0,177
MRT Glc	0,276 0,104	0,197 0,249	**-0,398** 0,016	-0,229 0,179	**0,517** 0,001	**0,618** 0,000	-0,049 0,776	-0,181 0,291	0,134 0,437	-0,252 0,138	**0,560** 0,000		-0,045 0,810	0,291 0,090	0,233 0,177	-0,004 0,983	0,112 0,523	-0,059 0,735	0,211 0,216	0,096 0,622	0,148 0,391	0,172 0,316	**0,424** 0,016	-0,202 0,239
Starch conc.	**-0,433** 0,012	-0,286 0,106	0,190 0,289	0,284 0,110	-0,296 0,094	**-0,394** 0,023	0,009 0,962	-0,184 0,305	0,233 0,192	-0,153 0,395	0,074 0,683	-0,045 0,810		-0,182 0,320	**-0,429** 0,014	0,084 0,647	-0,319 0,075	0,157 0,399	0,091 0,622	0,276 0,120	-0,060 0,741	-0,048 0,793	-0,174 0,367	-0,307 0,082
Cellulose conc.	**0,376** 0,020	**-0,534** 0,001	**-0,720** 0,000	**0,537** 0,001	**0,676** 0,000	**0,495** 0,002	**-0,724** 0,000	**-0,534** 0,001	0,017 0,920	0,162 0,330	-0,076 0,652	0,291 0,090	-0,182 0,320		**0,786** 0,000	**0,845** 0,000	**0,830** 0,000	-0,264 0,125	-0,166 0,333	**-0,387** 0,017	**-0,707** 0,000	**-0,667** 0,000	-0,325 0,057	-0,137 0,412
Lignin conc.	**0,557** 0,000	-0,290 0,078	**-0,679** 0,000	0,299 0,068	**0,686** 0,000	**0,586** 0,000	**-0,605** 0,000	**-0,371** 0,022	-0,071 0,674	0,164 0,325	-0,143 0,391	0,233 0,177	**-0,429** 0,014	**0,786** 0,000		**0,565** 0,000	**0,961** 0,000	-0,299 0,081	-0,167 0,331	**-0,458** 0,004	**-0,598** 0,000	**-0,512** 0,001	-0,183 0,293	-0,025 0,881
Cellulose:N	0,084 0,615	**-0,891** 0,000	**-0,629** 0,000	**0,896** 0,000	**0,492** 0,002	0,166 0,321	**-0,805** 0,000	**-0,529** 0,001	-0,103 0,540	**0,360** 0,027	**-0,364** 0,025	-0,004 0,983	0,084 0,647	**0,845** 0,000	**0,565** 0,000		**0,743** 0,000	**-0,397** 0,018	**-0,368** 0,027	**-0,516** 0,001	**-0,827** 0,000	**-0,806** 0,000	**-0,563** 0,000	-0,181 0,276
Lignin:N	**0,443** 0,005	**-0,526** 0,001	**-0,693** 0,000	**0,537** 0,001	**0,646** 0,000	**0,453** 0,004	**-0,712** 0,000	**-0,434** 0,007	-0,097 0,563	0,263 0,111	-0,283 0,085	0,112 0,523	-0,319 0,075	**0,830** 0,000	**0,961** 0,000	**0,743** 0,000		**-0,379** 0,025	-0,271 0,110	**-0,546** 0,000	**-0,726** 0,000	**-0,659** 0,000	**-0,358** 0,035	-0,068 0,684
Depoly	-0,212 0,214	**0,420** 0,011	**0,394** 0,017	**-0,435** 0,008	-0,306 0,070	-0,143 0,406	**0,344** 0,040	-0,178 0,299	**0,618** 0,000	**-0,495** 0,002	**0,727** 0,000	-0,059 0,735	0,157 0,399	-0,264 0,125	-0,299 0,081	**-0,397** 0,018	**-0,379** 0,025		**0,936** 0,000	**0,804** 0,000	**0,334** 0,047	**0,434** 0,008	-0,096 0,600	-0,148 0,390
Glc cons.	-0,156 0,355	**0,448** 0,005	0,282 0,091	**-0,471** 0,003	-0,185 0,273	-0,005 0,975	**0,338** 0,041	-0,225 0,180	**0,685** 0,000	**-0,584** 0,000	**0,896** 0,000	0,211 0,216	0,091 0,622	-0,166 0,333	-0,167 0,331	**-0,368** 0,027	-0,271 0,110	**0,936** 0,000		**0,805** 0,000	**0,357** 0,030	**0,474** 0,003	-0,024 0,897	-0,205 0,223
Cellulase	-0,252 0,116	**0,539** 0,000	**0,518** 0,001	**-0,553** 0,000	**-0,446** 0,004	-0,241 0,134	**0,540** 0,000	-0,105 0,518	**0,678** 0,000	**-0,712** 0,000	**0,653** 0,000	0,096 0,579	0,276 0,120	**-0,387** 0,017	**-0,458** 0,004	**-0,516** 0,001	**-0,546** 0,000	**0,804** 0,000	**0,805** 0,000		**0,577** 0,000	**0,633** 0,000	0,171 0,320	-0,123 0,450
Peroxid.	-0,097 0,553	**0,778** 0,000	**0,610** 0,000	**-0,764** 0,000	**-0,495** 0,001	-0,213 0,188	**0,824** 0,000	**0,447** 0,004	0,218 0,178	**-0,442** 0,004	0,273 0,089	0,148 0,391	-0,060 0,741	**-0,707** 0,000	**-0,598** 0,000	**-0,827** 0,000	**-0,726** 0,000	**0,334** 0,047	**0,357** 0,030	**0,577** 0,000		**0,898** 0,000	**0,649** 0,000	0,184 0,256
Phenolox.	-0,114 0,485	**0,775** 0,000	**0,576** 0,000	**-0,768** 0,000	**-0,447** 0,004	-0,157 0,332	**0,773** 0,000	**0,404** 0,010	0,223 0,166	**-0,482** 0,002	**0,368** 0,019	0,172 0,316	-0,048 0,793	**-0,667** 0,000	**-0,512** 0,001	**-0,806** 0,000	**-0,659** 0,000	**0,434** 0,008	**0,474** 0,003	**0,633** 0,000	**0,898** 0,000		**0,644** 0,000	0,140 0,390
Respiration	0,121 0,482	**0,648** 0,000	0,189 0,270	**-0,636** 0,000	-0,035 0,840	0,212 0,215	**0,436** 0,008	0,320 0,057	-0,064 0,710	-0,306 0,069	0,157 0,360	**0,424** 0,016	-0,174 0,367	-0,325 0,057	-0,183 0,293	**-0,563** 0,000	**-0,358** 0,035	-0,096 0,600	-0,024 0,897	0,171 0,320	**0,649** 0,000	**0,644** 0,000		0,237 0,164
Mass loss	0,304 0,057	0,200 0,216	0,165 0,309	-0,157 0,333	-0,116 0,476	-0,062 0,704	0,213 0,187	**0,333** 0,036	-0,197 0,223	0,110 0,499	-0,218 0,177	-0,202 0,239	-0,307 0,082	-0,137 0,412	-0,025 0,881	-0,181 0,276	-0,068 0,684	-0,148 0,390	-0,205 0,223	-0,123 0,450	0,184 0,256	0,140 0,390	0,237 0,164	

Table 3: Correlation analysis for harvests 2 and 3 (combined) between litter chemistry and stoichiometry, gross rates of glucan depolymerisation and glucose consumption and extracellular enzyme activities in litter mesocosms. C_{lit}, N_{lit}, P_{lit}, $C:N_{lit}$, $C:P_{lit}$, $N:P_{lit}$, Glc conc., Starch conc., Cell. conc. Lignin conc., Cellulose:N and Lignin:N represent the respective elemental and compound concentrations and ratios of beech litter, C_{mic}, N_{mic} and $C:N_{mic}$ the elemental content and ratio of the microbial biomass, $C:N_{imbalance}$ the ratio of $C:N_{litter}/C:N_{mic}$. Abbreviations for gross processes are Depoly, gross glucan depolymerisation; Glc cons., gross glucose consumption, Cellulase, Peroxid., Phenolox., the respective potential activities of cellulase, peroxidase and phenoloxidase; Respiration, microbial respiration in the mesocosms. Numbers represent correlation coefficients (upper lines) and p-values (lower lines). Correlation coefficients in bold denote significant relationships between variables ($p < 0.05$).

Appendix

1. A few examples of HPLC-IsoLink-IRMS chromatograms

1.1. Sample preparation and analysis

All shown chromatograms are beech litter extracts in high purity water (MilliQ, > 18.2 MOhm, Millipore), which were filtered, ion-exchanged and concentrated by freeze-drying prior to analysis.

The HPLC-IRMS system consisted of an ICS-3000 unit and an AS50 autosampler with a 25 µl injection loop (Dionex). The separation column was a HyperREZ XP Carbohydrate Ca^{2+} 8 µm column (Thermo Fisher Scientific), run at 85°C with 0.5 ml min^{-1} MilliQ water as eluent isocratic for 29 min. The HPLC was connected to an IRMS (Delta Advantage, Thermo Fischer Scientific) via a Finnigan LC IsoLink Interface (Thermo Fischer Scientific), in which glucose was oxidized to CO_2 by an acid persulfate digestion at 99.9°C. A 0.5 M solution of sodium persulfate was used as oxidant and 1.7 M phosphoric acid to acidify the solution. The resulting CO_2 was then transferred via a gas-permeable membrane to a stream of helium in a separation unit and the gas stream was dried over Nafion tubes. Before entering the IRMS, excess oxygen was removed as described elsewhere (Hettmann *et al.*, 2007). Subsequently, the sample stream was introduced into a Delta V Advantage Mass Spectrometer (Thermo Fisher Scientific) by an open split.

Figures A5-A8 show a comparison between normal and spiked samples. Spiking was performed whenever samples had a glucose concentration below the isotope quantiation limit (roughly, 20 mg glc l^{-1}) of the HPLC-IRMS system. In such a case, 90µl of sample were spiked with 10 µl of a 1 g l^{-1} D-glucose solution. The concentration of the spike in the sample therefore constituted 100 mg Glc l^{-1}. The $\delta^{13}C$ of the spiked sample was measured on the abovementioned HPLC-IRMS system. Additionally, the glucose concentration of samples was determined on a high performance anion exchange chromatography - pulsed amperometric detection system (HPAEC-PAD), which has a lower detection limit than the HPLC-IRMS system (0.024 mg l^{-1}). The

HPAEC-PAD system consisted of an ICS3000 SP-1 Pump, an AS50 Autosampler with a 10 µl injection loop and an ICS3000 DC-2 Detector/Chromatography Module (all provided by Dionex). As separation column a CarboPac PA20 Analytical Column (3 x 150 mm, Dionex) with a CarboPac PA20, Guard Column (3 x 30 mm, Dionex) was used with 0.5 ml min^{-1} 20 mM NaOH as eluent. The $\delta^{13}C$ value of the glucose in the sample were determined using an isotopic mixing model (1):

(1) $$\delta_{sample} = \frac{c_{all} * \delta_{all} - c_{spike} * \delta_{spike}}{c_{sample}}$$

(2) $$c_{all} = c_{sample} + c_{spike}$$

where c_{sample} is the concentration of glucose in the sample as measured by the HPLC-PAD system. For calibration, a glucose solution was used at concentrations between 0.1 and 50 mg l^{-1}. C_{spike} is the concentration of the C4 glucose standard, which is 100 mg l^{-1}, and c_{all} is the calculated concentration of the spiked sample as shown in equation (2). δ_{spike} and δ_{all} were measured on the HPLC-IRMS system. For calibration on the HPLC-IRMS, a glucose solution was used in concentration of 100 and 150 mg l^{-1} and injected at least eight times each, four times at the beginning and four times at the end of the measurement, with additional injections of the 100 mg l^{-1} standard every 15 samples. For δ_{spike}, the mean value of the 100 mg l^{-1} standard was taken. Equation (2) was then used to calculate the δ_{sample}.

1.2. Normal samples

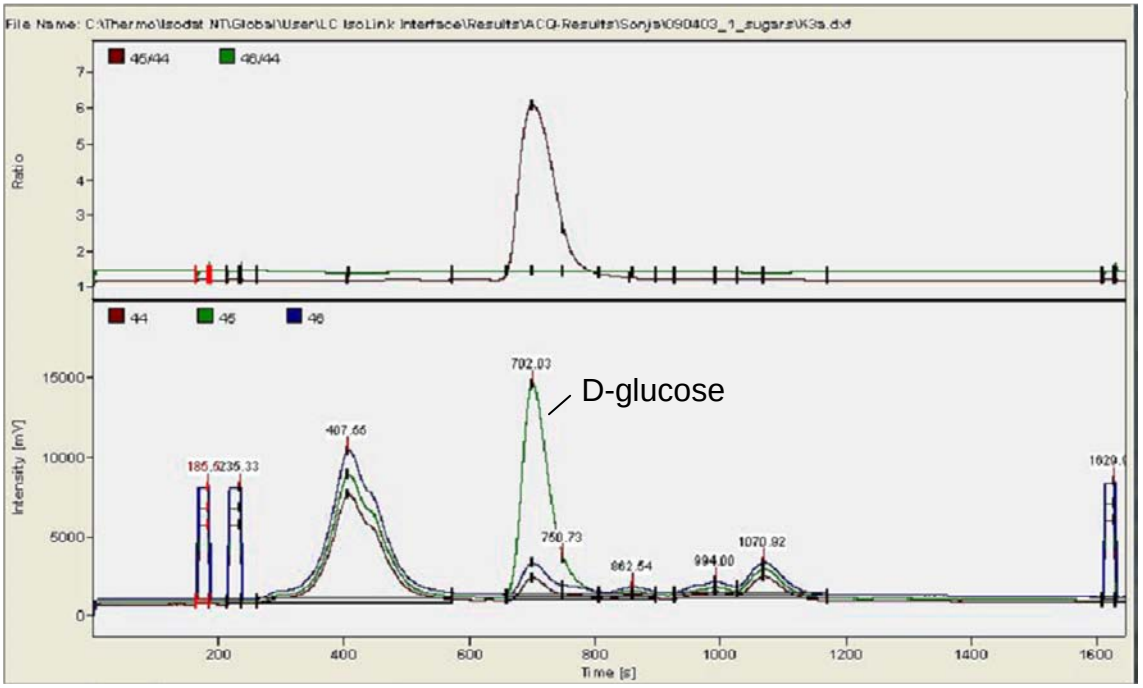

Figure A1: Harvest 1, Litter O, extracted after 30'
Concentration = 70 mg glucose l^{-1}, $\delta^{13}C$ = 11134

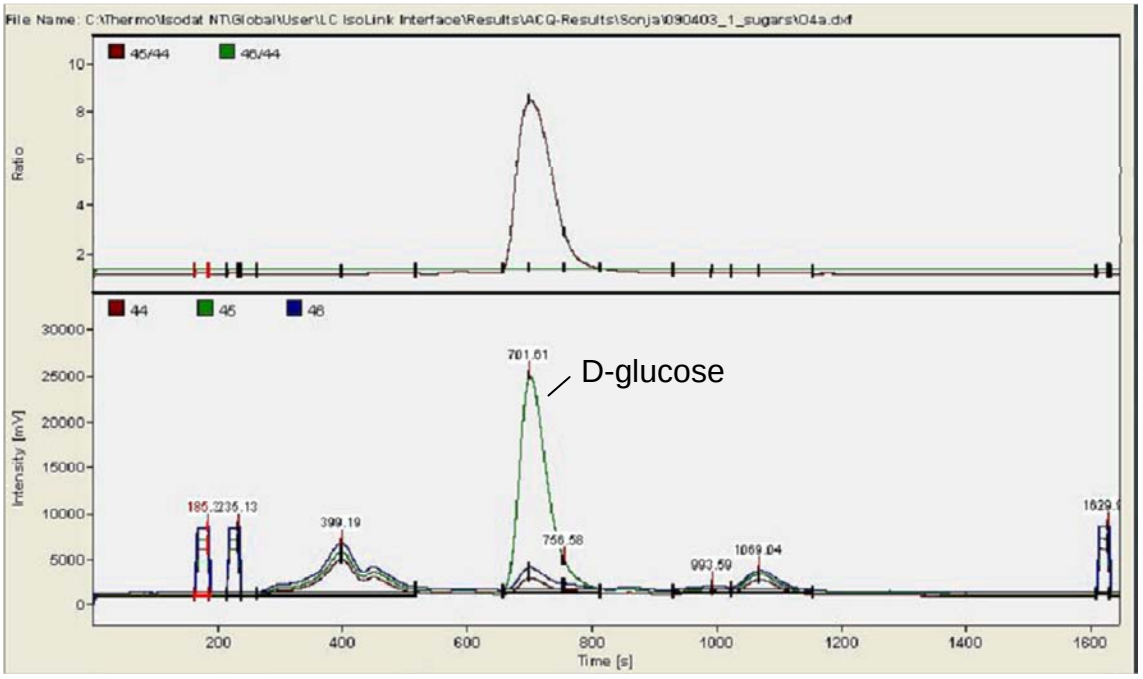

Figure A2: Harvest 1, Litter O, extracted after 120'
Concentration = 65 mg glucose l^{-1}, $\delta^{13}C$ = 6752

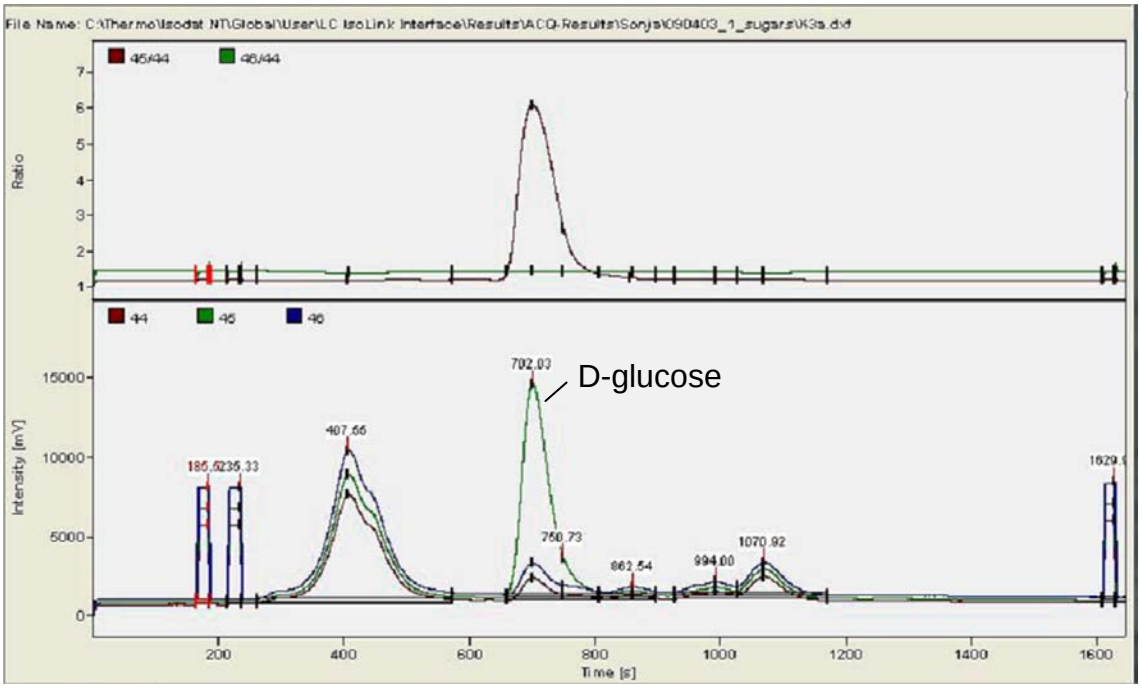

Figure A3: Harvest 1, Litter K, extracted after 30'
Concentration = 54 mg glucose l^{-1}, $\delta^{13}C$ = 7575

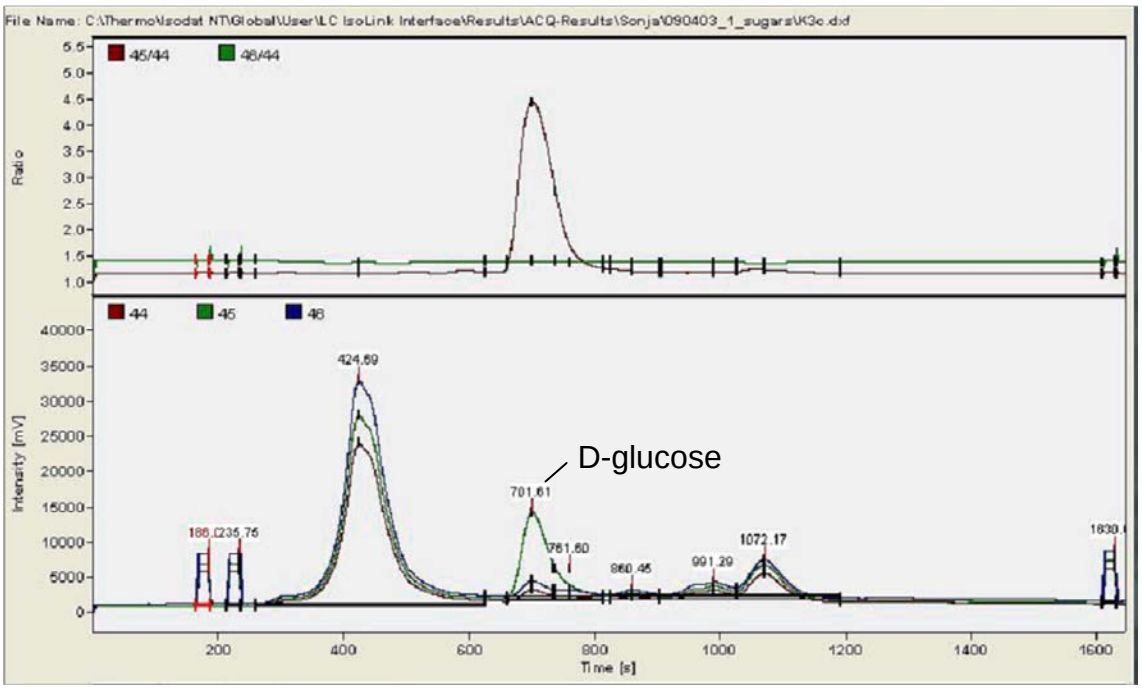

Figure A4: Harvest 1, Litter K, extracted after 120'
Concentration = 55 mg glucose l^{-1}, $\delta^{13}C$ = 5939

1.3. Spiked samples

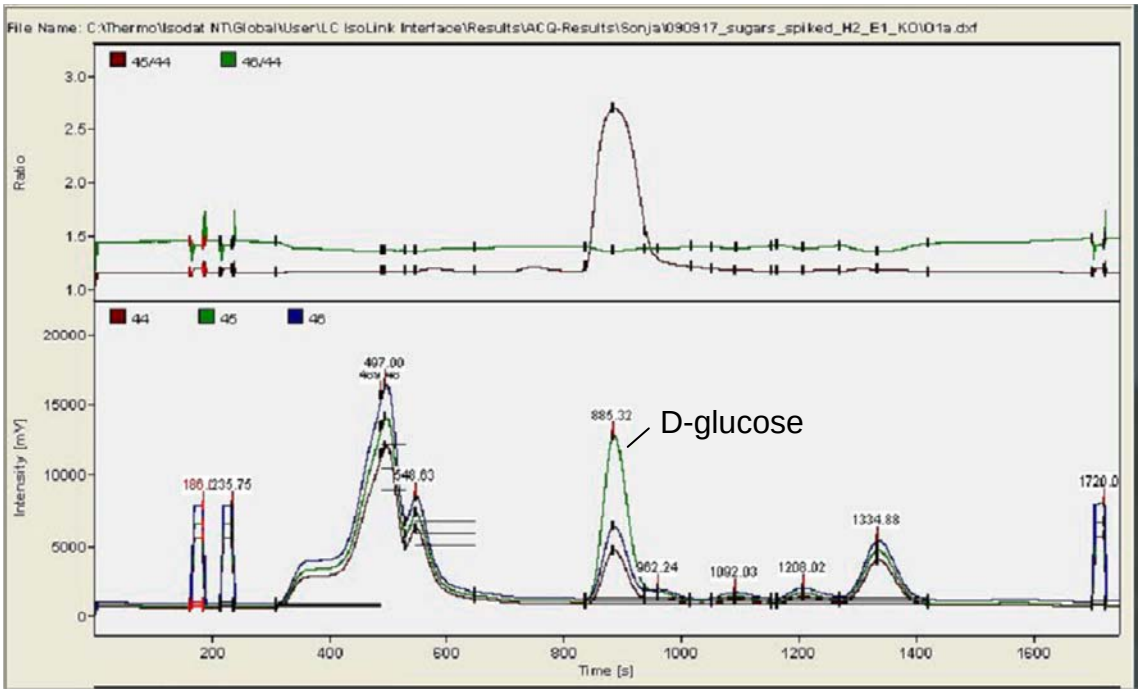

Figure A5: Harvest 2, Litter O, extracted after 30', spiked
Dilution 9:10 (90µl sample + 10 µl spike)

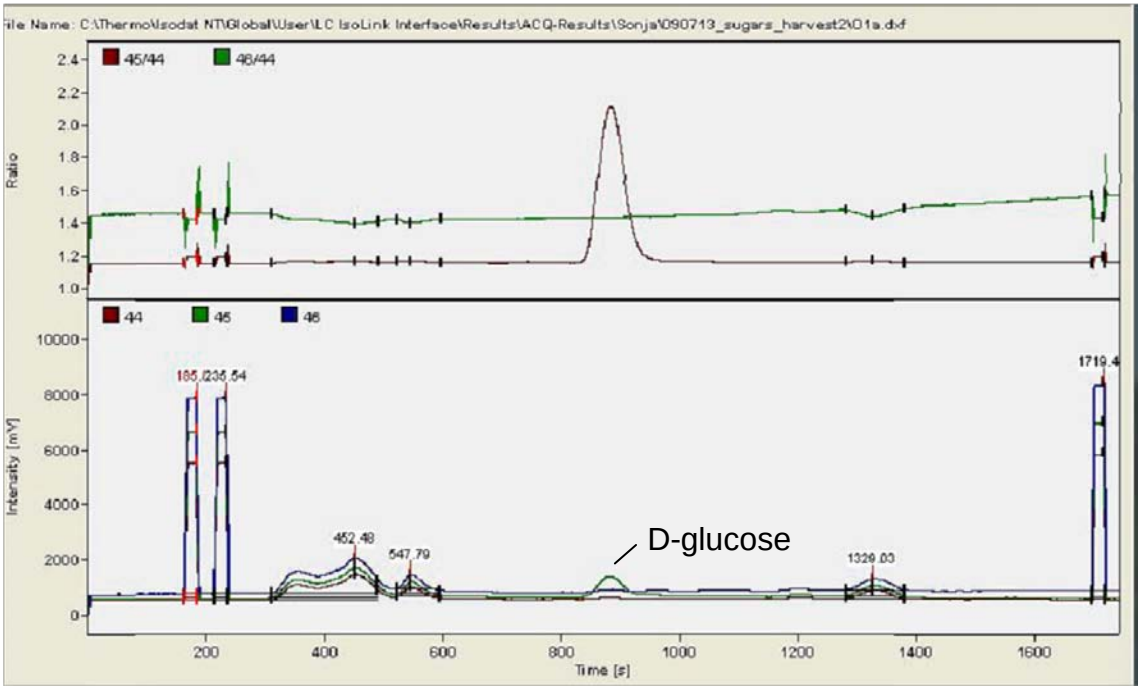

Figure A6. Harvest 2, Litter O, extracted after 30', not spiked
Dilution 1:10

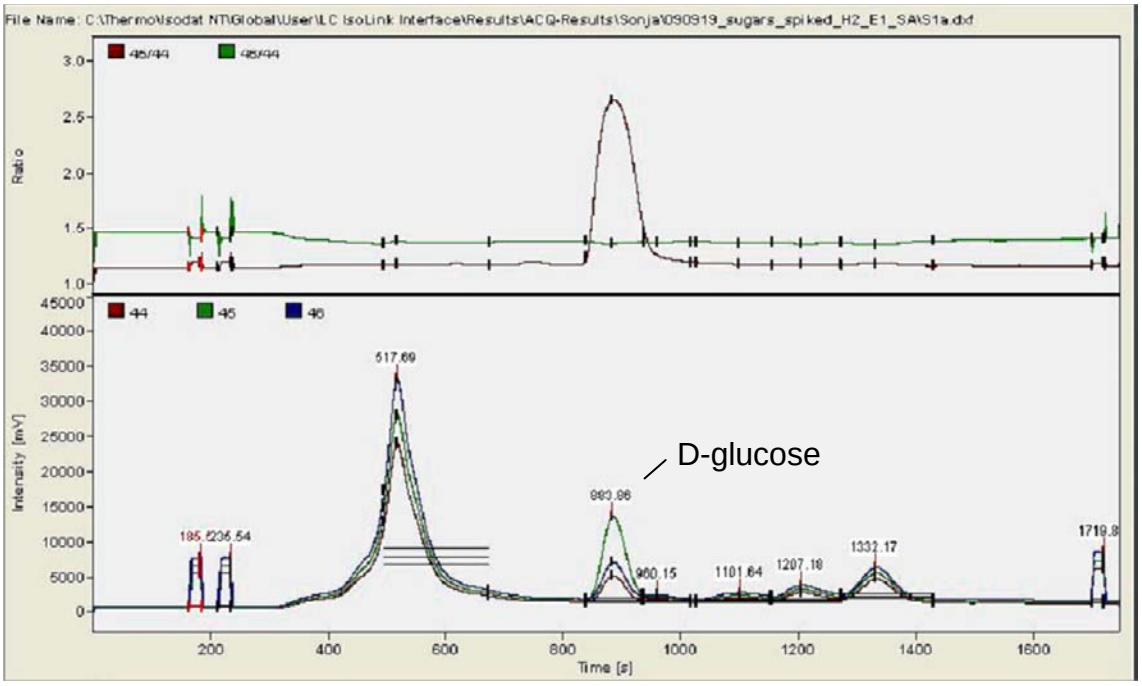

Figure A7: Harvest 2, Litter S, extracted after 30', spiked
Dilution 9:10 (90µl sample + 10 µl spike)

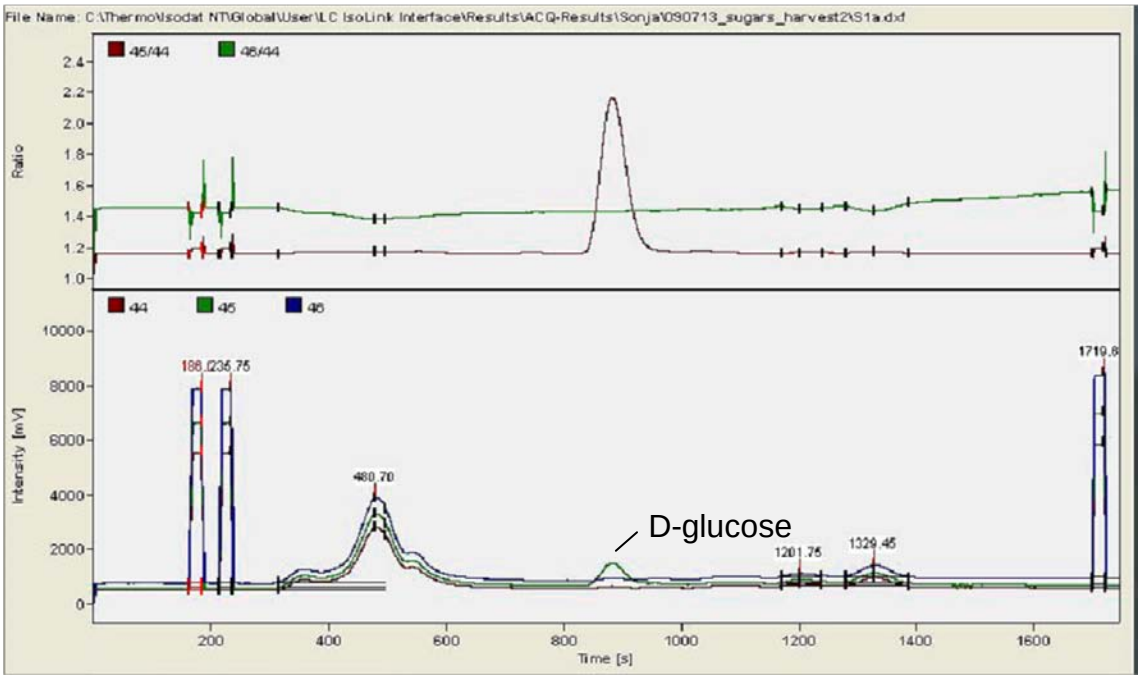

Figure A8: Harvest 2, Litter S, extracted after 30', not spiked
Dilution 1:10

2. Correction of $\delta^{13}C$ values

The HPLC-IsoLink-IRMS system exhibited a linear relationship between peak area and carbon concentration, but an offset of $\delta^{13}C$ values compared to referenced EA-IRMS standards was observed. This offset increased with decreasing concentrations. For correction, $\delta^{13}C$ standards of glucose and sucrose that had previously been measured on an EA-IRMS system were used in different concentrations between 25 and 300 mg l^{-1}. A multiple regression equation (3) was computed

(3) $$\delta_{offline} = a * \text{peak area} + b * \delta_{online} + c$$

where $\delta_{offline}$ and δ_{online} represent the standard's $\delta^{13}C$ values as measured by EA-IRMS and HPLC-IRMS, respectively. This equation can then used to calculate $\delta_{corrected}$, the corrected $\delta^{13}C$ values for the samples (Wild *et al.*, 2010).

3. References

Hettmann E, Brand WA, Gleixner G. 2007. Improved isotope ratio measurement performance in liquid chromatography/isotope ratio mass spectrometry by removing excess oxygen. *Rapid Communications in Mass Spectrometry* **21**(24): 4135-4141.

Wild B, Wanek W, Postl W, Richter A. 2010. Contribution of carbon fixed by Rubisco and PEPC to phloem export in the Crassulacean acid metabolism plant Kalanchoe daigremontiana. *Journal of Experimental Botany* **61**(5): 1375-1383.

Zusammenfassung

Um den Einfluss der chemischen Beschaffenheit und elementaren Zusammensetzung von Pflanzenstreu (Verhältnis von Kohlenstoff, Stickstoff und Phosphor, C:N:P) auf die Abbauraten von Glucanen zu untersuchen, wurde ein sechsmonatiges Streuabbauexperiment unter kontrollierten Bedingungen (Temperatur von 15°C, Feuchtegehalt der Streu von 60%) durchgeführt. Als Substrat wurde Buchenstreu (*Fagus sylvatica* L.) mit unterschiedlicher chemischer und elementarer Zusammensetzung verwendet, die sterilisiert und mit einer Suspension des organischen Horizonts eines Waldbodens beimpft worden war, um eine identische mikrobielle Gemeinschaft auf allen Streusorten herzustellen. Nach zwei Wochen sowie drei und sechs Monaten wurden Proben genommen und C-, N-, P-, Stärke-, Zellulose- und Ligningehalt der Streu, mirkobielle Respiration sowie Aktivitäten von Zellulasen, Peroxidasen und Phenoloxidasen gemessen. Bruttoraten der Glucoseproduktion durch Depolymerisierung von Glucanen und der Glucoseaufnahme durch Mikroorganismen wurden mit einer neu entwickelten Methode basierend auf der „Isotope Pool Dilution"-Technik bestimmt, bei welcher der Glucosepool in der Streu mit ^{13}C-Glucose markiert und die anschließende Verdünnung des Tracers durch Glucosefreisetzung aus Glucanen gemessen wird. Die Bruttoraten der Glucandepolymerisierung und Glucoseaufnahme waren höchstsignifikant miteinander korreliert, was eine gemeinsame Regulierung dieser Prozesse vermuten lässt, welche möglicherweise durch die Verknüpfung des mikrobiellen Bedarfs an Kohlenstoff und Energie mit der Zuteilung von Ressourcen zu Glucan abbauenden Enzymen erklärt werden kann. Des Weiteren fanden wir nach zwei Wochen Inkubation Korrelationen zwischen der Glucandepolymerisierung und dem Stärkegehalt der Streu, während die Depolymerisierung nach drei und sechs Monaten mit der Zellulosemenge und der Zellulaseaktivität korreliert war. Dies lässt vermuten, dass anfänglich hauptsächlich Stärke, mit fortschreitendem Streuabbau jedoch vermehrt Zellulose abgebaut wurde. Außerdem waren sowohl Glucandepolymerisierung als auch Glucoseaufnahme drei und sechs Monate nach der Beimpfung der Streu mit dem N-Gehalt sowie dem C:N und Lignin:N Verhältnis in der Streu korreliert. Die Glucoseaufnahme war zu keinem Zeitpunkt mit der Respiration korreliert, was darauf hindeutet, dass Glucose nicht das primäre Substrat für die Respiration war. Die Aktivitäten von Zellulasen, Peroxidasen und Phenoloxidasen waren nach

drei und sechs Monaten negativ mit C:N und C:P der Streu korreliert, was den Einfluss von N und P für den Glucanabbau zusätzlich hervorhebt. Die hier vorliegende Diplomarbeit konnte zum ersten Mal die Bedeutung der chemischen und elementaren Zusammensetzung der Streu für den Prozess der Glucan-Depolymerisierung nachweisen.

Curriculum Vitae

Personal information:

Name	Sonja Leitner
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Education:

Since October 2008	Diploma thesis, Department of Chemical Ecology and Ecosystem Research, Vienna University
August 2009	Study field trip to Siberia, Russia to study soil, vegetation and limnology on a transect from steppe to tundra
February 2008	Study field trip to Costa Rica to study tropical forest ecology
July 2007	Study field trip to the Austrian Alps to study alpine vegetation
26-09-2005	Intermediate degree in biology, Vienna University
Since 01-10-2003	University study in Biology, Vienna University
06-06-2003	Matura (final exam qualifying for university admission)
1995-2003	Secondary School St. Ursula in Vienna with focus on languages
1991-1995	Primary School Alt Erlaa in Vienna

Relevant work experience

Winter term 2010	Tutor for the course 'Interaction of Terrestrial and Aquatic Ecosystems', Department of Chemical Ecology and Ecosystem Research, Vienna University
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July – August 2010	Project assistant at the Institute of Silviculture, University of Natural Resources and Life Sciences, Vienna
March – August 2009	Research assistant at the Department of Chemical Ecology and Ecosystem Research, Vienna University
Winter term 2008	Tutor for the course 'Plant anatomy', former Department for Ecophysiology and Functional Anatomy of Plants, Vienna University