

# Carbohydrate and amino acid composition of dissolved organic matter leached from soil

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## Abstract

Low molecular weight organic substances (LMWOS) in soil and soil solution include mainly amino acids, carboxylic acids, and carbohydrates. Due to their high bioavailability they play a crucial role in the cycles of C and nutrients in soils. The variety of soil processes that involve LMWOS requires identifying their composition to elucidate reactions and transformations. In most studies, LMWOS are extracted under artificial conditions, e.g. batch experiments, which may overestimate the actual concentrations. This study measures the composition of carbohydrates and amino acids in solution of a Haplic Luvisol leached in a column experiment. A combined system for simultaneous leaching and blowout of CO<sub>2</sub> was used to estimate LMWOS decomposition. <sup>14</sup>C-labeled glucose was added as a highly sensitive tracer to control the efficiency of the LMWOS extraction by leaching and to estimate LMWOS decomposition during leaching. High performance liquid chromatography (HPLC), optimized for soil extracts, was used to analyze LMWOS composition. For HPLC optimization, different preparations of leached solutions (filtration vs. centrifugation, and drying vs. no-drying) were compared. For sugar determination, drying had no influence on the solution concentrations. In contrast, amino acid concentrations significantly decreased by drying LMWOS eluted substances. Combining the HPLC identification of eluted substances with <sup>14</sup>C tracer application revealed that about 5% of the glucose could be leached unchanged within 786 min (13.1 h), whereas about 84% remained in the soil, 9% were decomposed to CO<sub>2</sub>, and 2% were transformed to other LMWOS and recovered in the soil solution. The total amino acid concentration (TAC) in soil solution was about 8.2 μmol l<sup>-1</sup>, dominated by alanine (14.4% of TAC), glycine (13.4%), glutamic acid (9.9%), serine (9.4%), and leucine (9.3%). The total carbohydrate concentration was about 2.4 μM, dominated by glucose (29.9%), glucuronic acid (26.8%), and galacturonic acid (17.3%). Ratios of hexoses to pentoses, amino sugars glucosamine to galactosamine, and neutral sugars to uronic acids were determined. All three parameters pointed to the dominant influence of plants as the source of LMWOS in the leached soil solution. Within the small contribution of microorganisms, bacteria dominated over fungi. These used biomarker ratios as well as LMWOS concentrations differed widely from the ones obtained with conventional batch extraction. More research is necessary to evaluate the application of these biomarkers to soil solutions.

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## 1. Introduction

Sources of low molecular weight organic substances (LMWOS) in soil are the decomposition of diverse kinds of high molecular organic matter like soil organic matter, plant litter or microbial biomass, and root exudates (Kuzyakov and Domanski, 2000). LMWOS belong to the

water-soluble fraction and therefore leach out if not incorporated, degraded, or sorbed (Kalbitz et al., 2000).

Although LMWOS such as amino acids, carboxylic acids, and carbohydrates account only for less than 10% of the dissolved organic matter (DOM) in soil (Strobel, 2001; van Hees et al., 2005), they contribute strongly to nutrient cycling of C, N, and P and are the main C and energy source for soil microbial biomass. Nutrient transport in soil and uptake by plants and microorganisms is almost completely limited to the soluble fraction, i.e. DOM

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(Neumann and Römheld, 2002). LMWOS, as the most decomposable DOM fraction, are therefore predominantly mineralized by soil microorganisms. Several studies (Chapin et al., 1993; Jones et al., 2004; Kuzyakov and Jones, 2006) demonstrated that LMWOS such as amino acids and sugars can even be taken up directly by plants. Mineralization of LMWOS is fast and leads to short half-life times ranging from 1 h to 5 days (Jones and Darrach, 1994; Jones et al., 1996a, b; Kuzyakov and Demin, 1998).

On the other hand, charged LMWOS can be sorbed to clay minerals, sesquioxides, and soil organic matter, decreasing their effect on nutrient solubility and C turnover. Thus, Jones and Brassington (1998) and van Hees et al. (2003) found that from 50% to 95% of added LMW carboxylic acids and about 10% of amino acids (Jones and Hodge, 1999) were sorbed to the solid phase. These values strongly vary depending on the soil pH and the surface area of sesquioxides.

Rhizosphere soil solution concentrations can be as low as 0.1–100  $\mu\text{mol l}^{-1}$  for free amino acids (Jones and Willett, 2006; van Hees et al., 2005). While much information is available on the concentrations of amino acids and carboxylic acids in soil solution, and on extractable sugars (e.g. Hertenberg et al., 2002 with acetone/water), no study has been conducted on actual sugar concentrations in soil solution.

The composition of LMWOS gives evidence about their origin: since Oades (1984), it is known that the higher the ratio of hexoses to pentoses the higher is the contribution of microorganisms to the pool of sugars. Parsons (1981) found out that the ratio of some amino sugars elucidate whether they are produced mostly by bacteria or by fungi.

In most previous investigations, the LMWOS were extracted from the soil by batch experiments. During the standard shaking extraction, substances bound on organic matter and clay particles or stored in microbial cells can be released and then recorded as free LMWOS occurring in natural soils (Jones and Willett, 2006). In order to obtain the LMWOS, which are actually part of DOM, natural leaching conditions should be simulated (Kalbitz et al., 2000). To our knowledge, the composition of LMWOS leached from soil has never been investigated.

In contrast to the above-mentioned studies, we imitated natural leaching conditions instead of conducting batch extraction experiments to obtain LMWOS. The removal of substances from soil by leaching is correlated with their sorption by organic and mineral particles as well as with microbial utilization and decomposition. Thus, we obtained only the mobile fraction of LMWOS located in macro- and mesopores; under natural conditions, this fraction undergoes convective transport by seepage (Kalbitz et al., 2000).

Furthermore, to control the efficiency of the leaching process and to estimate LMWOS decomposition during leaching, we spiked the soil solution with  $^{14}\text{C}$ -labeled glucose. Microbial decomposition was estimated by measuring  $^{14}\text{C}$  activity in trapped  $\text{CO}_2$ . The extent of sorption

of glucose and derivatives as well as their retention in microbial biomass was assessed by measuring the  $^{14}\text{C}$  activity remaining in the soil.

Pre-column derivatization followed by ion-pair or reversed phase HPLC (RP-HPLC) as described by Fischer et al. (2003) and Meyer et al. (2001) as effective tool to measure LMWOS concentrations in seepage water and landfill leachates was used for determination of amino acids and carbohydrates.

The objectives of this study were

- to describe the composition of LMWOS (amino acids, sugars, and uronic acids) leached from soil,
- to determine the efficiency of LMWOS leaching by tracing  $^{14}\text{C}$ -labeled glucose and to determine the portion of glucose remaining in the soil and subjected to microbial decomposition, and
- to optimize sample preparation for HPLC analysis of LMWOS composition.

## 2. Materials and methods

### 2.1. Soil

Soil used in this experiment was a silty loamy Haplic Luvisol (FAO-UNESCO, 1997) from Heidfeldhof near Hohenheim University, Stuttgart, Germany (Table 1). Twelve years prior to sampling time, fruit trees stood on this site; thereafter, a continuous rotation of vegetables, legumes, and wheat was established. The first 10 cm of the Ap horizon were collected, air dried, and sieved (<2 mm). About 55 g soil was filled into 50 ml centrifuge tubes (VWR, Bruchsal, Germany), which were used for LMWOS leaching. The pore volume in these tubes was  $40.8 \pm 0.5\%$  (calculated by saturation of a defined volume of soil with water).

### 2.2. Experimental setup

The experimental setup is similar to that described by Kuzyakov and Siniakina (2001). A schematic is given in Fig. 1. In brief, the system consists of the container filled with soil (③), connected upstream with a flask filled with

Table 1  
Soil properties

Soil property	Value
pH ( $\text{H}_2\text{O}$ )	6.9
$\text{C}_{\text{tot}}$ (%)	1.5
$\text{N}_{\text{tot}}$ (%)	0.14
Mineral N ( $\text{mg kg}^{-1}$ )	14.6
Content of SOM (%)	2.2
Content of sand (%)	14.5
Content of silt (%)	62.8
Content of clay (%)	22.6

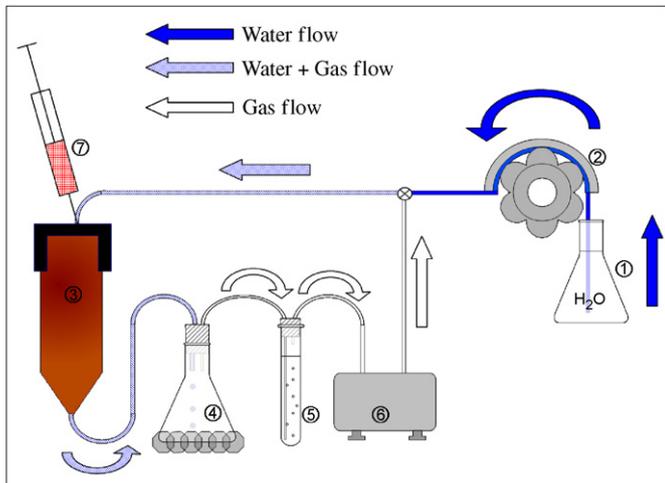


Fig. 1. Experimental setup: ① flask with deionized water for elution, ② peristaltic pump, ③ soil container (50 ml VWR centrifuge tube), ④ collection flask for leachate placed on ice to slow down decomposition, ⑤ test tube with NaOH for CO<sub>2</sub> trapping, ⑥ membrane pump, ⑦ place of glucose addition, and ⑧ joint where gas and water flows are connected.

deionized water for the elution (①) and downstream with a collection flask for the leachate (④) and a test tube (⑤) with NaOH solution to trap CO<sub>2</sub>. A membrane pump (wisa, Wuppertal, Germany; ⑥) propels the water–air mixture through these with silicon tubes connected compounds. As air is pumped through the soil container reductive conditions are avoided. Three modifications were made to the original setup: (1) A peristaltic pump (model GUV-150; meredos GmbH, Bovenden, Germany; Fig. 1, ②) was used to pump constant water into the tube system and the soil container. (2) Due to analytical interferences during HPLC measurements, no sterilization with HgCl<sub>2</sub> and/or NaN<sub>3</sub> was used. Therefore, the leachate collection flasks (④) were placed in ice during the leaching process. Afterward leaching, 0.05 vol% CHCl<sub>3</sub> was added to sterilize the sample. (3) Instead of the CombiStart filtration devices used by Kuzyakov and Siniakina (2001), 50 ml-centrifuge tubes (VWR) were used as soil containers (③). The lid and cone point of these tubes were drilled and connected with the tubes. The whole experiment was conducted in triplicates.

### 2.3. Labeling

One week prior to the experiment, the soil was moistened to about 75% water holding capacity (28% (w/w) corresponding to 15 ml) to allow development of a natural community of microorganisms. Via the opening in the lid, 1 ml solution of uniformly labeled <sup>14</sup>C glucose was added (37 μg (206 nmol) to each container, approximating a soil solution concentration of about 14 μmol l<sup>-1</sup> or 0.7 mg kg<sup>-1</sup> dry soil). Although this amount is well below “free sugar concentrations” extracted with an acetone/water mixture by Hertenberger et al. (2002) (40–392 mg kg<sup>-1</sup> soil), we assumed that this labeling would greatly affect the amounts

that we could extract with the presented technique. The added <sup>14</sup>C activity for each replication was 5.4 kBq <sup>14</sup>C.

### 2.4. <sup>14</sup>C measurement and sample preparation

Immediately after adding <sup>14</sup>C-glucose, the tubes were closed and the leaching started. The peristaltic pump was adjusted to a flow of 15 ml h<sup>-1</sup>. Samples of leachate and NaOH traps were taken simultaneously 100, 242, 387, 786, 979, 1129, and 1371 min after the start of the leaching.

The radioactivity of leachate and CO<sub>2</sub> trapped in NaOH solution was measured by adding 1 ml solution to 4 ml of scintillation cocktail EcoPlus (Roth Company, Germany). The remainder of leachate was frozen until preparation for HPLC analysis. NaOH traps were measured 1 day after mixing with the cocktail in order to allow chemiluminescence to decay. The <sup>14</sup>C counting efficiency ranged from about 85% for leachate samples to about 87% for NaOH traps.

For comparison of preparation methods for HPLC, 40 ml each of two representatives were pre-filtered through glass fiber filters (GF/D; Whatman, Brentford, UK). Then, both samples were divided into four aliquots and prepared in four different ways.

The first method consisted of a second filtration via a polycarbonate filter with pore size of 0.4 μm (Type 230 Sartorius, Göttingen, Germany) and a subsequent 10-fold concentration (from 10 to 1 ml) using a speedvac vacuum centrifuge (RVC 2–25, Chris GmbH). The sample was completely dried in the speedvac, and then 1 ml of deionized water was added to dissolve the precipitate.

In the second method, the sample was not completely dried after filtration but the procedure was stopped when less than 1 ml but more than 0.5 ml was left of each sample. They were then filled up to 1 ml again. Methods 3 and 4 corresponded to methods 1 and 2 except that, instead of filtration using polycarbonate filter, the samples were centrifuged for 10 min at 1500g. The other samples were filtered and completely dried. The obtained concentrated solutions were measured by HPLC.

After the end of the leaching, the soil was dried for 48 h at 50 °C and ground. The <sup>14</sup>C content of the soil was determined using an OX 400 Biological Oxidizer (Harvey Instruments Corporation, Hillsdale, NJ) and liquid scintillation counting (Wallac 1409; EG&G Ltd., Milton Keynes, UK).

All <sup>14</sup>C data are presented as a percentage of total recovered <sup>14</sup>C.

### 2.5. HPLC analysis

HPLC analyses on amino acids and carbohydrates (sugars and uronic acids) were done as described in Fischer et al. (2003) and Meyer et al. (2001). Only a summary is given in this text. All data were corrected by blank sample values, i.e. deionized water, which were prepared in the

same way (centrifugation, filtration, concentration via speedvac).

### 2.5.1. Amino acid analysis

After automatic derivatization (200  $\mu$ l of the OPA-derivatization solution were pipetted to 80  $\mu$ l sample in 160  $\mu$ l borate buffer (pH 10)), 50  $\mu$ l of the derivatized sample were injected onto the column (Hypersil ODS (150  $\times$  4 mm; 5  $\mu$ m) with a 4  $\times$  2 mm guard column (Phenomenex)). This was part of a Shimadzu HPLC system (autosampler SIL 10A, controller SCL-10AVP, gradient pump LC-10 ADVP and fluorescence detector SPD-10 AXL (340 nm; 445 nm)). Data acquisition and processing were accomplished with the Shimadzu CLASS VP 6.12 software.

### 2.5.2. Carbohydrate analysis

Fifty microliter of the sample were injected into the Dionex IC system (autosampler AS 50, gradient pump GP 40 and electrochemical detector ED 40 with a thin-layer type amperometric cell). Columns were CarboPac PA 20 (3  $\times$  150 mm) with 3  $\times$  30 mm CarboPac PA 20 guard column and an amino trap guard (4  $\times$  50 mm) (Dionex). Data acquisition and processing were done with the Dionex Chromeleon 6.70 software. The analytes were detected with a quadrupole-potential waveform on the gold-electrode.

### 2.6. Statistical analyses

Statistical analyses such as paired-samples *T* tests and correlation analyses were performed using SPSS 10.0.1 for windows (SPSS Inc., Chicago, USA). For correlation analysis the nonparametric Spearman coefficient was preferred to the more common Pearson coefficient for correlation analyses, when not stated otherwise. The Pearson coefficient is stricter than the Spearman coefficient. However, it requires normality of the data. Wherever possible we used the stricter Pearson coefficient. Nevertheless, when normality of the data set was not confirmed with the Kolmogorov–Smirnov test or when the data set was too small for this decision, we used the Spearman ranking coefficient. Data sets were in most cases too small ( $n < 15$ ) to reject the hypothesis of normal distribution at the necessary level of significance.

## 3. Results

### 3.1. Comparison of preparation methods

The combination of two steps of the sample preparation (centrifugation vs. filtration and complete drying vs. not complete drying) yielded four data sets for each sample.

Correlations between data sets of different sample preparations were highly significant for all four combinations ( $P < 0.01$ ). However, the coincidence of the individual

substances obtained by different methods was not very high ( $r^2 < 0.60$ ) (Fig. 2).

For sugars in general, drying had no significant influence on the solution concentrations ( $P > 0.05$ ). In contrast, amino acid concentrations were significantly lower in the dried and re-dissolved samples than in the incompletely dried samples ( $P < 0.01$ ). On average, amino acid concentrations of the undried samples were more than 25% higher than in the dried ones (Pearson, slope of regression equation = 1.28; Fig. 2). Some of the amino acids were clearly more affected by drying than others, among them leucine, glycine, and to a lesser extent serine and alanine.

No significant difference between filtration and centrifugation was observed ( $P > 0.05$ ), either for sugars or for amino acids. Filtration—and not complete drying—was chosen as a uniform method for all further samples.

### 3.2. Tracer recovery

Recovery rates of  $^{14}\text{C}$  in four individual replicated soil containers ranged from 89% to 119% of the input. The variation was connected with uneven distribution of  $^{14}\text{C}$  remaining in the soil. An average of  $10.1 \pm 0.5\%$  of the recovered  $^{14}\text{C}$  activity was measured in the leachate;  $6.0 \pm 1.3\%$  was trapped as  $\text{CO}_2$  in NaOH solution. The remainder,  $83.9 \pm 1.7\%$ , was recovered in the soil, where it may have been sorbed to the mineral or organic soil components or incorporated into microorganisms.

The dynamics of both leaching rate and  $\text{CO}_2$  efflux were characterized by a constant decline in  $^{14}\text{C}$ , which dropped below 0.5% per hour within 300 min after pulse labeling (Fig. 3). The overall recovery in  $\text{CO}_2$  was about half of the recovery in the leachate, indicating that, within the leaching time, about one-third of the not sorbed and not incorporated glucose (and decomposition products) was decomposed to  $\text{CO}_2$ . The longer the leaching lasted the higher became the relative contribution of  $^{14}\text{C}$  trapped in NaOH in comparison to leachate  $^{14}\text{C}$  (see Fig. 3).

### 3.3. Validation of $^{14}\text{C}$ as a proxy for eluted LMWOS

To evaluate the usefulness of the  $^{14}\text{C}$ -labeled glucose as an easily measurable proxy for LMWOS leaching, we examined the correlations between LMWOS concentrations and  $^{14}\text{C}$  activity in all eluate samples. The  $^{14}\text{C}$  in the eluate showed high and highly significant correlations with certain substances, among them threonine and arginine (both  $r^2 = 0.95$ ,  $P < 0.01$ ) arabinose ( $r^2_{\text{Pearson}} = 0.70$ ,  $P < 0.01$ ), and rhamnose ( $r^2_{\text{Pearson}} = 0.81$ ,  $P < 0.01$ ). Conversely, the correlations were surprisingly low for glucose ( $r^2_{\text{Pearson}} = 0.27$ ,  $P < 0.05$ ). For the remaining measured substances, however, no significant correlations to the  $^{14}\text{C}$  activity in the sample could be found.

The glucose and total carbohydrate concentrations dropped from first sampling to second sampling to only 10% of their initial value (Fig. 4). Although the glucuronic acid concentration did not decrease as fast as that of other

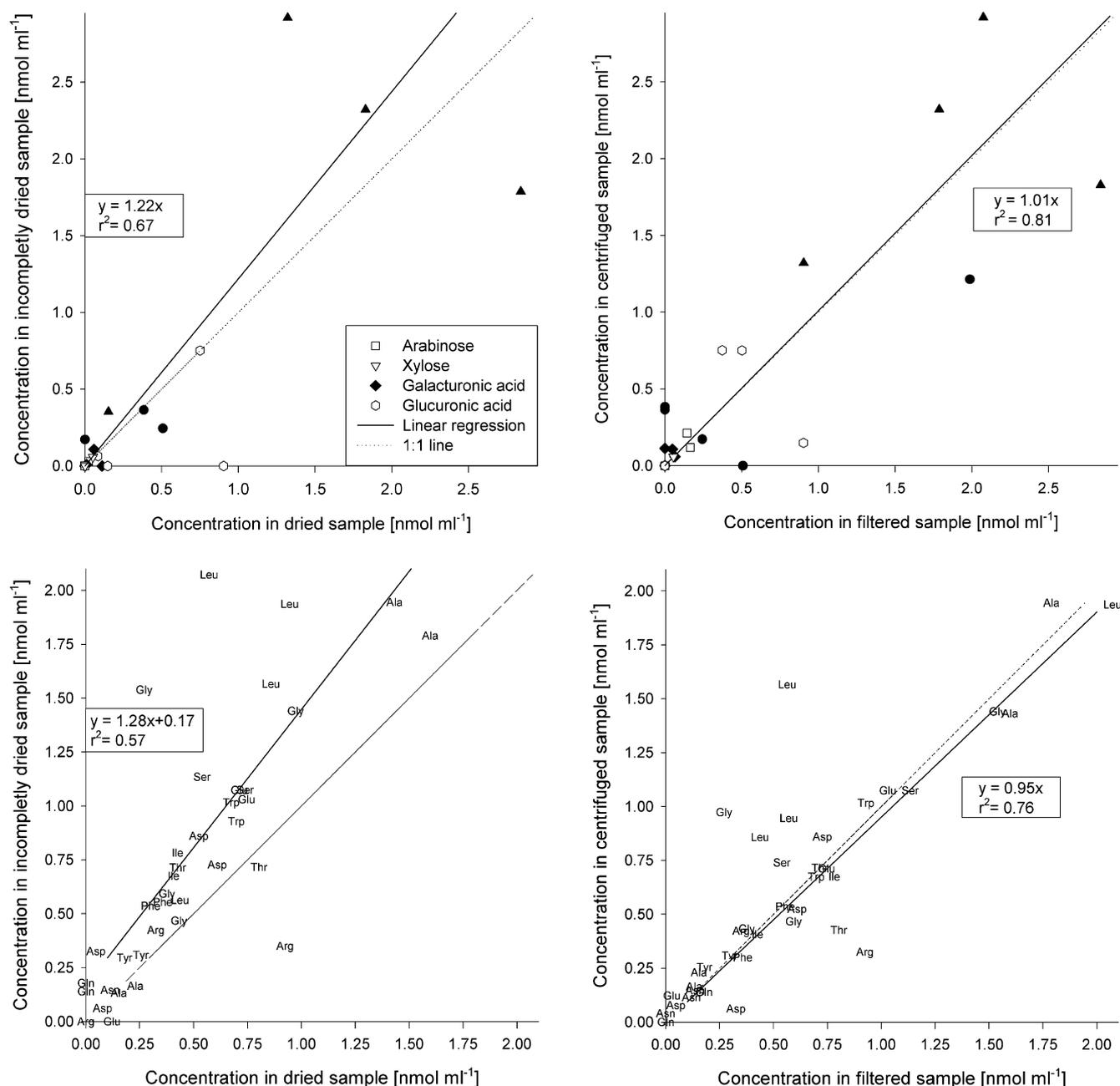


Fig. 2. Comparison of sampling procedures (drying vs. incomplete drying) (left) and filtering vs. centrifuging (right) of selected sugars (top) and amino acids (bottom). *Note:* Presented concentrations are sample concentrations and not soil solution concentrations.

carbohydrates, it dropped faster than the  $^{14}\text{C}$  activity concentration. In contrast, the total amino acid concentration decreased more slowly and remained at about 40% of the initial value after 300 min.

### 3.4. The amino acid and carbohydrate composition of DOM

In total, 122.7 nmol of amino acids and 35.7 nmol of carbohydrates were leached from one 55 g soil column within 786 min (13.1 h) (Table 2). As at the onset of leaching, one column had 15 ml water, the theoretical soil solution concentrations at the beginning were  $8.18 \mu\text{mol l}^{-1}$  for amino acids and  $2.41 \mu\text{mol l}^{-1}$  for carbohydrates. The

recovered amino acids were dominated by alanine (14.4% of all recovered amino acids), glycine (13.4%), glutamic acid (9.9%), serine (9.4%), and leucine (9.3%). The pool of eluted carbohydrates was dominated by glucose (29.9% of all recovered carbohydrates), probably mostly originating from the labeled glucose added to the soil. Apart from glucose, high concentrations were measured for the uronic acids glucuronic acid (26.8%) and galacturonic acid (17.3%). The amino sugars galactosamine and glucosamine made up 10.8%. In contrast, the hexoses mannose and galactose, mostly microbically produced, were partly below the detection limit and totaled only 0.8% of total carbohydrates.

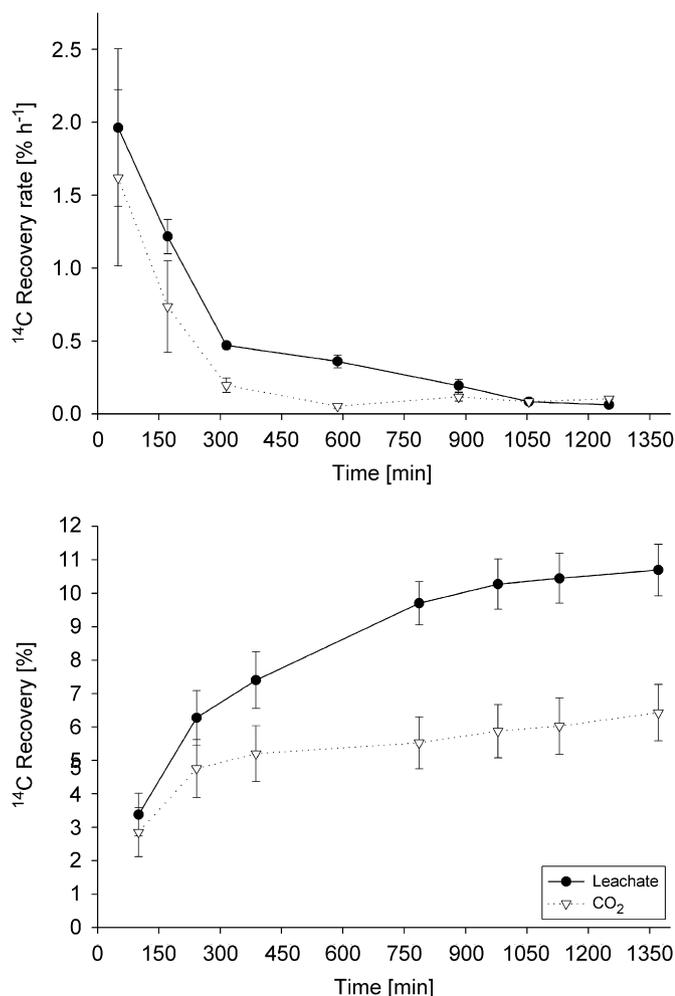


Fig. 3. Dynamics of  $^{14}\text{C}$  (top) and its cumulative activity (bottom) in the LMWOS leachate and  $\text{CO}_2$  trapped in  $\text{NaOH}$  ( $\pm$ S.E.) after adding  $^{14}\text{C}$ -glucose. Shown are mean values with S.E. ( $n = 4$ ).

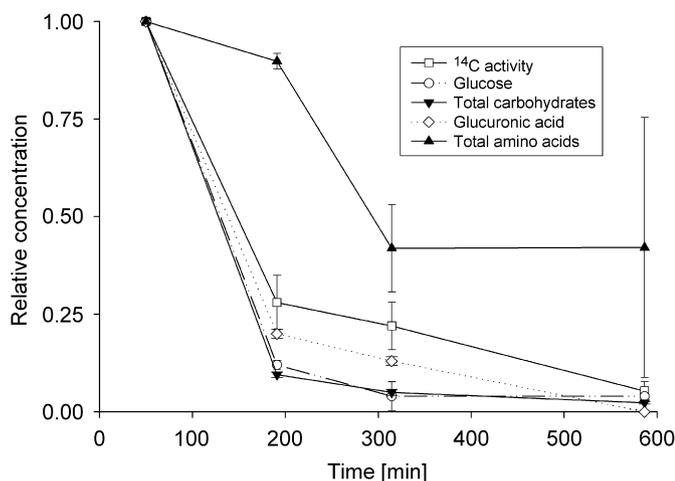


Fig. 4. Decrease in concentrations of glucose, glucuronic acid, total carbohydrates, total amino sugars, and  $^{14}\text{C}$  activity compared to the concentrations of the first leaching cycle. For all variables, data have been set to “1” for the sample of the first cycle. Shown are means with S.E. ( $n = 2-5$ ).

## 4. Discussion

### 4.1. Optimization of sample preparation

Complete drying of leached solutions before the HPLC analysis decreased the average amino acid concentrations by more than one-fourth (slope of regression equation = 1.28; Fig. 2). Carbohydrates were less affected than amino acids. However, the regression equation is strongly influenced by the high values of glucose, which suffered greater losses by drying than the other sugars.

The concentrations of aliphatic amino acids—leucine, glycine, alanine, and serine—are more affected by drying than those of other amino acids. The charge of the amino acids apparently played only a minor role with regard to retrieval in dried vs. undried samples. Decomposition experiments (Kuzuyakov and Demin, 1998) have shown that glucose (sugars) was decomposed to  $\text{CO}_2$  more slowly than simple amino acids, especially in the first days.

The incomplete dissolving of precipitated amino acid complexes, however, more likely explains their low recovery after drying. Engels et al. (2000) provided two reasons why carboxylic acids might not be detected by HPLC: (1) Citrate and other carboxylic acids form with Ca, Fe, and Al, metal complexes of partly low solubility which cannot be re-dissolved completely in water at neutral pH. (2) Other metal ions form soluble complexes, which can be measured by HPLC but are often not recognized as the uncomplexed substances due to changed retention time. These complexing abilities might be less distinctive for amino acids than for di- and tri-carboxylic acids. Nevertheless, they can also principally occur in amino acids (G. Neumann, Hohenheim University, personal communication). Additionally, amino acids may react with other components of DOC and form complexes that are insoluble when the dried samples are remoistened.

Jones and Willett (2006) found, in agreement with our results, no significant difference between filtering and centrifuging of soil extract samples. By using water elution instead of the traditional shaking, we mainly sampled the soil solution in macro- and mesopores. Substances located inside the aggregates might have been incompletely leached.

### 4.2. Tracer recovery

Considering that leaching was started with about  $15 \text{ ml h}^{-1}$  immediately after  $^{14}\text{C}$ -glucose addition, the recovery of  $^{14}\text{C}$  in eluate and as  $\text{CO}_2$  trapped in  $\text{NaOH}$  is low. As not only water but also air was pumped through the soil cores, it is very unlikely that lack of oxygen limited microbial decomposition of the glucose to  $\text{CO}_2$ . Within 90 min all the pore water in one container (22 ml) should be completely replaced. Nevertheless, only 16.1% of the added  $^{14}\text{C}$  activity were recovered outside the soil container after more than 20 h of leaching. For sugars, no sorption mechanism is known (Kuzuyakov and Jones, 2006). Never-

Table 2  
Amounts (nmol), soil contents ( $\mu\text{mol kg}^{-1}$  and  $\mu\text{g kg}^{-1}$ ), and concentrations in soil solution ( $\mu\text{mol l}^{-1}$ ) of amino acids and carbohydrates after 786 min (13.1 h) of leaching

Substance	Total amount in leachate (nmol)	Content ( $\mu\text{mol kg}^{-1}$ soil)	Content ( $\mu\text{g kg}^{-1}$ soil)	Concentration in soil solution ( $\mu\text{mol l}^{-1}$ ) <sup>a</sup>	Percentage (mol/mol)
Aspartic acid	9.1	0.17	21.9	0.61	7.4
Glutamic acid	12.2	0.22	32.6	0.81	9.9
Asparagine	2.5	0.05	6.0	0.17	2.0
Serine	11.6	0.21	22.1	0.77	9.4
Glutamine	6.1	0.11	16.2	0.41	5.0
Histidine	2.9	0.05	8.1	0.19	2.3
Glycine	16.5	0.30	22.4	1.10	13.4
Threonine	7.0	0.13	15.2	0.47	5.7
Arginine	3.9	0.07	12.4	0.26	3.2
Alanine	17.6	0.32	28.6	1.18	14.4
Tyrosine	2.8	0.05	9.4	0.19	2.3
Methionine	0.7	0.01	1.8	0.04	0.5
Tryptophan	8.2	0.15	30.4	0.55	6.7
Phenylalanine	4.3	0.08	13.0	0.29	3.5
Isoleucine	5.8	0.11	13.9	0.39	4.8
Leucine	11.4	0.21	27.1	0.76	9.3
Total amino acids	122.7	2.23	281.1	8.18	100.0
Fucose	0.9	0.02	3.0	0.06	2.5
Galactosamine	2.2	0.04	7.3	0.17	6.1
Rhamnose	2.4	0.04	7.3	0.18	6.8
Arabinose	1.5	0.03	4.0	0.13	4.3
Glucosamine	1.7	0.03	5.6	0.11	4.7
Galactose	0.3	0.01	1.1	0.02	0.8
Glucose	10.7	0.19	35.0	0.72	29.9
Xylose	0.3	0.01	0.7	0.02	0.8
Galacturonic acid	6.2	0.11	20.0	0.39	17.3
Glucuronic acid	9.6	0.17	31.1	0.61	26.8
Total carbohydrates	35.7	0.65	115.1	2.41	100.0
(Mannose + galactose)/ (arabinose + xylose) <sup>b</sup>	0.16		0.23		
GluN/GalN <sup>c</sup>	0.77		0.77		

<sup>a</sup>Calculated with 15 ml water.

<sup>b</sup>Mannose was below detection limit.

<sup>c</sup>GluN, glucosamine; GalN, galactosamine.

theless, we cannot rule out that some capillars were closed after leaching began due to aggregate swelling and colloid transport inside the pores. The remaining 83.9% of the glucose found in soil was either incorporated by soil microorganisms and thereby protected from leaching or decomposed to products that were subject to sorption. It is not surprising that glucose incorporation is preferred to decomposition into  $\text{CO}_2$ . Sugai and Schimel (1993) reported similar results: only 17% of added glucose was transformed to  $\text{CO}_2$  and 74% were incorporated in microbial biomass, whereas, of the phenolics salicylic acid and *p*-hydroxybenzoic acid, far more (67% and 33%, respectively) were decomposed to  $\text{CO}_2$ , albeit within 48 h.

The correlation between  $^{14}\text{C}$  recovery and glucose concentration in eluate was surprisingly low. This is a clear proof that eluted  $^{14}\text{C}$  was not the original glucose but its transformation products, other LMWOS and  $\text{CO}_2$ . Experiments with samples of similar origin (same soil, same

labeling, but different leaching rate) proved that the  $^{14}\text{C}$  activity of eluate samples is reduced by about one-third to half within 1 min when pH is lowered from around 5 to 0.5 (data not presented). We therefore assume that, instead of 10%, only 5–7% of the glucose was eluted as LMWOS (including glucose), whereas 9–11% (rather than only 6%) were decomposed to  $\text{CO}_2$ .

Concordantly, glucose in the leachate accounted for less than 11 nmol, while 20 nmol of tracer  $^{14}\text{C}$  was found there (10%  $^{14}\text{C}$  recovery in leachate of 200 nmol initially added glucose). Glucose decomposition before leaching and subsequent leaching of the decomposition products is probable. Consequently, almost 50% of the leachate  $^{14}\text{C}$  activity originates not from the added glucose but from its decomposition products. As mentioned above, there is evidence that between one-third and one-half of leachate  $^{14}\text{C}$  has to be allotted to  $\text{CO}_2$ . The rest has presumably been transformed to substances that are products in the

anabolism of microorganisms. We could not assess how much of the glucose was decomposed and what the decomposition products were based on the radioactivity measurements because  $^{14}\text{C}$  activity would have remained in solution.

Fig. 4, however, demonstrates that all carbohydrate concentrations decreased in the same pattern, while  $^{14}\text{C}$  activity did not drop as distinctly. For this reason, other carbohydrates including glucuronic acid are unlikely products of glucose decomposition. The high correlations for rhamnose and arabinose with the  $^{14}\text{C}$  activity are easily explained: the concentrations of both sugars rapidly dropped to almost zero after the first sampling. In contrast, the amino acid concentrations decreased more slowly—not before 200 min of leaching. Next to sorption, which could have delayed leaching, continuous production of amino acids, e.g. from glucose decomposition, is a potential explanation.

#### 4.3. Composition of amino acids and carbohydrates in soil solution

About  $8.2\ \mu\text{mol l}^{-1}$  ( $0.281\ \text{mg kg}^{-1}$  soil) of total amino acids were leached from the soil. Our data agree with Christou et al. (2006), who found similar concentrations of free amino acids ( $16 \pm 1\ \mu\text{M}$ ) in Mediterranean vineyard soil; they are also in the range of other investigations on free amino acids, e.g. on free amino acids extracted via centrifugation (e.g. Jones and Willett (2006), mostly  $1\text{--}100\ \mu\text{mol l}^{-1}$ ).

On the other hand, these values are far below those obtained with batch studies even with mild extractants like 10% ethanol (Warman and Bishop, 1987) or acetone/water mixture (Hertenberger et al., 2002), where “free” amino acids contents ranged from  $6.3$  to  $22\ \text{mg kg}^{-1}$  soil.

As mentioned above, no data on sugars in soil solution was available. Only those values obtained from landfill leachates (Fischer et al., 2003) were in the same range ( $0.3\text{--}0.7\ \mu\text{mol l}^{-1}$  for xylose).

The contents of total leachable amino acids were more than 2.4 times higher than those of leachable total carbohydrates ( $281.1\text{--}115.1\ \mu\text{g kg}^{-1}$  soil); this is equivalent to a factor of almost 3.4 when transformed to concentrations in soil solution ( $8.2\text{--}2.4\ \mu\text{mol l}^{-1}$ ). This ratio in soil solution does not correspond to the findings of Hertenberger et al. (2002) and Martens et al. (2003), who extracted soil with an acetone/water mixture and acid, respectively. All found that the carbohydrate content exceeded the amino acid content by a factor of 2–10! Measurements in different environments using various sample preparation methods complicate direct comparisons. The composition of LMWOS clearly differs widely with factors like soil type or experiment type (pot vs. field scale). The greatest influence, however, is that most studies hydrolyze whole soil prior to LMWOS analysis, whereas we leached soil solution with water. Moreover, glucose addition may have an effect on the leachate composition: glucose is a strong

energy source for microorganisms, which can increase N immobilization in microorganisms and modify subsequently N composition of leachates. Also, the increase in production of microbial metabolites after glucose addition can modify the leachate composition.

Hexoses like galactose (gal) and mannose (man) are mostly produced by microorganisms, and pentoses like arabinose (ara) and xylose (xyl) by plants. Total pentose contents in leaves and needles of plants extracted with  $4\ \text{mol l}^{-1}$  trifluoroacetic acid are in the range of  $125\text{--}240\ \text{mg g}^{-1}$  (Sariyildiz and Anderson, 2003, 2005, 2006) and thus increase soil contents (about  $3\text{--}30\ \text{mg g}^{-1}$  soil, e.g. Guggenberger et al., 1994) for the 50-fold. Fast decrease of pentoses in litter of up to 37.5% within 6 months was found by Sariyildiz and Anderson (2003) and gives evidence of the origin of pentoses in soil. Oades (1984) suggested using the ratio  $(\text{gal} + \text{man})/(\text{ara} + \text{xyl})$  as an indicator for microbial (when  $>2$ ) or plantal origin ( $<0.5$ ). As gal occurs in plant material (Baldock et al., 1987) and some microorganisms can produce ara (Murayama, 1977), the ratio  $(\text{man}/\text{xyl})$  may be even more accurate. All these ratios, however, are based upon total sugar contents obtained by acidic hydrolysis. Galactose and mannose concentrations were near or even below the detection limit (combined  $<1\%$  of all carbohydrates), while arabinose and xylose totaled 5.1%. We therefore attributed the found sugars mainly to plant origin.

The two measured amino sugars galactosamine (GalN) and glucosamine (GluN) made up 10.8% of all recovered carbohydrates. These relatively high contributions concur with the results of Amelung et al. (2001) that amino sugars resist decomposition better than other organic residues and thus become enriched during decomposition.

While GalN is assumed to be entirely bacteria-derived, GluN can be found in chitin. As chitin input into soil is almost exclusively contributed to fungi (Parsons, 1981), the ratio GluN/GalN can be used to determine the relative contributions of bacteria and fungi to the amino sugar pool. The higher the GluN/GalN value, the greater is the fungal contribution. From the earliest works of Snowdon (1959), GluN/GalN values in soils have been reported to range from 1 upwards, with a range of 1.3–2.6 being common (e.g. Kogel and Bochter, 1985; Turrion et al., 2002). Amelung et al. (2002) even reported ratios of 6 in the sand fraction of a South African Plinthustalf. Values  $>7$  can be found in litter layer of forests (Turrion et al., 2002), indicating a strong contribution of fungi.

The GluN/GalN ratio in the leachate recovered in the present experiment was 0.77 and is therefore outside the range known to the authors. The contribution of fungal amino sugars to the soil solution is clearly far below that of bacteria. Note again that the comparative data was gained by acidic hydrolysis (vs. leached soil solution date here). Nevertheless, fungi probably play a minor role (if any) in the turnover of organic matter in our soil containers. Furthermore, our samples originated from soil used for 12 years for vegetable cultivation. A shift from bacterial to

more fungal residues may indicate declining N availability in soil (Amelung et al., 2002). Reciprocally, our high bacteria input values in the soil solution match well with the high N input in intensive cropping at the sampling site via legumes.

Uronic acids are oxidized forms of monosaccharides. Galacturonic acid is a main component of pectin, which builds up cell walls of higher plants. Glucuronic acid is a possible decomposition product of glucose because it is created when the sixth C-atom of a glucose molecule is oxidized. After acidic hydrolysis of forest floor leachates and soil solution, Kaiser et al. (2004) reported ratios of neutral sugars to uronic acids of about 7–50. In our study the ratio is almost 1:1. Again, we assume that the different DOM sampling approach is the main reason for different LMWOS compositions.

## 5. Conclusions

The presented leaching approach and the sample preparation for HPLC enabled the amino acids and carbohydrates in DOM to be measured while it must be admitted that glucose as tracer did show differences in leaching from most of the other measured LMWOS.

Sample preparation by filtration and centrifugation had no effect on HPLC results. Complete drying should be avoided when concentrating the samples, especially prior to amino acids analyses.

Amino acid and carbohydrate concentrations in the leachate were much lower than those extracted in batch experiments. Our use of leaching to obtain LMWOS differs completely from batch extraction, complicating direct comparisons. In our opinion, however, our results show representative amounts of the mobile and thus active fraction of LMWOS.

All three parameters—the pentose-to-hexose ratio, GluN/GalN, and the neutral sugar-to-uronic acid ratio—point to the dominant influence of plants as the source of LMWOS in the leached soil solution. Compared to plants, microorganisms play a minor role based on the hexose-to-pentose ratio and the high level of galacturonic acid. Within the (small) contribution of microorganisms, the amino sugar ratio underlines the dominance of bacteria over fungi.

We showed that soil solution concentrations, composition, as well as the biomarker ratios differed greatly from those values obtained from whole soil with acidic hydrolysis. Determining the importance of soil solution in the ecosystem will require further research, with a focus on evaluating the named biomarkers.

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