

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 CO₂ was used to estimate LMWOS decomposition. ¹⁴C-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 ¹⁴C 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 CO₂, 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⁻¹, 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}C -labeled glucose. Microbial decomposition was estimated by measuring ^{14}C activity in trapped CO_2 . The extent of sorption

of glucose and derivatives as well as their retention in microbial biomass was assessed by measuring the ^{14}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}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 ($<2\text{ mm}$). 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 (H_2O)	6.9
C_{tot} (%)	1.5
N_{tot} (%)	0.14
Mineral N (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

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