Rhizodeposition of maize: Short-term carbon budget and composition

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Abstract

The aim of this study was to assess differences in rhizodeposition quantity and composition from maize cropped on soil or on 1:1 (w/w) soil–sand mixture and distribution of recently assimilated C between roots, shoots, soil, soil solution, and CO_2 from root respiration. Maize was labeled in $^{14}CO_2$ atmosphere followed by subsequent simultaneous leaching and air flushing from soil. ^{14}C was traced after 7.5 h in roots and shoots, soil, soil solution, and soil-borne CO_2. Rhizodeposits in the leachate of the first 2 h after labeling were identified by high-pressure liquid chromatography (HPLC) and pyrolysis–field ionization mass spectrometry (Py-FIMS). Leachate from soil–sand contained more ^{14}C than from soil (0.6% *vs.* 0.4%) and more HPLC-detectable carboxylates (4.36 *vs.* 2.69 μM), especially acetate and lactate. This is either because of root response to lower nutrient concentrations in the soil–sand mixture or decreasing structural integrity of the root cells during the leaching process, or because carboxylates were more strongly sorbed to the soil compared to carbohydrates and amino acids. In contrast, Py-FIMS total ion intensity was more than 2 times higher in leachate from soil than from soil–sand, mainly due to signals from lignin monomers. HPLC-measured concentrations of total amino acids (1.33 μM [soil] *vs.* 1.03 μM [soil–sand]) and total carbohydrates (0.73 *vs.* 0.34 μM) and $^{14}CO_2$ from soil agreed with this pattern. Higher leachate concentrations from soil than from soil–sand for HPLC-measured carbohydrates and amino acids and for the sum of substances detected by Py-FIMS overcompensated the higher sorption in soil than in sand-soil. A parallel treatment with blow-out of the soil air but without leaching indicated that nearly all of the rhizodeposits in the treatment with leaching face decomposition to CO_2. Simultaneous application of three methods—^{14}C-labeling and tracing, HPLC, and Py-FIMS—enabled us to present the budget of rhizodeposition (^{14}C) and to analyze individual carbohydrates, carboxylates, and amino acids (HPLC) and to scan all dissolved organic substances in soil solution (Py-FIMS) as dependent on nutrient status.

Key words: amino acids / carbohydrates / carboxylates / ^{14}C budget / maize / HPLC / rhizodeposition / composition / Py-FIMS / methods

Accepted March 25, 2009

1 Introduction

The root–soil interface—the rhizosphere—is an important hotspot of microbial activity in the soil. A high and diverse community of soil microorganisms is fed by root exudation. This involves the continuous active (secretion) and passive (diffusion) release of organic substances from undamaged roots cells into soil and C input *via* release from damaged cells (lysates), *i.e.*, rhizodeposition (*Neumann* and *Römheld*, 2007). Rhizodeposits consist of over 200 organic compounds, mainly low-molecular-weight organic substances (LMWOS) such as amino acids (AA), carbohydrates (CH) and carboxylic acids (CA), lipids and phenols (*Farrar* et al., 2003; *Kraffczyk* et al., 1984). The LMWOS concentrations in the rhizosphere are one order of magnitude higher than in bulk soil and range from 0.1 to 1000 μM (*Christou* et al., 2006; *Jones*, 1998; *Strobel*, 2001; *van Hees* et al., 2002, 2005).

Highly soluble LMWOS are rapidly mineralized by soil microorganisms. This leads to short half-life times that range from <1 min to a few hours in solution (*Boddy* et al., 2007; *Hill* et al., 2008) and from 1 h to a few days in microbial biomass (*Jones* and *Darrah*, 1994; *Jones* and *Shannon*, 1999; *Kuzyakov* and *Demin*, 1998). Half-lives of sorbed LMWOS can be considerably longer ((*Kuzyakov* and *Demin*, 1998).

Low-molecular-weight organic substances play a crucial role in plant nutrition because they significantly increase the concentrations of dissolved ions such as Fe^{2+}, Cu^{2+}, Mn^{2+}, and PO_4^{3-}. They are also important in the detoxification of Al^{3+} (*Dakora* and *Phillips*, 2002; *Jones* et al., 2003). Low nutrient supply frequently boosts rhizodeposition (*Neumann* and *Römheld*, 2002). An increase takes place in root exudation of CA (especially under deficiency of P [*Gerke* et al., 1994; *Neu-*

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mann and *Römheld*, 1999; *Wittenmayer* et al., 1995]) or Fe (*Jones* et al., 1996; *Römheld*, 1991). For maize, *Kraffczyk* et al. (1984) showed that exudation of CH, CA, and AA increased with decreasing K supply. Concordantly, *Werth* and *Kuzyakov* (2006) demonstrated that more recently assimilated C was present in exudates of maize grown under low than under high nutrient supply.

On the other hand, charged LMWOS can be sorbed by clay minerals and soil organic matter (SOM), thus decreasing their effect on nutrient solubility and C turnover. Accordingly, *Jones* and *Brassington* (1998) and *van Hees* et al. (2003) found that from 50% up to 95% of added LMW CA and of AA (*Jones* and *Hodge*, 1999) was sorbed to the solid phase. However, these values may strongly vary depending on the soil pH, surface of clays, and sesquioxides.

Many investigations on rhizodeposition involve plants grown in nutrient solutions. The significance of such results in real soil–root systems is under debate. For example, *Barber* and *Lynch* (1977) and *Meharg* and *Killham* (1991) found that root exudation was up to 5 times lower in sterile media than when microorganisms were present. Moreover, roots grown in solution instead of soil might be morphologically different because of lacking mechanical impedance. This would result in different branching systems, *e.g.*, less root hairs—all factors that influence rhizodeposition (*Jones*, 1998).

Low-molecular-weight organic substances (especially CH) released by roots into soil are frequently recovered by acidic extractants, such as 4 M trifluoracetic acid (*e.g., Sariyildiz* and *Anderson*, 2006) or 0.17 M H_2SO_4 (*Martens* and *Loeffelmann*, 2002). These acidic solutions extract not only dissolved substances but also sorbed and polymeric material with low bioavailability; they may lead to partial hydrolysis of LMWOS. The recommendation when estimating concentrations in soil solution is to use water as an extractant in combination with centrifugation techniques (*e.g., Zabowski*, 1989). *Fischer* et al. (2007) found that the concentrations and composition of LMWOS leached directly from soil with water differed considerably from those obtained by batch experiments.

Analyzing soil solution or soil extracts *via* high-pressure liquid chromatography (HPLC) or gas chromatography (GC) standard methods with selective sample pretreatments usually revealed the substances of the expected classes (usually CH, AA, and CA). Other ecosystem-relevant compound classes such as lipids, fatty acids, peptides, and aromatic substances, however, are frequently not considered. Another approach for identifying a broad range of substances is pyrolysis–field ionization mass spectrometry (Py-FIMS). Py-FIMS has already been used to analyze aquatic humic substances and dissolved organic matter (DOM) (*Schulten* et al., 2002), physical fractions and bulk soil organic matter (SOM) (*Schulten* and *Leinweber*, 1999), and rhizodeposits from maize (*Kuzyakov* et al., 2003a; *Melnitchouck* et al., 2005) and potato plants (*Melnitchouck* et al., 2006). Py-FIMS has the advantage that expected substances such as AA, CH, and CA but also unexpected compounds can be detected in soil extracts and solid samples without derivatization or other pretreatments.

Leinweber et al. (2008) developed a Py-FIMS method to measure minimal amounts as small as 5 μL of leachates from soil with maize plants. They demonstrated that the new micro-method reflected the influence of substrate (soil or sand–soil) on the mass-spectrometric "fingerprint" of leachates. This yielded a pattern of molecule ions detected by the Py-FIMS. That study, however, evaluated neither the compound classes that can be derived from the summed Py-FI mass spectra, nor the thermal evolution curves of individual compounds, nor compound classes that indicate the stability of chemical bonds. Furthermore, that study did not include data from HPLC analyses of the samples.

Therefore, our objectives in the current study were:

(1) to assess the effect of nutrient level on

   a) short-term LMWOS distribution by tracing ^{14}C in the respective pools, and

   b) LMWOS composition by complementary analysis of HPLC and Py-FIMS;

(2) to estimate the rhizodeposition by parallel treatment, where only CO_2 was collected and the soil was not leached;

(3) to elucidate the advantages and shortcomings of each method (^{14}C, HPLC, and Py-FIMS).

2 Materials and methods

2.1 Soil

A Haplic Luvisol (*IUSS Working Group WRB*, 2007) from the experimental field Heidfeldhof of the University of Hohenheim, Stuttgart (SW Germany) was used. The soil had the following properties: pH 6.9, C_{tot} 1.5%, N_{tot} 0.14%, mineral N 14.6 mg kg^{-1}, SOM 2.2%. Concentrations of plant-available nutrients (CAL: Ca-acetate-lactate extract) were 250 mg kg^{-1} K, 160 mg kg^{-1} Mg, 225 mg kg^{-1} P. The first 10 cm of the Ap horizon were taken, air-dried, and sieved (<2 mm). For half of the treatments, the soil was mixed with an equal amount of quartz sand, changing the texture from 22.6% clay, 62.9% silt, 14.5% sand (silty loam, *FAO-UNESCO*, 1997) to 11.3% clay, 31.4% silt, 57.3% sand (sandy loam). Quartz-sand addition also reduced all macro- and micronutrients in the soil by 50%.

The soil and the soil–sand mixture were filled in 50 mL centrifuge tubes (VWR, Bruchsal, Germany). The lid and cone point of these tubes were drilled and connected with PVC tubes. In the center of the lid, 2.5 mL reaction vials (Eppendorf, Hamburg, Germany) were inserted. Their rounded bottoms and their lids were removed. Hot glue (Pattex, Henkel KG, Germany) was used to connect the vials with the lids of the centrifuge tubes. These planting containers were filled

with 58 g dry soil or with 69 g of the 50:50 mixture of quartz sand and soil.

2.2 Plants

Seeds of maize (*Zea mays* L. cv. "Amadeo") were germinated at 20°C for 2 d. Then, equally developed seedlings were selected: All had one main root axis of length. One seedling was placed in the middle of the centrifugation tubes.

Soil moisture was kept at 21% (w/w). Plants were watered daily with deionized water and grown for 20 d in a day/night rhythm of 24°C/18°C, 50% relative humidity, 12 h photoperiod, with a light intensity of 300 μmol m^{-2} s^{-1}. On the day before the labeling (day 19), 16 equally developed plants (eight on soil, eight on soil–sand) with five leaves and an average height of (range 17.0–19.5 cm) were selected. No visible signs of any nutrient deficiency were detected.

2.3 Carbon-14 labeling

One day before the labeling, the holes of the reaction vials were sealed above the seeds with a two-component, nonphytotoxic silicone rubber paste (NG 3170; Thauer & Co., Dresden, Germany). All seals were tested for air-tightness: Air was pumped into the sealed containers, and the seals were covered with small amounts of water. No evolution of bubbles was considered as sign for air-tightness. The two other openings were closed with plugs. The pots were put into an airtight Plexiglas chamber (0.5 × 0.5 × 0.6 m^3). A flask with 124 kBq ^{14}C per plant (1.984 MBq total) in form of $Na_2{}^{14}CO_3$ solved in water (pH 10) was connected *via* a tube. Adding 6 mL 2 M H_2SO_4 caused the evolution of $^{14}CO_2$, which was channeled completely into the Plexiglas chamber, where it was equally mixed in the air *via* a fan. After 2 h, air was pumped out of the chamber for 45 min by two membrane pumps (Wisa, Wuppertal, Germany). CO_2, including not assimilated $^{14}CO_2$ of the labeling, was trapped in a NaOH flask to evaluate the efficiency of the labeling.

2.4 Experimental set-up

Four treatments were established in four replicates: 1) soil simultaneously leached with water and blown out with air (SOIL), 2) soil–sand mixture simultaneously leached with water and blown out with air (SAND), 3) soil blown out with air (AIR-SOIL), and 4) soil–sand mixture blown out with air (AIR-SAND).

The eight pots of SOIL and SAND were then connected to an experimental set-up similar to that described by *Kuzyakov* and *Siniakina* (2001) with the modifications made by *Fischer* et al. (2007) (Fig. 1). Deionized water (1) was pumped by a peristaltic pump (model GUV-150, Meredos GmbH, Bovenden, Germany, (2) into a PVC-tube system with circulating air driven by a membrane pump (Wisa, Wuppertal, Germany, (6) in Fig. 1). The air-water mixture was piped to soil-filled containers (50 mL centrifuge tubes, VWR, Bruchsal, Germany, (3) in Fig. 1). Downstream of these containers a collection flask for the leachate (4) and a test tube (5) with NaOH solution (1 M) to trap CO_2 was connected. Pumping air through the soil container prevented reductive conditions. To minimize microbial decomposition of the leachate, the collection flasks were placed on ice during the leaching period. When leaching was finished, for sterilization $CHCl_3$ was added to reach final concentration 0.05%$_{vol}$ $CHCl_3$.

The other eight plant pots (AIR-SOIL and AIR-SAND) were connected to the same experimental set-up without water. Thus, neither the peristaltic pump nor the collection flask for the leachate was built in the air current cycle. Merely the test tube with NaOH solution was installed to trap CO_2.

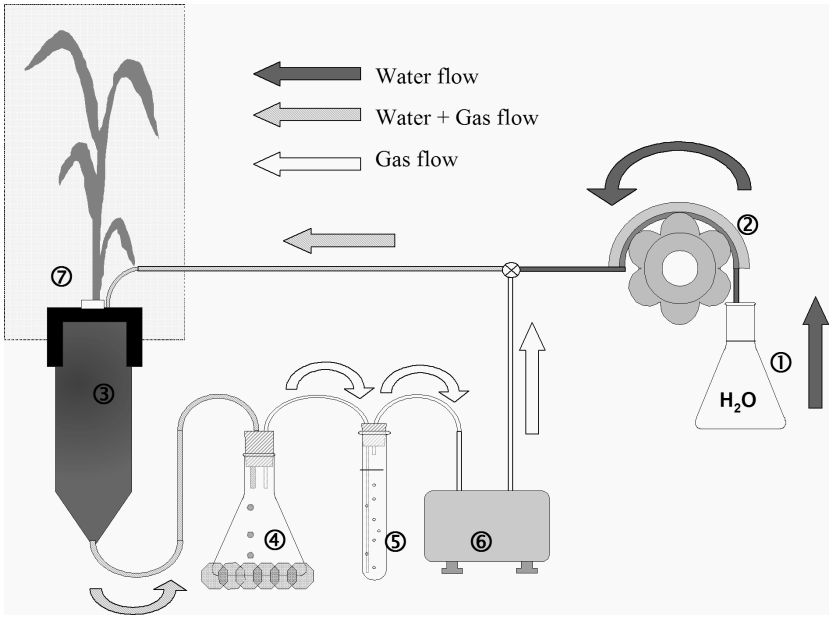

Figure 1: Experimental setup: ① Flask with deionized water for elution, ② peristaltic pump, ③ soil container (50 ml VWR centrifuge tube), ④ collection flask for leachate placed on ice to slow down decomposition, ⑤ test tube with NaOH for CO_2 trapping, ⑥ membrane pump, ⑦ labeling chamber (removed for leaching), ⊗ joint where gas and water flows are connected.

2.5 Sample preparation and ^{14}C analyses

Immediately after removing the labeling chamber, the leaching and blow-out apparatus was connected. The peristaltic pump rate was adjusted to a water flow of 50 mL h^{-1}. Samples of leachate and NaOH were taken simultaneously at 30 min, 110 min, 240 min, 448 min, and 523 min after begin of the leaching period. In this process, the sampling bottles were emptied completely.

Carbon-14 activity of leachate and CO_2 trapped in NaOH solution was measured by adding 1 mL solution to 4 mL of scintillation cocktail EcoPlus (Roth Company, Germany). NaOH samples were measured 1 d after mixing with the cocktail in order to allow decay of chemiluminiscence. The ^{14}C counting efficiency ranged from for leachate samples to for NaOH samples. NaOH traps worked with efficiencies > 90% (checked by two in series connected NaOH traps). CO_2 not adsorbed during the first passing through the NaOH trap is inert in the tubes and in the soil and thus will be trapped during the next passing through the NaOH trap. For this reason, values for ^{14}C from the NaOH traps did not need to be corrected.

After the end of the leaching, the shoots were cut and roots were separated from the soil by washing. All three compartments (soil, shoots, and roots) were dried for 48 h at 50°C and then ground with a ball mill (Retsch, Reutlingen, Germany). The ^{14}C of these solid samples was measured by liquid scintillation counting after combustion of 200 mg of plant samples or 1 g of soil samples within an oxidizer unit (Model 307, Canberra Packard Ltd., Meriden, USA), absorption of the ^{14}C in Carbo-Sorb E (Perkin Elmer, Inc., Boston, USA), and addition of the scintillation cocktail Permafluor E^+ (Perkin Elmer, Inc.).

For measurement with HPLC and Py-FIMS, sample pretreatment was performed as described by *Fischer* et al. (2007): filtration *via* glass-fiber filter (GF/D, Whatman, Brentford, UK) and subsequently *via* 0.4 μm polycarbonate filter (Type 230 Sartorius, Göttingen, Germany), 10-fold concentration (from 10 mL to 1 mL) using a speedvac vacuum centrifuge (Savant, Richmond, CA, USA). Subsequently, samples were kept in the dark at –18°C until analysis with HPLC and Py-FIMS.

2.6 HPLC and Py-FIMS measurements

Leachate samples of the first and the second sampling were measured. Concentrations of LMW CA in the first leaching fraction were expected to be largely dominated by existent organic matter accumulated in the existing substrate, while the influence of recent rhizodeposition is greater in the composition of the second leaching fraction.

For AA and CH, the methods and chemicals presented in *Meyer* (2008) were used. Carboxylic acids were measured according to *Neumann* (2007) after isocratic separation on a reversed-phase C-18 column (GROM-SIL 120 ODS-5 ST, particle size 5 μm; length 250 mm, inner diameter 4.6 mm) with a Hypersil ODS guard column (20 mm, ID 4.6 mm; GROM, Herrenberg, Germany) in the ion-suppression mode (*Neumann*, 2007); 18 mM KH_2PO_4 pH 2.1 was used as eluent with a flow rate of 0.5 mL min^{-1} and column temperature of 35°C. The carboxylates were identified and quantified by comparing the retention times and peak areas with those of known standards and by recording absorption characteristics.

For temperature-resolved Py-FIMS, we used a new rapid micro-method that involves the following steps: (1) injection of 5 μL aliquots of the concentrated leachates into the quartz crucible and drying over silica gel in a desiccator for 6 h, (2) insertion of the quartz crucible with dry leachate into the ion source of a Finnigan MAT 731 (Finnigan, Bremen, Germany) modified high-performance (AMD Intectra GmbH, Harpstedt, Germany) mass spectrometer, (3) heating the sample under high vacuum (10^{-4} Pa) from 50°C to 700°C in steps of 10 K per magnetic scan, (4) soft ionization of the evolved molecules at the carbon needles of the field-ionization (FI) emitter at a potential difference of 14 kV, (5) recording of about 60 magnetic scans in the range *m/z* 15 to 900 during 18 min of total registration time, and (6) data processing such as calculation of the relative abundance of ten important compound classes of organic matter for every single spectrum based on the indicator signals and integration of the single scan spectra over the whole temperature range to obtain one summed spectrum (*Leinweber* et al., 2008).

2.7 Statistics

Significant differences in ^{14}C recovery between treatments were determined by one-way ANOVA (SPSS) followed by *post-hoc* Tukey HSD test when homogeneity of variances was confirmed. If not, the nonparametric Dunnet T3 test was applied. To compare mean LMWOS concentrations of the soil treatment with the soil–sand treatment independent samples, t-tests with $p < 0.05$ were conducted. Outliers were eliminated according the procedure of Grubbs (*Hartung* et al., 1995).

3 Results

3.1 Plants

Differences in dry mass of shoots or roots were not significant after 20 d between plants grown on soil (SOIL and AIR-SOIL) and those grown on soil–sand mixture (SAND and AIR-SAND) (Tab. 1).

Table 1: Dry masses of maize roots and shoots grown on soil (SOIL, AIR-SOIL) and soil–sand (SAND, AIR-SAND). Mean values ± SE, $n = 8$.

	Shoot dry mass / mg	**Root dry mass** / mg	**Roots-to-shoots**
SOIL, AIR-SOIL	213 ± 20	225 ± 22	1.06 ± 0.13
SAND, AIR-SAND	199 ± 13	258 ± 24	1.30 ± 0.13

3.2 Carbon-14 recovery

About 99.3% of the applied 1.984 MBq ^{14}C were assimilated by the plants within the 2 h of labeling. The remaining 0.7% (13.8 kBq) was found after labeling in the labeling chamber and in the container with the labeling solution. Amounts of recovered ^{14}C in all compartments are given as mean recovery per pot in percent of assimilated amount of ^{14}C (Tab. 2). Thus, 100% refers to 123 kBq (= 1.970 MBq/16). On average, 65.0% of the assimilated ^{14}C was found in the monitored compartments (soil, roots, shoots, leachate, and soil-borne CO_2). As shoot respiration was not measured here, ^{14}C not recovered was probably lost by aboveground respiration of the plants.

Table 2: Carbon-14 recovery after 7.5 h in all compartments of plant–soil–atmosphere (mean values ± SE, 2–4 replicates, in% of the allotted amount of ^{14}C per plant, = 124 kBq). Different letters indicate significant difference ($p < 0.05$).

	Leached and blow-out		**Only blow-out**	
Compartment	SOIL	SAND	AIR-SOIL	AIR-SAND
Shoots	63.0 ± 19.2	52.5 ± 2.5	7.7	52.6 ± 2.0
Roots	n.m.	6.8 ± 1.5	8.3 ± 1.6^{c}	3.2 ± 1.3^{d}
Soil	02.8 ± 0.2	4.8 ± 0.6	3.3 ± 0.4	4.2 ± 1.1
CO_2 from soil	01.0 ± 0.1	0.6 ± 0.1^{a}	1.8 ± 0.3	2.2 ± 0.6^{b}
Leachate	00.4 ± 0.0	0.6 ± 0.1	–	–
Total	**67.1 ± 19.2**	**65.3 ± 5.4**	**65.4 ± 7.8**	**62.1 ± 2.7**

After leaching for 7.5 h, most ^{14}C was found in the plants (shoots and roots, >55.8%), while the other compartments (soil, CO_2, leachate) contributed within this short time only little to total ^{14}C recovery (< 6.0%). The mean ^{14}C recovery in leachate from SAND was higher than in leachate from SOIL at all sampling times, whereas trapped $^{14}CO_2$ was higher in SOIL than in SAND (Fig. 2, left). In the treatments AIR-SOIL and AIR-SAND, the ^{14}C amount was higher than the sum of ^{14}C amounts in leachate and CO_2 of SOIL and SAND during the whole sampling time (Fig. 2, right).

3.2 HPLC analyses of leachates

3.2.1 Amino acids

Leachate AA concentrations significantly decreased from the first to the second leaching fraction in both treatments for Glu, Ser, Thr, Phe, and total AA ($p < 0.05$) (Fig. 3 and Fig. 4).

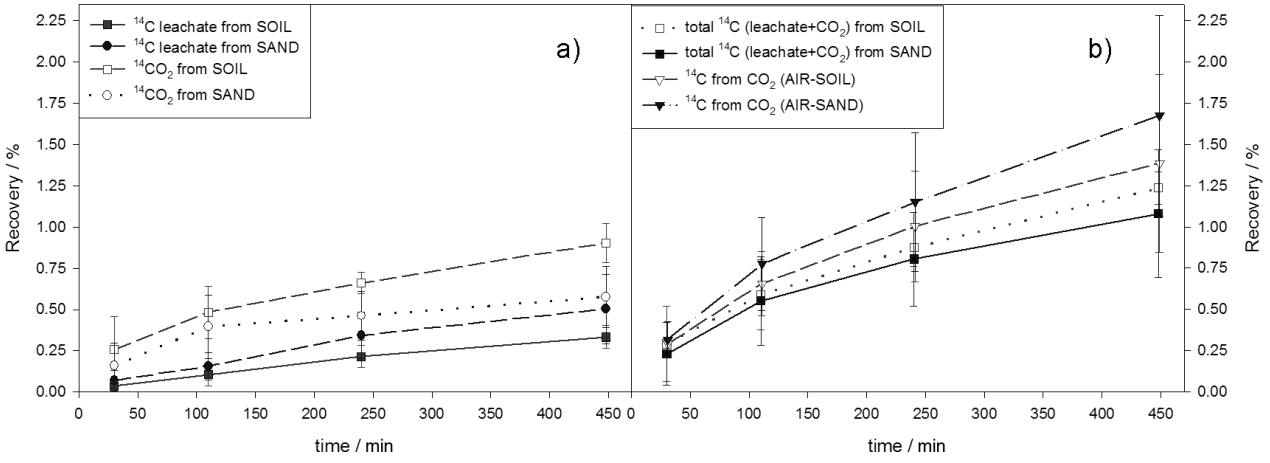

Figure 2: Cumulative ^{14}C in a) leachate (full symbols, straight line) and CO_2 (open symbols, dotted line) in soil (treatment A) and soil–sand (treatment B), b) sum of leachate and CO_2 of treatments A and B (straight line, squares), and CO_2 solely from blow-out treatments C and D (triangles, dotted line). Mean values ± SE, $n = 4$.

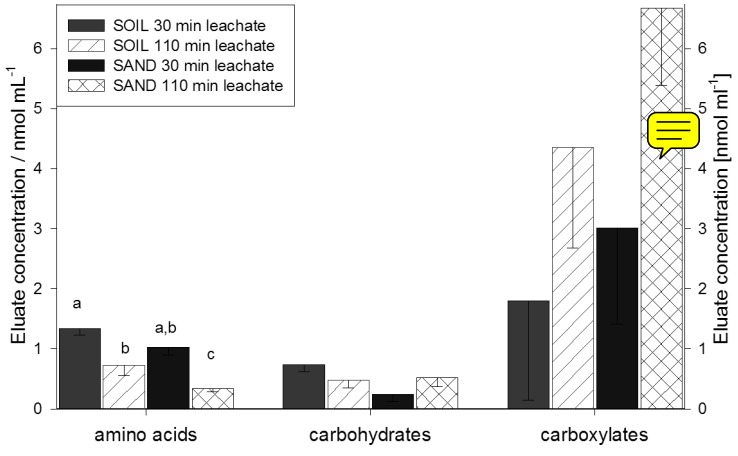

Figure 3: Substance classes in leachates of soil and sand–soil treatments at two leaching fractions determined by HPLC: total amino acids, carbohydrates, and carboxylates [nmol mL^{-1}]. Mean values ± SE, n = 2–4. Different letters indicate significant difference ($p < 0.05$). Note: Depicted concentrations are eluate concentrations, *i.e.*, measured concentrations were divided by 10 (due to 10-fold concentration of the samples during preparation).

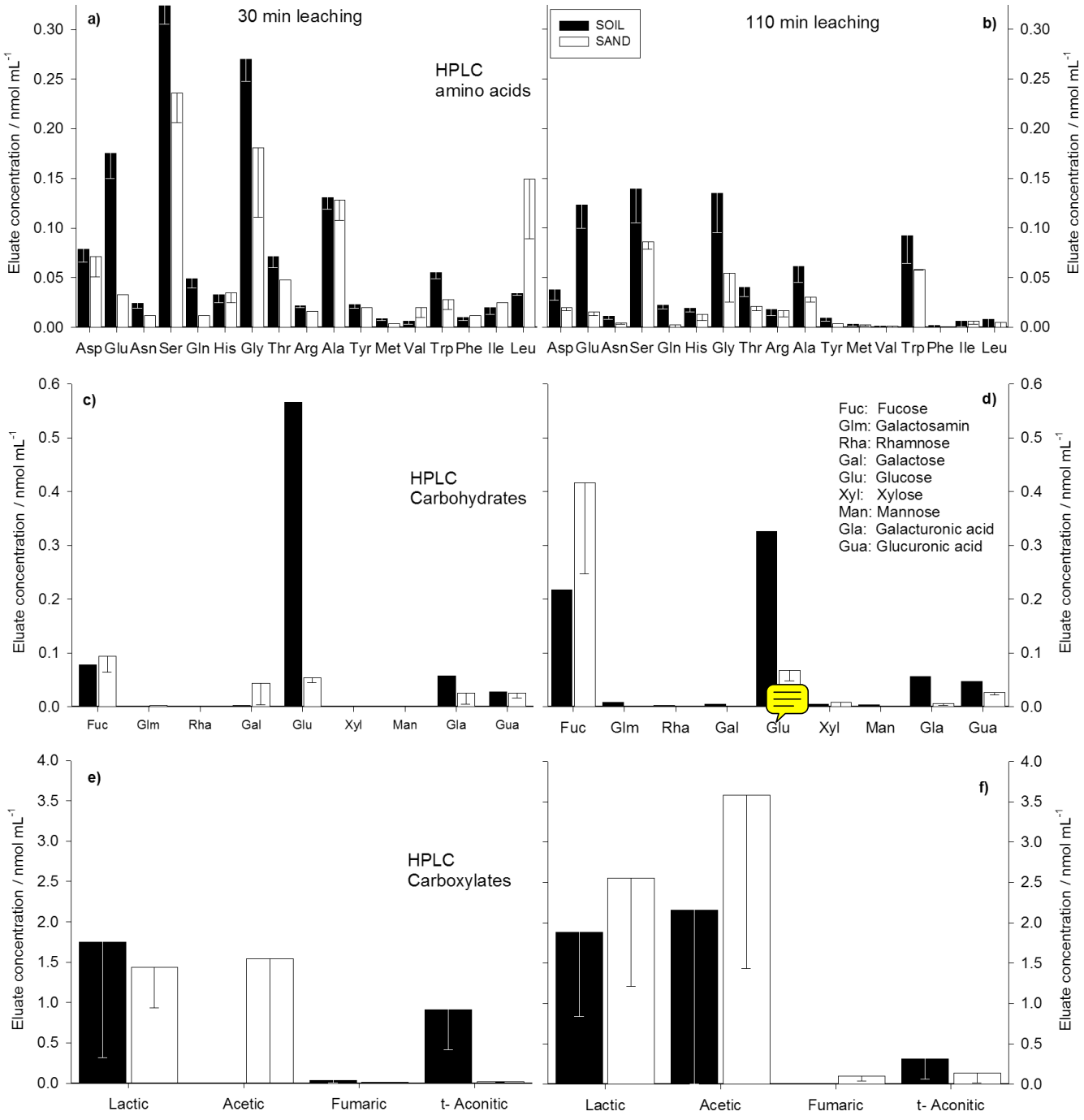

Figure 4: Concentrations of amino acids (above), carbohydrates (middle), and carboxylic acids (bottom) in LMWOS leached from soil and sand–soil treatments at two leaching fractions of individual amino acids (above), carbohydrates (middle), and carboxylates [nmol mL^{-1}] as measured with HPLC (Mean values ± SE, n = 2–4). Note: Depicted concentrations are eluate concentrations. Measured concentrations were divided by 10 (due to 10-fold concentration of the samples during preparation). The legend applies to all three subplots.

In the first leaching fraction, the total AA concentrations were higher in leachate from SOIL (1.33 ± 0.11 μM) than in leachate from SAND (1.03 ± 0.13 μM). In the second leaching fraction, the concentrations of Glu, Ser, Thr, and total AA (0.73 ± 0.12 *vs.* 0.34 ± 0.06 μM) were significantly higher in SOIL than in SAND. The AA composition in leachates of the treatment SOIL was dominated by Ser (21.7%), Gly (19.4%), Glu (15.0%), and Ala (9.1%). In leachates from the treatment SAND, Ser (24.0%), Gly (16.7%), Ala (10.6%), and Leu (8.0%) had the highest proportions of total AA.

3.2.2 Carbohydrates

Similar to AA, in the first leaching fraction the total CH concentrations were higher in SOIL than in SAND (0.73 ±

0.21 μM *vs.* 0.25 ± 0.17 μM). In the second leaching fraction, the total CH concentrations in the treatment SAND increased to 0.52 ± 0.30 μM. Among individual CH, fucose (*fuc*) increased in the second leaching fraction (Fig. 4b). *Fuc* dominated SAND total CH, while glucose had the highest concentration in SOIL (especially in the first leaching fraction with 0.57 ± 0.08 μM). In addition to these two carbohydrates, uronic acids (galacturonic acid and glucuronic acid) contributed to total CH, while other carbohydrates were near the detection limit.

3.2.3 Carboxylic acids

In contrast to AA, CA leachate concentrations were higher in SAND than in SOIL and increased from the first to the second leaching fraction (Fig. 3). Acetic and lactic acid dominated with ≈ 1.5 to 3.5 μM and were especially responsible for the increase from first to second leaching fraction.

Low-molecular-weight organic substances—as the sum of CA, CH, and AA—were dominated by CA (Fig. 6). This dominance was greater in SAND than in SOIL and increased from the first to the second leaching fraction. In relation to AA, CH became more important in the second leaching fraction.

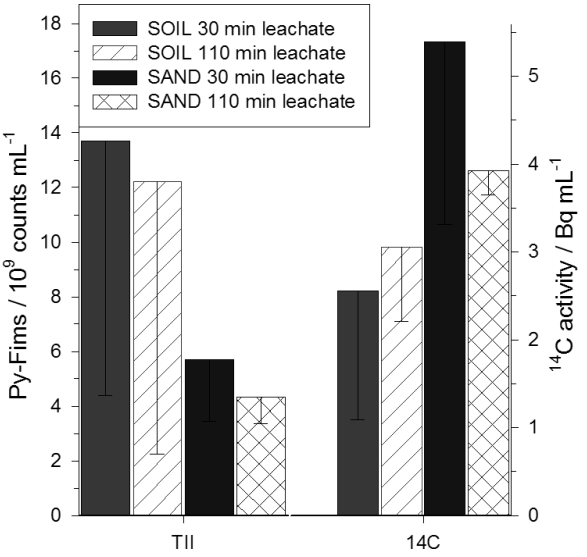

Figure 5: Substance classes in leachates of soil and sand–soil treatments at two leaching fractions determined by Py-FIMS and ^{14}C analysis: Total Ion Intensity (TII) [10^9 counts mL^{-1}], ^{14}C [Bq mL^{-1}]. Mean values ± SE, n = 2–4.

3.3 Py-FIMS analyses of leachates

The total ion intensity (TII) of all samples ranged from 1.5 × 10^6 to 32.5 × 10^6 counts mL^{-1} in the leachate. In contrast to ^{14}C, the TII and the indicator signals of almost all compound classes decreased from the first to the second leaching fraction and were higher in SOIL than in SAND (Fig. 5). Diluting the soil with 50% sand resulted in lower signal intensity for phenols, lignin monomers, and akylaromatics (Fig. 6).

These compounds together comprised about half of all assigned substances in all leachates. By contrast, the class with *m/z* < 56 (mainly inorganic substances and nonspecific fragments) increased in SOIL from the first to the second leaching fraction. Among individual *m/z* signals, the indicator ions for CH, phenols, and lignin monomers were most abundant in all samples (data not shown). *m/z* signals which can be attributed to hexoses (*m/z* 126, 144, 162) and pentoses (*m/z* 114, 132) served for estimating the ratio of hexoses to pentoses (*hex* to *pent*) in the Py-FI mass spectra. In the first leaching fraction, the *hex*-to-*pent* ratios were ≈ 2 for both treatments. In the second leaching fraction, the *hex*-to-*pent* ratio of SOIL increased to ≈ 4 and slightly decreased in the SAND treatment. The ion intensities for peptides in the leachates from SOIL slightly exceeded those leached from SAND (Fig. 6).

As the class "peptides" involves free amino acids and peptides, temperature-resolved Py-FIMS can distinguish free amino acids from peptidic-bound amino acids. As an example, Fig. 7a shows the different thermal volatilization of

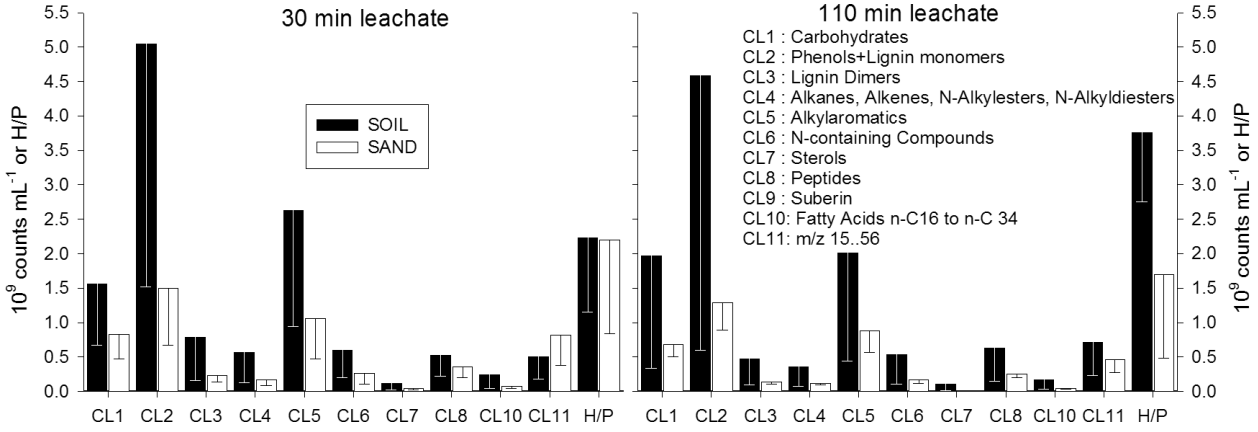

Figure 6: Py-FIMS Ion intensity [10^9 counts mL^{-1}] in the eluate of 11 substance classes (see graph, CL9 Suberin was not detectable) and ratios of hexoses to pentoses (right axis), Mean values (n = 3 or 4) ± SE. Depicted concentrations are eluate concentrations. Measured concentrations were divided by 10 (10-fold concentration of the samples during preparation).

glycine (as the sum of its molecular ion *m/z* 75 and the protonated ion *m/z* 76) from glycine and from the pentapeptide Tyr-Gly-Gly-Phe-Leu. It takes more thermal energy to break the peptidic bond than to merely vaporize the free glycine. At a pyrolysis temperature of ≈ 170°C the thermogram of glycine reveals a maximum. The graph for Tyr-Gly-Gly-Phe-Leu shows no signal until ≈ 270°C is reached. The sensitivity (counts [μg glycine]$^{-1}$) is ≈ 10 times higher for the free amino acid than for Tyr-Gly-Gly-Phe-Leu. The lower signal for polypeptides is caused by rapid decarboxylation as soon as the peptidic bond breaks up. Although Py-FIMS shows a higher sensitivity for free amino acids, all thermograms from leached rhizodeposits of the present experiment showed mainly peptidic-bound amino acids. This is demonstrated for glycine in Fig. 7b, but the same was observed for all amino acid markers.

4 Discussion

4.1 Carbon-14 distribution in the plant–soil system

In the monitored compartments (soil, roots, shoots, leachate, and soil-borne CO_2), 65% of the assimilated ^{14}C was found. Thus, aboveground respiration of the plants accounted for up to 35% of the applied ^{14}C. Fast respiration of recently assimilated C was previously reported from several researchers: *Lehmeier* et al. (2008) found that recently assimilated carbon in ryegrass is stored in three pools. The two faster ones had half-lives of 0.1 and ≈ 3 h and accounted for ≈ 44% of the respiration. In addition, *Ryle* et al. (1976) and *Schnyder* et al. (2003) reported for recently assimilated half-life times of some hours. *Werth* and *Kuzyakov* (2008) found in a labeling experiment similar to the present study that maize respired ≈ 30%–40% of assimilated $^{14}CO_2$ within < 6 d. Loss of ^{14}C due to inefficient trapping of belowground CO_2 can be ruled out as described in section 2.5.

Carbon-14 assimilation and ^{14}C rhizodeposition were affected by the different nutrient status: Total ^{14}C recovery in the soil variants (SOIL and AIR-SOIL) was higher than in SAND and AIR-SAND (in some compartments significantly, see Tab. 2). We assume that the lower nutrient supply in the SAND and AIR-SAND led to a shortage of nutrients, like N, for photosynthesis, causing slower $^{14}CO_2$ assimilation than in SOIL.

More ^{14}C was found in the leachates from SAND than from SOIL. This agrees with the findings by *Werth* and *Kuzyakov* (2006) that exudation of recently assimilated C (^{14}C) is higher when nutrient supply was lower (SAND).

Compared to *Werth* and *Kuzyakov* (2006), more ^{14}C was found in the shoots and less as in CO_2 from the roots. In the present experiment, the time between labeling and sampling was only 7.5 h instead of days. Thus, translocation of assimilates to the roots and then either (1) respiration by roots with subsequent release of CO_2 or (2) exudation of LMWOS from the roots and fast microbial decomposition was not completed. Belowground CO_2 production peaked between 30 and 110 min leaching fraction. This dynamic and also the amount of $^{14}CO_2$ from soil (0.5%–0.75% h^{-1} of added ^{14}C) is in the range of previous studies with ryegrass (*Rattray* et al. 1995; 0.3% in 0.5 h) and maize (*Kuzyakov* et al., 2003b; 0.7% h^{-1}).

Data from experiments with isotope dilution and the ^{14}C-dynamics method (*e.g., Kuzyakov*, 2002; *Werth* et al., 2006) suggest that within 2 d after assimilation, the CO_2 released from soil originated mostly from root respiration. *Rattray* et al. (1995) found that ^{14}C from $^{14}CO_2$ shoot assimilation of ryegrass needed to arrive to soil microbial biomass. We can therefore assume that in our experiment in the leached treatments—with a short time between labeling and sampling of CO_2 (7.5 h)—the contribution of $^{14}CO_2$ originating from microorganisms decomposing rhizodeposits was negligible. Before microorganisms can evolve $^{14}CO_2$, plant exudation and microbial uptake have to take place.

The $^{14}CO_2$ recovery from the only blow-out treatments AIR-SAND and AIR-SOIL, was at least as high as the combined $^{14}CO_2$ and ^{14}C leachate recovery from the SOIL and SAND treatments (Fig. 2, right). We therefore conclude that leached organic ^{14}C compounds face fast decomposition under non-leaching conditions (as, *e.g.*, in moderately dry soil). With half-lives of LMWOS in microbial biomass typically ranging from 1 h to a few days (*e.g., Jones* and *Darrah*, 1994; *Jones* and *Shannon*, 1999), some of the ^{14}C evolved at the end of the experiment was probably from microbial decomposition.

While leachate ^{14}C was lower, $^{14}CO_2$ evolution was higher in SOIL than in SAND. The sums of both fluxes were equal and were released from soil at the same rate (Fig. 2b). This indicates that both pools were derived from the same source and that decomposition to CO_2 was faster in SOIL *vs.* SAND. In addition, higher lactate and acetate concentrations in SAND than SOIL were observed and both concentrations increased from the first to the second leaching fraction. The lower nutrient supply in SAND *vs.* SOIL could have led to higher root exudation, especially of carboxylic acids, as has been previously observed in several studies.

4.2 Composition of LMWOS in the 30 min leaching fraction

At the onset of leaching, the pots contained ≈ 12 mL of water. Within the first 30 min, ≈ 27 mL were leached. Thus, original soil-solution concentrations were at least twice as high as the concentration of the leached solution. This results in total AA concentrations of roughly 2.7 μM in soil solution (SOIL) and 1.5 μM (SAND), total CH 1.5 μM (SOIL) and 1.4 μM (SAND), and CA 3.6 μM (SOIL) and 6.0 μM (SAND). These substances were certainly not completely leached within the first 30 min of leaching (first leaching fraction). For this reason, these values are the lowest estimates. Hence, our values were in the lower range of values from literature (0.1–1000 μM, mostly 10 to 100 μM; *Christou* et al., 2006; *Jones*, 1998; *Strobel*, 2001; *van Hees* et al., 2002, 2005).

Among the three LMWOS classes detectable with HPLC, CA concentrations were always highest and CH concentrations

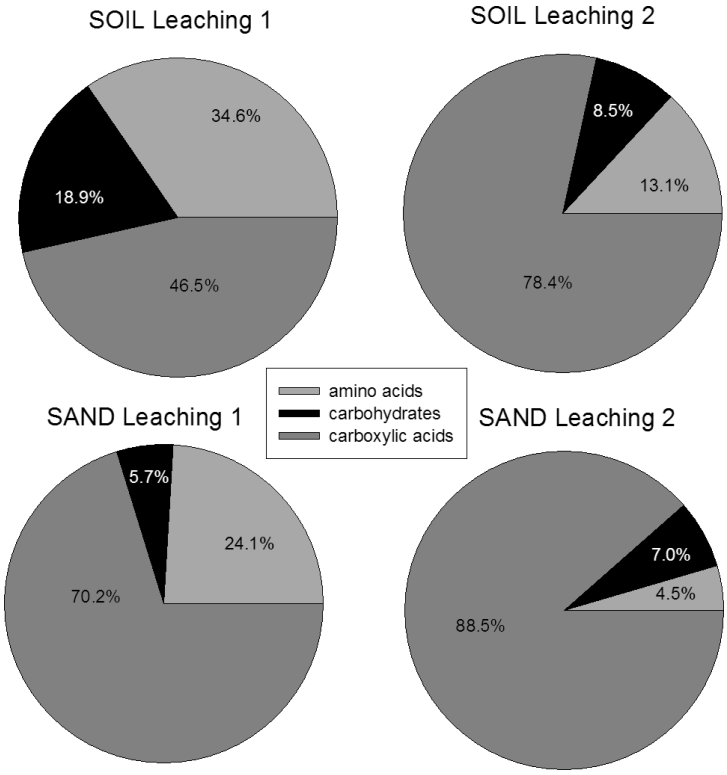

Figure 7: Distribution of amino acids, carbohydrates, and carboxylic acids in soil and soil–sand treatments at both leaching fractions (mean values [in mol%]).

were mostly the lowest (Fig. 7). This contrasts with results by *Merbach* et al. (1999) for several species (wheat, amaranth, alfalfa, and pea): Among CH, AA, and CA in root-near soil, CH dominated with ≈ 50%. On the other hand, the same study determined that, for *Chenopodium* and oil radish, AA (24%–30%) was second to CA (40%–57%), leaving only ≈ 15% for CH. This distribution resembled the one we found. Clearly, the distribution among AA, CH, and CA is highly variable in species, space, and time. *Jones* and *Brassington* (1998) and *van Hees* et al. (2003) showed that especially negatively charged CA are subject to sorption in the presence of Fe/Al oxides/hydroxides. Without a high content of Fe/Al oxides, Ca^{2+} ions can promote sorption of negatively charged CA to predominantly negatively charged soil particles. This sorption apparently leads to an underestimation of CA in leachates, moreover, when hot spots of CA in microsites are not sampled (*Jones* et al., 2003). In our experiment, less sorption sites in soil–sand contributed to a higher CA concentration than in soil.

Figure 6 shows that percentages of AA and CH were higher in SOIL than in SAND for both leaching fractions. *Bowen* (1969) found for *Pinus radiata* seedlings under N deficiency, a decrease of AA exudation while P deficiency led to an increase of AA rhizodeposition. Thus, lower percentage of AA in SAND suggests N deficiency.

4.3 Origin of carbohydrates

While HPLC-measured *fuc* was high, the other CH, especially *xyl* and *ara*, were near or below the detection limit. Certainly, hexoses outbalanced pentoses. In acidic extracts from soil, pentoses like arabinose (*ara*) and xylose (*xyl*) have been assigned to plant origin, derived from polysaccharides and structural material of the plants. In contrast, hexoses like galactose (*gal*), mannose (*man*), rhamnose (*rha*), and fucose (*fuc*) are the main CH compounds of microorganisms. Hence, several ratios like (*man* + *gal*) to (*ara* + *xyl*) or (*fuc* + *rha*) to (*ara* + *xyl*) have been used to determine the origin of soil CH (*Bock* et al., 2007; *da Cunha* et al., 2002; *Glaser* et al., 2000; *Moers* et al., 1989; *Oades*, 1984). Low ratios (<0.5) were considered to be characteristic for plant-derived material and high ratios (>2) were allotted to microbial input or degraded organic matter (OM). However, *Bacic* et al. (1987) and *Nguyen* (2003) found that *fuc* was also an important constituent of maize mucilage. In all cited studies, CH were extracted by acidic extractants; these studies therefore dealt with the origin of total OM in soils, not the composition of root exudates. The CH signature of root exudates may differ from acidic soil extracts: *Kraffczyk* et al. (1984) found mainly glucose and arabinose, and lesser amounts of fructose and sucrose, to be released by maize roots. Since the early work of *Vancura* (1967), glucose and fructose are known to be the most abundant among CH exudates of maize, while "signal hexoses" for microbial biomass *gal* and *rha* outnumber pentoses. There is no tool like the (*man* + *gal*)-to-(*ara* + *xyl*) ratio to distinguish between root-exudated and microbial produced CH.

The patterns of HPLC-measured CH (Fig. 3) and Py-FIMS-measured CH (CL 1, Fig. 6) were not similar. Py-FIMS detects not only free CH in solution but also polymers such as cellulose. Thus, the different CH measured by HPLC and Py-FIMS can be explained by the pyrolysis and mass-spectral detection of polymers. Hence, in contrast to HPLC,

hex-to-*pent* ratios can be used to determine the source of Py-FIMS measured CH. The pattern of CH increase for SOIL and decrease for SAND from the first to the second leaching fraction was retraced by the ratio of hexoses to pentoses (Fig. 6). This indicated that differences in the supply of microbial hexoses and plant-related pentoses affected the leachate composition. In a similar experimental setup, *Melnitchouck* et al. (2005) observed diurnal differences in *hex*-to-*pent* ratio as determined by Py-FIMS (*m/z* 126, 144, 162 for hexoses divided by *m/z* 114, 132 for pentoses). They found ratios of for night-rhizodeposits and 1 for day-rhizodeposits. Maize roots and leaves had values of 1.41 ± 0.07 ($n = 6$). They concluded that the night-rhizodeposits were dominated by microbial CH and the day-rhizodeposits by plant pentoses. Correspondingly, the Py-FIMS indicates that CH from the first leaching fraction were mostly bacterially derived (values ≈ 2) (Fig. 6). Higher input of organics by roots as proven by the ^{14}C values explains the decrease in *hex*-to-*pent* ratio in the second leaching fraction of SAND samples. In SOIL, *hex*-to-*pent* ratio increased, indicating that microbial input prevailed.

Melnitchouck et al. (2005) observed a diurnal change for the release of free amino acids from maize: They were mainly released during the day and presumably decomposed by microbes over night. In contrast to that study, the present leachates were collected at noon and were dominated by bound peptides as indicated by the thermograms, which showed volatilization at ≈ 300°C and higher temperatures (Fig. 8b). This explains the differences between HPLC and Py-FIMS in detecting amino acids in the present leachates. The HPLC measured free amino acids only, while the Py-FIMS detected mostly amino acids bound in peptides.

5 Conclusions

The multimethodological approach involving ^{14}C tracer, HPLC, and Py-FIMS was well suited to estimate the budget of assimilated C (by ^{14}C), to identify individual AA, CH, and CA (HPLC), and to quantify other main compound classes of DOM (Py-FIMS). Differences between the methods reflect the detection of individual AA, CH, and CA by HPLC, and of free soluble and polymeric compounds (cellulose, peptides) by Py-FIMS.

Table 3 comprises the advantages and shortcomings of each of the applied methods.

The chasing of the ^{14}C signal in soil, in maize roots and shoots, in CO_2 from the soil, and in the leachate revealed the distribution of recently assimilated C in the monitored compartments. The extreme low detection limit of ^{14}C also permitted the rapid tracing in compartments with a lower percentage of C distribution, for example in leachate. However, it

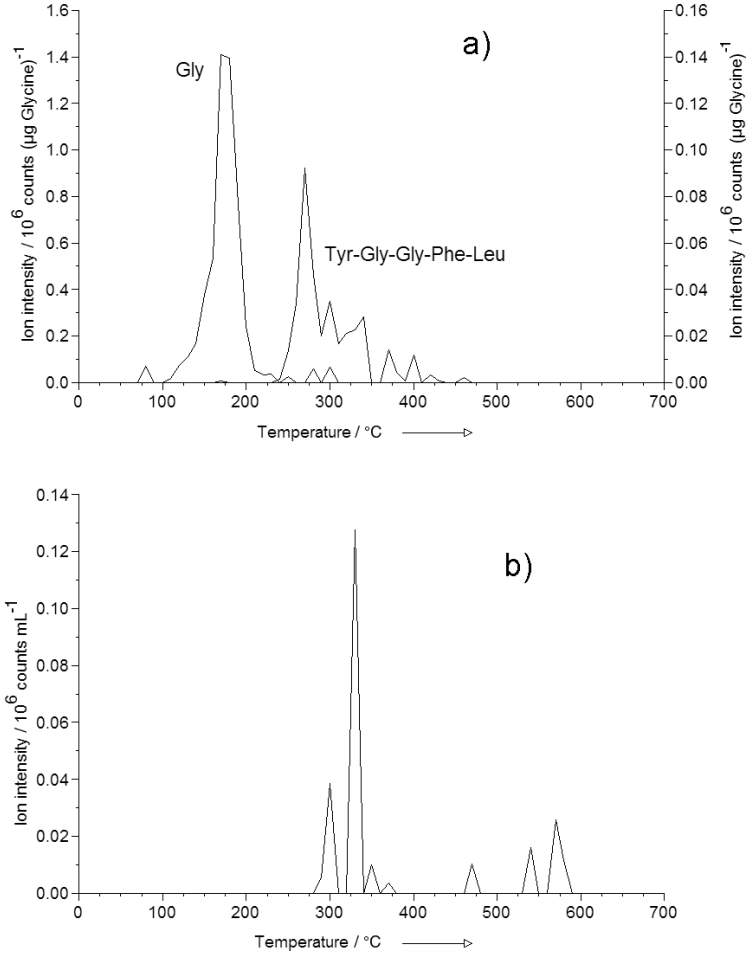

Figure 8: Py-FIMS thermograms for glycine molecules volatilized from free glycine (left axis) and glycine a) bound in the pentapeptide Tyr-Gly-Gly-Phe-Leu (right axis) and b) from leachate rhizodeposits of the present experiment.

Table 3: Comparison of advantages and shortcomings of the three applied methods: ^{14}C chasing, HPLC, and Py-FIMS for budgeting LMWOS in the rhizosphere.

	^{14}C-labeling/chasing	HPLC	Py-FIMS
Budgeting of assimilated CO_2	yes	no	no
Identification of specific substances (LMWOS)	no	yes	not directly
Identification of polymer sources of LMWOS	no	no	yes
Sensitivity for short-term changes in the rhizosphere	very high	high	low
Detection limit	<< 0.001% of input*	≈ 0.1 μmol L^{-1}§	not applicable
Combined set-up with the other two methods	very easy	possible	possible
Work effort	low	high	intermediate

* detectable: 0.5 Bq, applied 124 kBq per plant, potential increase of sensitivity by 3 orders of magnitude when higher ^{14}C activities are applied
§ detection limit obtained from *Mayer* et al. (2008)

was not possible to determine which substances were exudated to the soil solution by the plants.

HPLC was very suitable for the exact determination of individual LMWOS. With HPLC, various individual CH, CA, and AA could be identified and their concentrations determined.

In contrast to HPLC, Py-FIMS enabled the scanning for all dissolved substances in the filtrate from soil solution (not only AA, CH, and CA). This allowed signals from, *e.g.*, phenols, lignin monomers, and akylaromatics to be interpreted. With temperature-resolved Py-FIMS, it was even possible to distinguish between free amino acids and peptidic-bound amino acids. However, Py-FIMS information on these substances was always limited to *m/z* signals and their intensity, depending on the pyrolysis temperature. Several substances can cause similar signals in Py-FIMS, *e.g.*, galactose and glucose (both *m/z* 180). In terms of combining the three methods in one setup, ^{14}C labeling and chasing proved to be the easiest as needed sample amount was small (0.5 mL) and no extra sample preparation was necessary.

Mixing soil with sand had three consequences: (1) lower nutrient concentrations, (2) fewer sorption sites, resulting in higher concentrations of leached substances, and (3) increased root exudation that partly compensates or amplifies the effect of (1) or (2). Since the TII of Py-FIMS and the CH and AA concentrations detected by HPLC in the first leaching fraction were higher in SOIL than in SAND, we concluded that diluting soil with quartz sand decreased the total amount of leached organic substances. The CH and AA were less influenced by sorption than CA. Thus, more water (leaching 6 h instead of 2 h after the labeling) and fewer sorption sites (SAND *vs.* SOIL) led to higher CA concentrations. The higher nutrient content in SOIL than in SAND led to higher microbial activity, which increased AA and CH concentrations.

For further studies on rhizodeposition, we recommend applying labeling by ^{14}C or ^{13}C to estimate the C budget, HPLC analysis to precisely determine individual organic substances, and Py-FIMS to investigate the whole spectrum of soluble substances in soil solution. The combined application of the three presented methods (^{14}C, HPLC, Py-FIMS) yields more detailed information on the substance composition of the rhizosphere and its budget.

Acknowledgments

This study was funded by the *Deutsche Forschungsgemeinschaft (DFG)*.

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