Qualitative assessment of rhizodeposits in non-sterile soil by analytical pyrolysis

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Summary – Zusammenfassung

Estimation of the amount of root exudates and simultaneous identification of their composition in non-sterile soil is a challenging objective in rhizosphere research. We coupled 3 methods: (1) labeling of corn in $^{14}CO_2$ atmosphere to separate root-derived and soil-derived organic substances in the rhizosphere, (2) a previously developed leaching method to collect rhizodeposits, and (3) pyrolysis field ionization mass spectrometry (Py-FIMS) to investigate the molecular-chemical composition of rhizodeposits. Eluted rhizodeposits accounted for 2.8 % (Loam) and 0.97 % (nutrient solution in quartz sand) of recovered ^{14}C and showed clear differences in composition between the growth substrates. The $^{14}CO_2$ evolved mostly by root respiration accounted for 3.5–4.0 % without significant differences according to growth substrate or diurnal dynamics. Principal component analysis of the Py-FI mass spectra of leachates showed a clear diurnal dynamics of the amount and the composition of corn rhizodeposits collected during day-time and night-time. Differences originated mostly from signals assigned to carbohydrates, sterols, and peptides. This approach is recommended for forthcoming studies of rhizodeposition in different soil substrates, crops grown, and time-series of exudate sampling.

Qualitative Untersuchung der Rhizodeposition im nicht sterilen Boden mit analytischer Pyrolyse

Quantifizierung und gleichzeitige chemische Identifikation der Rhizodeposite im nicht sterilen Boden sind nach wie vor eine Herausforderung in der Rhizosphärenforschung. Als neuen methodischen Ansatz kombinierten wir (1) die Markierung von Mais in einer $^{14}CO_2$-Atmosphäre zur Trennung würzelbürtiger und bodenbürtiger organischer Substanzen in der Rhizosphäre mit (2) einer neuen Perkolationsmethode zur separaten Erfassung der Rhizodeposite und (3) Pyrolyse-Feldionisations-Massenspektrometrie (Py-FIMS) zur Untersuchung der molekular-chemischen Zusammensetzung der Rhizodeposite. Zwischen 2.8 % (Toniger Schluff) und 1.0 % (Nährlösung in Quarzsand) des wiedergefundenen ^{14}C entfiel auf die eluierten Rhizodeposite. Das überwiegend durch Wurzelatmung freigesetzte $^{14}CO_2$ betrug 3.5–4.0 % des wiedergefundenen ^{14}C, wobei kaum Unterschiede hinsichtlich Substrat bzw. Tageszeit deutlich wurden. Auswertung der Py-FI-Massenspektren der Eluate mit Hauptkomponentenanalyse zeigte deutliche Unterschiede in der Zusammensetzung der Eluate aus den beiden unterschiedlichen Substraten sowie aus der Deposition am Tage und in der Nacht. Diese Unterschiede wurden vor allem durch die Intensitäten von Markersignalen für Kohlenhydrate, Sterole und Peptide hervorgerufen. In weitergehenden Untersuchungen soll dieser experimentelle Ansatz zur Erforschung der Rhizodeposition unter verschiedenen Bodenbedingungen, mit verschiedenen Pflanzenarten und in Zeitreihen der Beprobung genutzt werden.

Key words: analytical pyrolysis / mass spectrometry / Py-FIMS / root exudates / rhizodeposition / rhizosphere / ^{14}C PNSS P03/63P

1 Introduction

Quantification of root exudates is one of the key points for estimating their role for rhizosphere microorganisms as carbon (C) and energy sources and their contribution to C turnover in soil (*Kuzyakov* and *Domanski*, 2000; *Hütsch* et al., 2002). For quantification of total amount of exudates, important progress was achieved in the last two decades by using different C isotope techniques (^{14}C or ^{13}C pulse and continuous labeling, as well as $\delta^{13}C$ natural abundance). Until now, for qualitative investigations, root exudates were collected from plants grown in axenic nutrient solutions or quartz sand and subsequently analyzed by liquid chromatography (*Wiren* et al., 1994; *Gransee* and *Wittenmayer*, 2000). Unfortunately, the wide spectrum of methods developed in plant physiology for investigations of root-derived organic substances in nutrient solution and artificial substrates cannot be directly applied to soils because of the strong effects of rhizosphere microorganisms on the amount and the composition of root exudates. Our knowledge on the quantity and quality of exudate input by roots growing in soil is still incomplete because of (1) low concentrations of root-derived organic substances in comparison to the concentrations of other organic substances, (2) rapid decomposition ($T_{½}$ = 0.5–10 days) of organic substances released from roots by soil microorganisms (*Kuzyakov* and *Demin*, 1998), (3) occurrence of the rhizodeposits in the narrow zones of soil adhering to the root surface (*Kuzyakov* et al., 2003), and (4) difficulties in distinguishing between the organic substances originating from the roots and those originating from microbial utilization of soil organic matter.

The objective of the present study was to use an alternative approach consisting of (1) quantification of exudate-^{14}C with a newly developed siphon system for sampling rhizodeposits by elution and simultaneous trapping of ^{14}C-labeled CO_2 respired by roots (*Kuzyakov* and *Siniakina*, 2001), and (2) qualitative chemical characterization of eluted rhizodeposits by pyrolysis field ionization mass spectrometry (Py-FIMS),

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which was shown to be a rapid and sensitive method for dissolved organic matter (DOM) characterization (*Schulten* at al., 2002). The coupling of these approaches is sensitive enough to prove quantitative (^{14}C) and qualitative (Py-FIMS) differences in rhizodeposition by plants growing in quartz sand with nutrient solution and loamy soil. Our previous studies show significant differences in the amount of organic substances exuded by roots during the day- and night phases (*Kuzyakov* and *Siniakina*, 2001; *Kuzyakov* and *Cheng*, 2001). Therefore, the second aim of the study was to use the new approach to verify the earlier results that the composition of exudates differs between day and night.

2 Material and methods

Young corn plants were grown in (1) quartz sand with nutrient solution and (2) a loamy soil sample from a Haplic Luvisol-Ap horizon (long-term field experimental station Karlshof of the Hohenheim University). The soil sample contains no $CaCO_3$ and has the following characteristics: pH 6.0, C_t 1.2 %, N_t 0.13 %, clay 23 %, silt 73 %, and sand 4.4 %. 500 g air-dried soil (< 2 mm) or quartz sand was used to fill each container (Polycarbonate filtration device "CombiSart", volume 250 ml, Merck®-Laborkatalog, 2000).

One germinated seed of corn (*Zea mays* L.) was put into each container and grown under 27/22 °C day/night temperature, 12 hours photoperiod and 400 μmol m^{-2} s^{-1} photosynthetic active radiation. About 10 ml of nutrient solution (*Smith* et al., 1983) was added daily to the plants grown in quartz sand. The plants were pulse-labeled in $^{14}CO_2$ atmosphere (256 kBq $plant^{-1}$) on day 28 and harvested on day 31. After one hour of labeling, the rhizodeposits were eluted from the rhizosphere and, simultaneously, CO_2 originating from root respiration was forced out and collected in NaOH using a siphon-elution system developed earlier (*Kuzyakov* and *Siniakina*, 2001). Elution was done 6 times a day using 400 ml of de-ionized water. The total amount of exudates as percentage of C assimilated by plants was estimated by ^{14}C activity. The ^{14}C-CO_2 trapped in the NaOH solution and ^{14}C in eluted organic compounds were determined on 2-ml aliquots added to 3 ml scintillation cocktail EcoPlus (Roth) on ß-spectrometer Rackbeta (Mod. 1419, LKB). The total content of CO_2-C collected in NaOH solution was determined by titration with 0.2 *M* HCl against phenolphthalein (pH change 8.2–9.8) after addition of 2.0 *M* $BaCl_2$. The preparation of soil samples, the main soil characteristics, and the growing conditions are the same as described in *Domanski* et al. (2001). The siphon-elution system and ^{14}C labeling procedure is described in detail by *Kuzyakov* and *Siniakina* (2001).

Eluted exudates were analyzed for ^{14}C and for composition by pyrolysis field ionization mass spectrometry (Py-FIMS). The experimental conditions for Py-FIMS were similar as described recently by *Leinweber* et al. (2001). Three replicates of each sample were analyzed. The mass spectra recorded were tested for differences between groups of samples by principal component analysis (PCA). The PCA reduces the dimensionality of the data set to a few principal components (PC) containing most of the total variance in original variable space.

3 Results and discussion

The first samples of eluted rhizodeposits obtained 6 hours after the labeling had the highest ^{14}C activity and subsequently diminished during the 2.5 days of the experiment. The ^{14}C in eluted rhizodeposits indicated diurnal dynamics that were much more pronounced for maize grown in the loamy soil than for that grown in quartz sand (Fig. 1, top). Respired $^{14}CO_2$ generally indicated a similar trend over time, but the diurnal dynamics were less pronounced (Fig. 1, bottom). The day-night dynamics of CO_2 efflux has been frequently measured under field (e.g. *Kim* and *Verma*, 1992; *Baldocchi* et al., 1986) or laboratory conditions (*Kuzyakov* and *Cheng*, 2001). However, it was previously impossible to separate CO_2 originating from root respiration and CO_2 originating from rhizomicrobial respiration. Our results show that the diurnal CO_2 dynamics were due to changing root exudation intensity rather than to varying root respiration. Generally, these findings with corn confirm previous results obtained with the elution method for ryegrass (*Kuzyakov* and *Siniakina*, 2001).

The cumulative root exudation to the loamy soil was greater by a factor of 2.9 than to the quartz sand (compare Fig. 1, top and bottom) and amounted to 2.77 % ± 0.27 and 0.97 % ± 0.48, respectively. This agrees with many studies showing larger total amounts of exudates from plants grown under soil conditions compared to nutrient solution cultures (*Meharg* and *Killham*, 1991). In most studies, sterilization of soil or growing of plants on nutrient solution cultures decreased the total exudate amount by about 1.5 to 3 times compared to non-sterilized soil. Microbial communities in the rhizosphere are a large sink for root-derived organic C. Thus, the absence of rhizosphere microorganisms strongly modifies C flows on the root-soil surface, resulting in a reduction in the amount of exudation in quartz sand, as well, as compositional changes. As shown in our experiment, the substrate (loamy soil or quartz sand) had no effect on root respiration (Fig. 1).

The Py-FIMS of all samples gave highly complex spectra with *m/z* up to > 600 (not shown). The PCA of all 24 recorded spectra distinguished four groups of samples according to the substrates: quartz sand and loamy soil, and to the periods of exudation: day-time and night-time. The 43 signals with the highest discriminating power were then used to separate samples according to both substrates for plant growth (Fig. 2a) and period of exudation (Fig. 2b). The plots of the first versus the second principal components show that about 58 % of variance between samples resulted from different growth substrates. About 33 % of variance between day-time and night-time exudates could be explained by the first two principal components calculated from the Py-FI mass spectra. So, rhizodeposits differed in chemical composition according to substrate on which the crops were grown, and according to photoperiod (day-time *vs.* night-time). Signals tentatively assigned to sterols (*m/z* 372, 390, 400, 416, 430) were among the 20 signals with the largest variance weights. They were used for the discrimination between samples from quartz sand and loamy soil. The samples from day- and night-time rhizodeposits could be separated by Py-FI mass spectrometry, in parti-

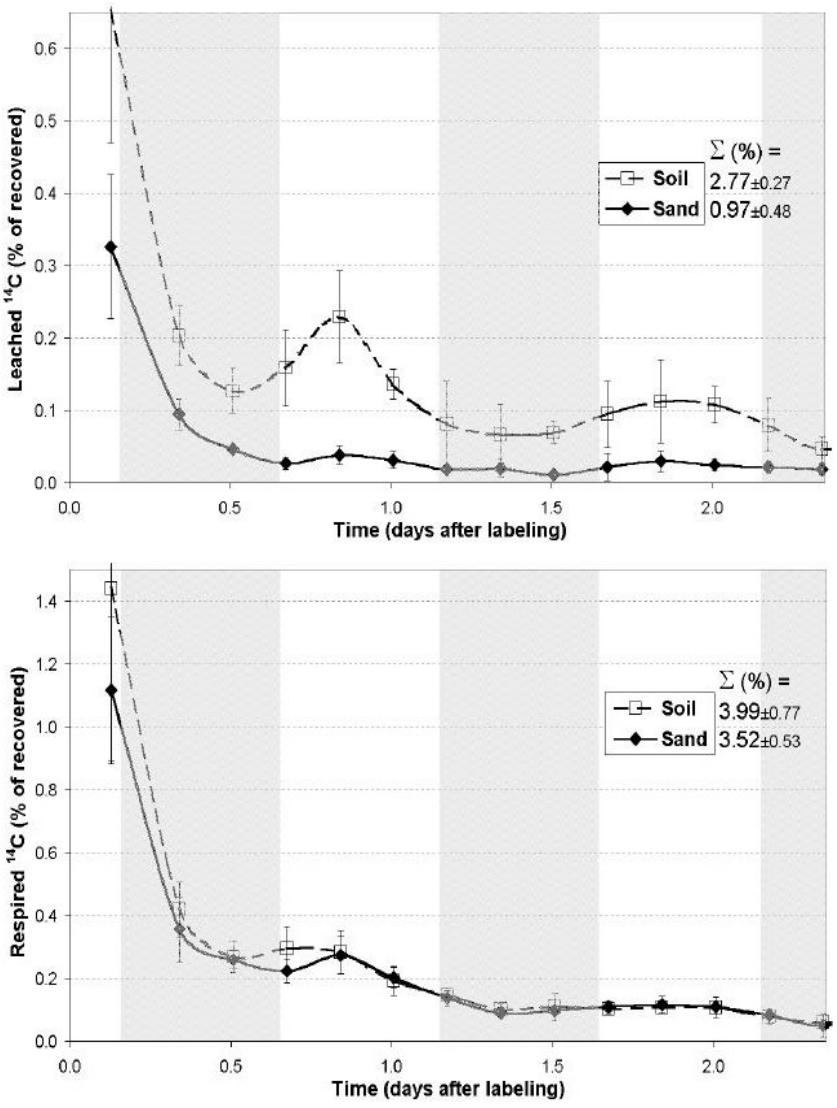

Figure 1: Dynamics and total amount of ^{14}C (± SD) in eluted exudates (top) and in CO_2 trapped in NaOH (bottom) during 2.5 days after labeling of corn shoots. Grey area shows the night-time.
Abbildung 1: Dynamik und Gesamtmenge von ^{14}C (± SD) in ausgewaschenen Exsudaten (oben) und in CO_2, das in NaOH aufgefangen wurde (unten) im Verlaufe von 2,5 Tagen nach der Markierung von Maispflanzen. Graue Flächen zeigen die Nachtzeit.

cular by signals assigned to peptides (*m/z* 75, 87), odd-numbered *m/z*, which possibly indicate other N-containing compounds (*m/z* 361, 317, 93, 347, 305, 351, 89), and by signals assigned to carbohydrates (*m/z* 72, 126).

To the best of our knowledge, there is no publication comparing the composition of root exudates from nutrient solution studies and natural soil in the literature. This is due to the previous impossibility to collect root exudates from non-sterilized soil. The present study demonstrates significant differences in the composition of substances eluted from non-sterilized soil and from quartz sand with nutrient solution. These differences could originate from (1) differences in the exudation of roots grown in different substrates, at different nutrient supply, and by different microbial communities, and (2) the elution and analysis of substances originating from the interactions of exudates with soil organic matter. In the present study, both sources can contribute to the differences between the composition of organic substances eluted from rhizosphere of plants grown on quartz sand with nutrient solution and on the loamy soil.

In most previous studies of rhizodeposition in nutrient solutions, only the three main substance classes of root exudates, i.e. sugars, carboxylic acids, and amino acids were analyzed (*Gransee* and *Wittenmayer*, 2000). One reason is the methodological restriction of the liquid chromatography systems used in these studies. The common liquid chromatography systems consist of cationic–anionic exchange resin columns, that enable the retention of amino acids (cationic), carboxylic acids (anionic), and sugars (pass both columns). Other substance classes were not considered with this traditional method.

Using Py-FIMS, sterols were suggested to contribute to differences between exudates in quartz sand and the loamy soil (Fig. 2a). *Svenningsson* et al. (1990) and *Zenoff* et al. (1994) showed that there were different free sterols in roots of rape and soybean, and the concentrations of sterols increased strongly under stress conditions. *Thompson* and *Hale* (1983) found free sterols and fatty acids in root exudates. However, all these results were obtained in axenic nutrient solution studies. Cell walls of fungi are another important source of ste-

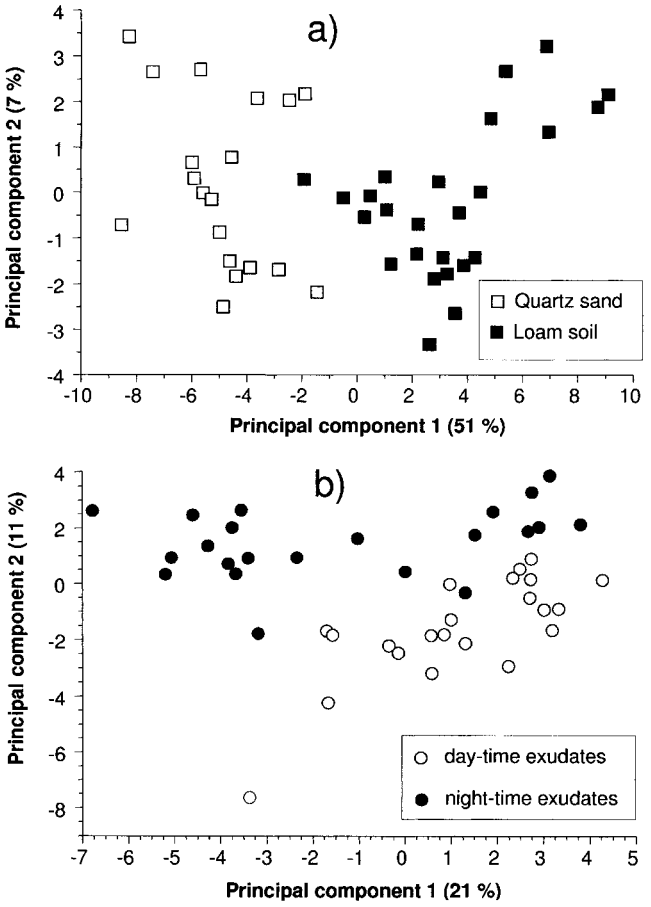

Figure 2: Principal component analysis of Py-FI mass spectra using 43 *m/z* with the highest discriminating power between rhizodeposits eluted (a) from quartz sand and loamy soil, and (b) at day-time and night-time.
Abbildung 2: Hauptkomponentenanalyse der 43-*m/z*-Signale aus den Py-FI-Massenspektren mit der höchsten Diskriminierungskraft zwischen den ausgewaschenen Rhizodepositen aus (a) Quarzsand und lehmigem Oberboden, und (b) während des Tages (mit Licht) und der Nacht (ohne Licht).

rols in natural soil. Therefore, the stress of plants grown in quartz sand and the occurrence of fungi in non-sterilized soil may be explanations for the discriminating power of sterol signals.

In our study, we observed different substance composition from day and night exudates. This result could be expected because many plant physiology studies showed diurnal changes in the composition of carbohydrates in shoots and phloem (*Buchi* et al., 1998; *Klages* et al., 2001). However, there are only few studies indicating different exudate composition during day and night (*Walter* et al., 1995), but these results were also observed under axenic conditions.

The novel approach of sampling rhizodeposits by elution, simultaneous trapping of labeled $^{14}CO_2$ respired by roots and characterization of samples by Py-FIMS was proved to be highly sensitive in quantitative (^{14}C) and qualitative (Py-FIMS) differences of rhizodeposition according to substrate and period of exudation. Ongoing analyses using the same approach confirmed that N-containing compounds, including free amino acids and carbohydrates were main components determining differences between day- and night-time rhizodeposits. For these reasons, this approach is recommended to be widely explored in rhizodeposition studies with varying soil substrates, different crops and time-series of rhizodeposition sampling.

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