Short Communication
Spatial distribution of root exudates of five plant species as assessed by ^{14}C labeling

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1 Introduction

Rhizodeposition of plants is the main carbon (C) and energy source in the rhizosphere and is responsible for fast turnover, high diversity, and abundance of microorganisms in the rhizosphere (*Kuzyakov*, 2002a). In contrast to the root-free soil, the processes close to the root surface are not C-limited because of continuous release of easily available organic compounds from the roots. With increasing distance to the root surface, the amount of rhizodeposits decreases because of slow diffusion and rapid microbial decomposition. Soluble constituents of rhizodeposition—root exudates—have the maximal diffusion range. Therefore, they determine the rhizosphere extension—the distance from the root surface that is affected by input of the easily available C and energy sources. The extension of the rhizosphere can be determined by concentration profiles of nutrients in the vicinity of the root surface (*Jones* et al., 2004). This is the approach based on nutrient **depletion** by the roots and is not directly connected with C dynamics. Another approach is to measure the **accumulation** profile of substances released by roots, *i.e.*, exudates. This approach was tested only in few studies (*Kuchenbuch* and *Jungk*, 1982; *Helal* and *Sauerbeck*, 1983; *Kuzyakov*, 2002b; *Kuzyakov* et al., 2003). One of the difficulties of this approach is to distinguish between soluble organic substances released by roots from chemically identical substances released by microorganisms by decomposition of dead-plant residues and soil organic matter (SOM). This difficulty can be solved by labeling the plants in $^{14}CO_2$ atmosphere and tracing of ^{14}C in different proximity to the root surface. The other difficulty is connected with continuous microbial decomposition of released exudates by microorganisms. This is the main mechanism limiting the infinite exudates diffusion in the nonsterile soil.

As the rhizosphere extension is crucial for microbial-turnover processes in soil, we compared the diffusion of root exudates of five agricultural plant species in one study.

2 Materials and methods

Plants were grown in vessels divided by a gauze (clear mesh: 30 μm) into an inner rooted zone and, to the left and right, two root-free zones (Fig. 1). The central part of the vessels was ∅ 50 mm and 56 mm long. It was filled with 110 g air-dried soil from the Ap horizon of a Luvisol from loess. The lateral parts were ∅ 45 mm and 85 mm long. They were filled each with 135 g of soil from a Fluvisol of similar texture (silt loam). The bulk density of the soil was 1.0 g cm^{-3}. Four vessels each were planted with maize (*Zea mays* L., Tassilo), wheat (*Triticum aestivum* L., Thasos), and sunflower (*Helianthus annuus* L., San Luca), so that eight pseudo-replicates per species were obtained. Two vessels each were planted with lettuce (*Lactuca sativa* var. capitata L., Mona) and spinach (*Spinacia oleracea* L., Matador), and two were left without plants. During plant growth, the soil water content was kept between 50%–60% of the available field capacity.

As soon as the gauze was completely covered by roots, the aboveground plant parts were pulse-labeled in $^{14}CO_2$ atmosphere. This was the case after 38 d for maize, after 43 d for wheat, after 44 d for sunflower, after 49 d for lettuce, and after 56 d for spinach. Activity of ^{14}C used for labeling varied be-

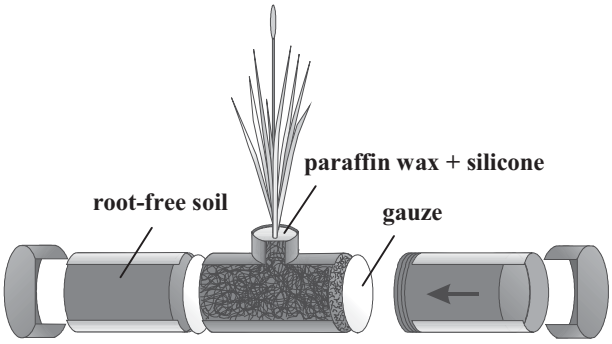

Figure 1: Experimental air-tight vessels divided by gauze into three parts: a central part containing rooted soil and two lateral parts with root-free soil separated by gauze with a clear mesh of 30 μm. The arrow indicates the treatment of the two lateral soil columns, which were separated from the central part, frozen, pushed out (arrow) of the vessel, and cut into 2 mm slices.

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tween 35 and 70 × 10^6 DPM per plant. For the calculation of the results, all data were referred to labeling activity of 100 × 10^6 DPM per plant. Before labeling, the openings of the vessels were sealed by a layer of paraffin wax and another layer of silicone rubber. Hence, ^{14}C could only get into the soil *via* $^{14}CO_2$ assimilation of the plants and subsequent root exudation. For the labeling procedure, the plants were exposed to a $^{14}CO_2$-enriched atmosphere in a plexiglass chamber for 1 h. The growth conditions, labeling procedure, and ^{14}C analysis were described in detail by *Kuzyakov* et al. (2003).

Four days after labeling, the lateral root-free vessel parts were removed and immediately deep-frozen by dipping them into liquid N. The frozen soil column was pushed out of the tube-shaped outer part of the vessel until it projected 12 mm. The projecting part was cut into 2 mm slices by deep-freeze microtome. The soil slices were dried at 40 °C, and subsamples were combusted in an oxidizer unit (Canberra Packard, Model 307). The released CO_2 was trapped in a scintillation cocktail (Rotiszint Eco Plus, Carl Roth, Karlsruhe, Germany), in which ^{14}C activity was determined by a liquid scintillation counter (Tri-Carb 2000CA, Canberra Packard).

3 Results and discussion

3.1 Differences in root exudation between the five plant species

The ^{14}C activity in the nonrooted zone, caused by diffusion of root exudates, was greatest for spinach, lettuce, and sunflower, with no significant differences between these plants (Fig. 2). For maize and spinach, it was significantly smaller (maize: 27% and wheat: 13% of the ^{14}C activity in the root-free zone of spinach). The difference between maize and wheat was not significant. Possible reasons for the differences in the total amount of ^{14}C recovered in the root-free soil could be that the tested plants released different amounts of organic substances into the rhizosphere. Secondly, the exudate composition of the different plant species varies (*Neumann* and *Römheld*, 2001), which may lead to differences in microbial decomposition of the exudates affecting the total amount recovered 4 d after labeling. A third reason could be different amounts of root hairs (in the first 2 mm).

Lettuce, sunflower, and maize showed the maximal range of exudates diffusion reaching up to 12 mm. For these plants, the ^{14}C activities in the soil between 10 and 12 mm from the root surface still differed significantly from zero (likelihood ≥75%). Wheat exudates were recovered up to 10 mm and spinach exudates up to 8 mm from the root surface. These various maximal ranges suggest different intensity of exudation by the investigated plant species.

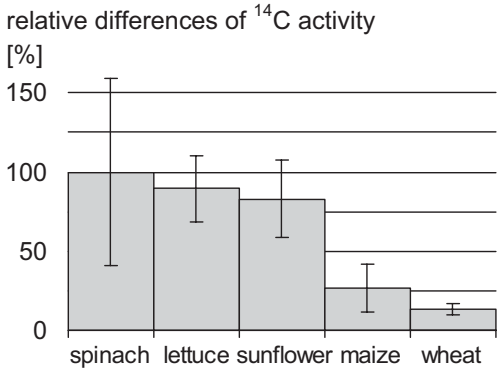

Figure 2: Relative ^{14}C activity found outside the rooted zone of spinach, lettuce, sunflower, maize, and wheat (mean values and standard errors). The total ^{14}C activity was related to the amount of exudates recovered by spinach which was set as 100%. Significant differences (error probability < 5%): spinach > wheat, lettuce > wheat, lettuce > maize, sunflower > wheat, sunflower > maize.

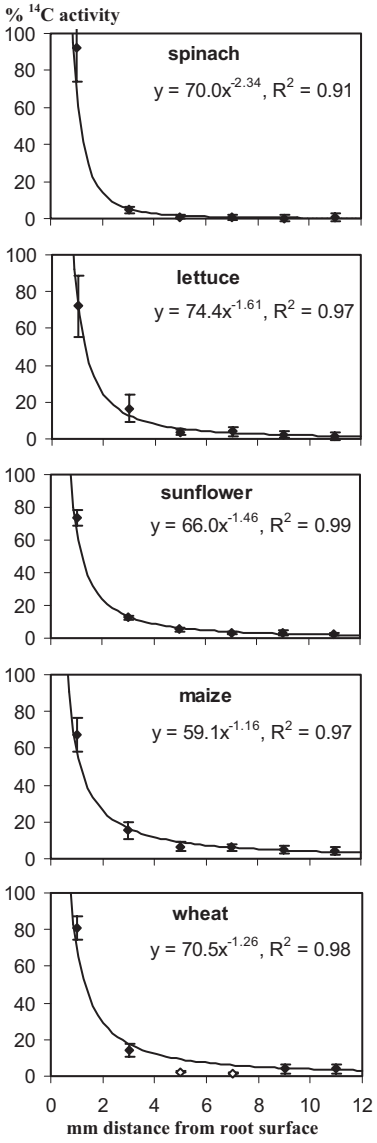

Figure 3: Relative distribution of ^{14}C activity with increasing distance from the root surface of spinach, lettuce, sunflower, maize, wheat (mean values and standard errors). N = 8 for maize, wheat, and sunflower, N = 4 for lettuce and spinach. The white diamonds (wheat) indicate that the data for 4–6 and 6–8 mm distance were outliers and therefore excluded from the calculation.

3.2 Spatial distribution of ^{14}C-labeled exudates with increasing distance to the roots

More than two thirds of the total ^{14}C activity detected outside the rooted zone were found within 2 mm distance from the root surface (Fig. 3). For spinach, the ^{14}C activity in the first 2 mm amounts to 92.5% of the total ^{14}C activity in the non-rooted zone, for lettuce it was 72.1%, for sunflower 73.7%, for maize 67.6%, and for wheat 80.9%. This high percentage of ^{14}C recovered in the first 2 mm is mainly connected with root hairs, which may easily penetrate the gauze because of their small diameters of 5–17 μm and which usually are about 0.08–2 mm long (*Esau*, 1965; *Zhu* et al., 2005). The rapid decrease of ^{14}C activity with distance from the root surface is a result of two concurrent processes: (1) diffusion of root exudates and (2) microbial decomposition. Both processes may be described by differential equations (*e.g.*, *Darrah*, 1991a, b; *Gräfe* and *Kuchenbuch*, 2002). However, we found that the spatial distribution of root exudates, resulting from the interaction of exudate diffusion and microbial decomposition, could be described with maximal accordance by potential equation

$$Y = A_1 \cdot X^{-B},$$

where Y is the ^{14}C activity representing the amount of exudates at the distance X from the root surface, A_1 is the ^{14}C activity in the first millimeters (upper boundary), and B is steepness of the concentration curve.

Fitting of the A_1 and B parameters for individual exudation profiles gave determination coefficients above $R^2 = 0.9$. Therefore, we conclude that this simplifying approach based on potential equations is sufficient to describe—and predict—the exudation profile and the rhizosphere extension under nonsterile conditions.

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