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Turnover and distribution of root exudates of *Zea mays*

Y. Kuzyakov[1,4], A. Raskatov[1,2] & M. Kaupenjohann[3]
[1]*Department of Soil Science and Land Evaluation, Institute of Soil Science and Land Evaluation (310), Hohenheim University, Emil-Wolff-Strasse 27, D-70599 Stuttgart, Germany.* [2]*Department of Ecology, Moscow Agricultural Academy, Timiryazevsky proezd 2, 127550 Moscow, Russia.* [3]*Department of Soil Science, Institute of Ecology, Salzufer 12, D-10587 Berlin, Germany.* [4]*Corresponding author**

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Abstract

Decomposition and distribution of root exudates of *Zea mays* L. were studied by means of $^{14}CO_2$ pulse labeling of shoots on a loamy Haplic Luvisol. Plants were grown in two-compartment pots, where the lower part was separated from the roots by monofilament gauze. Root hairs, but not roots, penetrated through the gauze into the lower part of the soil. The root-free soil in the lower compartment was either sterilized with cycloheximide and streptomycin or remained non-sterile. In order to investigate exudate distribution, 3 days after the ^{14}C labeling, the lower soil part was frozen and sliced into 15, one-mm thick layers using a microtome. Cumulative $^{14}CO_2$ efflux from the soil during the first 3 days after ^{14}C pulse labeling did not change during plant growth and amounted to about 13–20% of the total recovered ^{14}C (41–55% of the carbon translocated below ground). Nighttime rate of total CO_2 efflux was 1.5 times lower than during daytime because of tight coupling of exudation with photosynthesis intensity. The average CO_2 efflux from the soil with *Zea mays* was about 74 μg C g^{-1} day^{-1} (22 g C m^{-2} day^{-1}), although, the contribution of plant roots to the total CO_2 efflux from the soil was about 78%, and only 22% was respired from the soil organic matter. *Zea mays* transferred about 4 g m^{-2} of carbon under ground during 26 days of growth. Three zones of exudate concentrations were identified from the distribution of the ^{14}C-activity in rhizosphere profiles after two labeling periods: (1) 1–2 (3) mm (maximal concentration of exudates) 2) 3–5 mm (presence of exudates is caused by their diffusion from the zone 1); (3) 6–10 mm (very insignificant amounts of exudates diffused from the previous zones). At the distance further than 10 mm no exudates were found. The calculated coefficient of exudate diffusion in the soil was 1.9×10^{-7} cm^2 s^{-1}.

Introduction

The rhizosphere is the site of intensive interactions between plant roots, microorganisms and soil constituents (Curl and Truelove, 1986; Kuzyakov, 2002; Marschner, 1995). Organic substances released by plant roots – exudates – play an important role in these interactions. Root exudates may affect the turnover processes in the rhizosphere in different ways. They may (i) modify the solubility, sorption and transport of mineral elements to the roots through change of pH, redox potential and organic complexation (Dinkelaker et al., 1989; Marschner, 1995; Merbach et al., 1999; Norvell et al., 1993; Schilling et al., 1998; Uren and Reisenauer, 1988; Zhang, 1993), (ii) affect the microbial activity and turnover of microbial biomass (Deubel, 1996; Deubel et al., 2000; Helal and Sauerbeck, 1986; Kuzyakov, 2002), and (iii) improve the soil structure. The total concentration of organic substances in the rhizosphere soil depends on three main interacting processes: (i) the release of water soluble exudates and insoluble C compounds by roots, (ii) the transport by diffusion of water soluble compounds into surrounding soil, and (iii) the microbial decomposition of the released and transported organic compounds which leads to microbial growth. The first and the

* Fax No: +49-711-459-3117.
E-mail: kuzyakov@uni-hohenheim.de

third processes can be summarized as turnover of root exudates, and this turnover can be estimated according to CO_2 efflux coming from rhizosphere.

Studies of the rhizosphere under natural conditions are technically difficult and special methods are required for such investigations. Farr et al. (1969) held onion roots between two soil blocks and sliced the blocks to obtain rhizosphere soil. This method worked well only for roots without root hairs due to their damage during placement of the onion roots between the soil blocks. Boero and Thien (1979) used plants grown in concentric cylinders made of nylon net to simulate the rhizosphere and to separate it from root-free soil. However, preparing accurate cylinders from nylon gauze as well as maintaining nutrient supply were difficult in these systems, which limited the applicability of the method. Kuchenbuch and Jungk (1982) used a screen to separate the roots mat from a soil column. At harvest the plants were separated by cutting between the roots and screen, the soil column was rapidly frozen and sliced into thin layers with a microtome. However, this method was used only with very young seedlings, which could survive on the small amount of water and nutrients from the soil column. To overcome these difficulties Gahoonia and Nielsen (1991) studied P depletion and pH changes in the rhizosphere with a very similar method, but with externally regulated nutrient and water supply.

The application of C isotopes (^{14}C and ^{13}C) in rhizosphere studies has led to significant progress in the understanding of C cycling within the root–soil system. The results of experiments, in which plants were labeled, have shown that the amounts of root-derived C are much higher than observed with root washing methods or root growth estimation. For example, root weight estimated by means of ^{14}C was about 20–60% higher than that measured using the root washing method (Sauerbeck and Johnen, 1976), which may be attributed to high losses of C during the root washing procedure (Swinnen et al., 1994; Kuzyakov et al., 1999). Root exudate concentrations calculated with tracer techniques were 20–100 times higher (Cheng et al., 1993) than the concentrations calculated according to the exudation rates of roots (Darrah, 1991a, b; Newman and Watson, 1977), which may be the result of fast microbial utilization of organic substances released by the roots (Kuzyakov, 1997; Kuzyakov and Demin, 1998; Paul and Clark, 1996).

Investigating C distribution in the rhizosphere, Helal and Sauerbeck (1983, 1986) used ^{14}C labeling to quantify root exudates remaining in the soil in different proximities to the roots. In this study, however, the rhizosphere sections, in which concentrations of root exudates were measured were too large to establish exact sizes of the rhizosphere and exudate distribution. Helal and Sauerbeck (1983) divided the soil with vertical screens into three quite large zones (ca. 10 mm) of differing root proximity and grew maize for 25 days in a ^{14}C-growth chamber. They found 0.3 and 0.1% of total ^{14}C assimilated by plants only in the first and the second 10 mm of soil from maize roots, respectively. However, the system used did not allow for the separation of the effects of exudate diffusion and the uptake of exudates by microorganisms. Moreover, this method is restricted to small pots for two maize plants, with quite large distances between the screens and no possibility to slice the rhizosphere soil. For better understanding of process scales and rhizosphere effects, detailed knowledge and a complete picture of exudate distribution in the rhizosphere are necessary.

We combined the methods mentioned above to (a) study $^{14}CO_2$ evolution from rhizosphere of maize to estimate turnover of exudates, (b) examine the distribution of root-derived C as a function of root proximity, (c) evaluate the effect of microorganisms on distribution of root exudates in the soil, and (d) study diffusion of root exudates in the rhizosphere soil.

Materials and methods

Soil, pots and plant growing conditions

We studied the C rhizodeposition and distribution of root exudates of *Zea mays* L. by means of $^{14}CO_2$ pulse labeling of shoots in two growth stages of the plants (19- and 26-day-old). The plants were grown in two-compartment pots (Figure 1) under controlled laboratory conditions. The soil was taken from the top 10 cm (Ah horizon) of a loamy Haplic Luvisol at the experimental station Karlshof (South Germany, Stuttgart), air-dried, mixed, and passed through a 5-mm sieve (Table 1).

The two-compartment PVC container consisted of (i) a upper part (160 mm height, Ø57 mm) and (ii) a lower part (40 mm height, Ø57 mm, Figure 1). A monofilament screen manufactured by the Büttner GmbH (Sefar Filtration, Ruschlikon, Switzerland) separated the two parts from each other. The gauze had 153 threads per cm, the clear mesh is 30 μm, and 21% of the total area is open. Roots did not penetrate

Table 1. Main characteristics of the loamy Haplic Luvisol (0–10 cm depth, Ap)

pH	Sand	Silt	Clay	Field capacity	Available water capacity	C_{org}	N_t	C/N	$N–NO_3$	$N–NH_4$
$CaCl_2$	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(mg × 100 g^{-1} soil)	
6.8	4.4	73	23	33	24	1.2	0.13	9.3	0.32	0.15

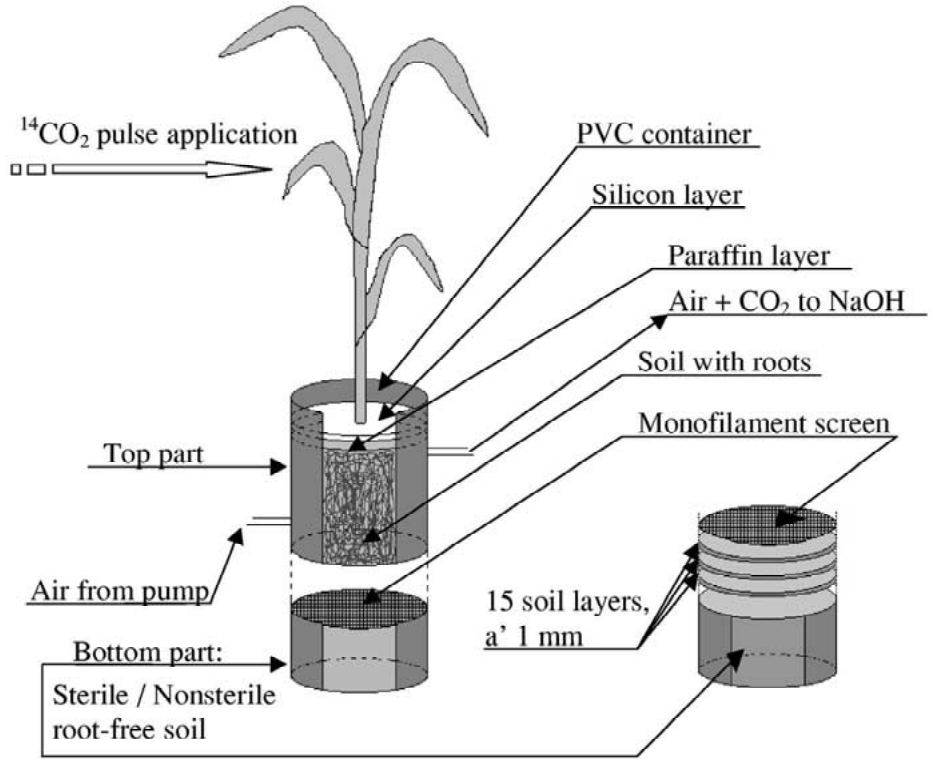

Figure 1. Two-compartment pot for studying distribution of root-derived carbon as a function of root proximity and presence of soil microorganisms.

through the screen, but root hairs were easily able to grow through the screen into the soil (Kuchenbuch and Jungk, 1982).

Each pot was filled with 476 g of air-dried soil (371 g in the upper and 105 g in the lower part). One seedling of *Zea mays* (spec. Benicia) was grown in each pot at a 26–28 °C day and 22–23 °C night temperature, with a day-length of 14 h and light intensity of approximately 400 μmol m^{-2} s^{-1} at the top of the canopy (two lamps of type SON-T Agro NYAS TB 15, Phillips, Holland). The soil water content of each pot was determined by weighing the pot and was adjusted daily to about 60% of the available field capacity.

To compare total unlabeled CO_2 evolution from soil with and without *Zea mays*, soil without plants was also incubated in similar pots and under the same experimental conditions.

To suppress microbial decomposition of exudates in the lower soil part, the soil in the lower part of half of the pots was treated with 3.75 mg g^{-1} soil of cycloheximide against fungi and 5 mg g^{-1} soil of streptomycin against bacteria (Lin and Brookes, 1999). The sterilization took place 1 day before $^{14}CO_2$ pulse was applied. The sterilization was carried out by means of injecting an aqueous solution of cycloheximide and streptomycin into the soil, with a syringe. The sterility of the soil was not checked. The rooted soil (upper part of the pots) was not sterilized. In this way we reduced the effect of cycloheximide and strep-

tomycin on the roots. Eight replications were made of the sterilized and non-sterilized pots in the first labeling and four in the second labeling.

^{14}C labeling

One day before labeling, the root and shoot zones of the plants were separated by low melting point Paraffin (m.p. 42–44 °C; Merck Eurolab GmbH, Bruchsal) and overlaid with Silicon paste (NG 3170 of Fa. Thauer and Co.Dresden). The labeling was applied to two growth stages (19- and 26-day-old plants) for a period of 1 h. Eight pots were labeled simultaneously in a Plexiglas chamber. 460 kBq of ^{14}C as $Na^{14}CO_3$ solution were used for each pot, the chamber was then closed and 3 mL of lactic acid were added to the $Na^{14}CO_3$ solution in a tube through a pipe on the plexiglas chamber. This allowed complete evolution of $^{14}CO_2$ into the chamber atmosphere. In order to remove the remaining unassimilated $^{14}CO_2$, 1 h after labeling began, CO_2 from the Plexiglas chamber was trapped by continuously pumping (100 cm^3 min^{-1}) the air from the chamber through 10 mL of 1 *M* NaOH solution using a membrane pump. Then the upper part of the chamber was opened and the plants were grown under normal conditions.

Sample collection, analysis and calculations

Air cycling through the bottom compartments was started immediately after labeling. During the experiment, CO_2 released from the soil compartments containing soil and roots was trapped in 20 cm^3 of 0.5 *M* NaOH solution by continuously pumping (100 cm^3 min^{-1}) with a membrane pump. As checked in previous studies, the NaOH washing flasks used, enable the trapping of more than 95% of CO_2 released. The tubings to and from soil pots were connected in a circle, and the air was circulated between soil pot and NaOH trapping flask continuously. This kind of air circulation allows for the trapping of 100% of CO_2 evolved. The trap for CO_2 evolved from the upper part of the soil was changed twice a day during 4 days after labeling. Four days after each labeling, the lower parts of the pots were removed and frozen at –25 °C. At the same time all plants of variants with sterilized lower parts were cut, root–soil column (upper part) pulled out, and the roots were washed from the soil by hand. The second labeling took place 7 days after the first one. For the second labeling, the previously non-sterilized pots remaining from the first labeling were used. Half a day before the second labeling, new lower parts with moist soil were inserted. The pots were divided into two treatments (four pots per treatment), and the lower parts of four of the pots were sterilized as described above. The other four pots remained unsterilized.

The lower PVC containers (of first and second labeling) were removed and the soil blocks were inserted in the sample holder of a microtome. The frozen soil blocks were cut into 15 slices about 1 mm thick. The dry weight of one slice was about 3.0 g. Shoots, roots and soil were dried at 60 °C. Dry samples of shoots, roots and soil were pulverized in a ball mill prior to the analysis of radioactivity.

Radioactivity of shoots, roots and soil samples were measured with the scintillation cocktail Permafluor E+ (Canberra Packard) by a liquid scintillation counter (Tri-Carb 2000CA, Canberra Packard), after combustion of 1 g of sample within an oxidizer unit (Canberra Packard, Model 307). The ^{14}C in CO_2 collected in the NaOH solution was measured with the scintillation cocktail Rothiscint-22x of Roth Company with 2-mL aliquots of NaOH after the decay of chemiluminescence. The ^{14}C counting efficiency was about 89% and the ^{14}C-activity measurement error did not exceed 2%. The absolute ^{14}C-activity was standardized by addition of NaOH solution as quencher to the scintillation cocktail and using a two-channel ratio method of extended standard (tSIE).

We labeled 8 plants together in one chamber. Therefore, the single plants could assimilate different amounts of $^{14}CO_2$. In order to correct for differences in plant sizes and photosynthetic rates, rates of $^{14}CO_2$ evolution from the soil were calculated on the basis of total ^{14}C recovered from plants, soil respiration, and soil (^{14}C net assimilation), for each plant–soil microcosm. All results regarding the ^{14}C distribution in the maize rhizosphere were presented as percentage of ^{14}C recovered in the lower part of soil.

Total content of CO_2 collected in NaOH solution was measured by titration with 0.2 *M* HCl against phenolphthalein after addition of 0.5 *M* $BaCl_2$ solution (Black, 1965). Total C concentration in shoots was considered to be 40 ± 3% of dry mass and was accepted as a constant. Because of different content of mineral soil particles as result of the root washing procedure, carbon content in the roots was considered by all calculations according to C analysis.

For the estimation of carbon flows that *Zea mays* transfers below the soil we used the following equation

(Kuzyakov et al., 1999):

$$C_{UG}\ (\text{g plant}^{-1}) = C_{shoots}\ (\text{g plant}^{-1}) \times {}^{14}C_{UG}(\%) \,/\, {}^{14}C_{shoots}\ ([\%), \quad (1)$$

where C_{UG} (g plant^{-1}) is carbon amount transferred underground, C_{shoots} (g plant^{-1}) is carbon content in the shoots, $^{14}C_{UG}$ (%) is ^{14}C content in the soil or roots 6 days after labeling, $^{14}C_{shoots}$ (%): ^{14}C content in the shoots 6 days after labeling.

For the calculation of the diffusion coefficient of root exudates we used the diffusion equation in the form (Darrah, 1991d):

$$\frac{\varphi C_s}{\varphi_t} = D_e \frac{\varphi^2 C_s}{\varphi_\chi^2} \quad o < x < L$$

$$\frac{\varphi C_s}{\varphi_t} = 0 \quad x = 0, x = L, \quad (2)$$

where C_s is the concentration of diffusing exudates per unit volume of soil at a distance x from the root surface; D_e id the effective diffusion coefficient; L is length of the soil cylinder.

Results and discussion

Zea mays assimilated practically the total amount of $^{14}CO_2$ applied to the shoots during one hour. Only 0.88–1.82% of the $^{14}CO_2$ input remained in the chamber with plants was trapped in NaOH solution after the labeling.

Root derived $^{14}CO_2$ and total CO_2 efflux from soil

$^{14}CO_2$ evolution from the soils reached its maximum within the first day after labeling (Figure 2, left). Some investigations (Cheng et al., 1993; Gregory and Atwell, 1991; Kuzyakov et al., 1999) show that assimilation of CO_2 and the downward transport of assimilated C in plants are very rapid processes. Biddulph (1969) estimated the rate of flow of assimilates towards the roots to be about 100 cm h^{-1}. These high transport rates confirm the fact that the downward transport of assimilates is an active process that is much faster than diffusion by a concentration gradient (Waremburg and Morral, 1978).

Cumulative $^{14}CO_2$ efflux from the soil in the first 3 days after ^{14}C pulse labeling was 13–20% of recovered ^{14}C and did not change between the first and the second labeling. These values of root derived CO_2 correspond to 41–55% of the ^{14}C label translocated

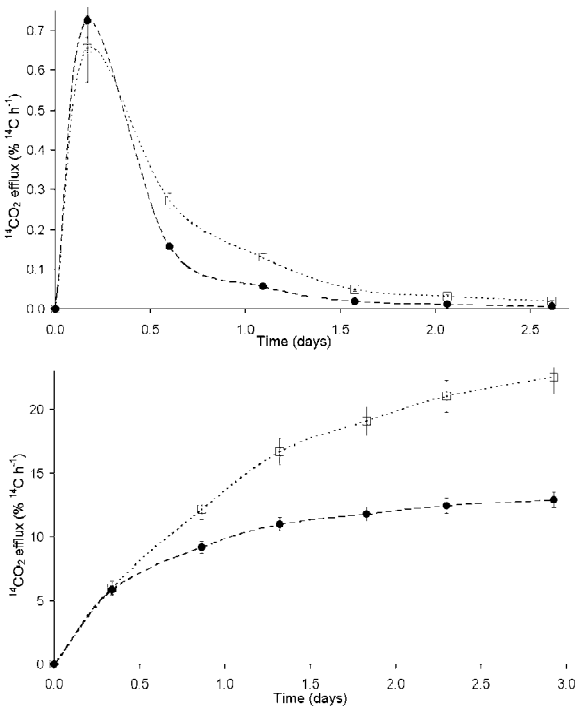

Figure 2. $^{14}CO_2$ efflux rate (top) and cumulative $^{14}CO_2$ efflux (bottom) (± SE) from the soil in the first 3 days after ^{14}C pulse labelling of 19-day-old (top) maize plants. n = 8. (- - ●- -) not sterile; (- - □ - -) sterilized.

below ground and included both: root respiration and microbial decomposition of rhizodeposits. However, rather large variations were reported in the literature: 16–40% of the below ground translocated carbon was released as root-soil respiration from maize. The fraction related to C amount translocated below ground increased with plant age (Whipps, 1990). Martens (1990) found that approximately 45% of carbon translocated below ground by maize roots was respired in a few days. Also, in studies in nutrient solution, 49% of the carbon translocated to roots was respired (Veen, 1980). This means that similar to other experiments, about one-half of organic substances translocated below ground by plants were respired as CO_2 in the rhizosphere during few days in our study. According to Domanski et al. (2001), few days after a pulse of $^{14}CO_2$, the release of exudates by roots is finished.

Cumulative $^{14}CO_2$ efflux from the variant with non-sterized soil in the lower pot part was much less than that of soil with a sterilized lower part (Figure 2). Probably, cycloheximide or streptomycin affected the roots in such a way that the below ground C trans-

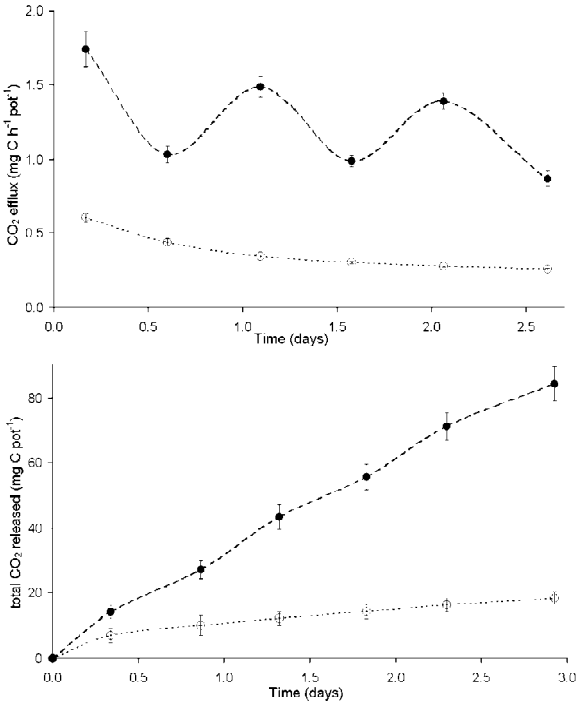

Figure 3. Rate of total (left) and cumulative (right) CO_2 efflux (±SE) from the unplanted soil and soil planted with maize during a period of three days (19-day-old maize).

location strongly increased. This fact needs further investigation.

Total CO_2 evolution from the soil and contribution of Zea mays to soil respiration

We compared the total unlabeled CO_2 evolution from the soil with *Zea mays* and from the bare soil incubated under the same conditions (the same soil amounts in the pot, the same soil moisture, the same air temperature and airflow). Depending on the time of the day, the rate of total CO_2 efflux from the unplanted soil is 2–5 times less that from the soil planted with 19-day-old maize (Figure 3). As expected, the night time rate of CO_2 efflux from planted soil plants was significantly lower than that of day time, although no day/night changes were measured from unplanted soil. Kuzyakov and Cheng (2001) showed that the rate of root-derived CO_2 efflux from rhizosphere depends on diurnal cycles of photosynthesis. Similar diurnal cycles of bacterial activities (higher during the day time and lower during night) were observed in seagrass sediments (Moriarty and Pollard, 1982; Welsh et al., 1997). This shows indirectly that exudation, and not root respiration increases during the day time, because bacterial activity is unaffected by root respiration. Later, Kuzyakov and Siniakina (2001) experimentally proved that exudation, and not the root respiration, increased during the day time.

In this study, we accept that the difference between CO_2 efflux from the soil with plants and from the bare soil is equal to the contribution of plant roots to the CO_2 efflux. The average CO_2 efflux from the soil with *Zea mays* is about 5 times higer than that from the bare soil. The CO_2 evolution from bare soil was about 16.5 μg C-CO_2 day^{-1} g^{-1} (5.0 g C-CO_2 m^{-2} day^{-1}, calculated from thickness of arable horizon 25 cm and density 1.2 g cm^{-3}) and was decreased slightly during the incubation. The average CO_2 efflux from the soil with *Zea mays* was about 73.7 μg C-CO_2 g^{-1} day^{-1} (22 g C-CO_2 m^{-2} day^{-1}). Therefore, the contribution of plant roots to the total CO_2 efflux from the soil (calculated as the difference) was about 78%. In spite of low total C content in soil used in the experiment, a high level of total CO_2 efflux was detected. Liljeroth et al. (1994) has reported much lower rates of total unlabeled CO_2 evolution from the soil with *Zea mays* (9.0 μg C-CO_2 g^{-1} day^{-1}). This difference can be explained by the smaller amount (7 times less) of soil used in our experiment, because the smaller amount of soil used in the pot experiments usually increased the turnover rates of soil organic matter. Additionally, a small soil volume is unfavorable especially for maize roots and may decrease the total below ground C translocation as well as root respiration and exudation.

Carbon transfer into the soil

The total ^{14}C content in the soil (soil and roots) after 6 days ranged from 12.3 to 18.5% of recovered carbon. The data for ^{14}C incorporation in the roots of *Zea mays* reported by Liljeroth et al. (1994), is in the same order (16–23%). However, Helal and Sauerbeck (1983) found that *Zea mays* transferred about 33% of recovered carbon under the soil surface for a long time. Only a fifteenth of this was in the soil and the rest in the roots (Helal and Sauerbeck, 1983). The similar distribution (1:9) of ^{14}C between soil and roots was found in our study.

According to the equation one, 26-day-old *Zea mays* transferred about 0.56 g $plant^{-1}$ or 4 g m^{-2} of carbon into the soil. The total root mass measured after root washing amount to 0.42 ± 0.07 g $plant^{-1}$ (±SE). It means, that the total transfer of organic C into the soil is at least 33% higher than that

measured by root washing procedure. Considering the data of root-derived CO_2 efflux (41–55% of below ground translocated C) we conclude that about two-thirds of root-derived CO_2 was produced by microbial decomposition of exudates and about one-third by root respiration.

The amount of carbon transferred into the soil may be slightly higher than calculated because maize can still transfer a part of the assimilated carbon from the shoots to the roots, after the 6 days after labeling.

Distribution of root-derived C as a function of root proximity and presence of soil microorganisms

Results of the present study showed that the quantity of labeled C remaining in the lower soil part separated from roots by gauze was much smaller (0.03–0.06% of ^{14}C recovered) compared to the amount of root-derived C released from the soil during the experiment (13–20% of ^{14}C recovered). After separation of the rooted and root-free soil parts, it was definitely possible to see that the roots had covered the whole monofilament screen. Therefore, the root length on the screen had no effect on the observed results of ^{14}C exudate distribution in the root-free soil part. Although rhizodeposition occurred mainly in the upper parts and in the first two layers of the lower parts of the pots, diffusion of root-derived carbon into deeper layers was also significant (Figure 4).

The distribution of native root exudates and their diffusion into the soil has not been investigated up till now because of the lack of suitable methods. Newman and Watson (1977) and Rovira (1969) concluded that the zone of influence of root exudates in soil is narrow because the exudates are rapidly utilized and thus have little opportunity to diffuse far from the root. But other investigations showed that the rhizosphere microorganisms do not constitute a continuous layer on the root surface. Bacteria occur in microcolonies (Newman and Bowen, 1974; Rovira and Campbell, 1974) and the microbial cover usually does not exceed 5–15% of root surface (Bowen and Rovira, 1976; Bowen and Theodorou, 1979; Rovira et al., 1974). Therefore, we expected a detectable diffusion of root exudates from the root surface.

Slicing of 1-mm layers with increasing proximity to the roots and analyzing ^{14}C distribution in 15 such soil layers shows exponentially decreasing exudate concentration with distance from the roots, both in the sterilized and non-sterilized variants (Figure 4). Because of rapid microbial utilization, the decreasing of the concentration of ^{14}C labeled exudates is much faster in non-sterilized variants compared to the sterilized soil. The amount of ^{14}C-exudates in the soil layer nearest to the roots of the non-sterilized soil was higher than that of the sterilized soil. It shows the stimulation of exudation by microorganisms frequently observed in other studies (Martin, 1977; Meharg and Killham, 1995; Merbach and Ruppel, 1992).

In spite of on almost continuous gradient of exudate distribution as a function of the distance from the root surface, three zones of exudate concentrations could by identified according to the distribution of the ^{14}C-activity in rhizosphere after two labeling periods: (1) 1–2 (3) mm (maximal concentration of exudates around root hairs. In these layers there are also root hairs, which could penetrate into the synthetic screen); (2) 3–5 mm (presence of exudates is caused by their diffusion from the zone); (3) 6–10 mm (very insignificant amounts of exudates diffused in this zone.) At the distance further than 10 mm, no significant ^{14}C activity was observed, indicating the complete absence of exudates. This exudate distribution indicated that the maximal extension of the rhizosphere is about 10 mm.

Diffusion of root exudates in the rhizosphere

The following processes control the distribution and the local concentration of exudates in the rhizosphere: exudation intensity, uptake of exudates by roots, diffusion from the root surface, and microbial utilization. In this study we cannot distinguish between exudation and uptake of exudates by roots, and thus we define the difference between these processes as the net exudation. Under soil conditions, especially in the presence of rhizosphere microorganisms, the uptake of exudates by roots is negligible in comparison to nutrient solution studies, in which the most results about root uptake of exudates were obtained (Paterson and Sim, 1999). Therefore we ignored the uptake of exudates by roots in this study.

Some studies of exudate diffusion were made using mathematical simulation models (Gardner et al., 1983; Darrah, 1991c, d) or as investigations with some individual substances that are components of root exudates (Darrah, 1991a, b). However, the important limitation of these studies is the fact that they were not experimentally proved within soil–plant system and with natural composition of exudates.

The microcosm design used in this experiment allows the investigation of the diffusion and micro-

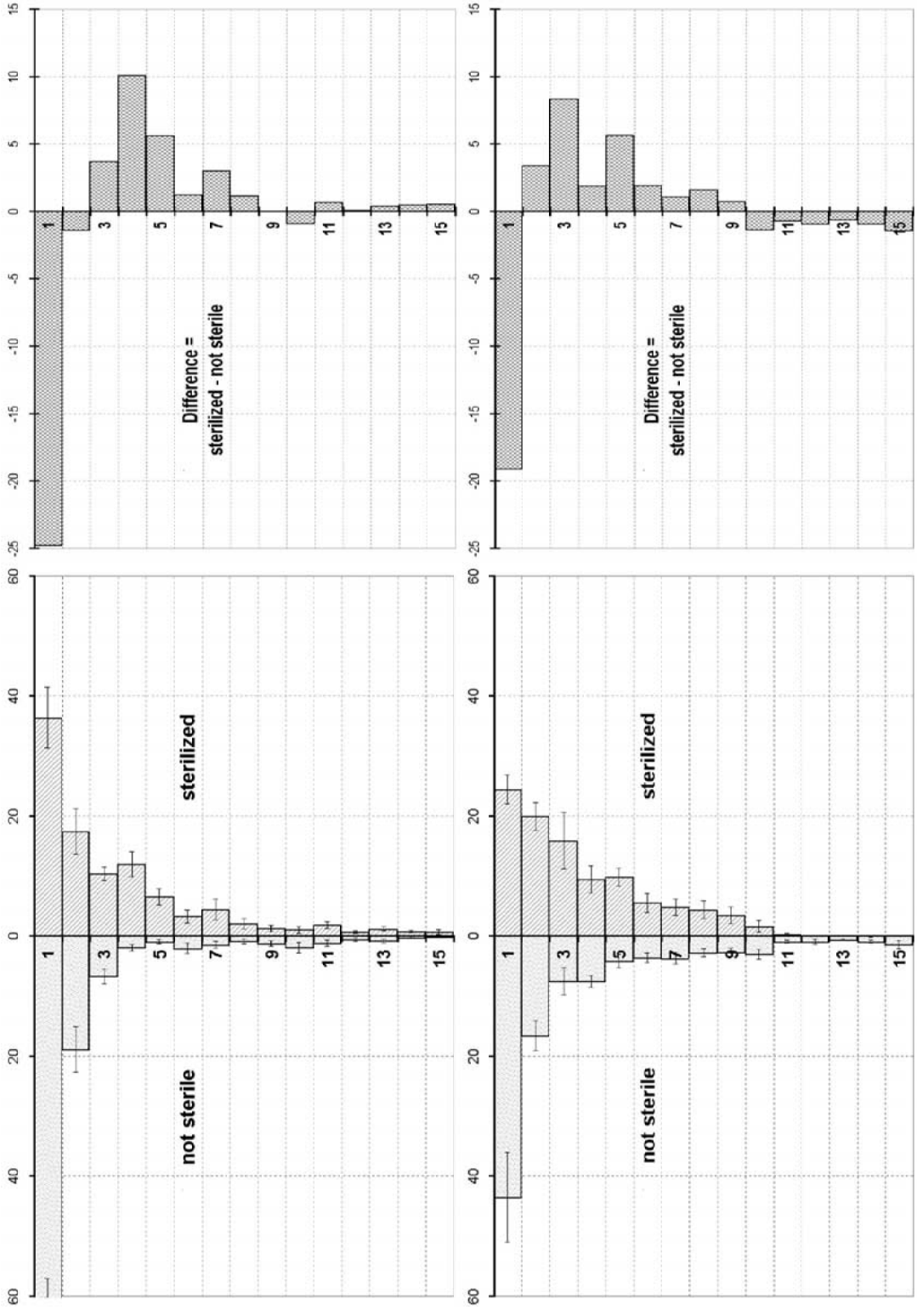

Figure 4. Exudate distribution (% of total ^{14}C found in all 15 layers, ±SE) depending on the distance from roots of 19- (top) and 26-day-old (bottom) maize, 4 days after ^{14}C pulse labelling. Left: exudate distribution in 'non-sterile' and 'sterile' soil. Right: difference between amounts of exudates in 'sterile' and 'non-sterile' soil. Vertical scale: mm from roots.

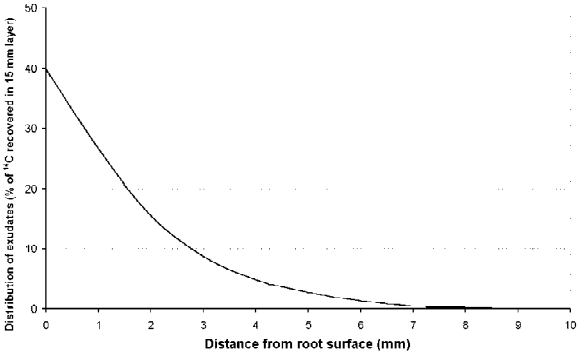

Figure 5. Diffusion profile of exuded organic substances in sterilized soil, 4 days after assimilation of C from atmosphere calculated on measured ^{14}C distribution.

bial utilization of exudates. Separation of root zone and root-free zone by the microfilament gauze impermeable for roots (Helal and Sauerbeck, 1983) enables the study of exudate distribution depending on different proximity to root surface. Use of the variant with sterilization of soil in the root-free compartment allows the comparison of exudate distribution in sterilized and microorganism colonized soil (Figure 4, right). ^{14}C pulse labeling permits tracing of organic substances exuded in a definite period. We combined the three methods to estimate the diffusion of root-borne organic substances in rhizosphere and assumed that the diffusion rate in the sterilized and non-sterilized soil was of the same order.

Diffusion of soluble organic compounds from a single root in the soil occurs in both radial and vertical directions. Additionally, any vertical movement is affected by the mass flow of soil solution, which occurs radially towards the root. In our study, only one-dimensional diffusion was calculated because the roots covered the whole surface of the screen. Under such conditions the radial diffusion is absent. As shown in Figure 5, the concentration profile of exudates with increasing proximity from root surface is steep. Moreover, it becomes less steep as D_e increases under higher relative concentration of diffused substance (Gardner et al., 1983). Coefficient of exudate diffusion in the soil calculated according to Darrah (1991d) was 1.89×10^{-7} cm^2 s^{-1}. It is important to mention, that the diffusion coefficient calculated is a parameter of a complex cocktail of exudates and it is not a parameter of a single substance, which can be obtained in a study with individual compounds. In most rhizosphere and ecological studies, the information about the real distribution of easily available C sources exuded by roots as a function of root proximity is necessary and not the distribution of one or some substances.

Diffusion coefficients obtained in some of the studies mentioned above varied from 1×10^{-4} to 1×10^{-7} cm^2 s^{-1}. Higher coefficients were frequently measured for substances non-adsorbed in soil such as glucose or sucrose (Darrah, 1991a). Substances, which are more strongly adsorbed by soil such as citric or glutamic acids are characterized by lower diffusion coefficients (Darrah, 1991b). The diffusion coefficient of root exudates obtained in our study is of a low order. The reasons for this could be (1) a low concentration C_s of exudates in the soil compared to other studies with artificial input of substances into the soil, and (2) partial sorption of exudates by the soil solid phase, which can reach up to 99% for some organic substances (Darrah, 1991b). The majority of organic solutes found in soil solutions are absorbed by the solid phase of the soil. It means that the substance composition and availability of C changed dramatically close to the root surface and at few mm from it. Commonly, solutes will interact with the solid phase by sorbing to it by mechanisms such as inner- and outer-sphere complexation or as part of the diffuse ion swarm, involving ionic, covalent and H bonding (Sposito, 1989).

Such sorption reactions usually depend on concentration and are reversible if the solution concentration decreases. Reversible sorption slows the rate of diffusion of the solute by a factor $\theta dC_L/dC$, where θ is the volumetric moisture content and C_L and C are the concentration of the solute in the soil solution and in the soil as a whole, respectively (Nye and Tinker, 1977). Darrah (1991a) found that glucose and sucrose behaved as non-sorbing substances in the soil, while acetate can interact with the solid phase and thus have its mobility decreased. In addition, other organic substances exuded by maize roots, such as carboxylic (18% of exudates) and amino acids (22% of exudates) (Gransee and Witterenmayer, 2000) are sorbed in the soil. Therefore, sorption of exudates by soil colloids could be responsible for the low value of the diffusion coefficient observed in this study.

We conclude that the combination of methods (^{14}C pulse labeling, root separation by a screen, and slicing of root-free soil at different proximities to the root surface) was an adequate tool for determination of root exudate distribution and also for studying exudate diffusion. The total ^{14}C content in the soil with roots and exudates after 6 days was about 24% of assimilated

carbon, and corresponds to an underground carbon transfer by *Zea mays* of 4 g m^{-2} during first 26 days of growth. The maximal extension of the rhizosphere as soil volume affected by root exudates and as measured with sensitive ^{14}C technique, was not more than 10 mm. The calculated coefficient of exudate diffusion in the soil was 1.9×10^{-7} cm^2 s^{-1}.

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References

Biddulph O 1969 Mechanisms of translocation of plant metabolites. *In* Physiological Aspects of Crop Yield. Eds. J D Eastin, F A Haskins, C Y Sullivan and C H N Van Bavel. pp. 143–164. Am. Soc. Agron. Crop. Sci. Soc. Am. Inc., Madison, WI.

Black C A (Ed.) 1965 Methods of Soil Analysis, Part 2, pp. 1562–1565. Am. Soc. Agron., Inc. Publisher, Madison, WI.

Boero G and Thien S 1979 Phosphatase activity and phosphorus availability in the rhizosphere. *In* The Soil Root Interface. Eds J L Harley and R S Russell. pp. 231–242, Academic Press, London.

Bowen G D and Rovira A D 1976 Microbial colonization of plant roots. Annu. Rev. Phytopathol. 14, 121–144.

Bowen G D and Theodorou C 1979 Interaction between bacteria and ectomycorrhizal fungi. Soil Biol. Biochem. 11, 119–126.

Cheng W, Coleman D C, Carroll C R and Hoffman C A 1993 In situ measurement of root respiration and soluble C concentrations in the rhizosphere. Soil Biol. Biochem. 25, 1189–1196.

Curl E A and Truelove B 1986 The Rhizosphere. Springer, Berlin.

Darrah P R 1991a Measuring the diffusion coefficient of rhizosphere exudates in soil. I. The diffusion of non-sorbing compounds. J. Soil Sci. 42, 413–420.

Darrah P R 1991b Measuring the diffusion coefficients of rhizosphere exudates in soil. I. The diffusion of sorbing compounds. J. Soil Sci. 42, 421–434.

Darrah P R 1991c Models of the rhizosphere. II. A quasi three-dimensional simulation of the microbial population dynamics around a growing root releasing soluble exudates. Plant Soil 138, 147–158.

Darrah P R 1991d Models of the rhizosphere. I. Microbial population dynamics around a root realising soluble and insoluble carbon. Plant Soil 133, 187–199.

Deubel A 1996 Einfluss wurzelbürtiger organischer Kohlenstoffverbindungen auf Wachstum und Phosphatmobilisierung verschiedener Rhizosphärenbakterien. Dissertation, Martin-Luther-Universität Halle-Wittenberg.

Deubel A, Gransee A and Merbach W 2000 Transformation of organic rhizodeposition by rhizosphere bacteria and its influence on the availability of tertiary calcium phosphate. J. Plant Nutr. Soil. Sci. 163, 387–392.

Dinkelaker B, Römheld V and Marschner H 1989 Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus L.*). Plant Cell Environ. 12, 285–292.

Domanski G, Kuzyakov Y, Siniakina SV, Stahr K 2001 Carbon flows in the rhizosphere of *Lolium perenne*. J. Plant Nutr. Soil Sci. 164, 381–387.

Farr E, Vaidyanathan L V and Nye P H 1969 Measurement of ionic concentration gradients in soil near roots. Soil Sci. 107, 385–391.

Gahoonia T S and Nielsen N E 1991 A method to study rhizosphere processes in thin soil layers of different proximity to roots. Plant Soil 135, 143–146.

Gardner W K, Parbery D G, Barber D A and Swinden L 1983 The acquisition of phosphorus by *Lupinus albus* L.V. The diffusion of exudates away from roots: a computer simulation. Plant Soil 72, 13–29.

Gransee A and Wittenmayer L 2000 Qualitative and quantitative analysis of water-soluble root exudates in relation to plant species and development. J. Plant Nutr. Soil. Sci. 163, 381–385.

Gregory P J and Atwell B J 1991 The fate of carbon in pulse labelled crops of barley and wheat. Plant Soil 136, 205–213.

Helal H M and Sauerbeck D 1983 Method of study turnover processes in soil layers of different proximity to roots. Soil Biol. Biochem. 15, 223–225.

Helal H M and Sauerbeck D 1986 Effect of plant roots on carbon metabolism of soil microbial biomass. Z. Pflanzenernähr. Bodenk. 149, 181–188.

Kuchenbuch R and Jungk A 1982 A method for determining concentration profiles at the soil–root interface by thin slicing rhizosphere soil. Plant Soil 68, 391–394.

Kuzyakov Y 1997 Abbau von mit ^{14}C spezifisch markierten Aminosäuren im Boden und Decarboxylierung als einer der natürlichen Neutralisationmechanismen. Arch. Acker- Pflanzenbau Bodenk 41, 335–343.

Kuzyakov Y 2002 Review: Factors affecting rhizosphere priming effects. J. Plant Nutr. Soil Sci. 165, 382–396.

Kuzyakov Y and Cheng W 2001 Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biol. Biochem. 33, 1915–1925.

Kuzyakov Y and Demin V V 1998 CO_2 efflux by rapid decomposition of low molecular organic substances in soils. Science of Soils, 3. http://link.springer.de/link/service/ journals /10112/fpapers /8003001/80030002.htm.

Kuzyakov Y and Siniakina SV 2001 Siphon method of separating root-derived organic compounds from root respiration in non-sterile soil. J. Plant Nutr. Soil Sci. 164, 511–517.

Kuzyakov Y, Kretzschmar A and Stahr K 1999 Contribution of *Lolium perenne* rhizodeposition to carbon turnover of pasture soil. Plant Soil 213, 127–136.

Kuzyakov Y, Ehrensberger H and Stahr K 2001 Carbon partitioning and below-ground translocation by *Lolium perenne*. Soil Biol. Biochem. 33, 61–74.

Liljeroth E, Kuikman P and Van Veen J A 1994 Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organic matter at different soil nitrogen levels. Plant Soil 161, 233–240.

Lin Q and Brookes P S 1999 An evaluation of the substrate-induced respiration method. Soil Biol. Biochem. 31, 14, 1969–1983.

Marschner H 1995 Mineral Nutrition of Higher Plants. 2nd Edition. Academic Press, London.

Martens R 1990 Contribution of rhizodeposits to the maintenance and growth of soil microbial biomass. Soil Biol. Biochem. 22, 141–147.

Martin J K 1977 Factors influencing the loss of organic carbon from wheat roots. Soil Biol. Biochem. 9, 1–7.

Meharg A A and Killham K 1995 Loss of exudates from the roots of perennial ryegrass inoculated with a range of microorganisms. Plant Soil 170, 345–349.

Merbach W and Ruppel S 1992 Influence of microbial colonization on $^{14}CO_2$assimilation and amounts of root-borne ^{14}C compounds in soil. Photosynthetica 26, 551–554.

Merbach W, Mirus E, Knof G, Remus R, Ruppel S, Russow R, Gransee A and Schulze J 1999 Release of carbon and nitrogen compounds by plant roots and their possible ecological importance. J. Plant Nutr. Soil Sci. 162, 373–383.

Moriarty D J W and Pollard P C 1982 Diel variation of bacterial productivity in seagrass (*Zostera capricorni*) beds measured by rate of thymidine incorporation in DNA. Mar. Biol. 72, 165–173

Newman E I and Bowen H J 1974 Patterns of distribution of bacteria on root surface. Soil Biol. Biochem. 6, 205–209.

Newman E I and Watson A 1977 Microbial abundance in the rhizosphere – a computer model. Plant Soil 48, 17–56.

Norvell WA, Welch R M, Adams M L and Kochlan L V 1993 Reduction of Fe(III), Mn(III), and Cu(II) chelates by roots of pea (*Pisum sativum L.*) or soybean (*Glycine max.*). Plant Soil 155/156, 123–126.

Nye P H and Tinker P B 1977 Solute Movement in the Soil-Root System. Blackwell Scientific Publications, Oxford. Paterson E and Sim A 1999 Rhizodeposition and C-partitioning of *Lolium perenne* in axenic culture affected by nitrogen supply and defoliation. Plant Soil 216, 155–164.

Paul E A and Clark F E 1996 Soil Microbiology and Biochemistry. Academic Press, London. Rovira A D 1969 Plant root exudates. Bot. Rev. 35, 35–57.

Rovira A D and Campbell R 1974 Scanning electron microscopy of microorganisms on the roots of wheat. Microbial Ecol. 1, 15–23.

Sauerbeck D and Johnen B 1976 Der Umsatz von Pflanzenwurzeln im Laufe der Vegetationsperiode und dessen Beitrag zur 'Bodenatmung'. Z. Pflanzenernähr. Bodenk. 3, 315–328.

Schilling G, Gransee A, Deubel A, Lezovic G and Ruppel S 1998 Phosphorus availability, root exudates, and microbial activity in the rhizosphere. J. Plant Nutr. Soil Sci. 161, 465–478.

Sposito G 1989 The Chemistry of Soils. Oxford University Press, Oxford.

Swinnen J 1994 Evaluation of the use of a model rhizodeposition technique to separate root and microbial respiration in soil. Plant Soil 165, 89–101.

Uren N C and Reisenauer H M 1988 The role of root exudates in nutrient acquisition. Adv. Plant Nutr. 3, 79–114.

Veen B W 1980 Energy cost of ion transport. *In* Genetic Engineering of Osmoregulation. Impact on Plant Productivity for food, Chemicals and Energy. Eds D W Rains, R S Valentine and A Hollaender. pp. 187–195. Plenum Press, New York.

Waremburg F R and Morral R A A 1978 Energy flow in the plant – microorganism system. *In* Interactions between Non-Pathogenic Soil Microorganisms and Plants. Eds Y R Dommergues and S V Krupa. pp. 205–242. Elsevier, Amsterdam.

Welsh D T, Bourgues S, de Wit R and Aubi I 1997 Effect of plant photosynthesis, carbon sources and ammonium availability on nitrogen fixation rates in the rhizosphere of *Zostera noltii*. Aquat. Microb. Ecol. 12, 285–290.

Whipps J M 1990 Carbon economy. *In* The Rhizosphere. Ed J M Lynch. pp. 59-97. John Wiley and Sons Ltd., Chichester.

Zhang F S 1993 Mobilization of iron and manganese by plant-borne and synthetic metal chelators. Plant Soil 155/156, 111–114.

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