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REGULAR ARTICLE

Spatial distribution and turnover of root-derived carbon in alfalfa rhizosphere depending on top- and subsoil properties and mycorrhization

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Abstract
Aims This study analyzed the extent to which root exudates diffuse from the root surface towards the soil depending on topsoil and subsoil properties and the effect of arbuscular mycorrhizal fungal hyphae on root-derived C distribution in the rhizosphere.
Methods Alfalfa was grown in three-compartment pots. Nylon gauze prevented either roots alone or roots and arbuscular mycorrhizal fungal hyphae from penetrating into the rhizosphere compartments. $^{14}CO_2$ pulse labeling enabled the measurement of ^{14}C-labeled exudates in dissolved (DOC) and total organic carbon (TOC) in the rhizosphere, distributed either by diffusion alone or by diffusion, root hair and hyphal transport.
Results Root exudation and microbial decomposition of exudates was higher in the rhizosphere with topsoil compared to subsoil properties. Exudates extended over 28 mm (DOC) and 20 mm (TOC). Different soil properties and mycorrhization, likely caused by the low arbuscular mycorrhizal colonization of roots (13±4 % (topsoil properties) and 18±5 % (subsoil properties)), had no effect.
Conclusions Higher microbial decomposition compensated for higher root exudation into the rhizosphere with topsoil properties, which resulted in equal exudate extent when compared to the rhizosphere with subsoil properties. Higher ^{14}C activity used for labeling compared with previous studies enabled the detection of low exudate concentrations at longer distances from the root surface.

Keywords Plant-soil-microorganism interactions · $^{14}CO_2$ pulse labeling · C partitioning · Subsoil · Topsoil · *Medicago sativa*

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Introduction

Soils are characterized by complex physical, chemical and biological properties. Various resources such as nutrients or water are heterogeneously distributed in soil, resulting in hot spots, i.e. in microsites with increased resource availability (Beare et al. 1995). Higher microbial abundance and activity in these hot spots

compared to bulk soil increase organic matter turnover and nutrient mineralization (Cheng 2009; Kuzyakov 2010). One of these hot spots in soil is the rhizosphere, which was first mentioned by Hiltner in 1904, and is defined as the soil volume surrounding the root that is affected by root activity (Darrah 1993).

The soil volume affected by plant roots, i.e. the extent of the rhizosphere, depends on the processes and parameters considered (Gregory 2006). Most previous studies have determined the extent of various parameters in the rhizosphere of topsoil A_p or A_h horizon (WRB IUSS-ISRIC-FAO 2006; Kuzyakov et al. 2003; Schenck zu Schweinsberg-Mickan et al. 2012). However, the spatial distribution of e.g. rhizodeposits and therefore the soil volume affected by rhizodeposition can be assumed to change with increasing soil depth, because of changing pedological, environmental, physicochemical (Salomé et al. 2010) and biological features.

Radial gradients that develop in the vicinity of the root can be used to describe the extent of the rhizosphere (Uren 2007). The size of the gradients ranges from less than 1 mm for microbial populations up to tens of millimeters for volatile compounds (Gregory 2006). Gradients develop due to nutrient uptake, which causes their depletion from bulk soil to root surface (Jungk 2002) or due to the release of rhizodeposits from the root into the soil (Sauer et al. 2006). Rhizodeposits comprise a wide range of organic compounds (Rovira 1956), which can be divided into water-soluble exudates such as e.g. sugars, amino acids and low molecular organic acids and water-insoluble components such as decaying fine-roots, root hairs, cell walls, sloughed cells and mucilage (Wichern et al. 2008). Root exudates have been reported to diffuse up to 12 mm from the root surface (Sauer et al. 2006), indicating that a relatively large soil volume is affected. Root exudates are released from the root into the soil due to: 1) passive diffusion, which is mainly controlled by concentration differences of individual solutes in the cytoplasm and in the soil and the permeability of cell membranes of the solute; and 2) possible additional active release due to the opening of membrane pores (Jones et al. 2004). In turn, this affects the distribution of the respective compound from the root surface into bulk soil. Once exuded into the soil, the spatial distribution of the solute depends on the diffusivity of the solute in water (Watt et al. 2006), the soil water content (Olesen et al. 2000; Watt et al. 2006), re-uptake by roots (Jones and Darrah 1993), uptake by microorganisms (Hill et al. 2008; Fischer et al. 2010) and adsorption to the soil matrix (Jones and Edwards 1998).

The properties that affect the distribution of root exudates from the root surface to bulk soil change with increasing soil depth. C, N, C/N ratios (Salomé et al. 2010) and nutrient availability decrease with soil depth (Jobbagy and Jackson 2001). As an increase in the exudation of organic compounds by roots was observed under low N or P supply (Paterson and Sim 1999; Neumann and Römheld 1999), the same can be expected in subsoil. Due to a decrease in microbial biomass with soil depth (Fierer et al. 2003), the decomposition of the released root exudates will be slower. Changes in soil texture and the amount and distribution of soil organic matter with depth affect the sorption of root exudates to the solid phase. In turn, the distribution of root exudates in the rhizosphere is affected by differences in sorption as it reduces their bioavailability and biodegradation (Jones and Edwards 1998).

Arbuscular mycorrhizal (AM) symbioses are formed between the majorities of land plants and AM fungi (Smith and Smith 2011). The symbiosis is based on the exchange of C and nutrients between the host plant and the AM fungi (Smith and Smith 2011). AM symbioses affect rhizosphere extent by C translocation from the host plant to the AM fungi and exudation by external AM fungal hyphae that extend the rhizosphere into the mycorrhizosphere (Jones et al. 2004). The extent of the mycorrhizosphere depends on the spatial distribution of the AM fungal hyphae. Hyphal density and the spread of external hyphae from the root surface into soil differ between AM fungi and depend on the time of existing symbiosis (Jakobsen et al. 1992). Moreover, root exudation is changed in quality and quantity, as the colonization of roots by AM fungi changes the permeability of root membranes (Ratnayaker et al. 1978; Mada and Bagyaraj 1993).

Generally, soil composition, moisture, temperature, pH, nutrient availability and anthropogenic stressors affect the formation and function of AM symbiosis (Entry et al. 2002). With increasing soil depth, the mentioned soil conditions change, spore abundance decreases and AM fungi species composition changes (Oehl et al. 2005).

We hypothesize that: 1) root exudation into the soil with subsoil properties is higher per unit of root mass due to lower nutrient content compared to the soil with topsoil properties; 2) the diffusion distance of root exudates is longer in the soil with subsoil properties,

because of lower microbial biomass content and consequently lower decomposition of root exudates; 3) mycorrhization further extends the rhizosphere due to the transport and exudation of assimilated C by AM fungal hyphae; 4) the effect of mycorrhization on rhizosphere extent differs between the soil with top- and subsoil properties, because soil properties affect the formation and function of AM symbiosis.

To test these hypotheses, alfalfa was grown in three-compartment pots filled with either homogenized top- or subsoil (Fig. 1). Two months old plants were pulse labeled with $^{14}CO_2$ to distinguish root-derived C (^{14}C) in dissolved (DOC) and total organic carbon (TOC) from all other C sources. Due to the installation of nylon gauze root exudates were distributed in the rhizosphere either by diffusion alone (1 μm gauze) or by diffusion, root hair and hyphal transport (30 μm gauze). To identify AM symbiosis, the colonization of roots by AM fungi was measured.

Material and methods

Experimental setup

Alfalfa (*Medicago sativa*) was grown in three-compartment pots (Sauer et al. 2006) (Fig. 1). The pots consisted of T-shaped tubes with an inner diameter of 83 mm. The three-compartment pots were filled with either homogenized topsoil (sampled from 0 to 30 cm, A_p horizon) or subsoil (45–75 cm, B_t horizon) of a Haplic Luvisol (WRB IUSS-ISRIC-FAO 2006) collected from the field trial of the DFG research group 1320 in Klein Altendorf (06° 59′ 29″ E, 50°37′21″ N) (Gaiser et al. 2012) (Table 1). Both topsoil and subsoil samples were taken from three replicate plots of alfalfa cultivated in the second year. Replicate samples were combined and sieved to 2 mm for homogenization prior to setting up the three-compartment experiment.

The side rhizosphere compartments were filled by adding the required amount of soil needed to adjust a bulk density of 1.2 g cm^{-3}. To avoid heterogeneities related to filling, soil was pressed into the rhizosphere compartment by applying pressure once from one side. After filling, the nylon gauze was first fixed to the opposite side of the rhizosphere compartment using an elastic band and then the rhizosphere compartments were connected to the root compartment. The three-compartments were put into a vise, which was built specifically for the pots, to fix the connection. The soil was filled into the root compartment after fixing both rhizosphere compartments to ensure connectivity. A total of 0.5 g of alfalfa seeds per pot was sown directly into the soil of the root compartment.

The plant roots grew in the root compartment, but could not penetrate into the rhizosphere compartments due to nylon gauze (Kuchenbuch and Jungk 1982). Within one pot, two mesh sizes were used. As root hairs are approximately 10 μm in diameter (Gahoonia et al. 1997; Grierson and Schiefelbein 2002) and the diameter of AM fungal hyphae ranges from 2 to 20 μm (Smith and Smith 2011), one side was covered with 30 μm gauze to allow AM fungal hyphae and root hairs to

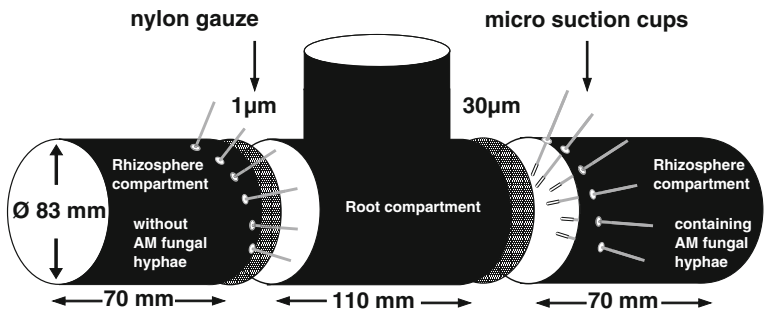

Fig. 1 T-shaped three-compartment pots, containing a central root compartment and two side compartments that represent the rhizosphere of alfalfa. The rhizosphere compartments are separated from the root compartment by nylon gauze to prevent either roots alone or roots and arbuscular mycorrhizal (AM) fungal hyphae from penetrating into the rhizosphere compartments. One rhizosphere compartment was separated by 30 μm gauze from the root compartment to allow AM fungal hyphae and root hairs to penetrate into the rhizosphere compartment. The other rhizosphere compartment was separated from the root compartment by nylon gauze with a mesh size of 1 μm in order to exclude AM fungal hyphae, roots and root hairs. Micro suction cups were installed at a distance of 3, 6, 9, 13, 19 and 28 mm from the nylon gauze, i.e. the root surface

Table 1 Properties of the homogenized soil sampled from top- (A_p) and subsoil (B_t) horizon of a Haplic Luvisol, including soil organic carbon (SOC) content, C/N ratio and texture, before the cultivation of alfalfa. Shoot and root dry weight (DW) of alfalfa grown in top- or subsoil pots 2 months after sowing are presented. Comparison of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) content and C/N ratio of the soil solution and average pH between the rhizosphere with top- or subsoil properties 2 months after sowing

		Topsoil	Subsoil
Soil properties			
SOC	(g kg^{-1})	9.5±0.1 a*	5.1±0.1 b
C/N		8.8±0.1 a	7.7±0.1 b
Texture[a]	Sand (%)	16	12
	Silt (%)	67	59
	Clay (%)	17	29
Plant biomass 2 months after sowing			
Shoot	(g DW pot^{-1})	18.1±0.8 a	18.7±0.9 a
Root	(g DW pot^{-1})	4.4±0.5 a	2.7±0.2 b
Rhizosphere compartment[b]			
DOC	(mg C l^{-1})	98.1±2.1 a	88.2±1.6 b
TDN	(mg N l^{-1})	23.2±3.9 a	4.6±0.1 b
C/N		11.1±1.5 a	20.0±0.5 b
pH (H_2O)		7.5±0.0 a	7.7±0.0 a

Values are given as means and standard errors of the means

[a] Texture values originate from Uteau Puschmann et al. (*Oxygen and redox potential gradients in the rhizosphere of alfalfa grown on a loamy soil*. Under Review)

[b] DOC, TDN, C/N ratio and pH values are means of the rhizosphere compartments with top- or subsoil properties

*Different letters indicate significant differences between top- and subsoil properties (Mann–Whitney *U* test; $p<0.05$)

penetrate into the rhizosphere compartment. However, roots were not able to penetrate this gauze. The second rhizosphere compartment was divided from the root compartment by 1 μm nylon gauze to exclude AM fungal hyphae and root hairs.

The experiment was conducted under controlled conditions. Water content was daily adjusted to 80 % of the water holding capacity and was checked gravimetrically. Plants were watered from the top of the root compartment. The photoperiod was 14 h light to 10 h dark. Light intensity was 300 μmol m^{-2} s^{-1} and the room temperature was 23 °C during light and 20 °C during dark periods.

To gain insight into changes in C partitioning over time, pots were harvested destructively 1, 3 and 6 days after ^{14}C labeling (see below). In total, the experiment consisted of 24 experimental units (homogenized soil sampled from A_h or B_t horizon, three sampling dates and four replicates, each).

$^{14}CO_2$ pulse labeling

Two months after sowing, plants were pulse-labeled with $^{14}CO_2$ to trace root exudates in the rhizosphere compartments. The pulse-labeling procedure was previously described by e.g. Cheng et al. (1993) and Gocke et al. (2011). For the ^{14}C pulse labeling, four pots were placed into a sealed transparent acrylic glass chamber (length × width × height; 0.5×0.5×0.7 m). Two replicate pots containing homogenized soil from A_h horizon and two replicate pots containing homogenized soil from B_t horizon of the same sampling date were labeled simultaneously. The $^{14}CO_2$ pulse was applied by adding 5 M sulfuric acid (H_2SO_4) to the labeling solution containing $Na_2{}^{14}CO_3$ diluted in 10 ml of deionized water. Evolving $^{14}CO_2$ was pumped into the labeling chamber and a 12 V fan enabled the uniform distribution of the labeled CO_2. The added ^{14}C activity of the labeling solution was adjusted to 1.85 MBq per three-compartment pot. Before the $^{14}CO_2$ pulse, the chamber was closed and plants assimilated the label for 3 h. The unassimilated $^{14}CO_2$ in the chamber air prior to opening was trapped in 15 ml of 1 M sodium hydroxide solution (NaOH).

Soil solution sampling

Soil solution was sampled in the rhizosphere compartments at various distances to the gauze, i.e. to the root surface, to detect root exudates in the DOC pool ($DO^{14}C$) (Fig. 1). For this purpose, micro suction cups (PI Ceramic, Lederhose, Germany) (Göttlein et al. 1996) were installed at distances of 3, 6, 9, 13, 19 and 28 mm from the root surface 2 weeks before labeling to minimize disturbance. After the ^{14}C labeling, the micro suction cups were directly connected to a vacuum collection device using polytetrafluoroethylene (PTFE) tubes (Göttlein et al. 1996). The micro suction cups were set to a suction of 400 hPa for 3.5 h and soil solution was collected once in 2 ml reaction vials.

Destructive sampling of the three-compartment pots

After soil solution sampling, the three-compartment pots were carefully opened. Shoots were cut at the soil surface. Roots of the root compartment were removed

and put into a beaker containing deionized water to separate soil from roots. To improve separation, the beaker was put into an ultrasonic bath for 5 min. After removing the roots, the soil of the root compartment, roots and shoots were dried at 60 °C, weighed and ball milled (ball mill, Retsch MM2).

The rhizosphere compartments were cut at room temperature into slices at distances of 2, 4, 6, 8, 10, 12, 14, 16, and 20 mm from the previous root surface (i.e. the nylon gauze) using a microtome. Each obtained soil slice was mixed for homogenization, dried at 60 °C and milled for the measurement of ^{14}C activity in total organic carbon (TOC).

^{14}C analysis

^{14}C activity was measured in soil solution from micro suction cups, in TOC of every soil slice as well as in shoots, roots and TOC of the root compartment by Liquid Scintillation Counting (LS 6500 Multi-154 Purpose Scintillation Counter, 217 Beckman, USA). Before measuring, milled plant biomass and soil samples were combusted at 600 °C and evolving CO_2 was trapped in 10 ml of 1 M NaOH. An aliquot of 2 ml was transferred to scintillation vials and mixed with 4 ml of the scintillation cocktail (Rothiszint eco plus, Carl Roth GmbH & Co. KG, Germany). Samples were measured 24 h after mixing with the scintillation cocktail, enabling the decay of chemoluminescence. Soil solution samples were mixed with the scintillation cocktail at a ratio of 1:5 and measured directly after collection.

Colonization of roots by arbuscular mycorrhizal fungi

In order to identify the colonization of roots by AM fungi, AM fungi structures in root tissue were first stained using ink and vinegar (Vierheilig et al. 1998). Second, the proportion of root length containing arbuscles, the arbuscular colonization, was determined (McGonigle et al. 1990).

Calculation of ^{14}C partitioning

Measured ^{14}C activity of the samples was multiplied by the correspondent pool size (shoot and root dry weight, soil dry weight of the root compartment or of the considered soil slice of the rhizosphere compartment). The resulting ^{14}C activity of the C pools is presented as percentage of the total ^{14}C activity (the sum of ^{14}C activity in plant biomass, soil and DOC) in the three-compartment pots harvested after 1 day. For the pots harvested after 1 day, total ^{14}C activity was calculated for each pot.

Due to the destructive sampling, the ^{14}C activity in a C pool of the pots harvested 3 or 6 days after labeling was normalized to the average total ^{14}C activity of the replicate topsoil and subsoil pots after 1 day.

The recovered ^{14}C in TOC of the soil slices (% of recovered ^{14}C) was related to a 1 mm distance as the thickness of the soil slices varied in the experimental setup. The recovered ^{14}C in DOC was related to 1 mL of soil solution and a 1 mm distance, because the soil solution volume obtained and the distance between the suction cups was different. The volume for each suction cup was defined as the distance between the middle to the previous adjacent and to the next adjacent suction cups.

Spatial distribution of root-derived C in the rhizosphere

The spatial distribution of root-derived C in the rhizosphere compartments in DOC and TOC was described on the basis of ^{14}C recovery in DOC (% of recovered ^{14}C ml^{-1} mm^{-1}) and TOC (% of recovered ^{14}C mm^{-1}). An exponential decay function (one phase decay) or linear regression using the least squares fitting method was used to obtain the best fitting result for ^{14}C in DOC and TOC. Functions were fitted to the means of ^{14}C recovery in TOC of associated soil slices and in DOC of associated micro suction cups of the four replicate rhizosphere compartments.

The distance from the root surface ($x_{1/2}$), at which the ^{14}C recovery in DOC and TOC was half that of the initial ^{14}C recovery at $x=0$ cm, was calculated using the obtained rate constants (k) after the exponential fitting:

$$x_{\frac{1}{2}} = \frac{ln\,2}{k}$$

Dynamics of root-derived C in DOC and TOC of the rhizosphere

Changes in ^{14}C recovery over the 6 days lasting chase period in DOC and TOC within the whole rhizosphere compartments were used to describe the dynamics of C exuded and translocated to the AM fungi. The functions fitted to the distribution of $DO^{14}C$ and $TO^{14}C$ in the

rhizosphere compartments were integrated. Limits of integration were 0 to 28 mm for DO^{14}C and 0 to 20 mm for TO^{14}C. The integrals, R, (the areas under the curves) obtained 1, 3, and 6 days after labeling were compared between the rhizosphere compartments filled with either homogenized top- or subsoil. For comparison, the integrals determined 1 or 3 days after labeling were set to 100 %, to calculate the relative changes of the integral between the time steps.

The decrease –R (% d^{-1}) or increase rates +R (% d^{-1}) of the integral were calculated for the periods between 1 and 3 and 1 and 6 or between 3 and 6 days after labeling. Changes between the time steps were assumed to decrease or increase exponentially:

$$A(t) = A(0)\cdot e^{kt}$$
$$R = \left(e^{k}-1\right)\cdot 100$$

where, A (t) (%) is the percentage of the integral after 3 or 6 days t (d) related to the integrals after 1 or 3 days A (0) after labeling, respectively. k is the rate constant of the exponential decrease (−k) or increase (+k) between the individual time steps.

Statistics

To determine if the fitted parameters of the exponential model, the rate constant (k) and the y-intercept (Y_0) differ between the rhizosphere with top- or subsoil properties as well as between with and without AM fungi, the extra-sum-of-squares F test ($p<0.05$) was used. The non-parametric Mann–Whitney *U* test was applied to reveal significant differences of 1) soil organic carbon (SOC) content and C/N ratio ($n=5$, $p<0.05$) between the top- and subsoil used for the experiment before the cultivation of alfalfa; 2) root ($n=7$, $p<0.05$) and shoot biomass ($n=12$, $p<0.05$) between the topsoil and subsoil pots after 2 months of growth; 3) DOC and total dissolved nitrogen (TDN) content and C/N ratio ($n=40$, $p<0.05$) of the soil solution of the rhizosphere compartments; and 4) ^{14}C allocation in shoots, roots, soil of the root compartment and the rhizosphere compartments with 1 μm and 30 μm gauze ($n=4$, $p<0.05$) between topsoil and subsoil pots at 1, 3 and 6 days after labeling and between the time steps. Means and standard errors of the means are presented in the figures and tables. Statistical analyses of significant differences were carried out using STATISTICA for Windows (version 10.0; StatSoft Inc., Tulsa, OK, USA). Fitting of exponential decay functions and comparison of fits as well as the integration of the functions were carried out using Graph Pad Prism (version 6; GraphPad Software, Inc., La Jolla, CA, USA)

Results

Bulk elemental analyses

Before the experimental cultivation of alfalfa, SOC content and C/N ratio of the homogenized soil sampled from the A_p horizon were significantly higher compared to the homogenized soil sampled from the B_t horizon of a Haplic Luvisol (WRB IUSS-ISRIC-FAO 2006) (Table 1). The textural differences were mainly expressed by the clay content that was almost twice as high in the B_t compared to the A_p horizon (Table 1).

After 2 months of alfalfa growth under controlled conditions, all nylon gauzes were completely covered by roots. The average shoot biomass per pot did not differ between the topsoil and subsoil pots after 2 months (Table 1). However, root biomass was significantly lower in the subsoil compared to the topsoil pots. The average DOC and TDN contents were significantly higher in the soil solution of the rhizosphere with topsoil properties (TP) compared to the rhizosphere with subsoil properties (SP). However, the average C/N ratio of dissolved organic matter in the TP rhizosphere was much lower compared to the SP rhizosphere (Table 1). The average pH in the rhizosphere compartments did not differ between the top- and subsoil pots.

^{14}C partitioning

The average total ^{14}C activity per pot was 1.4±0.1 MBq in topsoil pots and 1.3±0.2 MBq in subsoil pots 1 day after labeling. Comparison of ^{14}C recovery in C pools of the root compartment (shoots, roots and TOC) did not reveal any significant differences between the top- and subsoil pots 1, 3 or 6 days after labeling (Table 2). Likewise, total ^{14}C recovery in TOC did not differ significantly for any sampling dates between the TP and SP rhizosphere (Table 2).

After 6 days, ^{14}C recovery in shoots in top- and subsoil pots was higher compared to all other C pools. In root biomass, only 2.5±0.7 and 2.7±0.2 % of ^{14}C were recovered after 6 days in top- and subsoil pots, respectively. In TOC of the root compartment, 16.3±

Table 2 Partitioning of assimilated ^{14}C between shoots, roots and total organic carbon (TOC) in the root compartment and TOC and dissolved organic carbon (DOC) in the rhizosphere compartments 1, 3 and 6 days after $^{14}CO_2$ pulse labeling of alfalfa plants

	Pool	Days after labeling	Topsoil pot		Subsoil pot	
			^{14}C recovery (% of recovered ^{14}C)		^{14}C recovery (% of recovered ^{14}C)	
Root compartment	Shoot	1	80.6±3.2	a*	86.0±1.0	a
		3	49.8±8.1	b	49.1±4.5	b
		6	53.1±9.9	ab	46.7±6.7	b
	Root	1	8.6±2.7	a	3.6±1.2	a
		3	6.2±0.9	a	2.6±0.5	a
		6	2.5±0.7	a	2.7±0.2	a
	TOC	1	10.7±0.9	a	10.3±0.9	a
		3	15.5±1.3	ab	13.6±1.5	ab
		6	16.3±0.6	b	15.0±0.4	b
Rhizosphere −AM fungal hyphae	TOC	1	0.07±0.03	a	0.05±0.02	a
		3	0.06±0.03	a	0.06±0.03	a
		6	0.05±0.02	a	0.09±0.05	a
	DOC	1	0.0030		0.0005±0.0001	a
		3	0.0009		0.0013±0.0005	a
		6			0.0005±0.0001	a
Rhizosphere + AM fungal hyphae	TOC	1	0.06±0.02	a	0.04±0.01	a
		3	0.03±0.01	a	0.06±0.01	a
		6	0.08±0.05	a	0.06±0.02	a
	DOC	1	0.0020		0.0007±0.0001	a
		3	0.0010		0.0016±0.0006	a
		6			0.0005±0.0001	a

Values are given as means and standard errors of the means

* Significant differences between the time steps are indicated by different letters (Mann–Whitney *U* test, $p<0.05$, $n=4$). Not any significant differences were found between the topsoil and subsoil pots for any sampling date

0.6 % of ^{14}C was recovered in topsoil and 15.0±0.4 % in subsoil pots 6 days after labeling. ^{14}C recovery was lowest in the C pools of the rhizosphere compartments compared to all other C pools after 6 days (Table 2).

In shoots and roots, the ^{14}C recovery decreased during the experiment, whereas ^{14}C recovery in TOC of the root compartment increased (Table 2). No significant changes of total ^{14}C recovery in the rhizosphere compartments were determined between the first and the sixth day after labeling (Table 2).

Effect of top- and subsoil properties on spatial distribution of root exudates

The ^{14}C distribution in DOC in all rhizosphere compartments decreased exponentially with increasing distance to the root surface (Fig. 2). In the SP rhizosphere, ^{14}C-labeled root exudates in DOC were lacking at a distance of 28 mm after 1 and 6 days, because ^{14}C recovery was insignificant. However, after 3 days, the spatial extent of ^{14}C-labeled root exudates in DOC exceeded the experimental sampling distance, because ^{14}C was recovered even at a distance of 28 mm (Fig. 2). Likewise, the spatial extent of ^{14}C in DOC exceeded the experimental sampling distance of 28 mm in the TP rhizosphere 1 and 3 days after labeling. Therefore, no maximal spatial extent of root exudate C (^{14}C) in DOC was obtained. $DO^{14}C$ gradients from the root surface to bulk soil were compared between the TP and SP rhizosphere based on fitted rate constants (k) (Fig. 2). No significant differences between the TP and SP rhizosphere were observed. However, after 1 day $DO^{14}C$ gradients from the root surface to bulk soil were steeper in the TP compared to SP rhizosphere, whereas these gradients were uniform for the TP and SP rhizosphere after 3 days (Fig. 2).

^{14}C recovery in DOC at the root surface (Fig. 2, y-intercept) was three times higher in the TP compared to the SP rhizosphere after 1 day. In contrast, after 3 days,

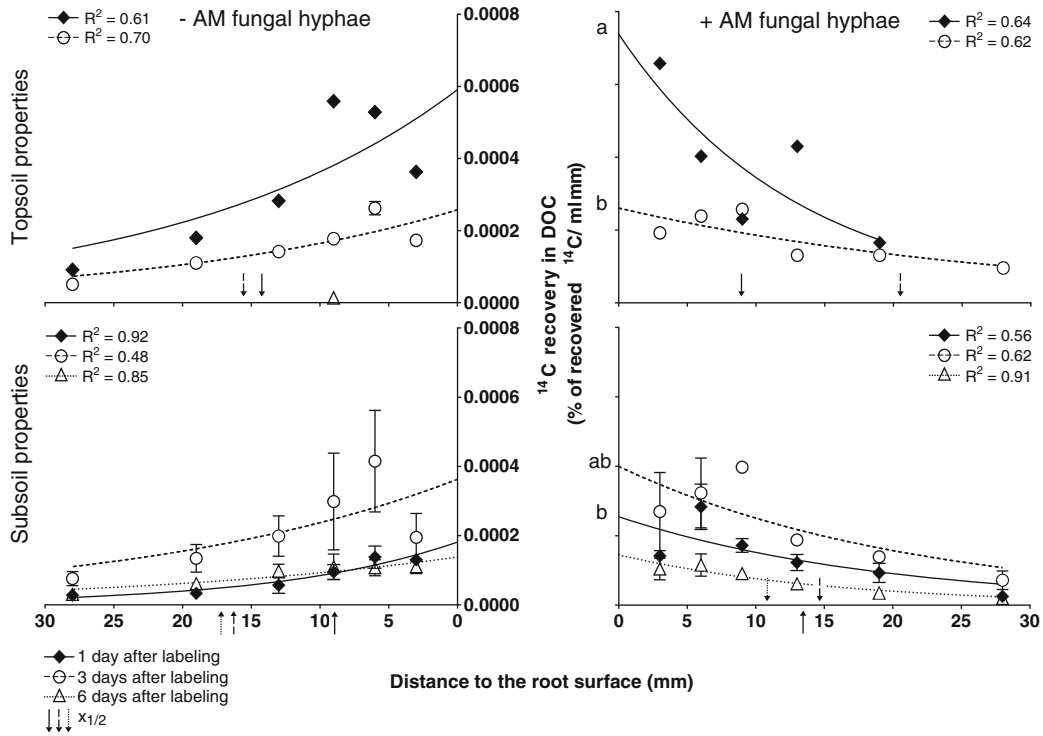

Fig. 2 Spatial distribution and dynamics of exudates (^{14}C) in DOC (% of recovered ^{14}C ml^{-1} mm^{-1}) in the rhizosphere with topsoil and subsoil properties. Both the $DO^{14}C$ distribution in the rhizosphere with (+) and without (−) arbuscular mycorrhizal (AM) fungal hyphae are shown. *Arrows* indicate the distance from the root surface at which the ^{14}C recovery is half that of the initial ^{14}C recovery at the distance x=0 cm. Different *letters* indicate significant differences at $p<0.05$ in ^{14}C recovery at the root surface

the ^{14}C recovery at the root surface was higher in the SP compared to the TP rhizosphere (Fig. 2).

$TO^{14}C$ distribution in the rhizosphere commonly decreased exponentially with increasing distance to the root surface (Fig. 3). Exceptions were the $TO^{14}C$ distribution after 3 and 6 days in the TP rhizosphere with AM fungal hyphae and after 6 days in the TP rhizosphere with and without AM fungal hyphae. $TO^{14}C$ distribution in the SP rhizosphere without AM fungal hyphae did not decrease exponentially 1 and 3 days after labeling; hence, a linear regression was used instead.

Within 0–20 mm distance from the root surface, ^{14}C recovery in TOC was significant for all treatments and time steps (Fig. 3). Consequently, spatial extent of ^{14}C-labeled root exudates in TOC exceeded 20 mm in TP and SP rhizosphere. $TO^{14}C$ gradients (with exponential decrease) from the root surface to bulk soil did not differ significantly in rate constant (k). The fitted value of ^{14}C recovery at the root surface was three times higher in TP compared to SP rhizosphere after 1 day. However, the differences disappeared after 3 days.

Effect of mycorrhization on spatial distribution of root-derived C in the rhizosphere

Roots in all pots were colonized by AM fungi (Table 4). Arbuscular and hyphal colonization of the roots growing in subsoil were higher compared to that of the roots growing in topsoil. 18±5 % of root length was colonized by arbuscles in the roots growing in subsoil and 13±4 % in the roots growing in topsoil.

Comparing the TP rhizosphere containing AM fungal hyphae to that without AM fungal hyphae, no significant differences between ^{14}C recoveries at the root surface in DOC were obtained after 1 day (Fig. 2). Likewise, ^{14}C recovery at the root surface in TOC did not differ between the TP rhizosphere with and without AM fungal hyphae (Fig. 3). Equal results were obtained for the effect of AM on ^{14}C recovery at the root surface in SP rhizosphere. Rate constants (k) of $DO^{14}C$ and $TO^{14}C$ gradients from the root surface into bulk soil did not differ in any treatment or sampling time due to the presence of AM fungal hyphae. An exception to this general pattern was the distribution of ^{14}C in TOC in the

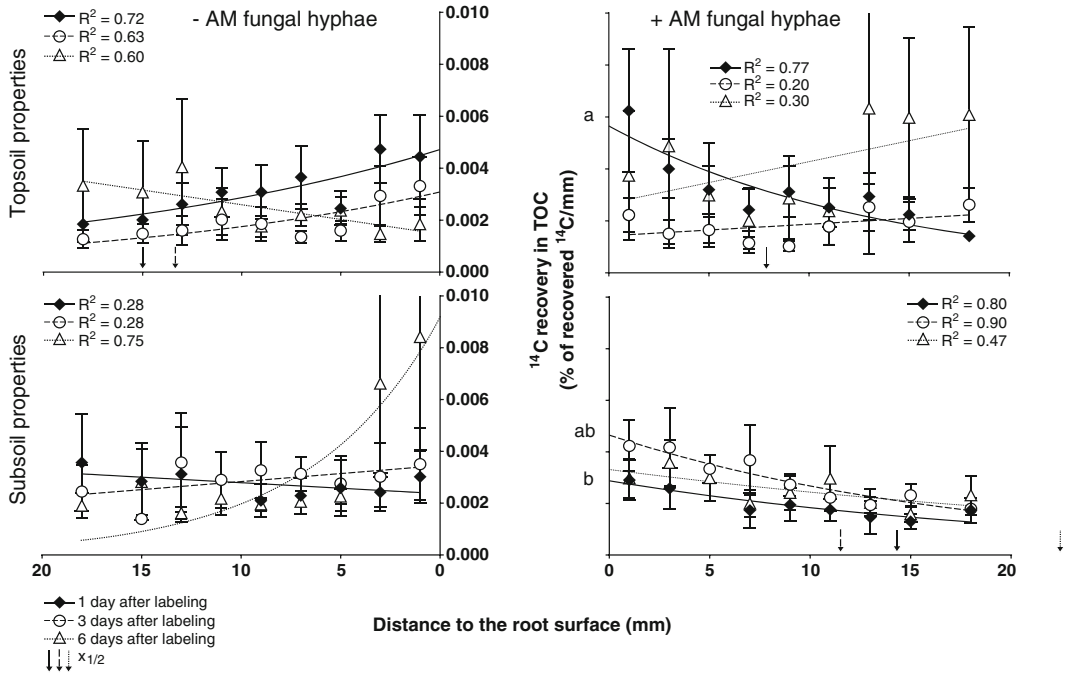

Fig. 3 Spatial distribution and dynamics of root-derived C (^{14}C) in TOC (% of recovered ^{14}C mm^{-1}) in the rhizosphere with topsoil and subsoil properties. Both the TO^{14}C distribution in the rhizosphere with (+) and without (−) arbuscular mycorrhizal (AM) fungal hyphae are shown. *Arrows* indicate the distance from the root surface at which the ^{14}C recovery is half that of the initial ^{14}C recovery at the distance x=0 cm. Different *letters* indicate significant differences at $p<0.05$ in ^{14}C recovery at the root surface

TP rhizosphere with AM fungal hyphae, which was no longer exponential after 3 days (Fig. 3).

Dynamics of ^{14}C in DOC and TOC of the rhizosphere

The integrals of the DO^{14}C and TO^{14}C distribution from the root surface into bulk soil were used to compare the dynamic of root-derived C between the TP and SP rhizosphere (Table 3). Total ^{14}C recovery in DOC in both TP rhizosphere compartments was approximately four times higher compared to the SP rhizosphere after 1 day. In contrast, after 3 days, total ^{14}C recovery in DOC in both SP rhizosphere compartments was higher compared to the TP rhizosphere (Table 3). From the first to third day, total ^{14}C recovery in DOC decreased in the TP rhizosphere without AM fungal hyphae by 32.5 % d^{-1}, whereas an increase of 68.3 % d^{-1} was observed in the SP rhizosphere without AM fungal hyphae (Table 3). The decrease and increase rates in the rhizosphere were similar with and without AM fungal hyphae. From the third to sixth day, a further decrease in total ^{14}C recovery in DOC was observed in the SP rhizosphere.

Total ^{14}C recovery in TOC was higher in both TP rhizosphere compartments compared to the SP rhizosphere with AM fungal hyphae after 1 day. In contrast, after 3 days, total ^{14}C recovery was higher in the SP compared to the TP rhizosphere (Table 3). Total ^{14}C recovery in TOC of the TP rhizosphere decreased from the first to the third day by 21.5 % d^{-1} with and 20.8 % d^{-1} without AM fungal hyphae. In contrast, in the SP rhizosphere with AM fungal hyphae, an increase of 21.4 % d^{-1} occurred (Table 3).

Discussion

Effect of top- and subsoil properties on spatial distribution and turnover of root exudates

The extent to which root exudates diffused from the root surface of alfalfa to bulk soil did not depend on top- or subsoil properties in our study, but exceeded previously reported distances. ^{14}C-labeled root exudates were found at a distance of 28 mm (DOC) and 20 mm (TOC) from the root surface after 1, 3 and 6 days in

Table 3 Dynamic of root-derived C in DOC and TOC in the rhizosphere of alfalfa. Percentage change of the integral and decrease or increase rate (% d^{-1}) of total ^{14}C recovery in TOC and DOC between: (1) the first and third or the first and sixth day after the labeling and; (2) the third and sixth day after the labeling, depending on rhizosphere properties are presented

	Rhizosphere properties	Days after labeling	Integral	Percentage change of the integral		[a] De- (−) or increase (+) rate	
		(d)	(mm·% of recovered ^{14}C mm^{-1})	(1) (%)	(2) (%)	(1) (% d^{-1})	(2) (% d^{-1})
TO^{14}C	Topsoil properties						
	−AM fungal hyphae	1	0.060	100.0			
		3	0.037	61.7	100.0	−21.5	
		6	0.052	86.7	140.5	−2.8	12.0
	+AM fungal hyphae	1	0.059	100.0			
		3	0.037	62.7	100.0	−20.8	
		6	0.086	145.8	232.4	7.8	32.5
	Subsoil properties						
	−AM fungal hyphae	1	0.056	100.0			
		3	0.057	101.8	100.0	0.9	
		6	0.057	101.8	100.0	0.4	0.0
	+AM fungal hyphae	1	0.038	100.0			
		3	0.056	147.4	100.0	21.4	
		6	0.049	128.9	87.5	5.2	−4.4
			(mm·% of recovered ^{14}C mm^{-1} ml^{-1})				
DO^{14}C	Topsoil properties						
	−AM fungal hyphae	1	0.009	100.0			
		3	0.004	45.6		−32.5	
		6					
	+AM fungal hyphae	1	0.009	100.0			
		3	0.005	55.3		−25.6	
		6					
	Subsoil properties						
	−AM fungal hyphae	1	0.002	100.0			
		3	0.006	283.3	100.0	68.3	
		6	0.002	110.8	39.1	2.1	−26.9
	+AM fungal hyphae	1	0.004	100.0			
		3	0.006	165.6	100.0	28.7	
		6	0.002	50.0	30.2	−13.0	−32.9

[a] De- and increase rate of the integrals were calculated based on the assumption of exponential de- or increase of root-derived C in the rhizosphere between the time steps

both TP and SP rhizosphere (Figs. 2 and 3). Therefore, the extent of root exudates into bulk soil was larger than the considered distance in the experiment, which was contrary to previous findings (Kuzyakov et al. 2003; Sauer et al. 2006; Schenck zu Schweinsberg-Mickan et al. 2012). The concentration of root exudates was found to be highest within 3 mm of the root surface (Kuzyakov et al. 2003). However, root exudates were detected in previous studies from 6 mm (Schenck zu Schweinsberg-Mickan et al. 2012), and 10 mm (Kuzyakov et al. 2003), up to a distance of 12 mm from the root surface (Sauer et al. 2006). In our experiment, ^{14}C input was 1.85 MBq per pot and therefore higher compared to the previous studies that used either 0.46 MBq (Kuzyakov et al. 2003) or between 0.6 and 1.2 MBq per pot (Sauer et al. 2006).

Coinciding with higher ^{14}C input, the detection of root exudates was not limited by low concentrations at larger distances from the root surface compared to previous studies.

Fischer et al. (2010) showed that microbial uptake and subsequent decomposition outcompeted the sorption of low molecular weight organic substances. Therefore, we assume that microbial utilization mainly determined the distribution of root exudates and the differences in sorption between the TP and SP rhizosphere can be ignored. Likewise, the re-uptake of root exudates is of minor importance for their distribution under soil conditions compared to fast microbial utilization (Biernath et al. 2008).

Microbial utilization of root exudates was higher in the TP compared to the SP rhizosphere after 2 months of alfalfa growth. The higher amount of exudates at the root surface in the TP rhizosphere after 1 day indicated higher root exudation compared to the SP rhizosphere. Steeper gradients of exudates in DOC from the root surface into bulk soil revealed that higher exudation was compensated by increased microbial utilization of root exudates in the TP rhizosphere (Fig. 2). The increase of root exudates in DOC and TOC in the SP rhizosphere in comparison to a decrease in the TP rhizosphere between day one and three (Table 3) further indicated the higher microbial utilization of root exudates, which could be related to a higher microbial biomass in the TP rhizosphere (Marschner and Kalbitz 2003). Higher microbial abundance and activity could be expected in the TP rhizosphere, due to the higher SOC content in the topsoil A_p horizon compared to the subsoil B_t horizon used in the experiment (Fierer et al. 2003; Gaiser et al. 2012) (Table 1). Significantly higher activities of leucin-amino-peptidase, ß-glucosidase and ß-N-acetylglucosaminidase in the TP compared to the SP rhizosphere further indicated higher microbial activity in the TP rhizosphere (see Fig. 1; online supplementary material). De Nobili et al. (2001) showed that the input of trace amounts of low molecular weight organic substances caused an increase in metabolic activity of the microbial biomass. This effect was sustained and even more pronounced if a more substantial substrate for microbes like cellulose was present (De Nobili et al. 2001). Consequently, increased microbial utilization of root exudates in the TP rhizosphere was caused by higher substrate availability due to higher SOC content and higher input of root exudates compared to the SP rhizosphere.

Root exudation rates of ^{14}C-labeled organic compounds are highest within hours after the $^{14}CO_2$ pulse (Rattray et al. 1995; Dilkes et al. 2004). The utilization of exudates by microorganisms is fast in the rhizosphere, as the half-life of glucose in soil solution was reported to be several minutes (Hill et al. 2008; Fischer et al. 2010) and a similar half-life was obtained for acetate and alanine at concentrations that were relevant for the rhizosphere (Fischer et al. 2010). Due to the fast input of ^{14}C-labeled root exudates into the rhizosphere and their fast microbial utilization, a decrease of root exudates in DOC between day one and three should occur, which was true for the TP rhizosphere (Fig. 2, Table 3). In contrast, an increase of root exudates in DOC of the SP rhizosphere between day one and three was observed. Lower microbial utilization of root exudates and ongoing root exudation could have caused the increase of root exudates in DOC between day one and three, despite the expected highest exudation within hours (Rattray et al. 1995; Dilkes et al. 2004).

The hypothesized higher exudation into the TP rhizosphere was not found. The nylon gauze was completely covered by roots in both treatments, but the amount of root exudates in DOC at the root surface 1 day after labeling was significantly lower in the SP rhizosphere (Fig. 2). Exudate amounts in DOC at the root surface did not reflect total exudation, due to fast translocation of recent photosynthates and uptake by microorganisms (Rattray et al. 1995; Dilkes et al. 2004; Fischer et al. 2010). However, the lower exudate amount in DOC at the root surface in combination with the lower microbial utilization of root exudates (more gentle gradients of ^{14}C in DOC), indicated lower root exudation into the SP rhizosphere. In conclusion, higher exudation into the TP rhizosphere could have been caused by the higher abundance of microbial biomass, because it was observed in other studies that microorganisms increase rhizodeposition (Meharg and Killham 1991; Schönwitz and Ziegler 1994). Consequently, the abundance of microorganisms rather than soil nutrient content (Paterson and Sim 1999; Neumann and Römheld 1999) affected root exudation.

Due to the fast decomposition of root exudates, their concentrations in soil solution are generally very low (μmol l^{-1} soil solution) (Fischer et al. 2007; Fischer and Kuzyakov 2010). Therefore, fast translocation of photosynthates and fast decomposition of root exudates (Dilkes et al. 2004; Hill et al. 2008) resulted in very low ^{14}C recovery in DOC 1 day after the $^{14}CO_2$ pulse

(Fig. 2). The half-life of root exudates taken-up by microorganisms ranges from hours to several days (Kuzyakov and Demin 1998; Rangel-Castro et al. 2005). Therefore, ^{14}C recovered in TOC of the rhizosphere, partly reflecting ^{14}C taken up by microorganisms, was ten times higher compared to DOC in both treatments after 1 day (Figs. 2 and 3). As the obtained input of root exudates and the microbial biomass was higher in the TP rhizosphere, higher ^{14}C recovery in TOC resulted after 1 day in the TP compared to the SP rhizosphere.

Effect of mycorrhization on spatial distribution of root-derived C in alfalfa rhizosphere

Our study did not reveal any clear effects of mycorrhization on rhizosphere extent. Against initial expectations, the distribution and gradients of root-derived C in the rhizosphere with AM fungal hyphae were similar to those without hyphae (Figs. 2 and 3). After 2 months of alfalfa growth, the proportion of root length colonized by AM fungi was low (13±4 % and 18 ±5 % in TP and SP rhizosphere, respectively) compared to other studies (Table 4). Inoculation of pure cultures of AM fungi resulted in colonization between 44 and 95 % of root length (Jakobsen and Rosendahl 1990; Li et al. 1991; Jakobsen et al. 1992).

Low AM colonization of alfalfa roots could have been caused by several factors. First, homogenization of the soil by mixing and sieving destroyed the existing hyphal network. Colonization of roots by AM can be caused by spores, infected root fragments and AM fungal hyphae, whereas the relative importance of every single inoculum for colonization potential is difficult to determine (Smith and Read 2008). However, it was shown that after the destruction of the hyphal network in soil, AM colonization of roots was strongly reduced (Merryweather and Fitter 1998; Evans and Miller 1990). Second, 2 months could have been an insufficient period for the establishment of AM, as the colonization of *Medicago sativa* roots by *Glomus caledonius* increased from 35 % after 6 weeks to 78 % after 18 weeks of growth under low P supply (Nielsen and Jensen 1983). Third, low AM colonization of alfalfa roots could have been dependent on the AM fungi involved in the symbiosis. The development of root colonization by AM fungi follows a sigmoidal increase with time, but the time to reach maximum colonization differs between AM fungi (Sanders et al. 1977). Further environmental factors affecting colonization like temperature and light (Smith and Read 2008) are negligible, as they were comparable to field conditions within the growing season of alfalfa.

Table 4 Proportion of alfalfa root length colonization by arbuscular mycorrhizal fungi. Arbuscular, Vesicular and Hyphal colonization of roots are presented

	Arbuscular colonization	Vesicular colonization	Hyphal colonization
Topsoil pots	0.13±0.04	0.00	0.30±0.08
Subsoil pots	0.18±0.05	0.01±0.004	0.50±0.08

Even though AM colonization is not an indicator for the effect of the symbiosis on plant growth (Smith and Read 2008), low AM colonization did not affect the extent or the gradients of root-derived C from the root surface into bulk soil (Fig. 3). The ^{14}C activity in the rhizosphere compartment was not determined in the AM fungal hyphae themselves after separation from soil, but in the soil slices containing hyphae. Although 0.7–0.8 % of assimilated C can be incorporated into external hyphae and the total C usage of AM is much higher (Jakobsen and Rosendahl 1990; Pearson and Jakobsen 1993; Johnson et al. 2002), no effect on ^{14}C in TOC was obtained. The developed extraradical mycelium of AM fungi can be expected to be small due to low AM colonization. Therefore, ^{14}C activity in AM fungal hyphae and microorganisms due to hyphal exudation was too low to be detected in TOC.

The allocation of assimilated C into the extraradical mycelium of AM fungi was shown to be fast, as the maximum respiration of $^{13}CO_2$ by AM fungal mycelium was reached 9–14 h after labeling of pasture plants (Johnson et al. 2002). In conclusion, small extraradical mycelium of AM fungi associated with fast allocation and turnover of assimilated C in AM fungal mycelium (Johnson et al. 2002) could have caused the absence of the effect of mycorrhization on the extent of root-derived C in alfalfa rhizosphere.

Conclusions

The extent and turnover of root-derived C from the root surface into soil was assessed in a laboratory experiment using three-compartment pots, grown with alfalfa, following $^{14}CO_2$ pulse labeling. Root exudates extended to

a distance longer than 28 mm in DOC and 20 mm in TOC in the rhizosphere of alfalfa with topsoil and subsoil properties. The diffusion distance of root exudates observed here exceeded previously reported distances due to larger amounts of label (^{14}C) used in the current study. However, differing properties of the homogenized soil sampled from a top- (A_h) and subsoil (B_t) horizon of a Haplic Luvisol did not affect the diffusion distance of root exudates. Against initial expectations, root exudation per root mass was lower in the rhizosphere with subsoil compared to topsoil properties. Our results suggest that the diffusion distance of root exudates is independent from top- and subsoil properties, because higher root exudation into the rhizosphere with topsoil properties is compensated by the higher microbial utilization of root exudates.

Effects of mycorrhization on rhizosphere extent of alfalfa were not identified as the recovery of root-derived C in DOC and TOC was not affected by AM fungal hyphae. The absence of an effect was due to low root colonization by AM fungi, and the consequently expected low AM fungal hyphae biomass in the rhizosphere. To determine the effects of mycorrhization on the spatial distribution of root-derived C, ^{14}C activity needs to be measured in external AM fungal hyphae extracted from the soil.

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