A novel method for separating root-derived organic compounds from root respiration in non-sterilized soils

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Summary – Zusammenfassung

A novel method of separating exudates from root respiration in non-sterilized soils has been developed. The method is based on a simultaneous elution of exudates from rhizosphere and the blowout of CO_2 originating from root respiration. The innovation of the method lies in the function of a membrane pump to drive the movement of air and simultaneously the circulation of water according to the Siphon principle.

The separation method was tested by means of ^{14}C pulse labeling of *Lolium perenne* to track the C dynamics in the production of rhizosphere CO_2 and of exudates, which were eluted. The total ^{14}C activity of rhizosphere CO_2 and of eluted exudates was found to be 8.5 % and 2.3 % of total assimilated ^{14}C, respectively. Thus, at least 19 % of root-derived C can be accounted to root exudation. However, the suggested Siphon method underestimates the amount of exudates and shows only a minimum of organic substances exuded by roots.

The diurnal dynamics of exudation was detected, but no significant day-night changes were measured in root and microbial respiration. Tight coupling of assimilation with exudation, but not with root and microbial respiration, was observed. The advantages, shortcomings, and possible applications of the Siphon method are discussed.

Key words: root and microbial respiration / exudation / rhizosphere / *Lolium perenne* / separation method / ^{14}C pulse labeling / CO_2

Eine neue Methode zur Trennung der wurzelbürtigen organischen Substanzen von der Wurzelatmung im nicht-sterilen Boden

Es wurde eine neue Methode zur Trennung der Exsudate von der Wurzelatmung und separaten Quantifizierung des wurzelbürtigen C in einem nicht sterilen Boden erarbeitet. Die Methode basiert auf der Auswaschung der Exsudate aus der Rhizosphäre beim gleichzeitigen Ausblasen von CO_2 aus der Wurzelatmung. Ein wesentlicher Teil der Methode besteht darin, dass die Membranpumpe, die den Luftstrom fördert, auch für die Wasserzirkulation nach dem Siphon-Prinzip verantwortlich ist. Die Trennungsmethode wurde durch ^{14}C-Pulsmarkierung von *Lolium perenne* überprüft, um die ^{14}C-Dynamik in Exsudaten und im CO_2-Efflux aus dem Boden verfolgen zu können. Im CO_2-Efflux aus dem Boden wurden 8,5 % des gesamten von der Pflanze assimilierten ^{14}C gefunden und in den ausgewaschenen Exsudaten hingegen nur 2,3 %. Die Siphon-Methode unterschätzt die Exsudatmenge und zeigt nur eine minimale Menge an wasserlöslichen Exsudaten. Folglich entsprechen die 81 % des im CO_2-Efflux aus dem Boden gemessen ^{14}C nicht allein der Wurzelatmung, sondern zum Teil auch der mikrobiellen Veratmung der Exsudate.

Die ^{14}C-Dynamik der ausgewaschenen Exsudate und des CO_2-Effluxes aus dem Boden zeigte, dass die Exsudation einem Tag-Nacht-Zyklus folgt, dagegen wurde bei der Wurzelatmung keine diurnale Dynamik festgestellt. Eine enge Kopplung der Exsudation mit der Assimilation, jedoch nicht mit der Wurzelatmung, wurde beobachtet. Die Vor- und Nachteile und mögliche Anwendungsbereiche der Siphon-Methode werden diskutiert.

1 Introduction

The partitioning of root-derived CO_2 efflux from the soil in actual root respiration and respiration of microorganisms utilizing the exudates and root residues is very important for the C and energy balance of the soil. Exudates and root residues are energy-rich; they enhance the underground C stock and are metabolized by soil microflora. These C sources, which are easily available to microorganisms, contribute to fast C turnover of the soil, and to higher microbial activity in the rhizosphere when compared with root-free soil. Stimulation of microbial growth and activity around roots increases mineralization of native soil organic matter and subsequently increases the availability of mineral nutrients, following the turnover of microbial biomass (*Clarholm*, 1985a; 1985b). In contrast to root exudates, CO_2 originating from root respiration cannot be used since it is energy-poor and does not affect the turnover of microbial biomass and soil organic matter. Nevertheless, CO_2 originating from root respiration is part of the total CO_2 evolution from the soil and has been frequently measured in field experiments studying soils as a source of increased CO_2 concentration in the atmosphere. Therefore, accurate C and energy budget of the soil cannot be determined without the separate estimation of root respiration and microbial utilization of root exudates.

The term 'root-derived CO_2' is used here to describe the sum of root respiration and CO_2 evolved by microbial decomposition of exudates, secretes as well as root residues

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such as sloughed root cells, root hairs, and dead roots. The term 'rhizosphere CO_2', frequently used in literature, is aimed at the location of CO_2 production and from this point of view must include not only root respiration and CO_2 evolved by microbial utilization of exudates, but also the CO_2 derived by microbial decomposition of rhizosphere soil organic matter.

Nutrient solution cultures (*Helal* and *Sauerbeck*, 1991; *Meharg* and *Killham*, 1991; *Hodge* et al., 1996; *Groleau-Renaud* et al., 1998), soil sterilization (*Barber* and *Martin*, 1976; *Martin*, 1977; *Merbach* et al., 1990; *Merbach* and *Ruppel*, 1992), and fumigation techniques (*Helal* and *Sauerbeck*, 1991) related to ^{14}C or ^{13}C labeling were used for the investigation of microbial respiration of root exudates. The results of these studies show that investigations based on artificial environments for the roots like hydroponic cultures or sterile soils give unreal figures for C partitioning (*Warembourg*, 1975; *Bowen*, 1980; *Schönwitz* and *Ziegler*, 1988; *Merbach* et al., 1990; *Meharg* and *Killham*, 1991; *Schulze* et al., 1994). This makes them unsuitable for the prediction of C flows under natural conditions.

More recently efforts have been made to divide root-derived CO_2 (as a sum of root respiration and microbial respiration of root-derived organic C) in CO_2 originating from root respiration and that from microbial respiration of root-borne substances during plant growth on non-sterile soils. The first method, called isotope dilution, is based on the addition of a solution of unlabeled glucose to the soil and simultaneous ^{14}C pulse labeling of growing plants (*Cheng* et al., 1993). With this method, root respiration of 3-weeks-old wheat plants was found to account for about 41 % of the root-derived CO_2. The second method based on the addition of ^{14}C-labeled model rhizodeposits to soil (*Swinnen*, 1994) shows that the contribution of root respiration of 30-days-old wheat and barley to the total root-derived CO_2 was between 89 % and 95 %. The third method based on the kinetics of $^{14}CO_2$ efflux from the soil after ^{14}C pulse labeling (*Kuzyakov* et al., 1999; 2001) has shown that root respiration and rhizomicrobial respiration of growing *Lolium perenne* amounts to 41 % and 59 % of root-derived CO_2 efflux from the soil, respectively. The contribution of root respiration varied from 17 % to 61 % of total CO_2 efflux from the soil depending on the age of the *Lolium* plants. A detailed description of these three methods, their shortcomings and advantages are discussed in *Kuzyakov* (2001) and *Kuzyakov* and *Domanski* (2001).

The original method of *Hodge* et al. (1996) for the elution of exudates from the rhizosphere can be used for the separation of root respiration and exudation, but it was presented only for exudate collection. This work here describes a new method for the extraction of root exudates from the rhizosphere and for the immediate estimation of root respiration in a non-sterile soil. The method is based on simultaneous elution of exudates from the rhizosphere with a continuous water stream and blowout of CO_2 originating from root respiration, both driven by a membrane pump that moves air and water according to the Siphon principle. Some preliminary results from the use of this microcosm are presented and its application discussed.

2 Materials and Methods

2.1 Soil and test crops

The soil, a loamy Haplic Luvisol, was taken from the top 10 cm (Ap horizon) of the long-term field experimental station Karlshof of the University of Hohenheim. The soil contains no $CaCO_3$ and has the following characteristics: pH 6.0, C_t 1.2 %, N_t 0.13 %, clay 20 %, silt 8 %. About 400 g soil (air-dried and sieved on a 2-mm screen) was filled in each container. One pre-germinated seed of *Lolium perenne* L. was put in each container and grown under 27/22 °C day/night temperature, 14 hours photoperiod and 400 µmol m^{-2} s^{-1} light intensity. The plants were labeled on day 64 and harvested on day 69.

The preparation of soil samples, the main soil characteristics, and the growing conditions are same as described in *Domanski* et al. (2001).

2.2 Experimental setup

The experimental setup consists on a two-compartment chamber, two flasks, one test tube with NaOH aqueous solution, and a membrane pump connected to other parts by PVC tubes (Fig. 1). The upper part of the chamber (dashed line in Fig. 1) was covered only for a short period of time (four hours) to allow pulse ^{14}C-labeling of shoots. In the bottom part of the chamber (Fig. 1, ②) a Polycarbonate filtration device "CombiSart" was fitted (volume = 250 ml, *Merck®-Laborkatalog*, 2000). However, the real volume of the device including the space under the lid is about 340 ml. The bottom chamber contained soil with roots. There were three inlets in the lid of the CombiSart device (only two inlets are shown in Fig. 1). Prior to

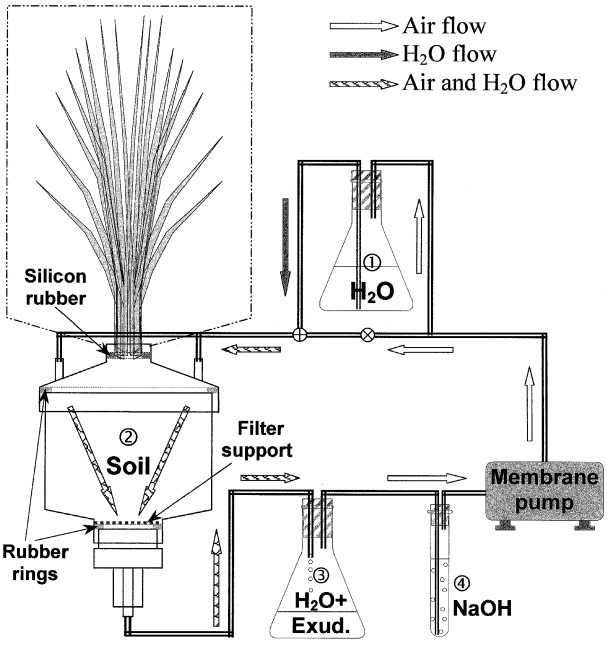

Figure 1: Experimental setup for separate measurement of root respiration and exudation. ① – flask with water for elution, ② – Polycarbonate filtration device CombiSart (*Merck®-Laborkatalog*, 2000) with soil and roots, ③ – collection flask with eluted exudates, ④ – test tube with NaOH solution for CO_2 trapping. ⊗ – regulation clamp, ⊕ – joint where air and water flows are connected.

Abbildung 1: Experimentelle Anlage zur getrennten Messung von Wurzelatmung und Exudation. ① – Kolben mit Wasser für Auswaschung, ② – Polycarbonat-Filtrationsgerät CombiSart (*Merck®-Laborkatalog*, 2000) für Boden und Wurzeln, ③ – Sammelkolben für ausgewaschene Exsudate, ④ – Waschflasche mit NaOH-Lösung für CO_2-Sorption. ⊗ – Regulationsklemme, ⊕ – Verbindungsstelle, an der Luft mit Wasser vermischt wird.

labeling, the soil surface under the hole of the lid was sealed with a 2 mm layer of silicone paste that overlaid a 2 mm layer of low melting point Paraffin. PVC tubes brought air and deionized water through the three inlets into the CombiSart device from the output of the membrane pump and flask ①, respectively. The soil was separated from the outlet in the bottom of the CombiSart device by a perforated filter support, delivered together with the filtration device, overlain by two layers of perforated (holes = 0.5 mm) polyethylene. The outlet was connected in series with a PVC tube to the flask ③ and test tube ④ with NaOH. Water with released exudates and air with $^{14}CO_2$ coming from root respiration passed through the outlet, then dropped into another flask (see ③ Fig. 1) and was separated by adsorbing $^{14}CO_2$ in NaOH in the test tube ④. Micropur® containing Ag^+ was added to the flask ③ to suppress the microbial decomposition of leaked exudates before analysis (*Deubel*, 1996; *Gransee* and *Wittenmayer*, 2000). The test tube (see ④ Fig. 1) was connected to the input of the pump thus creating air circulation in the whole closed system.

2.3 Labeling and sampling

A day before labeling, every hole with a plant was sealed with low melting point Paraffin and a two-component Silicon paste (NG 3170 from Thauer & Co. Dresden). The seal was tested for air leaks. The labeling took place 64 days after *Lolium* seeds had been planted. Each chamber was labeled separately. A label 118 kBq of ^{14}C as $Na_2{}^{14}CO_3$ solution was put in a 2-ml Eppendorf micro test tube in the upper compartment of the chamber and the chamber was then closed. The addition of 1 ml of 5 M H_2SO_4 to the $Na_2{}^{14}CO_3$ solution in the micro test tube through a Teflon tube allowed complete evolution of $^{14}CO_2$ into the chamber. Assimilation took place within 4 hours after the $^{14}CO_2$ pulse had been applied. Four hours before the trapping of CO_2 from the upper compartment begun, any unassimilated $^{14}CO_2$ was removed by pumping the air from the upper chamber through 20 ml of a 0.25 M NaOH solution. The top part of the chamber was then removed.

The traps for $^{14}CO_2$ evolving from root respiration and those for eluted exudates were started at the beginning of labeling. Flask ① was filled up regularly with water and flask ③ emptied. At the same time the NaOH solution in the test tube ④ was exchanged. 400 ml of distilled water was used to fill up flask ① and 20 ml of 0.25 M NaOH was used to fill up test tube ④. To obtain the dynamics of root respiration and exudation, the exchange of solutions was done 5 times on the first day after the labeling and only two times on the 4th day. At the start of elution, a short closing (10–20 seconds) of the tube at the clamp ⊗ was necessary to increase the pressure in the flask ① and to start the movement of water into the tube behind flask ①. When the water level in the tube was lower than the water level in flask ①, then the Siphon principle began to work. The clamp ⊗ could then be opened and the water continues to move according to the Siphon principle. At the joint ⊕, the water from the flask mixes with the air coming from the pump. The height of the water level in the upper flask above the joint ⊕ is the main factor regulating the rate of water circulation. The rate of air circulation through the system is also responsible for the water transport. The clamp ⊗ is also useful in regulating the air circulation speed. Between the joint ⊕ and flask ③ the movement of water with air as bubbles in the tube occurs simultaneously (dashed arrows in Fig. 1). Behind the flask ③ only air is pumped (blank arrows in Fig. 1). Fresh air was introduced into each container once daily to compensate for the O_2 consumed by soil microorganisms and roots.

2.4 Sample analysis and calculations

^{14}C in CO_2 collected in the NaOH solution and ^{14}C in eluted organic compounds were measured on 2-ml aliquots added to 3 ml scintillation cocktail EcoPlus (Roth Company, Germany) after the decay of chemiluminiscence (for NaOH). 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 an extended standard (tSIE).

The total content of C-CO_2 collected in the NaOH solution was measured by titration with 0.2 M HCl against phenolphthalein after addition of 0.25 M $BaCl_2$ solution (*Black*, 1965).

All the ^{14}C data are presented as percentages of total assimilated ^{14}C. The total assimilated C was calculated according to the equation:

$$^{14}C_{ass} = {}^{14}C_{input} - {}^{14}C_n - {}^{14}C_r,$$

with $^{14}C_{ass}$: activity of total assimilated ^{14}C; $^{14}C_{input}$: total activity introduced as $Na_2{}^{14}CO_2$; $^{14}C_n$: activity of the NaOH solution after flushing the upper chamber; $^{14}C_r$: ^{14}C not volatilized after H_2SO_4 addition.

The ^{14}C labeling, chemical and radiochemical analyses as well as the calculations are described in detail in *Kuzyakov* et al. (1999; 2001).

3 Results

The start of air pumping and short closure of the clip ⊗ led to the movement of air and water into the container with the soil and roots. A few minutes later, water drops appeared in the outlet of the container and they were collected in the flask ③. Only a few visible mineral soil particles (less than 0.1 g per 400 ml water) were eluted together with the water and they appeared only in the first sample. The subsequent solution samples were a little murky, but they contained no sediment.

^{14}C activity was found in the $^{14}CO_2$ coming from the rhizosphere and in the water with eluted organic compounds. It is important to note that the eluted ^{14}C-labeled compounds stem from original root exudates as well as from organic compounds metabolized and modified by microorganisms during elution. Here, the term 'exudates' is used for both the unchanged organic substances exuded by roots and any other root-derived organic compounds modified by microorganisms.

The first samples taken 1 hour 40 minutes after the start of labeling showed that in $^{14}CO_2$ there was nine times more ^{14}C compared to ^{14}C in eluted root-derived organic compounds. The dynamics of $^{14}CO_2$ coming from the rhizosphere and eluted organic compounds during four-and-half days after the labeling is presented in Fig. 2. The maximum intensity of $^{14}CO_2$ efflux occurred roughly 12 hours after the labeling. However, there are two maxima of the intensity of ^{14}C in eluted root-derived organic compounds. The first one appeared 5 hours after labeling and the second between 20 and 24 hours. Noteworthy is that both maxima of ^{14}C activity in eluted exudates do correspond with the light phases (Fig. 2), that means during photosynthesis. On the third day after the labeling (rd. 40 hours after the labeling) a smaller third maximum appeared also during the light phase, although this was not significant. In contrast, both minima of ^{14}C in eluted root-derived organic compounds measured during the first two days occurred mainly at night.

The intensity of exudation decreased after the second maximum and was two days after the labeling less than 0.01 % ^{14}C h^{-1}. This suggests that 2 days after assimilation most of the ^{14}C activity was located in the non-soluble organic compounds, such as root hairs, sloughed cells and partially in mucigels, microbial biomass, etc. Therefore, the

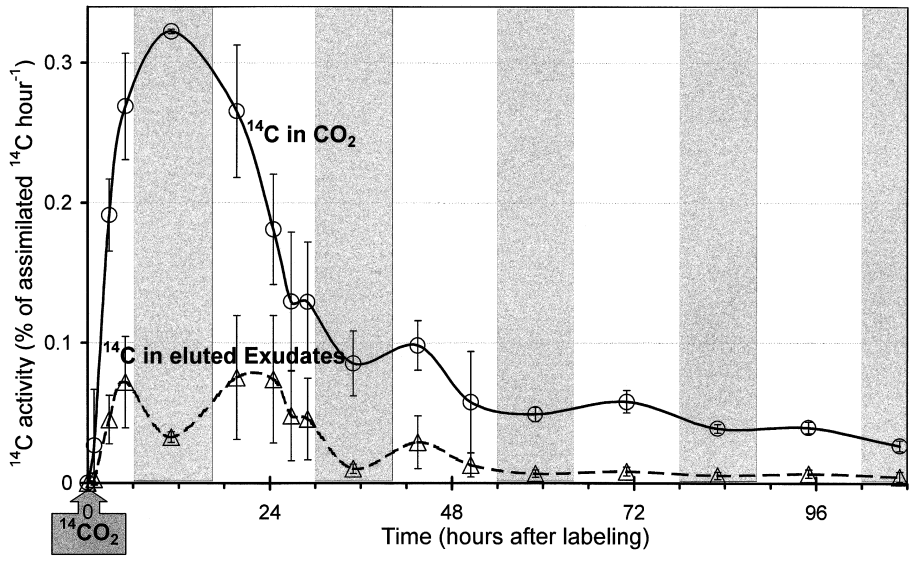

Figure 2: Dynamics of ^{14}C in root respiration and exudates separated with the Siphon method after ^{14}C pulse labeling of shoots. Vertical bars show standard deviation. Grey area shows the nighttime.
Abbildung 2: Dynamik von ^{14}C in Wurzelatmung und Exsudation (± SD) – aufgetrennt mit der Siphon-Methode nach der oberirdischen ^{14}C-Pulsmarkierung der Pflanzen. Graue Flächen zeigen die Nachtzeit.

$^{14}CO_2$ coming from the rhizosphere two days after the labeling mainly originates from microbial decomposition of root hairs, sloughed cells and turnover of microorganisms. Strictly speaking, it is no longer connected with the mineralization of actual root exudates.

The total ^{14}C activity found in $^{14}CO_2$ coming from the rhizosphere corresponds to 8.5 % and the ^{14}C activity in eluted organics corresponds to 2.3 % of total assimilated ^{14}C. So, the ratio of ^{14}C activity measured in $^{14}CO_2$ coming from the rhizosphere to ^{14}C in eluted organic compounds during the whole observation period is about 4.5. However, this ratio strongly depends on sampling time, especially during the first 2 days after start of assimilation (labeling). The ratio of $^{14}CO_2$: ^{14}C in eluted root-derived organic compounds is about 6 to 9 in the nighttime and diminishes to about 2 to 3 during the assimilation time (Fig. 3). Thus, the exudation intensity is much higher during the daytime than nighttime. Two days after assimilation the ratio shows no diurnal dynamics and remains on the level of 5 to 6.

4 Discussion

4.1 The Siphon method

The Siphon method presented here allows the separation of carbon (C) coming from root respiration from the C passing through exudates in a non-sterilized soil. The separation is based on the blowout of $^{14}CO_2$ coming from root respiration by continuous air pumping and simultaneous leakage of original root exudates and organic compounds modified by microorganisms by continuous water flow through the rhizosphere soil. The innovation of the method lies in the installation of a membrane pump for air circulation and simultaneously for the water transport in the system. The use of non-sterilized soil as a growth matrix ensures that the plant roots are subjected to mechanical impedance and microbial stimulation of exudation and respiration. The system allows a continuous flow of nutrient and O_2 and removal of exudates and CO_2 without disturbance of the plants. The continuous flow of water or the nutrient solution also reduces the re-sorption of exudates by roots (*Paterson* and *Sim*, 1999), utilization by microorganisms and sorption on clay particles.

The use of the commercially available filtration device CombiSart (*Merck®-Laborkatalog*, 2000) in the present approach makes the preparation of microcosms much easier compared to many hand-made devices described in the literature (*Swinnen*, 1994; *Hodge* et al., 1996; reviewed by *Kuzyakov* and *Domanski*, 2000). The CombiSart device is developed for filtration under subpressure or overpressure, so it is airtight. It consists of three main parts that can be connected to each other and to the tube in a few minutes. The dismantling of the system and the removal of the soil with roots is also very easy. The device is not expensive; it costs about 80 EUR including all the components and can be used repeatedly.

Four main points distinguish this system from all the systems presented in literature before:

1) The whole system is closed in a circuit; therefore there are no losses of C or air.
2) Shoot growth takes place in an open environment. Therefore there are no disturbing effects of closed chambers, which usually have higher temperatures and higher air moisture.
3) The water transport is driven by air circulation.
4) The roots and microorganisms have aerobic conditions despite the water saturation of the soil.

Limitations of the method are:

1) Only water-soluble exudates can be eluted with this method.
2) The exudates are likely to be decomposed or modified by microorganisms during the leakage.
3) Preferential flow of water in the soil.
4) Flow of water and nutrients may change the amount and composition of C released by the roots (*Johnes* and *Darrah*, 1993).

The first shortcoming is connected with the limited elution of some mucigels secreted by roots as well as with the ^{14}C incorporated in root hairs and sloughed root cells. The ^{14}C

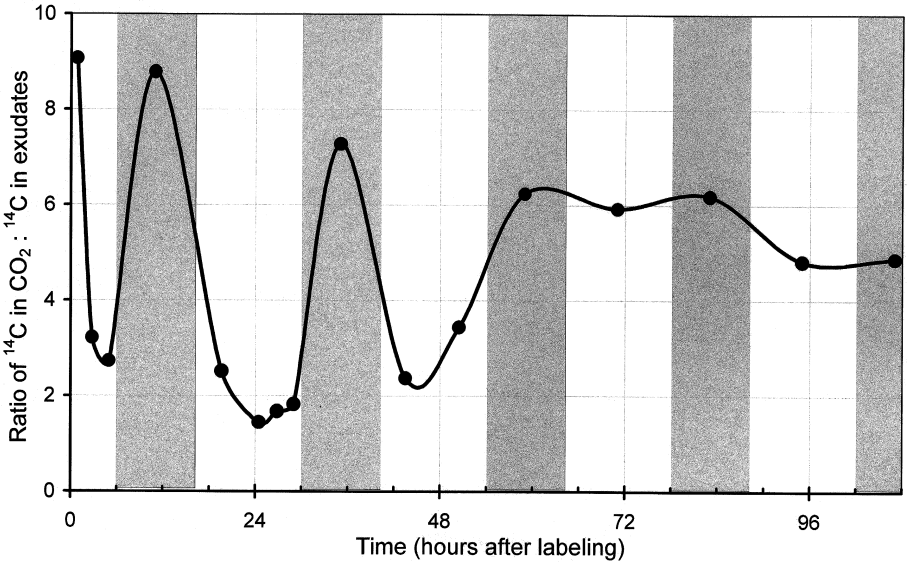

Figure 3: Quotient between ^{14}C in root respiration and in exudates during four and a half days after assimilation. Grey area shows the nighttime.
Abbildung 3: Quotient zwischen ^{14}C in der Wurzelatmung und in Exsudaten im Laufe der ersten 4,5 Tage nach der Assimilation. Graue Flächen zeigen die Nachtzeit.

incorporated in mucigels is mostly accepted as ^{14}C in exudates, although ^{14}C evolved by microbial decomposition of root hairs and sloughed root cells is a part of root turnover. However, *Merbach* et al. (1999) showed that up to between 60 % and 80 % of the root-borne organic compounds found in the immediate vicinity of the roots was mainly water-soluble. *Jones* and *Darrah* (1993) found that, depending on the removal of nutrient solution, between 48 % and 86 % of root-derived organic compounds were soluble low molecular weight exudates.

The exudates in the suggested system are mainly eluted by preferential flow. Therefore, some differences arise from the exudation of organic substances near the preferential flow pathway and at some distance from it. So, microorganisms can decompose the exudates during their diffusion from the root to the exudate collector (flask ③ in Fig. 1). Therefore, the eluted organics consist not only of the original exudates but also include substances modified by microorganisms during elution. This shortcoming may depend on the soil texture. Sandy soils may probably be more suitable for the separation with this method than the clayey soils. This is hypothesized because of the relatively smaller sorption of organic compounds and higher infiltration rates in a sandy soil than in a clayey soil.

Continuous water flow in the microcosm may change the amount and composition of the C released by the roots. *Johnes* and *Darrah* (1993) reported an up to 98 % re-uptake of maize exudates in a sterile static nutrient solution culture. On an example of ^{14}C labeled glucose *Paterson* and *Sim* (1999) show a 75 % re-uptake of exudates by roots of *Lolium perenne* in a sterilized nutrient solution culture. However, it is doubtable whether the re-uptake of released organic substances by roots plays a significant role under non-sterilized soil conditions. Under field conditions microorganisms located on root surface strongly compete with roots for exudates. Continuous removal of exudates should decrease the re-absorption by roots. In our system the removal of exudates from roots by water flow may be accepted as uptake by microorganisms.

In this study only the plant derived C in rhizosphere was of interest. The used ^{14}C labeling of plants with subsequent tracing of root and microbial respiration as $^{14}CO_2$ and exudation as eluted ^{14}C-labeled organic compounds allowed a reliable separation between soil-derived and plant-derived C. However, for studies without plant labeling a control microcosm without plants must be included to estimate the leakage of organic compounds and nutrients from a bare soil.

4.2 Separation

The above-described shortcomings will have the effect of increasing the ^{14}C part in CO_2, thereby decreasing it in exudates. Therefore, the results for the part of ^{14}C measured in eluted organic compounds appear to be underestimated and the ^{14}C part in CO_2 overestimated. Hence it can be concluded that the method shows only the **minimal amount of water-soluble exudates** released from roots. However, this minimal amount of exudates is about two to three times higher than that estimated with the model rhizodeposits method (*Swinnen*, 1994). Therefore, the addition of ^{14}C-labeled model rhizodeposits to soil (*Swinnen*, 1994) cannot be accepted as a satisfactory method for separation of root respiration from microbial respiration of exudates.

The amount of eluted root-derived organic substances measured with the Siphon method is about 2 times smaller than that measured with the isotope dilution (*Cheng* et al., 1993) or kinetics method (*Kuzyakov* et al., 1999; 2001). A similar factor of about 2 was found out when the dipping method (almost 100 % of cold water soluble exudates) was compared with the percolation method (comparable with the presented Siphon method) for the extraction of exudates from the rhizosphere (*Gransee* and *Wittenmayer*, 2000). So, we can estimate that roughly about half of the substances exuded by roots are water-insoluble and microorganisms will mineralize a part (not more than a half) of the exuded organic compounds during the leaching process through our loamy soil. *Merbach* et al. (1999) found that the amount of water-insoluble secretes comprises less than 40 % of root-

borne organic compounds. Both, the sorption of exudates on mineral particles and the microbial decomposition must be smaller in a sandy soil or under a stronger H_2O stream.

Despite the underestimation of exudation by the Siphon method, the diurnal dynamics of exudation was observed. Similar day-night changes for total CO_2 evolved from a planted soil as well as ^{13}C and ^{14}C labeled root-derived CO_2 were detected by *Kuzyakov* and *Cheng* (2001). No separation of root respiration and exudation was undertaken in that study. The results of this separation show that the diurnal dynamics measured before for total root-derived CO_2 is based mostly on exudation dynamics and not on the root respiration. So both, root and microbial respiration are heterotrophic respiration and are not closely connected with plant photosynthesis. In contrast, exudation is an active process, which is closely connected with assimilation. Whereas in the experiment by *Kuzyakov* and *Cheng* (2001) day-night temperature was constant, the daytime temperature in the current experiment was about 5 °C higher than the nighttime temperature. In spite of that, the diurnal dynamics was not found in the rhizosphere CO_2 but in exudates.

5 Outlook

The separation system presented in this study can be easily used for other kinds or similar investigations of simultaneous measurements of gaseous and liquid phases of soil or soil-plant-systems. If the experimentation duration is longer than a couple of days, then nutrient solutions should be used instead of water. All parts of the Merck®-CombiSart device are autoclavable, so the experiments could also be conducted under sterile conditions. Therefore, this system could be used for investigations of the effect of various environmental factors (pH, nutrient availability, soil temperature, presence of microorganisms, oxygen status, light intensity, etc.) on the qualitative and quantitative composition of root exudates and rhizosphere gases. However, drought and water stress may not be investigated with this system.

The suggested Siphon system can be used for long-term elution or extraction of some substances or elements from the soil with a small volume of water or other solution (similar to the Soxhlet apparatus for repeated extraction (*Birioukova* and *Kozlovskaya*, 1985)) by simultaneous collection of soil gases. A small change in the system is necessary for that purpose. The outlet tube must be connected directly to the first flask to close the water circulation in the system. The air circulation would be slightly different from that shown in Fig 1. For the collection of the investigated substance, the solution must pass through an absorber binding the substance. This system of simultaneous and partly connected circulation of air and water is now under construction and optimization.

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