J. Plant Nutr. Soil Sci. 2005, *168,* 503–520 **DOI:** 10.1002/jpln.200421703

Root and rhizomicrobial respiration: A review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil

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Accepted April 28, 2005 PNSS P170/3B

Summary—Zusammenfassung

Partitioning the root-derived CO_2 efflux from soil (frequently termed rhizosphere respiration) into actual root respiration (RR, respiration by autotrophs) and rhizomicrobial respiration (RMR, respiration by heterotrophs) is crucial in determining the carbon (C) and energy balance of plants and soils. It is also essential in quantifying C sources for rhizosphere microorganisms and in estimation of the C contributing to turnover of soil organic matter (SOM), as well as in linking net ecosystem production (NEP) and net ecosystem exchange (NEE). Artificial-environment studies such as hydroponics or sterile soils yield unrealistic C-partitioning values and are unsuitable for predicting C flows under natural conditions. To date, several methods have been suggested to separate RR and RMR in nonsterile soils: 1) component integration, 2) substrate-induced respiration, 3) respiration by excised roots, 4) comparison of root-derived $^{14}CO_2$ with rhizomicrobial $^{14}CO_2$ after continuous labeling, 5) isotope dilution, 6) model-rhizodeposition technique, 7) modeling of $^{14}CO_2$ efflux dynamics, 8) exudate elution, and 9) $\delta^{13}C$ of CO_2 and microbial biomass. This review describes the basic principles and assumptions of these methods and compares the results obtained in the original papers and in studies designed to compare the methods.
The component-integration method leads to strong disturbance and non-proportional increase of CO_2 efflux from different sources. Four of the methods (5 to 8) are based on the pulse labeling of shoots in a $^{14}CO_2$ atmosphere and subsequent monitoring of $^{14}CO_2$ efflux from the soil. The model-rhizodeposition technique and exudate-elution procedure strongly overestimate RR and underestimate RMR. Despite alternative assumptions, isotope dilution and modeling of $^{14}CO_2$-efflux dynamics yield similar results. In crops and grasses (wheat, ryegrass, barley, buckwheat, maize, meadow fescue, prairie grasses), RR amounts on average to 48±5% and RMR to 52±5% of root-derived CO_2.
The method based on the ^{13}C isotopic signature of CO_2 and microbial biomass is the most promising approach, especially when the plants are continuously labeled in $^{13}CO_2$ or $^{14}CO_2$ atmosphere. The "difference" methods, *i.e.*, trenching, tree girdling, root-exclusion techniques, etc., are not suitable for separating the respiration by autotrophic and heterotrophic organisms because the difference methods neglect the importance of microbial respiration of rhizodeposits.

Key words: root respiration / rhizomicrobial respiration / rhizosphere CO_2 / partitioning methods / ^{14}C, $\delta^{13}C$, ^{13}C natural abundance / autotrophic respiration / heterotrophic respiration

Wurzelatmung und rhizomikrobielle Atmung: Übersicht über verschiedene Ansätze zur Abschätzung der Atmung autotropher und heterotropher Organismen im Boden

Trennung des wurzelbürtigen CO_2-Effluxes aus dem Boden (oft als Rhizosphärenatmung bezeichnet) in die eigentliche Wurzelatmung (= Atmung der Autotrophen) und die rhizomikrobielle Atmung (= ein Teil der Atmung der Heterotrophen) ist entscheidend für die Bestimmung des Kohlenstoff (C)- und Energiehaushaltes von Pflanzen und Böden. Sie ist unentbehrlich für die Quantifizierung der C-Quellen für Rhizosphären-Mikroorganismen und die organische Bodensubstanz, aber auch zur Kopplung der Netto-Ökosystemproduktion (NEP) mit dem Netto-Ökosystemaustausch (NEE). Künstliche Wachstumsmedien für Pflanzen (Hydrokultur oder Bodensterilisation) lieferten für die C-Partitionierung unrealistische Ergebnisse und sind für die Vorhersage der C-Flüsse unter natürlichen Bedingungen kaum anwendbar. Bis heute sind folgende Methoden zur Trennung der Wurzelatmung und der rhizomikrobiellen Atmung in nicht sterilen Böden vorgeschlagen worden: 1) Komponenten-Integration, 2) substratinduzierte Atmung, 3) Messung der Atmung von abgeschnittenen Wurzeln, 4) Vergleich des $^{14}CO_2$-Effluxes aus dem wurzelbürtigen CO_2 mit dem aus der rhizomikrobiellen Atmung, 5) Isotopen-Verdünnung, 6) Anwendung von künstlichen Rhizodepositen, 7) Modellierung der Dynamik des $^{14}CO_2$-Effluxes, 8) Auswaschung der Exsudate und 9) $\delta^{13}C$ in CO_2 und mikrobieller Biomasse. Dieser Übersichtsartikel beschreibt die Grundlagen und Annahmen dieser Methoden und vergleicht die Ergebnisse aus den Originalpublikationen mit Studien, die auf einen Vergleich der Methoden ausgerichtet waren.
Die Methode der Komponenten-Integration führt zu einer starken Störung und zu einem nicht proportionalen Anstieg des CO_2-Effluxes aus unterschiedlichen Quellen. Vier Methoden (5–8) basieren auf einer Pulsmarkierung von Pflanzen in einer $^{14}CO_2$-Atmosphäre und anschließendem Monitoring des CO_2-Effluxes aus dem Boden. Die Methoden der künstlichen Rhizodeposite und der Auswaschung von Exsudaten überschätzen die Wurzelatmung stark und unterschätzen die rhizomikrobielle Atmung. Trotz gegensätzlicher Annahmen zeigen die Isotopen-Verdünnung und die Modellierung der Dynamik des $^{14}CO_2$-Effluxes ähnliche Ergebnisse. In den untersuchten Pflanzen (Weizen, *Lolium perenne*, Gerste, Buchweizen, Präriegräser) betrug die Wurzelatmung 48±5 % und die rhizomikrobielle Atmung ca. 52±5 % des wurzelbürtigen CO_2-Effluxes.
Die Methode, die auf der ^{13}C-Isotopensignatur des CO_2 und der mikrobiellen Biomasse basiert, scheint viel versprechend zu sein, insbesondere wenn Pflanzen kontinuierlich in der $^{13}CO_2$- oder $^{14}CO_2$-Atmosphäre markiert werden. Differenzmethoden wie Trenching, Baumgürteln (tree girdling) und Wurzelausschlusstechnik (root exclusion) sind für eine Trennung der Atmung von autotrophen und heterotrophen Organismen nicht anwendbar, weil sie die Wichtigkeit der mikrobiellen Veratmung der Rhizodeposite vernachlässigen.

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 1436-8730/05/0408-503

1 Introduction

1.1 Why we need separate evaluation of root and rhizomicrobial respiration

After photosynthesis, CO_2 flux from soil is the second largest carbon (C) flux in most ecosystems, amounting to 60%–90% of total ecosystem respiration (*Goulden* et al., 1996; *Longdoz* et al., 2000; *Schlesinger* and *Andrews*, 2000). On a global scale, soil respiration produces 80.4 Pg C-CO_2 annually (*Raich* et al., 2002). This is more than 11 times the current rate of industrial CO_2 emissions produced by fossil-fuel combustion (*Marland* et al., 2001). The realization that soils are a potential source of atmospheric CO_2 has given rise to numerous methods to quantify this input. Subsequently, the total CO_2 efflux from soil has been measured in ecosystems all over the world. In contrast to other greenhouse gases (CH_4, N_2O), values for total CO_2 efflux from soil do not provide sufficient information to determine whether the soil is a net source or net sink for atmospheric CO_2. This uncertainty is connected with a specific feature of C turnover and CO_2 efflux from soil: the total amount of CO_2 coming from soil is not all soil-derived, *i.e.*, it is not all produced by the decomposition of soil organic matter (SOM). Most soils are covered with vegetation, and this vegetation can contribute considerably to the total CO_2 efflux (*Bond-Lamberty* et al., 2004). The earliest studies concluded that the contribution of vegetation (*Reiners*, 1968; *Anderson*, 1973), as well as vegetation-induced changes in SOM turnover (*Dormaar*, 1990; *Kuzyakov*, 2002; *Cheng* and *Kuzyakov*, 2005), strongly limit the reliability of total CO_2 efflux to determine whether the soil is a net source or a net sink of CO_2. This fact is reflected in the great amount of research designed to evaluate the contribution of different C sources to the total CO_2 efflux from soil (for forest ecosystems reviewed by *Hanson* et al., 2000). The most progress in the partitioning of CO_2-efflux sources was achieved in separating the CO_2 evolved by microbial decomposition of SOM from root-derived CO_2. This separation is crucial to estimate the decomposition rates of SOM and its contribution to the total CO_2 efflux. This achievement was mainly based on plant labeling in a $^{14}CO_2$ or $^{13}CO_2$ atmosphere or on the use of differences in the natural abundance of ^{13}C in plants having C_3 or C_4 photosynthetic pathways (reviewed by *Hanson* et al., 2000; *Kuzyakov*, 2005). Different methods allowing for the separate estimation of SOM-derived and root-derived CO_2 fluxes were developed and used under both laboratory and field conditions. The most important methods as well as the results for forest ecosystems were reviewed by *Hanson* et al. (2000).

The next logical step in separating the CO_2 sources is to differentiate the root-derived CO_2 efflux (frequently termed rhizosphere respiration) into actual root respiration (RR) on the one hand and into microbial respiration of exudates and of other rhizodeposits on the other hand. Despite great progress in plant physiology, **we still do not know which part of C compounds synthesized from assimilated C is used by plants grown under soil conditions for RR and which for rhizodeposition**. This differentiation is crucial in quantifying the C and energy balance of plants, soils, and microorganisms. Exudates and root residues are energy rich; they enhance the underground C stock and are metabolized by soil microorganisms. These C sources, which are readily available to microorganisms, contribute to fast C turnover in the soil and to higher microbial activity in the rhizosphere when compared with root-free soil. Stimulation of microbial growth and activity around roots changes the mineralization rate of native SOM (reviewed by *Dormaar*, 1990; *Kuzyakov*, 2002; *Cheng* and *Kuzyakov*, 2005) and subsequently increases the availability of mineral nutrients. In contrast to root exudates, CO_2 originating from RR cannot be used by microorganisms for growth: it is an energy-poor mineralization product that does not affect the turnover of microbial biomass and SOM. Therefore, accurate C and energy budgets of the plants, soils, and rhizosphere microorganisms cannot be determined without separately estimating RR and microbial utilization of root exudates.

The ecosystem C balance results from the difference between C uptake by photosynthesis and C losses by respiration by autotrophic organisms (plants) plus respiration from heterotrophic organisms. This difference is defined as net ecosystem production (NEP) or net ecosystem exchange (NEE). Conceptually, both parameters are equivalents, but the estimates of NEP and NEE are based on different methodological approaches. NEP is calculated as the difference between net primary production (NPP = C uptake by photosynthesis – respiration by plant shoots and roots) and respiration by heterotrophs. The NPP estimates are based on the measurements of plant above- and belowground biomass dynamics at monthly (or similar) sampling intervals. These estimates neglect C pools with fast turnover rates such as root rhizodeposits, assuming that these have a negligible C input into the ecosystem (*Scurlock* et al., 2002). NEE assays are based on eddy covariance or micrometeorological measurements and provide therefore more frequent integration of vegetation and soil CO_2 fluxes at subhour intervals. Such subhour measurements consider the input and decomposition of C pools with short residence time. The use of different approaches to estimate NEP and NEE led to poor correlation between annual NEP and NEE in the same experiments despite being equivalent concepts (*Curtis* et al., 2002). Some of the deviations can be explained by technical problems: eddy-covariance measurements are unreliable at night and during precipitation. **The main reason for the poor correspondence of NEP and NEE, however, is firstly ignoring C input in the rhizosphere when estimating NPP and secondly disregarding respiration by heterotrophs decomposing rhizodeposits by separation of total soil CO_2 efflux into autotrophic and heterotrophic components.** Carbon loss from roots including exudates, lysates, mucilage, and dead root cells can account for up to 40% of dry matter produced by plants (*Lynch* and *Whipps,* 1991; *Kuzyakov* and *Domanski*, 2000; *Nguyen*, 2003). Neglecting this important part of C input by plants into the soil results in a critical underestimation of NPP. At the same time, C losses as CO_2 respired by heterotrophs decomposing rhizodeposits are very frequently measured (and accepted) as part of root (autotrophic) respiration by root exclusion, trenching, girdling, and other difference methods (see below), which are widely used in field studies. Hence, the use of difference methods leads to overestimation of autotrophic component and underestimation of heterotrophic component of CO_2 emission. To understand the degree to which NPP and respiration by heterotrophs are underestimated, there is a need for methods providing the assessment of C fluxes in the rhizosphere separating RR from rhizomicrobial respiration (RMR).

Separate estimation of RR and RMR is also a prerequisite for modeling CO_2 fluxes from soil (*Pumpanen* et al., 2003), as **different environmental variables control the intensity of the respective fluxes** (*Burton* et al., 2002; *Burton* and *Pregitzer*, 2003; *Lee* et al., 2003). The SOM-derived CO_2 efflux is known to depend mainly on the SOM content and its recalcitrance, soil temperature, moisture content, and aeration (*Raich* and *Schlesinger*, 1992; *Kirschbaum*, 2000). The response of root and microbial respiration may be different: respiration by heterotrophs is more sensitive to temperature changes than RR (*Boone* et al., 1998; *Epron* et al., 2001; *Dioumaeva* et al., 2002). Primary RR and RMR are driven by photosynthesis intensity. However, secondary controls may strongly differ: an important part of RR is respiration for maintenance, which strongly depends on the temperature, while RMR is controlled by the amount of easily decomposable rhizodeposits, which depends largely on photosynthetic active radiation and N content (*Whipps*, 1984; *Todorovic* et al., 1999; *Craine* et al., 1999; *Högberg* et al., 2001; *Kuzyakov* and *Cheng*, 2001). However, these observations about the factors controlling RR and RMR are based on limited studies and urgently require further investigation, which cannot be conducted without separate estimation of RR and RMR.

In summary, RR and RMR must be separately estimated to 1) evaluate the C and energy balance of plants, soils, and microorganisms, 2) link NEP and NEE, 3) understand the environmental factors controlling CO_2 efflux from different components, 4) successfully model CO_2 fluxes from soil, and 5) comprehend how the plant-soil system functions as a whole.

1.2 Definitions

The definitions of sources of CO_2 efflux from soil vary in different studies. This review uses the following definitions (*Kuzyakov*, 2005):

Root respiration (RR) is the actual respiration by roots to obtain energy for maintenance of the metabolism and concentration gradient in cells (maintenance respiration), growth, and active uptake of nutrients (*George* et al., 2003). RR is the only significant CO_2 flow of respiration by autotrophic organisms in soil. (The respiration by algae and chemolithotrophs can be neglected here because of their minor importance in most soils (*Paul* and *Clark*, 1996), as well as the same location of assimilation and CO_2 production).

Rhizomicrobial respiration (RMR) is the respiration by heterotrophic microorganisms decomposing organic substances released by living roots (rhizodeposits). RMR clearly belongs to respiration by heterotrophs. We do not consider here the contribution of soil macro- and mesofauna involved in predator-prey interactions with rhizosphere microorganisms, since their direct contribution to the CO_2 efflux is negligible (*Paul* and *Clark*, 1996; *Panikov*, 1995; *Ke* et al., 2005).

In some cases (see below), when the method does not allow separation of the respiration by rhizosphere microorganisms from the respiration by microorganisms decomposing SOM (in the bulk soil), the term **microbial respiration (MR)** is used. In this case, microbial respiration includes rhizomicrobial respiration.

"Root-derived CO_2" (in contrast to SOM-derived CO_2) is used to describe the sum of RR and RMR. The terms "rhizosphere CO_2" or "rhizosphere respiration" are frequently used in literature to refer to the sum of RR and RMR. Strictly speaking, the term "rhizosphere respiration" refers to the **location** of CO_2 production—not to the pool of C from which the CO_2 originates or to the agents of CO_2 production. Considering the location of CO_2 production, "rhizosphere respiration" should include not only RR and RMR, but also CO_2 derived by microbial SOM decomposition in the rhizosphere. In some studies (*Andrews* et al., 1999; *Lee* et al., 2003; *Bhupinderpal* et al., 2003), the term "root respiration" was used for the CO_2 evolved as the sum of RR and RMR. Such an exchange of terms, especially without prior definition, is strongly misleading.

1.3 Where is the boundary for respiration by autotrophs?—the nature of the problem

The inclusiveness of the term "respiration by autotrophs" is controversial. If we accept that plants are only one large group of autotrophic organisms living on and in the soil, we should also accept that respiration by rhizosphere microorganisms and different types of mycorrhizal fungi (vesicular-arbuscular, endo- and ectomycorrhizal fungi) does not belong to the term "respiration by autotrophs". Therefore, respiration by bacteria, fungi, actinomycetes, soil animals, etc. that inhabit the rhizosphere represents respiration by heterotrophs. Many studies passing over in silence accept that the respiration by autotrophs (designated as "autotrophic respiration") also includes the respiration by rhizosphere microorganisms, *i.e.*, microorganisms that use organic substances released by roots. In our opinion, this "inaccuracy" mainly reflects the impossibility or unwillingness to separate the respiration by autotrophs and heterotrophs; it also reflects a strong underestimation of the role of rhizodeposition in the C flows.

Another source of uncertainty here is the attempt to delimit the extension of the rhizosphere. However, *Jones* et al. (2004) show in their review that the rhizosphere extension differs for various nutrients and organisms. In our opinion, such fixed delimitation is unnecessary and clearly insufficient to define the respiration by autotrophic and heterotrophic organisms. Accordingly, it is inconsequential whether the microbe is located on the root surface, in the root, or 100 mm from the root. The key question is whether it is able to produce organics from mineral components or not.

Note here that the term "autotrophic respiration" is misleading because the terms "autotrophic" and "heterotrophic" show the way of substrate and energy assimilation by an organism and are not connected with the respiration themselves. Therefore, we use the terms "respiration by autotrophs" and "respiration by heterotrophs". Other suitable terms were suggested by *Bond-Lamberty* et al. (2004): "heterotrophic and autotrophic components of soil respiration".

To imagine the scientific challenge of RR and RMR separation, the intimate interactions between roots and rhizosphere microorganisms should be visualized. The challenge is to separate the CO_2 evolved from root cells (root respiration) from the CO_2 respired by microorganisms located directly on these roots and utilizing organics released from the roots (rhizomicrobial respiration). These organics will be captured by microorganisms immediately or shortly after release into the rhizosphere. Some microorganisms, mainly mycorrhiza fungi, spread their hyphae into the roots or even in the root cells. Small changes in the rhizosphere environment lead to changes in 1) root respiration, 2a) amount of released organic compounds and 2b) their composition, and 3a) microbial community and 3b) its activity. Considering these problems and also the importance of separation RR and RMR, *Killham* and *Yeomans* (2001) underlined that: "Discriminating between CO_2 which is directly derived from root respiration and that which is derived from mineralization of the components of C-flow is exceptionally difficult and has presented one of the greatest challenges to quantifying rhizosphere C-flow".

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 have been used in the past to evaluate RR and the amount of root exudates. The results show that investigations based on artificial root environments (like hydroponics or sterile soils) yield unrealistic values for C partitioning (*Bowen*, 1980; *Schönwitz* and *Ziegler*, 1988; *Merbach* et al., 1990; *Meharg* and *Killham*, 1991; *Schulze* et al., 1994). This makes them unsuitable for predicting C flows under natural conditions.

More recently, efforts have been made to divide root-derived CO_2 (the sum of RR and RMR) into CO_2 originating from RR and that originating from microbial respiration of root-derived substances during plant growth on nonsterile soils. Different methods, ranging from simple physical separation of C pools to sophisticated isotopic applications, have been proposed to estimate RR and RMR under soil conditions. Unfortunately, these methods yielded different results in original publications. It remains unclear whether these discrepancies reflect the use of different plants, soils, environmental conditions, and experimental equipment or whether they are a methodological artifact due to their different principles and assumptions. Moreover, some methods purport to evaluate the respiration by autotrophic organisms and to allow the separate estimation of RR and RMR. All these approaches and methodological developments of the last decade have not been reviewed. The recent review of *Hanson* et al. (2000) focuses on the methods applied mainly for forest ecosystems that separate the SOM-derived, root-derived, and litter-derived CO_2 efflux. Only three methods allowing a separation of RR and RMR were briefly referred to, but without explanations. Therefore, in contrast to the review by *Hanson* et al. (2000), the present contribution reviews all the separation methods that enable or claim to enable estimation of RR and RMR. We describe the principles behind the methods, their assumptions, advantages, and shortcomings, as well as the results presented in original publications and in studies designed to compare existing methods.

2 Methods: background and assumptions

2.1 Nonisotopic methods

2.1.1 Component integration

Except soil sterilization, the component-integration method (Tab. 1) is the first one designed to separate RR and RMR (*Edwards* and *Sollins*, 1973; *Singh* and *Gupta*, 1977; *Gloser* and *Tesarova*, 1978; *Singh* and *Shekhar*, 1986). The method is based on manual separation of roots, soil, and plant residues (*e.g.*, from the O horizon) from soil samples taken from the field or laboratory. Subsamples of these pools are separately incubated under controlled conditions from 2 to 48 h with trapping of evolved CO_2; the longer incubation results in an increased contribution of root respiration (*Crapo* and *Bowmer*, 1973), caused by the autolysis of root cells. The specific respiration rates (sR) of each component are calculated based on the evolved CO_2 and the mass of the incubated component. The contribution of root respiration (%RR), as well as that of any other component is calculated according to Eq. 1:

$$\%RR = sR_r \cdot M_r / ([sR_r \cdot M_r + sR_s \cdot M_s + sR_o \cdot M_o] \cdot 100) \quad \text{Eq. 1}$$

Where sR_r, sR_s, sR_o are specific respiration rates of roots, soil, and organic residues, respectively, and M_r, M_s, and M_o are the amounts of roots, soil, and organic residues in the sample studied. In some studies, the soil is additionally divided into root-free soil and soil adhering to roots (rhizosphere soil) (*Panikov* et al., 1989; *Sapronov* and *Kuzyakov*, 2004).

In earlier studies, roots were washed from the soil (*Edwards* and *Sollins*, 1973; *Crapo* and *Coleman*, 1972). However, manual separation was used in later studies to avoid highly moistening the roots (*Coleman*, 1973; *Gloser* and *Tesarova*, 1978; *Burton* and *Pregitzer*, 2002). Due to high losses of fine roots during both manual separation and washing procedure, the incubation of rooted soil was compared with root-free soil, and the RR was calculated as the difference (*Larionova* et al., 1998, 2001, 2003). Similarly, the RR was calculated as the difference between total CO_2 efflux from soil and the specific CO_2 efflux obtained by litter and SOM decomposition (*Ewel* et al., 1987).

The assumptions of the component-integration method are: 1a) physical separation of soil components does not significantly change respiration rates or 1b) the effect on of physical separation is the same on respiration from all components; 2) the decrease of respiration rates after the start of incubation is the same for each component. To check the first assumption, CO_2-efflux rates should be measured under field conditions and compared to the sum of CO_2 efflux from each component obtained under controlled conditions. The incubation should take place at similar temperatures to that of the field soil or recalculated. Ideally, the CO_2-efflux rates should be equal under field and laboratory conditions. In reality, the disturbance by component separation increases the CO_2 effluxes from the soil (*Powlson*, 1980; *Larionova* et al., 2001). Under these circumstances, assumption 1b is relied on that the increase in CO_2 efflux is uniform for all components. To check the second assumption, the dynamics of CO_2-efflux rates during 9 d were measured for each component (*Sapronov* and *Kuzyakov*, 2004). The results show that, after disturbance, the decrease is maximal for the rhizosphere soil without roots (up to 6 times compared to initial CO_2 efflux) and for mixed rooted soil (up to 4–5 times). The decrease of CO_2-efflux rates from washed or picked roots was much less (up to 1.5–3 times). Relatively constant CO_2-efflux rates were reached after 1 d for roots and rhizosphere soil, but after 2–3 d for root-free soil and mixed rooted soil. Therefore, the second assumption of the method is also inconclusive.

Besides the strong disturbance due to separation, another shortcoming is that rhizosphere and rhizoplane microorganisms remaining after the washing or hand picking decompose a part of exudates released by roots and so contribute to the CO_2 efflux. To more precisely assess the root contribution to total soil respiration, component integration is generally combined with substrate induced respiration.

Table 1: Methods suggested for separation of root and rhizomicrobial respiration, their background, suitability for partitioning of different CO_2 fluxes and applicability.
Tabelle 1: Vorgeschlagene Methoden zur Trennung von Wurzelatmung und rhizomikrobieller Atmung, Grundlagen und Anwendbarkeit für die Trennung unterschiedlicher CO_2-Flüsse.

Method	Background	Partitioning of CO_2 fluxes [1]	Field/Lab[2]	Reference[3]	Comp./ Comb.[4]
Non isotopic methods:		RR/MR of [Rhiz + SOM]/Litter	FS+L, L		
– Component integration	CO_2 from manually separated individual components	RR/MR of [Rhiz + SOM]/Litter	FS+L, L	a b c	A D
– Substrate-induced respiration	Increase of MR after glucose addition, but constant RR	RR/MR of [Rhiz + SOM]	FS+L, L	d e f	
– Respiration of excised roots	CO_2 efflux from RR only, MR as difference to total CO_2	RR/MR of [Rhiz + SOM]	FS+L, L	g h i j k	C
Isotopic methods:		RR/RMR/(SOM)	L (F)		
– Isotope dilution	Dilution of $^{14}CO_2$ by CO_2 of added unlabelled glucose	RR/RMR	L	l	B
– Model-rhizodeposition method	Addition of ^{14}C labelled artificial rhizodeposits	RR/RMR	L	m	B
– Root-derived $^{14}CO_2$ – RMR-$^{14}CO_2$	Difference: Root-derived $^{14}CO_2$ – RMR-$^{14}CO_2$	RR/RMR/SOM	L	n	E
– Dynamics of $^{14}CO_2$ efflux	Delay of $^{14}CO_2$ evolved from RMR compared to RR	RR/RMR	L (F)	o p	B D
– Exudate elution	Elution of ^{14}C exudates + simultaneous $^{14}CO_2$ trapping	RR/RMR	L	q	B
– $\delta^{13}C$ of microbial biomass and CO_2	C_4-C_3 transition or continuous labelling or FACE	RR/RMR/SOM	F, L	r	
Difference methods[5]:		[RR + RMR] / SOM/(Litter?)	F, L		
– Root exclusion	Comparison or rooted and bare soil	[RR + RMR] / SOM	F, L	e s	A D
– Trenching	Comparison or rooted soil and soil with trenched roots	[RR + RMR] / SOM + Litter	F, L	t u	
– Shading or clipping, gap formation	Shading or clipping of above ground plant parts	[RR + RMR] / SOM + Litter	F, L	v x	C
– Tree girdling	Interruption of assimilate transport to roots by girdling	[RR + RMR] / SOM + Litter	F	y	

[1] CO_2 fluxes partitioned by the method: RR/MR of [Rhiz + SOM] / Litter – separation of 1) RR and 2) microbial respiration of the sum of rhizodeposits and SOM and 3) of litter

[2] Suitability for field (F) or laboratory only (L); FS+L: samples taken from field, but measuring CO_2 in laboratory

[3] Recent references: a: *Larionova* et al., 1998, 2001, 2003; b: *Burton* and *Pregitzer*, 2002; c: *Irwine* and *Law*, 2002; d: *Panikov* et al., 1991; e: *Larionova* et al., 2005; f: *Ekblad* and *Högberg*, 2000; g: *Reich* et al., 1998; h: *Craine* et al., 1999; i: *Burton* and *Pregitzer*, 2002; j: *Burton* et al., 2002; k: *Lipp* and *Andersen*, 2003; l: *Cheng* et al., 1993; m: *Swinnen*, 1994; n: *Johansson*, 1992; o: *Kuzyakov* et al., 1999, 2001; p: *Kuzyakov* and *Domanski*, 2002; q: *Kuzyakov* and *Siniakina*, 2001; r: *Kuzyakov*, 2004; s: *Edwards*, 1991; t: *Bowden* et al., 1993; u: *Epron* et al., 2001; v: *Craine* et al., 1999; x: *Brumme* 1995; y: *Hogberg* et al., 2001.

[4] Studies compared or combined different methods: A: *Larionova* et al., 2005; B: *Kuzyakov*, 2002; C: *Craine* et al., 1999; D: *Sapronov* and *Kuzyakov*, 2004; E: *Johansson*, 1992.

[5] Difference methods are not suitable for separation of root and rhizomicrobial respiration and therefore not for separation of respiration by autotropic and heterotrophic organisms; only selected references for difference methods are presented.

2.1.2 Substrate-induced respiration

Substrate-induced respiration (SIR) of microorganisms, an approach frequently used to estimate the microbial biomass in the soil (*Anderson* and *Domsch*, 1978), has also been applied to estimate RR and RMR (*Panikov* et al., 1991) (Tab. 1). In the original method (*Anderson* and *Domsch*, 1978), the addition of glucose to soil leads to a strong increase in microbial respiration (MR), which was limited before glucose addition by easily available substrate. In 2–4 hours immediately after the addition, the substrate-induced CO_2 increase is proportional to the amount of microbial biomass present and so allows its calculation. The same SIR method with small modifications can be used to estimate RR and RMR. The idea is that after addition of glucose to rooted soil, the microbial respiration (MR, here including RMR) strongly increases, while the RR remains at the same level (*Panikov* et al., 1991). The CO_2 efflux is measured before and after glucose addition, enabling the respiration activity of roots, and microorganisms is calculated from the following equation system:

$$\begin{cases} R_1 = RR + MR_1 \\ R_2 = RR + k \cdot MR_1 \\ k \;\; = MR_2/MR_1 \end{cases} \qquad \text{Eq. 2}$$

where R_1 and R_2 are respiration rates before and after glucose addition, and k is the magnification factor, which is equal to the ratio between respiration rates before and after glucose addition to the soil with carefully removed roots.

The assumption of this method is that RR does not increase after glucose addition. In testing this assumption, *Larionova* et al. (2005) found that the k factor strongly varies depending on the component analyzed. After adding glucose to the soil, k was between 2 and 10. After addition to dead shoot and root residues (O horizon), k varied between 1.5 and 3. After adding glucose solution to the living roots, k was about 1.02–1.05. The same result showing absence of increase of RR was obtained by *Ekblad* and *Högberg* (2000) after addition of sucrose to the nonmycorrhizal and ectomycorrhizal roots of *Pinus silvestris.* These strong differences between the k of roots and of the microbial biomass allow RR and MR to be calculated more exactly than by the component-integration method.

The critical factor of this method is the concentration of added glucose. It should be lower than the sugar content in roots and much higher than the sugar concentration in the soil solution. The amount of nonstructural carbohydrates in root tissues ranges from 50 to 5 mg (g d. m.)$^{-1}$, whereas the concentration of water-soluble carbohydrates in the soil does not exceed 12 mg kg^{-1} (*Sikora* and *McCoy*, 1990; *DeLuca*, 1998; *Larionova* et al., 2005). Based on this range of concentrations between water-soluble carbohydrates in soil and roots, aqueous glucose solution of 0.5–1.0 mg glucose (g soil)$^{-1}$ is usually added to rooted soil.

One limitation of this method involves soil moisture: if the soil moisture is low, then adding water with glucose results in moistening of the roots, boosting RR. The recommendation was therefore to add dry glucose powder (*Larionova* et al., 2005). If the soil moisture is above the field capacity, then the k value drops below 1. The addition of glucose as a powder or solution to water-saturated soil led to rapid consumption of oxygen, resulting in anaerobic glucose oxidation with a slow rate of CO_2 production. The second reason for the drop in k is that the evolved CO_2 dissolves in the soil water and decreases the CO_2 efflux. This method is therefore useful only at moisture levels between 10% and 80% of WHC. Equal distribution of added dry glucose is a prerequisite of the method for undisturbed soil samples. A nonuniform distribution of glucose has a minor effect on CO_2 efflux when added to disturbed samples because the amount of added glucose is much higher than the microorganisms can utilize in the short period (other factors limit microbial growth in the rhizosphere; *Cheng*, 1996). To achieve a better distribution, talcum or fine sand can be added together with glucose to the soil and then mixed.

This modified SIR method applied for estimation of RR and RMR is a further development of the component-integration method (see above). However, the main shortcomings remain: 1) disturbance of the soil sample and 2) nonproportional changes of respiration rates of different components after disturbance.

Advanced development of the SIR method was suggested by *Ekblad* and *Högberg* (2000). They applied C_4 sugar (instead of glucose) to a soil developed under C_3 vegetation and measured total CO_2 efflux and its $\delta^{13}C$ value. The $\delta^{13}C$ value allowed partitioning of the CO_2 evolved from the endogenous C_3 sources and added C_4 sugar. Also the estimation of the magnification factor (k in Eq. 2) is more precise by separating C_3 and C_4 sources, and the effect of the added sugar on the turnover of microbial biomass was estimated. Despite in the original study, the RR of *Pinus silvestris* was not calculated, the high potential of the SIR method with C_4 sugar and its application under field conditions was shown (*Ekblad* and *Högberg*, 2000).

2.1.3 Respiration by excised roots

In order to estimate RR only, a few grams of excised and washed roots were incubated (*Reich* et al., 1998; *Craine* et al., 1999; *Burton* and *Pregitzer*, 2002; *Burton* et al., 2002). The roots were incubated between a few minutes (*Burton* et al., 2002) and 24 h (*Lipp* and *Andersen*, 2003), and CO_2 was then analyzed by IRGA or absorbed in alkali. When measuring the RR of grasses and crops, the incubation can proceed in special chambers without removal of above-ground plant parts (*Naumov*, 1988; *Golovko*, 1999). If the soil temperature differs from the incubation temperature, then corrections are made according to Q_{10} estimates. As described above, manual brushing or shaking is preferable to root washing, especially for roots collected from dry soil, the small amount of mineral soil remaining on the roots does not significantly increase the measured RR because the specific respiration rates of soil are 2–3 orders lower than those of roots (*Zak* et al., 1999; *Larionova* et al., 2005).

The advantages of the method are that it is simple, it can be used for field studies, and it allows estimations involving tree roots. The shortcomings are similar to that of the component-integration approach: an increase of RR by 1.5–3 times after the separation from soil and subsequent decrease of RR (*Sapronov* and *Kuzyakov*, 2004). Therefore, different periods of measurement after excising will lead to different estimations of RR. As mentioned above, RR results from the energy demand to maintain metabolism and the concentration gradient in cells, root growth, and active uptake of nutrients. Therefore, along side the loss of small roots and root hairs during separation, the excised roots extracted from the soil do not have sufficient energy for growth or for the active uptake of nutrients. RR used for growth was estimated to lie between half (aspen, *Desrochers* et al., 2002) and two thirds of total RR (Ponderosa pine, *Lipp* and *Andersen*, 2003), and the remainder being necessary for maintenance. The contribution of growth respiration to total RR can be higher for grasses. Additionally, strong stress situations such as the extraction from soil may increase the portion of maintenance respiration by roots to a value of up to 99% of total root respiration (*Golovko*, 1999). Therefore, these two factors affecting respiration in different directions have an unpredictable effect on the obtained RR: disturbance and injury of roots during separation may increase RR, but the absence of root growth and of nutrient uptake can decrease RR.

2.2 Isotopic methods

Several methods that separate root and rhizomicrobial respiration are based on the application of C isotopes, mainly ^{14}C (Tab. 1). Importantly, the application of C isotopes allows researchers to overcome the problems relating to the contribution of microbial decomposition of SOM to the CO_2 efflux (see section 1). The separation of RR and RMR *in situ*, however, requires special experiment layouts and creative ideas.

2.2.1 Comparison of root-derived $^{14}CO_2$ with $^{14}CO_2$ evolved by decomposition of ^{14}C-labeled rhizodeposits

The principle of the method suggested by *Johansson* (1992) looks simple: root-derived $^{14}CO_2$ evolved from the rhizosphere of plants continuously labeled in the $^{14}CO_2$ atmosphere is compared with $^{14}CO_2$ evolved by decomposition of uniformly ^{14}C-labeled rhizodeposits obtained from the same plants. The difference corresponds to RR. The labeling (as in the other methods) is necessary to avoid interference between RMR and respiration of microorganisms decomposing SOM.

Despite being simple in principle, the practical application of the method is complex. Besides the technical difficulties of continuous labeling, when compared to pulse labeling used in other approaches (see below), the greatest problem lies in the exact estimation of the initial amount of rhizodeposits released by the roots into the soil. The amount of rhizodeposits is not known *a priori*, as in many classical incubation studies, and will be estimated according the nondecomposed residue of rhizodeposits as ^{14}C activity remaining in soil after long-term incubation (61 weeks) of soil after the growth of continuously labeled plants (*Johansson*, 1992). This remaining ^{14}C activity is one of the parameters used to estimate the "degree of stabilization" of rhizodeposits:

$$\text{Degree of stabilization} = (C_{added} - C_{mineralized}) / C_{added} \cdot 100 \qquad \text{Eq. 3}$$

Since C_{added} (initial amount of rhizodeposits) – $C_{mineralized}$ = $C_{remained}$, the initial amount of rhizodeposits:

$$C_{added} = C_{remained} / \text{degree of stabilization} \cdot 100 \qquad \text{Eq. 4}$$

The degree of stabilization is not known, but it is to be proportional to the ratio between nonhydrolizable and hydrolizable C, which depends on the composition of the material. Therefore, the soil after incubation of the rhizodeposits and of some reference materials (uniformly ^{14}C-labeled finely ground shoots, roots, and glucose) will be hydrolyzed in the 12.5 M H_2SO_4 to obtain the amount of nonhydrolizable ^{14}C residue. This nonhydrolizable ^{14}C residue of rhizodeposits and of reference materials can then be compared with the total ^{14}C residue after the incubation by linear regression:

$$\text{Degree of stabilization (\%)} = k \cdot {}^{14}\text{C after hydrolysis} / {}^{14}\text{C before hydrolysis} \cdot 100 \qquad \text{Eq. 5}$$

where k is slope of the regression between total ^{14}C residue and nonhydrolizable ^{14}C after the incubation of reference materials of different composition.

Using this approach, *Johansson* (1992) found that RMR amounted to 32% and RR amounted to 68% of total rhizosphere respiration for 7 weeks after germination of meadow fescue (*Festuca pratensis* L.).

The main shortcomings of this approach are in the indirect assessment of decomposition of rhizodeposits and the very long incubation period (61 weeks). The approach is also laborious, since at least three reference substances should be incubated. Furthermore, the assumed linear relationship between ^{14}C remaining after the incubation to the ratio between acid hydrolyzed and nonhydrolized ^{14}C was proven only in *Johansson's* (1992) study. However, the hydrolyzed : nonhydrolized ratio showed that the decomposability of rhizodeposits is slightly less than that of glucose, but higher than that of shoots and much higher than root residues.

2.2.2 Isotope dilution

The isotope-dilution method is based on the addition of a solution of unlabeled glucose to the soil with growing plants that were pulse-labeled in a $^{14}CO_2$ atmosphere. The added unlabeled glucose dilutes the ^{14}C-labeled rhizodeposits (*Cheng* et al., 1993) (Fig. 1). The underlying assumption is that the dilution of ^{14}C by ^{12}C in the CO_2 originating from microbial respiration of rhizodeposits is proportional to the amount of unlabeled glucose added. Thus, only the microbial respiration of exudates is diluted, but the $^{14}CO_2$ evolved by RR remains constant. This principle is very similar to that used for SIR, but the glucose is added to plants labeled in a $^{14}CO_2$ atmosphere. Originally (*Cheng* et al., 1993), two glucose concentrations were used: 171 and 881 µg C (g soil)$^{-1}$.

In order to test the higher amount of added glucose, *Kuzyakov* (2002) suggested a function of evolved $^{14}CO_2$ depending on the amount of added glucose ($^{14}CO_2$% [Glucose]), using the parameter (*p*) in an exponential equation with constant RR:

$$^{14}CO_2\% \, (\text{Glucose}) = (100 - \text{RR}) \cdot \exp(-p \cdot \text{Glucose}) + \text{RR} \quad \text{Eq. 6}$$

where: RR is root respiration, Glucose is the concentration of added unlabeled glucose, *p* is a proportionality coefficient of decreasing specific activity of $^{14}CO_2$ (Fig. 1).

Some assumptions of this method were discussed by *Cheng* et al. (1993, 1994, 1996): (1) injection of glucose does not produce short-term effects on plant physiology other than diluting the root exudates, (2) glucose is compatible with root exudates in terms of substrate specificity, (3) adding glucose does not stimulate or suppress the microbial growth in the rhizosphere during the experiment for ~4 h (*Cheng* et al., 1993), (4) the dilution of $^{14}CO_2$ evolved from the soil shows a simple and proportional relationship to the amounts of added glucose. The last assumption enables calculating the ratio of root respiration to rhizomicrobial respiration. All these assumptions are acceptable because they have no effect on the separation results. However, one very important hidden assumption not discussed in the original paper must be considered (*Kuzyakov*, 2002): the ratio of ^{14}C in root respiration to ^{14}C in rhizomicrobial respiration is accepted as fixed during the experiment and is extrapolated for the whole period of rhizosphere respiration. Based on the $^{14}CO_2$ efflux, *Warembourg* and *Billes* (1979), *Nguyen* et al. (1999), and *Kuzyakov* et al. (1999, 2001) have indirectly demonstrated that this ratio changes during the ^{14}C chase after a ^{14}C pulse. A later study with exudate elution (see below) directly showed that the RR : RMR ratio changes during the light and dark phase (*Kuzyakov* and *Siniakina*, 2001).

A shortcoming of this method is that the measurements can be conducted only during a short period (about 4–5 h) after supplying the soil with glucose. After this lag-period, the microorganisms begin to grow exponentially (as in the substrate-induced-respiration method of microbial-biomass estimation; *Anderson* and *Domsch*, 1978; *Blagodatsky* et al., 2002), and the third and fourth assumptions can no longer be accepted. Peak growth depends on the amount of glucose added and should differ in the treatments with increasing amounts. At the low-addition levels, the glucose is insufficient for exponential growth.

Using this method, *Cheng* et al. (1993) found that RR of 3-week-old wheat plants accounts for about 41% of the root-derived CO_2, and RMR accounts for 59%.

2.2.3 Model-rhizodeposition technique

The model-rhizodeposition method is based on adding artificial ^{14}C-labeled rhizodeposits to the soil (*Swinnen*, 1994). The $^{14}CO_2$ efflux from this soil is then compared with the $^{14}CO_2$ efflux from plants labeled previously in a $^{14}CO_2$ atmosphere. The idea here is that the $^{14}CO_2$ efflux from the soil with labeled plants consists of the sum of RR and RMR, but that the $^{14}CO_2$ efflux from the soil with unlabeled plants and added labeled model rhizodeposits consists only of RMR. The calculation of RR and RMR by the model-rhizodeposition method assumes a constant ratio between microbially respired ^{14}C (^{14}C-MR) and ^{14}C remaining in the soil (^{14}C-Soil) in both the treatment with natural rhizodeposits and the treatment with model rhizodeposits (*Swinnen*, 1994):

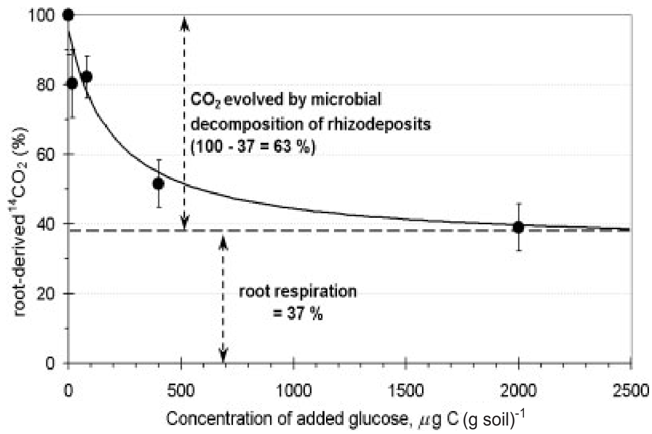

Figure 1: Principle of separation of root respiration and rhizomicrobial respiration using the isotope-dilution method (*Cheng* et al., 1993): dilution of $^{14}CO_2$ efflux (± SD) from soil with $^{12}CO_2$ coming from increased amounts of added unlabeled glucose has a limit corresponding to root respiration (from *Kuzyakov*, 2002).
Abbildung 1: Prinzip der Trennung von Wurzelatmung und rhizomikrobieller Atmung mit der Isotopen-Verdünnungsmethode (*Cheng* et al., 1993): Verdünnung des $^{14}CO_2$-Effluxes (± SD) aus dem Boden mit $^{12}CO_2$ aus der Veratmung von ansteigenden Mengen der zugegebenen unmarkierten Glukose erfolgt maximal bis zum Niveau der Wurzelatmung (aus *Kuzyakov*, 2002).

$$^{14}\text{C-MR}_C \,/\, ^{14}\text{C-Soil}_C = {}^{14}\text{C-MR}_{Glu} \,/\, ^{14}\text{C-Soil}_{Glu} \quad \text{Eq. 7}$$

where: ^{14}C-MR_C and ^{14}C-MR_{Glu} are ^{14}C activity of CO_2 evolved by microbial respiration from the soil of the control treatment with labeled plants and from the soil with added ^{14}C glucose, and ^{14}C-$Soil_C$ and ^{14}C-$Soil_{Glu}$ are ^{14}C activity remaining in soil residue in the treatment with labeled plants and in the treatment with added ^{14}C glucose.

Using this model-rhizodeposits method, *Swinnen* (1994) showed that the contribution of RR in 30 d old wheat and barley to the total root-derived CO_2 was between 89% and 95%; RMR contributed only 5%–11%. We suggest that microbial respiration was underestimated because of the following shortcomings of this method:

Many rhizosphere microorganisms are located directly on the rhizoplane at the exudation sites (*Grayston* and *Jones*, 1996) where the microbial activity in the rhizosphere is much higher when compared to the root-free soil. The organic substances released from roots are directly taken up by microorganisms and thus are practically not absorbed by clay minerals and SOM. By artificial addition of model rhizodeposits to soil, only a part falls into root-affected soil volume with the associated high microbial activity. An important part of model rhizodeposits remains in root-free soil and, therefore, their utilization by microorganisms as well as interactions with clay minerals and SOM is different compared to the rhizosphere.

Values of yield factor for microbial growth (Y) are variable depending on the physiological state of the microbial community, the availability of nutrients in the soil, and the quantity of the C source added (*Coody* et al., 1986; *Blagodatsky* et al., 1993, 2002; *Nguyen* and *Guckert*, 2001). The higher the concentration of C added, and the higher its availability, the lower the measured Y values (*Bremer* and *Kuikman*, 1994) and thus the higher the determined microbial contribution to CO_2 efflux by the model-rhizodeposition method (*Kuzyakov*, 2002).

Uptake of labeled C by roots and its translocation into shoots and respiration by aboveground plant parts was not taken into account. In the meantime, the phenomenon that roots may take up low-molecular organic substances (amino acids, sugars, organic acids) has become well known (*Jones*, 1998; *Jones* and *Darrah*, 1993, 1996). Even small absolute inputs of labeled C (either as model rhizodeposits or as the products of microbial metabolism, lysates of microbial cells, etc.) could decrease the apparent value of microbial respiration because the concentrations of model rhizodeposits used by *Swinnen* (1994) were as low as 1.2–12 $\mu g\ g^{-1}$. In other words, Eq. 4 works only if ^{14}C uptake by roots is equal to 0. However, the contribution of this shortcoming to the separation results is minor (see also 2.2.4).

2.2.4 Dynamics of $^{14}CO_2$ efflux

This method is based on modeling the dynamics of $^{14}CO_2$ efflux from soil after pulse labeling (*Kuzyakov* et al., 1999, 2001). It assumes that, after pulse labeling, the $^{14}CO_2$ evolved by RR appears earlier than that of RMR-derived $^{14}CO_2$ (Fig. 2). This delay reflects the time necessary for the synthesis of exudates, for the exudation and secretion processes, and for the uptake and utilization of rhizodeposits by microorganisms (*Warembourg* and *Billes*, 1979). However, this time delay was not introduced in the model artificially (*i.e.*, no time lags in the model), and the rates responsible for RR and root exudation are of the same order. This delay reflects the chain of successive transformation processes of released organic substances in the rhizosphere.

The following other assumptions were used in the C-flow model of the dynamics-separation method (*Kuzyakov* and *Domanski*, 2002): 1) the plant biomass does not significantly change during the whole $^{14}CO_2$-monitoring period until the end of C allocation after the ^{14}C pulse (~2–3 d), 2) the influence of plant growth on partitioning processes was omitted from the model (reverse transport of ^{14}C-labeled compounds from roots to shoots), 3) the model does not consider the diurnal changes in assimilation, translocation, and respiration activity, 4) all ^{14}C flows in the model are described by first-order kinetics. All these assumptions are used in developing the model and have no short-term effects (several days after ^{14}C-pulse labeling) on the separation results.

The $^{14}CO_2$-efflux rate from soil after pulse labeling is monitored for at least 7 d (Fig. 2). The model parameters responsible for the exudation rate and for root respiration intensity are fitted on the measured $^{14}CO_2$-efflux rate. The other parameters responsible for mineralization of roots and exudates as well as biomass respiration rates are taken from the literature. Based on the parameters fitted in the experiment, RR and RMR are separately simulated by the model and integrated to calculate the C amounts passed through RR and RMR. In the second version of the model (*Kuzyakov* and *Domanski*, 2002), all model parameters were fitted in a special ^{14}C-labeling experiment (*Domanski* et al., 2001).

The most important shortcomings of the approach based on the dynamics of $^{14}CO_2$ efflux are connected with the model and its assumptions. 1) The model (as with most other models) implies C flows between limited stated pools, and the rates are parameterized according to the ^{14}C dynamics in measurable pools: shoots, roots, microbial biomass, DOC, SOM, and CO_2. The goodness of fit of the model parameter and therefore of the subsequent simulation of RR and RMR strongly depends on the number of pools and flows considered. Additionally, some parameters may be interrelated. This results in uncertainty in the subsequent simulation of RR and RMR. 2) Although the model itself can be used for any plant, the labeling of large plants in a $^{14}CO_2$ atmosphere is infeasible. Therefore, the applicability of the approach is limited to smaller plants and laboratory conditions. 3) Diurnal dynamics of CO_2 efflux from soil (*Baldocchi* et al., 1986; *Kim* and *Verma*, 1992) and of $^{14}CO_2$ (*Kuzyakov* and *Cheng*, 2001), which is not considered in the model, may strongly affect the parameterization and subsequent simulation of RR and RMR.

In the original study conducted with *Lolium perenne* (*Kuzyakov* et al., 1999), the RR varied between 17% and 61% of

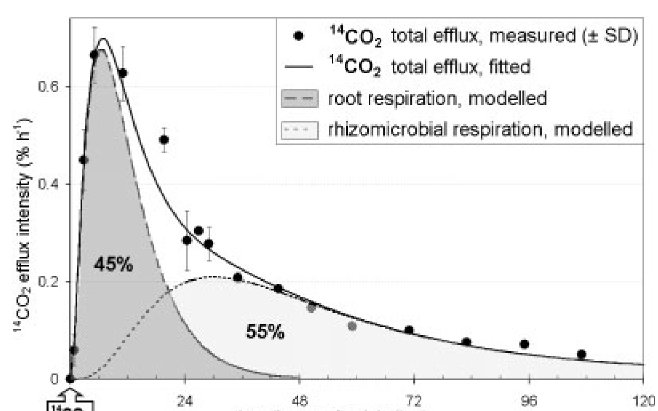

Figure 2: Principle of separation of root respiration and rhizomicrobial respiration using the $^{14}CO_2$-efflux-dynamics method (*Kuzyakov* et al., 1999, 2001; *Kuzyakov* and *Domanski*, 2002): $^{14}CO_2$ efflux (±SD) evolved by root respiration appears earlier than $^{14}CO_2$ respired by rhizomicrobial respiration (from *Kuzyakov*, 2002).

Abbildung 2: Prinzip der Trennung von Wurzelatmung und rhizomikrobieller Atmung mit der Methode der Modellierung der Dynamik des $^{14}CO_2$-Effluxes (*Kuzyakov* et al., 1999, 2001; *Kuzyakov* und *Domanski*, 2002): $^{14}CO_2$ (± SD), das bei der Wurzelatmung ausgeschieden wird, erscheint früher als $^{14}CO_2$, das durch die Rhizosphären-Mikroorganismen veratmet wird (aus *Kuzyakov*, 2002).

root-derived $^{14}CO_2$ depending on the plant-growth stage. On average, 41% were accounted by RR and 59% by RMR. Later experiments with *Lolium perenne* grown at two different N levels showed the average contribution of RR to be about 46% of root-derived CO_2 (*Kuzyakov* et al., 2001). The conclusion was that young roots have higher specific respiration rates and that total rhizodeposition strongly increases during the plant development. Using a similar approach, without modeling and under axenic conditions, *Warembourg* (1975) found that the ratio between RR and RMR for wheat and grass was about one.

2.2.5 Exudate elution

This method is based on the elution of exudates from soil before microorganisms can utilize them (*Kuzyakov* and *Siniakina*, 2001). Rooted soil is flushed by a water-air mixture and eluted exudates are collected in a flask separately from the alkali traps for CO_2. To separate root-derived organic substances as well as CO_2 of RR from soil-derived organic substances and CO_2 evolved by SOM decomposition, plants should be labeled in a $^{14}CO_2$ (or $^{13}CO_2$) atmosphere. Therefore, in this approach, the actual exudate elution is combined with plant labeling.

The first shortcoming of the method involves the limited elution of certain mucigels secreted by roots as well as of the ^{14}C incorporated in root hairs and sloughed root cells. However, *Merbach* et al. (1999) showed that up to 60%–80% of the root-borne organic compounds were water soluble. Similarly, *Jones* and *Darrah* (1993) found that, depending on the removal of nutrient solution, soluble low-molecular-weight exudates account for between 48% and 86% of root-derived organic compounds. Secondly, the exudates in this method are mainly eluted by preferential flow. The mean time for exudate elution by preferential flow is about 5–10 min. The elution time of organic substances exuded away from the water streams is longer, but is difficult to estimate. Thus, microorganisms can decompose some of the exudates during their transport from the root to the exudate collector, which contains Ag^+ for sterilization. The eluted organics therefore consist not only of the original exudates, but also include substances modified by microorganisms during elution. Thirdly, continuous water flow in the microcosm may change the amount and composition of the C released by the roots. *Jones* and *Darrah* (1993) reported up to 98% re-uptake of maize exudates in a sterile, static nutrient-solution culture. Using ^{14}C-labeled glucose, *Paterson* and *Sim* (1999) showed a 75% re-uptake of exudates by *Lolium perenne* roots in a sterile nutrient-solution culture. However, it is doubtful whether such re-uptake plays a significant role under non-sterilized soil conditions. Under field conditions, microorganisms on the root surface strongly compete with roots for exudates. In the exudate-elution system, the removal of exudates from roots by water flow has a similar effect as uptake by microorganisms. This method is better suited to sandy soils than to clay soils.

These shortcomings increase the ^{14}C in CO_2, thereby decreasing it in exudates. Therefore, the ^{14}C measured in eluted organic compounds is underestimated, and the ^{14}C in CO_2 is overestimated. This method therefore shows only the **minimal amount of water-soluble exudates** released from roots. The separation of root-derived CO_2 efflux from *Lolium* rhizosphere by this method showed that the ^{14}C in CO_2 (accepted as RR) accounted for 81% of root-derived CO_2 and the ^{14}C in eluted exudates (accepted as the amount which would be respired by microorganisms in the absence of elution ~RMR) for 19% (*Kuzyakov* and *Siniakina*, 2001).

Despite such shortcomings, one key advantage over the other tested methods deserves mention: The exudate-elution method is the only available technique allowing physical separation of different CO_2 sources (RR and decomposition of root exudates). Except for the component-integration method, all other methods are based on calculations and not on physical separation. Therefore, their results cannot be verified directly and remain hypotheses. This important advantage allowed the exudate-elution technique to be used to characterize the chemical composition of organic substances released by roots growing in nonsterilized soil (*Kuzyakov* et al., 2003; *Melnitchouck* et al., 2005).

2.2.6 $\delta^{13}C$ of microbial biomass and CO_2

Recently, a theoretical background for a new method based on ^{13}C natural abundance by growing C_4 plants on C_3 soil or *vice versa* was suggested (*Kuzyakov*, 2004). Four $\delta^{13}C$ values are necessary: that of the SOM (δ_3^{SOM}), of the roots (δ_4^{Rhiz}), of soil microbial biomass (δ^{MO}), and of CO_2 efflux (δ^{CO2}) from the soil:

$$RR = \frac{(\delta^{CO_2} - \delta^{MO}) \cdot (\delta_3^{SOM} - \delta_4^{Rhiz})}{(\delta_4^{Rhiz} - \delta^{MO}) \cdot (\delta_3^{SOM} - \delta^{CO_2})} \quad \text{Eq. 8}$$

$$RMR = \frac{(\delta_3^{SOM} - \delta^{MO}) \cdot (\delta_4^{Rhiz} - \delta^{CO_2})}{(\delta_4^{Rhiz} - \delta^{MO}) \cdot (\delta_3^{SOM} - \delta^{CO_2})} \quad \text{Eq. 9}$$

The new method is based on two assumptions concerning ^{13}C-isotopic discrimination during RR and microbial respiration:

The $\delta^{13}C$-isotope signature of CO_2 released as RR and of rhizodeposits C is the same as the $\delta^{13}C$ value of the roots. Up to now, this assumption was used in most rhizosphere-CO_2 studies. *Cheng* (1996) grew winter wheat on C-free vermiculite and a vermiculite-sand mixture and proved this assumption.

The $\delta^{13}C$-isotope signature of CO_2 respired by microorganisms corresponds with the $\delta^{13}C$ value of microbial biomass. This assumption was checked in the literature, but the results vary strongly. According to *Santruckova* et al. (2000), who measured the $\delta^{13}C$ of CO_2 respired from 21 Australian soils with C_3 and C_4 vegetation, the microbially respired CO_2 is depleted on average by 2.2‰ compared to $\delta^{13}C$ of microbial biomass. Similar $\delta^{13}C$ difference between SOM and microbial biomass was found by *Potthoff* et al. (2003). However, the $\delta^{13}C$ difference between microbial biomass and respired CO_2 varied between 0.1‰ and 7.7‰ (*Santruckova* et al., 2000). According to the principle of the ^{13}C-natural-abundance method suggested in the present study to separate CO_2

sources, the unconsidered isotopic effect (approximately ±1‰) during microbial decomposition of SOM to CO_2 results in an error of about 7% when calculating the contribution of C_3–C or C_4–C sources to the CO_2 efflux from soil. The differences between $\delta^{13}C$ of SOM and that of respired CO_2 was found to vary from –3.2‰ to +2.1‰ (references in *Santruckova* et al. (2000).

The first assumption can be checked by introducing one treatment with plants growing on a C-free substrate and measuring the $\delta^{13}C$ value of the CO_2 evolved from roots (*Cheng*, 1996). Measuring the $\delta^{13}C$ value of microbial biomass and of CO_2 from unplanted soil is necessary to prove the second assumption. If the isotopic effects are significant, they should be considered in the equations above. Importantly, these two assumptions are more realistic than the assumptions accepted by the four methods based on ^{14}C-pulse labeling described above for RR and RMR separation. Moreover, it is easy to check these assumptions in each experiment.

We conclude that despite some advantages of isotopic methods, they are based on sophisticated techniques and until now were only applied under laboratory conditions. The isotopic methods are mainly suitable for short-stature plants and for short-term studies and are unfeasible for bigger plants (*e.g.*, trees, shrubs, etc.). Except the last method based on $\delta^{13}C$ of microbial biomass and CO_2, which is not experimentally proven yet, all isotopic methods are mainly limited for laboratory conditions (Tab. 1).

2.3 Other methods

Beside the above-mentioned approaches to separate RR and RMR, some other methods claim to enable the estimation of RR and RMR (Tab. 1). Most of these methods are based on the exclusion of root contributions (RR+RMR) to the CO_2 efflux and comparison of CO_2 from this treatment to the CO_2 originated from planted untreated soil. All these methods are actually based on indirect estimations and belong to different variations of the difference or root-exclusion method.

2.3.1 Difference method or root-exclusion method

The root-exclusion method is based on the comparison (difference) of total CO_2 efflux from rooted and root-free soil (*Hall* et al., 1990; *Hanson* et al., 2000). This method was also used to separate the CO_2 efflux under trees (*Edwards* and *Norby*, 1998). We mention this method here only because in many (earlier) studies, the difference between these fluxes was accepted as "root respiration". Clearly, the exclusion of roots removes not only RR but also RMR. Additionally, the presence of rhizodeposits of living roots may induce rhizosphere priming effects (RPE) (*Dormaar*, 1990; *Cheng* and *Kuzyakov*, 2005)—additional CO_2 evolved by SOM decomposition due to higher microbial activity in the rhizosphere compared to root-free soil. Based on the absence of real separation of RR and RMR, as well as the possibility of RPE, we cannot accept this method as one allowing the separation of respiration by autotrophic and heterotrophic organisms.

2.3.2 Trenching

The trenching method is based on cutting of roots in a soil volume and subsequent comparison of total CO_2 efflux from nontrenched and trenched plots (*Ewel* et al., 1987; *Bowden* et al., 1993). Root in-growth should be inhibited after trenching (*Son* and *Kim*, 1996; *Buchmann*, 2000). Trenching is one of the most frequently used methods to separate CO_2 flows under forest. This method has shortcomings similar to those of the root-exclusion method and, here as well, the difference was frequently termed "root respiration" (*Epron* et al., 1999; *Lee* et al., 2003). One advantage over the root-exclusion method is that trenched plots contain dying roots, which will be decomposed with CO_2 release. However, decomposition CO_2 should be corrected for, *e.g.*, by the buried-root-bag method (*Epron* et al., 1999, 2001; *Lee* et al., 2003). Also, dying roots with an absence of water uptake lead to changed environmental conditions, especially to an increase in soil moisture (*Fisher* and *Gosz*, 1986; *Staples* et al., 2001; *Ross* et al., 2001), decreases in extractable C, microbial C and N (*Ross* et al., 2001), and increased net N mineralization (Fisher and Gosz, 1986).

2.3.3 Shading and clipping

The other two methods frequently used to separate CO_2 efflux are shading of plants or clipping of the aboveground plant parts in grasslands and clear-cutting in forests. These methods are based on stopping leaf photosynthesis and therefore excluding new assimilate transport to the roots. The disadvantages of these methods are similar to those of trenching and root exclusion: they do not separate the actual RR and RMR. In grassland dominated by *Schizachyrium scoparium,* 2 d of shading reduced the soil CO_2 flux by 40%, while clipping led to a 19% reduction (*Craine* et al., 1999). The reduction of the CO_2 efflux from the soil after clipping corresponded with the CO_2 efflux from excised roots (which is RR).

Gap formation, *i.e.*, the removal of aboveground vegetation in a large forest area, reduced CO_2 emission from the soil surface by 40%–50% (*Brumme*, 1995; *Nakane* et al, 1996). This decrease was attributed to the root contribution (root-derived CO_2), but up to 20% of the remaining flux were produced by the decomposition of roots that died after gap preparation (*i.e.*, forest clear-cutting).

Also, a study by *Kuzyakov* and *Cheng* (2001) showed a strong reduction of the CO_2 efflux of about one-third to one-half after 2 d and 4 d shading, respectively. However, the $\delta^{13}C$ clearly showed that, despite this drop, more than half of the total CO_2 efflux originated from wheat roots (the sum of RR and RMR) and not from SOM. Shading alone is therefore insufficient to separate RR and RMR.

2.3.4 Tree-girdling method

Most of the above-described separation methods are useless for forest soils because of very large above- and belowground tree biomass. Recently, tree girdling was used to estimate CO_2 fluxes originating from SOM and root-derived CO_2

in forests (*Keutgen* and *Huysamer*, 1998; *Högberg* et al., 2001). The girdling of phloem interrupts the flow of assimilates from leaves to the roots. Shortly after girdling, the interrupted flow leads to exclusion of RR and RMR. This technique was also used in earlier studies to investigate C flow in the rhizosphere of legumes for N_2 fixation (*Walsh* et al., 1987; *Vessey* et al., 1988).

Compared to the root-exclusion method, the main advantage of girdling is that the moisture and temperature of the soil under girdled trees remains similar to nongirdled trees. Therefore, the method does not affect the CO_2 efflux (at least for 1–2 months). One important problem does remain unsolved: if the rhizodeposition including root exudation decreases, then the additional decomposition of SOM in the rhizosphere (rhizosphere priming effect) also declines very quickly. In the original paper as well as in subsequent studies, girdling was proposed as a method allowing the respiration by autotrophic and heterotrophic organisms to be separated (*Högberg* et al., 2001; *Bhupinderpal* et al., 2003). This, however, is misleading because it does not allow the separation of RR (the only autotrophic source of respiration in soil) from RMR (respiration by heterotrophs). As the assimilate transport into the roots is interrupted, both sources of root-derived CO_2 decrease strongly.

We conclude that the following methods—root exclusion, trenching, shading, clipping, and girdling—are various treatments of the difference approach and consequently have similar shortcomings as the difference approach. These methods can be acceptable for estimating the SOM-derived and root-derived CO_2—if the absence of RPE is assumed (Tab. 1). However, because of the short-term link between plant photosynthesis and rhizodeposition (*Hodge* et al., 1997; *Craine* 1999; *Kuzyakov* and *Cheng*, 2001), all of these methods are unacceptable for evaluating RR and therefore cannot be used to estimate the respiration by autotrophic organisms. Additionally, root exclusion, trenching, shading, and clipping lead to changed environmental conditions, especially to an increase of soil moisture (*Staples* et al., 2001; *Ross* et al., 2001) and to decreases in extractable C, microbial C and N (*Ross* et al., 2001).

3 Combination of methods and comparison of results observed by different methods

3.1 Combination of methods

Presenting nonisotopic methods (sections 2.1 and 2.3), we mentioned that some of these methods cannot separate root-derived CO_2 into actual RR and RMR (difference methods), and other methods (component integration, excised roots, and SIR) cannot separate RMR from MR. It means that in these approaches, RMR remains as nonseparated subpool of root-derived CO_2 **or** MR. This fact was used in several studies to assess RMR by combination of two methods.

Larionova et al. (2005) combined **component-integration and root-exclusion methods** to estimate RR and RMR of different crops under field conditions (Fig. 3). The root-derived CO_2 was measured as the difference between the CO_2 efflux from cropped and bare soil (actually RR + RMR) and was much higher than the RR determined by incubation in the component-integration method. The difference between the obtained root-derived CO_2 and RR values constitutes a rough assessment of RMR, which for maize, barley, and buckwheat comprised respectively of 28%, 15%, and 68% of total CO_2 efflux from the soil surface or 40%, 39%, and 77% of root-derived CO_2 (Tab. 2) (*Larionova* et al., 2005). These values clearly show the significance of contribution of rhizodeposits to the annual CO_2 efflux from soil.

Interesting comparative results for grassland dominated by *Schizachyrium scoparium* were observed by *Craine* et al. (1999), who investigated sources of CO_2 efflux from soil by **shading, clipping, and incubation of excised roots**. Two days of shading caused a 40% drop in soil CO_2 flux, while clipping led to a 19% reduction (*Craine* et al., 1999). Thus, shading (absence of photosynthesis) results in a 2-fold stronger decline than clipping the shoots 2 cm above the soil surface. The same study revealed that the reduced CO_2 efflux from the soil after clipping corresponds with the CO_2 efflux from excised roots (equaling RR). Should this correspondence be supported in the further studies with other plants, it might give rise to an easy field method of estimating RR.

Kelting et al. (1998) combined **trenching with the excised-roots method** to partition the total CO_2 efflux in forest with *Quercus rubra.* They showed that root-derived CO_2 in

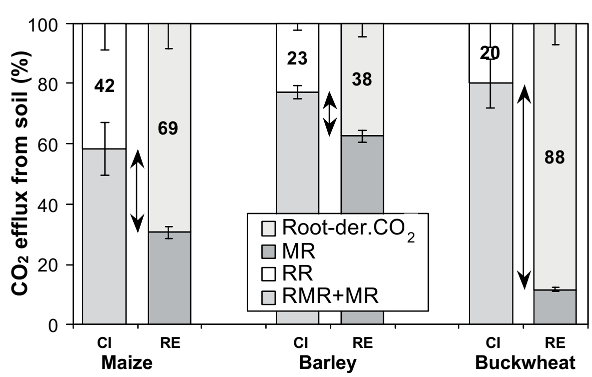

Figure 3: The share of root respiration (RR) and microbial respiration (MR) to the annual CO_2 efflux from soil surface obtained by combination of component-integration (CI) and root-exclusion (RE) methods. Bars indicate SD (from *Larionova* et al., 2005, modified). RR: root respiration, RMR: rhizomicrobial respiration, MR: microbial respiration of SOM, Root-der. CO_2: root-derived CO_2 (= RR+RMR). ↕: RMR calculated as difference between the sum of MR + RMR obtained by component-integration and MR obtained by root-exclusion method.

Abbildung 3: Anteile der Wurzelatmung (RR) und der mikrobiellen Atmung (MR) am jährlichen CO_2-Efflux (± SD) von der Bodenoberfläche als Ergebnis der Kombination von Komponenten-Integrationsmethode (CI) und Wurzelausschlussmethode (RE) (aus *Larionova* et al., 2005, geändert). RR: Wurzelatmung, RMR: rhizomikrobielle Atmung, MR: mikrobielle Veratmung der organischen Bodensubstanz, Root-der.CO_2: wurzelbürtiges CO_2 (= RR+RMR). ↕: RMR rhizomikrobielle Atmung berechnet als Differenz der Summe der MR + RMR aus der Komponenten-Integrationsmethode und der MR aus der Wurzelausschlussmethode.

Table 2: Results (± SE) of studies which combined or compared different methods for estimation of root respiration (RR) and rhizomicrobial respiration (RMR).
Tabelle 2: Ergebnisse (± SE) von Untersuchungen mit Kombination oder Vergleich von Methoden zur Trennung der Wurzelatmung (RR) und rhizomikrobiellen Atmung (RMR).

Plant	Field/ Lab	Method	RR (%)*	RMR (%)*	Reference
		Combination of methods			
Festuca pratensis	Lab	Difference: Root-derived $^{14}CO_2$ – RMR-$^{14}CO_2$	68	32	*Johansson*, 1992
Quercus rubra **	Field	Combination of trenching with excised roots	62	38	*Kelting* et al., 1998
Prairie grasses, predominant *Schizachyrium scoparium* (Michx.)	Field	Combination of excised roots with shading and clipping	48	53	*Craine* et al., 1999
Maize, integrated for growth season	Field	Combination of root exclusion with component integration	60±8	40±8	*Larionova* et al., 2005
Spring barley, integrated for growth season			61±3	39±3	
Buckwheat, integrated for growth season			23±10	77±10	
		Comparison of methods			
Lolium perenne, 2 months old	Lab	Isotopic dilution	37	63	*Kuzyakov*, 2002
		Model rhizodeposits	71–83±2	17–29±2	
		$^{14}CO_2$ dynamics	45	55	
		Exudate elution	81±3	19±3	
Maize, 1.5 months old	Lab	Component integration	44± 9	56±17	*Sapronov* and *Kuzyakov*, 2004
		$^{14}CO_2$ dynamics	44±13	56±11	
Average* ± SE******			**48± 5**	**52± 5**	

* the sum of RR and RMR is equal to 100%. The contribution of SOM, dead roots, and aboveground litter is not included here.
** for the calculation of the average values of RR and RMR, the results for *Quercus rubra* were not considered because of very different root physiology compared to grasses (all other studies).
*** for the calculation of the average values of RR and RMR, the results obtained by model-rhizodeposits and exudate-elution methods were not considered because of important underestimation of RMR by these methods (see text).
**** for the calculation of SE, the average values of RR and RMR were used; the error of individual values was not considered.

trenched plots comprised 52% of total CO_2 efflux, while the respiration of excised roots amounted to 32%. Hence, RMR contributed to 20% of total CO_2 efflux from soil or 38% of root-derived CO_2. Therefore, the RR amounts to 62% of root-derived CO_2.

These three studies (*Kelting* et al., 1998; *Craine* et al., 1999; *Larionova* et al., 2005) clearly showed that the partitioning of total CO_2 efflux from soil into three sources: SOM-derived CO_2, RR, and RMR, and quantitative estimation of RR and RMR is possible under field conditions and can be achieved by a combination of methods. The results demonstrated the significance of rhizodeposition as a source for CO_2 efflux under field conditions. The combination of methods and calculation of RMR by the difference between two CO_2 fluxes is an assessment needing further improvement. This can be done by comparison of results obtained by different methods under the same experimental conditions and by use of isotopic approaches.

3.2 Comparison of results obtained by different methods

Despite the urgent necessity, only a few studies have compared the methods designed to separate RR and RMR. To our knowledge, only one study focused on comparing the isotopic methods based on the pulse labeling of shoots in $^{14}CO_2$ atmosphere and subsequent tracing of ^{14}C evolved from the soil (*Kuzyakov*, 2002). Four methods, 1) the isotope-dilution method, 2) the model-rhizodeposition technique, 3) modeling of $^{14}CO_2$-efflux dynamics, and 4) the exudate-elution procedure, were compared under the same experimental conditions: *Lolium perenne* was grown on a loamy Haplic Luvisol for 2 months under 27°C/22°C day/night temperature. The comparison showed (Fig. 4) that, despite different assumptions and principals, the isotope dilution and the $^{14}CO_2$-dynamics methods resulted in a similar level of RR, 37% and 45%, respectively, of total root-derived CO_2 efflux. The remaining 63% and 55% were accepted as RMR. The exudate-elution method, which underestimates total rhizodeposition, showed that at least 19% of the root-derived CO_2 was produced by exudate decomposition. The MR of rhizodeposits calculated using the model-rhizodeposition technique (17%–29%) was also underestimated. Considering the

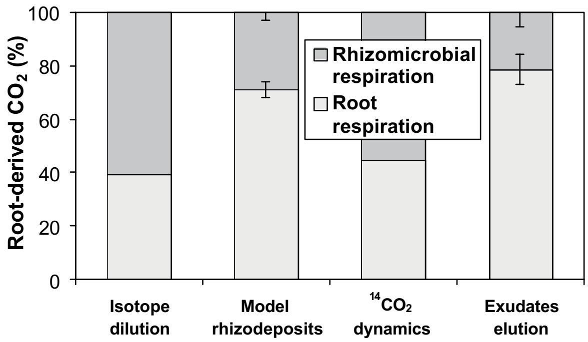

Figure 4: Comparison of the results obtained by four isotopic (^{14}C) methods separating RR and RMR of 64 d old *Lolium perenne* grown in nonsterile loamy Haplic Luvisol: 1) isotope dilution, 2) model rhizodeposits, 3) $^{14}CO_2$ efflux dynamics, and 4) exudate elution. Mean values ± SD are shown (from *Kuzyakov*, 2002).

Abbildung 4: Vergleich der Ergebnisse (± SD) aus der Anwendung von vier Isotopenmethoden (^{14}C) zur Trennung der Wurzelatmung und der rhizomikrobiellen Atmung am Beispiel des 64 Tage alten *Lolium perenne* auf nicht sterilem lehmigen Haplic Luvisol: 1) Isotopenverdünnung, 2) Modell-Rhizodeposition, 3) Dynamik des $^{14}CO_2$-Effluxes und 4) Auswaschung der Exsudate (aus *Kuzyakov*, 2002).

underestimation of RMR by the model-rhizodeposition technique and by the exudate-elution method, it was concluded that RR of *Lolium* contributes about 40%–45% to the root-derived CO_2 efflux (*Kuzyakov*, 2002). The remaining 55%–60% comprises of the microbial decomposition of root exudates and other rhizodeposits. It was also concluded that the longer the monitoring period of the CO_2 efflux after the pulse labeling is, the higher the contribution of RMR to the total root-derived CO_2 efflux from soil.

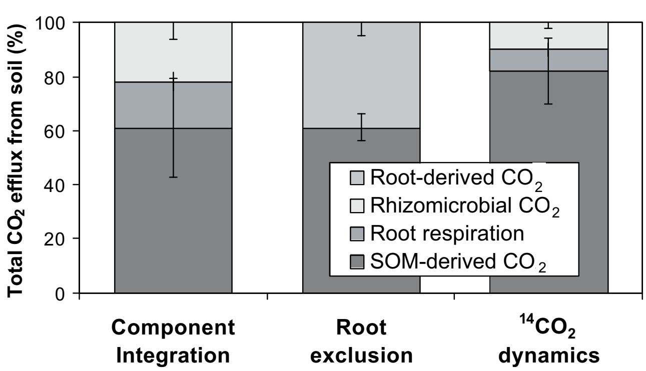

Figure 5: Comparison of the results of separation of RR and RMR of maize grown on nonsterile loamy Haplic Luvisol obtained by three methods: 1) component integration, 2) root exclusion, and 3) $^{14}CO_2$ dynamics after ^{14}C-pulse labeling (from *Sapronov* and *Kuzyakov*, 2004, modified).

Abbildung 5: Vergleich der Ergebnisse der Trennung von Wurzelatmung und rhizomikrobieller Atmung am Beispiel von Mais auf nicht sterilem lehmigen Haplic Luvisol aus drei Methoden: 1) Komponentenintegration, 2) Wurzelausschlussmethode und 3) Dynamik des $^{14}CO_2$-Effluxes nach ^{14}C-Pulsmarkierung (aus *Sapronov* und *Kuzyakov*, 2004, verändert).

By cultivating maize on a loamy Haplic Luvisol, *Sapronov* and *Kuzyakov* (2004) compared RR, RMR as well as SOM-derived CO_2 by three methods: 1) component integration, 2) root exclusion, and 3) $^{14}CO_2$ dynamics after ^{14}C-pulse labeling. Even though component integration and $^{14}CO_2$ dynamics showed very similar results for the separation of root-derived CO_2 (44% for RR and 56% for RMR; Fig. 5), the estimation of the actual root-derived CO_2 was different in comparison with the results of the root-exclusion technique, the root-derived CO_2 estimated by component integration was 59%–61% of the total efflux. However, the root-derived CO_2 estimated by ^{14}C was only 18% (Fig. 5). This underestimation probably reflects the noneven distribution of ^{14}C in plant tissues after pulse labeling and shows an important shortcoming of pulse labeling for estimation of belowground C flows including RR and RMR.

4 Conclusions

The analysis of different nonisotopic and isotopic methods as well as their comparisons under the same experimental conditions showed that the methods themselves are the main cause for the different RR and RMR estimates obtained in the original studies. This reflects the very different principles behind the approaches as well as their obvious and hidden assumptions. This calls for urgent experimental proof of the principles and assumptions of existing methods. Additionally, the elaboration of new approaches could improve the separate estimation of RR and RMR. Beside future development of isotopic methods mentioned above, simple approaches useful for field studies as well as for C-balance estimations are urgently required. For C-balance assessments, the ratio of RR and RMR as well as the amount of rhizodeposits typical for agricultural, grassland, and forest plants estimated by isotopic methods would be very helpful. We showed that separate estimation of RR and RMR can be done under field conditions by a combination of methods. We envisage that rapid and different responses of RR and RMR to different environmental variables such as PAR, temperature, and moisture could be the next clue for separate estimation of RR and RMR that may be useful also for field studies. Furthermore, the comparison of root-derived CO_2 efflux from plants grown on soils with different nutrient level (mainly N and P), as well as mycorrhized and nonmycorrhized plant species may help for evaluation of RR and RMR.

Based on the results obtained by combining and comparing different methods separating RR and RMR in young cereal plants, we conclude that their RR amounts to about 48% of root-derived CO_2 and RMR to 52% (Tab. 2). These values of RMR show that the rhizodeposition and its contribution to the CO_2 efflux from the soil should not be neglected. However, these very preliminary values need to be proven in future studies on wider range of agricultural, grassland, and forest plants.

Acknowledgments

This paper was prepared with financial assistance provided by the *German Research Council* (DFG) (to Yakov Kuzyakov) and by *Russian Foundation for Basic Researches* (RFFI) and

Russian Ministry of Science and Education (to Alla A. Larionova). The authors are greatly thankful to *Elizabeth Boddy* for linguistic corrections.

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