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

Yakov Kuzyakov1 and Alla A. Larionova2

1 Institute of Soil Science and Land Evaluation, Hohenheim University, D-70593 Stuttgart, Germany
2 Institute of Physico-Chemical and Biological Problems of Soil Science, Puschino, Russia

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Summary—Zusammenfassung

Partitioning the root-derived CO2 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 14CO2 with rhizomicrobial 14CO2 after continuous labeling, 5) isotope dilution, 6) model-rhizodeposition technique, 7) modeling of 14CO2 efflux dynamics, 8) exudate elution, and 9) δ13C of CO2 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 CO2 efflux from different sources. Four of the methods (5 to 8) are based on the pulse labeling of shoots in a 14CO2 atmosphere and subsequent monitoring of 14CO2 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 14CO2-efflux dynamics yield similar results. In crops and grasses (wheat, ryegrass, barley, buckwheat, maize, meadow fescue, prairie grasses), RR amounts on average to ±5% and RMR to 52±5% of root-derived CO2. The method based on the 13C isotopic signature of CO2 and microbial biomass is the most promising approach, especially when the plants are continuously labeled in 13CO2 or 14CO2 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 CO2 / partitioning methods / 14C, δ13C, 13C natural abundance / autotrophic respiration / heterotrophic respiration

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


1 Introduction

1.1 Why we need separate evaluation of root and rhizomicrobial respiration

After photosynthesis, CO₂ 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- CO₂ annually (Raich et al., 2002). This is more than 11 times the current rate of industrial CO₂ emissions produced by fossil-fuel combustion (Marland et al., 2001). The realization that soils are a potential source of atmospheric CO₂ has given rise to numerous methods to quantify this input. Subsequently, the total CO₂ efflux from soil has been measured in ecosystems all over the world. In contrast to other greenhouse gases (CH₄, N₂O), values for total CO₂ efflux from soil do not provide sufficient information to determine whether the soil is a net source or net sink for atmospheric CO₂. This uncertainty is connected with a specific feature of C turnover and CO₂ efflux from soil: the total amount of CO₂ 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 CO₂ 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 CO₂ efflux to determine whether the soil is a net source or a net sink of CO₂. This fact is reflected in the great amount of research designed to evaluate the contribution of different C sources to the total CO₂ efflux from soil (for forest ecosystems reviewed by Hanson et al., 2000). The most progress in the partitioning of CO₂-efflux sources was achieved in separating the CO₂ evolved by microbial decomposition of SOM from root-derived CO₂. This separation is crucial to estimate the decomposition rates of SOM and its contribution to the total CO₂ efflux. This achievement was mainly based on plant labeling in a ¹⁴CO₂ or ¹³CO₂ atmosphere or on the use of differences in the natural abundance of ¹³C in plants having C₃ or C₄ photosynthetic pathways (reviewed by Hanson et al., 2000; Kuzyakov, 2005). Different methods allowing for the separate estimation of SOM-derived and root-derived CO₂ 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 CO₂ sources is to differentiate the root-derived CO₂ 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, CO₂ 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 CO₂ 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 CO₂ 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 CO₂ respired by heterotrophs decomposing rhizodeposits are very frequently measured (and accepted) as part of root (auto- trophic) 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 underesti-
mation of heterotrophic component of CO₂ 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 CO₂ 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 CO₂ 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 photosynthetically 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 CO₂ efflux from different components, 4) successfully model CO₂ fluxes from soil, and 5) comprehend how the plant-soil system functions as a whole.

1.2 Definitions

The definitions of sources of CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂" (in contrast to SOM-derived CO₂) is used to describe the sum of RR and RMR. The terms “rhizosphere CO₂” 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 CO₂ production**—not to the pool of C from which the CO₂ originates or to the agents of CO₂ production. Considering the location of CO₂ production, “rhizosphere respiration” should include not only RR and RMR, but also CO₂ 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 CO₂ 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 heterotrophs (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 rhizosphere 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
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 CO₂ evolved from root cells (root respiration) from the CO₂ 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) underline that: “Discriminating between CO₂ 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”.

More recently, efforts have been made to divide root-derived CO₂ (the sum of RR and RMR) into CO₂ 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 CO₂ 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; Groller 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 CO₂; 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 CO₂ 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:

\[
\%RR = \frac{sR_R \cdot M_R}{(sR_R \cdot M_R + sR_S \cdot M_S + sR_L \cdot M_L)} \cdot 100 \quad \text{Eq. 1}
\]

Where \(sR_R\), \(sR_S\), and \(sR_L\) are specific respiration rates of roots, soil, and organic residues, respectively, and \(M_R\), \(M_S\), and \(M_L\) 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; Groller 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 CO₂ efflux from soil and the specific CO₂ 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.


Table 1: Methods suggested for separation of root and rhizomicrobial respiration, their background, suitability for partitioning of different CO\(_2\) fluxes and applicability.

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\(^1\) CO\(_2\) fluxes partitioned by the method: RR/RMR 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


\(^4\) 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 autotrophic and heterotrophic organisms; only selected references for difference methods are presented.

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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 CO₂ 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 CO₂ efflux is measured before and after glucose addition, enabling the respiration activity of roots, and microorganisms is calculated from the following equation system:

\[
\begin{align*}
R_1 &= RR + MR_1 \\
R_2 &= RR + k \cdot MR_1 \\
k &= MR_2 / MR_1
\end{align*}
\]  
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 Högborg (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 CO₂ production. The second reason for the drop in \(k\) is that the evolved CO₂ dissolves in the soil water and decreases the CO₂ 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 CO₂ 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 Högborg (2000). They applied C₄ sugar (instead of glucose) to a soil developed under C₃ vegetation and measured total CO₂ efflux and its δ¹³C value. The δ¹³C value allowed partitioning of the CO₂ evolved from the endogenous C₃ sources and added C₄ sugar. Also the estimation of the magnification factor (\(k\) in Eq. 2) is more precise by separating C₃ and C₄ 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 C₄ sugar and its application under field conditions was shown (Ekblad and Högborg, 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 CO₂ 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 Q₁₀ 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 14C (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 CO₂ 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 14CO₂ with 14CO₂ evolved by decomposition of 14C-labeled rhizodeposits

The principle of the method suggested by Johansson (1992) looks simple: root-derived 14CO₂ evolved from the rhizosphere of plants continuously labeled in the 14CO₂ atmosphere is compared with 14CO₂ evolved by decomposition of uniformly 14C-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 14C activity remaining in soil after long-term incubation (61 weeks) of soil after the growth of continuously labeled plants (Johansson, 1992). This remaining 14C activity is one of the parameters used to estimate the “degree of stabilization” of rhizodeposits:

\[
\text{Degree of stabilization} = \frac{C_{\text{added}} - C_{\text{mineralized}}}{C_{\text{added}}} \times 100 \quad \text{Eq. 3}
\]

Since \( C_{\text{added}} \) (initial amount of rhizodeposits) – \( C_{\text{mineralized}} \) = \( C_{\text{remaining}} \) (the initial amount of rhizodeposits):

\[
C_{\text{added}} = C_{\text{remaining}} / \text{degree of stabilization} \times 100 \quad \text{Eq. 4}
\]

The degree of stabilization is not known, but it is to be proportional to the ratio between nonhydrolyzable and hydrolyzable C, which depends on the composition of the material. Therefore, the soil after incubation of the rhizodeposits and of some reference materials (uniformly 14C-labeled finely ground shoots, roots, and glucose) will be hydrolyzed in the 12.5 M H₂SO₄ to obtain the amount of nonhydrolyzable 14C residue. This nonhydrolyzable 14C residue of rhizodeposits and of reference materials can then be compared with the total 14C residue after the incubation by linear regression:

\[
\text{Degree of stabilization (\%) } = k \cdot \frac{14\text{C after hydrolysis}}{14\text{C before hydrolysis}} \times 100 \quad \text{Eq. 5}
\]

where \( k \) is slope of the regression between total 14C residue and nonhydrolyzable 14C 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 14C remaining after the incubation to the ratio between acid hydrolyzed and nonhydrolyzed 14C was proven only in Johansson’s (1992) study. However, the hydrolyzed : nonhydrolyzed 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 unlabeled glucose to the soil with growing plants that were pulse-labeled in a 14CO₂ atmosphere. The added unlabeled glucose dilutes the 13C-labeled rhizodeposits (Cheng et al., 1993) (Fig. 1). The underlying assumption is that the dilution of 14C by 12C in the CO₂ 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 14CO₂ 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 14CO₂ 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:

\[
^{14}\text{CO}_2\% \text{(Glucose)} = (100 - RR) \cdot \exp(-p \cdot \text{Glucose}) + RR \quad \text{Eq. 6}
\]

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 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):

\[
^{14}\text{C-MR}_{\text{Soil}} / ^{14}\text{C-Soil}_{\text{Soil}} = ^{14}\text{C-MR}_{\text{Glucose}} / ^{14}\text{C-Soil}_{\text{Glucose}} \quad \text{Eq. 7}
\]

where: \(^{14}\)C-MR\(_{\text{Soil}}\) and \(^{14}\)C-MR\(_{\text{Glucose}}\), 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\(_{\text{Soil}}\) and \(^{14}\)C-Soil\(_{\text{Glucose}}\) 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 CO₂ 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 μg g⁻¹. In other words, Eq. 4 works only if ¹⁴C 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 ¹⁴C₀₂ efflux

This method is based on modeling the dynamics of ¹⁴C₀₂ efflux from soil after pulse labeling (Kuzyakov et al., 1999, 2001). It assumes that, after pulse labeling, the ¹⁴C₀₂ evolved by RR appears earlier than that of RMR-derived ¹⁴C₀₂ (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 ¹⁴C₀₂-monitoring period until the end of C allocation after the ¹⁴C pulse (~2–3 d), 2) the influence of plant growth on partitioning processes was omitted from the model (reverse transport of ¹⁴C-labeled compounds from roots to shoots), 3) the model does not consider the diurnal changes in assimilation, translocation, and respiration activity, 4) all ¹⁴C 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 ¹⁴C-pulse labeling) on the separation results.

The ¹⁴C₀₂-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 ¹⁴C₀₂-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 ¹⁴C-labeling experiment (Domanski et al., 2001).

The most important shortcomings of the approach based on the dynamics of ¹⁴C₀₂ 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 ¹⁴C dynamics in measurable pools: shoots, roots, microbial biomass, DOC, SOM, and CO₂. 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 ¹⁴C₀₂ atmosphere is infeasible. Therefore, the applicability of the approach is limited to smaller plants and laboratory conditions. 3) Diurnal dynamics of CO₂ efflux from soil (Baldochchi et al., 1986; Kim and Verma, 1992) and of ¹⁴CO₂ (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
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 Sinia- kina, 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 mucigel 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; Melnitchouk 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 ($\delta^{13}$SOM), of the roots ($\delta^{13}$Rhiz), of soil microbial biomass ($\delta^{13}$MO), and of CO$_2$ efflux ($\delta^{13}$CO$_2$) from the soil:

$$RR = \left(\frac{\delta^{13}CO_2 - \delta^{13}MO}{\delta^{13}MO - \delta^{13}Rhiz}\right) \cdot \left(\frac{\delta^{13}SOM - \delta^{13}Rhiz}{\delta^{13}Rhiz - \delta^{13}CO_2}\right)$$  \hspace{1cm} (Eq. 8)

$$RMR = \left(\frac{\delta^{13}SOM - \delta^{13}MO}{\delta^{13}MO - \delta^{13}Rhiz}\right) \cdot \left(\frac{\delta^{13}Rhiz - \delta^{13}CO_2}{\delta^{13}Rhiz - \delta^{13}CO_2}\right)$$  \hspace{1cm} (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 microbial 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 CO₂ results in an error of about 7% when calculating the contribution of C₃–C or C₄–C sources to the CO₂ efflux from soil. The differences between δ¹³C of SOM and that of respired CO₂ 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 δ¹³C value of the CO₂ evolved from roots (Cheng, 1996). Measuring the δ¹³C value of microbial biomass and of CO₂ 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 ¹⁴C-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 unsuitable for bigger plants (e.g., trees, shrubs, etc.). Except the last method based on δ¹³C of microbial biomass and CO₂, 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 CO₂ efflux and comparison of CO₂ from this treatment to the CO₂ 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 CO₂ efflux from rooted and root-free soil (Hall et al., 1990; Hanison et al., 2000). This method was also used to separate the CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ release. However, decomposition CO₂ 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 CO₂ 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 CO₂ flux by 40%, while clipping led to a 19% reduction (Craine et al., 1999). The reduction of the CO₂ efflux from the soil after clipping corresponded with the CO₂ efflux from excised roots (which is RR).

Gap formation, i.e., the removal of aboveground vegetation in a large forest area, reduced CO₂ emission from the soil surface by 40%–50% (Brumme, 1995; Nakane et al, 1996). This decrease was attributed to the root contribution (root-derived CO₂), 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 CO₂ efflux of about one-third to one-half after 2 d and 4 d shading, respectively. However, the δ¹³C clearly showed that, despite this drop, more than half of the total CO₂ 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 CO₂ fluxes originating from SOM and root-derived CO₂.
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 N₂ 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 CO₂ 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 transported into the roots is interrupted, both sources of root-derived CO₂ 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 CO₂—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 CO₂ into actual RR and RMR (difference methods), and other methods (component integration, excised roots, and SIIR) cannot separate RMR from MR. It means that in these approaches, RMR remains as nonseparated subpool of root-derived CO₂ 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 CO₂ was measured as the difference between the CO₂ 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 CO₂ and RR values constitutes a rough assessment of RMR, which for maize, barley, and buckwheat comprised respectively of 28%, 15%, and 68% of total CO₂ efflux from the soil surface or 40%, 39%, and 77% of root-derived CO₂ (Tab. 2) (Larionova et al., 2005). These values clearly show the significance of contribution of rhizodeposits to the annual CO₂ efflux from soil.

Interesting comparative results for grassland dominated by Schizachyrium scoparium were observed by Craine et al. (1999), who investigated sources of CO₂ efflux from soil by shading, clipping, and incubation of excised roots. Two days of shading caused a 40% drop in soil CO₂ 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 CO₂ efflux from the soil after clipping corresponds with the CO₂ 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 CO₂ efflux in forest with Quercus rubra. They showed that root-derived CO₂ in

![Figure 3: The share of root respiration (RR) and microbial respiration (MR) to the annual CO₂ 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. CO₂: root-derived CO₂ (= RR+RMR). ↑↓: RMR calculated as difference between the sum of MR + RMR obtained by component-integration and MR obtained by root-exclusion method.](attachment:figure3.png)
trenched plots comprised 52% of total CO₂ efflux, while the respiration of excised roots amounted to 32%. Hence, RMR contributed to 20% of total CO₂ efflux from soil or 38% of root-derived CO₂. Therefore, the RR amounts to 62% of root-derived CO₂.

These three studies (Kelting et al., 1998; Craine et al., 1999; Larionova et al., 2005) clearly showed that the partitioning of total CO₂ efflux from soil into three sources: SOM-derived CO₂, 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 CO₂ efflux under field conditions. The combination of methods and calculation of RMR by the difference between two CO₂ 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 ¹⁴CO₂ atmosphere and subsequent tracing of ¹⁴C evolved from the soil (Kuzyakov, 2002). Four methods, 1) the isotopic dilution method, 2) the model-rhizodeposition technique, 3) modeling of ¹⁴CO₂-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 isotopic dilution and the ¹⁴CO₂-dynamics methods resulted in a similar level of RR, 37% and 45%, respectively, of total root-derived CO₂ 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 CO₂ was produced by exudate decomposition. The MR of rhizodeposits calculated using the model-rhizodeposition technique (17%–29%) was also underestimated. Considering the

### Table 2: Results (± SE) of studies which combined or compared different methods for estimation of root respiration (RR) and rhizomicrobial respiration (RMR).

<table>
<thead>
<tr>
<th>Plant Field/Lab</th>
<th>Method</th>
<th>RR (%) RMR (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Festuca pratensis</td>
<td>Lab</td>
<td>Difference: Root-derived ¹⁴CO₂ – RMR-¹⁴CO₂</td>
<td>68 32</td>
</tr>
<tr>
<td>Quercus rubra</td>
<td>Field</td>
<td>Combination of trenching with excised roots</td>
<td>62 38</td>
</tr>
<tr>
<td>Prairie grasses, predominant Schizachyrium scoparium (Michx.)</td>
<td>Field</td>
<td>Combination of excised roots with shading and clipping</td>
<td>48 53</td>
</tr>
<tr>
<td>Maize, integrated for growth season</td>
<td>Field</td>
<td>Combination of root exclusion with component integration</td>
<td>60±8 40±8</td>
</tr>
<tr>
<td>Spring barley, integrated for growth season</td>
<td>Field</td>
<td>Combination of trenching with excised roots</td>
<td>61±3 39±3</td>
</tr>
<tr>
<td>Buckwheat, integrated for growth season</td>
<td>Field</td>
<td>Combination of trenching with excised roots</td>
<td>23±10 77±10</td>
</tr>
<tr>
<td>Comparison of methods</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lolium perenne, 2 months old</td>
<td>Lab</td>
<td>Isotopic dilution</td>
<td>37 63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Model rhizodeposits</td>
<td>71–83±2 17–29±2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>¹⁴CO₂ dynamics</td>
<td>45 55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exudate elution</td>
<td>81±3 19±3</td>
</tr>
<tr>
<td>Maize, 1.5 months old</td>
<td>Lab</td>
<td>Component integration</td>
<td>44±9 56±17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>¹⁴CO₂ dynamics</td>
<td>44±13 56±11</td>
</tr>
<tr>
<td>Average*** ± SE****</td>
<td></td>
<td></td>
<td>48±5 52±5</td>
</tr>
</tbody>
</table>

* 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.
By cultivating maize on a loamy Haplic Luvisol, Sapronov and Kuzyakov (2004) compared RR, RMR as well as SOM-derived CO₂ by three methods: 1) component integration, 2) root exclusion, and 3) ¹⁴CO₂ dynamics after ¹⁴C-pulse labeling. Even though component integration and ¹⁴CO₂ dynamics showed very similar results for the separation of root-derived CO₂ (44% for RR and 56% for RMR; Fig. 5), the estimation of the actual root-derived CO₂ was different in comparison with the results of the root-exclusion technique, the root-derived CO₂ estimated by component integration was 59%–61% of the total efflux. However, the root-derived CO₂ estimated by ¹⁴C was only 18% (Fig. 5). This underestimation probably reflects the noneven distribution of ¹⁴C 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 CO₂ 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 CO₂, and RMR to 52% (Tab. 2). These values of RMR show that the rhizodeposition and its contribution to the CO₂ 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.

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References


