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Original article

Theoretical background for partitioning of root and rhizomicrobial respiration by $\delta^{13}C$ of microbial biomass

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Abstract

Separate estimation of sources of root-derived CO_2 efflux from the soil into actual root respiration and microbial respiration of rhizodeposits is very important for determining the carbon (C) and energy balance of soils and plants, C sources for rhizosphere microorganisms, sources of soil organic matter (SOM), etc. Besides component integration, to date, only four adequate methods based on the pulse labeling of shoots in a $^{14}CO_2$ atmosphere and subsequent monitoring of $^{14}CO_2$ efflux from the soil have been suggested: 1) the isotope dilution, 2) the model rhizodeposition technique, 3) modeling of $^{14}CO_2$ efflux dynamics, and 4) the exudate elution procedure. These methods are based on different assumptions and principles that are very difficult to check experimentally and have different results. Therefore, none of these methods can be accepted as a standard procedure allowing quantitative separate estimation of root respiration and rhizomicrobial respiration.

This contribution provides an elaboration of the theoretical background of a procedure allowing quantitative separate estimation of root respiration and rhizomicrobial respiration in non-sterile soils. The method is based on ^{13}C natural abundance by growing C_4 plant on C_3 soil or vice versa. Four $\delta^{13}C$ values are necessary: of the SOM, of the roots, of soil microbial biomass, and of CO_2 efflux from the soil. The advantages and assumptions of the new approach, as well as possible applications including FACE systems and continuous labeling experiments are discussed.
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1. Introduction

"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" [25]. This separate estimation of root respiration and rhizomicrobial respiration is necessary for:

1) quantitative estimation of the amounts of C sources easily available for rhizosphere microorganisms [2,19,32];
2) quantification of C turnover of rhizosphere microorganisms and their physiological state [33,46];
3) estimation of food web and relations between organisms in the rhizosphere [6];
4) quantification of C sources for dissolved organic matter [4] and soil organic matter (SOM) [22];
5) investigation of the changes of microbial SOM decomposition in the rhizosphere compared to root-free soil, so called priming effects, their mechanisms and their magnitude [5,11,14];
6) study of the mechanisms of nutrient mobilization in the rhizosphere [20,32];
7) modeling of rhizosphere processes, especially estimation of rhizodeposition rates [41];
8) separation and estimation of respiration of autotrophic and heterotrophic organisms [25].

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Therefore, the separate estimation of C passed through root respiration and through rhizomicrobial respiration is very important and quantitative results are urgently necessary. To date, only four adequate methods have been suggested to separate root respiration and rhizomicrobial respiration in non-sterile soils: i) the isotope dilution method [12]; ii) the model rhizodeposition technique [40]; iii) modeling of $^{14}CO_2$ efflux dynamics [27,29], which is partly based on the idea of Warembourg (1975) [43] and Warembourg and Billes (1979) [44]; and iv) the exudate elution procedure [28]. In the original publications, the contribution of root respiration to the root-derived CO_2 was estimated as 41%, 89–95%, 17–61% and less than 81% for the isotope dilution, model rhizodeposition, modeling of $^{14}CO_2$ dynamics, and exudate elution, respectively. The methods and basic assumptions, as well as possible error sources were described in detail earlier [26]. All four methods 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. However, the basic assumptions and principles of these methods, as well as the results observed in the original papers, all differ from one another. The comparison of all four methods under equal conditions did show that $^{14}CO_2$ efflux coming from *Lolium perenne* rhizosphere grown on a loamy Haplic Luvisol consists of about 40–50% of root respiration and on about 50–60% of rhizomicrobial respiration [26]. The comparison shows that despite mutual exclusive assumption, the isotope dilution method [12] and the method based on the modeling of $^{14}CO_2$ efflux dynamics [27,29] are the most reliable methods and they show similar separation results. However, because of many difficulties and some untestable assumptions, none of the four methods could be fully accepted as an easy standard procedure to separate root respiration and rhizomicrobial respiration. Therefore, the development of other methods is crucially important.

Two other methods: component integration and girdling have also proposed to separate root and rhizomicrobial respiration. The so called component integration method [3,16] is based on physical separation of roots, root-free soil, rhizosphere soil, and sometimes litter layer [30] with subsequent measurement of specific respiration activity of each component part. The component integration method disturbs very strongly the intact soil and therefore mechanically disturbed soil components have a different respiration rate compared to undisturbed conditions. Additionally, the rates of exponential decrease of CO_2 efflux during incubation are strongly different for the components. Therefore, the results are strongly dependent on the time period of CO_2 trapping [38]. Recently, a principally new method based on the interruption of assimilate transport to the roots by girdling of pine trees was proposed by Högberg et al. (2001) [21]. However, the interruption of the assimilate transport to the roots stopped not only the root respiration, but also the root exudation and secretion, and subsequently rhizomicrobial respiration. Therefore, the obtained decrease of CO_2 efflux from soil with girdled plants is not only the result of interrupted root respiration (actual respiration of autotrophs). It is also the result of decreased rhizomicrobial respiration (actually, it belongs to respiration of heterotrophs).

This communication presents a theoretical background of a new approach allowing separate estimation of root respiration and rhizomicrobial respiration in non-sterile soils. Basically, the method is based on ^{13}C natural abundance technique and does not require any artificial labeling. To increase the sensitivity of the method, FACE with depleted ^{13}C in CO_2 or continuous labeling of plants in $^{13}CO_2$ or $^{14}CO_2$ atmosphere could be of advantage.

2. Theoretical background

The total CO_2 efflux (CO_2^{Total}) from vegetated soil consists on three sources (Fig. 1): 1) CO_2 originated from microbial decomposition of SOM (CO_2^{SOM}), 2) CO_2 originated from microbial decomposition of rhizodeposits = rhizomicrobial respiration (CO_2^{RMR}), and 3) CO_2 originated from actual root respiration (CO_2^{RR}),

$$CO_2^{\text{Total}} = CO_2^{\text{SOM}} + CO_2^{\text{RMR}} + CO_2^{\text{RR}} \tag{1}$$

The CO_2 efflux evolved by decomposition of plant residues is not considered here.

The sum of rhizomicrobial respiration and root respiration is equivalent to root-derived CO_2 (CO_2^{RD}),

$$CO_2^{\text{RD}} = CO_2^{\text{RMR}} + CO_2^{\text{RR}} \tag{2}$$

The term 'root-derived CO_2' is used here to describe the sum of root respiration and CO_2 evolved by microbial decomposition of exudates, secretions as well as root residues such as sloughed root cells, root hairs, and dead roots. Strictly speaking, the term 'rhizosphere CO_2' or 'rhizosphere respiration', frequently used in the literature, refers to the location of CO_2 production. From this point of view it must include not only root respiration and CO_2 evolved by microbial utilization of exudates, but also the CO_2 originated by microbial decomposition of rhizosphere SOM.

If a C_3 plant is growing on a C_3 soil (SOM was produced from remainders of C_3 plants), then the $\delta^{13}C$ isotope signature of all three CO_2 sources assumed to be the same. Isotopic effects are not considered in the equations below [10,17]. Actually, the isotopic effects should be measured in the experiment and considered in the calculations (see Section 3) (Fig. 1).

2.1. Calculation step 1

If a C_4 plant (i.e. corn) is newly growing on a "C_3 soil" (or vice versa), then the contribution of microbial SOM decomposition (cC_3^{CO2}) and contribution of root-derived CO_2 to total CO_2 efflux from the soil will be calculated according to the $\delta^{13}C$ isotope signature of the total CO_2 efflux (δ^{CO2}), and the $\delta^{13}C$ isotope signature of both CO_2 sources: SOM (δ_3^{SOM}) and rhizodeposits (δ_4^{Rhiz}) [9,10,31],

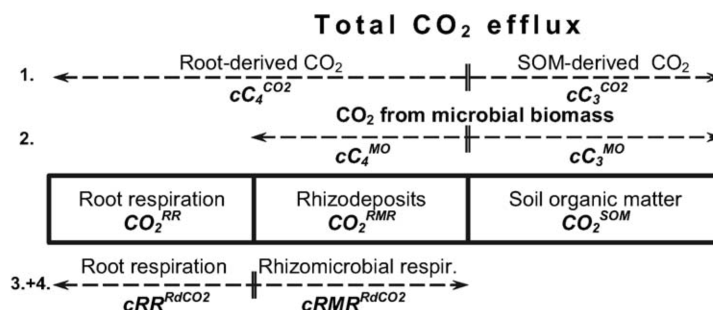

Fig. 1. Three main sources of CO_2 efflux from planted soil and the calculation steps for estimation of their contributions to the total CO_2 efflux. The labels are the same as used in the text and equations.

$$cC_3^{CO_2} = \frac{\delta^{CO_2} - \delta_4^{Rhiz}}{\delta_3^{SOM} - \delta_4^{Rhiz}} \quad (3)$$

(This equation was developed from the main mass balance equation $\delta^{13}C_t \cdot C_t = \delta^{13}C_1 \cdot C_1 + \delta^{13}C_2 \cdot C_2$ for calculation of the isotopic composition of a pool (C_t) consisting from two sources (C_1 and C_2).)

The contribution of root-derived CO_2 to the total CO_2 efflux (cC_4^{CO2}) will be calculated as follow,

$$cC_4^{CO_2} = 1 - cC_3^{CO_2} \quad (4)$$

or

$$cC_4^{CO_2} = \frac{\delta^{CO_2} - \delta_3^{SOM}}{\delta_4^{Rhiz} - \delta_3^{SOM}} \quad (5)$$

This separation of contribution of SOM (cC_3^{CO2}) and root-derived CO_2 (cC_4^{CO2}) to the total CO_2 efflux from planted soil is presented as ‖ on the upper arrow on Fig. 1.

2.2. Calculation step 2

As analogy to Eq. (3), the contribution of a C_3 source (i.e. SOM) to microbial nutrition (cC_3^{MO}) in the rhizosphere of a C_4 plant based on the $\delta^{13}C$ isotope signature of microbial biomass (δ^{MO}) will be calculated:

$$cC_3^{MO} = \frac{\delta^{MO} - \delta_4^{Rhiz}}{\delta_3^{SOM} - \delta_4^{Rhiz}} \quad (6)$$

The contribution of a C_4 source (rhizodeposition of the C_4 plant) to microbial nutrition (cC_4^{MO}) will be calculated as analogy to the Eqs. (4) and (5),

$$cC_4^{MO} = 1 - cC_3^{MO} \quad (7)$$

or

$$cC_4^{MO} = \frac{\delta^{MO} - \delta_3^{SOM}}{\delta_4^{Rhiz} - \delta_3^{SOM}} \quad (8)$$

The separation of the contributions of rhizodeposition (cC_4^{MO}) and of SOM (cC_3^{MO}) to the nutrition of microorganisms is shown with ‖ on the second arrow (Fig. 1). As the result of Eqs. (3)–(8), the contribution of both C sources: SOM and rhizodeposition to the total CO_2 efflux and to the nutrition of soil microorganisms can be calculated according to the $\delta^{13}C$ isotope signature of CO_2 efflux, microbial biomass, and both C sources.

2.3. Calculation step 3

The $\delta^{13}C$ value of CO_2 efflux evolved by microbial respiration corresponds roughly to $\delta^{13}C$ value of microbial biomass [15,37]. Therefore according to Fig. 1, if the contribution of SOM to the microorganisms' nutrition (cC_3^{MO}) corresponds to the contribution of SOM to CO_2 total efflux from the soil (cC_3^{CO2}), than the contribution of rhizodeposition to the microorganism nutrition (cC_4^{MO}) corresponds to the contribution of rhizomicrobial respiration to the CO_2 total efflux from the soil ($cRMR^{CO2}$),

$$cC_3^{MO} \rightarrow cC_3^{CO_2}$$

$$cC_4^{MO} \rightarrow cRMR^{CO_2}$$

The contribution of rhizomicrobial respiration to the total CO_2 efflux from the soil ($cRMR^{CO2}$) is only one unknown parameter. The other parameters were calculated according to the Eqs. (3), (6) and (8). Therefore, the contribution of rhizomicrobial respiration to the total CO_2 efflux from the soil ($cRMA^{CO2}$) will be calculated as follows,

$$cRMR^{CO_2} = \frac{cC_4^{MO} \cdot cC_3^{CO_2}}{cC_3^{MO}} \quad (9)$$

The contribution of root respiration to the total CO_2 efflux (cRR^{CO2}) will be calculated as difference (Fig. 1),

$$cRR^{CO_2} = 1 - cC3^{CO_2} - cRMR^{CO_2} \quad (10)$$

As a result, the contributions of all three CO_2 sources were calculated.

2.4. Calculation step 4

Finally, the contribution of root respiration (cRR^{RdCO2}) and rhizomicrobial respiration ($cRMR^{RdCO2}$) to the root-derived CO_2 will be calculated as follow:

$$cRR^{RdCO_2} = \frac{cRR^{CO_2}}{cRR^{CO_2} + cRMR^{CO_2}} \quad (11)$$

and

$$cRMR^{RdCO_2} = \frac{cRMR^{CO_2}}{cRR^{CO_2} + cRMR^{CO_2}} \quad (12)$$

The variables in the Eqs. (11) and (12) can be substituted according the Eqs. (3)–(8). After transformation and simplification, the contribution of root respiration (cRR^{RdCO2}) and rhizomicrobial respiration ($cRMR^{RdCO2}$) to the root-derived CO_2 will be calculated as follow,

$$cRR^{RdCO_2} = \frac{(\delta^{CO_2} - \delta^{MO}) \cdot (\delta_3^{\mathrm{SOM}} - \delta_4^{\mathrm{Rhiz}})}{(\delta_4^{\mathrm{Rhiz}} - \delta^{MO}) \cdot (\delta_3^{\mathrm{SOM}} - \delta^{CO_2})} \quad (13)$$

and

$$cRMR^{RdCO_2} = \frac{(\delta_3^{\mathrm{SOM}} - \delta^{MO}) \cdot (\delta_4^{\mathrm{Rhiz}} - \delta^{CO_2})}{(\delta_4^{\mathrm{Rhiz}} - \delta^{MO}) \cdot (\delta_3^{\mathrm{SOM}} - \delta^{CO_2})} \quad (14)$$

These are two final equations for quantification of contributions of root respiration (cRR^{RdCO2}) and rhizomicrobial respiration ($cRMR^{RdCO2}$) to the root-derived CO_2. They are based on the $\delta^{13}C$ isotope signature of: 1) the total CO_2 efflux (δ^{CO_2}), 2) the microbial biomass (δ^{MO}), 3) the SOM (δ_3^{SOM}), and 4) the rhizodeposition (δ_4^{Rhiz}).

In most studies, fumigation–extraction procedure is used to measure microbial biomass C in soils. By using fumigation–extraction procedure, the $\delta^{13}C$ isotope signature of microbial biomass can be calculated according to the following mass balance equation [7,35,36],

$$\delta^{MO} = \frac{\delta C_f \cdot C_f - \delta C_e \cdot C_e}{C_f - C_e} \quad (15)$$

where, δC_f and δC_e are the $\delta^{13}C$ values of fumigated and extracted soil samples, respectively; C_f and C_e are the C amounts of fumigated and extracted soil samples, respectively. Different methods for analyzing $\delta^{13}C$ of microbial biomass obtained by fumigation–extraction were compared earlier and showed no significant differences [34].

It is important to note here, that the equilibrium between the contribution of both C sources and $\delta^{13}C$ of microbial biomass will not be reached immediately after the start of the rhizodeposition. However, this period necessary for the changes is not longer that one turnover time of rhizosphere microorganisms. It takes about 8 days for the change of generation of rhizosphere microorganisms [33]. Carbon turnover in the hyphae of mycorrhizal fungi measured by accelerator mass spectrometry after plant labeling with fossil carbon dioxide was also of about 5–6 days [39]. It means, that the time necessary for the equilibration of $\delta^{13}C$ value of microbial biomass or mycorrhizal fungi is about 1 week.

3. Assumptions and sensitivity analysis

The suggested method is based on two assumptions concerning extraction and yield factor of microbial biomass and two assumptions concerning ^{13}C isotopic effects during root and microbial respiration:

1) The first assumption of the method is the application of the same extraction factor for rhizosphere microorganisms and microorganisms living in root-free soil obtained by using fumigation–extraction method. Actually, the factor converting the amount of extracted carbon to microbial carbon (is about 0.45) is less for more active microorganisms (i.e. living in the rhizosphere) comparing to dormant microorganisms (i.e. living in root-free soil). However, the most studies used the same factor for fumigation–extraction method independently on the origin and physiological state of the microorganisms.
2) The second assumption (which is actually a hidden one) concerns the equal yield factor of microbial biomass using rhizodeposits (easily available C source) and utilizing SOM (hardly available C source). If the yield factor is the same, the $\delta^{13}C$ value of CO_2 respired by microbial community using both C sources is equal the $\delta^{13}C$ value of microbial biomass. In some studies it was concluded that rhizosphere microorganisms utilize rhizodeposits with lower efficiency than the C sources in the root-free soil [20]. Such lower efficiency can shift the $\delta^{13}C$ value of CO_2 closer to the root $\delta^{13}C$ from $\delta^{13}C$ of the actual microbial biomass and the contribution of root respiration and rhizomicrobial respiration can be overestimated. In my opinion, the frequently observed lower efficiency of rhizodeposits utilization has the following reason. In contrast to the microorganisms living in the root-free soil which are strongly limited by available C (Wardle, 1992) [42], rhizosphere microorganisms are not limited by easily available C sources [13], but by other factors, i.e. N availability. This limitation by N or other nutrients and high availability of C substrates lead to very fast turnover rates of microorganisms in the rhizosphere amounting for few days [33,39] in contrast to the turnover rates in the root-free soil amounting for between weeks and months. This difference in the turnover rates leads to strong underestimation of yield factor of rhizosphere microorganisms obtained in the studies where the same sampling time scale was used for rooted and root-free soil. However, this underestimation of the yield factor is apparent and is connected with different turnover rates. Surely, the rhizosphere microorganisms evolve more CO_2 per unit of time and microbial C, but this is connected not with different yield factors but with different turnover rates. The turnover rates of microbial biomass are not included in the equations above and therefore should have no effect on the separation results obtained by the suggested method.
3) The $\delta^{13}C$ isotope signature of CO_2 released as root respiration and of rhizodeposits C is the same as $\delta^{13}C$ value of the roots (no ^{13}C discrimination by respiration). Up to now,

this assumption ($\delta^{13}C$ of root-derived CO_2 = $\delta^{13}C$ of the roots) was accepted for the most CO_2 and rhizosphere studies [1,8,18]. The study of Cheng [10] growing winter wheat on C-free vermiculite and vermiculite-sand-mixture proves this assumption. The first assumption can be checked by introduction of one variant with growing plants on a C-free substrate and measuring the $\delta^{13}C$ value of CO_2 evolved from roots [10].

4) The $\delta^{13}C$ isotope signature of CO_2 respired by microorganisms corresponds with $\delta^{13}C$ value of microbial biomass. This assumption was checked in the literature, but the results vary strongly. According to the results of Santruckova et al. [37] measured $\delta^{13}C$ 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 microbial biomass. However, the $\delta^{13}C$ difference between microbial biomass and respired CO_2 varied between 0.1‰ and 7.7‰.

To evaluate the effect of unconsidered isotopic discrimination by respiration (or the effect of erroneous estimation of $\delta^{13}C$ of CO_2 or of microbial biomass) on the standard error (S.E.) of the calculated contribution of the three CO_2 sources, the changes in the contribution of C_3–C or C_4–C sources to the CO_2 efflux were simulated. Sensitivity analysis of the S.E. depending on the error of $\delta^{13}C$ of CO_2 or of microbial biomass was conducted. For the simulation, SOM and roots were chosen as end-members and their $\delta^{13}C$ values were set as constant for –29 ± 0‰ and –11 ± 0‰, respectively. For the first part of the sensitivity analysis, the $\delta^{13}C$ of microbial biomass was set as constant at –17‰ and $\delta^{13}C$ of CO_2 was changed starting from –22‰ to –11‰ (Fig. 2, top). For the second part of the sensitivity analysis, the $\delta^{13}C$ of CO_2 was set as constant at –22‰ and $\delta^{13}C$ of microbial biomass was changed starting from –29‰ to –17‰ (Fig. 2, bottom). In this simulations one of the both variables: $\delta^{13}C$ of CO_2 or $\delta^{13}C$ of microbial biomass was set as constant value showed by bold arrows on Fig. 2. The unconsidered isotopic effect (or the error of estimation of one of two parameters) was increasing from zero to ± 5‰ (± 7‰).

For this sensitivity analysis of the S.E. of partitioning, four replicates with two maximal possible differences between the true $\delta^{13}C$ value and erroneously estimated ^{13}C value of CO_2 or of microbial biomass were used. Thereafter, the contribution of the three CO_2 sources were calculated by Eqs. (11)–(14). Such simulated dependence of the S.E. of calculated contribution of three CO_2 sources on the error of the estimation of $\delta^{13}C$ values of the CO_2 efflux and of microbial biomass is presented in Fig. 2 (changed from [45]). The sensitivity analysis showed that the unconsidered isotopic effect (or erroneous estimation of $\delta^{13}C$ value) of about ± 1‰ during microbial decomposition of SOM to CO_2 results in a S.E. between 1% and 3% of the contribution of C_3–C or C_4–C sources to the CO_2 efflux from soil. As expected, the S.E. of estimation strongly increases with increasing $\delta^{13}C$ error. However, the maximal S.E. simulated is less than ± 15% for root respiration and less than ± 10% for CO_2 derived from SOM or rhizodeposits decomposition if the estimation error of the $\delta^{13}C$ of CO_2 is about 6‰ (Fig. 2, top). The differences between $\delta^{13}C$ of SOM and that of respired CO_2 were found to vary from –3.2‰ to +2.1‰ (Ref. in [37]). It means that according to this sensitivity analysis, the S.E. of CO_2 partitioning by the method suggested here will be less than ± 5% for rhizomicrobial and SOM-derived CO_2 and less than ± 8% for root respiration. This sensitivity analysis also showed that the S.E. of estimation is slightly higher, if not the $\delta^{13}C$ of CO_2 but the $\delta^{13}C$ value of microbial biomass will be estimated with the same error. So, an error up to 7‰ of microbial biomass led to the S.E. of obtained contributions of all three variables up to ± 15% (Fig. 2, bottom). In this simple simulation one of the both inaccurate variables: $\delta^{13}C$ of CO_2 or $\delta^{13}C$ of microbial biomass was set as constant ($\delta^{13}C$ of microbial biomass = –22‰ or $\delta^{13}C$ of CO_2 = –17‰), whereas the second was changed gradually (in 1 per mil steps). Surely, if both variables ($\delta^{13}C$ of CO_2 and of microbial biomass) will be estimated with errors, then the S.E. will increase compared to the one-variable-change sensitivity analysis presented in Fig. 2. However, these 10–15% of errors of estimation are worst-case scenarios associated with 5–7‰ error of $\delta^{13}C$ value. Such high errors of $\delta^{13}C$ value are not expected. It is important to underline here that by this simulation, the $\delta^{13}C$ of SOM and of roots were set as fixed values (–29‰ and –11‰, respectively) as estimated without errors. It means that the analytical error of $\delta^{13}C$ of SOM and of roots was accepted as disregarding small compared to the error of $\delta^{13}C$ of microbial biomass or of CO_2 estimation. If the error of estimation of $\delta^{13}C$ of SOM and/or of roots is comparable with that of the estimation error of $\delta^{13}C$ of microbial biomass or of CO_2 then the final error of the partitioning will be much higher as calculated here. This sensitivity analysis showed that it is very important to measure the isotopic effects in the specific study.

If the isotopic effects will be significant, they should be considered in the equations above. The isotopic effects can be considered by adding specific terms corresponding to the discrimination values to the total CO_2 efflux (δ^{CO2}), or/and the microbial biomass (δ^{MO}), or/and the rhizodeposition (δ_4^{Rhiz}). Therefore it is important to note here, that these assumptions are more realistic than the assumptions accepted by the four methods based on ^{14}C pulse labeling used earlier for separation of root and rhizomicrobial respiration [26]. Additionally, it is comparatively easy to check these assumptions in each experiment conducted for the separation.

4. Advantages and difficulties of the method

Compared to the four previous methods for estimation of the contributions of root respiration and rhizomicrobial respiration to the rhizosphere CO_2, there are many advantages of the suggested approach. The approach is easy in application. Only five $\delta^{13}C$ values are necessary to calculate the contribution of root and rhizomicrobial respiration to root-derived CO_2. To calculate the amount of C passed through

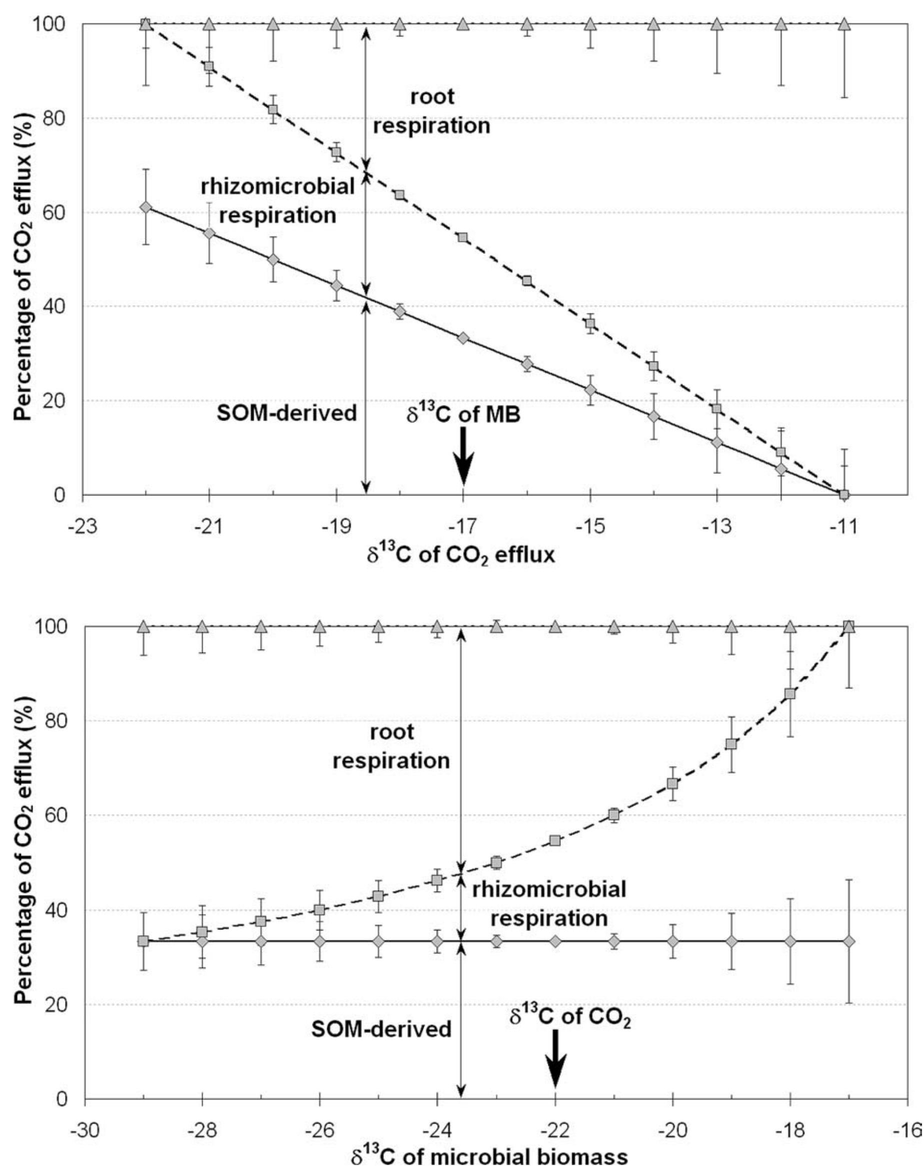

Fig. 2. Sensitivity analysis of the error of CO_2 efflux partitioning (error bars) into root and rhizomicrobial respiration as well as of SOM decomposition simulated by changing $\delta^{13}C$ values of CO_2 efflux (top) or microbial biomass (bottom). Here the $\delta^{13}C$ of SOM was set as –29 ± 0‰ and the $\delta^{13}C$ of roots was set as –11 ± 0‰. For the simulation, one of the both variables: $\delta^{13}C$ of CO_2 or $\delta^{13}C$ of microbial biomass was set as constant value showed by bold arrows ($\delta^{13}C$ of microbial biomass = –22‰ or $\delta^{13}C$ of CO_2 = –17‰). The other variable was changed in one per mil steps from –22‰ to –11‰ for $\delta^{13}C$ of CO_2 (top) or from –29‰ to –17‰ for $\delta^{13}C$ of microbial biomass (bottom). The simulated S.E.s showed the worst-case scenario calculated for four replicates with two pairs of values with maximal estimation errors of $\delta^{13}C$ of CO_2 or of microbial biomass.

each flow, the multiplication of the contributions estimated by Eqs. (11)–(14) with the total CO_2 efflux is necessary. It is important that the contribution of SOM and root-derived CO_2 to the total CO_2 efflux from soil will be calculated simultaneously to the separation of root-derived CO_2.

Other advantages are connected with the ^{13}C natural abundance. Because the ^{13}C natural abundance is used, no artificial ^{14}C labeling is necessary. Therefore all shortcomings and difficulties connected with ^{14}C application are excluded. Additionally, the distribution of ^{13}C among the C pools in the plant is much more uniform compared to the artificial ^{14}C pulse labeling. The method can be applied under field conditions. The other four methods were suitable only for laboratory conditions. For application in the field a C_4 plant has to be grown on a C_3 soil (or vice versa) and the $\delta^{13}C$ values of CO_2 emission from soil and microbial biomass has to be measured. Compared to previous methods, the new one is not destructive: Small amount of soil sample is enough to measure $\delta^{13}C$ values of microbial biomass and SOM. Therefore many measurements are possible in one canopy (e.g. during vegetation

period). Until now, there are not any suggested non-destructive methods (four described above, as well as soil sterilization/ fumigation or nutrient solution studies) allowing quantification of root and rhizomicrobial respiration.

The new approach has some difficulties. The separation of the soil air from atmosphere to trap CO_2 emission from soil is necessary. This difficulty however, is the same as in the other four ^{14}C pulse labeling methods. Actually, all isotopic methods estimating CO_2 flows in soil and in the rhizosphere need airtight separation of the soil compartment to avoid dilution with atmosphere air. However, this difficulty can be solved by calculating 'Keeling plots' showing the dependence of the $\delta^{13}C$ on the reciprocal of the CO_2 concentration in the mixture between soil CO_2 and atmosphere CO_2 [23,24]. This presentation form of $\delta^{13}C$ values of CO_2 emission from the soil is based in an easy two-component (soil CO_2 and atmosphere CO_2) mixture model of CO_2 sources with different $\delta^{13}C$ values and allows calculation of the $\delta^{13}C$ values of CO_2 respired from the soil without sealing the soil from the atmospheric CO_2.

Soil–plant pairs impose limitations to the ^{13}C natural abundance method through C_3 plants growing in a C_4 soil or vice versa are unusual. Hence, the field application of this method is restricted to places where soils developed under C_3 vegetation allow the growth of C_4 plants and vice versa. It is important to note, that this difficulty can be overcome in the free air carbondioxide enrichments (FACE). For the CO_2 enrichments under FACE, the CO_2 from combustion of fossil C sources is frequently used. This CO_2 has the $\delta^{13}C$ value of about –45‰ (it is varying between –40‰ and –50‰ depending on the CO_2 source; in the most cases methane oxidation). After the mixing of the supplied CO_2 with CO_2 of the atmosphere, the $\delta^{13}C$ of the mixed CO_2 becomes about 25–30‰. This $\delta^{13}C$ value is significantly less than that of the atmosphere air ($\delta^{13}C \approx -7.5$‰). Therefore, the released rhizodeposits as well as CO_2 originated from root respiration will have $\delta^{13}C$ values strongly different of that of SOM. This fact reveals a new principle that has until now not been used as a possibility to estimate the ratio between root respiration and rhizomicrobial respiration for the most important plants under FACE, by applying the suggested $\delta^{13}C$ method. Surely, isotopic effects described above could affect the results of the application of FACE for the suggested separation of root and rhizomicrobial respiration.

At the end it is important to underline, that the most problems concerning low resolution of natural abundance method as well as possible uncertainties around isotopic discrimination by microbial respiration can be easily overcome by using continuous labeling of plants in $^{13}CO_2$ or $^{14}CO_2$ atmosphere.

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Appendix A

The symbols and abbreviation of all equations and Fig. 1 are,

CO_2^{Total} total CO_2 efflux from soil

CO_2^{SOM} CO_2 originated from SOM

CO_2^{RMR} CO_2 originated from rhizomicrobial respiration

CO_2^{RR} CO_2 originated from root respiration

CO_2^{RD} root-derived CO_2 (CO_2 originated from the sum of root and rhizomicrobial respiration)

cC_3^{CO2} contribution of a C_3 source (SOM) to the total CO_2 efflux (the contribution here and in the following abbreviations is presented as a portion, not as percentage)

cC_4^{CO2} contribution of a C_4 source (rhizodeposition) to the total CO_2 efflux

cC_3^{MO} contribution of a C_3 source (SOM) to the C in microorganisms and their nutrition

cC_4^{MO} contribution of a C_4 source (rhizodeposition) to the C in microorganisms and their nutrition

δ^{CO2} $\delta^{13}C$ of the total CO_2 efflux from soil with a C_4 plant

δ^{MO} $\delta^{13}C$ of the microorganisms

δ_4^{Rhiz} $\delta^{13}C$ of rhizodeposition (= $\delta^{13}C$ of root; = $\delta^{13}C$ of root respiration); C_4 source

δ_3^{SOM} $\delta^{13}C$ of SOM

$cRMR^{CO2}$ contribution of rhizomicrobial respiration to the CO_2 efflux

cRR^{CO2} contribution of root respiration to the CO_2 efflux

$cRMR^{RdCO2}$ contribution of rhizomicrobial respiration to the root-derived CO_2

cRR^{RdCO2} contribution of root respiration to the root-derived CO_2

δC_f $\delta^{13}C$ value of C from fumigated soil sample

δC_e $\delta^{13}C$ value of C from extracted soil sample

C_f and C_e C amounts of fumigated and extracted soil samples, respectively

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