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CARBON AND NITROGEN CYCLES AND THE COMPOSITION OF GREENHOUSE GASES

Separation of Root and Rhizomicrobial Respiration by Natural ^{13}C Abundance: Theoretical Approach, Advantages, and Difficulties[1]

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Abstract—This contribution provides an elaboration of the theoretical background of an easy procedure allowing quantitative separate estimation of root respiration and rhizomicrobial respiration in nonsterile soils. The method is based on ^{13}C natural abundance by growing C_4 plants on C_3 soil or vice versa. Four $\delta^{13}C$ values are necessary: of the soil organic matter, of the roots, of the 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 (^{13}C or ^{14}C) experiments are discussed. It is expected that the new method will become the standard procedure for separate estimation of root respiration (=respiration of autotroph organisms) and rhizomicrobial respiration (=respiration of heterotroph organisms) in nonsterile soils.

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

- quantitative estimation of the amounts of C sources easily available for rhizosphere microorganisms;
- quantification of C turnover of rhizosphere microorganisms and their physiological state;
- estimation of the food web and relations between organisms in the rhizosphere;
- quantification of C sources for soil organic matter (SOM);
- 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;
- study of the mechanisms of nutrient mobilization in the rhizosphere;
- modeling of rhizosphere processes, especially estimation of rhizodeposition rates;
- separation and estimation of respiration of autotrophic and heterotrophic organisms.

Therefore, the separate estimation of C that has 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 nonsterile soils:

(1) the isotope dilution method [6];

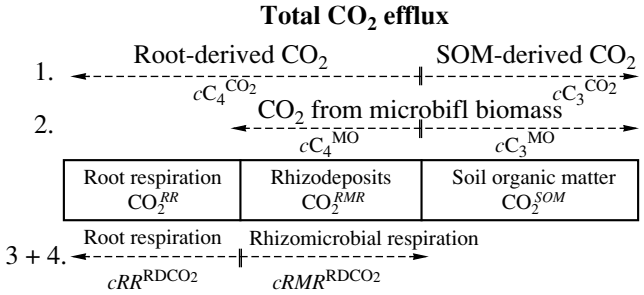
(2) the model rhizodeposition technique [23];

(3) modeling of $^{14}CO_2$ efflux dynamics [13, 15, 16];

(4) the exudate elution procedure [14].

The methods and their basic assumptions, as well as possible sources of error were described in detail earlier [12]. 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 in one experiment under equal conditions shows 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 about 50–60% of rhizomicrobial respiration [12]. The comparison shows that, despite mutual exclusive assumptions, the isotope dilution method [6] and the method based on the modeling of $^{14}CO_2$ efflux dynamics [13, 15, 16] are the most reliable methods and they show similar separation results. However, it could be concluded that, because there are many difficulties and some assumptions are not possible to test, none of the four methods could be accepted as an easy and standard procedure allowing separate estimation of root respiration and rhizomicrobial respiration. Therefore, the elaboration of other methods is crucially important.

[1] This article was submitted by the author in English.



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.

Here, it is important to note that two other methods, component integration and girdling, were also tested to separate root and rhizomicrobial respiration. However, the so-called component integration method [1, 8] based on physical separation of roots, root free soil, rhizosphere soil, and sometimes a litter layer [17] with subsequent measurement of the specific respiration activity of each component part disturbs very strongly the intact soil and the balance between the components. Additionally, the rates of exponential decrease of CO_2 efflux differ strongly for the components and, therefore, the results depend greatly on the time period of incubation and CO_2 trapping. Recently, a principally new method based on the interruption of assimilate transport to the roots by girdling of trees was suggested and tested on pine by Högberg *et al.* [10]. 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 (respiration of autotrophs), but also the result of interrupted rhizomicrobial respiration (respiration of heterotrophs).

This communication presents the theoretical background of a new approach allowing separate estimation of root respiration and rhizomicrobial respiration in nonsterile soils. Basically, the method is based on the ^{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 a $^{13}CO_2$ or $^{14}CO_2$ atmosphere could be of advantage.

THEORETICAL BACKGROUND

The total CO_2 efflux (CO_2^{Total}) from planted soil (without plant remainders of previous crops) consists of three sources (figure):

(1) CO_2 originating from microbial decomposition of soil organic matter (CO_2^{SOM});

(2) CO_2 originating from microbial decomposition of rhizodeposits = rhizomicrobial respiration (CO_2^{RMR});

(3) CO_2 originating from actual root respiration (CO_2^{RR}).

So,

$$CO_2^{Total} = CO_2^{SOM} + CO_2^{RMR} + CO_2^{RR}. \quad (1)$$

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

$$CO_2^{RD} = CO_2^{RMR} + CO_2^{RR}. \quad (2)$$

The term "root-derived CO_2" is used here to describe the sum of root respiration and CO_2 evolving 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 evolving from microbial utilization of exudates, but also the CO_2 originating from microbial decomposition of rhizosphere soil organic matter.

If a C_3 plant is growing on a C_3 soil (soil organic matter was produced from remains of C_3 plants), then the $\delta^{13}C$ isotope signature of all three CO_2 sources is the same (isotopic effects less than ±1–2° are not considered here) [5, 9].

Calculation step 1. If a C_4 plant (i.e., corn) is growing on a "C_3 soil" (or vice versa), then the contribution of microbial SOM decomposition ($cC_3^{CO_2}$) and the contribution of root-derived CO_2 to the total CO_2 efflux from the soil will be calculated according to the ^{13}C isotope signature of the total CO_2 efflux (δ^{CO_2}) and the ^{13}C isotope signature of both CO_2 sources: SOM (δ_3^{SOM}) and rhizodeposits (δ_4^{Rhiz}) [4, 5, 18]:

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

(This equation was developed from the main mass balance equation $\delta^{13}C_tC_t = \delta^{13}C_1C_1 + \delta^{13}C_2C_2$ for calculation of the isotopic composition of a pool (C_t) consisting of two sources (C_1 and C_2).)

The contribution of root-derived CO_2 to the total CO_2 efflux ($cC_4^{CO_2}$) will be calculated as follows:

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

or

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

This separation of the contribution of SOM ($cC_3^{CO_2}$) and root-derived CO_2 ($cC_4^{CO_2}$) to the total CO_2 efflux from planted soil is presented as || on the upper arrow in the figure.

Calculation step 2. Analogous 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 the 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 analogous to 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 (figure).

As the result of equations (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 ^{13}C isotope signature of the CO_2 efflux, microbial biomass, and both C sources.

Calculation step 3. The $\delta^{13}C$ value of the CO_2 efflux evolving from microbial respiration corresponds roughly to the $\delta^{13}C$ value of the microbial biomass [7, 22]. Therefore, according to the figure, if the contribution of SOM to the microorganisms' nutrition (cC_3^{MO}) corresponds to the contribution of SOM to the CO_2 total efflux from the soil ($cC_3^{CO_2}$), then 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 ($cRMR^{CO_2}$):

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

$$cC_4^{MO} \longrightarrow cRMR^{CO_2}.$$

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

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

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

$$cRR^{CO_2} = 1 - cC_3^{CO_2} - cRMR^{CO_2}. \quad (10)$$

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

Calculation step 4. Finally, the contribution of root respiration (cRR^{RdCO_2}) and rhizomicrobial respiration ($cRMR^{RdCO_2}$) to the root-derived CO_2 will be calculated as follows:

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

and

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

The variables in equations (11) and (12) can be substituted according to equations (3)–(8). After transformation and simplification, the contribution of root respiration (cRR^{RdCO_2}) and rhizomicrobial respiration ($cRMR^{RdCO_2}$) to the root-derived CO_2 will be calculated as follows:

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

and

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

These are the two final equations for quantification of the contributions of root respiration (cRR^{RdCO_2}) and rhizomicrobial respiration ($cRMR^{RdCO_2}$) to the root-

derived CO_2. They are based on the $\delta^{13}C$ isotope signature of the following:

(1) the total CO_2 efflux (δ^{CO_2});

(2) the microorganisms (δ^{MO});

(3) the soil organic matter (δ_3^{SOM}); and

(4) the rhizodeposition (δ_4^{Rhiz}).

By using the fumigation–extraction procedure to obtain C of the microbial biomass, the $\delta^{13}C$ isotope signature of microorganisms can be calculated according to the following mass balance equation [2, 20, 21]:

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

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. It is important to note here that the equilibrium between the contribution of both C sources and ^{13}C of the microbial biomass will not be reached immediately after the start of the rhizodeposition. However, this period that is necessary for the changes is not longer than one generation of rhizosphere microorganisms. Neergaard and Magid [19] show that it takes about 8 days for the change of generations of rhizosphere microorganisms. This means that the time necessary for the equilibration of the $\delta^{13}C$ value of the microbial biomass is no longer than 8 days.

ASSUMPTIONS

The new method is based on two assumptions concerning ^{13}C isotopic effects during root and microbial respiration.

(1) The $\delta^{13}C$ isotope signature of CO_2 released as root respiration and of rhizodeposits C is the same as the $\delta^{13}C$ value of the roots. Up to now, this assumption ($\delta^{13}C$ of root-derived CO_2 = $\delta^{13}C$ of the roots) was used in most rhizosphere CO_2 studies. The study of Cheng [5] growing winter wheat on C free vermiculite and a vermiculite–sand mixture proves this assumption.

(2) The ^{13}C isotope signature CO_2 respired by microorganisms corresponds with the $\delta^{13}C$ value of the microbial biomass. This assumption was checked in the literature, but the results vary strongly. According to the results of Santruckova *et al.* [22], the 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‰ $\delta^{13}C$ compared to microbial biomass. However, the $^{\delta 13}C$ difference between microbial biomass and respired CO_2 varied between 0.1 and 7.7‰. According to the principle of $\delta^{13}C$ natural abundance method suggested in this work for separation of CO_2 sources, the unconsidered isotopic effect of about ±1‰ during microbial decomposition of SOM to CO_2 results in an error of about 7% by calculation of the contribution of C_3–C or C_4–C sources to the CO_2 efflux from soil. The differences between ^{13}C of SOM and that of respired CO_2 were found to vary from –3.2‰ to +2.1‰ (references in [22]).

By the calculation of the contribution of root and rhizomicrobial respiration to the root-derived CO_2, this error per 1 ‰ may increase to 10–15% or more depending on the contribution of C_3–C or C_4–C sources to the nutrition of microbial biomass and on the contribution of SOM and rhizosphere originated CO_2 to the total CO_2 emission from the soil. Therefore, it is very important to measure the isotopic effects in the specific study. The first assumption can be checked by introduction of one variant with growing of the investigated plant on a C-free substrate and measuring the $\delta^{13}C$ value of CO_2 evolved from roots [5]. Measuring of $\delta^{13}C$ value of CO_2 from unplanted soil can check the second assumption. Therefore, it is important to note here that these two 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 [12]. Additionally, it is very easy to check these assumptions in each experiment conducted for the separation.

One additional 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) (Blagodatsky, 2003, personal communication). However, the most previous studies used the same factor for fumigation–extraction method independently on the origin and physiological state of the microorganisms.

ADVANTAGES AND DIFFICULTIES OF THE METHOD

The advantages of the new method for estimation of the contributions of root respiration and rhizomicrobial respiration to the rhizosphere CO_2 compared to the four other methods are:

The method 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 each flow, the multiplication of the contributions with the total CO_2 efflux is necessary.

The contribution of SOM and root-derived CO_2 to the total CO_2 efflux from soil will be calculated simultaneously.

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.

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 application of this method is not limited for growth of a C_4 plant on a C_3 soil. It is possible to apply the same method on a C_4 soil planted with C_3 plants.

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.

It is not destructive method: small amount of soil sample is enough to measure ^{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 nondestructive methods (four described above, as well as soil sterilization/fumigation or nutrient solution studies) allowing quantification of root and rhizomicrobial respiration.

DIFFICULTIES OF THE METHOD

Studies based on isotope application and especially based on natural abundance with very low differences in $\delta^{13}C$ values have some difficulties for the field and laboratory studies. The difficulties of the new method are:

separation of the soil air from atmosphere to trap CO_2 emission from soil is necessary; this difficulty remains the same as in the other four methods that are based on ^{14}C pulse labeling;

spatial and seasonal variations of the $\delta^{13}C$ value of CO_2 may affect the results of the separation [3];

for investigations using this method, growing of a C_4 plant on a C_3 soil or vice versa is necessary.

It is important to note that this difficulty can be overcome in the free air carbon dioxide 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). After the mixing of the supplied CO_2 with CO_2 of the atmosphere, the $\delta^{13}C$ of the mixed CO_2 is about 25–30‰. This ^{13}C value is significantly less than that of the atmosphere air ($^{13}C \approx -7$‰). 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 principal 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. To overcome possible problems with isotopic effects, continuous labeling of plants in $^{13}CO_2$ or $^{14}CO_2$ atmosphere is useful.

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APPENDIX

The symbols and abbreviation of all equations and the figure are:

CO_2^{Total}—total CO_2 efflux from soil;

CO_2^{SOM}—CO_2 originated from soil organic matter;

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);

$cC_3^{SO_2}$—contribution of a C_3 source (SOM) to the total CO_2 efflux (the contribution here and in the following is presented as a portion, not as percentage);

$cC_4^{SO_2}$—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^{CO_2}—$\delta^{13}C$ of the total CO_2 efflux from soil with a C_4 plant;

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

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

δ_3^{SOM}—^{13}C of soil organic matter;

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

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

$cRMR^{RdCO_2}$—contribution of rhizomicrobial respiration to the root-derived CO_2;

cRR^{RdCO_2}—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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