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SOIL BIOLOGY

Separation of Root and Microbial Respiration: Comparison of Three Methods

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Abstract—In a laboratory experiment, the following methods of separating the soil CO_2 flux into the root respiration and the respiration of the rhizosphere and nonrhizosphere microorganisms were compared: (1) root exclusion, (2) component integration, and (3) ^{14}C pulse labeling. Depending on the method used, the combined contribution of the rhizosphere microorganisms and roots varied from 18 to 40% of the total CO_2 emission; the contribution of the roots alone was 8–19%, and that of the nonrhizosphere microorganisms was 51–82%. The nonisotope methods (1 and 2) gave similar results of the separation. The pulse labeling of plants satisfactorily separated the root and microbial respiration, but it is unsuitable for determining the respiration of the nonrhizosphere microorganisms. Advantages and disadvantages of each method are discussed.

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INTRODUCTION

The major sources of carbon dioxide (CO_2) in the soil are plant root respiration (**RR**) and soil microbial respiration (**MR**). The respiration of soil fauna and the emission of CO_2 due to physical and chemical processes are less important than root and microbial respiration. Microbial respiration is subdivided into the respiration of rhizosphere microorganisms (**RMR**) decomposing root rhizoexcreta (exudates, secrets, lysates, necrotic cells, etc.) and the respiration of soil nonrhizosphere microorganisms (**NMR**) decomposing humus and plant residues.

The partitioning of the soil CO_2 flux into the RR and MR is necessary to calculate the budget of carbon (C) in the soil and to assess the soil as a sink or source of CO_2. The separation of the RR and RMR is especially important and methodologically difficult, because the root respiration has no effect on the carbon budget in the soil. Exudates, on the contrary, are an important source of C for the soil organic matter pool and microbial activity.

The CO_2 flux is divided by different methods, which subdivide the soil respiration into root and microbial respiration with different degrees of accuracy. They include methods based on the chemical sterilization of the soil with roots [5, 26, 27], growing of plants under sterile conditions [39], root exclusion [23, 24], and the separate incubation of selected roots, the rhizosphere, and the nonrhizosphere soil [1, 4, 18].

Methods based on the use of ^{13}C and ^{14}C isotopes have also seen considerable recent development. These are the ^{13}C natural abundance method [12, 44], ^{14}C pulse and continuous labeling of plants [33], isotope dilution [11], exudate elution [36, 37], and model rhizodepositon [47].

The methods based on root exclusion, the separate incubation of soil CO_2 sources, and the pulse labeling of plants with ^{14}C find wide use.

The root exclusion method involves the comparison of CO_2 effluxes from a rooted soil and from a soil after the removal of roots. The main disadvantage of the procedure is that it does not separate the contributions of roots and rhizosphere microorganisms. This results in an overestimation of the root respiration. In addition, an initial peak of CO_2 emission appears because of the sample disturbance and enhanced destruction of root residues, regardless of the root removal method [24, 30]. However, this method can be used under field conditions to determine the total contribution of the roots and the rhizosphere microorganisms to the CO_2 efflux from the soil surface.

The component integration method involves the mechanical separation of a soil sample into the components contributing to the soil respiration and the measurement of the rate of the CO_2 emission by each component [1, 4, 18]. For this purpose, roots are separated by hand [13, 22, 45] or washed from the soil [20, 40]; the roots and the soil are then incubated separately (rhizosphere and nonrhizosphere soils are sometimes also separated). Next, the specific rates of the CO_2 emission are multiplied by the weights of the components and summed to obtain the total CO_2 efflux [4, 24].

Table 1. CO_2 sources determined by different methods

Method	Treatments	CO_2 sources
Root exclusion	(a) rooted soil or loess	NMR + RhR
	(b) root-free soil or loess	NMR
Separate incubation of CO_2 sources	(a) soil or loess + roots	NMR + RhR
	(b) rhizosphere soil or loess	RMR
	(c) nonrhizosphere soil or loess	NMR
	(d) selected roots	RR
	(e) washed roots	RR
^{14}C pulse labeling; $^{14}CO_2$ flux simulation	(a) soil with labeled plants	$^{14}CO_2$ from RMR and RR, total CO_2 from soil
	(b) loess with labeled plants	

Some important advantages of the method are its simplicity and lack of need for specialized equipment. Its disadvantage is the disturbance of the soil sample, which affects the CO_2 emission rate and the ratio of the contributions of the different sources after the separation of the sample into components.

The methods based on the use of ^{13}C and ^{14}C isotopes are presently considered the most accurate, because they do not involve the assumptions used in the nonisotope methods [30]. The ^{14}C pulse labeling method is suitable for tracing the emission dynamics of assimilated CO_2 from the rhizosphere and is based on the supposition that, after plant labeling in an atmosphere with $^{14}CO_2$, its emission from the soil due to root respiration begins earlier than that produced by the respiration of the rhizosphere microorganisms [32, 34, 49]. The isotope procedures, their advantages and disadvantages, and the assumptions behind the separation of the total CO_2 efflux into components are discussed in some reviews [24, 30, 35].

The isotope methods are mainly used under laboratory conditions. At the same time, the methods such as root exclusion or separate incubation are most frequently used under field conditions. All the above methods are comprehensively described in the literature; however, no experimental comparison of the most common procedures has been performed until now. In this context, the aim of this work was to simultaneously separate the soil CO_2 flux by three methods under similar experimental conditions. The root exclusion method, the separate incubation method, and the pulse labeling of plants were compared.

EXPERIMENTAL

Soil. The experiment was carried out with a loamy Haplic Luvisol (the soil treatment) and a soil–loess mixture (the loess treatment) under laboratory conditions. Soil samples (Ap, 0–10 cm, C org 1.4%) were taken from a fallow plot of a long-term filed experiment at the Karlshof Experimental Station (University of Hohenheim, Germany). The soil samples were air-dried and sieved through a 2-mm sieve to remove roots and plant residues. Loess (C org 0.3%) was also sampled at the Karlshof Experimental Station from a depth of 150 cm and prepared in the same manner as the soil. A soil–loess mixture was prepared from 5% soil and 95% loess. The soil was added to introduce microorganisms from the natural soil into the loess. The loess treatment was used to decrease the respiration of the nonrhizosphere microorganisms decomposing soil organic matter. Each experimental pot was filled with 2.4 kg of air-dry soil or loess.

Plant growing conditions. The experiments were performed with corn (*Zea mays* L., var. Tassilo). Corn seeds were germinated for 5 days on wet filter paper. Three seedlings were planted in each experimental pot. The plants were grown at 27/20°C (day/night) with a lighting duration of 12 h and at an intensity of 800 mmol/(m^2s). To feed the plants with nutrients, the plants on the loess were irrigated with Ruakura solution [46] at a rate of 1 mg N/kg of soil beginning from the 10th day after the bedding. The plants on the soil were irrigated at the same rate beginning from the 33rd day. Before the beginning of the experiment, the plants were 44 days old. The soil water content in each pot was daily adjusted to 74% of the maximum moisture capacity with distilled water.

Methods of separating the root and microbial respiration. We compared three methods used for separating the root and microbial respiration: (1) root exclusion, (2) component integration, and (3) ^{14}C pulse labeling followed by the simulation of the root and rhizomicrobial respiration dynamics (Table 1). The first method can separate the soil CO_2 flux into the respiration of the nonrhizosphere microorganisms and the respiration of the roots together with the rhizosphere microorganisms. The second method can separately estimate the RR, RMR, and SMR and calculate the combined respiration of the roots and rhizosphere microorganisms. The third method enabled separating the root respiration and rhizosphere microbial respiration; their total contribution to the soil respiration was estimated by calculation.

The root exclusion method. Experiments were performed using 4 experimental treatments: (1) rooted soil, (2) root-free soil, (3) rooted loess, and (4) root-free loess. The root-free soil and loess were incubated under the same conditions as the rooted substrates. The day before measuring the CO_2 flux, the experimental pots were made airtight using NG 3170 silicon paste (Thauer and Co., Dresden). During the experiment, the CO_2 released from the soil and loess was absorbed with a 1 M NaOH solution. The NaOH solution (20 ml) was changed twice a day immediately after turning the light on/off to estimate the daily and nightly emissions of

CO_2. The CO_2 emission rate was measured for 5 days and 12 h. A continuous air flow through the pots with the soil was maintained with membrane pumps (100 cm^3/min). The rate of the CO_2 emission from the soil was calculated using a modified equation [6]:

$$F = 6C_{HCl}(G - G_1)/V_{tit}V_t/t/m, \quad (1)$$

where F is the CO_2 flux (mg C/(g/h), 6 is the scaling coefficient for C (mg), C_{HCl} is the concentration of HCl (mol/l), G is the HCl volume (ml) used for the blank titration, G_1 is the HCl volume (ml) used for the sample titration, V_{tit} is the volume of the NaOH titrated (ml), V_t is the total NaOH volume used for the CO_2 absorption (ml), t is the time of the CO_2 absorption (h), and m is the mass of the soil (g).

The rhizosphere respiration was calculated as the difference between the CO_2 flux from the rooted and root-free soils and expressed as a percentage of the total flux from the rooted soil:

$$C_{CO_2} = (F - G)/F \times 100, \quad (2)$$

where C_{CO_2} is the contribution of the rhizosphere respiration to the total flux (%), F is the total CO_2 flux from the rooted soil (mg), and G is the total CO_2 flux from the root-free soil (mg).

The component integration method. After the end of the pulse labeling experiment, the plants were cut and five combinations of the CO_2 sources were separated: (1) soil + selected roots (S + R sel), (2) rhizosphere soil (RS), (3) nonrhizosphere soil (NS), (4) selected roots (R sel), and (5) washed roots (R wash). The rhizosphere and the nonrhizosphere soils were separated by shaking: the soil remaining on the roots after a slight shaking was taken as the rhizosphere soil. A sample of about 45 g was taken to incubate the S + R sel, RS, and NS. To measure the root respiration, 0.33–1.44 g of selected or washed roots were incubated. The incubation was performed in sealed glass vessels for 8 days 15 h.

The CO_2 released during the incubation was absorbed by 4 ml of 1 M NaOH in a glass beaker placed on the soil surface. The alkali solution was changed at increasing intervals of 7, 14, 48, 50, and 90 h.

The amount of CO_2 released by each pool was calculated using Eq. (1). The contributions of the RS, NS, and roots were determined as the percentages of the total CO_2 flux from the soil. The total CO_2 flux was calculated by two methods: (1) as the CO_2 flux in the treatment with S + R sel and (2) as the sum of the CO_2 fluxes from the RS, NS, and R sel. The contribution of the root respiration was also determined by two methods: (1) by measuring the root respiration (R sel and R wash) and (2) as the difference between the respiration of the treatment with S + R sel and the combined respiration of RS and NS. The contributions of all the CO_2 sources were calculated with account for the mass of each component in the original soil sample:

$$N = K/F \times 100, \quad (3)$$

where N is the contribution of the components (the soil microorganisms, roots, etc.) (%), K is the mean CO_2 flux from each component during the incubation period (mg C/(g/h)), and F is the CO_2 flux from the S + R sel treatment or the calculated flux (mg C/(g/h)).

The ^{14}C pulse labeling method was described in detail by Kuzyakov et al. [2, 34] and Domanskii et al. [15]. The day before the plant labeling, the experimental pots were sealed with silicon NG 3170 paste (Thauer and Co., Dresden). Then, the plants were placed into a Plexiglas chamber [11] for 1 h to assimilate the labeled carbon dioxide. $^{14}CO_2$ was prepared in the reaction of $Na_2{}^{14}CO_3$ with 2.5 M H_2SO_4. For this purpose, the acid was added to a test flask with an $Na_2{}^{14}CO_3$ solution connected to the chamber by a silicon tube and $^{14}CO_2$ was released into the chamber atmosphere. After labeling the plants (1 h), the air from the chamber was pumped through 20 ml of 1 M NaOH for 30 min to determine the amount of unassimilated $^{14}CO_2$. The plants were then removed from the chamber, and the total and labeled CO_2 released from the soil were measured.

The carbon dioxide flux was determined in the same manner as in the root exclusion method. The rate of the CO_2 emission from the soil was calculated using Eq. (1). The amount of ^{14}C released as $^{14}CO_2$ was calculated as a percentage of the total assimilated ^{14}C:

$$A = B \times 100/((C - (R + L))/4), \quad (4)$$

where A is the amount of released ^{14}C (% of the assimilated ^{14}C), B is the activity of the $^{14}CO_2$ sorbed in 20 ml of 1 M NaOH, C is the initial activity of the $Na_2{}^{14}CO_2$, R is the activity of the unassimilated $^{14}CO_2$, L is the residual activity of the $Na_2{}^{14}CO_2$, and 4 is the number of pots with simultaneously labeled plants.

The amount of CO_2 released due to the respiration of the rhizosphere microorganisms and roots was calculated using the following equation [32]:

$$\text{C root} = \text{C shoot}\,{}^{14}C_{CO_2}/{}^{14}\text{C shoot}/T, \quad (5)$$

where C root is the amount of CO_2 released due to the rhizosphere respiration (mg C/(kg h)), C shoot is the amount of C in the above-ground plant organs (g C/kg), $^{14}C_{CO_2}$ is the content of ^{14}C released from the soil as CO_2 (%), ^{14}C shootis content of ^{14}C in the above-ground plant organs (%), and T is the time between the planting and labeling of the plants (44 days).

To separate the fluxes of root and microbial CO_2, the model proposed by Kuzyakov and Domanskii [33] was used. The model consists of 11 carbon pools in the atmosphere–plant–soil system. It was developed for the separation of root and rhizomicrobial respiration fluxes. The use of the model involves the optimization of the exudate release rate and the root respiration rate

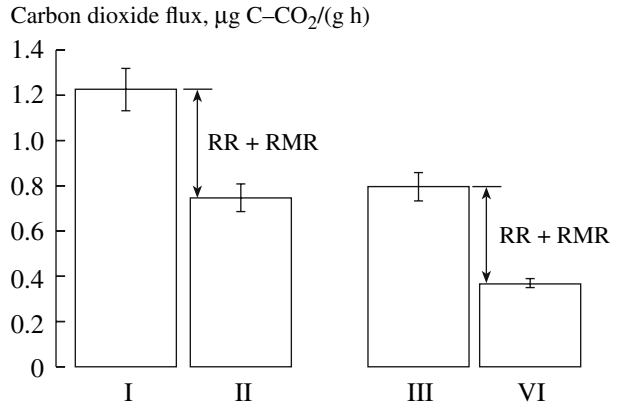

Fig. 1. Rate of carbon dioxide emission from (I, II) soil and (III, IV) loess (I, III) with and (II, IV) without corn plants: average values for 5.5 days (± standard deviation); (RR + RMR) combined respiration of roots and rhizosphere microorganisms.

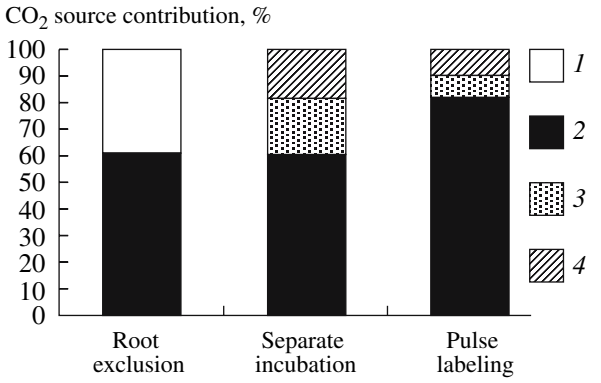

Fig. 2. Contributions of different sources to the total CO_2 flux from soil determined by different methods: (*1*) roots together with rhizosphere microorganisms; (*2*) nonrhizosphere microorganisms; (*3*) rhizosphere microorganisms; (*4*) roots.

on the basis of experimental data on the emission of $^{14}CO_2$ from the soil with plants labeled under a $^{14}CO_2$ atmosphere. Other parameters (13 parameters of the rates and distribution portions) were optimized in a special experiment by Kuzyakov and Domanskii [33] and were considered constant in our study. Simulation after the optimization of all the parameters allows the root respiration and the microbial decomposition of exudates to be calculated independently.

Analytical methods. The total amount of CO_2 absorbed by the NaOH solution was determined by titration with 0.2 M HCl using phenolphthalein as an indicator after the addition of an excess 0.5 M $BaCl_2$ solution [6]. The amount of total nitrogen and carbon in the above- and underground parts of the plants was determined using a Carlo-Erba C–N analyzer.

The ^{14}C activity was measured in 1 ml of NaOH with the addition of 4 ml of Rothiscint-22x scintillation cocktail (Roth Company, Germany) using a liquid scintillation counter (1411 Rackbeta, Wallac) with correction for the chemiluminescence. The efficiency of the ^{14}C count was about 89%; the error of the activity measurements was no higher than 2%.

The radioactivity of the above- and underground plant organs and soil was measured after annealing a 1-g sample in oxygen (using a Canberra Packard Model 307 sample oxidizer) using a scintillation cocktail (Permafluor E+, Canberra Packard Co. Ltd.).

The experiment was performed in four replicates for the treatments with plants and in three replicates for the treatments without plants. The arithmetic mean and standard deviation were calculated for each treatment.

RESULTS AND DISCUSSION

The separation of the soil respiration by the root exclusion method showed that the average rate of the CO_2 flux from the rooted soil was 1.2 ± 0.09 μg C/(g h) over the entire period of the measurements (5 days 12 h). In the treatment without plants, the CO_2 emission rate corresponding to the respiration rate of the nonrhizosphere microorganisms was 0.75 ± 0.06 μg C/(g h) (Fig. 1). The combined respiration rate of the rhizosphere microorganisms and roots calculated as the difference between the treatments with and without plants was 0.48 μg C/(g h). Thus, the combined contribution of the rhizosphere microorganisms and roots in the soil treatment was 39%, and the contribution of the nonrhizosphere microorganisms was 61% of the total CO_2 flux from the soil (Fig. 2). Similar results for different plant species were reported in a review by Hanson et al. [24]. This method was used for a soil with herbaceous plants [43] and a soil under a forest [8, 10, 25, 48]. The carbon dioxide emission rate from the loess was reliably lower than that from the soil. The average rate of the CO_2 emission was 0.8 ± 0.06 and 0.37 ± 0.02 μg C/(g h) for the rooted and root-free loess, respectively. The combined respiration of the rhizosphere microorganisms and roots in the loess (0.43 μg C/(g h)) was only slightly lower than that in the soil. This difference may be attributed to the additional decomposition of humus in the rhizosphere due to the priming effect of the soil treatment. As a result, the contribution of the nonrhizosphere microorganisms and the combined contribution of the roots and rhizosphere microorganisms in the loess were 47 and 53% of the total CO_2 flux from the rooted loess, respectively.

The difference between the soil and loess respiration (0.43 and 0.38 μg C/(g h) in the treatments with and without plants, respectively) was due to the decrease in the respiratory activity of the nonrhizosphere microorganisms decomposing soil organic matter. This indicates that soils with different humus content can have different ratios between the contributions of the root and microbial respiration. The respiration of the nonrhizosphere microorganisms in the loess was lower

than in the rooted soil by a third and lower than in the root-free soil by half.

The root exclusion method gives very approximate estimates of the root respiration. In many earlier studies, the difference between the respiration of rooted and root-free soils was taken to be equal to the root respiration. This is obviously not true, because the exclusion of the roots excludes both the RR and RMR. Even more, the presence of root exudates in the soil stimulates the additional mineralization of soil organic matter related to the so-called priming effect [16, 31]. In our opinion, this method determines the total respiration of the plant roots and rhizosphere microorganisms.

The main advantages of the root exclusion method are its simplicity, its lack of need for specialized equipment, and the possibility of using it under field conditions. The main disadvantage is that the method does not separately estimate the contributions of the RR and RMR; i.e., it does not separate the respiration of the autotrophic and heterotrophic organisms. In addition, an initial peak of the CO_2 emission appears regardless of the method of the root removal. Some authors relate this phenomenon to the enhanced destruction of mobile soil organic matter [7] or to the destruction of root residues [24]. Both processes are possible. The return of the system to the equilibrium state after the disturbance of the soil sample requires time.

It is believed that measurements of the CO_2 flux over a year would decrease the effect of the soil disturbance compared to the measurements performed immediately after the removal of the roots [8, 21]. However, new roots can grow under conditions of long-term field observations [19]. The preliminary removal of the plant residues and the long-lasting incubation of the soil in experimental pots (without disturbance of their equilibria) before the beginning of the measurements allowed us to avoid the appearance of the initial peak of the CO_2 emission and the growth of roots. However, we considered neither the additional emission of CO_2 due to the effect of the root exudates on the decomposition of the soil organic matter nor the effect of the rhizosphere microorganisms on the root activity.

In most of the earlier studies, the separation of the soil respiration by the separate incubation method involved incubation for 2–48 h [4, 14], because a longer incubation can increase the contribution of roots due to the autolysis of root cells. It was also shown that the disturbance of the soil sample entails an initial increase in the CO_2 emission rate, and the return of the system to the equilibrium state requires time. To estimate the change in the carbon dioxide emission rate during the incubation, measurements were performed for 8 days 15 h. The carbon dioxide flux rates are given in Table 2. Observations of the CO_2 emission dynamics after the beginning of the incubation showed that the disturbance resulted in a nonuniform increase in the respiratory activity of each source. In the first 7 h, the respiration rate of each source exceeded its average rate for the entire incubation period (8 days 15 h) by a factor of 1.1–3. Then, the flux rate decreased, the highest decrease being observed during the first day after the beginning of the incubation. According to Edwards [17], the return of the system to the equilibrium state takes 2 days. In our case, the decrease in the emission rate to its average value occurred within 1.5–3 days and varied among the sources. The greatest decreases in the respiratory activity were observed for the rhizosphere soil (by 5.4 times over the incubation period) in the soil treatments and for the rhizosphere (by 3.1 times) and nonrhizosphere soil (by 4.5 times) in the loess treatments (Fig. 3). These abrupt decreases in the respiratory activity were due to the consumption of readily

Table 2. Rate of CO_2 emission from different sources (μg C/(g h), the average value for the entire incubation period)

CO_2 source	Treatment	
	soil	loess
S + R sel	3.0 ± 0.9	1.5 ± 0.7
NMR	1.2 ± 0.5	1.1 ± 0.4
RMR	1.7 ± 0.8	1.1 ± 0.3
R sel	211 ± 66	135 ± 30
R wash	177 ± 66	136 ± 31

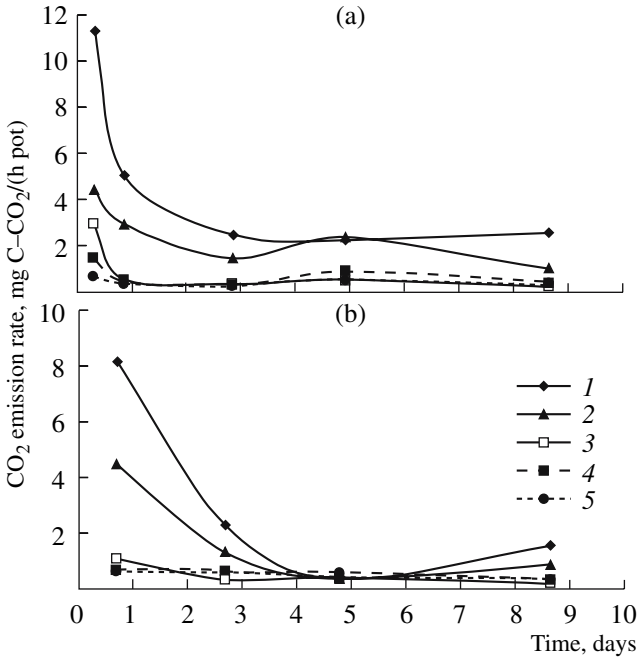

Fig. 3. Dynamics of CO_2 emission by incubated components from (a) soil and (b) loess: (*1*) soil + roots; (*2*) nonrhizosphere soil; (*3*) rhizosphere soil; (*4*) selected roots; (*5*) washed roots.

Table 3. Contribution (%) of different sources to the CO_2 emission from soil (above the line) and loess (under the line) depending on the flux taken as 100% and the period of CO_2 measuring

CO_2 source	Over the entire incubation period		In the first 7 h	
	S + R sel	total flux (RS, NS, and R sel)	S + R sel	total flux (RS, NS, and R sel)
Rhizosphere soil (RS)	17.5/17.8	20.9/15.7	26.4/13.3	33.4/14.9
Nonrhizosphere soil (NS)	50.9/75.4	60.5/66.9	39.4/67.5	49.9/75.5
Selected roots (R sel)	15.7/19.6	18.6/17.4	13.2/8.6	16.7/9.6
Total	81.4/112.8	100/100	79.0/89.0	100/100
Washed roots (R wash)	12.6/19.6	15.6/17.4	8.0/7.9	10.9/8.9

available organic matter. Approximately on the fifth day of the incubation, an increase in the respiratory activity was noted for the treatments with washed roots or roots selected from the soil, but it was not statistically reliable.

In the treatments with rooted loess, a gradual decrease in the respiratory activity was observed during the entire incubation period, which was also within the standard deviation of single measurements. Thus, the autolysis of cells had no significant effect on the contribution of the roots during a long-lasting incubation.

Powlson [42], Larionova [3], and some other authors showed that the disturbance of the sample increases the release of CO_2. To assess the changes in the respiration of the disturbed soil sample, the total flux from the incubated sources should be compared with the flux from the undisturbed soil under natural conditions [24]. We compared the emission of CO_2 from the incubated treatments with that from the pots with undisturbed soil. It was shown that the average flux rate from the S + R sel treatment exceeded the average rate of CO_2 emission from the undisturbed substrate by 2 times for the loess and by 2.5 times for the soil. The total flux from the RS, NS, and R sel also exceeded the flux from the undisturbed treatment by 1.3 and 1.9 times. The respiration of the nonrhizosphere soil exceeded the flux from the undisturbed root-free substrate by 1.7 and 3 times for the soil and loess, respectively. Thus, the disturbance of the soil sample significantly affected the CO_2 emission rate and resulted in a disproportional change in the respiratory activity of each source.

The results of the determination of the contribution from each source to the total soil CO_2 flux are significantly affected by the method of calculating the total CO_2 flux, which is taken as 100%, and the duration of the measuring period (Table 3). In our work, the total flux was calculated by two methods: (1) as the CO_2 flux from the S + R sel treatment and (2) as the sum of the fluxes from the RS, NS, and R sel. When the respiration of the S + R sel treatment was considered as the total soil CO_2 flux, the sum of the obtained contributions was not equal to 100%. The disturbance of the soil sample affected the respiratory activity and the ratio of the CO_2 sources; therefore, their total respiration was not equal to the respiration of this treatment. When the sum of the CO_2 fluxes was considered to be the total soil flux, it was initially taken as 100% (Fig. 2).

The contributions calculated from the average emission rates for the entire incubation period and for the first 7 h are given in Table 3. It was found that the contributions of the rhizosphere and nonrhizosphere soils in the soil treatment strongly depended on the measuring period. The contribution of the rhizosphere soil to the total flux was higher during the first 7 h and decreased when it was calculated for the entire incubation period. This was due to the consumption of all the root exudates present in the rhizosphere by microorganisms. The average contribution of the other CO_2 sources, on the contrary, was higher than its value calculated for the first 7 h. In the loess, the average contributions of all the sources were higher than their values calculated for the first 7 h; the contributions of the selected and washed roots changed by almost two times.

The roots were selected from the soil by hand [13, 22, 45] or washed [20, 40]. The washing removed the rhizosphere microorganisms from the root surface, but the roots were overmoistened. The selection by hand prevented overmoistening, but the presence of soil particles and rhizosphere microorganisms on the surface of the roots was ignored in this case [9, 13, 22]. To avoid the overmoistening of the roots, Larionova [3, 4, 38] calculated the root respiration as the difference between the CO_2 fluxes from rooted and root-free soils. In this case, the root respiration could be overestimated because of the RMR, and the effect of the root exudates on the additional mineralization of the soil organics could not be assessed. In our work, the calculated root contribution was 32%, which agrees with the results of Larionova. Selection and washing result in the loss of the fine and most actively respiring roots. We found that the respiration of the selected and washed roots differed only in the soil treatment: the respiration of the washed roots was lower. This result showed that the method of root isolation had no significant effect on the contribution of the roots to the total CO_2 flux under the experimental conditions.

Table 4. Distribution of assimilated ^{14}C among the pools (mean ± standard deviation), % of the total ^{14}C

Treatment	C pools				
	leaves	roots	soils	released CO_2	total
Soil	74.4 ± 3.6	11.6 ± 2.2	3.9 ± 0.2	10.1 ± 1.4	100
Loess	73.4 ± 2.9	13.9 ± 3.2	3.2 ± 0.7	9.4 ± 4.4	100

The analysis of our results and the data of other authors show that the important advantages of the selected incubation method are its simplicity and no need for specialized equipment. This procedure allows a separate, although approximate, estimation of the respiration of autotrophic and heterotrophic organisms. A disadvantage of the method is the disturbance of the soil sample. This produces some adverse consequences, including changes in the CO_2 emission rate compared to the undisturbed soil and in the contributions of different sources after the separation of the sample into components.

The use of the pulse labeling procedure showed that the plants assimilated 99.9% of the $^{14}CO_2$ introduced into the chamber. The distribution of ^{14}C among the pools is given in Table 4. At the end of the experiment, a significant part of the ^{14}C (75%) was found in the above-ground plant organs. About 12% of the labeled carbon got into the plant roots, and only 4% got into the soil.

The beginning of the ^{14}C emission from the soil as CO_2 was already noted in the first samples of the NaOH taken 9 h after the labeling; the $^{14}CO_2$ flux from the soil reached its maximum values in 12–24 h (Fig. 4). This agrees with the literature data. Thus, Cheng [11] showed that $^{14}CO_2$ began to be released from the soil as early as 30 min after the labeling. The total amount of ^{14}C released from the soil as CO_2 for 5 days was about 10% of the assimilated ^{14}C. About half this amount was released in the first two days. This showed that the assimilated C was rapidly utilized for the respiration of the roots and rhizosphere microorganisms.

According to the Kuzyakov and Domanskii model [33], the contribution of the root respiration prevailed in the $^{14}CO_2$ flux from the soil during the first 24 days after the assimilation. The microbial release of $^{14}CO_2$ began 6–12 h after the assimilation and reached its maximum two days after (Fig. 4). The experimentally measured total $^{14}CO_2$ flux from the soil was used as an initial parameter for the model. On its basis, the root respiration and the respiration of the rhizosphere microorganisms were simulated. Taking into account the period of the $^{14}CO_2$ absorption, the first model was calculated for 5.5 days after the labeling. It was found that the root respiration reached 54%, and the respiration of the rhizosphere microorganisms reached 46% of the total $^{14}CO_2$. The further development of the root and microbial respiration was also calculated for 12 days, because the contribution ratio of the roots and microorganisms to the labeled CO_2 flux changed if the period of absorption was prolonged [24, 41]. On the 12th day after the ^{14}C assimilation, the contribution of the roots decreased to 46% (by 10%) and the contribution of the rhizosphere organisms increased to 54% of the total CO_2 (Fig. 5). Thus, the contributions changed depending on the duration of the CO_2 flux measuring period. From the reported data obtained using this method, the root respiration varied from 17 to 61% (the average value being 41–45%) and the respiration of the rhizosphere microorganisms varied in the range of 44–60% of the total $^{14}CO_2$ flux [15, 32–34].

The combined contribution of the rhizosphere microorganisms and the roots to the CO_2 flux from the soil was estimated using Eq. (5). The combined respiration of the rhizosphere microorganisms and the roots in the soil treatment made up 18% of the total CO_2 flux, and the respiration of the nonrhizosphere microorganisms made up 82%. In the loess treatment, the total respiration of the roots and the rhizosphere microorganisms reached 27%, and the contribution of the nonrhizosphere microorganisms was 73%. Analogously to the two above methods, the contribution of the nonrhizosphere microorganisms in the loess treatment decreased, and the ratio of the root and microbial respiration changed. With account for the ratio of the root

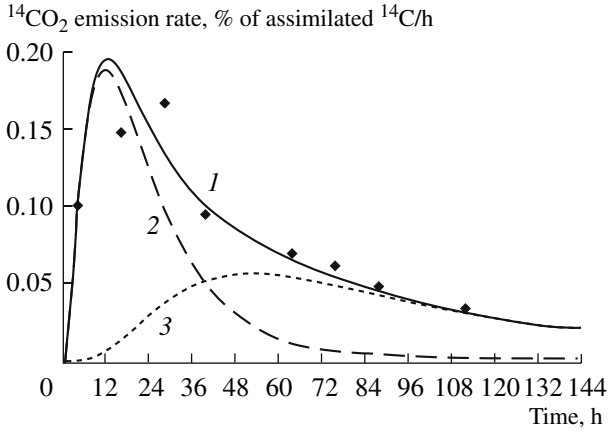

Fig. 4. Dynamics of CO_2 emission from rooted soil after pulse labeling and the simulation of root and microbial contributions to the total rhizosphere $^{14}CO_2$ flux: (*1*) total $^{14}CO_2$ flux (experimental); (*2*) root $^{14}CO_2$ flux; (*3*) microbial $^{14}CO_2$ flux.

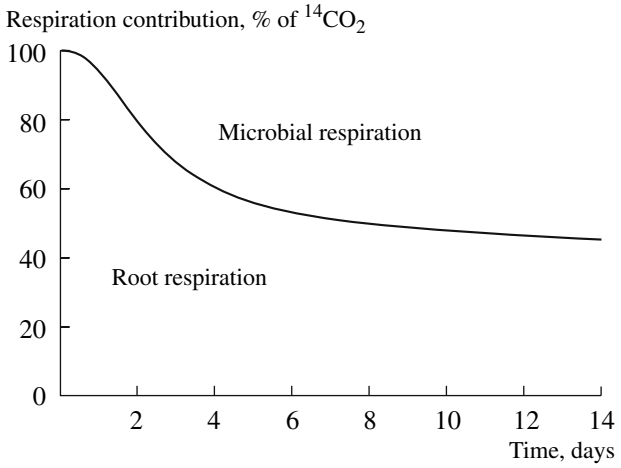

Fig. 5. Contributions of root and rhizosphere microbial respiration to the $^{14}CO_2$ flux from the rhizosphere as a function of the measurement time.

and microbial respiration derived from the model, their contributions to the total CO_2 flux from the soil made up 9.7 and 8.3% of the total CO_2 flux from the soil (Fig. 2) and 15 and 12% from the loess.

The methods based on the use of ^{13}C and ^{14}C are considered presently most accurate. The ^{14}C pulse labeling method has an important advantage over the other, nonisotope methods; it permits one to separate the rhizosphere CO_2 flux into the root and rhizomicrobial respiration, which is difficult without isotope techniques. In addition, this method is possible without disturbing the natural state of the soil [24, 33, 35].

The use of specialized equipment and high-priced analyses is a common disadvantage of all isotope-indicator methods. An important disadvantage of pulse labeling is that the contribution of the root respiration and the respiration of the rhizosphere microorganisms can be estimated only against the $^{14}CO_2$ flux and not against the total CO_2 flux from the soil. Thus, the contribution of the rhizosphere to the emission of CO_2 from the soil surface cannot be directly estimated. This can be related to the fact that the distribution of ^{14}C from the labeling moment to the end of measuring the CO_2 flux does not correspond to the long-term distribution of C among the plant pools [28, 47, 50]. Therefore, the contribution of the rhizosphere to the total CO_2 flux can be estimated only approximately under the pulse labeling conditions.

CONCLUSIONS

The comparison of three methods of separating the total soil CO_2 flux into the component sources showed that the combined contribution of the rhizosphere microorganisms and roots to the total CO_2 flux from the soil surface varied from 18 to 50% and the contribution of the plant roots themselves varied from 8 to 32% depending on the method used. The emission of CO_2 by the nonrhizosphere microorganisms made up 50–80% of the total CO_2 flux from the soil.

The ratio of the root and microbial respiration can vary depending on the content of organic matter in the soil.

The results obtained in the separation of the CO_2 flux by the separate incubation method and the root exclusion method well agree and can be used for comparing the results of different authors.

The ^{14}C pulse labeling method can be used for separating the CO_2 flux into the root and rhizomicrobial respiration. However, only approximate separation of the total CO_2 flux from the soil into the respiration of the rhizosphere microorganisms together with the roots and the respiration of the nonrhizosphere microorganisms can be achieved.

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