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SOIL BIOLOGY

Contribution of Rhizomicrobial and Root Respiration to the CO_2 Emission from Soil (A Review)

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Abstract—Separate determination of root respiration and rhizomicrobial respiration is one of the most interesting, important, and methodologically complicated problems in the study of the carbon budget in soils and the subdivision of the CO_2 emission from soils into separate fluxes. In this review, we compare the main principles, the advantages and disadvantages, and the results obtained by the methods of component integration, substrate-induced respiration, respiratory capacity, girdling, isotope dilution, model rhizodeposition, modeling of the $^{14}CO_2$ efflux dynamics, exudates elution, and the $\delta^{13}C$ measurements of the microbial biomass and CO_2. Summarizing the results of the determinations performed by these methods, we argue that about 40% of the rhizosphere CO_2 efflux is due to root respiration and about 60% of this efflux is due to the respiration of microorganisms decomposing root exudates.

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INTRODUCTION

The separation of root respiration from rhizomicrobial respiration is one of the most interesting and methodologically complicated problems in the study of the carbon budget in soils and the subdivision of the CO_2 emission from soils into separate fluxes [6, 27]. Root respiration exerts no effect on the balances of energy and carbon in soils. Rhizomicrobial respiration, being associated with microbial decomposition or root exudates to CO_2, makes it possible to judge the amount of root exudates that serve as an important substrate for microorganisms and a source of substances for the renewal of soil humus. Separate quantitative evaluation of these two fluxes of the rhizosphere CO_2 and, hence, the determination of the amount of root exudates are necessary for solving the following problems:

- the assessment of the carbon balance in soils and plants,
- the assessment of the amount of organic substances available for microorganisms in the rhizosphere,
- a full account of humification sources,
- the distinction between respirations of the heterotrophic and autotrophic organisms,
- the study of mechanisms responsible for the priming effect in the rhizosphere, and
- the modeling of the carbon cycling in ecosystems.

Despite the importance of the problem, at present, there are no commonly accepted methods for the separate determination of root respiration and rhizomicrobial respiration.

In previous years, the respiration of the rhizosphere microorganisms was determined after the sterilization or fumigation of the soil together with plants [5]. It was assumed that the sterilizing substances do not have a significant effect on root respiration. In the course of special studies of the effect of fumigation, it was found that the absence of the rhizosphere microorganisms leads to considerable changes in the amount and qualitative composition of root exudates [5, 23]. Therefore, the methods based on the soil sterilization cannot be used for an adequate evaluation of root exudates and root respiration. Later, in the 1990s, a number of approaches were suggested to distinguish between root and rhizomicrobial respiration in a nonsterile soil [7].

According to different estimates, the portion of root respiration in the sum of root and rhizomicrobial respiration varies from 16% [25] to 94% [42]. Surely, the values obtained by different methods fit this huge diapason. The great variability of the values obtained can be partly explained by the diversity of the studied types of soils and ecosystems. However, as different methods were used in these studies, it is impossible to estimate the effects of particular methods of the determination of root respiration on the results obtained. A valid comparison of the methods is only possible on the basis of simultaneous determinations of the root and rhizomicrobial respiration by different methods on the same objects. In this context, one of the goals of our review of the methods is a comparison of the results obtained by different methods.

Various approaches that are used for the separate determination of the root respiration and rhizomicro-

bial respiration are analyzed in this paper; their advantages and disadvantages are discussed in order to suggest the most reasonable methods for further studies. The review of the earlier obtained results will make it possible to give a more adequate assessment of these two CO_2 fluxes and, thus, to facilitate expert evaluation of the processes mentioned above.

TERMS

The definitions of the particular components of the total CO_2 emission from soil are somewhat different in different works. In our review, we use the terms and their definitions suggested in [36].

Root respiration (RR) is the respiration of roots proper necessary as the source of energy for metabolic processes and for sustaining the gradient in concentrations of substances in root cells, the growth of the roots, and the active uptake of nutrients by them. Root respiration represents the only significant respiratory flux from autotrophic organisms in soil.

Rhizomicrobial respiration (RMR) is the respiration of microorganisms decomposing rhizodepositions, including their most labile part, root exudates.

Basal respiration (BR) is the respiration of microorganisms decomposing soil organic matter without plant roots.

Microbial respiration (MR) is the sum of the RMR and basal respiration.

Priming CO_2 is the CO_2 flux releasing upon the altered (accelerated or decelerated) decomposition of soil organic matter in the rhizosphere.

Rhizosphere respiration is the sum of RR, RMR, and priming CO_2.

THE ROOT EXCLUSION METHOD

The first attempts to separate the total efflux of CO_2 from soil into separate fluxes were generally based on the comparison of the respiration intensities in soils with plants and in fallow soils [37]. This method makes it possible to separate the basal soil respiration from the sum of the other three fluxes (i.e., from rhizosphere respiration). It should be noted that, earlier, the notion of root respiration was often applied to denote the rhizosphere respiration (see below). Lundegardth [37] found that the total efflux of CO_2 from a soil under oats exceeds the soil respiration under fallow by 30%. Investigations performed by Makarov [12] demonstrated that the CO_2 emission from a soil under crops and perennial herbs exceeds the CO_2 emission from the soil under fallow by 1.2–2.0 times. As well as in the study by Lundegardth [37], the average rhizosphere respiration in soddy-podzolic and peat bog soils during the growing period was estimated at about one-third of the total CO_2 emission from the soil surface [13].

To distinguish between the RR and MR, as well as to determine the total CO_2 emission from the soil surface, it is necessary to conduct measurements directly in the field with minimal disturbances of the soil. On the cropland, the determination of the soil respiration under fallow makes it possible to estimate the contribution of the MR to the total soil respiration without additional soil disturbances. In the natural forest and meadow cenoses, the preparation of a fallow plot—the setting of some restraints stopping the growth of roots or soil digging with the removal of living roots—involves considerable disturbances in the water, air, and temperature regimes of the soil [24]. Thus, the main advantage of the root exclusion method—the possibility to determine the RR directly in the field—vanishes upon the transition from agrocenoses to natural phytocenoses.

The major disadvantage of this method in both cases (in agrocenoses and in natural phytocenoses) is that it cannot take into account the contribution of the rhizosphere soil to the MR. The respiration of soil microorganisms under plants and under fallow plots, even in the case of an agrocenosis, may only be similar at the very beginning of the growing season. With the growth of plants and their root systems, the difference between the soil respiration under the plants and under the fallow is not only due to the RR under the plants but also due to the additional CO_2 flux from the decomposition of the root exudates; in other words, this difference is the sum of the RR and RMR. The basal respiration of a soil under plants may also differ from the basal respiration of the same soil under fallow due to positive and negative priming effects on the soil humus [31]. Hence, the determination of the RR from the difference between the soil respiration under plants and under fallow is not quite correct. In fact, the rhizosphere respiration is determined by this method. However, the values of the RR (30–50% of the total CO_2 emission from the soil surface) obtained by this method in the 1950s–1970s are still used as expert estimates of the contribution of the RR to the total soil respiration [2, 6, 14]. This method is still used in field studies of the carbon budget in soils and ecosystems. The new averaged estimates of the contribution of the RR to the total soil respiration that were obtained by Hansson with coauthors [24]—45% in forest ecosystems and 60% in nonforest phytocenoses—reflect the summary effect of the RR and RMR rather than give their separate estimates.

In the present review, we shall compare the field and laboratory methods making it possible to separate the rhizosphere flux of CO_2 from soils into the RR and RMR (the respiration of rhizosphere microorganisms decomposing rhizodepositions).

THE COMPONENT INTEGRATION METHOD

The method of separate incubation, or the method of component integration (as it is referred to in foreign

works), has become popular in the 1970s as an alternative to the root exclusion method [21, 22, 40, 41]. The subdivision of soil respiration into separate fluxes by the method of component integration is performed in laboratories on fresh soil samples taken under plants, including rhizosphere soil and plant litter. The samples are manually separated into roots, soil, and plant remains. Weighed portions are subjected to incubation in hermetically sealed vessels under laboratory conditions, and the specific respiration of each of the components (respiration per unit mass) is determined from the amounts of CO_2 accumulated in the vessels during 2–24 h. The contribution of the RR is calculated according to the following equation:

$$\%RR = r_r m_r/(r_r m_r + r_s m_s + r_{pr} m_{pr}) \times 100, \quad (1)$$

where r_r, r_s, and r_{pr} are the specific respirations of the roots, soil, and plant remains, respectively; and m_r, m_s, and m_{pr} are the masses of the roots, soil, and plant remains in the sampled soil monolith, respectively. The contributions of the soil and plant remains to the integral emission are calculated in the same manner.

Initially, the weighed portion of roots was washed with water [21]. In order to avoid additional moistening of the root tissues and the loss of some roots in the course of the washing, this procedure was later replaced by the manual separation of the roots [22, 41]. In the latter case, as well as in the case of the soil washing off from the roots, the finest and metabolically active roots cannot but be torn off. Therefore, in our investigations, we also used the whole soil sample with the roots. To estimate the RR, the difference between the respiration of the soil with the roots and the soil with manually removed roots was determined [8–10].

The calculations of the root growth and the RR measured by the incubation method showed that the RR constitutes about 20–50% of the total carbon consumed in the course of the growth and respiration of the roots, i.e., of the carbon of photosynthates translocated into the roots [9]. The contribution of the RR to the CO_2 emission from the soil surface varied from 10 to 58% in dependence on the soil type and the character of vegetation [9]. The annual contribution of the RR to the total CO_2 emission from the soil surface under a meadow cenoses comprised 33%, which was close to the value of 37% obtained by Tesarova [43] in the course of similar determinations of the annual dynamics of the RR under meadow vegetation by the incubation method and close to the results obtained by the isotope dilution method (see below) [19]. The annual contribution of the RR under a mixed forest comprised 13% of the total CO_2 emission from the soil surface [10]. This value was significantly lower than the values reported in other works, because we took into account only the respiration of the fine roots of herbs, shrubs, and trees. The specific respiration of the coarse roots in the studied ecosystem comprised 0.03–0.05 mg C–CO_2/g per hour, and it was an order of magnitude lower than the specific respiration of the fine roots (0.1–0.3 mg C–CO_2/g per hour [10]. However, the contribution of the coarse roots to the total RR was significant, because the mass of coarse roots exceeded the mass of the fine roots by ten times [1, 44]. Hence, the contribution of fine roots to the total RR is comparable with the contribution of the coarse roots. In this context, the value of the RR obtained in [10] by the incubation method has to be increased by two times to take into account both the fine and coarse roots.

The seasonal dynamics of the RR in the mixed forest (Fig. 1) was very distinct. The maximum contribution of the RR to the total CO_2 emission from the soil surface was observed in June and July; in the winter, the contribution of the RR was negligibly small. In the meadow cenosis, the RR comprised nearly 50% of the total CO_2 emission during the period of active growth of meadow herbs; in the winter, it comprised 10.6% of the CO_2 emission from the soil surface. A relatively high value of the RR under the meadow cenosis in the winter season could be due to the methodological limitations of the method, as it was necessary to separate the plant roots into the groups of living roots and dead roots. This was done manually on the basis of the visual examination of the character of the roots. It is probable that a considerable part of the RR in the winter period was due to the respiration of soil microorganisms decomposing dead roots.

In general, the incubation method does not allow one to clearly distinguish between the RR and MR, because, upon all the techniques to determine the RR by this method (the determination of the respiration of the washed roots or of the manually separated roots and the determination of the respiration in the bulk soil sample with roots and in the soil sample with manually removed roots), the MR on the surface of living and dead roots is registered as RR. To distinguish between the RR and MR, the method of substrate-induced respiration can be used.

THE METHOD OF SUBSTRATE-INDUCED RESPIRATION

The determination of the RR with the use of a glucose solution in concentrations of about 0.5–1.0 mg/g of soil with roots was suggested by Panikov with coauthors [18]. In essence, this is a modification of the incubation method: the RR is estimated on the basis of a comparison of the soil respiration with the roots and soil respiration with thoroughly removed roots. The content of soluble carbohydrates in the root tissues varies from 5 to 50 mg/g of dry roots [10, 16], which is much higher than the concentration of added glucose. The separation of the RR from the MR is based on the fact that the respiration of the soil microorganisms limited by the amount of easily available substrate increases by several times upon the addition of glucose, whereas the RR remains at the same level. The root res-

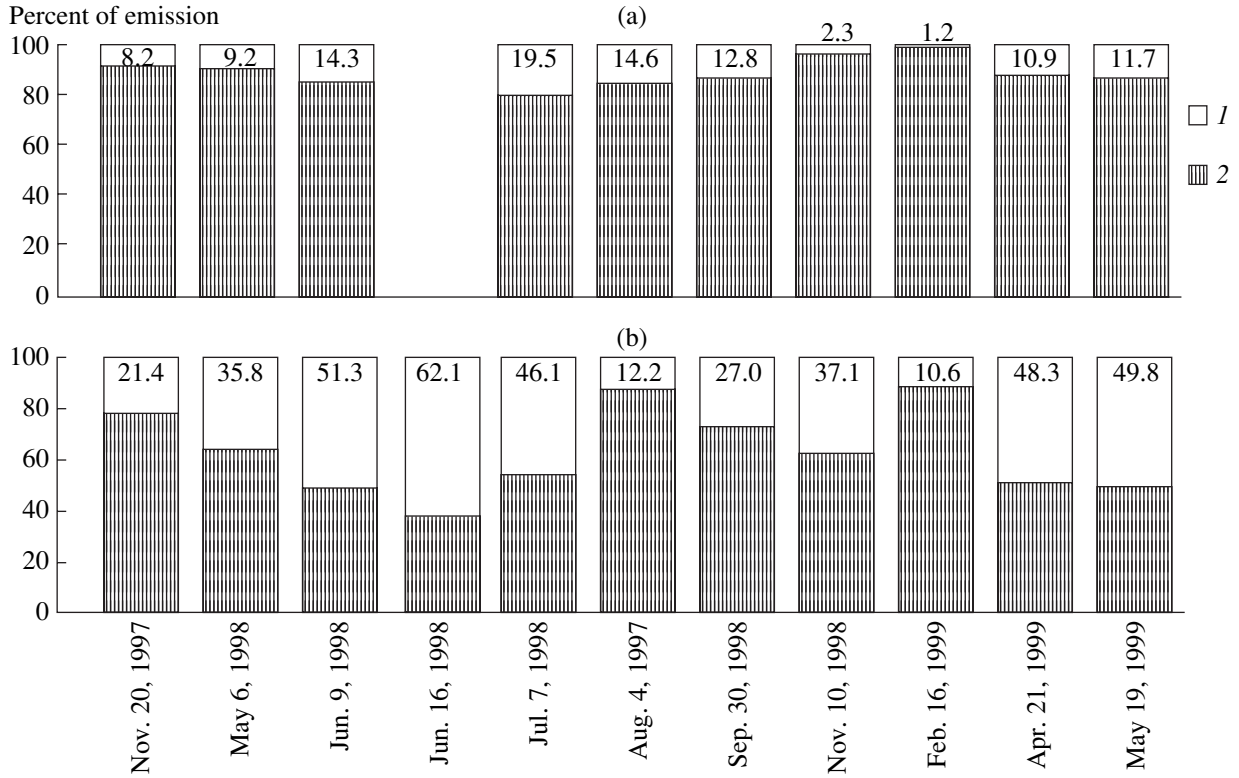

Fig. 1. Contributions of (*1*) root respiration and (*2*) microbial respiration to the CO_2 emission from soddy podzolic soils under (a) forest and (b) meadow (according to [9]).

piration and MR are calculated via the following system of equations [18]:

$$V1 = V_r + V_{mb},$$
$$V2 = V_r + kV_{mb}, \quad (2)$$

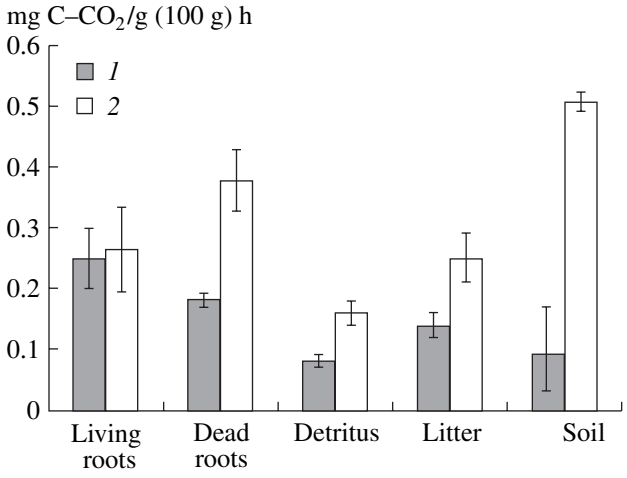

Fig. 2. Specific soil respiration (mg CO_2–C/h × 100 g), root respiration, and microbial respiration upon the decomposition of plant remains (mg CO_2–C/h g) (*1*) before and (*2*) after the addition of glucose (1 mg/g). The vertical lines correspond to the standard deviation values (according to [10]).

where $V1$ and $V2$ are the intensities of the CO_2 efflux before and after the addition of glucose to the soil sample with roots, respectively; and V_r and V_{mb} are the intensities of the RR and MR, respectively. Soils samples with thoroughly removed roots are used to determined the coefficient of the increase in the soil respiration after the addition of glucose:

$$k = V2_{mb}/V1_{mb}, \quad (3)$$

where $V1_{mb}$ and $V2_{mb}$ are the intensities of the CO_2 efflux before and after the addition of glucose into the soil without roots.

The addition of a glucose solution to the weighed portions of soil, roots, and plant remains led to the increase in the CO_2 emission (Fig. 2), but the coefficients characterizing this increase were different for the different components. Thus, the coefficient for the decomposing plant remains (plant litter, phytodetritus, and dead roots) varied from 1.5 to 3.0; for the soil, it varied from 2 to 10 [10]. The addition of glucose to living roots increased their respiration by only 2–5%. Such a low increase was due to the decomposition of glucose by microorganisms and the to activity of exoenzymes left on the surface of the roots after their washing.

Under similar ecological conditions, the coefficient of the increase in respiration after the addition of glucose is generally lower for plants than for the soil. In the

soils of natural cenoses with considerable amounts of phytodetritus and dead roots, the k value should be lower than in the soils with thoroughly removed roots. Therefore, to distinguish between the separate CO_2 fluxes from the soil, some averaged coefficient necessary for solving the system of equations (2) has to be found. In order to find it, soil monoliths with a natural proportion between the soil mass and the mass of plant remains should be used. To determine the k in soil samples with plant remains, the living roots should be thoroughly removed from the samples. As this procedure is labor-consuming, the soil respiration before and after the addition of glucose can be determined in small weighed portions of the soil without roots and of the plant remains. Then, taking into account the masses of the soil and of the plant remains in the monolith, k can be calculated according to the following equation:

$$k = (v2_s m_s + v2_{pr} m_{pr})/(v1_s m_s + v1_{pr} m_{pr}), \quad (4)$$

where $v1_s$ and $v2_s$ are the specific respirations of the soil before and after the addition of glucose, respectively; $v1_{pr}$ and $v2_{pr}$ are the specific respirations upon the decomposition of plant remains before and after the addition of glucose, respectively; and m_s and m_{pr} are the masses of the soil and of the plant remains in the soil monolith, respectively.

Under conditions of the low field moisture of the soil, the addition of a glucose solution changes the content of water in root tissues and, hence, affects the RR. The addition of dry glucose increases the intensity of the soil and litter respiration within a sufficiently large range of the water content values (from 10 to 80% of the field capacity) [10], which allows us to consider this way of the addition of glucose as the optimum way that does not affect the natural moisture content in the soil and in the plant litter. The highest coefficient k was observed at the moisture content of 60% of the field capacity both for the soil and for the forest litter. At the very low moisture content (about the permanent wilting point), as well as the high moisture content (>100% of the field capacity), the k values for the soil and for the forest litter were about 1.0 and lower. The absence of an increase in the respiration intensity after the addition of glucose does not make it possible to apply the method of substrate-induced respiration under conditions of a long-term drought or to waterlogged soils.

The contribution of herb roots to the total CO_2 emission as determined by the incubation method with glucose application proved to be higher for the meadow cenosis (>40%) than for the agrocenoses (about 30%) [17, 18]. The determination of the RR immediately after the addition of dry glucose in a series of soil wetting–drying cycles showed that the RR of the meadow vegetation is highly tolerant towards the soil drying [11]. In the periods of short-term droughts, it amounted to 65% of the total CO_2 emission, whereas in the periods with optimum soil moistening, it was equal to 35–40% of the total CO_2 emission.

The incubation method does not allow a researcher to judge reliably about the RMR, which is a significant disadvantage of this method. In agrocenoses, it is possible to remove the rhizosphere soil from the main soil mass via mechanical shaking off of the soil from the roots. This technique, having its own limits, allows one to separate the rhizosphere soil as a particular source of the CO_2 emission. In meadow ecosystems, the mechanical shaking is inefficient, because the entire upper horizon is densely penetrated by roots. In the gray forest soil under meadow vegetation, the rhizosphere respiration exceeded the basal respiration only in the layer of 10–20 cm; in the layer of 0–10 cm, these two respiration fluxes were almost equal [10]. The substrate-induced respiration method also does not make it possible to separate the rhizosphere respiration from the respiration in the nonrhizosphere soil. The coefficient of the increase in the respiration intensity after the addition of glucose in the rhizosphere and nonrhizosphere soil samples was equal to 6.1 and 5.3, respectively. These values are relatively close to one another and do not make it possible to reliably determine the rhizosphere and nonrhizosphere respirations.

The substrate-induced respiration method is an advanced modification of the incubation method. However, it does not eliminate the main problem related to this method, which is the determination of the RR and MR in the disturbed soil samples. The disturbance of the natural soil morphology immediately before the incubation leads to an increased CO_2 production by the soil microorganisms and to changes in the RR [2, 8]. The extent of these changes in the respiration activity of the soil microorganism and roots upon the disturbance of the soil remains unknown. In the corresponding calculations, it is assumed that both respiratory fluxes increase in the same way. This supposition has to be verified in further studies. It is necessary to compare the portions of the RR and MR in the total CO_2 emission from the surface of the disturbed and undisturbed soils and to trace the dynamics of changes in these values during the long-term (for several days) incubation of the soil and roots. This comparison may be performed via simultaneous determination of the RR and MR by the incubation and isotope dilution methods. The latter is based on labeling carbon assimilated by plants with $^{14}CO_2$ with further study of the translocation of the labeled carbon into the roots and into the soil. At present, field methods to distinguish between the RR and MR and isotopic methods that are mostly used to study the RR and MR under laboratory conditions are being developed in parallel. To validate the RR values obtained in the field experiments, it will be reasonable to compare the results obtained by the field method and by the laboratory isotopic method under controlled conditions.

DETERMINATION OF THE RESPIRATORY CAPACITY AND THE RESPIRATORY COSTS OF ROOT RESPIRATION

The method of determination of the respiratory capacity of roots and other parts of plants in order to calculate the use of assimilated carbon for plant respiration is discussed here. This method is used in field studies of the role of respiration in the photosynthetic production of phytocenoses [3, 15, 16]. On experimental plots, 25–30 plants are carefully excavated, together with their roots, down to a depth of 20 cm. The monoliths of soil, together with the plants, are transported to a laboratory, and the soil is washed off from the roots. In the course of this washing, dead roots and lignified parts of the subsurface plant organs are removed. In order to determine the respiration intensity, the aboveground and underground parts of the plants are placed in a chamber, and the CO_2 evolution in the air flux is measured with the help of an infrared gas analyzer at 25°C within 10–20 min [15, 16]. To calculate the respiration activity of the roots under field conditions, the measurements are repeatedly performed with time intervals of 10–15 days. In parallel, the study of the temperature dependence of root respiration in the interval from 10 to 30°C is performed. The respiratory costs (RCs) between two consecutive measurements are calculated according to the following equation:

$$RCs = (RC_i + RC_e)(M_i + M_e)\, QT(n/2), \qquad (5)$$

where RC_i and M_i are the respiratory costs and the biomass of the roots at the beginning of the experiment, respectively; RC_e and M_e are the respiratory costs and the biomass of the roots at the end of the experiment, respectively; Q is the ratio of the intensity of the root respiration at the average temperature of the root tissues to the intensity of the root respiration at 25°C; T is the duration of the dark period (for the roots, it is equal to 24 h); and n is the duration of the period between two separate measurements. The Q value is determined from the temperature curve; the average temperature of the root tissues is taken equal to the average temperature of the soil.

With the help of this method, the dynamics of the root respiration were studied in the agrocenoses of wheat [15] and oats [3]; the root respiration dynamics of steppe grasses were studied during the entire growing season [16]. In the course of these experiments, the root respiration was studied as a part of the respiration of the entire plant; the basal soil respiration and microbial respiration of the rhizosphere microorganisms were not measured. The results obtained by this method can be compared with the results obtained by other methods if we recalculate them as the portion of the root respiration in the total amount of the carbon assimilated by the plants, or the amount of carbon translocated into the roots. The respiratory costs of oat roots during the growing season comprised 3% of the total amount of the assimilated carbon [3]. The respiratory costs of wheat roots comprised 10% of the assimilated carbon and 30–45% of the carbon translocated into the roots [15]. The respiratory costs of the roots of steppe grasses were higher than those of the crops. During the growing season, 60% of the carbon translocated into the roots of feather grasses (*Stipa rubens* and *S. lessingiana*) and Koeleria (*Koeleria gracilis*) was spent for root respiration; for fescue (*Festuca sulcata*), this value was even higher (76%) [16]. During the growing season, the respiratory costs of the root respiration in the agrocenoses of wheat and in the steppe phytocenoses varied from 30 to 113% of the amount of translocated carbon in dependence on the particular phase of the plant development. The values exceeding 100% were associated with the beginning of the decomposition of the roots against the background of the low translocation of carbon or its absence in the periods after flowering and during ripening. It should also be noted that the root exudates were not taken into account during the determination of the carbon translocation by this method.

We argue that this method determines the potential rather than the actual root respiration. The potential root respiration is significantly higher than the actual root respiration under the field conditions, because of the certain disturbances of the roots during their preparation for incubation and because of the unnatural conditions for the roots in the incubation chamber.

GIRDLING METHOD

The method of girdling was recently suggested by Hogberg with coauthors [26]. It is based on paring the phloem of a tree trunk, so that the flux of photoassimilates from the leaves into the roots and soil is stopped. Though the authors of this method suggest that it makes it possible to determine the root respiration, we argue that the girdling method ensures the determination of the rhizosphere respiration without its separation into the root respiration and the rhizomicrobial respiration.

The experiment was performed on large (900 m^2) plots on which 120 trees were growing. The phloem was pared on all the trees at a height of 1.5 m above the soil surface. The calculation of the rhizosphere respiration in this case is based on the determination of the difference between the CO_2 emission from soil on the control plot (without girdling) of the same size and on the test plot. Thus, in essence, the girdling method is a variant of the root exclusion method, and it has all the advantages and disadvantages of the latter. An important advantage of the girdling method is that this method is performed in the field over relatively large areas without the soil cover disturbance. Taking into account the great variability of the vegetation in forest ecosystems, an assessment of the root respiration on an area with 120 trees is an important advantage in comparison with the incubation method. At the same time, this method is not quite satisfactory, as the cessation of

the flux of assimilates into the soil reduces not only the root respiration but also the root exudation. Hence, this method, as well as the root exclusion method based on the determination of the soil respiration on a plot with roots and on a plot without roots, allows a researcher to evaluate the total effect of the root respiration and the respiration of rhizosphere microorganisms. In forest ecosystems, the latter are mainly represented by mycorrhizal fungi. In five days after the beginning of the experiment, the rhizosphere respiration in the investigated pine forest comprised 23–37% of the total CO_2 emission. In longer experiments (two–seven weeks), this portion increased up to 52–56% CO_2, which means that the cessation of the translocation of assimilates into the roots and into the soil is a gradual process that develops for several weeks. Therefore, this method is unsuitable to study root respiration and rhizomicrobial respiration in their dynamics (for example, their study with the use of the same plants during the growing season is impossible). The contribution of the root respiration and the respiration of the rhizosphere microorganisms to the total CO_2 emission from the soil surface was assessed by the authors of this method at 54%. This value can be considered a tentative estimate of the rhizosphere respiration. The cessation of the flux of assimilates into the roots stimulates the mobilization of reserved carbohydrates. According to the authors of this method [26], the decrease in the starch content in the root tissues and the starch utilization for the respiratory activity of the roots after the girdling correspond to carbon losses equal to 10–20% of the CO_2 efflux from the soil. The cessation of the translocation of assimilates into the roots and the depletion of the reserved carbohydrates favor the intensive decay of the roots, which leads to the dissimilar heterotrophic respiration on the control and test plots. Herbs and shrubs also contribute to root respiration. In the discussed experiment, this contribution remained unknown, because only the trees were girdled. Thus, a costly and labor-consuming girdling procedure that can only be used in countries with large forest areas does not make it possible to distinguish between the root respiration and rhizomicrobial respiration; more over, even the sum of these two respiration fluxes cannot be reliably determined.

Below, a series of methods based on the use of carbon isotopes is described. Except for the natural ^{13}C abundance method, both the ^{14}C and ^{13}C isotopes can be used in these methods. The application of ^{14}C is more feasible because of the higher sensitivity and lower cost of the corresponding analyses. Most of these methods are still used under laboratory conditions only, which is related to the impossibility to control some of the parameters in the field and due to the labor intensiveness of the methods.

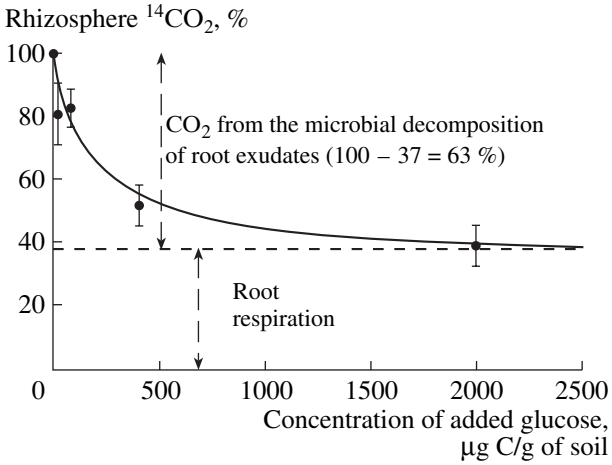

Fig. 3. The principle of the separation of the rhizosphere CO_2 into the root respiration and rhizomicrobial respiration using the method of isotope dilution with unlabeled glucose (according to [33]).

ISOTOPE DILUTION METHOD

This method is based on the isotope dilution of ^{14}C-labeled exudates of plants that assimilated $^{14}CO_2$ with unlabeled glucose [19]. The plants are labeled in a chamber with different concentrations of $^{14}CO_2$ in the air; the soil is treated with increasing amounts of unlabeled glucose. The $^{14}CO_2$ releasing from the soil upon the microbial decomposition of the labeled root exudates is diluted with unlabeled CO_2 appearing in the soil upon the decomposition of the unlabeled glucose (Fig. 3). The soil microorganisms decompose both the labeled exudates and the unlabeled glucose. At the same time, the $^{14}CO_2$ released upon the root respiration is not diluted (see the discussion on substrate-induced respiration above). Therefore, increasing amounts of added glucose lead to some limit of the $^{14}CO_2$ dilution, which corresponds to the root respiration (Fig. 3). Taking into account the experimental data on the decrease in the $^{14}CO_2$ emission with the increasing concentration of added glucose, the theoretical curve can be calculated according to the following equation [32]:

$$^{14}CO_2\ (\%) = (100 - RR)\exp(-k[Glu]) + RR, \qquad (6)$$

where $^{14}CO_2$ (%) is the decrease in the $^{14}CO_2$ evolution in percent of the control without glucose, RR is the root respiration, and k is an experimentally determined coefficient linking the decrease in the $^{14}CO_2$ evolution with the glucose concentration [Glu].

The basic principle of this method is relatively simple. However, its use involves a number of assumptions and difficulties. One of the main problems is to ensure an even distribution of added glucose in the soil with roots. Also, this method assumes that the ratio between the root respiration and the rhizomicrobial respiration should be constant after the $^{14}CO_2$ labeling. In fact, it

was found that this ratio is changeable and depends on the time elapsed after the labeling [28, 30] and on the time of day [29]. If the ratio between the root respiration and the rhizomicrobial respiration is not constant but changes with time, the results of the determination should depend on the particular time and duration of the measurements of the $^{14}CO_2$ efflux.

According to this method, the root respiration of wheat plants comprises 41% of the rhizosphere respiration, the portion of the rhizomicrobial respiration being equal to 59% [19].

THE MODEL RHIZODEPOSITION TECHNIQUE

The model rhizodeposition technique is based on a comparison of the $^{14}CO_2$ emission from the soil with plants labeled with $^{14}CO_2$ and the soil with added artificial rhizodepositions labeled with $^{14}CO_2$ [42]. In the first case, the $^{14}CO_2$ emission represents a sum of the root respiration and the microbial decomposition of the $^{14}CO_2$-labeled root exudates. In the second case, it only consists of the flux resulting from the microbial decomposition of the $^{14}CO_2$-labeled rhizodepositions. It is assumed that, in both cases, the ratio between the $^{14}CO_2$ released due to the microbial respiration and the $^{14}CO_2$ remaining in the soil (i.e., incorporated into the microbial biomass and the soil organic matter) is the same:

$$^{14}CO_2\text{–}MR_{RZD}/^{14}C\text{–}Soil_{RZD} = {}^{14}CO_2\text{–}PMR_{Plant}/^{14}C\text{–}Soil_{Plant}, \quad (7)$$

where $^{14}CO_2$–MR_{RZD} and $^{14}CO_2$–PMR_{Plant} are the activities of the emitted CO_2 in the variants with the model rhizodepositions and with the labeled plants, respectively; and ^{14}C–$Soil_{RZD}$ and ^{14}C–$Soil_{Plant}$ are the activities of the ^{14}C remaining in the soil after the experiment. The CO_2 of the rhizomicrobial respiration in the variant with the labeled plants is calculated according to Eq. (7) from the data on the activities of the $^{14}CO_2$ and ^{14}C in the soil with the artificially added rhizodepositions and on the amount of ^{14}C remaining in the soil in the variant with the labeled plants. The difference between the total amount of the released CO_2 and the calculated amount of CO_2 from the rhizomicrobial respiration in the variant with the labeled plants indicates the contribution of the root respiration.

The use of this method showed that the root respiration constitutes 89–94% of the rhizosphere respiration, the portion of the rhizomicrobial respiration being equal to 5–11%. We suppose that this method overestimates the root respiration and underestimates the rhizomicrobial respiration because of the following reasons. First, the spatial distribution of the added model rhizodepositions in the soil is different from the localized distribution of the natural exudates. This leads to difference in the rate and completeness of the consumption of the natural exudates and the artificially added rhizodepositions. The rhizosphere microorganisms stimulate the release of root exudates, whereas the addition of model rhizodepositions is independent of the activity of the rhizosphere microorganisms. The consumption of root exudates by the rhizosphere microorganisms takes place on the surface of the roots, which minimizes the loss of this substrate upon its adsorption by clay minerals and the soil organic matter. Second, as shown in our work [32] and as follows from the method of isotope dilution [19], the effect of the dilution depends on the amount of artificially added rhizodepositions. Thus, the principle of the method of isotope dilution contradicts the basic principles of the model rhizodeposition technique.

As well as in the previous method, the even distribution of the added rhizodepositions should be ensured.

EXUDATES' ELUTION PROCEDURE

This method is based on elution of exudates from the soil with a constant flow of water and the parallel trapping of the CO_2 emitted due to the root respiration [29]. It is supposed that the exudates are eluted from the soil before being decomposed by microorganisms. Exudates' elution and the trapping of the $^{14}CO_2$ from the root respiration are performed in a hermetic system, which makes it possible to calculate the carbon balance in it. As well as in the two previous methods, the plants are preliminarily labeled in air with $^{14}CO_2$ in order to distinguish between the C from the soil and the C from the plants in the eluted exudates and in the emitted CO_2.

This method proved that the contribution of the rhizomicrobial respiration is no less than 19%, and the contribution of the root respiration does not exceed 81%. The proportion between the ^{14}C in the root exudates and in the emitted CO_2 varied considerably in dependence on the time of the day [29].

A serious disadvantage of this method is that the exudates are incompletely eluted from the soil; some part of the exudates may be partially decomposed during the elution, which leads to the overestimation of the root respiration and the underestimation of the rhizomicrobial respiration. However, this is the only method that allows one to separate the two carbon fluxes in reality (physically). All the other methods are based on calculations rather than on the physical separation of the CO_2 fluxes. This is an important advantage of the elution of the exudates technique; it can be used for the separation and identification of root exudates in soil rather than in a nutrient medium, as it is often done in the studies on plant physiology.

MODELING OF THE $^{14}CO_2$ EFFLUX DYNAMICS

This method is based on the study of the dynamics of the $^{14}CO_2$ efflux from the rhizosphere of plants labeled with $^{14}CO_2$ [28, 30]. It is hypothesized that after

the one-time labeling of the plants in the air with $^{14}CO_2$, the $^{14}CO_2$ emission from the soil due to the respiration takes place earlier that the $^{14}CO_2$ emission due to the rhizomicrobial respiration (Fig. 4). This lag in time appears because the second process includes several stages: (1) the release of exudates from the roots into the rhizosphere, (2) the consumption of the exudates by microorganisms, and (3) the decomposition of the exudates by the microorganisms to $^{14}CO_2$. Therefore, the final emission of the $^{14}CO_2$ due to the rhizomicrobial respiration takes place later than the emission due to the root respiration.

The partitioning of the $^{14}CO_2$ emission flux into the particular components is based on the dynamic model of carbon fluxes in the rhizosphere [32] after the proper selection of the parameters characterizing the rate of the root respiration and the rate of the release of the root exudates. These parameters of the model should fit the experimental data on the dynamics of the $^{14}CO_2$ emission from the soil. After the parameters are selected, the dynamics of the root respiration and the rhizomicrobial respiration are modeled by separate models, and the ratio between them is determined.

The use of this method showed that the root respiration constitutes from 41 [28] to 56% [32] of the rhizosphere respiration.

In contrast to the method of isotope dilution (see above), this approach suggests certain changes in the relative contributions of the root respiration and the rhizomicrobial respiration to the total $^{14}CO_2$ emission with time elapsed after the one-time assimilation of $^{14}CO_2$ by the plant leaves. The use of variable values of the root respiration during the calculations makes this method an alternative to the method of isotope dilution. However, it should be noted that the experimental validation of this basic assumption of the method has yet to be performed.

NATURAL ^{13}C ABUNDANCE METHOD

Recently, a new method based on the natural differences in the concentrations of ^{13}C isotope in plants with C_3 and C_4 photosynthesis has been suggested [34, 35]. This method assumes the growing of some C_4 plants (e.g., corn) on soil that was formed under the impact of C_3 vegetation, or vice versa. The $\delta^{13}C$ values have to be determined in the four major pools of carbon in the system: the soil organic matter (formed under the impact of C_3 plants, δ_3^{SOM}); the plant (C_4) roots, δ_4^{Roots}; the microbial biomass (δ^{MB}, and the emitting CO_2 (δ^{CO_2}) [34, 35]:

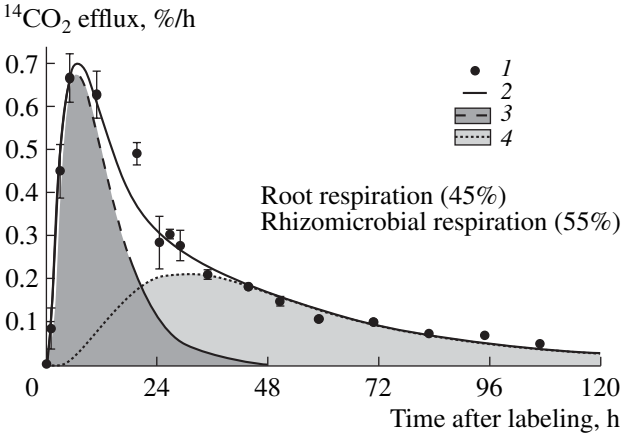

Fig. 4. The principle of the separation of the rhizosphere CO_2 into the root respiration and rhizomicrobial respiration using the $^{14}CO_2$ efflux dynamics modeled after the impulse labeling of the plant (according to [33]). Designations: (*1*) $^{14}CO_2$ efflux in the experiment, (*2*) the modeled values and the separation of the emitted $^{14}CO_2$ into (*3*) the root respiration, and (*4*) the rhizomicrobial respiration.

$$\%RR = \frac{(\delta^{CO_2} - \delta^{MB})(\delta_3^{SOM} - \delta_4^{Roots})}{(\delta_4^{Roots} - \delta^{MB})(\delta_3^{SOM} - \delta^{CO_2})} \times 100,$$
$$\%RZR = \frac{(\delta_3^{SOM} - \delta^{MB})(\delta_4^{Roots} - \delta^{CO_2})}{(\delta_4^{Roots} - \delta^{MB})(\delta_3^{SOM} - \delta^{CO_2})} \times 100, \quad (8)$$

where %RR and %RZR are the portions (%) of the root respiration and the rhizomicrobial respiration in the rhizosphere respiration, respectively. These equations were theoretically derived from the fact that the ratios between ^{13}C and ^{12}C isotopes in the CO_2 released upon the root respiration and upon the rhizomicrobial respiration should be equal to the ratios between these isotopes in the roots and in the microorganisms, respectively. The correspondence of $\delta^{13}C$ in the root respiration to $\delta^{13}C$ in the roots themselves was experimentally proved by Cheng [20] for wheat grown on sand without organic matter. A study by Santruckova with coauthors [38] stated that the $\delta^{13}C$ values in the CO_2 released due to the respiration of the soil microorganisms may be somewhat different from the $\delta^{13}C$ in the microbial biomass; however, this difference normally does not exceed 2‰.

This method has none of the disadvantages of the previous methods; it can be used under laboratory conditions and in the field. The main drawback of this method is the complexity of the separation of the rhizosphere microbial biomass from the nonrhizosphere microbial biomass, which is important as these biom-

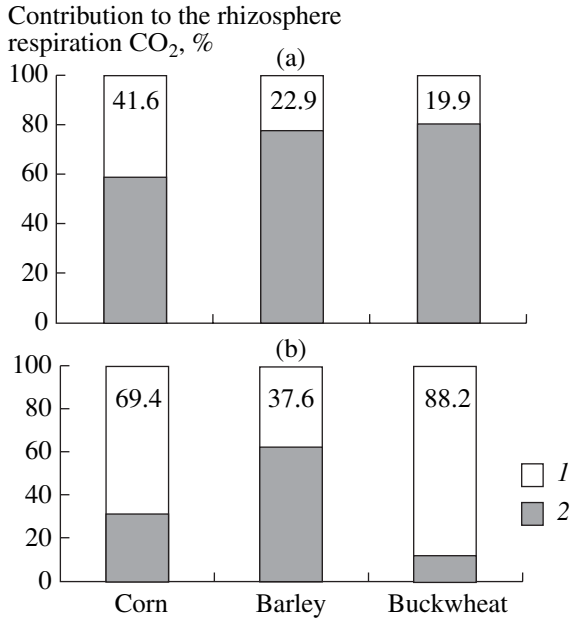

Fig. 5. The contribution of (*1*) root respiration and (*2*) rhizomicrobial respiration to the CO_2 emission from the gray forest soil as determined with the (a) incubation and (b) root exclusion methods (according to [10]).

asses should be characterized by different $\delta^{13}C$ values. Also, the accuracy of the method is relatively low, because of the natural impact of the environment on the isotope discrimination processes, including the variation of $\delta^{13}C$ in the CO_2 derived from the microbial respiration. However, the low accuracy of the method may be eliminated if we use continuous artificial labeling of grown plants in the atmosphere with $^{14}CO_2$ or $^{13}CO_2$. Continuous artificial labeling of the plants offers an opportunity to use this method not only for the C_4 plant–C_3 soil pair (or vice versa) but also for any combination of plants and soils. Currently, this approach is being subjected to experimental testing.

COMPARISON OF THE METHODS

A comparison of the root exclusion method with the incubation method applied on the same cropland demonstrated a significant difference between the values of the RR obtained by these methods (Fig. 5). The intensity of the respiration in the soil under the fallow was significantly lower than the intensity of the microbial respiration determined by the incubation method. Therefore, the contribution of the RR to the total CO_2 emission from the soil determined by the root exclusion method (Fig. 5) was considerably higher than that determined by the incubation method [10]. The difference between the RR values obtained by these methods is a tentative estimate of the rhizomicrobial respiration. For the soils under corn, barley, and buckwheat, this difference was equal to 28, 15, and 68%, respectively (Fig. 5). These are approximate estimates of the rhizomicrobial respiration, because the respiration of the nonrhizosphere microorganisms (the basal respiration) upon the decomposition of the soil organic matter under the crop may differ from that under the fallow [31].

The intensity of the CO_2 emission from the fallow soil depends on the duration of the fallow stage, i.e., the duration of the period during which the soil does not receive easily decomposable organic substances. In the determination of the RR by the root exclusion method under buckwheat and barley, the fallow of the same year was used; for buckwheat, a ten-year-old fallow was used [10]. The soil respiration from the short-term fallow was 1.5–3.0 lower than that under the crop; in the case of the long-term fallow, it was almost ten times lower. Under the long-term fallow, the mineralization of the labile soil organic matter takes place, which leads to a significant decrease in the basal respiration. Hence, the high contribution of the rhizomicrobial respiration under the buckwheat (68%) is explained by the low intensity of the basal respiration under the compared long-term fallow rather than by the specific biological properties of this crop. Thus, the root exclusion method overestimates the contribution of root respiration to the total CO_2 emission due to the lower activity of the basal respiration in the soil of the long-term fallow. The determination of the root respiration and the microbial respiration by the incubation method is free from this drawback of the root exclusion method, because freshly sampled soil under plants is used for the incubation.

To compare the methods of isotope dilution, model rhizodeposition, exudates' elution, and the $^{14}CO_2$ efflux dynamics, an experiment was performed with plants (*Lolium perenne* L.) grown on gray forest soil (a Haplic Luvisol). In different variants of the experiment, different methods were used to discriminate between the root respiration and the rhizomicrobial respiration [33]. It was shown that the portion of the root respiration comprised 40% (and, hence, the portion of the rhizomicrobial respiration comprised 60%) in the experiment based on the method of isotope dilution with unlabeled glucose (Fig. 6). In the case of the method of model rhizodepositions, the root respiration decreased from 83 to 71% of the rhizosphere respiration with an increasing dose of glucose. The portion of the root respiration determined by the method of exudates' elution did not exceed 79% of the total *Lolium perenne* rhizosphere CO_2 flux. The method based on modeling of the $^{14}CO_2$ efflux dynamics gave a value of the root respiration of 45%. With an increase in the duration of $^{14}CO_2$ measurements, the portion of the root respiration somewhat decreased. Thus, despite the different principles of the methods of the isotope dilution and the $^{14}CO_2$ efflux dynamics, these two methods give similar and the most probable results. According to them, the root respiration constitutes about 40% of the rhizosphere

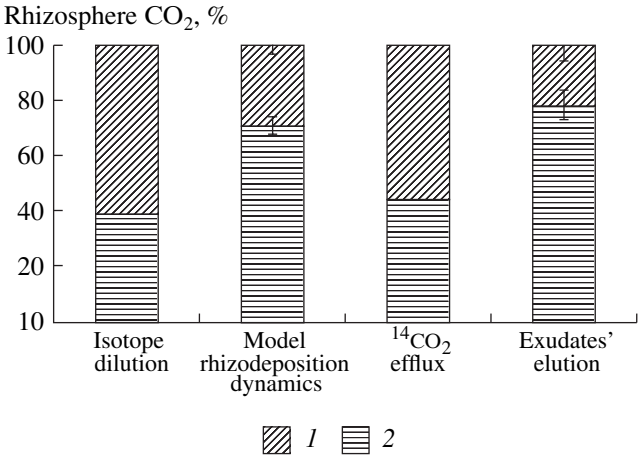

Fig. 6. Separation of the rhizosphere CO_2 into (*1*) the rhizomicrobial respiration and (*2*) the root respiration as determined by the methods of isotope dilution, modeling of the rhizodeposition, the $^{14}CO_2$ efflux dynamics, and the exudates' elution (according to [33]).

respiration, the remaining 60% being due to the rhizomicrobial respiration.

The comparison of both the isotopic and nonisotopic methods for similar experimental conditions showed that the methods themselves may be an important source of considerable differences between the values of the root and rhizomicrobial respirations obtained by different authors. Another source of these differences is related to natural factors (the differences in the types of soils and plants, the phases of the vegetation, etc.). Therefore, the experimental validation of the methods is necessary.

The comparison between these two groups of methods in the experiment with corn [39] demonstrated that the method based on the $^{14}CO_2$ efflux dynamics gives results similar to those obtained by the component integration method (incubation method). In both cases, the portions of the root respiration and rhizomicrobial respiration were approximately equal to 50% of the rhizosphere respiration. However, the contribution of the rhizosphere respiration to the total CO_2 emission from the soil as determined by the isotopic method was lower than that determined by the root exclusion and incubation methods.

Hence, further experiments are necessary to compare the different methods for separate determination of the root respiration and the rhizomicrobial respiration.

CONCLUSIONS

Nine methodological approaches used to discriminate between root respiration and rhizomicrobial respiration have been evaluated. Some of them are relatively simple, which makes it possible to apply them directly in the field to study the carbon budget in different ecosystems. Other methods are complicated and labor-consuming, and they can only be applied in laboratories and for herbaceous plants. Each of the methods has its own advantages and disadvantages, and it is difficult to indicate a universal method suitable for different purposes. All the methods are based on certain assumptions that can hardly be verified in the experiments.

Thus, in order to develop a detailed model of the carbon budget in the rhizosphere, the search for new methods should be continued. It is also important to compare the existing methods on the same object and to estimate the possibility of extrapolation of the results obtained in laboratories to field conditions.

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