PARTITIONING OF ROOT AND MICROBIAL RESPIRATION IN SOIL: COMPARISON OF THREE METHODS

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ABSTRACT
Three techniques for separation of total CO_2 efflux from soil into root and microbial respiration were compared: component integration, root exclusion and pulse labelling of shoots in $^{14}CO_2$ atmosphere. The contribution of rhizosphere to total CO_2 efflux from soil varied from 19 to 49% (including root respiration amounted to 9-32%). The share of non-rhizosphere respiration was 51-80%. The results obtained by component integration and root exclusion techniques were similar. Rhizosphere respiration estimated by pulse labelling were less as estimated by two non-isotopic methods.

INTRODUCTION
Two main agents are responsible for CO_2 efflux from soil: roots and soil microorganisms. Separation of soil CO_2 efflux into actual root respiration and microbial respiration is very important for evaluation of the soil as source or sink of atmospheric CO_2. For partitioning of CO_2 efflux from soil, various methods are used and obtained results differ one from others. The aim of this study was to compare three methods allowing partitioning soil CO_2 efflux into root and microbial respiration: root exclusion, component integration, and ^{14}C pulse labelling.

MATERIALS AND METHODS
Maize (*Zea maize* L., var, Tassilo) was grown on loamy Haplic Luvisol (Ap, 0-10 cm, C_{opr} 1.4%, 2.4 kg per pot) under 27/20 °C day/night temperature and 12 h photoperiod. The soil water content was adjusted daily to 74% of the WHC. Before the start of the method's evaluation, maize was 44 days old.

The component integration method involves physical separation of the soil constituents contributing to CO_2 efflux followed by measurements of specific CO_2 efflux rates of each constituent. Following constituents were separated and tested by incubation in closed jars: soil with roots (S+R), rhizosphere soil (RS), non-rhizosphere soil (NRS), separated roots (SR), washed roots (WR). Total CO_2 efflux considered as 100% was calculated by two ways: 1) as CO_2 efflux from variant S+R, and 2) as the sum of CO_2 efflux from RS, NRS and SR.

The root exclusion method is based on the comparison of CO_2 effluxes from rooted and root-free soil. The contribution of C fluxes was expressed as percentage of total CO_2 efflux from planted soil.

The ^{14}C pulse labelling method is based on the dynamics of $^{14}CO_2$ efflux from rooted soil after ^{14}C pulse labelling of shoots and modelling of C fluxes in the rhizosphere. The method assumes that $^{14}CO_2$ respired by roots appears earlier than $^{14}CO_2$ respired by microorganisms decomposing rhizodeposits.

RESULTS AND DISCUSSION
Three different methods allowing the separation of soil CO_2 efflux into root respiration and microbial respiration were compared under the same environmental and experimental conditions. Thus, the observed differences between investigated methods can be only attributed to the methods themselves.

Component integration method. CO_2 efflux rates from individual constituents averaged over incubation period (8.6 days) from S+R, RS, NRS, SR, WR were 3.0, 2.0, 1.3, 211.2, and 172.5 µg C g^{-1} h^{-1}, respectively. During the first 7 hours, the rates from the individual CO_2 sources exceeded their average rates for 1.6-3 times. The CO_2 efflux rate decreased to average values during 1.5 – 3 days. The strongest decrease of CO_2 efflux rates was recorded during the first day after the start of incubation and was maximal for RS (5.4 times between the first and the last days). We compared the sum of CO_2 efflux from each constituent with the CO_2 efflux from undisturbed pots. The average rate of CO_2 efflux from S+R exceeded the total CO_2 efflux from undisturbed rooted soil by 2.5 times. The sum of CO_2

efflux from RS, NRS and SR also exceeded total CO_2 efflux from undisturbed rooted soil by 1.9 times. The CO_2 efflux from NRS exceeded that from root-free soil by 1.7 times.

The component integration method showed that contribution of constituted CO_2 fluxes varied depending on many factors. Other studies used component integration showed that root contribution varies from 5 – 10% [*Phillipson*, 1975; *Nakatsubo et al.,* 1998] to about 90% [*Flanagan and Van Cleve*, 1977; *Johnson et al.,* 1994]. If CO_2 efflux from S+R was considered as 100%, the contribution of NRS amounted to 50.9% and the contribution of RS was 17.5% (Fig. 1). The share of picked roots was 15.7% and was close to that of washed roots (12.6%). However, if the sum of CO_2 efflux from all constituents was used as 100%, the contribution of NRS amounted to 60.5%, the contribution of RS was 20.9%, the share of SR in total efflux was 18.6%, and WR amounted to 15%.

Root exclusion method. Over the CO_2 trapping period (5.5 days), average CO_2 efflux rate from planted soil was 1.2±0.09 μg C g^{-1} h^{-1} and that from unplanted soil (corresponding to non-rhizosphere microorganisms (SMR) respiration) was 0.75±0.06 μg C g^{-1} h^{-1}. Therefore, the rhizosphere respiration (RhR) rate calculated as difference was 0.48 μg C g^{-1} h^{-1} (RhR = 39% and SMR = 61%; Fig. 1). Other studies showed that contribution of rhizosphere CO_2 estimated by this method varied from 13% of total soil CO_2 efflux [*Catricala et al.,* 1997] to 90% (*Thierron and Laudelout,* 1996], and amounts on average to 54%. However, the most studies used root exclusion has been conducted under forest. Because of difficulty of partitioning RR and RS, this method does not allow separate estimation of root respiration. Only Kelting et al. [1998] separated total soil CO_2 efflux into RR (32%), RS (20%), and NRS (48%). One important shortcomings of the root exclusion is initial CO_2 flush following mechanical disturbance. By using pre-treated soil we avoided the initial flush by removing plant residues.

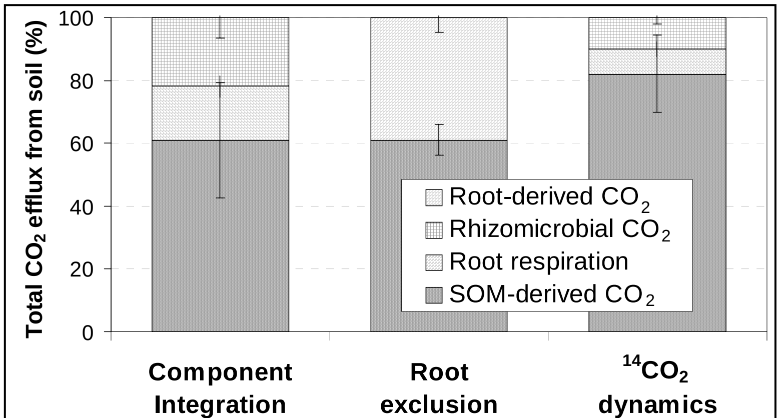

Fig. 1. Comparison of the results of separation of root respiration and rhizomicrobial respiration of maize grown on non-sterile loamy Haplic Luvisol obtained by three methods: 1) component integration, 2) root exclusion and 3) $^{14}CO_2$ dynamics after ^{14}C pulse labeling

^{14}C pulse labeling method. The $^{14}CO_2$ efflux from the soil after pulse labeling of shoots reached maximum 12-24 hours after assimilation. Total amount of $^{14}CO_2$ respired from the soil over 5.5 days was 9-10% of recovered ^{14}C. According to the model [*Kuzyakov and Domanski*, 2002], RR predominated in the $^{14}CO_2$ efflux during the first 24 h after assimilation. Microbial respiration started mainly 6-12 hours after the assimilation and 2 days later reached the maximum. In the first 5.5 days, the RR amount for 54% and RMR for 46% of respired $^{14}CO_2$. Additionally, we calculated further development of RR and RMR contributions until day 12. Recalculation of the percentage of $^{14}CO_2$ on the weight units showed that the contribution of RhR to total efflux from soil was 18% and the share of SMR was 82% (Fig. 1). These results agreed with previously published, obtained by this mehtod: the contribution of RR accounted for 17 – 61%, and average – 41-45% from total $^{14}CO_2$, and the contribution of RS amounted to 44-60%. [*Кузяков* 2001; *Cheng et al* 1993; *Kuzyakov, Kretzschmar, Stahr* 1999; *Kuzyakov, Domanski* 2002; *Kuzyakov* 2002]. One important shortcomings of this method is the estimation of the contribution of RhR only because $^{14}CO_2$ efflux is related to root-derived CO_2 and has no connection with CO_2 efflux by microbial decomposition of soil organic matter.

Comparing the results obtained by three CO_2 partitioning methods, we found that the contribution of the maize rhizosphere estimated by component integration and root exclusion methods has similar values and amounted about to 40% of total soil CO_2 efflux. The contribution of rhizosphere CO_2 estimated by ^{14}C pulse labelling was two times less.

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