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NEW ASPECT OF SOIL RESPIRATION ACTIVITY MEASURING

Teresa Włodarczyk, Alicja Księżopolska, Jan Gliński

Institute of Agrophysics, Polish Academy of Sciences
Doświadczalna str. 4, 20–290 Lublin
t.wlodarczyk@ipan.lublin.pl

Summary. The purpose of this paper was to introduce a new method for measuring soil organic matter mineralisation at the start of incubation (*start respiration – SR)*, as a supplement for other types of respiration, like *reactive respiration (RR)*, *basal respiration* (*BR*) and *long term respiration* (*LTR*). The effect of temperature and time of lessive soil incubation on CO_2 evolution was determined. Soil samples were incubated for 7 days at 28°C to determine *SR, RR and BR*, and for 35 days at 25°C to determine *LTR*. Statistical analysis showed that the total amount of CO_2 released from soil as well as the rate of organic carbon (C_{org}) mineralisation were significantly dependent on the time of soil incubation in all types of measured respiration. The results obtained confirm our hypothesis that determination of *start respiration* is justified when respiration methods for investigation of C_{org} mineralisation in soil are used. In this case dissolved fraction of C_{org} in soil solution is taken into account. Other types of respiration (*RR, BR and LTR*) expressed in time of incubation give us information about soil organic matter degradability and availability.

Key words: soil respiration, types of respiration, CO_2 release

INTRODUCTION

Respiration activity of soil is the most important characteristic of the soil biological activity. It depends on many factors like the kind of substrate, soil moisture, temperature, texture, structure, density, nutrient availability, pH, presence of heavy metals and pesticides [Gliński and Stępniewski 1973, Andersen 1982, Fang and Moncrieff 2001, Vanhala 2002, Hong *et al*. 2006].

The amount CO_2 produced and released is a very strong indicator of the soil microbiological metabolisms. It reflects the intensity of soil organic matter decomposition and mineralisation and the activity of microorganisms in soil, and it is often used for biomass determination [Anderson and Domsh 1990, Brookes 1995, Kubat *et al*. 1999, 2002, Brzezińska 2006].

There is a close relationship between the soil respiration activity, microbial biomass C and total organic C content [Włodarczyk *et al*. 2003, Růžek *et al*. 2004, 2005]. Soil respiration activity is, however, rather variable depending on the number of biotic and abiotic factors, mainly on the physical and chemical properties of soil, abundance and diversity of soil microorganisms, substrate availability, aeration, soil temperature and moisture. *In situ* measurements of soil respiration activity (CO_2 production) belong among the basic characteristic of the carbon cycle and its sequestration to soil [Nowak and Apfelthaler 1964, Klimanek *et al*. 1979, Mercik *et al*. 1999, Ryan and Law 2005, Cerhanová *et al*. 2006].

Soil temperature and incubation time influences soil microbial CO_2 evolution. Soil temperature affects microbial processes activity and organic substrate decomposition, but soil microbial respiration (CO_2 evaluation) expressed in time of incubation informs us about organic matter degradability and availability.

There are some incubation methods for measuring the intensity of organic matter mineralisation (expressed by CO_2 evaluation). Apfelthaler [1994] suggested CO_2 evaluation measurement after 3 days of incubation (*reactive respiration*) and after 7 days of incubation (*basal respiration*) at 28°C. Klimanek [1994] proposed five-week incubation at 25°C (*long-term respiration*). In our opinion the easiest available organic substrate is utilised immediately after vessels closed during laboratory incubation. Because of that we suggest CO_2 evaluation measuring more frequently during the first day of incubation and call it *start respiration*. In our opinion only these three methods give us the possibility to measure the whole spectrum of organic matter availability.

The main purpose of this study was to introduce a new method for measuring organic matter mineralisation at the start of incubation (*start respiration – SR*), as a supplement to *reactive respiration* (*RR*), *basal respiration* (*BR*) and *long-term respiration* (*LTR*).

MATERIALS AND METHODS

For the investigations, samples derived from Ap horizon of lessive soil (Haplic Luvisol) were used. The granulometric composition of the soil was sandy-silty loam, pH in H_2O 5.19 and in 1M KCl 4.46, organic carbon content 4.65 g kg^{-1}.

The respiration activity of soil was measured as CO_2 production after incubation of 2.5 g soil portions which were placed in glass bottles and watered to 20% of moisture content, then tightly covered with rubber stoppers and incubated in three steps. The gases evolved from the soil were collected with a syringe.

The 1st incubation step was at 28°C for 24 h, with CO_2 collection after 5, 15, 30, 45 minutes and 1, 2, 6, 12 and 24 hours. The authors termed this step *start respiration* (SR). It determines dissolved organic C used by microorganisms.

The 2nd step was at 28°C for seven successive days. The value of respiration after the first three days is named by Apfelthaler [1994] *reactive respiration*

(*RR*), and after the next four days – *basal respiration* (*BR*). Gases were collected each day at a previously established fixed time.

The 3rd step – at 25°C for five weeks, with respiration measurements at one week intervals, was named according to Klimanek [1994] *long term respiration* (*LTR*). Gases were collected at the end of each week.

Temperatures of 25°C and 28°C were selected as being optimal for the development of bacteria [Kobus 1995].

The quantitative index of organic carbon mineralisation in soil (soil respiration) was CO_2 released from it. Carbon dioxide was determined by means of a gas chromatograph (Shimadzu GC-14A) equipped with thermal conductivity detector (TCD) on column filled with Porapak (TCD temperature 60°C, column temperature 40°C, gas flow 60 ml min^{-1}) [Włodarczyk 2000].

The intensity of CO_2 release was calculated as follows: data from each successive period of incubation were subtracted from those of the preceding one and then divided by incubation time expressed in minutes for SR and in days for RR, BR and LTR. These data were then used to calculate the per cent CO_2 release from soil in individual periods of incubation. The sum activity of all the periods of incubation was equal to 100%.

STATISTICAL ANALYSIS

The results were statistically elaborated including analysis of variation using the Statgraff software (95% LSD), and analysis of regression using MS Excel. The calculated relations were then described with linear, power, logarithm and exponential equations. The description of the relationship analysis was made using the best fitted function.

RESULTS

Cumulative soil microbial CO_2 evolution as a function of time of incubation

Figure 1 (a, b, c) shows the sum of CO_2 released from the soil during the first 24 h of incubation at 28°C (*SR*), after the first week of incubation at 28°C (*RR and BR*), and during 5 weeks of soil incubation at 25°C as a function of time (*LTR*). The relationship between the cumulative amount of CO_2 released from the soil and incubation time is described by a positive linear function for all types of respiration, with coefficient of determination (R^2) of 0.960, 0.979 and 0.976 ($P < 0.001$) for *SR*, *RR+ BR* and *LTR*, respectively. Statistical analysis shows that the sum of CO_2 released from the soil is strongly connected with time of soil incubation, which is confirmed in all types of respiration.

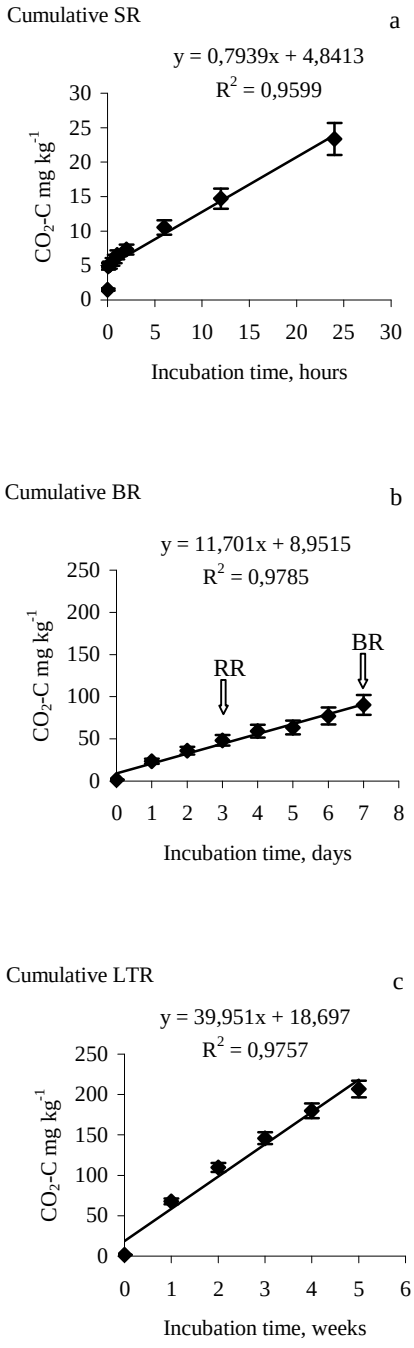

Fig. 1. Cumulative amount of CO_2 release during (a) *start respiration* (*SR*), (b) *reactive respiration* (*RR*) and *basal respiration* (*BR*) and (c) *long term respiration* (*LTR*)

Activity of soil microbial respiration as a function of time of respiration

Figure 2 presents the amount of CO_2 released from the soil during the first 24 h of incubation at 28°C (*SR*) converted to 1 min of incubation (data of each successive day of incubation were subtracted from those of previous data and divided by the number of minutes).

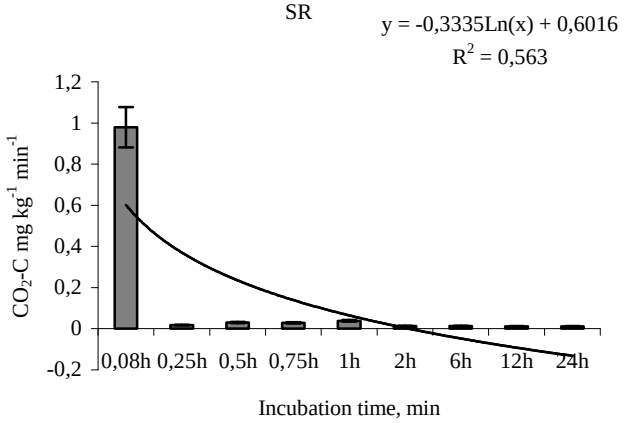

Fig. 2. CO_2 release from soil during *start respiration* (*SR*) converted to 1 min of incubation time

Figures 3a and 3b show the amount of CO_2 released from the soil during the first week of incubation at 28°C (*RR and BR*) converted to one day of incubation (from the second day the first was subtracted, from the third – the second, etc.) as a function of time.

In Fig. 4 we can see the amount of CO_2 released from the soil during five weeks of incubation at 25°C (*LTR*) converted to one day of incubation (first week of incubation was divided into 7; from the second week of incubation the first week was subtracted and divided by 7, etc.) as a function of time. Activity of soil microbial CO_2 evolution is described by negative curvilinear functions for *SR*, *RR* and *LTR*, and a positive rectilinear function for *BR*. Negative functions indicate different quality of C_{org} and different susceptibility to biological decomposition. A significant positive relationship was proved for *SR*, *RR* and *LTR* and not significant for *BR*. It means that the activity in CO_2 release from the soil is strongly connected with time of soil incubation and is inversely proportional to the length of this time, except for *BR*.

For *SR* the rate of CO_2 release from soil during the first 24 h of incubation was very high already in the first 5 min and rapidly dropped in further time of incubation. This proves that microbes use, at the lowest energy consumption, C_{org} as dissolved form in soil solution.

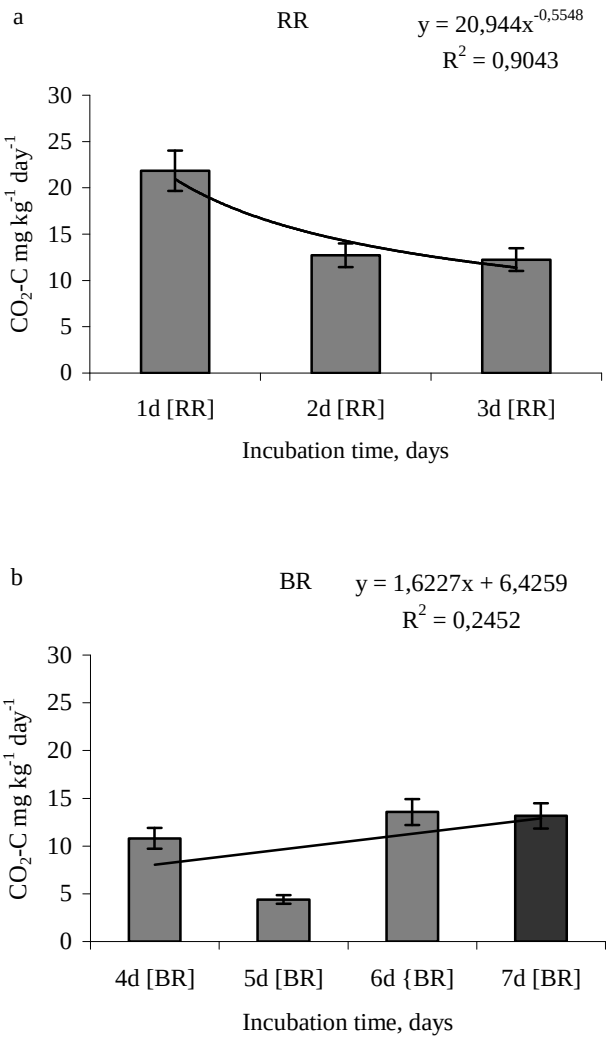

Fig. 3. CO_2 release from soil during the first 3 days (a) *reactive respiration* (*RR*) and following 4 days (b) *basal respiration* (*BR*) of soil incubation converted to 1 day

For *RR* and *BR* (treated as the first week of incubation) two peaks of soil microbial respiration were observed after the first and the sixth day of incubation. This indicated that during the first 5 days of incubation easily available organic carbon was used, and after that time followed microbes adaptation to decomposition of hardly decomposed C_{org}.

The third type of microbial respiration (*LTR*), prolonged in time and at lower temperature, shows an inhibited rate of C_{org} decomposition and the possibility

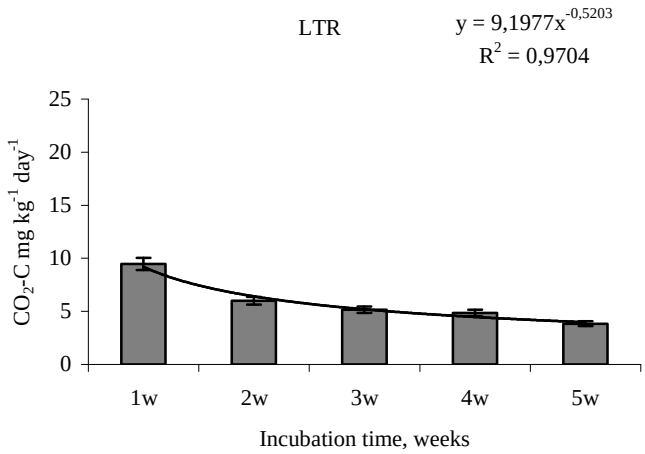

Fig. 4. CO_2 release from soil during *long term respiration* (*LTR*) of soil incubation converted to 1 day

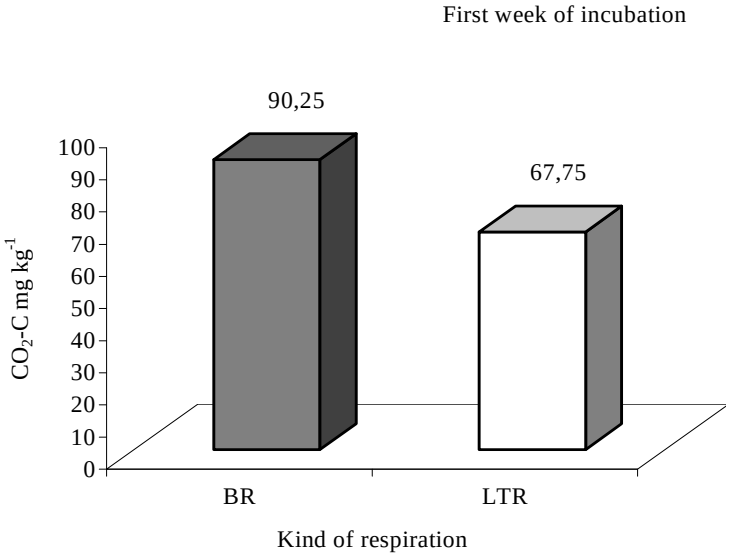

Fig. 5. Cumulative amount of CO_2 release from soil during first week of incubation at the temperature of 28°C (*BR*) and at 25°C (*LTR*)

of using hardly available carbon. Cumulative amount of CO_2 released from soil at the first week of incubation during *LTR* was about 25% lower than during analogous time of *RR* + *BR* (Fig. 5). *LTR* is characterised by the highest coefficient of correlation with time of incubation.

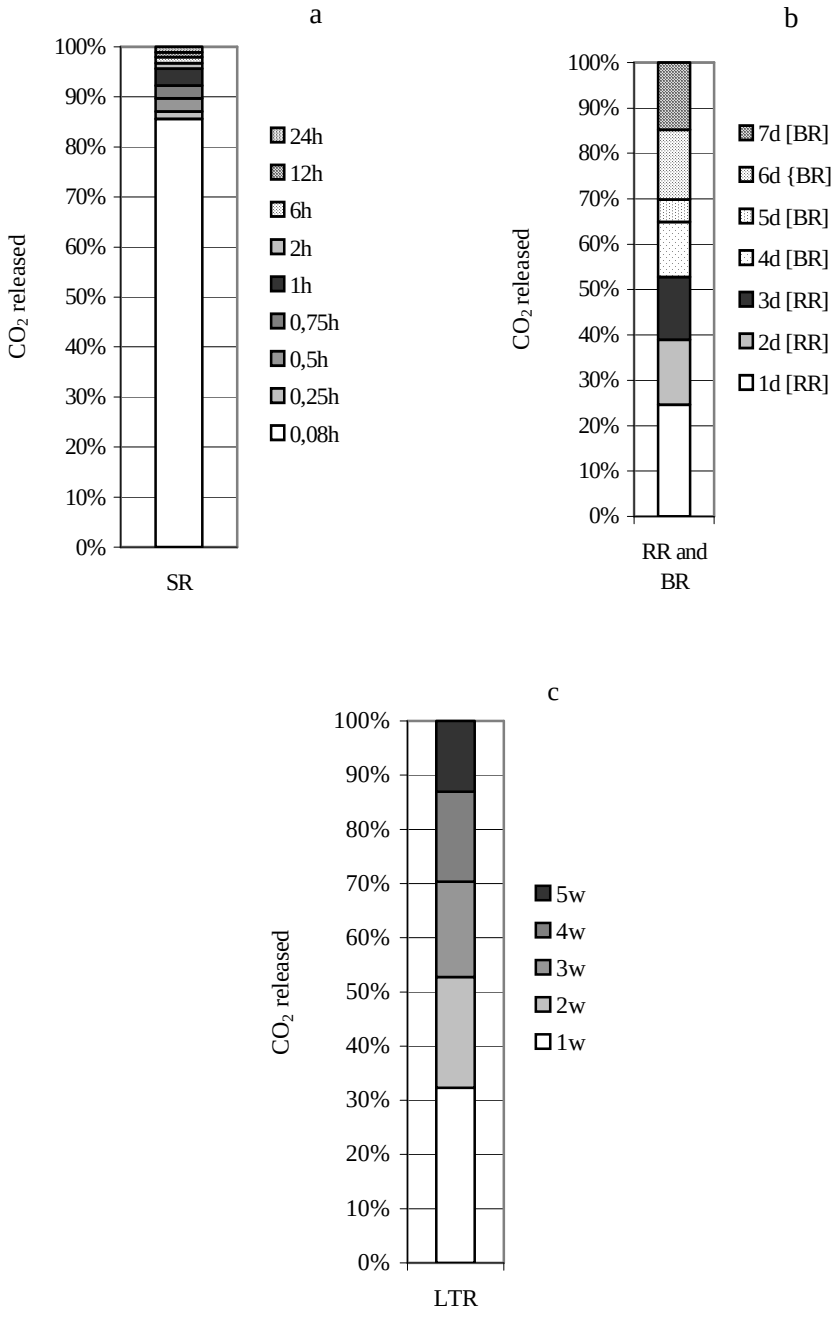

Fig. 6. Percent of CO_2 release per min during (a) *start respiration* (*SR*), per day (b) during *reactive respiration* (*RR*) *and basal respiration* (*BR*) and per week (c) during *long term respiration* (*LTR*) as a function of time of incubation

Figures 6a, b, c show percentage share of CO_2 release from soil for particular hours of *SR* (a) converted to 1 min of incubation (the sum is 100%), for particular days during *RR + BR* (b) (% was counted for daily incubation; from each successive day of incubation the preceding day was subtracted; the sum of 7 days was 100%) and for particular week of incubation during *LTR* (c) (% was counted for weekly incubation; from each successive week of incubation the preceding week was subtracted; the sum of 5 weeks was 100%). Considering % share of CO_2 released from the soil, the results obtained show that pool of carbon very easily available during the first 24 h was used by microorganisms in the amount of 86% already during the first 5 min of incubation. This confirms our hypothesis concerning the proposal of the new term *start respiration* (*SR*) when respiration methods are used for quantitative determination of intensity of C_{org} mineralisation in soil. In this case it is not only the carbon fraction that is subject to easy mineralisation that is measured, but also the fraction already mineralised and dissolved in soil solution. It seems to be that for practical use of this method measurements of CO_2 released from soil after 5, 15, 30, 45 and 120 min are enough.

Data presented in Fig. 6b show that during *RR* about 53% C_{org} has been mineralised. If we add to this result the amount of CO_2 released from soil during the 4^{th} day of incubation, during which microbes probably use carbon of similar susceptibility to mineralisation (except for *SR* intensity of CO_2 released from soil slightly decreased in time), mineralisation of about 65% was obtained. It seems that adoption of 3 days for *RR* provides sufficient conventional time for comfortable comparison of results obtained for different soils. However, this time does not reflect mineralisation of C_{org} of various susceptibility to microbial decomposition and can be different for various soils. The time of 4 days for *RR* could be accepted when mineralisation rate decreases considerably. It is probably from the fifth day of incubation that microbes start mineralisation of not easy available $C_{org.}$

Quite a different situation for *LTR* is presented in Fig. 6c where the rate of $C_{org.}$ mineralisation decreased almost monotonously with time of soil incubation.

Summing up we can say that the proposed method of *SR* determination can be a valuable complement for soil characteristics with respect to $C_{org.}$ content of different susceptibility to microbial mineralisation. For this reason it can be complementary to the methods used so far (*RR, BR* and *LTR)* for quantitative determination of intensity of C_{org} mineralization in soils, based on the use of incubation methods in carbon mineralisation in fixed time and temperature [Apfeltaler 1994, Klimanek 1994, Mercik *et al*. 1999, Cerhanova *et al*. 2006].

The results obtained show that for the determination of intensity of C_{org} mineralisation in soils with the use of incubation method, the amount of CO_2 released from soil should be converted to unit of time: for *SR* in minutes, and for other types of respiration – in days. Such a form of result presentation gives us the possibility to compare them in different soils.

CONCLUSIONS

1. The results obtained confirm our hypothesis that determination of *start respiration* is justified when respiration methods for quantitative determination of $C_{org.}$ mineralisation intensity in soil is used, because it takes into account dissolved carbon fraction in soil solution.

2. The other types of respiration (*RR*, *BR* and *LPR*) expressed in time of incubation inform us about organic matter decomposition and availability.

3. Statistical analysis showed that the sum of CO_2 released from soil as well as the rate of C_{org} mineralisation are significantly related to the incubation time of soil in all types of respiration.

4. For better understanding susceptibility of C_{org} mineralisation in soils, results should be presented per a unit of time.

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NOWY ASPEKT POMIARU AKTYWNOŚCI ODDYCHANIA GLEBY

Streszczenie. Celem pracy było wprowadzenie nowej metody pomiaru stopnia mineralizacji węgla organicznego w początkowej fazie inkubacji (*oddychanie startowe – OS*), jako uzupełnienie do *oddychania reaktywnego* (*OR*), *oddychania podstawowego* (*OP*) oraz *oddychania długotrwałego* (*OD*). W pracy badano wpływ czasu i temperatury na ilość wydzielonego CO_2 w warunkach doświadczenia inkubacyjnego gleby płowej bez wzbogaceń. Próbki glebowe inkubowano przez 7 dni w temperaturze 28ºC, gdzie badano *OS*, *OR* i *OP*, oraz w temperaturze 25ºC przez 35 dni, gdzie badano *OD*. Analiza statystyczna wykazała, że zarówno sumaryczne wydzielanie CO_2, jak i tempo mineralizacji C_{org} jest istotnie związane z czasem inkubacji we wszystkich typach mierzonego oddychania. Przeprowadzone badania potwierdzają naszą hipotezę, że wyznaczenie *oddychania startowego* ma swoje uzasadnienie przy wykorzystaniu metod respiracyjnych w określaniu ilościowego oznaczenia intensywności mineralizacji C organicznego w glebie, ponieważ uwzględnia pomiar frakcji rozpuszczonej w roztworze glebowym. Ilość wydzielonego CO_2 w pozostałych typach oddychania (*OR*, *OP* i *OD*) wyrażona w czasie informuje o stopniu rozkładu oraz przyswajalności węgla organicznego.

Słowa kluczowe: oddychanie gleb mineralnych, oddychanie startowe, reaktywne, podstawowe, długoterminowe, wydzielanie CO_2