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Original Research

Respiration of Soil Enriched with Manure and Mineral Materials (Methodical Aspects)

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Abstract

Methods of start (as a new one), reactive, basal, and long-term respiration determination in laboratory conditions were used for evaluating C_{org} transformation with CO_2 release from sandy soil (Haplic Luvisol) enriched in field experiments with organic (manure) and mineral (clay, lime) materials. They proved to be useful in such experiments. The objective of the investigation was to find which treatments stimulate or inhibit C_{org} mineralization. Of all treatments used, the least susceptible to C_{org} mineralization in soil appeared to be the soil enriched with manure. The most susceptible was soil enriched with soil+manure+clay+lower dose of lime.

Keywords: manure, clay, lime, incubation, respiration, CO_2

Introduction

Soil respiration depends on many factors such as organic substrate, moisture, temperature, texture, and structure of soil, available nutrients, pH, heavy metal content, pesticides, and time of incubation, etc. [1-4].

To achieve optimal fertility it is necessary to maintain the correct proportion between the amount of organic substance and mineral components in soil. Various substances are introduced to soils to improve their fertility and increase crop production, including manure, clay, lime, and mineral fertilization [5-8]. Nearly all processes that regulate soil fertility are based mainly on the transformation of organic matter [2, 3, 9-11]. However, these processes have occurred in Poland too intensively because of the types of soils (mostly sandy), climatic conditions, and intensive agriculture that decreases humus resources [12, 13]. This is a harmful effect for agricultural ecosystems and for the environment (increase of CO_2 evolution).

To counteract this effect it has been proposed to create organo-mineral complexes in soils that can stabilize organic substance levels [14] by enriching soil with organic and mineral matter resistant to decomposition [15].

One of the most frequently used methods to determine organic carbon mineralization in soil is to measure the amount of CO_2 released during incubation for a definite time and temperature [16-19].

The aim of this paper is to test various methods of the determination respiration activity of sandy soil (Haplic Luvisol) enriched in with organic and inorganic materials on the course and rate of organic carbon (C_{org}) transformation. The objective of the investigation was also to find which treatments stimulate or inhibit C_{org} mineralization.

Materials and Methods

Field Experiment

For investigations soil samples were taken from six 2 m^2 plots from experimental plots with soil enriched with

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Table 1. Fertility substance and their doses on the plot.

Experimental plots – Treatments	Fertility substance, dose
I	No treatment (untreated soil as control)
II	Soil + manure (8 kg per plot)
III	Soil + manure (8 kg per plot) + clay (50 kg per plot)
IV	Soil + manure (8 kg per plot) + clay (50 kg per plot) + post-flotation lime (1.12 kg per plot)
V	Soil + manure (8 kg per plot) + clay (50 kg per plot) + post-flotation lime (2.24 kg per plot)
VI	Soil + manure (8 kg per plot) + clay (50 kg per plot) + $Ca(OH)_2$ (2 kg per plot)

All plots were covered with the grass *Dactylis glomerata*.

organic and inorganic materials (6 treatments, Table 1). Plots were located in Kolonia Boniewo near Lublin, Poland. They were established in 2001 on Haplic Luvisol sandy texture, pH in 1M KCl 4.46, with 0.46% of organic C. Haplic Luvisols in field culture are the majority in Poland.

Cattle manure (M) was taken from an agricultural farm and added to all soils except the control. M contains in dry mass: 2-2.5% N, 0.6% P, 2.8% K, and 0.8% Mg. Clay (Cl) came from the sulfur mine "Jeziórko" where it is used for soil improvement around the area of the mine [20]. Post-flotation lime (L) was a waste product from sulfur processing.

Laboratory Experiment

In the autumn of 2002 soil samples were taken (14 months from the addition to soil of the mentioned substances) from the upper horizons (0-25 cm) of the plots of field experiment to the laboratory, where 2.5 g of each soil portion (well homogenized in three replications) was placed in (60 ml) glass bottles, watered to 20% moisture content, and closed with rubber stoppers. The resulting soils were then incubated in three levels of time and temperature. The cumulative gases evolved from soils were collected with a syringe in amounts of 0.05 ml, and four methods of respiration measurements [21] were used.

The 1st incubation level was at 28ºC for 24 h with CO_2 collection after 5 min, 6 h, 12 h, and 24 h. The authors term this step start respiration (SR). It determines the use of dissolved organic C by microorganisms [21].

The 2nd level was at 28ºC for seven successive days. The value of respiration after the first three days is named by Appfelthaler [16] reactive respiration (RR) and, after the next four days, basal respiration (BR). Gases were collected each day at a previously established fixed time (without washing flask by CO_2-free air).

The 3rd level at 25ºC for five weeks with respiration measurements at one-week intervals was named according to Klimanek's [19] long-term respiration (LTR). Gases were collected at the end of each week.

Temperatures of 25ºC and 28ºC were selected as being optimal for the development of bacteria [1, 15].

Measurement of CO_2

Carbon dioxide was determined by a Shimadzu GC-14A gas chromatograph equipped with thermal conductivity detector (TCD) on a column filled with Porapak (TCD temperature 60ºC, column temperature 40ºC, gas flow 60 ml min^{-1}) [21].

Treatment of Results

CO_2 data from each successive period of incubation were subtracted from those of the previous one and then divided by the incubation time expressed in minutes for SR and in days for RR, BR, and LTR. Figs. 1, 3, and 5 show cumulative amounts of CO_2 released from soil.

The rate of CO_2 release from soil during respiration is shown in Figs. 2, 4, and 6.

Statistical Analysis

The results were analyzed statistically using analysis of variation (Statgraphics; 95% LSD) and regression (Excel).

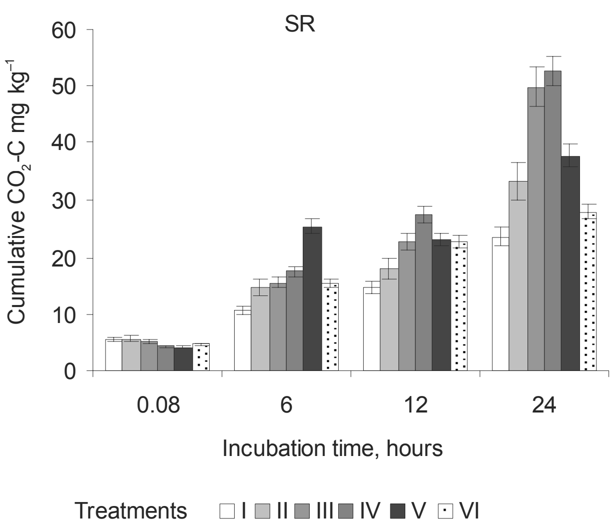

Fig. 1. Cumulative amount of the first day of CO_2 release during start respiration (SR).

Results

Start Respiration

The cumulative amount of CO_2 released from soil after 5 min of SR incubation was in the range 4.21 to 5.53 CO_2-C mg·kg^{-1} (Fig. 1). In the subsequent hours of incubation, the respiration activity of enriched soils distinctly increased in relation to the control soil (from 1.2 to 2.3 times). After the first 24 h of incubation the highest rate of CO_2 production (52.7 mg CO_2-C kg^{-1}) was observed in treatment IV (soil +M+Cl+ 1.12 kg L), and the lowest (23.4 mg CO_2-C kg^{-1}) in the control soil. The data set was used for regression analysis of possible correlations between the amount of CO_2 release and time of incubation during particular types of respiration (SR, RR, BR, and LTR). During the first day of incubation no significant differences in an average value of CO_2 release between treatments were found.

Fig. 2 showes the rate of soil respiration (amount of CO_2 per min) for the first day of incubation and shows that the highest respiration activity resulting from additives occurs during the first 5 min of incubation. Microbes in the control soil were the most active in the use of native C_{org} while the weakest carbon mineralization was found in treatment V (soil+M+CL+2.24 kg L). Clay and lime together with manure added to soil treatments III-V slowed this process only negligibly. In the next hours of incubation the rate of C_{org} mineralization was noticeably lower.

Respiratory activity dropped after 6 h of incubation, to increase again at 24 h.

In summary we can state that, in comparison to the control soil, the most intensive C_{org} mineralization during SR was in treatment IV (soil+M+Cl+ 1.12 kg L), and the weakest (not statistically significant) was in treatment VI (soil +M+L+ $Ca(OH)_2$). The addition of M+Cl to soil stimulated the mineralization process, but the addition of clay, a higher dose of 2.24 kg L, and $Ca(OH)_2$ slowed it.

Reactive and Basal Respirations

The cumulative amount of CO_2 released from soil during RR and BR types is shown in Fig. 3. The progressive increase (similar to SR) of the cumulative amount of CO_2 release during soil incubation is clearly visible, during both RR and BR. Significantly higher cumulative evolution from soil during the first 3 days of incubation (RR) and the next 4 days (BR) was observed in treatments III (soil+M+Cl) and IV (soil+M+Cl+1.12 kg L).

During RR (Fig. 4) the highest respiration activity of soil was in the first day of incubation with a clearly visible effect of our additives. The rate of C_{org} mineralization dropped in the second day of incubation and increased slightly in the third day. High respiration activity was seen in treatments III and IV (soil+M+Cl and soil+ M+Cl+1.12 kg L) during the whole period of RR. Soil treated with other materials showed lower rates of C_{org} mineralization, close to that of the control soil. In all treatments the amount of mineralized C_{org} after three days of incubation was higher, ranging from 1.1 times in treatment VI (soil+M+Cl+$Ca(OH)_2$) to 2.5 times in treatment IV (soil+M+Cl+1.12 kg L) compared to the control.

During BR (Fig. 4) the rate of CO_2 production diminished considerably in comparison to RR, but toward the end of the experiment there was still a high daily rate of mineralization in treatments III (soil+M+Cl) and IV (soil+M+Cl+1.12 kg L). The lowest was in treatment II (soil+M). Therefore, nearly all materials used in field experiments stimulated the rate of CO_2 production during BR. Additions of manure and lime distinctly prolonged stimulation of the respiratory process in soil toward the first day of soil incubation. However, similarly to SR, a dose of 2.24 kg lime and $Ca(OH)_2$ introduced to soil inhibited CO_2 release in comparison to treatments III and IV (soil+M+L).

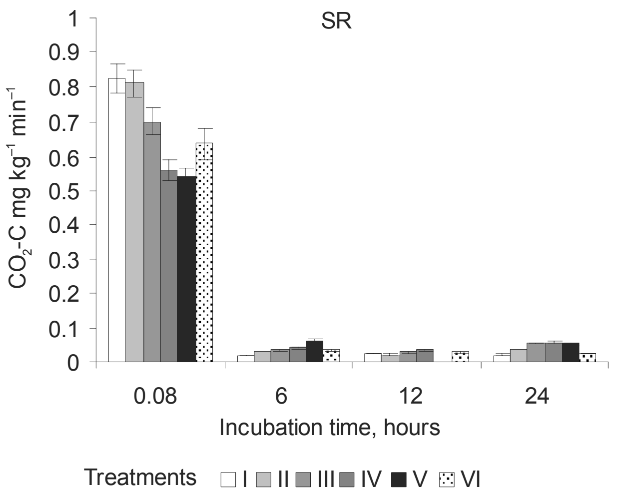

Fig. 2. The rate of CO_2 release from soil during start respiration (SR)

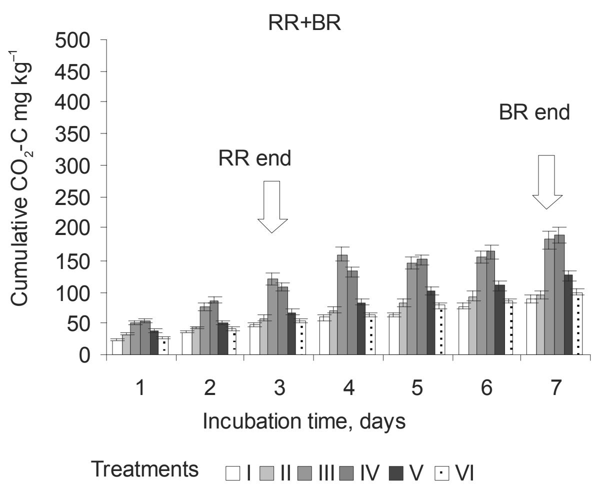

Fig. 3. Cumulative amount of CO_2 released from soil during the first 3 days (reactive respiration RR) and the following 4 days (basal respiration (BR) of soil incubation.

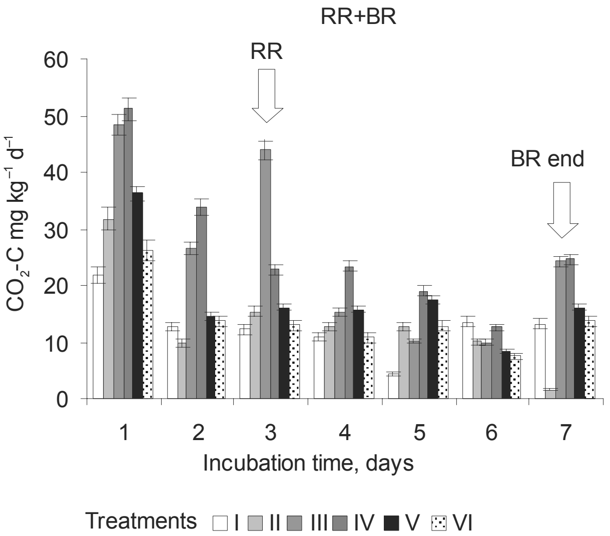

Fig. 4. The rate of CO_2 release from soil during reactive respiration (RR) and basal respiration (BR).

Long-Term Respiration

The highest cumulative amount of CO_2 release from soil (449 mg CO_2-C kg^{-1}) was in treatment IV (soil + M+Cl+1.12 kg L) (Fig. 5). The lowest amount was found in the control soil (207 mg CO_2-C kg^{-1}). Therefore, all materials used in the experiment stimulated CO_2 release from soil during the whole period of C_{org} mineralization, including LTR. Differences between the mean values of CO_2 release from soil treatments during LTR are evident.

The highest respiration activity of soil was observed during the first week of incubation and decreased systematically during the course of soil incubation (Fig. 6). This testifies to the varying susceptibility of C_{org} to mineralization.

In all experimental treatments the amount of C_{org} mineralization after 5 weeks of soil incubation was 1.4 times higher in treatment VI (soil+M+Cl+ $Ca(OH)_2$) and 2.2 times higher in treatment IV (soil+M+Cl+1.12 kg L) than in the control soil.

Discussion

Respiration activity of soil is the most important characteristic of soil biological activity and depends on many factor [18], including carbon availability, temperature, and humidity. Our result showed that during the first 5 min of incubation (SR), microbes in the control soil were the most active in the use of native C_{org}, while the weakest carbon mineralization was found in treatment V (soil+M+Cl+ 2.24 kg L). In the next hours of incubation the rate of C_{org} mineralization was noticeably lower. This means that 6 h of incubation was sufficient for microbial adaptation to growth in experimental conditions and resulted in a higher respiration intensity of enriched soil in comparison to the control. Respiratory activity dropped after 6 h of incubation, to increase again at 24 h. These convertible cycles in the increase and decrease of CO_2 evolution from soil were probably due to the exhaustion of a very easy oxidized C fraction and the consequent necessity of using C-bearing material more resistant to biological oxidation. These probably reflect differences in its availability as well as the adaptation of microbes to decomposition in subsequent C fractions. The smoothest dynamics of C_{org} decomposition was observed in the control soil. The greatest fluctuations were in treatment V (soil+M+Cl+2.24 kg L). Therefore, we have concluded that the term start respiration is justifiable and can be useful in discussing the susceptibility of C_{org} to mineralization. The C_{org} content and its two fractions (easily available and not easy accessible) in the soil taken from the field experiment presented by Księżopolska [22] was very different (Table 2). A significant very close positive correlation between the rate of CO_2 release and easily available carbon for the SR (r=0.92** for n=6) and negative correlation with not easy accessible fraction (r=0.868** for

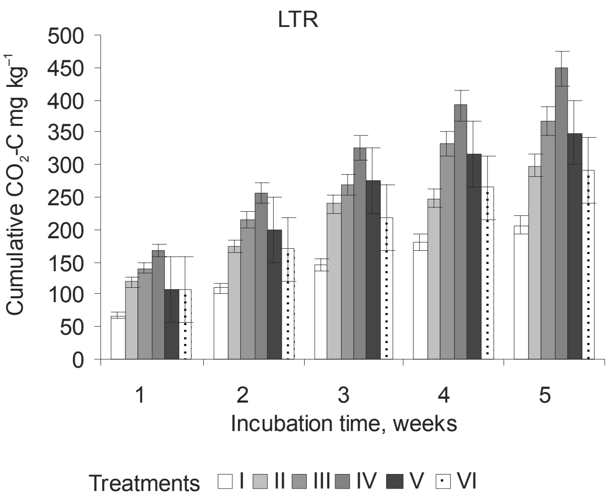

Fig. 5. Cumulative amount of CO_2 release from soil during long-term respiration (LTR) of soil incubation.

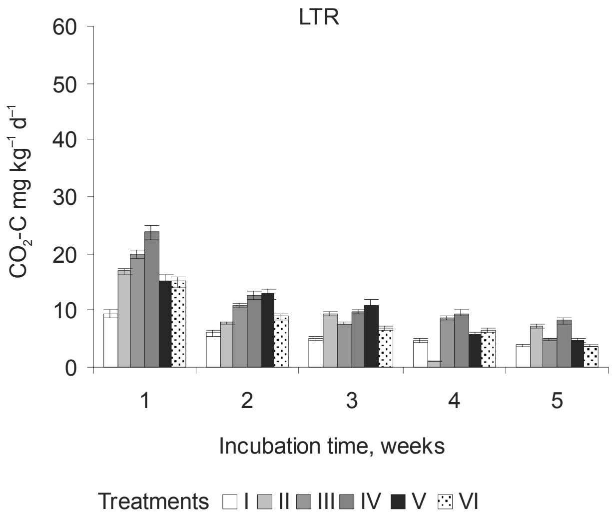

Fig. 6. The rate of CO_2 release from soil during long-term respiration (LTR).

Table 2. Total, easily available, and not easily available C_{org} [22].

Plots-treatments	Organic carbon	Easily available	Not easily available
	(g·kg^{-1})	C_{org} (%)	C_{org} (%)
I	6.0	35.0	65.0
II	7.5	20.2	79.8
III	8.2	18.4	81.6
IV	7.6	9.9	90.1
V	8.1	9.3	90.7
VI	7.8	9.6	90.4

n=6) was found. It confirmed our presumption that measuring SR determined the use of dissolved organic C by microorganisms.

During reactive and basal respiration significantly higher average value of CO_2 evolution from soil was observed in treatments III (soil+M+Cl) and IV (soil+M+Cl+1.12 kg L) in RR, and additionally in treatment V in BR. These additions significantly stimulated development of bacteria responsible for C_{org} mineralization compare to mineralization of native carbon in control soil. Much lower values were noted for remaining treatments and the lowest one for control soil. However, the addition of lime and hydrated calcium significantly decreased CO_2 release from soil in comparison to its treatment with manure and lime. This can be important in the storage of native and organic carbon introduced to soil. The stimulating effect of clay in C_{org} mineralization found in our experiment appeared to be in contradiction to the inhibiting effect obtained by several other authors [6, 15]. They suggest this effect to be due to the formation of organo-mineral complexes resistant to carbon mineralization. In a laboratory such phenomena probably did not exist. However, Księżopolska [22] found slightly lower CO_2 emissions from plots with clay additions compared to plots without the addition under field experiment (the average value from three years lasted for the field experiment).

The addition of manure to soil slowed the rate of organic matter decomposition during BR in comparison to the control soil and to soils with other additives. The results obtained show that materials added to soil distinctly affected the availability of C_{org}. During the first day of incubation, microbes were the most active in the use of C_{org} introduced to soil of treatment II (soil+M). Similar results were obtained by Cerhanova et al. [17]. The addition of clay to soil only gradually slowed this process. The addition of lime to soil had no significant effect on the rate of C_{org} use.

During RR the rate of C_{org} mineralization was higher than during the consecutive four days (BR) of organic carbon. mineralization and the differences in carbon availability were found. In the 7th day of BR, respiratory activity was relatively high (compared to the previous three days) with the exception of soil+M, where the share of CO_2 released from soil was the lowest, which may be explained by the exhaustion of easily available carbon.

Similarly to SR, the variability of time maxima and minima in CO_2 evolution from soil during RR and BR indicates differing carbon availability and may also reflect adaptation of microbes to decomposition of consecutive carbon fractions. However, the dynamics of the processes in comparison to SR is different but more stable. In the case of RR and BR there wasn't any significant correlation between C_{org} content and CO_2 release.

Lowering temperature as well as prolonging the incubation time (LTR) significantly affected differences between C_{org} mineralization in control soil and soil modified by addition of improving materials. In all enriched soils the amount of CO_2 released was significantly higher than in control soil. The effect of carbon mineralization delay observed during BR in treatments II and VI (soil+M and soil+M+Cl+Ca(OH)$_2$) in relation to control soil was canceled during LTR through the time factor.

The greatest fluctuations in C_{org} mineralization rate during LTR was in treatment III (soil+M+Cl). This probably reflects the much differentiated availability of carbon introduced to soil in this treatment. The addition of lime to soil with manure and clay distinctly affected the stabilization of C_{org} mineralization rate at longer times and lower incubation temperatures in comparison to other types of respiration (SR, RR, and BR).

The rate of C_{org} mineralization in soil during LTR is more stable than during the SR, RR, and BR phases of incubation. The exceptions are treatments II and III (soil+M and soil+M+Cl). This means that reducing the incubation temperature by 3ºC distinctly slows C_{org} mineralization and allows its available pool to be used for a longer time.

The most stable C_{org} mineralization rate in soil is observed in the control; this probably implies the best microorganism adaptation for C_{org} decomposition as well as the lower availability of carbon compounds subject to mineralization.

The highest and the most even equal respiration activity was found in the first 2 weeks of the experiment. From the 3rd week of incubation onwards, greater differences in the respiration activity of soil were observed, depending on the type of enrichment used. This was confirmed by regression analysis, which showed very close positive correlation between cumulative CO_2 release from the soil and total C_{org} content (r=0.875** for n=6), and not easily available C_{org} (r=0.91** for n=6).

Reversible cycles of the increase and decrease in the amount of CO_2 released from soil seem to indicate the adaptation of different microorganisms to variations in C_{org} availability. Similar results were obtained by Klimanek [19], who found that during daily measurements of CO_2 over 35 days of incubation, the highest soil respiration was during the first 10-15 days. It then diminished until the end of incubation.

Conclusions

1. Four methods of determining respiration activity of soil (start, reactive, basal, and long-term respirations) appeared to be useful in evaluating C_{org} transformation with CO_2 release in sandy soil enriched with various organic and mineral materials.
2. The amount of CO_2 released from soil in all experimental treatments and of respiration types was in the range of about 10 to about 27 mg –C day^{-1}.
3. Of all treatments used, the least susceptible to C_{org} mineralization in soil appeared to be soil enriched with manure, and the most susceptible was soil enriched with soil+manure+clay+lower dose of lime.
4. A significant positive correlation between the rate of CO_2 release as easily available C_{org} and negative correlation with not easily available C_{org} for start respiration was found. Also, positive correlation appeared between mean values of CO_2 release as total and not easily available C_{org} for long-term respiration.
5. The amount of CO_2 release from soil enriched with manure, clay, and the lower dose of lime was more than two-fold higher in comparison to control soil, as well during basic and long-term respiration.
6. The highest intensity of CO_2 release from soil was found during start respiration and the lowest during long-term respiration.

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