Examination of four methods for measuring soil respiration

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Abstract

We compared the accuracy of four methods for measuring soil respiration: the open-flow infra-red gas analyzer method (OF-method); the closed chamber method (CC-method); the dynamic closed chamber method (DC-method); and the alkali absorption method (AA-method), using artificial soil medium (vermiculite with glucose and mineral nutrients) inoculated with Trichoderma sp. as respiring microorganisms. The CO₂ emission rates from the medium were measured by the four methods once a day for 10 days. We compared the estimated amounts of glucose respired by Trichoderma sp. (EGR, an integration of measured CO₂ emission rates for 10 days) with the actual amounts of glucose respired (AGR, calculated from weight loss of the medium during the same period). In the AA-method, the EGR value was 1.3 times larger than the AGR value, while in the OF-, DC- and CC-methods EGR values were almost the same as the AGR (0.95, 0.95 and 0.94 times respectively). Higher CO₂ emission rates obtained by the AA-method were attributed to low CO₂ concentration (20–250 μl l⁻¹) in the chamber, because at such low CO₂ levels the respiration rate of Trichoderma sp. was enhanced by 20–70%. These results indicate that the OF-, DC- and CC-methods are more suitable for soil respiration measurement than the conventional AA-method. © Elsevier Science B.V. All rights reserved

Keywords: CO₂ concentration; Measurement method; Soil respiration

1. Introduction

Methods for measuring soil respiration can be classified into the following three methods: First, the alkali absorption method (AA-method). Carbon dioxide evolved from soil in a closed chamber is absorbed in a caustic solution (Witkamp, 1966; Kirita, 1971; Anderson, 1973; Edwards and Ross-Todd, 1983; Buyanovsky et al., 1986). Second, the open flow infra-red gas analyzer method (OF-method) whereby ambient air flows through a chamber, and CO₂ flux is calculated from the concentration difference between inlet- and outlet-air (Witkamp and Frank, 1969; Garret and Cox, 1973; Nakadai et al., 1993). Third, the closed chamber method (CC-method), where CO₂ in a closed chamber is sampled periodically and the efflux is computed from the rate of increase of CO₂ concentration in chamber (Matthias et al., 1980; Hutchinson and Mosier, 1981; Rolston, 1986; Mariko et al., 1994; Bekku et al., 1995). More recently, another type of the closed chamber method has been used; the dynamic closed chamber method (DC-method), in which air is circu-
lated from the gas analyzer and returned to the chamber (Rochette et al., 1991; Rochette et al., 1992).

The AA-method has been used by many researchers because of its convenience and the ability to measure many points. However, it has been suggested that the AA-method may underestimate actual soil respiration because the CO₂ absorption efficiency of the alkali solution in a dish or jar decreases when the solution is neutralized (Haber, 1958; Kucera and Kirkham, 1971; Freijer and Bouten, 1991). Kirita (1971) modified this method, and proposed to use an alkali-soaked sponge disc instead of a dish to increase the surface area of the trapping solution and the CO₂ absorption efficiency. Although the modified AA-method may allow a more accurate measurement of the soil respiration rate, the values measured by this method are still two- to three-fold larger than those by the OF-method and the CC-method (Kirita, 1983; Nakadai et al., 1993). On the other hand, Rochette et al. (1992) indicated that the AA-method produced lower soil respiration rates than did the DC-method. Though there were many comparative studies among these methods under field conditions and laboratory experiments, no direct comparison of the values measured by these four methods and the absolute amounts of CO₂ evolved have yet been reported.

In the present study the validity of the four methods was examined with an experiment using an artificial soil medium inoculated with Trichoderma sp., in which the absolute CO₂ emission rate could be estimated by measuring the weight loss of the medium. The causes of the variations specific to the methods are also discussed.

2. Materials and methods

2.1. Artificial soil medium

We used an artificial soil medium inoculated with Trichoderma sp. as a source of CO₂ emission. When glucose is used as the only carbon source in soil, part of the glucose is incorporated into biomass, and the other part is respired as CO₂ and H₂O under aerobic conditions. The latter process can be expressed as follows:

\[ C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \]  

The weight loss of the artificial soil medium is associated with the emission of CO₂ and H₂O, and the small amount of H₂O liberated in the process of biosynthesis. As the amount of the latter is assumed to be negligible, the weight loss of the medium corresponds to the amount of glucose respired by Trichoderma sp., and is proportional to the cumulative amount of CO₂ evolved from the soil.

The artificial medium consisted of 100 g DW vermiculite sterilized at 160°C for 1 h, 5 g glucose and 0.6 g mineral nutrients (NH₄Cl, 0.5 g; NH₄H₂PO₄, 0.05 g; NaH₂PO₄, 0.05 g). Spores of Trichoderma sp. (about 1.2 × 10⁸) were scattered uniformly on the surface of the medium in a glass pot (11 cm in diameter, 6 cm in height). The water content of the medium was maintained at 30% of dry weight by irrigation with deionized water every 12 h. The experiment was conducted in an incubator kept at 20°C and CO₂ concentration of about 370 μl 1⁻¹ for 10 days with five replications for each measurement method. Moreover, five blanks which were not inoculated were prepared to determine a weight loss from the pots which was not due to the decomposition of glucose. Five controls, which were inoculated but not measured with the four methods, were made to determine the effect of the measurement on the decomposition of glucose. The measurement of CO₂ emission from each artificial medium was carried out once a day using the four methods described below. At the end of the experiment, each medium was air-dried at 80°C for 3 days, and then weighed.

2.2. Measurement of CO₂ emission rate

The CO₂ emission rate from the artificial medium was measured using four methods described below (Fig. 1).

The alkali absorption method (AA-method): The procedure of the AA-method followed that of Kirita (1971). A cylindrical chamber of polyvinyl chloride, 12 cm in diameter and 17 cm in height, was used for this method. A sponge containing 25 ml of 1 N KOH was used for CO₂ absorbant. The solution of KOH squeezed from the sponge was titrated with 0.1 N HCl using phenolphthalein and methyl orange as indicators.

The open-flow infra-red gas analyzer method (OF-method): The measurement system is the same
as described in the previous study (Nakadai et al., 1993). A cylindrical chamber of polyvinyl chloride, 12 cm in diameter and 6 cm in height, was used for the OF-method. The pot was covered with the chamber, and then ambient air stored in a balloon was passed through the chamber. The air flow rate in the

![Diagram of experimental setup]

Fig. 1. Experimental setup to compare four methods for measuring CO₂ emission rates. AA-method: A disk made of sponge containing a KOH solution was placed at a height of 10 cm. The air was sampled with a micro-syringe from a port (1 cm above the soil surface) covered with rubber for measurement of CO₂ in the chamber. OF-method: The air stored in balloon was passed through the chamber at flow rate of 0.8 l min⁻¹, then dried with perma pure drier, and passed into IRGA at 0.5 l min⁻¹. P: air pump; F: flow meter; PD: perma pure drier; IRGA: infra-red gas analyzer. CC-method: The air sampled by a micro-syringe was inserted into a gas line in which CO₂ free air streamed at 0.5 l min⁻¹, and the CO₂ concentration was measured by IRGA. The chart speed of the recorder was 120 mm h⁻¹; scale: 200 mV. DC-method: The air circulated from the chamber to the IRGA at the air flow rate of 0.6 l min⁻¹. The CO₂ concentration in the chamber was monitored and the rate of increase was computed. P: air pump; F: flow meter.
chamber was regulated at 0.8 1 min\(^{-1}\). A part of the air (0.5 1 min\(^{-1}\)) was passed through a dehumidifier to an infrared gas analyzer. (IRGA: Fuji Electric, Model ZRC). The CO\(_2\) emission rate from the medium was determined from the difference of CO\(_2\) concentrations at the inlet and outlet of the chamber.

The closed chamber method (CC-method): Details of procedure were described in the previous paper (Bekku et al., 1995). A cylindrical chamber of polyvinyl chloride, 12 cm in diameter and 6 cm in height, was used for the CC-method. The medium pot was covered with the chamber, and then 2 ml air was sampled from the chamber with a micro-syringe from a rubber septum at intervals of 0.5–2 min. The CO\(_2\) concentration in the sampled air was determined with IRGA. Soil respiration rate was calculated from the concentration increase in the chamber.

The dynamic closed chamber method (DC-method): The soil respiration chamber (6000-09, LI-COR Inc., Lincoln, NE, USA), 9.5 cm in diameter and 12 cm in height, was connected to the LI-6200 portable leaf photosynthesis system (LI-COR Inc., Lincoln, NE, USA). Air from the chamber circulated to the gas analyzer (LI-6250, LI-COR Inc., Lincoln, NE, USA) in the LI-6200. The CO\(_2\) concentration in the chamber was monitored for 45 s and the rate of increase was computed every 15 s. Soil respiration rate was determined by average of the three trials. More details are given in Rochette et al. (1991).

Each method was applied to five replicate pots. Measurements were conducted for 4 h using the AA-method, 10–20 min using the OF-method, 2–4 min using the CC-method and 45 s using the DC-method. Each measurement was carried out once every day under the same temperature conditions (20°C). This experimental design was chosen to keep the pots under the ambient CO\(_2\) concentration most of the time.

CO\(_2\) concentration in the chambers was monitored during the measurements of the CO\(_2\) emission rate. In the AA-method, 2 ml air at 1 cm above the medium was sampled with a micro-syringe, and the CO\(_2\) concentration was measured by IRGA. In the OF-method, the CO\(_2\) concentration in the chamber was calculated as a geometrical average of CO\(_2\) concentrations of inflow and outflow air. In the CC-method and the DC-method, the CO\(_2\) concentration at the end of the measurement was used.

2.3. Effects of CO\(_2\) concentration on respiration of Trichoderma sp.

Effects of CO\(_2\) concentration on respiration of Trichoderma sp. in the pots were examined using OF-method. Another medium inoculated with spores of Trichoderma sp. (3.2 \(\times\) 10\(^7\)) was exposed to different CO\(_2\) concentrations on the 5th day after the inoculation. The respiration rate was measured at 20°C and water content of 30%. The CO\(_2\) concentration of the air was changed at about 100 \(\mu l\) 1\(^{-1}\) interval from 0 to 580 \(\mu l\) 1\(^{-1}\).

2.4. Calculations

Estimates of the amount of glucose respired by Trichoderma sp. (EGR) in each pot were obtained by integrating the CO\(_2\) emission rates measured by each method over the 10 day period. Actual amounts of glucose respired by Trichoderma sp. (AGR) in the pots were determined based on the difference between the dry weight of the medium at the beginning of the experiment (IDW) and that at the end of the experiment (FDW). There might be, however, a slight weight loss from the pots during the experiment which was not due to glucose respiration. These weight losses were determined by calculating the difference between the IDW and the FDW of the blanks. The AGR for each medium was determined by correcting the above weight loss. The effect of the measurement by the four methods on the glucose decomposition was examined through the comparison between the mean AGR of the controls and those of the four methods. The accuracy of the four methods was evaluated by comparing the EGR with the AGR in each method. Significant differences between the AGR and the EGR were determined using \(t\) test.

3. Results

Fig. 2 represents time courses of CO\(_2\) emission rates from the medium during the experimental period. The pattern of the time courses was similar for the four measurement methods. The CO\(_2\) emission rates increased from the beginning of the experiment to around 100 h, and then gradually decreased. How-
ever, the rates measured by the AA-method were 1.3–9 times larger than those by the other three methods in the first half stage of the experiment, and they were 1.1–1.8 times larger in the later stage. The OF- and DC-methods showed similar CO$_2$ emission rates. The rates measured by the CC-method were slightly lower than those by the OF- and DC-method, but not significantly different.

Table 1 represents relationships between AGR and EGR during the experiment. The weight loss from blank pots which was not due to glucose respiration was negligibly small. There were no significant differences between AGR of the control and those of the four methods. The EGR values for the OF-, CC- and DC-methods were not significantly different from the AGR values, but those for the AA-method was significantly higher ($P < 0.01$, $t$-test). The average value of the EGR/AGR was 95.1% (SD = 2.1, $n = 5$) for the OF-method, 95.4% (SD = 1.3, $n = 5$) for the DC-method, 93.5% (SD = 3.7, $n = 5$) for the CC-method and 130.3% (SD = 2.1, $n = 5$) for the AA-method.

The CO$_2$ concentrations in the chamber during measurement of CO$_2$ emission rate were different among the four methods (Fig. 3). During the experimental period, the CO$_2$ concentration ranged from 380 to 450 $\mu$L $\text{l}^{-1}$ for the OF-method, 380 to 470 $\mu$L $\text{l}^{-1}$ for the DC-method and 440 to 540 $\mu$L $\text{l}^{-1}$ for the CC-method. The average CO$_2$ concentration during 4 h measurement at 1 cm above the medium surface in the AA-method chamber was lower than other methods, and varied between 20 and 250 $\mu$L $\text{l}^{-1}$ during the experiment. All the pots were exposed to the CO$_2$ concentration of about 400 $\mu$L $\text{l}^{-1}$ at the medium surface during the 10 day experiment except for the measuring period.

Table 1

<table>
<thead>
<tr>
<th>Method</th>
<th>AGR (g)</th>
<th>SD</th>
<th>EGR (g)</th>
<th>SD</th>
<th>EGR/AGR (%)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA-method</td>
<td>2.24</td>
<td>0.10</td>
<td>2.92</td>
<td>0.12</td>
<td>130.3</td>
<td>2.15</td>
</tr>
<tr>
<td>OF-method</td>
<td>2.16</td>
<td>0.13</td>
<td>2.05</td>
<td>0.16</td>
<td>95.1</td>
<td>2.05</td>
</tr>
<tr>
<td>CC-method</td>
<td>2.16</td>
<td>0.27</td>
<td>0.93</td>
<td>0.16</td>
<td>93.5</td>
<td>3.67</td>
</tr>
<tr>
<td>DC-method</td>
<td>2.26</td>
<td>0.16</td>
<td>0.17</td>
<td>0.17</td>
<td>95.4</td>
<td>1.29</td>
</tr>
<tr>
<td>Control</td>
<td>2.21</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AGR was calculated from the difference between the dry weight of medium at the beginning of the experiment (IDW) and that at the end (FDW). EGR values were estimated by converting the integrated CO$_2$ emission rates measured into the amount of glucose.
The respiration rate of *Trichoderma* sp. in the artificial medium was affected by the CO₂ concentration in the air ventilated through the chamber (Fig. 4). The respiration rates exponentially decreased with increasing CO₂ concentrations. The regression curve was fitted as \( y = 476.5 \cdot e^{-0.00138x} \). The respiration rates at 20 \( \mu l \cdot l^{-1} \) and 250 \( \mu l \cdot l^{-1} \) were 70% and 20% higher than that at 400 \( \mu l \cdot l^{-1} \) which was ambient CO₂ concentration at the medium surface in the incubator.

![Fig. 3. Time courses of CO₂ concentrations in the chamber during the measurement of soil respiration and ambient CO₂ concentrations during the experiment. The values are mean of five replicates. Bars indicate SD. ●, OF-method; △, CC-method; ○, DC-method; ■, AA-method; .... , ambient CO₂ level in the incubator.](image)

![Fig. 4. Relationships between CO₂ concentrations and respiration rates of *Trichoderma* sp. in an artificial medium. The measurement was conducted on the 5th day at 20°C. Regression curve was fitted: \( y = 476.5 \cdot e^{-0.00138x} (r = 0.990) \).](image)

4. Discussion

The percentages of the EGR to the AGR were 95% for the OF- and DC-method, 94% for the CC-method, and 130% for the AA-method. These results indicate that the OF-, CC- and DC-methods provided a close approximation of the actual CO₂ emission rates occurring under ambient air, while the AA-method overestimated the rates by 30% (Table 2). The differences in the CO₂ emission rates by the four methods (Fig. 2) would be caused by the differences in the CO₂ concentration in the chamber (Fig. 3). The CO₂ concentrations in the AA-method chamber were low (20–250 \( \mu l \cdot l^{-1} \)). Under such low CO₂ concentrations, the CO₂ emission rate was enhanced by 70–20% as compared with that under the ambient CO₂ of 400 \( \mu l \cdot l^{-1} \) (Fig. 3). This result indicates that a depression of CO₂ concentrations in the AA-method chamber most likely causes acceleration of CO₂ emission rates and overestimation of those under ambient air. On the other hand, CO₂ concentrations in the chamber of the OF-method were stable and nearly equal to those of the ambient air (Fig. 3). With the DC- and CC-method, CO₂ concentration in the chamber increased from 380 to 470, 380 to 540 \( \mu l \cdot l^{-1} \) during the measurement, respectively. Freijer and Bouten (1991) reported that in a longer time measurement up to 1 h, the increase of CO₂ concentration in the CC-method chamber decreases the concentration gradient in the soil, resulting in a decreased CO₂ flux. However, in our results, the CO₂ emission rate was little influenced by the changing CO₂ concentrations, because the measurement time of these two methods were so short (CC-method, 2–4 min; DC-method, 45 s) that the CO₂ emission rate would be little affected by changing the soil CO₂ gradient.

The degree of the overestimation by the AA-method would differ among soil type. In the present laboratory experiment the total amount of CO₂ emission estimated by the AA-method was 1.3-fold of that under ambient air (Table 2), and the rates by the AA-method were 1.1–9 times larger than those by the OF-method (Fig. 2). Nakadai et al. (1993) reported that soil respiration rates obtained by the AA-method were about two or three times as large as those by the OF-method in cultivated land. These differences could be attributed to the differences in
Table 2
Carbon balance sheet in each method

<table>
<thead>
<tr>
<th>Inputed glucose-C (mg C)</th>
<th>Emitted CO₂-C</th>
<th>Estimated/Actual (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimated *  (mg C)</td>
<td>Actual ** (mg C)</td>
</tr>
<tr>
<td>AA</td>
<td>2000</td>
<td>896</td>
</tr>
<tr>
<td>OF</td>
<td>2000</td>
<td>864</td>
</tr>
<tr>
<td>CC</td>
<td>2000</td>
<td>864</td>
</tr>
<tr>
<td>DC</td>
<td>2000</td>
<td>904</td>
</tr>
<tr>
<td>Control</td>
<td>2000</td>
<td>884</td>
</tr>
</tbody>
</table>

* Emitted CO₂-C that was calculated by integration of CO₂ emission rates in Fig. 2. ** Emitted CO₂-C that was calculated from weight loss of glucose.

The CO₂ concentration in the chamber which depends on soil structure, diffusivity, microbial respiration rates, and their sensitivity to the CO₂ concentration.

The effects of CO₂ concentrations on soil respiration have often been explained by diffusion processes of CO₂ (Freijer and Bouten, 1991; Naganawa and Kyuma, 1991). The CO₂ flux is determined by the diffusivity and the concentration gradient of CO₂ between soil and the atmosphere (Fick’s law). Thus, if the CO₂ concentration in the soil respiration chamber is decreased, the CO₂ flux from soil will be increased because of the greater CO₂ gradient between soil and the atmosphere. However, the acceleration of soil respiration rate in low CO₂ concentration may also be explained by the CO₂ dependence of the microbial respiration. In the present results the respiration rate of Trichoderma sp. was enhanced at low CO₂ levels and decreased with increasing CO₂ levels (Fig. 4). Previous study also demonstrated that respiration rates of bacteria and fungi were accelerated at low CO₂ levels below 300 μl l⁻¹ (Koizumi et al., 1991). However, the respiratory response to CO₂ concentration would differ among microbes. Koizumi et al. (1991) indicated that respiratory activities at 0 μl l⁻¹ CO₂ was 3.0-fold that at 350 μl l⁻¹ in the bacteria-actinomycetes group and 1.2-fold in the filamentous fungi group respectively, but there was little influence of CO₂ levels at above 200 μl l⁻¹ in the fungi group. Our results, however, indicated that the respiration of Trichoderma sp., (fungi), was depressed at higher CO₂ levels (200–580 μl l⁻¹). Moreover, metabolic inhibition at higher CO₂ concentration has been reported. MacFadyen (1973) showed that respiratory activities in soil samples from diverse areas were inhibited at high CO₂ concentrations above 2500 μl l⁻¹ and ceased completely at levels greater than 10000 μl l⁻¹ CO₂. Sierra and Renault (1995) observed that oxygen consumption by microorganisms in soil aggregates was inhibited by CO₂ at higher than 40000 μl l⁻¹ CO₂. The CO₂ concentration which accelerates or inhibits the microbial respiration would differ among soil microorganisms and their environments. More detailed investigations are required to understand the extent and mechanisms of CO₂ dependence of soil and microbial respiration at various CO₂ levels.

In conclusion, our results demonstrated that it is necessary to maintain the CO₂ concentration in the chamber at that of the ambient air to determine accurate soil respiration rates, since the CO₂ concentration affects the rates through both the diffusion process and the biological process. The AA-method, which isolates the soil surface in the chamber from the outside for a long time, greatly decreases the CO₂ concentration in the chamber and is therefore not a suitable procedure to measure soil respiration rates under natural conditions. On the other hand, the OF-, DC- and CC-methods, which can measure the soil respiration with little effect on the CO₂ concentration in the chamber, are more suitable.

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