Dynamics of Organic Matters in the Root-rhizoplane-soil System of Maize

I. A simple and rapid method for measuring root respiration*

Kensuke OKADA**, and Atsuhiko KUMURA***

(Faculty of Agriculture, the University of Tokyo,
Bunkyo-Ku, Tokyo 113, Japan)

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Abstract : In the analysis of dynamics of organic matter in the root-rhizoplane-soil system, it is essential to estimate various kinds of carbon flows in the system separately. Since a simple and rapid method for measuring root respiration was needed for this purpose, the authors developed the following method.

A plastic syringe is used as the chamber. Sample roots are put into a syringe, in which the air is replaced with air of known CO₂ concentration and the syringe is kept at a constant temperature for a certain time. A volume of the air in the syringe is injected into the flow of N₂ gas in the tube which is connected to an infrared gas analyzer. The CO₂ concentration in the syringe is directly related to the reading of the analyzer. From the difference of the CO₂ concentration in the syringe before and after the incubation, the respiration rate of the roots is calculated. The details of the procedure were determined by the results of experiments regarding the effects of factors concerned.

Key words : Carbon dioxide, Infrared gas analyzer, Root respiration.

トウモロコシの根—根面—土壌系における有機物の動態 第1報 根の呼吸の簡便で迅速な測定法の開発
岡田謙介・玖村敦彦 (東京大学農学部)

要 旨 : 根流出有機物の定量には、根自身の呼吸と、根面あるいは根圏微生物の呼吸を分離測定することが不可欠である。この方法を確立するにあたって、少量の切断根 (生体重約 1 g) の呼吸を簡単かつ迅速に測定する方法が必要であったので、これを開発した。

プラスチック注射器を呼吸室としてそのなかに根を入れ、中の空気を一定 CO₂ 濃度の空気で置き換えたのち、密閉して定温下に置く。そののち、中の空気の一部を赤外線ガス分析計に接続したビニール管の中に注射する。ビニール管のなかには N₂ ガスを一定速度で流しておく。注射後に分析計の出力が示す山型の高さは、注入空気の CO₂ 濃度と直線関係にあったので、それから注射器内の CO₂ 濃度が求められる。注射器内の CO₂ 濃度の、根密閉以前に比べての上昇から、根の呼吸速度を計算できる。測定手順の各ステップにおける諸要因の影響につき検討し、その結果をもとに、測定手順の細部を定めた。

キーワード : 赤外線ガス分析計, 二酸化炭素, 根の呼吸。

Many papers report that the organic matter exuded from plants roots to soil amounts to a considerable magnitude^{3,6,8,9,10,11,15,18,19,20}. This means that this kind of carbon flow cannot be neglected in the studies on carbon balance of plants, carbon cycling in terrestrial ecosystem, soil fertility, interaction between plants and soil microorganisms, and so on.

In this series of studies, the authors intended to accurately estimate the amount of

organic matter exuded from the roots of a maize plant and chase the time course of mineralization of the exuded organic matter through microbial respiration. We think such data offer the basic information to the research field described above.

There are some methodological problems in the quantitative estimation of root exudates. It is well known that the organic matter exuded from roots to soil is rapidly mineralized through microbial respiration and only a small part can remain as organic matter for a relatively long time. Thus, to estimate the total amount of root exudation, we have to estimate both the amount of remaining organic matter and that of the organic matter mineralized by microorganisms, that is, microbial respiration.

However, it is not easy to estimate microbial

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** Present address : International Crops Research Institute for the Semi-Arid Tropics, Patancheru P.O., Andhra Pradesh 502324 India.

*** Present address : Kokushikan University, Machida, Tokyo 194—01, Japan.

respiration because a greater part of the rhizoplane organisms exist within the mucigel on the root surface^{2,4,5,17}) and therefore the CO₂ evolved by the microorganisms cannot be separated from CO₂ produced through root respiration. Rhizoplane organisms are not completely removed even after thorough rinsing^{12,16}).

Therefore, in this series of studies, the authors intended to estimate microbial respiration as the difference between total respiration (root respiration + microbial respiration) and root respiration which was measured after treating roots with fungicide suppressing microbial respiration without affecting respiration of the root itself.

In order to select a suitable fungicide and determine an appropriate procedure for the treatment, a simple and rapid method for measuring root respiration is needed, because very many comparisons should be made about the effects of fungicides or procedures on microbial and root respiration.

Further, the method should be suitable for a small amount of the fresh root material which is determined by two factors; one is the amount of materials available in our experiments, and the other is the amount of materials needed to cancel the effect of heterogeneity of the material.

However, no appropriate method has been found so far.

The open cycle gas exchange method with an infrared gas analyzer (IRGA), which is widely used for the measurement of photosynthesis and respiration, requires relatively a large amount of plant material in order to detect the difference in the CO₂ concentration between the inlet and outlet gas flow. It also takes time before the equilibrium establishes.

The oxygen electrode (here, widely used Clark type will be considered) is also used for measuring photosynthesis and respiration of excised leaf discs⁷. In this method, the material is submerged in water, so that CO₂ exchange can be measured in unnatural conditions. In addition, when the roots of the plant grown in the sand or soil are used as the material, sand or soil particles inevitably damage the teflon membrane.

Warburg manometer is also commonly used for measuring respiration of small pieces of tissue. Yet it takes time, and handling of the

equipment is relatively complicated.

Thus, the authors tested a new method, in which sample roots are incubated in a plastic syringe, and the CO₂ concentration of the air in the syringe before and after the incubation is measured by an IRGA as in the method of Atkins and Pate¹¹.

The objectives of this study were to examine the effects of factors concerned at each measurement step and to establish a new method based on the results obtained in the examinations.

Materials and Methods

1. Outline of the method

Roots were washed and severed at the base, and blotted with paper towels. A portion of 0.5–1.0 g fresh weight was taken, weighed and wrapped in a wet paper towel for a certain period. The roots are then transferred into a plastic syringe of 50 ml volume in flushing air of known CO₂ concentration which has been stored in a balloon and taken up by an air pump. By pushing and pulling the inner cylinder several times with the needle of the syringe holding in the flushing air, the air in the syringe is completely replaced by the air of known CO₂ concentration in the balloon. With the tip of the needle inserted into silicone stopper, the syringe is wrapped with a black vinyl sheet and submerged in water of constant temperature for a certain period. Then the concentration of the air in the syringe is measured as follows.

The sample cell of an infrared CO₂ gas analyzer (Fuji Electronics IRP-11 with the cell volume of 212 ml was used in this experiment) is connected to one end of a Perspex tube filled with CaCl₂ to remove water, and the other end of which is connected to a vinyl tube (Fig. 1). N₂ gas flow was held at a rate of 0.5 l/min in both the control and sample cells. The needle of the syringe is thrust into the vinyl tube connected to the sample cell at the point shown in Fig. 1 and a certain amount of the air in the syringe is injected into the N₂ flow. An example of the output record is shown in Fig. 2. The height or the area of the peak are correlated to the CO₂ concentration by the calibration curve.

2. Methods for growing non-sterile and sterile plant materials

Maize plants (*Zea mays* L. var Koh 7) were

Fig. 1. Equipment for measuring CO_2 concentration.

Fig. 2. An example of the recorder chart.

used in all experiments.

(1) Non-sterile plants

Plants were grown for 8 day in soil in a 0.5l plastic beaker under natural light at 30/25°C (day/night). This material was used for Experiment 5.

(2) Sterile plants

Seeds were immersed in 0.1% AgNO_3 solution and shaken for 5 minutes vigorously. After being kept in the solution for 55 minutes, the seeds were rinsed with sterile distilled water five times, transferred to the surface of BCP (Bromo Cresol Purple)-added Plate Count Agar¹⁴ (0.5% agar) in petri dishes, and kept for 2 days at 27°C. Non-contaminated seeds were then selected, and three seeds each were transferred into a long test tube (26.5 mm i.d. $\times$ 450mm) (Fig. 3), which had been filled with vermiculite up to 250 mm from the bottom and 70 ml of tap water, and then autoclaved. The surface of vermiculite was covered with 5 ml of BCP-added Plate Count Agar for detecting microbial contamination. The test tubes were covered at the portion where vermiculite was stuffed and transferred to a growth chamber. Plants were grown for 5–6 days at 30/25°C (day/night) under 10 klx light for 12 hours per day.

Fig. 3. Culture vessel for the growth of maize seedlings in sterile conditions.

Then, the test tubes with no visible microbial colony were selected and used for the Experiment 6. The final sterility check was also done about the materials used for the experiment in the following way. After 80 ml of sterile distilled water was added into the test tube, the test tube was shaken vigorously. And the rinsing was served for plate counting. A subsample (1 ml) of the rinsing was incubated at 26°C with egg albumin agar medium or Tryptic Soy Broth agar medium for 7–8 days, and with Rose Bengal agar medium for 4 days. The data obtained from contaminated plant materials were all discarded.

Results and Discussion

1. The rate of injecting sample gas into the tube (Experiment 1)

Since it usually takes some time to inject gas in a syringe into vinyl tube, the effect of the

Fig. 4. The effect of the time spent for injecting sample gas on the output of gas analyzer (the peak height of the output voltage).

length of the time needed for injection on the result was investigated. The investigation revealed that the time length less than 10 seconds did not affect the result (Fig. 4), on the conditions that the flow rate of the carrier gas was 0.5 l/min, the volume of the sample gas was 20 ml and the CO_2 concentration of the sample gas was 808 ppm (v/v). The cell volume of the IRGA was 212 ml, and so the exchange time for the carrier gas which flowed at the rate of 0.5 l/min was 25 seconds. The results suggested that when the length of the time for the injection was much smaller than the exchange time, the obtained value was not affected because all the sample gas stayed in the cell for some time, but when the time for injection became closer to the exchange time, the obtained value decreased because a part of the sample gas flowed out of the cell before the last part of the sample gas was transported

Fig. 5. The relationship between the amount of sample gas injected and the output of analyzer. (Vertical bars indicate SE ($n=4$)).

into the cell.

2. The relationship between the amount of sample gas and the result (Experiment 2)

The effect of the amount of the sample gas injected at a time on the result was investigated.

The obtained value linearly correlated to the amount of the gas injected in the range of less than 40 ml (Fig. 5), when the flow rate of the carrier gas was 0.5 l/min, the CO_2 concentration was 808 ppm (v/v) and the time spent for the injection was 1 second.

3. The linearity between the CO_2 concentration of sample gas and the result (Experiment 3)

On the conditions that the flow rate was 0.5 l/min, the amount of sample gas was 20 ml and the injection time was 1 second, the obtained value was linearly correlated to the CO_2 concentration of the sample gas (mixture

Fig. 6. The relationship between the CO_2 concentration of sample gas injected and the output of gas analyzer. (Vertical bars indicate SE ($n=3$)).

Table 1. The effect of water vapor in sample gas on the output of gas analyzer.

Condition of sample air	Output of analyzer (mV)
Dry air	$1.99 \pm 0.04^*$
Humidified air	2.04 ± 0.02

*Mean $\pm$ SE (n=4).

Table 2. The effect of water rinsing on respiration of sterile roots.

Treatment	CO ₂ evolution rate per tissue fresh weight (mg g ⁻¹ h ⁻¹)
Unrinsed	$0.58 \pm 0.17^*$ (100)
Rinsed	0.41 ± 0.08 (71)

*Mean $\pm$ SE (n=5).

of the CO₂ and N₂ gas) up to 2000ppm (v/v) (Fig. 6).

4. The effect of water vapor in the sample gas on the result (Experiment 4)

The humidity of the sample gas in the syringe must be high when fresh root material is used. There is a possibility that the humidity interferes CO₂ measurement by IRGA since water vapor has an absorption band near that of CO₂. The ability of the CaCl₂ column (20 mm i.d. $\times$ 300 mm), which was inserted in the system in order to prevent the interferences, was tested. The injection of the humidified sample air gave almost the same output as that of the dry air (Table 1). From this, it was concluded that the effect of water vapor in the sample air was negligible.

5. The effect of the time lapse after the excision of roots on the respiration rate (Experiment 5)

Because there is usually a time interval between cutting off roots from shoots and the start of incubating the roots, the effect of the length of the time lapse on the obtained respiration rate was investigated. The excised roots were kept in a wet paper towel for a certain time before measurement. Then the roots were transferred into a syringe and incubated for 5 minutes at 25°C.

The results were shown in Fig.7. Measurements were taken 3 times of a day to check the possible diurnal changes of root respiration. There was no consistent difference between the rates obtained at different times in a day.

Fig. 7. The effect of the time lapse after detaching roots on the respiration rate.

Measured at 13:00—15:00.

Measured at 18:00—19:30.

Measured at 20:30—22:00.

That is, the roots did not show diurnal changes in the respiration rate in this experiment. One reason may be that plants were still living depending on the seed reserves at that stage and so their root respiration was not affected by diurnal changes in the light regime.

After the top being detached, the respiration rate of the roots gradually decreased with time. This suggests that the interval between root excision and the start of incubation should be standardized. Considering the effect of the time lapse and convenience in handling sample roots, the interval was decided to be 20 min.

6. The effect of rinsing with water on the root respiration rate (Experiment 6)

In the measurement of root respiration, it is inevitable to rinse sample roots to remove soil or sand particles attaching to the roots. Thus, the effect of this procedure on respiration was investigated. If non-sterile roots were used in this experiment, there was a possibility that the rinsing procedure removed some microorganisms on the root surface and affected the total respiration of the root system. In such a condition, it is impossible to examine the real effect of rinse on root respiration. Considering this, sterile roots were used for this experiment. In the "rinsed" treatment, plants were repeatedly dipped into water for 4 minutes. Then after being detopped, the roots were blotted by paper towels and weighed. They were kept in paper towels for 20 minutes and their respiration rate was measured during the

incubation for 15 min at 25°C. In the “unrinsed” treatment, plants were detopped without rinsing roots, and handled as in the “rinsed” treatment.

As shown in Table 2, the “rinsed” treatment reduced root respiration rate by 29%.

Ohmura and Howell examined the effect of drenching samples on the respiration rate in several plant tissues and concluded that the respiration rate of maize roots 4–7 days after germination was reduced by 37% after getting drenched. They clarified that it was due to the water film on the tissue which hindered diffusion of oxygen into the tissue¹³. The reduction of root respiration rate by “rinsed” treatment may be ascribed to this effect and to some mechanical damages created by frequent dipping.

Considering the above mentioned, the rinsing procedure should be standardized.

7. *The details of the procedure established by the authors*

From the above described results, the details of the procedures were determined as follows.

- (1) The flow rate of the carrier gas is 0.5 l/min.
- (2) The amount of the gas injected at a time is less than 40 ml, and should be injected within 10 seconds.
- (3) The CO₂ concentration of the sample gas should be less than 2000 ppm (v/v).
- (4) The detopped roots are kept for 20 minutes before the incubation.
- (5) The rinse of the roots is done according to a standardized procedure.

8. *Characteristics of the method established*

This method for measuring root respiration has the following characteristics.

- (1) It is rapid. It takes 45–50 minutes to measure the root respiration of one sample, including root rinsing (4 min), excision, blotting and weighing of the roots (1–2 min). But it includes the time when no manipulation is done (after excision, 20 min; incubation time for the measurement, 15 min). Therefore the measurements can be started at the intervals of 7–8 min successively and several samples can be treated at the same time.
- (2) Easy to handle. It does not use chemical solutions; it saves time and labor for the preparation and washing of implements; no glass implement is used.
- (3) It can measure the respiration of roots

with soil particles which might damage instruments in some other methods.

(4) It can measure respiration of samples of a wide range of amount by using syringes of different sizes.

(5) Excision or imbibing of sample roots affects the root respiration rate to some extent, but other methods do not solve these problems, either.

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