Soil Biol. Biochem. Vol. 9, pp. 193 to 196. Pergamon Press 1977. Printed in Great Britain.

A DEVICE TO STUDY METABOLIC GASES IN THE RICE RHIZOSPHERE

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(*Accepted* 7 *July* 1976)

Summary—A simple apparatus was developed to study metabolic gases in the rice rhizosphere. With this device, a positive rhizosphere effect of rice was demonstrated on three soil microbial activities: N_2 fixation, CH_4 formation and denitrification.

INTRODUCTION

Several devices have been used to study metabolic gases in the soil–plant system. Ross *et al.* (1964) and Martin and Ross (1968) developed a gas-lysimeter for a N balance study, using labelled fertilizer. Stefanson used sealed growth chambers to study the effects of plants on denitrification (1970) and N_2 fixation (1973), during long-term incubation.

The C_2H_2–C_2H_4 assay has been applied to measure N_2 fixation in the rhizosphere. The *in situ* measurement of nitrogenase activity developed by Balandreau and Dommergues (1971) was of particular interest because it did not disturb the soil–plant system. This method was used in the case of rice (Balandreau *et al.*, 1975; 1976); it utilized the peculiarity of rice to allow gaseous exchange between the external atmosphere and the rhizosphere (Luxmoore *et al.*, 1970).

To study biological phenomena involving the consumption or evolution of gases in the rice rhizosphere, such as N_2 fixation, CH_4 formation and denitrification, we have developed a simple piece of equipment that allows comparative measurements of these processes during a short period of incubation in the rhizosphere of 3-week old rice plants and unplanted soil.

MATERIALS AND METHODS

One seed of rice variety IR 8 was sown in a 14 × 200 mm Pyrex tube containing 13 g clay soil collected from a rice field. The C and N contents of this soil were 1.35 and 0.6% respectively and its pH was 6.3. For comparison soil only was placed in a second identical tube. The tubes were maintained under artificial illumination with a light intensity of 20,000 lux and a 14 h photoperiod. Three weeks later, the contents of each tube were transferred to a device made from two glass tubes joined together by fusion (Fig. 1). The top Pyrex tube (200 × 14 mm i.d.) had a wider upper end (60 × 16 mm i.d.). The lower Pyrex tube (180 × 18 mm i.d. was joined by fusion to the upper Pyrex tube and had two side arms closed by Vacutainer stoppers (Becton Dickinson, ref. 4827 0–0) and Vigreux points above the lower side arm, in order to hold the soil core (Fig. 1).

After joining the lower part of the culture tube to the upper part, a partial vacuum was applied to the lower compartment using a 10 ml syringe inserted through one of the Vacutainer stoppers. The vacuum caused the water-saturated soil core of the culture tube to slide into the top compartment of the device. The vacuum was released when the lower end of the soil core reached the joint between the two compartments. The lower compartment was then flushed with pure N_2 for 5 min. The N_2 was admitted through hypodermic needles inserted in the Vacutainer stoppers.

Then the soil core was allowed to slide into the lower compartment until it rested on the Vigreux points. A few ml of a solution made of $\frac{1}{3}$ solid paraffin and $\frac{2}{3}$ paraffin oil was applied to the top of the soil core, at a temperature slightly higher than the melting point of the paraffin. Pure N_2 was again flushed into the lower compartment. A 1 cm layer of water was

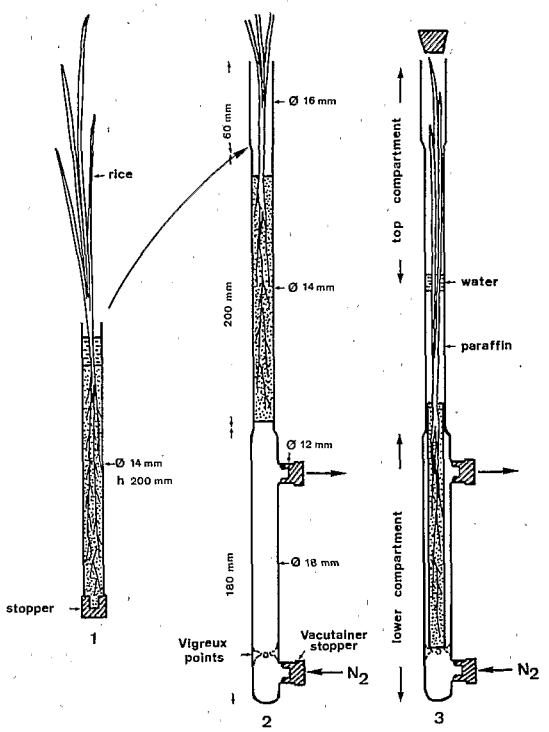

Fig. 1. Device to study metabolic gases in the rice rhizosphere. 1—Culture tube. 2—Transfer of the soil core of the culture tube into the top compartment of the device. 3—Device ready for use.

added above the solidified paraffin to test for leaks by the appearance of bubbles during flushing and to detect any possible defects in the apparatus. The gaseous volume in the device was about 22 ml per compartment. The efficiency of flushing with N_2 was checked by withdrawing and analysing gas samples for presence of O_2 by gas chromatography. Where needed the top part of the tube was sealed with a rubber stopper.

N_2 fixation

N_2 fixation was estimated by C_2H_2–C_2H_4 assay. The upper part of the apparatus was closed by a Vacutainer stopper and 2.2 ml C_2H_2 (10% of the total volume) were injected simultaneously into the two compartments. For diurnal measurements, the devices were illuminated at the beginning of the light period during 6.5 h; measurements during the dark period were also performed during the 5 h before illumination period. These measurements were made with simultaneous removal of 0.5 ml samples from the atmospheres of the two compartments with a 1 ml plastic syringe. Analysis (6 replicates) were made with a flame ionisation chromatograph (Varian Aerograph 1200) using the following conditions: Porapak R 100–120 mesh, 150 × 0.3 cm stainless steel column monitored about 60°C; injector temperature, 105°C; detector temperature, 180°C; N_2 flow, 30 ml·min^{-1}; H_2 flow, 30 ml·min^{-1}; compressed air flow, 300 ml·min^{-1}.

CH_4 formation

The top compartment was closed by a rubber stopper. Every hour, 0.5 ml gas samples were withdrawn from both compartments. These samples were analysed by flame ionisation gas chromatography as described for N_2 fixation. The results represented the total amount of CH_4 evolved in the two compartments, the greater part (about 80%) being in the lower compartment, and were the mean of 10 replicates.

To prevent CH_4 formation, 1 ml (5%) C_2H_2 was injected into the lower compartment before measurements were made (Raimbault, 1975). To study CH_4 formation under light, the soil cores of the culture tubes were placed in the devices after 2 h illumination and were incubated under light during measurement. To measure activity during the dark period, the soil cores were transferred 2 h before illumination began and incubated continued in the dark.

Denitrification

Denitrifying activity was estimated by a N_2O reduction method (Garcia, 1974). Unlike the previous measurements, the apparatus was only used to incubate rhizosphere soil (*R*) and unplanted soil (*S*) in the presence of N_2O so as to induce N_2-reductase in the soils under ecological conditions.

To do this, 2.5 ml N_2O were injected into the lower compartment through one of the Vacutainer stoppers. The plant canopy was exposed to air (the upper compartment was left open) and the samples were illuminated under standard conditions for 14 h. Then the soil cores were extracted and put into 250 ml serum flasks, with 3 cores per flask and 5 replicates. The flasks were evacuated and flushed with He to ensure anaerobiosis and 80 parts/10^6 N–N_2O (2.5 ml) and 1 ml krypton as an internal standard was injected into the flasks with plastic syringes. Measurements of N_2O reduction were made with a thermal conductivity gas chromatograph Varian Aerograph 90 P4 (Garcia, 1974) during the next 24 h.

RESULTS AND DISCUSSION

N_2 fixation

Figure 2 shows C_2H_2 reduction rates of rice rhizosphere soils incubated during the dark period (*RD*), at the beginning of the light period (*RL*) and those for unplanted soils (*S*). Most of the C_2H_4 produced (about 90%) had diffused through the soil core in the lower compartment of the apparatus.

The C_2H_2 reduction rates are linear after a 2 h lag. Because of the small amounts of C_2H_4 evolved (10 nmoles C_2H_4 corresponded to 0.224 µl C_2H_4), we assumed that this initial lag was caused by poor C_2H_2 or C_2H_4 diffusion between the atmosphere and the soil during the first hours of incubation.

N_2 fixation in unplanted soils was very slight (0.6 nmoles $C_2H_4 \cdot h^{-1}$) compared to planted soils. For *R*, a greater activity was observed during the light period (21.6 nmoles C_2H_4 evolved·h^{-1}) than during darkness (8.8 nmoles C_2H_4 evolved·h^{-1}). One of possible causes would be more organic C exudation as a result of photosynthesis.

These agree with those of Balandreau *et al.* (1975). However, we noted that the activity they measured in darkness was very weak due to the technique they used, where C_2H_2 was injected only in the presence of the plant canopy. Their method only allowed the demonstration of activity if stomata were open, whereas the activity we measured during the dark period, represented about 50% of the maximum diurnal activity.

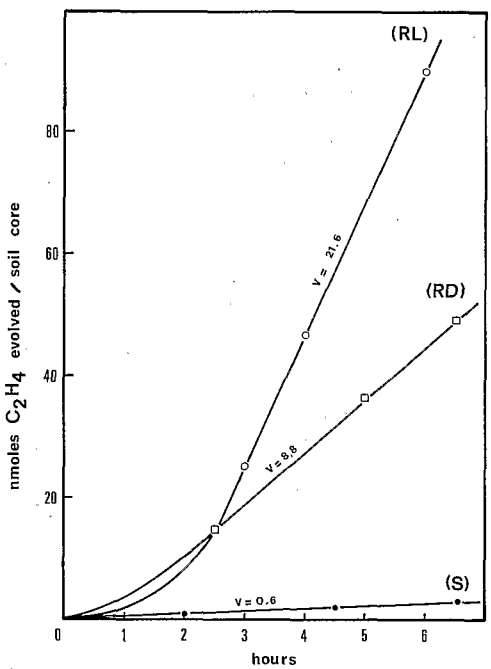

Fig. 2. N_2 fixation estimated by the C_2H_2–C_2H_4 assay. (*RL*) rhizosphere incubated at the beginning of the light period; (*RD*) rhizosphere incubated during the dark period; (*S*) unplanted soil.

CH_4 *formation*

Our apparatus satisfactorily measured CH_4 formation in planted soils. Figure 3 shows that rice seedlings during the light period produced practically twice as much CH_4 (8 μl CH_4 evolved·h^{-1}·soil $core^{-1}$) as during dark incubation (4.2 μl CH_4 evolved·h^{-1}·soil $core^{-1}$).

With 5% C_2H_2, CH_4 evolution was completely inhibited as demonstrated previously (Raimbault, 1975). CH_4 formation in unplanted soils could not be estimated since CH_4 evolution was approximately the same with the addition of C_2H_2 as without (Fig. 3). In unplanted soils, CH_4 remained confined in the soil during the 3 weeks of flooding, but diffused into the atmosphere surrounding the soil core when placed in the lower compartment of the device. If so, gas evolution measured by this method will not agree with the CH_4 actually produced during incubation. On the other hand, in planted soils, CH_4 would diffuse through the gas spaces of the rice seedlings during the 3 weeks growth. This lack of accumulation permitted us to report the measured amounts as CH_4 production in the device during the incubation of the samples.

To measure the activity of unplanted soil, we made a vacuum in sealed test tubes enclosing the soil core, followed by flushing with pure N_2. This was repeated several times. A linear evolution rate of 0.7 μl CH_4·h^{-1}·soil $core^{-1}$ was then measured. These results show the strong positive effect of the rice rhizosphere on CH_4 formation.

Denitrification

Reduction of N_2O was much greater in the rice rhizosphere soil that in the unplanted soil (Fig. 4). The initial denitrifying activity that represented induced activity in ecological conditions, was measured during the first 6 h of incubation (Garcia, 1974). This activity was found to be 4 times higher for *R* than for *S*; these results agreed with those of a previous experiment in which the soils were preincubated in a stainless steel sieve of 30 μm to permit the diffusion of N_2O (Garcia, 1975).

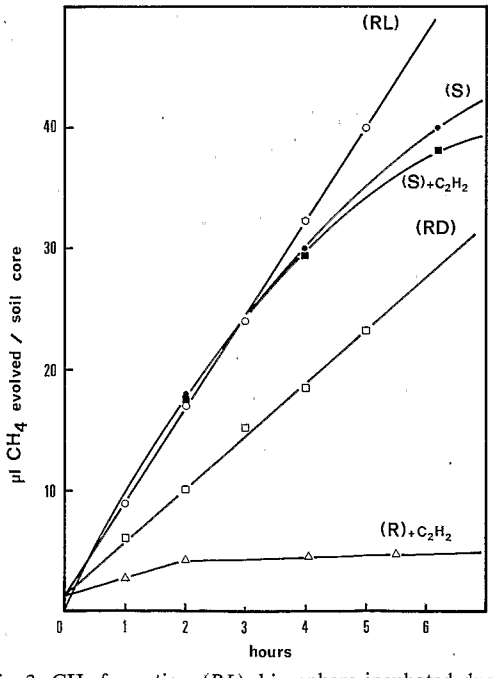

Fig. 3. CH_4 formation. (*RL*) rhizosphere incubated during the light period; (*RD*) rhizosphere incubated during the dark period; (*S*) unplanted soil.

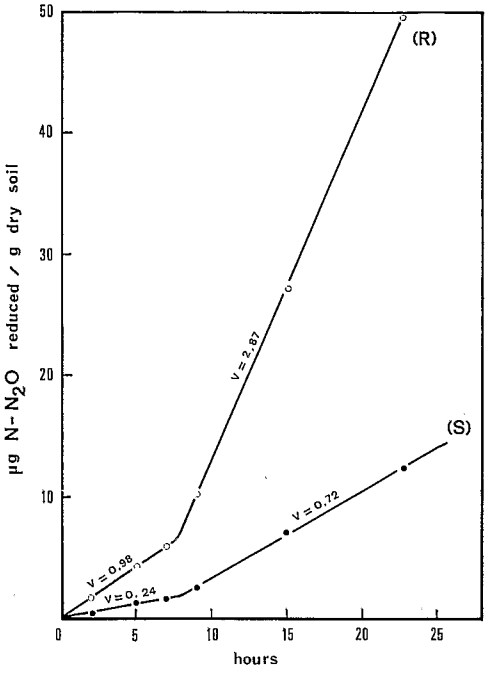

Fig. 4. Denitrification estimated by the N_2O reduction method. (*R*) rhizosphere; (*S*) unplanted soil.

On the other hand, the potential denitrifying activity developed by *de novo* synthesis of enzyme induced by the injected N_2O, was measured during the last 18 h of incubation in the flasks (Garcia, 1974). This potential activity was also found to be 4 times higher for *R* than for *S*. This confirms the positive effect of the rice rhizosphere on denitrification. As the rice seedlings were incubated with their canopies in open air during the light period—during a period of O_2 formation—the induction of a higher N_2O reductase activity in *R* than in *S*, strictly inhibited by O_2, shows again the existence of an anaerobic zone in the rice rhizosphere under the influence of root exudates. Such exudation of organic C could represent one possible cause of the increase in denitrification in the rhizosphere of rice.

CONCLUSION

This device to study gaseous metabolism in the rice rhizosphere permitted the demonstration of a strong positive effect of the rice rhizosphere on three microbial activities involving the evolution or consumption of gas: N_2 fixation, CH_4 formation and denitrification. As CH_4 formation and denitrification are strictly anaerobic processes, the rice rhizosphere effect resulted from the existence of anaerobic zones directly influenced by root exudates. Concerning N_2 fixation, the diffusion of air through the rice seedlings permitted the activity of both anaerobic and aerobic micro-

organisms. Finally, in this apparatus gas diffusion was independent of movement within the rice plant and through stomata.

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