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Mechanism of Methane Transport from the Rhizosphere to the Atmosphere through Rice Plants[1]

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ABSTRACT

To clarify the mechanisms of methane transport from the rhizosphere into the atmosphere through rice plants (*Oryza sativa* L.), the methane emission rate was measured from a shoot whose roots had been kept in a culture solution with a high methane concentration or exposed to methane gas in the gas phase by using a cylindrical chamber. No clear correlation was observed between change in the transpiration rate and that in the methane emission rate. Methane was mostly released from the culm, which is an aggregation of leaf sheaths, but not from the leaf blade. Micropores which are different from stomata were newly found at the abaxial epidermis of the leaf sheath by scanning electron microscopy. The measured methane emission rate was much higher than the calculated methane emission rate that would result from transpiration and the methane concentration in the culture solution. Rice roots could absorb methane gas in the gas phase without water uptake. These results suggest that methane dissolved in the soil water surrounding the roots diffuses into the cell-wall water of the root cells, gasifies in the root cortex, and then is mostly released through the micropores in the leaf sheaths.

Recent studies of ancient air trapped in polar ice cores (7, 17) have shown that the concentration of atmospheric methane has more than doubled during the past 200 years and that during the last decade atmospheric methane has increased approximately 1% per year (4). Because methane is one of the so-called greenhouse gases, in addition to CO_2, N_2O, O_3, and chlorofluorocarbons, the increase in atmospheric methane may cause an increase in the globally averaged surface temperature (19, 25). About 80% of methane emissions are produced biologically by methanogenic bacteria in flooded soils and in the intestines of domestic animals (9). Rice (*Oryza sativa* L.) paddy fields are known to be a major source of methane (5) and the area of rice paddy fields in the world averaged over the last 35 years has increased 1.6% per year (13). Although a full explanation of increasing atmospheric methane concentration remains uncertain, the increasing area of rice paddy fields in the world is considered to be an important cause of the recent shifts in the atmospheric methane balance.

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Studies have found that methane emission from vegetated plots in rice paddy fields were much higher than from unvegetated plots (6, 14). Therefore, Cicerone and Shetter (6) proposed that methane emitted to the atmosphere from rice paddy fields is transported mostly through rice plants and not across the water-air interface via bubbles or molecular diffusion.

In rice and other hydrophytes, it is well known that atmospheric O_2 is transported to the submerged organs from the leaf parts above water through the aerenchyma and intercellular gas space systems by diffusion (2, 10, 15, 24) or by mass flow (8). Since these internal air spaces in rice plants are particularly well developed in the culm (1) and roots (16), the ventilation system in rice plants plays an important role in transport of gases between the rhizosphere and the atmosphere. The absorption and release sites of gases are generally considered to be the stomata which are linked to intercellular air spaces in the leaf blade. However, Seiler *et al.* (22) reported that stomatal closing by exposure to darkness or increased CO_2 in the atmosphere did not significantly affect the methane emission rate from rice plants. Similar results have been observed using exogenously applied ethylene (18) and CO_2 (12) passed through the rice plants from the roots to the shoots. These observations indicate that the stomata are not the major release site of methane or other gases from rice plants. Thus, little is known about the mechanism of methane transport from the rhizosphere to the atmosphere through rice plants in paddy fields. Furthermore, the primary site of methane release from rice plants has not been identified.

The present study was initiated to determine the site of methane release from the rice plant and the process of methane uptake by rice roots. To accomplish these goals the profile of methane concentration in the paddy soil layer, the methane concentration in the rice body, the relationship between methane emission and transpiration rates from a shoot, site of methane release, and the mechanism of methane uptake by roots were examined.

MATERIALS AND METHODS

Field Studies

Measurements of methane concentration in soil water and in the medullary cavity of rice plants (*Oryza sativa* L.) were carried out in two rice plots (2.5 × 4.0 m) grown in lysimeters

2.0 m deep. The soil in the lysimeters was an Andosol fertilized with 9.0 g of nitrogen, 9.0 g of P_2O_5, and 9.0 g of K_2O per square meter. The two plots were treated with different organic substrates, rice straw and compost. Prior to planting, the rice straw-treated and compost-treated plots received rice straw at the rate of 0.6 kg m^{-2} and compost at the rate of 2.0 kg m^{-2}, respectively. The rice paddy was flooded on May 18, 1988 and rice plants (cultivars, Nipponbare) were transplanted at the rate of about 20 stubs per square meter on May 20. The field was irrigated as needed to maintain water depth at about 5 cm throughout the growing season.

Profile of Methane Concentration in Paddy Soil Layer

Soil water extractors were constructed using 8 mm diameter by 100 mm long hollow cylinders of highly permeable sintered polyethylene filter cups (23). The polyethylene filter cups were connected to Teflon tubes (1 mm in diameter) and were inserted at 5, 10, 20, 30, 40, and 50 cm depths by pushing a pointed steel rod into the flooded soil on July 20. Three polyethylene filter cups were installed at each depth. Soil water was extracted based on the siphon theory. The profile of methane concentration in the soil water layer was measured four times between August 15 and September 20.

Methane Concentration in Medullary Cavity (Internodal Lacunae) of Rice

The collection of gas from the medullary cavity of rice was carried out according to the method of Higuchi (11). At the middle ripening stages of rice (about 120 d after transplant), methane in the rice body was collected directly from the medullary cavity of the rice plant. The culm and leaf sheath of rice were excised with a razor blade at the zone of partition. The needle of a microsyringe was inserted into the medullary cavity which was the third from the top node, and 50 μL of gas was collected. After gas collection at the third internode (upper), gas in the medullary cavity of the fourth internode (lower) was collected by the same method. The position of an internode was counted from the top internode. These internodes, which were about 6 to 10 cm above ground level, were chosen for this experiment because their volumes were larger than the other internodes and facilitated the collection of gas.

Laboratory Experiments

Plant Materials

Rice seeds (cultivars, Koshihikari) were germinated and grown on a Saran net floating in a vat filled with distilled water for 1 week. Seedlings were grown with Kimura's B culture solution (3) in a naturally lit, environmentally controlled glass chamber with a constant temperature of 25 ± 0.1°C and RH of 75 ± 5%. Rice plants at the tillering stage were used for determinations of methane emission rates. All experiments were performed with 50- to 70-d-old plants.

Measurements of Methane Flux from Shoots

The experimental set-up for measurements of methane emission rates is shown in Figure 1. The apparatus consists

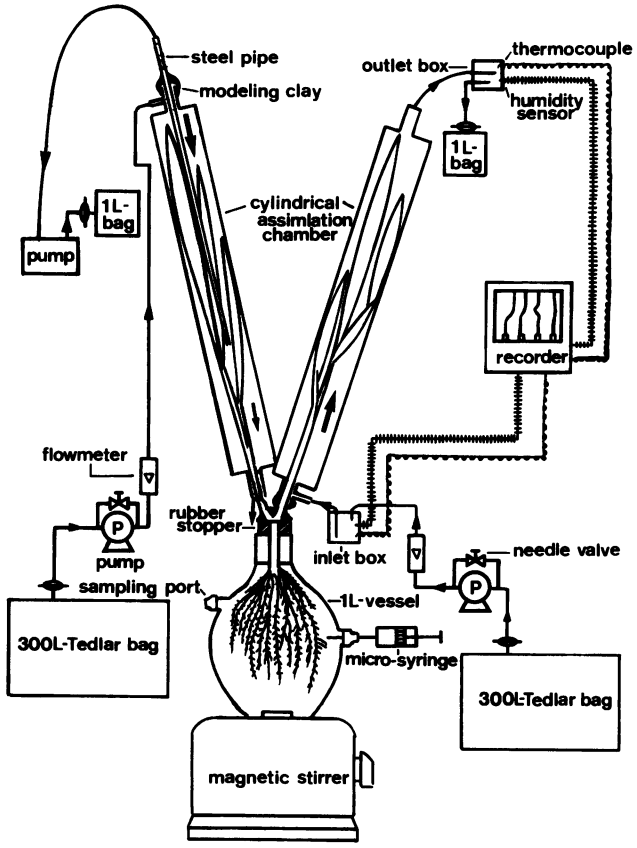

Figure 1. Experimental set-up for measurement of methane emission.

of a 1 L glass vessel for the root zone with two sampling ports and a magnetic stirrer. The glass vessel is attached to cylindrical acrylic chambers for shoots with flowmeters, pumps, and other environmental control devices. A rice plant was placed in the vessel and held in place by sealing at the base of the culm with modeling clay and a rubber stopper divided into two pieces. The methane emission rate from a shoot was determined by sampling the air in the cylindrical acrylic chambers (3 cm in diameter and 50 cm in length). Ambient air stored in a 300 L Tedlar bag was continuously introduced into the cylindrical chamber by a pump. The air flow rate was monitored by a flowmeter and was adjusted to 1.0 L min^{-1}. The effluent air from the cylindrical chamber was collected in a 1 L Tedlar bag for 1 min.

The methane emission rate and the transpiration rate were calculated by comparing the differences in concentration of methane and water vapor in the air entering and leaving the chamber, respectively. The amount of methane and water was determined with a gas chromatograph and thin-film capacitive humidity sensors (Visala Co.), respectively. The temperature of the inlet and outlet air was measured with thermocouples.

In liquid phase experiments, a culture solution with a high methane concentration was prepared by bubbling air with a particular concentration of methane into Kimura's B culture

solution for at least 3 h with an air stone placed at the bottom of the vessel. The solution in the vessel, which was covered with aluminum foil, was slowly stirred during the measurements. Water samples (10 μL) were taken with a microsyringe through a silicon septum. In gas phase experiments, air at different concentrations of methane in a 300 L Tedlar bag was introduced into the vessel holding a rice seedling shoot through the inlet port and ejected from the other one using a pump (4.0 L min^{-1}). After 4 min, the flow rate was lowered to 0.40 L min^{-1} and the methane enriched air was continuously passed through the vessel during the measuring periods.

These experiments were conducted in an artificially lit, environmentally controlled chamber (1.3 m [d] × 1.0 m [w] × 1.1 m [h]) equipped with 15 metal halide lamps (seven 400-W Yoko lamps and eight 400-W BOC lamps, Toshiba). The light intensity was about 700 μmol m^{-2} s^{-1} at the center of plant height. The light was passed through a heat absorbing water filter. The conditions in the chamber were kept at 25 ± 0.5°C and 70 ± 5% for temperature and RH, respectively. All measurements of methane emission rate were performed under light except for the light-dark treatments.

Injection of Air into the Medullary Cavity of Rice

Measurements of air released from the shoots by injecting air with a syringe were carried out according to the method of Higuchi *et al.* (12). The main stem of the rice was cut off near the root and the upper part with leaves was exposed to water. Air was injected into the medullary cavity and the release of the air through the shoot was monitored by the appearance of air bubbles.

Observation of Surface Structure of Leaf Sheath with Scanning Electron Microscopy

For scanning electron microscopy, some sections of the leaf sheath releasing air bubbles were cut off with a razor blade. The sample pieces were freeze-dried, coated with gold and examined with a scanning electron microscope (Hitachi, X-650). Observations and photographs with the scanning electron microscope were made at 15 KeV.

Methane Gas Analysis

The methane concentration was determined by a gas chromatograph equipped with hydrogen flame ionization detectors (Shimadzu, GC-9A), a gas sampler (Shimadzu, MGS-4) and an integrator (Shimadzu, Chromatopac C-R3A). Separations of methane in air and in water were carried out at 90°C using a glass column (3 mm in diameter and 2 m in length) packed with molecular sieve 5A (60–80 mesh) and activated carbon (80–100 mesh), respectively. The gas chromatograph was calibrated every day using 10 μL/L standard methane gas.

RESULTS

Profile of Methane Concentration in the Soil Water Layer

To determine whether methane abundantly dissolves in the soil water of rhizospheres, the profiles of methane concentration in the soil water layer of both the rice straw-treated and compost-treated plots were measured on August 15, August 26, September 8, and September 20. Water samples were taken regularly each day at noon. The profile of methane concentration in the soil water layer had almost the same pattern during each of the four measurements. Figure 2 shows a typical profile of methane concentration in the soil water layer of the rice straw-treated and compost-treated plots on August 26, 1988. In both plots, the concentration of methane dissolved in the water was highest at the 5 cm depth, followed by that at the 10 cm depth. At 20 cm and below, there were very low levels of methane dissolved in the water. Since organic materials (rice straw and compost) which are substrates for methane production were mixed into the soil between the surface and the 10 cm depth, it is not surprising that the profiles of methane concentration in the soil water layer correlated positively with the presence of these organic materials.

The concentration of methane dissolved at the 5 cm depth in the rice straw-treated and compost-treated plots from August 15 to September 20 ranged between 7.9 to 15.0 μL/mL and 0.9 to 2.7 μL/mL, respectively. The concentration of methane dissolved in the soil water at the 5 cm depth of the rice straw-treated plot was about 10 times higher than that of the compost-treated plot, indicating that the kind of organic substrate greatly affects methane production in flooded soil.

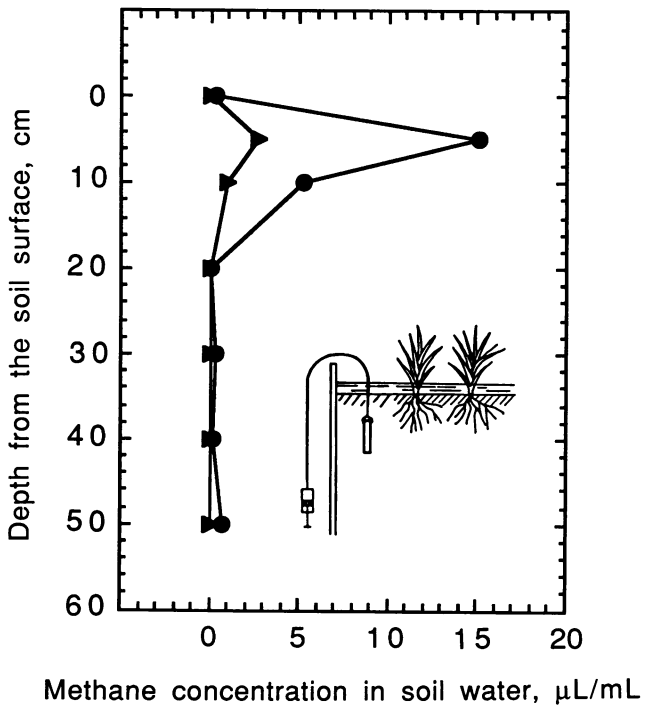

Figure 2. Profile of methane concentration in soil water layer in fertilized straw (●) and compost plots (▲). The polyethylene filter cups were connected to Teflon tube and were inserted at 5, 10, 20, 30, 40, and 50 cm depths. Soil water was extracted by siphoning and taken with a microsyringe.

Methane Concentration in Medullary Cavity of Rice

Figure 3 shows the diurnal changes in methane concentration in the medullary cavities of the rice plants in the rice straw-treated plot on September 19 to 20. The methane concentration in these cavities was 500 to 5000 μL/L. The methane concentration in the lower medullary cavity was always 1.6 to 2.4 times higher than that in the upper one.

Relationship between Methane Emission Rate and Transpiration Rate

To clarify the relationship between stomatal openings and methane emission through rice plants, methane emission rate and transpiration rate were measured simultaneously from a rice shoot having three leaves whose roots had been exposed to a culture solution with a high methane concentration. Figure 4 shows a typical temporal response of the methane emission rate during the following schedule of light-dark treatments: 70 min light → 70 min darkness → 60 min light → 60 min darkness → 60 min light. The axis of the abscissa was the time after soaking in the culture solution with the high methane concentration (prepared by bubbling 40% methane through the culture solution). Since the change in transpiration rate is dependent on stomatal opening, the transpiration rate was enhanced by light and decreased by darkness. On the other hand, the methane emission rate generally dropped immediately after changes in exposure to light and darkness except for a slight increase during the first 5 min following exposure to light. When the light was turned on after about 20 min or turned off after 8 min, the methane emission rate tended to gradually return to the values which were observed immediately before the light-dark treatment. Thus, the change in methane emission rate was similar to the change in transpiration rate only immediately after the light-dark transitions. During the remainder of the exposure to light or darkness the change in methane emission rate did not coincide with that of the transpiration rate. This experiment was repeated five times with similar results. Although this phenomenon may occur due to some physiological responses in rice plants or due to a physical reaction such as a thermal effect from the heat due to the light, the reason is unknown why the methane emission rate remarkably decreased immediately after exposure to light or darkness.

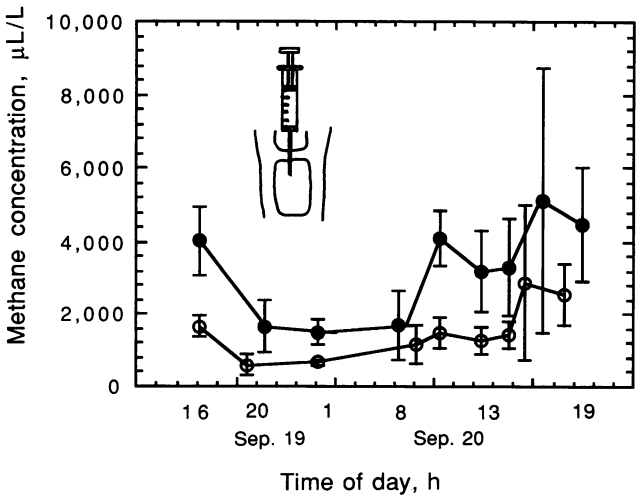

Figure 3. Diurnal changes of methane concentration in medullary cavity. Measurements of methane concentration in the upper medullary cavity at the third internode (○) and the fourth internode (●) were taken at ripening stage of rice plants in the rice straw-treated plot on September 19–20.

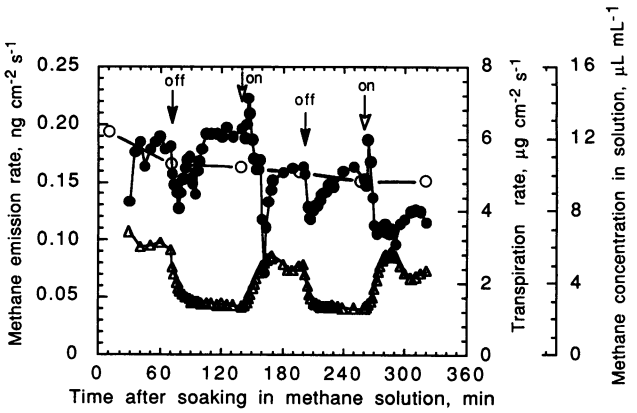

Figure 4. Relationship between methane emission rate (●) and transpiration rate (△) from a rice shoot whose roots were exposed to a culture solution with a high methane concentration. The culture solution with the high methane concentration was prepared by bubbling 40% methane into the solution for 3 h. The concentration of methane dissolved in the culture solution (○) was measured a total of six times immediately before the light (white arrows) dark (black arrows) treatments.

Next, a comparison was made of the amount of methane which would be emitted by transpiration and of methane actually emitted using the data above (Fig. 4). In this experiment, the concentration of methane dissolved in the culture solution with the high methane concentration was measured a total of six times immediately before the light-dark treatments. The calculated concentration of dissolved methane at each period was estimated by exponential function. The methane emission rate by transpiration was calculated as a function of the amount of transpiration and the methane concentration in the culture solution. Figure 5 shows the ratio of measured methane emission rate to calculated methane emission rate due to transpiration at each time. Under illumination the measured methane emission rates were 8 times higher than the calculated rates that would result from transpiration. During the dark periods (except for immediate transition periods) the measured methane emission rates were about 20 times higher than the calculated rates that would result from transpiration.

Release Site of Methane from a Shoot

To locate the site of methane release from a shoot, air surrounding the leaf blade or culm of the shoot was collected and the methane concentration at each point was measured (Fig. 1). A shoot having three leaves whose roots were soaked

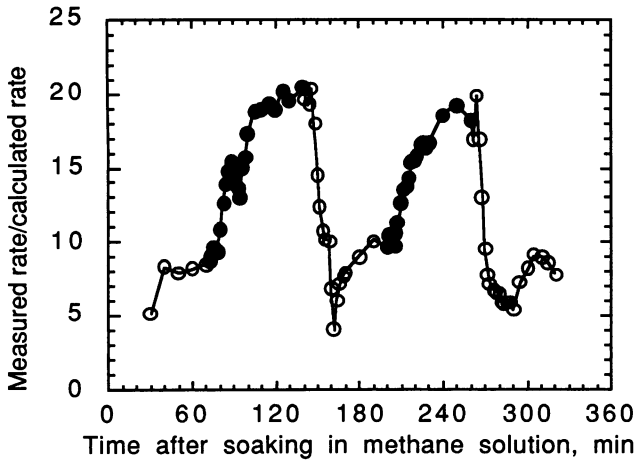

Figure 5. Ratio of the measured methane emission rate to the methane emission rate calculated for transpiration. The methane emission rate due to transpiration was calculated from the amount of transpiration and the methane concentration in the culture solution (using data in Fig. 4). The calculated concentration of dissolved methane at each time was estimated by the following equation

$$Y = 11.566 \exp(-0.000597X) \quad r = 0.93$$

where y is the concentration of methane dissolved in the solution and X is the time (min).

in a culture solution with a high methane concentration (25 μL/mL) was placed within the cylindrical chamber without sealing the base of the culm. A long stainless steel pipe (1 mm in diameter and 60 cm in length) connected to a silicon tube (0.9 mm in diameter) was inserted into the top of the chamber and held in position and sealed with modeling clay on the top of the chamber. Air was pumped into the chamber and flowed from the top of the leaf blade to the base of the leaf sheath at a rate of 1.0 L min^{-1}. Air samples were collected in a 1 L Tedlar bag for 2 min at a rate of 0.40 L min^{-1} by a mini-pump (Shibata, MP-2N) through the long stainless steel pipe. Figure 6 shows data representative of the methane concentration at each height of the shoot and the increase in methane emission rate at given intervals. The shoot was 54 cm in length from the base of the culm to the top of the leaf blade. Although the methane emission rate was approximately zero from the 53 to the 18 cm position (leaf blade), it abruptly increased from the 13 to the 6 cm position of the culm (leaf sheath). Furthermore, the methane emission from the culm was uneven, peaking at two sites, 6 and 13 cm from the base of the culm. This experiment was repeated five times with similar results.

Site of Appearance of Air Bubbles by Injection of Air into the Medullary Cavity of Rice

To more precisely determine the site of methane release, air was injected into the medullary cavity. Many air bubbles were observed from both the abaxial epidermis of the lower portion of the leaf sheath and the top gap between the epidermis of culm and the leaf sheath. Small air bubbles from the abaxial epidermis of the lower portion of the leaf sheath were released in a line. On the other hand, relatively large air bubbles from the top gap were released from near the junction of the nodal plate and the leaf sheath when the leaf sheath was stripped off carefully from the culm. In addition, air bubbles were sometimes also observed adhering to the stomata in the leaf blade.

Examination of Surface Structure of Abaxial Epidermis of Leaf Sheath by Scanning Electron Microscopy

A scanning electron microscope was used to examine the surface structure of the portions of the shoot where air bubbles appeared during air injection. Micropores shown in Figure 7 were observed in these portions. Hook-shaped micropores, 4 μm in diameter, were arranged regularly on a vein about 50 μm wide and frequently existed about 80 μm apart from each other. Stomata were also observed in the leaf sheath, but there were only about one-fifth as many stomata in the leaf sheath as in the leaf blade.

Uptake of Methane in the Gas Phase by Rice Roots

To examine whether rice roots can absorb methane in the gas phase and the methane absorbed by roots can then be released from a shoot, the methane emission rate was measured from a shoot whose roots were surface-dried by shaking the water off the roots and exposed to gas phase methane. Figure 8 shows the time course of the methane emission rate from a shoot whose roots were exposed to 10, 40, 70, and 100% methane in the gas phase. The result from the culture solution with the high methane concentration (liquid phase) into which 100% methane was bubbled was inserted in Figure

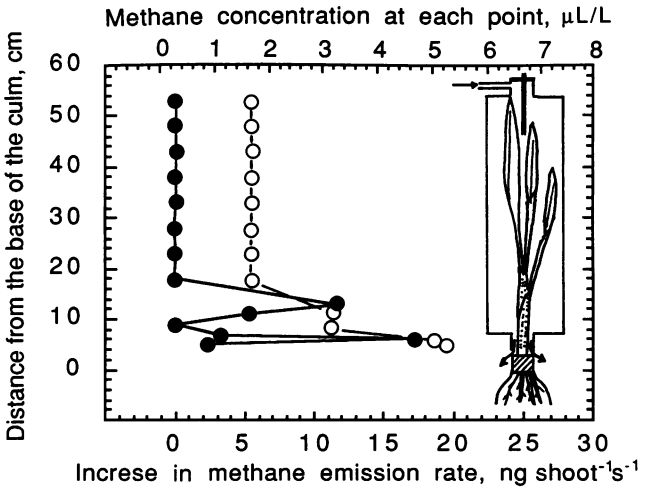

Figure 6. Methane concentration at each position of a shoot (○) in the cylindrical chamber and increase of methane emission at given intervals (●). Air was introduced into the top of a cylindrical chamber and expelled from the lower part at a rate of 1.0 L min^{-1}. Periodically, air samples for methane measurements were collected in a 1 L-Tedlar bag for 2 min through a long stainless steel pipe connected to a silicon tube at a rate of 0.40 L min^{-1}. Height was measured from the base of the culm (including the portion of the rubber stopper).

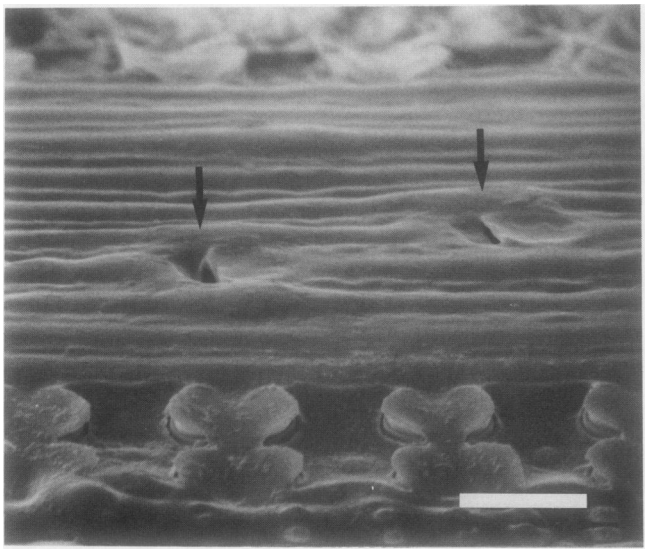

Figure 7. Scanning electron micrograph of micropores which are thought to be the site of methane release in the abaxial epidermis of the lower portion of the leaf sheath. The arrows indicate micropores which exist in a vein. Scale bar, 20 μm, magnification, ×2,000.

8 for comparison. The methane emission rate gradually increased with time and reached steady state within 30 min after the start of methane exposure. The methane emission rate from the gas phase increased with an increase in methane concentration. Next, the maximum values of methane emission rate from the gas phase were compared with that from the liquid phase at four concentrations of methane (10, 40, 70, and 100%). Methane emission rates from the gas phase at all four methane concentrations were 10 to 33% lower than those from the liquid phase (Table I). The time courses of both the methane emission rates from the liquid phase and the gas phase were almost the same (Fig. 8).

DISCUSSION

Since the gas diffusion rate of methane is very slow in the liquid phase, much of the methane produced by methanogenic bacteria within the surface layer (0–10 cm) of the paddy soil dissolves in the soil water (Fig. 2). The maximum concentration of methane dissolved in soil water in the rice straw-treated plot at the 5 cm depth was 15 mL methane per liter of water, corresponding to a solution 267,000 times concentrated than would normally result in solutions exposed to atmospheric concentration of methane (1.7 μL/L). In other words, these conditions corresponded to bubbling about 45% methane gas into soil water (by calculation using 4.1×10^4 atoms as the Henry constant for methane). This calculation confirms that methane abundantly dissolved in the soil water of rice paddy fields. In addition, this work demonstrates that the methane emission rate through rice plants is proportional to the methane concentration in the culture solution (Table I). In previous work it was also reported that the methane emission rate from pots with rice plants was 5 to 20 times higher than that from pots without plants during the entire growing season (manuscript in preparation). Therefore, as has already been pointed out by Cicerone and Shetter (6) and Seiler *et al.* (22), we also conclude that methane from rice paddy fields is mostly emitted to the atmosphere through rice plants.

The air space of the medullary cavity in rice plants is linked to the lysigenous air space in the roots through the aerenchyma in the node (1). The internal air spaces can act as a ventilation system. It was found that the methane concentration in the medullary cavities of rice plants in the field was about 2900 times higher than that of ambient air (Fig 3). Higuchi (11) and Higuchi *et al.* (12) have stated that absorbed CO_2 accompanying water absorption by rice roots may be gasified in the root cortex and can move to the shoots in the gaseous state. Since methane is less soluble in water and more easily gasified than CO_2, it can also be assumed that the methane absorbed by the roots can be gasified in the root cortex and transported in the gaseous state to the shoots via the aerenchyma and the lysigenous intercellular space. Thus, methane can diffuse and move upward through the shoots via the internal air spaces along concentration gradients. On the other hand, Raskin and Kende (20, 21) found that air is moved to the submerged organs of the partially flooded deep water rice through the external air layer, which is trapped between the hydrophobic surface of rice leaves and surrounding water, and internal air spaces, primarily by mass flow caused by the difference in solubility of CO_2 and O_2 in water. However, such mass flow should not significantly affect methane movement by diffusion in the experiments in this paper because the plants were not flooded. Even though the plants were not deep water rice plants, mass flow could also be produced by respiration of the roots. For the case of O_2 diffusion movement from the leaves to the roots, flow of bulk air will be generated with O_2 diffusion and the O_2 flux will increase compared with when only diffusion is considered. The maximum increased value of O_2 flux is W_{O_2}/W_{N_2}, assuming that diffusion is 1, where W_{O_2} and W_{N_2} are mass fractions

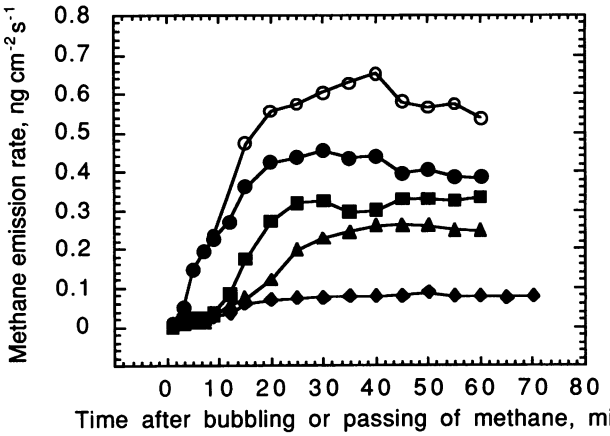

Figure 8. Time course of methane emission rate from a shoot whose roots were exposed to 10% (♦), 40% (▲), 70% (■), and 100% (●) methane gas in the gas phase or soaked in a solution prepared by bubbling 100% methane (○).

Table I. *Comparison of Maximum Methane Emission Rate from Gas Phase and Liquid Phase at Four Concentrations of Methane*

Methane Concentration	Concentration in the Solution	Emission Rate	
		Liquid phase	Gas phase
%	μL/mL	ng cm^{-2} s^{-1}	
10	4.4 ± 0.9	0.12 ± 0.05	0.08 ± 0.06
40	12.8 ± 1.1	0.29 ± 0.07	0.26 ± 0.08
70	30.2 ± 4.3	0.48 ± 0.07	0.32 ± 0.18
100	37.3 ± 1.5	0.65 ± 0.10	0.54 ± 0.08

Values are mean ± SD of 3 replicates.

of O_2 (0.23) and N_2 (0.77), respectively. On the other hand, methane diffuses along the same pathway but moves in the opposite direction to O_2, from the roots to the leaves. In this case, diffusion does not generate a flow of bulk air because the mass fraction of methane is much smaller than that of O_2. However, if O_2 and methane are diffusing at the same time, the bulk air caused by O_2 moving toward the roots will decrease the methane flux and the maximum value would be $W_{O_2}/W_{N_2} = 0.30$. The value 0.30 is small compared with 1. The difference between the diffusion coefficients of O_2 and methane was neglected in this evaluation. Therefore, it can be concluded that mass flow from the atmosphere to the roots scarcely suppresses methane transport by diffusion through the internal air spaces of rice plants.

No clear correlation between the change in transpiration rate and that in the methane emission rate was observed (Fig. 4). This indicates that methane transport from the roots to the atmosphere does not depend on opening or closing of stomata. In another experiment, the methane emission rate through rice plants was not affected by applications of abscisic acid, which is known to close stomata, to both leaf blades and leaf sheaths of rice plants (data not shown). These results indicate that the stomata do not play a major role in methane release from rice plants and that another pathway must exist which includes release sites for methane. Furthermore, methane is mostly released from the culm which is an aggregation of leaf sheaths; methane release from the leaf blade was found to be scarce (Fig. 6). These results demonstrate that the main site of methane release is the leaf sheath, not the leaf blade. In addition, many air bubbles were observed from both the abaxial epidermis of the lower portion of the leaf sheath and near the junction of the nodal plate and the leaf sheath of the lower leaf position, after air injection into medullary cavity of rice plants. The two release locations of air bubbles approximately coincided in position with the increase in methane emission rate (Fig. 6). Furthermore, micropores (Fig. 7), which are distinct from stomata, were observed in that portion of the leaf sheath by scanning electron microscopy. We believe that these micropores have not been reported previously. The results indicate the possibility that the micropores are the main site of methane release from rice plants. However, since the portion where the micropores exist is surrounded by sclerenchyma, there is no evidence that the micropores are linked to the lysigenous intercellular space. Further anatomical work is needed to elucidate whether the micropores are the main site of methane release from rice plants.

Since the uptake of water by the roots mainly depends upon transpiration, changes in the pattern of the methane emission rate should coincide with that of the transpiration rate by exposure to light and darkness. However, in these experiments the methane emission rate increased even when the transpiration rate decreased (Fig. 4). These results indicate that other uptake processes might exist than methane uptake mediated by water uptake by roots. In addition, the measured methane emission rate was much higher than the calculated methane emission rate that could result from transpiration (Fig. 5). These results suggest that much of the methane transport through the rice plants to the atmosphere does not depend on water flow driven by transpiration. As shown in Table I, the methane emission rate in the gas phase was fairly equal even under conditions different to that of the plants in culture solution containing a methane concentration corresponding to the concentration in the gas phase. Although the mechanism of gas uptake is not well understood, this result indicates that rice roots can absorb methane gas without water uptake.

We propose the following mechanisms of methane transport through rice plants. First, dissolved methane in the soil water surrounding the roots dissolves into the surface water of the roots, diffuses into the cell-wall water of root epidermis cells, and then diffuses through the cell-wall water of the root cortex, depending upon the concentration gradient between the soil water surrounding the roots and the lysigenous inter-

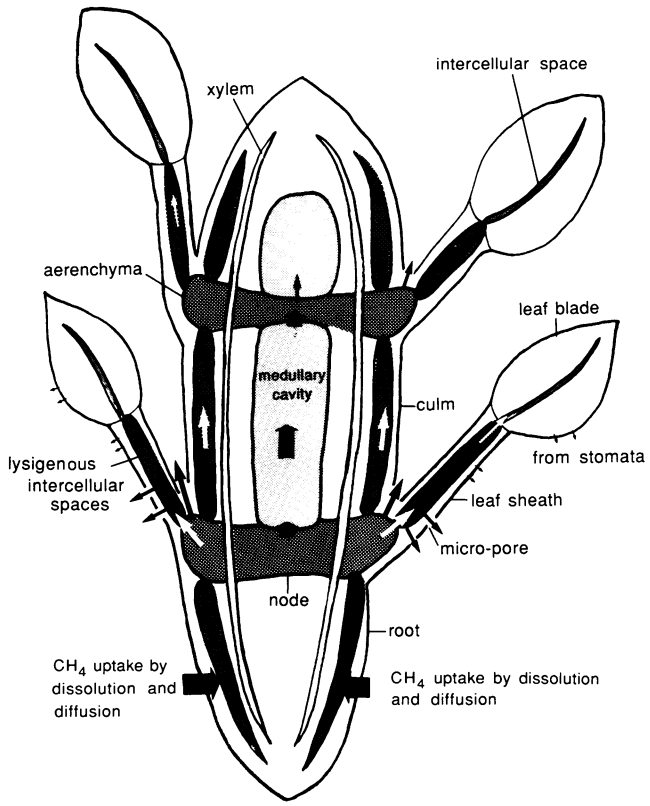

Figure 9. A hypothetical pathway of methane transport from the rhizosphere to the atmosphere. Both black and white arrows represent methane flow.

cellular spaces in the roots. Methane is then gasified in the root cortex and transported to the shoots via the lysigenous intercellular spaces and aerenchyma. Eventually, methane is released primarily through the micropores in the leaf sheath of the lower leaf position and released secondarily through the stomata in the leaf blade. Based on these considerations, we propose the hypothetical pathway of methane transport as shown in Figure 9. This pathway may also operate for other gases dissolved in paddy soil water.

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