Increasing measurement throughput of methane emission from rice paddies with a modified closed-chamber method

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Abstract
Manually operated closed-chamber methods have been used extensively to measure CH₄ emissions from rice paddies, but the long chamber deployment time (e.g., 30 min), together with the labor required for gas sampling and subsequent analysis by gas chromatography, severely limit their usefulness for multiple, high-throughput measurements. The objective of this study was to develop a modified closed-chamber method suitable for high-throughput measurements. The design of the modified system is based on a conventional method, but uses a portable spectroscopic gas analyzer and a newly developed dehumidification system. Results of a series of test operations showed that the use of a portable gas analyzer substantially reduced the time needed for each chamber deployment to as little as 4–5 min. A simple dehumidification system employing a selective water vapor-permeable tube was introduced to effectively dehumidify the sample gas so that the risk of water condensation was removed without attenuating the CH₄ concentration signal. The chamber must be equipped with a fan to ensure that the chamber air is well mixed and to attain representative CH₄ concentration values. The new method is suitable for high-throughput CH₄ emission measurements and can also be used for studying bubbling CH₄ emissions.

Key words: Dehumidification, High throughput, Methane flux, Mobile gas analyzer

1. Introduction
Field measurements of methane (CH₄) emission are the basis of CH₄ emission estimates and are a means of evaluating possible countermeasures for reducing emission from anthropogenic sources. Because of their high logistical feasibility, manually operated closed-chamber methods are commonly used to quantify the rate of CH₄ emission in rice paddies (Minamikawa et al., 2015), one of the most important anthropogenic sources of CH₄ (Ciais et al., 2013). In most cases, headspace gas is sampled manually, and the CH₄ concentration ([CH₄]) of the samples is analyzed by means of gas chromatography with a flame ionization detector (FID-GC) in the laboratory. The rate of CH₄ emission is determined by calculating the increase in the chamber [CH₄] against elapsed time. With this method, each chamber measurement typically takes about 30 min during which chamber air is sampled several (generally three or four) times (Sander and Wassmann, 2014). Reduction of this long chamber deployment time, together with the labor required in the FID-GC analysis, represents one of the major challenges to increase the throughput of CH₄ flux measurement.

Recently, commercially available CH₄ analyzers based on spectroscopic techniques have become more affordable, providing the opportunity for real-time in-situ measurement (e.g., Ishikura et al., 2019). These new gas analyzers have a number of advantages over the traditional FID-GC method, including better accuracy and higher temporal resolution (Zellweger et al., 2016). Therefore, one may expect a shorter time for each chamber deployment with a spectroscopic gas analyzer, leading to a higher measurement throughput than can be achieved with the conventional method.

There are, however, issues that need to be considered when a spectroscopic analyzer is to be used, especially for CH₄ measurement in rice paddies. First, there is a risk of water condensation along a gas flow line. Unlike the conventional method in which the chamber air is collected by a syringe and stored in glass vials, the chamber air is pumped and circulated through the analyzer and back into the chamber during the real-time measurement. Because the chamber is placed on the surface paddy water and encloses rice plants, the relative humidity inside the chamber is almost always 100%. Therefore, a dehumidification system is necessary to avoid condensation. However, installing a simple moisture trap into the gas flow line may not be a good solution because it may cause gas dispersion and severely attenuate the gas concentration fluctuations, making short-time measurements impossible. Second, a thorough mixing of the chamber air is a prerequisite for precise and representative concentration measurements, especially when the chamber deployment time is short. Because rice plant-mediated transport is the dominant pathway of CH₄ release (Denier van der Gon and van Breezen, 1993), the flux chamber needs to enclose the rice plants, but the rice plants can obstruct air circulation inside the chamber. Therefore, a battery-driven fan may be required for a rapid and thorough mixing of the gases inside the chamber.

The objective of this study was to develop a modified closed-chamber method for high-throughput CH₄ emission measurement.
measurements suitable for rice paddy studies. The design of the modified system is based on the conventional closed-chamber method and uses a spectroscopic gas analyzer instead of FID-GC, and on a simple invention of a dehumidification system. Here I evaluated the performance of the dehumidification system, examined the need for a fan inside the chamber to mix the air, and evaluated the practicality and throughput of flux measurements under field conditions.

2. Materials and Methods

2.1 Measurement system

The method presented here is a modification of a conventional closed-chamber measurement method that has been implemented in numerous studies of CH₄ emission from rice paddies (Minamikawa et al., 2015). The most important modification is that [CH₄] is measured on site with a portable gas analyzer instead of the gas being collected and then subsequently analyzed by FID-GC back in laboratory. Here I used the GasScouter™ G4301 (Picarro Inc., CA, USA) portable gas analyzer, but similar instruments are commercially available from different companies and could be used for the same purpose. The G4301 measures concentrations of CH₄, CO₂, and H₂O based on laser-absorption (cavity ring-down) spectroscopy. The precision of the CH₄ measurement (3 ppb, s.d.) is 10 times better than the conventional FID-GC method (usually >30 ppb, s.d.) and measurement (recording) frequency (1 Hz) is more than 100-fold greater than that of the normal manual gas sampling rate (typically 1 sample per 10 min). During the test and field trial described below, a transparent, acrylic cuboid chamber with a basal area of 30 cm × 60 cm was used. The top of the chamber was equipped with two quick connectors (Light Coupling E3 series, 4 mm o.d., Nihon Pisco Co., Ltd., Japan) through which a closed loop, including the gas analyzer apparatus, was made with PTFE tubes (4 mm o.d., 2 mm. i.d.). Sample air was pumped from the chamber by a vacuum pump built into the analyzer. As described below, dehumidification units were connected in between the chamber and the gas analyzer. A disposable filter unit (9933-05-BQ, Parker Hannifin Corp. NY, USA) was placed just before the inlet of the gas analyzer. The air was circulated through the gas analyzer and back into the chamber at a rate of 1.0 L min⁻¹.

2.2 Test for water vapor removal

To prevent potential condensation without attenuating fluctuations in the gas concentrations, I prepared a dehumidification unit consisting of an air-tight box (1.2 L, Sanplatec Corp., Japan) filled with a desiccant (silica gel) and a water vapor-permeable tube (Nafion dryer, ME-110, 0.11 inch [2.8 mm] i.d., Perma Pure LLC, NH, USA) (Photograph 1, 2d). The gas stream from the chamber was dehumidified according to the humidity gradient between the inside and the outside of the tube, which is kept dry by the desiccant. Attenuation of the gas concentration fluctuations was minimized by keeping the cross section of the flow line as small as possible by using a small diameter Nafion tube. The inlet (connected to the outlet of the chamber) and the outlet (directed to the gas analyzer) are equipped with quick connectors (CPSE3M-4, Nihon Pisco) so that the dehumidification unit is easily removable (Photograph 1b).

To test the performance of the dehumidification unit and the effect of tube length, I prepared two units: one with a 64-cm tube and the other one with a 120-cm tube. A cubic chamber (30 cm × 60 cm, 60 cm in height) was placed onto a vat filled with 3 cm of standing water, so that the humidity inside the chamber was kept at nearly 100%. The test began with the gas analyzer directly connected to the chamber without the dehumidification unit for a couple of minutes. The test for the dehumidification units was then conducted according to the following sequence: (i) a dehumidification unit with a 64-cm tube was connected and tested for 20 minutes, (ii) the first dehumidification unit was replaced with a unit with a 120-cm tube and tested for 23 minutes, and (iii) two dehumidification units (one with a 64-cm tube and one with a 120-cm tube) were connected sequentially and tested for 23 minutes. After the initial measurements for these three steps, I kept the chamber in place and continued recording for 5 h to evaluate the capacity and durability of the units (64-cm + 120-cm tubes) for day-long operation.

2.3 Test for chamber air mixing

I also conducted a test to determine whether air mixing by a
battery-operated fan inside the chamber affected the time course of CH₄ concentration. The test was conducted in an experimental paddy field at the Institute for Agro-Environmental Sciences, NARO, located in Tsukuba, Ibaraki, Japan. A chamber was placed in the field while the gas analyzer was loaded in a vehicle (Photograph 2); the chamber and analyzer were connected via two PTFE tubes (4 mm o.d., 2 mm i.d., 10 m long) to make a closed loop. The gas analyzer (Picarro G4301) provides a wi-fi connection, and real-time visual monitoring was possible on a mobile device (in this case, a tablet; Photograph 2b). A small vehicle was used to simplify logistics and as a shelter from sunshine and/or rain for the gas analyzer (Photograph 2a, b). The chamber was the same as that used for the water vapor test, and it enclosed four hills of rice plants (Oryza sativa L. cv. Takanari) at the grain-filling stage. The field was submerged for 3 months, so the soil had already been reduced and become methanogenic. The four hills (spacings: 15 cm × 30 cm) were softly bound with a plastic net (originally designed for pest prevention) for ease of chamber placement (Photograph 2c). To prevent soil disturbance during chamber deployment, which may cause the release of CH₄-containing bubbles trapped in the soil, four aluminum pedestals (aluminum plate: 100 mm × 100 mm, 12 mm thick, with a handle) were placed at the corners of the test area prior to chamber placement (Photograph 2c); the chamber was then placed on the pedestals. The pedestals were immersed in the surface water so that the chamber was sealed (i.e., gas tight).

To prevent water condensation, two dehumidification units (one on top of the chamber [Photograph 2d] and the other just before the inlet of the gas analyzer) were used. A fan (San Ace 80, 109R0805F401, Sanyo Denki Co., Ltd., Japan) was installed inside the chamber (attached to the top side), and the fan was powered by a rechargeable battery placed on top of the outside of the chamber (QE-AL101, Panasonic Corporation, Japan) (Photograph 2d).

After the chamber was set up, the test was conducted according to the following procedure: (i) the fan was kept off (13 min), (ii) the fan was turned on (12 min), (iii) the fan was turned off again (12 min), and (iv) the fan was turned on again (12 min). The chamber was removed after the test.

2.4 Test for high-throughput field measurement

The trial for the high-throughput measurement in the field was conducted in the same experimental field as the air-mixing test. Although the basic setup was similar to that of the fan test, I prepared two chambers (A and B) to make the most efficient use of the gas analyzer. When Chamber A was connected to the gas analyzer, Chamber B was prepared for the next measurement. When the preparations for Chamber B and the Chamber A test were completed, the gas analyzer was connected to Chamber B and the operator would prepare Chamber A for the next test. This procedure was repeated six times for each box, so that the gas analyzer yielded data continuously for 12 chamber
measurements. For each test, the chamber was placed on different but adjacent rice hills. The rice plants (Oryza sativa L. cv. IR64) had reached the grain-filling stage at the time of the testing.

3. Results and Discussion: Performance of the CH$_4$ flux measurement system

3.1 Performance of the dehumidification system

When the chamber was directly connected to the analyzer, the water vapor reading was nearly 4% (in mole fraction), suggesting 100% relative humidity. Soon after the dehumidification unit with a 64-cm Nafion tube was connected, the water vapor reading dropped to as low as 0.33% and then gradually increased and leveled off at 0.70% (Fig. 1-i). When the unit was replaced with one with a 120-cm Nafion tube, the water vapor reading dropped sharply to 0.20% and then showed a similar increase and leveling off trend as in the case with the shorter tube (Fig. 1-ii). Finally, when the two dehumidification units were attached sequentially, the water vapor readings gradually decreased to 0.33% (Fig. 1-iii). Continued monitoring showed a gradual rise, but the level never exceeded 0.45% in the 5-h monitoring period (data not shown).

Based on these results, I concluded that the experimental apparatus sufficiently removed water vapor such that the risk of water condensation was eliminated. Use of a single dehumidification unit (with either the 64-cm or 120-cm Nafion tube) may be sufficient for a short-term measurement, but operation with two units may be a better practice for a longer period (e.g., a day). The use of a longer tube (e.g., 180 cm) with a larger box may be another solution, but if the chamber and the gas analyzer are spatially separated (which was the case here), two separate units might be easier to handle; for example, in this study, the size of one unit was small enough to be placed on top of the chamber (Photograph 2d) and the other one was placed just before the inlet of the gas analyzer in the vehicle.

3.2 Chamber air mixing

Results showed that when the fan was turned on, the CH$_4$ concentrations increased monotonically at a constant rate (Fig. 2-ii, iv), except for several stepwise increases (gray shaded areas, Fig. 2). With the fan off, the CH$_4$ concentration frequently fluctuated up and down although the overall trend was up. The resultant slope of the chamber [CH$_4$] at 1 min interval (as an example) was variable (9.01 ± 5.15 ppm h$^{-1}$, CV = 57.2%) when the fan was off (Fig. 2-iii), while it was stable (8.10 ± 0.34 ppm h$^{-1}$, CV = 4.16%) with the fan on (Fig. 2-ii, except for the periods of stepwise [CH$_4$] increase). These results clearly demonstrated the need to use a fan inside the chamber to achieve precise and representative concentration measurements, especially when the chamber deployment time is short.

The stepwise increases in CH$_4$ concentration when the fan was on were probably caused by episodic emissions of CH$_4$-containing bubbles (Tokida et al., 2013; Wassmann et al., 1996). When the fan was off, this type of bubbling emission (ebullition) might not have been detected because any sudden increase in CH$_4$ concentration could have been masked by background fluctuations caused by poor mixing of the chamber air. The use of a real-time gas analyzer combined with the fan thereby appears to allow for the separate quantification of bubbling CH$_4$ emissions and non-bubbling emissions, most of which should be plant-mediated transport (Nouchi et al., 1990; Wang et al., 1997).

3.3 Field test for high-throughput measurement

Using the new closed-chamber method combined with the alternating chamber strategy, one person was able to conduct 12 chamber measurements in one hour (Fig. 3). The time for each individual chamber measurement (4–5 min) was determined by the setup time required for the next chamber, which is much shorter than the time required using the conventional method.

**Fig. 1.** Time course of water vapor concentration (mole fraction, %) during the dehumidification test: (i) one unit with a 64-cm Nafion tube, (ii) one unit with a 120-cm Nafion tube, and (iii) two units connected sequentially (64- + 120-cm tubes).
(about 30 min). Accordingly, the new method enabled much quicker measurements and was more suitable for multipoint measurements than the conventional method.

As was observed in the fan test, the CH$_4$ concentration exhibited a sudden jump in most chamber experiments, indicating occurrence of ebullition (the gray shaded areas in Fig. 3). For short-term measurements, it is difficult to calculate time-representative total CH$_4$ emission including the bubbling.

**Fig. 2.** Time course of CH$_4$ concentration (dry mole fraction, ppm) during the fan test for chamber air mixing: (i) fan off, (ii) fan on, (iii) fan off, and (iv) fan on. Time zero indicates the timing of the chamber deployment, and the vertical arrow indicates the timing of chamber removal. Gray shaded areas indicate a stepwise increase in CH$_4$ concentration.

**Fig. 3.** Time course of CH$_4$ concentration (dry mole fraction, ppm) during the test for high-throughput flux measurement. One person set up and operated two chambers (alternating them one at a time as described in the text) for a total of 12 chamber measurement periods in 1 h. Gray shaded areas indicate a stepwise increase in CH$_4$ concentration caused by putative ebullition (bubbling release).
component, and one can only estimate non-bubbling flux by a linear fitting from the non-bubbling period. The high-frequency measurement of the chamber CH$_4$ concentration made possible by the new chamber method revealed that bubbling CH$_4$ emissions occur more frequently and may make a greater contribution to CH$_4$ emission than has previously been thought. Additional studies should be made with regard to the magnitude and timing of ebullition as well as for relevant controlling factors using the new method presented here.

4. Conclusions

The use of a portable gas analyzer substantially reduced the time needed for each chamber measurement down to 4–5 min, which represents a substantial reduction from the time required with the original closed-chamber method. The newly proposed simple dehumidification system effectively dehumidified the sample gas without attenuating the CH$_4$ concentration signal. For short-time measurements, a fan must be equipped inside the chamber to ensure that the air inside the chamber is well mixed and attain representative CH$_4$ concentration measurements. The method presented here is suitable for multipoint, high-throughput CH$_4$ emission measurements from rice paddies and also for the study of bubbling CH$_4$ transport.

Acknowledgements

I thank Takeru Saito of Ibaraki University, Japan, Xuping Ma of the Institute for Agro-Environmental Sciences, NARO, Japan, and Loc Nguyen of Can Tho University, Vietnam, for their support during the field measurements. This study was supported by JSPS KAKENHI Grant Numbers JP19K22921, JP19H03096, and JP16H06204.

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