Appropriate chamber deployment time for separate quantification of CH₄ emissions via plant and ebullition from rice paddies using a modified closed-chamber method

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Abstract

A modified closed-chamber method for estimating total, plant-mediated, and bubbling (ebullition) emissions of CH₄ from rice paddies has been developed to use high-time-resolution CH₄ concentration data (~1 Hz) obtained by a spectroscopic mobile gas analyzer. Here we aimed at determining an appropriate minimum time length of chamber closure for accurate flux measurement by investigating 3255 datasets obtained from a 2-year field survey. To investigate the minimum time length for each chamber measurement, we generated a series of datasets from each measurement: by setting the hypothetical termination time of the chamber closure ahead in 1-min intervals, we obtained various chamber CH₄ concentration time series with different durations of chamber closure, and separately estimated CH₄ emissions via rice plants and bubbling from each. The estimated flux was sensitive to time length with short closure times, but became less sensitive with longer closure. We defined the minimum time length at which the difference in estimated flux between adjacent time windows was small enough (<10% of plant-mediated emission). The estimated minimum time length differed from one measurement to another, but 10 min was sufficient for >99% of cases. Detailed analysis showed a positive correlation between minimum time length and frequency of bubbling events; the time length needed to be longer as bubbling events became more frequent. From this relationship, we computed the appropriate chamber-duration time as a function of bubbling frequency. In the absence of ebullition, 4–5 min was sufficient, but as the bubbling frequency increased to 2.5 times per minute 15–20 min was necessary for accurate pathway-dependent flux measurements.

Key words: Bubbling frequency, CH₄ flux, High time resolution, Measurement time, Portable gas analyzer

1. Introduction

Rice paddies are an important source of atmospheric CH₄, a highly potent greenhouse gas (Ciais et al., 2013). Most methane produced in rice paddies is transported to the atmosphere either through aerenchymous tissues of rice plants or by upward migration of gas bubbles, i.e. ebullition (Komiya et al., 2020). Recently, we developed a protocol for separately determining plant-mediated flux and bubbling flux from high-time-resolution CH₄ concentration ([CH₄]) data (~1 Hz) obtained by a modified closed-chamber method in combination with a portable spectroscopic gas analyzer (Kajiura and Tokida, 2021; Tokida, 2021). The separate quantifications revealed that plant-mediated emission and ebullition had different sensitivities to temperature and rice growth stages, clearly demonstrating the need to determine CH₄ fluxes via each emission pathway for accurate observation and modeling (Kajiura and Tokida, 2021).

High-time-resolution and precise determination of chamber CH₄ may also enable much faster flux determination than the conventional closed-chamber protocols, in which >60% of studies waited for ≥30 min to manually take 3 or 4 gas samples (Sander and Wassmann, 2014). However, information about appropriate chamber deployment time for accurate CH₄ flux measurement is lacking (Minamikawa et al., 2015). Here, we assessed the minimum chamber deployment time necessary for accurate flux estimation by analyzing 3255 datasets of chamber [CH₄] obtained from rice paddies during 2 years of field surveys.

2. Methods

2.1. Dataset

2.1.1. Experimental field and rice cultivation

We conducted field experiments in 2019 and 2020 in rice paddies at the National Agriculture and Food Research Organization (NARO) in Japan (36°01′28″N, 140°06′30″E). In both years we planted various rice genotypes, including members of the World Rice Core Collection (Kojima et al., 2005; Tanaka et al., 2020) and chromosome segment substitution lines (CSSL) in an elite Koshihikari background (Fukuoka et al., 2010; Kobayashi et al., 2018; Takai et al., 2014). Each plot consisted of 24 hills (1.2 m × 0.9 m) of one genotype, replicated three to eight times depending on the genotype. In total, we prepared 765 plots in 2019 and 448 plots in 2020. Genotypic differences in CH₄ emissions have been detected, but this topic is beyond the scope of this study; here we used the whole dataset to assess appropriate chamber closure time. We sowed pre-germinated rice seeds in seedling trays (in April or May), raised the seedlings
in the open field, and then transplanted them at the 5-leaf age at a spacing of 15 cm × 30 cm with three seedlings per hill (late May to early June). The field was continuously flooded from transplanting until the middle of the grain-filling stage.

2.1.2. Measurement of CH₄ emission

Methane emission was measured by a modified closed-chamber method in which high-time-resolution [CH₄] (at intervals of 0.9 s) was measured with a mobile gas analyzer (G4301, Picarro Inc., CA, USA) (Kajiura and Tokida, 2021; Tokida, 2021). The chamber air was circulated through the gas analyzer and back into the chamber at a rate of 1.0 L min⁻¹. We used an acrylic closed-top chamber (basal area of 30 cm × 60 cm) to enclose four hills of rice plants at the center of each plot. Measurements were made at the panicle formation (PF), booting (BT), and heading (HD) stages to cover important CH₄-emitting periods (Tokida et al., 2014). In total, 3255 chamber measurements were conducted in the daytime during the two years. Development stages varied among genotypes, but plants headed from late July to mid August in most genotypes. At the PF stage, a 60-cm-tall chamber equipped with a fan was used, but at the BT and HD stages, the height of the chamber was increased by adding bottom chambers (with 3-cm chamber collar) to make double-deck (120 cm) or triple-deck (140 cm) chambers. We set the chamber a few minutes before each measurement and kept the chamber closed until we obtained a steady [CH₄] increase, not interrupted by a bubbling event, for at least 40 s. Therefore, the actual chamber deployment time increased as bubbling events increased.

2.2. Analysis

We first estimated minimum time length (TL_min) necessary to accurately determine each type of flux (total flux, F_total; plant-mediated flux, F_plant; bubbling flux, F_bubble) for each single chamber measurement (section 2.2.1). From the estimated TL_min, we calculated the success rate—i.e., percentage of accurate flux estimates as a function of chamber deployment time (section 2.2.2). Next, we estimated the frequency of bubbling events (section 2.2.3) and investigated its relationship with TL_min. From the relationship, we proposed an appropriate chamber deployment time as a function of bubbling frequency (section 2.2.4).

2.2.1. Estimation of the minimum time length for each chamber measurement

We prepared a series of datasets from each single chamber measurement after removing unstable periods (gray areas in Fig. 1), bringing the end point of the chamber closure ahead in intervals of 1 min to create [CH₄] time series with different chamber closure times (0–1, 0–2, 0–3, 0–4; TL1–TL4; Fig. 1). The datasets unlikely included the artificial bubbling stimulated by the chamber deployment because bubbling frequency was not reduced as measurement time progressed. Using each [CH₄] time series, we estimated F_total, F_plant, and F_bubble as in Kajiura and Tokida (2021) with slight modifications (Fig. S1). In brief, F_plant, corresponding to a period of steady [CH₄] increase, was determined as the lowest flux intensity showing local maxima in the frequency distribution of the CH₄ flux. F_total was estimated from the slope of the linear regression between [CH₄] times series and elapsed time. Finally, F_bubble, which was reflected as a “jump” in the [CH₄] time series, was calculated as F_total − F_plant.

The estimated flux generally fluctuated with short time lengths (TLs), but the dependency on TL became small with longer TL. Therefore, we defined TL_min the minimum time-length necessary for accurate flux estimation, for each chamber measurement, as the TL at which the estimated flux did not differ substantially from that of the next TL (1 min longer), and judged whether the difference in the flux (ΔF_i = F_i + 1

![Fig. 1. How to determine TL_min (minimum time length needed) for each flux component (F_total, F_plant and F_bubble). Step 1: Preparation of a set of [CH₄] time series with different TLs (TL1–TL4). Step 2: Flux (F) estimation for each dataset and calculation of the difference in F (ΔF) between adjacent TLs (ΔF_i = F_i + 1 − F_i). The superscript denotes the TL of the corresponding dataset, and the subscript indicates the type of flux. A grey (black) “ΔF” indicates that ΔF was smaller (greater) than the threshold (0.30 mg-C m⁻² h⁻¹). Step 3: Selection of TL_min for each type of flux (TL at which ΔF is smaller than the threshold). In this example, TL_min is the same for F_total and F_bubble (3 min), but shorter for F_plant (2 min).](Image 75x134 to 235x262)
was smaller than a threshold value (Fig. 1). Clearly, a tradeoff exists between the accuracy of the flux estimation and the necessary chamber deployment time: the smaller the $\Delta F$, the longer the $T_{\text{L, min}}$ would be. We used a moderate threshold (0.30 mg-C m$^{-2}$ h$^{-1}$) based on comparisons of fluxes estimated using different thresholds (see Fig. S2). Note that $T_{\text{L, min}}$ is determined in increments of 1 min.

### 2.2.2. Frequency of bubbling events

The estimated $T_{\text{L, min}}$ for each chamber measurement showed a strong correlation with the frequency of bubbling events (see Results and Discussion). Therefore, the appropriate chamber deployment time for accurate flux estimation may be better defined as a function of bubbling frequency rather than as a constant value. We therefore conducted linear regression analysis of the relationship between $T_{\text{L, min}}$ and bubbling frequency for each flux type ($F_{\text{total}}$, $F_{\text{plant}}$, and $F_{\text{bubble}}$). To achieve homoscedasticity, the data were log-transformed ($\log (T_{\text{L, min}})$, $\log (\text{bubbling frequency} + 1)$), and we obtained 95% and 99% prediction intervals by using the `predict` function in the stats package in R. The upper limits of the intervals as a function of bubbling frequency can be used as the appropriate time length for accurate flux estimation ($T_{\text{L, opt}}$). Finally, a common unstable period-time length (median value of the unstable period of all data) was added to yield the appropriate chamber deployment time-length ($T_{\text{L, opt}}$).

### 3. Results and Discussion

A series of datasets generated by sequential earlier termination of chamber closure showed that the estimated fluxes were sensitive to TL with short chamber closure, but became less sensitive with longer TL (Fig. S4). $T_{\text{L, min}}$, the time length at which estimated flux stabilized, differed from one chamber measurement to another, but it was <10 min in most cases (Fig. 2). Consequently, the success rate of accurate flux estimation increased with increasing TL and reached 99.7%–100% at 10 min (Fig. 3). $F_{\text{total}}$ and $F_{\text{bubble}}$ required longer $T_{\text{L, min}}$ than $F_{\text{plant}}$; for example, 8 min was necessary for $F_{\text{total}}$ and $F_{\text{bubble}}$ to reach 99% success rate, while 4 min was enough for $F_{\text{plant}}$.

Estimated $T_{\text{L, min}}$ showed a strong dependency on bubbling frequency for $F_{\text{total}}$ and $F_{\text{bubble}}$ (Fig. 4), presumably because longer TL may be necessary for the slope of the linear regression to stabilize for their estimation (Fig. S5). For $F_{\text{plant}}$, on the other hand, the dependency of $T_{\text{L, min}}$ on bubbling frequency was much weaker although still statistically significant (Fig. 4). Longer time-period may be necessary to correctly capture the baseline in the time-series flux data (i.e., $F_{\text{plant}}$) when bubbling emissions occurred frequently. Taking longer time would not underestimate $F_{\text{plant}}$ (Wassmann et al., 2018) because an increase in chamber $[\text{CH}_4]$ within a few hours would hardly affect $[\text{CH}_4]$ gradient between the soil and the atmosphere, considering very high $[\text{CH}_4]$...
found in typical paddy soils (>10% in trapped gas phase) (Tokida et al., 2013).

On the basis of the dependency of TL_{min} on bubbling frequency (Fig. 4), we propose the appropriate chamber deployment time length (Chamber-TL_{ap}, including typical unstable period of 1 min) to be 4–15 min by 95% and 5–20 min by 99% prediction intervals, depending on bubbling frequency (Table 1). In the absence of a bubbling event, a short chamber closure time (e.g., 4 min) was sufficient, but if bubbling emission occurred frequently (e.g., 2.5 times per minute), a much longer deployment time (e.g., 15 min) was necessary for accurate flux measurements. In our observations, bubbling events were rare (<0.5 min⁻¹ in many cases) during the PF stage (Fig. S6); hence, Chamber-TL_{ap} can be ≤ 6 min (Fig. 2, Table 1). However, they were much more frequent at the HD stage, as reported previously (Tokida et al., 2013; Wassmann et al., 1996), and longer Chamber-TL_{ap} may be necessary (Figs. 2, S6).

The appropriate chamber deployment time length may vary with other factors because bubbling frequency explains less than half of TL_{min} variability (Fig. 4). Potential factors include the intensity of bubbling emissions. If a large bubbling emission occurred, the regression lines to estimate F_{total} would not stabilize for a long time, leading to a longer Chamber-TL_{ap}. Therefore, the results summarized in Table 1 might not be directly applicable to other fields where bubbling intensity is substantially different. Nevertheless, given the large dataset used in this study, Chamber-TL_{ap} proposed here can be used as a guideline. At the same time, users of the new closed-chamber method should assess site-specific chamber deployment time for themselves, as we have done here.

### 4. Conclusion

We assessed the appropriate chamber-deployment time length for accurate estimation of F_{total}, F_{plant}, and F_{bubble} from rice paddies by using 3255 high-time-resolution [CH_{4}] datasets (0.9 s interval) obtained during two years of field measurements. The appropriate time length depended on the frequency of bubbling events: 5 min was enough in the absence of ebullition, and at most 20 min was necessary when bubbling occurred frequently.

### Acknowledgement

We thank Takeru Saito of Ibaraki University, Japan, Xuping Ma of the Institute for Agro-Environmental Sciences, NARO, Japan, for their support during the field measurements. This study was supported by JSPS KAKENHI Grant Numbers JP19K22921, JP19H03096, JP2040189, and also based on results obtained from a project, JPNP18016, commissioned by the New Energy and Industrial Technology Development...
M. Kajiura and T. Tokida: Chamber closure time for CH₄ flux measurement

Organization (NEDO). The authors declare no conflicts of interest associated with this manuscript.

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