Uncertainty of methane emissions coming from the physical volume of plant biomass inside the closed chamber was negligible during cropping period

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Abstract

In rice paddy, the closed chamber method is broadly used to estimate methane (CH₄) emission rate. Since rice plants can significantly affect CH₄ production, oxidation and emission, rice plantation inside the chamber is standardized in IPCC guidelines. Methane emission rate is calculated using the increased concentration inside the headspace. Biomass growth might decrease the headspace volume, and thus CH₄ emission rates might be overestimated. To evaluate the influence of chamber headspace decreased by rice plant development on CH₄ emission rates, five Korean rice cultivars were cultivated in a typical rice paddy, and physical volume changes in rice biomass were assayed using water displacement method. The recommended acrylic closed chambers (H. 1.2 m x W. 0.6 m x L. 0.6 m) were installed, and eight rice plants were transplanted inside the chamber with the same space interval with the outside. Biomass growth significantly decreased the headspace volume of the chamber. However, this volume covered only 0.48–0.55% of the closed chamber volume at the maximum growth stage. During the whole cropping period, mean 0.24–0.28% of chamber headspace was allocated by plant biomass, and thus this level of total CH₄ emissions was overestimated. However, this overestimation was much smaller than the errors coming from other investigation processes (i.e., chamber closing hour, temperature recording, inconstant flooding level, different soil environments, etc.) and rice physiological changes. In conclusion, the influence of physical biomass volume inside the closed chamber was negligible to make the error in total CH₄ emission assessment in rice paddies.

Introduction

Methane (CH₄) is the second potent greenhouse gas (GHG) after carbon dioxide (CO₂) and contributed to approximately 18% of total global warming over the last 50 years [1]. Over 60%
of the total CH\textsubscript{4} emissions originated from human activities like agriculture, industry, and waste management [2]. In particular, the agriculture sector solely contributes to almost half of the anthropogenic CH\textsubscript{4} emissions, including rice production which accounts for more than 10\% of anthropogenic sources [3].

Methane exists in the rice fields either as gas or in the dissolved phase [4,5]. However, dissolved CH\textsubscript{4} concentration is minimal due to its low water solubility and the lack of ionic form [6]. Three possible mechanisms are known as the CH\textsubscript{4} emission pathway from rice paddy soil to the atmosphere namely, diffusion, ebullition, and plan-mediated transport. However, rice plant-mediated transport via aerenchyma tissue is accepted as the main CH\textsubscript{4} emission route in rice fields [4,7,8]. This pathway is known to cover approximately 80–90\% of CH\textsubscript{4} emission from rice cropping fields [9–12].

To estimate CH\textsubscript{4} emission rates in rice cropping fields, two different methods such as closed-chamber and micrometeorological techniques (e.g., eddy covariance or gradient techniques) were generally used [13–15], but the former is mainly utilized since it is relatively easier and less expensive for installation and operation. Transparent materials like acrylic sheet, perspex or rigid plastics are commonly used to make the closed chamber having the dimension big enough to cover rice plants [16–18]. In addition, since CH\textsubscript{4} is transported from the rhizosphere to the atmosphere via rice aerenchyma tissues [19], rice planting inside the chamber is recommended to have equal space with the outside [18,20].

Gas samples are collected by closing the chamber lid for a period of time, and CH\textsubscript{4} emission rate (mg m\textsuperscript{-2} h\textsuperscript{-1}) is calculated by assaying the increased CH\textsubscript{4} concentration inside the chamber [21]. The reliability of closed-chamber method in estimating CH\textsubscript{4} emission rates has been verified through a number of studies [22–25]. Various designs of the chamber have been developed to stabilize microclimate conditions (e.g., light, wind, CO\textsubscript{2} concentration, temperature, etc.) inside the chamber comparable to the outside condition [26–29].

Methane emission rate (F, mg m\textsuperscript{-2} h\textsuperscript{-1}) is calculated using the increased CH\textsubscript{4} concentration per hour inside the chamber [21]. In this equation, the headspace volume of the chamber was fixed without considering rice plant volume allocation. As rice plants matured, the developed rice biomass visually occupied a big part of chamber headspace, and then the void headspace appeared to become smaller. However, there was no study that evaluated the influence of physical allocation of rice plant biomass inside the closed chamber on the estimation of CH\textsubscript{4} emission rates during rice cultivation.

We hypothesized that as rice plants grow, developed plant biomasses occupy significant headspace volumes of chambers, and thus total CH\textsubscript{4} emissions (kg ha\textsuperscript{-1}) might be overestimated due to use of fixed chamber volume in calculation rather than the real headspace volume. In this field test, to evaluate the influence of rice biomass allocation inside the closed chamber on uncertainty of CH\textsubscript{4} emission rates, the physical volumes of five different rice cultivars were periodically evaluated using water displacement method during rice cultivation period. The uncertainty of total CH\textsubscript{4} emissions which come from the decrease of chamber volume with plant biomass developing was evaluated.

**Materials and methods**

**Experimental site selection**

The experimental plots were installed at the agronomic rice field of Gyeongsang National University (35°08′56″N and 128°05′46″E), Jinju, South Korea. The region has a typical temperate monsoon climate. For the last 30 years, the mean temperature and annual precipitation were 13 °C and 1513 mm, respectively [30]. The soil was classified as Pyeongtaeg series (fine-silty, mixed, nonacid, mesic Typic haplaquent) and the field was exclusively utilized for rice...
cultivation for over 40 years. Before the experiment, soil had slightly acidic pH (5.9±0.2, 1:5 with H$_2$O) and low fertility (21.5±2.3 g kg$^{-1}$ of organic matter, 0.71±0.06 g kg$^{-1}$ of total N, and 41±4.1 mg kg$^{-1}$ of available P).

**Rice cultivar selection and cultivation**

To investigate the influence of rice plant biomass volume inside the closed chamber on calculating CH$_4$ emission rates, five Korean rice cultivars (Chuchung, Dongjin, Ilmi, Junam, and Saenuri) were selected, and the physical volume changes of rice plants were periodically monitored during cropping period. The selected cultivars belong to Japonica rice and the late-maturing species (130–140 days of cultivation period).

Total 15 experimental plots (5 cultivars × 3 replications) having 100 m$^2$ area per plot (10 m × 10 m size) were arranged in a randomized block design in the experimental fields. Twenty-one-day old seedlings were manually transplanted with a spacing of 30 cm × 15 cm. Single rice seedling per hill was manually transplanted to accurately compare the changes in plant biomass volume and harvested after 130 days.

Rice were cultivated under the same condition. Nutrients were managed by chemical fertilization (N-P$_2$O$_5$-K$_2$O = 90-45-57 kg ha$^{-1}$) based on the Korean fertilization standard for rice [31]. Weeds and pathogens were properly controlled. The irrigation water level was automatically controlled with a depth of 5–7 cm throughout cropping season, and water was drained four weeks before harvesting.

**Evaluation of rice plant biomass volume and soil properties**

To determine the changes in headspace volume inside the closed chamber, rice plants outside the chamber were sampled total 12 times during cropping season (one-week interval during vegetative growth period from transplanting to panicle initiation stage, and thereafter two-weeks interval to harvesting stage). Five hills of rice plants per plot were sampled at every sampling stage. Whole rice plant (above- and below-ground biomass) were uprooted and kept in an icebox to minimize changes in physical biomass volume. The plant biomass above the flooded water level was carefully separated by distinguishing green and white color differences that represent the upper and lower parts of the irrigated water table, respectively. The physical volume of plant biomass was determined by the water displacement method, which was modified from the methodology to measure soil particle density [32]. Rice growth characteristics (i.e., tiller number per hill, plant height, and above-ground biomass productivity) were simultaneously evaluated base on the Korean rice sampling standard [33].

During rice cultivation period, soil redox potential (Eh value) was consistently monitored by using Eh meter. The electrodes were permanently installed at 10 cm of soil depth. Soil temperature was also monitored with a thermometer at every sampling.

**Gas sample collection and analysis**

A closed-chamber method was used to estimate CH$_4$ emission rates [16–18,20]. Three pairs of transparent acrylic chambers (length 60 cm × width 60 cm × height 120 cm) were permanently installed in each plot. Eight rice plants were transplanted inside the chambers with the same space interval (30 cm × 15 cm) on the outside. Static chambers were kept open during the entire cropping season, except for gas sampling. Each chamber contained two electric fans and a thermometer inside to mix air and monitor temperature.

Before starting regular gas samplings, the research protocol was established to set sampling conditions such as sampling hour, interval, and times. In the preliminary measurement, 30 minutes was selected as chamber lid closing hour to collect gas samples, since the positive
linear relationships between CH$_4$ concentration and chamber closing hour (0, 10-, 20-, 30-, and 60-min. chamber closing) were observed. Gas sampling time was fixed at 10:30 am, because the mean daily emission rate was detected in this time via 12 times of gas sampling a day. Gas samples were collected once a week during rice cultivation. At 0 and 30 min after chamber closing, gases were sampled from the headspace using a 50 mL gas-tight syringe and immediately transferred to a 30 mL vacuum glass vial.

Methane concentrations were analyzed using gas chromatography (Shimadzu, GC-2010 PLUS, Japan) equipped with Porapak NQ column (Q 80–100 mesh) and a flame ionization detector (FID). Column, injector and detector temperatures were controlled at 35, 200 and 250˚C, respectively. Helium and hydrogen gases were used as carrier and combustion gases, respectively. Methane emission rate (F, mg m$^{-2}$ h$^{-1}$) was calculated using the increased CH$_4$ concentration inside the chamber for a specific time interval (Eq 1) [21].

$$F \text{ (mg m}^{-2} \text{ h}^{-1}) = \left(\frac{\Delta C}{\Delta t}\right) \times \frac{V}{A} \times \rho \times \left(\frac{273}{T}\right) \quad (\text{Eq 1})$$

where $\Delta C$ is the increased CH$_4$ concentration (mg m$^{-3}$), $\Delta t$ is the chamber closing hour, $V$ is the chamber volume (m$^3$), $A$ is the chamber surface area (m$^2$), $\rho$ is the gas density (0.714 mg cm$^{-3}$) of CH$_4$ at the standard state, and $T$ (K) is the absolute temperature (273 + temperature in the chamber,˚C).

To assess the influence of real chamber headspace which might be reduced by plant biomass development on CH$_4$ emission rates, net CH$_4$ emission rate ($F_i$, mg m$^{-2}$ h$^{-1}$) was calculated by the newly developed equation (Eq 2) following as.

$$F_i \text{ (mg m}^{-2} \text{ h}^{-1}) = \left(\frac{\Delta C}{\Delta t}\right) \times \frac{V_{\text{net}}}{A} \times \rho \times \left(\frac{273}{T}\right) \quad (\text{Eq 2})$$

where $V_{\text{net}}$ means the real headspace volume of chamber which considered rice plant biomass volume from the growth chamber volume.

The total CH$_4$ emissions for the entire cropping period were calculated using the following formula (Eq 3) [34].

$$\text{Total CH}_4 \text{ emission (kg ha}^{-1}) = \sum^n_{i=1} (R_i \times D_i) \quad (\text{Eq 3})$$

where $R_i$ is the daily CH$_4$ emission rate (g m$^{-2}$ d$^{-1}$) in the $i^{th}$ sampling interval. $R_i$ is calculated by multiplying CH$_4$ emission rate ($F$ & $F_i$, mg m$^{-2}$ h$^{-1}$) and 24 hours. $D_i$ is sampling day interval between the $i^{th}$ and $(i-1)^{th}$ day. $n$ is the number of samplings.

Statistical analysis

The statistical analysis was carried out using the SPSS package (IBM SPSS Statistics 23). All datasets were subjected to variance analysis. Mean differences of plant volumes, growth characteristics, and total CH$_4$ emissions among rice cultivars were assessed through one-way ANOVA, Tukey HSD test. The differences of total CH$_4$ emissions between calculation methods (with and without plant volume consideration) were evaluated by independent-sample t-test. In order to estimate relationship between CH$_4$ emission rates and variables, correlation and linear regression analyses were performed by Pearson correlation analysis (SPSS and Sigma Plot software).

Results

Changes in soil temperature and redox potential (Eh value)

Soil temperature was similarly changed with air temperature during rice cultivation (Fig 1). However, it was not different among the selected rice cultivars. Soil temperature increased
after rice transplanting and peaked at approximately 25 to 60 days after transplanting, and thereafter, rapidly decreased.

Soil Eh values dramatically decreased to less than negative 150 mV within one week after rice transplanting (Fig 1). This anaerobic soil condition was maintained for over 100 days, but Eh values rapidly increased after drainage for harvesting. However, soil Eh values were not discriminated among rice cultivars.

**Changes in rice biomass volumes and growth characteristics**

The biomass volume of rice cultivars was changed with a sigmoid pattern over the whole rice life cycle (Fig 2). After transplanting, the physical volume of rice plants gradually increased up to 90 days, and thereafter, it sharply decreased to the harvesting phase. The allocation of biomass volume inside the chamber was negligible at the early rice-growing stage but slightly increased with plant growth. It was maximized at the panicle initiation stage.

Single rice seedling was transplanted in this field test, but it propagated to the mean 8–11 tillers per hill at the early tillering stage (S2 Fig). In this period, the above-ground biomass of rice plants had 15–20 cm\(^3\) of physical volumes per hill, but only 40–45% (6–9 cm\(^3\) per hill) of biomass was placed over the flooded water table. Thus, the physical volume of eight rice plants inside the...
chamber was 48–72 cm³ and covered only 0.012–0.018% of the chamber headspace (Fig 3). Plant height and biomass productivity were gradually increased with plant growth (S2 Fig), and the above-ground biomass was maximized at the panicle initiation stage, having 309–352 cm³ of physical volume per hill. In this stage, approximately 80% (245–281 cm³ per hill) of above-ground biomass was placed above the flooded water table. Eight hills of rice plant inside the chamber had the maximum physical volume (1,960–2,248 cm³ over the water table) and occupied 0.48–0.55% of the chamber headspace. Thereafter, plant biomass volume slightly decreased with maturing to 166–248 cm³ per hill at the harvesting stage. A total of eight hills of rice plant inside the chamber had 1,328–1,984 cm³ of physical biomass volume and occupied approximately 0.31–0.46% of chamber headspace. As a result, the physical volume of rice plant biomass inside the chamber occupied average 0.24–0.28% of chamber headspace during the whole rice cropping season, and thus, this level of total CH₄ emissions was overestimated.

The physical rice growth characteristics (tiller number, plant height, fresh and dry weight) were gradually developed to the heading stage, and thereafter, slightly decreased towards the harvesting stage (S2 Fig). Single rice seedling was transplanted, but the number of tillers increased up to 20–22 per hill at the end of tillering stage. However, the effective tiller number decreased to 14–18 per hill at the harvesting stage. Plant height increased by the flowering stage and thereafter, stabilized. Fresh biomass weight was changed with sigmoid pattern, which was similar with plant volume changes. Among rice cultivars, these growth characteristics were not discriminated at early growing stage but showed significant difference at the maximum growth stage (S1 Table). However, plant volume and fresh weight were not significantly different among rice cultivars. The physical volume of rice plants showed highly positive correlation with plant height and biomass weight (fresh and dry) (Fig 4).

**Difference of methane emission rates with/without considering plant biomass volume inside the chamber**

Irrespective of rice cultivars, CH₄ emission rates similarly changed during rice cultivation (Fig 5). Low CH₄ emission rates were observed at the early growing stage, but it was highly
increased with developing anaerobic soil condition and rice plants. Two peaks of CH$_4$ emission rates were observed at approximately 50 and 80 days after transplanting in all rice cultivars. After panicle initiation stage, CH$_4$ emission rates rapidly decreased with plant maturing to the background level at harvesting stage.

Total CH$_4$ emissions were slightly different among the selected rice cultivars (Fig 5). Junam and Dongin cultivar had the highest total CH$_4$ emissions with 475 and 445 kg ha$^{-1}$, respectively, and then followed by Chuchung, Saenuri, and Ilmi cultivar with 373, 354 and 318 kg ha$^{-1}$ of total CH$_4$ emissions, respectively. Among rice growth characteristics, the number of tillers showed a highly positive correlation with total CH$_4$ emissions (Table 1).

The uncertainty of total CH$_4$ emissions coming from rice plant biomass occupying chamber headspace was negligible. The allocation of rice plant biomass clearly increased with plant growth (Fig 3), but this volume occupied only 0.48–0.55% of closed chamber headspace at the
maximum plant biomass developing stage. This allocation decreased with maturing to 0.31–0.46% at the harvesting stage. During the whole rice cultivation period, rice plant biomass occupied only 0.24–0.28% of closed chamber headspace on average. Therefore, these small levels of total CH$_4$ emissions might be overestimated more than the CH$_4$ emissions conventionally calculated by the IPCC investigation protocol.

**Discussion**

Methane is biologically formed from the anaerobic decomposition of organic matter [35,36]. Methane production is initiated under extremely reduced soil condition having negative 150–160 mV of soil redox potential (Eh value) [37,38]. Therefore, methanogenesis can be influenced mainly by organic substrates and oxygen contents in soil. In this field study, soil Eh values were dramatically decreased with flooding from 100 mV to negative 150 mV within a week after transplanting and stabilized within minus 180–220 mV until drainage for harvesting (Fig 1). However, we could not find any difference of soil Eh values between inside and outside the chamber.

In the flooded rice cropping fields, closed chamber techniques have broadly used to determine CH$_4$ emission rates due to its minimal cost for installation and management, easy manipulation, and high efficiency to detect low CH$_4$ fluxes [17,39–43]. To calculate CH$_4$ emission rates in closed chamber method (Eq 1) [21], the increased CH$_4$ concentration and temperature for chamber closing hour were considered as the variables. Chamber headspace volume was calculated by considering the flooded water level. In addition, gas density was designed as the fixed value. Rice plantation inside the chamber is essential to make the same condition with outside since rice plants strongly influence CH$_4$ production and emission characteristics.

However, this closed chamber technique has several limitations in accurately characterizing CH$_4$ emission rates, since the environment condition might be big different between inside and outside the chamber. Firstly, the net chamber headspace could be significantly decreased by plant biomass development, and therefore, the CH$_4$ emission rates calculated by the fixed headspace volume considering only water table changes might be largely overestimated. However, we found the change of rice biomass volume negligibly influenced on evaluating CH$_4$ emissions (Fig 5). During the whole rice cropping period, rice plant biomass occupied only 0.2–0.3% of closed chamber headspace, and therefore, only these levels of total CH$_4$ emissions can be overestimated. This overestimation might be much less than the errors caused by other practices on chamber management (i.e., chamber closing hour, inconstant flooding level, temperature rising) and changes of plant mediating physiological characteristics.

In the guidelines for measuring CH$_4$ emission [18], chamber closing for 30 minutes is recommended, but the exact time control is difficult under the manual system with many chamber replications. For example, closing time error (longer or shorter closing) of 2 minutes can generate an error from approximately ±6.7% on a daily CH$_4$ emission rate (Fig 6). In rice cropping fields, it is not easy to control the irrigated water level constantly even under automatically controlled irrigation systems. The flooded water table difference can change the headspace volume inside the chamber. Two cm over- or less measurement of flooding water

### Table 1. Correlation between total CH$_4$ emissions and rice growth characteristics at harvesting stage.

| Volume | Height | Tiller no. | Fresh biomass weight | Dry biomass weight |
|--------|--------|------------|----------------------|--------------------|
| -0.518 | -0.393 | 0.916***   | -0.136               | 0.558              |

***Significant differences (P<0.001).

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table can make the error approximately ±1.77% on the daily CH$_4$ emission rate. During this field investigation, the temperatures between inside and outside of the chamber showed a big difference on gas sampling stage, particularly in hot summer season (S1 Fig). Air temperature inside the closed chamber was average 5°C higher than the outside during the investigation period. The enhanced temperature was considered to calculate CH$_4$ emission rates as a variable. However, 2°C over- or less measurement of headspace temperature at gas sampling time can generate approximately -0.65% to +0.66% of calculation error on the daily CH$_4$ emission rate.

Furthermore, temperature increase inside the closed chamber could influence rice plant development and soil microbial characteristics which are fundamental traits to CH$_4$ production and emission. In fact, at the harvesting stage, above-ground biomass productivity of rice in a closed chamber was minimum 5–10% higher than that of rice outside the chamber. Given that methanogenesis activity showed a very high positive correlation with soil temperature [44–47] and rice biomass productivity [48], the enhanced soil temperature and improved rice biomass productivity inside the chamber may significantly increase CH$_4$ production and emission activity and then make a big difference in total CH$_4$ emissions between inside and outside the chamber. However, the temperature effect induced by chamber has not yet been properly evaluated. It may be possible that the error from chamber enclosure is far more influential than chamber handling errors (i.e., closing time, water table, and temperature measurement).

The content and lability of soil organic matter might be similar between inside and outside the chamber, but rice root activity is probably much stronger inside than outside, which can be presumed from higher rice above-ground biomass development inside the chamber. In general, higher rice biomass can release more root exudates [49] and then produce more CH$_4$ during rice cropping. Root exudates contain various metabolites such as organic acids (acetic, 

![Fig 6. Potential error (%) in daily CH$_4$ emission rates coming from chamber management practices during gas sampling.](https://doi.org/10.1371/journal.pone.0256796.g006)
citric, oxalic acid, etc.), sugars (glucose, sucrose, etc.) and amino acids [50], and they can be important organic substrates for methanogens [51]. We confirmed that CH$_4$ emission rates showed highly positive correlation with rice growth characteristics like biomass productivity (Table 2). The higher biomass inside the chamber might increase CH$_4$ production by minimum 5–10% over out of the chamber. Furthermore, dead root biomass is also an important organic substrate for methanogenesis, so greater biomass yields inside the chamber can produce more organic residues thus increase CH$_4$ production.

Methane is emanated through three different pathways in rice fields: transport through aerenchyma channel, ebullition as gas bubbles, and diffusion through the flooded water and soil interfaces [52]. However, in the rice paddy, most of CH$_4$ emitted is diffused via aerenchyma as both water-dissolved and gas phases from the flooded soils to the atmosphere [4,9,10,53]. Simply, we can think that rice plants with greater aerenchyma might emit more CH$_4$ from rhizosphere soils to the atmosphere [9]. However, aerenchyma channel can also transport oxygen from the atmosphere to plant and rhizosphere [53,54]. Thus, at the rhizosphere of greater aerenchyma having rice, more CH$_4$ may be oxidized by methanotrophs which use oxygen to metabolize CH$_4$ as their carbon and energy source. However, the net effect of aerenchyma size on CH$_4$ production and consumption was not clear yet. Therefore, we need to study more the influence of the chamber enclosure on methanogenesis to improve the accuracy of estimating CH$_4$ emissions in rice cropping fields.

Conclusion

During the whole rice cropping period, rice plant biomasses occupied the mean 0.24–0.28% of chamber headspace volume, and thus this level of total CH$_4$ emissions was overestimated in the conventional calculation (proposed by IPCC Guidelines). However, this overestimation was much less than the errors which can be generated from incorrect chamber managements such as closing time control, flooding water table management, temperature misreading, etc. Chamber effect which can increase temperature and differentiate plant and microbial activities inside the chamber might lead to more serious errors in estimating CH$_4$ emissions in rice paddies. In conclusion, the uncertainty coming from the occupation of rice plant biomass volumes inside the closed chamber was negligible to estimate total CH$_4$ emissions in rice paddy fields.

Supporting information

S1 Fig. Changes in air temperatures inside and outside the closed chamber during rice cropping period.

(DOCX)
S2 Fig. Changes in plant growth characteristics during rice cropping season.

S3 Fig. Static chamber installation and rice plant growth inside the closed chamber.

S1 Table. The maximum plant volumes and growth characteristics with statistical significance between rice cultivars.

S1 Data. Changes in CH$_4$ emission rates (mg m$^{-2}$ hr$^{-1}$) which were calculated without considering biomass volume inside chamber during rice cropping season.

S2 Data. Changes in rice biomass volume (cm$^3$ hill$^{-1}$) during rice cultivation.

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