Rice cultivation reduces methane emissions in high-emitting paddies [version 1; peer review: 2 approved with reservations, 1 not approved]

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Abstract

Background: Rice is typically understood to enhance methane emissions from paddy fields. However, rice actually has two separate functions related to methane: i) emission enhancement, such as by providing emission pathways (aerenchyma) and methanogenetic substrates; and ii) emission suppression by providing oxygen pathways, which suppress methanogenesis or enhance methane oxidation. The overall role of rice is thus determined by the balance between its enhancing and suppressing functions. Although existing studies have suggested that rice enhances total methane emissions, we aimed to demonstrate that the balance between rice’s emitting and suppressing functions changes according to overall methane emission levels, which have quite a large range (16–500 kg methane ha\(^{-1}\) crop\(^{-1}\)).

Methods: Using PVC chambers, we compared methane emissions emitted by rice paddy fields with and without rice plants in rice fields in the Mekong Delta, Vietnam. Samples were analyzed by gas chromatograph.

Results: We found high overall methane emission levels and our results indicated that rice in fact suppressed methane emissions under these conditions. Emission reductions increased with the growth of rice, up to 60% of emission rate at the maximum tillering stage, then decreased to 20% after the heading stage, and finally recovering back to 60%.

Discussion: It is known that methane is emitted by ebullition when the emission level is high, and methane emission reductions in rice-planted fields are thought to be due to oxidation and methanogenesis suppression. However, although many studies have found that the contribution of soil organic matter to methanogenesis is small, our results suggested that methanogenesis depended mainly on soil...
organic matter accumulated from past crops. The higher the methane emission level, the lower the contribution of rice-providing substrate.

**Conclusion:** As a result, during the growing season, rice enhanced methane emissions in low-emission paddy fields but suppressed methane emissions in high-emission paddy fields.

**Keywords**
Greenhouse gases, Mekong Delta, Methane oxidation, Methanogenesis inhibition, Rice paddy, Triple cropping

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**Competing interests:** No competing interests were disclosed.

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Introduction
The role of rice in methane (CH$_4$) emissions changes according to emission levels. Rice performs three key functions related to CH$_4$ emissions: i) providing a CH$_4$ pathway through a well-developed system of intercellular air spaces (aerenchyma), ii) providing a substrate for methanogenesis, and iii) oxidising CH$_4$ in rhizosphere by supporting O$_2$ counter-transport through aerenchyma system$^{[4,5]}$. The level of contribution of these functions varies with the overall emissions, and the total amount of CH$_4$ emitted to the atmosphere is thus a balance between CH$_4$ production and oxidation$^1$. To the best of our knowledge, all studies have concluded that rice enhances overall CH$_4$ emissions from paddy fields; however, these studies have mostly disregarded overall emission levels and their potential impact on the role of rice in enhancing or suppressing CH$_4$ emissions.

Cicerone and Shetter measured CH$_4$ emissions with a closed chamber on the water surface of a paddy field, and found that 4 hours after starting measurement, CH$_4$ concentration was 290 ppm over rice plants and only 4 ppm over open water$^6$. Further studies have revealed that CH$_4$ produced under methanogenesis diffuses through the soil, which is oxidised by the surface barrier before reaching the atmosphere$^7$. Rice absorbs diffused CH$_4$ from its roots and emits CH$_4$ through aerenchyma$^{[4,5,7]}$. Therefore, an established theory has emerged that CH$_4$ is not emitted from the soil without rice. Other recent studies have provided additional evidence that the primary source of CH$_4$ is current-season photosynthates—specifically, root exudates or decaying tissues$^{[8,9,11]}$. This results in CH$_4$ emissions that peak during the late stage of rice growth. Thus, the presence of rice plants has been determined to be the cause of CH$_4$ emissions in paddy fields.

Wassmann et al.$^{[12]}$ measured CH$_4$ emissions on the water surface of a paddy field amended with organic matter. They found that organic matter incorporation increased total CH$_4$ emission levels from 27–90 to 160–240 kg CH$_4$ ha$^{-1}$ crop$^{-1}$, and ebullition increased from 15–23% to 35–62%, respectively$^{[12]}$. Since it is known that organic matter incorporation causes CH$_4$ emissions to peak during the early stages of rice growth, when the rice is still small and the aerenchyma is not well developed, the results of Wassmann et al.$^{[12]}$ should be closely examined to determine whether ebullition increased with total emissions. According to the 2006 Intergovernmental Panel on Climate Change (IPCC) guidelines, CH$_4$ emissions for 100 days of rice cropping are 130 kg CH$_4$ ha$^{-1}$ crop$^{-1}$; however, emissions were observed at almost twice this value by Wassmann et al.$^{[12]}$. Average CH$_4$ emissions from rice paddies in Asia without and with organic matter incorporation ranged from 16 to 200 and 250 to 500, respectively$^{[13]}$. Although ebullition has been little studied$^{[14]}$, ebullition must occur at high emission levels. Furthermore, in the study by Wassmann et al.$^{[12]}$, twin CH$_4$ emissions peaks appeared, with an early peak corresponding to the organic matter amendment and a later peak corresponding to rice-originated substrate$^{[12]}$. An alternate interpretation of these results is that the twin peaks could reflect the oxidation performance of rice, since CH$_4$ oxidation is known to increase with rice growth, up to the maximum tillering stage, and then decrease$^{[15]}$.

We monitored CH$_4$ emissions in the paddy fields for 5 years (total 15 crops) in triple rice cropping fields in the Mekong Delta, Vietnam. The CH$_4$ emission level was an order of magnitude higher than IPCC standards. These high CH$_4$ emissions suggest that ebullition must have been occurring.

Results and discussion
In the present study, we compared CH$_4$ emissions on the water (soil) surface of paddy fields with and without rice in paddy fields. Our results showed that rice decreased CH$_4$ emissions by half relative to paddy fields without rice (see Figure 1, Figure 2). Complete, unprocessed data are available on figshare$^{[16]}$. There was no marked CH$_4$ emissions peak in the

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**Figure 1.** Seasonal CH$_4$ emissions in paddy fields. Average CH$_4$ emissions of rice-planted areas and no-rice-planted areas in a triple cropping rice field in Mekong Delta. Winter–Spring season (2017). The paddy fields did not receive rice straw incorporation. Error bars are s.d. (n = 3).
late-stage of the rice field. This suggests that the amount of methanogenesis from rice-providing substrate was relatively small. Note the high emission levels (500–1400 kg CH\textsubscript{4} ha\textsuperscript{-1} crop\textsuperscript{-1}). These findings suggest that total CH\textsubscript{4} emissions are reduced by oxidation or methanogenesis inhibition associated with growing the rice plant.

We also found that the reduction rate of CH\textsubscript{4} emissions increased up to 60%, at maximum tillering stage, then decreased to 20% after the heading stage, and then finally recovered to 60% (see Figure 3). The decrease around the heading stage was caused partially by an increase of emissions in rice-planted fields, and mainly by the erratic emissions in the unplanted area (see Figure 1).

No consensus has yet been reached on the extent to which methanotrophs or rice roots attenuate CH\textsubscript{4} emissions\textsuperscript{17}. Using the N\textsubscript{2} atmosphere technique, CH\textsubscript{4} oxidation ratios have been found to be around 40% on average and were relatively stable throughout the rice growing season\textsuperscript{17}. However, genuine CH\textsubscript{4} oxidation, as measured using inhibitors, tend to decrease with rice growth, and the reduction rate for total CH\textsubscript{4} emissions can reach up to 20%\textsuperscript{17}. Although, most of those studies assume plant-mediated transportation\textsuperscript{17}, in general, our results roughly matched the results using the N\textsubscript{2} atmosphere technique. This suggests that the reduction in CH\textsubscript{4} emissions was not due to genuine oxidation and was more likely to be due to methanogenesis inhibition by oxygen from aerenchyma, which lasts until harvesting\textsuperscript{18}.

We found high CH\textsubscript{4} emissions in paddy fields that were not planted with rice and did not incorporate rice straw or other organic material. Despite this lower input of methanogenesis substrate, CH\textsubscript{4} emission levels were 12 times higher than the IPCC guidelines. The emission levels remained almost stable after reaching maximum. This suggests that methanogenesis mainly depends on soil organic matter that has been accumulated from past rice crops. Prior studies have suggested that the contribution of soil organic matter to methanogenesis is small; however, these studies also indicated that higher emission levels tend to be associated with higher contribution rates of soil organic matter\textsuperscript{8–11,19}. Therefore, our results are consistent with prior studies that assume that emission levels are proportional to the amount of soil organic matter, which can be a methanogenesis substrate. Hotspots of CH\textsubscript{4} emissions, which exist widely across tropical Asia, would have huge soil organic matter stock formed by sequential rice cropping under flooded conditions.

To summarise, most studies of CH\textsubscript{4} emissions in paddy fields have been conducted in fields with low overall emission levels. Since the role of rice in CH\textsubscript{4} emissions varies according to the overall emission levels, these results cannot be appropriately generalised to rice paddies with high emission levels. The results of our study suggest that rice reduces CH\textsubscript{4} emissions in hotspot paddy fields.

**Methods**

**Study site**

Experimental fields were in Tan Loi 2 Hamlet, Thuan Hung village, Thot Not district, Can Tho city, Vietnam. Farmers conducted triple rice cropping by direct seeding and full flooding. This area receives almost 2 months of floods annually from the Mekong River. The flood decomposes rice straw underwater to the extent that it is no obstacle for seeding. Therefore, farmers start the rice cropping by levelling the fields, without incorporating rice straw. We observed CH\textsubscript{4} emissions in 18 paddy fields (26 × 17 m each) under several conditions for 5 years from September 2011. A preliminary study was conducted with the rice variety OM501 (suitable for the season) in the Summer-Autumn season of 2016 by the same methods and paddies of the main study (see Figure 4). In the present study, we used three such fields (managed with straw return under flooded conditions) for replication. We conducted the main experiment after the annual floods (4 November 2016–12 February 2017); these fields did not incorporate rice straw.

**Treatment**

We compared CH\textsubscript{4} emissions in paddy fields with and without rice. We set 2 × 2 m squares of plastic films on each field just before seeding, then carefully removed them with seeds on
the films immediately after seeding. In other plots, there was no difference with farmers’ conventional rice-growing procedures. Farmers spread 230 g m\(^{-2}\) (in dry weight) of germinated rice seed (variety Jasmine) on drained wet paddy field surfaces on 5th November. This wet condition was maintained for 7 days, then the field was kept flooded until 89 days after seeding (DAS), and the rice harvested on 100 DAS. The farmers applied fertiliser, which included 76 kg of urea on 12 November, 53 kg each of urea and NPKS (16-16-8-13), diammonium phosphate on 19 November, and 53 kg each of urea and NPKS on 15 December. The daily average water level was monitored with a water level logger (HOBO U20; Onset Computer Corporation, Bourne, Massachusetts) at the corner of the field was 2.0 cm (±0.6–6.1 cm) until drained.

Measurement of \(\text{CH}_4\) emissions

We set an approximately 2 m long and 0.5 m wide ladder from the centre of the shorter bund to allow measurement of \(\text{CH}_4\) without touching the paddy soil surface. This ladder was on the border of the non-planted area in each plot. We set PVC chamber bases on the paddy field of both sides of the ladder to avoid measurement perturbation. Chambers (60 × 80 cm and 100 cm high, transparent acryl) were set on a watertight chamber base for every measurement. Measurements were taken at 8 a.m. because previous research has indicated that emissions at this time have a high correlation (ca. 90% of average emission) with average daily emissions\(^{20}\). We mixed the air in the chamber with a fan for 5 min after setting the chamber, then sampled the first gas, then sampled the second gas 20 min later.
later. We conducted the measurements once a week throughout the rice growing stage, but every 3 days for 2 weeks after seeding, heading stage, and around draining. The samples were analysed by gas chromatograph (GC-14B, Shimadze, Kyoto). The cumulative CH$_4$ emissions were calculated by linear interpolation.

**Ethics statement**
This study was conducted with the approval of the farmer.

**Statistical analysis**
Data were processed using Microsoft Excel 2016.

**References**


**Data availability**
Raw data of this article is available from figshare: https://doi.org/10.6084/m9.figshare.6916277.v1. Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

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Version 1

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Dung Duc Tran
Center of Water Management and Climate Change (WACC), Vietnam National University – Ho Chi Minh City (VNU – HCM), Ho Chi Minh City, Vietnam

The authors of "Rice cultivation reduces methane emissions in high-emitting paddies" present an interesting manuscript with great effort to demonstrate the balance between rice's emitting and suppressing function changes according to overall methane emission levels. In general, the manuscript is well-written. However, I recommend the authors to do a major revision to the manuscript before being potentially accepted for indexing by the Journal.

1. The Introduction section should add research objectives or in terms of research questions that help readers to promptly understand the paper's content.

2. In the Methods section, description and activities are needed in more detail; additionally a map of the case study would be useful.

3. In the Results and Discussion section, I expected more discussion that enhance more work on comparing findings with previous studies. In addition, the limitations and future outlooks need to be elaborated upon.

4. A Conclusion is important to add into the manuscript since it presents the real implication of the research based on the authors' perspective.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly
If applicable, is the statistical analysis and its interpretation appropriate?  
Yes

Are all the source data underlying the results available to ensure full reproducibility?  
Yes

Are the conclusions drawn adequately supported by the results?  
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Hydrology and Agricultural Water Management

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 17 Jun 2019  
Masato Oda, Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

Thank you very much for your precious suggestions.

1. We improved the abstract and the introduction to describe more clearly the aim of this study.
2. We corrected the method section and clarified the details. For the site location map, we think it can be found on the internet nowadays.
3. We added the latest reference which is expected to give readers the deeper understanding of our findings to the result and discussion section.
4. For the limitations and future outlooks, we described them at the end of the discussion section and the newly added conclusion section.

See the revised manuscript, please.

Competing Interests: No competing interests were disclosed.
Department of Geography, College of Life and Environmental Sciences, University of Exeter, Exeter, UK

Oda and Chiem present CH$_4$ fluxes from Vietnamese rice paddies with- and without rice plants, and conclude that the presence of rice plants reduces CH$_4$ emissions. Their results are interesting and convincing, and the experimental design seems solid. That said, I have several serious reservations about this paper that need to be addressed before it can be approved.

1. The authors need to explain more clearly the relevance of their research. It might be true that the presence of rice plants reduces CH$_4$ emissions, but the sole purpose of paddies is to support rice agriculture. Thus, the statement that rice cultivation reduces methane emissions from paddies seems a bit strange. Does the IPCC provide estimates of emissions from rice paddies without rice plants? If not, how exactly would the results from this study contribute to understanding the contribution of rice agriculture to CH$_4$ emissions?

2. The experimental design does not allow the authors to test their hypothesis as stated in the abstract. The authors aim to demonstrate that the effect of rice plants on methane emissions depends on the background emissions (i.e. without plants). However, the authors only test the effect of rice plants in paddies with high background emissions.

3. The authors did not test the significance of differences between plots with- and without rice plants.

4. The reference list is quite outdated; only 2 of the cited studies were published in the last 5 years. Several relevant recent papers are ignored by the authors. For instance, Jiang et al. 2017 reported that in paddies with high soil C contents, large rice plants reduce CH$_4$ emissions compared to small rice plants. Furthermore, they show the opposite effect in paddies with low soil C contents. Please note that I am a co-author on that paper; I don't suggest that the authors need to include it, but please consider this as just one example of relevant recent research.

5. The first sentence of the introduction is essentially an hypothesis. Yet, the authors provide no clear support to back up this statement.

6. In the last sentence of the introduction, what does “all studies” refer to? The studies 1-6? Did these studies compare CH$_4$ emissions with and without rice plants? This is not clear.

7. The text is slightly confusing in some places. For instance, in the “background” section in the abstract it’s not clear whether the authors refer to methane emissions with or without rice plants. The same is true for the last paragraph of the introduction. On page 4, second paragraph, the discussion of changes in “reduction rates” is quite confusing. Does a decrease in reduction rates mean relatively low or high rates in plots with plants?

8. Some basic info about the field site is missing. For instance, MAT and MAP data would be useful, as well as soil C contents.

9. I spotted a few grammatical errors. For instance, in the background section of the abstract, “existing studies” should be “previous studies”. In the discussion section of the abstract “rice-providing” should be “rice-derived”. The first sentence of the results and discussion section should read “In the present study, we compared CH$_4$ emissions on the water (soil) surface of paddy fields with and without rice”. These are just a couple of examples. Perhaps the authors could contact a native speaker to have a quick look at their paper?

References
Full Text

Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Agronomy, biogeochemistry, greenhouse gas fluxes.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 14 Jun 2019

Masato Oda, Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

1. Thank you for your precious suggestion! We changed the word "cultivation" to "plants" in the title. We think the word is more suitable to the content.
2. Thank you again. We aim to demonstrate rice plants are suppressing overall methane emission in high CH4 emission paddy fields. We changed the last sentence of the background as follows. "that the balance..." to "that the overall methane emission is decreased by rice plants." We expect that this change clarifies some other points that you indicate. In association with, we think the experiment in the low background is not necessary because they have been conducted by plenty of studies.
3. We think it's obvious from Figure 1 and 2; for your information, the p-value by one-sided Welch's t-test for the total emissions is 0.013.
4. Thank you very much for introducing the study to support our conclusion. We referred to that in the last paragraph of the discussion. We have referred to rather old-dated studies because we aim to mention the role of rice in the context of the history of the study for paddy fields CH4 emission.
5. The sentence indicates common sense; therefore, we would like to change as follows:
"Rice is understood to enhance methane emissions from paddy fields as mentioned in IPCC guidelines."
6. We would like to change the sentence ", all studies ... these studies" to "rice enhances overall CH4 emissions from paddy fields. In addition, previous studies".
7. We added the formula, and we clarified the sentences in the results section.
8. We think the soil carbon contents is important; however, that is out of range of this study and we don't have the map.
9. Thank you so much! We had used an English editing service, but that was not enough. We have revised the English.

**Competing Interests:** No competing interests were disclosed.
Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Greenhouse gas monitoring in agricultural systems.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 21 May 2019

Masato Oda, Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

Thank you very much for reviewing our manuscript and giving precious comments.

I think your comments can be summarized to three points: “uniformity of conditions”, “the effect of early stage rice plants”, and “the reason of high CH4 emission in the first week”.

1) For the first, we would like to apologize for the incomplete explanation and mistype in the treatment subsection that leads readers to misunderstanding for the above points. The sentences and the correct version are as follows:

“We set 2 × 2 m squares of plastic films on each field” → “We set 2 × 2 m squares of plastic films on a part of each three fields”

“In other plots, there was no difference with farmers’ conventional” → ”In other points, there was no difference with farmers’ conventional”

These corrections will make clear that the experiment was conducted under completely uniformed conditions of three pair. These corrections are also the answer for the suspicion of uniformity of conditions.

2) For the second, we think the effect of rice is large even in the early growth stage of rice because of the high plant density (230 kg ha-1) and the rapid growth of them. We would like
to apologize again for mistype of the unit.

3) For the third, we think the high CH4 emission in the first week was caused by the erratic character of “ebullition”. You can check there is no difference between “no rice” and “rice” in the 7th and 9th day’s emission by raw data (https://f1000research.com/articles/7-1349/v1#ref-16). For straws, this is not a treatment but the conventional management of triple cropping rice cultivation in Mekong Delta. They were decomposed in the natural flooding water and not incorporated into the soil (however, straws were incorporated in the preliminary study).

Following the editorial team’s recommendation, we are waiting for additional peer review reports before starting on any article revisions.

**Competing Interests:** No competing interests were disclosed.

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