Review of “Straw application in paddy soil enhances methane production also from other carbon sources” by Yuan *et al.*, submitted to *Biogeosciences*.

In this study Yuan et al. investigate how rice straw application affect methane ($CH_4$) production from other carbon sources, i.e. soil organic matter and organic matter supply from rice plant. They use isotope-labeling technique to differentiate three carbon sources (ref. 1). The authors find priming effect of straw application on $CH_4$ production, i.e. stimulation of $CH_4$ production from the other two sources.

The topic of this study is of great importance as rice paddies might contribute up to 20% of global $CH_4$ emission and straw management is attracted attention as a potential mitigation measure. The results of the study are novel and biogeochemical aspect of this study is definitely of interest to readers of *Biogeosciences*. Howerver I am concerned about experimental design, reliability of the calculation formulae, and interpretation of the results.

My first major concern is about potential artifact in $CH_4$ production experiment. As the authors pointed out, organic materials from rice root can serve as a major source of substrate for $CH_4$ production as reported in previous studies (ref. 2, 3). In this paper, the authors use the term “rice root organic carbon, ROC (the last line in p14170)” for designating such materials, including “root exudates, sloughed-off dead root (line 1, p14171).”

Since the rate of ROC supply has closely related to rice activities including above-ground photosynthesis and exudation processes from living root, rice must be kept “alive” when one wants to investigate ROC (as is the case in ref. 2, 3). However, in the present study (and previous one (ref. 1)), production rate and isotopic signature of $CH_4$ was measured in a “soil incubation experiment” of which soil were sampled from rice pot after cutting off the rice plant (2.1.2). Therefore actual carbon flow associated with ROC cannot be evaluated properly in the experimental setup.

My second concern relates to the validity of the assumptions used to calculate relative contribution (fraction) of $CH_4$ production from ROC ($f_{ROC}$). The formulation was mainly described in their previous paper (equations 1-4 in ref. 1), but I feel the necessity to discuss it here because the validity of $f_{ROC}$ estimation can have significant consequences on results of current study.

They used a mass balance equation to compute $f_{ROC}$ ($f_{ROC}$ is implicitly defined),

$$\delta^{13}C_{CH4} = f_{ROC}\delta^{13}C_{CH4-ROC} + (1 - f_{ROC})\delta^{13}C_{CH4-SOR}, \text{ (eq. 1 in ref. 1)}$$

where variables are defined as follows: $\delta^{13}C_{CH4}$ is $\delta^{13}C$ of total $CH_4$, $\delta^{13}C_{CH4-ROC}$ is $\delta^{13}C$ of $CH_4$ produced from ROC, $\delta^{13}C_{CH4-SOR}$ is $\delta^{13}C$ of $CH_4$ formed from soil organic matter (SOM) plus rice straw (RS).

Clearly this equation has three unknown variables, i.e. $f_{ROC}$, $\delta^{13}C_{CH4-ROC}$, and $\delta^{13}C_{CH4-SOR}$. Therefore, the "two" equations (eq. 2 and 3 in ref. 1, derived from two RS-treatments having different $^{13}C/^{12}C$ ratio) cannot be solved for the "three." In order to get the solution they put another assumption, i.e. $\delta^{13}C_{CH4-SOR}$ is the same across rice-planted and unplanted treatments (ref. 1) but I doubt this assumption is reasonable because it is well known that the presence of rice can dramatically change physicochemical environments in the soil-plant-atmosphere system. This is especially important when considering $^{13}C/^{12}C$ ratio of $CH_4$ because isotopic discrimination occurs in production, consumption and transport processes of $CH_4$, all of which are sensitive to chemical and physical conditions of the system. Unlike the equations used to compute $f_{RS}$ (eq. 5, 6 in ref. 1), isotopic fractionation factors are not explicitly defined in eq. 1 and therefore included in each term of $\delta^{13}C_{CH4}$.

My third concern is about assumptions regarding redox condition of the soil. The main conclusion of the current study "positive priming effect of RS on SOM and ROC" depends on the assumption that "soil conditions were reduced and methanogenesis was the exclusive terminal decomposition process of organic matter (lines 13-14, p14179)." However, this assumption may be open to debate. In some soils Fe(III) reduction may last over 16 weeks even at higher incubation temperature (30 degC, ref. 4) than this study (25 degC). Furthermore some results of this study clearly indicate that the soil is not completely reduced. As the authors noted, organic matter is eventually degraded to equal amount of $CH_4$ and $CO_2$ under purely methanogenic environment (eq. R1, p14181). However, results of $CH_4$ and $CO_2$ production suggest that the amount of $CH_4$

accounts for only a quarter of $CO_2$ production (Fig. 4-B, D). It is likely that the rest of $CO_2$ production should be coupled to reductions of inorganic electron acceptors, most probably Fe (III) (ref. 5). Therefore, their observation of increased $CH_4$ production from SOM can be best explained by accelerated soil reduction by RS treatment, not by the priming effect *per se*. Absence of the '*priming effect*' on $CH_4$ plus $CO_2$ production (lines 5-7, p14182) can be explained in the same manner, that is, in non-amended soil, $CO_2$ production coupled to reduction of inorganic electron acceptors was larger because soil condition was less reduced.

More specific comments are itemized below:

Non linearity of $\delta^{13}C$ notation: By definition, δ-notation lacks linearity (e.g. –100‰ + 100‰ ≠ 0‰ in a strict sense). I guess omission of the non-linearity does not have significant consequences on the results of computations, but it would be better to show maximum error associated with the non-linearity effects.

Please add more information of rice phenology (day of heading etc) as growth stage is very important factor affecting C flow from rice to soil.

Rice straw enriched in $^{13}C$ was used in this study. I wonder if the labeling was homogeneous across rice-straw components having different degradability. For example, if labeling was conducted in a rather short time period, easily-degradable component (such as non-structural carbon hydrate) might be preferentially labeled. In that case, average $^{13}C/^{12}C$ of rice straw and that of decomposed C could differ.

References

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3. Tokida et al (2011) Global Change Biology, 17, 3327.
4. Cheng et al. (2007) Journal of Environmental Quality, 36, 1920.
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