Reply to anonymous referee #1 and #2

We thank the two referees for their expert comments that will be very helpful in improving our manuscript. Referee #2 who posted the comments after referee #1 basically addressed the same potential problems. Nevertheless, we will answer the comments of the two referees separately.

Referee #1

Referee #1 raised three major points concerning the method of determining the relative fractions of CH_4 originating from root (ROC), straw (RC) and soil organic matter (SOM). These determinations were done according to a protocol that had been presented in an earlier publication (Yuan et al. 2012) and were based on measurement of $\delta^{13}C$ of CH_4 produced in anoxically incubated slurried soil cores taken from planted and unplanted rice microcosms treated and untreated with rice straw labeled with ^{13}C at two different levels. These $\delta^{13}C$ data allowed the calculation of the fractions of CH_4 produced from ROC, RS and SOM. Using these fractions and the CH_4 production rates, it was possible to detect priming effects, i.e., stimulation of rates of CH_4 production from ROC and SOM by the addition of rice straw. The determination of priming effect critically depends on the determination of the fraction of CH_4 produced from ROC and SOM. In the first part of our study we addressed the possible priming effect of rice straw on CH_4 production from both ROC and SOM using planted microcosms, while in the second part we only addressed the priming effect on CH_4 production from SOM using unplanted anoxic soil slurries. The first and second major concerns of referee #1 only refer to the first part of our study.

The **first** major concern of referee #1 was about a potential artifact in CH_4 production determined during the rice microcosm experiments. 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. Therefore the carbon flow from the root into the soil was interrupted. This was indeed the case and could have resulted in underestimation of the rate of CH_4 production. However, the soil slurry still contained root exudates previously excreted and cut roots. Literature data and own experience show that there is always a delay between carbon input and CH_4 production, as for example shown after addition of root exudates or glucose (Lu et

al. 2000). Therefore, we are confident that our CH_4 production measurements, which were done within 24 h, covered CH_4 production from ROC (in addition to that from RS and SOM) quite well. This is actually indicated by the fact that the fractions of ROC-dependent CH_4 production determined by this approach were quite high (>60%; Yuan et al. 2012) and thus, CH_4 production from ROC was not or at least not much underestimated.

Second, the fraction of CH_4 production from ROC was calculated from $\delta^{13}C$ values, which were quite stable, as seen from the $\delta^{13}C$ in control (no straw addition) rice microcosms during the whole season (Yuan et al. 2012). This stability comes from the fact that the $\delta^{13}C$ value of rice plant biomass was stable and that the methanogenic community in rice field soil was also stable over such short incubation times. Therefore, the short time (24 hours) soil core incubation method which was applied in our study should be suitable for determining the isotopic signatures of CH_4 in rice field soil. Furthermore, we would like to emphasize that for assessing the priming effect it is mainly the comparison of treatment with and without rice straw rather than with and without plants that matter (compare point (3) of referee #2).

The **second** major concern of referee #1 addresses the mass balance equations used for calculation of the fraction of ROC-derived CH_4, in particular whether our assumption is true that $\delta^{13}C_{CH4\text{-}SOR}$ is the same across rice-planted and unplanted treatments. $\delta^{13}C_{CH4\text{-}SOR}$ is the $\delta^{13}C$ of CH_4 produced from both SOM and RS (Yuan et al. 2012). Referee #1 is concerned since 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 and these conditions could be different in the presence and absence of plants. In addition, the newly produced CH_4 is always pooled with formerly accumulated CH_4 in the soil pore water. In order to avoid most of these problems, we did not use the $\delta^{13}C$ of the CH_4 that was emitted from the microcosms, but instead collected soil cores and incubated them under anoxic condition. Before the anoxic incubation, the previously accumulated CH_4 was removed, and there was no consumption and transport of CH_4 during the incubation, so that our measurements recorded the $\delta^{13}C$ of the newly produced CH_4. As a result, the variables ($\delta^{13}C_{CH4}$, $\delta^{13}C_{CH4\text{-}ROC}$ and $\delta^{13}C_{CH4\text{-}SOR}$) in eq. 1 (Yuan et al. 2012) are free from isotopic fractionation factors due to consumption and transport of CH_4 and only comprise fractionation factors involved in CH_4 production from organic matter. Still the isotopic fractionation during production of CH_4 from SOR could be affected by the presence or absence of plants. However, this is not very likely, since there was no significant difference in the

abundance of the methanogenic populations between planted and unplanted treatments (abundance of methanogenic community was only enhanced by addition of straw). Therefore, it was reasonable to assume that $\delta^{13}C_{CH4\text{-}SOR}$ was the same across rice-planted and unplanted treatments. However, even when it was different, the difference had probably not a large influence on the determination of f_{ROC}, since according to eq. 2 (Yuan et al. 2012):

$$f_{ROC} = (\delta^{13}C_{CH4\text{-}I} - \delta^{13}C_{CH4\text{-}SOR\text{-}I})/(\delta^{13}C_{CH4\text{-}ROC} - \delta^{13}C_{CH4\text{-}SOR\text{-}I}),$$

and a small fluctuation of $\delta^{13}C_{CH4\text{-}SOR}$ will not change f_{ROC} significantly when there is a relatively large difference between $\delta^{13}C_{CH4\text{-}SOR}$ and $\delta^{13}C_{CH4\text{-}ROC}$ and between $\delta^{13}C_{CH4\text{-}SOR}$ and $\delta^{13}C_{CH4}$. Such large difference was created by the application of ^{13}C-labeled rice straw (Fig. 4 in Yuan et al. 2012). Therefore, our assumptions in mass balance calculation should be rather robust and the thus calculated values of f_{ROC} should be valid.

The **third** major concern of referee #1 addressed the soil conditions, i.e., whether the soils studied were sufficiently reduced so that methanogenesis was the exclusive terminal decomposition process of organic matter. This comment concerns the studies of both microcosm and soil slurries. Referee #1 argues that Fe(III) may not have been completely reduced (in some soils Fe(III) reduction may last over 16 weeks). Referee #1 also points out that the amount of CH_4 produced was often smaller than that of CO_2. Therefore, the observation of increased CH_4 production from SOM could be best explained by accelerated soil reduction in RS treatment, not by the priming effect per se. Although we agree that some soils may take a long time to reach reduced condition, this was not the case for our Vercelli rice field soil, in which Fe(III) reduction is generally finished within 15 days of anoxic incubation at 25°C. Furthermore, we have measured Fe(III) reduction in our experiments (albeit not explicitly reported) and found that the reduction was complete and that soil conditions were reduced after preincubation. Ratios of CO_2 to CH_4 production in rice field soil incubations are frequently somewhat higher than 1.0 (e.g., Yao and Conrad 2000). Furthermore, CO_2 production was probably partially due to dissolved CO_2 and bicarbonate in the water, which could not be completely removed by the flushing with N_2 after soil preincubation.

In addition to these three major concerns, referee #1 also had a few specific comments.

(1) The non-linearity of the $\delta^{13}C$ notation.

Response: The non-linearity is only a problem for very high $\delta^{13}C$ values. Then, it is better to use isotope fractions ($F = {}^{13}C/({}^{13}C+{}^{12}C)$. The values of $\delta^{13}C$ in CH_4 were all <400‰, so that the

error caused by using the delta notation is small compared to experimental errors. Mass balance calculations based on the F-notation would be prohibitively difficult.

(2) 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.

Response: we will add information of rice phenology into the paper.

(3) 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.

Response: we agree that homogeneous labeling across rice-straw components is very important. If easily degradable straw components were preferentially labeled, they would be decomposed faster, resulting in $\delta^{13}C$ values of CH_4 and CO_2 that are higher (more ^{12}C) than the labeled rice straw itself. However, this was never the case, even when we used conditions (addition of large amounts of RS) in which virtually all of the CH_4 and CO_2 (90%-100%) was derived from the RS.

In summary, we thank referee #1 for the valuable comments and suggest mentioning and discussing these points in a revised manuscript.

Referee #2

We also appreciate the comments of referee #2. The comments are generally quite positive and basically address the same points as done by referee #1. We will answer these comments in the following.

(1) Referee #2 also notes certain limitations in the way to calculate the contribution of CH_4 production from ROC, in particular our assumption that the contribution of CH_4 produced from SOM (and from RS) is the same in both planted and unplanted rice microcosms. Referee #2 would not completely deny this assumption, but thinks that we should discuss and mention this limitation openly to enable the reader to decide whether he or she may follow our assumption or not. Referee #2 suggests doing a sensitivity analysis to help in judging whether our assumptions may lead to a total reversal of the findings in case of uncertainty here, or whether the presented results are robust despite this limitation.

Response: We have already explained the calculation of f_{ROC} in our reply to the second major point of referee #1. We also emphasized that our calculations should be rather robust even when small uncertainties in the determination of $\delta^{13}C_{CH4\text{-}SOR}$ exist.

(2) Referee #2 felt somewhat confused by the fact that we used mathematical formulae for which we referred to a previously published paper (Yuan et al. 2012).

Response: In fact there are many different formulae and data used for calculation of f_{ROC} and f_{SOM}. These are all detailed in our previously published paper (Yuan et al. 2012). We found a repeated presentation of them not really useful and thus did not show them again in the present paper. However, upon the comment, we will add the equations to a revised paper.

(3) Referee #2 agreed with the first concern of reviewer #1 about artifacts in determining the CH_4 production from ROC. However, referee #2 suggested that we may consider focusing even more on the comparison of the treatments with and without RS. If both treatments (+ and – RS) are treated similarly, the effect of how and if the rice plant was removed is the same for both treatments. Thus it would be valid to derive the difference here and derive a "priming effect", although as stated by reviewer #1 the exact determination of CH_4 from ROC remains uncertain.

Response: We agree with this opinion. In fact, the positive priming effect of RS on CH_4 production from ROC was determined in two different ways: First, calculation from the total CH_4 production rates (p_{CH4}) and f_{ROC} (calculated from the $\delta^{13}C$ of CH_4 produced) (eq. 1 and Fig. 1). Second, mass balance calculation of CH_4 production rates in microcosms that were planted or unplanted and treated or untreated with RS (Table 1). The results from both methods are consistent with each other.

(4) Referee #2 suggests considering showing first the results from the slurry experiments (which are easier to support the "priming effect") and then introduce the assumptions made for the microcosms.

Response: We considered this suggestion, but still think it is better to present the data in the order in which they were generated. In particular, after quantification of abundance of methanogens and bacteria in the rice microcosms, we refer to these results in the slurry experiments. Therefore, we should present the experiments with rice microcosms first.

(5) Referee #2 suggests reconsidering using the term "priming effect" in general, and argues that it is more a redirection of the total carbon flow towards CH_4 production, away from CO_2 production, rather than an enhancement of total carbon flow.

Response: In principle we agree with the interpretation of referee #2. It is a matter of definition and nomenclature. The original definition of priming effect is enhancement of SOM degradation, with SOM degradation being determined from CO2 production. Under anoxic conditions there are two gaseous products (CO_2 and CH_4) of mineralization of organic matter. Thus, one might specifically look at enhancement of CH_4 production. We always described in the paper "priming effect of RS addition on CH_4 production". This was to emphasize the specific role of production and emission of CH_4, as outlined in the Introduction. In this context we found the term priming effect quite useful. We propose adding clarifying statements to the Introduction and Discussion.

(6) Referee #2 (compare third comment of referee #1) does not see so much of a problem in the comparably high ratio of CO_2 to CH_4 production in the anaerobic incubations, as such high ratios have frequently been observed in many studies.

Response: We agree and refer to our response to referee #1. In particular, we would like to mention that we tested the reduction of Fe(III).

In summary, we thank referee #2 for the valuable comments and suggest mentioning and discussing these points in a revised manuscript.

References:

Lu et al. (2000) *Soil Biology & Biochemistry* 32, 1683-1690

Yao & Conrad (2000) *Eur J. Soil Sci.* 51, 369-378

Yuan et al (2012) *PLoS ONE*, 7, e49073.