

## ***Interactive comment on “Pathway of CH<sub>4</sub> production, fraction of CH<sub>4</sub> oxidized, and <sup>13</sup>C isotope fractionation in a straw incorporated rice field” by G. B. Zhang et al.***

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We are grateful for the constructive and detailed expert comments of referee #3, which will help to improve our manuscript. In the following we will consecutively address each point raised and, if appropriate, will make suggestions how to change the manuscript.

Firsly, the referee #3 criticized that the sentences in the original manuscript were too long and redundant, and they were hard to find the logic clearly. So, we asked an expert for a full-scale revision, such as:

1. Sentences “Straw incorporation is regarded as a key practice in management of organic fertilizer in rice cultivation, and proved to be able to gradually improve soil

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structure (Wang et al., 2010)...” (Page 14177, Line 8~11 in the BGD) were changed to “Straw incorporation is regarded as a key practice in management of organic fertilizer in rice cultivation. Moreover, it proves to be able to gradually improve soil structure (Wang et al., 2010)...”.

2. Sentences “Organic matter is fermented into acetate, CO<sub>2</sub>, H<sub>2</sub>, propionate as well as other fatty acids, while acetate, CO<sub>2</sub> and H<sub>2</sub> are the main substrates methanogenic bacteria use for production of CH<sub>4</sub> (Krüger et al., 2002; Conrad et al., 2010)” (Page 14177, Line 22~25 in the BGD) were changed to “Organic matter is fermented into acetate, CO<sub>2</sub>, H<sub>2</sub>, propionate as well as other fatty acids. However, acetate, CO<sub>2</sub> and H<sub>2</sub> are the main substrates methanogenic bacteria use for production of CH<sub>4</sub> (Krüger et al., 2002; Conrad et al., 2010)”

3. Sentences “On the other hand, rice roots themselves not only excrete organic acid and slough off old or dead tissues as sources of carbon and energy for CH<sub>4</sub> production, but also act as an important site in the rhizosphere where CH<sub>4</sub> is oxidized by oxygen available from root secretion, which probably stimulates growth and activity of methanotrophs, and consequently increases the potential of CH<sub>4</sub> oxidation.” (Page 14178, Line 8~13 in the BGD) were revised to “On the other hand, rice roots themselves not only excrete organic acid and slough off old or dead tissues as sources of carbon and energy for CH<sub>4</sub> production, but also act as an important site where CH<sub>4</sub> is oxidized by oxygen available from root secretion in the rhizosphere. Therefore, the growth of rice roots may probably stimulate growth and activity of methanotrophs, and consequently increase the potential of CH<sub>4</sub> oxidation.”

4. Sentences “CH<sub>4</sub> produced in paddy soil usually has a high proportion being oxidized in the rhizosphere and at the soil-water interface, and then the remaining CH<sub>4</sub> is emitted from the soil to the atmosphere mainly through the aerenchyma of rice plants.” (Page 14179, Line 1~4 in the BGD) were revised to “Usually a high proportion of the CH<sub>4</sub> produced in the paddy fields is oxidized in the rhizosphere and at the soil-water interface. The remaining CH<sub>4</sub> is emitted from the soil to the atmosphere mainly through

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the aerenchyma of rice plants.”

5. Sentences “The  $\delta^{13}\text{C}$ -value of  $\text{CO}_2$  produced in the paddy soil was relatively stable in the two treatments ( $\sim -18\text{‰}$  over the four rice growth stages, while the one on rice roots decreased obviously from the tillering and booting stages ( $\sim -15\text{‰}$  to the grain filling and ripening stages ( $\sim -25\text{‰}$  (Fig. 2c, f).” (Page 14185, Line 8~12 in the BGD) were changed to “The  $\delta^{13}\text{C}$ -value of  $\text{CO}_2$  produced in the paddy soil was relatively stable in the two treatments ( $\sim -18\text{‰}$  over the four rice growth stages. On rice roots, however, it decreased obviously from the tillering and booting stages ( $\sim -15\text{‰}$  to the grain filling and ripening stages ( $\sim -25\text{‰}$  (Fig. 2c, f).”

6. Sentences “. . . which probably increased the population and activity of methanogens on the surface of rice roots, thus increasing  $\text{CH}_4$  production (Fig. 2d).” (Page 14188, Line 11~12 in the BGD) were changed to “. . . which probably increased the population and activity of methanogens on the surface of rice roots. As a result,  $\text{CH}_4$  production was increased (Fig. 2d).”

7. Sentences “. . . and straw incorporation highly increased their  $\text{CH}_4$  production potentials at those periods, whereas a little was measured at the graining filling and ripening stages (Fig. 2a, d).” (Page 14188, Line 23~25 in the BGD) were changed to “. . . and straw incorporation highly increased their  $\text{CH}_4$  production potentials at those periods. However, a little was measured at the graining filling and ripening stages (Fig. 2a, d).”

8. Sentences “Since the temperature of incubation in this study was in the range from  $17.6\text{ }^\circ\text{C}$  to  $29.7\text{ }^\circ\text{C}$  and averaged  $25.1\text{ }^\circ\text{C}$ ,  $\alpha_{\text{CO}_2/\text{CH}_4} = 1.079$  was hence considered to be reasonable for calculation of  $\delta^{13}\text{CH}_4$  ( $\text{H}_2/\text{CO}_2$ ), which has been validated in other experiments (Zhang et al., 2011c, 2012).” (Page 14190, Line 21~24 in the BGD) were changed to “Since the incubation in this study varied in the range from  $17.6\text{ }^\circ\text{C}$  to  $29.7\text{ }^\circ\text{C}$  and averaged  $25.1\text{ }^\circ\text{C}$  in temperature,  $\alpha_{\text{CO}_2/\text{CH}_4} = 1.079$  was hence considered to be reasonable for calculation of  $\delta^{13}\text{CH}_4$  ( $\text{H}_2/\text{CO}_2$ ). Fortunately, it has been validated in the other experiments we conducted (Zhang et al., 2011c, 2012).”

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9. Sentences “A similar temporal variation was observed by Krüger et al. (2002) who reported that acetate-dependent methanogenesis was dominant at the end of the season, whereas H<sub>2</sub>/CO<sub>2</sub>-dependent methanogenesis was very important at the beginning of the season.” (Page 14191, Line 11~14 in the BGD) were changed to “Similarly, Krüger et al. (2002) also found that acetate-dependent methanogenesis dominated the end of the season, whereas H<sub>2</sub>/CO<sub>2</sub>-dependent methanogenesis was very important at the beginning of the season.”

10. Sentence “, thus increasing the CH<sub>4</sub> oxidation capacity.” (Page 14193, Line 5 in the BGD) was deleted .

11. Sentences “The fraction of CH<sub>4</sub> oxidized (Fox) was calculated using. . . , and results of the calculation are shown in Table 7” (Page 14193, Line 25 in the BGD) were changed to “The fraction of CH<sub>4</sub> oxidized (Fox) was calculated using. . . and shown in Table 7.”

12. Sentences “Therefore, straw incorporation increases  $\alpha_{ox}$ , which is probably ascribed to its stimulation of methanotrophic bacteria in the soil by promoting CH<sub>4</sub> oxidation in Treatment WS relative to Treatment CK (Fig. 4a).” (Page 14196, Line 20~23 in the BGD) were revised to “Therefore, straw incorporation increased  $\alpha_{ox}$ , which is probably ascribed to its promotion of CH<sub>4</sub> oxidation by stimulating methanotrophic bacteria in Treatment WS relative to Treatment CK (Fig. 4a).”

Secondly, the referee #3 recommended us to supplement a conclusion in the manuscript. We agreed with the suggestion which was able to better demonstrate our original questions and the important findings. So, a discussion section (5 Conclusions) was added:

## 5 Conclusions

The study of biogeochemical mechanism of CH<sub>4</sub> emission from a Chinese rice field demonstrated that straw incorporation obviously increased CH<sub>4</sub> production and oxida-

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tion potentials in paddy soil and on rice roots. What is more, the effect of straw incorporation on methanogenic pathways and fraction of CH<sub>4</sub> oxidized was quantified by measuring stable carbon isotopic signatures. The results show that straw incorporation increased the contribution of acetate to methanogenesis in paddy soil, but decreased on rice roots, and acetoclastic methanogenesis was more important in the former than in the latter. Furthermore, the fraction of CH<sub>4</sub> oxidized was significantly decreased by straw incorporation, which is likely ascribed to the fact that CH<sub>4</sub> production potential was increased to a larger extent than CH<sub>4</sub> oxidation potential was. This indicates that the production of CH<sub>4</sub> increased directly by straw incorporation will indirectly reduce the fraction of CH<sub>4</sub> oxidized, thus significantly decreasing CH<sub>4</sub> emission from the rice fields. Although it may be difficult to estimate its exact effects on CH<sub>4</sub> production and oxidation on a regional or a global scale, the variation pattern of methanogenesis being increased while the fraction of CH<sub>4</sub> oxidized being reduced by straw incorporation is probably not changed. Certainly, more relevant investigations in different conditions should be done in the future.

This study also contributes another important question related to carbon isotope fractionation during the processes of CH<sub>4</sub> oxidation and transport. Although it is very limited, to our knowledge so far, in paddy soil, the fractionation factor  $\alpha_{ox}$  varied in the range of 1.025–1.038, which was well in agreement with previous reports on different soils. What is more, it was increased by straw incorporation, which probably resulted from the effects of straw incorporation stimulating methanotrophs and hence promoting CH<sub>4</sub> oxidation in the soil. Difference between  $\delta^{13}C$ -values of the emitted and aerenchymatic CH<sub>4</sub> indicates a fractionation factor  $\epsilon_{transport}$  of –12.0‰ to –8.0‰ which was to some extent controlled by straw incorporation. The reason is likely that the diffusion process in transport is affected by growth of rice plants and pressure in the rhizosphere, though the processes to cause the difference are not clearly known. As a traditional practice in the rice cultivation in China, straw incorporation is very important to the global carbon cycles involved in source and sink of the greenhouse gas CH<sub>4</sub>. Therefore, it is essential to contribute more efforts to the study on methanogenic and

methanotrophic microbial communities, and fractionation factors  $\alpha_{ox}$  and  $\epsilon_{transport}$  to better elucidate the processes of CH<sub>4</sub> emission from rice fields as affected by straw incorporation.

Thirdly, in order to more desirable to express the variables like soil Eh and isotopic composition, the referee #3 recommended us to change the modes of Fig. 1b, Fig. 2b-c-e-f, and Fig. 3b-d. Thanks very much! They were revised carefully, and we hoped that they could meet the requirement. Please see the attached files below.

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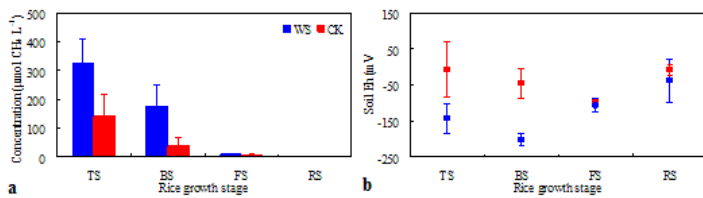


Fig. 1.

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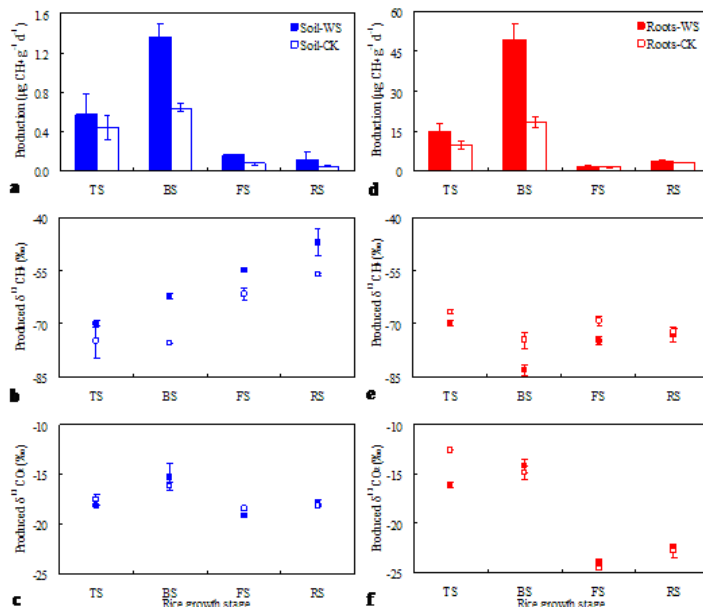


Fig. 2.



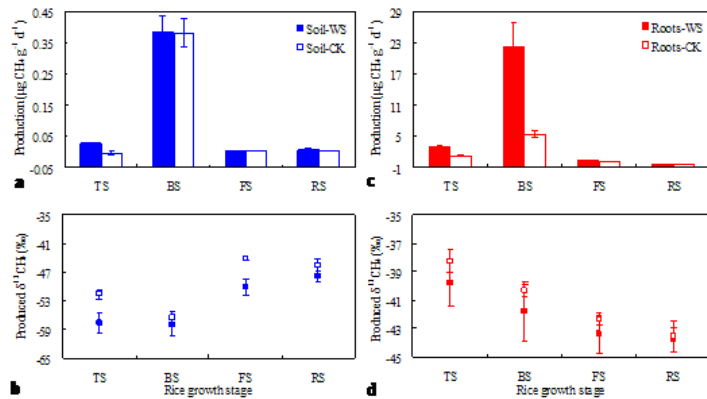
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Fig. 3.

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