Biogeosciences Discuss., 9, 14175–14215, 2012
www.biogeosciences-discuss.net/9/14175/2012/
doi:10.5194/bgd-9-14175-2012

Biogeosciences Discussions

Pathway of CH_4 production, fraction of CH_4 oxidized, and ^{13}C isotope fractionation in a straw incorporated rice field

G. B. Zhang[1], Y. Ji[1,2], J. Ma[1], G. Liu[1,2], H. Xu[1], and K. Yagi[3]

[1]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[2]Graduate University of Chinese Academy of Sciences, Beijing 100049, China
[3]National Institute of Agro-Environmental Sciences, 3–1-3, Kannondai, Tsukuba Ibaraki 305–8604, Japan

Received: 7 August 2012 – Accepted: 2 October 2012 – Published: 15 October 2012

Correspondence to: H. Xu (hxu@issas.ac.cn)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Straw incorporation generally increases CH_4 emission from rice fields, but its effects on the mechanism of CH_4 emission, especially on the pathway of CH_4 production and the fraction of CH_4 oxidized are not well known. To investigate the methanogenic pathway, the fraction of CH_4 oxidized as well as the stable carbon isotope fractionation during the oxidation and transport of CH_4 as affected by straw incorporation, production and oxidation of CH_4 in paddy soil and rice roots and δ^{13}C-values of produced CH_4 and CO_2, and emitted CH_4 were observed in incubation and field experiments. Straw incorporation significantly enhanced CH_4 production potentials of the paddy soil and rice roots. However, it increased the relative contribution of acetate to total CH_4 production (F_{ac}) in the paddy soil by ~ 10–30 %, but decreased F_{ac}-value of the rice roots by ~ 5–20 %. Compared with rice roots, paddy soil was more important in acetoclastic methanogenesis, with F_{ac}-value being 6–30 % higher. Straw incorporation highly decreased the fraction of CH_4 oxidized (F_{ox}) by 41–71 %, probably attributed to the fact that it increased CH_4 oxidation potential whereas CH_4 production potential was increased to a larger extent. There was little CH_4 formed during aerobic incubation, and the produced CH_4 was more ^{13}C-enriched relative to that of anaerobic incubation. Assuming δ^{13}C-values of CH_4 aerobically produced in paddy soil to be the δ^{13}C-values of residual CH_4 after being oxidized, F_{ox}-value still appeared to be 45–68 % lower when straw was incorporated. Oxidation fractionation factor (α_{ox}) was higher with straw incorporation (1.033) than without straw incorporation (1.025). The δ^{13}C-values of CH_4 emitted after cutting of the plants (−50−−43 ‰) were more positive than those of before (−58−−55 ‰), suggesting a transport fractionation factor ($\varepsilon_{transport}$) was −8.0 ‰ with straw incorporation and −12.0 ‰ without straw incorporation. Reasons for this difference may be related to the decrease in growth of the rice crop as a result of straw incorporation. The experiment shows that straw incorporation increases the contribution of acetate to total methanogenesis in paddy soil but decreases it on rice roots, and it significantly

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decreases the fraction of CH_4 oxidized in the field, and expands oxidation fractionation while reducing transport fractionation.

1 Introduction

Atmospheric methane (CH_4), the second most important greenhouse gas next to carbon dioxide (CO_2), reached 1808 $nl\,l^{-1}$ in 2010 (WMO, 2010). As an important source of anthropogenic CH_4, paddy field is responsible for ~5–19 % of the global CH_4 emission (IPCC, 2007). China, being one of the most important rice producing countries in the world, has an abundant straw resource, which reaches 6.4×10^8 $t\,yr^{-1}$ and even up to 7.3×10^8 t in 2010 (Xiong et al., 2010). 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 structure (Wang et al., 2010), increase soil organic carbon (Deng et al., 2010; Ma et al., 2010; Wang et al., 2010), raise soil fertility (Deng et al., 2010; Ma et al., 2010; Wang et al., 2010), and in the long run promote the agro-ecosystem into a benign cycle. However, in the short term, it highly increases CH_4 emission from the paddy fields (Cai, 1997; Jiang et al., 2003; Ma et al., 2009), causes microbial immobilization of soil mineral N (Tanaka et al., 1990; Jensen, 1997), and accumulates organic acids (Tanaka et al., 1990; Shan et al., 2006), thus affecting growth of the crop. Incorporation of straw significantly increases CH_4 emission from paddy fields, which has been considerably reported (Jiang et al., 2003; Ma et al., 2008, 2009), but its effects on production and oxidation of CH_4 are not clear yet.

In paddy fields, CH_4 is an important end product of the degradation of organic matter under anaerobic conditions (Cicerone and Oremland, 1988; Conrad, 2007). Organic matter is fermented into acetate, CO_2, H_2, propionate as well as other fatty acids, while acetate, CO_2 and H_2 are the main substrates methanogenic bacteria use for production of CH_4 (Krüger et al., 2002; Conrad et al., 2010). The relative contribution of acetoclastic ($CH_3COOH \rightarrow CH_4 + CO_2$) and hydrogenotrophic ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$) methanogenesis to total CH_4 formation is sensitive to availability of the substrates and

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varies with the growth of rice plants (Whiticar et al., 1986; Conrad, 1999; Krüger et al., 2002). Degradation of straw generates abundant organic acids, such as formic, acetic, propionic and butyric acids (Shan et al., 2006), which is likely to increase the contribution of acetoclastic methanogenesis. Moreover, it provides methanogenic bacteria with plenty of precursors to produce CH_4 and accelerates the decline of soil Eh, thus forming a favorable environment condition for growth of methanogens, which in turn promotes formation of CH_4 and further affects CH_4 oxidation capacity in the field (Bender and Conrad, 1995; Arif et al., 1996). 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_4 production, but also act as an important site in the rhizosphere where CH_4 is oxidized by oxygen available from root secretion, which probably stimulates growth and activity of methanotrophs, and consequently increases the potential of CH_4 oxidation. A considerable number of studies have reported production and oxidation of CH_4 in paddy soil or on rice roots (Frenzel and Bosse, 1996; Bosse and Frenzel, 1997; Conrad and Klose, 1999; Lehmann et al., 1999; Dan et al., 2001; Krüger et al., 2002; Zhang et al., 2011a), but little has focused on effect of straw incorporation on CH_4 production and CH_4 oxidation separately, let alone on pathway of CH_4 production and fraction of CH_4 oxidized.

In the study of CH_4 production and oxidation processes in the rice ecosystem, the stable carbon isotope technique, deemed to be a feasible and very effective method, has been widely used (Sugimoto and Wada, 1993; Chanton et al., 1997; Tyler et al., 1997; Bilek et al., 1999; Krüger et al., 2002; Conrad and Klose, 2005). Relative contribution of the two methanogenic pathways and proportion of CH_4 oxidized in the field can be estimated if δ^{13}C-values of CH_4, CO_2 and acetate are measured and relevant fractionation factors ($\varepsilon_{acetate}$, α_{CO_2/CH_4}, α_{ox} and $\varepsilon_{transport}$) are known. The carbon isotope fractionation factors of CH_4 oxidation (α_{ox}) and CH_4 transport ($\varepsilon_{transport}$) are crucial to quantification of the fraction of CH_4 oxidized in applying the stable carbon isotope technique (Tyler et al., 1997; Bilek et al., 1999; Krüger et al., 2002; Zhang et al., 2009). Therefore, the acquisition of reliable and exact fractionation factors (α_{ox} and $\varepsilon_{transport}$)

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is an important precondition for use of the method in studying CH_4 oxidation. CH_4 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_4 is emitted from the soil to the atmosphere mainly through the aerenchyma of rice plants. Due to the fact that $^{12}CH_4$ is consumed faster than $^{13}CH_4$ by methane-oxidizing bacteria as well as $^{12}CH_4$ transports faster than $^{13}CH_4$ in the process of CH_4 transport (Whiticar, 1999; Chanton, 2005; Venkiteswaran and Schiff, 2005), significant isotopic fractionation is observed during these processes. Previous studies mainly focused on fractionation factor α_{ox} in the landfill cover soils (see Chanton et al., 2008a, 2008b), rather than in paddy soil (Krüger et al., 2002). Moreover, early reports showed that fractionation factor $\varepsilon_{transport}$ varied with rice growth and with rice variety as well (Chanton et al., 1997; Tyler et al., 1997; Bilek et al., 1999; Krüger et al., 2002). Further study is necessary to discuss the effect of straw incorporation on growth of rice plants and eventually on fractionation factors, α_{ox} of paddy soil and $\varepsilon_{transport}$ of rice plants.

In recent studies, CH_4 concentration in soil solution of the field, δ^{13}C-values of CH_4 emitted before and after cutting of the plants, production and oxidation of CH_4 in paddy soil and fresh rice roots, and δ^{13}C-values of CH_4 and CO_2 produced in anaerobic and aerobic incubations, were measured in field and incubation experiments to clarify the effect of straw incorporation on CH_4 production and oxidation, respectively, especially on pathway of CH_4 production and fraction of CH_4 oxidized in the field. Moreover, stable carbon isotope fractionation factors α_{ox} and $\varepsilon_{transport}$ were investigated to exactly estimate the effect of straw incorporation on them and finally on the fraction of CH_4 oxidized.

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2 Methods

2.1 Designing of the experiment

The experimental field was located at Baitu Town, Jurong City, Jiangsu Province, China (31° 58′ N, 119° 18′ E). The main characteristics of this site have been described in detail before (Zhang et al., 2012). Two treatments, WS (straw incorporation) and CK (without straw incorporation), were designed with experimental plots in triplicate. Stubbles and weeds were all removed from the experimental plots. Then, dry wheat straw (C/N = 85, $\delta^{13}C_{org}$ = −28.3 ‰), chopped to ∼ 1 cm in length, was evenly spread over the plots of Treatment WS, and raked into the topsoil (0–15 cm) Rice seedlings (Huajing 3), 32 days old, were transplanted on 26 June in all the plots and the crop was harvested on 3 November. The same fertilization and water management practices were adopted in the experiment as in the local rice cultivation. For details, please refer to Table 1.

2.2 Field experiment

Soil solution was collected from each plot using a Rhizon soil moisture sampler (Zhang et al., 2012). Prior to sampling, about 5 ml soil solution was extracted using 18 ml vacuum vial to flush and purge the sampler. Approximate 10 ml water was then drawn into another vial for further analysis. CH_4 concentration in the headspace of the vial was measured on a GC-FID. The CH_4 concentration (C_{CH_4}) in soil solution was calculated using:

$$C_{CH_4} = \frac{m \times G_V}{G_L \times M_V} \ (\mu mol\,l^{-1}) \quad (1)$$

where m stands for mixing ratio of CH_4 in the headspace of a vial ($\mu l\,l^{-1}$), M_V for gas volume of an ideal gas (24.78 $l\,mol^{-1}$ at 25° C), G_V for volume of the gas headspace of the vial (l), and G_L for volume of liquid in the vial (l) Simultaneously, soil redox potential

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(*E*h) at the depth of 10 cm was measured, using Pt-tipped electrodes (Hirose Rika Co. Ltd., Japan) and an oxidation-reduction potential meter with a reference electrode (Toa PRN-41). Soil temperature at the depth of 10 cm was measured with a hand-carried digital thermometer (Yokogawa Meter and Instruments Corporation, Japan).

Triplicate soil cores were collected from each experimental plot using a stainless steel corer with an inner diameter of 7 cm and a length of 25 cm (Zhang et al., 2011a) and then prepared into mixture on a plot basis. Samples from the mixture, each about 50 g (dry weight), were promptly taken and transferred into the 250 ml Erlenmeyer flask separately, and turned into slurries with N_2-flushed de-ionized sterile water in the ratio of 1 : 1 (soil/water). During the whole process, the samples were constantly flushed with N_2 to remove O_2 and CH_4, and the flasks containing these samples were then sealed for anaerobic incubation. Other flasks with air headspace were sealed directly for aerobic incubation. All the flasks were sealed with rubber stoppers fitted with silicon septum that allowed sampling of headspace gas. Finally, they were stored in N_2 at 4 °C and transported back to the lab as soon as possible for further analysis. A portion of soil sample was dried for 72 h at 60 °C for determination of isotopic composition of the organic carbon.

Rice plants complete with roots were carefully collected from the plots at each of the four rice growth stages, i.e. tillering stage (TS, 16 July), booting stage (BS, 15 August), grain filling stage (FS, 22 September), and ripening stage (RS, 12 October) (Zhang et al., 2011a). The roots were washed with N_2-flushed demineralized water and cut off from the green shoots at a point, 1–2 cm from the root with a razor blade. The fresh roots, 20 g each portion, were then put into flasks Further preparation and processes of the roots were the same as for the soil and detailed in the preceding paragraph The shoots were dried at 60 °C for 72 h for dry weight measurement, and then stored at room temperature for determination of isotopic composition of the organic carbon. Rice grain yield was measured at harvest.

Gas samples of emitted CH_4 were taken simultaneously before and after the plants were cut at the late booting stage (28 August) using specially designed PVC bottomless

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pots for determining stable carbon isotope fractionation during CH_4 transport ($\varepsilon_{transport}$) through the aerenchyma of rice plants (Zhang et al., 2012b). The pot, 30 cm in height and 17 cm in diameter, was designed to have a water-filled trough around its top, allowing a chamber to rest on the pot with the joint completely sealed to avoid any possible gas exchange during the sampling times. Each plot had two such pots installed ~ 7–10 days before gas sampling began, in a way to keep the plants in the center of each pot. A PVC plate (18 cm in diameter) with a hole (4 cm in diameter) in the center was placed on top of each pot, allowing the plant to grow through the hole and keep divided into two parts. Then, one plant inside the pot was cut right above the plate while the other remained intact as the control. Finally, chambers (30 × 30 × 100 cm) were laid on the pots, and gas samples in the headspace of the chambers were collected simultaneously using 500 ml bags (aluminium foil compound membrane, Delin gas packing Co., Ltd, Dalian, China) with a small battery-driven pump for measurement of $\delta^{13}C$-value of the emitted CH_4 (Zhang et al., 2011c).

2.3 Incubation experiment

Potential CH_4 production rates in paddy soil and rice roots were determined anaerobically (Zhang et al., 2011b). Flasks used for anaerobic incubation were flushed with N_2 consecutively for six times through double-ended needles connecting a vacuum pump to purge the air in the flasks of residual CH_4 and O_2. Simultaneously, CH_4 production was determined aerobically and flasks with air headspace were used directly in the experiment. They were then incubated at a temperature the same as measured in the field for 50 h in darkness. Gas samples were collected twice with a pressure lock syringe, 1 h and 50 h later after the flasks were heavily shaking by hand, and analyzed for CH_4 on a GC-FID. CH_4 production was calculated using the linear regression of CH_4 increasing with the incubation time.

Potential CH_4 oxidation rates in paddy soil and rice roots were determined aerobically, using the same equipments as described above but with air headspace in the flasks. Into each flask pure CH_4 was injected to make a high concentration inside

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($\sim 10\,000\,\mu l\,l^{-1}$). Then the flasks were incubated in dark under the same temperature as in the field and shaken at 120 r.p.m. CH_4 depletion was measured by sampling the headspace gas in the flask after vigorously shaking for subsequent GC-FID analysis. The first sample was collected generally 30 min after pure CH_4 was injected and made homogeneously distributed inside the flask. Samples were then taken in 2–3 h intervals during the first 8 h of the experiment. The flasks were left over night and sampled the next day in 2 h intervals again. CH_4 oxidation was calculated using the linear regression of the CH_4 depletion with incubation time.

2.4 Analytical methods

CH_4 concentration in gas samples was analyzed with a gas chromatograph (Shimadzu GC-12A, Kyoto, Japan) equipped with a flame ionization detector (FID). $\delta^{13}C$-values were determined with a Finnigan MAT-253 Isotope Ratio Mass Spectrometer (IRMS, Thermo Finnigan, Bremen, Germany) using the continuous flow technique. The IRMS had a fully automatic interface for pre-GC concentration (PreCon) of trace gases (Cao et al., 2008, Zhang et al., 2011c). Isotope ratios were expressed in the standard delta notation: $\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000$ (‰) with $R = {}^{13}C$ / ${}^{12}C$ of sample and standard, respectively. Precision of the repeated analyses was ±0.196 ‰ ($n = 9$) with $2.02\,\mu l\,l^{-1}$ CH_4 injected. Samples of dried soil and plants were analyzed for carbon isotope composition with a Finnigan MAT-251 Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany).

2.5 Statistical analysis

Statistical analysis was done using the SPSS 18.0 software for Windows (SPSS Inc., Chicago). Least significant difference (LSD) tests were used to compare means between treatments. Standard deviation of the means was calculated using the Microsoft Excel 2003 software for Windows.

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3 Results

3.1 CH_4 concentration in soil solution

CH_4 concentration in soil solution of the two treatments dropped sharply over the season, from as high as 150–300 $\mu mol\,l^{-1}$ at the tillering stage to $\sim 1\ \mu mol\,l^{-1}$ at the ripening stage (Fig. 1a). Obviously, CH_4 concentration in soil solution was much higher in Treatment WS (180–330 $\mu mol\,l^{-1}$) than in Treatment CK (40–140 $\mu mol\,l^{-1}$) at the tillering and booting stages. However, no significant discrepancy between the two treatments was observed at the graining filling and ripening stages (Fig. 1a). Similarly, soil *E*h was significantly lower in Treatment WS (−200 to −150 mV) than in Treatment CK (−50 to −10 mV) at the tillering and booting stages while no obvious difference was detected between the two treatments at the graining filling and ripening stages (Fig. 1b). Soil temperature ranged from 17.6 °C to 29.7 °C during the whole season, with an average of 25.1 °C.

3.2 CH_4 production potential in paddy soil and rice roots under anaerobic incubation

The CH_4 production potentials of paddy soil and rice roots in the two treatments (Fig. 2a, d) varied in similar patterns, rising up to the peak (soil: 0.6–1.4 $\mu g\,CH_4\,g^{-1}\,d^{-1}$; roots: 18.5–49.4 $\mu g\,CH_4\,g^{-1}\,d^{-1}$) at the booting stage, and declined rapidly towards the valley at the ripening stage. The potential was significantly higher in Treatment WS than in Treatment CK at the booting stage ($P < 0.05$). As a whole, straw incorporation increased CH_4 production potential in paddy soil and rice roots obviously, with the mean increased by 95 % and 134 % relative to those in Treatment CK, respectively (Fig. 2a, d).

With the growth of rice plants, $\delta^{13}C$-value of the CH_4 produced in paddy soil became more positive in both treatments (Fig. 2b). The produced CH_4 was more ^{13}C-enriched in Treatment WS (−70 to −47 ‰) than in Treatment CK (−75 to −56 ‰) at all the four rice

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growth stages (Fig. 2b), with δ^{13}C-values being 5 ~ 13 ‰ higher in Treatment WS than in Treatment CK. On the contrary, CH_4 produced on rice roots was gradually becoming ^{13}C-depleted from the tillering stage to the ripening stage, and it was relatively more obvious in Treatment WS (−83 to −70 ‰) than in Treatment CK (−75 to −67 ‰) (Fig. 2e). Compared with Treatment CK, Treatment WS was negative by 1–9 ‰ in $\delta^{13}CH_4$-value over the season. CH_4 produced on rice roots was more depleted in ^{13}C, on average, by ~ 16 ‰ (Treatment WS) and ~ 4 ‰ (Treatment CK) relative to that produced in paddy soil (Fig. 2b, e) during the four rice growth stages for analysis. The δ^{13}C-value of CO_2 produced in paddy soil was relatively stable in the two treatments (~ −18 ‰) over the four rice growth stages, while the one on rice roots decreased obviously from the tillering and booting stages (~ −15 ‰) to the grain filling and ripening stages (~ −25 ‰) (Fig. 2c, f). No significant difference was observed in δ^{13}C-value of CO_2 produced either in paddy soil or on rice roots between the two treatments ($P > 0.05$).

3.3 CH_4 production in paddy soil and rice roots under aerobic incubation

Little CH_4 production was observed in aerobic paddy soil at the tillering, grain filling and ripening stages, although relatively visible CH_4 production was at the booting stage (~ 0.4 μg CH_4 g^{-1} d^{-1}) (Fig. 3a). Significant CH_4 production on aerobic rice roots was measured at the tillering and booting stages, especially at the booting stage, reaching as high as 5.4–22.2 μg CH_4 g^{-1} d^{-1}, but little was at the grain filling and ripening stages (Fig. 3c). CH_4 production in aerobic incubation (Fig. 3a, c) was significantly lower than in anaerobic incubation (Fig. 2a, d, $P < 0.05$). The δ^{13}C-value of CH_4 produced in paddy soil ranged from −58 ‰ to −48 ‰ in Treatment WS, being slightly more negative than that in Treatment CK (−56 to −44 ‰). Moreover, δ^{13}C-value of the CH_4 produced on rice roots also appeared to be negative in Treatment WS (−44 to −38 ‰) relative to that in Treatment CK (−44 to −40 ‰) at all the four rice growth stages (Fig. 3b, d). Compared with what was observed in anaerobic incubation (Fig. 2b, e), CH_4 produced in aerobic incubation was significantly enriched in ^{13}C (Fig. 3b, d, $P < 0.05$), with the

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average $\delta^{13}CH_4$-value higher by ~ 10‰ in paddy soil and by ~ 30‰ on rice roots during all the four rice growth stages.

3.4 CH_4 oxidation potential in paddy soil and rice roots under aerobic incubation

A similar pattern in variation of the CH_4 oxidation potential in paddy soil was observed for both treatments during the four rice growth stages (Fig. 4a), showing a relatively high beginning (4.7–8.6 $\mu g\,CH_4\,g^{-1}\,d^{-1}$) at the tillering stage, and a peak (8.1–10.9 $\mu g\,CH_4\,g^{-1}\,d^{-1}$) at the graining filling stage. However, CH_4 oxidation was very important only at the tillering stage (over 600 $\mu g\,CH_4\,g^{-1}\,d^{-1}$) on rice roots, and weakened towards the ripening stage (~ 200 $\mu g\,CH_4\,g^{-1}\,d^{-1}$) (Fig. 4b). Compared with Treatment CK, Treatment WS showed higher CH_4 oxidation potentials in both paddy soil and rice roots at all the four rice growth stages (Fig. 4a, b).

3.5 δ^{13}C-value of CH_4 emitted before and after cutting of the plants

As shown in Table 2, the average δ^{13}C-value of CH_4 emitted before cutting of the plants in Treatment CK was –55‰, being slightly higher than that in Treatment WS (–58‰). Similarly, the mean δ^{13}C-value of CH_4 emitted after cutting of the plants in Treatment CK (–43‰) was more positive than that in Treatment WS (–50‰). Compared with the δ^{13}C-value of CH_4 emitted before cutting of the plants in both treatments, the value after cutting of the plants was significantly more positive, especially in Treatment CK (Table 2). Collectively, the latter was 8.0‰ in Treatment WS and 12.0‰ in Treatment CK higher the former (Table 2).

3.6 Organic carbon in soil and plant samples

Contents of organic carbon in soil increased slightly towards the end of the rice season, being 1.69 % and 1.85 % in Treatment CK, and 1.73 % and 1.89 % in Treatment

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WS at the tillering and ripening stages, respectively. However, δ^{13}C-value of soil carbon remained stable over the season, being −27.6 ‰ and −27.8 ‰ in Treatment CK, and −27.3 ‰ and −28.0 ‰ in Treatment WS at the tillering and ripening stages, respectively. Organic carbon in plant samples was slightly lighter, with δ^{13}C-value being −28.9 ‰, −29.3 ‰ and −28.6 ‰ at the tillering, booting and ripening stages, respectively. It also showed little change throughout the whole season.

3.7 Biomass and rice grain yield

Dry matter accumulations of rice plants (aboveground and underground parts) in the two treatments are presented in Table 3. They increased obviously from the tillering stage to the ripening stage and tended to be higher in Treatment CK than in Treatment WS at all the four growth stages. Grain yield did not differ much between Treatment WS and Treatment CK ($P > 0.05$) though it also tended to be higher in Treatment CK (8.89 t ha^{-1}) than in Treatment WS (8.63 t ha^{-1}).

4 Discussion

4.1 Effect of straw incorporation on CH_4 production

CH_4 is an end product of methanogenic bacteria acting on methane-producing substrates under strict anaerobic conditions. Sufficient substrates and a favorable habitat for growth of methanogenic bacteria are prerequisites for CH_4 generation (Conrad, 2007). Straw incorporation provides methanogenic bacteria with abundant methane-producing substrates. Meanwhile, flooding accelerates decomposition of straw and fall in soil redox potential (Eh) (Fig. 1b), creating a favorable environment for growth of methanogens, which in turn promotes CH_4 production in the paddy field (Fig. 2a, d). Shangguan et al. (1993) found that CH_4 production rate in a straw incorporated paddy soil, collected from a double rice cropping field in Hunan, was 1–2 times higher than

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that in the soil without straw incorporated. Moreover, they demonstrated that straw incorporation would be an important cause for the appearance of the first CH_4 production peak just ~20 days after the field was flooded. The findings in this study also show that straw incorporation increased CH_4 production remarkably in paddy soil (Fig. 2a). Furthermore, fresh rice roots produced considerable CH_4 in an extremely anaerobic environment, showing a variation pattern similar to that in paddy soil (Fig. 2d). Previous studies have demonstrated that excised fresh rice roots themselves could produce CH_4 (Frenzel and Bosse, 1996; Conrad and Klose, 1999; Lehmann et al., 1999; Krüger et al., 2001, 2002; Zhang et al., 2011a). Straw incorporation accelerated the formation of an extremely anaerobic condition in the soil and hence the decline of soil *E*h (Fig. 1b), which probably increased the population and activity of methanogens on the surface of rice roots, thus increasing CH_4 production (Fig. 2d).

In field conditions, variation of CH_4 concentration in soil solution reflects changes in CH_4 production of the paddy field to some extent. Under continuous flooding, CH_4 concentration in soil solution generally increased with the extension of the flooding time, and continued until the field was drained up before the crop was harvested (Cai et al., 2009). Under intermittent irrigation in this experiment, however, it was very high at the tillering stage (before midseason aeration) and at the booting stage (the first re-flooding period) while very low at the graining filling and ripening stages (Fig. 1a). On the other hand, straw incorporation profoundly increased CH_4 concentration in soil solution at the tillering and booting stages (Fig. 1a, $P < 0.05$). This is because that considerable CH_4 production in paddy soil and on rice roots was observed at the tillering and booting stages and straw incorporation highly increased their CH_4 production potentials at those time periods, whereas a little was measured at the graining filling and ripening stages (Fig. 2a, d). Alberto et al. (1996) measured concentration of the CH_4 entrapped in paddy soil in Philippines, and results also showed that it was significantly higher in plots incorporated with green manure and straw than in plots applied with chemical fertilizer.

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The δ^{13}C-value of organic carbon was relatively lower in straw samples (−28.3‰) than in paddy soil (−27.4‰), but slightly higher than in rice roots (−28.9‰). In addition, it was very stable over the season both in paddy soil and rice roots. Therefore, straw per se would neither be a ^{13}C-enriched source for methanogenesis in paddy soil nor a ^{13}C-depelted source for methanogenesis on rice roots for the whole season. As a consequence, incorporation of straw increased δ^{13}C-value of the CH_4 produced in paddy soil but decreased that of the CH_4 derived form rice roots (Fig. 2b, e), which is probably attributed to its different effects on pathways of CH_4 production in paddy soil and rice roots. Microbial CH_4 production per se is a process, which exhibits completely different fractionation factors depending on the pathway of methanogenesis (Games et al., 1978; Gelwicks et al., 1994; Krüger et al., 2002). In paddy fields, total CH_4 production is mainly done through acetate fermentation and H_2/CO_2 reduction (Conrad, 1999), and acetate-dependent methanogenesis is more ^{13}C-enriched than CO_2-dependent methanogenesis (Sugimoto and Wada, 1993; Whiticar, 1999). In the processes of acetate and H_2/CO_2 producing CH_4, fractionation factors were defined by Hayes (1993):

$$\varepsilon_{\text{acetate}/CH_4} = (1 - \alpha_{\text{acetate}/CH_4}) \times 1000 \approx \delta^{13}CH_{4\,(\text{acetate})} - \delta^{13}C_{\text{acetate}} \quad (2)$$

$$\alpha_{CO_2/CH_4} = (\delta^{13}CO_2 + 1000)/(\delta^{13}CH_{4\,(H_2/CO_2)} + 1000) \quad (3)$$

where $\delta^{13}CH_{4\,(\text{acetate})}$ and $\delta^{13}CH_{4\,(H_2/CO_2)}$ are δ^{13}C-values of the CH_4 produced from acetate and H_2/CO_2, and $\delta^{13}C_{\text{acetate}}$ is δ^{13}C-value of the acetate.

Acetate is an important intermediate in anaerobic degradation of organic matter, which is relatively stable in δ^{13}C-values in the form of soil and plant organic carbon during the whole season (Conrad et al., 2002; Krüger et al., 2002). Generally, methyl carbon of the acetate is thought to be converted into CH_4 (Krzycki et al., 1982; Conrad et al., 2002), and an isotope fractionation factor of $\varepsilon_{\text{acetate}/CH_4} = -21$‰ was measured for the transformation of acetate methyl carbon into CH_4 (Gelwicks et al., 1994). Based on that, Krüger et al. (2002) estimated a measurement of $\delta^{13}CH_{4\,(\text{acetate})}$ between

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−43 ‰ and −37 ‰ over the season with δ^{13}C-value of the acetate (−22 to −16 ‰) extracted from the soil pore water of an Italian rice field. Therefore, $\delta^{13}CH_{4(acetate)}$ = −43 ‰ and −37 ‰ was assumed in this study, mainly because the δ^{13}C-value of soil organic carbon (−27.4 ‰) is close to what Krüger et al. (2002) reported (−26.7 ‰). The $\delta^{13}CH_{4(acetate)}$ ranging from −43 ‰ to −37 ‰ is also in good agreement with those used earlier (Sugimoto and Wada, 1993; Tyler et al., 1997; Bilek et al., 1999; Conrad et al., 2002). To our knowledge, measurements of fractionation factor $\varepsilon_{acetate/CH_4}$ during acetoclastic methanogenesis are still scarce (Conrad, 2005). Moreover, $\delta^{13}C_a$cetate changes with the growth of rice plants (Krüger et al., 2002) or the temperature of incubations (Fey et al., 2004), and it may be different from that of rice roots (Conrad et al., 2002). The constant values (−43 ‰ and −37 ‰) for $\delta^{13}CH_{4(acetate)}$ were applied both in paddy soil and on rice roots in this study due to the lack of measurements of $\varepsilon_{acetate/CH_4}$ and $\delta^{13}C_{acetate}$.

It is also essential to set a fractionation factor α_{CO_2/CH_4} that occurs during H_2/CO_2 reduction to CH_4 for this experiment. Previous studies have showed that hydrogenotrophic methanogenesis expresses a stronger kinetic isotope effect than acetoclastic methanogenesis does (Whiticar 1999; Conrad 2005). By summarizing the reported measurements Zhang et al. (2009) showed that α_{CO_2/CH_4} was in the range of 1.025–1.083. Fey et al. (2004) found α_{CO_2/CH_4} in flooded paddy soil decreased with rising temperature in anaerobic incubation, with value of 1.083 at 10 °C, 1.079 at 25 °C, and 1.073 at 37 °C. Since the temperature of incubation in this study was in the range from 17.6 °C to 29.7 °C and averaged 25.1 °C, α_{CO_2/CH_4} = 1.079 was hence considered to be reasonable for calculation of $\delta^{13}CH_{4(H_2/CO_2)}$, which has been validated in other experiments (Zhang et al., 2011c, 2012). It is much higher than those in earlier reports (Sugimoto and Wada, 1993; Tyler et al., 1997; Bilek et al., 1999; Chidthaisong et al., 2002; Krüger et al., 2002; Valentine et al., 2004), indicating fractionation factor α_{CO_2/CH_4} should not be assumed to be the same for different experiments and situations. Such differences may result from variation in the community structure of methanogens with

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the soil and the duration and condition of incubation (Chin et al., 1999; Lueders and Friedrich, 2000; Chidthaisong et al., 2002; Conrad et al., 2002; Krüger et al., 2002; Ramakrishnan et al., 2002).

When $\delta^{13}CH_{4\,(acetate)}$, $\delta^{13}CH_{4\,(H_2/CO_2)}$, and δ^{13}C-value of CH_4 ($\delta^{13}CH_4$) produced in paddy soil and rice roots were obtained, relative contribution of acetate to total CH_4 production (F_{ac}) could be estimated in line with Eq. (4) (Tyler et al., 1997; Bilek et al., 1999; Krüger et al., 2002):

$$\delta^{13}CH_4 = F_{ac} \times \delta^{13}CH_{4\,(acetate)} + (1 - F_{ac}) \times \delta^{13}CH_{4\,(H_2/CO_2)} \quad (4)$$

As shown in Table 4, hydrogenotrophic methanogenesis in paddy soil was very important at the tillering and booting stages (~ 50–70 %) while acetoclastic methanogenesis dominated at the grain filling and ripening stages (~ 60–90 %). 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_2/CO_2-dependent methanogenesis was very important at the beginning of the season. Expectedly, acetoclastic methanogenesis was more important in Treatment WS than in Treatment CK at all the four rice growth stages, with F_{ac}-value higher by ~ 10–30 %. It shows that incorporation of straw supplies abundant substrates for soil CH_4 production, thus promoting acetate-dependent methanogenesis. Wang et al. (1995) found that application of the organic fertilizers significantly increases the content of soil organic acid which is positively related to the rate of CH_4 emission from rice field. Unfortunately, contents of the organic acids, especially acetate, in soil solution were not measured simultaneously. The pathways of methanogenesis in paddy soil have been considerably observed in Italy and Japan, and they are similar to the measurements in this study (Table 5).

For rice roots, however, hydrogenotrophic methanogenesis was dominant (~ 50–90 %) in both treatments at all the four rice growth stages and more important in Treatment WS than in Treatment CK, with a mean F_{ac}-value being ~ 5–20 % lower (Table 6). Additionally, the average F_{ac}-value was 6 % higher (Treatment CK) and 30 % higher (Treatment WS) in paddy soil than on rice roots (Table 6). A similarly high contribution

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of hydrogenotrophic methanogenesis to total CH_4 production on rice roots was observed in radiotracer experiments (Conrad et al., 2000, 2002). Measurements of stable carbon isotope also showed that methanogenesis on excised rice roots was mostly from H_2/CO_2 throughout the season (Krüger et al., 2002). The methanogens population on rice roots is different from that of surrounding soil (Grosskopf et al., 1998a, 1998b; Lehmann et al., 1999), which may be the reason for the high hydrogenotrophic methanogenesis. Previous studies demonstrated that CH_4 was predominantly produced from H_2/CO_2 on rice roots by Rice Cluster I (RC-I) methanogens (Richter et al., 1999; Lueders et al., 2001; Lu et al., 2005; Lu and Conrad, 2005; Conrad et al., 2006). Therefore, straw incorporation would accelerate the growth and activity of RC-I methanogens, hence promoting CO_2-dependent methanogenesis relative to Treatment CK (Table 6). Organic carbon being slightly lighter in plant samples than in soil samples might be another possible reason for F_{ac}-value being lower in paddy soil than on rice roots (Tables 4 and 6). Compared with previous measurements, the relative contribution of acetate to total methanogenesis on fresh roots in this study appeared to be slightly lower, particularly in Treatment WS (Table 5).

4.2 Effect of straw incorporation on CH_4 oxidation

Straw incorporation not only provides sufficient substrates for methanogens in paddy soil, thus promoting methanogenesis directly, but also affects soil CH_4 oxidizing capacity indirectly. Straw incorporation affects CH_4 oxidation mainly through its influence on methanotrophic population and activity. Previous studies have shown that a high concentration of CH_4 stimulates growth of methanotrophs and their activity in oxidization (Bender and Conrad, 1995; Arif et al., 1996). As shown in Figs. 2a and 4a, it increased the CH_4 production capacity in paddy soil, as a consequence, the CH_4 oxidizing ability, as well. On the other hand, it is not only the oxygen secreted from the roots that oxidize CH_4 in the rhizosphere (Butterbach et al., 1997), but also the roots per se that have a strong CH_4 oxidization capacity (Bosse and Frenzel, 1997; Dan et al., 2001). Krüger et al. (2002) found that CH_4 oxidation rates on excised fresh rice roots were

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highest at the beginning of the season and then declined, which is in agreement with the measurements in this study. Straw incorporation enhanced CH_4 oxidation potential on rice roots to some extent (Fig. 4b), which is possibly attributed to the growth and activity of methanotrophs on the surface of rice roots stimulated by straw decomposition, thus increasing the CH_4 oxidation capacity. Unfortunately, community structure of the methanotrophs in the soil was not analyzed in this study, and therefore, further research is needed in this aspect.

Besides the direct in vitro measurements of CH_4 oxidation above, δ^{13}C-values of the CH_4 from various compartments of the rice field were applied to estimation of the fraction of CH_4 oxidized (F_{ox}) by using the following steady-state mass balance equation (Stevens and Engelkemeir, 1988; Tyler et al., 1997):

$$F_{ox} = (\delta^{13}CH_{4(original)} - \delta^{13}CH_{4(oxidized)})/[(1/\alpha_{ox} - 1) \times (\delta^{13}CH_{4(oxidized)} + 1000)] \quad (5)$$

where α_{ox} stands for fractionation factor during CH_4 oxidation, $\delta^{13}CH_{4(original)}$ for δ^{13}C-value of the initial pool of CH_4 that is produced in soil under aerobic incubation (Fig. 2a), and $\delta^{13}CH_{4(oxidized)}$ is the δ^{13}C-value of remaining unoxidized CH_4, generally estimated from the measurements of $\delta^{13}CH_{4(emitted)}$ corrected with plant transport fractionation (Tyler et al., 1997; Krüger et al., 2002):

$$\delta^{13}CH_{4(oxidized)} = \delta^{13}CH_{4(emitted)} - \varepsilon_{transport} \quad (6)$$

where $\delta^{13}CH_{4(emitted)}$ stands for δ^{13}C-value of CH_4 emitted from rice field, and $\varepsilon_{transport}$ for transport fractionation factor, with a range of −12.0 to −8.0 ‰ in the present study (for detailed description, please see 4.3. below).

The fraction of CH_4 oxidized (F_{ox}) was calculated using Eq. (5) based on the values of $\delta^{13}CH_{4(original)}$, $\delta^{13}CH_{4(oxidized)}$ and α_{ox} (1.025 in Treatment CK and 1.033 in Treatment WS) subsequently referred to (for detailed description, please see 4.3. below), and results of the calculation are shown in Table 7. It peaked at the tillering stage (60–101 %), declined gradually and reached the lowest (−19–45 %) at the ripening stage

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both in Treatment CK and Treatment WS (Table 7). This was well in agreement with the findings of Krüger et al. (2001, 2002) who found the value of F_{ox} was the highest at the beginning of the season, turned lower and lower towards the end of the season, and even dropped below zero as depicted in the present study at last (Table 7). Contrary to its effect on CH_4 oxidation potential of paddy soil, straw incorporation reduced the value of F_{ox}, in terms of percentage, by 41–71 % during the four rice growth stages (Table 7). A possible explanation was that straw incorporation increased both CH_4 production and oxidation potentials, particularly the former, to a larger extent (Figs. 2a and 4a), which eventually caused a decrease in F_{ox}-value (Table 7). It was more visible when the situations of high CH_4 production potential and low CH_4 oxidation potential appeared simultaneously at the booting stage (Figs. 2a and 4a, Table 7), with value of F_{ox} 71 % lower in Treatment WS than in Treatment CK.

CH_4 production rates in aerobic incubation were very low relative to those in anaerobic incubation (Figs. 2a, d, 3a and c), which suggests that strong oxidation happens therein. On the other hand, it is quite clear in this study that CH_4 produced in aerobic incubation was much more positive than that in anoxic incubation (Figs. 2b and 3b). This further shows that CH_4 in the former has been oxidized intensively relative to that in the latter. So the $\delta^{13}C$-values of CH_4 aerobically produced in paddy soil were likely to represent $\delta^{13}CH_{4\,(oxidized)}$. An analogous calculation was tentatively conducted with Eq. (5), using the stable carbon isotope technique in the present study. Similar temporal variation of F_{ox}-value in the two treatments was observed (Table 8), being the highest (41–101 %) at the tillering stage and the lowest at the ripening stage (−3–45 %). Moreover, F_{ox}-value was 45–68 % lower in Treatment WS than in Treatment CK, which was consistent with the results reported before (Tables 5 and 8). It indicates that the $\delta^{13}CH_{4\,(oxidized)}$ obtained in this way, to some extent, may be used represent the $\delta^{13}C$-value of CH_4 that remains after being oxidized and has not yet been emitted to the atmosphere if it is hard to measure $\varepsilon_{transport}$ in rice fields.

Compared with the former reports, the measurements of F_{ox}-value in this study were significantly higher (Table 5). The differences in α_{ox} between these studies were a

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possible reason, because the α_{ox} used in this experiment was 1.025–1.033, while in others 1.038 was used to calculate F_{ox}-value (Krüger et al., 2002; Krüger and Frenzel, 2003; Zhang et al., 2012a). Therefore, α_{ox} = 1.038 was used instead in estimating F_{ox} in order to offset the discrepancy caused by different values of α_{ox}. For the present study, F_{ox}-values were still reduced by ~20–40 % in Treatment WS relative to that in Treatment CK (Tables 7 and 8), further suggesting that straw incorporation is an important factor, instead of α_{ox}, influencing the measurement of F_{ox}-value. More interestingly, it was still much higher than the measurements in previous studies in Italy (Krüger et al., 2002; Krüger and Frenzel, 2003), especially in Treatment CK (Tables 7 and 8). However, it was similar to the data reported by Zhang et al. (2012a) who found F_{ox}-value was ~20–70 % in continuous flooding plots and ~50–80 % in intermittent irrigation plots. This indicates that the fields under the special water management in China, i.e. intermittent irrigation (this study and Zhang et al., 2012a), would increase CH_4 oxidation in comparison with those under continuous flooding (Krüger and Frenzel, 2003) or those that had just a brief period of drainage (Krüger et al., 2002). Therefore, α_{ox} itself in the present study may not be a key factor to influence F_{ox}-value relative to early reports in different conditions (Tyler et al., 1997; Bilek et al., 1999; Krüger et al., 2002; Krüger and Frenzel, 2003; Conrad et al., 2005).

4.3 Effect of straw incorporation on carbon isotope fractionation during CH_4 oxidation and transport

When the stable carbon isotope method is used for calculating F_{ox}, oxidation fractionation factor (α_{ox}) has to be taken into consideration (Tyler et al., 1997; Krüger et al., 2002). The oxidation fractionation is caused by methanotrophs. In the closed-system incubation, fractionation factor α_{ox} is known to be calculated according to the Rayleigh Equation (Coleman et al., 1981; Liptay et al., 1998):

$$\alpha_{ox} = 1 + [\log(\delta^{13}CH_{4\,(initial)} + 1000) - \log(\delta^{13}CH_{4\,(final)} + 1000)] / \log f \quad (7)$$

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where $\delta^{13}CH_{4\,(initial)}$ stands for δ^{13}C-value of CH_4 at time 0, $\delta^{13}CH_{4\,(final)}$ for δ^{13}C-value of CH_4 at time t, and f (%) for percentage of CH_4 remaining at time t.

In paddy soil 28.3 °C in temperature, α_{ox} = 1.025 in Treatment CK and 1.033 in Treatment WS was observed in this study, which was well in agreement with the measurements before (1.025–1.038, Coleman et al., 1981; Chanton and Liptay, 2000). To our knowledge, α_{ox}, was firstly measured in methanotrophs enriched cultures (Coleman et al., 1981) and then mainly in landfill cover soils (Liptay et al., 1998; Chanton et al., 1999; Chanton and Liptay, 2000; Mahieu et al., 2006; Chanton et al., 2008a, 2008b). Although nothing was known about α_{ox} in paddy soil before, it (1.025–1.038) was adopted considerably in paddy field experiments (Tyler et al., 1997; Bilek et al., 1999; Krüger et al., 2002; Krüger and Frenzel, 2003; Conrad and Klose, 2005). It is affected by temperature (Chanton and Liptay, 2000; Chanton et al., 2008a), methanotrophs (Coleman et al., 1981; Krüger et al., 2002), and soils (Tyler et al., 1994; Chanton and Liptay, 2000; Snover and Quay, 2000). The differences in α_{ox} between Treatment CK and Treatment WS might be attributed to methanotrophic bacteria in the soil. Methanotrophs preferentially oxidize $^{12}CH_4$, leaving the residual CH_4 ^{13}C-enriched (Whiticar, 1999; Venkiteswaran et al., 2005), which results in a shift in the isotopic fractionation. There is such a possibility that the higher the population and activity of methanotrophs, the more the $^{12}CH_4$ being preferentially consumed. Subsequently, the more the residual CH_4 enriched in ^{13}C, the bigger the fractionation after CH_4 oxidation. Therefore, straw incorporation increases α_{ox}, which is probably ascribed to its stimulation of methanotrophic bacteria in the soil by promoting CH_4 oxidation in Treatment WS relative to Treatment CK (Fig. 4a). Although it has been reported considerably, the lack of knowledge on α_{ox} in paddy soil calls for more efforts in the further study.

In rice fields, most unoxidized CH_4 escapes into the atmosphere through the aerenchyma of rice plants. In the process of CH_4 transport via plants, significant transport fractionation is observed (Chanton et al., 2005). During the rice season, the CH_4 transport fractionation factor $\varepsilon_{transport}$ is known to be equivalent to the difference between δ^{13}C-values of the emitted and aerenchymatic CH_4 (Tyler et al., 1997; Bilek

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et al., 1999; Krüger et al., 2002). A value of $\varepsilon_{transport}$ was −8.0‰ in Treatment WS and −12.0‰ in Treatment CK estimated accordingly from the data of Table 2. Similar differences (∼ −12‰) were observed in former reports (Chanton et al., 1997; Tyler et al., 1997; Bilek et al., 1999). It is a matter of fact, in general, that both CH_4 transport efficiency (Jia et al., 2002) and value of $\varepsilon_{transport}$ (Krüger et al., 2002; Conrad and Klose, 2005) are significantly affected by the growth of plants during the rice season. The biomass of rice plants including both aboveground and underground parts at all the four rice growth stages was lower in Treatment WS than in Treatment CK (Table 3), which suggests that the growth of rice crop has been controlled by straw incorporation. As a result, the capacity of CH_4 transport from soil to the atmosphere was probably lower, thus causing the carbon isotope fractionation much stronger in Treatment CK than in Treatment WS. This indicates that the difference in $\varepsilon_{transport}$ between the two treatments may be ascribed to the influence of straw incorporation on growth of rice plants.

Acknowledgements. We are grateful to Han Yong Cao Yacheng, and Sun Guoqing, etc. in Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China for they help in isotopic data analysis. The authors are also grateful to our anonymous reviewers for their helpful comments. This research work was financially supported by the National Natural Sciences Foundation of China (Grant No. 41071169) the "Strategic Priority Research Program – Climate Change: Carbon Budget and Relevant Issues" of the Chinese Academy of Sciences (XDA05020200), and the Ministry of Science and Technology of China (S2012GR0080)

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Table 1. Schedule of fertilizer application and water management during the rice season.

Fertilizer application	Date	Water management	Date
Organic fertilizer: wheat straw, 4.8 t ha^{-1}	19 Jun	Continuous flooding	23 Jun
Basal N-fertilizer: Urea, 150 kg N ha^{1}	26 Jun	Midseason aeration	23 Jul
Tillering N-fertilizer: Urea, 75 kg N ha^{1}	18 Jul	Alternation of drying and wetting	25 Aug
Panicle N-fertilizer: Urea, 75 kg N ha^{1}	16 Aug	Final drainage	12 Oct

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Table 2. δ^{13}C-values of CH_4 (‰) emitted before and after cutting of the plants. WS: straw incorporation, CK: without straw incorporation.

Sampling plot	Before		After	
	CK	WS	CK	WS
1	−56.8	−55.6	−44.8	−47.1
2	−54.2	−56.8	−42.7	−49.8
3	−54.8	−61.1	−42.3	−52.6
Mean	−55.3	−57.8	−43.3	−49.8
SD	1.4	2.9	1.4	2.6

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Table 3. Dry matter accumulation of rice plants aboveground and underground; mean ± SD, $n = 3$. WS: straw incorporation, CK: without straw incorporation.

Rice growth stage	Aboveground ($g\,m^{-2}$)		Underground ($g\,m^{-2}$)	
	CK	WS	CK	WS
Tillering stage	198 ± 15	194 ± 15	51 ± 0	44 ± 3
Booting stage	1006 ± 43	951 ± 124	158 ± 5	132 ± 2
Grain filling stage	2603 ± 5	2464 ± 178	207 ± 7	182 ± 4
Ripening stage	3452 ± 87	3325 ± 148	216 ± 8	202 ± 3

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Table 4. Contribution of acetate-dependent CH_4 production (F_{ac}) in paddy soil at the four rice growth stages; mean ± SD, n = 3. WS: straw incorporation, CK: without straw incorporation.

Rice growth stage	δ^{13}C-values of produced CH_4 (‰)	δ^{13}C-values of produced CO_2 (‰)	δ^{13}C-values of CH_4 from H_2/CO_2 (‰)[a]	F_{ac} calculated with $\delta^{13}CH_{4\,(acetate)}$ = −37 ‰[b]	F_{ac} calculated with $\delta^{13}CH_{4\,(acetate)}$ = −43 ‰[b]
CK					
Tillering stage	−75.0 ± 4.5	−17.5 ± 0.6	−89.5 ± 0.6	0.28 ± 0.07	0.31 ± 0.08
Booting stage	−75.4 ± 0.0	−16.2 ± 0.4	−88.2 ± 0.4	0.25 ± 0.00	0.28 ± 0.00
Grain filling stage	−61.5 ± 1.6	−18.4 ± 0.2	−90.3 ± 0.2	0.56 ± 0.03	0.63 ± 0.04
Ripening stage	−56.1 ± 0.5	−18.2 ± 0.1	−90.1 ± 0.1	0.64 ± 0.01	0.72 ± 0.01
WS					
Tillering stage	−69.9 ± 1.0	−18.1 ± 0.0	−90.0 ± 0.0	0.38 ± 0.02	0.43 ± 0.02
Booting stage	−62.2 ± 0.8	−15.2 ± 1.4	−87.3 ± 1.3	0.50 ± 0.04	0.57 ± 0.05
Grain filling stage	−54.9 ± 0.2	−19.2 ± 0.2	−91.0 ± 0.2	0.67 ± 0.04	0.75 ± 0.04
Ripening stage	−47.1 ± 3.9	−17.7 ± 0.2	−89.7 ± 0.2	0.81 ± 0.07	0.91 ± 0.08

[a]Calculated with Eq. (3) using the δ^{13}C-values of CO_2 produced in paddy soil and α_{CO_2/CH_4} = 1.079.

[b]Calculated with Eq. (4) using different $\delta^{13}CH_{4\,(acetate)}$-values for CH_4 from acetate.

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Table 5. Overview of the contribution of acetate-dependent CH_4 production (F_{ac}) and the fraction of CH_4 oxidized (F_{ox}) in paddy fields calculated with Eqs. (4) and (5).

Experimental site	Straw incorporation	Incubation sample	F_{ac}	F_{ox}	Reference
Japan	No	Air-dried soil	0.12–1.00	–	Sugimoto and Wada, 1993
Vercelli, Italy	No	Air-dried soil	0.30–0.80	–	Conrad et al., 2002
	No	Fresh roots	0.05–0.65	–	
Vercelli, Italy	No	Fresh soil	0.27–0.67	0.02–0.36	Krüger et al., 2002
	No	Fresh roots	0.36–0.58	–	
Vercelli, Italy	No	Fresh soil	–	0.04–0.45	Krüger and Frenzel, 2003
Vercelli, Italy	No	Air-dried soil	0.40–0.80	–	Fey et al., 2004
Jurong, China	No	Fresh soil[CF]	0.49–0.76	0.19–0.66	Zhang et al., 2012
	No	Fresh soil[II]	0.45–0.76	0.46–0.83	
Jurong, China	Yes	Fresh soil	0.43–0.91	−0.19–0.60	This study
	No	Fresh soil	0.28–0.72	0.45–1.01	
	Yes	Fresh roots	0.07–0.41	–	
	No	Fresh roots	0.28–0.51	–	

[CF] the field was under continuous flooding,
[II] the field was under intermittent irrigation.

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Table 6. Contribution of acetate-dependent CH_4 production (F_{ac}) on rice roots at the four rice growth stages; mean ± SD, n = 3. WS: straw incorporation, CK: without straw incorporation.

Rice growth stage	δ^{13}C-values of produced CH_4 (‰)	δ^{13}C-values of produced CO_2 (‰)	δ^{13}C-values of CH_4 from H_2/CO_2 (‰)[a]	F_{ac} calculated with $\delta^{13}CH_{4\,(acetate)}$ = −37 ‰[b]	F_{ac} calculated with $\delta^{13}CH_{4\,(acetate)}$ = −43 ‰[b]
CK					
Tillering stage	−66.7 ± 0.7	−12.6 ± 0.0	−84.9 ± 0.3	0.38 ± 0.02	0.43 ± 0.02
Booting stage	−74.6 ± 2.2	−14.8 ± 0.7	−87.0 ± 0.5	0.25 ± 0.04	0.28 ± 0.05
Grain filling stage	−69.2 ± 1.4	−24.5 ± 0.1	−96.0 ± 1.3	0.45 ± 0.01	0.51 ± 0.02
Ripening stage	−72.1 ± 1.1	−22.8 ± 0.6	−94.3 ± 0.7	0.39 ± 0.06	0.43 ± 0.06
WS					
Tillering stage	−69.9 ± 0.7	−16.1 ± 0.3	−88.1 ± 0.1	0.36 ± 0.09	0.40 ± 0.09
Booting stage	−83.2 ± 1.7	−14.2 ± 0.8	−86.4 ± 1.0	0.06 ± 0.00	0.07 ± 0.00
Grain filling stage	−74.8 ± 1.1	−23.9 ± 0.2	−95.4 ± 0.5	0.35 ± 0.03	0.39 ± 0.04
Ripening stage	−73.3 ± 1.8	−22.5 ± 0.4	−94.1 ± 0.4	0.36 ± 0.04	0.41 ± 0.05

[a]Calculated with Eq. (3) using the δ^{13}C-values of CO_2 produced in paddy soil and α_{CO_2/CH_4} = 1.079.

[b]Calculated with Eq. (4) using different $\delta^{13}CH_{4\,(acetate)}$-values for CH_4 from acetate.

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Table 7. Fraction of CH_4 oxidized (F_{ox})[a] in paddy fields at the four rice growth stages; mean ± SD, n = 3. WS: straw incorporation, CK: without straw incorporation.

Rice growth stage	α_{ox} = 1.025		α_{ox} = 1.033		α_{ox} = 1.038	
	CK	WS	CK	WS	CK	WS
Tillering stage	1.01 ± 0.13	0.78 ± 0.01	0.77 ± 0.10	0.60 ± 0.01	0.68 ± 0.08	0.52 ± 0.01
Booting stage	0.86 ± 0.03	0.19 ± 0.02	0.66 ± 0.02	0.15 ± 0.02	0.57 ± 0.02	0.13 ± 0.01
Grain filling stage	0.50 ± 0.07	0.02 ± 0.09	0.38 ± 0.05	0.02 ± 0.07	0.33 ± 0.05	0.01 ± 0.06
Ripening stage	0.45 ± 0.15	−0.25 ± 0.02	0.34 ± 0.12	−0.19 ± 0.01	0.30 ± 0.10	−0.16 ± 0.01

[a]Calculated with Eq. (5) using the δ^{13}C-values of CH_4 anoxically produced in paddy soil (Fig. 2b) for $\delta^{13}CH_{4(original)}$ and the δ^{13}C-values of emitted CH_4 (data taken from Zhang et al., 2012) minus −8.0 ‰ (Treatment WS) and −12.0 ‰ (Treatment CK) for $\delta^{13}CH_{4(oxidized)}$.

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Table 8. Fraction of CH_4 oxidized (F_{ox})[a] in paddy fields at the four rice growth stages; mean ± SD, n = 3. WS: straw incorporation, CK: without straw incorporation.

Rice growth stage	α_{ox} = 1.025		α_{ox} = 1.033		α_{ox} = 1.038	
	CK	WS	CK	WS	CK	WS
Tillering stage	1.01 ± 0.08	0.53 ± 0.17	0.77 ± 0.06	0.41 ± 0.13	0.67 ± 0.05	0.36 ± 0.11
Booting stage	0.82 ± 0.21	0.19 ± 0.18	0.63 ± 0.16	0.14 ± 0.13	0.55 ± 0.14	0.12 ± 0.12
Grain filling stage	0.71 ± 0.05	0.20 ± 0.08	0.54 ± 0.04	0.15 ± 0.06	0.47 ± 0.03	0.14 ± 0.05
Ripening stage	0.45 ± 0.03	−0.03 ± 0.09	0.35 ± 0.02	−0.03 ± 0.07	0.30 ± 0.02	−0.02 ± 0.06

[a]Calculated with Eq. (5) using the δ^{13}C-values of CH_4 anoxically produced in paddy soil (Fig. 2b) for $\delta^{13}CH_{4\,(original)}$ and the δ^{13}C-values of emitted CH_4 (data taken from Zhang et al., 2012) minus −8.0 ‰ (Treatment WS) and −12.0 ‰ (Treatment CK) for $\delta^{13}CH_{4\,(oxidized)}$.

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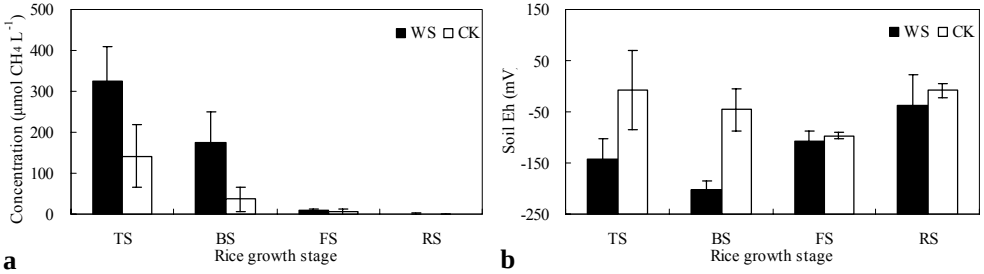

Fig. 1. CH_4 concentration in soil solution **(a)** and soil *E*h of the field **(b)** at the four rice growth stages. TS: tillering stage, BS: booting stage, FS: grain filling stage, RS: ripening stage. WS: straw incorporation, CK: without straw incorporation. Mean ± SD, $n = 3$.

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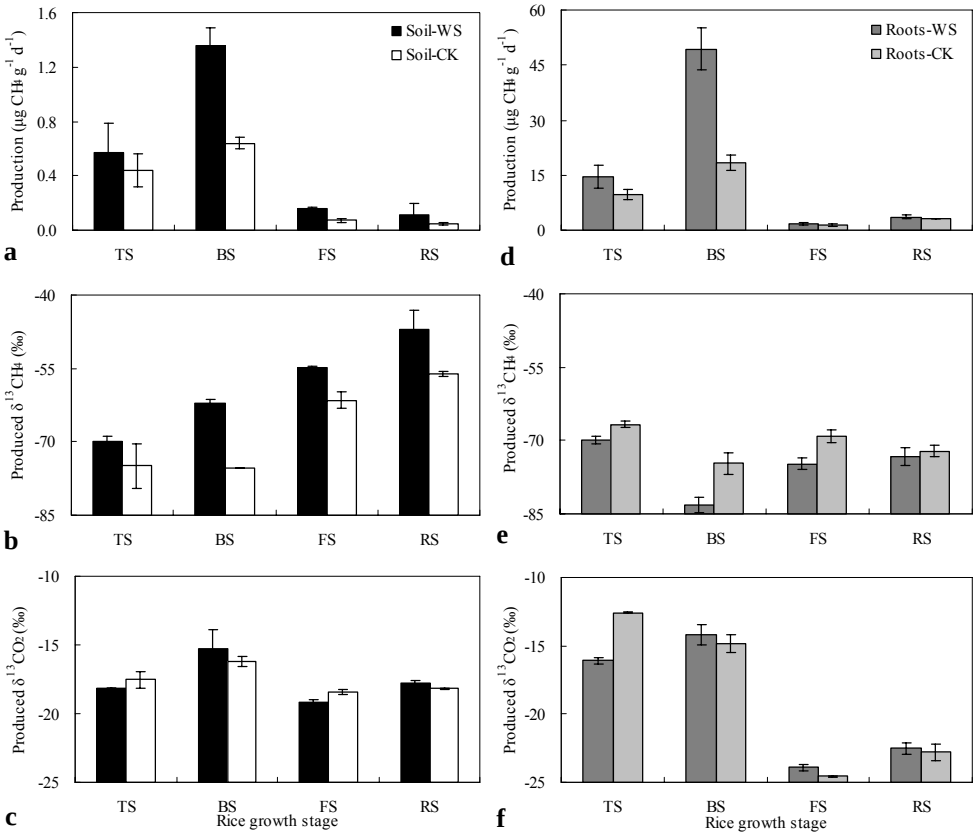

Fig. 2. Potential CH_4 production rates in anaerobically incubated paddy soil **(a)** and rice roots **(d)**, and corresponding δ^{13}C-values of CH_4 **(b)** and **(e)** and CO_2 **(c)** and **(f)** at the four rice growth stages. TS: tillering stage, BS: booting stage, FS: grain filling stage, RS: ripening stage. WS: straw incorporation, CK: without straw incorporation. Mean ± SD, n = 3.

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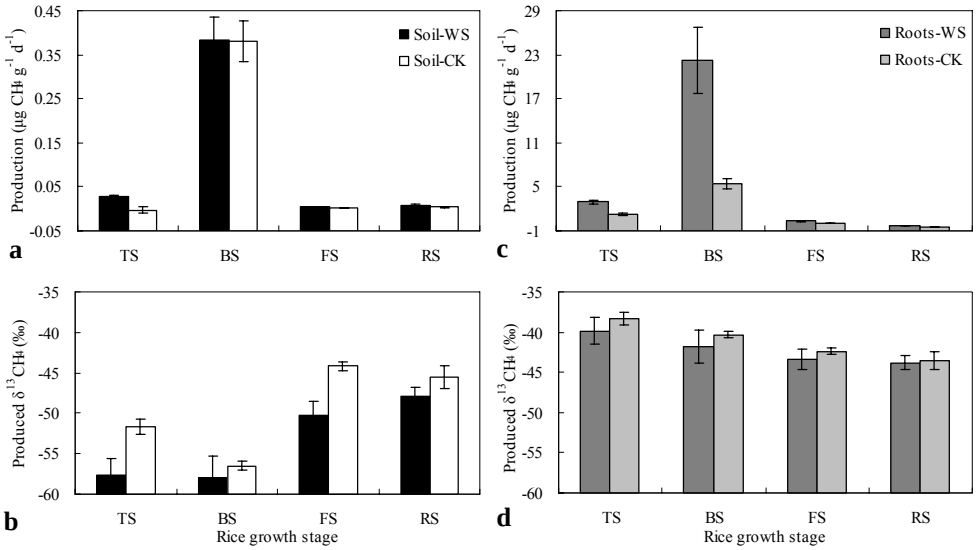

Fig. 3. CH_4 production in aerobically incubated paddy soil **(a)** and rice roots **(c)**, and corresponding δ^{13}C-values of CH_4 **(b)** and **(d)** at the four rice growth stages. TS: tillering stage, BS: booting stage, FS: grain filling stage, RS: ripening stage. WS: straw incorporation, CK: without straw incorporation. Mean ± SD, n = 3.

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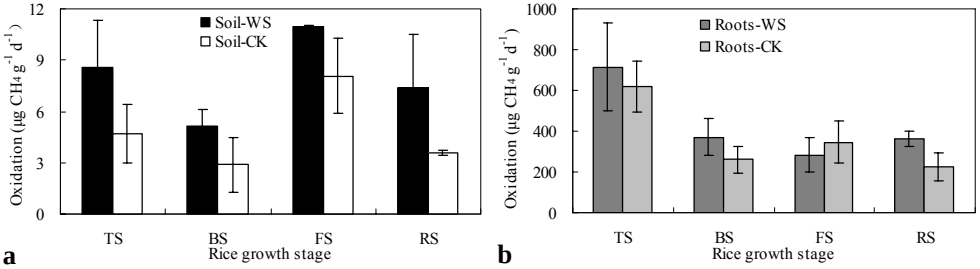

Fig. 4. Potential CH_4 oxidation rates in aerobically incubated paddy soil **(a)** and rice roots **(b)** at the four rice growth stages. TS: tillering stage, BS: booting stage, FS: grain filling stage, RS: ripening stage. WS: straw incorporation, CK: without straw incorporation. Mean ± SD, n = 3.

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