



Pathway of CH₄ production, fraction of CH₄ oxidized, and ¹³C isotope fractionation in a straw-incorporated rice field

G. B. Zhang¹, Y. Ji^{1,2}, J. Ma¹, G. Liu^{1,2}, H. Xu¹, and K. Yagi³

¹State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

²University of Chinese Academy of Sciences, Beijing 100049, China

³National Institute of Agro-Environmental Sciences, 3-1-3, Kannondai, Tsukuba Ibaraki 305-8604, Japan

Correspondence to: H. Xu (hxu@issas.ac.cn) and G. B. Zhang (gbzhang@issas.ac.cn)

Received: 7 August 2012 – Published in Biogeosciences Discuss.: 15 October 2012

Revised: 12 April 2013 – Accepted: 28 April 2013 – Published: 22 May 2013

Abstract. Straw incorporation generally increases CH₄ emission from rice fields, but its effects on the mechanism of CH₄ emission, especially on the pathway of CH₄ production and the fraction of CH₄ oxidized, are not well known. To investigate the methanogenic pathway, the fraction of CH₄ oxidized as well as the stable carbon isotope fractionation during the oxidation and transport of CH₄ as affected by straw incorporation, observations were conducted of production and oxidation of CH₄ in paddy soil and rice roots and $\delta^{13}\text{C}$ -values of produced CH₄ and CO₂, and emitted CH₄ in incubation and field experiments. Straw incorporation significantly enhanced CH₄ production potentials of the paddy soil and rice roots. However, it increased the relative contribution of acetate to total CH₄ 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 CH₄ oxidized (F_{ox}) by 41–71%, probably attributed to the fact that it increased CH₄ oxidation potential whereas CH₄ production potential was increased to a larger extent. There was little CH₄ formed during aerobic incubation, and the produced CH₄ was more ¹³C-enriched relative to that of anaerobic incubation. Assuming $\delta^{13}\text{C}$ -values of CH₄ aerobically produced in paddy soil to be the $\delta^{13}\text{C}$ -values of residual CH₄ 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 $\delta^{13}\text{C}$ -values of CH₄ emitted after cutting of the

plants (–50 to –43‰) were more positive than those of before (–58 to –55‰), suggesting a transport fractionation factor ($\varepsilon_{\text{transport}}$) was –8.0‰ with straw incorporation and –12.0‰ without straw incorporation. Causes of this difference may be related to the diffusion process in transport as affected by growth of rice plants and pressure in the rhizosphere. 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 decreases the fraction of CH₄ oxidized in the field and expands oxidation fractionation while reducing transport fractionation.

1 Introduction

Atmospheric methane (CH₄), the second most important greenhouse gas next to carbon dioxide (CO₂), reached 1808 nL L^{–1} in 2010 (WMO, 2010). As an important source of anthropogenic CH₄, paddy field is responsible for ~5–19% of the global CH₄ 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 × 10⁸ t yr^{–1} and reached even up to 7.3 × 10⁸ t in 2010 (Xiong et al., 2010). Straw incorporation is regarded as a key practice in management of organic fertilizer in rice cultivation. And it proves 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 agroecosystem into a benign cycle. However, in the short term, it highly increases CH₄ 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 CH₄ 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 CH₄ are not clear yet.

In paddy fields, CH₄ 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, CO₂, H₂, propionate as well as other fatty acids. However, acetate, CO₂ and H₂ are the main substrates that methanogenic bacteria use for production of CH₄ (Krüger et al., 2002; Conrad et al., 2010). The relative contribution of acetoclastic (CH₃COOH → CH₄ + CO₂) and hydrogenotrophic (CO₂ + 4H₂ → CH₄ + 2H₂O) methanogenesis to total CH₄ formation is sensitive to availability of the substrates and 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 CH₄ and accelerates the decline of soil *Eh*, thus forming a favorable environment condition for growth of methanogens, which in turn promotes formation of CH₄ and further affects CH₄ 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 CH₄ production, but also act as an important site where CH₄ is oxidized by oxygen available from root secretion in the rhizosphere. Therefore, the growth of rice roots probably stimulates growth and activity of methanotrophs, and consequently increase the potential of CH₄ oxidation. A considerable number of studies have reported production and oxidation of CH₄ in paddy soil or on rice roots (Frenzel and Bosse, 1996; Bosse and Frenzel, 1997; Conrad and Klose, 1999; Lehmann-Richter et al., 1999; Dan et al., 2001; Krüger et al., 2002; Zhang et al., 2011c), but little has focused on the effect of straw incorporation on CH₄ production and CH₄ oxidation separately, let alone on pathway of CH₄ production and fraction of CH₄ oxidized.

In the study of CH₄ 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 CH₄ oxidized in the field can be estimated if δ¹³C-values of CH₄, CO₂

and acetate are measured and relevant fractionation factors (ε_{acetate}, α_{CO₂/CH₄}, α_{ox} and ε_{transport}) are known. The carbon isotope fractionation factors of CH₄ oxidation (α_{ox}) and CH₄ transport (ε_{transport}) are crucial to the quantification of the fraction of CH₄ 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 ε_{transport}) is an important precondition for use of the method in studying CH₄ oxidation. Usually a high proportion of the CH₄ produced in the paddy fields is oxidized in the rhizosphere and at the soil–water interface. The remaining CH₄ is emitted from the soil to the atmosphere mainly through the aerenchyma of rice plants. Due to the fact that ¹²CH₄ is consumed faster than ¹³CH₄ by methane-oxidizing bacteria as well as ¹²CH₄ transports faster than ¹³CH₄ in the process of CH₄ 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, b), rather than in paddy soil (Krüger et al., 2002). Moreover, early reports showed that fractionation factor ε_{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 ε_{transport} of rice plants.

In recent studies, CH₄ concentration in soil solution of the field, δ¹³C-values of CH₄ emitted before and after cutting of the plants, production and oxidation of CH₄ in paddy soil and fresh rice roots, and δ¹³C-values of CH₄ and CO₂ produced in anaerobic and aerobic incubations were measured in field and incubation experiments to clarify the effect of straw incorporation on CH₄ production and oxidation, respectively, especially on pathway of CH₄ production and fraction of CH₄ oxidized in the field. Moreover, stable carbon isotope fractionation factors α_{ox} and ε_{transport} were investigated to exactly estimate the effect of straw incorporation on them and finally on the fraction of CH₄ oxidized.

2 Methods

2.1 Design 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. Stubble and weeds were all removed from the experimental plots. Then, dry wheat straw (C/N = 85, δ¹³C_{org} = −28.3‰), chopped to ~10 cm in length, was evenly spread over the plots of

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 June	Continuous flooding	23 June
Basal N-fertilizer: urea, 150 kg N ha ⁻¹	26 June	Mid-season aeration	23 July
Tillering N-fertilizer: urea, 75 kg N ha ⁻¹	18 July	Alternation of drying and wetting	25 August
Panicle N-fertilizer: urea, 75 kg N ha ⁻¹	16 August	Final drainage	12 October

Treatment WS, and raked into the topsoil (0–15 cm). Rice seedlings (Huajing 3), 32-days-old, were transplanted on 26 June in all 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 of soil solution were extracted using 18 mL vacuum vial to flush and purge the sampler. Approximate 10 mL of water were then drawn into another vial for further analysis. CH₄ concentration in the headspace of the vial was measured on a GC-FID. The CH₄ concentration (C_{CH_4}) in soil solution was calculated using

$$C_{\text{CH}_4} = (m \times G_V) / (G_L \times M_V) \left(\mu\text{mol L}^{-1} \right), \quad (1)$$

where m stands for mixing ratio of CH₄ in the headspace of a vial ($\mu\text{L L}^{-1}$), 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 (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 Meters & 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 N₂-flushed de-ionized sterile water in the ratio of 1 : 1 (soil/water). During the whole process, the samples were constantly flushed with N₂ to remove O₂ and CH₄, and the flasks containing these samples were then sealed for anaerobic incubation. Other flasks with air headspace were sealed directly for aerobic incubation. All flasks were sealed with rubber stoppers fitted with silicon septum that allowed sampling of headspace gas. Finally, they were stored in N₂ 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: 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., 2011c). The roots were washed with N₂-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 CH₄ were taken simultaneously before and after the plants were cut at the late booting stage (28 August) using specially designed PVC bottomless pots for determining stable carbon isotope fractionation during CH₄ transport ($\epsilon_{\text{transport}}$) through the aerenchyma of rice plants (Zhang et al., 2013). 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 $\delta^{13}\text{C}$ -value of the emitted CH₄ (Zhang et al., 2011b).

2.3 Incubation experiment

Potential CH₄ production rates in paddy soil and rice roots were determined anaerobically (Zhang et al., 2011c). Flasks

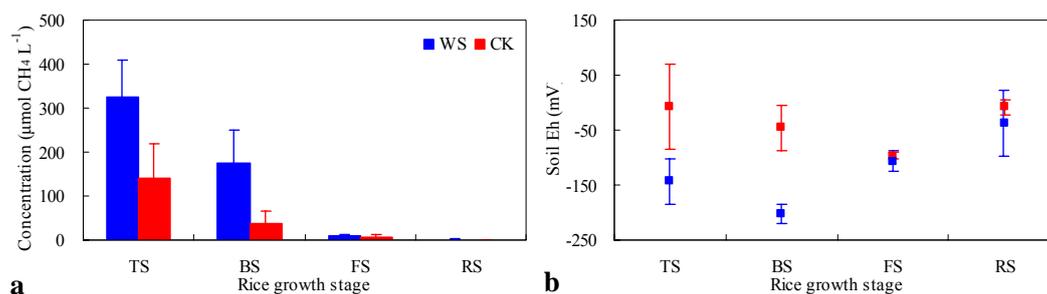


Fig. 1. CH₄ concentration in soil solution (a) and soil *Eh* of the field (b) at the four rice growth stages. TS: tillering stage, BS: booting stage, FS: grain-filling stage, RS: ripening stage. WS: straw incorporation, CK: without straw incorporation. Mean ± SD, *n* = 3.

used for anaerobic incubation were flushed with N₂ consecutively six times through double-ended needles connecting a vacuum pump to purge the air in the flasks of residual CH₄ and O₂. Simultaneously, CH₄ 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 shaken by hand, and analyzed for CH₄ on a GC-FID. CH₄ production was calculated using the linear regression of CH₄ increasing with the incubation time.

Potential CH₄ 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 CH₄ was injected to make a high concentration inside (~10 000 µL L⁻¹). Then the flasks were incubated in dark under the same temperature as in the field and shaken at 120 rpm. CH₄ 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 CH₄ 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. CH₄ oxidation was calculated using the linear regression of the CH₄ depletion with incubation time.

2.4 Analytical methods

CH₄ concentration in gas samples was analyzed with a gas chromatograph (Shimadzu GC-12A, Kyoto, Japan) equipped with a flame ionization detector (FID). δ¹³C-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 (Pre-Con) of trace gases (Cao et al., 2008; Zhang et al., 2011b). Isotope ratios were expressed in the standard delta notation: δ¹³C = [(R_{sample}/R_{standard}) - 1] × 1000 (‰) with R = ¹³C/¹²C of sample and standard, respectively. Preci-

sion of the repeated analyses was ±0.196 ‰ (*n* = 9) with 2.02 µL L⁻¹ CH₄ 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.

3 Results

3.1 CH₄ concentration in soil solution

CH₄ concentration in soil solution of the two treatments dropped sharply over the season, from as high as 150–300 µmol L⁻¹ at the tillering stage to ~1 µmol L⁻¹ at the ripening stage (Fig. 1a). Obviously, CH₄ concentration in soil solution was much higher in Treatment WS (180–330 µmol L⁻¹) than in Treatment CK (40–140 µmol L⁻¹) at the tillering and booting stages. However, no significant discrepancy between the two treatments was observed at the grain-filling and ripening stages (Fig. 1a). Similarly, soil *Eh* 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 grain-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 CH₄ production potential in paddy soil and rice roots under anaerobic incubation

The CH₄ 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 µg CH₄ g⁻¹ d⁻¹; roots:

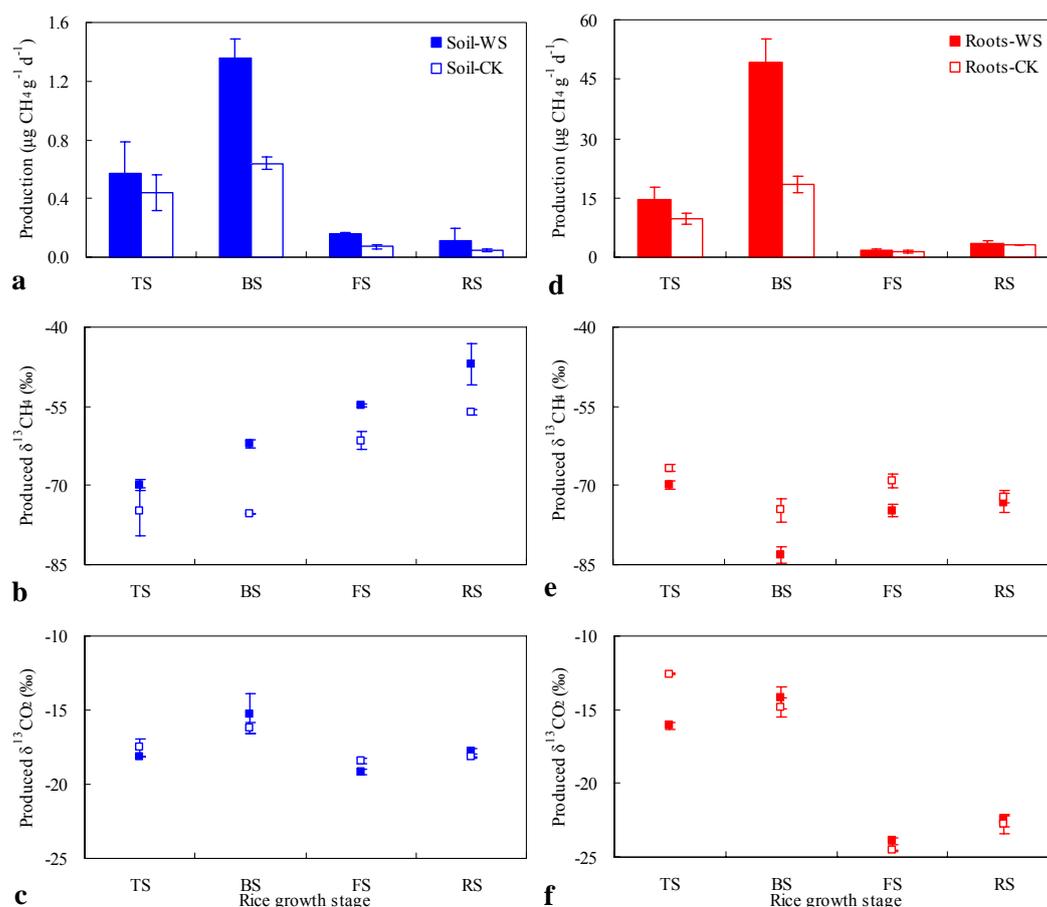


Fig. 2. Potential CH₄ production rates in anaerobically incubated paddy soil (a) and rice roots (d), and corresponding δ¹³C-values of CH₄ (b) and (e) and CO₂ (c) and (f) at the four rice growth stages. TS: tillering stage, BS: booting stage, FS: grain-filling stage, RS: ripening stage. WS: straw incorporation, CK: without straw incorporation. Mean ± SD, *n* = 3.

18.5–49.4 μg CH₄ 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 CH₄ 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, δ¹³C-value of the CH₄ produced in paddy soil became more positive in both treatments (Fig. 2b). The produced CH₄ was more ¹³C-enriched in Treatment WS (–70 to –47 ‰) than in Treatment CK (–75 to –56 ‰) at all four rice growth stages (Fig. 2b), with δ¹³C-values being 5–13 ‰ higher in Treatment WS than in Treatment CK. On the contrary, CH₄ produced on rice roots was gradually becoming ¹³C-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 δ¹³CH₄-value over the season. CH₄ produced on rice roots was more de-

pleted in ¹³C, 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 δ¹³C-value of CO₂ produced in the paddy soil was relatively stable in the two treatments (~ –18 ‰) over the four rice growth stages. On rice roots, however, it 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 δ¹³C-value of CO₂ produced either in paddy soil or on rice roots between the two treatments (*P* > 0.05).

3.3 CH₄ production in paddy soil and rice roots under aerobic incubation

Little CH₄ production was observed in aerobic paddy soil at the tillering, grain-filling and ripening stages, although relatively visible CH₄ production was at the booting stage (~ 0.4 μg CH₄ g⁻¹ d⁻¹) (Fig. 3a). Significant CH₄ production on aerobic rice roots was measured at the tillering and booting stages, especially at the booting stage, reaching as

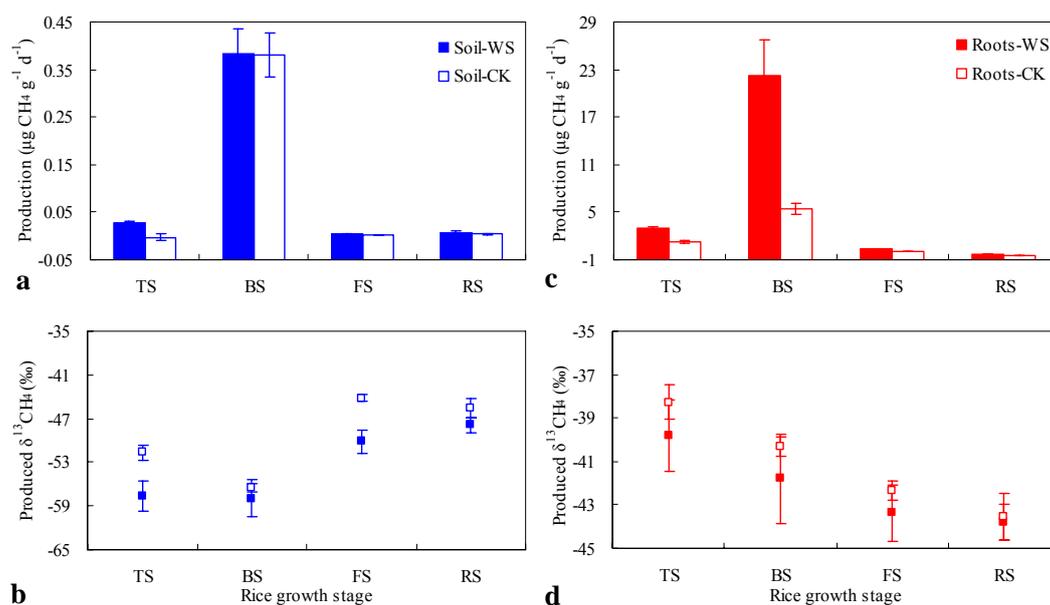


Fig. 3. CH₄ production in aerobically incubated paddy soil (a) and rice roots (c), and corresponding δ¹³C-values of CH₄ (b) and (d) at the four rice growth stages. TS: tillering stage, BS: booting stage, FS: grain-filling stage, RS: ripening stage. WS: straw incorporation, CK: without straw incorporation. Mean ± SD, *n* = 3.

Table 2. δ¹³C-values of CH₄ (‰) 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

high as 5.4–22.2 μg CH₄ g⁻¹ d⁻¹, but little was at the grain-filling and ripening stages (Fig. 3c). CH₄ production in aerobic incubation (Fig. 3a, c) was significantly lower than in anaerobic incubation (Fig. 2a, d, *P* < 0.05). The δ¹³C-value of CH₄ 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, δ¹³C-value of the CH₄ 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 four rice growth stages (Fig. 3b, d). Compared with what was observed in anaerobic incubation (Fig. 2b, e), CH₄ produced in aerobic incubation was significantly enriched in ¹³C (Fig. 3b, d, *P* < 0.05), with the average δ¹³CH₄-value higher by ~10 ‰ in paddy soil and by ~30 ‰ on rice roots during all four rice growth stages.

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

3.4 CH₄ oxidation potential in paddy soil and rice roots under aerobic incubation

A similar pattern in variation of the CH₄ 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 μg CH₄ g⁻¹ d⁻¹) at the tillering stage, and a peak (8.1–10.9 μg CH₄ g⁻¹ d⁻¹) at the grain-filling stage. However, CH₄ oxidation was very important only at the tillering stage (over 600 μg CH₄ g⁻¹ d⁻¹) on rice roots, and weakened towards the ripening stage (~200 μg CH₄ g⁻¹ d⁻¹) (Fig. 4b). Compared with Treatment CK, Treatment WS showed higher CH₄ oxidation potentials in both paddy soil and rice roots at all four rice growth stages (Fig. 4a, b).

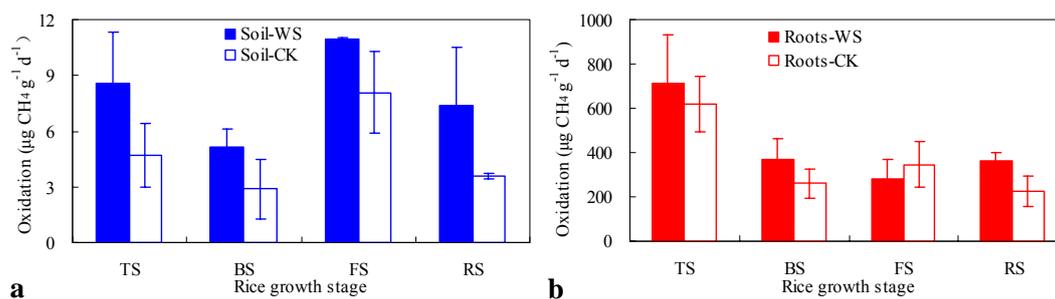


Fig. 4. Potential CH₄ oxidation rates in aerobically incubated paddy soil (a) and rice roots (b) at the four rice growth stages. TS: tillering stage, BS: booting stage, FS: grain-filling stage, RS: ripening stage. WS: straw incorporation, CK: without straw incorporation. Mean \pm SD, $n = 3$.

3.5 $\delta^{13}\text{C}$ -value of CH₄ emitted before and after cutting of the plants

As shown in Table 2, the average $\delta^{13}\text{C}$ -value of CH₄ emitted before cutting of the plants in Treatment CK was -55% , being slightly higher than that in Treatment WS (-58%). Similarly, the mean $\delta^{13}\text{C}$ -value of CH₄ emitted after cutting of the plants in Treatment CK (-43%) was more positive than that in Treatment WS (-50%). Compared with the $\delta^{13}\text{C}$ -value of CH₄ 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 WS at the tillering and ripening stages, respectively. However, $\delta^{13}\text{C}$ -value of soil carbon remained stable over the season, being -7.6% 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 $\delta^{13}\text{C}$ -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 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 CH₄ production

CH₄ 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 CH₄ 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 (E_h) (Fig. 1b), creating a favorable environment for growth of methanogens, which in turn promotes CH₄ production in the paddy field (Fig. 2a, d). Shangguan et al. (1993) found that CH₄ production rate in a straw-incorporated paddy soil, collected from a double rice cropping field in Hunan, was 1–2 times higher than that in the soil without straw incorporated. Moreover, they demonstrated that straw incorporation would be an important cause for the appearance of the first CH₄ production peak just ~ 20 days after the field was flooded. The findings in this study also show that straw incorporation increased CH₄ production remarkably in paddy soil (Fig. 2a). Furthermore, fresh rice roots produced considerable CH₄ 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 CH₄ (Frenzel and Bosse, 1996; Conrad and Klose, 1999; Lehmann-Richter et al., 1999; Krüger et al., 2001, 2002; Zhang et al., 2011c). 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. As a result, CH₄ production was increased (Fig. 2d).

In field conditions, variation of CH₄ concentration in soil solution reflects changes in CH₄ production of the paddy field to some extent. Under continuous flooding, CH₄ 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 mid-season aeration) and at the booting stage (the first re-flooding period) while very low at the grain-filling and ripening stages (Fig. 1a). On the other hand, straw incorporation profoundly increased CH₄ concentration in soil solution at the tillering and booting stages (Fig. 1a, $P < 0.05$). This is because considerable CH₄ production in paddy soil and on rice roots was observed at the tillering and booting stages and straw incorporation highly increased their CH₄ production potentials at those periods. However, a little was measured at the grain-filling and ripening stages (Fig. 2a, d). Alberto et al. (1996) measured concentration of the CH₄ entrapped in paddy soil in the 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.

The $\delta^{13}\text{C}$ -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 be neither a ¹³C-enriched source for methanogenesis in paddy soil nor a ¹³C-depleted source for methanogenesis on rice roots for the whole season. As a consequence, incorporation of straw increased $\delta^{13}\text{C}$ -value of the CH₄ produced in paddy soil but decreased that of the CH₄ derived from rice roots (Fig. 2b, e), which is probably attributed to its different effects on pathways of CH₄ production in paddy soil and rice roots. Microbial CH₄ production per se is a process that 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 CH₄ production is mainly done through acetate fermentation and H₂/CO₂ reduction (Conrad, 1999), and acetate-dependent methanogenesis is more ¹³C-enriched than CO₂-dependent methanogenesis (Sugimoto and Wada, 1993; Whiticar, 1999). In the processes of acetate and H₂/CO₂ producing CH₄, fractionation factors were defined by Hayes (1993):

$$\varepsilon_{\text{acetate}/\text{CH}_4} = (1 - \alpha_{\text{acetate}/\text{CH}_4}) \times 1000 \quad (2)$$

$$\approx \delta^{13}\text{CH}_4(\text{acetate}) - \delta^{13}\text{C}_{\text{acetate}}$$

$$\alpha_{\text{CO}_2/\text{CH}_4} = \left(\delta^{13}\text{CO}_2 + 1000 \right) / \left(\delta^{13}\text{CH}_4(\text{H}_2/\text{CO}_2) + 1000 \right), \quad (3)$$

where $\delta^{13}\text{CH}_4(\text{acetate})$ and $\delta^{13}\text{CH}_4(\text{H}_2/\text{CO}_2)$ are $\delta^{13}\text{C}$ -values of the CH₄ produced from acetate and H₂/CO₂, and $\delta^{13}\text{C}_{\text{acetate}}$ is $\delta^{13}\text{C}$ -value of the acetate.

Acetate is an important intermediate in anaerobic degradation of organic matter, which is relatively stable in $\delta^{13}\text{C}$ -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 CH₄ (Krzycki et al., 1982; Conrad et al., 2002), and an isotope fractionation factor of $\varepsilon_{\text{acetate}/\text{CH}_4} = -21\text{‰}$ was measured for the transformation of acetate methyl carbon into CH₄ (Gelwicks et al., 1994). Based on that, Krüger et al. (2002) estimated a measurement of $\delta^{13}\text{CH}_4(\text{acetate})$ between -43‰ and -37‰ over the season with $\delta^{13}\text{C}$ -value of the acetate (-22 to -16‰) extracted from the soil pore water of an Italian rice field. Therefore, $\delta^{13}\text{CH}_4(\text{acetate}) = -43\text{‰}$ and -37‰ was assumed in this study, mainly because the $\delta^{13}\text{C}$ -value of soil organic carbon (-27.4‰) is close to what Krüger et al. (2002) reported (-26.7‰). The $\delta^{13}\text{CH}_4(\text{acetate})$ 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 $\varepsilon_{\text{acetate}/\text{CH}_4}$ during acetoclastic methanogenesis are still scarce (Conrad, 2005). Moreover, $\delta^{13}\text{C}_{\text{acetate}}$ 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 $\delta^{13}\text{CH}_4(\text{acetate})$ were applied both in paddy soil and on rice roots in this study due to the lack of measurements of $\varepsilon_{\text{acetate}/\text{CH}_4}$ and $\delta^{13}\text{C}_{\text{acetate}}$.

It is also essential to set a fractionation factor $\alpha_{\text{CO}_2/\text{CH}_4}$ that occurs during H₂/CO₂ reduction to CH₄ 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 $\alpha_{\text{CO}_2/\text{CH}_4}$ was in the range of 1.025–1.083. Fey et al. (2004) found $\alpha_{\text{CO}_2/\text{CH}_4}$ 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 incubation in this study varied in the range from 17.6 °C to 29.7 °C and averaged 25.1 °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., 2011b, 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 $\alpha_{\text{CO}_2/\text{CH}_4}$ 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 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 $\delta^{13}\text{CH}_4(\text{acetate})$, $\delta^{13}\text{CH}_4(\text{H}_2/\text{CO}_2)$, and $\delta^{13}\text{C}$ -value of CH₄ ($\delta^{13}\text{CH}_4$) produced in paddy soil and rice roots were obtained, relative contribution of acetate to total CH₄ production (F_{ac}) could be estimated in line with Eq. (4) (Tyler

Table 4. Contribution of acetate-dependent CH₄ production (F_{ac}) in paddy soil at the four rice growth stages; mean \pm SD, $n = 3$. WS: straw incorporation, CK: without straw incorporation.

Rice growth stage	$\delta^{13}\text{C}$ -values of produced CH ₄ (‰)	$\delta^{13}\text{C}$ -values of produced CO ₂ (‰)	$\delta^{13}\text{C}$ -values of CH ₄ from H ₂ /CO ₂ (‰) ^a	F_{ac} calculated with $\delta^{13}\text{CH}_4(\text{acetate}) = -37\text{‰}^b$	F_{ac} calculated with $\delta^{13}\text{CH}_4(\text{acetate}) = -43\text{‰}^b$
CK					
Tillering stage	-75.0 \pm 4.5	-17.5 \pm 0.6	-89.5 \pm 0.6	0.28 \pm 0.07	0.31 \pm 0.08
Booting stage	-75.4 \pm 0.0	-16.2 \pm 0.4	-88.2 \pm 0.4	0.25 \pm 0.00	0.28 \pm 0.00
Grain-filling stage	-61.5 \pm 1.6	-18.4 \pm 0.2	-90.3 \pm 0.2	0.56 \pm 0.03	0.63 \pm 0.04
Ripening stage	-56.1 \pm 0.5	-18.2 \pm 0.1	-90.1 \pm 0.1	0.64 \pm 0.01	0.72 \pm 0.01
WS					
Tillering stage	-69.9 \pm 1.0	-18.1 \pm 0.0	-90.0 \pm 0.0	0.38 \pm 0.02	0.43 \pm 0.02
Booting stage	-62.2 \pm 0.8	-15.2 \pm 1.4	-87.3 \pm 1.3	0.50 \pm 0.04	0.57 \pm 0.05
Grain-filling stage	-54.9 \pm 0.2	-19.2 \pm 0.2	-91.0 \pm 0.2	0.67 \pm 0.04	0.75 \pm 0.04
Ripening stage	-47.1 \pm 3.9	-17.7 \pm 0.2	-89.7 \pm 0.2	0.81 \pm 0.07	0.91 \pm 0.08

^a Calculated with Eq. (3) using the $\delta^{13}\text{C}$ -values of CO₂ produced in paddy soil and $\alpha_{\text{CO}_2/\text{CH}_4} = 1.079$. ^b Calculated with Eq. (4) using different $\delta^{13}\text{CH}_4(\text{acetate})$ -values for CH₄ from acetate.

Table 5. Overview of the contribution of acetate-dependent CH₄ production (F_{ac}) and the fraction of CH₄ oxidized (F_{ox}) in paddy fields calculated with Eqs. (4) and (5).

Experimental site	Straw incorporation	Incubation sample	F_{ac}	F_{ox}	Reference
Konosu, 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	–	-0.13–0.45	Krüger and Frenzel (2003)
Vercelli, Italy	No	Air-dried soil	0.40–0.80	–	Fey et al. (2004)
	No	Fresh soil ^{CF}	0.49–0.76	0.19–0.66	
Jurong, China	No	Fresh soil ^{II}	0.45–0.76	0.46–0.83	Zhang et al. (2012)
	Yes	Fresh soil	0.43–0.91	-0.19–0.60	
	No	Fresh soil	0.28–0.72	0.45–1.01	
Jurong, China	Yes	Fresh roots	0.07–0.41	–	This study
	No	Fresh roots	0.28–0.51	–	

^{CF} the field was under continuous flooding, ^{II} the field was under intermittent irrigation.

et al., 1997; Bilek et al., 1999; Krüger et al., 2002):

$$\delta^{13}\text{CH}_4 = F_{ac} \times \delta^{13}\text{CH}_4(\text{acetate}) + (1 - F_{ac}) \delta^{13}\text{CH}_4(\text{H}_2/\text{CO}_2), \quad (4)$$

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 %). Similarly, Krüger et al. (2002) also found that acetate-dependent methanogenesis dominated the end of the season, whereas H₂/CO₂-dependent methanogenesis was very important at the beginning of the season. Acetoclastic methanogenesis was more important in Treatment WS than in Treatment CK at all four rice growth stages, with F_{ac} -value higher by ~ 10–30 %. It shows that incorporation of straw supplies abundant substrates for soil CH₄ 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 CH₄ 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 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 of hydrogenotrophic methanogenesis to total CH₄ 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 H₂/CO₂ throughout the season (Krüger et al., 2002). The methanogens population on rice roots is different from that of surrounding soil (Grosskopf et al., 1998a, b; Lehmann-Richter et al., 1999), which may be the reason for the high hydrogenotrophic methanogenesis. Previous studies demonstrated that CH₄ was predominantly produced from H₂/CO₂ on rice roots by Rice Cluster I (RC-I) methanogens (Lehmann-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 CO₂-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 CH₄ oxidation

Straw incorporation not only provides sufficient substrates for methanogens in paddy soil, thus promoting methanogenesis directly, but also affects soil CH₄ oxidizing capacity indirectly. Straw incorporation affects CH₄ oxidation mainly through its influence on methanotrophic population and activity. Previous studies have shown that a high concentration of CH₄ 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 CH₄ production capacity in paddy soil, and as a consequence, the CH₄ oxidizing ability, as well. On the other hand, it is not only the oxygen secreted from the roots that oxidize CH₄ in the rhizosphere (Butterbach et al., 1997), but also the roots per se that have a strong CH₄ oxidization capacity (Bosse and Frenzel, 1997; Dan et al., 2001). Krüger et al. (2002) found that CH₄ oxidation rates on excised fresh rice roots were highest at the beginning of the season and then declined, which is in agreement with the measurements in this study. Straw incorporation enhanced CH₄ 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. 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 CH₄ oxidation above, ¹³C-values of the CH₄ from various compartments of the rice field were applied to estimation of the fraction of CH₄ oxidized (*F_{ox}*) by using the following steady-state mass balance equation

(Stevens and Engelkemeir, 1988; Tyler et al., 1997):

$$F_{\text{ox}} = \frac{\left(\delta^{13}\text{CH}_4(\text{original}) - \delta^{13}\text{CH}_4(\text{oxidized})\right)}{\left[\left(1/\alpha_{\text{ox}} - 1\right) \times \left(\delta^{13}\text{CH}_4(\text{oxidized}) + 1000\right)\right]}, \quad (5)$$

where α_{ox} stands for fractionation factor during CH₄ oxidation, $\delta^{13}\text{CH}_4(\text{original})$ for ¹³C-value of the initial pool of CH₄ that is produced in soil under aerobic incubation (Fig. 2a), and $\delta^{13}\text{CH}_4(\text{oxidized})$ is the ¹³C-value of remaining unoxidized CH₄, generally estimated from the measurements of $\delta^{13}\text{CH}_4(\text{emitted})$ corrected with plant transport fractionation (Tyler et al., 1997; Krüger et al., 2002):

$$\delta^{13}\text{CH}_4(\text{oxidized}) = \delta^{13}\text{CH}_4(\text{emitted}) - \varepsilon_{\text{transport}}, \quad (6)$$

where $\delta^{13}\text{CH}_4(\text{emitted})$ stands for ¹³C-value of CH₄ emitted from the rice field, and $\varepsilon_{\text{transport}}$ for transport fractionation factor, with a range of −12.0 to −8.0% in the present study (for detailed description, please see Sect. 4.3. below).

The fraction of CH₄ oxidized (*F_{ox}*) was calculated using Eq. (5) based on the values of $\delta^{13}\text{CH}_4(\text{original})$, $\delta^{13}\text{CH}_4(\text{oxidized})$ and α_{ox} (1.025 in Treatment CK and 1.033 in Treatment WS) subsequently referred to (for detailed description, please see Sect. 4.3. below) and shown in Table 7. It peaked at the tillering stage (60–101%), declined gradually and reached the lowest (−19–45%) at the ripening stage both in Treatment CK and Treatment WS (Table 7). This was well in agreement with the findings of Krüger et al. (2001, 2002), who reported that 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 CH₄ 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 CH₄ 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 CH₄ production potential and low CH₄ oxidation potential appeared simultaneously at the booting stage (Figs. 2a and 4a, Table 7), with *F_{ox}*-value of 71% lower in Treatment WS than in Treatment CK.

CH₄ 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 CH₄ produced in aerobic incubation was much more positive than that in anoxic incubation (Figs. 2b and 3b). This further shows that CH₄ in the former has been oxidized intensively relative to that in the latter. So the ¹³C-values of CH₄ aerobically produced in paddy soil were likely to represent $\delta^{13}\text{CH}_4(\text{oxidized})$. An analogous calculation was tentatively conducted with Eq. (5), using the stable carbon isotope technique in the present study. Similar temporal variation

Table 6. Contribution of acetate-dependent CH₄ production (F_{ac}) on rice roots at the four rice growth stages; mean \pm SD, $n = 3$. WS: straw incorporation, CK: without straw incorporation.

Rice growth stage	$\delta^{13}\text{C}$ -values of produced CH ₄ (‰)	$\delta^{13}\text{C}$ -values of produced CO ₂ (‰)	$\delta^{13}\text{C}$ -values of CH ₄ from H ₂ /CO ₂ (‰) ^a	F_{ac} calculated with $\delta^{13}\text{CH}_4(\text{acetate}) = -37\text{‰}$ ^b	F_{ac} calculated with $\delta^{13}\text{CH}_4(\text{acetate}) = -43\text{‰}$ ^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 $\delta^{13}\text{C}$ -values of CO₂ produced on rice roots and $\alpha_{\text{CO}_2/\text{CH}_4} = 1.079$. ^b Calculated with Eq. (4) using different $\delta^{13}\text{CH}_4(\text{acetate})$ -values for CH₄ from acetate.

Table 7. Fraction of CH₄ oxidized (F_{ox})^a in paddy fields at the four rice growth stages; mean \pm SD, $n = 3$. WS: straw incorporation, CK: without straw incorporation.

Rice growth stage	$\alpha_{ox} = 1.025$		$\alpha_{ox} = 1.033$		$\alpha_{ox} = 1.038$	
	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 $\delta^{13}\text{C}$ -values of CH₄ anoxically produced in paddy soil (Fig. 2b) for $\delta^{13}\text{CH}_4(\text{original})$ and the $\delta^{13}\text{C}$ -values of emitted CH₄ (data taken from Zhang et al., 2012) minus -8.0‰ (Treatment WS) and -12.0‰ (Treatment CK) for $\delta^{13}\text{CH}_4(\text{oxidized})$.

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 (Table 7). It indicates that the $\delta^{13}\text{CH}_4(\text{oxidized})$ obtained in this way, to some extent, may be used to represent the $\delta^{13}\text{C}$ -value of CH₄ that remains after being oxidized but has not yet been emitted to the atmosphere if it is hard to measure $\epsilon_{\text{transport}}$ 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 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., 2012). Therefore, $\alpha_{ox} = 1.038$ 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 in-

terestingly, 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. (2012), 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., 2012), would increase CH₄ 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 influencing 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 CH₄ 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.,

Table 8. Fraction of CH₄ oxidized (F_{ox})^a in paddy fields at the four rice growth stages; mean \pm SD, $n = 3$. WS: straw incorporation, CK: without straw incorporation.

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

^a Calculated with Eq. (5) using the $\delta^{13}\text{C}$ -values of CH₄ anaerobically produced in paddy soil (Fig. 2b) for $\delta^{13}\text{C}_{\text{CH}_4(\text{original})}$ and the $\delta^{13}\text{C}$ -values of CH₄ aerobically produced in paddy soil (Fig. 3b) for $\delta^{13}\text{C}_{\text{CH}_4(\text{oxidized})}$.

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):

$$\alpha_{\text{ox}} = 1 + \left[\log \left(\delta^{13}\text{C}_{\text{CH}_4(\text{initial})} + 1000 \right) - \log \left(\delta^{13}\text{C}_{\text{CH}_4(\text{final})} + 1000 \right) \right] / \log f, \quad (7)$$

where $\delta^{13}\text{C}_{\text{CH}_4(\text{initial})}$ stands for $\delta^{13}\text{C}$ -value of CH₄ at time 0, $\delta^{13}\text{C}_{\text{CH}_4(\text{final})}$ for $\delta^{13}\text{C}$ -value of CH₄ at time t , and f (%) for percentage of CH₄ remaining at time t .

In paddy soil 28.3 °C in temperature, $\alpha_{\text{ox}} = 1.025$ 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 methanotroph-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, b). 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 ¹²CH₄, leaving the residual CH₄ ¹³C-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 ¹²CH₄ being preferentially consumed. Subsequently, the more the residual CH₄ enriched in ¹³C, the bigger the fractionation after CH₄ oxidation. Therefore, straw incorporation increased α_{ox} , which is probably ascribed to its promotion of CH₄ oxidation by stimulating methanotrophic bacteria in Treatment WS relative to Treatment CK (Fig. 4a). Although it has been reported consider-

ably, the lack of knowledge on α_{ox} in paddy soil calls for more efforts in the further study.

In rice fields, most unoxidized CH₄ escapes into the atmosphere through the aerenchyma of the rice plants. In the process of CH₄ transport via plants, significant transport fractionation is observed (Chanton, 2005). During the rice season, the CH₄ transport fractionation factor $\epsilon_{\text{transport}}$ is known to be equivalent to the difference between $\delta^{13}\text{C}$ -values of the emitted and aerenchymatic CH₄ (Tyler et al., 1997; Bilek et al., 1999; Krüger et al., 2002). A value of $\epsilon_{\text{transport}}$ 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 CH₄ transport efficiency (Jia et al., 2002) and value of $\epsilon_{\text{transport}}$ (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 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. On the other hand, CH₄ transportation by plants is basically a diffusion process, and a small difference in condition may bring about a great difference in isotopic composition (Chanton, 2005). Pressure and partial pressure in the rhizosphere is possibly higher in Treatment WS than in Treatment CK as well as the $\delta^{13}\text{C}$ -value of CH₄ in the soil. Therefore, those differences in physical condition may cause differences in transport fractionation. Although the processes resulting in the difference in $\epsilon_{\text{transport}}$ are not fully understood, the differences in physical conditions, such as growth of rice plants and pressure in the rhizosphere, are likely to affect the diffusion process and consequently the $\epsilon_{\text{transport}}$.

5 Conclusions

The study on the mechanism of CH₄ emission from a Chinese rice field demonstrated that straw incorporation obviously increased CH₄ production and oxidation potentials in

paddy soil and on rice roots. What is more, the effect of straw incorporation on methanogenic pathways and fraction of CH₄ 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 by ~10–30 %, but decreased on rice roots by ~5–20 %, and acetoclastic methanogenesis was more important in the former than in the latter. Furthermore, straw incorporation significantly decreased the fraction of CH₄ oxidized by 41–71 %, which is likely ascribed to the fact that CH₄ production potential was increased to a larger extent than CH₄ oxidation potential was. This indicates that the production of CH₄ increased directly by straw incorporation will indirectly reduce the fraction of CH₄ oxidized, thus significantly decreasing CH₄ emission from the rice fields. These findings are beneficial to enhance the understandings of the effect of straw incorporation on CH₄ emission and the mechanism at the soil micro-process level and further to supply scientific advice and available options for mitigating CH₄ emissions from rice-based ecosystems. Although it may be difficult to estimate its quantitative effects on CH₄ production and oxidation on a regional or a global scale, the variation pattern of methanogenesis being increased while the fraction of CH₄ oxidized being reduced by straw incorporation is probably not changed. 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 CH₄ oxidation and transport. Although it is very limited, to our knowledge so far, in paddy soil, the fractionation factor α_{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 CH₄ oxidation in the soil. Difference between $\delta^{13}\text{C}$ -values of the emitted and aerenchymatic CH₄ indicates a fractionation factor $\epsilon_{\text{transport}}$ 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 causing the difference are not clearly known. As a traditional agricultural management, straw incorporation is estimated to be the most sensitive factor influencing CH₄ emission from rice fields. Produced CH₄ partially oxidized by the paddy fields has a very important effect on regulating the atmospheric CH₄. By quantifying the fractionation factors (α_{ox} and $\epsilon_{\text{transport}}$), one can not only better understand the kinetic isotope effect in the processes of CH₄ emission but also more accurately estimate CH₄ oxidation, which will help to put constraints on global CH₄ sinks. Therefore, it is essential to contribute more efforts to the study on methanotrophic microbial communities and fractionation factors α_{ox} and $\epsilon_{\text{transport}}$ to better elucidate the processes of CH₄ emission from rice fields as affected by straw incorporation.

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 their help in isotopic data analysis. The authors are also very 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).

Edited by: N. Saigusa

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