Methanogenic Pathway and Fraction of CH₄ Oxidized in Paddy Fields: Seasonal Variation and Effect of Water Management in Winter Fallow Season

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Abstract

A 2-year field and incubation experiment was conducted to investigate δ¹³C during the processes of CH₄ emission from the fields subjected to two water managements (flooding and drainage) in the winter fallow season, and further to estimate relative contribution of acetate to total methanogenesis (F_ac) and fraction of CH₄ oxidized (F_ox) based on the isotopic data. Compared with flooding, drainage generally caused CH₄ to be aerobically or anaerobically produced, depleted in ¹³C. There was no obvious difference between the two in transport fractionation factor (r transport) and δ¹³C-value of emitted CH₄. CH₄ emission was negatively related to its δ¹³C-value in seasonal variation (P<0.01). Acetate-dependent methanogenesis in soil was dominant (60–70%) in the late season, while drainage decreased F_ac-value by 5–10%. On roots however, CH₄ was mostly produced through H₂/CO₂ reduction (60–100%) over the season. CH₄ oxidation mainly occurred in the first half of the season and roughly 10–90% of the CH₄ was oxidized in the rhizosphere. Drainage increased F_ox-value by 5–15%, which is possibly attributed to a significant decrease in production while no simultaneous decrease in oxidation. Around 30–70% of the CH₄ was oxidized at the soil-water interface when CH₄ in pore water was released into floodwater, although the amount of CH₄ oxidized therein might be negligible relative to that in the rhizosphere. CH₄ oxidation was also more important in the first half of the season in lab conditions and about 5–50% of the CH₄ was oxidized in soil while almost 100% on roots. Drainage decreased F_ox-value on roots by 15% as their CH₄ oxidation potential was highly reduced. The findings suggest that water management in the winter fallow season substantially affects F_ac in the soil and F_ox in the rhizosphere and roots rather than F_ac on roots and F_ox at the soil-water interface.

Introduction

Paddy fields are an important source of the greenhouse gas, methane (CH₄), contributing to 5–19% of the total global CH₄ emission [1]. Proper water management is considered to be one of the most important options for regulating CH₄ emission from paddy fields [2,3]. Generally, the fields are either intermittently irrigated or continuously flooded during the rice-growing season, and either drained without any irrigation except for rain water or kept flooded in the winter fallow season. Compared with continuous flooding, intermittent irrigation significantly decreases CH₄ emission from rice fields during the rice-growing season by 40–70% [4–6]. Similarly, drainage, relative to flooding, in the winter fallow season not only prevents CH₄ emission from the fields directly in the current season, but also sharply reduces CH₄ emission indirectly during the following rice-growing season [7–9]. Although effects of water management in the winter fallow season on CH₄ flux from the fields are considerably reported, its effect on the processes of CH₄ emission, including CH₄ production, oxidation and transportation, remains unclear. The stable carbon isotope technique, an important method for identifying processes of CH₄ emission from rice fields, has been widely used through measuring carbon isotopic ratios [10–12]. In addition, it can be used to quantify contributions of various CH₄ sources and provide information about carbon isotopes for global CH₄ budget [13,14]. To our knowledge so far, very little study has been done on the measurement of stable carbon isotopes in the fields during the rice-growing season as affected by water management in the winter fallow season.

Methanogenesis is the precondition of CH₄ emission from paddy fields and mainly occurs through two pathways. One is H₂/CO₂ reduction with the participation of specific hydrogenotrophic methanogens that use H₂ or organic molecules as H donor (CO₂+H₂ → CH₄+2H₂O). The other is acetate fermentation with the participation of acetotrophic methanogens (CH₃COOH → CH₄+CO₂). In general, the latter plays a more important role than the former in CH₄ formation [15,16]. If δ¹³C-values of the CH₄, CO₂ and acetate involved in methanogenesis are measured, contributions of the two pathways can be estimated by using the stable carbon isotope technique [17,18]. Theoretically, acetate...
fermentation and H₂/CO₂ reduction accounts for 67% and 33%, respectively, of the total methanogenesis. Practically, relative contributions of the two pathways vary with rice cultivar, rice growth, water management, and environmental conditions, etc. [4,10,11,19]. During the rice-growing season, drainage can significantly enhance soil Eh, causing increase in oxidizing substances like Fe³⁺, sulphate and nitrate, and their inhibition of acetotrophic methanogens, thus reducing acetate-dependent methanogenesis [4,20]. In the winter fallow season, water management also significantly affects soil Eh, CH₄ production and then CH₄ emission from the fields during the following rice-growing season [8], but its impact on relative contributions of the two main pathways of methanogenesis remains poorly known.

CH₄ oxidation, which occurs at the root–soil interface and soil–water interface, is very important to regulating paddy CH₄ emission. By comparing CH₄ emission from the field or CH₄ production from aerobic incubation with methanogenesis in the strict anaerobic environment at the early stage, it was found that as much as 50–90% of the CH₄ was oxidized before escaping into the atmosphere [21–23]. By using the stable carbon isotope method to quantify the fraction of CH₄ oxidized in the paddy fields, recent studies in America and Italy indicated that it was less than 50% [10,12,24,25]. In China however, the fraction of CH₄ that was oxidized in a paddy field under intermittent irrigation during the rice-growing season was measured by this means to be up to 80% [4]. It was significantly higher than those in the fields under continuous flooding as above mentioned. Moreover, CH₄ oxidation potential was relatively higher in intermittently irrigated paddy soil than in continuously flooded soil [4], which suggests that CH₄ oxidation is highly impacted by water management during the rice-growing season. It is further indicated that oxidation of endogenous CH₄ in the paddy fields seems to be more obvious in China, particularly in the fields that are intermittently irrigated during the rice-growing season. Although CH₄ oxidation potential in paddy soil in a whole year has been reported [9], the percentage of CH₄ oxidized in the field as affected by water management in the winter fallow season is still unknown.

Therefore, a 2-year field and incubation experiment was carried out in the paddy fields subjected to two types of water management (flooding and drainage) in the winter fallow season. Seasonal CH₄ emission fluxes, CH₄ in soil pore water and floodwater, CH₄ in the aerenchyma of the plants, CH₄ production and oxidation in fresh paddy soil and rice roots, and their respective δ¹³CH₄-values during the rice-growing season were measured. The objectives of this study were: (1) to investigate impact of water management in the winter fallow season on CH₄ production, oxidation and emission and their δ¹³CH₄; and (2) further to evaluate its effect on pathways of CH₄ production and fraction of CH₄ oxidized in the fields by using the isotopic measurements.

Materials and Methods

Field Description and Experimental Design

With the authorization of the Institute of Agricultural Science, Zhenjiang City, the experiment was carried out at Baitu Town, Jurong City, Jiangsu Province, China (31°58’N, 119°18’E). Main features of the experiment field have already been described in detail before [8]. The experiment was designed to have two treatments, three replicates each, i.e. winter fallow under continuous flooding (Flooding) and winter fallow without irrigation except for rain water (Drainage). Measurements of this study were performed during the 2008 and 2009 rice-growing seasons. Rice stubble and wild weeds were all removed from the experimental plots after rice harvest in the winter fallow season. For rice transplanting in the next rice-growing season, all the plots were ploughed the way the local farmers do. During the rice-growing season, they were continuously flooded and only drained for rice harvest. Rice seedlings (Cultivar ‘Oryza sativa L. Huajing 3’) were transplanted at their 3–4-leaf stage on June 22 and 26 in 2008 and 2009, respectively. Urea was applied at a rate of 300 kg N ha⁻¹, of which 50% was done as basal fertilizer, 25% as tillering fertilizer, and 25% as panicle fertilizer. Both Calcium superphosphate and Potassium chloride were applied as basal fertilizer at a rate of 450 and 225 kg ha⁻¹, respectively. For further details of the farming practices during the two years, please see Zhang et al. [8].

Field Sampling and Measuring

CH₄ flux was observed using the static closed chamber method [8]. To measure the flux, gas samples were collected at 4–7-day intervals using 18 mL vacuum vials. For determining isotopic signature (δ¹³C) of the emitted CH₄, gas samples were taken at 10–20-day intervals using 500 mL bags (Aluminium foil compound membrane, Delin gas packing Co., Ltd, Dalian, China) with a small battery-driven pump [26]. The first sample was collected after the chamber was closed for 3–5 min, and the second at the end of the 2 h closure. Isotopic signature (S) of the emitted CH₄ was calculated using the equation below:

\[ S = \frac{B \times b - A \times a}{B \times A} \]

where A and B stands for CH₄ concentration (µL L⁻¹) in the samples at the beginning and at the end, respectively, while a and b for the corresponding δ¹³CH₄-values (%) of the gas samples, separately. Simultaneously, soil redox potential (Eh) 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).

Soil pore water samples were collected using a Rhizon soil moisture sampler (10 RHIZON SMSMOM, Eijkelkamp Agri-search Equipment, Giesbeek, Netherlands) [26]. The samplers were installed (in triplicate) in the plots prior to rice transplanting and then left in the soil over the whole observational periods. Samples (~5 mL) were firstly extracted using 18 mL vacuum vials to flush and purge the sampler before sampling. Then ~10 mL of soil solution was drawn into another vial for further analysis. Simultaneously, 10 mL of floodwater was collected using a plastic syringe and then transferred in to an 18 mL vacuum vial. Finally, the pressure of all sampling vials was equilibrated by filling in pure N₂ gas. After heavy shaking by hand, the airs in the headspace of the vials were directly analyzed for CH₄ on the GC-FID, and their corresponding δ¹³CH₄-values were determined using the isotope ratio mass spectrometer. CH₄ concentrations (CCH₄) in pore water and floodwater were calculated using the following equation:

\[ C_{\text{CH}_4} = \frac{m \times G_{\text{CH}_4}}{G_f \times M_v} \text{ (µmol L}^{-1} \text{)} \]

where m stands for mixing ratio of CH₄ in the headspace of a vial (µL L⁻¹), Mᵥ for volume of an ideal gas (24.78 L mol⁻¹ at 25°C), Gᵥ for volume of the gas headspace of the vial (L), and Gₘ for volume of the liquid in the vial (L).
The pot, 30 cm in height and 17 cm in diameter, was designed to have a water-filled trough around its top, avoiding any possible gas exchange during the sampling times. A PVC plate (18 cm in diameter) with a hole (the diameter could be adjusted to the growing plant) 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 with a small battery-driven pump.

Triplicate soil cores were collected from each experimental plot using a stainless steel core (7 cm diameter × 25 cm length) and then prepared into mixture [9]. Samples (in triplicate) from the mixture, about 50 g (dry weight) each, were taken and promptly transferred into 250-mL Erlenmeyer flasks separately, and turned into slurries with N2-flushed de-ionized sterile water at 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 sealed for anaerobic incubation. Some other flasks with air headspace were sealed directly for aerobic incubation. They were all stored in N2 at 4 °C for further analysis within 8 h. A small portion of the soil sample was dried for 72 h at 60 °C for determination of isotopic composition of the organic carbon.

Complete rice plants together with roots were carefully collected from the experimental plots at each main rice growth stage, i.e., tillering (TS), booting (BS), grain-filling (FS) and ripening (RS) stages, in 2009 [8]. The roots were washed clean with N2-flushed demineralized water and cut off from the green shoots at 1–2 cm from the root with a razor blade. The fresh roots, 20 g each portion, were then put into flasks, separately, for further preparation and processing in the same way as for the soil. 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.

### Fresh Soil and Roots Incubations

CH4 production potentials were measured for the paddy soil and rice roots under anaerobic incubation. The flasks were flushed with N2 consecutively for six times through double-ended needles connecting a vacuum pump to purge the air in the flasks of residual CH4 and O2. Simultaneously, methanogenesis was determined aerobically using flasks with air headspace directly. They were subsequently 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, and analyzed 1 h and 50 h later after the flasks were heavily shaken by hand, for CH4 on the GC-FID. CH4 production was calculated using the linear regression of CH4 increasing with the incubation time.

CH4 oxidation potentials were determined for the paddy soil and rice roots under aerobic incubation with high CH4 concentration supplemented, using equipment the same as described above. Firstly, pure CH4 was injected into each flask to make a high concentration inside (~10000 μL L−1). Then, the flasks were incubated in dark under the same temperature as measured in the field and shaken at 120 r.p.m. CH4 depletion was measured by sampling the headspace gas in the flask after vigorous shaking for subsequent GC-FID analysis. The first sample was collected generally 30 min after pure CH4 was injected. Samples were then taken in 2–3 h intervals during the first 8 h of the experiment. They were left in the flasks over night and measured the next day in 2 h intervals again. CH4 oxidation was calculated by linear regression of CH4 depletion with incubation time.

### Analytical Methods

CH4 was quantified using the gas chromatograph (GC) equipped with a flame ionization detector (FID) [28]. The isotopic composition (δ13C) of CH4 and CO2 was determined with a Finnigan MAT-253 Isotope Ratio Mass Spectrometer (IRMS, Thermo-Finnigan, Bremen, Germany) using the continuous flow technique [26,29]. The IRMS had a fully automatic interface for pre-GC concentration (Pre-Con) of trace gases, and the precision of repeated analyses was ±0.196‰ (n = 9) with 2.02 μL L−1 CH4 injected. Gas samples were first blown into the chemical trap with MgCl2 and ascarite by He flow (20 mL min−1). Over 99.99% of the CO2 and H2O in the samples was absorbed and removed. CH4 in the samples was then converted into CO2 in a combustion reactor at about 1000 °C. Subsequently, it was flowed into the freezing traps with liquid nitrogen (−196 °C) and the GC for further separation. The separated gases were finally transferred into the mass spectrometer for δ13C determination. The dried soil and plant samples were analyzed for carbon isotope composition with a Finnigan MAT-251 Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany). Soil organic carbon contents were measured by wet oxidation using dichromate in acid medium followed by the FeSO4 titration method.

### Calculations

Isotope ratios are expressed in the standard delta notation:

\[ \delta^{13}C = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \% \text{o} \]  \hspace{1cm} (3)

where R stands for 13C/12C of the sample and the standard, respectively, using DDB carbonate for the standard. Carbon isotope fractionation factor during acetate fermentation (\( \delta_{\text{acetate/CH4}} \)) or H2/CO2 reduction (\( \delta_{\text{CO2/CH4}} \)) for methanogenesis was defined by Hayes [30]:

\[ \delta_{\text{acetate/CH4}} = \left( 1 - \frac{2_{\text{acetate}}}{2_{\text{CH4}}} \right) \times 1000 \approx \delta^{13} \text{CH}_4(\text{acetate}) - \delta^{13} \text{C}_{\text{acetate}} \]  \hspace{1cm} (4)

\[ \delta^{13} \text{CO}_2/\text{CH}_4 = \frac{\delta^{13} \text{CO}_2 + 1000}{\delta^{13} \text{CH}_4(\text{H}_2/\text{CO}_2) + 1000} \]  \hspace{1cm} (5)

where \( \delta^{13} \text{C}_{\text{acetate}} \), \( \delta^{13} \text{CH}_4(\text{acetate}) \), and \( \delta^{13} \text{CH}_4(\text{H}_2/\text{CO}_2) \) are the δ13C values of acetate, CH4 produced from acetate and from H2/CO2, respectively.

Relative contribution of acetate to total CH4 (Fac) was calculated using the following mass balance, assuming that acetate fermentation and H2/CO2 reduction were the only sources of methanogenesis in the rice fields [10-12]:

\[ Fac = \frac{CH_4(\text{acetate})}{CH_4(\text{acetate}) + CH_4(\text{H}_2/\text{CO}_2)} \]  \hspace{1cm} (6)

\[ \delta^{13} \text{CH}_4 = Fac \times \delta^{13} \text{CH}_4(\text{acetate}) + (1 - Fac) \times \delta^{13} \text{CH}_4(\text{H}_2/\text{CO}_2) \]  \hspace{1cm} (7)

where \( \delta^{13} \text{CH}_4 \) stands for δ13C value of total CH4. In addition, the fraction of CH4 that was oxidized (Fac) in the fields was estimated using the equation given by Stevens and Engelke [13] and
where $\delta^{13} \text{CH}_4(\text{original})$ stands for carbon isotopic signature of the primarily produced CH$_4$, $\delta^{13} \text{CH}_4(\text{oxidized})$ for carbon isotopic signature of the residual CH$_4$ after oxidation, of which the calculation was done using a semi-empirical equation [12]:

$$F_{\text{ox}} = \frac{\delta^{13} \text{CH}_4(\text{original}) - \delta^{13} \text{CH}_4(\text{oxidized})}{(1/\alpha_{\text{ox}} - 1) \times (\delta^{13} \text{CH}_4(\text{oxidized}) + 1000)} \tag{8}$$

and $\alpha_{\text{ox}}$ stands for isotope fractionation factor due to CH$_4$ oxidation by the methanotrophs, and $\epsilon_{\text{transport}}$ for isotope fractionation factor due to CH$_4$ transport by the plants.

**Statistics**

Statistical analysis was done using the SPSS 18.0 software for Windows (SPSS Inc., Chicago). Differences between the two treatments in mean ($n$ = 3) CH$_4$ concentration, mean CH$_4$ production and oxidation potentials, and mean soil Eh were determined through one-way analysis of variance (ANOVA) and least significant difference (LSD) test. Relationships between CH$_4$ fluxes and emitted $\delta^{13}$CH$_4$ ($n$ = 16), between mean CH$_4$ production potential and soil Eh ($n$ = 11), and between CH$_4$ oxidation potential and soil temperature ($n$ = 11) were assessed using correlation analysis. Statistical significant differences and correlations were set at $P<0.05$.

**Results**

**CH$_4$ Emission and $\delta^{13}$C**

CH$_4$ emissions (Fig. 1a, c) were significantly higher from flooded fields than from drained fields as had been reported before [8]. Different variation patterns were observed in the $\delta^{13}$C of the emitted CH$_4$ in 2008 and 2009 seasons (Fig. 1b, f). Generally, the emitted CH$_4$ tended to be $^{13}$C-enriched in 2008 with its $\delta^{13}$C-value increased from −69 to −51% in Treatment Flooding, and from −65 to −47% in Treatment Drainage (Fig. 1b). However, more complicated changes were observed in 2009, showing that the emitted CH$_4$ was relatively enriched in $^{13}$C at the beginning and at the end of the season, and relatively $^{13}$C-depleted in the middle of the season (Fig. 1f). However, little difference was found between Treatments Flooding and Drainage, with $\delta^{13}$C-values being in the range of −68 to −48% and −71 to −53%, respectively (Fig. 1b, f, $P>0.05$). Although more measurements were performed in 2009 than in 2008, the mean $\delta^{13}$C-value seemed to be more positive in 2009 (−58 to −55%) than in 2009 (−62 to −61%). Notably, negative relationship was observed between CH$_4$ flux and $\delta^{13}$C in the seasonal variation in 2008 ($r = -0.695$, $P<0.01$) and 2009 ($r = -0.546$, $P<0.01$).

Significant difference in soil Eh was observed between the two treatments (Fig. 1c, g, Table 1). Soil Eh was very close to 0 mV at the beginning of the season and remained much lower in Treatment Flooding than in Treatment Drainage throughout the two seasons, with the averaged value of −165 and −88 mV in 2008, and −133 and −26 mV in 2009, respectively. Soil temperature at the depth of 10 cm generally peaked around D25 (25 days after rice transplanting) and then gradually declined till the end of the season (Fig. 1d, h), fluctuating within the range from 16 to 30.1°C in 2008 and from 17.2 to 29.7°C in 2009, and being averaged 24.3 and 24.4°C, respectively.

**CH$_4$ Concentration and $\delta^{13}$C**

CH$_4$ concentration in pore water for Treatment Flooding was relatively high (200–400 μmol L$^{-1}$) at the beginning of the season, dropped subsequently to 20–200 μmol L$^{-1}$ and then turned upwards again to 350–450 μmol L$^{-1}$ at the end of the season in both 2008 and 2009 (Fig. 2a, c). For Treatment Drainage however, CH$_4$ concentration decreased gradually from 300 to 200 μmol L$^{-1}$ during the 2008 season (Fig. 2a), whereas it was the highest (~200–250 μmol L$^{-1}$) in the middle and the lowest (<50 μmol L$^{-1}$) at the beginning and the end of the 2009 season (Fig. 2c). The averaged CH$_4$ concentration during the two seasons was generally higher in Treatment Flooding than in Treatment Drainage (Table 1). $\delta^{13}$C-value of the CH$_4$ was relatively stable during the 2008 season though it increased and then slightly decreased (Fig. 2b). As a whole, CH$_4$ was much more $^{13}$C-enriched in Treatment Flooding (~56%) than in Treatment Drainage (~67%) over the 2008 season (Fig. 2b, $P<0.05$). In the 2009 season however, $\delta^{13}$C-value fluctuated sharply within the range from −65 to −55% to −70‰ or so (Fig. 2d). No obvious difference in mean $\delta^{13}$C-value (~−60‰) was observed between the two treatments in 2009 (Fig. 2d, $P>0.05$).

CH$_4$ concentration in floodwater of the field in 2009 was measured simultaneously. No more than 7 μmol L$^{-1}$ of CH$_4$ was detected though little data were obtained (Fig. 2e). On the other hand, CH$_4$ in floodwater became more and more $^{13}$C-enriched towards the end of the season, with the $\delta^{13}$C-value increased from −50 to −40‰ (Fig. 2d). Little difference in $\delta^{13}$C-value was observed as well between Treatments Flooding and Drainage (Fig. 2d, $P>0.05$). Compared with porewater CH$_4$, floodwater CH$_4$ was much more enriched in $^{13}$C (Fig. 2d, $P<0.05$).

**Plants Emitted and Aerenchymatic CH$_4$ and $\delta^{13}$C**

To quantify stable carbon isotope fractionation during the CH$_4$ emitted through the aerenchyma of the plants, $\delta^{13}$C-values of the emitted CH$_4$ and aerenchymatic CH$_4$ were measured simultaneously. On the three sampling days during the 2009 season, the emitted CH$_4$ was relatively stable with its $\delta^{13}$C-value stable around −60‰ (Table 2). The aerenchymatic CH$_4$ as expected, was significantly $^{13}$C-enriched compared to the emitted CH$_4$, with the $\delta^{13}$C-values varying in the range of −51 to −42‰, and being about −47‰ on average for the two treatments (Table 2). As a result, the isotope fractionation factor due to CH$_4$ transport ($\epsilon_{\text{transport}}$) was determined to be in the range from −16 to −11‰ in Treatment Flooding and from −14 to −12‰ in Treatment Drainage. As a whole, no obvious difference in mean value of $\epsilon_{\text{transport}}$ (~−13‰) was observed between the two treatments (Table 2, $P>0.05$).

**CH$_4$ Production Under Anaerobic Incubation and $\delta^{13}$C**

CH$_4$ production potentials of the slurries of paddy soil were measured during the 2008 and 2009 rice seasons (Fig. 3a, d). Methanogenesis started more quickly and became more intense in Treatment Flooding than in Treatment Drainage over the two seasons (Fig. 3a, d), peaked around D40–60 for the former and around D80 for the latter. Mean production potential was significantly higher in Treatment Flooding than in Treatment Drainage during the two seasons (Table 1, $P<0.05$). The produced CH$_4$ was relatively stable in $\delta^{13}$C (~−60‰) in Treatment Flooding while fluctuated sharply (from −72 to −53‰) in Treatment Drainage in 2008 (Fig. 3b). In 2009 however, it was gradually enriched in $^{13}$C for the two treatments, with $\delta^{13}$C-value ranging from −70 to −60‰ (Fig. 3c). In addition, the mean $\delta^{13}$CH$_4$-value in Treatment Flooding appeared to be slightly more positive than that in Treatment Drainage over the two seasons, varying in the range of −63 to −58‰ and −66 to −63‰.
Figure 1. Seasonal variations of CH₄ emission, δ¹³C-value of emitted CH₄, soil Eh and soil temperature. (a, b, c and d) 2008, (e, f, g and h) 2009. TS, BS, FS and RS represent tillering, booting, grain-filling and ripening stages, respectively. Bars represent standard errors (n = 3).

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Table 1. Mean CH₄ concentration (µmol L⁻¹) in soil pore water, mean CH₄ production and oxidation potentials (µgCH₄ g d⁻¹), and mean soil Eh (mV) during the 2008 and 2009 rice seasons (mean ± SD, n = 3).

| Treatment | Concentration | Production | Oxidation | Eh |
|-----------|---------------|------------|-----------|----|
|           | Soil          | Roots      | Soil      | Roots |
| 2008      |               |            |           |      |
| Flooding  | 340±47 a      | 2.96±0.30 a| 8.66±1.64 a| -165±15 a |
| Drainage  | 281±63 a      | 1.16±0.26 b| 8.37±1.24 a| -88±26 b |
| 2009      |               |            |           |      |
| Flooding  | 170±6 a       | 1.99±0.11 a| 27.2±3.3 a| 649±88 a |
| Drainage  | 119±12 b      | 1.15±0.02 b| 11.9±2.1 b| 308±59 b |

Means in the same column followed by different letters between the two treatments indicate significant difference at P<0.05.
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respectively. The produced CO₂ became isotopically heavier step by step, causing δ¹³C-value to decrease from −20 to −15% at the beginning of the season to around −10% at the end of the season, and it was relatively more ¹³C-enriched in Treatment Flooding than in Treatment Drainage over the two seasons (Fig. 3c, f, P<0.05).

Abundant methanogenesis was measured on the fresh rice roots under anaerobic incubation in 2009 (Fig. 4a). The production of CH₄ increased sharply and peaked around D60, just like the soil (Fig. 3d). Then it decreased gradually till the end of the season. As a whole, it was significantly higher in Treatment Flooding than in Treatment Drainage (Fig. 4a, Table 1, P<0.05). Similar to CH₄ produced in the soil, CH₄ produced on the roots was depleted in ¹³C at the beginning of the season (Fig. 4b). Subsequently, it became more ¹³C-enriched, with its δ¹³C-values ranging from −90 to −75%. No significant difference was observed in mean δ¹³C-value between Treatment Flooding (−83%) and Treatment Drainage (−81%). However, it was much more negative compared to the CH₄ produced in the soil in δ¹³C-value (Figs. 3e and 4b, P<0.01). The δ¹³C-value of produced CO₂ ranged from −22 to −17% over the two seasons and no obvious difference was observed between the two treatments (Fig. 4c, P>0.05).

CH₄ Production Under Aerobic Incubation and δ¹³C
Less than 0.3 μg CH₄ g⁻¹ d⁻¹ was produced in the soil under aerobic condition over the 2009 season (Fig. 5a), and 1.0–1.5 μg CH₄ g⁻¹ d⁻¹ was on the roots at the beginning of the season and below 0 μg CH₄ g⁻¹ d⁻¹ at the end of the season (Fig. 5c). The produced CH₄ was very stable over the season both in the soil and on the roots, with δ¹³C-values around −58 to −55% and −44 to −41%, respectively. Opposite to the CH₄ produced under anaerobic condition, it was significantly more enriched in ¹³C on the roots than in the soil (Fig. 5b, d, P<0.01). Generally, the δ¹³C-value was more positive in Treatment Flooding than in Treatment Drainage (Fig. 5b, d). In addition, the CH₄ produced under aerobic condition was significantly ¹³C-enriched relative to that produced under anaerobic condition, especially those from the roots (Figs. 3e, 4b and 5b, d, P<0.01).

CH₄ Oxidation Under Aerobic Incubation Amended with High CH₄ Concentration
Similar variation patterns of the CH₄ oxidation potentials in the paddy soil during the 2008 and 2009 seasons were observed, showing a peak at the beginning of the season and a steep slope till the end of the season (Fig. 6a, b). Although the potential was relatively lower in Treatment Flooding than in Treatment

| Days after rice transplanting (d) | Aerenchymatic CH₄ (a) | Emitted CH₄ (b) | Emitted CH₄ (b) | Emitted CH₄ (b) |
|----------------------------------|----------------------|----------------|----------------|----------------|
|                                  | Flooding | Drainage | Flooding | Drainage |
| 37                               | −48.46   | −51.49   | −60.94   | −63.82   |
| 62                               | −42.44   | −43.58   | −58.77   | −57.84   |
| 98                               | −48.56   | −47.32   | −59.51   | −59.18   |
| **Mean**                         | −46.49   | −47.46   | −59.74   | −60.28   | −12.48   | −12.33   |
| **SD**                           | 3.50     | 3.96     | 1.10     | 3.14     | 1.28     | 2.77     |

Table 2. δ¹³C-values of CH₄ (%) in the aerenchyma of and emitted from the plants during the 2009 rice season.

![Figure 2](https://example.com/fig2.png)
Drainage in the middle of the season but slightly higher both at the beginning and at the end of the season (Fig. 6a, b), no significant difference was observed between the two treatments in mean of the potential (Table 1, \(P > 0.05\)). Notably, a significant positive relationship was observed between CH4 oxidation potential and soil temperature in temporal variation (\(r = 0.703–0.859, P < 0.05\)). Intensive oxidation signal on the fresh roots was observed, which was also the strongest (400–600 \(\mu g CH_4 groots^{-1} d^{-1}\)) at the beginning of the season and declined to the lowest (150–400 \(\mu g CH_4 groots^{-1} d^{-1}\)) at the end of the season (Fig. 6c). Throughout the 2009 season, CH4 oxidation potential on the roots was significantly higher in Treatment Flooding than in Treatment Drainage (Fig. 6c, \(P < 0.05\)).

Organic Carbon in Soil and Plant Samples

During the 2008 season, the content of organic carbon in the soil was 1.02 ± 0.08% in Treatment Flooding and 1.11 ± 0.05% in Treatment Drainage, and it seemed to increase during the 2009 season, reaching 1.65 ± 0.01% and 1.83 ± 0.10%, respectively. Soil organic carbon in Treatment Drainage was very stable in \(^{13}C\) (–27.9\%) during the two rice seasons, whereas it was slightly \(^{13}C\)-enriched in Treatment Flooding, with \(^{13}C\)-value increasing from –28.1\% in 2008 to –27.0\% in 2009. Organic carbon in the plant samples showed little change throughout the 2009 season, with \(^{13}C\)-value of –28.9\%, –29.2\% and –28.7\% on D27, D66 and D108, respectively, although it was slightly lighter in contrast to the organic carbon in the soil.

Discussion

Effects on Stable Carbon Isotopes

The processes of CH4 emission involved in CH4 production, oxidation and transportation in the fields were well identified by measuring stable carbon isotopes (Fig. 7). In paddy fields, besides the applied organic fertilizers, plant photosynthesis and degradation of soil organic carbon are the two most important substrate sources for methanogenesis [31]. As substrates for CH4 production, \(^{13}C\)-value of organic carbon in the plant samples (–29\%) seemed to be slightly negative than that in the soil samples (–27\%) (Fig. 7). Previous observations also showed that organic carbon was slightly lighter in the plant than in the soil [10,27,32]. Intensive carbon isotope fractionation generally happens when methanogenic precursors form CH4. Around 10–20\% occurs during CH4 production through acetate fermentation while 50–70\% during CH4 production through H2/CO2 reduction [16,33]. As a consequence, CH4 from the former (–60–50\%) is usually more positive than that from the latter (as negative as –110\%) [34]. The CH4 produced in the soil was more \(^{13}C\)-enriched in Treatment Flooding than in Treatment Drainage (Fig. 3b, c) and also more (–65\%) than that on the roots (–63\%) in both treatments (Fig. 7). This shows that flooding, compared with drainage in the winter fallow season, increased the relative contribution of acetate to CH4 production and that acetoclastic methanogenesis in the soil was more important than that on the roots (Fig. 8). Early anaerobic measurements indicated that CH4 from the roots was more depleted in \(^{13}C\) than that from the soil [17,27].
Figure 4. CH₄ production potential and δ¹³C-values of CH₄ and CO₂ anaerobically produced on rice roots. (a) Potential, (b) δ¹³CH₄, (c) δ¹³CO₂. TS, BS, FS and RS represent tillering, booting, grain-filling and ripening stages, respectively. Bars represent standard errors (n = 3).
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Figure 5. Temporal variation of CH₄ production and corresponding δ¹³C-value in aerobic incubation. (a, b) Paddy soil, (c, d) Rice roots. TS, BS, FS and RS represent tillering, booting, grain-filling and ripening stages, respectively. Bars represent standard errors (n = 3).
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Figure 6. Temporal variation of CH₄ oxidation potential in paddy soil and on rice roots. (a) 2008, (b and c) 2008 and 2009. TS, BS, FS and RS represent tillering, booting, grain-filling and ripening stages, respectively. Bars represent standard errors (n = 3).
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In rice-based ecosystems, the produced CH$_4$, except for the portions oxidized and emitted into the atmosphere, is temporarily retained in the soil as entrapped CH$_4$ and dissolved CH$_4$ in soil pore water [35]. As mainly in the form of bubbles, CH$_4$ in soil pore water probably remains unoxidized and is usually considered to be the original CH$_4$ produced in the field in many reports [11,12,36]. However, Krüger et al. [10] found that CH$_4$ in soil pore water poorly represented the produced CH$_4$. In the present study, it might be partially oxidized as well in the rhizosphere during the periods of D30–60 in the 2009 season (Fig. 6d), though its mean $\delta^{13}$C-value lingered around $-60\%$ and close to that of the CH$_4$ produced in anaerobic soil over the season (Fig. 7). When porewater CH$_4$ is released through the soil-water interface in paddy fields, it will be considerably oxidized, leaving the remainder temporarily in the floodwater. Since $^{12}$CH$_4$ is consumed faster than $^{13}$CH$_4$ by soil microbes, the residual CH$_4$ is then $^{13}$C-enriched [34]. As a consequence, floodwater CH$_4$ ($-45\%$) was more $^{13}$C-enriched than porewater CH$_4$ ($-60\%$). The observation of $\delta^{13}$C-value of the CH$_4$ produced in aerobic soil being more positive than that in anaerobic soil (Fig. 7) further demonstrates that CH$_4$ oxidation is intensive at the soil-water interface. In addition, rice roots can excrete O$_2$ thus forming an important CH$_4$-oxidizing zone in the rhizosphere. What is more, fresh rice roots per se have a strong CH$_4$ oxidation capacity [10,27,37]. CH$_4$ aerobically produced on the roots appeared to be more enriched in $^{13}$C ($-45\%$) than that in the soil ($-55\%$). It suggests that rice roots in the rhizosphere may be more important than the soil per se as driving force for CH$_4$ oxidation, thus causing more $^{12}$CH$_4$ consumed and leaving more $^{13}$CH$_4$ remained (Fig. 7).

Aerenchymatic CH$_4$ ($\sim -47\%$) was similar to oxidized CH$_4$ in $\delta^{13}$C-value (Fig. 7), which demonstrates that it has been strongly oxidized in the rhizosphere. In Italian paddy fields, Krüger et al. [10] also found that it stayed around $-50\%$ throughout the rice-growing season. After being emitted through transportation of the plants, aerenchymatic CH$_4$ was much heavier than emitted CH$_4$ (Fig. 7), due to the fact that $^{12}$CH$_4$ was transported from the plants at a faster rate than $^{13}$CH$_4$ [38]. By subtracting $\delta^{13}$C-value of aerenchymatic CH$_4$ from $\delta^{13}$C-value of emitted CH$_4$ the transport fractionation by the plants is quantified [10–12]. In theory, the transport fractionation is relatively small due to small CH$_4$ transport capacity of the plants at the beginning of the season. It gets strengthened together with the growth of the plants during the middle of the season but weakens again till the end of the season because of aging of the roots and plants. Consequently, value of fractionation ($\varepsilon_{\text{transport}}$) was the lowest during the middle of the season, and relatively high at the beginning and the end of the season because it was shown as negative (Table 2). Moreover, it was averaged around $-13\%$, suggesting that water management in the winter fallow season has little effect on CH$_4$ transport fractionation during the following rice-growing season. Similar $\varepsilon_{\text{transport}}$ was also observed in other field experiments [10–12]. The $\delta^{13}$C-value of emitted CH$_4$ fluctuated largely during the 2008 and 2009 rice seasons (Fig. 1b, f), and they were negatively related to CH$_4$ emission in seasonal variation ($r=-0.695 \sim -0.346, P<0.01$). An analogous relationship between them was also observed in other experiments [4,26], which was considered
to be the combined effect of CH₄ production, oxidation and transport in the fields [26,39,40]. Although water management in the winter fallow season played a key role in CH₄ emission from the rice fields (Fig. 1a, e), it had little impact on δ¹³C-value of emitted CH₄ (Fig. 1b, f). For the two seasons, mean value was ~ −60‰, being in the range of the measurements in previous report [26]. Compared with flooding, drainage had CH₄ relatively more depleted in ¹³C (Fig. 3b, e), but the CH₄ would become enriched was 5–15% higher (for detailed description, please see Section Effects on CH₄ oxidation below). In addition, there was no obvious difference in Δ¹³C between the two treatments (Table 2). Therefore, the ¹³C-depleted CH₄ in Treatment Drainage was supposed to be offset by the higher fraction of CH₄ oxidation, thus making the δ¹³C-value of emitted CH₄ from the two treatments similar.

Effects on CH₄ Production
Previous studies demonstrated that water management in the winter fallow season significantly affected CH₄ production during the following rice-growing season [8,9]. In the present study, it showed an important effect on CH₄ production of the fields by significantly affecting soil Eh. Methanogens are a kind of extreme anaerobic bacteria, which produce CH₄ under strict reductive conditions. Compared to the fields flooded in the winter fallow season, the fields drained were probably much lower in population and activity of methanogens [41–43] and it generally took a longer time for methanogens to revive during the following rice-growing season [44]. Therefore, drainage delayed and decreased CH₄ production in soil by significantly increasing soil Eh (Figs. 1c, g and 3a, d, Table 1). On roots as well, the lower the soil Eh, the higher the CH₄ production (Table 1) because roots get to age and decay faster if they are constantly under a stronger reductive condition [45,46]. A significant negative correlation between mean CH₄ production and soil Eh (Table 1, r = −0.805, P<0.01 for soil and r = −0.994, P<0.01 for roots, respectively) better demonstrated that soil Eh significantly affected by water management in the winter fallow season was a key factor that influenced CH₄ production. CH₄ concentration in soil pore water being generally lower in Treatment Drainage than in Treatment Flooding (Table 1) further showed that drainage decreased CH₄ production in the fields.

In paddy fields, CH₄ mainly comes from acetotrophic and hydrogenotrophic methanogenesis. Methanol-dependent methanogenesis may possibly be another contributor to the total CH₄ production, though, insignificant [47]. Relative contribution of acetotrophic methanogenesis (Facet) to the total CH₄ production can be calculated by following Eq. (7) if a fractionation factor of \( \alpha_{\text{CO}_2/\text{CH}_4} = 1.079 \) is used for CO₂-dependent methanogenesis and \( \delta^{13}\text{CH}_4 (\text{acetate}) \approx -43\% \) is for acetate-dependent methanogenesis based on the following reports [17,19]. In an Italian paddy soil, Fey et al. [19] found that \( \alpha_{\text{CO}_2/\text{CH}_4} \) decreasing with increasing temperature, was 1.083 at 10°C, 1.079 at 25°C, and 1.073 at 50°C, which was in good agreement with the relationships in marine sediment [48] and methanogenic cultures [49]. Therefore, \( \alpha_{\text{CO}_2/\text{CH}_4} = 1.079 \) was applied because the temperature during the two seasons varied in the range of 20–30°C with an average of 24°C. On the other hand, Fey et al. [19] demonstrated that \( \delta^{13}\text{CH}_4 (\text{acetate}) \) increased with increasing temperature, e.g., from −50 to −46‰ at 10°C to −45 to −36‰ at 25°C, and to −43 to −31‰ at 37°C. Moreover, the \( \delta^{13}\text{CH}_4 (\text{acetate}) \) values of −43 to −36‰ have even been applied considerably to experiments in the fields during the rice-growing season [10–12,36]. Due to lack of measurements, a constant value of −43‰ was hence used in the present study for better comparison with these reports. What is more important, it was more reasonable and suitable because \( \delta^{13}\text{C} \)-value of the soil organic carbon–substrate for methanogenesis, in this study was similar to those observed before [10,17,19]. Although they might be different in microbe habitats and varied with temperature and rice growth [10,17,19], the same values of \( \delta^{13}\text{CH}_4 (\text{acetate}) \) above mentioned have also been used [4,26,27].

The findings show that variation of \( F_{\text{acet}} \)-value in paddy soil during the 2008 rice season was similar to that during the 2009 rice season in pattern. That is, acetate-dependent methanogenesis dominated in the late season, while it was not so much important in the mid season, with \( F_{\text{acet}} \)-value being over 60–70% and less than 40%, respectively (Fig. 6a, b). In Italian paddy fields, measures also show that it was dominant at the end of the season [10]. Water management in the winter fallow season had an important impact on methanogenic pathways of paddy soil during the following rice-growing season. Generally, CH₄ from acetate cleavage dominated in Treatment Flooding during the two rice seasons, having a mean \( F_{\text{acet}} \)-value of 53–65%, which was 5–10% higher than in Treatment Drainage (Fig. 6a, b). Drainage increased production of oxidants, such as Fe³⁺ or sulphate, along with the increase in soil Eh [20,50]. As a result, the growth and activity of methanogens was probably out-competed by iron- or sulphate-reducing bacteria [31,32]. More importantly, acetotrophic methanogens seemed to be inhibited to a larger extent than hydrogenotrophic methanogens [20,53]. This suggests that soil Eh is an important indicator of pathways of methanogenesis in paddy fields—the higher the soil Eh, the more inhibited the acetotrophic methanogens, and the less the contribution of acetate to the total methanogenesis. In the present study therefore, drainage in the winter fallow season significantly increased soil Eh (Fig. 1c, g) and obviously decreased methanogenesis in paddy soil compared to flooding (Fig. 3a, d), and hence the contribution of acetate-dependent methanogenesis, probably ascribed to the fact that acetate-utilizing methanogens was more inhibited by any increase in soil Eh (Fig. 1c, g) [20]. Intermittent irrigation during the rice-growing season significantly reduced the contribution of acetate to CH₄ production, of which the finding could further verify this point of view [4].

The relative contribution of acetate-dependent methanogenesis on rice roots was similar to that in paddy soil in 2009, which was the lowest (almost 0%) in the mid season but rose up to the highest (~40%) at the end of the season (Fig. 6c). In total, \( F_{\text{acet}} \)-value was 1–32% in Treatment Flooding and 0–38% in Treatment Drainage, being much lower than that in the soil (Fig. 6). It indicates that methanogenesis on fresh rice roots is mostly from H₂/CO₂, and it is little affected by water management in the winter fallow season. Previous reports also show that \( F_{\text{acet}} \)-value of rice roots was less than 40% in most of the season [10]. In an incubation experiment with rice roots D75–80 old, Conrad et al. [17] found that CH₄ mainly came from H₂/CO₂-dependent methanogenesis as well throughout the entire observation, with an average \( F_{\text{acet}} \)-value of 47%. Compared with that of soil, the relative contribution of acetate to the total methanogenesis on the roots was lower by approximately 30% (Fig. 8), which is likely attributed to the difference in population of their dominant methanogens [54–56]. More exact measurements using stable isotope probing techniques have further demonstrated that CH₄ production on roots depends mainly on H₂/CO₂ reduction triggered by RC-I methanogens (Rice Cluster I Archaea) [57,58]. On the other hand, organic carbon slightly lighter in plant samples than in soil samples might be a possible reason for \( F_{\text{acet}} \)-value being much lower in paddy soil than on rice roots.
Effects on CH₄ Oxidation

CH₄ oxidation in soil seemed to be highly influenced by soil temperature rather than water management in the winter fallow season. Firstly, no significant difference was observed between flooding and drainage in mean oxidation potential during the 2008 and 2009 seasons (Table 1). Secondly, it varied with soil temperature (Figs. 1d, h and 6a, b), and a positive relationship was observed over the two seasons (r = 0.703–0.859, P<0.05), which is in good agreement with the previous report [9]. An appropriate soil temperature favors growth of methane-oxidizing bacteria, thus enhancing their capacity of CH₄ oxidation [59]. The higher the soil temperature within the range of 12.5–34.8°C, the higher the CH₄ oxidation rate [60], which is consistent with our observations. Considering measurements on fresh roots have shown that the roots per se have a high CH₄ oxidation capacity [10,37,61]. In the present study, CH₄ oxidation on the roots was the strongest at the beginning of the season and weakened later on (Fig. 6c), being in agreement with the previous reports [10,27]. Drainage compared to flooding in the winter fallow season significantly decreased CH₄ oxidation potential on the roots (Fig. 6c), probably attributed to the effect of flooding high CH₄ production (Fig. 4a). Higher concentration of CH₄ stimulated growth and activity of the methanotrophs on the surface of the roots, thus raising their CH₄ oxidation capacity [59].

The fraction of CH₄ oxidation (Fₑ) can be quantified by measuring δ¹³C-value of CH₄ from various compartments of the paddy fields with a special model in case some parameters (xₑ and k_transport) are already available [10–13]. The potential shift in the carbon isotopes during the CH₄ oxidation (fractionation factor xₑ = 1.025–1.030) was firstly determined in methanotrophs enriched cultures [62] and then considerably in landfill cover soils at a temperature of about 25°C [63–65]. Interestingly, the value of 1.025–1.038 has been widely applied to field conditions [10–12,24,25] though the knowledge of xₑ in paddy soil is still incomplete. Very recently, we have found xₑ = 1.025–1.033 at 28.3°C in a Chinese paddy soil [27]. Consequently, the value of 1.038 was used in the present study due to the similar temperature during the seasons, and more reasonable results would be obtained (Fig. 9). On the other hand, the transport fractionation factor k_transport was equivalent to the difference in ¹³C between emitted and aerenchymatic CH₄ (Table 2), ranging from –16 to –11% in the 2009 season. In 2008 however, no corresponding measurements were performed. Nevertheless, the averaged value of –13% in 2009 was applied to the 2008 field data (Fig. 9), because it was also very close to previous observations [10–12]. Since fractionation factors (xₑ and k_transport) are influenced by temperature, microbes, soil property, and rice growth [10,64], more attention thereby need further be paid to getting reliable and exact values of CH₄ oxidation in paddy fields.

Similar to the potentials of CH₄ oxidation, the fraction of CH₄ oxidized in the rhizosphere was relatively high (as high as 60–90%) in the first half of the rice growth period during the 2008 and 2009 seasons and relatively low (~10–30%) in the remainder periods (Fig. 9a, b). In Italian paddy fields, measurements also show that CH₄ oxidation was very important at the beginning of the season but became slight later, with Fₑ-value decreasing rapidly from approximately 40 to 0% [10,20,24]. Under unfertilized microcosms, Conrad and Klose [25] obtained that Fₑ-value decreased from about 15% in the beginning to about 5% at the end, which was probably attributed to nitrogen-limitation of the methanotrophs [10,20,24]. On the whole, mean value of Fₑ was 35–55% in Treatment Drainage, being 5–15% higher than that in Treatment Flooding during the 2008 and 2009 seasons (Fig. 9a, b). It suggests that compared to flooding in the winter fallow season drainage can increase the proportion of CH₄ oxidized during the following rice-growing season. Probable reason was that drainage significantly decreased CH₄ production (Fig. 5a, d) while it did not simultaneously affect CH₄ oxidation in the field (Fig. 6a, b).

When CH₄ in soil pore water passed through the soil-water interface into the floodwater, intensive signals of CH₄ oxidation were observed by following changes in isotopic signature between them (Fig. 7). An obvious oxidation signal was also observed of the CH₄ production in a Chinese paddy soil [27]. Consequently, the value of δ¹³C in a Chinese paddy soil [27]. Interestingly, the value of 1.025–1.038 has been widely applied to field conditions [10–12,24,25] though the knowledge of xₑ in paddy soil is still incomplete. Very recently, we have found xₑ = 1.025–1.033 at 28.3°C in a Chinese paddy soil [27]. Consequently, the value of 1.038 was used in the present study due to the similar temperature during the seasons, and more reasonable results would be obtained (Fig. 9). On the other hand, the transport fractionation factor k_transport was equivalent to the difference in ¹³C between emitted and aerenchymatic CH₄ (Table 2), ranging from –16 to –11% in the 2009 season. In 2008 however, no corresponding measurements were performed. Nevertheless, the averaged value of –13% in 2009 was applied to the 2008 field data (Fig. 9), because it was also very close to previous observations [10–12]. Since fractionation factors (xₑ and k_transport) are influenced by temperature, microbes, soil property, and rice growth [10,64], more attention thereby need further be paid to getting reliable and exact values of CH₄ oxidation in paddy fields.

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When CH₄ in soil pore water passed through the soil-water interface into the floodwater, intensive signals of CH₄ oxidation were observed by following changes in isotopic signature between them (Fig. 7). An obvious oxidation signal was also observed of the dissolved CH₄ approaching to soil surface [11,12]. Therefore, Fₑ-value was reasonably calculated based on δ¹³C-value of CH₄ in pore water for δ¹³CH₄ (initial) and on δ¹³C-value of CH₄ in floodwater for δ¹³CH₄ (final). It was high on D16 and D88, but relatively low on D47 and D50, especially in Treatment Flooding (Table 3). As the emission of CH₄ in the fields goes absolutely incomplete. Very recently, we have found xₑ = 1.025–1.033 at 28.3°C in a Chinese paddy soil [27]. Consequently, the value of 1.038 was used in the present study due to the similar temperature during the seasons, and more reasonable results would be obtained (Fig. 9). On the other hand, the transport fractionation factor k_transport was equivalent to the difference in ¹³C between emitted and aerenchymatic CH₄ (Table 2), ranging from –16 to –11% in the 2009 season. In 2008 however, no corresponding measurements were performed. Nevertheless, the averaged value of –13% in 2009 was applied to the 2008 field data (Fig. 9), because it was also very close to previous observations [10–12]. Since fractionation factors (xₑ and k_transport) are influenced by temperature, microbes, soil property, and rice growth [10,64], more attention thereby need further be paid to getting reliable and exact values of CH₄ oxidation in paddy fields.
Methanogenic Pathway and Fraction of CH$_4$ Oxidized

Therefore, the fraction of CH$_4$ that was oxidized in aerobic conditions could be estimated directly by using Eq. (8) based on $\delta^{13}$C-values of aerobic CH$_4$ produced in soil (Fig. 3b, e) for $\delta^{13}$CH$_4$$_{\text{initial}}$, and $\delta^{13}$C-values of emitted CH$_4$ (Fig. 1b) minus –13.0% for both treatments in 2008 but (Fig. 1f) minus –13.3% for flooding and –12.8% for drainage in 2009 for $\delta^{13}$CH$_4$$_{\text{final}}$. $F_{ox}$ in (c) and (d) was calculated in 2009 with Eq. (8) using 1.038 for $\delta^{13}$CH$_4$$_{\text{initial}}$, and $\delta^{13}$C-values of CH$_4$ aerobically produced in soil (Fig. 5b) and on roots (Fig. 5d) for $\delta^{13}$CH$_4$$_{\text{final}}$. TS, BS, FS and RS represent tillering, booting, grain-filling and ripening stages, respectively. Bars represent standard errors (n = 3).

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Figure 9. Temporal variation of the fraction of CH$_4$ oxidized ($F_{ox}$) in the rhizosphere and at the surfaces of paddy soil and rice roots. $F_{ox}$ in (a) and (b) was calculated with Eq. (8) using 1.038 for $\delta^{13}$C-values of CH$_4$ anaerobically produced in soil (Fig. 3b, e) for $\delta^{13}$CH$_4$$_{\text{initial}}$, and $\delta^{13}$C-values of emitted CH$_4$ (Fig. 1b) minus –13.0% for both treatments in 2008 but (Fig. 1f) minus –13.3% for flooding and –12.8% for drainage in 2009 for $\delta^{13}$CH$_4$$_{\text{final}}$. $F_{ox}$ in (c) and (d) was calculated in 2009 with Eq. (8) using 1.038 for $\delta^{13}$C-values of CH$_4$ anaerobically produced in soil (Fig. 3e) and on roots (Fig. 4b) for $\delta^{13}$CH$_4$$_{\text{initial}}$, and $\delta^{13}$C-values of CH$_4$ aerobically produced in soil (Fig. 5b) and on roots (Fig. 5d) for $\delta^{13}$CH$_4$$_{\text{final}}$. TS, BS, FS and RS represent tillering, booting, grain-filling and ripening stages, respectively. Bars represent standard errors (n = 3).

In lab conditions, the difference between anaerobic and aerobic CH$_4$ productions in the soil was apparently attributed to CH$_4$ oxidation at the soil-water interface. Notably, Kruger et al. [10] had even pointed out that porewater CH$_4$ was a poor indicator of produced CH$_4$. Therefore, CH$_4$ in soil pore water on D47 and D50 in the present study might be oxidized partially as well. As a result, its $\delta^{13}$C-value would be significantly lower than those in the rhizosphere, and may be negligible.

In lab conditions, the difference between anaerobic and aerobic CH$_4$ productions in the soil was apparently attributed to CH$_4$ oxidation at the soil-water interface. Thus, it can be estimated that CH$_4$ oxidation in the fields.

Conclusions

Through the field and laboratory experiments, we investigated $\delta^{13}$C in every process of CH$_4$ emission from rice fields as affected by water management in the winter fallow season and further estimated pathways of CH$_4$ production and fraction of CH$_4$ oxidation using the stable carbon isotope technique. Compared...
CO2-dependent methanogenesis occurred mostly on the rice roots oxidized in the soil, while almost all on the roots. Moreover, CH4 production by 5–10%. In field conditions, CH4 was oxidized in the rhizosphere, while drainage increased the fraction of CH4 oxidation was more important in the first half of the season as well as in the rhizosphere. Drainage increased the fraction of CH4 oxidized in the rhizosphere by 5–15%, which is possibly attributed to the fact that CH4 production decreased significantly while CH4 oxidation did not simultaneously. Measuring δ13C-values of the CH4 from different pools in the rice fields is useful for quantifying the methanogenic pathway and the fraction of CH4 oxidized in these fields. More importantly, it is useful for better understanding the processes of CH4 emission, which may provide useful information for setting up an isotope model. Such a model may be of a great help to national or global CH4 budget. Therefore, more attentions should be paid to the paddy fields with more different patterns of agricultural management at a larger scale.

**Author Contributions**

Conceived and designed the experiments: HX JM GZ. Performed the experiments: GZ JM GL YZ. Analyzed the data: HX GZ. Contributed reagents/materials/analysis tools: HX KY. Wrote the paper: GZ HX. Obtained permission for use: HX GZ.

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**Table 3. Fraction of CH4 oxidized (Fo) at the soil-water interface during the 2009 rice season (mean ± SD, n = 3).**

| Days after rice transplanting (d) | δ13CH4_initial (%a) | δ13CH4_final (%b) | Fo (%c) |
|----------------------------------|---------------------|-------------------|---------|
| Flooding                         | Drainage            | Flooding           | Drainage|
| 16                               | −65.98 ± 1.58       | −50.24 ± 1.66     | 45 ± 9  |
| 47                               | −54.43 ± 0.21       | −43.13 ± 1.27     | 32 ± 3  |
| 50                               | −56.52 ± 0.72       | −43.09 ± 1.25     | 38 ± 4  |
| 88                               | −65.79 ± 3.37       | −40.34 ± 5.15     | 72 ± 0  |

*δ13C-values of CH4 in soil pore water (Fig. 2d); δ13C-values of CH4 in floodwater (Fig. 2d).
*Calculated with Eq. (8) using \( z_{avg} = 1.038 \).
*\( \alpha \) indicates for use: HX GZ.
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