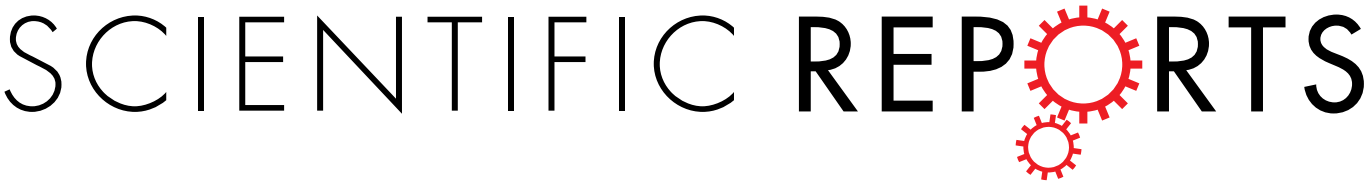

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# Carbon isotope fractionation reveals distinct process of $CH_4$ emission from different compartments of paddy ecosystem

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Guangbin Zhang[1], Haiyang Yu[1,2], Xianfang Fan[1,2], Jing Ma[1] & Hua Xu[1]

**Carbon isotopic fractionations in the processes of $CH_4$ emission from paddy field remain poorly understood. The $\delta^{13}C$-values of $CH_4$ in association with production, oxidation and transport of $CH_4$ in different pools of a paddy field were determined, and the stable carbon isotope fractionations were calibrated to assess relative contribution of acetate to $CH_4$ production ($f_{ac}$) and fraction of $CH_4$ oxidized ($f_{ox}$) by different pathways. The apparent isotope fractionation for $CO_2$ conversion to $CH_4$ ($\alpha_{app}$) was 1.041–1.056 in the soil and 1.046–1.080 on the roots, indicating that $f_{ac}$ was 10–60% and 0–50%, respectively. Isotope fractionation associated with $CH_4$ oxidation ($\alpha_{ox}$) was $1.021 \pm 0.007$ in the soil and $1.013 \pm 0.005$ on the roots, and the transport fractionation ($\varepsilon_{transport}$) by rice plants was estimated to be $-16.7‰$ ~ $-11.1‰$. Rhizospheric $f_{ox}$ was about 30–100%, and it was more important at the beginning but decreased fast towards the end of season. Large value of $f_{ox}$ was also observed at the soil-water interface and soil and roots surfaces, respectively. The results demonstrate that carbon isotopic fractionations which might be different in different conditions were sensitive to the estimations of $f_{ac}$ and $f_{ox}$ in paddy field.**

Methane ($CH_4$) is the second most important greenhouse gas after carbon dioxide ($CO_2$). On a 100-year horizon, $CH_4$ has 25 times the global warming potential of $CO_2$. Paddy fields are one of the largest anthropogenic sources of atmospheric $CH_4$, contributing to 33–40 Tg $yr^{-1}$ during the 2000–2009[1]. The global $CH_4$ emission from paddy fields will continually increase by intensification of rice cultivation and expansion of planting area to meet the demands of the growing populations[2–4]. Paddy $CH_4$ emission is an integrated effect of the production, oxidation and transport of $CH_4$ in the field. A better knowledge of these processes affecting $CH_4$ emission may provide more information for effectively mitigating $CH_4$ emission in agricultural ecosystems.

The technique of stable carbon isotopes has been proved to be a useful tool in studying the processes of $CH_4$ emission[5–9]. Isotope fractionation happens in all the major processes $CH_4$ emission, namely, $^{12}C$-substrate is preferentially utilized by methanogens for $CH_4$ production, and once formed, $^{12}CH_4$ is consumed faster than $^{13}CH_4$ by methanotrophs, and $^{12}CH_4$ is transported faster than $^{13}CH_4$ as well[10–12]. Thereby, measurements of the $\delta^{13}C$ in production, oxidation and transport of the $CH_4$ from different pools of the field are benefical to support a process-based model for $CH_4$ emission[8,13,14]. Moreover, the relative contribution of acetate to $CH_4$ production ($f_{ac}$) and the fraction of $CH_4$ oxidized ($f_{ox}$) can be quantitatively estimated[5–7] using mass balance equations based on the measurements of $\delta^{13}C$ in $CH_4$, $CO_2$ and acetate, and of the isotope fractionation factors ($\alpha_{CO_2/CH_4}$, $\varepsilon_{acetate/CH_4}$, $\alpha_{ox}$ and $\varepsilon_{transport}$). Investigations on $\alpha_{CO_2/CH_4}$, $\varepsilon_{acetate/CH_4}$ and $\varepsilon_{transport}$ of paddy fields were carried out greatly[5,15–18], however, few data are available on $\alpha_{ox}$ for $CH_4$ oxidation by methanotrophs in paddy soils, in particular $\alpha_{ox}$ on rice roots[19].

Some uncertainties also exist in the $\delta^{13}CH_4$ that are used as newly produced $\delta^{13}CH_4$ ($\delta^{13}CH_{4\,(original)}$) and finally oxidized $\delta^{13}CH_4$ ($\delta^{13}CH_{4\,(oxidized)}$) in different studies for quantifing $f_{ac}$ and $f_{ox}$. For example, former reports in USA using porewater $\delta^{13}CH_4$ as $\delta^{13}CH_{4\,(original)}$[6,7] whereas anaerobically produced $\delta^{13}CH_4$ was used in Italy[5,20] and China[19,21]. They believed that porewater $CH_4$ was a poor proxy for $\delta^{13}CH_{4\,(original)}$ as it was potentially affected by $CH_4$ oxidization and transport in field conditions[11,22]. Similarly, various $\delta^{13}CH_4$, such as rhizospheric $\delta^{13}CH_4$,

[1]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China. [2]University of Chinese Academy of Sciences, Beijing 100049, China. Correspondence and requests for materials should be addressed to H.X. (email: hxu@issas.ac.cn)

| Days after rice transplanting (d) | Production | | $\delta^{13}CH_4$ | | $\delta^{13}CO_2$ | | $\alpha_{app}$ | |
|---|---|---|---|---|---|---|---|---|
| | Soil | Root | Soil | Root | Soil | Root | Soil | Root |
| 20 | 0.13 ± 0.18 | 3.4 ± 0.7 | −71.1 ± 2.4 | −69.4 ± 2.8 | −18.8 ± 2.9 | −15.0 ± 2.3 | 1.056 ± 0.005 | 1.058 ± 0.002 |
| 50 | 2.15 ± 0.21 | 11.1 ± 2.2 | −64.4 ± 0.4 | −86.9 ± 3.5 | −17.0 ± 1.8 | −14.1 ± 1.7 | 1.051 ± 0.002 | 1.080 ± 0.003 |
| 88 | 0.38 ± 0.12 | 4.5 ± 0.6 | −57.5 ± 1.1 | −66.6 ± 2.7 | −15.1 ± 0.5 | −24.0 ± 2.9 | 1.045 ± 0.001 | 1.046 ± 0.005 |
| 108 | 0.22 ± 0.03 | 3.2 ± 0.9 | −53.9 ± 0.2 | −72.5 ± 2.9 | −14.9 ± 0.4 | −23.3 ± 2.8 | 1.041 ± 0.000 | 1.053 ± 0.001 |

**Table 1. $CH_4$ production potentials ($\mu$g $CH_4$ $g^{-1}$ $d^{-1}$), $\delta^{13}C$-values (‰) of $CH_4$ and $CO_2$ in the soil and on the roots under anaerobic incubation, and the corresponding apparent fractionation ($\alpha_{app}$) between $CO_2$ and $CH_4$ calculated by the ratio of ($\delta^{13}CO_2 + 1000$)/($\delta^{13}CH_4 + 1000$).**

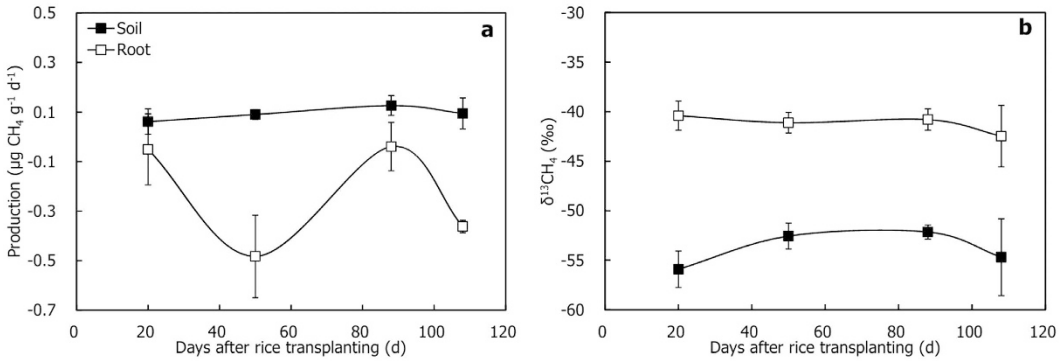


**Figure 1.** Temporal variations of $CH_4$ production rates in the soil and on the roots under aerobic (**a**,**b**) incubation, and corresponding $\delta^{13}CH_4$.

aerobically produced $\delta^{13}CH_4$, porewater $\delta^{13}CH_4$ or floodwater $\delta^{13}CH_4$ sometimes, have been regarded as $\delta^{13}CH_{4\,(oxidized)}$ for estimation of $f_{ox}$ in the rhizosphere or at the soil-water interface[5–7,9,19,23]. More importantly, large differences were observed in the estimated $f_{ac}$ and $f_{ox}$ if different $\delta^{13}CH_{4\,(original)}$ and $\delta^{13}CH_{4\,(oxidized)}$ was assumed in the same study[5,7]. Therefore, more comparable observations in different conditions with corresponding isotope fractionation factors are needed to discuss $\delta^{13}CH_{4\,(original)}$ and $\delta^{13}CH_{4\,(oxidized)}$ in accurate estimations of $f_{ac}$ and $f_{ox}$.

In this study, field and incubation experiments were conducted to observe the process of $CH_4$ emission closely related to the production, oxidation and transport of $CH_4$, including $CH_4$ fluxes emitted from the field and via the plants, $CH_4$ concentrations in the aerenchyma of the plants, and in soil pore water and floodwater, $CH_4$ production and oxidation rates in the soil and on the roots, and all the corresponding $\delta^{13}CH_4$. The objectives of the present study were (1) to improve our understanding of the processes in $CH_4$ emission by measurements of the stable carbon isotopes, (2) to investigate the isotope fractionation factor $\alpha_{ox}$ in the soil and on the roots, and (3) to discuss the availabilities in the estimation of $f_{ac}$ and $f_{ox}$ associated with different pools of $\delta^{13}CH_4$ in the field.

## Results

**$CH_4$ production and $\delta^{13}C$ of $CH_4$ and $CO_2$.** In anaerobic incubation, both $CH_4$ production potentials in the soil and on the roots were relatively low on 20 days after rice transplanting (D20), peaked (2.2 $\mu$g $CH_4$ g $soil^{-1}$ $d^{-1}$ and 11.1 $\mu$g $CH_4$ g $root^{-1}$ $d^{-1}$) on D50, and then turned downwards gradually to the bottom on D108 (Table 1). For $\delta^{13}C$-value of the produced $CH_4$, it was more and more positive in the soil during the whole observational period, being from −71.1‰ to −53.9‰ (Table 1). For $CH_4$ produced on the roots however, it was most $^{13}C$-depleted on D50 and then $^{13}C$-enriched again on D88, with $\delta^{13}C$-value ranging between −86.9‰ and −66.6‰ (Table 1). Throughout the whole season, $\delta^{13}C$-value of produced $CO_2$ increased from −18.8‰ to −14.9‰ in the soil while decreased from −15.0‰ to −23.3‰ on the roots (Table 1). $CH_4$ production under aerobic incubation was hardly observed in the soil (0.06 to 0.13 $\mu$g $CH_4$ g $soil^{-1}$ $d^{-1}$), particularly on the roots, which was lower than 0 $\mu$g $CH_4$ g $root^{-1}$ $d^{-1}$ over the whole season (Fig. 1a). The $\delta^{13}C$-value of $CH_4$ produced in the soil was relatively stable around −55‰ while on the roots it was about −40‰ (Fig. 1b). Apparently, the $\delta^{13}C$-values of $CH_4$ produced in aerobic incubation were significantly higher ($P < 0.05$) than those of the $CH_4$ that was produced in anaerobic incubation (Table 1 and Fig. 1).

**$CH_4$ concentration and $\delta^{13}C$ of $CH_4$ and $CO_2$.** $CH_4$ concentration in soil pore water was more than 100 $\mu$M $L^{-1}$ in most part of the season (Fig. 2), and it was highest (~120 $\mu$M $L^{-1}$) on D50. $CH_4$ concentration in floodwater was in the range of 0.21–2.6 $\mu$M $L^{-1}$, being significantly lower than that of soil pore water over the season ($P < 0.01$). The $\delta^{13}C$-values of $CH_4$ in soil pore water and floodwater appeared to increase simultaneously (Fig. 2), from ~−70‰ to −60‰ and from ~−50‰ to −40‰, respectively. Obviously, $CH_4$ in soil pore water was more depleted in $^{13}C$ than that of floodwater $CH_4$ ($P < 0.05$), indicating that porewater $CH_4$ was intensively affected by $CH_4$ oxidation at the soil-water interface when it released into the atmosphere. $CO_2$ in soil pore water tended to $^{13}C$-enriched gradually during the rice season, with $\delta^{13}C$-values ranging from −20.0‰ to −14.5‰ (Fig. 2).

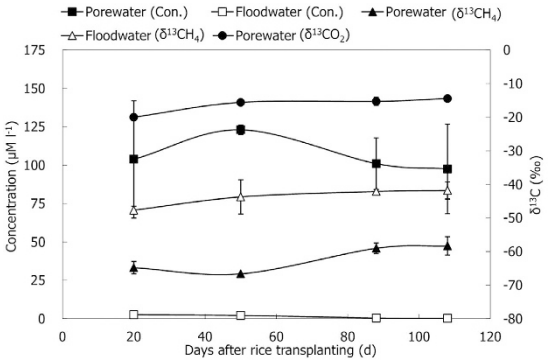


**Figure 2. Temporal variations of $CH_4$ concentrations in soil pore water and floodwater, and corresponding $\delta^{13}C$-values of $CH_4$ and $CO_2$.**

| Days after rice transplanting (d) | Oxidation | | $\delta^{13}CH_{4\,(initial)}$ | | $\delta^{13}CH_{4\,(final)}$ | | $\alpha_{ox}$ | |
|---|---|---|---|---|---|---|---|---|
| | Soil | Root | Soil | Root | Soil | Root | Soil | Root |
| 20 | 4.4 ± 1.4 | 580 ± 116 | −38.4 ± 1.6 | −38.7 ± 1.9 | −35.6 ± 2.2 | −34.0 ± 2.8 | 1.014 ± 0.002 | 1.019 ± 0.005 |
| 50 | 6.9 ± 1.1 | 335 ± 84 | −41.0 ± 0.4 | −40.4 ± 0.7 | −35.0 ± 1.7 | −26.5 ± 1.9 | 1.020 ± 0.002 | 1.012 ± 0.007 |
| 88 | 5.1 ± 1.9 | 454 ± 68 | −38.7 ± 1.1 | −40.5 ± 2.6 | −32.5 ± 1.0 | −30.6 ± 2.2 | 1.030 ± 0.004 | 1.015 ± 0.003 |
| 108 | 2.3 ± 1.3 | 258 ± 78 | −40.3 ± 0.0 | −36.0 ± 0.2 | −38.4 ± 0.2 | −31.2 ± 3.4 | 1.021 ± 0.009 | 1.008 ± 0.009 |

**Table 2. $CH_4$ oxidation potentials ($\mu$g $CH_4$ $g^{-1}$ $d^{-1}$), $\delta^{13}C$-values (‰) of $CH_4$ at time 0 ($\delta^{13}CH_{4\,(initial)}$) and at time t ($\delta^{13}CH_{4\,(final)}$) in the soil and on the roots under aerobic incubation with high $CH_4$ concentration supplemented, and the corresponding $CH_4$ oxidation fractionation factor ($\alpha_{ox}$) calculated by the Equation (5).**

**$CH_4$ oxidation and $\delta^{13}C$ of $CH_4$.** $CH_4$ oxidation potential in the soil peaked on D50 (6.9 $\mu$g $CH_4$ g $soil^{-1}$ $d^{-1}$), and then it dropped gradually to the lowest on D108 (Table 2). In contrast, it was highest on the roots (580 $\mu$g $CH_4$ g $root^{-1}$ $d^{-1}$) on D20 and decreased sharply on D50. After an increase on D88, it decreased again to the lowest on D108 (Table 2). The $\delta^{13}C$-values of $CH_4$ before oxidization were −41.0‰ ~ −38.4‰ in the soil and −40.5‰ ~ −36.0‰ on the roots. After $CH_4$ oxidization, the $CH_4$ both in the soil and on the roots were more enriched in $^{13}C$, with $\delta^{13}C$-values of −38.4‰ ~ −32.5‰ and −34.0‰ ~ −26.5‰, respectively (Table 2).

**Plants emitted and aerenchymatic $CH_4$.** On the three sampling days (D37, D62 and D98) during the season, $CH_4$ emitted via the plants was relatively stable with $\delta^{13}C$-values of −63.9‰, −62.6‰ and −63.5‰, respectively. For $\delta^{13}C$-values of aerenchymatic $CH_4$, they were −49.2‰, −45.9‰ and −52.4‰, respectively, being significantly higher in comparison of the emitted $CH_4$ ($P < 0.05$). As a result, the isotope fractionations due to $CH_4$ transport ($\varepsilon_{transport}$) were measured to be −14.7‰, −16.7‰ and −11.1‰, respectively, with a mean value of −14.2‰.

**$CH_4$ emission and $\delta^{13}C$ of $CH_4$.** The $CH_4$ flux varied significantly, with the highest value appeared on D50 and the lowest on D108, ranging from 0.4 to 11.5 mg $CH_4$ $m^{-2}$ $h^{-1}$ during the observational period (Fig. 3a). The $\delta^{13}CH_{4\,(emission)}$ varied between −68.7‰ and −61.5‰ with the variation pattern just opposite to that of $CH_4$ flux (Fig. 3a). It is noteworthy that a significant negative relationship between $CH_4$ flux and corresponding $\delta^{13}CH_{4\,(emission)}$ was observed (Fig. 3b). Soil temperature ranged from 17.2 °C to 30.5 °C during the rice season, with a value of 24.5 °C on average.

**$\delta^{13}C$ of organic carbon in soil and plant samples.** The values of $\delta^{13}C$ in soil organic carbon did not show much variation during the rice season, being −26.84‰ on D37 and −27.66‰ on D108, respectively. The organic carbon in the plant samples also remained relatively stable over the season, with $\delta^{13}C$-values being −29.19‰ on D37 and −28.70‰ on D108, respectively, although they were slightly lighter than those of the soil organic carbon.

## Discussion

**$CH_4$ production.** The processes of $CH_4$ production, oxidation, transport and emission from paddy field were well presented by the measurements of stable carbon isotopes in $CH_4$ from different pools of the field (Fig. 4). The decomposition of plants debris and root exudates, besides soil organic matters in the bulk soil, is very important to methanogenesis in paddy field[24]. As a key precursor for methanogens, it was slight $^{13}C$-depletion on the roots relative to soil organic carbon (Fig. 4). Previous studies also showed $\delta^{13}C$-value of organic carbon relatively negative in the plant than in the soil[5,19]. Paddy field $CH_4$ is mainly produced out of either cleavage of acetate ($f_{ac}$) or reduction of $H_2/CO_2$ ($1 - f_{ac}$), and the $\delta^{13}C$-value of produced $CH_4$ primarily depends on relative contribution of the two main methanogenic pathways[10,25]. The $f_{ac}$ was calculated by the following mass balance[6,7]:

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

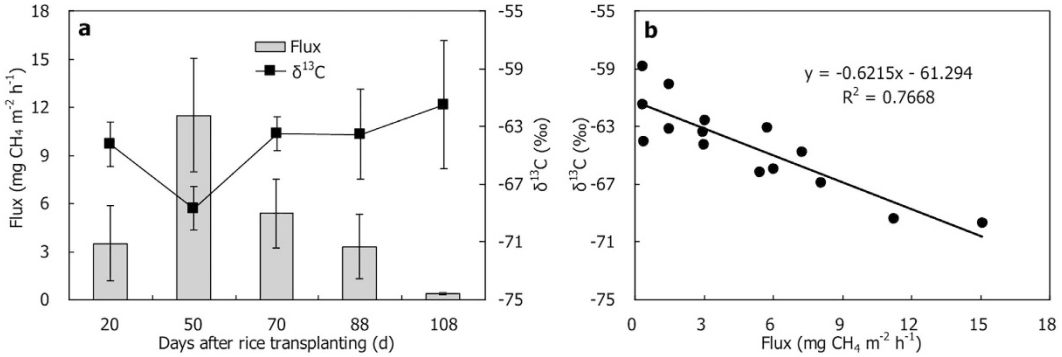


**Figure 3.** Temporal variations of $CH_4$ flux and $\delta^{13}CH_4$ (**a**) and they relationship (**b**).

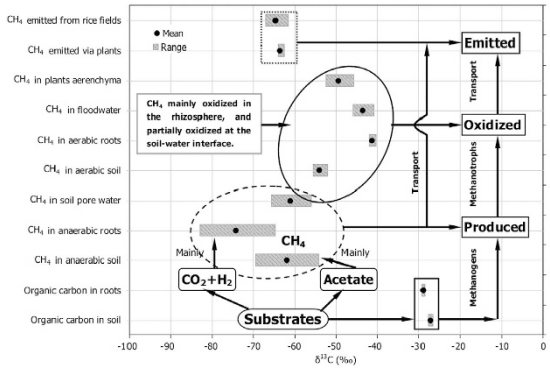


**Figure 4. Stable carbon isotopes in the processes of $CH_4$ emission from the paddy field.** Note: each $\delta^{13}C$-value was given in arithmetic mean of the rice season.

| Days after rice transplanting (d) | $f_{ac}{}^{a}$ | | | | $f_{ac}{}^{b}$ | | | |
|---|---|---|---|---|---|---|---|---|
| | $\delta^{13}CH_{4\,(acetate)} = -37‰$ | | $\delta^{13}CH_{4\,(acetate)} = -43‰$ | | $\delta^{13}CH_{4\,(acetate)} = -37‰$ | | $\delta^{13}CH_{4\,(acetate)} = -43‰$ | |
| | $\alpha_{CO_2/CH_4}$=1.050 | $\alpha_{CO_2/CH_4}$=1.060 | $\alpha_{CO_2/CH_4}$=1.050 | $\alpha_{CO_2/CH_4}$=1.060 | $\alpha_{CO_2/CH_4}$=1.070 | $\alpha_{CO_2/CH_4}$=1.080 | $\alpha_{CO_2/CH_4}$=1.070 | $\alpha_{CO_2/CH_4}$=1.080 |
| 20 | −21 ± 4 | 8 ± 3 | −27 ± 4 | 9 ± 3 | 24 ± 6 | 37 ± 7 | 28 ± 7 | 41 ± 8 |
| 50 | −2 ± 6 | 23 ± 3 | −3 ± 7 | 28 ± 3 | −20 ± 1 | 0 ± 0 | −23 ± 2 | 1 ± 0 |
| 88 | 18 ± 3 | 39 ± 2 | 24 ± 4 | 48 ± 3 | 42 ± 2 | 50 ± 15 | 47 ± 2 | 56 ± 17 |
| 108 | 32 ± 0 | 50 ± 0 | 42 ± 0 | 61 ± 0 | 29 ± 0 | 39 ± 11 | 33 ± 0 | 44 ± 12 |

**Table 3. Relative contribution of acetate to total $CH_4$ production (%) in the soil ($f_{ac}{}^{a}$) and on the roots ($f_{ac}{}^{b}$).** $f_{ac}{}^{a}$ and $f_{ac}{}^{b}$ was calculated with Equation (2) using $\delta^{13}C$-values of $CH_4$ anaerobically produced in the soil and on the roots (Table 1) as originally produced $\delta^{13}CH_4$, respectively.

During the process of acetate fermentation forming $CH_4$, isotopic fractionation occurs and the fractionation factor is generally expressed to $\varepsilon_{acetate/CH_4}$. It was found to be −21‰ in pure cultures of acetoclastic *Methanosarcina barkeri*[26] and −18‰ for acetoclastic *Methanosaeta concilii*[15,27]. Using $\varepsilon_{acetate/CH_4} = -21‰$, Krüger *et al.*[5] estimated $\delta^{13}C$ of $CH_4$ produced from acetate ($\delta^{13}CH_{4\,(acetate)}$) between −43‰ and −37‰ according to the measurements of $\delta^{13}C_{acetate}$ (−22‰ ~ −16‰) in soil pore water of an Italian rice field. Meanwhile, both values of −43‰ and −37‰ have well been applied in many studies[6,7,9,16,19,21]. Due to a lack of knowledge on $\varepsilon_{acetate/CH_4}$ and in order to compare the data interpretation with those of above mentioned, both $\delta^{13}CH_{4\,(acetate)}$ of −43‰ and −37‰ were used in the present study (Table 3).

When $H_2/CO_2$ reduction produces $CH_4$, isotopic fractionation factor $\alpha_{CO_2/CH_4}$ is defined by Hayes[28]:

$$\alpha_{CO_2/CH_4} = (\delta^{13}CO_2 + 1{,}000)/(\delta^{13}CH_{4(H_2/CO_2)} + 1{,}000) \quad (2)$$

where $\delta^{13}CH_{4(H_2/CO_2)}$ is $\delta^{13}C$ of the $CH_4$ produced from $H_2/CO_2$ reduction. In addition, based on the ratio of $\delta^{13}CO_2$ to $\delta^{13}CH_4$ in anaerobic incubation (Table 1), an approximation of apparent fractionation ($\alpha_{app}$) between $CO_2$ and $CH_4$ can be calculated by using $\alpha_{app} = (\delta^{13}CO_2 + 1000)/(\delta^{13}CH_4 + 1000)$. The $\alpha_{app}$ is calculated from the isotopic signatures of total $CH_4$ produced from $H_2/CO_2$ reduction and acetate cleavage, and theoretically, it is lower than $\alpha_{CO_2/CH_4}$. Results of 16 different lake sediments from tropical freshwater wetlands in Brazil[29] have well

demonstrated that $\alpha_{CO_2/CH_4}$ (1.075 ± 0.008) is much higher than $\alpha_{app}$ (1.059 ± 0.009). In this study (Table 1), the $\alpha_{app}$ decreased gradually from 1.056 on D20 to 1.041 on D108 for the soil. In contrast, it increased sharply from 1.058 on D20 to 1.080 on D50, and then decreased again to 1.053 for the roots. Totally, $\alpha_{app}$ was relatively lower in the soil (1.041–1.056) than on the roots (1.046–1.080). The $\alpha_{CO_2/CH_4}$ was hence assumed to be 1.050–1.060 in the soil and 1.070–1.080 on the roots (Table 3). Incubating three different soils, Conrad *et al.*[17] also found that $\alpha_{CO_2/CH_4}$ was between 1.050 and 1.060 for two paddy soils. Additionally, previous studies approved the relatively larger $\alpha_{CO_2/CH_4}$ (≥1.070) on the roots than in the soil due to their methanogenic populations were different[16,30].

The $CH_4$ produced in anaerobic incubation changed significantly during the rice season, and it was much more $^{13}C$-enriched in the soil than on the roots (Table 1). It indicates that methanogenic pathway was changed with rice growing, and also demonstrates that acetate-dependant methanogenesis was more important in the soil. In this study, $f_{ac}$ in the soil was initially very low (<10%) on D20, but it increased obviously with the rice growing and reached over 60% on D108. In contrast, $f_{ac}$ on the roots was relatively high (~30–40%) on D20. It decreased sharply in the middle of the season (near 0%) and then increased again to about 50% on D108. As a whole, $f_{ac}$ was relatively higher in the soil than that on the roots (Table 3). Previous study in Italian paddy soil has also demonstrated that acetoclastic methanogenesis was higher than 60% at the end of the season[5]. High contribution of $H_2/CO_2$-dependent methanogenesis to total $CH_4$ production on rice roots was considerably reported[5,9,16,19], and the major reasons were supposed to be the methanogens population on rice roots dominated by Rice Cluster I archaea[31–33]. Methanogenic substrates of organic carbon in the plant appeared to be slightly $^{13}C$-depleted relative to those of the bulk soil (Fig. 4), which might be a potential reason for the lower $f_{ac}$ in the soil.

On the other hand, Belik *et al.* and Tyler *et al.*[6,7] estimated $f_{ac}$ of the USA paddy fields by using porewater $\delta^{13}CH_4$ as $\delta^{13}C$-value of the produced $CH_4$, and they found that it was as high as 80% when $\alpha_{CO_2/CH_4} = 1.045 - 1.060$. However, Canadian field data have showed that porewater $CH_4$ is possibly influenced by $CH_4$ oxidation and transport[22], and in an Italian paddy field Krüger *et al.*[5] also considered that porewater $CH_4$ was a poor proxy for produced $CH_4$ due to the potential $CH_4$ oxidation therein. Recently, a pot experiment in Germany suggested that porewater $CH_4$ could be used as newly produced $CH_4$ after tillering stage since they were similar in $\delta^{13}C$[34]. In this study, both porewater $\delta^{13}CH_4$ and produced $\delta^{13}CH_4$ generally tended to be enhanced during the rice season (Table 1 and Fig. 2), and on average they were similar with each other (Fig. 4). According to $\delta^{13}C$-values of porewater $CH_4$ and $CO_2$ (Fig. 2), it was found that the $\alpha_{app}$ in soil pore water was from 1.047 to 1.054. Therefore, $\alpha_{CO_2/CH_4} = 1.050 - 1.060$ was assumed for comparing with former reports[6,7] and present data of the paddy soil. Hydrogenotrophic methanogenesis was estimated to be dominated over the season (~60–80%), which differed much from the field data in USA[6,7]. More importantly, the methanogenic pathway in soil pore water was different from that in paddy soil (Table 3). Although reasons for the difference in $f_{ac}$ between paddy soil and porewater were not clear, it is not recommended here that porewater $CH_4$ was absolutely regarded as newly produced $CH_4$.

**$CH_4$ oxidation.** The produced $CH_4$ in paddy field is mainly oxidized in the rhizosphere and at the soil-water interface, and accurate estimation of the $CH_4$ oxidation is one of the major aims of this study. Compared to $\delta^{13}C$ of newly produced $CH_4$ ($\delta^{13}CH_{4\,(original)}$), $\delta^{13}C$ of remaining $CH_4$ after it has undergone oxidization ($\delta^{13}CH_{4\,(oxidized)}$) was significant $^{13}C$-enriched (Fig. 4). Therefore, fraction of the $CH_4$ that is oxidized ($f_{ox}$) in the field can be estimated by the mass balance equation[6,7]:

$$f_{ox} = (\delta^{13}CH_{4(original)} - \delta^{13}CH_{4(oxidized)})/[(1/\alpha_{ox} - 1) \times (\delta^{13}CH_{4(oxidized)} + 1{,}000)] \quad (3)$$

In general, anaerobically produced $\delta^{13}CH_4$ is regarded as $\delta^{13}CH_{4\,(original)}$ and $\delta^{13}CH_{4\,(oxidized)}$ is estimated by the measurements of $\delta^{13}CH_{4\,(emission)}$ corrected with transport fractionation factor ($\varepsilon_{transport}$) using a semi-empirical equation[5,7,16]:

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

In the closed-system incubation, $CH_4$ oxidation fractionation factor $\alpha_{ox}$ is known to be calculated according to the Rayleigh equation[35,36]:

$$\alpha_{ox} = 1 + [\log(\delta^{13}CH_{4(initial)} + 1{,}000) - \log(\delta^{13}CH_{4(final)} + 1{,}000)]/\log f \quad (5)$$

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

To our knowledge, $\alpha_{ox} = 1.025 - 1.038$ at a temperature of 12–35 °C is initially measured in methanotrophs-enriched cultures[35] and then widely in landfill cover soils[36–38], and it has substantially been used in the studies of paddy soil[5–7,9,20,21,23]. Recently, $\alpha_{ox} = 1.025 - 1.033$ was found in a Chinese paddy soil at 28.3 °C[19]. By far, reports on $\alpha_{ox}$ in paddy soil, in particular on rice roots, are very few available. In the present study (Table 2), $\alpha_{ox}$ in the soil firstly increased from 1.014 on D20 to the highest value of 1.030 on D88, and then it decreased again to 1.021 on D108. In contrast, $\alpha_{ox}$ on the roots generally declined from 1.019 on D20 to the lowest 1.008 on D108. As a whole, it was higher in the soil (1.021 ± 0.007) than on the roots (1.013 ± 0.005) at 24.5 °C, being much lower than those of measured or used in previous studies under a similar temperature as above mentioned. In addition to $\alpha_{ox}$-value of 1.021 ± 0.007 in the soil and 1.013 ± 0.005 on the roots was used, we made an alternative calculation using $\alpha_{ox} = 1.038$ for better comparable to the previous studies (Table 4). Reasons for the difference in $\alpha_{ox}$ between paddy soils and rice roots are not understood, but Jahnke *et al.*[39] found that there were complex factors influencing the isotopic fractionation in $CH_4$ oxidation and carbon assimilation

| Days after rice transplanting (d) | $\alpha_{ox}=1.021$ | | | $\alpha_{ox}=1.013$ | $\alpha_{ox}=1.038$ | | | |
|---|---|---|---|---|---|---|---|---|
| | $f_{ox}^{a}$ | $f_{ox}^{b}$ | $f_{ox}^{c}$ | $f_{ox}^{d}$ | $f_{ox}^{a}$ | $f_{ox}^{b}$ | $f_{ox}^{c}$ | $f_{ox}^{d}$ |
| 20 | 108 ± 16 | 88 ± 16 | 78 ± 18 | 235 ± 26 | 61 ± 9 | 49 ± 7 | 44 ± 10 | 82 ± 9 |
| 50 | 51 ± 6 | 116 ± 25 | 61 ± 6 | 372 ± 43 | 29 ± 3 | 65 ± 14 | 34 ± 3 | 130 ± 16 |
| 88 | 42 ± 16 | 86 ± 22 | 27 ± 3 | 209 ± 27 | 23 ± 9 | 48 ± 12 | 15 ± 2 | 73 ± 10 |
| 108 | 33 ± 22 | 84 ± 12 | −4 ± 13 | 244 ± 24 | 19 ± 12 | 47 ± 7 | −2 ± 8 | 86 ± 9 |

**Table 4. Fraction of $CH_4$ oxidized (%) in the rhizosphere ($f_{ox}^{a}$) and at the soil-water interface ($f_{ox}^{b}$) in field conditions, and at the surfaces of soil ($f_{ox}^{c}$) and rice roots ($f_{ox}^{d}$) in lab conditions.** $f_{ox}^{a}$ was calculated with Equation (5) using $\delta^{13}C$-values of $CH_4$ anaerobically produced in the soil (Table 1) as $\delta^{13}CH_{4\,(original)}$ and $\delta^{13}CH_{4\,(emission)}$ (Fig. 3a) minus −14.2‰ as $\delta^{13}CH_{4\,(oxidized)}$; $f_{ox}^{b}$ was calculated with Equation (5) using $\delta^{13}C$-values of $CH_4$ in soil pore water (Fig. 2) as $\delta^{13}CH_{4\,(original)}$, and $\delta^{13}C$-values of $CH_4$ in floodwater (Fig. 2) as $\delta^{13}CH_{4\,(oxidized)}$; $f_{ox}^{c}$ and $f_{ox}^{d}$ were calculated with Equation (5) using $\delta^{13}C$-values of $CH_4$ anaerobically produced in the soil and on the roots (Table 1) as $\delta^{13}CH_{4\,(original)}$ and $\delta^{13}C$-values of $CH_4$ aerobically produced in the soil and on the roots (Fig. 1) as $\delta^{13}CH_{4\,(oxidized)}$, respectively.

in various methanotrophs. Besides, main groups of methanotrophs in rice microcosm (*Methylococcaceae* and *Methylocystaceae*) are active, but their dominance may change depending upon substrate supply and nutrient status[40,41]. Therefore, it is no wonder that our measurement of $\alpha_{ox}$ in paddy soil was different from that on rice roots, and that both of them differed much from that found in different environments and habitats as above mentioned. On the other hand, $\varepsilon_{transport}$ is equivalent to the difference between emitted and aerenchymatic $\delta^{13}CH_4$[5,7], and it was estimated to be −14.2‰ on average (Detailed descriptions please see below).

Rhizospheric $CH_4$ oxidation was the most important on D20 (Table 4). At that time, almost the produced $CH_4$ was oxidized before it was emitted into the atmosphere. With the rice growing, the $f_{ox}$ decreased fast to ~30% in the end. Both $CH_4$ oxidation potentials in the soil and on the roots were highest between D20 and D50, and decreased gradually towards the end of the season (Table 2), which might be the important reason. *In situ* inhibitor experiments, Krüger *et al.*[42] also found that $f_{ox}$ was highest just at the beginning of the season with a peak of ~40%, and then it became negligible at the end of the season. Soon later, it was reported that $f_{ox}$ was no more than 50% over the season and it decreased rapidly from the beginning till the end of the season[5,20]. They further concluded the possible reason was that activities of the methanotrophs were limited by nitrogen consumption with the rice growing under field conditions[5,20,42].

When porewater $CH_4$ released into the floodwater of the paddy fields, it was strongly oxidized at the soil-water interface since floodwater $CH_4$ was much more $^{13}C$-enriched than porewater $CH_4$ (Fig. 2). So, $f_{ox}$ in this oxidizing area, in principle, can be estimated using porewater $\delta^{13}CH_4$ as $\delta^{13}CH_{4\,(original)}$ and floodwater $\delta^{13}CH_4$ as $\delta^{13}CH_{4\,(oxidized)}$. Value of $f_{ox}$ was found to be over 80% throughout the whole season, which was generally higher than that of $f_{ox}$ in the rhizosphere (Table 4). Although $f_{ox}$ at the soil-water interface appeared to be much high, the amount of the $CH_4$ oxidized must be significantly lower than that in the rhizosphere. Because produced $CH_4$ is mostly oxidized in the rhizosphere during the rice-growing season as over 90% of the $CH_4$ emits to the atmosphere through the aerenchyma of the plants while less than 0.1% releases via ebullition and diffusion[24,43,44]. In addition, it was reported that the absolute $CH_4$ oxidation rate at the soil-water interface was much lower than that in the rhizosphere[24,45,46].

Compared to methanogenesis in anaerobic soil, that was in aerobic soil significantly lower in $CH_4$ production rate but more positive in $\delta^{13}C$ (Fig. 4). The findings demonstrate that intensive $CH_4$ oxidization happened at the soil surface in lab conditions. As a result, $f_{ox}$ at the soil surface (Table 4) was estimated using anaerobically produced $\delta^{13}CH_4$ as $\delta^{13}CH_{4\,(original)}$ and aerobically produced $\delta^{13}CH_4$ as $\delta^{13}CH_{4\,(oxidized)}$[19]. It was the highest (~80%) at the beginning of the season and decreased rapidly later (<0%). In field conditions, $CH_4$ oxidation in paddy field without rice plants occurs mainly at the soil-water interface, which is similar to $CH_4$ oxidation under aerobic incubation in lab conditions. Therefore, it is feasible to quantitatively estimate $f_{ox}$ in paddy fields during the non-rice-growing season or at the soil-water interface during the rice-growing season based on the difference in $\delta^{13}CH_4$ between anaerobic and aerobic incubations. What is more, $f_{ox}$ at the root surface was also estimated by comparing $\delta^{13}C$-value of the $CH_4$ produced under aerobic conditions with those under anaerobic conditions (Table 4). It was found that $f_{ox}$ at the root surface stayed over 100% throughout the whole season. Even if the $\alpha_{ox}=1.038$ was used, it was still as high as 100% (Table 4), further suggesting that $CH_4$ oxidation on rice roots was extremely strong indeed. $CH_4$ oxidation rate much higher on the roots (Table 2) was supposed to be the main reason for the $f_{ox}$ was higher than that in the soil.

**$CH_4$ transport and emission.** Transporting $CH_4$ is the last step of $CH_4$ emission from paddy field to the atmosphere. Although $CH_4$ oxidation leads to the produced $CH_4$ obviously enriched in $^{13}C$, isotope fractionation in $CH_4$ transport offsets the positive effect on $\delta^{13}CH_4$, causing the $CH_4$ $^{13}C$-depleted again[13,22]. As a result, the $\delta^{13}C$-values of emitted $CH_4$ were close to the produced $\delta^{13}CH_4$ (Fig. 4). The isotope fractionation changes with the efficiency of $CH_4$ transport in growth of the plants[5,9,23]. In the middle of the season, $CH_4$ transport capacity of the plants should get to highest because of full-developed rice plants and roots. Transport fractionation at that time is believed to be strongest and a value of −16.7‰ for $\varepsilon_{transport}$ was measured on D62. At the beginning of the season or aging in the late part of the season, transport fractionation would be relatively weak due to the undeveloped plants with low $CH_4$ transport capacity. Therefore, the $\varepsilon_{transport}$ was found to be −14.7‰ on D37 and −11.1‰ on D98. Many reports have shown a similar variation and it is generally between −16‰ and −11‰[5–7,9,13,19].

Similar to $CH_4$ emission from paddy fields, $\delta^{13}CH_{4\,(emission)}$ is significantly affected by all each process of $CH_4$ production, oxidation and transport. At the beginning of the season, the high $\delta^{13}CH_{4\,(emission)}$ was most likely ascribed to the relatively low transport fractionation and the highest $f_{ox}$. Subsequently, it became lowest, which was supposed to be the biggest transport fractionation and significantly decreased $f_{ox}$. At the late period of the season, the emitted $CH_4$ was $^{13}C$-enriched again, mainly due to an obvious increase of $f_{ac}$ at this moment. The $\delta^{13}CH_{4\,(emission)}$ was negatively correlated with $CH_4$ emission (Fig. 3b), which further indicates that the higher the $CH_4$ flux, the lower the $f_{ox}$, thus causing the lower the emitted $\delta^{13}CH_4$. Similar relationships were also observed in the previous studies[9,21,47].

In conclusion, this study well showed each process of $CH_4$ emission by the measurements of $\delta^{13}CH_4$ from various pools of the paddy field, and found that stable carbon isotope fractionation occurred in $CH_4$ production, oxidation and transport. Compared to the roots (1.046–1.080 and $1.013 \pm 0.005$), $\alpha_{app}$ was lower (1.041–1.056) whereas $\alpha_{ox}$ was greater ($1.021 \pm 0.007$) in the soil. This suggests that acetate-dependent methanogenesis was more important in paddy soil whereas $CH_4$ oxidation was much stronger on the roots. Rice plant-mediated $CH_4$ transport fractionation ($\varepsilon_{transport}$) was found to be $-16.7‰ \sim -11.1‰$. Temporal variation of $CH_4$ emission negatively correlated with $\delta^{13}CH_{4\,(emission)}$ indicates the important relationships of $CH_4$ emission with production, oxidation and transport of the $CH_4$, which could be demonstrated by the changes of pathway of $CH_4$ production and fraction of $CH_4$ oxidation. Besides related newly produced $\delta^{13}CH_4$ and finally oxidized $\delta^{13}CH_4$, available carbon fractionation factors were needed to estimate relative contribution of acetate to total $CH_4$ production and fraction of $CH_4$ oxidized.

## Methods

**Experimental site.** The experimental plots are located at Baitu Town, Jurong City, Jiangsu Province, China (31°58′N, 119°18′E). Soil of the field is classified as Typic Haplaquepts, with 11.1 g $kg^{-1}$ in total C, 1.3 g $kg^{-1}$ in total N and −26.8‰ in $\delta^{13}C$-value of soil carbon. After wheat was harvested on June 13, 2009, wheat straw with stubble and even wild weeds were all removed from the plots. Then the plots were kept flooded from June 24 to October 15 and drained on October 16 before rice harvest. Seeds of the rice ("*Oryza sativa L.* Huajing 3") crop were sown into the nursery bed on May 25, seedlings were transplanted into the field on June 26, and the crop was harvested on November 3. Urea was applied at a rate of 300 kg N $ha^{-1}$, 50% as basal fertilizer on June 26, 25% as tillering fertilizer on July 17, and 25% as panicle fertilizer on August 16. $Ca(H_2PO_4)_2$ (450 kg $ha^{-1}$) and KCL (225 kg $ha^{-1}$) was applied with urea just as basal fertilizer on June 26.

**Field sampling.** $CH_4$ flux was monitored using the static chamber technique. The flux chambers ($0.5 \times 0.5 \times 1$ m), made of plexiglass, covered six hills of rice plants each. Plastic bases for the chambers were installed before rice transplantation in the plots. Removable wooden boardwalks (2 m long) were set up at the beginning of the rice season to avoid soil disturbance during sampling and measuring. To measure $CH_4$ flux, gas samples were usually collected once every 4–7 days. Four gas samples from each chamber were collected using 18 mL vacuum vials at 15 min intervals between 09:45 and 10:30 in the morning on each sampling day. To determine carbon isotope composition of the $CH_4$ gas ($\delta^{13}CH_{4\,(emission)}$), samples were taken at 15–30 day intervals. Only two gas samples were collected using 0.5 L bags (aluminium foil compound membrane, Delin gas packing Co., Ltd, Dalian, China) with a small battery-driven pump[47]. The first sample was taken after the chamber was closed for 3–5 min, and the second at the end of the 2 h closure period. When $CH_4$ flux was monitored, soil temperature at 10 cm depth was simultaneously measured with a hand-carried digital thermometer (Yokogawa Meter and Instruments Corporation, Japan).

Soil pore water samples, 10 cm in depth, were collected using a Rhizon soil moisture sampler (10 RHIZON SMSMOM, Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands)[47]. The samplers were installed (in triplicate) prior to rice transplanting and then left in the soil throughout the whole season. Samples (about 5 mL) were firstly extracted using 18 mL vacuum vials to flush and purge the sampler before sampling. Then about 10 mL of soil solution was drawn into another vial. Simultaneously, 10 mL of floodwater was collected using a plastic syringe and then transferred in to an 18 mL vacuum vial. Subsequently, all sampling vials were equilibrated by filling in pure $N_2$ gas for further analysis with a GC-FID.

$CH_4$ emitted via rice plants and the aerenchymatic $CH_4$ was measured using specially designed PVC bottomless pots[19]. 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 adjustable in diameter to fit the growing plant in the center was placed on top of the pot, allowing the plant to grow through the hole and keeping the plant into two parts. Then, the plant in one pot was cut right above the plate while the plant in the other pot remained intact as control. Finally, chambers ($0.3 \times 0.3 \times 1$ m) were laid on the pots, and gas samples in the headspace of the chambers were collected simultaneously with a small battery-driven pump.

Soil cores of the top layer (0–15 cm) were collected at about 15–30-day intervals, and samples of the same plot were first mixed together[48]. Two samples from the mixture, about 50 g each (dry weight), were then taken and transferred promptly into two 250-mL Erlenmeyer flasks separately. Samples in the flasks were prepared into slurries with $N_2$-flushed de-ionized sterile water at a soil/water ratio of 1:1. During the whole process, $N_2$ was constantly flushed through the samples to remove $O_2$ and $CH_4$. One flask was sealed for anaerobic incubation. Other flask with air headspace was sealed directly for aerobic incubation. Simultaneously, rice plants together with roots were carefully collected from the plots[48]. The roots were washed clean with $N_2$-flushed demineralized water and cut off at 1–2 cm from the root with a razor blade. The fresh roots, about 20 g each portion, were put into flasks, separately, for further preparation and processing in the same way as for the soil samples. All the flasks were sealed with rubber stoppers fitted with silicon septum that allowed sampling of headspace gas. Finally, they

were stored under $N_2$ at 4 °C and transported back to the lab as soon as possible for further analysis. A small portion of the soil and plant samples were dried for 72 h at 60 °C for determination of isotopic composition of the organic carbon.

**Lab incubations.** $CH_4$ production potentials were measured under anaerobic incubation. The flasks were flushed with $N_2$ consecutively for six times through double-ended needles connecting a vacuum pump to purge the air in the flasks of residual $CH_4$ and $O_2$. Simultaneously, methanogenesis was determined aerobically using flasks with air headspace directly. They were incubated in darkness at a temperature the same as measured in the field for 50 h. Gas samples were analyzed 1 h and 50 h later after heavily shaking the flasks by hand. $CH_4$ production rates were calculated using the linear regression of $CH_4$ increasing with the incubation time.

$CH_4$ oxidation potentials were determined under aerobic incubation with high concentration of $CH_4$ supplemented, using equipment the same as described above. Firstly, pure $CH_4$ was injected into each flask to make a high concentration inside (~10,000 $\mu$L $L^{-1}$). Then, the flasks were incubated in darkness under the same temperature as measured in the field and shaken at 120 r.p.m. $CH_4$ depletion was measured by sampling the headspace gas in the flask after vigorous shaking for subsequent analysis. The first sample was collected generally 30 min after pure $CH_4$ was injected. Samples were then taken at 2–3 h intervals during the first 8 h of the experiment. The flasks were left over night and sampled the next day at 2 h intervals again. $CH_4$ oxidation rates were calculated by linear regression of $CH_4$ depletion with incubation time.

**Chemical measurements.** $CH_4$ concentrations were analyzed with a gas chromatograph (Shimadzu GC-12A, Kyoto, Japan) equipped with a flame ionization detector. For analysis of carbon isotope composition, the continuous flow technique and a Finnigan MAT 253 isotope ratio mass spectrometer was used (Thermo Finnigan, Bremen, Germany)[47,49]. $CO_2$ in gas samples was directly analyzed while $CH_4$ in gas samples was converted into $CO_2$ and separated primarily on a PreCon (pre-concentration device). Then, the gas was piped into a GC equipped with a Pora PLOT Q column (25 m length; 0.35 mm i.d.) at 25 °C under $2.0 \times 10^5$ Pa for further separation. The separated gases were finally transferred into the mass spectrometer for $\delta^{13}C$ determination. The reference and carrier gases used were $CO_2$ (99.999% in purity and −23.73‰ in $\delta^{13}C_{PDB}$-value) and He (99.999% in purity, 20 mL $min^{-1}$), respectively. The precision of the repeated analysis was ±0.2‰ when 2.02 $\mu$L $L^{-1}$ $CH_4$ was injected. 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).

**Statistical analyses.** Statistical analysis was done using the SPSS 18.0 software for Windows (SPSS Inc., Chicago). Differences between the four treatments were determined through one-way analysis of variance (ANOVA) and least significant difference (LSD) test. Relationships were assessed using correlation analysis. Significant differences and correlations were set at $P < 0.05$.

## References


1. Ciais, P. *et al.* (ed [Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V. & Midgley, P. M.]) (Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2013).
2. Bouwman, A. F. Agronomic aspects of wetland rice cultivation and associated methane emissions. *Biogeochemistry* **15,** 65–88 (1991).
3. Ali, M. A., Lee, C. H., Lee, Y. B. & Kim, P. J. Silicate fertilization in no-tillage rice farming for mitigation of methane emission and increasing rice productivity. *Agr Ecosyst Environ* **132,** 16–22 (2009).
4. Linquist, B., van Groenigen, K. J., Adviento-Borbe, M. A., Pittelkow, C. & van Kessel, C. An agronomic assessment of greenhouse gas emissions from major cereal crops. *Global Change Biol* **18,** 194–209 (2012).
5. Krüger, M., Eller, G., Conrad, R. & Frenzel, P. Seasonal variation in pathways of $CH_4$ production and in $CH_4$ oxidation in rice fields determined by stable carbon isotopes and specific inhibitors. *Global Change Biol* **8,** 265–280 (2002).
6. Bilek, R. S., Tyler, S. C., Sass, R. L. & Fisher, F. M. Differences in $CH_4$ oxidation and pathways of production between rice cultivars deduced from measurements of $CH_4$ flux and $d^{13}C$ of $CH_4$ and $CO_2$. *Global Biogeochem Cy* **13,** 1029–1044 (1999).
7. Tyler, S. C., Bilek, R. S., Sass, R. L. & Fisher, F. M. Methane oxidation and pathways of production in a Texas paddy field deduced from measurements of flux, $\delta^{13}C$, and $\delta D$ of $CH_4$. *Global Biogeochem Cy* **11,** 323–348 (1997).
8. Bergamaschi, P. Seasonal variations of stable hydrogen and carbon isotope ratios in methane from a Chinese rice paddy. *J Geophys Res* **102,** 25383–25393 (1997).
9. Zhang, G. B. *et al.* Methanogenic pathway and fraction of $CH_4$ oxidized in paddy fields: Seasonal variation and effect of water management in winter fallow season. *PLoS one* **8,** e73982 (2013).
10. Whiticar, M. J. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chem Geol* **161,** 291–314 (1999).
11. Chanton, J. P. The effect of gas transport on the isotope signature of methane in wetlands. *Org Geochem* **36,** 753–768 (2005).
12. Venkiteswaran, J. J. & Schiff, S. L. Methane oxidation: isotopic enrichment factors in freshwater boreal reservoirs. *Appl Geochem* **20,** 683–690 (2005).
13. Chanton, J. P., Whiting, G. J., Blair, N. E., Lindau, C. W. & Bollich, P. K. Methane emission from rice: Stable isotopes, diurnal variations, and $CO_2$ exchange. *Global Biogeochem Cy* **11,** 15–27 (1997).
14. Marik, T., Fischer, H., Conen, F. & Smith, K. Seasonal variations in stable carbon and hydrogen isotope ratios in methane from rice fields. *Global Biogeochem Cy* **16,** Artn 1094 (2002).
15. Fey, A., Claus, P. & Conrad, R. Temporal change of $^{13}C$-isotope signatures and methanogenic pathways in rice field soil incubated anoxically at different temperatures. *Geochim Cosmochim Ac* **68,** 293–306 (2004).
16. Conrad, R., Klose, M. & Claus, P. Pathway of $CH_4$ formation in anoxic rice field soil and rice roots determined by $^{13}C$-stable isotope fractionation. *Chemosphere* **47,** 797–806 (2002).
17. Conrad, R., Klose, M., Lu, Y. & Chidthaisong, A. Methanogenic pathway and archaeal communities in three different anoxic soils amended with rice straw and maize straw. *Fron microb* **3,** 4–4 (2012).
18. Conrad, R. & Klose, M. Stable carbon isotope discrimination in rice field soil during acetate turnover by syntrophic acetate oxidation or acetoclastic methanogenesis. *Geochim Cosmochim Ac* **75,** 1531–1539 (2011).

19. Zhang, G. B. *et al.* Pathway of $CH_4$ production, fraction of $CH_4$ oxidized, and $^{13}C$ isotope fractionation in a straw incorporated rice field. *Biogeosciences* **10,** 3375–3389 (2013).
20. Krüger, M. & Frenzel, P. Effects of N-fertilisation on $CH_4$ oxidation and production, and consequences for $CH_4$ emissions from microcosms and rice fields. *Global Change Biol* **9,** 773–784 (2003).
21. Zhang, G. B. *et al.* Intermittent irrigation changes production, oxidation, and emission of $CH_4$ in paddy fields determined with stable carbon isotope technique. *Soil Biol Biochem* **52,** 108–116 (2012).
22. Popp, T. J., Chanton, J. P., Whiting, G. J. & Grant, N. Methane stable isotope distribution at a Carex dominated fen in north central Alberta. *Global Biogeochem Cy* **13,** 1063–1077 (1999).
23. Conrad, R. & Klose, M. Effect of potassium phosphate fertilization on production and emission of methane and its $^{13}C$-stable isotope composition. *Soil Biol Biochem* **37,** 2099–2108 (2005).
24. Kimura, M., Murase, J. & Lu, Y. H. Carbon cycling in rice field ecosystems in the context of input, decomposition and translocation of organic materials and the fates of their end products ($CO_2$ and $CH_4$). *Soil Biol Biochem* **36,** 1399–1416 (2004).
25. Whiticar, M. J., Faber, E. & Schoell, M. Biogenic methane formation in marine and freshwater environments: $CO_2$ reduction vs. acetate fermentation—isotopic evidence. *Geochim Cosmochim Ac* **50,** 693–709 (1986).
26. Gelwicks, J. T., Risatti, J. B. & Hayes, J. M. Carbon isotope effects associated with aceticlastic methanogenesis. *Appl Environ Microb* **60,** 467–472 (1994).
27. Valentine, D. L., Chidthaisong, A., Rice, A., Reeburgh, W. S. & Tyler, S. C. Carbon and hydrogen isotope fractionation by moderately thermophilic methanogens. *Geochim Cosmochim Ac* **68,** 1571–1590 (2004).
28. Hayes, J. M. Factors controlling $^{13}C$ contents of sedimentary organic compounds: principles and evidence. *Mar Geol* **113,** 111–125 (1993).
29. Conrad, R. *et al.* Stable carbon isotope discrimination and microbiology of methane formation in tropical anoxic lake sediments. *Biogeosciences* **8,** 795–814 (2011).
30. Conrad, R., Klose, M. & Claus, P. Phosphate inhibits acetotrophic methanogenesis on rice roots. *Appl Environ Microb* **66,** 828–831 (2000).
31. Conrad, R., Erkel, C. & Liesack, W. Rice Cluster I methanogens, an important group of Archaea producing greenhouse gas in soil. *Curr Opin Biotech* **17,** 262–267 (2006).
32. Lu, Y. H. & Conrad, R. *In situ* stable isotope probing of methanogenic archaea in the rice rhizosphere. *Science* **309,** 1088–1090 (2005).
33. Lu, Y. H., Lueders, T., Friedrich, M. W. & Conrad, R. Detecting active methanogenic populations on rice roots using stable isotope probing. *Environ Microbiol* **7,** 326–336 (2005).
34. Yuan, Q., Pump, J. & Conrad, R. Partitioning of $CH_4$ and $CO_2$ production originating from rice straw, soil and root organic carbon in rice microcosms. *PloS one* **7,** e49073 (2012).
35. Coleman, D. D., Risatti, J. B. & Schoell, M. Fractionation of carbon and hydrogen isotopes by methane-oxidizing bacteria. *Geochim Cosmochim Ac* **45,** 1033–1037 (1981).
36. Liptay, K., Chanton, J., Czepiel, P. & Mosher, B. Use of stable isotopes to determine methane oxidation in landfill cover soils. *J Geophys Res* **103,** 8243–8250 (1998).
37. Chanton, J. P. & Liptay, K. Seasonal variation in methane oxidation in a landfill cover soil as determined by an *in situ* stable isotope technique. *Global Biogeochem Cy* **14,** 51–60 (2000).
38. Chanton, J. P., Powelson, D. K., Abichou, T., Fields, D. & Green, R. Effect of temperature and oxidation rate on carbon-isotope fractionation during methane oxidation by landfill cover materials. *Environ Sci Technol* **42,** 7818–7823 (2008).
39. Jahnke, L. L., Summons, R. E., Hope, J. M. & Des Marais, D. J. Carbon isotopic fractionation in lipids from methanotrophic bacteria II: The effects of physiology and environmental parameters on the biosynthesis and isotopic signatures of biomarkers. *Geochim Cosmochim Ac* **63,** 79–93 (1999).
40. Bodelier, P. L. E., Roslev, P., Henckel, T. & Frenzel, P. Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. *Nature* **403,** 421–424 (2000).
41. Eller, G. & Frenzel, P. Changes in activity and community structure of methane-oxidizing bacteria over the growth period of rice. *Appl Environ Microb* **67,** 2395–2403 (2001).
42. Krüger, M., Frenzel, P. & Conrad, R. Microbial processes influencing methane emission from rice fields. *Global Change Biol* **7,** 49–63 (2001).
43. Holzapfel-pschorn, A., Conrad, R. & Seiler, W. Effects of vegetation on the emission of methane from submerged paddy soil. *Plant Soil* **92,** 223–233 (1986).
44. Schütz, H., Seiler, W. & Conrad, R. Processes involved in formation and emission of methane in rice paddies. *Biogeochemistry* **7,** 33–53 (1989).
45. Gilbert, B. & Frenzel, P. Methanotrophic bacteria in the rhizosphere of rice microcosms and their effect on porewater methane concentration and methane emission. *Biol Fert Soils* **20,** 93–100 (1995).
46. Kumaraswamy, S. *et al.* Spatial distribution of methane-oxidizing activity in a flooded rice soil. *Plant Soil* **191,** 241–248 (1997).
47. Zhang, G. B. *et al.* Carbon isotopic composition, methanogenic pathway, and fraction of $CH_4$ oxidized in a rice field flooded year-round. *J Geophys Res* **116,** G04025 (2011).
48. Zhang, G. B., Zhang, X. Y., Ma, J., Xu, H. & Cai, Z. C. Effect of drainage in the fallow season on reduction of $CH_4$ production and emission from permanently flooded rice fields. *Nutr Cycl Agroecosys* **89,** 81–91 (2011).
49. Cao, Y. C., Sun, G. Q., Han, Y., Sun, D. L. & Wang, X. Determination of N, C and O stable isotope ratios in $N_2O$, $CH_4$ and $CO_2$ at natural abundance levels by mass spectrometer. *Ac Pedol Sin* (*In Chinese*) **45,** 249–258 (2008).


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## Author Contributions

G.Z. and H.X. conceived and designed the research; G.Z., J.M., H.Y. and X.F. performed the experiment; G.Z., J.M. and H.X. analyzed data; G.Z. and H.X. wrote the main manuscript text; G.Z., J.M. and H.X. reviewed the manuscript.

## Additional Information

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