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Straw application in paddy soil enhances methane production also from other carbon sources

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Abstract. Flooded rice fields are an important source of the greenhouse gas methane. Methane is produced from rice straw (RS), soil organic matter (SOM), and rice root organic carbon (ROC). Addition of RS is widely used for ameliorating soil fertility. However, this practice provides additional substrate for CH_4 production and results in increased CH_4 emission. Here, we found that decomposing RS is not only a substrate of CH_4 production, but in addition stimulates CH_4 production from SOM and ROC. Apart from accelerating the creation of reduced conditions in the soil environment, RS decomposition resulted in enhancement of SOM-derived CH_4 production. In particular, hydrogenotrophic methanogenesis from SOM-derived CO_2 was stimulated, presumably by H_2 released from RS decomposition. On the other hand, the enhancement of ROC-derived CH_4 production after RS application was probably caused by the significant increase of the abundance of methanogenic Archaea in the RS treatment compared with the untreated control. Our results show that traditional management of rice residues exerts a positive feedback on CH_4 production from rice fields, thus exacerbating its effect on the global CH_4 budget.

1 Introduction

Flooded rice fields are one of the largest sources of atmospheric CH_4, the second most important greenhouse gas (Lelieveld et al., 1998). Estimates of rice fields CH_4 emission range from 31 to 112 Tg yr^{-1}, accounting for up to 19 % of global total emissions (Forster et al., 2007). Change in CH_4 cycling due to agroecosystem management has an immediate impact on climate due to the relatively short lifetime of CH_4 in the atmosphere (Montzka et al., 2011). Methane and CO_2 are end products of decomposition of organic matter in anoxic rice field soil (Kimura et al., 2004). The organic materials available for anaerobic decomposition are mainly derived from three sources (Chidthaisong and Watanabe, 1997; Watanabe et al., 1999): (1) soil organic matter (SOM), (2) root organic carbon (ROC) including root exudates and sloughed-off dead root, and (3) incorporated organic material such as rice straw (RS), which is often applied in large amounts (up to 12 t ha^{-1} annually) to maintain soil fertility. Knowledge of the partitioning the CH_4 production among these three types of organic materials is important for improving process-based modeling of CH_4 emission from rice fields, which is the basis for predicting methane flux and assessing the impact of agricultural management and global change (Fumoto et al., 2008; Li et al., 2004; Tokida et al., 2010). So far, there are only few studies that have quantified the relative contribution of each individual source to total CH_4 production and emission (Tokida et al., 2011; Watanabe et al., 1999; Yuan et al., 2012). It is possible that contribution of each individual source could change greatly with the amount of RS applied (Watanabe et al., 1998).

More interestingly, the RS applied may not only serve as the substrate for CH_4 production, but might also affect CH_4 production from the other (SOM, ROC) carbon sources (Chidthaisong and Watanabe, 1997; Watanabe et al., 1998). It has been argued that the decomposed RS may promote CH_4 production from the other carbon sources by accelerating the creation of reduced soil conditions (Tokida et al., 2010; Watanabe et al., 1998). However, there is an alternative possibility. Labile carbon addition (such as straw or cellulose) could stimulate decomposition of more recalcitrant SOM (De Troyer et al., 2011; Guenet et al., 2012) eventually resulting in stimulated CH_4 production. Such stimulation of

SOM decomposition is called a priming effect. Priming effects have frequently been reported in upland soils where CO_2 is the only end product of decomposition of organic matter (Kuzyakov and Bol, 2006; Zhu and Cheng, 2011), but they have rarely been studied in rice field soils where both CO_2 and CH_4 are the end products of anaerobic decomposition of organic matter (Conrad et al., 2012b).

In this study, we explored the possibility of enhancing CH_4 production from SOM and ROC by RS application. Moreover, we investigated whether there is a priming effect of RS on anaerobic SOM decomposition. These objectives required the quantification of the partitioning of CH_4 and CO_2 production from the individual carbon sources (i.e., from ROC, SOM and RS). Recently, we introduced a novel technique by treating rice microcosms with rice straw that was enriched in ^{13}C so that it was possible to differentiate between the C-flux derived from either RS or from ROC and SOM (Yuan et al., 2012). Applying this technique, we were able to detect the enhancement of RS on CH_4 production from both ROC and SOM using rice field soil from Vercelli, Italy.

2 Material and methods

2.1 Greenhouse experiment

2.1.1 Planted and unplanted rice microcosms

Soil was taken from a drained paddy field of the Italian Rice Research Institute in Vercelli, Italy, in spring 2009 and was air-dried and stored at room temperature. The soil was sieved (< 2 mm) prior to use. The characteristics of the soil have been described previously (Holzapfel-Pschorn and Seiler, 1986). Planting pots (upper diameter = 19 cm; lower diameter = 14 cm; height = 16 cm) were filled with 2 kg dry soil and turned into a slurry with demineralized water.

Preparation of such microcosms has been described previously (Yuan et al., 2012). In brief, 48 pots were prepared for planted rice microcosms: 16 pots for the unamended control, and 16 pots each for RS treatment I and RS treatment II. For both RS treatments, 10 g powder of RS was added to each pot and mixed thoroughly. The $\delta^{13}C$ values of RS added in treatment I and II were 213.0 ‰ and 474.7 ‰, respectively. These $\delta^{13}C$ values were obtained by mixing ^{13}C-labeled ($\delta^{13}C$= 1859.9 ‰) and unlabeled ($\delta^{13}C$= −27.6 ‰) RS. The $\delta^{13}C$ values of these RS mixtures were always higher than those of the produced CH_4 and CO_2, even when these gases were almost exclusively (90–100 %) produced from the added RS. Therefore, the RS mixtures were sufficiently homogeneous to prevent preferential decomposition of ^{13}C-labeled (and presumably labile) components of RS. After 3 days of incubation in the greenhouse, all the pots were planted with one 12-day-old rice seedling (*Oryza sativa* var. KORAL type *japonica*), and were flooded with demineralized water to give a water depth of 5 cm above the soil surface. The water depth was maintained throughout the experimental period. The rice microcosms were incubated in the greenhouse with a relative humidity of 70 %, a 12 h photoperiod and a 28/22 °C day/night temperature cycle. The day of transplantation was taken as day zero. At each sampling time (day 41, 55, 70 and 90), 12 rice microcosms were sacrificed (4 replicates for control and for each treatment). For unplanted microcosms, the preparation was the same as for planted ones, but without rice plant in the pots. In total, 12 pots were prepared with 4 pots each for the unamended control, RS treatment I and RS treatment II.

2.1.2 CH_4 flux and production rates

Rates of CH_4 emission were measured on day 41, 55, 70 and 90 of incubation in the greenhouse. For flux measurements, planted rice microcosms were covered by flux chambers, and gas samples were taken every 30 min for 2 h. CH_4 emission rates were determined from the slope of the linearly increasing CH_4 mixing ratio and expressed in mmol CH_4 m^{-2} h^{-1}.

Production rates of CH_4 and respective $\delta^{13}C$ values were determined by collecting soil core samples in rice microcosms on day 41, 55, 70 and 90 of incubation in the greenhouse (Krüger et al., 2001; Yuan et al., 2012). After cutting off the rice plant, the surface water layer was removed. Soil cores were taken with a stainless steel corer (22 mm in diameter, 210 mm in length). Two to three soil cores (about 100 g in total) were collected from each pot and transferred into a 250 mL bottle. The soil samples were turned into slurry using N_2-gassed deionized sterile water so that the ratio of dry weight of soil to water was 1 : 1. After flushing the samples with N_2, the bottles were sealed with rubber stoppers and, after shaking, flushed again with N_2 to remove residual O_2 and CH_4. Incubation was performed statically at 25 °C in darkness for 24 h. Headspace samples were taken every 12 h after shaking the bottles, and analyzed for concentration of CH_4 and $\delta^{13}C$ of CH_4. The CH_4 production from planted soil microcosms was due to decomposition of SOM plus ROC (unamended control) or of SOM, ROC plus RS (RS treatments). CH_4 production rates were calculated by linear regression of the CH_4 increase with incubation time, and expressed in nmol CH_4 g_{dw}^{-1} h^{-1} of soil.

For unplanted soil microcosms, the methods for collection and incubation of soil core samples were similar, but these pots were not sacrificed. At each sampling day (day 41, 55, 70 and 90), a 60 g soil core was taken from the pot. After removal of the soil core the residual soil in the pot was compacted, and water was added to maintain a water level of 5 cm depth. Using this procedure about 2.1 % of the total amount of soil in the pot was collected during each sampling. The CH_4 production from unplanted soil microcosms was only due to decomposition of SOM (unamended control) and of SOM plus RS (RS treatments).

During each sampling, small amounts of soil were collected from the homogenized soil cores before anoxic incubation and stored at −80 °C for later molecular analysis.

2.2 Laboratory incubation of soil with RS application

After 0.5 mm sieving, 5 g dry Vercelli soil was mixed with 5 mL anoxic water in 26 mL pressure tubes. Tubes were closed with butyl rubber stoppers, sealed with aluminum crimps, then flushed with N_2 and incubated statically at 25 °C in darkness. RS treatments I and II were done at the beginning of the anoxic incubation or after 40 days. The $\delta^{13}C$ values of RS added in treatment I and II were 596.1 ‰ and 885.0 ‰, respectively. The preparation of RSI and RSII was as described above. For RS treatments at the beginning of anoxic incubation, 25 mg (0.5 %) unlabeled RS or labeled RS (RSI or RSII) powder was added to each tube. For RS treatments after 40 days of anoxic incubation, 5 mg (0.1 %) or 10 mg (0.2 %) RSI or RSII powder was added to each tube. Immediately after RS addition, the tubes were sealed again and flushed with N_2 and, after shaking, re-flushed with N_2 to remove the residual O_2 and CH_4. Then the tubes were incubated statically at 25 °C. Besides the RS treatments, methyl fluoride (CH_3F) was added to the headspace of several incubation batches to give the desired concentration of 1.0 %. All the treatments were prepared in triplicates. After RS application in each RS treatment, the RS-derived CH_4 and CO_2 production rate was calculated by linear regression of the CH_4 and CO_2 increase from RS within 3 days, and expressed in nmol CH_4 or CO_2 h^{-1} g_{dw}^{-1} of soil. The calculations of CH_4 and CO_2 derived from RS were done as explained below.

2.3 Analytical techniques

The gas samples were analyzed for CH_4 and CO_2 using a gas chromatograph (GC) equipped with flame ionization detector (FID) (Bodelier et al., 2000). Stable isotopic analyses of CH_4 and CO_2 were performed using a gas chromatograph combustion isotope ratio mass spectrometer (GC-C-IRMS) (Finnigan, Bremen, Germany) (Penning and Conrad, 2007). The determination of the stable isotopic signatures of dried plant (RS) and soil samples (SOM) was carried out at the Institute of Soil Science and Forest Nutrition (IBW) at the University of Göttingen, Germany.

2.4 Quantification of microbial abundance

DNA from the soil samples was extracted according to the lysis protocol described in the NucleoSpin® soil kit (Macherey-Nagel, Germany). Quantifications of bacterial 16S rRNA gene and gene (mcrA) coding for a subunit of the methyl coenzyme M reductase were done via iCycler Thermal Cycler (Bio-Rad, Germany) using SYBR® Green JumpStart™ Taq ReadyMix™ (Sigma). This enzyme is characteristic and unique for methanogenic Archaea. For the quantitative polymerase chain reaction (PCR) of bacterial 16S rRNA, the primer pair (519f and 907r) and parameters followed the protocol described in Stubner (2002), for mcrA gene, the primer pair (mlas-mod and mcrA-rev), and parameters followed the protocol described in Angel et al. (2011). The gene copy numbers detected are a proxy for the abundance of the respective microbes.

2.5 Calculations

2.5.1 Fraction of CH_4 production from ROC (f_{ROC}), RS carbon (f_{RS}) and SOM (f_{SOM}) in planted rice microcosms with RS application

The fraction of CH_4 derived from ROC (f_{ROC}) can be determined from the following mass balance equation:

$$\delta^{13}C_{CH_4} = f_{ROC}\delta^{13}C_{CH_4\text{-}ROC} + (1 - f_{ROC})\delta^{13}C_{CH_4\text{-}SOR}, \quad (1)$$

where $\delta^{13}C_{CH_4} = \delta^{13}C$ of CH_4 produced in the planted rice microcosms at each sampling time; $\delta^{13}C_{CH_4\text{-}ROC} = \delta^{13}C$ of CH_4 formed from ROC (determination see below); $\delta^{13}C_{CH_4\text{-}SOR} = \delta^{13}C$ of CH_4 formed from SOM plus RS (i.e., the CH_4 produced in the unplanted soil treated with RS). The equation can be transformed into the following two equations for RS treatment I and II, respectively:

$$f_{ROC} = (\delta^{13}C_{CH_4\text{-}I} - \delta^{13}C_{CH_4\text{-}SOR\text{-}I}) / (\delta^{13}C_{CH_4\text{-}ROC} - \delta^{13}C_{CH_4\text{-}SOR\text{-}I}), \quad (2)$$

$$f_{ROC} = (\delta^{13}C_{CH_4\text{-}II} - \delta^{13}C_{CH_4\text{-}SOR\text{-}II}) / (\delta^{13}C_{CH_4\text{-}ROC} - \delta^{13}C_{CH_4\text{-}SOR\text{-}II}). \quad (3)$$

Since f_{ROC} and $\delta^{13}C_{CH_4\text{-}ROC}$ should be the same in treatment I and II, $\delta^{13}C_{CH_4\text{-}ROC}$ can be calculated by solving Eqs. (2) and (3). Then, f_{ROC} can be calculated from either Eqs. (2) or (3). The fraction of CH_4 derived from RS (f_{RS}) can be determined from the following mass balance equation:

$$f_{RS} = (\delta^{13}C_{CH_4\text{-}I} - \delta^{13}C_{CH_4\text{-}II}) / (\delta^{13}C_{RS\text{-}I} - \delta^{13}C_{RS\text{-}II}), \quad (4)$$

of which $\delta^{13}C_{CH_4\text{-}I}$ and $\delta^{13}C_{CH_4\text{-}II}$ were determined experimentally, and RS-I and RS-II were mixtures of labeled and unlabeled RS, of which the $\delta^{13}C$ were determined experimentally. Details of calculations of f_{ROC} and f_{RS} have been described previously (Yuan et al., 2012). Finally, the fraction of CH_4 production from SOM (f_{SOM}) can be calculated, since

$$f_{RS} + f_{ROC} + f_{SOM} = 1. \quad (5)$$

2.5.2 CH_4 production rates from SOM and ROC in planted rice microcosms with RS application

The effect of RS on CH_4 production from SOM and ROC was determined in the RS treatments with rice plants. The

rates of CH_4 production from ROC (p_{ROC,CH_4}) and from SOM (p_{SOM,CH_4}) were calculated from the total CH_4 production rates (p_{CH_4}) and the fractions of CH_4 production from ROC (f_{ROC}) and SOM (f_{SOM}) as below:

$$p_{ROC,CH_4} = f_{ROC}\, p_{CH_4}, \quad (6)$$

$$p_{SOM,CH_4} = f_{SOM}\, p_{CH_4}. \quad (7)$$

2.5.3 Contribution of RS and SOM to CH_4 in soil slurries with RS application

The $\delta^{13}C$ values of the CH_4 produced in the two RS treatments are given by the following two mass balance equations:

$$\delta^{13}C_{CH_4\text{-I}} = f_{RS}\delta^{13}C_{RS\text{-I}} + f_{SOM}\delta^{13}C_{SOM} + \Delta CH_4, \quad (8)$$

$$\delta^{13}C_{CH_4\text{-II}} = f_{RS}\delta^{13}C_{RS\text{-II}} + f_{SOM}\delta^{13}C_{SOM} + \Delta CH_4, \quad (9)$$

with f_{RS} and f_{SOM} denoting fractions of CH_4 produced from RS and SOM, respectively; $\delta^{13}C_{RS\text{-I}}$ and $\delta^{13}C_{RS\text{-II}}$ are $\delta^{13}C$ of the rice straw carbon in treatment I (596.1 ‰) and II (885.0 ‰), respectively; $\delta^{13}C_{SOM}$ is $\delta^{13}C$ of SOM (−25.8 ‰), ΔCH_4 designates the overall isotopic enrichment factors involved in the conversion of RS and SOM to CH_4. Since the terms $f_{SOM}\delta^{13}C_{SOM}$ and ΔCH_4 should be the same in treatment I and II, the combination of Eqs. (3) and (4) and solving for f_{RS} results in

$$f_{RS} = (\delta^{13}C_{CH_4\text{-I}} - \delta^{13}C_{CH_4\text{-II}})/ (\delta^{13}C_{RS\text{-I}} - \delta^{13}C_{RS\text{-II}}), \quad (10)$$

of which $\delta^{13}C$ can be determined experimentally. Here, $\delta^{13}C_{CH_4\text{-I}}$ and $\delta^{13}C_{CH_4\text{-II}}$ were determined experimentally, and RS-I and RS-II were mixtures of labeled and unlabeled RS, of which the $\delta^{13}C$ were determined experimentally (see above). Finally, the fraction of CH_4 production from SOM (f_{SOM}) can be calculated, since

$$f_{RS} + f_{SOM} = 1. \quad (11)$$

Then, the amount of CH_4 production from RS (p_{RS,CH_4}) and from SOM (p_{SOM,CH_4}) was calculated from the total amount of CH_4 produced (p_{CH_4}) and the fractions of CH_4 production from ROC (f_{RS}) and SOM (f_{SOM}):

$$p_{RS,CH_4} = f_{RS}\, p_{CH_4}, \quad (12)$$

$$p_{SOM,CH_4} = f_{SOM}\, p_{CH_4}. \quad (13)$$

Analogous equations are valid for the fractions and amount of CO_2 produced from RS and SOM in rice soil.

2.5.4 Amount and $\delta^{13}C$ of total inorganic carbon (TIC)

Total amounts of gases in the headspace of the tubes were calculated from the partial pressures using the volume of the gas space and the gas constant. The amounts of CH_4 dissolved in the liquid were less than 3 % of the total and were neglected. The amounts of CO_2 (aq) dissolved in the liquid were calculated from the solubility constant of CO_2 ($1 \times 10^{-1.47}$ mol L^{-1} bar^{-1}), those of bicarbonate (HCO_3^-) from the solubility constant of CO_2, the pH (measured), and the dissociation constant ($10^{-6.35}$) of bicarbonate (Stumm and Morgan, 1981). Total inorganic carbon (TIC) was defined as the sum of bicarbonate, gaseous, and dissolved CO_2. The $\delta^{13}C$ of dissolved CO_2 ($\alpha_{CO_2(aq)} = 0.9990$) and bicarbonate ($\alpha_{HCO_3} = 1.0075$) were calculated from the $\delta^{13}C$ of gaseous CO_2 and the corresponding fractionation factors α (Stumm and Morgan, 1981), which are

$$\alpha_{CO_2(aq)} = (\delta^{13}C_{CO_2(aq)} + 1000)/(\delta^{13}C_{CO_2(g)} + 1000), \quad (14)$$

$$\alpha_{HCO_3} = (\delta^{13}C_{HCO_3} + 1000)/(\delta^{13}C_{CO_2(g)} + 1000). \quad (15)$$

The values of $\delta^{13}C_{CO_2(g)}$, $\delta^{13}C_{CO_2(aq)}$, and $\delta^{13}C_{HCO_3}$ were used to calculate $\delta^{13}C_{TIC}$ using the mole fractions of the different CO_2 species (Penning and Conrad, 2006).

2.6 Statistical analysis

The significance of differences between treatments over time for various variables was determined by one-way analysis of variance (ANOVA) followed by multiple comparisons (Duncan's post hoc test) using SPSS 13.0. To test the significance of the differences between control and RS treatment on CH_4 or TIC production from SOM, two-tailed independent *t* tests were applied using Microsoft Excel 2007.

3 Results

3.1 Enhancement of CH_4 production from both SOM and ROC by RS application in planted rice microcosms

Application of rice straw increased the rates of both CH_4 production and CH_4 emission in a proportional way (Fig. 1). In the rice microcosms without RS, CH_4 production rates increased from the tillering stage (day 41) to the booting stage (day 55) and the flowering stage (day 70), then peaked at the ripening stage (day 90) (Table 1). Methane production rates were increased by RS application throughout the growth period, but particularly during the tillering and booting stages. The $\delta^{13}C$ values of the CH_4 produced in microcosms amended with ^{13}C-labeled RS were used for calculation of the fractions of total CH_4 derived from RS, ROC and SOM. ROC was found to make a major contribution (41–63 %) to CH_4 production over the entire vegetation period.

Table 1. CH_4 production rates in soil sampled from microcosms with and without rice plant and rice straw (RS), mean ±SD ($n = 4$).

Time (days)	(A)[a] Planted soil without RS (nmol g_{dw}^{-1} h^{-1})	(B)[a] Planted soil with RS (nmol g_{dw}^{-1} h^{-1})	(C)[a] Unplanted soil without RS (nmol g_{dw}^{-1} h^{-1})	(D)[a] Unplanted soil with RS (nmol g_{dw}^{-1} h^{-1})	A–C[b] (nmol g_{dw}^{-1} h^{-1})	B–D[b] (nmol g_{dw}^{-1} h^{-1})
41	5.5 ± 1.0	58.1 ± 11.2**	0.1 ± 0.0	21.3 ± 2.3	5.4 ± 1.0	36.9 ± 11.4$^{\#}$
55	13.0 ± 1.3	41.8 ± 2.5**	2.4 ± 0.9	21.5 ± 2.1	10.6 ± 1.6	20.2 ± 3.3$^{\#\#}$
70	16.1 ± 4.5	33.4 ± 11.7*	3.9 ± 2	20.1 ± 1.2	12.2 ± 4.9	13.2 ± 11.8
90	25.1 ± 1.1	42.1 ± 10.4*	3.6 ± 0.4	10.4 ± 2.1	21.6 ± 1.1	31.7 ± 10.6

[a] Data taken from Yuan et al. (2012).
[b] The values give the apparent contribution of rice plants to CH_4 production in microcosms without (A–C) and with (B–D) rice straw. The differences between planted soil without (A) and with RS (B) were tested by two-tailed independent t test, indicated by ** when $P < 0.01$ or * when $P < 0.05$. The differences between A–C and B–D were also tested with two-tailed independent t test, indicated by ## when $P < 0.01$ or # when $P < 0.05$.

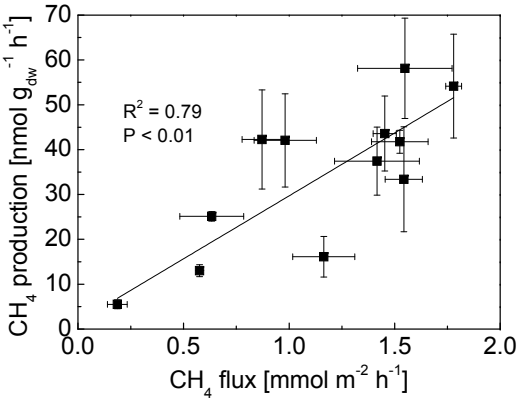

Fig. 1. Rates of CH_4 production and CH_4 emission measured during incubation of planted rice microcosms without and with addition of rice straw; means ±SD ($n = 4$).

SOM contributed about 23–35 %, and RS accounted for the rest (12–24 %) (Yuan et al., 2012).

Knowing the percentage contribution of SOM and ROC and the total CH_4 production rates, the individual production rates of CH_4 from ROC (p_{ROC}) and SOM (p_{SOM}) could be calculated in the RS-treated microcosms (Fig. 2a). Production rates of total CH_4 were also determined for unamended control microcosms, in which CH_4 was produced from ROC and SOM only. The results showed that SOM-derived plus ROC-derived CH_4 production rates were higher in the RS-treated microcosms than in the untreated controls during the entire vegetation season. Specifically at the tillering stage, both the SOM-derived and the ROC-derived CH_4 production rates were increased in the presence of RS. At the booting stage, the ROC-derived CH_4 production was still substantially increased (Fig. 2a). Hence, the RS treatment exerted a positive feedback on the CH_4 production from SOM and ROC. The positive feedback on CH_4 production from ROC after RS application was consistent with mass balance calculations of CH_4 production in microcosms that were planted or unplanted and treated or untreated with RS (Table 1).

Microcosms treated with RS exhibited a higher abundance of mcrA copies than untreated microcosms, and planted microcosms generally contained more mcrA copies than unplanted ones (Fig. 2b). By contrast, addition of RS did not significantly affect the abundance of Bacteria (Fig. 2c).

3.2 Stimulation of CH_4 production from SOM by RS application in soil slurry

Rice field soil was amended with 0.5 % ^{13}C-labeled RS I or II and then preincubated for 40 days under anoxic conditions to ensure that soil conditions were reduced and methanogenesis was the exclusive terminal decomposition process of organic matter. Methane production was higher in the RS-treated soil than in the untreated control (Fig. 3a). The SOM-derived CH_4 production after 40 days of anoxic pre-incubation was calculated from the amount (Fig. 3a) and $\delta^{13}C$ (Fig. 3b) of the released CH_4 using Eq. (13). The results showed that CH_4 production from SOM was always higher in the RS-treated than in the untreated control soil (Fig. 3c).

In a second experiment, unamended rice field soil was preincubated for 40 days under anoxic conditions and then treated with either 0.1 % or 0.2 % ^{13}C-labeled RS. Methane production rates were higher in the RS-treated soil than in the untreated control and were higher in the treatment with 0.2 % than 0.1 % RS (Fig. 4a). After about 10 days of anaerobic decomposition of RS, the accumulated CH_4 derived from SOM was higher in the RS treatments than in the untreated control and further increased gradually afterwards (Fig. 4b). The stimulation of SOM degradation by RS was also seen when methanogenesis was partially inhibited by CH_3F, a specific inhibitor of aceticlastic methanogenesis (Janssen and Frenzel, 1997) (Fig. 4c). The residual CH_4 production observed in the presence of CH_3F was assigned to hydrogenotrophic methanogenesis. While hydrogenotrophic methanogenesis accounted for about 25 % of total CH_4 production in the untreated control soil, it accounted for about 50% in the RS treatment, indicating that RS stimulated hydrogenotrophic methanogenesis in particular. Carbon dioxide (quantified as

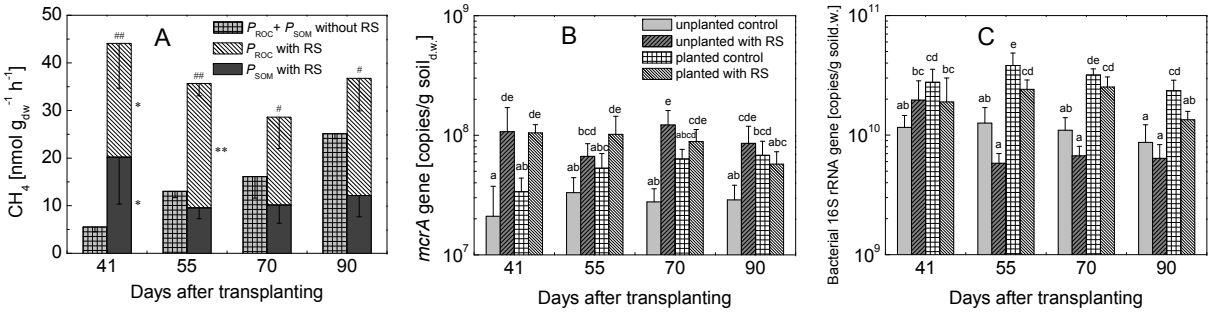

Fig. 2. Production rates of CH_4 and abundance of methanogens and Bacteria in planted microcosms without and with RS application. **(A)** Individual CH_4 production derived from ROC (p_{ROC}) and SOM (p_{SOM}) with RS application compared to total CH_4 production ($p_{ROC} + p_{SOM}$) without RS addition. The differences between $p_{ROC} + p_{SOM}$ without RS and p_{ROC} or p_{SOM} with RS were tested by one-tailed independent t test, indicated beside the bars by ** when $P < 0.01$ or * when $P < 0.05$. The differences between $p_{ROC} + p_{SOM}$ without and with RS were tested as described above, indicated on the top of the bars by ## when $P < 0.01$ or # when $P < 0.05$; **(B)** mcrA gene (characteristic for methanogenic Archaea) and **(C)** bacterial 16S rRNA gene copy numbers without and with RS application; means ±SD ($n = 4$). The differences between the treatments over time were examined using Duncan's post hoc test of a one-way analysis of variance (ANOVA). Different letters on the top of bars indicate significant difference ($P < 0.05$) between the data.

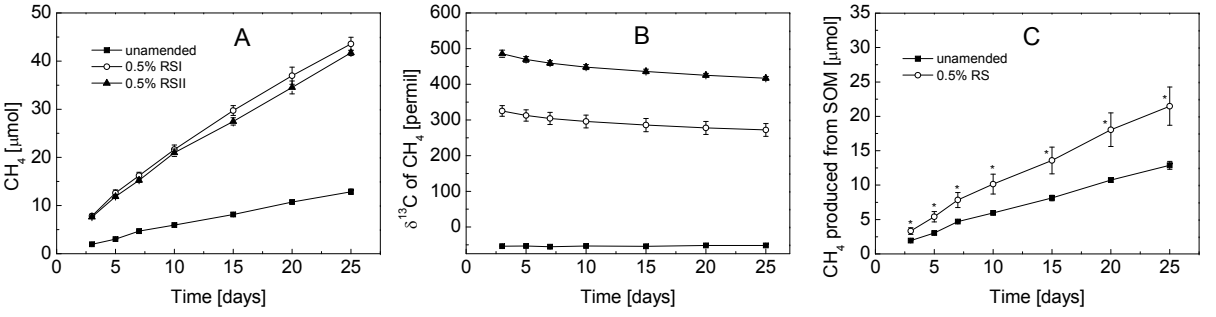

Fig. 3. Production of CH_4 **(A)**, $\delta^{13}C$ value of produced CH_4 **(B)** and SOM-derived CH_4 production **(C)** in control soil and treatments with 0.5 % ^{13}C-labeled RS I or II after 40 days of anoxic pre-incubation. The RS was applied at the beginning of anoxic incubation. The headspace of all bottles was re-flushed with N_2 after 40 days of anoxic incubation. Therefore, "day 0" on the x axis corresponds to the actual date of "day 40" in the entire incubation period. Data are means ±SD ($n = 3$). The differences between control and RS treatment in SOM-derived CH_4 production were tested by two-tailed independent t test, indicated by * when $P < 0.05$.

TIC) is besides CH_4 the end product of anaerobic degradation of organic matter. At the end of incubation (day 25), there were no significant differences in the total amount of CH_4 plus TIC derived from SOM between RS treatments and control (Fig. 4d).

3.3 Methanogenic decomposition of RS in anoxic soil slurry with different abundance of methanogenic community

Degradation of RS was studied in soil that had or had not previously been treated with RS. For this purpose, control soil or soil amended with 0.5 % unlabeled RS was again treated with 0.1 % or 0.2 % ^{13}C-labeled RS. The production rates of TIC and CH_4 derived from the newly applied RS were calculated from the total production rates of TIC and CH_4 and their $\delta^{13}C$ values. The results showed that previous RS treatment resulted in strong increase (at least doubling) of the production rates of both TIC and CH_4 derived from newly applied ROC (Table 2). In addition, the larger amount of newly added RS also resulted in a proportionally larger amount of RS-derived TIC and CH_4 produced (Table 2).

4 Discussion

4.1 Reliability of design and calculations used for the rice microcosm experiments

Isotopic discrimination of ^{13}C occurs in production, consumption and transport processes of CH_4 in rice field soil, all of which are sensitive to chemical and physical conditions, and these conditions could be different in the presence and absence of plants. Therefore, we did not use the $\delta^{13}C$ of the CH_4 that was emitted from the microcosms, but instead collected soil cores and incubated them under anoxic conditions. Prior to incubation, the CH_4 accumulated was

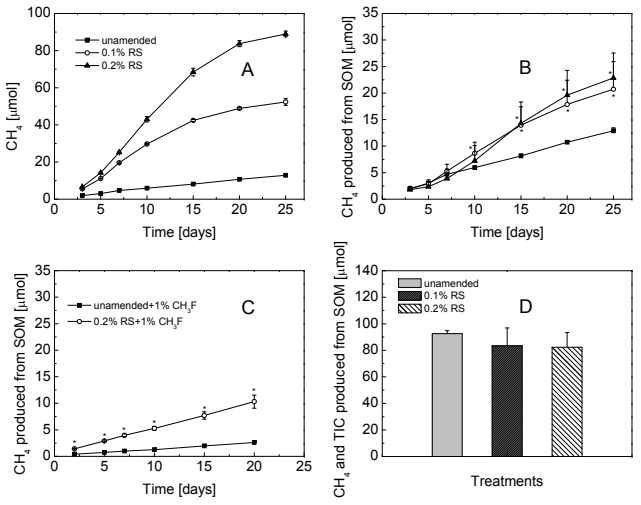

Fig. 4. Production of CH_4 **(A)**, SOM-derived CH_4 production **(B)**, SOM-derived CH_4 production in the presence of 1 % CH_3F **(C)** and total amount of SOM-derived CH_4 and TIC **(D)** in control soil and treatments with 0.1 % or 0.2 % ^{13}C-labeled RS I or II. The RS was applied after 40 days of anoxic pre-incubation of rice soil, and then the headspace of all bottles was re-flushed with N_2. Therefore, "day 0" on the *x* axis corresponds to the actual date of "day 40" in the entire incubation period. Data are means ±SD ($n = 3$). The total amount of SOM-derived CH_4 and TIC were calculated at day 25 after RS application. The differences between control and RS treatments were tested by two-tailed independent *t* test only in **(B)** and **(D)**, indicated by * when $P < 0.05$.

removed so that the $\delta^{13}C$ of the CH_4 measured was that of newly produced CH_4 and unbiased from isotope fractionation other than during CH_4 production. The soil was sampled from rice pots after cutting off the rice plant. Therefore the carbon flow from the root (ROC) into the soil was interrupted and could have resulted in underestimation of CH_4 production. However, our measurement period was short (24 h) to avoid depletion of root exudates previously excreted and of cut roots. Literature data (Lu et al., 2000) and our own experience indicate that CH_4 production rates under such conditions cover methanogenesis from root exudates quite well. Indeed, the f_{ROC} measured by this procedure was 41–63 % and thus quite high. Therefore, the measurement procedure most probably did not result in substantial underestimation of f_{ROC}.

The measurement procedure also ensured that variables ($\delta^{13}C_{CH_4}$, $\delta^{13}C_{CH_4-ROC}$ and $\delta^{13}C_{CH_4-SOR}$) associated with Eq. (1) were free from isotopic fractionations due to consumption and transport of CH_4 and from the influence of formerly accumulated CH_4. In addition, there was no significant difference in the abundance of the methanogenic populations between planted and unplanted treatments (abundance of methanogenic community was only enhanced by addition of straw) (Fig. 2b). Therefore, it was reasonable to assume that $\delta^{13}C_{CH_4-SOR}$ (the $\delta^{13}C$ of CH_4 produced from

Table 2. Production rates of TIC and CH_4 derived from 0.1 % or 0.2 % ^{13}C-labeled RS applied after 40 days of anoxic incubation of untreated control soil or RS-treated soil. For RS-treated soil, rice soil was amended with 0.5 % unlabeled RS at the beginning of anoxic incubation. The headspace of all bottles was re-flushed with N_2 after addition of ^{13}C-labeled RS. This labeled RS was used as proxy of ROC in this experiment. Data are means ±SD ($n = 3$). The differences in RS-derived TIC or CH_4 production rates among the treatments were examined using Duncan's post hoc test of ANOVA. Different letters indicate significant difference ($P < 0.05$) between the data.

Treatments	RS-derived TIC ($nmol\,h^{-1}\,g^{-1}$)	RS-derived CH_4 ($nmol\,h^{-1}\,g^{-1}$)
Control + 0.1 % RS	23.58 ± 2.77^{a}	11.51 ± 0.57^{a}
Control + 0.2 % RS	55.32 ± 0.88^{b}	16.77 ± 0.52^{b}
RS-treated + 0.1 % RS	50.06 ± 2.21^{b}	40.09 ± 2.26^{c}
RS-treated + 0.2 % RS	109.46 ± 8.67^{c}	87.09 ± 2.53^{d}

both SOM and RS) was the same across rice-planted and unplanted treatments. However, even if there was an effect of plants on $\delta^{13}C_{CH_4-SOR}$, such an effect would not result in a large error of f_{ROC} determined in Eqs. (2) or (3), since compared to the possible error in $\delta^{13}C_{CH_4-SOR}$, the difference between $\delta^{13}C_{CH_4-SOR}$ and $\delta^{13}C_{CH_4-ROC}$ and between $\delta^{13}C_{CH_4-SOR}$ and $\delta^{13}C_{CH_4}$ was rather large. Such a large difference was created by the application of ^{13}C-labeled rice straw (Yuan et al., 2012). Therefore, our assumptions in mass balance calculation should be rather robust, and thus calculated values of f_{ROC} and enhanced CH_4 production rates from ROC after RS application should be valid.

Furthermore, for assessing the enhanced CH_4 production rates from ROC, it is mainly the comparison of treatment with and without rice straw rather than with and without plants that is important (Table 1). The enhancement obtained from such comparison was consistent with the calculation from the total CH_4 production rates (p_{CH_4}) and f_{ROC} (Fig. 2a).

4.2 Positive feedback of RS addition on CH_4 production from both SOM and ROC

Our results quantified the positive feedback of RS addition on CH_4 production from both SOM and ROC during the rice growth season. Stimulation of CH_4 production by RS has commonly been observed as straw serves as a relatively labile organic substrate that is readily degraded to CH_4 (Chidthaisong and Watanabe, 1997; Kimura et al., 2004; Sass et al., 1991; Schütz et al., 1989; Denier van der Gon and Neue, 1995; Yagi and Minami, 1990). It is known that at the beginning of flooding of rice fields, electrons derived from organic matter degradation are mainly used for the creation of reduced soil conditions (e.g., reduction of O_2, NO_3^-, Fe(III) and SO_4^{2-}), while only the residual electrons can be

used for CH_4 production (Tokida et al., 2010; Yao and Conrad, 2000). Addition of RS would increase the supply of electrons and thus allow a larger portion of the electrons (both from RS and SOM) being used for CH_4 production. Such a mechanism has been already incorporated into process-based models of CH_4 emission (Fumoto et al., 2008). However, such a mechanism could only explain the enhancement of RS on SOM-derived and ROC-derived CH_4 production immediately after flooding of rice soil, since inorganic electron acceptors (e.g., O_2, NO_3^-, Fe(III) and SO_4^{2-}) present in the soil are usually completely reduced after a few days or weeks (Yao and Conrad, 1999; Yao et al., 1999). This was also the case in our experiments (data not shown). However, the stimulation of CH_4 production from ROC and SOM by RS was observed after 40 days of flooding of Vercelli soil when methanogenic conditions had well been established (Fig. 2a).

Methanogenic Vercelli soil slurry was used as a model system to study the priming effect (PE) of RS on anaerobic SOM decomposition production of CH_4 and CO_2 (TIC). It should be noted that organic matter is eventually degraded to equal amounts of CH_4 and CO_2 (e.g., cellulose):

$$C_6H_{12}O_6 \rightarrow 3CH_4 + 3CO_2. \quad (16)$$

However, CH_4 is only produced from acetate and from $H_2 + CO_2$ so that part of the primarily produced CO_2 is later reduced to CH_4:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2, \quad (17)$$

$$2CH_3COOH \rightarrow 2CH_4 + 2CO_2, \quad (18)$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O. \quad (19)$$

While process (17) is achieved by hydrolytic and fermenting Bacteria, processes (18) and (19) are achieved by hydrogenotrophic and aceticlastic methanogens, respectively. As a result, CO_2 is not only produced but also consumed during methanogenic degradation of organic matter. Therefore, priming effects in anoxic rice field soils may be different from those in oxic upland soils in which CO_2 is only produced and is the sole product (Cheng, 2009; Kuzyakov, 2010). Conventionally, "priming effect" (PE) is defined as the enhancement of SOM degradation by labile organic substrates, which in upland soil is equivalent to CO_2 production from SOM. However, in methanogenic rice field soil, production of both CH_4 plus CO_2 is equivalent to degradation of SOM. In our study we therefore differentiate between enhancement of CH_4 production, CO_2 production and SOM degradation (priming effect).

The following observations are noteworthy: (1) enhancement of CH_4 production was observed after a certain period of anoxic degradation of RS (Figs. 3c and 4b), while PE of CH_4 plus CO_2 production was not observed (Fig. 4d). (2) Enhancement of CH_4 production was mainly from hydrogenotrophic methanogenesis (Fig. 4c). (3) Degradation of RS resulted in an increased abundance of methanogens, but not of Bacteria (Fig. 2b). (4) Previous degradation of RS resulted in enhancement of RS degradation at later treatment as seen in enhanced production of both CH_4 and CO_2 (Table 2). These results are most parsimoniously interpreted as follows: anoxic degradation of rice straw in Vercelli rice field soil was not limited by the abundance of Bacteria but by the abundance of methanogenic Archaea, explaining why preincubation of soil with RS increased the number of methanogens and the rates of TIC and CH_4 production from new RS (Fig. 4b, Table 2) and also ROC (Fig. 2a), since both RS and ROC are rice-plant-derived labile carbon. Besides, preincubation of soil with RS resulted in the simultaneous enhancement of CH_4 production from SOM (Figs. 3c and 4b), which was mainly caused by enhancement of hydrogenotrophic CO_2 reduction (Fig. 4c), so a PE of CH_4 plus CO_2 production (i.e., SOM decomposition) was not observed (Fig. 4d).

Previous studies have shown that the fermenting microorganisms that colonize RS and cause the primary hydrolysis and fermentation of the straw polysaccharides release fermentation products into the soil environments, where they are further degraded to CH_4 and CO_2 (Glissmann et al., 2001). Here we have shown that methanogenic reduction of SOM-derived CO_2 is apparently also enhanced by fermentation products of RS degradation, presumably by H_2. It has previously been observed that increased abundance of methanogens is paralleled by increased CH_4 production (Liu et al., 2012). More specifically, it has been shown that RS treatment results in an increase of total CH_4 production and in the abundance of methanogenic Archaea but not of Bacteria, and that the increase is mostly due to *Methanosarcina* species that are both potentially hydrogenotrophic and aceticlastic methanogens (Conrad and Klose, 2006; Conrad et al., 2012a). Here we have shown that this increase in methanogenic abundance further stimulated additional CH_4 production from SOM.

In summary, our study demonstrated that RS is not only an additional substrate for CH_4 production and enhances the creation of a reduced soil environment, but also causes a positive feedback on the CH_4 production from both SOM and ROC, so the overall production of CH_4 is larger than expected from the methanogenic degradation of RS alone. As CH_4 emission increases with CH_4 production (Fig. 1), the widespread application of RS will produce a non-linear response of CH_4 emission to straw application, which will be important for process-oriented models of CH_4 emission (Fumoto et al., 2008) and the assessment of future climate change due to CH_4 (Montzka et al., 2011).

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