Soil bacterial community mediates the effect of plant material on methanogenic decomposition of soil organic matter

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Abstract

Input of plant material may strongly change decomposition rates of soil organic matter (SOM), i.e. causing priming effect (PE), but the underlying mechanisms are largely unknown. We show that rice straw addition in anoxic Fuyang (F) rice field soil stimulated CH_4 production from SOM at the expense of CO_2, whereas in Uruguay (U) soil it suppressed SOM degradation to CO_2 plus CH_4 (negative PE). Reciprocal inoculation experiments with non-sterile and sterile soils showed that the soils always displayed the effect of rice straw characteristic for the live microbial community rather than for the soil physicochemical properties. Pyrosequencing of 16S rRNA genes showed that bacterial communities in these soil samples were separated into two clusters (F and U). *Symbiobacterium* was abundant or dominant in microbiota from U soil, but negligible in those from F soil. Network analysis indicated that the bacterial populations involved in SOM decomposition were different between soils of F and U clusters; moreover, they were more tightly connected to methanogens in U than in F clusters. Ultimately, our results suggested that the PE of rice straw is mediated by the composition and activity of soil microbial community.

Keywords: Soil organic matter; Rice field soil; Anoxia; Priming effect; 16S rRNA gene; Microbial community

1. Introduction

Incorporation of plant material, such as litter, dead roots or root exudates into the soil is quite common in terrestrial ecosystems (Kramer et al., 2010; Yagi and Minami, 1990; Zhu and Cheng, 2011), and it is important for maintaining soil fertility (Sass et al., 1991; Schütz et al., 1989; Yagi and Minami, 1990). Moreover, input of fresh organic matter may accelerate or suppress soil organic matter (SOM) decomposition, causing a positive or negative priming effect (PE) (Guenet et al., 2010; Kuzyakov et al., 2000; Langley et al., 2009; Paterson et al., 2008; Wolf et al., 2007). A positive PE increases the rate of SOM decomposition (Chen et al., 2014; Paterson and Sim, 2013; Pausch et al., 2013; Zhu and Cheng, 2011). Negative PEs, which decrease the rate of SOM decomposition, are not reported quite as often as positive PEs (Cheng, 1996, 1999), but negative PEs are also of great significance to carbon balance, since slower decomposition leaves more C sequestered and not released as CO_2 (Kuzyakov et al., 2000). Over long time scales, PEs are thought to be able to influence ecosystem C balance (Wieder et al., 2013). In addition, soil C pools are larger than the pool of atmospheric CO_2, so that small changes in the rate of soil C decomposition could cause a profound impact on atmospheric CO_2 concentration (Davidson and Janssens, 2006; Smith et al., 2008).

Underlying mechanisms of PEs remain largely elusive. Soil microorganisms, including bacteria and fungi, are considered to play the key role in the process leading to PEs during decomposition of upland SOM (Fontaine and Barot, 2005; Kuzyakov, 2010; Nottingham et al.,

2009). It is widely accepted that the growth of microorganisms utilizing fresh organic matter (FOM degraders) is stimulated after substrate addition, followed by the gradual increase in the abundance of microorganisms utilizing polymerized SOM (SOM degraders), thus resulting in a positive PE (Fontaine and Barot, 2005; Fontaine et al., 2003; Perveen et al., 2014). In contrast, it is assumed that SOM degraders would preferentially utilize fresh organic matter, if it is available in excess, and thus lead to a negative PE, since competition between FOM and SOM degraders is negligible under this condition (Blagodatskaya et al., 2007; Cheng, 1999; Kuzyakov and Bol, 2006); however, experimental support remains ambiguous with some reports being inconsistent with these explanations (Rousk et al., 2015; Wild et al., 2014; Wu et al., 1993). Furthermore, recent studies suggest that there is a close correlation between PE and the soil microbial community composition. For example, diversity and composition of the soil microbial community were found to change in concert with negative or positive PE after single or repeated substrate amendments (Mau et al., 2015), and the magnitude of positive PE of fresh organic carbon on N mineralization from SOM increased in treatments with higher fungal dominance (Rousk et al., 2016). Despite this, it is still unclear what role the microbial community composition plays in causing a PE, in particular which microbial species are involved.

Current reports on PE and the plausible mechanisms were mostly targeted at various upland soils where CO_2 is the only end product of organic matter decomposition (Kuzyakov and Bol,

2006; Zhu and Cheng, 2011), but the PE has rarely been studied in flooded soil, such as rice field and wetland soils (Ye et al., 2015; Yuan et al., 2014), where both CO_2 and CH_4 are the end products of anaerobic degradation of organic matter. Anaerobic degradation is accomplished consecutively by a complex microbial community consisting of hydrolytic, fermentative, syntrophic, homoacetogenic bacteria and methanogenic archaea (Conrad, 1999; Glissmann et al., 2001). Anaerobic degradation of organic matter in rice fields is one of the most important sources of atmospheric CH_4 (Conrad, 2009), which has approximately 25 times the global warming potential of CO_2 (Forster et al., 2007). Rice provides the staple food for half the world population (Kalbitz et al., 2013). Input of plant material, such as rice straw (RS) is common in the management of rice field soils (Sass et al., 1991; Yagi and Minami, 1990; Yuan et al., 2012). Consequently, an effect of rice straw on rice field SOM degradation could influence the global budgets not only of CO_2, but also CH_4. While 80-90% of the RS is decomposed within the first year (Neue and Scharpenseel, 1987), the SOM in rice field soils is rather refractory, and was found to decrease only little (6-17%) within 120 days of anoxic incubation (Yao et al., 1999). The RS applied might be of significance for the decomposition rate of SOM in rice field soils exerting either a positive or negative PE. Previous studies indeed reported either negative (Conrad et al., 2012) or positive (Ye et al., 2015; Yuan et al., 2014) effects of RS on the production of CH_4 from SOM, but largely neglected the production of CO_2, which is an essential part of the PE on SOM in flooded soils.

In this study, we investigated the microbial mechanisms underlying the effect of RS on methanogenic SOM decomposition in rice field soils. We used two rice soil samples (Fuyang and Uruguay), which were selected based on the fact that they both had a relatively high CH_4 production potential (Fernandez Scavino et al., 2013; Yuan and Lu, 2009), and since RS additions resulted in different responses in SOM decomposition in the two soils. A ^{13}C-labeling technique was applied to determine the PE of RS on SOM decomposition (Yuan et al., 2014). Our hypothesis has been that microbial community composition is the key for the PE, i.e. differences in PE between soils are dependent on their distinct soil microbial community compositions rather than on their distinct soil physicochemical properties. To test this hypothesis, we manipulated the soil microbial community through reciprocal inoculation with non-sterile and sterile samples of Fuyang and Uruguay soils. In this way we intended to create the same microbial community (e.g., from Fuyang) in a soil background with different soil physicochemical characteristics (Fuyang versus Uruguay). Then, we analyzed the bacterial and archaeal community composition and abundance in these soil samples. Correlation-based co-occurrence networks analysis was employed to produce microbial functional modules, aiming to reveal the differences in functional groups between soils with and without PE.

2. Material and methods

2.1. Soil samples

Soils were collected from China (Fuyang) and Uruguay. The China soil (Fuyang) is a clay loam (soil type: hydrargic anthrosol) collected in 2007 from a rice field (30.1˚N, 119.9˚E) at the China National Rice Research Institute in Hangzhou (Rui et al., 2009). The Uruguay soil is a clay soil (soil type: planosol) sampled in 2011 from a field (32.49˚S, 53.49˚W) 70 km from the Instituto Nacional de Investigación Agropecuaria (INIA) at the city Treinta-y-Tres, Uruguay (Fernandez Scavino et al., 2013). The fields in Uruguay had a history in rotation management. The typical rotation is four consecutive years of cattle pasture followed by two consecutive years of flooded rice fields. The soil sample used in this experiment was taken after four years of cattle pasture prior to flooding. Nevertheless, the Uruguay soil can still be considered as a paddy soil, since a previous study has concluded that a stable methanogenic microbial community established in the Uruguay soil once pastures had been turned into management by pasture-rice alternation (Fernandez Scavino et al., 2013). The sampling for each soil was done by taking soil cores (0-10 cm depth) from the ploughing layer at three locations in the field. Since we did not intend to assess site variability within the original field sites, a composite sample was prepared by mixing the samples by hand from all the three sites. These composite samples were termed Fuyang (F) or Uruguay (U), respectively. The soil samples were air-dried and stored at room temperature (Frenzel et al., 1999; Ma et al., 2010). The storage of dried soil at room temperature has no significant effect on soil methane

production capacity (Mayer and Conrad, 1990). The dry soil lumps were broken using a mechanical grinder, and sieved through a 0.5-mm stainless steel sieve to homogenize sample (Chidthaisong et al., 1999; Roy and Conrad, 1999). Chemical characteristics of the soil samples are shown in Table S1. Part of each soil sample was sterilized by γ-irradiation (30 kGy; ^{60}Co) (McNamara et al., 2003; Philippot et al., 2013). The sterility of the γ-irradiated soil was checked by following CH_4 release upon flooding. No CH_4 production was detected during the whole experiment (62 days in total).

2.2. Preparation of the rice straw

Preparation of the ^{13}C-labeled rice straw (RSI and RSII) has been described previously (Yuan et al., 2012). The RSI and RSII were prepared for calculating the relative contributions of RS and SOM to CH_4 and CO_2 as described below. The $\delta^{13}C$ values of RSI (596.1‰) and RSII (885.0‰) were obtained by mixing desired amount of ^{13}C-labeled ($\delta^{13}C$= 1859.9‰) and unlabeled ($\delta^{13}C$= -27.6‰) RS. All the RS derived from rice plants grown in the greenhouse, ^{13}C-labeled RS was prepared by labeling the rice plants with $^{13}CO_2$ (Yuan et al., 2012). These rice plants were harvested at the late vegetative stage, then RS was dried and ground to powder. In soil applied with RSI or RSII, the $\delta^{13}C$ values of the produced CH_4 and CO_2 were always lower than that of the RS mixture even when both 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 (Yuan et al., 2014). The C/N ratio of labeled RS was 20. The determination of the soil organic carbon content and the stable isotopic signatures of dried plant (RS) were carried out at the Institute for Soil Science and Forest Nutrition (IBW) at the University of Göttingen, Germany.

2.3. Soil incubation and analytical techniques

Waterlogged soil microcosms were prepared not only from original Fuyang and Uruguay soil samples, but also from combinations of original and sterilized soils as follows: first, 5% original Fuyang soil was inoculated into 95% sterilized Fuyang soil (5%F+sF) and sterilized Uruguay soil (5%F+sU), respectively; also, 5% Uruguay soil was inoculated into 95% sterilized Uruguay soil (5%U+sU) and sterilized Fuyang soil (5%U+sF), respectively. Two further combinations were prepared as controls: 5% sterilized Uruguay soil was added into 95% original Fuyang soil (5%sU+F), and 5% sterilized Fuyang soil was added into 95% Uruguay soil (5%sF+U). For each soil microcosm, a total of 4 g dry weight soil was prepared and flooded with 6 ml anoxic water in 26-ml pressure tubes as described before (Yuan et al., 2014). Tubes were closed with butyl rubber stoppers, sealed with aluminum crimps, then flushed with N_2 and incubated statically at 25°C in darkness. The CH_4 and CO_2 production were measured during the incubation. The Fe (III) reduction was determined by measurement of the production of Fe (II) during the incubation (Yao et al., 1999). The soils were preincubated under anoxic conditions, in order to revive the microbial community and

establish relatively stable methanogenic conditions to mitigate handling effects (Teh and Silver, 2006). After 40 days of preincubation, RS treatments I and II were prepared by adding same amount (8 mg) of RSI or RSII powder into each tube. Immediately after RS addition, the tubes were sealed again and flushed with N_2, after shaking by vortexing, re-flushed with N_2 to remove the residual CH_4 and CO_2. Finally, the tubes were incubated statically at 25°C for 22 days. All the treatments were prepared in triplicate.

2.4. CH_4 and CO_2 analyses

At regular time intervals (day 3, 5, 7, 10, 15, 20 and 22), gas samples from the headspace of the tubes were analyzed for CH_4 and CO_2 using a gas chromatograph (GC) equipped with flame ionization detector (FID). The CO_2 was measured after conversion to CH_4 using a methanizer (nickel catalyst at 350°C) (Penning and Conrad, 2007). 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 tubes were opened at the end of the incubation, and the liquid was analyzed for pH. Then the total amounts of CO_2 dissolved (aq) in the liquid were calculated from the solubility constant of CO_2 ($1\times10^{-1.47}$ mol L^{-1} bar^{-1}), those of bicarbonate (HCO_3^-) were calculated from the solubility constant of CO_2, the pH, and the dissociation constant ($10^{-6.35}$) of bicarbonate (Stumm and Morgan, 1981). The sum of gaseous, dissolved and bicarbonate CO_2 was defined as total inorganic carbon (TIC).

190 Stable isotopic analyses of CH_4 and CO_2 were performed as described in an earlier study
191 (Penning and Conrad, 2007) using GC-combustion-isotope ratio mass spectrometer (GC-C-
192 IRMS) (Finnigan, Bremen, Germany). The $\delta^{13}C$ values of dissolved CO_2 ($\alpha_{CO2(aq)}$ = 0.9990)
193 and HCO_3^- (α_{HCO3^-} = 1.0075) were calculated from the $\delta^{13}C$ of gaseous CO_2 and the
194 corresponding fractionation factors α (Stumm and Morgan, 1981). The values of $\delta^{13}C_{CO2(g)}$,
195 $\delta^{13}C_{CO2(aq)}$ and $\delta^{13}C_{HCO3^-}$ were used to calculate $\delta^{13}C_{TIC}$ using the mole fractions of the
196 different CO_2 species (Penning and Conrad, 2006).
197 *2.5. Contribution of SOM and RS to CH_4 and CO_2 in soil slurries with RS application*
198 The calculations were the same as described earlier (Yuan et al., 2014). In brief, since the
199 only difference between the treatments RSI and RSII is the $\delta^{13}C$ of the RS applied, the
200 fraction of CH_4 produced from RS (f_{RS}) was calculated by:
201 $$f_{RS} = (\delta^{13}C_{CH4\text{-}I} - \delta^{13}C_{CH4\text{-}II}) / (\delta^{13}C_{RS\text{-}I} - \delta^{13}C_{RS\text{-}II}) \quad (1)$$
202 of which the $\delta^{13}C$ values were determined experimentally. The $\delta^{13}C_{CH4\text{-}I}$ and $\delta^{13}C_{CH4\text{-}II}$ were
203 the $\delta^{13}C$ values of the CH_4 produced in the RS treatment I and II, respectively; the $\delta^{13}C_{RS\text{-}I}$ and
204 $\delta^{13}C_{RS\text{-}II}$ are $\delta^{13}C$ of the RS carbon in treatment I (596.1‰) and II (885.0‰), respectively.
205 Next, the fraction of CH_4 production from SOM (f_{SOM}) can be calculated, since in the RS
206 treatment
207 $$f_{RS} + f_{SOM} = 1 \quad (2)$$

208 Finally, the amount of CH_4 production from SOM ($p_{SOM,CH4}$) and RS ($p_{RS,CH4}$) were
209 calculated from the total amount of CH_4 produced (p_{CH4}) and the fractions of CH_4 production
210 from SOM (f_{SOM}) and RS (f_{RS}), respectively:

211 $$p_{SOM,CH4} = f_{SOM}\, p_{CH4} \quad (3)$$

212 $$p_{RS,CH4} = f_{RS}\, p_{CH4} \quad (4)$$

213 Analogous equations are valid for the fractions and amounts of CO_2 produced from SOM
214 and RS in the rice soil microcosms.

215 *2.6. DNA extraction and quantification of microbial abundance*

216 DNA from the soil samples collected after the preincubation and at the end of incubation
217 was extracted according to the lysis protocol described in the FastDNA® Spin kit for soil
218 (Qbiogene, Germany). The quantitative PCR of bacterial 16S rRNA was performed following
219 a protocol described previously (Stubner, 2002); the quantitative PCR of archaeal methyl
220 coenzyme M reductase (*mcrA*) gene, which is characteristic and unique for methanogenic
221 archaea, was performed as described previously (Angel et al., 2011). The gene copy numbers
222 detected are a proxy for the abundance of the respective microbes.

223 *2.7. Pyrosequencing of bacterial and archaeal communities*

224 For tagged pyrosequencing of bacterial 16S rRNA gene fragments we used primers F515
225 and R806 (Bates et al., 2011), and primers Arch344F and Arch915 for archaeal 16S rRNA
226 gene fragments (Casamayor et al., 2002; Yu et al., 2008). The forward primer of each

227 combination contained a unique 6-bp barcode (Hernandez et al., 2015). The purified PCR
228 product of each sample was pooled in an equimolar concentration for pyrosequencing. The
229 454-pyrosequencing was carried out at the Max Planck Genome Centre in Cologne (Germany)
230 using a Roche 454 Genome Sequencer GS FLX+.
231 All raw sequences obtained from pyrosequencing were first analyzed with Mothur (v. 1.27)
232 software package (http://www.mothur.org/) (Schloss et al., 2009), and the OTU table was
233 created using the UPARSE pipeline (http://www.drive5.com/usearch/manual/uparse_cmds.
234 html) (Edgar, 2013). Within this pipeline, sequences were first sorted based on barcodes and
235 removed from further analysis if they were shorter than 200 bp, contained ambiguous bases or
236 homopolymers greater than 6 bp in length. Chimeras were removed using UCHIME (Edgar et
237 al., 2011). Operational taxonomic units (OTUs) were defined from the accepted sequences
238 with 97% sequence similarity. Taxonomic classification was carried out with the naïve
239 Bayesian classifier in Mothur using the Silva 16S rRNA reference database. Rarefaction
240 curves and diversity indices including microbial community richness (Chao1), diversity
241 (Shannon index) and coverage were calculated in Mothur. The OTU table was subsampled to
242 the minimum number of sequences obtained for a sample prior to downstream analysis. The
243 454 pyrosequencing reads (raw data) were deposited under the study number SRP058834 in
244 the NCBI Sequence Read Archive (SRA).
245 *2.8. Heatmap analysis*

246 The heatmap representation of the relative abundance of bacterial OTUs among samples
247 was built using R (http://www.r-project.org/), as described previously (Deng et al., 2014). The
248 OTU abundance table was Hellinger transformed (decostand function within the R vegan
249 package) to diminish the influence of zero values and to give low weights to rare species
250 (Legendre and Gallagher, 2001). Principal components analysis (PCA) was performed using
251 prcomp and the result indicated that for bacterial OTUs, PC1, PC2 and PC3 explained 40%,
252 18% and 13% of the variance, respectively. To select the OTUs explaining most of the
253 differences between samples, the 40 bacterial OTUs with highest loadings of PC1, 18 OTUs
254 of PC2 and 13 OTUs of PC3 were chosen to construct the heatmap. A total of 27 unique
255 OTUs were obtained because of the two reasons: first, some of the OTUs were selected from
256 more than one PC; second, OTUs with low averaged relative abundance (<1% in every
257 control or treatment) were removed (those removed OTUs also had relatively lower loading
258 values). The OTU abundances were converted to percentage of reads from each sample and
259 the heatmap constructed using the heatmap.2 function in gplots (Warnes et al., 2014). The
260 taxonomy of the selected OTUs was added separately.

261 *2.9. Co-occurrence network analysis*

262 The bacterial and archaeal OTU tables were randomly subsampled to the same sequence
263 depth using daisychopper.pl (http://www.festinalente.me/bioinf/downloads/daisychopper.pl).
264 These tables were combined so as to prepare the network analysis for prokaryotic OTUs in

soil samples. Cosmopolitan bacterial and archaeal OTUs, which occurred in more than half of the selected samples were used for network analysis. We calculated all possible Spearman's rank correlations between selected OTUs (Ju et al., 2014). We considered a valid co-occurrence event to be a robust correlation if the Spearman's correlation coefficient (ρ) was >0.6 and the P value was <0.01 (Barberan et al., 2012). Correlation networks were constructed with the robust correlations as weighted edges and visualized with Gephi software (https://gephi.github.io/). 10000 Erdös-Réyni random networks, which had the same number of nodes and edges as the empirical networks, were generated using the R package igraph (http://cran.rproject.org/web/packages/igraph/) (Ju et al., 2014).

2.10. Statistical analysis

To test the significance of the differences between control and RS treatment on various variables, two-tailed independent t-tests were applied using Microsoft Excel 2007. The significance of differences in relative abundance of OTU between treatments was determined by one-way analysis of variance (ANOVA) using SPSS 13.0. P values below 0.05 were considered statistically significant.

Overall structural changes of prokaryotic communities were evaluated by Principal Coordinate Analyses (PCoA) with Fast UniFrac distances (Lozupone et al., 2006). The statistical significance among datasets was assessed by PerMANOVA using the weighted PCoA scores in PAST (http://folk.uio.no/ohammer/past/). The Mantel test was applied to

evaluate the correlations between prokaryotic communities with environmental variables using the Mantel procedure in the R package Vegan. The Variance partitioning analysis (VPA) was performed to quantify the relative contributions of environmental variables to changes in the bacterial and archaeal community structures by the method described previously (Yao et al., 2014).

3. Results

3.1. Differential response of methanogenic decomposition of SOM to RS addition

The paddy soils were 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, which was confirmed by active CH_4 production (Fig. S1) and absence of Fe (III) reduction (data not shown). Subsequently, the soils were amended with 0.2% (2 mg straw g dw^{-1} soil) ^{13}C-labeled RS, and the amount of CH_4 and TIC produced from SOM in the treatment were calculated using eq. (1-3). The results showed that RS treatment enhanced the accumulation of SOM-derived CH_4 in Fuyang (F) soil sample (Fig. 1a). Nevertheless, there was no significant difference in the total amounts of SOM decomposition (SOM-derived CH_4 and CO_2) between RS treatment and control at the end of incubation (Fig. 1g), since the accumulation of CO_2 (quantified as TIC) was decreased (Fig. S2). In Uruguay (U) soil sample, however, the SOM-derived CH_4 was almost the same between treatment and

control (Fig. 1b), while SOM decomposition was significantly decreased in the RS treatment (Fig. 1g). Besides, the total amount of SOM decomposition in F soil (>183 μmol) was substantially larger than that in U soil (<130 μmol), which is consistent with the higher soil organic carbon content in F (2.38%) than U soil (1.57%) (Table S1).

In both combinations of 5% non-sterilized F with sterilized soil (sF or sU), the effects of RS addition on both SOM-derived CH_4 production and SOM decomposition (Fig. 1c, d, g) were similar to that in original F soil, although the amount of SOM decomposition in combination of 5%F with sU was similar with that in original U soil. For both combinations of 5% U with sterilized soil (sF or sU), the effects of RS were consistent with that in original U soil (Fig. 1e, f, g), although the amount of SOM decomposition in combination of 5%U with sF was similar with that in original F soil. On the other hand, for the combination of 5% sterilized U with original F, the effects of RS were consistent with that in original F soil (Fig. S3a, c), and vice versa (Fig. S3b, c). The experiment of sterilization and inoculation had little influence on the pH values of soil samples (Table S2). Besides, the amounts of RS decomposition were not significantly different between original F soil and U soil, and between sterilized soil inoculated with F and U (Fig. S4). However, CH_4 production was enhanced in sF inoculated with 5%F compared to the original F soil (Fig. 1a, c), while SOM degradation was similar (Fig. 1g). Sterilization of the F soil apparently resulted in enhanced production of CH_4. This was probably caused by a side effect of gamma irradiation. It has

been shown that decomposition rates in some γ-irradiated soils, after re-introduction of a microbial community, were greater than in the un-irradiated controls (McNamara et al., 2003).

3.2. Abundance of bacterial and archaeal communities

The abundances of bacteria and methanogenic archaea in most of the soil samples were in the similar range after preincubation before being used for the incubation experiments (Fig. S5). This was also the case for the sterilized soils that had been inoculated with 5% non-sterile soil, indicating that the microorganisms had increased in abundance during the preincubation time. The microbial abundances were again determined at the end of the incubation experiment using quantitative PCR. The abundance of the bacterial 16S rRNA gene ranged from about 3×10^9 to 9×10^9 copies g^{-1} soil (Fig. 2a). RS addition significantly stimulated the abundance of bacteria only in original F and in the combination of 5%U with sterilized F. The abundance of methanogenic archaea was determined by targeting the methyl coenzyme M reductase (*mcrA*) gene, which is characteristic and unique for methanogenic archaea. The results indicated that original U soil had the lowest abundance of *mcrA* (5.6×10^7 copies g^{-1} soil) (Fig. 2b), while the highest abundances of *mcrA* were found in 5%F+sF with RS treatment (3.3×10^8 copies g^{-1} soil). The RS treatment resulted in significant increase of the *mcrA* abundance in the original U soil only.

3.3. Bacterial and archaeal 16S rRNA genes diversity analysis

A similarity level of 97% was used to identify OTUs and to estimate diversity of bacterial and archaeal 16S rRNA genes in soil samples. A total of 122201 bacterial sequences corresponding to 7987 OTUs were obtained after quality filtering. In both control and RS treatment of F soil and control of U soil, about 2000 OTUs were found at a sequencing depth of about 7000 (Fig. S6a), while all other soil samples contained fewer than 900 OTUs at a sequencing depth ranging from 1272 to 2611. Rarefaction curves of Shannon indices almost approached plateaus (Fig. S6c) and the coverage was higher than 80% in each soil sample (Fig. S6d), although the rarefaction curves of OTUs and Chao1 were not saturated in any sample (Fig. S6a, b). The control and RS treatment of original F soil and the control of original U soil were sequenced to greater depth, since they displayed a higher bacterial α-diversity than the other samples. The dominant phyla were Firmicutes (39.9% on average) and Proteobacteria (12.6% on average) (Fig. 3a). The major difference between the original F and U soil was the relative abundance of Firmicutes being higher in F soil. The sterilization and inoculation treatments increased the relative abundance of Firmicutes in each soil. RS treatment increased the relative abundance of Acidobacteria and Firmicutes in original U soil, while there was no major difference between control and RS treatment in other soil samples.

For the archaea, a total of 106220 sequences belonging to 424 OTUs were obtained after quality filtering. The sequencing depths ranged from 1143 to 6123, and the sequences from different samples clustered into 73 to 173 OTUs. The sequence sampling effort was sufficient

to obtain coverages higher than 97% for every sample (data not shown). The archaeal communities were composed of both Crenarchaeota and Euryarchaeota phyla (Fig. 3b). Euryarchaeota-affiliated sequences were dominant in most of the samples, accounting for more than 90% of archaeal sequences except in the original Uruguay soil (82%).

3.4. OTU-level bacterial and archaeal diversity analysis

The bacterial communities in sterilized soils were highly correlated (79%) with the inoculum sources (Table 1). Variance partitioning analysis (VPA) also showed that inoculum source explained a higher ratio of the observed variation (37%) of bacterial communities relative to other variables including soil type, etc (Table S3). Indeed, soil type affected the soil bacterial community composition only little (17%) albeit significantly (Table S3). As a result, bacterial communities of the soil samples were separated into two clusters (F and U) in accordance with the inoculum sources based on Unifrac distances (PerMANOVA p=0.001) (Fig. 4). One replicate of 5%U+sF apparently deviated from the others and was close to cluster F. We assumed that this variation was caused by the treatment of sterilization and inoculation. In contrast to the Bacteria, the archaeal communities did not cluster according to the origin of the microbial communities (Fig. S7), and the inoculum source also explained less of the variance (26%) of the archaeal communities (Table S3). Next, the heatmap analysis was used to intuitively display the differences in relative abundances of bacterial OTUs among samples (Fig. 5). The OTUs with the highest contribution to the PCA ordination were

selected. The results indicated that the relative abundance of OTU2 and OTU52 (both belonging to *Symbiobacterium*) significantly increased in original U soil after RS addition (Table S4); moreover, both these OTUs were also much higher in sterilized soils inoculated with U than with F soil (Table S5). In contrast, the relative abundances of OTU8026 (*Clostridium*), OTU1 and OTU3 (both belonging to Sphingobacteriales) were significantly higher in original F compared with original U soil. OTU3 in particular was notably more abundant in sterilized soils inoculated with F than with U soil (Table S5).

3.5. Network analysis of cosmopolitan bacterial and archaeal OTUs in cluster F and U

Two positive correlation-based networks, named F and U (Fig. 6a, b), were constructed with these cosmopolitan prokaryotic OTUs of samples in cluster F and U (Fig. 4), respectively. The results showed that network F and U were similar in size and topology (Table S6), sharing about 40% of the bacterial nodes and about 90% of the archaeal nodes (Fig. S8). In each network, there were three major modules (Fig. 6 and Table 2). Among them, two major modules were mainly composed of bacterial nodes, i.e. FM1 and FM2 in network F, UM1 and UM2 in network U (Table 2), while another module in each network was almost exclusively composed of methanogenic archaea (FM3 and UM3). In network F, bacterial nodes in FM2 had numerous positive correlations with methanogens in FM3 (Fig. 6), the same as between UM2 and UM3 in network U. The modules FM1, FM2, UM1 and UM2

396 exhibited positive correlation with soil organic carbon (SOC) content and soil pH value but
397 not with RS treatment (Table 3).
398 *Symbiobacterium* was dominant in network U but substantially lower in network F (32%
399 versus 3%) (Table S7). Besides, in network F, the number of positive correlations between
400 bacteria and hydrogenotrophic methanogens was similar with that between bacteria and
401 acetoclastic methanogens (Table S8). In network U, however, both these correlations were
402 more numerous, and in addition the number of positive correlations between bacteria and
403 acetoclastic methanogens exceeded that between bacteria and hydrogenotrophic methanogens.
404

405 **4. Discussion**

406 Addition of fresh organic matter in form of RS to anoxic flooded soils affected SOM
407 degradation to CO_2 plus CH_4. In F soil, RS input had no effect on SOM decomposition (no PE;
408 Fig. 1g). However, the relative amount of CH_4 produced from SOM significantly increased
409 (Fig. 1a). Consequently, the production of CO_2 from SOM must have decreased. This
410 observation is best explained by assuming that hydrogenotrophic methanogenesis from SOM-
411 derived CO_2 was stimulated by H_2 released from RS decomposition, similarly as it had been
412 observed in Italian rice field soil (Yuan et al., 2014). By contrast, stimulation of acetoclastic
413 methanogenesis should have increased production of both CO_2 and CH_4. In U soil, however,

RS addition resulted in suppression of SOM degradation (negative PE) to CO_2 and CH_4 (Fig. 1g) and thus, decreased greenhouse gas production from SOM.

Reciprocal inoculation of sterilized soil with non-sterile soil from the same or the different type showed that the PE on SOM degradation was predominantly determined by the soil inoculum source. For example, both sterilized F and U soils showed negative PEs after inoculation with 5% U but not with 5% F (Fig. 1g). Therefore, the observed PE was not caused by the physicochemical soil characteristics but by the soil microbial community. This conclusion is at least true for the soils studied. However, we cannot exclude that in other rice cultivation areas soil physicochemical characteristics may be of greater importance for causing PE.

RS treatment significantly increased the abundance of bacteria or methanogens only in some of the soil samples, which did not necessarily have a PE (Fig. 2a, b). Hence, in our soils it is unlikely that the observed PE was caused by the biomass of bacteria or methanogenic archaea. This finding does not rule out, however, that in other soils acceleration or retardation of SOM decomposition may be due to the increase of soil microbial biomass after substrate addition (Kuzyakov et al., 2000).

We conclude that in our experiments it was the composition of the soil microbial communities rather than biomass abundance or physicochemical soil characteristics that did or did not cause PE(Fig. 1, 2 and 4). In order to characterize the correlation of bacterial and

433 archaeal microorganisms in methanogenic soils with and without a PE, two positive
434 correlation-based networks were constructed with cosmopolitan prokaryotic OTUs for cluster
435 F and U, respectively (Fig. 6a, b). Correlation-based co-occurrence network analysis can
436 produce microbial functional modules, which allows the interactions between different
437 functional groups in complex systems to be revealed (Barberan et al., 2012; Deng et al., 2012;
438 Ju et al., 2014). The prokaryotic community in each network was organized by three major
439 functional modules (Fig. 6 and Table 2). Values of modularity, average clustering coefficient
440 and two other parameters in both empirical networks were higher than those in random
441 networks (Table S6), suggesting that the empirical networks had common network
442 characteristics, such as modularity and hierarchy properties (Deng et al., 2012; Watts and
443 Strogatz, 1998).
444 The important function of each module could be inferred based on the prokaryotic
445 composition and their known physiological functions (Rui et al., 2015). Our results suggested
446 that each network probably included a primary fermentation module (FM1 or UM1),
447 methanogenic fermentation module (FM2 or UM2) and methanogenic module (FM3 or UM3).
448 This was consistent with studies showing that anaerobic methanogenic systems consist of
449 well-organized, closely interacting bacterial and archaeal populations (Kim and Liesack, 2015;
450 Rui et al., 2015). Most of the nodes in FM1 and UM1 belonged to Firmicutes, Actinobacteria
451 and Acidobacteria (Table 2), which usually are involved in hydrolysis of complex organic

452 matter (Kim and Liesack, 2015; Rui et al., 2015; Wegner and Liesack, 2016). The numerous
453 positive correlations between bacteria and methanogens in FM2 and UM2 were probably
454 caused by the bacterial production of methanogenic substrates, e.g., H_2/CO_2, formate and
455 acetate (Kim and Liesack, 2015; Rui et al., 2015), or because of multiple syntrophic
456 interactions between bacteria and hydrogenotrophic methanogens (Schink, 1997). In general,
457 positive co-occurrence of prokaryotic populations within or between modules could reflect
458 their similar niche adaptation or interspecies cooperation (Rui et al., 2015).
459 The network analysis mainly reflected the correlation of prokaryotic populations during
460 degradation of SOM rather than degradation of RS because of two reasons. First, RS addition
461 did not substantially change the bacterial and archaeal community compositions and
462 abundances in most of the soil samples (Fig. 2, 3 and S4). Second, the two types of
463 fermentation modules in both networks were highly correlated with the SOC content but not
464 with the RS treatment in each soil (Table 3). Therefore, we suggest that the network analysis
465 is helpful in the elucidation of differences in the microbial community composition between
466 soils with and without PE during SOM decomposition.
467 For example, the primary fermentation module (FM1) of network F contained more than 20
468 nodes of Clostridia (Table 2). However, in network U, OTUs of Clostridia were solely found
469 in the methanogenic fermentation module (UM2). Clostridia are of importance for the
470 anaerobic breakdown of polymers in flooded paddy soil, but could also participate in

fermentation and utilization of sugar (Kim and Liesack, 2015; Rui et al., 2015). Therefore, some major nodes of the Class Clostridia, such as the genus *Clostridium* (Fig. 6 and Table S7), probably were involved in the primary fermentation and methanogenic fermentation during consecutive degradation of SOM in soils of F and U clusters, respectively.

Analogously, *Symbiobacterium* was dominant in network U but substantially lower in network F (Table S7), which was also consistent with the heatmap analysis of the bacterial OTUs in soils of F and U clusters (Fig. 5). *Symbiobacterium* spp. are known as symbiotic bacteria (Ohno et al., 2000; Rhee et al., 2002), but also exhibit marked mono-growth if CO_2 or bicarbonate is available (Watsuji et al., 2006). *S. thermophilum* possesses a glucose degradation pathway and carries the genes for metabolizing gluconate, cellobiose and others (Ueda et al., 2004). Indeed, nodes of *Symbiobacterium* in network U had positive correlations with numerous bacteria and methanogenic archaea (Fig. 6). *Symbiobacterium* was actively involved in methanogenic fermentation in soils of U cluster, in accordance with its presence in modules UM2 and UM3, while this was not the case in soils of F cluster. Finally, compared with network F, bacteria in network U had more positive correlations with methanogens, especially having many more edges with acetoclastic than with hydrogenotrophic methanogens (Table S8). These results implied that the two networks probably differed in the role of hydrogenotrophic methanogenesis versus acetoclastic methanogenesis, which suggested that these soils of F and U clusters had different pathways of methanogenic

degradation of SOM (Conrad et al., 2009, 2010). Therefore, network analysis of prokaryotic community composition indicated that bacterial communities involved in consecutive anaerobic SOM decomposition were apparently different between soils of F and U clusters. The reason why such difference in microbial community composition and network clustering exists between F and U soils is unknown. In fact, it is generally not known how and why differences in the individual microbial communities arise between different soils. Generation of such knowledge needs much more research using a large variety of different soils.

The mechanism for the negative PE in soils of U cluster is not quite clear, but our results allow some speculation. It has been proposed that a negative PE may be due to a switch of the SOM-degrading microorganisms from degradation of SOM to degradation of the fresh organic matter added (Blagodatskaya et al., 2007). We speculate that *Symbiobacterium* and other Clostridia, which were prevalent in soils of U cluster and were probably involved in methanogenic fermentation, were able to switch to FOM degradation, while the bacteria that were characteristic for soils of F cluster were not able to do so. This interpretation is consistent with the observation that the bacteria in network U had more positive correlations with both hydrogenotrophic and acetoclastic methanogens than in network F (Table S8), so that a switch from SOM to FOM would have immediate effects on production of both CH_4 and CO_2, now being produced from FOM instead SOM. This was consistent with the negative PE in soils of U cluster after RS addition. In soils of F cluster, by contrast, FOM degradation

would only indirectly affect the methanogens in network F, resulting in stimulation of CH_4 production but not of SOM degradation. Besides, it's not likely that the accumulated intermediates inhibited decomposition of both RS and SOM in the soils of U cluster, since the amount of decomposition of RS in soils of U cluster were almost the same with that in soils of F cluster (Fig. S4).

In summary, the bacterial community composition (and its activity) was found to be crucial for establishment of a negative PE on SOM degradation in Uruguay rice field soil. This result is consistent with our hypothesis that the PE in a soil mainly depends on its soil microbial community rather than its physicochemical properties. It is unclear, however, whether this result is universal for rice field soils. Production of the greenhouse gas CH_4 is always considerably higher in the presence than in the absence of RS (Conrad and Klose, 2006; Kimura et al., 2004; Peng et al., 2008). Nevertheless, large amounts of SOM would be preserved and the emission of greenhouse gases (CH_4 and CO_2) reduced if the bacterial community composition would be optimal for avoiding a stimulating and causing a suppressing effect. Our results showed that tight networks between fermenting and methanogenic microorganisms may facilitate a switch between the degradation of SOM to degradation of fresh organic matter (e.g., RS) thus preserving SOM. Such tight methanogenic networks seem to depend on the presence or absence of particular bacterial genera, e.g. *Symbiobacterium*. On the other hand, previous studies in upland soils have shown that several

528 factors, including quality and quantity of SOM, do also matter for positive and negative PEs
529 (Kuzyakov et al., 2000). Similarly, there remain many unknowns regarding the PEs in rice
530 soils that cannot be understood from the two soils investigated for this study. Therefore,
531 further studies on more rice field soils are necessary for fully uncovering the mechanisms of
532 PE during methanogenic decomposition of SOM.
533
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542
543 **Conflict of Interest**
544 The authors declare no conflict of interest.
545
546 **References**

547 Angel, R., Matthies, D., Conrad, R., 2011. Activation of methanogenesis in arid biological
548 soil crusts despite the presence of oxygen. PLoS ONE 6, e20453.
549 Barberan, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to
550 explore co-occurrence patterns in soil microbial communities. The ISME Journal 6, 343-
551 351.
552 Bates, S.T., Berg-Lyons, D., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011.
553 Examining the global distribution of dominant archaeal populations in soil. The ISME
554 Journal 5, 908-917.
555 Blagodatskaya, E.V., Blagodatsky, S.A., Anderson, T.H., Kuzyakov, Y., 2007. Priming
556 effects in Chernozem induced by glucose and N in relation to microbial growth strategies.
557 Applied Soil Ecology 37, 95-105.
558 Casamayor, E.O., Massana, R., Benlloch, S., Ovreas, L., Diez, B., Goddard, V.J., Gasol, J.M.,
559 Joint, I., Rodriguez-Valera, F., Pedros-Alio, C., 2002. Changes in archaeal, bacterial and
560 eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting
561 methods in a multipond solar saltern. Environmental Microbiology 4, 338-348.
562 Chen, R.R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X.G.,
563 Blagodatskaya, E., Kuzyakov, Y., 2014. Soil C and N availability determine the priming
564 effect: microbial N mining and stoichiometric decomposition theories. Global Change
565 Biology 20, 2356-2367.

566 Cheng, W.X., 1996. Measurement of rhizosphere respiration and organic matter
567 decomposition using natural C-13. Plant and Soil 183, 263-268.
568 Cheng, W.X., 1999. Rhizosphere feedbacks in elevated CO_2. Tree Physiology 19, 313-320.
569 Chidthaisong, A., Rosenstock, B., Conrad, R., 1999. Measurement of monosaccharides and
570 conversion of glucose to acetate in anoxic rice field soil. Applied and Environmental
571 Microbiology 65, 2350-2355.
572 Conrad, R., 1999. Contribution of hydrogen to methane production and control of hydrogen
573 concentrations in methanogenic soils and sediments. Fems Microbiology Ecology 28, 193-
574 202.
575 Conrad, R., 2009. The global methane cycle: recent advances in understanding the microbial
576 processes involved. Environmental Microbiology Reports 1, 285-292.
577 Conrad, R., Claus, P., Casper, P., 2009. Characterization of stable isotope fractionation during
578 methane production in the sediment of a eutrophic lake, Lake Dagow, Germany.
579 Limnology and Oceanography 54, 457-471.
580 Conrad, R., Claus, P., Casper, P., 2010. Stable isotope fractionation during the methanogenic
581 degradation of organic matter in the sediment of an acidic bog lake, Lake Grosse
582 Fuchskuhle. Limnology and Oceanography 55, 1932-1942.
583 Conrad, R., Klose, M., 2006. Dynamics of the methanogenic archaeal community in anoxic
584 rice soil upon addition of straw. European Journal of Soil Science 57, 476-484.

585 Conrad, R., Klose, M., Yuan, Q., Lu, Y., Chidthaisong, A., 2012. Stable carbon isotope
586 fractionation, carbon flux partitioning and priming effects in anoxic soils during
587 methanogenic degradation of straw and soil organic matter. Soil Biology & Biochemistry
588 49, 193-199.
589 Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition
590 and feedbacks to climate change. Nature 440, 165-173.
591 Deng, Y., Jiang, Y.H., Yang, Y., He, Z., Luo, F., Zhou, J., 2012. Molecular ecological
592 network analyses. Bmc Bioinformatics 13, 113.
593 Deng, Y.C., Cui, X.Y., Hernandez, M., Dumont, M.G., 2014. Microbial diversity in hummock
594 and hollow soils of three wetlands on the Qinghai-Tibetan Plateau revealed by 16S rRNA
595 pyrosequencing. PLoS ONE 9, e103115.
596 Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads.
597 Nature Methods 10, 996.
598 Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves
599 sensitivity and speed of chimera detection. Bioinformatics 27, 2194-2200.
600 Fernandez Scavino, A., Ji, Y., Pump, J., Klose, M., Claus, P., Conrad, R., 2013. Structure and
601 function of the methanogenic microbial communities in Uruguayan soils shifted between
602 pasture and irrigated rice fields. Environmental Microbiology 15, 2588-2602.

603 Fontaine, S., Barot, S., 2005. Size and functional diversity of microbe populations control
604 plant persistence and long-term soil carbon accumulation. Ecology Letters 8, 1075-1087.
605 Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question
606 of microbial competition? Soil Biology & Biochemistry 35, 837-843.
607 Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D.W., Haywood, J.,
608 Lean, J., Lowe, D.C., Myhre, G., Nganga, J., Prinn, R., Raga, G., Schulz, M., Dorland,
609 R.V., 2007. Changes in atmospheric constituents and in radiative forcing, In: Solomon, S.,
610 Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.),
611 Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the
612 Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge
613 University Press, Cambridge, United Kingdom and New York, NY, USA., pp. 129-234.
614 Frenzel, P., Bosse, U., Janssen, P.H., 1999. Rice roots and methanogenesis in a paddy soil:
615 ferric iron as an alternative electron acceptor in the rooted soil. Soil Biology &
616 Biochemistry 31, 421-430.
617 Glissmann, K., Weber, S., Conrad, R., 2001. Localization of processes involved in
618 methanogenic degradation of rice straw in anoxic paddy soil. Environmental Microbiology
619 3, 502-511.

620 Guenet, B., Neill, C., Bardoux, G., Abbadie, L., 2010. Is there a linear relationship between
621 priming effect intensity and the amount of organic matter input? Applied Soil Ecology 46,
622 436-442.
623 Hernandez, M., Dumont, M.G., Yuan, Q., Conrad, R., 2015. Different bacterial populations
624 associated with the roots and rhizosphere of rice incorporate plant-derived carbon. Applied
625 and Environmental Microbiology 81, 2244-2253.
626 Ju, F., Xia, Y., Guo, F., Wang, Z.P., Zhang, T., 2014. Taxonomic relatedness shapes bacterial
627 assembly in activated sludge of globally distributed wastewater treatment plants.
628 Environmental Microbiology 16, 2421-2432.
629 Kalbitz, K., Kaiser, K., Fiedler, S., Kolbl, A., Amelung, W., Brauer, T., Cao, Z.H., Don, A.,
630 Grootes, P., Jahn, R., Schwark, L., Vogelsang, V., Wissing, L., Kogel-Knabner, I., 2013.
631 The carbon count of 2000 years of rice cultivation. Global Change Biology 19, 1107-1113.
632 Kim, Y., Liesack, W., 2015. Differential assemblage of functional units in paddy soil
633 microbiomes. PLoS ONE 10, e0122221.
634 Kimura, M., Murase, J., Lu, Y.H., 2004. Carbon cycling in rice field ecosystems in the
635 context of input, decomposition and translocation of organic materials and the fates of their
636 end products (CO_2 and CH_4). Soil Biology & Biochemistry 36, 1399-1416.

637 Kramer, C., Trumbore, S., Froberg, M., Dozal, L.M.C., Zhang, D.C., Xu, X.M., Santos, G.M.,
638 Hanson, P.J., 2010. Recent (< 4 year old) leaf litter is not a major source of microbial
639 carbon in a temperate forest mineral soil. Soil Biology & Biochemistry 42, 1028-1037.
640 Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter.
641 Soil Biology & Biochemistry 42, 1363-1371.
642 Kuzyakov, Y., Bol, R., 2006. Sources and mechanisms of priming effect induced in two
643 grassland soils amended with slurry and sugar. Soil Biology & Biochemistry 38, 747-758.
644 Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of
645 priming effects. Soil Biology & Biochemistry 32, 1485-1498.
646 Langley, J.A., McKinley, D.C., Wolf, A.A., Hungate, B.A., Drake, B.G., Megonigal, J.P.,
647 2009. Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed
648 to elevated CO_2. Soil Biology & Biochemistry 41, 54-60.
649 Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination
650 of species data. Oecologia 129, 271-280.
651 Lozupone, C., Hamady, M., Knight, R., 2006. UniFrac--an online tool for comparing
652 microbial community diversity in a phylogenetic context. Bmc Bioinformatics 7, 371.
653 Ma, K., Qiu, Q.F., Lu, Y.H., 2010. Microbial mechanism for rice variety control on methane
654 emission from rice field soil. Global Change Biology 16, 3085-3095.

Mau, R.L., Liu, C.M., Aziz, M., Schwartz, E., Dijkstra, P., Marks, J.C., Price, L.B., Keim, P., Hungate, B.A., 2015. Linking soil bacterial biodiversity and soil carbon stability. The ISME Journal 9, 1477-1480.

Mayer, H.P., Conrad, R., 1990. Factors influencing the population of methanogenic bacteria and the initiation of methane production upon flooding of paddy soil. Fems Microbiology Ecology 73, 103-111.

McNamara, N.P., Black, H.I.J., Beresford, N.A., Parekh, N.R., 2003. Effects of acute gamma irradiation on chemical, physical and biological properties of soils. Applied Soil Ecology 24, 117-132.

Neue, H.U., Scharpenseel, H.W., 1987. Decomposition pattern of C-14-labeled rice straw in aerobic and submerged rice soils of the Philippines. Science of the Total Environment 62, 431-434.

Nottingham, A.T., Griffiths, H., Chamberlain, P.M., Stott, A.W., Tanner, E.V.J., 2009. Soil priming by sugar and leaf-litter substrates: A link to microbial groups. Applied Soil Ecology 42, 183-190.

Ohno, M., Shiratori, H., Park, M.J., Saitoh, Y., Kumon, Y., Yamashita, N., Hirata, A., Nishida, H., Ueda, K., Beppu, T., 2000. *Symbiobacterium thermophilum* gen. nov., sp nov., a symbiotic thermophile that depends on co-culture with a Bacillus strain for growth. International Journal of Systematic and Evolutionary Microbiology 50, 1829-1832.

674 Paterson, E., Sim, A., 2013. Soil-specific response functions of organic matter mineralization
675 to the availability of labile carbon. Global Change Biology 19, 1562-1571.
676 Paterson, E., Thornton, B., Midwood, A.J., Osborne, S.M., Sim, A., Millard, P., 2008.
677 Atmospheric CO_2 enrichment and nutrient additions to planted soil increase mineralisation
678 of soil organic matter, but do not alter microbial utilisation of plant- and soil C-sources.
679 Soil Biology & Biochemistry 40, 2434-2440.
680 Pausch, J., Zhu, B., Kuzyakov, Y., Cheng, W.X., 2013. Plant inter-species effects on
681 rhizosphere priming of soil organic matter decomposition. Soil Biology & Biochemistry 57,
682 91-99.
683 Peng, J.J., Lu, Z., Rui, J., Lu, Y.H., 2008. Dynamics of the methanogenic archaeal community
684 during plant residue decomposition in an anoxic rice field soil. Applied and Environmental
685 Microbiology 74, 2894-2901.
686 Penning, H., Conrad, R., 2006. Carbon isotope effects associated with mixed-acid
687 fermentation of saccharides by *Clostridium papyrosolvens*. Geochimica Et Cosmochimica
688 Acta 70, 2283-2297.
689 Penning, H., Conrad, R., 2007. Quantification of carbon flow from stable isotope
690 fractionation in rice field soils with different organic matter content. Organic Geochemistry
691 38, 2058-2069.

692 Perveen, N., Barot, S., Alvarez, G., Klumpp, K., Martin, R., Rapaport, A., Herfurth, D.,
693 Louault, F., Fontaine, S., 2014. Priming effect and microbial diversity in ecosystem
694 functioning and response to global change: a modeling approach using the SYMPHONY
695 model. Global Change Biology 20, 1174-1190.
696 Philippot, L., Spor, A., Henault, C., Bru, D., Bizouard, F., Jones, C.M., Sarr, A., Maron, P.A.,
697 2013. Loss in microbial diversity affects nitrogen cycling in soil. The ISME Journal 7,
698 1609-1619.
699 Rhee, S.K., Jeon, C.O., Bae, J.W., Kim, K., Song, J.J., Kim, J.J., Lee, S.G., Kim, H.I., Hong,
700 S.P., Choi, Y.H., Kim, S.M., Sung, M.H., 2002. Characterization of *Symbiobacterium*
701 *toebii*, an obligate commensal thermophile isolated from compost. Extremophiles 6, 57-64.
702 Rousk, J., Hill, P.W., Jones, D.L., 2015. Priming of the decomposition of ageing soil organic
703 matter: concentration dependence and microbial control. Functional Ecology 29, 285-296.
704 Rousk, K., Michelsen, A., Rousk, J., 2016. Microbial control of soil organic matter
705 mineralization responses to labile carbon in subarctic climate change treatments. Global
706 Change Biology 22, 4150-4161.
707 Roy, R., Conrad, R., 1999. Effect of methanogenic precursors (acetate, hydrogen, propionate)
708 on the suppression of methane production by nitrate in anoxic rice field soil. Fems
709 Microbiology Ecology 28, 49-61.

Rui, J., Li, J., Zhang, S., Yan, X., Wang, Y., Li, X., 2015. The core populations and co-occurrence patterns of prokaryotic communities in household biogas digesters. Biotechnology for Biofuels 8, 1-15.

Rui, J.P., Peng, J.J., Lu, Y.H., 2009. Succession of bacterial populations during plant residue decomposition in rice field soil. Applied and Environmental Microbiology 75, 4879-4886.

Sass, R.L., Fisher, F.M., Harcombe, P.A., Turner, F.T., 1991. Mitigation of methane emissions from rice fields: Possible adverse effects of incorporated rice straw. Global Biogeochemical Cycles 5, 275-287.

Schütz, H., Holzapfel-Pschorn, A., Conrad, R., Rennenberg, H., Seiler, W., 1989. A 3-year continuous record on the influence of daytime, season, and fertilizer treatment on methane emission rates from an Italian rice paddy. Journal of Geophysical Research 94, 16405-16416.

Schink, B., 1997. Energetics of syntrophic cooperation in methanogenic degradation. Microbiology and Molecular Biology Reviews 61, 262-280.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75, 7537-7541.

729 Smith, P., Fang, C.M., Dawson, J.J.C., Moncrieff, J.B., 2008. Impact of global warming on
730 soil organic carbon. Advances In Agronomy, Vol 97 97, 1-43.
731 Stubner, S., 2002. Enumeration of 16S rDNA of Desulfotomaculum lineage 1 in rice field soil
732 by real-time PCR with SybrGreen (TM) detection. Journal of Microbiological Methods 50,
733 155-164.
734 Stumm, W., Morgan, J.J., 1981. Aquatic chemistry. An introduction emphasizing chemical
735 equilibria in natural waters. Wiley.
736 Teh, Y.A., Silver, W.L., 2006. Effects of soil structure destruction on methane production and
737 carbon partitioning between methanogenic pathways in tropical rain forest soils. Journal of
738 Geophysical Research-Biogeosciences 111.
739 Ueda, K., Yamashita, A., Ishikawa, J., Shimada, M., Watsuji, T., Morimura, K., Ikeda, H.,
740 Hattori, M., Beppu, T., 2004. Genome sequence of Symbiobacterium thermophilum, an
741 uncultivable bacterium that depends on microbial commensalism. Nucleic Acids Research
742 32, 4937-4944.
743 Warnes, G.R., Bolker, B., Bonebakker, L., Gentleman, R., Liaw, W.H.A., Lumley, T.,
744 Maechler, M., Magnusson, A., Moeller, S., Schwartz, M., Venables, B., 2014. gplots:
745 Various R programming tools for plotting data. The Comprehensive R - Archive Network.
746 Available: http://CRAN.R-project.org/package = gplots. Accessed 2014 January 10.

747 Watsuji, T.O., Kato, T., Ueda, K., Beppu, T., 2006. CO2 supply induces the growth of
748 Symbiobacterium thermophilum, a syntrophic bacterium. Bioscience Biotechnology And
749 Biochemistry 70, 753-756.
750 Watts, D.J., Strogatz, S.H., 1998. Collective dynamics of 'small-world' networks. Nature 393,
751 440-442.
752 Wegner, C.E., Liesack, W., 2016. Microbial community dynamics during the early stages of
753 plant polymer breakdown in paddy soil. Environmental Microbiology 18, 2825-2842.
754 Wieder, W.R., Bonan, G.B., Allison, S.D., 2013. Global soil carbon projections are improved
755 by modelling microbial processes. Nature Climate Change 3, 909-912.
756 Wild, B., Schnecker, J., Alves, R.J.E., Barsukov, P., Barta, J., Capek, P., Gentsch, N., Gittel,
757 A., Guggenberger, G., Lashchinskiy, N., Mikutta, R., Rusalimova, O., Santruckova, H.,
758 Shibistova, O., Urich, T., Watzka, M., Zrazhevskaya, G., Richter, A., 2014. Input of easily
759 available organic C and N stimulates microbial decomposition of soil organic matter in
760 arctic permafrost soil. Soil Biology & Biochemistry 75, 143-151.
761 Wolf, A.A., Drake, B.G., Erickson, J.E., Megonigal, J.P., 2007. An oxygen-mediated positive
762 feedback between elevated carbon dioxide and soil organic matter decomposition in a
763 simulated anaerobic wetland. Global Change Biology 13, 2036-2044.

764 Wu, J., Brookes, P.C., Jenkinson, D.S., 1993. Formation and destruction of microbial biomass
765 during the decomposition of glucose and ryegrass in soil. Soil Biology and Biochemistry 25,
766 1435-1441.
767 Yagi, K., Minami, K., 1990. Effect of organic matter application on methane emission from
768 some Japanese paddy fields. Soil Science and Plant Nutrition 36, 599-610.
769 Yao, H., Conrad, R., Wassmann, R., Neue, H.U., 1999. Effect of soil characteristics on
770 sequential reduction and methane production in sixteen rice paddy soils from China, the
771 Philippines, and Italy. Biogeochemistry 47, 269-295.
772 Yao, M., Rui, J., Li, J., Dai, Y., Bai, Y., Heděnec, P., 2014. Rate-specific responses of
773 prokaryotic diversity and structure to nitrogen deposition in the Leymus chinensis steppe.
774 Soil Biology & Biochemistry 79, 81-90.
775 Ye, R., Doane, T.A., Morris, J., Horwath, W.R., 2015. The effect of rice straw on the priming
776 of soil organic matter and methane production in peat soils. Soil Biology & Biochemistry
777 81, 98-107.
778 Yu, Z.T., Garcia-Gonzalez, R., Schanbacher, F.L., Morrison, M., 2008. Evaluations of
779 different hypervariable regions of archaeal 16S rRNA genes in profiling of methanogens
780 denaturing by Archaea-specific PCR and gradient gel electrophoresis. Applied and
781 Environmental Microbiology 74, 889-893.

Yuan, Q., Lu, Y., 2009. Response of methanogenic archaeal community to nitrate addition in rice field soil. Environmental Microbiology Reports 1, 362-369.

Yuan, Q., Pump, J., Conrad, R., 2012. Partitioning of CH_4 and CO_2 production originating from rice straw, soil and root organic carbon in rice microcosms. PLoS ONE 7, e49073.

Yuan, Q., Pump, J., Conrad, R., 2014. Straw application in paddy soil enhances methane production also from other carbon sources. Biogeosciences 11, 237-246.

Zhu, B., Cheng, W.X., 2011. Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. Global Change Biology 17, 2172-2183.

Table 1. Spearman's correlation of environmental variables with bacterial and archaeal communities of all soil samples treated with sterilization and inoculation. Mantel test was applied in this analysis. The relative abundances of OTUs were used as input. RS: rice straw.

	Bacterial community				Archaeal community			
	Soil type	Inoculum source	RS treatment	Averaged CH_4 production rate	Soil type	Inoculum source	RS treatment	Averaged CH_4 production rate
Correlation	0.33	0.786	-0.001	0.055	0.118	0.384	-0.025	0.014
p value	0.003	0.001	0.352	0.146	0.033	0.001	0.617	0.363

Table 2. Taxonomic information of dominant modules in the networks of F and U, the numbers of nodes affiliated with abundant class/order were shown in the table, the numbers in the parentheses were the total numbers of nodes in each module. FM: major module in network F, UM: major module in network U.

	Network F			Network U		
Abundant Class/Order	FM1 (66)	FM2 (30)	FM3 (44)	UM1 (40)	UM2 (57)	UM3 (44)
Clostridia	28	9			21	4
Acidobacteria	7	4		9	3	1
Actinobacteria	8	2		9	1	
Bacilli	5	3		5	1	
Sphingobacteriales	2				3	
Methanocellales	2		12	2	9	8
Methanosarcinales		1	30		8	24
Methanobacteriales		2	1			4

Table 3. Spearman's correlation of environmental variables to prokaryotic community structures of dominant modules in networks tested by Mantel test (permutations: 9999). RS: rice straw, SOC: soil organic carbon.

	pH	RS treatment	SOC content
(1) network F			
FM1	0.342**	-0.053	0.457**
FM2	0.482**	-0.035	0.694**
FM3	-0.03	-0.055	-0.016
(2) network U			
UM1	0.426**	-0.037	0.520**
UM2	0.405**	0.067	0.470**
UM3	-0.086	0.052	-0.099

* $p<0.05$, ** $p<0.01$

Figure legends

Figure 1. The SOM-derived CH_4 and total inorganic carbon (TIC) production in control and RS amended soils. Development of SOM-derived CH_4 production from Fuyang (a), Uruguay soil (b), from the combinations of 5%F with sterilized F (5%F+sF) (c) or with sterilized U (5%F+sU) (d), and from the combinations of 5%U with sterilized U (5%U+sU) (e) or with sterilized F (5%U+sF) (f) in control and rice straw (RS) treatment. Total amount of SOM-derived CH_4 and TIC at the end of incubation (day 22) from Fuyang, Uruguay, and from the combinations of original with sterilized soils in control and RS treatment (g). The data shown are from treatment RSI, which however was almost identical to the results from treatment RSII. Data are means ± SD (n=3). The differences between control and RS treatments were tested by two-tailed independent t-tests, indicated by * when $P<0.05$.

Figure 2. Bacterial 16S rRNA gene (a) and *mcrA* gene (characteristic for methanogenic archaea) (b) copy numbers in soils without and with RS application at the end of incubation (day 22); means ± SD (n = 3). The difference between control and RS treatments of each soil sample was examined by two-tailed independent t-tests, indicated by * when $P<0.05$.

Figure 3. Relative sequence abundances of bacterial phyla (a) and archaeal classes (b). Major taxa detected with average relative sequence abundances >1% are displayed; means ± SD (n = 3). Column “Bacteria-others” or “Archaea-others” indicate combined relative sequence abundances of all the rare phyla or classes, candidate divisions and of the taxonomically

824 unclassified sequences; rare phyla or classes are defined as having average relative sequence
825 abundances between all samples of <1%. The description of soil samples was the same as in
826 caption of Figure 1. There were control and RS treatment for each soil sample.
827 **Figure 4.** Principal coordinate analysis (PCoA) of bacterial communities based on weighted
828 UniFrac metrics. The description of soil samples was the same as in caption of Figure 1.
829 There were control and RS treatment for each soil sample.
830 **Figure 5.** Heatmap showing the relative abundance of selected bacterial OTUs. The colors
831 correspond to the relative abundance of the OTUs in the samples, as indicated by the color
832 legend. The samples are clustered according to Bray–Curtis distances. The taxonomy of each
833 OTU is provided to the lowest level obtained during the classification. Abbreviations are used
834 to indicate class (c), subclass (sc), order (o), suborder (so), family (f) and genus (g).
835 **Figure 6.** Networks of co-occurring prokaryotic OTUs in soil samples based on correlation
836 analysis. The network F (a) includes both the samples derived from original F soil and the
837 sterilized soils with 5% F, the network U (b) includes both the samples derived from original
838 U soil and the sterilized soils with 5% U. Nodes were colored by modularity class with
839 labeled taxonomic affiliation. The names of three major modules in each network are shown,
840 while those of other minor modules are neglected. A connection between two nodes (edge)
841 stands for a strong (Spearman's $\rho>0.6$) and significant ($p<0.01$) correlation. For each panel,
842 the size of each node is proportional to the number of connections (degree).