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Response of fermenting bacterial and methanogenic archaeal communities in paddy soil to progressing rice straw degradation

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ABSTRACT

Rice straw is one of the major organic materials introduced to rice field soils and its incorporation strongly enhances the emission of CH_4. We incubated unamended and straw-amended Italian paddy soil under anaerobic conditions and studied the functional (carbon source, rate, pathway) and structural (abundance, taxonomic composition) responses of methanogenic microbial communities progressively in samples taken after 0, 30, 60, 90, and 120 days. Initially, rice straw significantly enhanced CH_4 production rates. Later on, the values strongly decreased with the progress of rice straw degradation. The contribution of rice straw to CH_4 production decreased with progressing anaerobic incubation. This decrease was paralleled by an increase of the contribution of hydrogenotrophic methanogenesis to CH_4 production indicating a change in the network of bacterial and archaeal microbial communities. The methanogenic and bacterial communities indeed strongly responded to rice straw amendment and exhibited a distinct succession over the subsequent degradation periods. Network analysis of both 16S rRNA and Methyl coenzyme M reductase (*mcrA*) genes showed apparent co-occurrence of fermenting bacteria and CH_4-producing archaea belonging to distinct operational taxonomic units (OTU) demonstrating strong functional and structural responses of methanogenic microbial communities to progressing rice straw degradation. Clostridiales, Fibrobacterales, and two Bacteroidetes groups (WCHB1-32 and Sphingobacteriales), as well as Anaerolineales and Bacteroidetes environmental group vadinHA17 were important bacterial taxa. Acetoclastic *Methanosarcina* and *Methanothrix* ('*Methanosaeta*') as well as hydrogentrophic *Methanocella* were important archaeal taxa involved in rice straw degradation.

1. Introduction

Rice fields are one of the major sources in the global methane budget and contribute in the range of 25–300 Tg CH_4 per year (Bridgham et al., 2013; Chen and Prinn, 2005). The CH_4 is usually produced by acetoclastic and hydrogenotrophic methanogenic archaea (Conrad et al., 2009, 2012). It is produced from acetate or H_2/CO_2, the two major products of organic matter fermentation (Glissmann and Conrad, 2000). Rice straw is one of the major organic materials introduced to rice field soil (Kimura et al., 2004). It consists of different biopolymers, including cellulose (32–37%), hemicellulose (29–37%) and lignin (5–15%) and, in addition, contains inorganic components such as silica. The polysaccharides in the straw, in particular, serve as substrates for the complex microbial community that degrades organic matter to CO_2 and CH_4 (Kimura and Tun, 1999; Weber et al., 2001a, 2001b; Wegner and Liesack, 2016). Studies using isotopically labeled compounds have shown that the produced CH_4 is derived from the degradation of rice straw, root exudates and soil organic matter, and up to 42% of the carbon in CH_4 production is derived from rice straw (Glissmann et al., 2001; Watanabe et al., 1999; Yuan et al., 2012). It has frequently been demonstrated that incorporation of rice straw strongly enhances the emission of CH_4 from rice fields (Liu et al., 2014; Watanabe et al., 1995; Yan et al., 2005).

The relative contribution of acetoclastic versus hydrogenotrophic methanogenesis to CH_4 production and its response to rice straw amendment has been studied (Conrad et al., 2012; Penning and Conrad, 2007; Zhang et al., 2015). Complex microbial communities are involved in the anaerobic degradation of rice straw, consisting of hydrolytic and fermentative bacteria and methanogenic archaea (Conrad, 2007; Zinder, 1993). The methanogenic archaea include the acetoclastic

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families Methanosarcinaceae and Methanotrichaceae ('Methanosaetaceae') (Oren, 2014) as well as the hydrogenotrophic orders Methanocellales, Methanomicrobiales, and Methanobacteriales (Bao et al., 2016; Conrad et al., 2012; Lu et al., 2015). The bacterial communities in rice fields are even more diverse (Noll et al., 2005; Shrestha et al., 2009; Rui et al., 2009). The microorganisms involved in straw degradation have frequently been investigated in anoxic rice field soils (Bao et al., 2016; Conrad et al., 2012; Lu et al., 2015; Peng et al., 2008; Rui et al., 2009; Weber et al., 2001a, 2001b; Zhou et al., 2016). However, there is to our knowledge no study that addressed the structure (i.e., abundance and composition) of the microbial community together with the activity and the pathways of methanogenesis. Furthermore, it is unknown how structure and function of the methanogenic community changes with the progressing degradation of straw and its relative contribution to total CH_4 production. We hypothesized that the bacterial and archaeal methanogenic communities are organized in a manner that can be recognized by network analysis, and that these networks change with the progress of rice straw decomposition and are different for unamended soil and straw amended soil.

Therefore, we studied the dynamics of functional and structural responses of methanogenic microbial communities in paddy soils to progressing straw degradation. We determined methanogenic function by the carbon sources and rates of CH_4 production, and characterized the microbial community structure by the abundance and taxonomic composition of bacteria and methanogenic archaea.

2. Experimental procedures

2.1. Soil incubation and chemical analyses

Soil was sampled from rice fields at the Italian Rice Research Institute in Vercelli, Italy, in 2013 and was air dried and stored at room temperature. The soil was sieved (< 2 mm) prior to use. Rice straw and ^{13}C-labeled rice straw with two different ^{13}C-contents were prepared by growing rice plants in the greenhouse using Italian rice field soil (Pump and Conrad, 2014). The straw was air-dried and ground using a blender. The physicochemical properties of soil and rice straw are described in Table S1.

The incubation procedure has been described before (Conrad et al., 2011), and was the same as used in a parallel publication (Ji et al., 2018). Briefly, soil slurries were prepared by mixing dry soil with deionized, sterile, anoxic water at a ratio of 1:1, and incubating the mixture in bottles under a headspace of N_2 at 25 °C for 2 weeks of pre-incubation. After pre-incubation, triplicate treatments with just soil (SOIL) or soil plus rice straw amendment (RS) were established and incubated statically at 25 °C for 4 months. At each month, aliquots were subsampled and analyzed for production of CH_4, CO_2 and acetate as well as the $\delta^{13}C$ of these compounds, both in the presence and the absence of 2% CH_3F (vol/vol?), an inhibitor of acetoclastic methanogenesis (Conrad and Klose, 1999; Janssen and Frenzel, 1997). The data were used for the determination of the relative contribution of hydrogenotrophic methanogenesis to total CH_4 production (f_{H2}) by using the mass balance from the $\delta^{13}C$ of CH_4 in the presence ($\delta^{13}C_{CH4\text{-}mc}$) and absence ($\delta^{13}C_{CH4}$) of CH_3F and from the $\delta^{13}C$ of the methyl group of acetate ($\delta^{13}C_{ac\text{-}methyl}$): $f_{H2} = (\delta^{13}C_{CH4} - \delta^{13}C_{ac\text{-}methyl})/(\delta^{13}C_{CH4\text{-}mc} - \delta^{13}C_{ac\text{-}methyl})$. For molecular analyses, soil samples were stored frozen at −20 °C.

In the present study, we used two further treatments with ^{13}C-labeled rice straw at two different levels of ^{13}C (RSI, RSII). The RS, RSI and RSII treatments were only different in the extent of ^{13}C labelling, with −28‰, 222‰ and 88‰, respectively (Table S1). The fraction of CH_4 produced from RS (f_{RS}) was determined from the incubations with ^{13}C-labeled rice straw (RSI, RSII) using the same procedure as described above for SOIL and RS treatments (Ji et al., 2018). Soil slurries were prepared by mixing 600 g dry soil with 600 mL of deionized, sterile, anoxic water, and incubating the mixture in 2-L bottles under a headspace of N_2 at 25 °C for 2 weeks of pre-incubation. After pre-incubation, triplicates of treatments RSI and RSII were established by dispensing the slurries (about 100 ml) into 200-mL bottles. Labeled rice straw was added at a ratio of 5 mg dry ground straw per gram dry soil. Then the bottles were incubated statically at 25 °C for 4 months. At each month (0, 30, 60, 90 and 120 days), aliquots (10 g slurry) were dispensed into 26-mL pressure tubes, the time points corresponding to five subsequent incubation phases (in the following denominated phase D0, D30, D60, D90, D120). The tubes were closed with black rubber stoppers, flushed with N_2, pressurized to 0.5 bar overpressure, and incubated at 25 °C for about 20–30 days. For each phase, 2 replicate tubes with slurries were prepared from each of the triplicate incubations. One tube was amended with CH_3F to inhibit acetoclastic methanogenesis. The tubes were then incubated at 25 °C for about 20–30 days, and gas samples were analyzed at 2 to 3-day intervals for $\delta^{13}C$ of CH_4 and CO_2. In the end the tubes were opened and the slurries were analyzed for C, N and $\delta^{13}C$. The determination of f_{RS} used the following mass balance equation described in detail before (Yuan et al., 2012, 2014): $f_{RS} = (\delta^{13}C_{CH4}\text{-I} - \delta^{13}C_{CH4}\text{-II})/(\delta^{13}C_{RS\text{-}I} - \delta^{13}C_{RS\text{-}II})$, where $\delta^{13}C_{CH4\text{-}I} = \delta^{13}C$ values of CH_4 from RSI incubation, $\delta^{13}C_{CH4\text{-}II} = \delta^{13}C$ values of CH_4 from RSII incubation, $\delta^{13}C_{RS\text{-}I}$ = labeled values of RSI, $\delta^{13}C_{RS\text{-}II}$ = labeled values of RSII. Finally, the fraction of CH_4 production from soil organic matter (f_{SOM}) was calculated, as $f_{RS} + f_{SOM} = 1$.

2.2. Amplicon sequencing and sequence data processing

The DNA extraction procedure was the same as used in a parallel publication (Ji et al., 2018). Briefly, genomic DNA was extracted from about 0.4 g wet soil by using NucleoSpin™ Soil Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. Four combinations (SL1, SL2, SL1 plus enhancer and SL2 plus enhancer) of the lysis buffer were tested and SL1 was finally used. DNA concentration and purity were determined spectrophotometrically (NanoDrop Technologies). The extracted DNA was used for qPCR and ILLUMINA sequencing, and the procedure for methanogenic *mcrA* gene was the same with a parallel publication (Ji et al., 2018). The numbers of bacterial and archaeal 16S rRNA gene copies and of methanogenic *mcrA* gene copies were determined by qPCR with two technical replicate reactions (Angel et al., 2011, 2012; Kemnitz et al., 2005; Stubner, 2002).

ILLUMINA sequencing was carried out for the bacterial 16S rRNA gene and the methanogenic *mcrA* gene. Barcoded PCR amplicons from soil DNA were prepared by using a two-step PCR approach as described previously (Herbold et al., 2015). After quantification, two independent libraries were constructed by pooling equal amounts of individual barcoded amplicons of 16S rRNA and *mcrA* genes, respectively. For the 16S rRNA gene, the library was sequenced on an ILLUMINA HISEQ 2000 system using 2 × 250 cycle combination mode by Max Planck-Genome-Centre (Cologne, Germany). For *mcrA* gene, the library was sequenced on an ILLUMINA MISEQ system using 2 × 300 cycle combination mode by Microsynth (Balgach, Switzerland). Sequence datasets are available in the NCBI Sequence Read Archive under study accession number SRP103721. Paired-end reads were first merged by USEARCH and 16S rRNA gene and *mcrA* datasets were separated by primer sequences using CUTADAPT and demultiplexed using QIIME. All reads were subjected to quality control, de novo chimera filtering, singleton filtering and operational taxonomic units (OTU) clustering according to the UPARSE pipeline. Species level OTUs for the 16S rRNA genes were obtained at 97% sequence identity. Approximate species-level *mcrA* OTUs were obtained with the gene-specific OTU threshold (84% sequence identity) (Yang et al., 2014). Taxonomic identities of 16S rRNA gene OTUs were assigned with the Ribosomal Database Project (RDP) Classifier against the SILVA 123 SSU Ref database (Pruesse et al., 2007) at a confidence level of 80% (Wang et al., 2007). For *mcrA* gene, nucleotide sequences were initially translated into amino acid sequences using FrameBot (Wang et al., 2013) and aligned