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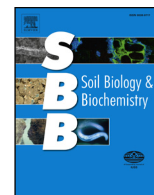

Temperature effects on structure and function of the methanogenic microbial communities in two paddy soils and one desert soil

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A B S T R A C T

Temperature is an important factor regulating the production of the greenhouse gas CH_4. Previous studies of temperate methanogenic paddy soils from Italy showed that structure and function of the soil microbial communities changed drastically when temperature was increased to values higher than about 40 °C. Since methanogenic archaea are ubiquitous in both wetland and upland soils, we wondered whether other soils would behave similarly. Therefore, we compared paddy soils from Italy and the Philippines, which have different microbial community structures, and also investigated a desert soil from Utah (USA), which expressed CH_4 production upon flooding. We incubated these soils under anoxic conditions at three different temperatures. We determined composition, abundance and function of the methanogenic archaeal and bacterial communities using illumina HiSeq sequencing, qPCR and analysis of activity and stable isotope fractionation, respectively. At moderate temperatures (25 °C and 35 °C), CH_4 was always produced by a combination of acetoclastic and hydrogenotrophic methanogenesis. However, at elevated temperature (45 °C) the combination of acetoclastic and hydrogenotrophic methanogenesis was only maintained in the Philippines soil, which contained hydrogenotrophic (*Methanobacteriales*, *Methanocellales*, *Methanosarcinaceae*) and acetoclastic (*Methanosarcinaceae, Methanotrichaceae*) methanogenic taxa under these conditions. In Italian and Utah soil by contrast, CH_4 production at 45 °C occurred by hydrogenotrophic methanogenesis, and the archaeal community was lacking acetoclastic methanogens. Acetate was instead oxidized by *Thermoanaerobacteraceae* (and perhaps *Heliobacteriaceae*) affiliated species which were syntrophically connected to hydrogenotrophic *Methanocellales* and *Methanobacteriales*. Our results showed that the different soils exhibited different structures and functions of the methanogenic archaeal and bacterial communities at elevated versus moderate temperatures. While acetoclastic methanogens in the Philippines paddy soil were able to tolerate elevated temperatures, those in Italian paddy soil and Utah desert soil were not. Instead, syntrophic acetate oxidation allowed the complete degradation of organic matter to CH_4 and CO_2.

1. Introduction

Methane is the second most important anthropogenic greenhouse gas (IPCC, 2013). With a contribution of about 10% to the annual CH_4 emissions, rice fields are one of the major sources in the global CH_4 budget (Conrad, 2009). Methane production is achieved by anaerobic degradation of organic matter (e.g. cellulose from dead plant material) involving a complex microbial community of hydrolytic bacteria, fermenting bacteria, syntrophic bacteria and methanogenic archaea, which accomplish the hydrolysis of polymers, the primary fermentation of monomers, the secondary fermentation of primary fermentation products, and finally the production of CH_4 from H_2/CO_2 and acetate, respectively (Schink and Stams, 2013). Temperature is a key factor that regulates CH_4 production in rice field soil and other wetland soils (VanGroenigen et al., 2013; Yvon-Durocher et al., 2014). However, temperature affects not only the rate of CH_4 production but also the pathway through acetoclastic or hydrogenotrophic methanogenesis (Conrad et al., 2009; Fey et al., 2004; Lu et al., 2015; Peng et al., 2008). Italian rice field soil has frequently been used as a model system to study the effects of temperature on CH_4 production in rice paddy soil. The methanogenic community was found to be able to produce CH_4 over a wide temperature range up to about 55 °C (Fey et al., 2001; Yao and Conrad, 2000).

Temperature was found to affect not only the rate and the pathway of CH_4 production but also the composition of the methanogenic community (Chin et al., 1999; Conrad et al., 2009; Fey and Conrad, 2000). At moderate temperatures, CH_4 is usually produced by hydrogenotrophic and acetoclastic methanogenic archaea, which frequently

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exhibit a rather large diversity in rice field soils, including acetoclastic *Methanosarcinaceae* and *Methanotrichaceae* ('*Methanosaetaceae*' (Oren, 2014)) as well as hydrogenotrophic *Methanocellales*, *Methanomicrobiales*, and *Methanobacteriales* (Conrad et al., 2009). However, structure and function of the Italian paddy soil community changed drastically when temperature was increased above about 40 °C (Conrad et al., 2009). Under these moderately thermophilic conditions, CH_4 was exclusively formed by hydrogenotrophic *Methanocellales* (Conrad et al., 2009; Fey et al., 2001). Similar temperature responses of the methanogenic community structures were found in two Chinese paddy soils (Lu et al., 2015; Peng et al., 2008), with *Methanocellales* and *Methanobacteriales* being enriched at 45 °C. Although moderately thermophilic methanogenic archaea seem to be ubiquitous in temperate anoxic soils (Wu et al., 2006), the prevalent thermophilic methanogens are not necessarily *Methanocellales*, but may also belong to *Methanobacteriales*, *Methanomicrobiales* or *Methanosarcinaceae* (Wu et al., 2006).

Deserts (semiarid, arid and hyperarid regions) span over 44 million km^2 and account for up to 33% of the earth's land surface (Verstraete and Schwartz, 1991). It has been shown that desert soils are frequently inhabited by methanogenic archaea and express CH_4 production upon flooding (Angel et al., 2011, 2012; Aschenbach et al., 2013). Unlike anoxic environments (e.g. paddy soil) which typically host a wide variety of methanogens, only a few lineages of methanogens were detected in oxic upland soils, i.e., mainly the genera *Methanosarcina* and *Methanocella* (Angel et al., 2011, 2012; Aschenbach et al., 2013). Desert soils typically experience large temperature changes during the day and over the season. However, the response of the methanogenic microbial community to temperature has not yet been studied.

Since desert soils apparently possess only a reduced diversity of methanogens (Angel et al., 2011, 2012), and since other soils may have moderately thermophilic methanogens of both putatively hydrogenotrophic and acetoclastic physiology (Wu et al., 2006), we wondered whether the functional and structural responses of such communities would be the same as in the Italian paddy soil. In particular, we hypothesized that soils exist which feature thermophilic acetoclastic methanogens, and consequently keep a combination of hydrogenotrophic and acetoclastic methanogenic pathways across a temperature range of 25 °C–45 °C. We also hypothesized that soils without moderately thermophilic acetoclastic methanogens would at 45 °C only exhibit hydrogenotrophic methanogenesis, similarly as observed in Italian paddy soil. Finally, we hypothesized that the desert soil with reduced diversity of methanogens would nevertheless adapt in some way to the elevation of temperature to allow complete degradation of organic matter to CH_4 and CO_2.

Therefore, we studied the structural and functional responses of the methanogenic archaeal communities to moderate (25 °C and 35 °C) and thermophilic (45 °C) temperatures in three different soils. We used the Italian soil, which had been studied before (Conrad et al., 2009), as well as a paddy soil from the Philippines and a desert soil from Utah. The Philippines paddy soil was tested, since the archaeal community structure was different from that in Italian soil, in particular having a relatively low abundance of hydrogenotrophic *Methanocellales* (Breidenbach and Conrad, 2015). The desert soil from Utah (USA) was studied, since it expressed CH_4 production upon water saturation and displayed a methanogenic archaeal community mainly consisting of the genera *Methanosarcina* (putatively acetoclastic) and *Methanocella* (hydrogenotrophic) (Angel et al., 2012). Since the methanogenic archaea depend on bacteria for providing the methanogenic substrates acetate and H_2/CO_2, we also studied the bacterial communities. We determined the methanogenic pathways by stable isotope fractionation and characterized the archaeal and bacterial community structures by taxonomic composition and abundance of Bacteria and Archaea.

2. Materials and methods

2.1. Soil sampling

Three different soils were tested in the present study. The first soil was collected from rice fields at the Italian Rice Research Institute in Vercelli in 2013. The physicochemical properties of Italian soil were described earlier (Pump and Conrad, 2014). The soil is a sandy loam with a pH of 5.75, total C of 1.1% and total N of 0.08%. The second soil was collected from the International Rice Research Institute (IRRI) in Los Baños, Philippines, in 2012. Philippines rice paddy soil is a silt loam with a pH of 6.3, total C of 1.9% and total N of 0.2% as measured by standard methods (Fu et al., 2018). Utah desert soil was collected from a natural site in Utah, USA in 2009. The same batch of desert soil had been used in a previous study (Angel et al., 2012). The soil is a silt loam with a pH of 6.5, total C_{org} of 4.71% and total N of 0.37%. Soils were air-dried and stored at room temperature. The soils were sieved (< 2 mm) prior to use.

2.2. Soil incubation and chemical analyses

Anoxic soil incubations were prepared by mixing 50 g dry soil with 50 ml of deionized, sterile, anoxic water and 100 mg cellulose in 150-ml bottles. Cellulose (Sigma) was added to serve as substrate in addition to the soil organic matter present in each soil. The addition of cellulose largely equalized the initial substrate availability in the different soils. Cellulose was used since it is the most important polysaccharide in dead plant material (e.g. rice straw), being applicable for desert soil as well as rice paddy soil. The bottles were sealed with rubber stoppers and flushed with N_2. Three temperature treatments (each with three replicates) were established for each soil: 25 °C, 35 °C and 45 °C. Treatments were designated as I25, I35 and I45 for Italian paddy soil, P25, P35 and P45 for the Philippines paddy soil and U25, U35 and U45 for Utah soil, respectively. After 250 days incubation, aliquots of soil slurries were collected and stored frozen at − 20 °C for later analysis of volatile fatty acids and for molecular analysis.

The accumulation of CH_4 and CO_2 was measured using a gas chromatograph equipped with methanizer and flame ionization detector as previously described (Yao et al., 1999). The $\delta^{13}C$ of CH_4 and CO_2 was measured by GC combustion isotope ratio mass spectrometry (GC-C-IRMS) (Conrad et al., 2012). The apparent isotopic fractionation factor for conversion of CO_2 to CH_4 was determined by $\alpha_{app} = (\delta^{13}CO_2 + 10^3)/(\delta^{13}CH_4 + 10^3)$ (Fey et al., 2004). Volatile fatty acids were measured by high-pressure liquid chromatography (Liu and Conrad, 2010).

2.3. DNA extraction and qPCR of bacterial and archaeal 16S rRNA gene

DNA was extracted from the soil samples using the NucleoSpin Soil Kit (Macherey-Nagel, Düren, Germany). Lysis buffer SL1 (the Philippines and Utah soils) or SL2 (Italian soil) and enhancer SX were used, and DNA was eluted in 100 μl of Elution Buffer. Extracted DNA was used as template for qPCR and Illumina HiSeq sequencing. The abundances of archaeal and bacterial 16S rRNA genes were determined by qPCR using the primer sets Arch364f/934br (Kemnitz et al., 2005) and Ba519f/907r (Stubner, 2002), respectively. The conditions of qPCR for the archaeal 16S rRNA genes were as following: 6 min at 94 °C, 45 cycles of 94 °C for 35 s, 66 °C for 30 s, 72 °C for 45 s, 86.5 °C for 10 s (data collection). The conditions of qPCR for the bacterial 16S rRNA genes were as following: 8 min at 94 °C, 50 cycles of 94 °C for 20 s, 50 °C for 20 s, 72 °C for 50 s (including data collection). Technical duplicates were performed for each of the replicates.

2.4. Illumina library preparation and sequencing

PCR primers (515F, 5′-GTGCCAGCMGCCGCGGTAA-3′ and 806R, 5′-

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