Temperature effects on structure and function of different soil methanogenic microbial communities

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Abstract:
Since methanogenic archaea are ubiquitous in soils, we wondered whether they would behave similarly. Therefore, we compared paddy soils from Italy and the Philippines, which have different microbial community structures, and 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) these pathways were 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, 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.

Keywords: Temperature; Methanogenesis pathway; Methanogenic community; Paddy soil; Desert soil

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1. Introduction
Temperature is an important factor regulating not only the rates of production of the greenhouse gas CH$_4$ (Yvon-Durocher et al., 2014), but also the structure of the methanogenic bacterial and archaeal communities and the pathway by which CH$_4$ is formed (Conrad, 2008). In most methanogenic environments there are two major pathways of CH$_4$ formation, either from the disproportionation of acetate (acetoclastic methanogenesis) or the reduction of CO$_2$ with H$_2$ (hydrogenotrophic methanogenesis). The proportion by which each pathway is used for CH$_4$ production depends on the extent by which the respective methanogenic substrates, i.e., acetate and H$_2$ + CO$_2$, are formed. Hence, the anaerobic degradation of organic matter by fermenting microorganisms to these compounds is crucial for the pathway of CH$_4$ production. Polysaccharides from dead plant material is the most important primary organic matter for CH$_4$ production. There are three basically different processes for degradation of polysaccharides:

(1) Fermentation to a mixture of acetate, H$_2$ and CO$_2$. Such fermentation encompasses many different species of fermenting bacteria.

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2 \text{H}_2\text{O} \rightarrow 2 \text{CH}_3\text{COOH} + 2 \text{CO}_2 + 4 \text{H}_2
\]

\[
2 \text{CH}_3\text{COOH} \rightarrow 2 \text{CH}_4 + 2 \text{CO}_2
\]

\[
4 \text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2 \text{H}_2\text{O}
\]

\[
\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 3 \text{CH}_4 + 3 \text{CO}_2
\]

The CH$_4$ production pathways are then 67% acetoclastic methanogenesis and 33% hydrogenotrophic methanogenesis.

(2) Fermentation to acetate only. Such fermentation encompasses only (homo) acetogenic fermenting bacteria.

\[
\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 3 \text{CH}_3\text{COOH}
\]

\[
3 \text{CH}_3\text{COOH} \rightarrow 3 \text{CH}_4 + 3 \text{CO}_2
\]

\[
\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 3 \text{CH}_4 + 3 \text{CO}_2
\]

The CH$_4$ production pathway is entirely by acetoclastic methanogenesis.
(3) Fermentation to $\text{H}_2+\text{CO}_2$ only. Such fermentation needs the operation of syntrophic acetate-oxidizing bacteria.

\[
\begin{align*}
\text{C}_6\text{H}_{12}\text{O}_6 + 2 \text{H}_2\text{O} & \rightarrow 2 \text{CH}_3\text{COOH} + 2 \text{CO}_2 + 4 \text{H}_2 \\
2 \text{CH}_3\text{COOH} + 4 \text{H}_2\text{O} & \rightarrow 4 \text{CO}_2 + 8 \text{H}_2 \\
12 \text{H}_2 + 3 \text{CO}_2 & \rightarrow 3 \text{CH}_4 + 6 \text{H}_2\text{O} \\
\text{C}_6\text{H}_{12}\text{O}_6 & \rightarrow 3 \text{CH}_4 + 3 \text{CO}_2
\end{align*}
\]

Methane is then entirely produced by hydrogenotrophic methanogenesis. The three different fermentation pathways may occur in mixture, so that the proportion of acetoclastic versus hydrogenotrophic methanogenesis may vary between 0 and 100%. Note however, that >67% acetoclastic methanogenesis requires the operation of homoacetogenesis and that >33% hydrogenotrophic methanogenesis requires the operation of syntrophic acetate oxidation.

Previous studies of paddy soils, lake sediments and peatlands have shown that polysaccharide degradation No.1 and No.2 are the most common processes at cold and moderate temperatures (Fey and Conrad, 2000; Kotsyurbenko et al., 2004; Schulz and Conrad, 1996; Takai, 1970; Winfrey and Zeikus, 1979). Process No.2 operates preferentially at cold (<20°C) temperatures so that the acetoclastic pathway of $\text{CH}_4$ production dominates (up to 100%) (Conrad et al., 1989; Kotsyurbenko et al., 1996; Phelps and Zeikus, 1984). As temperature increases, process No.1 becomes increasingly prominent so that $\text{CH}_4$ is eventually produced by a mixture of hydrogenotrophic (<33%) and acetoclastic (>67%) pathways (Fey and Conrad, 2000). However, when temperatures increases above about 45°C, paddy soils from Italy and China exhibit a drastic shift in structure and function of the soil microbial communities with process No.3 becoming the exclusive degradation pathway (Conrad et al., 2009; Lu et al., 2015; Noll et al., 2010; Rui et al., 2011). Under these conditions, acetoclastic methanogenesis is replaced by acetate oxidation syntrophically coupled to hydrogenotrophic methanogenesis. Since paddy soils may contain a variety of different thermophilic methanogens (Wu et al., 2006), we wondered whether thermophilic acetoclastic methanogenesis may operate in other soils and therefore, tested a paddy soil from the Philippines and a desert soil from Utah in comparison to the previously studied paddy soil from Italy.

2. Materials and methods

We used paddy soil from Vercelli, Italy and from the International Rice Research Institute at Los Banos, The Philippines, and also used a desert soil from Utah, USA. The soils were incubated under anoxic conditions at three different temperatures (25, 35, 45°C). 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. Details have been described (Liu et al., 2018).

3. Results and discussion

At moderate temperatures (25°C and 35°C), $\text{CH}_4$ was always produced by a combination of acetoclastic and hydrogenotrophic methanogenesis. Hydrogenotrophic methanogens (\textit{Methanobacteriales, Methanocellales}) were found in all soils and at all temperatures. However, at elevated temperature (45°C) the combination of acetoclastic and hydrogenotrophic methanogenesis was only maintained in the Philippines soil, which contained acetoclastic (\textit{Methanosarcinaceae, Methanosautaceae}) methanogenic taxa under these conditions (Table 1). In the Italian and Utah soil by contrast, $\text{CH}_4$ production at 45°C exclusively occurred by hydrogenotrophic methanogenesis, and the archaeal community was lacking acetoclastic methanogens (Table 1). Acetate was instead oxidized by \textit{Thermoanaerobacteraceae} (and perhaps \textit{Heliobacteriaceae}) affiliated species which were syntrophically connected to hydrogenotrophic \textit{Methanocellales} and \textit{Methanobacterialae}. Such syntrophic acetate oxidation has previously only been found in Italian and Chinese paddy soil (Liu and Conrad, 2010; Rui et al., 2011).
Table 1 Relative abundance (%) of Illumina 16S rRNA gene sequences of major microbial taxa in three different soils and three different incubation temperatures

<table>
<thead>
<tr>
<th>Origin of soil, Microbes</th>
<th>25°C</th>
<th>35°C</th>
<th>45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Italy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanothrix</td>
<td>15</td>
<td>16</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Methanosarcina</td>
<td>26</td>
<td>17</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Thermoanaerobacteraceae</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>6.3</td>
</tr>
<tr>
<td><strong>The Philippines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanothrix</td>
<td>3</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Methanosarcina</td>
<td>14</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Thermoanaerobacteraceae</td>
<td>&lt;0.4</td>
<td>&lt;0.4</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Utah</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanothrix</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Methanosarcina</td>
<td>30</td>
<td>22</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Thermoanaerobacteraceae</td>
<td>&lt;0.3</td>
<td>&lt;0.3</td>
<td>3.8</td>
</tr>
</tbody>
</table>

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. However, there were also changes in other bacterial taxa that were probably involved in hydrolytic and fermentation processes (Liu et al., 2018). There was a substantial taxonomic redundancy allowing for the different functions within the methanogenic pathways. We also realized an amazing individuality of the different soils with respect to microbial taxa, which were hardly shared. Even within shared taxa we realized different adaptabilities to temperature change suggesting the existence of ecotypes. In summary, our experiments showed that temperature is an important regulator for structure and function of methanogenic microbial communities, albeit taxonomic microbial community structures in each soil were different and reacted individually to temperature treatment.

4. Conclusion
Our study showed that the methanogenic archaeal and bacterial communities differ with soil type and temperature. Only the Philippines paddy soil contained thermophilic acetoclastic methanogens and thus maintained acetoclastic methanogenesis at elevated (45°C) temperature. In the other soils acetoclastic methanogens were missing at 45°C and consequently, acetoclastic methanogenesis ceased and was replaced by syntrophic acetate oxidation coupled to hydrognotrophic methanogenesis.

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References


Liu, P., Klose, M. and Conrad, R. 2018. Temperature effects on structure and function of the methanogenic microbial communities in two paddy soils and one desert soil. Soil Biology and Biochemistry, 124, 236-244.


