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RESEARCH ARTICLE

The linkage between methane production activity and prokaryotic community structure in the soil within a shale gas field in China

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Abstract

Soil methane generation mainly driven by soil prokaryotic microbes can be coupled with the degradation of petroleum hydrocarbons (PHCs); however, the relationship between prokaryotic community structure and methane production activity in soil with the potential risk of PHC contamination is seldom reported. In this study, 3 soil samples (CS-1 to CS-3) in the area nearby an exploratory gas well and 5 soil samples (DC-1 to DC-5) in a drill cutting dump area were obtained from the Fuling shale gas field (Chongqing City, China). Then, the prokaryotic community structure was examined by Illumina Miseq sequencing, and the linkage between soil methane production rate (MPR) and prokaryotic community composition was analyzed. The results indicated that 2 samples (DC-4 and DC-5) collected from the drill cutting dump area had significantly higher MPR than the other samples, and a significant and positive relationship ($r = 0.44$, $P < 0.05$) was found between soil MPR and soil organic matter (OM) content. The prokaryotic community composition in the sample (DC-5) with the highest MPR was different from those in the other samples, and soil OM and MPR were the major factors significantly correlated with the prokaryotic community structure in this soil. The samples (DC-4 and DC-5) with higher MPR had a higher relative abundance of Archaea and different archaeal community structures from the other samples, and the MPR was the sole factor significantly correlated with the archaeal genus composition in this soil. Therefore, both the prokaryotic and archaeal community structures are essential in the determination of soil MPR, and the bacterial genus of *Saccharibacteria* and the archaeal genus of *Methanolobus* might be the key contributors for methane generation in this soil from the shale gas field.

Keywords Shale gas field · Soil · Methane production rate · Prokaryotic community structure · Bacteria · Archaea

Introduction

Soils nearby the oil and gas fields have a high risk of petroleum hydrocarbons (PHCs) pollution, as high PHCs content can be usually detected in these soils (Liu et al. 2015; Sun et al. 2015; Wang et al. 2015). PHCs are a mixture of varied organic compounds, including alkanes, BTEX (benzene, toluene, ethylbenzene, and xylene), and polycyclic aromatic hydrocarbons (PAHs), which are toxic for ecosystem and human health due to their carcinogenicity, mutagenicity, and teratogenicity (Salanitro et al. 1997; Ritchie et al. 2001; Huang et al. 2016). Soil is considered as a major reservoir for PHCs, and PHCs in the soil can enter food chains, posing indirect threats to human health (Van-der-Oost et al. 2003; Xue and Warshawsky 2005; Gao et al. 2019). As such, soils in the region nearby the oil and gas fields, as aged soils with PHC pollution, should be very ideal objects for the study about the transformation of PHCs in soil.

At present, the investigation about the interaction between PHCs and soil microbes has been a hotspot. As soil microbes are not only the major consumers for soil PHCs but also can be used as sensitive indicators for the assessment of soil PHC contamination (Haritash and Kaushik 2009; Khan et al. 2013). In recent years, many investigators had pay attention to the responses of microbial community structure to PHC contamination in the soils within several important oilfields in China (Liao et al. 2015; Liu et al. 2015; Sun et al. 2015; Gao

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et al. 2016; Zhou et al. 2017; Gao et al. 2019). For example, in the soil collected from Dagang Oilfield (North China), it was reported that PHC pollution resulted in the increase of the gene copies of *AlkB* (alkane monooxygenase gene), while the decrease of the *Nah* (naphthalene dioxygenase gene) gene copies (Liu et al. 2015). Zhou et al. (2017) reported that, in the soil within the region of the Jianghan oilfield (Central China), the community structure of different microbes showed varied sensitivities to PHC contamination, with the order of bacteria > fungi > Archaea. Moreover, some bacterial genera, such as *Alkanindiges*, *Arthrobacter*, *Idiomarina*, *Lysobacter*, *Massilia*, *Micromonospora*, *Mycobacterium*, *Oxalobacteraceae*, and *Pseudomonas* were found to be potential PHC degraders in soil (Han et al. 2014; Li et al. 2015; Sun et al. 2015; Chen et al. 2016). Meanwhile, some fungal genera, such as *Trichoderma*, *Fusarium*, and *Pestalotiopsis* also, were often detected in soils contaminated by PHCs (Llado et al. 2013; Cebron et al. 2015; Kristanti and Hadibarata 2015; Andreolli et al. 2016; Zhou et al. 2017). Those studies identified some key microbes with the ability to degrade PHCs and confirmed the selection effect of PHCs on soil microbiota in the area of oilfields, which could be essential for the bioremediation and risk assessment of PHC contamination for local soils.

Methane, as the simplest alkane with a molecular structure of tetrahedron, is the main constituent of natural gas. Despite its strong greenhouse effect, methane has been considered as a clean energy source with high efficiency (Cornelissen et al. 2012; Kwietniewska and Tys 2014; Morel et al. 2019). Thus, the digestion of organic wastes or pollutants under methanogenic conditions is regarded as an ideal way, as this process can produce biogas mainly containing methane and carbon dioxide (Vergara-Fernandez et al. 2008). Furthermore, it has been confirmed that some PHCs, such as PAHs, long-chain alkane, and BTEX, can be degraded under methanogenic conditions (Ficker et al. 1999; Chang et al. 2006), and it had been reported that crude oil contamination could enhance soil methane emission (Dunfield et al. 1993; Le Mer and Roger 2001; Yang et al. 2018). The biological methane production in soil is a complex process driven by diverse prokaryotic microbes (bacteria and Archaea), whereas little information is available about the relationship between methane production activity and diversity of prokaryotic community in the soils with high risk of PHC pollution, which should be significant for the resource-based treatment of soil with PHC pollution.

In this study, soil samples were collected from the Fuling shale gas field, Chongqing City, China, which is the second-largest shale gas field in the world, and the relationship among soil properties, methane production rate (MPR), and diversity of prokaryotic community was investigated. The aim was to reveal the relationship between the MPR and the diversity of the prokaryotic community and identify the key microbes affecting MPR in this soil with a potential risk of PHC pollution, thus providing some useful microbial information for the resource-based treatment of PHC-polluted soil under methanogenic conditions.

Materials and methods

Site description and soil sampling

The Fuling shale gas field with gas storage of nearly 2.1 trillion cubic meters is the second-largest shale gas field in the world, located in Fuling District, Chongqing, China. Belonging to a subtropical monsoon moist climate, this region has a mean annual temperature of 18.1 °C and precipitation of 1072 mm, and the soils are classified as Cambisols (Chinese taxonomy) or Inceptisols (USDA taxonomy). In this study, 8 soil samples (triplicates for each sample) were obtained from this shale gas field in September 2018, and the geographic distribution for those sampling sites is presented in Fig. 1. Among those samples, 3 samples (CS-1, CS-2, and CS-3) and 5 samples (DC-1, DC-2, DC-3, DC-4, and DC-5) were obtained from the area nearby an exploratory shale gas well (CS) and a drill cutting dump (DC), respectively. At each sampling site, triplicate soil samples were collected, and each sample was obtained from a mixture of 8 soil cores (5 cm in diameter; 20 cm in depth) randomly selected within an area of 100 m^2. Then, the soil samples were kept in an icebox and taken back to the lab within 24 h. After passing through a 2-mm sieve, all soil samples were stored at 4 °C and − 20 °C for the following basic properties measurement and DNA extraction, respectively.

Measurement of soil basic properties and methane production rate

Soil pH was determined with soil to water ratio of 2:5. Soil organic matter (OM) and available phosphorus (AP) were determined according to the methods of Lu (1999). After extracted from 5 g of fresh soil with 2 M L^{-1} KCl, ammonium and nitrate were measured by a continuous flow analyzer (SAN++, Skalar, Holland). The contents of two representative PHCs (pyrene and benzopyrene) in those soil samples were measured according to the method of Cao et al. (2009), and the detailed procedure is available in the supplementary materials (Text S1).

Soil MPR was measured under a glucose-induced and anaerobic condition mainly according to the method of Liu et al. (2008) with some modification. For each soil sample, triplicates were set up, and the treatment without the addition of soil was used as control. The detailed procedure was as follows: 20 g fresh soil, 14 mL sterilized water, and 6 mL glucose

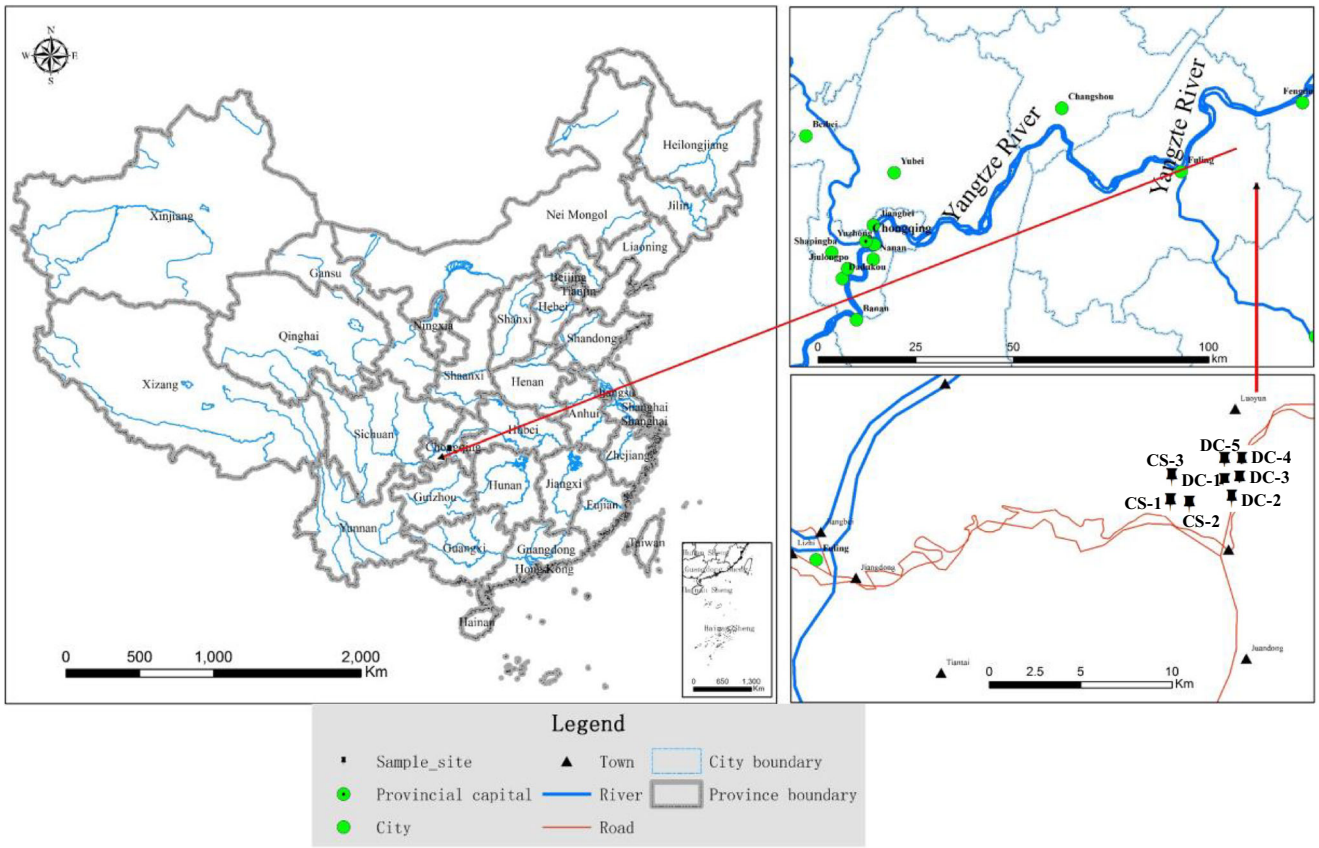

Fig. 1 The geographic information and location of the sampling sites in Fuling shale gas field

(0.1 M L^{-1}) was added into a serum bottle of 100 mL; after sealed with rubber stopper and aluminum cover, the serum bottle was filled with pure N_2 gas for 5 min to replace the air in the bottle; the serum bottle was placed in a shaker at 250 rpm for 30 min, and then was filled with pure N_2 gas for 3 min to ensure an absolutely anaerobic environment; after the serum bottle was incubated at 30 °C for 72 h in dark, the concentration of methane in the serum bottle was detected by a gas chromatograph (Agilent Technologies 7890A, USA). Finally, the MPR in each soil sample was calculated according to the method of Zhang et al. (2011).

Soil DNA extraction and Illumina MiSeq sequencing

A Fast DNA® SPIN Kit for Soil (Q-BIOgene, Carlsbad, CA, USA) was used to extract the total DNA from 0.5 g of frozen soil according to the manufacturer's protocol. The quantity and quality of the extracted DNA were checked using a 1% agarose gel electrophoresis and NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), respectively, and then was stored at − 20 °C before use.

In this study, the primer set of 515F (GTGCCAGC MGCCGCGGTAA) and 806R (GGACTACVSGGGTATC TAAT) was used to amplify the V4 hypervariable region of the prokaryotic 16S rRNA gene (Bates et al. 2011). After addition of the Illumina adaptors A and B to the forward and reverse primer sequences, respectively, the target gene was amplified in a 20 μL mixture with the following thermal profile: 3 min at 95 °C; 30 cycles of 30 s at 95 °C, 30 s at 62 °C, and 45 s 72 °C; 10 min at 72 °C. The PCR products were extracted and purified, and then were sequenced on an Illumina MiSeq platform (Illumina, San Diego, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The procedure of the raw data processing and operational taxonomic units (OTUs) identification was conducted according to the method described by Zhou et al. (2017) presented in the supplementary materials (Text S2). The taxonomy of each representative OTU was aligned by the RDP Classifier algorithm (http://rdp.cme.msu.edu/) based on the Silva ribosomal database (release128, http://www.arb-silva.de) using confidence threshold of 70% (Amato et al. 2013). The diversity indices of *Sobs* (species observed), *Chao*, *Shannon*, and *Simpson* were calculated by Mothur software (version v. 1.41.1, http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity) under a similarity cutoff of 97% (Schloss et al. 2011). The sequencing data in this study has been submitted into the Sequence Read Archive in the NCBI (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov) with the accession number PRJNA591071.

Data analysis

The quantitative difference about the properties or soil MPR between soil samples was examined by a Student-Newman-Keuls test in the one-way analysis of variance (ANOVA) using SPSS 24 (SPSS, Chicago, USA), and $P < 0.05$ was regarded as a significant difference. The correlation between MPR and prokaryotic diversity indices was analyzed using SPSS 24. In R software (http://www.r-project.org), the cluster analysis based on the microbial phyla was done with the method of "Jaccard distance" using the Vegan package, and the composition of the microbial genus in the samples was visualized by the heat map with the Pheatmap package. The redundancy analysis (RDA) profiles were generated based on the matrices of prokaryotic or archaeal composition and environmental variables using the CANOCO (version 5.0), and the detailed analysis steps were described in the supplementary materials (Text S3). Using OTU-based Bray-Curtis distance matrices, the correlation between prokaryotic community structures and environmental variables was evaluated by a Mantel test with 999 permutations in R software (http://www.r-project.org).

Results

Soil properties and MPR

The results of soil properties are listed in Table 1. In this region, the soil was alkaline with a narrow pH range of 8.09~8.34. Except DC-3, the samples (DC-1, DC-2, DC-4, and DC-5) from the drill cutting dump (DC) had significantly higher OM content than those samples (CS-1, CS-2, and CS-3) from the exploratory shale gas well (CS), and the OM contents in the DC-4 and DC-5 were significantly higher than all the other samples. The DC-4 and DC-5 had lower NH_4^+ content, which was significantly lower than that in the CS-1, while no significant difference was detected in the soil NO_3^- content among these soil samples. As for soil available phosphorus (AP), except DC-5, the other DC samples had significantly higher AP content than those CS samples. The result of soil MPR in these samples from the shale gas field is shown in Fig. 2. The significantly higher MPR values were detected in the DC-5 and DC-4, and the DC-1 and DC-2 showed significantly lower MPR values than the other samples.

The soil prokaryotic diversity indices

In these soil samples, a total of 344,805 available sequences were obtained from the Illumina Miseq Sequencing, and the diversity indices of prokaryotic microbes in them are presented in Table 2. The *coverage* of each soil sample was above 0.965, indicating that the result of Illumina Miseq Sequencing was successful and credible. Compared with the other samples, the DC-5 with the highest MPR had the lower values of *Sobs* and *Chao*, while it had a higher value of *Simpson*.

The prokaryotic community composition in the soil from the shale gas field

The cluster analysis and relative abundance of the prokaryotic phylum in these soil samples are shown in Fig. 3a. A total of 10 known bacterial phyla was detected in each soil sample, which occupied more than 90% of the total sequences obtained from the Illumina Miseq Sequencing. The cluster analysis indicated that those soil samples could be grouped into three clusters. In detail, four samples (DC-3, CS-2, DC-4, and CS-3) and three samples (DC-1, CS-1, and DC-2) were divided into two different clusters, whereas DC-5 formed an isolated cluster far away from the former two clusters, indicating that the DC-5 with the highest MPR had a unique phylum composition. Compared with the other samples, DC-5 had lower relative abundances of Acidobacteria, Actinobacteria, Chloroflexi, Cyanobacteria, and Planctomycetes, while had a higher relative abundance of Saccharibacteria (Fig. 3a). The heat map constructed from the dominant prokaryotic genera (top 50) in these soil samples is shown in Fig. 3b. Among these dominant microbial genera, there were 47 known bacterial genera distributed in 11 bacterial phyla, while there was only 1 archaeal genus (*Crenarchaeotic*). Similar to the result of cluster analysis based on the phylum level, the prokaryotic genus composition in DC-5 also was different from the other samples, and a higher relative abundance of norank *Saccharibacteria* was detected in the soil samples (DC-4 and DC-5) with higher MPR.

A low ratio (< 2%) of archaeal sequences to total sequences was detected in each soil sample from this shale gas field, and the soil samples (DC-4 and DC-5) with higher MPR had higher ratios of archaeal sequences to total sequences than the other samples (Fig. 4a). The relative abundance of archaeal phylum in each soil sample is shown in Fig. 4b. Four archaeal phyla of Bathyarchaeota, Euryarchaeota, Thaumarchaeota, and Woesearchaeota were detected in the soil from the shale gas region, and the soil samples (DC-4 and DC-5) with higher MPR had distinct archaeal phylum composition, which almost was solely occupied by Thaumarchaeota and Woesearchaeota, respectively (Fig. 4b).

Relationship between soil properties and prokaryotic community structure

The RDA profiles constructed from soil properties and the dominant prokaryotic OTUs (top 50) or archaeal genera in these soil samples are shown in Fig. 5. Based on the dominant OTUs composition, although a long distance was shown between the two samples (DC-4 and DC-5) with higher MPR,

Table 1 Basic properties of the soil samples in the shale gas field

Samples	pH	OM (g kg^{-1})	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	AP (mg kg^{-1})
CS-1	8.340 ± 0.121^{a}	0.143 ± 0.001^{e}	1.194 ± 0.099^{a}	0.433 ± 0.230^{a}	0.070 ± 0.001^{e}
CS-2	8.123 ± 0.021^{b}	0.142 ± 0.002^{e}	1.056 ± 0.017^{ab}	0.305 ± 0.018^{a}	0.120 ± 0.010^{d}
CS-3	8.310 ± 0.020^{a}	0.119 ± 0.004^{f}	1.091 ± 0.143^{ab}	0.239 ± 0.005^{a}	0.099 ± 0.006^{d}
DC-1	8.153 ± 0.038^{b}	0.231 ± 0.003^{c}	0.949 ± 0.148^{ab}	0.278 ± 0.017^{a}	0.168 ± 0.013^{bc}
DC-2	8.327 ± 0.012^{a}	0.169 ± 0.006^{d}	0.974 ± 0.088^{ab}	0.422 ± 0.106^{a}	0.239 ± 0.019^{a}
DC-3	8.183 ± 0.025^{b}	0.127 ± 0.006^{f}	1.013 ± 0.040^{ab}	0.294 ± 0.021^{a}	0.148 ± 0.011^{c}
DC-4	8.090 ± 0.010^{b}	0.339 ± 0.002^{a}	0.917 ± 0.110^{b}	0.253 ± 0.013^{a}	0.184 ± 0.020^{b}
DC-5	8.090 ± 0.010^{b}	0.314 ± 0.017^{b}	0.827 ± 0.102^{b}	0.418 ± 0.285^{a}	0.119 ± 0.005^{d}

OM, organic matter; *AP*, available phosphorus. Values are mean ($n = 3$), and values within the same column followed by the different letters indicate significant differences ($P < 0.05$)

they were separated from the other samples, indicating their distinct prokaryotic community structure (Fig. 5a). Additionally, the result of the mental test with 999 permutations indicated that soil MPR ($R^2 = 0.98$, $P < 0.01$) and OM content ($R^2 = 0.69$, $P < 0.05$) were significantly correlated with the soil prokaryotic community structure (Fig. 5a and Table S3). The RDA profile based on the archaeal genus showed that, except DC-3, the soil samples from the DC field could be separated from those from the CS field (Fig. 5b). The sample with the highest MPR (DC-5) was isolated from the other samples, and the archaeal genus of *Methanolobus* was the most closely related to soil MPR (Fig. 5b). Furthermore, the soil MPR ($R^2 = 0.95$, $P < 0.05$) was the only variable significantly correlated with soil archaeal genus composition (Fig. 5b and Table S3).

Discussion

In this study, the soil samples (DC-4 and DC-5) from the DC region had higher OM contents and higher MPR than the other samples, and a significantly positive correlation ($r = 0.44$, $P < 0.05$) was found between soil OM content and MPR (Table S1). Soil OM can supply the basic substrates for microbes to synthesize methane and has been considered as a crucial factor determining methane generation rate in both wetland and upland soils (Marschner et al. 2003; Bayer et al. 2012; Morin et al. 2014; Peng et al. 2015; Ye et al. 2016). Moreover, high OM contents were usually detected in soils with PHC pollution, and then OM content could be an important indicator for soil PHC contamination in oil and gas fields (Trofimov and Rozanova 2003; Ogboghodo et al. 2004; Zhou et al. 2017). In this study, compared with other samples, higher contents of two representative PHCs (pyrene and benzopyrene) were detected in DC-4 and DC-5 (Table S2). Therefore, the higher OM contents in DC-4 and DC-5 might be due to the PHC pollution, and the higher MPR in them confirmed that the increase in soil OM caused by PHC contamination could significantly enhance soil methane production (Yang et al. 2018).

In this study, the sample (DC-5) with the highest MPR had the lowest *Sobs*, *Chao*, and *Shannon* indices (Table 2). The negative correlation between soil PHCs content and bacterial diversity in the soils within several oilfields had been reported, which revealed the toxicity and selection of PHCs on soil microbes (Perez-Leblic et al. 2012; Elarbaoui et al. 2015;

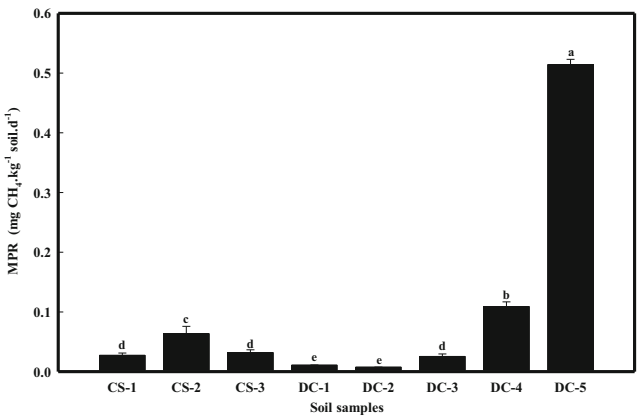

Fig. 2 The MPR in the soil samples from the shale gas field. Error bars indicate standard deviations ($n = 3$). The different letters above the columns indicate significant differences ($P < 0.05$)

Table 2 Diversity indices of prokaryotic microbes in soil samples in the shale gas field

Samples	Coverage	Sobs	Chao	Shannon	Simpson
CS-1	0.986	3803	4465.939	6.916	0.003
CS-2	0.981	3615	4403.889	6.645	0.005
CS-3	0.965	2820	3683.711	6.544	0.007
DC-1	0.976	3384	4143.523	6.798	0.003
DC-2	0.987	3401	4097.667	6.543	0.004
DC-3	0.973	3285	4153.081	6.359	0.015
DC-4	0.983	3613	4512.797	6.702	0.004
DC-5	0.990	2110	2791.055	5.394	0.021

The diversity indices all were calculated with 97% similarity cutoff

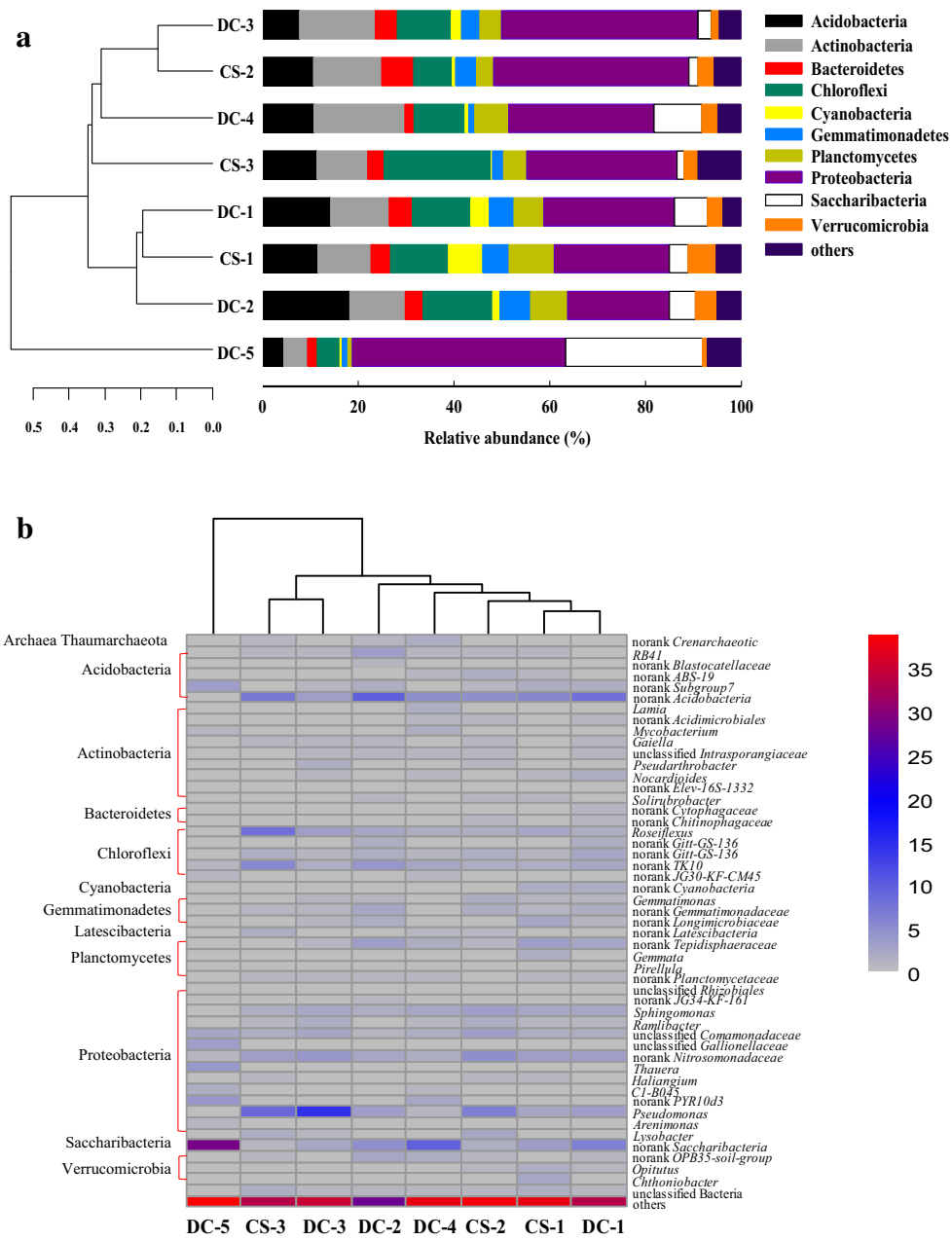

Fig. 3 Relative abundance of prokaryotic phylum and cluster analysis based on phylum composition in these soil samples (**a**), and heat map constructed from top 50 genera in these soil samples (**b**)

Abbasian et al. 2016; Zhou et al. 2017). No investigation had reported the relation between microbial diversity and MPR in those soils with PHC pollution, while Gebert and Perner (2015) found that the soil (collected from a municipal solid waste landfill region) with the highest OM and methane concentration also had the lowest bacterial diversity, which was consistent with the result in this study. Additionally, the result of this study showed that soil samples (DC-4 and DC-5) with higher MPR had higher portions of the archaeal sequence (Fig. 4a). In soil, those microbes directly participate in methane generation all belong to Archaea, which are not sensitive to both organic and inorganic pollutants due to their special structure and components in the cell wall (Valentine 2007; He et al. 2012). For example, it is ammonia-oxidizing Archaea but not ammonia-oxidizing bacteria can be responsible for the ammonia-oxidization process in some unfavorable environments, such as high temperature, low pH, low oxygen, and high salinity (Erguder et al. 2009; Hatzenpichler et al. 2008; Schauss et al. 2009). The major component is peptidoglycan in the bacterial cell wall, but it is pseudo-peptidoglycan or glycoprotein in Archaea; moreover, ester-linked lipids and ether-linked lipids are the major components in the cell membrane of bacteria and Archaea, respectively (Derosa et al. 1986; Kandler and Konig 1998). These differences in the cell structure might result in the low permeability for both nutrients and toxic pollutants in the archaeal cell, and thus ensure a

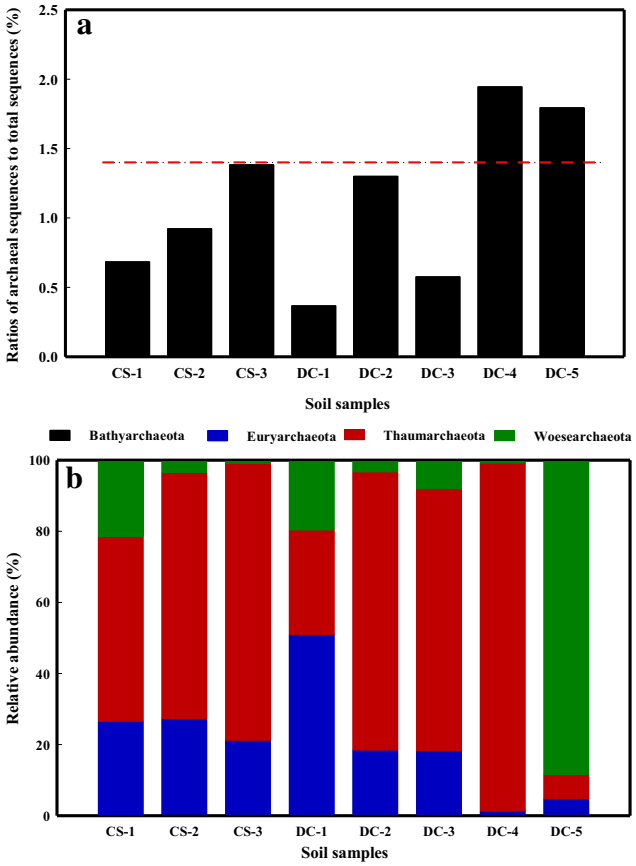

Fig. 4 Ratios of archaeal sequences to total prokaryotic sequences in these soil samples (**a**), and relative abundance of archaeal phylum in each soil samples (**b**)

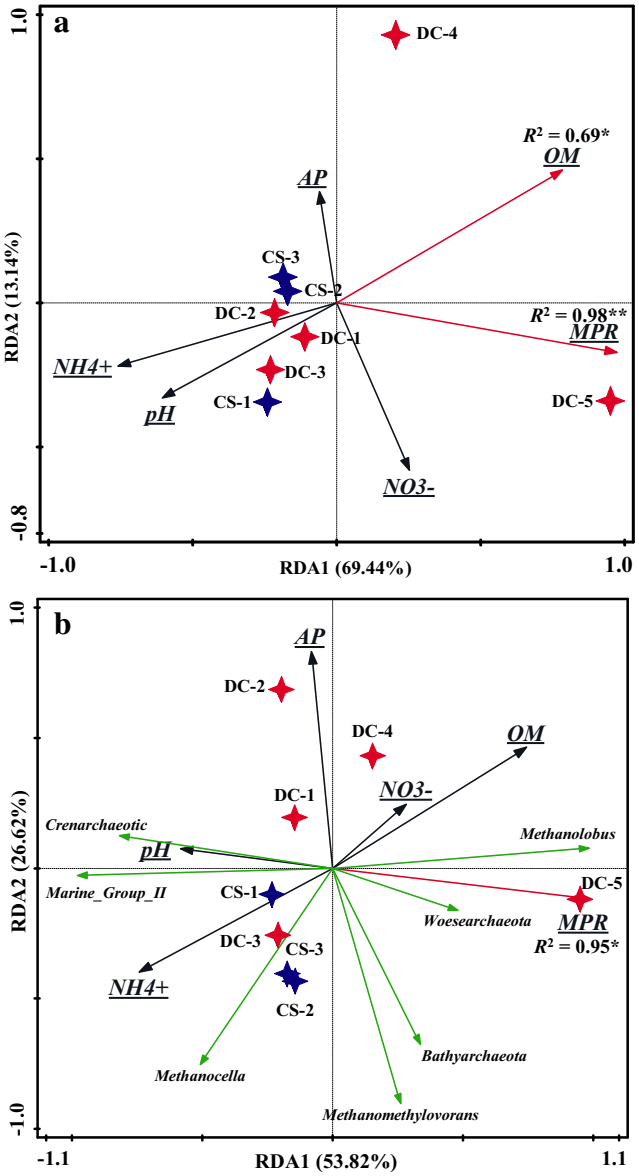

Fig. 5 RDA profiles constructed from soil properties and top 50 prokaryotic OTUs composition (**a**) and archaeal genus composition (**b**) in these soil samples, the significant variables significantly correlated to microbial community structure are shown by red arrows

decent environment for its energy metabolism and growth under the harsh environments (He et al. 2012; Valentine 2007). Consequently, in this study, the higher OM contents in these samples (DC-4 and DC-5) with higher MPR and higher contents of pyrene and benzopyrene (Fig. 2 and Table S2) might indicate their high risk of PHC contamination, which resulted in the decrease in prokaryotic diversity but the increase in the archaeal relative abundance in this soil.

In this study, those two samples (DC-4 and DC-5) with higher soil OM content and MPR all had a higher relative abundance of *Saccharibacteria* than the other samples (Fig. 3**)**. This kind of bacteria has been widely detected in soil, activated sludge, sediment, and other habitats (Bond et al. 1995; Borneman and Triplett 1997; Hugenholtz et al. 2001; Pace 2009), which can utilize various monosaccharides, amino acids, and proteins, and can ferment glucose and other sugars to produce lactate, playing an important role in mineralization of organic compounds (Ariesyady et al. 2007; Nielsen et al. 2009; Nielsen et al. 2010; Albertsen et al. 2013). More importantly, although it is unsure whether this bacterial genus can utilize complex PHCs, its ability to degrade some simple PHCs in soil, such as toluene and benzene, has been confirmed (Luo et al. 2009; Xie et al. 2011). Thus, we hypothesized that *Saccharibacteria* might play a critical role in the degradation of OM or PHCs in this gas field soil, then supplying organic substrates for methanogens. In addition, the RDA profile constructed from the dominant OTUs indicated that samples (DC-4 and DC-5) with higher MPR had different prokaryotic community structures from the other samples, and soil OM ($R^2 = 0.69$, $P < 0.05$) and MPR ($R^2 = 0.98$, $P < 0.01$) were the key factors influencing the soil prokaryotic community structure (Fig. 5a and Table S3). The soil OM content is not only an indicator for assessment of soil PHC contamination but also the important carbon and energy

sources for soil microbes, which has long been considered as one of the most important factors affecting soil microbial community structure (Perez-Leblic et al. 2012; Abbasian et al. 2016; Zhou et al. 2017; Gao et al. 2019). It has been previously reported that there was a significant correlation between the community structure of methanogens and soil MPR (Reim et al. 2017; Chen et al. 2018; Zhang et al. 2018), but the relationship between MPR and total prokaryotic community structure is still uncertain. For example, Wagner et al. (2017) reported that the prokaryotic community structure might control the MPR in Arctic Alaska soils, but no correlation was found between soil methane emission and prokaryotic community structure in the forest soil in Brazil (Cuer et al. 2018). Being consistent with the result of Wagner et al. (2017), our current result also found a significant correlation between the prokaryotic community structure and MPR in this soil from the shale gas field. In our opinion, besides a very small portion of Archaea in prokaryotic microbes directly participating in the methane biological synthesis, most of the prokaryotic microbes also might indirectly take part in the process of methane synthesis by supplying low molecular substrates degraded from complex organic compounds. Therefore, soil MPR ought to be tightly correlated to the prokaryotic community structure.

In this study, the RDA profile based on the archaeal genus revealed that the archaeal genus composition in DC-5 with the highest MPR was different from the other samples, and only MPR was significantly correlated with soil archaeal community structure (Fig. 5b and Table S3). Soil archaeal community should be important in determining the soil capacity of methane generation because the methanogens currently identified all belong to Archaea (Serrano-Silva et al. 2014). Ma and Lu (2010) also found archaeal community structure was an essential factor affecting methane emission in a rice paddy in Hangzhou City, China. Meanwhile, the samples (DC-4 and DC-5) with higher MPR and higher relative abundance of the archaeal phylum of Thaumarchaeota and Woesearchaeota, respectively (Fig. 4b). Presently, no methanogen has been found in these two archaeal phyla, and then, it is unsure whether these two archaeal phyla can directly participate in the process of soil methane synthesis. However, the result of RDA analysis indicated that DC-4 and DC-5 had a higher abundance of *Methanolobus*, which was the most closely correlated to soil MPR (Fig. 5b). *Methanolobus* is known as a methylotrophic methanogen that can only utilize methyl compounds to generate methane, which is the dominant methanogen in some oil or coal polluted habitats (Zhang et al. 2017; Bao et al. 2019; Okoro and Amund 2018). Therefore, our result reconfirmed that *Methanolobus* should be the critical methanogen directly responsible for methane production in those environments with the potential risk of PHC contamination.

Conclusions

In conclusion, in the soil from the shale gas field, OM content was significantly positively correlated with soil MPR. The sample with the highest MPR had the lowest prokaryotic diversity, and the samples with higher MPR had a higher ratio of archaeal sequences to the total prokaryotic sequences. Additionally, soil OM was a crucial parameter affecting soil prokaryotic community structure, and MPR was significantly correlated with both prokaryotic and archaeal community structure. The bacterial genus of *Saccharibacteria* and the archaeal genus of *Methanolobus* were the key microbes in determining soil MPR in the soil from the shale gas field. These results can be available for a deeper understanding of the mechanism of soil methane biological production in soil with the potential risk of PHC pollution and provide some useful microbial information for the resource-based treatment of the PHC-polluted soil.

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