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RESEARCH ARTICLE

Microbial community in produced water from typical coalbed methane wells and its geological significance in Guizhou and Yunnan Provinces, China

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Abstract The produced water from coalbed methane (CBM) wells contains abundant geochemical and microbiological information. The microbial communities in the produced water of 14 CBM wells from four coal-bearing synclines in Guizhou and Yunnan were successfully tested by using 16S rRNA amplicon sequencing technology. The results showed that the produced water contained a large number of archaea and bacteria. The bacteria mainly included the orders *Bacteroidales* and *Clostridiales*, accounting for 37.4% and 32.92%, respectively. The water contained more than 30 species of bacteria and 15 species of methanogens. *Macellibacteroides* was the dominant genus, followed by the genus *Citrobacter*. The methanogens mainly included the orders *Methanobacteriales* and *Methanosarcinales*, accounting for 57.46% and 26.49%, respectively. *Methanobacterium* was the dominant genus, followed by the genus *Methanothrix*. There were three kinds of metabolism: hydrogenotrophic methanogens, acetoclastic methanogens, and methylotrophic methanogens. The main influencing factors of archaea were coalbed properties, such as burial depth and $R_{o,max}$, while the influencing factors of bacteria were mainly the physical and chemical properties of groundwater, including Cl^-, total dissolved solids, and HCO_3^-. The microbial communities were segmented in the vertical direction of the coal measure strata, which can be consistent with the distribution characteristics of multiple superposed fluid systems, and the main microbial species in each section were preliminarily identified. Combining carbon and hydrogen isotopes of methane, and dissolved inorganic carbon stable carbon isotopes of produced water from CBM wells, the results showed that the microbial reduction in the Tucheng and Enhong synclines were strong and that there were obvious secondary biogases. A reduction in hydrogen-trophic methane bacteria is an important way to produce secondary biogases in the study area. These synclines are suitable to carry out microbially enhanced coalbed methane research, expanding and extending CBM stimulation technology in the later stage.

Keywords coalbed methane, produced water, 16S rRNA amplicon sequencing, environmental factors, secondary biogas

1 Introduction

Coalbed methane (CBM) well produced water contains rich geochemical and microbiological message. In recent years, with the progress of biotechnology, study of microbial communities in CBM formation water or produced water has been carried out worldwide, and a variety of microorganisms have been continuously detected (Li et al., 2014; Tian et al., 2023). A deep knowledge of the microbial communities in produced water from CBM wells plays an important part in understanding of the stratigraphic environment, recognition of the origin of CBM, and enhancement of methane production by microorganisms (Barnhart et al., 2016; Li et al., 2022; Shi et al., 2023).

The 16S rRNA amplicon sequencing technique is a useful tool for identifying uncultured or difficult to identify bacteria, which can greatly improve the ability to

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analyze the composition of complex microbial populations and provide more accurate taxonomic information for researchers (Stackebrandt and Goebel, 1994; Lu et al., 2022). Recently, 16S rRNA amplification and sequencing techique has been extensively applied to the study of microbial structure of coal-bearing basins (Park and Liang, 2016), such as Yubari, Japan (Shimizu et al., 2007); Surat Basin, Sydney Basin and Port Phillip Basin in Australia (Li et al., 2008; Vick et al., 2018); Illinois Basin (Strapoc et al., 2008; Zhang et al., 2015) and Porter River Basin (Klein et al., 2008) in the US; Waikato coalfields (Fry et al., 2009) in New Zealand; Alberta Basin (Penner et al., 2010) in Canada; and Ordos Basin (Guo et al., 2012) and Huaibei mine (Liu et al., 2019) in China. 16S rRNA amplicon sequencing studies have been conducted, and different bacteria and archaea have been found in these basins.

Shimizu et al. (2007) found the existence of hydrogenotrophic methanogens (genus *Methanoculleus*) and methylotrophic methanogens (genus *Methanolobus*) in the produced water from the Hokkaido CBM field in Japan. Mutual methanogen bacteria were also detected. Li et al. (2008) collected produced water samples and coal samples from 3 CBM fields of the Surat Basin, the Sydney Basin, and the Port Phillip Basin in eastern Australia. The results of the 16S rDNA gene library showed that the bacteria were mainly composed of phyla *Proteobacteria* and *Firmicutes*, and the latter was mainly confined to the genus *Clostridium*. The archaea contained the genera *Sulfophobococcus*, *Archaeoglobus*, and *Thermococcus*, while genuine methanogens were not detected. After inspecting the coal seam water in Powder River Basin, Klein et al. (2008) found that the water contained methane bacteria such as genera *Methanocaldococcus*, *Methanobacterium*, and *Methanomicrobium*. Midgley et al. (2010) detected the presence of bacteria and archaea in the produced water from a CBM field in the Gippsland Basin, Australia. These bacteria were also dominated by the phyla *Proteobacteria* and *Firmicutes*, while the genus *Methanobacterium* was only detected in archaea. Strapoc et al. (2011) conducted a systematic molecular biology and geochemical study on microbes in the produced water from the eastern CBM field in the Illinois Basin, US and concluded that the biogenetic gas was mainly a hydrogen nutrient and the methanogens were mainly genus *Methanocorpusculu*. Zhang et al. (2015) analyzed the produced water from CBM wells in the southern Illinois Basin and found that the microbes were mainly the hydrogen-nutrient order *Methanobacteriales*. Guo et al. (2012) carried out microbial community analysis on the produced water from CBM wells in the Liulin block of the eastern margin of the Ordos Basin, and the results showed that there were methanogens in the water and *Methanolobus* was the dominant genus. Liu et al. (2019) studied the microbial community compositions of the Luling coalfield in Anhui, China, and found that the produced water from CBM wells contained hydrogenotrophic, aciticlasitic, and methylotrophic methanogens, and the microbial community was diverse.

Eastern Yunnan and western Guizhou is a crucial CBM resources area in southern China. The geological resources of Upper Permian CBM account for about 10% of CBM resources in China (Strategy Research Center of Oil and Gas Resources Department, 2006). This area has the geological features of multiple-thin coal seams, wide coal rank range, medium to high stress, low water saturation, and complex coal structure (Qin et al., 2018; Yang et al., 2019a, 2019b, 2019c; Wang et al., 2021). It became a main CBM development area during the 13th and 14th Five-Year Plan period in China. At present, there are more than 400 CBM production wells in this region. In our previous work on the geochemical properties of the produced water from the CBM wells in this area, major elements, hydrogen and oxygen isotopes, and dissolved inorganic carbon stable carbon isotopes ($\delta^{13}C_{DIC}$) were analyzed (Wu et al., 2018; Yang et al., 2019b). The $\delta^{13}C_{DIC}$ value in some of the water samples were positively abnormal and microbial reduction was thus presumed responsible for it. However, so far, this area has not been subject to microbial detection and analysis of produced water from CBM wells.

Therefore, in this work, the typical synclines and their CBM development wells were selected, and 16S amplicon sequencing technology was used to perform microbial sequencing analysis of the produced water for the first time in this area. Different types of microorganisms were tested. This paper focuses on the analysis of the methanogens community and the characteristics of the microbial community, geological control factors, and its implications for the genesis of biogas and the microbially enhanced coalbed methane (MECBM).

2 Geological settings

The coal-bearing strata in eastern Yunnan and western Guizhou are the Late Permian Longtan Formation, with multiple coal seams and many synclines as the main coal-accumulating units. The CBM wells in western Guizhou are mainly distributed in Songhe, Zhijin, Faer and northern Guizhou blocks (Fig. 1). The CBM wells in the Songhe and Faer were selected as the research objects. The Songhe block has eight CBM wells, which form a clustered well group in the Tucheng (TC) syncline. Well GP-1 and GP-2 were put into production in January 2014, and wells GP-3 to GP-8 were put into production in January 2015. The commingled gas production is typically from 6 to 9 layers. The depth of the bottom coal seam is about 564.5–977.08 m. By August 2018, the maximum daily production rate was approximately 3000 m^3/d, and the stable production rate was approximately 500 m^3/d. Every single well had a cumulative

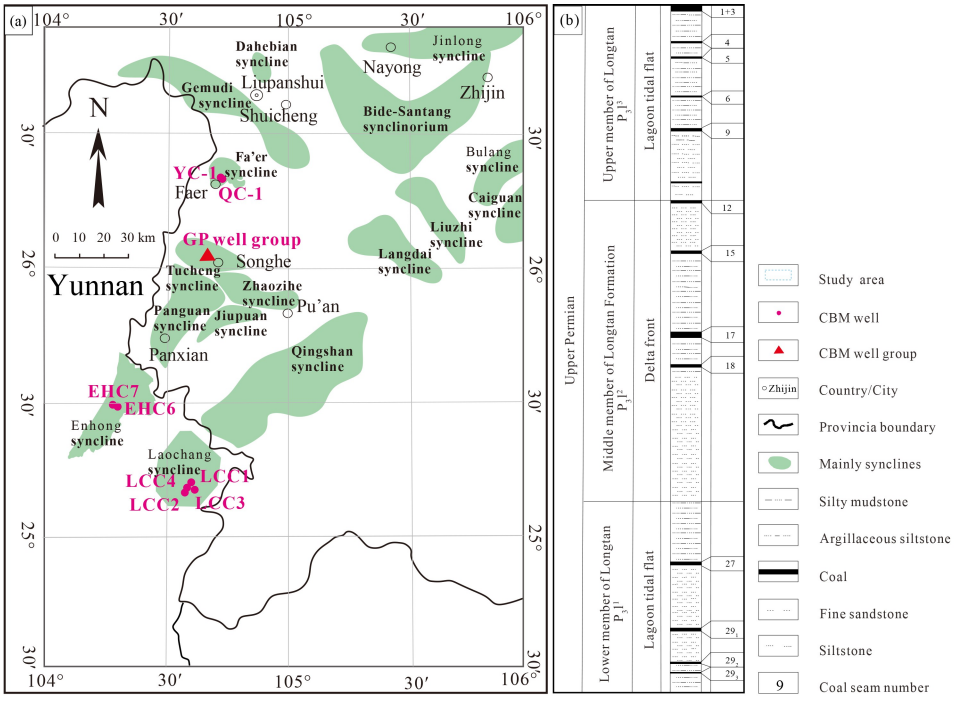

Fig. 1 The distribution map of CBM wells in the study area (a) and the stratigraphic map of the Upper Permian Longtan Formation (b).

water production of approximately 1400–3300 m^3. After January 2018, the GP-1 well was subject to secondary fracturing and was replaced by single-layer drainage of the 1 + 3 coal seam.

There are two CBM wells in the Faer (FE) syncline, wells YC-1 and FE-1. Both wells are multi-layer commingled production wells, with 3 combined production layers. The depth of the bottom coal seam is 659–739 m. Well YC-1 was put into operation in January 2017, with a maximum CBM production of 5400 m^3/d. At present, the YC-1 well has a stable production of 1400 m^3/d and a cumulative water production of approximately 1100 m^3. Well QC-1 was put into operation in September 2017, with a maximum CBM production of 1000 m^3/d. At present, the QC-1 well is stable at 420 m^3/d, and the cumulative water production is approximately 800 m^3.

There are eight CBM wells in eastern Yunnan, mainly located in the Enhong (EH) and Laochang (LC) synclines. The EHC6 and EHC7 wells are located in the EH syncline and are multi-layer combined mining wells with 3 to 4 production layers. The depth of the bottom coal seam is 1036–1182 m. The two wells were put into production in January 2018. By January 2019, the maximum CBM production reached 300 m^3/d, and the cumulative water production of each well ranged from 300 to 500 m^3.

LCC1, LCC2, LCC3, LCC4, LCS1, and LCS2 wells are located in the LC syncline, in which LCC4, LCS1, and LCS2 are in one well group. All the wells undergo multi-layer combined production, and the production layers are generally 3 to 4 layers. The depth of the bottom coal seam is 712.58–832 m. Most of them started drainage in April 2018. By January 2019, the maximum gas production reached 800 m^3/d, and the cumulative water production of each well ranged from 300 to 2000 m^3.

Among the four synclines, the EH and TC synclines mainly develop medium rank coal, the FE syncline mainly develops medium and high rank coal, while the LC syncline mainly develops high rank coal. The four synclines are adjacent to each other. The basic information of the CBM wells in the study area is shown in Table 1.

3 Experimental study

3.1 Conventional ion and dissolved inorganic carbon stable carbon isotope ($\delta^{13}C_{DIC}$) testing

The physical and chemical properties of water samples of 14 CBM production wells in the study area were tracked for 1 to 2 years. All water samples were gathered straight from the outlet of the CBM wells and loaded into 2.5 L cleaning bottles. Our process was to wash the plastic bottle with water 3 times, fill the bottle with water, discharge all the air in the bottle, seal with the cap. Lastly, the bottle were checked for leakage, and the sampling time and place were marked, which were sent to the State Key Laboratory of Environmental Geochemistry, Guiyang Institute of Geochemistry, Chinese Academy of Sciences for decetion within 72 h. The instruments used for the anion and the cation tests were ICS-90 and ICP-AES (USA Vista MPX), respectively, and the detection basis was the general principles of groundwater quality inspection methods (DZ/T 0064.1-1993). The total dissolved solids (TDS) was tested with a

Table 1 Basic information of the CBM wells in the study area

Syncline	Well name	Development coal seam	$R_{o,max}$/%	Depth/m	Temperature/°C	Starting time of production
TC	GP-1	6/9/12/13/15/16/29	1.4–1.7	847.00	31	2014.1
	GP-2	1 + 3/5/9/10/11/13/15/16	1.4–1.7	764.00	34	2014.1
	GP-3	6/9/12/13/15/29	1.4–1.7	610.00	37	2015.1
	GP-5	1 + 3/4/5/6/9/13/15/29	1.4–1.7	654.00	41	2015.1
	GP-7	1 + 3/4/5/12/15/27/29	1.4–1.7	902.00	48	2015.1
	GP-8	1 + 3/4/5/12/13/15/26/27/29	1.4–17	977.08	52	2015.1
FE	YC-1	5/7/13	1.8	659.00	29	2017.1
	QC-1	13/21	1.8	739.00	30	2017.9
EH	EHC6	7 + 8/9/16/21	1.2	1182.00	25	2018.2
	EHC7	16/17 + 18	1.2	1036.00	24	2018.1
LC	LCC1	7 + 8/13	3.0	778.30	26	2018.4
	LCC2	16/18/19	3.0	735.00	30	2018.4
	LCC3	13/14/19	3.4	832.00	36	2018.5
	LCC4	13/16/18/19	3.4	712.58	32	2018.5

Note: After January 2018, the GP-1 well was replaced by single-layer drainage of the 1 + 3 coal seam with a corresponding burial depth of 510.7 m.

TDS meter and the pH was tested with a pH meter at room temperature. The $\delta^{13}C_{DIC}$ values were determined according to Atekwana and Krishnamurthy's method (Atekwana and Krishnamurthy, 1998) using a gas isotope ratio mass spectrometer (MAT253, USA). Key test data are listed in Table 2.

3.2 CH_4 carbon ($\delta^{13}C(CH_4)$) and hydrogen ($\delta D(CH_4)$) isotope testing

The $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ test was carried out for some CBM wells, and the measurement was completed at the Key Laboratory of Petroleum Resources Research, Chinese Academy of Sciences. The $\delta^{13}C(CH_4)$ and $\delta D(CH_4)$ values were determined according to GB/T6041-2002 by using a gas chromatograph (HP6890) and an isotope ratio mass spectrometer (Delta plus XP). The main test data are shown in Table 2.

3.3 Enrichment cultures, amplification and sequencing of the microorganisms

3.3.1 Enrichment cultures

Water samples collected from CBM wells were stored under anaerobic and low temperature conditions. A total

Table 2 Chemical properties of the produced gas and water from the CBM wells in January 2019

Syncline	Well name	Cl^-/(mg·L^{-1})	SO_4^{2-}/(mg·L^{-1})	HCO_3^-/(mg·L^{-1})	TDS/(mg·L^{-1})	pH	$\delta^{13}C(CH_4)$/‰	$\delta D(CH_4)$/‰	$\delta^{13}C_{DIC}$(‰VPDB)
TC	GP-1	2560.19	0.02	136.74	2277	6.7	−39.0	−157.2	1.49
	GP-2	2887.75	0.00	615.33	3425	7.1	−41.9	−171.7	13.73
	GP-3	3438.14	0.03	972.38	3926	7.5	−40.4	−152.8	14.13
	GP-5	3605.64	0.02	562.15	4068	7.5	−40.7	−179.5	22.19
	GP-7	4818.57	0.05	387.43	4683	7.4	−41.0	−158.3	0.59
	GP-8	4963.84	0.00	516.57	4715	7.3	−39.7	−157.5	7.325
FE	YC-1	2441.16	0.08	668.51	3193	7.7	−35.5	−144.6	−1.00
	QC-1	4106.94	0.07	615.33	4589	7.6	−35.9	−139.8	−1.60
EH	EHC6	1514.89	0.46	1200.28	2101	8.0	−45.8	−202.9	13.61
	EHC7	821.34	0.00	1215.47	3630	8.2	−50.9.	−213.9	19.39
LC	LCC1	1805.99	8.13	1906.77	3442	7.8	−33.6	−127.8	−4.623
	LCC2	1916.15	10.10	2575.28	3730	7.8	−36.3	−130.8	−8.54
	LCC3	462.42	7.21	1093.92	1459	7.8	−34.7	−130.1	−8.78
	LCC4	2175.86	0.34	3422.31	4194	7.7	−34.9	−127.6	−4.45

Note: Carbon and hydrogen isotope of methane date of GP-8 for November 2018.

of 500 mL of each water sample was cultured in methanogenic culture medium at 35°C for enrichment of methanogenic bacteria. Methane production medium was prepared by adding 1.0 g NH_4Cl, 1.0 g $MgCl_2$, 0.4 g K_2HPO_4, 0.2 g KH_2PO_4, 0.1 g tryptone, 1.0 mL resazurin (0.1%), 1.0 g yeast extract, 2.0 g sodium acetate, 2.0 g sodium formate, 0.5 g cysteine, 0.2 g Na_2S, 2.0 g $NaHCO_3$, and 10 mL trace element solution (the 1000 mL distilled water was added with triglycolamic acid 1.5 g, $MnSO_4{\cdot}2H_2O$ 0.5 g, $MgSO_4{\cdot}7H_2O$ 3.0 g, $FeSO_4{\cdot}7H_2O$ 0.1 g, NaCl 1.0 g, $CoCl_2{\cdot}6H_2O$ 0.1 g,$CaCl_2{\cdot}2H_2O$ 0.1 g, $CuSO_4{\cdot}5H_2O$ 0.01 g, $ZnSO_4{\cdot}7H_2O$ 0.1 g, H_3BO_3 0.05 g, $AlK(SO_4)_2$ 0.01 g, $NiCl_2{\cdot}6H_2O$ 0.02 g, and Na_2MoO_4 0.05 g) into 1 L CBM-produced water. After 4–5 days of culture, 30 mL of the cultured sample was infunded into a centrifuge tube, subjected to de-oxidation sealing treatment, and sent to be tested.

3.3.2 16S rRNA gene amplification by PCR

The sequencing was accomplished at Sangon Biotech (Shanghai) Co., Ltd. DNA extraction was achieved using a kit (E.Z.N. ATM Mag-Bind Soil DNA Kit). The detection of archaea and bacteria was performed by polymerase chain reaction (PCR) with two rounds of amplification. The primers used in the first round were fused with V3−V4 universal primers of the MiSeq sequencing platform, including the primers 341F, CCCTACACGACGCTCTTCCGATCTG (barcode) CCT ACGGGNGGCWGCAG, and 805R, GACTGGAGTTC CTTGGCACCCGAGAATTCCAGACTACHVGGGTAT CTAATCC. A 30-μL reaction mixture for PCR amplification contained 15 μL of Taq master Mix, 1 μL of Bar-PCR primer F (10 μmol/L), 1 μL of primer R (10 μmol/L), 10−20 ng of bulk DNA solution, and water to 30 μL. PCR conditions were 94°C for 3 min followed by 5 cycles of 94°C for 30 s, 45°C for 20 s and 65°C for 30 s, followed by 20 cycles of 94°C for 20 s, 55°C for 20 s and 72°C for 30 s, and finally 72°C for 5 min.

Secondly, Illumina bridge PCR-compatible primers were introduced. After the initial PCR, a 30-μL reaction mixture for PCR amplification containing 15 μL of Taq master Mix, 1 μL of Bar-PCR primer F (10 μmol/L), 1 μL of primer R (10 μmol/L), the second round PCR primers, and water to 30 μL was prepared. PCR conditions consisted of an initial denaturation step of 94°C for 3 min, followed by 5 cycles of 94°C for 20 s, 55°C for 20 s and 72°C for 30 s, followed by a final extension of 72°C for 5 min. The PCR products were checked using electrophoresis with 1% (w/v) agarose gels in TBE buffer (Tris, boric acid, EDTA) stained with ethidium bromide (EB) and visualized under UV light.

3.3.3 Sequencing data analysis

Total DNA product recovery was performed using a magnetic bead nucleic acid purification kit. The concentration of the DNA was measured using a Qubit 2.0 (Life, USA). Sequencing was performed using the Illumina MiSeq system (Illumina MiSeq, USA).

After the sample was sequenced, a high-quality sequence was obtained through quality control (QC) processing. Operational taxonomic units (OTU) clustering analysis was carried out for each sample sequence according to 97% sequence similarity. The Chao and ACE indexes were used to evaluate the richness of the microbial community. The larger the Chao 1 and ACE indexes were, the greater the microbial biomass was. The Shannon and Simpson indexes were used to evaluate the diversity of the microbial community. The larger the Shannon index value was, the richer the community diversity became, while it was the opposite for the Simpson index. Key test data are listed in Table 3 and Table 4.

The RDP classifier was used for species classification, according to Bergey's taxonomy. In this method, naïve Bayesian assignment was used to calculate the probability value of each sequence assigned to this rank at different levels. Bergey's taxonomy was divided into six layers: domain, phylum, class, order, family and genus. According to the results of taxonomic analysis, the community composition of each sample at each taxonomic level was calculated.

4 Results

4.1 Types of microbial communities

4.1.1 Types of archaea

The results of archaea community analysis display that there are a large amount of methanogens in the produced water from 14 CBM wells of four synclines (Table 2, Fig. 2). They mainly belong to phylum *Euryarchaeota*, accounting for 97.83%. It includes many types of methanogens such as the classes *Methanobacteria*, *Methanomicrobia*, and *Thermoplasmata*, and contains four orders of *Methanobacteriales*, *Methanomicrobiales*, *Methanosarcinales*, and *Methanococcales*. However, in these samples, there are mainly two classes *Methanobacteriales* and *Methanosarcinales*, accounting for 57.46% and 26.49%, respectively. They contain more than 15 species of methanogens, of which *Methanobacterium* is dominant, accounting for 57.01%, followed by the genus *Methanothrix*, accounting for 25.22%. Consistent with this, more than 89.8% of the bacteria in the coal-bearing formation water in the southern Illinois Basin, US also belong to the class *Methanobacteriales* (Zhang et al., 2015).

The genus *Methanobacterium* is the main hydrogentrophic methanogen, which can metabolize H_2/CO_2 to form CH_4. The genus *Methanothrix* is a methanogen of the acetic acid type, which can produce methane and

Table 3 Number of reads after sequencing, OTUs and diversity index (archaea)

Sample ID	Seq num	OTU num	Chao1 index	ACE index	Shannon index	Simpson index
GP-1	70429	442	459.98	487.04	1.68	0.38
GP-2	89486	166	274.97	443.87	2.18	0.71
GP-3	81272	169	331.88	433.04	2.10	0.49
GP-5	87554	203	261.16	285.92	0.74	0.32
GP-7	76157	314	544	811.02	1.08	0.25
GP-8	79982	244	330.62	354.61	1.54	0.15
QC-1	79183	404	591.31	828.52	1.70	0.42
YC-1	78040	326	441.55	489.45	0.74	0.35
LCC1	82672	556	642.36	687.51	1.77	0.58
LCC2	78876	179	270.12	383.36	2.18	0.56
LCC3	65189	506	660.73	740.44	1.17	0.48
LCC4	77317	106	185.57	217.83	1.08	0.52
EHC6	73039	188	458.04	1117.63	1.63	0.27
EHC7	84097	165	207.09	258.79	1.72	0.17

Table 4 Number of reads after sequencing, OTUs and diversity index (bacteria)

Sample ID	Seq num	OTU num	Chao1 index	ACE index	Shannon index	Simpson index
GP-1	77049	70	107.40	156.62	1.34	0.31
GP-2	61144	208	442.85	830.32	1.57	0.38
GP-3	58580	228	282.47	334.01	1.90	0.26
GP-5	68276	249	391.88	416.48	1.55	0.33
GP-7	70555	88	132.00	129.46	0.23	0.93
GP-8	80696	172	174.01	180.61	0.57	0.78
QC-1	65394	133	308.20	577.74	1.46	0.34
YC-1	77382	359	375.24	396.75	2.20	0.19
LCC1	65978	227	375.90	585.62	1.96	0.23
LCC2	79426	241	413.86	763.28	1.75	0.27
LCC3	80349	184	374.00	536.02	1.77	0.28
LCC4	87511	256	487.67	799.16	1.76	0.36
EHC6	77923	286	448.98	486.91	2.20	0.23
EHC7	73015	211	321.68	342.32	1.78	0.33

Note: The OTUs were defined with 97% similarity.

carbon dioxide through anaerobic metabolism (Papendick et al., 2011). In addition, the hydrotrophic genera *Methanocorpusculum*, *Methanoregula*, *Methanospirillum*, and *Methanoculleus*, the methanotrophic genera *Methanomassiliicoccus* and *Methanolobus*, the mixed genus *Methanococcus* and other genera were detected in the water samples. Although the proportion of these methanogens is small, it shows that there are three types of methanogens in the well group of the study area: hydrogen-trophic, acetic acid-trophic, and methyl-trophic. There are many ways to produce methane. Among them, the hydrogen-nutrient *Methanobacterium* is the main genus.

4.1.2 Types of bacteria

The results of bacterial community analysis show that the produced water from 14 CBM wells of four synclines contain a number of methanogens (Table 2, Fig. 3). They mainly belong to three phyla, *Bacteroidetes* (38.07%), *Firmicutes* (33.52%), and *Proteobacteria* (27.93%), followed by the phylum *Spirochaetes*. The *Bacteroidetes* identified mainly includes the class *Bacteroidia.* The *Firmicutes* identified mainly includes the classes *Clostridia* and *Negativicutes*. The *Proteobacteria* identified mainly includes the classes *Gammaproteobacteria, Betaproteobacteria*, *Deltaproteobacteria*, and *Alphaproteobacteria.* The *Spirochaetes* identified mainly

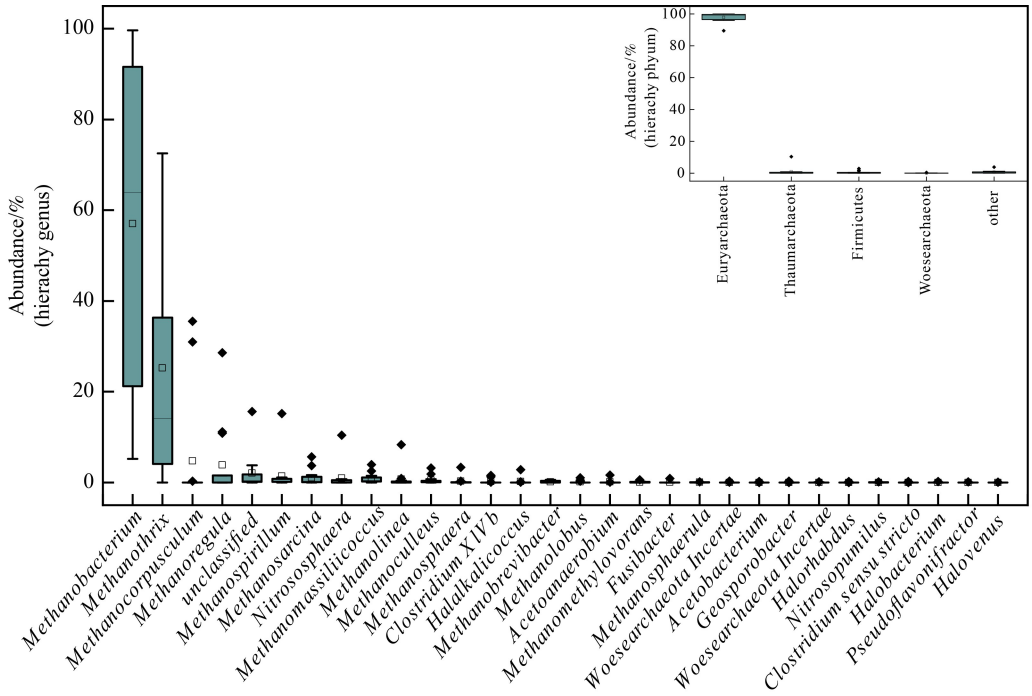

Fig. 2 Abundance of archaea.

includes the class *Spirochaetia.* The bacteria identified mainly belong to the orders *Bacteroidales*, *Clostridiales*, *Enterobacteriales*, *Pseudomonadales*, *Rhodocyclales*, *Vibrionales*, *Aeromonadales*, *Alteromonadales*, *Oceanospirillales*, *Desulfovibrionales*, *Spirochaetales*, and *Rhodobacterales. Bacteroidales* and *Clostridiales* are the dominant orders, accounting for 37.4% and 32.92%, respectively. The water contains more than 30 species of bacteria. *Macellibacteroides* is the dominant genus, accounting for 34.07%, followed by the genus *Citrobacter,* accounting for 7.27%.

The detected bacteria can participate in the microbial degradation of coal seams, thus providing a small molecular carbon source for methanogens. The phylum *Firmicutes* is important for biogenic methane production. The family *Clostridiaceae* contains spore-producing

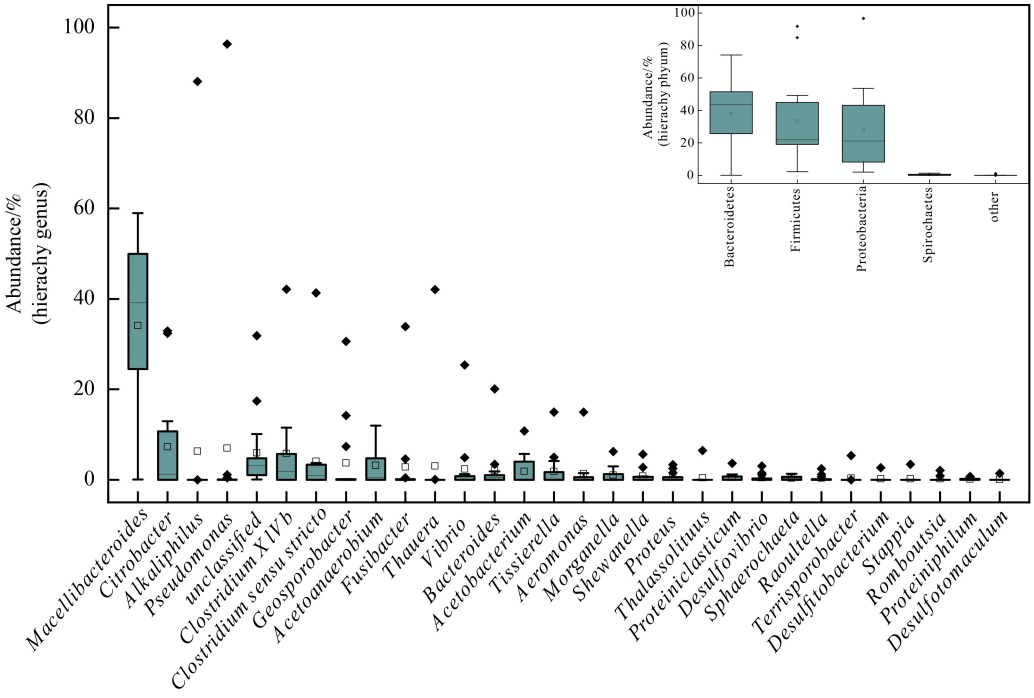

Fig. 3 Abundance of bacteria.

anaerobic bacteria with a wide range of catalytic mechanism (Kusel et al., 2000). The genus *Clostridium* can produce butyric acid, acetic acid, propionic acid, and lactic acid directly from carbohydrates, such as glucose. The genus *Bacillus* contains aerobic or facultative anaerobic bacteria and is only detected in small amounts in GP-7 and LCC3. Bacteria of the phylum *Bacteroidetes* can degrade macromolecular substances such as glue, agar, starch, or cellulose. The genus *Bacteroides* is the most widely distributed and is detected in all samples. The abundance of the genus *Macellibacteroides,* belonging to the phylum *Bacteroidetes,* is high. The genus *Pseudomonas,* belonging to the phylum *Proteobacteria,* is detected in most samples. The genus *Pseudomonas* contains bacteria that can degrade organic hydrocarbons. Some *Pseudomonas* strains can secrete surfactants that accelerate the decomposition and release of organic compounds in coal (Singh et al., 2012). Liu et al. (2019) found that the genus *Pseudomonas* was the dominant bacteria in a study of the microbial community in the Luling coalfield, China.

4.2 Distribution of microbial communities

4.2.1 Distribution characteristics of archaeal communities

Microbial communities have obvious regional distribution characteristics, namely, TC, FE, LC, and EH synclines that have their own characteristics (Fig. 4). The dominant genus in the TC syncline is genus *Methanobacterium*, accounting for 60.46%, followed by the genus *Methanothrix*, accounting for 23.26%, the genus *Methanocorpusculum*, accounting for 5.16%, and the genus *Methanoregula*, accounting for 4.15%.

The dominant genus of the FE syncline is genus *Methanothrix*, accounting for 68.14%, followed by the genus *Methanobacterium*, accounting for 13.20%, the genus *Methanospirillum*, accounting for 7.5%, and the genus *Methanolinea*, accounting for 4.23%.

The dominant genus of the LC syncline is the genus *Methanobacterium*, accounting for 87.69%, followed by the genus *Methanothrix*, accounting for 6.98%.

The dominant genus of the EH syncline is the genus *Methanobacterium*, accounting for 29.08%, followed by the genus *Methanothrix*, accounting for 24.68%, the genus *Methanocorpusculum* accounting for 17.90%, and the genus *Methanoregula* accounting for 14.36%. The dominant genus is not obvious.

Taking a single well as an example (Fig. 5), the composition and relative abundance of the archaea community at the genus level is shown in Fig. 5. The GP-2, GP-3, GP-5, LCC1, LCC2, LCC3, and LCC4 wells contain mainly genus *Methanobacterium*, accounting for more than 70%, among which the LCC4 well is the highest at 99.61%. The GP-1, QC-1, and YC-1 wells are dominated by the genus *Methanothrix*, accounting for more than 60%. Among these, the GP-1 well is the highest at 72.5%. The dominant genus of the GP-7, GP-8, EHC6, and EHC7 wells is not obvious because there are around 3 genera with similar percentage. For the EHC6 well, *Methanocorpusculum* is the highest, accounting for 35.52%.

4.2.2 Distribution characteristics of bacterial communities

From a regional distribution perspective, *Macellicharacters* is the dominant genus in TC, FE, LC, and EH, accounting for 21.02%, 34.44%, 43.07%, and 49.37%, respectively. Except for the genus *Macellicharacters*, the characteristics of the bacterial community in each syncline are complex, and there are more bacterial species whose abundance is more than 1% (Fig. 6).

In the syncline, the characteristics of the bacterial community vary greatly among wells. Taking TC syncline as an example, the bacterial communities of GP-1, GP-7, and GP-8 wells are relatively unique, while those of the other wells are relatively similar. The genus *Macellicharacters* of the GP-1, GP-7, and GP-8 wells are universally less than 1%. The dominant genera of the GP-

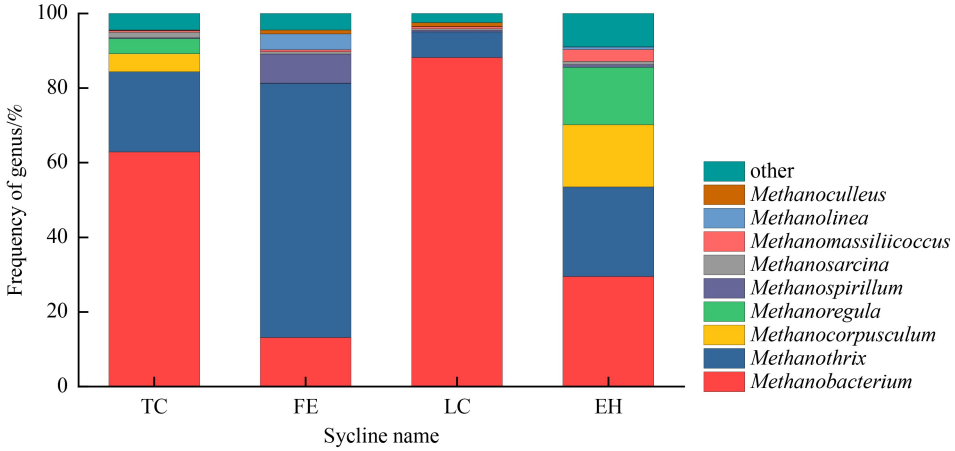

Fig. 4 Distribution barplot of archaea community at the genus level in each syncline.

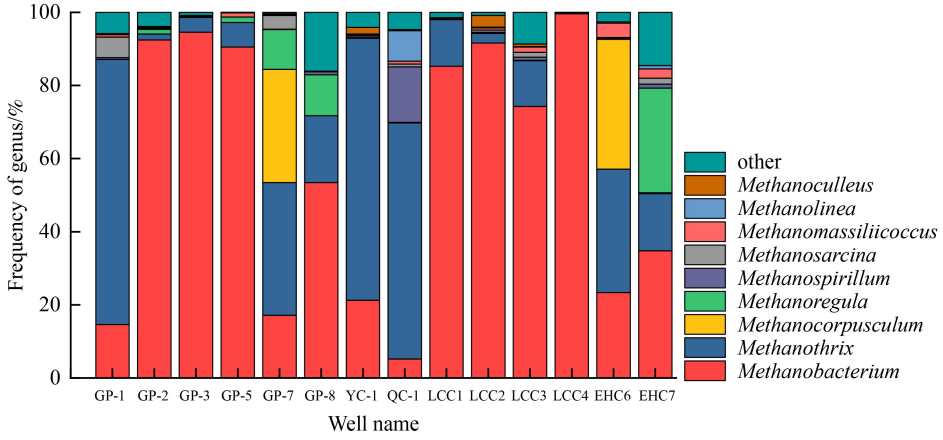

Fig. 5 Composition and relative abundance of the archaea community at the genus level of typical wells in the study area.

1 well are *Clostridium xlvb*, *Clostridium sensu stricto*, and *Aeromonas*, accounting for 42.14%, 41.3%, and 14.96%, respectively. The dominant genus of the GP-7 well is *Pseudomonas*, accounting for 96.38%. The dominant genus of the GP-8 well is *Alkaliphilus,* accounting for 88.09% (Fig. 7).

Macellicharacters are widely detected in most of the produced water of the CBM wells. In addition, the dominant genera of each well are different, but the bacterial community in each syncline is basically similar.

5 Discussion

5.1 Geological control of microbial community distribution

5.1.1 Regional environmental factor control of microbial community distribution

The main geological control factors affecting the microbial community distribution include coal reservoir material, physical properties, and formation water environment. The characteristics of the coal reservoir material mainly refer to the coal-rank reflectivity. The physical properties of the coal reservoir mainly refer to the permeability and reservoir pressure state, which is related to burial depth. The formation water environment mainly refers to the water temperature, salinity, pH, anions, cations, and so on. These are known as generalized environmental factors that affect the distribution of the microbial community.

The number of sequencing reads from the top 10 archaeal and bacterial genera with the highest abundance were used for analysis. Considering the difficulty of obtaining environmental parameters, the following nine parameters were used to explore the impact of environmental factors on the distribution of microbial communities: depth, temperature, $R_{o,max}$, pH, TDS, Cl^-, SO_4^{2-}, HCO_3^-, and $\delta^{13}C_{DIC}$ (Table 1 and Table 2).

The influence of environmental factors on the distribution of the microbial community was analyzed using the redundancy analysis (RDA) method. RDA, based on a linear model, can detect the relations among environmental factors, samples, and microbial communities. The distance between sample points indicates the similarity between samples. The angle between species

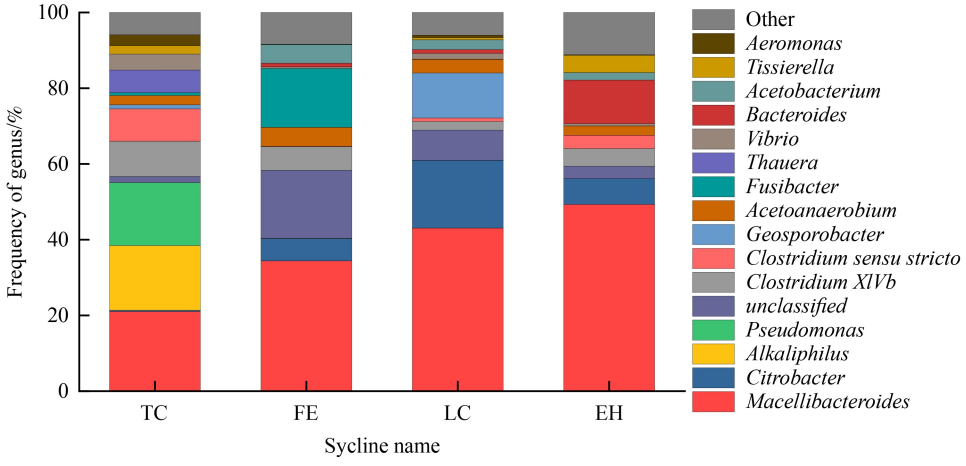

Fig. 6 Distribution bar plot of the bacterial community in each syncline at the genus level.

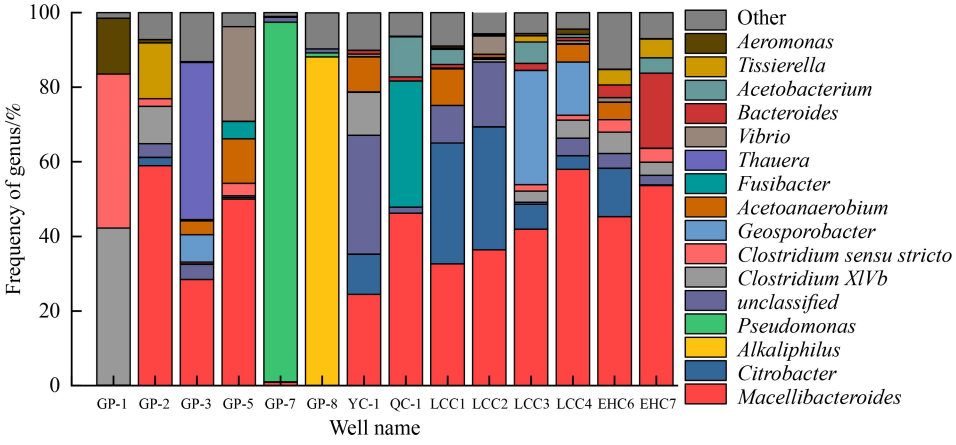

Fig. 7 Composition and relative abundance of the bacterial community of typical wells in the study area at the genus level.

and environmental factors indicates the positive or negative relationship between species and environmental factors (acute angle: positive correlation; obtuse angle: negative correlation; right angle: no correlation). A vertical line is drawn from different sample points to the environmental factor, and the distance between the projection points indicates the similarity of different samples on this environmental factor. The closer the distance is, the more similar the influence of the environmental factor is. There is a positive correlation between the length of the arrow corresponding to the environmental factor and the influence of the environmental factor on the diversity of the microbial community.

The comparison of the two maps shows that the environmental factors have different effects on archaea and bacteria (Fig. 8). In Fig. 8(a), the main environmental factors are depth, $\delta^{13}C_{DIC}$ and $R_{o,max}$, followed by HCO_3^- > temperature > SO_4^{2-} > pH > TDS > Cl^-. As a dominant genus, *Methanobacterium* has a strong positive correlation with temperature, followed by TDS, HCO3$^-$, and Cl^-. Some genera belonging to *Methanobacterium* are more likely to be present in water with high temperature and are often found in hot springs (Wasserfallen et al., 2000) (Table 5). The abundance of this genus is the highest in the LC and TC synclines, which is related to the higher formation temperature of these two synclines. The formation temperatures of the TC and LC synclines are highly abnormal, and the geothermal gradient is greater than 3°C/100 m in some regions. The formation temperatures of the FE and EH synclines are relatively low, especially for the latter. The formation temperature is only 25°C at a depth of 1182 m, indicating a low anomaly. This explains why in the RDA figure, GP-2, GP-3, GP-5, and LCC4 are located in the positive direction of the arrow of the genus *Methanobacterium*. The genera *Methanoregula* and *Methanocorpusulum* have a strong positive correlation with depth and pH and a negative correlation with $R_{o,max}$. The abundance of the genera *Methanoregula* and *Methanocorpusculum* is higher at deep depth and in medium rank coal seams, and the arrows corresponding to GP-7, GP-8, EHC6, and EHC7 are closest in the RDA figure. There is a strong positive correlation between the genus *Methanoculleus* and SO_4^{2-}, exhibited in well LCC2. In addition, the angle between the genus *Methanobacterium* and other archaea is obtuse because the genus *Methanobacterium* is one of the most widely distributed archaea in most samples, and its abundance is inversely proportional to that of other methanogens. This is also the main reason why the genus *Methanobacterium* has a positive correlation with most of the environmental factors, while most of the other methanogens have a negative correlation with the environmental factors.

As shown in Fig. 8(b), the main environmental factors are Cl^-, TDS, HCO_3^-, followed by pH > $R_{o,max}$ > SO_4^{2-} > temperature > depth > $\delta^{13}C_{DIC}$. The factors pH and HCO_3^{2-} are the main environmental factors that affect the dominant genera *Macrocharacters*, *Acetoaerobium*, *Citrobacter*, and *Geosporobacter*, which are positively correlated. The genera *Macrocharacters*, *Acetoaerobium*, *Citrobacter*, and *Geosporobacter* are abundant in most CBM wells of LC and EH synclines, while HCO_3^- and pH are higher in CBM wells of LC and EH synclines, such as LCC2, LCC4, and EHC6. The genus *Pseudomonas* is positively correlated with Cl^- and TDS, and the genus *Alkaliphilus* is also closely related to these two environmental factors as well as temperature; the typical wells are GP-7 and GP-8. The correlation between other bacteria and environmental factors is poor.

The main influencing factors of archaea are coalbed properties, such as burial depth and $R_{o,max}$, while the influencing factors of bacteria are mainly the physical and chemical properties of groundwater, including Cl^-, TDS, and HCO_3^-. These findings indicate that the distribution of the archaeal community is more stable, and the distribution of the bacterial community is more sensitive to changes in the water environment. To further distinguish the differences between samples, we used nonmetric multidimensional scaling (NMDS) for analysis

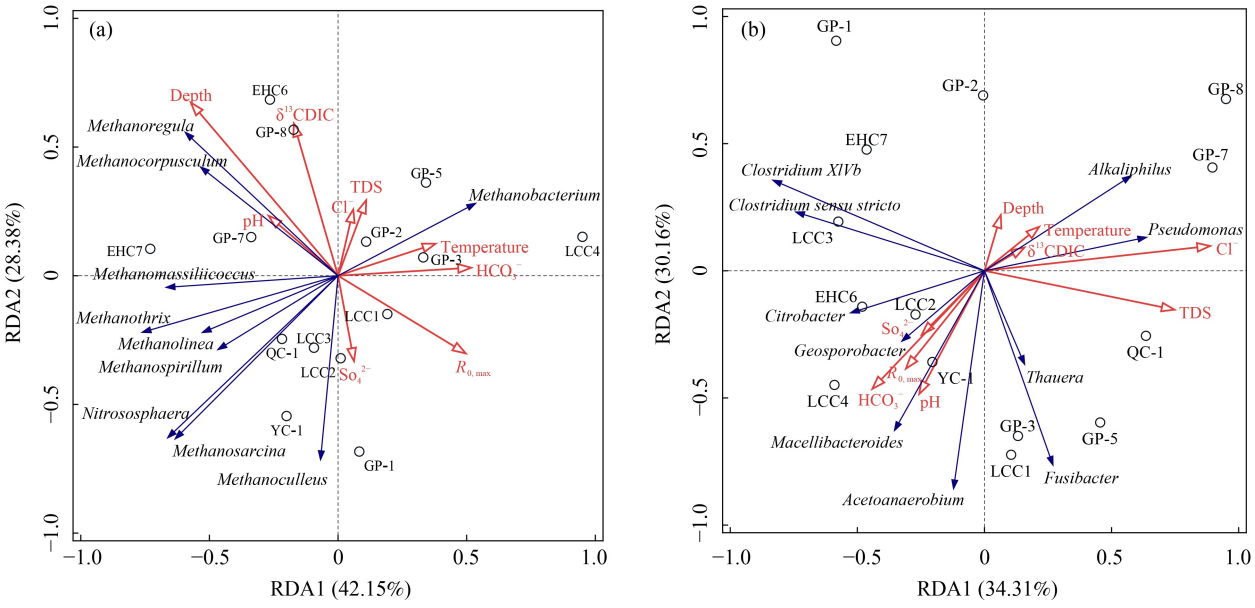

Fig. 8 RDA of samples ((a) archaea; (b) bacteria).

Table 5 Suitable environment for the main methanogen genera

Genus name	Suitable environment	Type of methanogens			
		HM	AM	MM	Other
Methanobacterium	In some hot, low-oxygen environments, such as anaerobic digestors, their waste water, and hot springs (Wasserfallen et al., 2000).	√		√	
Methanothrix	The temperature optimum for growth and methane formation is 37°C and the optimal pH range is 7.4–7.8 (Huser et al., 1984). Strictly anaerobic.		√		
Methanocorpusculum	They grow fastest at temperatures of 30–40°C, pH is approximately 7 (Zellner et al., 1989).	√			
Methanoregula	Optimal growth conditions were near pH 5.1 and 35°C (Bräuer et al., 2011).	√			
Methanospirillum	They are strictly anaerobic.	√			
Methanosarcina	The optimal growth temperature was 35°C to 40°C. The optimal pH range was 6.5 to 7.0. Both NaCl and Mg^{2+} were required for growth, with maximum growth rates at 0.2 M NaCl and 0.05 M $MgSO_4$ (Sowers et al., 1984).	√	√	√	
Methanomassiliicoccus	Grew optimally with 0.5%–1.0% (w/v) NaCl at pH 7.6 and at 37°C. It requires tungstate-selenite for growth (Dridi et al., 2012).			√	
Methanolinea	Growth was observed between 35°C and 55°C (optimum 50°C) and pH 6.7 and 8.0 (optimum pH 7.0) (Imachi et al., 2008).				formate
Methanoculleus	The optimum temperature and pH were 25°C–50°C and 6.7–7.8 (Dianou,et al., 2001; Cheng et al., 2008).	√			ethanol, secondary alcohols

Notes: hydrogenotrophic methanogens (HM), acetoclastic methanogens (AM), methylotrophic methanogens (MM).

(Fig. 9). To some extent, archaea demonstrate regional differences, but the differences between samples are not significant. In addition to the regional characteristics of bacteria, there are huge differences between samples, especially in the GP well group, and these differences are mainly due to the sensitivity of bacteria to the water environment.

5.1.2 Section control of layers in the vertical direction

There are several CBM wells with multi-coal seams in the study area (Table 1). Each well has different production layers, which may lead to the differences in the microbial community of the produced water. The reason for the difference is that there are multiple superimposed CBM-bearing systems in the vertical direction of the coal measure strata (Qin et al., 2018). The material composition and reservoir physical properties of each fluid system may be different, leading to the differences in the living environment of microorganisms. We focus on the GP well group in the TC syncline, and the upper, middle and lower members of the Longtan Formation in this area corresponding to three superimposed CBM-bearing systems (Qin et al., 2018; Yang et al., 2019b, 2020)

According to the principle of liquid level constraint, the existence of microbial reduction in the upper and middle member of the Longtan Formation (Yang et al., 2019b), production layers of the GP well group were further

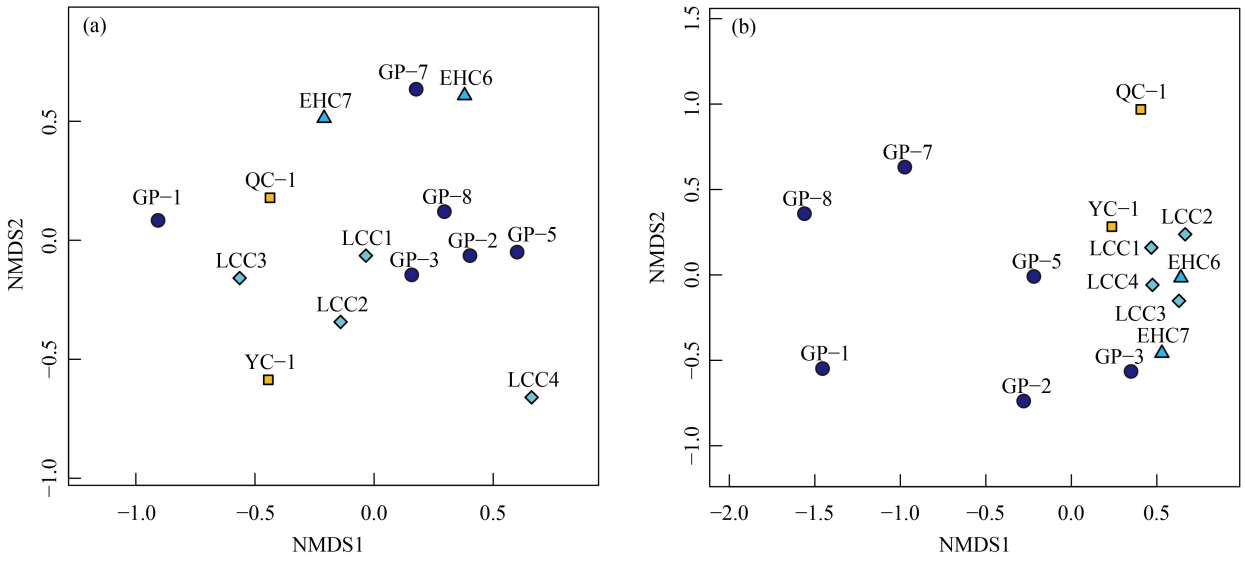

Fig. 9 NMDS results of samples ((a) archaea; (b) bacteria).

identified (Table 6). The production layers in well GP-1 were No. 1 + 3 coal seams, and those in well GP-2 were No. 13, 15, and 16 coal seams. GP-5 was more unique; its previous liquid level was in the No. 13 coal seam, and the production layers in GP-5 were the Nos. 13, 15, and 29 coal seams. Later, due to the second fracturing of GP-1, the production layers in well GP-1 were the No. 1 + 3 coal seams. GP-5 was adjacent to GP-1, which was vulnerable to indirect enhanced modification in the upward direction. As a result, the liquid level of GP-5 increased up to the Nos. 1 + 3 coal seam, which stopped CBM production of the No. 29 coal seams. At present, the production layers in well GP-5 were the Nos. 13 and 15 coal seams (Yang et al., 2020). Those in well GP-7 were the Nos. 27 and 19 coal seams, and those in well GP-8 were the Nos. 13, 15, 26, 27, and 29 coal seams.

Table 6 Developed horizon, liquid level position and main coal seams contributing to the production of all the wells

Coal seam segment	Coal seam number	Development coal seam and liquid level of well					
		GP-1	GP-2	GP-3	GP-5	GP-7	GP-8
P_3l^3	1 + 3	♂♂♂					
	4						
	5						
	6						
	9						
	10						
P_3l^2	11						
	12						
	13		♂♂♂		♂♂♂		♂♂♂
	15		♂♂♂	♂♂♂	♂♂♂		♂♂♂
	16		♂♂♂				
P_3l^1	26						♂♂♂
	27					♂♂♂	♂♂♂
	29			♂♂♂		♂♂♂	♂♂♂

Notes: Development coal seam; Dynamic liquid level; ♂♂♂ Gas symbol

Based on the above result, the single layer from the upper member of GP-1 made a higher CBM and water contribution, the 2−3 layers from the middle members of GP-2 and GP-5 made a higher CBM and water contribution, the two layers from the lower member of GP-7 made a higher CBM and water contribution, and the layers from the middle and lower members of GP-3 and GP-8 made a higher CBM and water contribution.

Principal component analysis (PCA) was used to analyze the differences in microorganisms in the GP well group. This method can reduce the dimensionality of data and reveal the simpler structure hidden behind the data. The distance between sample points in the graph indicates the similarity between samples. The archaeal community of the GP well group was analyzed (Fig. 10), and the archaeal PCA results showed that GP-1 and GP-7 were more independent than other wells. GP-2, GP-3, GP-5, and GP-8 were closer and more similar, especially GP-2, GP-3, and GP-5. This characteristic corresponds to the difference in the production layers of the GP well group and further reflects the difference in the microbial community in the production water of the upper, middle, and lower members of the GP well group. Taking GP-1, GP-2, and GP-7 as the level wells, it can be initially considered that the genus *Methanobacterium* was mainly developed in the middle member of the Longtan Formation, the genus *Methanothrix* was mainly developed in the upper member of the Longtan Formation, and the genera *Methanothrix* and *Methanocorpusculum* were mainly developed in the lower member of the Longtan Formation.

The bacterial PCA (Fig. 11) results of the GP well group showed that compared with archaea, the bacterial community had similar weak characteristics. GP-1, GP-7, and GP-8 were relatively independent compared with other wells. GP-1 and GP-7 were affected by the development layer, while GP-8 was located at the bottom of the well group, with a large buried depth in the wells group and a high TDS value. The difference in the groundwater chemical field will affect the distribution of the bacterial community. GP-2, GP-3, and GP-5 were closer and more similar. The above characteristic is consistent with the difference in the production layers of the GP well group. In general, stratigraphic segmentation has a certain impact on the distribution of the bacterial community. Taking GP-1, GP-2, and GP-7 as the level wells, it can be considered that the genus *Macellibacteroides* is mainly developed in the middle member of the Longtan Formation, the genera *Clostridium XlVB* and *Clostridium sense stricto* are mainly developed in the lower member of the Longtan Formation, and the genus *Pseudomonas* is mainly developed in the lower member of the Longtan Formation.

5.2 Indicative significance of biogas

The $\delta^{13}C$ and δD of CBM can be used to identify types of methane. According to Whiticar's template (Whiticar and Faber, 1986), the gas production causes have been identified in some wells of the TC, FE, EH, and LC synclines (Fig. 12). Most types of the gas in the study area are thermogenic gas. Gas produced from some wells in EH syncline falls in the range of mixed gas. Gas produced from wells in TC syncline is close to the range of mixed gas. Therefore, the produced gas in the study area is mainly thermogenic gas, but some synclines have obviously secondary biogas. Secondary biogas is mainly distributed in TC and EH synclines. A positive value of $\delta^{13}C_{DIC}$ is considered to be an important indicator of microbial reduction (Yang et al., 2019b, 2020). The value of $\delta^{13}C_{DIC}$ in some produced water of TC and EH synclines is far more than 10% (Fig. 13), which further implies that there is obviously secondary biogas in these two synclines.

Combined with this premise that many types of archaea and bacteria are successfully detected in the four synclines, especially the methanogens playing a key role,

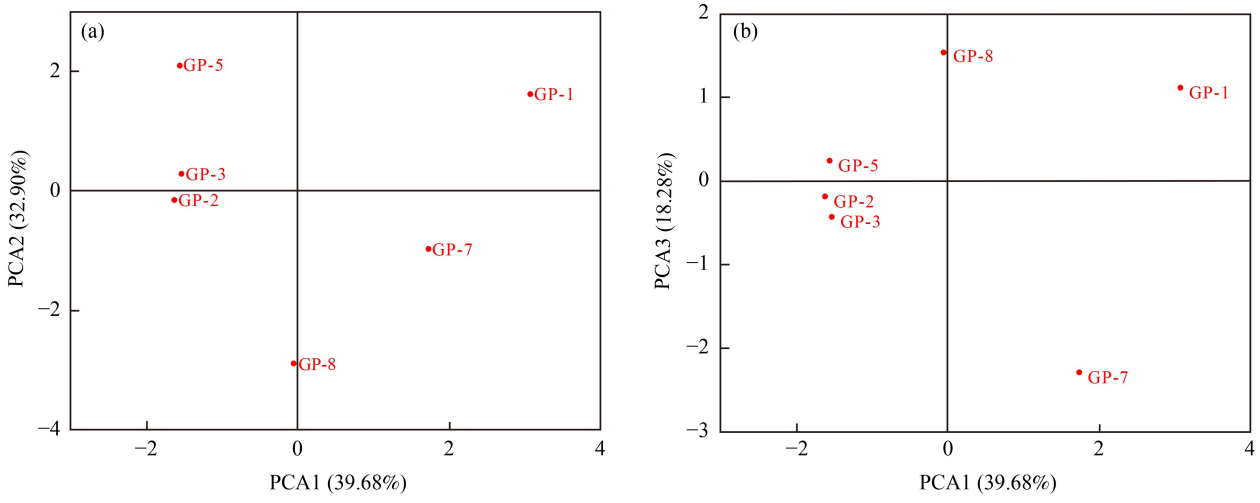

Fig. 10 PCA of archaea.

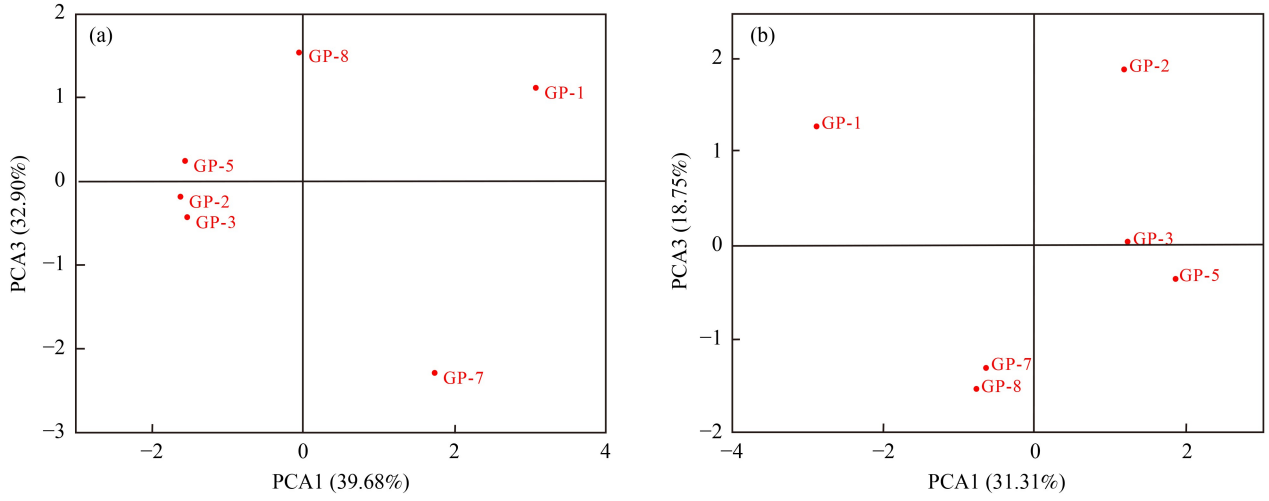

Fig. 11 PCA of bacteria.

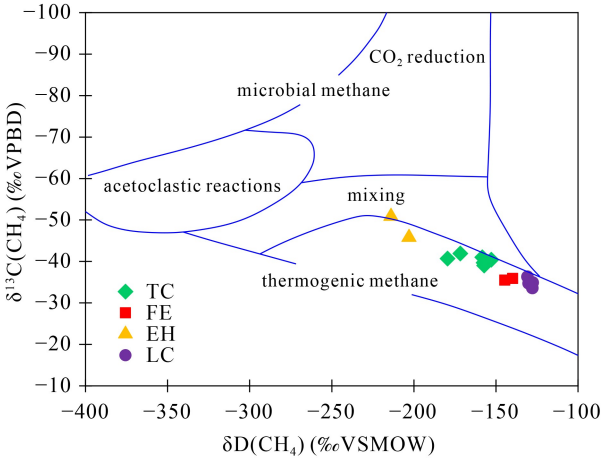

Fig. 12 Methane $\delta^{13}C$ versus δD for CBM.

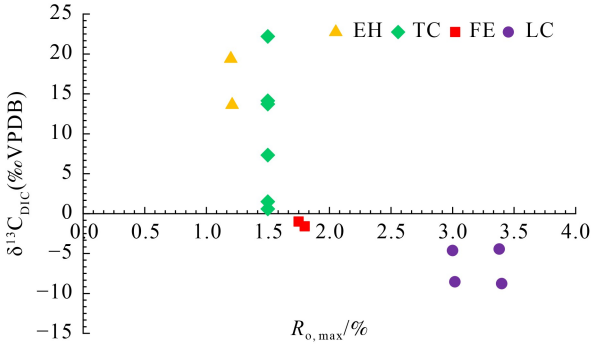

Fig. 13 $R_{o,max}$ verus $\delta^{13}C_{DIC}$ of water from CBM wells.

it can be concluded that the four synclines have the conditions to produce secondary biogas, but differences in microbial reduction are caused by the differences in the coal and rock materials and the stratigraphic environment in each syncline. Where microbial reduction is strong, there are obvious secondary biogases such as in the TC and EH synclines. Where microbial reduction is weak, there are weak secondary biogases in the LC and FE synclines. The coal rank is an important factor.

The correlations between the sequence reads numbers of the dominant genera, *Methanobacterium* and *Methanothrix*, and the average $\delta^{13}C_{DIC}$ are also analyzed (Fig. 14). The former is a hydrogen-trophic microorganism and the latter is an acetic acid-trophic microorganism. The results show that there is generally a positive correlation between these sequence reads numbers of dominant genus *Methanobacterium* and $\delta^{13}C_{DIC}$, while the sequence reads numbers of genus *Methanothrix* and $\delta^{13}C_{DIC}$ are generally negatively correlated, or not significantly correlated, except for the FE syncline. This shows that the reduction of hydrogen-trophic methanogens is an important way to produce secondary biogas in the study area, which is consistent with the conclusion that most biogas is produced by hydrogen-trophic methanogens reduced by carbon dioxide (Kirk et al., 2012; Singh et al., 2012). Meanwhile, the dominant genus, *Methanobacterium*, plays a major role in the formation of secondary biogas by microbial reduction of medium and high ranks of coal in the study area.

5.3 Indicative significance of MECBM

MECBM is a technology that stimulates the growth of native bacteria by injecting foreign microorganisms or nutrient solutions into the coal seam, thereby enhancing the degradative effect and metabolism of methane bacteria on the coal seam, increasing the biogas production (Park and Liang, 2016). Currently, Luca Technologies, Inc., Ciris Energy and Next Fuel, Inc. have conducted research and field experiments involving this technology (Ritter et al., 2015). In the Antelope Valley block of the Powder Basin in the US, the methanogen culture solution was injected into the coal seams of 4 wells, and after 3 months, 13 surrounding CBM wells were re-produced. After 4 months, the methane production of CBM increased rapidly by 2 to 5 times compared

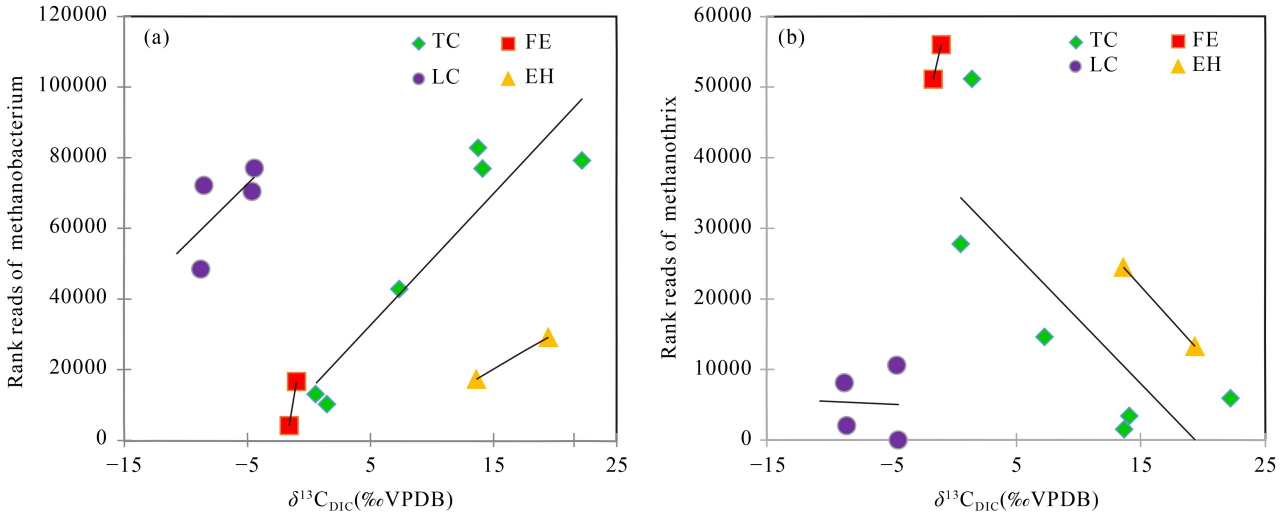

Fig. 14 Sequence reads number of dominant genus versus $\delta^{13}C_{DIC}$.

with that before injection. Recently, Australia has also conducted MECBM field trials (Vick et al., 2018). Some Chinese scholars also conducted a preliminary theoretical and experimental study in the Inner Mongolia low-ranking Erlian Basin (Sun et al., 2019).

In western Guizhou and eastern Yunnan, there are many coal seams and large variation in coal ranks, ranging from low to high ranks. However, the coal seam permeability is low. With conventional straight well fracturing, gas production is generally low (Yang et al., 2019b). Large-scale commercial development has not yet been achieved. From this study, it can be seen that there are a large number of multi-type methanogens in the four representative synclines. From the hydrogen-based dominant species of genus *Methanobacterium* to the acetic acid-type genus *Methanothrix* and the mixed genus *Methanosarcina*, there exists a distribution. This provides a precondition for the development of MECBM technology by injecting nutrient solution to stimulate the growth of native bacteria. According to the analysis of the carbon and hydrogen isotopes of methane and $\delta^{13}C_{DIC}$, it is revealed that the TC and EH synclines have strong microbial action. The material composition of the coal seam and stratum environment are relatively suitable for microbial survival. The coal is fertilizer and coking coal, and the formation temperature is between 20°C and 50°C. The formation water with a pH value of 7−8 has a low TDS of less than 7000 mg/L, low salinity, low sulfate concentration and weak runoff. Therefore, it is suitable and beneficial to select favorable sections, carry out MECBM research, and extend CBM stimulation in the two synclines in the future.

6 Conclusions

1) There are more than 30 kinds of bacteria and 15 kinds of archaea in the produced water of 14 coalbed methane wells in 4 synclines. The bacteria mainly included *Bacteroidetes* and *Clostridiales*, among which *Macellibacteroides* was the dominant genus, accounting for 34.07%. The archaea mainly included *Methanobacteriales* and *Methanosarcinales*, of which *Methanobacteria* was the dominant genus, accounting for 57.01%. There are including three kinds of methanogens: hydrogenotrophic, anaerobic, and methylotrophic methanogens.

2) The distribution characteristics of the microbial communities are mainly influenced by coal and rock composition, reservoir physical properties, and formation water environment, and have differentiation in vertical direction. The main influencing factors of archaea are coalbed properties such as buried depth and $R_{o,max}$, while the influencing factors of bacteria are mainly the physical and chemical properties of groundwater, including Cl^-, TDS, and HCO_3^-.

3) The microbial reduction in the TC and EH synclines is strong, and there is obvious evidence of secondary biogases; while the microbial reduction in the LC and FE synclines is weak, and there are weak levels of secondary biogases. Coal rank is an important factor affecting the strength of the secondary biogases. There is a significant positive correlation between the high-quality sequence numbers of the hydrotrophicgenus *Methanobacterium* and $\delta^{13}C_{DIC}$ in the study area, which indicates that the reduction by hydrogen-trophic methane bacteria is an important mechanism for the production of secondary biogases in the study area. The TC and EH synclines are suitable for the selection of favorable sections to carry out microbially MECBM research later.

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Competing interests The authors declare that they have no competing interests.

References

Atekwana E A, Krishnamurthy R V (1998). Seasonal variations of dissolved inorganic carbon and $\delta^{13}C$ of surface waters: application of a modified gas evolution technique. J Hydrol (Amst), 205(3−4): 265–278

Barnhart E P, Weeks E P, Jones E J P, Ritter D J, McIntosh J C, Clark A C, Ruppert L F, Cunningham A B, Vinson D S, Orem W, Fields M W (2016). Hydrogeochemistry and coal-associated bacterial populations from a methanogenic coal bed. Int J Coal Geol, 162: 14–26

Bräuer S L, Cadillo-Quiroz H, Ward R J, Yavitt J B, Zinder S H (2011). *Methanoregula boonei* gen. nov, sp. nov, an acidiphilic methanogen isolated from an acidic peat bog. Int J Syst Evol Microbiol, 61: 45–52

Cheng L, Qiu T L, Li X, Wang W D, Deng Y, Yin X B, Zhang H (2008). Isolation and characterization of *Methanoculleus receptaculi* sp. nov. from Shengli oil field, China. FEMS Microbiol Lett, 285(1): 65–71

Dianou D, Miyaki T, Asakawa S, Morii H, Nagaoka K, Oyaizu H, Matsumoto S (2001). *Methanoculleus chikugoensis* sp. nov, a novel methanogenic archaeon isolated from paddy field soil in Japan, and DNA-DNA hybridization among Methanoculleus species. Int J Syst Evol Microbiol, 51: 1663–1669

Dridi B, Fardeau M L, Ollivier D B, Raoult D, Drancourt M (2012). *Methanomassiliicoccus luminyensis* gen. nov, sp. nov, a methanogenic archaeon isolated from human faeces. Int J Syst Evol Microbiol, 62: 1902–1907

Fry J C, Horsfield B, Sykes R, Cragg B A, Heywood C, Kim G T, Mangelsdorf K, Mildenhall D C, Rinna J, Vieth A, Zink K, Sass H, Weightman A J, Parkes R J (2009). Prokaryotic populations and activities in an interbedded coal deposit, including a previously deeply buried section (1.6–2.3 km) above ~150 Ma basement rock. Geomicrobiol J, 26: 163–178

Guo H G, Liu R Y, Yu Z S, Zhang H X, Yun J L, Li Y M, Liu X, Pan J G (2012). Pyrosequencing reveals the dominance of *Methylotrophic methanogenesis* in a coal bed methane reservoir associated with eastern Ordos Basin in China. Int J Coal Geol, 93: 56–61

Huser B A, Wuhrmann K, Zehnder A J B (1984). *Methanotrix soehngenii* gen. nov. sp. nov, a new acetotrophic non-hydrogen-oxidizing methan bacterium. Arch Microbiol, 132: 1–9

Imachi H, Sakai S, Sekiguchi Y, Hanada S, Kamagata Y, Ohashi A, Harada H (2008). *Methanolinea tarda* gen. nov, sp. nov, a methane-producing archaeon isolated from a methanogenic digester sludge. Int J Syst Evol Microbiol, 58: 294–301

Kirk M F, Martini A M, Breecker D O, Colman D R, Takacs-Vesbach C, Petsch S T (2012). Impact of commercial natural gas production on geochemistry and microbiology in a shale-gas reservoir. Chem Geol, 332–333: 15–25

Klein D A, Flores R M, Venot C, Gabbert K, Schmidt R, Stricker G D, Stricker G D, Pruden A, Mandernack K (2008). Molecular sequences derived from Paleocene Fort Union Formation coals vs. associated produced waters: implications for CBM regeneration. Int J Coal Geol, 76: 3–13

Kusel K, Dorsch T, Acker G, Stackebrandt E, Drake H L (2000). *Clostridium scatologenes* strain SL1 isolated as an acetogenic bacterium from acidic sediments. Int J Syst Evol Microbiol, 50(2): 537–546

Li D M, Hendry P, Faiz M (2008). A survey of the microbial populations in some Australian coalbed methane reservoirs. Int J Coal Geol, 76: 14–24

Li Y, Pan S Q, Ning S Z, Shao L Y, Jing Z H, Wang Z S (2022). Coal measure metallogeny: metallogenic system and implication for resource and environment. Sci China Earth Sci, 65(7): 1211–1228

Li Y, Tang D Z, Fang Y, Xu H, Meng Y J (2014). Distribution of stable carbon isotope in coalbed methane from the east margin of Ordos Basin. Sci China Earth Sci, 57(8): 1741–1748

Liu Y F, Wang B B, Zhang H X, Yu Z S, Zhang H (2019). Study on the mMicrobial community and the type of methanogenesis associated with biogenic gas in Luling Coalfield, China. Acta Microbiol Sin, 59(06): 1174–1187

Lu B J, Xia D P, Zhao S P, Yang Z B, Chen Z H, Huang S, Li S S, Zhang Z G (2022). The influence mechanism of ethylenediaminetetraacetic acid (EDTA) and ferrous iron on the bioavailability of Fe in the process of low rank coal fermentation. Biochem Eng J, 185: 108520

Midgley D J, Hendry P, Pinetown K L, Fuentes D, Gong S, Mitchell D L, Faiz M (2010). Characterisation of amicrobial community associated with a deep, coal seam methane reservoir in the Gippsland Basin, Australia. Int J Coal Geol, 82(3/4): 232–239

Papendick S L, Downs K R, Vo K D, Hamilton S K, Dawson G K W, Golding S D, Gilcrease P C (2011). Biogenic methane potential for Surat Basin, Queensland coal seams. Int J Coal Geol, 88: 123–134

Park S Y, Liang Y N (2016). Biogenic methane production from coal: a review on recent research and development on microbially enhanced coalbed methane (MECBM). Fuel, 166: 258–267

Penner T J, Foght J M, Budwill K (2010). Microbial diversity of western Canadian subsurface coal beds and methanogenic coal enrichment cultures. Int J Coal Geol, 82: 81–93

Qin Y, Moore T A, Shen J, Yang Z B, Shen Y L, Wang G (2018). Resources and geology of coalbed methane in China: a review. Int Geol Rev, 60(5−6): 777–812

Ritter D, Vinson D, Barnhart E, Akob D M, Fields M W, Cunningham A B, Orem W, Mclntosh J C (2015). Enhanced microbial coalbed methane generation: a review of research, commercial activity, and remaining challenges. Int J Coal Geol, 146: 28–41

Shi W, Tang S H, Zhang S H (2023). Microbiome of high-rank coal reservoirs in the high-production areas of the southern Qinshui Basin. Microorganisms, 11: 497

Shimizu S, Akiyama M, Naganuma T, Fujioka M, Nako M, Ishijim Y (2007). Molecular characterization of microbial communities in deep coal seam groundwater of northern Japan. Geobiology, 5: 423–433

Singh D N, Kumar A, Sarbhai M P, Tripathi A K (2012). Cultivation-independent analysis of archaeal and bacterial communities of the formation water in an Indian coal bed toenhance biotransformation

of coal into methane. Appl Microbiol Biotechnol, 93(3): 1337–1350

Sowers K R, Baron S F, Ferry J G (1984). *Methanosarcina acetivorans* sp. nov, an acetotrophic methane-producing bacterium isolated from marine sediments. Appl Environ Microbiol, 47(5): 971–978

Stackebrandt E, Goebel B M (1994). Taxonomic note a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Evol Microbiol, 44(4): 846–849

Strapoc D, Mastalerz M, Dawson K, Macalady J, Callaghan A V, Wawrik B, Turich C, Ashby M (2011). Biogeochemistry of microbial coal-bed methane. Annu Rev Earth Planet Sci, 39: 617–656

Strapoc D, Picardal F W, Turich C, Schaperdoth I, Macalady J L, Lipp J S, Lin Y S, Ertefai T F, Schubotz F, Hinrichs K U, Mastalerz M, Schimmelmann A (2008). Methane-producing microbial community in a coal bed of the Illinois Basin. Appl Environ Microbiol, 74: 2424–2432

Strategy Research Center of Oil and Gas Resources Department (2006). The Ministry of Land and Resources. Assessment of Coalbed. Beijing: China Land Press

Sun B, Li J S, Cheng L, Yang Q, Tian W G, Li X, Chen H, Qi L (2019). The feasibility of biological gas recovery in low-rank coal: a case study of Jiergalangtu depression in Erlian Basin. Acta Petrol Sin, 39(11): 1272–1278

Tian W G, Yang Z B, Qin Z H, Qin Y, Li C L, Lu B J, Li Y C (2023). Characteristics of microbial communities in water from CBM wells and biogas production potential in eastern Yunnan and western Guizhou, China. Front Earth Sci, 17: 180–196

Vick S H W, Greenfield P, Tran-Dinh N, Tetu S G, Midgley D J, Paulsen I T (2018). The coal seam microbiome (CSMB) reference set, a lingua franca for the microbial coal-to-methane community. Int J Coal Geol, 186: 41–50

Wang Z W, Qin Y, Li T, Zhang X Y (2021). A numerical investigation of gas flow behavior in two-layered coal seams considering interlayer interference and heterogeneity. Int J Min Sci Technol, 31: 699–716

Wasserfallen A, Nölling J, Pfister P, Reeve J, Conway de Macario E (2000). Phylogenetic analysis of 18 thermophilic Methanobacterium isolates supports the proposals to create a new genus, Methanothermobacter gen. nov, and to reclassify several isolates in three species, *Methanothermobacter thermautotrophicu*s comb. nov, *Methanothermobacter wolfeii* comb. nov, and *Methanothermobacter marburgensis* sp. nov. Int J Syst Evol Microbiol, 50(1): 43–53

Whiticar M J, Faber E (1986). Methane oxidation in sediment and water column environments-isotope evidence. Org Geochem, 10: 759–768

Wu C C, Yang Z B, Qin Y, Chen J, Zhang Z G, Li Y Y (2018). Characteristics of hydrogen and oxygen isotopes in produced water and productivity response of coalbed methane wells in Western Guizhou. Energy Fuels, 32(11): 11203–11211

Yang Z B, Li Y Y, Qin Y, Sun H S, Zhang P, Zhang Z G, Wu C C, Li C L, Chen C X (2019a). Development unit division and favorable area evaluation for joint mining coalbed methane. Pet Explor Dev, 46(3): 1–10

Yang Z B, Qin Y, Qin Z H, Yi T S, Li C L, Zhang Z G (2020). Characteristics of dissolved inorganic carbon in produced water from coalbed methane wells and its geological significance dissolved inorganic carbon in water produced from coalbed methane wells and its geological significance. Pet Explor Dev, 47(4): 1–9

Yang Z B, Qin Y, Wu C C, Qin Z H, Li G, Li C L (2019b). Geochemical response of produced water in the CBM well group with multiple coal seams and its geological significance-A case study of Songhe well group in western Guizhou. Int J Coal Geol, 207: 3–51

Yang Z B, Qin Y, Yi T S, Tang J, Zhang Z G, Wu C C (2019c). Analysis of multi-coalbed CBM development methods in western Guizhou, China. Geosci J, 23(2): 315–325

Zellner G, Stackebrandt E, Messner P, Tindall B J, Conway de Macario E C D, Kneifel H, Sletr U B, Winter J (1989). *Methanocorpusculaceae* fam. Nov. , represented by *Methanocorpusculum parvum*, *Methanocorpusculum sinense* spec. nov. and *Methanocorpusculum bavaricum* spec. nov. Arch Microbiol, 151: 381–390

Zhang J, Liang Y N, Pandey R, Harpalani S (2015). Characterizing microbial communities dedicated for conversion of coal to methane *in situ* and *ex situ*. Int J Coal Geol, 146: 145–154