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Characteristics of Microbial Communities in Deep Geothermal Water and their geothermal Significance

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ABSTRACT

Understanding the characteristics of microbial communities is quite important for the study of deep geothermal water microbiology. However, the characteristics of microbial communities in deep carbonate karst-fissure geothermal storage environment remain unclear. In order to explore the abundance, composition and function of microbial communities in the deep geothermal water from the deep carbonate rock karst-fissure geothermal storage environment, this study, taking shallow water and soil as the references, collected deep geothermal water samples by the pumping tests using the geothermal scientific drilling holes located in Jizhong geothermal area, China and also collected shallow water samples from farm irrigation wells and soil samples near the top of each drill hole, respectively for droplet digital PCR (ddPCR) assay, 16S rRNA gene high-throughput sequencing and functional predictions using PICRUSt software. The results show that deep geothermal water was rich in sulfate-reducing bacteria compared with shallow water and soil with an average of 4.0×10^3copies/mL$\pm8.4\times10^3$copies/mL, $1.6\times10^2\pm3.5\times10^2$ copies/mL and $1.5\times10^3\pm1.2\times10^3$copies/g dw, respectively. There exist 38 phyla and 541 genera in the deep geothermal water of Jizhong geothermal area, of which bacteria are dominant (97.5%), whereas archaea are rare (2.5%), and characteristic microbial communities comprise mainly Firmicutes, Nitrospirae, Thermotogae and Euryarchaeota. The dominant bacterial genera include sulfate-reducing bacteria. These microbial communities in the deep geothermal water have relatively strong carbon fixation, fermentation and sulfate reduction, and weak methanogenesis and denitrification. Aerobic, mesophilic bacteria occurring in the deep geothermal water indicate that the deep geothermal water is recharged by the shallow water rich in proteobacteria under the condition of large flow pumping. This study has revealed that the deep carbonate rock karst-fracture geothermal reservoirs contain abundant microbial communities with various functions in Jizhong geothermal area, China. These microbial communities have great significance for the formation of chemical compositions of deep geothermal water and controlling corrosion and scaling during the development and utilization of geothermal energy.

1. INTRODUCTION

Microorganisms are widely distributed in the shallow environment and also exist in the deep underground, forming a deep biosphere. Its upper boundary is the bottom of the shallow underground water flow system (lovley and Chapelle, 1995) where water actively alternates and is closely related to the surface hydraulics. Its lower boundary can extend to several kilometers underground until the depth of the temperature limit that microorganisms can tolerate. At present, it is known that the maximum temperature that microorganisms can tolerate is 121 ℃ (KASHE fi and lovley, 2003). It is estimated that the lower limit of the deep biosphere can reach 5-10km deep underground (gold, 1992). The deep underground is a dark environment without sunlight and photosynthesis. 1km below the surface is often a reducing environment with high temperature, high pressure and high salt. Microorganisms that can adapt to this extreme environment have unique composition and functions. This extreme environment is similar to the environment of the early Earth and other planets. Exploring deep microbes helps to reveal the origin of early life on the earth and the movement law of deep life underground on other planets. The unique functions of deep microbes can be widely used in environmental remediation, improving oil recovery, biotechnology and bioenergy utilization (Dong Hailiang et al., 2009). Therefore, the study of deep microorganisms has become the frontier and hotspot in the field of Geoscience.

The characteristics of microbial communities in the deep underground are controlled by geological and hydrogeological conditions. In igneous areas, there are few microbial species in groundwater, mainly methanogenic archaea with hydrogen nutrition (Chapelle et al., 2002); In the metabasalt area, the microbial diversity in groundwater is very low, mainly a single type of thermophilic sulfate reducing bacteria belonging to Firmicutes (Lin et al., 2006); In the sandstone aquifer, the groundwater is rich in microorganisms, and archaea and bacteria are detected. Most of the Archaea are methanogens, and the bacteria are mainly sulfate reducing thermophiles, hydrogen trophic thermophiles and other chemoautotrophic bacteria (Kimura et al., 2005); In sandstone and dolomitic limestone aquifers with different lithology, the microbial composition of groundwater is very different. The former is mainly bacterial Proteobacteria and abundant archaeal methanogens, while the latter is mainly bacterial Firmicutes and a small amount of archaea, and the microbial diversity is related to the shallow water recharge of the aquifer (chiriac et al., 2018).

Some boreholes in xiong'an new area were selected for the study of deep hot water microbial community. In the previous work, the digital PCR detection technology of sulfate reducing bacteria droplets in deep hot water was established, and the technology was used to detect deep hot water, shallow water and soil, and it was found that deep hot water was rich in sulfate reducing bacteria (Zhao Jiayi et al., 2020). On this basis, this study continues to take the deep hot water in xiong'an New Area as the research object, adopts the new generation of high-throughput sequencing technology and function prediction technology, and

uses shallow water and soil as reference to study the characteristics and functional characteristics of the microbial community in the deep hot water, so as to provide scientific basis for improving the understanding of the deep biosphere and rational development and utilization of the deep hot water.

2. MATERIALS AND METHODS

2.1 Overview of the study area

Xiongan new area is located in the central part of Jizhong depression of Hebei plain. The structure is developed. The faults are distributed in NNE, NW and near EW directions (Figure 1). The basement is concave and convex. Among them, Niutouzhen bulge, Rongcheng bulge and Gaoyang low bulge are rich in geothermal resources, shallow in burial, high in water temperature and large in water volume. Geothermal scientific drilling holes are deployed in these bedrock bulges. The thermal reservoirs in this area are mainly Neogene Minghuazhen Formation sandstone thermal reservoirs and Jixian system Wumishan formation and Gaoyuzhuang Formation carbonate karst fissure bedrock thermal reservoirs. At present, geothermal resources are mainly developed from the carbonate karst fissure bedrock thermal reservoirs in the upper part of Jixian system Wumishan formation (Ma Feng et al., 2020). The thermal reservoir caprock is the Cenozoic, the uppermost stratum, of which the quaternary system is thick and has poor thermal conductivity. Thick clay layers are generally found in the lower part, which has the function of thermal insulation. There are four aquifer groups distributed in the quaternary system. At present, the groundwater of the second aquifer group is mainly exploited for industrial and agricultural production and domestic water. Groundwater is divided according to the groundwater flow system. The first and second aquifer groups belong to the shallow groundwater flow system, in which the groundwater stored is shallow water. The lower aquifer groups, including the bedrock thermal reservoirs, belong to the regional groundwater flow system. The hot water stored in the carbonate karst fissure thermal reservoirs of Wumishan formation and Gaoyuzhuang Formation of Jixian system belongs to the deep hot water. The shallow water is fresh water, and the hydrochemical type is HCO_3-Ca • Mg water, while the deep hot water is brackish water, originating from atmospheric precipitation. The hot water age is 20000-40000 years, and the hydrochemical type is mainly Cl • HCO_3-Na water and a small amount of Cl-Na water.

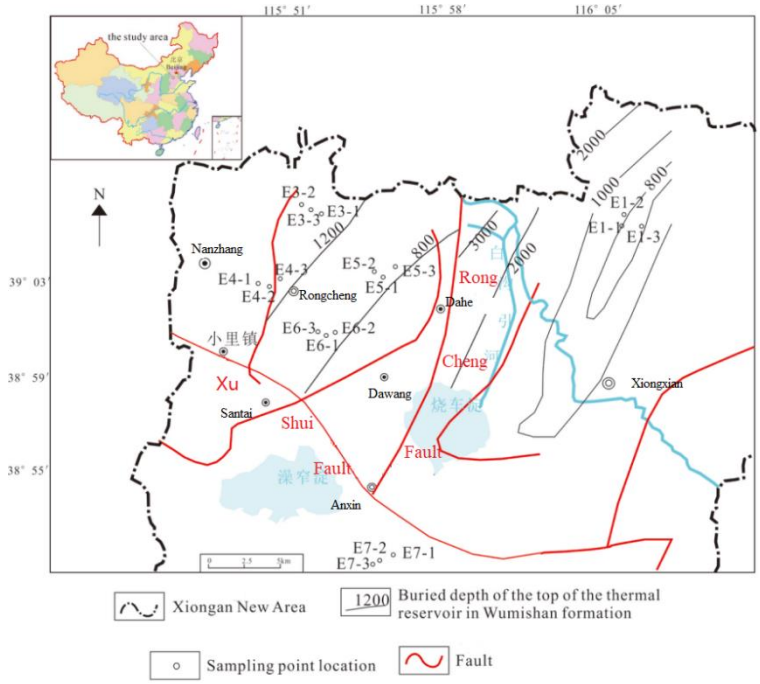

Figure 1: Sampling point map of study area

2.2 Sample collection and pretreatment

In this study, six groups of deep hot water samples (E1-1, E3-1 − E7-1) were collected from the pumping water in the last stage of the pumping test of geothermal scientific drilling hole in xiong'an New District, and samples were taken from the pump port of the well. At the same time, six groups of shallow water (E1-2,E3-2 − E7-2) and soil (E1-3, E3-3 − E7-3) samples from the Datian agricultural irrigation well near each borehole were collected as reference. A total of 18 groups of samples were used for high-throughput sequencing and functional prediction of 16S rRNA genes of microorganisms, The soil samples were also tested for water content. The hole depth of geothermal scientific drilling varies between 2506 − 3853m. the water producing interval of pumping test is the carbonate bedrock thermal reservoir of Wumishan formation or Gaoyuzhuang Formation of Jixian system, and the sampling depth is 1500 − 3853m. The depth of shallow water well varies from 70 to 120m, and the depth of soil sample collection is 0.5m. Field test shall be conducted during sampling, and the test items are GPS position, pH, temperature T and TDS of total dissolved solids. The water sample collection container is an 18 L high-temperature resistant sterile plastic barrel. Immediately after the sample is transported back to the laboratory, it is filtered onto a plurality of polytetrafluoroethylene filter membranes with a pore size of 0.22 M. the filter membranes with filter materials are stored in a -70 ℃ refrigerator. Luoyang shovel is used for core collection of soil samples, and sterile knife is used to dig the soil samples from the middle of the core, put them into sterile plastic bags, store them in the ice incubator on site, and transport them back to the laboratory as soon as possible. They are also stored in the - 70 ℃ refrigerator.

The filter membranes of deep hot water and shallow water samples and soil samples were frozen with dry ice and sent to Shanghai Meiji Biomedical Technology Co., Ltd. for 16S rRNA gene high-throughput sequencing. The moisture content of the soil sample is tested by drying method. Weigh a certain amount of wet soil and bake it at 105-115 ℃ for 8 h. The percentage of the difference between the weight of wet soil and dry soil and the ratio of dry soil is the moisture content. For the pH test of soil samples, the water solution with the volume ratio of soil to water of 1:2.5 shall be used.

3. RESULTS AND ANALYSIS

3.1 High throughput sequencing results and evaluation

The pH value of deep hot water varies between 6.77 − 8.75, which belongs to neutral and weakly alkaline water. Its pH value is similar to that of shallow water (7.50 − 7.87), but slightly lower than that of soil extract (8.55 − 8.94). The temperature of deep hot water varies between 56 − 105 ℃, which belongs to medium and low temperature hot water. Its temperature is much higher than that of shallow water (13 − 17 ℃) and soil (8 − 13 ℃), and the difference is large. The variation of TDS of deep hot water is between 1990 − 2677 mg / L, which belongs to brackish water. Its TDS is also much higher than that of shallow water (202 − 503 mg / L) and soil extract (72 − 178 mg / L), with great difference. It can be seen that the deep hot water in this area is significantly different from the shallow water and soil due to its high temperature and salinity.

Through high-throughput sequencing of 18 samples, 2560782 original sequences were obtained. After quality control filtering, 1146829 optimized sequences were obtained. After removing single sequences and chimeras, 362386 effective sequences were obtained. The taxonomic OTUs were clustered at 97% similarity level, resulting in 1131 OTUs. See Table 1 for the effective sequences and OTUs of each sample. The number of effective sequences of deep hot water (46740 − 69863) is similar to that of shallow water (48811 − 68666) and soil (51533-69835), but the number of OTUs varies greatly. The number of OTUs of deep hot water varies between 284 − 564, with a median of 292, while that of shallow water and soil is 112 − 3148 and 2133 − 3004, with a median of 1539 and 2394, respectively, It indicates that the microbial species in deep hot water are less than those in shallow water and soil.

Table 1: Results of measurement on site and high-through sequencing from deep thermal water, shallow water and soil

Sample ID	Depth (m)	pH	T/℃	TDS/mg • L-1	Number of valid sequences	OTU
Deep thermal water						
E1-1	2511	7.33	64.0	2197	46753	292
E3-1	2520.8	7.20	70.5	2677	61244	383
E4-1	2608.5	7.49	59.5	1990	68522	284
E5-1	2506	6.90	61.6	2063	46740	292
E6-1	2518.2	6.77	56.0	2620	69863	286
E7-1	3853	8.75	105.0	1998	69264	564
Shallow water						
E1-2	100	7.78	17.0	303	53932	1955
E3-2	70	7.60	16.0	470	48811	112
E4-2	100	7.74	15.0	304	51600	2119
E5-2	100	7.87	14.0	202	67725	1124
E6-2	70	7.50	14.0	548	68666	351
E7-2	120	7.42	13.0	503	62421	3148
Soil						
E1-3	0.5	8.94	12.5	85	69835	2923
E3-3	0.5	8.73	8.0	64	66503	3004
E4-3	0.5	8.56	9.0	73	65940	2133
E5-3	0.5	8.57	9.0	85	66432	2437
E6-3	0.5	8.55	13.0	178	65887	2352
E7-3	0.5	8.61	9.0	114	51533	2151

In order to compare the diversity index, the minimum sample sequence number of 46740 was used to draw the OTU of the classification unit, and the dilution curve and diversity index were calculated. The dilution curve of sobs index (Figure 2-A) shows that with the increase of sequencing depth, all dilution curves tend to plateau, indicating that the amount of sequencing this time is sufficient to evaluate microbial richness; The dilution curve of Shannon index (Figure 2-B) shows that when the number of sequences reaches 5000, all curves tend to plateau, indicating that the amount of sequencing this time is sufficient to evaluate the diversity of microorganisms. The community coverage of the samples is very high, all of which are greater than 98% (Table 2), indicating that the sequencing amount represents the real situation of the sample microorganisms.

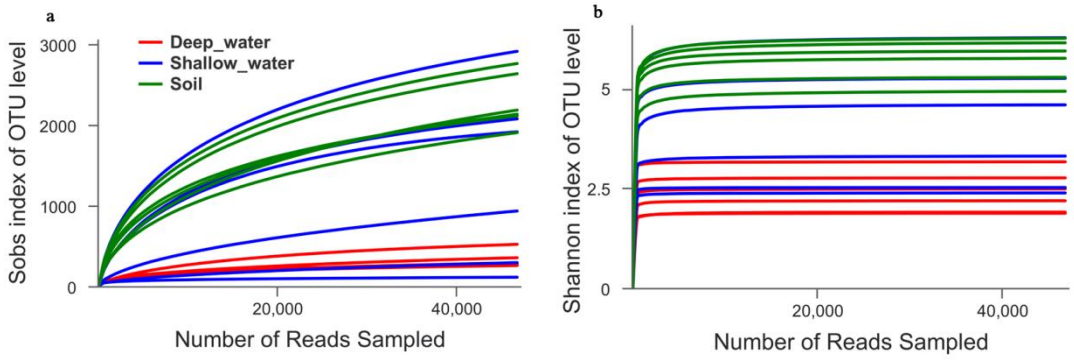

Figure 2: Rarefaction curves for Sobs (a) and Shannon (b) index of OTU level in Deep geothermal water, Shallow and Soil from Jizhong geothermal area

3.2 Analysis of microbial diversity in deep hot water

The richness of microbial communities is expressed by sobs, Chao1 and ACE indexes. The larger the value, the higher the richness; The diversity of microbial communities is expressed by Shannon and Simpson indexes. The larger the value of the former, the higher the diversity, while the latter is opposite. The diversity index results (Table 2) show that the mean values of the richness indices sobs, ACE and Chao of deep hot water are 325, 469 and 427 respectively, which are significantly lower than those of shallow water (137519711777) and soil (227430022970), indicating that the microbial richness of deep hot water is significantly lower than that of shallow water ($P <= 0.05$) and much lower than that of soil ($P <= 0.001$). Shannon's diversity index of deep hot water is 2.39, which is significantly lower than that of shallow water (4.06) and soil (5.73), while Simpson's average value of deep hot water is 0.23, which is significantly higher than that of shallow water (0.08) and soil (0.05). All these indicate that the microbial diversity of deep hot water is significantly lower than that of shallow water ($P < 0.05$) and soil ($P < 0.001$).

Table 2: Alpha-diversity indices of deep geothermal water, sallow water and soil samples from Jizhong geothermal area

Sample ID	Sobs	Shannon	Simpson	Ace	Chao	Coverage
E1-1	292	3.16	0.07	441.62	421.89	1.00
E3-1	345	2.48	0.27	632.23	494.73	1.00
E4-1	243	1.86	0.31	312.13	299.00	1.00
E5-1	292	2.76	0.16	509.16	454.30	1.00
E6-1	271	2.19	0.23	308.25	306.00	1.00
E7-1	509	1.90	0.35	610.26	590.28	1.00
maxum	509	3.16	0.35	632.23	590.28	1.00
minum	243	1.86	0.07	308.25	299.00	1.00
means	325	2.39	0.23	468.94	427.70	1.00
E1-2	1907	5.26	0.02	2155.68	2095.57	0.99
E3-2	111	2.52	0.16	156.38	141.00	1.00
E4-2	2076	4.60	0.08	2509.13	2478.44	0.99
E5-2	941	3.31	0.08	2406.14	1732.69	0.99
E6-2	299	2.38	0.16	910.54	593.53	1.00
E7-2	2920	6.28	0.01	3687.49	3623.55	0.98
maxum	2920	6.28	0.16	3687.49	3623.55	1.00
minum	111	2.38	0.01	156.38	141.00	0.98
means	1375	4.06	0.08	1970.89	1777.46	0.99
E1-3	2625	6.13	0.01	3310.38	3310.54	0.98
E3-3	2756	6.27	0.01	3457.61	3365.44	0.98
E4-3	1900	5.30	0.02	2726.64	2707.83	0.99
E5-3	2146	4.93	0.05	3040.82	2995.44	0.98
E6-3	2123	5.77	0.01	2817.28	2741.83	0.99
E7-3	2094	5.95	0.01	2660.65	2699.36	0.99
maxum	2756	6.27	0.05	3457.61	3365.44	0.99
minum	1900	4.93	0.01	2660.65	2699.36	0.98
means	2274	5.73	0.02	3002.23	2970.07	0.98

3.3 Characteristics of microbial community composition in deep hot water

3.3.1 Domain level

The primers for high-throughput sequencing are universal primers for bacterial archaea, and the sequencing results are sequences related to bacterial archaea. After the high-throughput sequencing results were optimized by quality control, 362386 effective sequences were obtained from 18 samples. After flattening, they were compared with the Silva / 16S database. A total of 3 biological domains, 62 phyla, 150 classes, 400 orders, 667 families, 1280 genera, 2494 species, and 7883 OTUs were detected, including 2 deep hydrothermal domains, 38 phyla, 74 classes, 178 orders, 293 families, 541 genera, 757 species, and 1079 OTUs; There are 3 shallow aquatic biological domains, 61 phyla, 142 classes, 363 orders, 594 families, 1080 genera, 2005 species and 5194 OTUs; There are 2

soil biological domains, 41 phyla, 108 classes, 282 orders, 458 families, 795 genera, 1513 species and 4766 OTUs. It can be seen that the deep hot water has the least microbial species, the shallow water has the most, and the soil has the second.

Most of the two domains of deep hot water belong to bacteria (97.5% of the six samples), and a small number of Archaea (2.5%). In addition to the bacterial domain (96.4%) and archaeal domain (3.6%), there are unclassified biological domains in shallow water, with only four sequences. The soil also has two domains, most of which are bacteria (90.9%) and a small number of Archaea (9.1%), but Archaea is higher than deep hot water and shallow water, and the sequence change is between 2178 − 5651. The above results show that, at the domain level, the deep hot water, like the shallow water and soil, is dominated by bacteria with a small amount of archaea, but the Archaea in the deep hot water is less abundant than that in the soil and is similar to that in the shallow water.

3.3.2 Phyla level

According to the statistics of Archaea and bacteria after leveling, the bar graph of community composition abundance of phylum level Archaea (the top 2 in abundance) and bacteria (the top 8 in abundance) (Fig. 3) shows that the deep hot water like Archaea is mainly composed of Euryarchaeota (6561 total sequences of six samples) and Crenarchaeota (442 sequences) of quanarchaea. The former accounts for 93% of the deep hot water Archaea sequence, and is mainly distributed in e5-1 and e6-1, while the latter accounts for 6%, The shallow water Archaea is mainly thaumarchaeots (9137 total sequences of 6 samples), accounting for 91% of the shallow water archaea, and mainly distributed in E4-2 and e7-2 samples; The Archaea of soil is also mainly thaumarchaeots (25231 total sequences of 6 samples), accounting for 99% of the soil archaea, and the Archaea is evenly distributed in all samples. Euryarchaeota is also found in shallow water (65 sequences) and soil (217 sequences), but its abundance is very low, accounting for only 1% of their Archaea groups. The above results show that the horizontal dominant Archaea of the deep hot water phylum in this area is very different from the shallow water and soil. The deep hot water is mainly Euryarchaeota, while the shallow water and soil are mainly thaumarchaeots.

Figure 3 also shows that the deep hot water bacteria phylum is mainly composed of Firmicutes (105811 total sequences of 6 samples, accounting for 39% of the deep hot water bacteria phylum), Proteobacteria (102831 sequences, 38%), nitrospirae (37304 sequences, 14%), aquae (6551, 2%), thermotogae (6412 sequences, 2%), Bacteroidetes (4107 sequences, 2%), chloroexi (3535 sequences, 1%) and Deinococcus Thermus (2412 sequences, 1%), Firmicutes and Proteobacteria are distributed in all samples. Firmicutes are abundant in E1-1, E3-1, E4-1, E5-1 and E6-1 samples except e7-1 samples. Proteobacteria are abundant in e3-1, e6-1 and e7-1 samples. The most abundant bacterial phyla in shallow water is Proteobacteria (172352 total sequences in 6 samples, accounting for 64% of the bacterial phyla group in shallow water), followed by Bacteroidetes (35571 sequences, 13%), and Firmicutes are few (2478 total sequences in 6 samples), accounting for only 3% (after the top 8 bacterial phyla abundances, they are not shown); Actinobacteria (79035 total sequences of 6 samples, accounting for 31% of the total number of soil bacteria) is the most abundant bacterial phyla in the soil, followed by Proteobacteria (51459 sequences, 20%), Firmicutes (24894 sequences), which is higher than shallow water, accounting for 10%. It can be seen that the horizontal dominant bacterial species of the deep hot water gate in this area are not the same as those of the shallow water and soil. Firmicutes and Proteobacteria have high abundances in the deep hot water, Proteobacteria have the highest abundances in the shallow water, actinobacteria have the highest abundances in the soil, and Firmicutes have low abundances in the shallow water and soil.

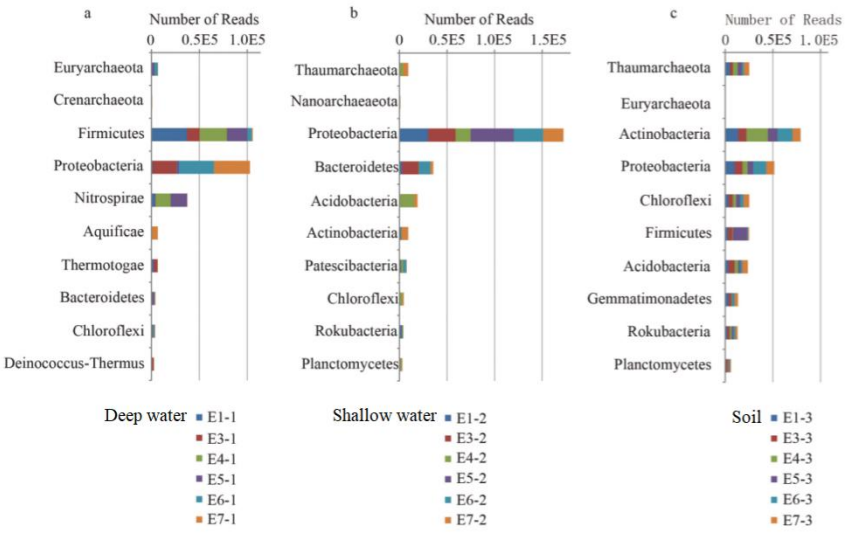

Figure 3: Composition and abundance bar chart of Archaea (top 2 abundance) and bacteria (top 8 abundance) at phylum level in deep geothermal water, shallow water and soil samples from Jizhong geothermal area

3.3.3 Genus level

After flattening, the Archaea and bacteria were statistically sorted respectively. The composition and abundance bar graph of genus level Archaea (the top 2 in abundance) and bacteria (the top 10 in abundance) showed that, The Archaea of deep hot water samples are mainly composed of methanothermobacter (6553 total sequences of 6 samples, accounting for 93% of the total sequences of deep hot water Archaea) of Euryarchaeota, a phylum of guangarchaea, and bathyarchaea (428, 6%) of unnamed deep Archaea of Crenarchaeota, a phylum of quanarchaea. The former is mainly distributed in e5-1 (2980 sequences) and e6-1 (3417 sequences), The latter was only distributed in e6-1 (427 sequences). The most abundant Archaea in shallow water is the unnamed nitrosopumilaceae

of thaumarchaeota (2636 total sequences of 6 samples, accounting for 26% of the total sequence of Archaea in shallow water), which is mainly distributed in E4-2 (2552 sequences), followed by nitrososphaeraceae (1971 sequences, 20%), which is only distributed in e7-2 (1971 sequences); The most abundant Archaea genus in the soil is also the unnamed nitrosopumilaceae (15075 total sequences of 6 samples, accounting for 59% of the total number of Archaea in the soil), which belongs to thaumarchaeota genus of the Archaea phylum, followed by nitrosophageaceae (5542, 22%) and is evenly distributed in all samples. The above results indicate that the dominant Archaea species in deep hot water are completely different from those in shallow water and soil.

Figure 4 also shows that the composition of the genus of deep hot water bacteria is mainly thermodesulfonbrio (37289 total sequences of 6 samples, accounting for 14% of the total sequence of deep hot water bacteria), a thermodesulfonbrio of nitrospirae, γ- Pseudomonas (27082 sequences, 10%), δ- Thermodesulfobacterium of Proteobacteria (23840 sequences, 9%), thermoanaerobacteraceae of Firmicutes (22381 sequences, 8%), γ- Hydrogenophilus of Proteobacteria (17516 sequences, 6%), caldicobacteria of Firmicutes (15019 sequences, 5%), γ- Thiofaba of Proteobacteria (14019 sequences, 5%), desulfovirgula of Firmicutes (8128 sequences, 3%), clostridia of Firmicutes (7926 sequences, 3%), and γ- The genus of Proteobacteria is the unnamed burkholderiaceae (7756 sequences, 3%). The most abundant bacterial genus in shallow water is γ- The genus of Proteobacteria is the unnamed burkholderiaceae (43502 total sequences of 6 samples, accounting for 16% of the genus of shallow water bacteria), followed by Flavobacterium of Bacteroidetes (29909 sequences, 11%); The most abundant bacterial genus in the soil is Actinobacteria, an unnamed genus of actinobacteria (22280 total sequences of 6 samples, accounting for 9% of the soil bacterial genus), followed by Bacillus, a genus of Firmicutes (16334 total sequences, 6%). Through comparison, the dominant bacteria in deep hot water are completely different from those in shallow water and soil, and the distribution of the dominant bacteria in deep hot water samples is extremely uneven, similar to that in shallow water, but relatively uniform in soil, which indicates that the composition of the dominant bacteria in deep hot water has large spatial variation, which is similar to that in shallow water and larger than that in soil.

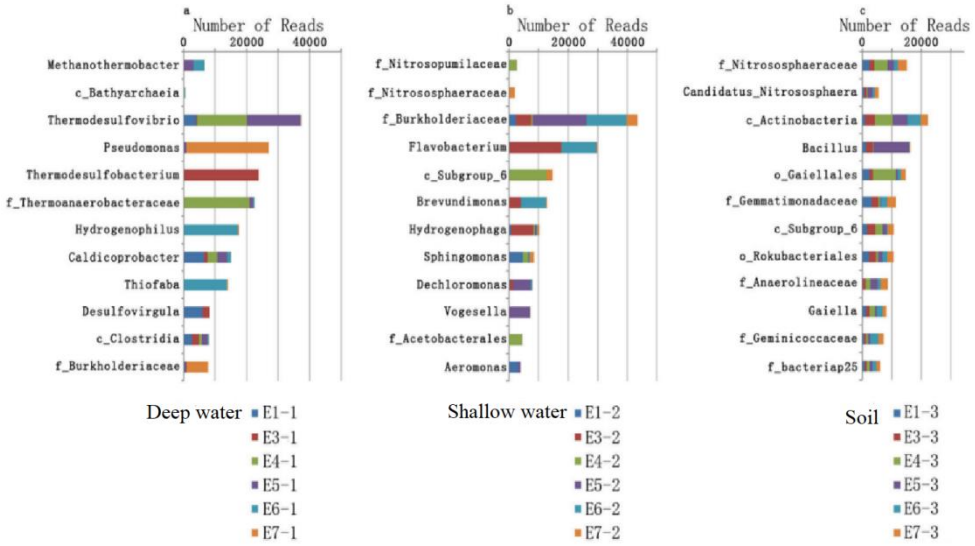

Figure 4: Composition and abundance bar chart of Archaea (top 2 abundance) and bacteria (top 10 abundance) at genus level in deep geothermal water (a), shallow water (b) and soil (c) samples from Jizhong geothermal area

3.3.4 Sample level clustering analysis

The hierarchical cluster diagram of samples can reveal the similarity of microbial community structure of samples. The results shown in Figure 5 show that, from the phylum level, the deep hot water samples and soil samples belong to different branches, while the deep hot water samples E3-1, E6-1 and E7-1 belong to the same branch as the shallow water, indicating that the microbial community structure of the deep hot water is quite different from that of the soil, while the microbial community structure of some samples E3-1, E6-1 and E7-1 is relatively similar to that of the shallow water. From the subordinate level, most of the deep hot water samples are the same branch, and only E7-1 sample is still the same branch as the shallow water, which indicates that the horizontal community structure of most of the deep hot water is quite different from that of the shallow water and soil, but the genus level community structure of E7-1 sample is still similar to that of the shallow water.

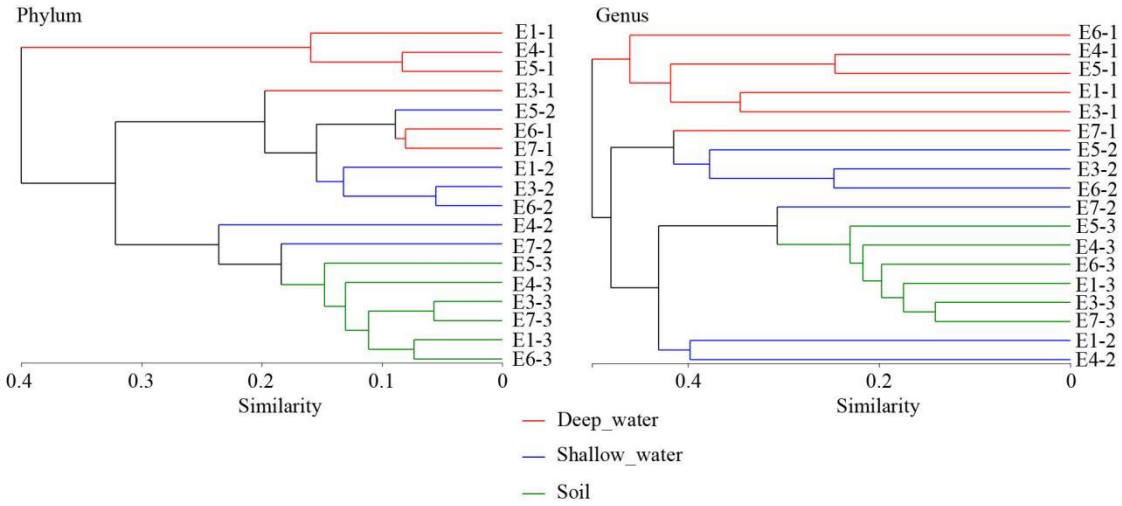

Figure 5: Hierarchical cluster analysis at phylum (left) and genus (right) levels in deep geothermal water, shallow water and soil sample from Jizhong geothermal area

3.3.5 Microflora typing analysis

Flora typing analysis mainly studies the typing of the dominant flora structure of samples through statistical clustering, and takes the group with the highest abundance as the typing name. Figure 6 shows that at the phylum level, the flora structure of shallow water is mainly represented by Proteobacteria, and that of soil is represented by Actinobacteria, while the flora structure of deep hot water is composed of two types of flora, one is represented by Firmicutes, and the other is represented by proteobacteria. Among them, the flora of deep hot water samples E3-1, E5-1 and E7-1 represented by Proteobacteria tends to be shallow water, It shows that the flora structure of these samples is similar to that of shallow water, which is consistent with the results of hierarchical cluster analysis at the phylum level.

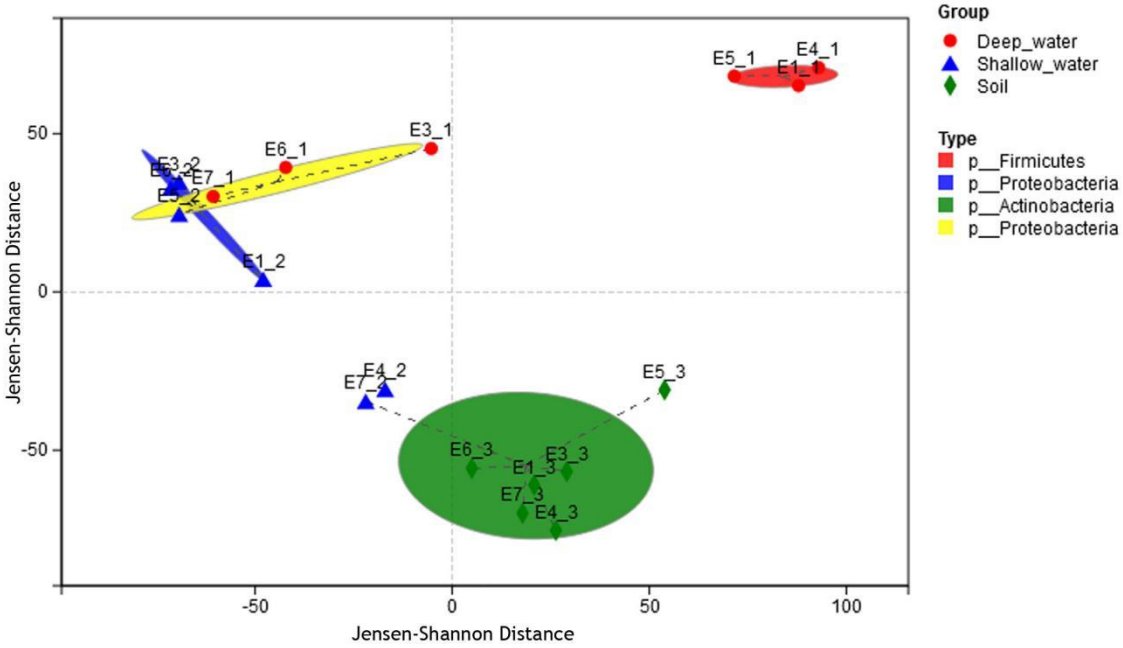

Figure 6: Structural typing of microbial communities at phylum level in deep geothermal water, shallow water and soil samples from Jizhong geothermal area

3.3.6 Analysis of differences between groups

The significance of the difference in flora structure among groups was further tested by the method of Kirschner rank sum. Figure 7 shows the main bacteria and genera that cause the difference in the microbial community structure between deep hot water, shallow water and soil. Among them, Firmicutes, Nitrospirae, Euryarchaeota, Aquificae and Thermotoae are relatively abundant in deep hot water, and Firmicutes and Thermotoae are significantly different in the abundance between groups ($P<0.01$); Thermodesulfovibrio γ-Pseudomonas, Hydrogenophilus and Thiofaba δ- Thermodesulfobacter of Proteus, Thermoanaerobacteraceae and Caldicoprobacters of Firmicum have high relative abundance in deep hot water, and Thermodesulfovibrio, Thermoanaerobacteraceae, Hydrogenophilus and Caldicoprobacters have significant differences among groups ($P<0.01$).

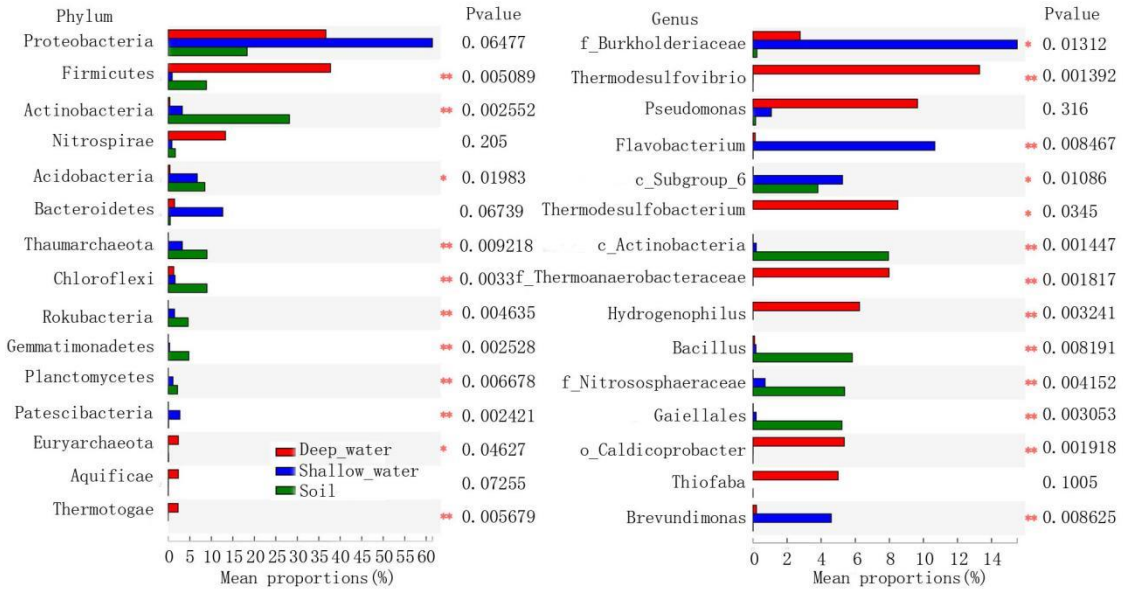

Figure 7: Kruskal-Wallis H test bar plot of microbial community structural difference at phylum level（left）and at genus level（right） among deep geothermal water, shallow water and soil samples from Jizhong geothermal area

4. DISCUSSION

The purpose of this study is to explore the microbial community characteristics and functional characteristics of deep hot water by using high-throughput sequencing technology and functional prediction technology, with shallow water and soil as reference. In order to achieve this goal, the primary task is to determine whether the collected deep hot water sample is polluted by drilling fluid. According to the design requirements of this geothermal scientific drilling, clean water drilling shall be used when drilling to deep bedrock thermal reservoir to avoid drilling mud polluting the thermal reservoir; In addition, the well shall be washed before the pumping test to ensure that the well is clean and the content of suspended solids in the fluid is less than 1/2000 mass ratio. According to research, when the water volume pumped exceeds 50 borehole volumes, microbial contamination from drilling fluid can be minimized (Davidson et al., 2011). This sampling is the final stage of 2-3 days of pumping test. At this time, the water pumped has greatly exceeded the volume of 50 boreholes. Therefore, the pollution from drilling fluid may not be considered in this microbial analysis.

Compared with shallow water and soil, the microbial community richness and diversity of deep hot water is the lowest (Table 2), which is closely related to nutrient richness and diversity and environmental conditions. The soil is rich in organic and inorganic substances, followed by shallow water. The deep hot water is oligotrophic, while the deep hot water is a high-pressure, high-temperature and high salinity environment, which is not conducive to biological survival. Therefore, the richness and diversity of the deep hot water microbial community is the lowest, which conforms to the environmental characteristics.

The horizontal microbial composition of the deep hot water area in this area is mainly composed of bacteria (97.5% of the total sequenced quantity), with few archaea (2.5%), which is similar to the horizontal microbial composition of the shallow water (96.4% bacteria, 3.6% archaea) 70-120m deep and the soil (90.92% bacteria, 9.08% archaea) 0.5m deep in this area; It is also similar to the domain level microbial composition in basalt and granite cores obtained by scientific deep drilling in western India (bacteria 92.36% - 99.36%, archaea 0.12% - 6.33%) (Dutta et al, The latter is mostly archaea (>90%) (Chapelle et al., 2002). The difference of microbial composition is closely related to local environmental conditions.

The most abundant archaea in the deep hot water in this area is Euryarchaeota (93% of the deep hot water archaea), and the dominant archaea is Methanothermobacter (93% of the deep hot water archaea), which is a thermophilic, anaerobic and hydrogen trophic methanogenic bacterium and has been found in high-temperature reservoir formation and natural gas field formation water and other environments (Cheng et al. 2012; Nakamura et al. 2013). The shallow water (91% of the shallow water archaea) and soil (99% of the soil archaea) archaea are both the rare archaea Thaumarchaeots, and the dominant genera are the unnamed bacteria Nitrosopumilaceae (26% of the shallow water archaea) and the bacteria Nitrososphaeraeae (59% of the soil archaea), which are thermophilic and aerobic ammonia oxidizing bacteria (Qin et al., 2017; Stieglmeier et al., 2014).

The most abundant bacteria in the deep hot water in this area is Firmicutes (accounting for 39% of the deep hot water bacteria), while it is less in the shallow water (accounting for 3% of the shallow water bacteria), and more abundant in the soil (accounting for 10% of the soil bacteria) (Figure 2), that is, this area has the characteristics of less Firmicutes in the shallow water and soil, but more in the deep hot water. Although there are Firmicutes in the shallow water, soil and deep hot water in this area, their dominant bacteria at the genus level are different. The most abundant genus of bacteria in the shallow water Firmicutes is Sporosarcina (0.21% of the shallow water bacteria), which is a thermophilic anaerobic bacteria; The most abundant genus of soil bacteria is Bacillus (accounting for 6.41% of soil bacteria), which is strictly aerobic or facultative anaerobic thermophilic bacteria. There are many dominant genera of bacteria in deep hot water Firmicutes, mainly belonging to the unnamed Thermoanaerobacteraceae (accounting for 8% of deep bacteria), Calcicobacteria (5%) and Desulfovirgula (3%). Desulfovirgula is a kind of thermophilic sulfate reducing bacteria, which can produce hydrogen sulfide gas with hydrogen or organic matter as electron donor and sulfur and its compounds as electron acceptor. Its strain has been isolated from the black sediment of hydrothermal activity in deep mine (Kaksonen et al., 2007). It can be seen from the above that the dominant genera of Firmicutes in shallow water and soil are thermophilic bacteria, while the dominant genera of Firmicutes in deep hot water are thermophilic and anaerobic bacteria in deep or thermal environment.

Proteobacteria are the dominant phylum in shallow water, soil and deep hot water in this region, especially rich in shallow water

(61.53% of shallow water bacterial phylum), but the genus level species are not the same. The dominant genera belonging to Proteobacteria in shallow water are more diverse, and the most abundant dominant genus is the genus unnamed Burkholderiaceae family of γ-Proteobacteria members (6% of the total sequence of shallow water bacteria), followed by Hydrogenphaga of γ-Proteobacteria (6% of the total sequence of shallow water bacteria), followed by Hydrogenphaga, Dechloromonas, Vogesella and Aeromonas of γ-Proteobacteria, Brevundimonas, Sphingomonas of α-Proteobacteria and Acetobacterales-incerfae-sedis of undetermined genus, all of which are thermophilic (<45 ºC). . The dominant genera in the soil belonging to Proteobacteria were few and low, mainly α-Proteobacteria of the unnamed genus Geminicoccaceae (3%) and δ-Proteobacteria of the unnamed genus Bacteriap25 (2%), the former being exclusively aerobic and thermophilic. The latter is an uncultured bacterium. In contrast, the dominant genus of deep hot water Proteobacteria was more diverse, and the most abundant was the genus Pseudomonas belonging to γ-Proteobacteria (9.64% of the total sequence of deep hot water bacteria), which is a Gram-negative, aerobic, thermophilic bacterium with a temperature range of 18-37 ºC and pH 6-10; followed by Thermodesulfobacterium (8.72% of the total sequence of deep hot water bacteria), which belongs to δ-Proteobacteria, was mainly distributed in sample E3-1. The strains of this genus were first isolated from volcanically active thermal environments and grew in the temperature range of 45-85 ºC, with an optimum of 70 ºC, as thermophilic Sulfate-reducing bacteria (Zeikus and Dawson, 1983). The third most abundant were Hydrogenophilus, Thiofaba and Burkholderiaceae belonging to γ-Proteobacteria (all >1%), of which Hydrogenophilus is a strictly aerobic thermophilic bacterium; Thiofaba is a group of specialized chemoautotrophic, sulfur-oxidizing bacteria, whose strains were isolated from Japanese hot springs, with an optimal growth temperature of 45 ºC, and use thiosulfate, sulfur and hydrogen sulfide as electron donors and CO_2 as a carbon source to oxidize low-valent sulfur to high-valent sulfur (Mori and Suzuki, 2008). The genus Burkholderiaceae is also a specialized aerobic thermophilic bacterium, widely distributed in soil and water environments, growing at 30-37 ºC. Thus, it can be seen that the species of each genus belonging to the Proteobacteria phylum in deep hot water are different from those in shallow water and soil, but most of the genera are aerobic thermophilic bacteria, similar to those in soil and shallow water. The presence of aerobic thermophilic bacteria in the deep hot water may be due to the recharge of the deep hot water by the shallow water under high flow pumping conditions. The results of the sample hierarchical clustering analysis were that, at the gate level, part of the deep hot water samples E3-1, E6-1 and E7-1 clustered with shallow water; at the genus level, sample E7-1 still clustered with shallow water. These results all indicate that the deep hot water is recharged by shallow water. The results of the fractionation analysis were that the dominant flora of deep hot water was divided into two groups, one represented by Firmicutes and the other by Proteobacteria similar to shallow water, and the latter tended to shallow water, which also indicated that deep hot water was influenced by the recharge of shallow water. Similarly, Hubalek et al. showed that microbial diversity in aquifers as deep as 455 m depends on the degree of connectivity between deep and shallow water (Hubalek et al., 2016).Chiriac et al. suggested that the increase in abundance of thermophilic bacteria and microbial community diversity in deep thermal waters may be caused by shallow water recharge (Chiriac et al, 2018).

The phylum Nitrospirae is rare in both shallow water and soil in the region, but is the third most abundant phylum in deep hot water (13.7% of the total sequence of deep hot water bacteria), mainly in samples E1-1, E4-1 and E5-1. Thermodesulfovibrio, a member of this phylum, is the most abundant genus of deep hot water bacteria in the region and It is an anaerobic, thermophilic sulfate-reducing bacterium. The strains of this genus isolated from the hot vent waters of Yellowstone Park, USA, have an optimum growth temperature of 65 ºC and use sulfate, thiosulfate and sulfite as electron acceptors and lactate, pentose, hydrogen and acetate as electron donors to reduce sulfur oxides to hydrogen sulfide gas (Henry et al., 1994). Aquificae is also rare in shallow water and soil, but is the dominant phylum in deep hot water (2.40% of the total sequence of deep hot water bacteria), and the dominant genus is Sulfurihydrogenibium (2%), which is a thermophilic, chemoautotrophic, parthenogenetic anaerobic bacterium found in deep hot aquifers in Japanese pits, oxidizing hydrogen to water It can oxidize hydrogen to water, sulfur and thiosulfate to sulfate, with an optimal growth temperature of 60-65 ºC, and cannot utilize complex organic matter, using carbohydrates, amino acids and organic acids as the only energy and carbon source (Takai et al., 2003). Thermotogae is also rare in shallow water and soil in this area, but it is the dominant phylum in deep hot water (2.34% of the total sequence of deep hot water bacteria), and is a class of super thermophilic anaerobic bacteria, which can ferment and degrade lactic acid and acetic acid to CO2 and H2O, and reduce sulfur to H2S at growth temperatures up to 90 ºC, with an optimum temperature of 80 ºC (Huber et al., 2003). (Huber et al., 1986). From the above, it can be seen that the above phyla are mainly found in deep hot water, and most of them are thermophilic anaerobes.

Based on the above analysis results and the analysis results of the structural difference of flora among groups (Figure 7), the characteristic flora of deep hot water in this area is mainly Archaeota, Firmicutes, Nitrospira, Aquificae and Thermotogae.

The characteristics and functional properties of the deep hot water microbial community in this area have significant geothermal significance. The deep hot water in this area is inhabited by a large number of microorganisms, and the metabolic activities of microorganisms will change the chemical composition of the deep hot water. The dominant genus in deep hot water is mainly sulfate-reducing bacteria, and the metabolic activity of this bacteria will reduce the SO_4^{2-}content of deep hot water, and the H_2S produced will combine with metal ions such as Fe to produce FeS precipitation, which will accelerate the corrosion and scaling of underground well pipes and shorten the service life of geothermal facilities, and the results of this study provide basic data for the prevention and control of sulfate-reducing bacteria. Usually, the CH_4 content of underground hot water is high, and the results of this study are that the biological methanogenesis of deep hot water in this area is very weak, implying that most of the CH_4 in deep hot water is non-biogenic methane, and the results of this study are helpful to the analysis of the genesis of deep hot water. The occurrence of aerobic thermophilic bacteria in the deep hot water of this area, as well as the results of various analyses such as sample stratification clustering, bacterial group typing and functional gene prediction, show that the deep hot water in this area is recharged by shallow water under high flow pumping conditions. Since the dominant genus (Pseudomonas Pseudomonas) of the anamorphic phylum in the deep hot water is different from the dominant genus (Burkholderiaceae) of the anamorphic phylum in the shallow water, it indicates that this recharge is not from the vertical recharge of shallow water above the borehole, but may be from the lateral recharge of shallow water, gaining thermal energy through deep circulation, or getting through the fracture zone connection recharge from shallow water in other parts, and the deep hot water in this area has the renewability of resources.

5. CONCLUSION

In this study, the microbial community characteristics and functional properties of deep hot water in the Jizhong geothermal zone were explored by using high-throughput sequencing technology and functional prediction technology, with shallow water and soil as reference. The deep hot water in Jizhong geothermal area contains 38 phyla and 541 genera, mainly bacteria (97.5%) and few archaea

(2.5%); the characteristic groups are mainly Euryarchaeota, Firmicutes, Nitrospirae, Aquificae and Thermotogae. Most of the dominant genera of deep hot water are sulfate-reducing bacteria, such as Thermodesulfonbrio, Thermodesulfobacterium, Thermoanaerobacteraceae, Desulfovirgula, and Desulfovirgula. Desulfovirgula and Desulfotomaculum, etc.

The presence of thermophilic and aerobic bacteria in the deep hot water indicates that the deep hot water is recharged by the shallow water under high flow pumping conditions. The results of sample hierarchical clustering analysis, bacteriophage typing and functional gene prediction further indicated that deep hot water was recharged by shallow water. The dominant genera of deep hot water anamorphic phylum are different from the dominant genera of shallow water anamorphic phylum above the borehole, indicating that the deep hot water gets shallow water recharge not from the vertical recharge of shallow water above the borehole, but from the lateral recharge of shallow water, and gets thermal energy through deep circulation, or may get shallow water recharge from other parts through the fracture zone connection, and the deep hot water has the renewability of resources. The characteristics and functional properties of the deep hot water microbial community in this area have significant geothermal significance.

In conclusion, the deep carbonate karst-fracture thermal reservoir contains a rich variety of microbial communities with diverse functions, and the microbial community structure and functional characteristics can be used as an indicator of the hydraulic connection between deep water and shallow water.

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