

Article

Characteristics of Groundwater Microbial Community Composition and Environmental Response in the Yimuquan Aquifer, North China Plain

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Abstract: To explore the intricate interrelationship between groundwater microbial communities and hydrochemical characteristics in the Mancheng District, Baoding City, samples from karst groundwater and porous groundwater were collected. Hydrochemical analysis and the 16s RNA gene V4-V5 region sequencing method were used to detail the water environmental characteristics and microbial community distribution pattern in the study area. The hydrochemical results reveal notable differences in major ion contents and other hydrochemical indices between karst water and porous water. Microbial community analysis demonstrates that *Proteobacteria*, *Actinobacteria*, and *Firmicutes* are the dominant taxa in both types of water bodies, albeit with significant variations in their proportions across distinct water bodies. The observed disparities in microbial communities between karst water and porous water underscore the influence of environmental factors such as ion concentration and pH on microbial distribution. Redundancy Analysis (RDA) indicates notable associations between microbial community composition and environmental factors in both types of groundwater environments. Studying the distribution pattern of microbial communities and the connection with environmental factors in two types of groundwater lays a crucial foundation for interpreting the functional roles of microbes in groundwater ecosystems.

Keywords: environmental microbiology; hydrogeology; hydrochemical characteristics; microbial ecology; environmental response characteristics



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1. Introduction

Natural spring areas, as the unique zones where groundwater and surface water converge, boast abundant water resources and distinct ecological environments [1,2]. Research on environmental microbial communities and their functions holds significant implications worldwide [3]. In recent years, it has been discovered that microbes in groundwater environments play vital roles in maintaining aquatic ecological stability, protecting water resources, and safeguarding ecosystem health [4–6]. Studies have shown that groundwater microbes can degrade organic matter, remove heavy metals, and eliminate pollutants such as nitrogen and phosphorus, offering biotechnological methods for protecting and purifying water resources [7]. Additionally, groundwater microbes play key roles in the Earth's biogeochemical cycles, such as those of carbon, nitrogen, and sulfur [8,9]. In-depth exploration into groundwater microbial communities and their functions is of great theoretical

significance for understanding global ecosystem functions and elucidating Earth's ecological evolution. The investigation of groundwater microbes provides new perspectives and methods for ecological protection and the sustainable use of water resources.

The Yimuquan Spring area in Baoding City is located at the southern margin of the North China Plain, in the pre-mountain area. It occupies the upstream area of the groundwater dynamics system in central Hebei, boasting abundant groundwater resources and complex hydrogeological conditions. According to the data from the China Water Resources Yearbook (2021), the groundwater resources of Baoding City account for 79.4% of the city's total water resources, playing a crucial role in agricultural production, ecological environment protection, and residential water supply [10]. However, as the population grows and economic development advances, the water resources of the Yimuquan Spring area in Baoding city are facing increasingly severe pressure, and the scientific management and protection of groundwater resources have become increasingly important. Although groundwater microorganisms play an important role in biogeochemical processes, the relationship between the microbial community structure in the groundwater layer of this area and environmental characteristics has not yet been thoroughly studied [11,12]. Therefore, this study aims to systematically survey the microbial communities in the groundwater layer of the Yimuquan Spring area in Baoding City. Specifically, our objectives are as follows: (i) comprehensively investigate the distribution and diversity of microbial communities in groundwater; (ii) analyze the relationships between these communities and environmental factors such as ion concentration and pH values, to uncover their structure and environmental response characteristics; and (iii) explore the ecological roles of groundwater microorganisms, providing a scientific basis for the protection and rational utilization of groundwater resources. Through this research, we hope to offer robust scientific support for the sustainable development of this region, the improvement of the ecological environment, and the advancement of the livelihood and the local citizens' well-being.

2. Materials and Methods

2.1. Study Area Overview

The study area is primarily located in Mancheng District, Baoding City, Hebei Province, encompassing parts of the upstream and downstream spring areas. It spans a range between 38°56'0"–38°58'30" N and 115°12'30"–115°24'30" E (Figure 1). The climate is a warm temperate semi-humid continental climate, with an average annual temperature of 13 °C and an average annual precipitation of 550 mm [13]. The terrain is predominantly plain, representing various geological origins such as ancient river floodplains, alluvial fans, and aeolian terraces (Figure 2). The topography is high in the north and low in the south, with altitudes ranging between 50 and 100 m [14]. The western part of the area is characterized by bedrock predominantly consisting of dolomitic stone, where geological layers alternating between mud and gravel and pebble and gravel. These layers are surmounted by a loess-shaped loam surface layer. With increasing stratal thickness, intermittent clay lenses and loess-shaped sub-sandy soil lenses become discernible. The hydrogeological conditions are complex. The main aquifers include the Quaternary alluvial layer and the Tertiary sedimentary layer. The former is the main aquifer, with a thickness of 20–100 m. The latter has a thickness of about 50–150 m and slightly less water content [15].

The study area has abundant overall resources, with annual available groundwater resources of around 936 million cubic meters. Among this available groundwater, 70% is used for agricultural irrigation, followed by industrial production and domestic water use [10]. The groundwater quality is safe with low nitrite, nitrate, and heavy metal contents. The concentrations of the former two are far below the values stipulated in the "Groundwater Quality Standards (GB/T 14848-2017)" [16,17]. In addition, the total hardness of groundwater in the study area is relatively high (average 450 mg/L) and exhibits alkaline characteristics (pH values are between 7.5 and 8.5). The main cations are calcium, mag-

nesium, and sodium, while the main anions are carbonate, bicarbonate, and sulfate. The water chemical type is primarily $\text{HCO}_3\text{-SO}_4\text{-Ca}\cdot\text{Mg}$ [18].

2.2. Sample Collection and Testing

In January 2022, considering the groundwater flow direction and actual water usage in the study area, we collected nine groundwater samples from the Yimuquan area in Mancheng District, Baoding City. The sampling depth ranged from 90 to 200 m. Prior to sampling, we pumped the well for 0.5–2 h until the water quality was clear and stable. Samples were then taken from the water outlet directly above the submersible pump at the ground surface, without pipe aeration or disinfection. Samples were collected in three parts: Firstly, on-site measurement of water temperature, pH, electrical conductivity (Ec), and oxidation–reduction potential (ORP) was performed using a Hach HQ40D portable multi-parameter water quality analyzer. Secondly, the samples were preserved according to the “Standard Test Methods for Drinking Water” (GB/T 5750-2006) [19], and then the total dissolved solids (TDS) were measured using the dry weighing method. An ICS-600 Ion Chromatograph was used to determine Cl^- , SO_4^{2-} , NO_3^- , NO_2^- , and F^- . Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used to determine Na^+ , Al^{3+} , Fe^{3+} , Mn^{2+} , and TDS. After the tests were completed, the relative measurement error was calculated to be 1.7% based on the standards announced in the “Standard Test Methods for Drinking Water—Quality Control in Water Quality Analysis (GB/T 5750-2006)” and DD2014-2015 “Technical Requirements for Quality Control of Sample Analysis in Groundwater Pollution Investigation and Evaluation” [20]. Thirdly, 1 L of each water sample was collected and filtered using a $0.22\ \mu\text{m}$ water system filter membrane using a suction pump, then stored in a dry ice box. These samples were finally transported to Shanghai Personal Biotechnology Co., Ltd., (Shanghai, China) for testing.

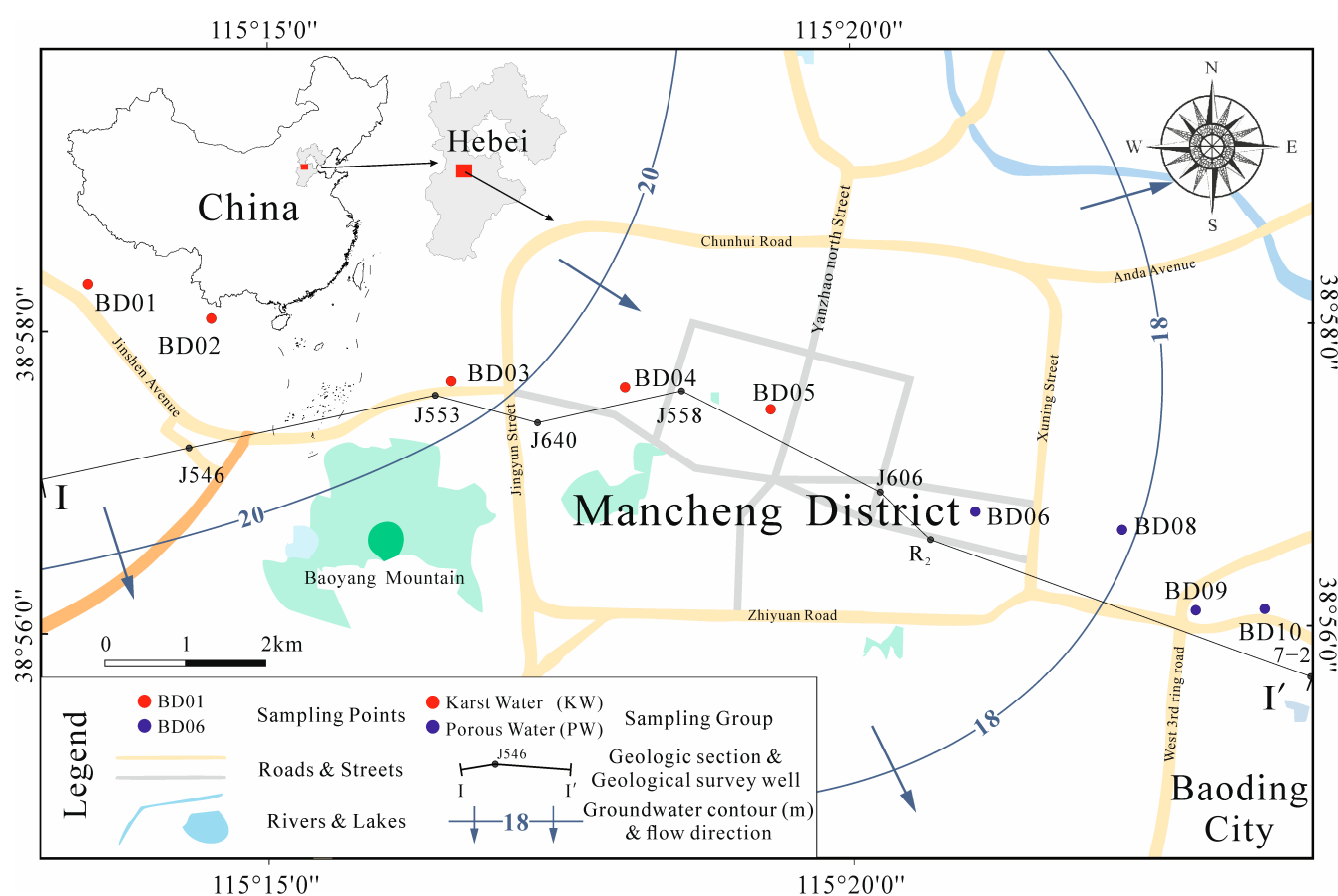


Figure 1. Geographical location and distribution of sampling points.

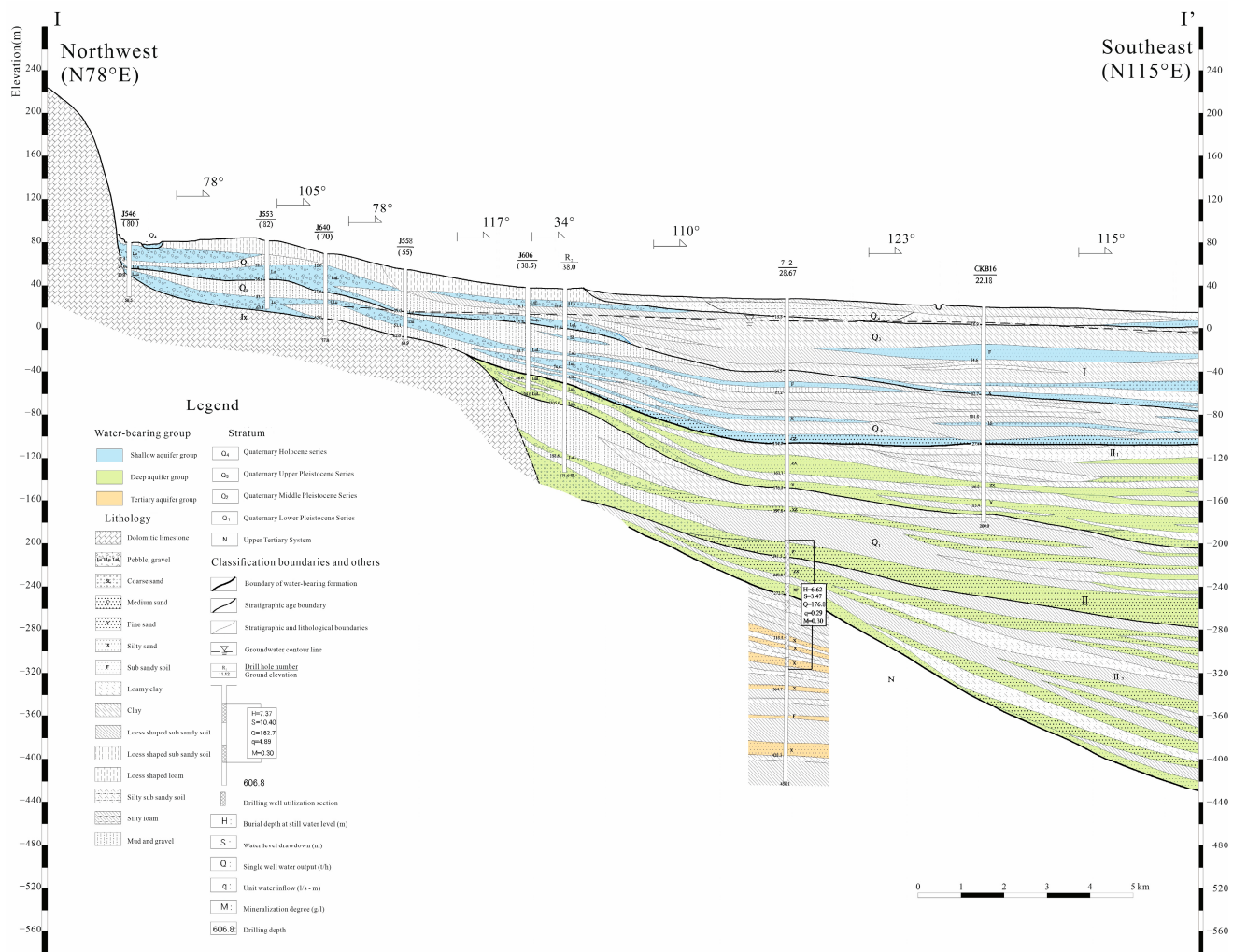


Figure 2. Geological profile and hydrological characteristics of the study area.

DNA extraction was performed using the OMEGA Soil DNA Kit (D5625-01) from Omega Bio-Tek, and the extracted DNA was tested. The DNA concentration was measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 260 nm and 280 nm, and the quality of the DNA was assessed using 1% agarose gel electrophoresis. The concentration of the DNA solution was adjusted, with the working DNA solution stored at 4 °C and the storage solution stored at −20 °C. The V4-V5 region of the bacterial 16S rRNA gene was amplified with PCR using the forward primer 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and the reverse primer 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). A 7 bp barcode specific to the samples was incorporated into the primers for multiplex sequencing. The PCR amplification products were purified using Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, the amplified products were pooled in equimolar amounts and sequenced on the Illumina MiSeq platform using the MiSeq Reagent Kit v3 for 2 × 250 bp paired-end sequencing.

2.3. Sequence and Data Analysis

Sequence data analyses were mainly performed using QIIME2 and R packages (v3.2.0). ASV-level alpha diversity indices, such as Chao1 richness estimator, Observed species, Shannon diversity index, Simpson index, Faith's PD, Pielou's evenness, and Good's coverage were calculated using the ASV table in QIIME2 and visualized as box plots. ASV-level

ranked abundance curves were generated to compare the richness and evenness of ASVs among samples. Beta diversity analysis was performed to investigate the structural variation of microbial communities across samples using Jaccard metrics, Bray–Curtis metrics and UniFrac distance metrics and visualized via principal coordinate analysis (PCoA). The statistical significance of alpha diversity variations among sample groups was assessed using Kruskal–Wallis rank-sum test and Dunn’s test for post-hoc analysis. For pairwise comparisons, the Kruskal–Wallis test was utilized, equating to the Wilcoxon test. Box plot components included interquartile range (IQR), median, max/min values, and outliers. Kruskal–Wallis p -values and Dunn’s test results were annotated for clarity [21]. In this study, we also employed the Random Forest algorithm, a decision-tree-based machine learning method known for its efficiency and effectiveness in analyzing non-linear data sets, such as our microbial community data [22]. Random Forest is a machine learning algorithm that employs a multitude of individual decision trees for prediction or analysis. This algorithm constructs an ensemble of decision trees, each trained on a random subset of the data and a random subset of features. It then amalgamates predictions or analytical outcomes from each tree to make a final prediction or analysis. In the computation process, the importance of individual variables is assessed by randomly permuting the values of each variable within the Random Forest model and comparing the error rates before and after permutation. The calculation of the importance of individual variables provides an in-depth method to understand the contribution of each variable to the model’s predictive performance, which is crucial for simplifying the model, enhancing interpretability, and guiding data-driven decision making. The analysis was conducted using the QI-ME2 (2019.4) software, specifically utilizing the ‘classify_samples_ncv’ function within the q2-sample-classifier for the Random Forest analysis and nested stratified cross-validation.

Water chemistry data and their correlation with species were compiled and analyzed using Excel 2007, SPSS 25.0, and Canoco5 for data collection, collation, and statistical analysis. Levins’ niche breadth was utilized to quantify the ecological niche width of microbial species within the groundwater communities. Niche breadth indicates the range of resources used by a species within an environment, reflecting its ecological versatility. The calculation of Levins’ niche breadth (B_i) was based on the following formula:

$$B_i = 1 / \sum_{j=1}^n (p_{ij})^2$$

where p_{ij} is the proportion of the i -th species using the j -th resource. Higher values of B_i suggest that a species has a broader niche, utilizing a wider array of resources, whereas lower values indicate specialization.

3. Results and Analysis

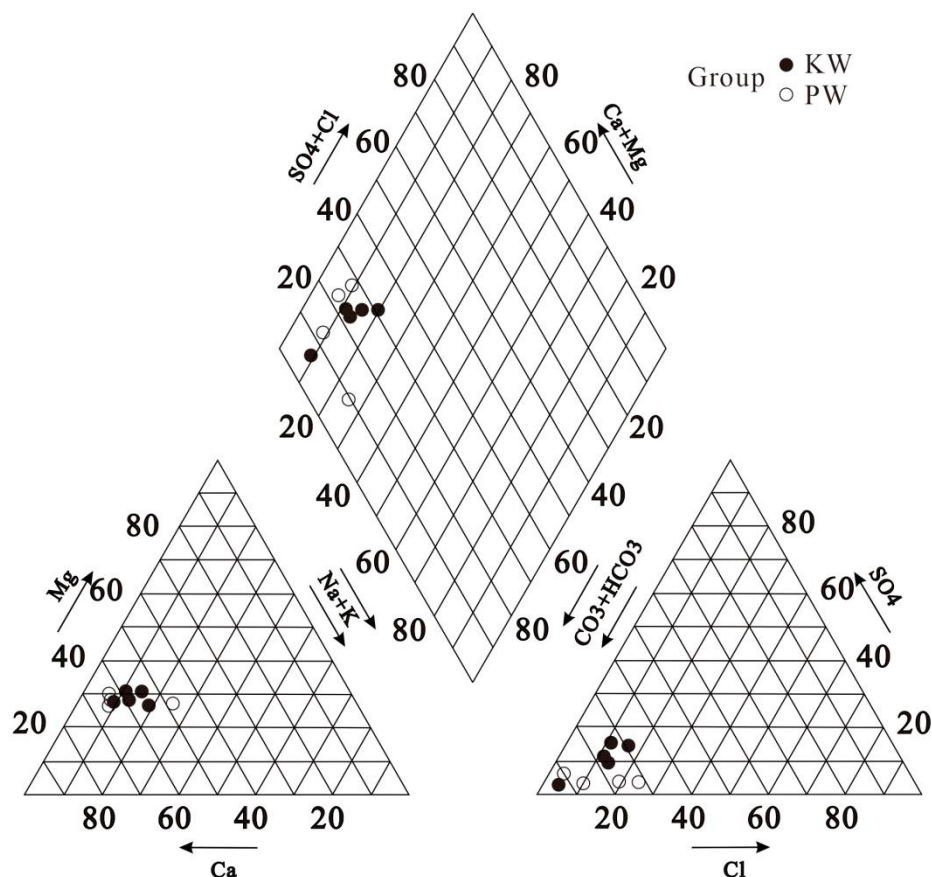
3.1. Hydrochemical Characteristics

According to the analysis, the collective groundwater in the study area is weakly alkaline, and there is not much difference in the basic characteristics of the porous water in the upper reaches and the karst water in the middle, with the pH ranging from 7.44 to 7.88 and an average value of 7.71 (Table 1). The TDS value is low, with the highest value being only 572.47 mg·L^{−1} and an average value of 408.99 mg·L^{−1}. In terms of composition, there are little differences in the cation content of the two, with high levels of Ca²⁺ and Mg²⁺, reflecting the lithological conditions of the strata in the area. The content of K⁺, Na⁺, and Mg²⁺ in porous water is slightly lower than that in karst water. In terms of anionic composition, the SO₄^{2−} and NO₃[−] levels in karst water are more than twice as high as in porous water, while Cl[−] and HCO₃[−] are relatively low. The hydrochemical type of all five karst water samples is represented as HCO₃–Ca·Mg; while in porous water samples, two types, HCO₃–Ca·Mg (BD6 & BD10) and HCO₃–Cl–Ca·Mg (BD8 & BD9), are present (Figure 3).

Table 1. Hydrochemical characteristics and major ion contents of groundwater in the Yimuquan area.

		pH	Ec ($\mu\text{S}/\text{cm}$)	ORP (mv)	DO (mg/L)	TDS (mg/L)	K ⁺ (mg/L)	Na ⁺ (mg/L)	Ca ²⁺ (mg/L)	Mg ²⁺ (mg/L)	Cl ⁻ (mg/L)	SO ₄ ²⁻ (mg/L)	HCO ₃ ⁻ (mg/L)	NO ₃ ⁻ (mg/L)	NO ₂ ⁻ (mg/L)	F ⁻ (mg/L)	H ₂ SiO ₃ (mg/L)
Karst Water (n = 5)	Avg	7.72	863.8	132.36	8.976	405.23	1.62	21.33	87.86	45.13	46.99	46.21	316.30	68.16	0.01	0.17	13.63
	Max	7.83	1032	163.8	9.9	487.21	3.44	35.75	106.92	54.79	74.87	71.07	344.68	109.75	0.04	0.30	15.56
	Min	7.49	550	96.2	7.46	277.03	0.84	8.58	64.10	28.40	11.73	8.96	300.23	8.64	0.00	0.11	12.35
Porous Water (n = 4)	Avg	7.70	812.75	166.1	6.92	413.69	1.22	17.24	93.64	43.75	70.67	18.36	362.19	26.42	0.01	0.17	18.29
	Max	7.88	1129	190.3	9.09	572.47	1.56	29.26	135.75	56.96	139.40	22.59	418.98	41.04	0.02	0.25	23.96
	Min	7.44	581	128.8	5.18	275.33	0.86	9.95	56.36	32.68	12.66	11.93	310.58	10.65	0.00	0.14	14.85

Note: ORP: Oxidation–Reduction Potential; DO: dissolved oxygen; TDS: total dissolved solids.

**Figure 3.** Piper diagram of groundwater in the Yimuquan area.

In addition, the redox environments of the two are different, with karst water having a higher amount of dissolved oxygen and slightly weaker oxidizing properties, and with porous water having a lower amount of dissolved oxygen and stronger oxidizing properties relative to karst water. This is consistent with the findings of C Q Qin et al., 2020 [23].

3.2. Microbial Community Structure

3.2.1. Diversity Analysis

In this study, 142,847–206,200 pairs of sequences were obtained, and 117,067–168,692 groups of high-quality sequences were analyzed and tested. From the rarefaction curves (Figures 4 and 5), it can be seen that, with the increase in drawdown depth, the rarefaction curves of Chao1 and Shannon indices gradually become noticeably flat. This suggests that the samples are sufficiently representative in terms of microbial quantity and community richness, and the sequencing depth has met the requirements to reflect the diversity and quantity of samples.

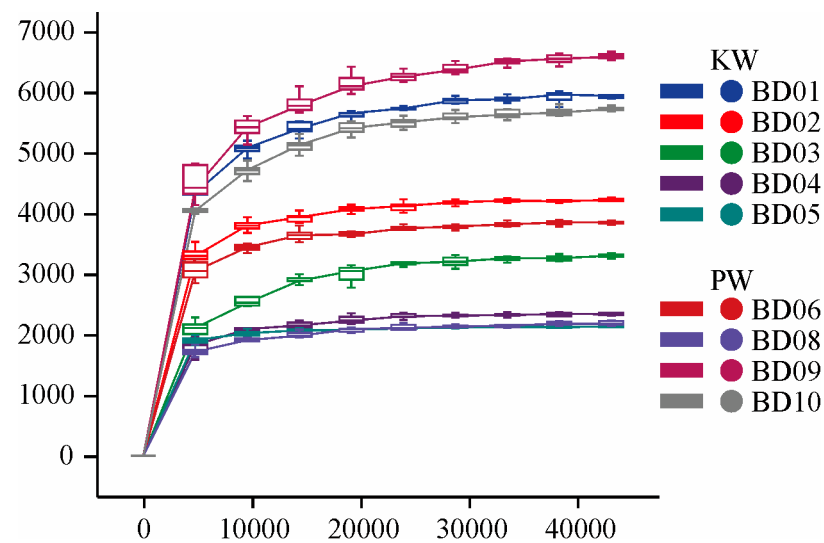


Figure 4. Chao1 exponential sparse curve.

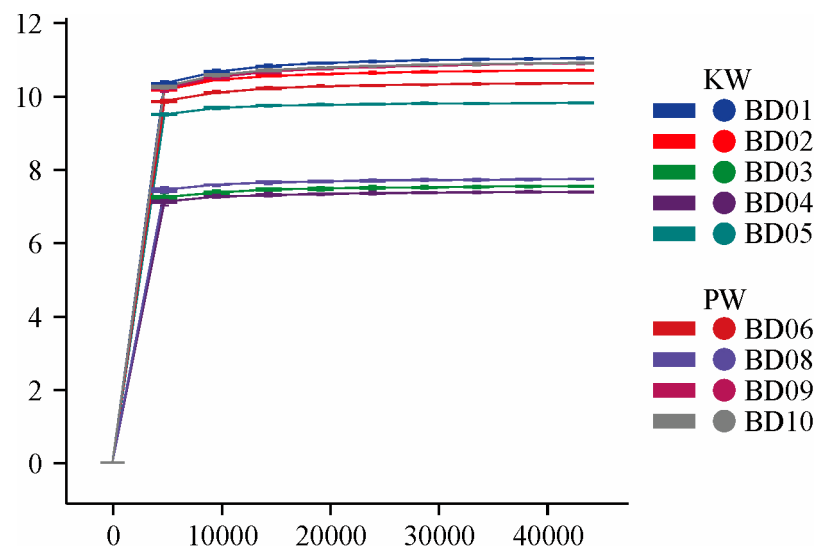


Figure 5. Shannon exponential sparse curve.

From the Alpha index box plot, it can be seen that the Goods_coverage index in the sequenced samples ranges from 0.969 to 0.998, reflecting a high and reliable sequencing depth (Figure 6). Upon scrutinizing the alpha diversity metrics in Figure 6, the data illustrate a nuanced ecological narrative between the KW group and the PW group. The Goods_coverage index, with values between 0.969 and 0.998, affirms a comprehensive sequencing depth. The Chao1 index reveals a higher mean species richness in the PW group (4586.25) as opposed to the KW group (3583.7), indicating a potentially more variable microbial community. The Shannon index, with close means across both groups, and the Simpson index reinforce the view of a similar overall diversity profile. The analysis of the Faith PD index, a widely recognized measure in microbial diversity studies, reveals that the samples from the Porous Water (PW) group exhibit a notably higher evolutionary-based diversity and species evenness in their microbiota [24]. This suggests a more complex and phylogenetically varied microbial community within the PW group, potentially indicative of a broader range of functional capabilities and ecological adaptability. While the alpha diversity analysis did not reveal statistically significant differences, the preliminary observations suggest distinctions in the microbial communities of the KW group and the PW group. These findings, though initial, lay the groundwork for further exploration of the ecological and functional dynamics underlying these two microbial ecosystems.

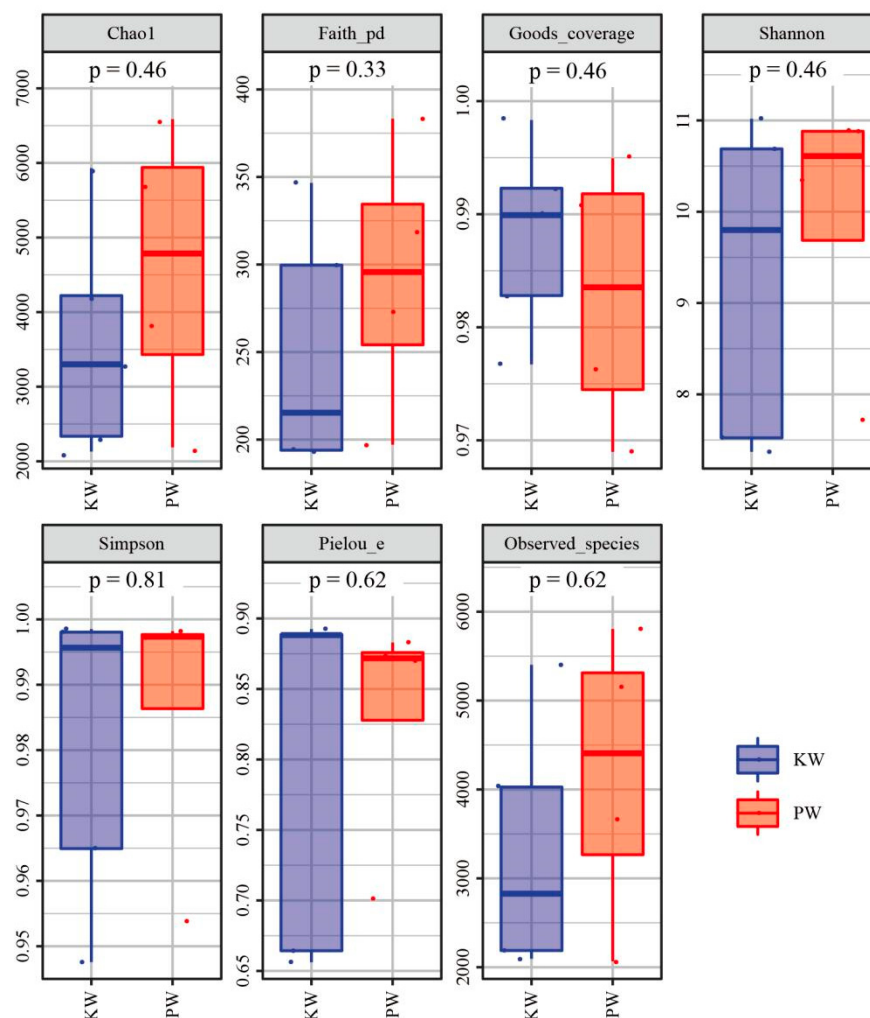


Figure 6. Box chart of Alpha index of groundwater microbial community. Note: p -values reflect the likelihood of the observed differences occurring by chance.

3.2.2. Microbial Composition Analysis

At the phylum level, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* are the bacterial species with the highest relative abundance in the groundwater in the study area, with all average contents above 6%, and single relative abundances accounting for 76.13~38.53%, 22.45~3.74%, and 22.67~2.49% of the total community, respectively (Figure 7 top). In addition, species with an average relative abundance greater than 3% include *Planctomycetes* (4.06%), *Chloroflexi* (4.04%), *Omnitrophicaeota* (3.95%), and *Acidobacteria* (3.26%). The groundwater in the study area moves from west (BD01) to east (BD10), resulting in gradient differences in water composition and underground environmental conditions, and the species composition changes accordingly [25]. In this process, the relative abundances of *Proteobacteria* and *Omnitrophicaeota* decreased, with a drop of 10.26% and 40.94%, respectively. Nonetheless, the relative abundances of *Actinobacteria*, *Bacteroidetes*, *Planctomycetes*, *Chloroflexi*, and *Acidobacteria* increased to varying degrees (20.93~95.52%). This growth in species relative abundance may imply a series of diversified ecological niches that have arisen or have been enhanced due to the gradual changes in water composition and underground environmental conditions. *Bacteroidetes* are famous for their ability to decompose polysaccharides, which helps in the degradation of organic matter [26]. Some studies have shown that *Planctomycetes* can degrade organic substances such as polysaccharides and polyphenols [27]. When groundwater flows from mountains to plains, it may change in the types and contents of organic matter, thereby benefiting the growth of *Planctomycetes*. *Chloroflexi* can also play a key role in the degradation of organic matter and

denitrification process [28]. *Acidobacteria* are involved in various biogeochemical processes such as organic matter degradation and nitrate reduction [29].

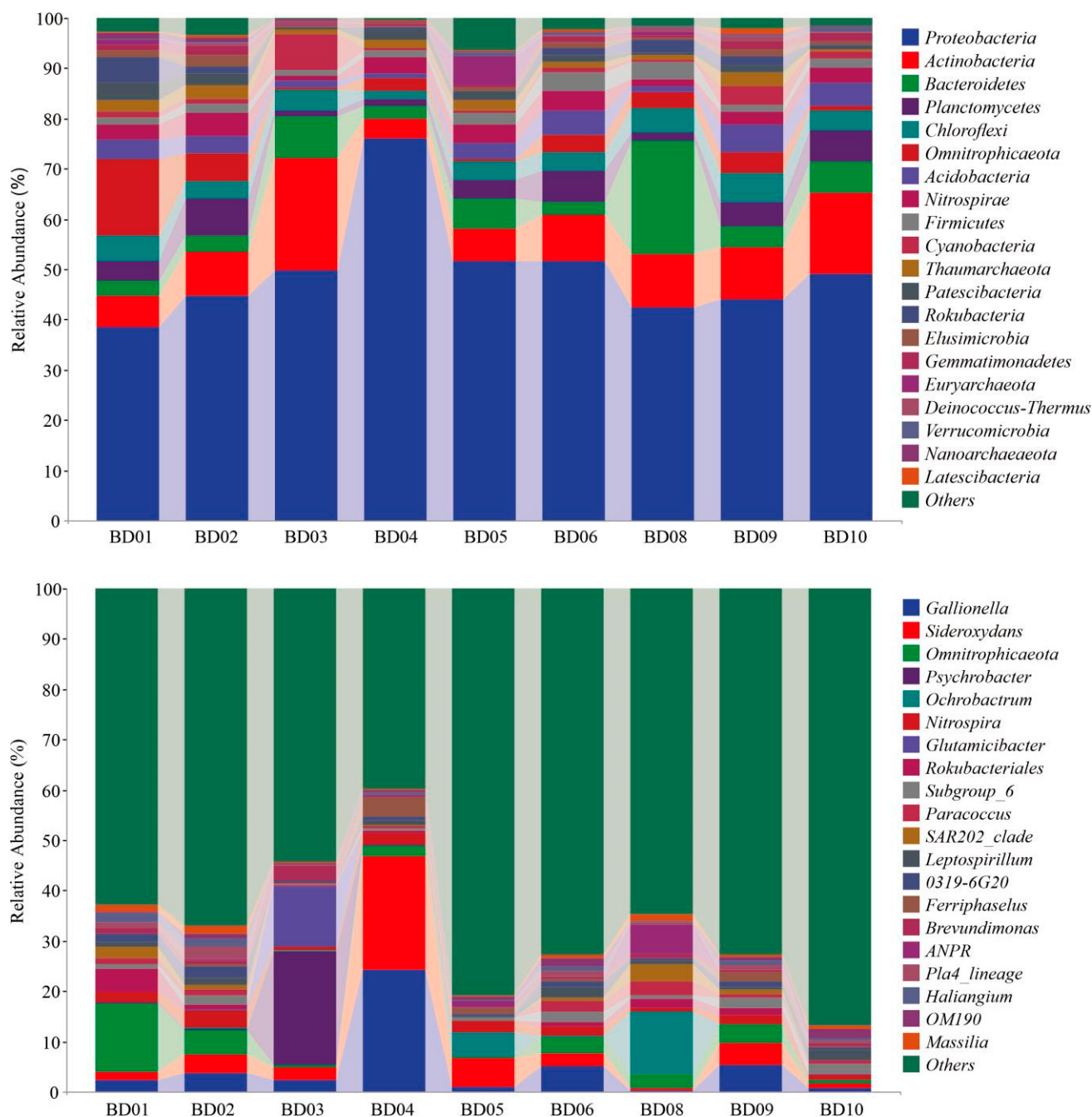


Figure 7. The phylum and genus composition of the groundwater microbial community.

The content of *Nitrospirae*, *Rokubacteria*, and *Elusimicrobia* are relatively uniform, with contents in the KW group of 3.06%, 1.42%, and 1.02%, respectively, and with contents in the PW group of 2.63%, 1.47%, and 0.9%. This finding indicates that they have potential adaptability to environmental changes, or that they are involved in basic biogeochemical processes that are common throughout the groundwater ecosystem. The ecological functions and relative abundance of *Rokubacteria* and *Elusimicrobia* in groundwater environments are relatively stable, which may be related to their physiological characteristics and ecological niches [30,31]. The similar relative abundance of *Nitrospirae* in karst water and porous water may imply that the nitrogen cycling processes in these two ground-

water environments are relatively similar. Furthermore, the compositional differences between the KW group and PW group are the most evident in *Actinobacteria*, *Bacteroidetes*, *Planctomycetes*, and *Acidobacteria* with the above four types of bacteria contributing 9.09% of the positive compositional difference to the PW group. This result indicates that they may be better adapted to the environmental conditions of the PW group.

At the genus level, the composition varies considerably among different samples, and there is no obvious dominant genus (Figure 7 bottom). The dominant genus of BD1 is *Omniitrophicaeota* (13.49%), that of BD3 is *Psychrobacter* (22.66%) and *Glutamicibacter* (12.14%), that of BD4 is *Gallionella* (24.26%) and *Sideroxydans* (22.47%), and that of BD8 is *Ochrobactrum* (12.38%). There is no obvious dominant genus in other samples. Overall, the microbial community structure of the KW group shows a more obvious dominant genus, while the microbial community structure of the PW group is more balanced. These differences may reflect the diversity of environmental conditions and microbial ecological adaptability, suggesting the complexity and uniqueness of microbial ecosystems in each groundwater sample [32–34].

3.2.3. Analysis of Differences in Microbial Community Composition

Based on the high dispersion at the genus level and no obvious dominant genus in the microbial taxa of the study area's groundwater, PCA analysis was carried out based on the species matrix at the phylum level [32]. As can be seen from the figure, PCo1 and PCo2 can represent 30.1% and 19.2% of the variation characteristics, respectively (Figure 8). The distribution of samples in the KW group is generally to the left, mainly concentrated in the area of the PCo1 axis from -0.2 to 0.05 . Due to the higher community relative abundance and diversity in the PW group compared to the KW group, all samples fall within the -0.1 to 0.1 range on the PCo2 axis.

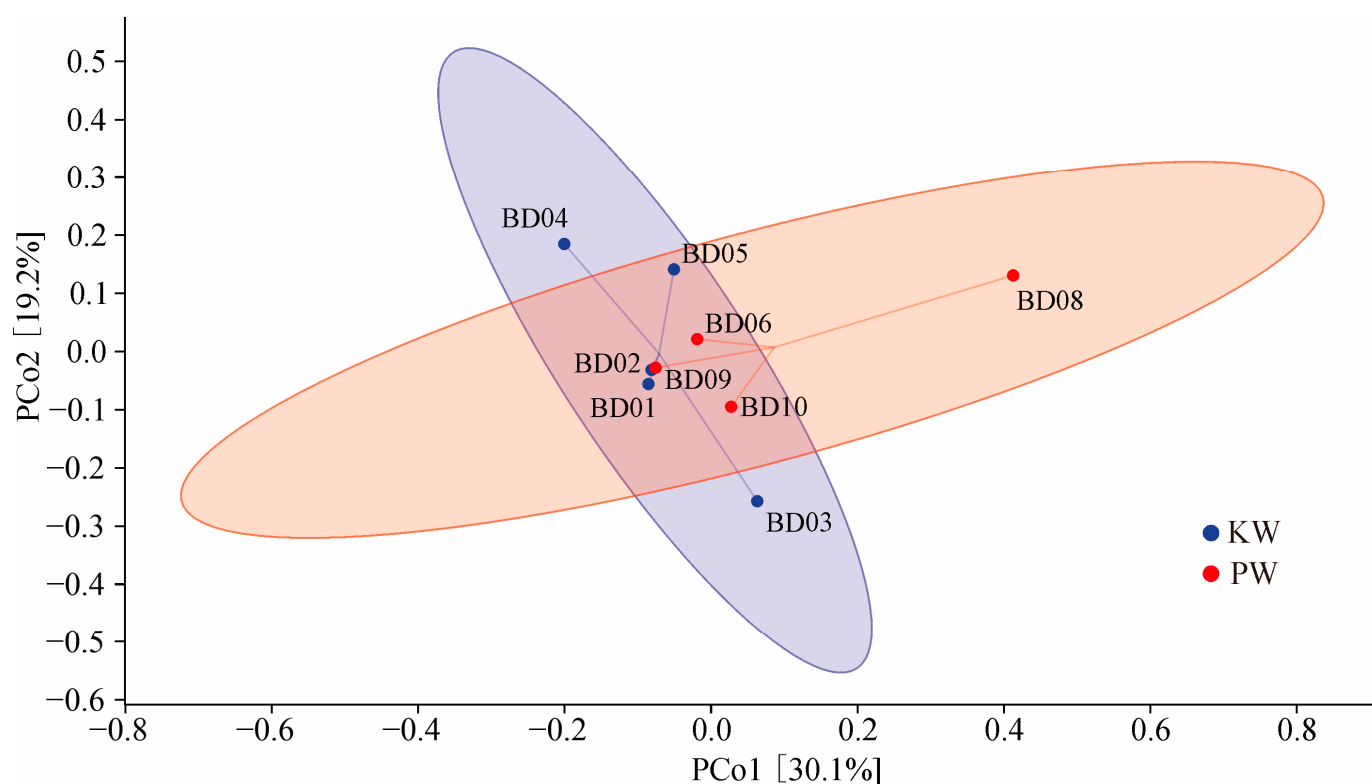


Figure 8. Principal coordinate analysis of samples in the Yimuquan area.

Given the nature of microbial community characteristic data, which often exhibit discontinuous or discrete distributions, this study employs heatmaps and Random Forest analyses (Figures 9 and 10). These methods leverage decision trees to create nonlinear classifiers for sample categorization, facilitating the identification of marker species [35,36]. Such approaches are instrumental in environmental microbial analysis, where they enable a nuanced exploration of complex data sets, highlighting key species that may serve as indicators of environmental conditions or ecological processes. From the heatmaps of community composition at the phylum level, it can be seen that *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Deinococcus-Thermus* are more abundant in the PW group, while *Omnitrophicaeota* and *Patescibacteria* are more indicative in the KW group (Figure 9). This is associated with environmental tolerance as well as dissolved oxygen concentration and organic matter content [37,38].

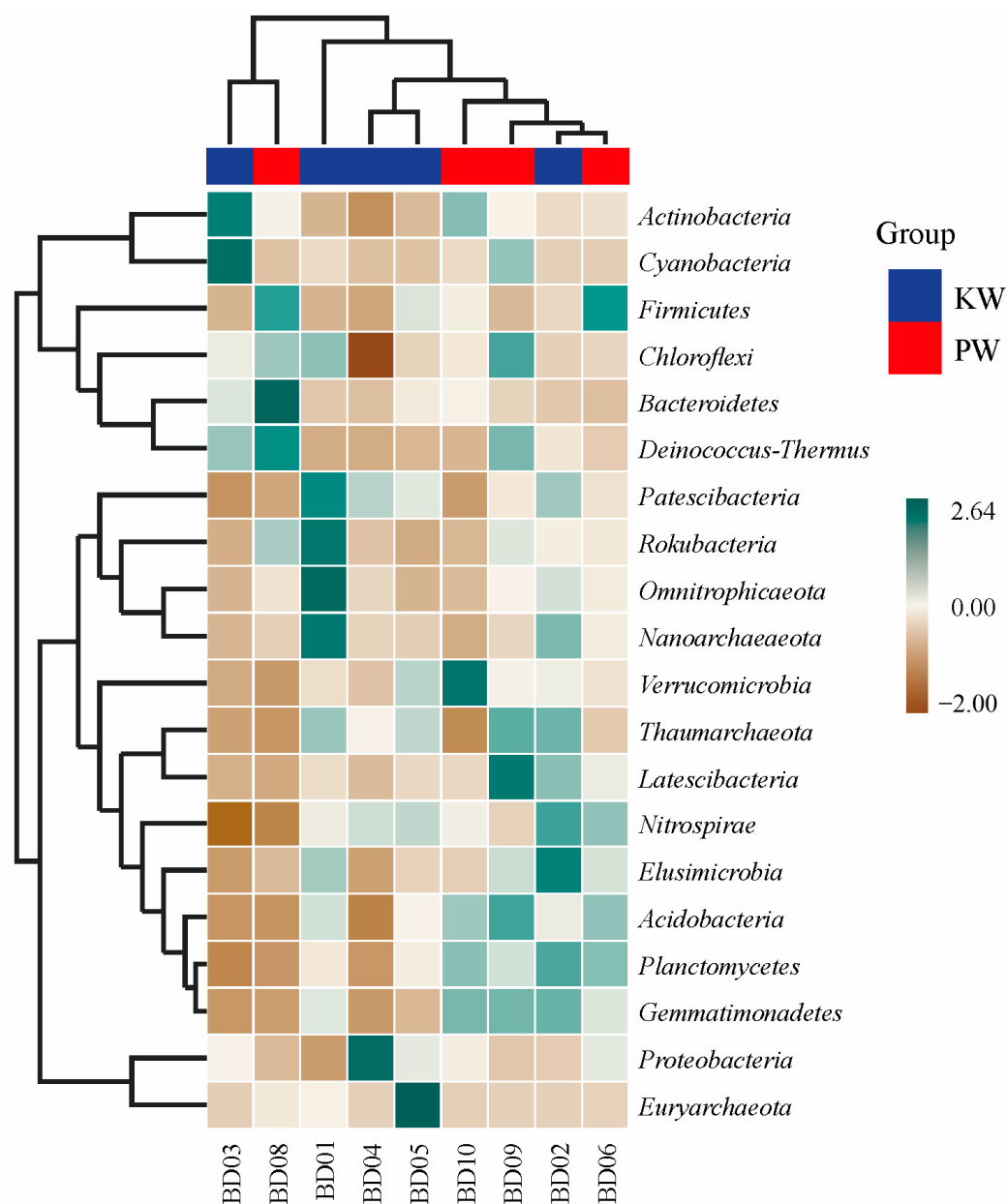


Figure 9. Heat map of samples on the phylum level.

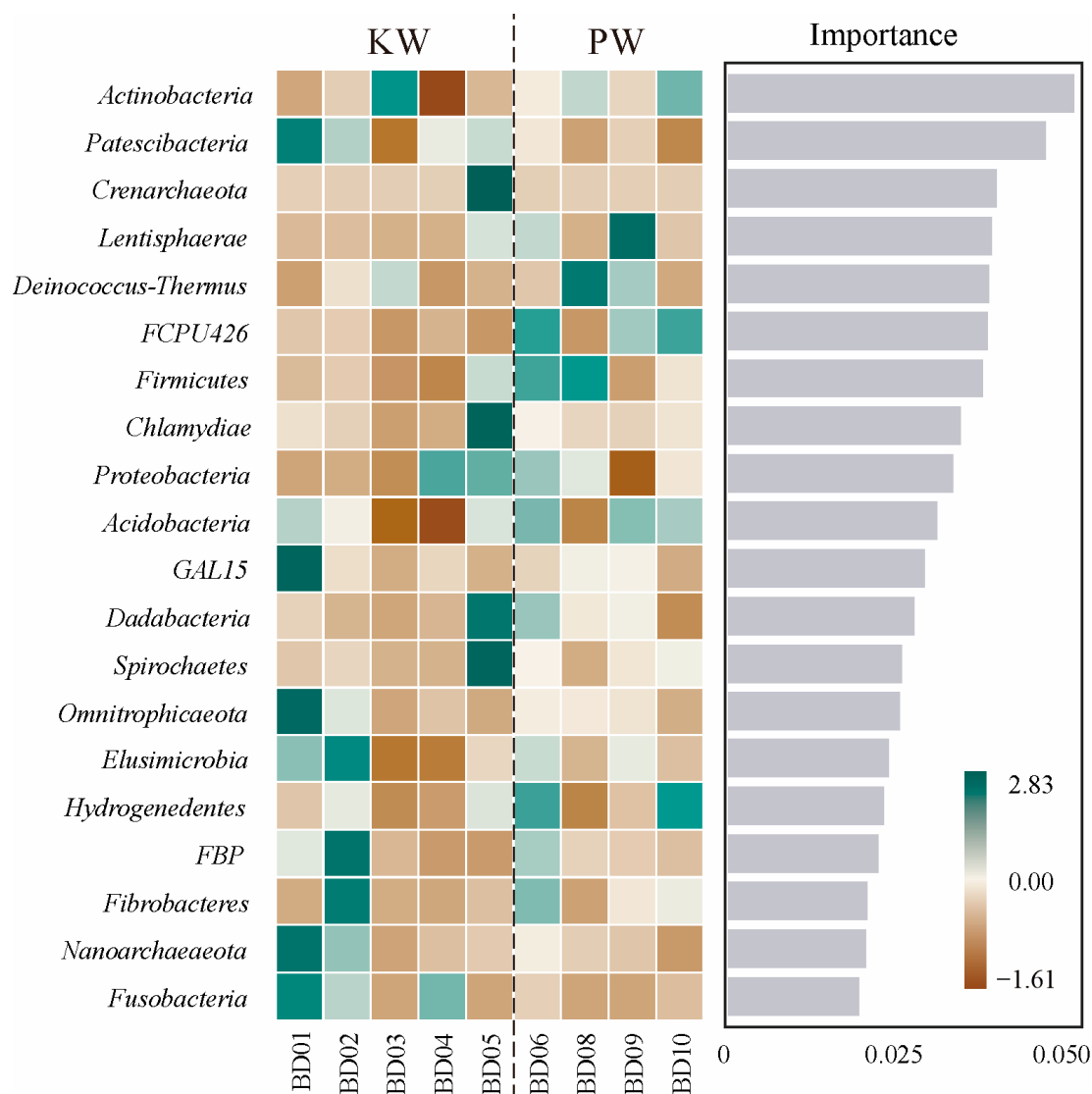


Figure 10. Assessment of bacterial phyla contributions through Random Forest analysis.

In the analysis of Figure 9, we discern the heatmap that illustrates the importance of various bacterial phyla in distinguishing between sample groups. The heatmap explicitly marks the phyla *Actinobacteria*, *Patescibacteria*, *Firmicutes*, and *Deinococcus-Thermus* with positive values, indicating their significant roles in differentiating sample clusters. The disparity in importance scores suggests the differential abundance or unique presence of these bacterial phyla within certain sample groups, which may reflect the ecological or environmental specificity of the sampled environments. Consequently, these bacteria may serve as pivotal indicators of the latent conditions within each environment. The pronounced significance of *Actinobacteria* and *Patescibacteria* could suggest their paramount role in defining microbial community structures. Notably, the biological traits of *Patescibacteria*, as documented by C J Castelle et al. (2017), indicate their robust survival in anoxic or microaerophilic conditions, utilizing a variety of organic substances for carbon and energy sources, and potentially justifying their marked importance in specific sample groups [39]. The analysis of metagenomic and metatranscriptomic sequences from microbial mats has revealed that *Patescibacteria*, a minor fraction, showed significant spatial and seasonal variations in composition, suggesting an active response to environmental conditions. The metagenome-assembled genomes (MAGs) of *Patescibacteria* indicated their potential importance in the ecosystem, despite their lower abundance [40]. Conversely, the adaptability of *Firmicutes* and *Deinococcus-Thermus* depends on nutrient-scarce and extreme environments,

which might suggest their distinguishing role in such conditions, in line with the findings of S Maloy (2013) and K S Makarova et al. (2001) [41,42]. Synthesizing these observations, it could be inferred that nutritional variations and dissolved oxygen levels are notable factors in differentiating microbial communities between the KW group and the PW group. This inference is in concordance with the broader research perspectives outlined by D C White et al. (1995) and K Manoj et al. (2016), underscoring the influence of environmental conditions on microbial diversity and distribution [43,44].

4. Discussion

Within the realm of hydrological characteristics, our analysis unveils a statistically significant correlation between the relative abundance of different microbial species and specific hydrological indicators. This correlation accentuates the intricate linkages between microbial diversity and hydrological parameters, highlighting the influence of environmental factors such as ion concentration, pH, and redox potential on microbial community dynamics within aquatic ecosystems. As can be seen from the correlation heatmap, in the KW environment, various ions in the water body support the relative abundance of species and to some extent support the development of species evolution and diversity. Among them, the higher ORP indicates that the level of oxidation is closely associated with the improvement of species diversity (Figure 11). The Chao1 diversity index is significantly positively correlated with ORP (Oxidation–Reduction Potential) and Na^+ , and significantly negatively correlated with pH. This implies that microbial relative abundance will be higher in environments with high oxidation–reduction potential and high Na^+ concentration. Species relative abundance will decrease in environments with low pH values. The Shannon diversity index is extremely positively correlated with ORP. This indicates that the diversity of microbial species will increase in environments with high ORP values. The PW environment is different, the overall relative abundance of species relatively prefers a weakly oxidizing environment, and the diversity and evolution level of species relatively depend on a high dissolved oxygen alkaline environment. The Shannon diversity index is significantly positively correlated with pH and DO (dissolved oxygen), and significantly negatively correlated with Ec (conductivity), Ca^{2+} , Cl^- , HCO_3^- , NO_3^- , and other factors. This implies that in environments with high pH values and high dissolved oxygen concentrations, the diversity of microbial communities will increase, while in environments with high conductivity and high concentrations of ions, such as Ca^{2+} , Cl^- , HCO_3^- , and NO_3^- , the diversity of microbial communities will decrease. The Pielou_e uniformity index is significantly positively correlated with pH and DO, and significantly negatively correlated with Ec, Ca^{2+} , Cl^- , HCO_3^- , NO_3^- , and other factors. This means that in environments with high pH values and high dissolved oxygen concentrations, the uniformity of microbial communities will increase, while in environments with high conductivity and high concentrations of ions, such as Ca^{2+} , Cl^- , HCO_3^- , and NO_3^- , the uniformity of microbial communities will decrease.

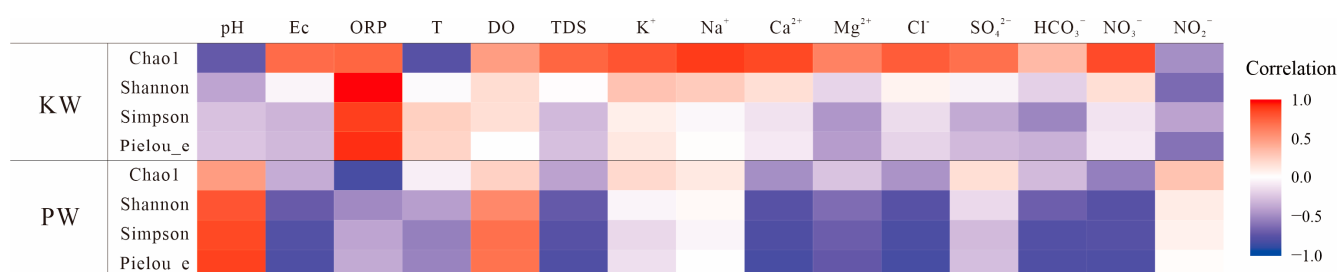


Figure 11. Heat map of correlation between environmental factors and alpha index.

In this study, Redundancy Analysis (RDA) was conducted on the top 20 taxa at the species level, aiming at elucidating their relationship with key hydrological and hydrochemical characteristics. The results, illustrated in Figures 12 and 13, offer detailed insight

on the specific interactions between these taxa and environmental factors. It is implicit in this analysis that the findings are taxon-specific, reflecting the unique ecological dynamics and environmental interactions of each species.

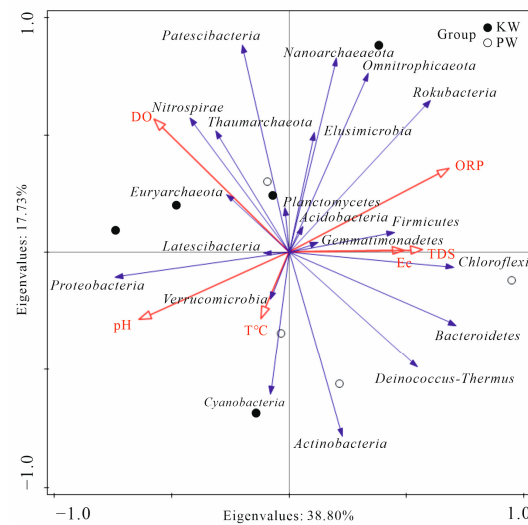


Figure 12. Redundancy Analysis (RDA) of the relationships between hydrological characteristics and bacterial phyla.

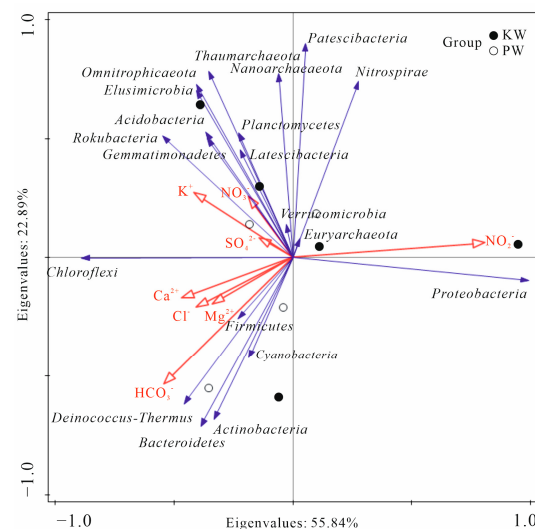


Figure 13. Redundancy Analysis (RDA) of the relationships between hydrochemical characteristics and bacterial phyla.

In our RDA within the KW group, significant insights emerged. *Proteobacteria* exhibited a positive correlation with NO_2^- , potentially linked to nitrate respiration capabilities in some of its members. *Actinobacteria*, *Cyanobacteria*, and *Deinococcus-Thermus* showed positive associations with HCO_3^- , Na^+ , Ca^{2+} , Mg^{2+} , and SO_4^{2-} , notably with HCO_3^- reaching significance, suggesting these ions might be crucial for their growth and metabolism. For instance, *Actinobacteria* are known to utilize bicarbonate ions as a carbon source [45]. *Omnitrophicaeota*, *Rokubacteria*, and *Nanoarchaeaeota*'s negatively correlated with pH and temperature and positively correlated with ORP, DO, and primary water ions, with significant associations with pH, ORP, K^+ , and Cu, indicating a preference for lower pH and temperatures and a requirement for higher oxidation–reduction potential and dissolved oxygen concentrations. In contrast, *Firmicutes*, *Euryarchaeota*, and *Verrucomicrobia* displayed negative correlations with Ec, DO, and main ion content. Nevertheless, *Firmicutes*, *Euryarchaeota*, and *Verrucomicrobia* showed positive correlations with ORP, temperature, F^- ,

silicic acid, and Fe content, as well as significant correlations with Ec, Mg^{2+} , Cl^- , NO_3^- , F^- , silicic acid, and Fe, suggesting an adaptation to environments rich in these elements. Lastly, *Planctomycetes*, *Nitrospirae*, *Thaumarchaeota*, and *Latescibacteria* were positively correlated with ORP and silicic acid, while negatively correlated with Ba, Cr, and the main ions in the water body. Additionally, *Planctomycetes*, *Nitrospirae*, *Thaumarchaeota*, and *Latescibacteria* showed significant correlations with ORP, Ba, and Cr. This indicates a stronger survival ability in high oxidation–reduction potential and silicic acid environments, but a possible sensitivity to elements like Ba and Cr.

In the PW group, *Proteobacteria*, *Planctomycetes*, *Acidobacteria*, and *Nitrospirae* were positively correlated with pH, DO, and F^- , while negatively correlated with Ec, temperature, and other main ions. Among them, pH, HCO_3^- , Mg^{2+} , Ba, Co, etc., reached extremely significant levels, which implies that these microbes are adapted to high pH, high dissolved oxygen, and F^- environments, but are not adapted to high conductivity and temperature, or have difficulty surviving in environments with high main ion concentration. This finding is consistent with the study conducted by T M Lapara, et al. 2001 [46]. These microbes may also use these environmental factors as energy or nutrients through some special biochemical pathways. In addition, *Bacteroidetes*, *Chloroflexi*, *Omnitrophicaeota*, *Rokubacteria*, *Euryarchaeota*, and *Deinococcus-Thermus* were negatively correlated with pH, DO, and F^- , while positively correlated with Ec, Ba, Co, Sr, and the main ions in the water body. Among them, Ec, Ca^{2+} , Mg^{2+} , Cl^- , SO_4^{2-} , NO_3^- , Co, and Sr showed extremely significant correlations. This indicates that these microbes may be adapted to low pH, low dissolved oxygen, and low F^- environments and have stronger survival capabilities in high-conductivity and high-ion-concentration environments or can metabolize some substances in high ion environments [47]. Also, *Cyanobacteria*, *Thaumarchaeota*, and *Latescibacteria* were significantly negatively correlated with ORP while significantly positively correlated with NO_2^- . This may mean that these microbes are more likely to survive in environments with lower oxidation–reduction potential and can use NO_2^- as an energy source or nutrient [48].

The categorization of resources utilized by microbial communities, as detailed in Table 2, delineates the diversity of dissolved ions, organic compounds, and environmental variables in the groundwater ecosystem. This table crucially outlines the shifts in niche breadth and resource utilization patterns among key species such as *Omnitrophicaeota* and *Euryarchaeota*. For instance, *Omnitrophicaeota* demonstrates a noteworthy shift from a positive correlation to a negative correlation with basic hydrochemical ions when transitioning from karst to porous water. Similarly, *Euryarchaeota*'s response to environmental conductivity and ion concentrations like Ca^{2+} and Mg^{2+} reverses from karst to porous water. These changes underscore the dynamic nature of microbial interactions with their environment, highlighting a complex interplay between microbial ecology and hydrochemical factors. Detailed insights into these transformations, as provided in Table 2, are instrumental in understanding the ecological adaptability of these species and their roles in groundwater ecosystems. Among them, *Omnitrophicaeota* and *Euryarchaeota* have changed in their response to basic hydrochemical ions. The correlation of *Omnitrophicaeota* with K^+ , Na^+ , and SO_4^{2-} changed from a positive correlation in karst water to a negative correlation ($0.767 \rightarrow -0.81^*$). The correlation of *Euryarchaeota* with Ec, TDS, Ca^{2+} , Mg^{2+} , Cl^- , and NO_3^- changed from a negative correlation in karst water to a positive correlation ($-0.82^* \rightarrow 0.756$). The response changes of *Thaumarchaeota* and *Latescibacteria* are consistent, affecting the correlation with ORP ($0.803 \rightarrow -0.81^*$) and NO_2^- ($-0.347 \rightarrow 0.883^*$). These microbes have shown different ecological niche widths in different water body environments, reflecting their outstanding environmental adaptability [49]. Specifically, the ecological niche widths of *Omnitrophicaeota*, *Thaumarchaeota*, and *Euryarchaeota* in porous water significantly exceed that of those in karst water, implying that the porous water environment may have larger environmental fluctuations, forcing these microbes to expand their resource acquisition strategies [50]. Similarly, the resource selection of these microbes in different water bodies also showed differences, possibly due to the different competitive pressures they face. For example, *Omnitrophicaeota* mainly uses S5 and S6 resources in karst water, while it mainly

uses S4, S8, and S9 resources in porous water, which may be a response to the competitive pressure of other microbial communities in the environment [51]. Moreover, the variability in niche width and resource selection is a critical factor contributing to ecosystem diversity. Microbes with broader ecological niches demonstrate robust resilience in fluctuating environments, thereby reinforcing the stability and diversity of their ecosystems. Such adaptability is evident in species like *Omnitrophicaeota* and *Euryarchaeota*, which exhibits remarkable flexibility in responding to hydrochemical changes in groundwater environments. This flexibility not only highlights their ecological plasticity but also underscores their crucial role in sustaining ecological diversity amidst environmental perturbations. These insights into microbial adaptability offer a profound understanding of the complex interplay between microbial communities and their changing deepening, advancing our comprehension of microbial contributions to ecosystem resilience.

Table 2. Changes in phylum niche in karst water and porous water.

Phylum	Karst Water		Porous Water	
	Levins Niche	Types of Commonly Used Resources	Levins Niche	Types of Commonly Used Resources
<i>Omnitrophicaeota</i>	11.1427	S5 = 11.32%, S6 = 10.62%	8.6607	S4 = 11.65%, S8 = 13.92%, S9 = 17.23%
<i>Thaumarchaeota</i>	4.1453	S1 = 34.59%, S3 = 17.67%, S5 = 25.14%	6.0497	S9 = 32.35%
<i>Euryarchaeota</i>	3.3207	S3 = 26.77%, S4 = 34.46%, S5 = 32.99%	9.355	S4 = 11.69%, S6 = 12.36%, S8 = 12.34%
<i>Latescibacteria</i>	5.1979	S2 = 32.32%, S4 = 18.00%, S8 = 14.49%	3.1762	S3 = 21.15%, S10 = 50.18%

5. Conclusions

Through hydrochemical analysis, it was observed that the groundwater in the study area was weakly alkaline. Karst water and porous water exhibit distinct differences in terms of major ion composition, dissolved oxygen content, and redox properties, reflecting two distinctly different environments due to the variations in stratum lithology and redox conditions. The alpha diversity indices, encompassing the Chao1, Shannon, and Simpson indices, collectively depict the biotic richness and evenness within the Yimuquan Spring groundwater ecosystem. Notably, the Chao1 index underscores a higher species richness in the PW group, suggesting a more diverse microbial assembly. Concurrently, the Faith PD index points to a greater evolutionary diversity and evenness within the PW group, implying a more intricate microbial network with potentially broader ecological functions and adaptability to environmental gradients such as ion concentration variations. These insights into microbial diversity and distribution are pivotal in mapping the ecological functions of groundwater microbiota. The groundwater microbial community is mainly composed of *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, etc., and their relative abundance undergoes noticeable changes in the process of groundwater migration from west to east, which reflects the influence of water composition and underground environmental conditions. It appears that different bacterial groups may demonstrate different ecological adaptability to changes in specific environmental conditions, suggesting the complexity and uniqueness of the underground water microbial ecosystem. Utilizing heat map and Random Forest analysis, it was found that *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, etc. tend to be more abundant in the PW group, while *Omnitrophicaeota* and *Cyanobacteria* were more commonly found in the KW group, potentially relating to environmental tolerance, dissolved oxygen concentration, and organic matter content. The observed prevalence of *Cyanobacteria* in the KW group could indicate interactions between surface and groundwater, given that *Cyanobacteria*'s light-dependent nature suggests an origin from surface waters [52]. Their ability to adapt to a range of conditions, including environments with variable dissolved oxygen levels and organic content, further highlights their potential as indicators

of hydrological interactions. The existence of these indicator species suggests that the differences in nutritional conditions and dissolved oxygen content are the main factors shaping the microbial community structure variations in the KW group and PW group of groundwater. Principal Component Analysis (PCA) and Redundancy Analysis (RDA) indicate that there is a noticeable correlation between the relative abundance and diversity of microbial communities and the hydrological features of the environment they inhabit. These environmental features include redox potential, ion concentration, dissolved oxygen concentration, conductivity, and pH. Individual microbial species such as *Omniitrophicaeota*, *Euryarchaeota*, *Thaumarchaeota*, and *Latescibacteria* demonstrate adaptive shifts in different water environments, which further emphasizes the crucial role of microbes in responding to environmental changes and in maintaining ecological diversity.

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