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Microbial community composition and environmental response characteristics of typical brackish groundwater in the North China Plain

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A B S T R A C T

To reveal the microbial community composition of regional shallow porous brackish groundwater and its response characteristics to groundwater environment, the first and second aquifers in Taocheng District, Hengshui City were selected, and 10 groundwater source samples were collected for hydrochemical analysis and microbial 16S RNA gene V4–V5 regional sequencing. The results showed that the shallow brackish groundwater in the study area is weakly alkaline and has high ion content. The hydrochemical types are SO_4·Cl-Na·Mg type and HCO_3·Cl-Na·Mg type as a whole. The spatial zonation of the abundance and diversity of groundwater microorganisms is obvious. The number of endemic bacteria in groundwater from upstream, midstream to downstream is 11, 135 and 22 respectively, with a total of 22 bacteria. *Proteobacteria* is the most dominant in groundwater level (38.82%–86.88%), and there are obvious differences in different sections. At the genus level, the main dominant species in each group and sample are *Pseudomonas* and *Hydrogenophaga*. In terms of composition difference, *Pseudohongiella*, *Pseudorhodobacter* and *Limnohabitans* are the representatives of UR, MR and LR. On the whole, the composition of flora in groundwater in the study area is sensitive and closely related to hydrochemical processes. Species abundance is affected by alkaline and high salinity environmental indicators, while species diversity is related to depth and dissolved oxygen in weak reduction environment.

1. Introduction

Brackish water resources are widely distributed in the alluvial proluvial plain of the Yellow River and the alluvial lacustrine plain in the middle of the piedmont plain, accounting for 32.26% of the total water resources in the North China Plain. Hengshui is a typical city in the alluvial lacustrine plain in the middle of the Piedmont. In terms of water resources, the annual exploitation resources and exploitation resource modulus of shallow groundwater in this area is at a low level (79.9×10^3 m^3/km^2), far less than Shijiazhuang, Baoding, Xingtai and other cities in the west of Piedmont Plain (131.4×10^3–254.8×10^3 m^3/km^2). In terms of water resources types, the total amount of groundwater exploitation resources in Hengshui City is 704×10^6 m^3/a, including fresh water resources of 82×10^6 m^3/a, brackish water resources 553×10^6 m^3/a, salt water resources 69×10^6 m^3/a, brackish water accounts for 78.55% of the total resources. Meanwhile, the exploitation potential coefficient of brackish water (0.86) is also higher than that of fresh water (0.71), and fresh water has no exploitation potential (Qian Y et al., 2014; Qi JF et al., 2016). In terms of hydrological characteristics, the aquifer in the salt water area has shallow burial depth, small water volume and easy drainage. Almost all the drainage methods are evaporation and drainage, and the current mining capacity is limited. However, the saline water area bears the ecological functions of shallow groundwater infiltration evaporation regulation and underground space regulation and storage, and plays an important role in the research and protection of ecological environment (Sakihara TS et al., 2015; Liu SY and Wang HQ,

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2016). Groundwater microorganism is one of the key indexes to measure the function of underground ecological space and observe the changes of environmental conditions (Liu YC et al., 2021). Existing researches use numerical simulation method to predict the microbial function and action process, so as to restore the biogeochemical process in groundwater environment from a certain angle (Shapiro B et al., 2018; Valsala R and Govindarajan SK, 2019; Shi ZQ et al., 2020).

Therefore, the study on the relationship between microbial group characteristics and environment in the study area is conducive to realize the multiple evaluation of the natural environment, improve the biochemical process of underground environment, and provide guidance for groundwater resources protection and ecological restoration. The shallow groundwater in the study area with gentle hydraulic gradient and single hydrogeological process is very suitable for the study of geological microorganisms.

2. Materials and methods

2.1. Overview of the study area

Taocheng District is subordinate to Hengshui City, Hebei Province, with longitude and latitude of 115°25′01″–115°51′12″E and 37°36′10″–37°49′55″N. It belongs to continental monsoon climate, with a total area of 951 km^2 (Fig. 1). Affected by sedimentary factors, the landform belongs to the slightly inclined flat land in the transition from gentle hill to depression. In terms of structural and hydrological conditions, since the late Yanshan movement, the study area has deposited extremely thick Tertiary (N) and Quaternary (Q) sedimentary accumulation strata. The Tertiary strata constitute the basement of the area, while the Quaternary strata are formed by the diluvium and alluvium of the river. Its thickness and distribution direction are mainly controlled and affected by the basement structure, paleoclimate and paleogeography (Li S et al., 2016; Zhang ZG et al., 2018). The shallow groundwater in Hengshui area is located at the bottom interface of shallow I aquifer, with a depth of 40–60 m, corresponding to Holocene Qh stratum, and its recharge source is atmospheric precipitation. Due to the long-term flooding and diversion of rivers in the area, sediments are staggered and form many micro landforms, but the overall fluctuation of surface morphology is not obvious, and the lateral runoff is very small (Fig. 2). The I aquifer in the central plain area is a multi-channel zone. The lithology is mainly fine sand and silty sand layer distributed in a strip in a NE direction. The general thickness is 10–30 m, the hydraulic conductivity coefficient is 100–300 m/d, and the unit water yield of a single well is 5–10 m^3/m per hour. The hydrochemical type is mainly $Cl·SO_4$-Na water, and the mineralization degree is mostly greater than 1 g/L, which has been used in industry and agriculture for decades (Zhang ZG et al., 2018; Liu B et al., 2019).

2.2. Sample collection

The samples collected in this study were shallow groundwater samples. A total of 10 groups were collected in December 2020. The source was national, local and factory

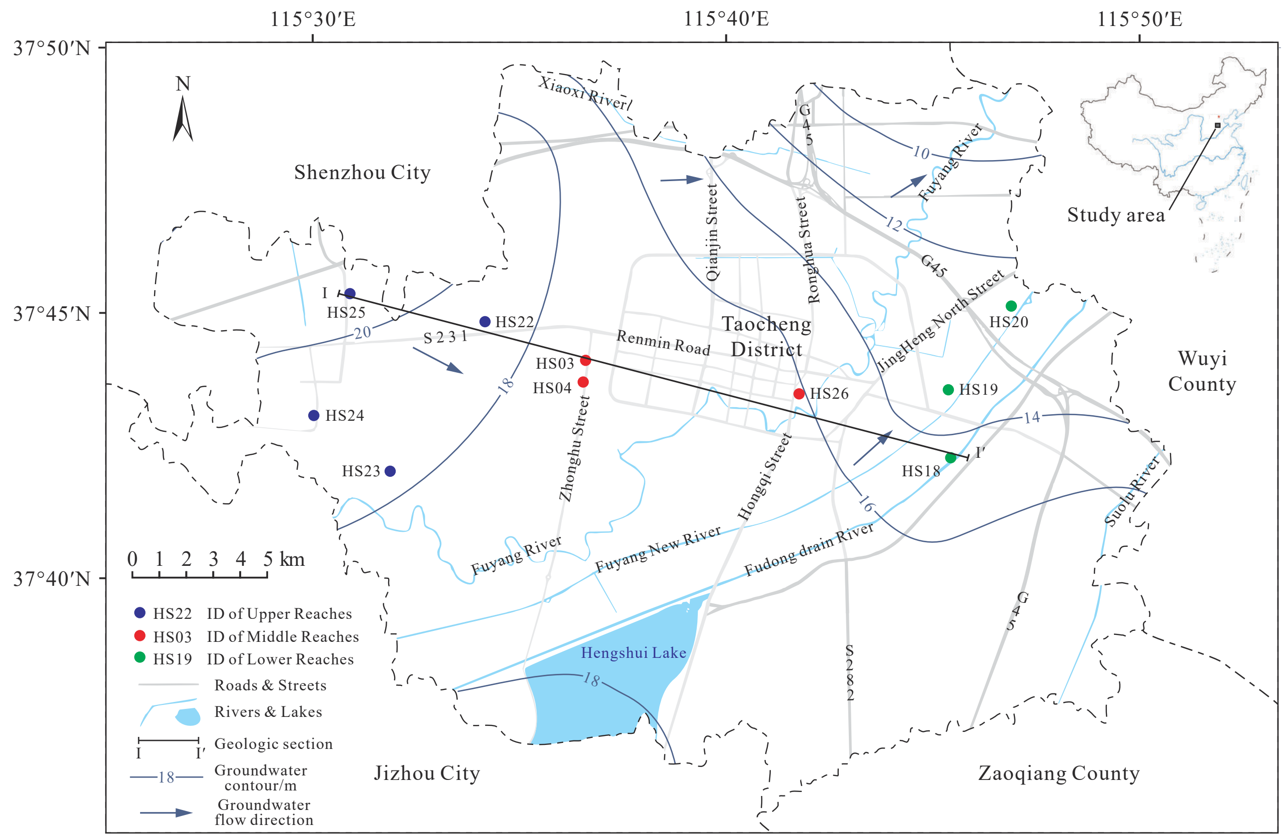

Fig. 1. Geographical location and distribution of sampling points.

monitoring wells. HS03 and HS04 were national monitoring wells, HS26 was local monitoring well with a depth of 30–78.56 m. Others (HS18–HS25) were newly built, with a depth of 100 m. The depth of filter pipe was 10–83.1 m and water table during sampling was 4.47–18.67 m (Table 1). Before sampling, we started the pump to wash the well for 0.5–2 hours. After the water quality was clear and stable, samples were taken at the *in-situ* surface outlet above the submersible pump directly. Samples were collected in three parts without pipeline aeration and disinfection. Groundwater collection and

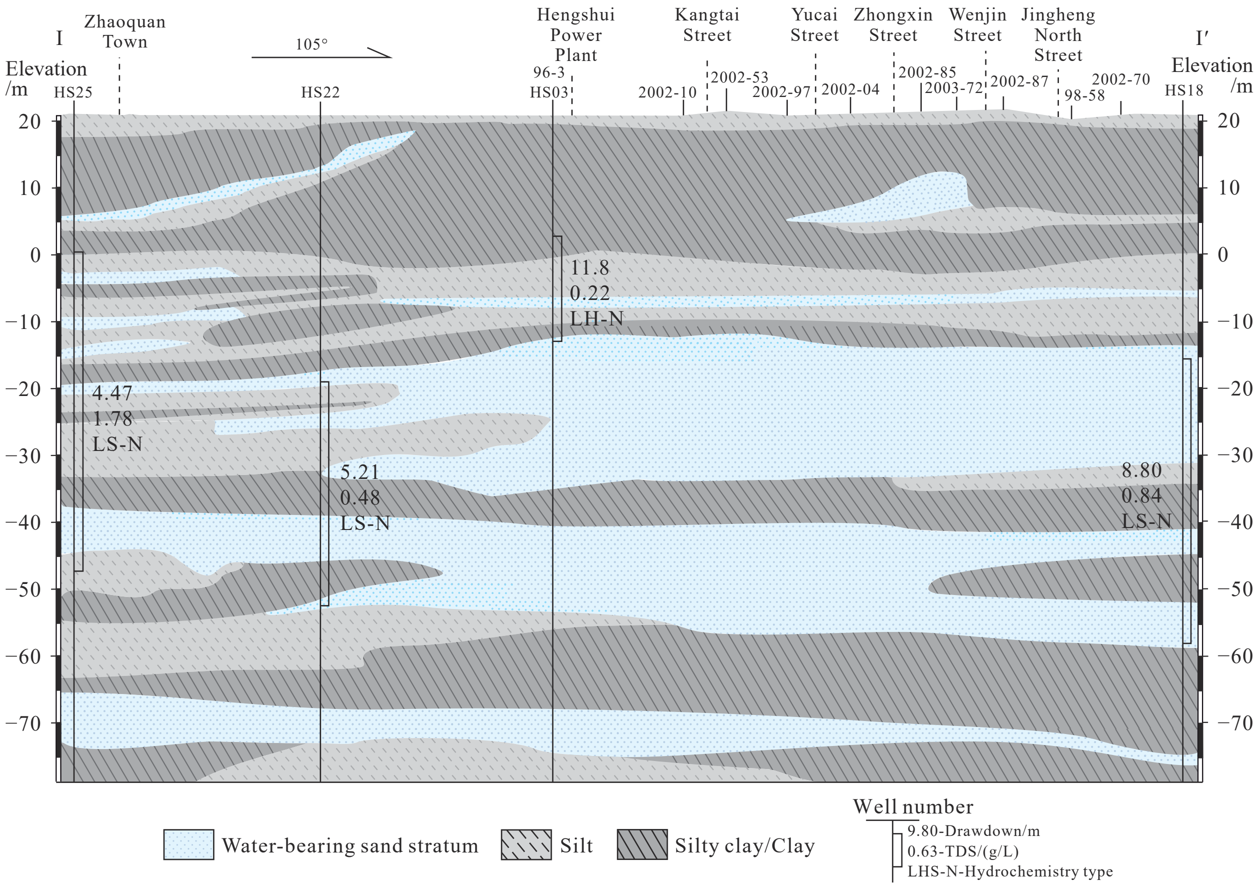

Fig. 2. Geological profile and hydrological characteristics of the study area.

Table 1. The location and environmental factors at the time of sampling.

Group	Station ID	Geographic coordinate	Depth of filter pipe/m	Water Tab./m	T/°C	pH	Ec/(μs/cm)	TDS/($\times10^{-6}$ mg/L)	ORP/mv	DO/%
Upper Reaches	HS22	115°34’11.4”E 37°44’50.5”N	42.8–76.3	5.21	14.7	7.78	6880	4780	75.8	6.93
	HS23	115°31’46.2”E 37°41’57.1”N	50.5–83.1	9.1	13.4	7.76	9930	6700	73	4.82
	HS24	115°30’01.8”E 37°43’03.0”N	24.9–36.5	4.9	13.4	7.51	8840	5580	70.8	5.77
	HS25	115°30’49.0”E 37°45’22.5”N	21.4–67.6	4.47	14.8	7.42	21750	17800	56.8	3.26
Middle Reaches	HS03	115°36’36.1”E 37°44’08.9”N	19.13–34.86	11.8	12.5	7.5	3670	2160	158.9	4.65
	HS04	115°36’33.0”E 37°43’38.4”N	10–30	12.79	11.5	7.17	2860	1880	188.9	4.96
	HS26	115°41’43.0”E 37°43’29.6”N	65.5–78.56	18.67	15.5	7.36	8300	5130	71.4	6.07
Lower Reaches	HS18	115°45’29.2”E 37°42’14.9”N	37.7–82.1	8.8	11.7	7.61	11540	8400	85.1	6.05
	HS19	115°45’12.5”E 37°43’28.0”N	22.4–77.2	7.3	12.8	7.42	12700	9420	88.3	6.77
	HS20	115°46’52.5”E 37°45’08.6”N	39.6–59.1	6.57	12.5	7.45	6160	4850	112.7	7.76

Notes: Ec–electrical conductivity; TDS–total dissolved solids; ORP–Oxidation-Reduction Potential; DO–dissolved oxygen.

preservation were carried out according to the requirements of "*Standard Test Method for Drinking Water*" (GB/T 5750-2006). For the first sample, the HQ40d Portable Dual Input Multi-parameter Meter was used to measure the water temperature, pH, EC (electrical conductivity) and ORP (oxidation-reduction potential) at the sampling site. The second sample was tested in the laboratory. The TDS was measured by dry weighing method. Cl^-, SO_4^{2-}, NO_3^-, F^- were determined by ICS-600 ion chromatograph, K^+, Na^+, Ca^{2+}, Mg^{2+} were determined by ICP-OES, HCO_3^- and CO_3^{2-} were determined by titration. For the third water sample, 1 L of water was collected and filtered with 0.22 μm water filter membrane by suction pump. It was then put into dry ice box for storage, and transported to Shanghai peseno Biotechnology Co., Ltd. for testing. After the completion of the hydrochemical tests, the reliability analysis was carried out according to the "*Standard Test Methods for Drinking Water Quality Analysis Quality Control*" (GB/T 5750.3-2006) standard issued by the Ministry of Health.

2.3. Sequence Analysis

Microbiome bioinformatics were performed with QIIME2 2019.4 with slight modification according to the official tutorials. Briefly, raw sequence data were demultiplexed using the demux plugin following by primers cutting with cutadapt plugin. Sequences were then quality filtered, denoised, merged and chimera removed using the DADA2 plugin. Non-singleton amplicon sequence variants (ASVs) were aligned with mafft and used to construct a phylogeny with fasttree2. Alpha-diversity metrics (Chao1, Observed species, Shannon, Simpson, Faith 's PD, Pielou 's evenness and Good 's coverage), beta diversity metrics, unweighted UniFrac, Jaccard distance, and Bray-Curtis dissimilarity) were estimated using the diversity plugin with samples were rarefied to 40666 sequences per sample. Taxonomy was assigned to ASVs using the classify-sklearn naïve Bayes taxonomy classifier in feature-classifier plugin against the SILVA Release 132 Database.

2.4. Bioinformatics and Statistical 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 unweighted pair-group method with arithmetic means (UPGMA) hierarchical clustering. Principal component analysis (PCA) was also conducted based on the genus-level compositional profiles. Venn diagram was generated to visualize the shared and unique genus among samples or groups using R package "Venn Diagram", based on the occurrence of genus across samples/groups regardless of their relative abundance. Random forest analysis was applied to discriminating the samples from different groups using QIIME2 with default settings.

Mapgis software was used to analyze spatial distribution characteristics. Excel 2007 and SPSS 25.0 were used for data collection and statistical analysis.

3. Analysis and discussion

3.1. Hydrochemical characteristics

The hydrochemical types in the study area are mainly $SO_4 \cdot Cl\text{-}Na \cdot Mg$ type and $HCO_3 \cdot Cl\text{-}Na \cdot Mg$ type (Fig. 3). In anions, the average contents of SO_4^{2-} and Cl^- are high, up to 1961.4 mg/L and 2300.7 mg/L respectively, followed by HCO_3^-, with the content between 282–1130 mg/L. In cations, the content of Na^+ is high, ranging from 475 to 3900 mg/L, with an average of 1464.8 mg/L, followed by Ca^{2+} and Mg^{2+}, reaching 317.87 mg/L and 463.6 mg/L respectively (Tables 1, 2). It can be seen that the hydrochemical content of pore water is high. In addition to dissolving some sediments, another reason is the compaction and release of water from the cohesive soil layers at the top and bottom of the aquifer caused by over-exploitation, and some soluble components (such as F^- etc.) of the ionic salt complex in the soil layer also enter the mining aquifer, resulting in corresponding changes in the chemical composition of groundwater.

Spatially, from the Piedmont to the middle of the plain, the hydrochemical types of the I and II aquifers are mainly from $HCO_3\text{-}Ca \cdot Mg$ type water and $HCO_3 \cdot Cl\text{-}Ca \cdot Mg$ type water to $Cl \cdot SO_4\text{-}Na \cdot Ca$ type water and $Cl \cdot SO_4\text{-}Na \cdot Mg$ type water. The ionic components of groundwater mainly come from minerals such as salt rock, calcite, dolomite, gypsum and silicate rock (Zhang ZG et al., 2018). According to the analysis of ion proportion coefficient, the groundwater dynamic conditions show obvious zoning in space. From the

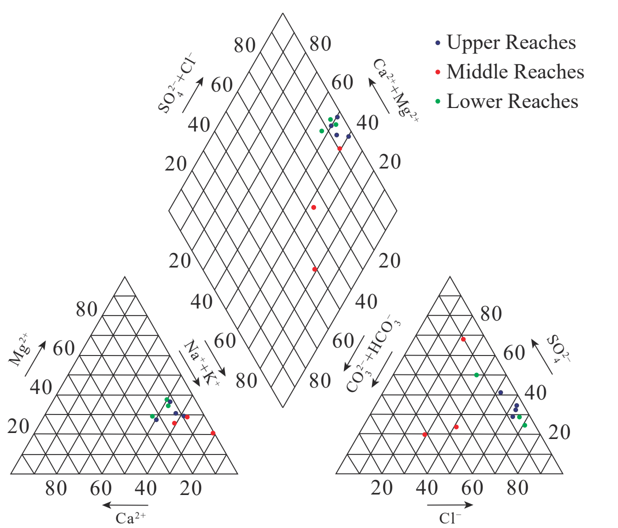

Fig. 3. Piper diagram of water type analysis based on the ionic composition.

piedmont plain to the central plain, the groundwater dynamic effect is weakened and the cation exchange adsorption is gradually enhanced (Hao QY et al., 2020).

3.2. Microbial community structure

3.2.1. Diversity analysis

In this study, 111457–135350 pairs of sequences were obtained, and 45528–82495 groups of high-quality sequences were analyzed and tested. The obtained samples are sufficiently representative in terms of microbial quantity and population richness, and the sequencing depth has met the needs of reflecting the diversity and quantity of samples.

The alpha index box chart shows that the coverage index in the sequenced samples is between 0.980–0.998, the sequencing depth is high, and the results are reliable (Fig. 4). Chao1, Observed_Species and faith_PD indexes show that MR group has obvious advantages in abundance level, and

Table 2. The main element of the water samples.

Group	Station ID	SO_4^{2-}	Cl^-	NO_3^-	HCO_3^-	K^+	Na^+	Ca^{2+}	Mg^{2+}	Hydrochemical types
Upper Reaches	HS22	1570	1490	1.98	330	6.45	974	368	286	SO_4·Cl-Na·Mg
	HS23	2000	2720	0.38	282	5.44	1660	240	422	SO_4·Cl-Na·Mg
	HS24	1390	2250	0	485	12.5	1260	275	364	SO_4·Cl-Na·Mg
	HS25	5150	6480	0	453	14.5	3900	807	1470	SO_4·Cl-Na·Mg
Middle Reaches	HS03	336	381	0	1130	2.75	729	10.6	102	HCO_3·Cl-Na·Mg
	HS04	350	445	0.04	655	1.11	475	54.1	116	HCO_3·Cl-Na·Mg
	HS26	2848	689	0	521	9.23	1450	335	329	SO_4-Na·Mg
Lower Reaches	HS18	1740	3610	0.85	410	8.52	1680	380	597	SO_4·Cl-Na·Mg
	HS19	2370	3950	0.14	478	9.48	1700	359	682	SO_4·Cl-Na·Mg
	HS20	1860	992	0.31	661	11.6	820	350	268	SO_4·Cl-Na·Mg

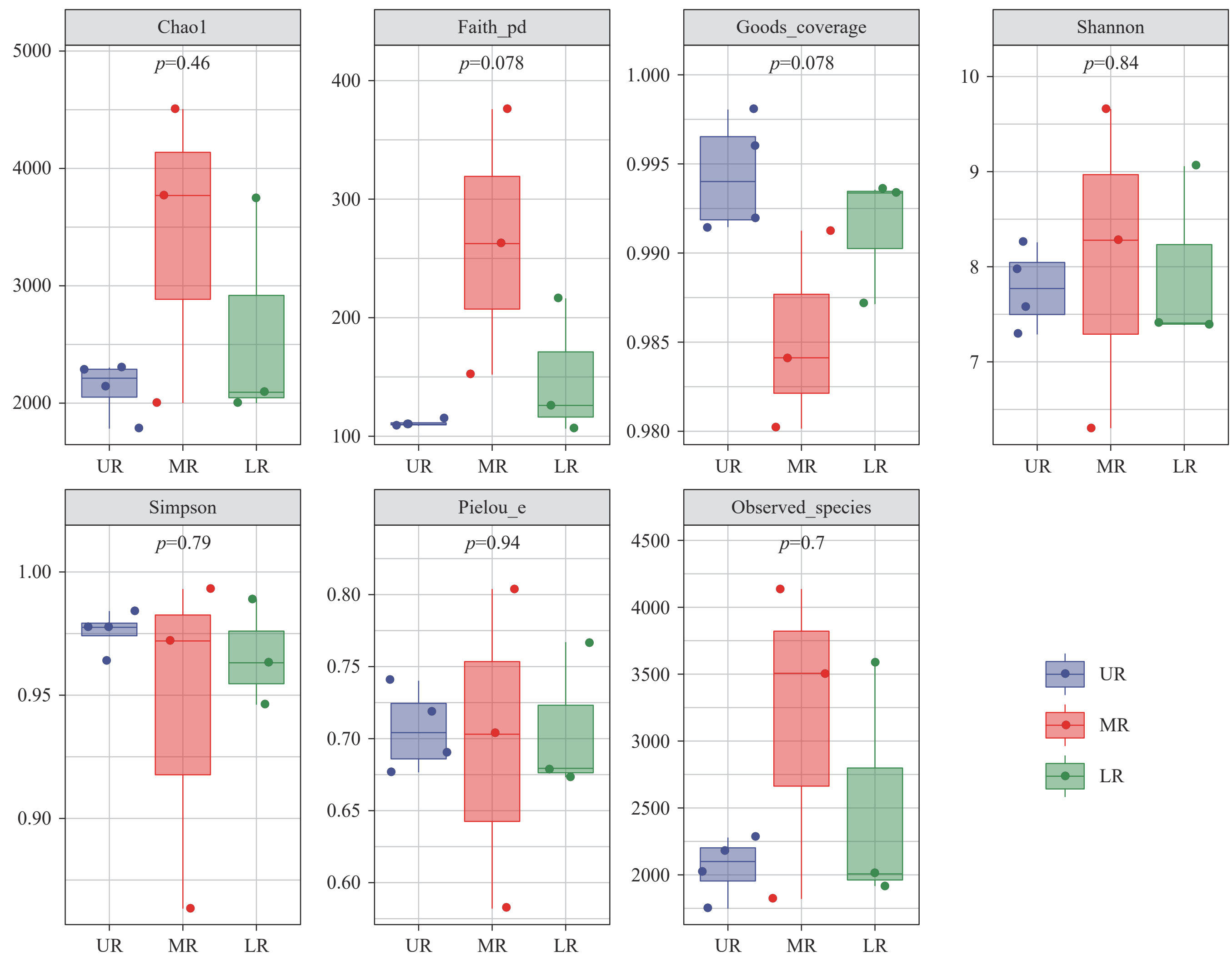

Fig. 4. Box chart of Alpha index of groundwater microbial community in brackish groundwater.

Simpson and Pielou_e indexes show that UR group has a slight advantage in species uniformity.

It can be seen from Table 3 that pH, EC, TDS and main ions (except HCO_3^-) are related to Chao1, observed species index and Faith_pd is negative correlation. Depth, DO, etc. are positively correlated with Shannon, Simpson and Pielou_e indexes, while ORP and HCO_3^- are on the contrary (Table 3). It can be seen that species abundance is affected by alkaline and high salinity environment, while species diversity is related to depth, dissolved oxygen and weak reduction environment.

3.2.2. Microbial composition analysis

At the phylum level, *Proteobacteria*, *Bacteroides*, *Epsilonbacteraeota*, *Actinobacteria*, *Firmicutes* and *Planctomycetes* are the most abundant bacterial species in the groundwater of the study area, with an average content of more than 2% (Fig. 5). In terms of specific composition, MR group is more complex. There are 11 species with an average content of more than 1% in MR group, which is much higher than UR (3 species) and LR group (6 species) (Figs. 5a, 5b and 5c). *Proteobacteria* and *Bacteroidetes* are the two species with the largest proportion, accounting for 38.82%–86.88% and 1.23%–32.05% respectively, accounting for 85% of the total composition. *Proteobacteria* is the largest branch of bacteria, including some pathogens and some nitrogen fixing bacteria. It plays an important role in nitrogen and phosphorus removal. It shows obvious inter group differences in groundwater in the study area. In UR and LR groups, the average content of *Proteobacteria* was 74.83%, the highest was 86.88%, while in MR group, the average content was only 57.25%, the lowest was 38.82%. *Bacteroidetes* accounted for a certain proportion (7.85%–19.32%) in the three groups and showed a positive correlation with EC, TDS, Na^+ and Cl^-. *Epsilonbacteraeota* content has little difference between groups, which is significantly correlated with aquifer depth and positively correlated with Fe^{3+} and Al^{3+}. Hou JL et al. (2018) showed that *Epsilonbacteraeota* plays a dominant role in the community of deep-sea chimneys. It has the metabolic potential of using reduced sulfur and hydrogen as energy autotrophic type, and also has a strong polymer transportation and secretion system. *Epsilonbacteraeota* can use hydrogen and reducing sulfide (hydrogen sulfide, thiosulfate) as energy, and fix CO_2 through rTCA pathway for chemoautotrophic growth. At the same time, the genome also has rich metal transport channels, secretory system and chemoflagellum movement system (Hou JL et al., 2018). *Actinobacteria*, *Firmicutes*, *Patescibacteria*, *Planctomycetes*, *Acidobacteria*, *Chloroflexi*, *Thaumarchaeota* and *Chlamydiae* are other important components of water microbial community in the study area. Their total proportion in the flow direction is 1.24% (UR), 26.98% (MR) and 5.53% (LR) respectively, which is related to the scope of regional socio-economic activities. Previous studies have shown that ammonium, nitrate, phosphorus, carbon and organic nutrients can be input into water bodies due to planting, aquaculture and the discharge of municipal sewage. These factors have been proved to be the direct cause of changes in water ecological structure (Chen Z et al., 2022; Zhang L et al., 2021; Mossa AW et al., 2017). Specifically, in the study area, the nutrient ions brought by planting and urban industrial activities have largely caused the changes in the microbial community structure of groundwater.

At the genus level, there are differences between groups and samples, but the main dominant species are *Pseudomonas* and *Hydrogenophaga*. They both belong to *Proteobacteria* and have aerobic denitrification function and have been proved to be involved in the denitrification process of groundwater (Ning Z et al., 2019). Represented by the species with large proportion and uniform distribution in each sample, the uniform dominant bacteria in UR group are *Pseudomonas* (17.74%), *Hydrogenophaga* (5.87%), *Flavobacterium* (7.3%), *Pseudohongiella* (3.26%). The dominant bacteria in MR group were *Pseudomonas* (0.43%), *Hydrogenophaga* (2.37%), *Sulfuritalea* (0.07%) and *Novosphingobium* (0.1%). The homogeneous dominant bacteria in LR group were *Pseudomonas* (3.64%), *Hydrogenophaga* (11.91%), *Limnohabitans* (13.08%), *Flavobacterium* (4.37%) and *Algoriphagus* (4.34%). *Pseudomonas* and *Flavobacterium* are common in the study of food spoilage and belong to aerobic bacteria (Negoro S et al., 1995).

It can be seen that there are certain similarities in composition and structure between UR group and LR group, and the composition proportion of species at all levels in MR group is more dispersed. At the phylum level, with the activity of the flow field, it shows a trend of first increasing and then decreasing. When *Proteobacteria* flows through the urban area and reaches the downstream, it increases by 2.69% on the basis of 75.71%, similar to *Actinobacteria* (+0.36%), *Firmicutes* (+1.22%), *Patescibacteria* (+2.61%), *Planctomycetes* (+0.02%), *Acidobacteria* (+0.03%), *Chloroflexi* (+0.02%), *Thaumarchaeota* (+0.01%), *Chlamydiae* (+0.02%), *Cyanobacteria* (+0.04%), *Nitrospirae* (+0.01%), *Omnitrophicaeota* (+0.03%), *Gemmatimonadetes*

Table 3. The Pearson correlation coefficient of microbial diversity indices and physicochemical parameters.

	pH	Depth	EC	TDS	ORP	DO	SO_4^{2-}	Cl^-	NO_3^-	HCO_3^-	K^+	Na^+	Ca^{2+}	Mg^{2+}
Chao1	−0.561	0.275	−0.242	−0.273	0.185	0.200	−0.068	−0.332	−0.264	−0.034	−0.251	−0.250	−0.252	−0.257
Shannon	−0.207	0.492	0.021	−0.030	−0.263	0.344	0.193	−0.079	0.050	−0.496	0.035	−0.038	0.080	−0.044
Simpson	−0.022	0.372	0.266	0.230	−0.504	0.321	0.378	0.210	0.175	−0.822**	0.367	0.179	0.390	0.203
Observ_s	−0.540	0.267	−0.216	−0.246	0.157	0.226	−0.046	−0.300	−0.222	−0.078	−0.230	−0.233	−0.217	−0.231
Faith_pd	−0.648*	0.290	−0.265	−0.290	0.240	0.080	−0.034	−0.406	−0.380	0.169	−0.230	−0.221	−0.250	−0.261
Pielou_e	−0.038	0.558	0.149	0.093	−0.450	0.361	0.309	0.047	0.170	−0.664*	0.172	0.077	0.233	0.066

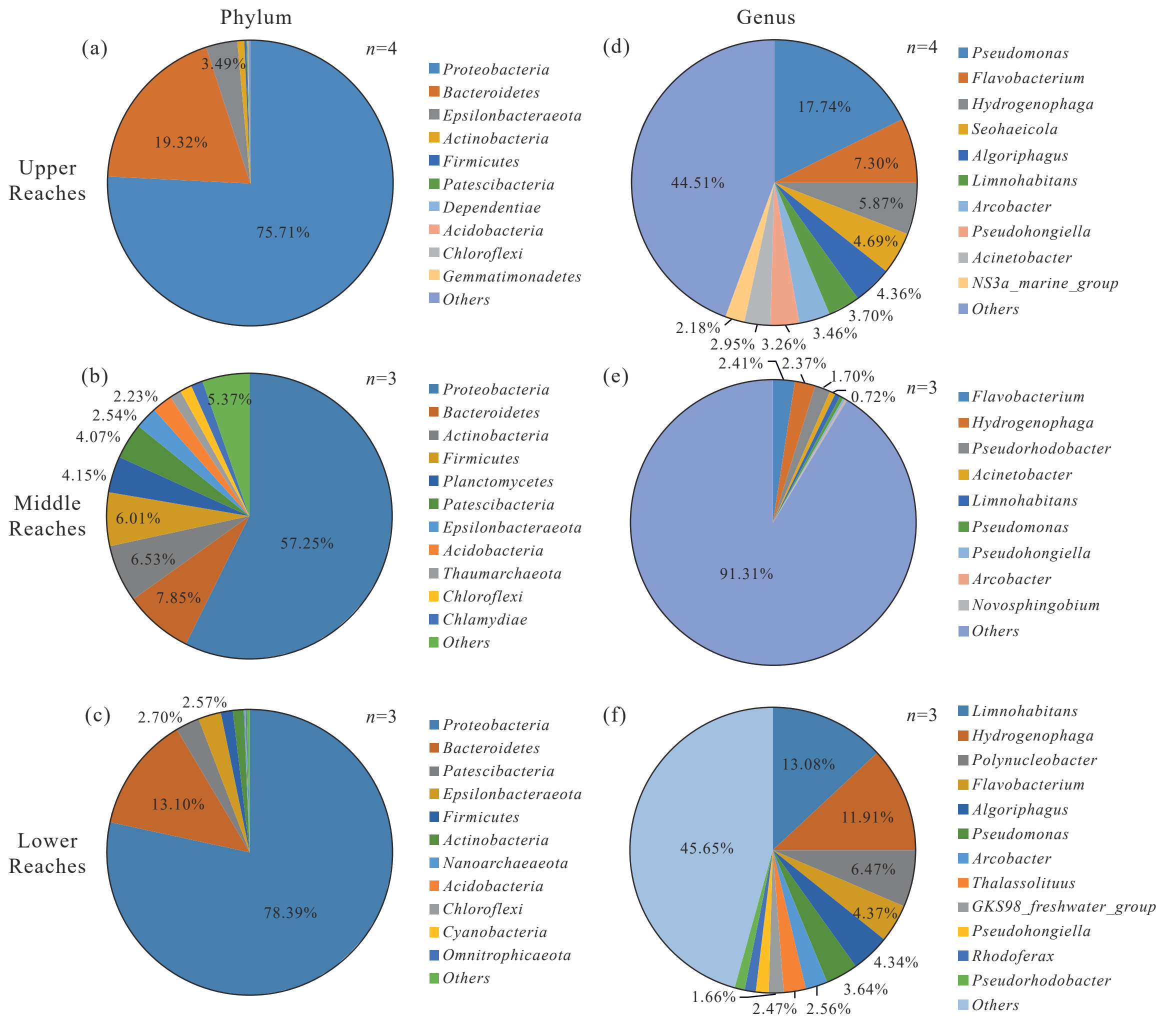

Fig. 5. The phylum and genus composition of the groundwater microbial community in brackish groundwater. a–phyla level bacterial composition in upper reaches; b–phyla level bacterial composition in middle reaches; c–phyla level bacterial composition in lower reaches; d–genus level bacterial composition in upper reaches; e–genus level bacterial composition in middle reaches; f–genus level bacterial composition in lower reaches

(+0.01%), *Verrucomicrobia* (+0.02%) and *Nanoarchaeaeota* (+0.05%). Some bacteria in *Planctomycetes* can use nitrite to oxidize ammonium ions to generate nitrogen under anaerobic conditions, so they are also called anaerobic ammonia oxidizing bacteria (Delmont TO et al., 2018). Nitrogen has an important impact on denitrifying bacteria community. Some studies have analyzed the impact of ion extracted nitrogen and weak acid extracted nitrogen on sediment denitrifying bacteria community. It is considered that these two kinds of nitrogen, as convertible nitrogen, are more conducive to the utilization of microorganisms (Chen ZY et al., 2022).

At the genus level, the increased species are *Limnohabitans* (+9.38%), *Algoriphagus* (+0.02%), *Polynucleobacter* (+5.79%), *Pseudorhodobacter* (+0.35%), *GKS98_freshwater_group* (+1.04%). The decreased species were *Pseudomonas* (−14.11%), *Flavobacterium* (−2.93%), *Seohaeicola* (−4.68%), *Pseudohongiella* (−1.84%), *Acinetobacter* (−2.11%), *Rhodoferax* (−0.34%), *RS62_marine_group* (−1.79%), *Sulfuritalea* (−1.00%), *Novosphingobium* (−0.48%), *Aquabacterium* (−0.93%). The flow process resulted in the increase of downstream NO_2^-, and the community as an energy source was improved. At the same time, the symbiotic *Algoriphagus* and *Pseudorhodobacter* also increased in varying degrees. On the contrary, the species partially dependent on NO_3^-, Zn^{2+}, Al^{3+} and alkaline environment decreased, and some symbiotic organisms such as *Seohaeicola* and *RS62_marine_Group* decreased with it.

3.2.3. Analysis of differences in microbial community composition

It can be seen in Fig. 6 that there are obvious differences in the composition of groundwater in UR, MR and LR groups. There are 29 genera in the groundwater of UR group and MR group, 41 genera in the groundwater of MR group and LR group, 40 genera in the groundwater of UR group and LR group, and 22 genera in the groundwater of the three groups (Fig. 6). There are 11, 135 and 22 endemic genera in UR, MR and LR groups respectively. Except that there are many endemic genera in MR group, the number of common genera among each group can account for 49.4%–69% of the total, it shows that the groundwater microbial community shows obvious continuity in the spatial scale of the study area, and the change of community structure can better reflect the influence of background environment and external factors. Combined with the regional groundwater flow field and surface social activities, the trend of microbial community in shallow groundwater in the study area is as follows: it starts from the environment with relative lack of materials in the upstream, then uses the materials produced by industry, agriculture and other human activities in the middle reaches, and species outbreak occurs, and finally decreases again in the downstream environment. In this process, there were 22 species in the three stages, and 18 species that disappeared in the middle reaches recovered when flowing through the downstream.

There are 22 species of bacteria in the shallow groundwater in the study area, of which more than 3% are 5 species, accounting for 30.52%, and the other 17 species account for 0.03%–0.99%, with an average of 0.35%. The composition is relatively balanced. Bacteria with a combined proportion of less than 1% are common in UR, MR and LR groups, as shown in Fig. 7. It can be seen that among the common bacteria, the bacteria with an average proportion of more than 3% are *unclassified_Burkholderiaceae* (9.21%), *Pseudomonas* (8.31%), *Hydrogenophaga* (6.63%), *unclassified_Rhodobacteraceae* (3.30%) and *Algoriphagus* (3.07%) were the relatively dominant genera. *Burkholderiaceae* is also an obligate aerobic thermophilic bacterium, widely distributed in soil and water environment, with a growth temperature of 30°C–37°C. In addition, *Pseudomonas* and *Hydrogenophaga* belong to *Proteobacteria*, which have the function of aerobic denitrification and are mainly involved in the denitrification process of groundwater (Huang Z et al., 2020). *Algoriphagus* belongs to *Bacteroidea*. Its characteristics of low temperature resistance and poor nutrition resistance are similar to *Pseudomonas*, and it has a certain degradation effect on organic matter (Sun QL, 2016).

PCA analysis of microbial groups in shallow groundwater at genus level was carried out. According to the genus level PCA analysis, PC1 and PC2 can represent 51.5% and 14.8%

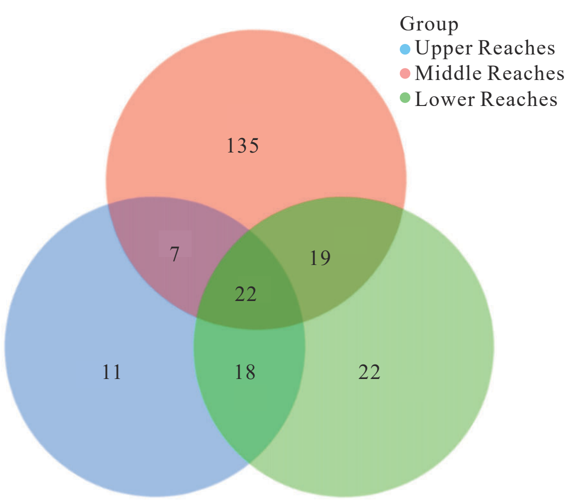

Fig. 6. Venn of brackish groundwater samples on genus level.

of the variation characteristics respectively (Fig. 8). MR samples are distributed on the positive axis of PC1 and the negative axis of PC2. UR and LR samples are distributed on the negative axis of PC1. LR is mainly positive on the PC2 axis and UR is mainly negative on the PC2 axis, mostly in the area of −0.04–−0.09.

Microbial community characteristic data used to show discontinuous distribution or even discrete characteristics, heat map established nonlinear sample classifier based on decision tree to find marker species, which is widely used in environmental microbial analysis (Robinson M et al., 2017). According to the thermal map analysis of community composition at the genus level, the population structure of microorganisms in shallow groundwater shows different characteristics with spatial changes (Fig. 9). Combined with the direction trend reflected in the groundwater flow field, *Pseudorhodobacter* increases and then decreases slowly. *Novosphingobium*, *NS3a_marine_group*, *Flavobacterium*, *Pseudohongiella*, *Pseudomonas*, *Sulfuritalea*, *Aquabacterium*, *Rhodoferax*, *Arcobacter*, *Herbaspirillum*, *Algoriphagus* increased after decrease, *Seohaeicola*, *Acinetobacter*, *RS62_*

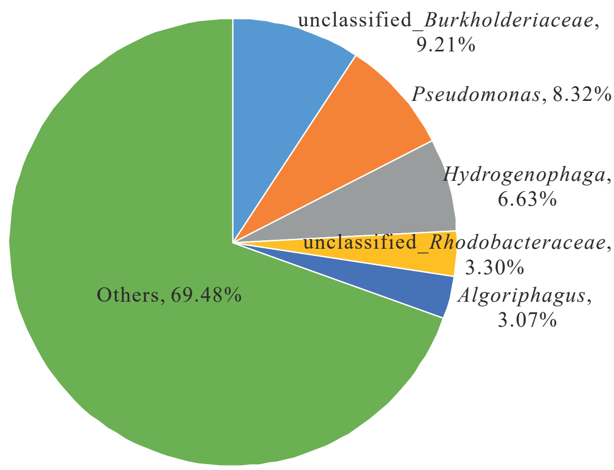

Fig. 7. Distribution of common genera of brackish groundwater samples.

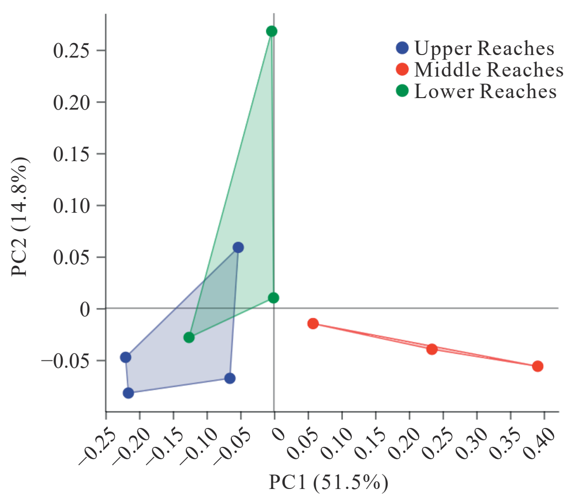

Fig. 8. Principal coordinate analysis of brackish groundwater samples at genus level.

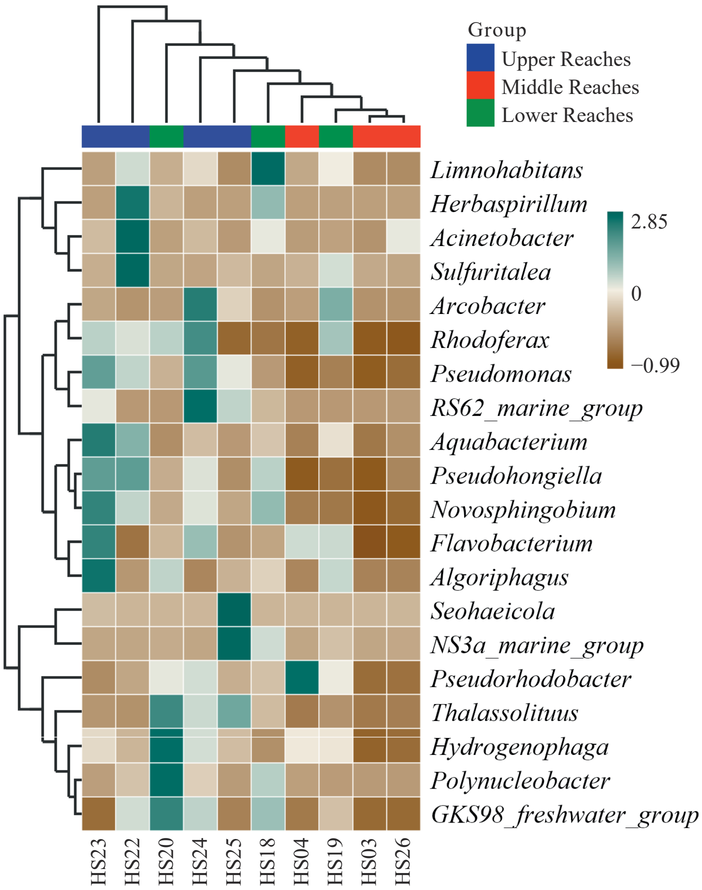

Fig. 9. Heat map of community structure of brackish groundwater samples on genus level.

marine_group recovered after decrease. *Polynucleobacter*, *Limnohabitans*, *Hydrogenophaga*, *GKS98_freshwater_group*, *Thalassolituus* reverses overload after reduction. Indicative, *Pseudorhodobacter* was the most abundant in MR group, while *Polynucleobacter*, *Limnohabitans*, *Hydrogenophaga* and *GKS98_freshwater_group* are the most abundant in LR group, while *Novosphingobium*, *NS3a_marine_group*, *Flavobacterium* and *Pseudohongiella* are prominent indicators of UR group.

3.3. Correlation Analysis between microbial community and environmental characteristics

Select the main environmental characteristic indicators, and conduct RDA redundancy analysis on the top 20 species in the abundance of phylum level and genus level in the sample, so as to intuitively show the relationship between each species and hydrological and hydrochemical characteristic factors (Figs. 10, 11).

In terms of hydrochemical characteristics, leaching process is the key process to change the hydrochemical characteristics of groundwater. A large amount of Ca^{2+} and SO_4^{2-} in groundwater in the study area come from the dissolution of fluorite and gypsum (Zhang ZG et al., 2018; Fuge R et al., 2019). At the phylum level, five of the six species with the highest relative abundance (except *Proteobacteria*) showed the same correlation in the relationship between them, and their abundance reached 78.8% of the remaining species, indicating that the main flora in the environment responded sensitively and closely to hydrochemical processes (Fig. 10a). *Proteobacteria* is positively correlated with pH and NO_2^- and may decompose C&N to supply other species. *Bacteroidetes* showed positive correlation with low valence anion and anion, significant in TDS, Ec, Cl^- and Na^+, and negative correlation with ORP, DO and HCO_3^-. *Epsilonbacteraeota* is positively correlated with HCO_3^- and high valence cations Fe^{3+} and Al^{3+}. *Actinobacteria* and *Firmicutes* are negatively correlated with pH and various main ions, and positively correlated with ORP, reflecting their resistance to alkaline environment and their need for environmental oxidation. At the genus level, *Pseudomonas* is positively correlated with pH and high valence ions, especially with Zn_2^+, but negatively correlated with ORP and HCO_3^-. *Hydrogenophaga* was positively correlated with DO and NO_2^-, and the correlation with NO_2^- was very significant.

In addition to environmental factors, symbiotic factors are also important. For example, *Limnohabitans*, *Flavobacterium* and *Algoriphagus* do not show obvious correlation with environmental indicators or other species. It is speculated that their material and energy sources are other species in the environment rather than the original environment itself.

In addition, the association between genus level species and hydrological and hydrochemical indicators is obvious, forming a relatively independent response group:

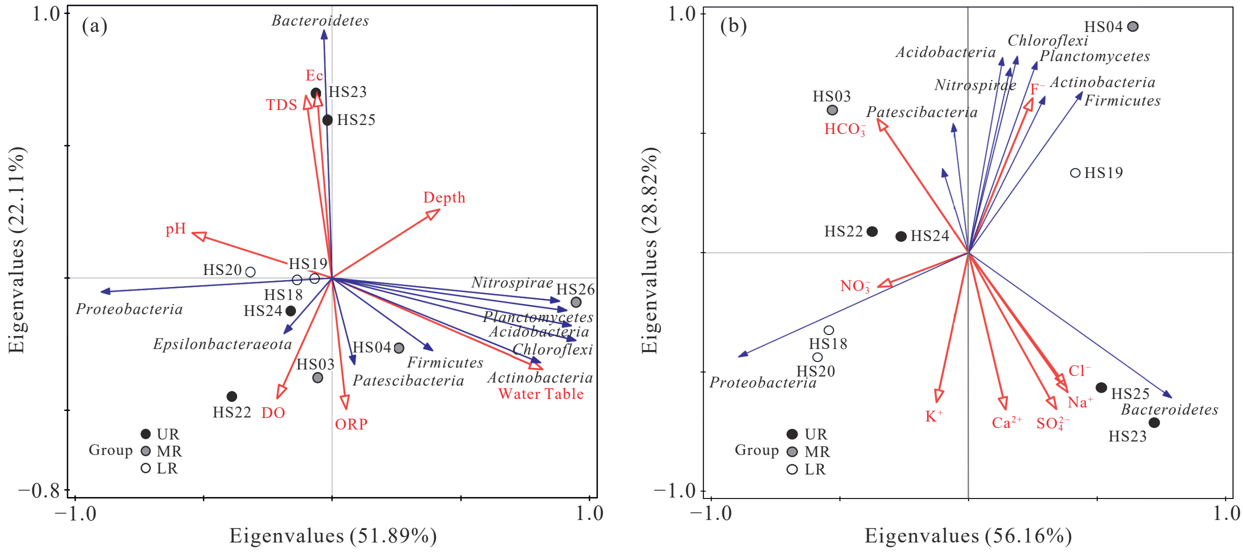

Fig. 10. RDA analysis on relationship between environmental factors and bacterial phyla. a–hydrological characteristic factors; b–hydrochemical characteristic factors

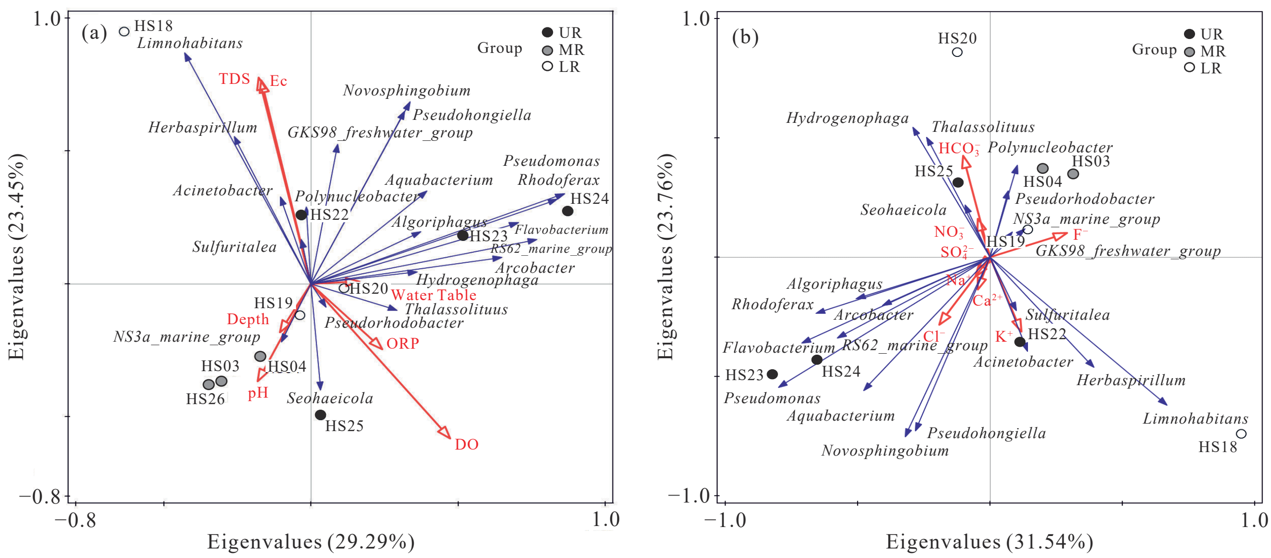

Fig. 11. RDA analysis on relationship between environmental factors and bacterial genera a–hydrological characteristic factors; b–hydrochemical characteristic factors.

Pseudomonas, *Pseudohongiella*, *Novosphingobium* and *Aquabacterium* have similar characteristics. They prefer the alkaline environment with deep horizon, which is positively correlated with pH, depth, NO_3^-, Zn_2^+ and Fe_3^+. Among them, they reach a very significant level with pH and Zn_2^+, and are significantly negatively correlated with ORP and HCO_3^-. In addition, *Arcobacter* and *Rhodoferax* are similar to the above communities. In addition to the above characteristics, their preference for cations is more inclined to high priced Fe^{3+}, Al^{3+}, K^+, etc.

Hydrogenophaga, *Polynucleobacter* and GKS98_freshwater_Group constitutes another group, which has higher correlation with ORP than all other species, and prefers higher DO and NO_2^- contents, indicating that it is more dependent on oxidizing groundwater environment. However, combined with the correlation level with other indexes (especially NO_3^-), it is considered that the weak oxidation environment with low NO_3^- synthesis is suitable for its survival. Although there are a lot of NO_2^- and O_2, due to the impact of the overall environment, the oxidation has not reached the level of large amount of NO_3^-.

Seohaeicola and NS3A_marine_Group constitutes a group that prefers an oxygen poor and high conductivity environment. Chlor alkali index is generally used to reflect the direction and intensity of cation exchange. The difference between the sum of Cl^- and K^+ + Na^+ in the study area is negative, which reflects the direction of cation alternation, that is, Ca^{2+} and Mg^{2+} migrate from groundwater to soil and replace K^+ and Na^+ in soil, so the content of Na^+ increases (Zhang HS et al., 2021). This process is closely related to the life activities of such groups.

Acinetobacter, *Sulfuritalea* and *Herbaspirillum* prefer nitrogen rich alkaline environment, which has a very significant positive correlation with nitrogen oxides NO_3^- and

NH_4^+ in oxidation environment, and has a positive correlation with pH and high valence alkali metal ions (such as Fe^{3+}, Al^{3+}). Studies have shown that metal ions participate in the formation of microbial protease structure, and can play a regulatory and catalytic role, and finally significantly improve the enzyme activity (Lerm S et al., 2013; Qu JY et al., 2021). It can be seen that the life activities of this group are related to the cation exchange of groundwater, and it is more suitable for salt water environment.

4. Conclusion

This research was carried out in the shallow groundwater in the middle of the North China Plain. In terms of hydrochemical characteristics, the ion content of shallow groundwater in the study area is high, with an average Ec of 9263 μS/cm, the average TDS is 6.67×10^{-3} mg/L, the hydrochemical types are mainly SO_4·Cl-Na·Mg and HCO_3·Cl-Na·Mg, and the average pH value is 7.5. From the piedmont plain to the central plain, the dynamic effect of groundwater is weakened, and the cation exchange adsorption is gradually enhanced. The compaction and water release of groundwater is also an important reason for the high ion content of groundwater in the study area.

Relatively special microbial population structures had emerged in this geologic environment. At the phylum level, *Proteobacteria*, *Bacteroidetes*, *Epsilonbacteraeota*, *Actinobacteria*, *Firmicutes* and *Planctomycetes* are the most abundant bacterial species in the groundwater of the study area, and the composition of MR group is more complex. At the genus level, the main dominant species in each group and sample are *Pseudomonas* and *Hydrogenophaga*. There are some similarities in composition and structure between UR group and LR group, and the species at all levels are more dispersed in the composition proportion of MR group. This study found that the abundance of microbial population is related to environmental indicators, indicating that most bacterial populations are sensitive to and closely related to hydrochemical processes. In general, species abundance is affected by alkaline and high salinity environment, while species diversity is related to depth, dissolved oxygen and weak reduction environment. In terms of composition differences, *Pseudohongiella*, *Pseudorhodobacter* and *Limnohabitans* at the genus level are the representatives of UR group, MR group and LR group respectively. Combined with species characteristics and hydrochemical characteristics, it is considered that the main factors causing the differences of community characteristics and groups are the differences of pH value, ORP and nitrogen oxides. Therefore, we believe that environmental factors are an important reason for the spatial heterogeneity of microbial communities and drive the adaptive evolution mechanism of microbial communities. Nutrient ions from planting and urban industrial activities have largely led to changes in groundwater microbial community structure. At the same time, symbiotic factors are also important factors affecting species types and community structure.

In the groundwater in the central part of the North China Plain, microorganisms have different adaptation modes and ecological functions. Based on the response of microorganisms to environmental characteristics, species at the genus level were divided into four typical characteristic groups: one kind prefers deep alkaline environment, mainly *Pseudomonas*, *Pseudohongiella*, *Novosphingobium* and *Aquabacterium*; the second type prefers oxygen enriched and NO_2^- environment, including *Hydrogenophaga*, *Polynucleobacter* and GKS98_freshwater_Group, etc; the third type prefers oxygen poor and high conductivity environment, with *Seohaeicola* and NS3A_marine_Group as the representative; the fourth type prefers alkaline environment rich in nitrogen, including *Acinetobacter*, *Sulfuritalea* and *Herbaspirillum*. However, the universality and two-way mechanism of this feature in deep groundwater environment needs to be further explored.

CRediT authorship contribution statement

Huai-sheng Zhang, Feng Guo and Chao Bian conceived of the presented idea. Huai-sheng Zhang, Feng Guo and Lei Zhang developed the theory and performed the computations. Wu-tian Cai, Lei Zhang and Fu-dong Liu helped supervise the project. Wu-tian Cai, Chao Bian, Jin-wei Liu and Miao Zhao contributed to the interpretation of the results. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Declaration of competing interest

The authors declare no conflicts of interest.

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