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Original Research

Comparison of Hydrochemical Characteristics and Biodiversity in Diverse Aquifers of Typical Coal Mines: A Case Study of the Huaibei Coalfield in China

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Abstract

This study aimed to investigate the hydrochemical characteristics of different aquifers, controlling factors, bacterial community structural characteristics, and their interaction with groundwater environmental factors in typical coal mines in the Huaibei Coalfield. A total of nine groundwater samples from the Cenozoic Loose Layer Pore (Second and Third), Carboniferous Taiyuan Group Tuff Karst Fissure, and Ordovician Tuff Karst Fissure aquifers were collected and used for water chemistry analysis and bacterial 16S rRNA gene sequencing. The overall groundwater in the study area was weakly alkaline, and the hydrochemical type of groundwater was dominated by the SO_4•Cl-Na type, followed by the SO_4•Cl-Ca•Mg and HCO_3-Mg•Ca types. The weathering of silicate and carbonate rocks controlled the formation of the chemical components of the groundwater, and the groundwater tended to develop from silicate to carbonate rocks. The dominant microbes in groundwater from different aquifers were Bacillaceae and Streptococcaceae, but their abundance was significantly affected by the depth; the abundance of the two bacterial groups was loose layer aquifer > Taiyuan limestone aquifer > Ordovician limestone aquifer. HCO_3^- and F^- were the main factors affecting the distribution of bacterial community structure. The study's results provide theoretical references for exploring the hydrochemical formation mechanisms and microbiological properties of different aquifers, as well as

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potential references for utilizing the differences in bacterial properties for identifying water sources during mine water emergencies.

Keywords: groundwater, hydrologic action, controlling actors, microbial communities, high-throughput sequencing

Introduction

Groundwater is an important component of natural water resources, and about one-third of the world's population uses groundwater sources for domestic production [1, 2]. Coal mining activities not only disturb groundwater flow and chemical fields but also cause groundwater pollution through various pathways, such as surface infiltration, overland flow, and direct discharge of mine wastewater [3, 4]. Due to comprehensive areal coverage of China's coal resources, complex geological conditions, and diverse mining methods, coal mining has caused different types of pollution, among which fluorine, acid drainage, heavy metals, and other groundwater pollution problems are prominent [5-7]. It is reported that the fluorine content of mine drainage is generally high in the pressurized aquifers in Northwest China [8] and North China [7]; acidic mine drainage and the resulting heavy metal contamination are more prominent in South China [7] and Southwest China [9]; Iron (Fe) and Manganese (Mn) contents are generally high in places such as Yunnan and Guizhou [7] and heavy metal contamination is present in the groundwater of the Liang huai Coalfield coalfields [10, 11].

Groundwater pollution itself is characterized by concealment, lagging, and the difficulty encountered in its management. With the extension of coal mining to the deep, the problem of deep groundwater pollution is bound to become more serious and is accompanied by changes in the aquatic environment of groundwater, which in turn triggers the reproduction and mutation of microbial community structure in groundwater [12, 13]. Therefore, a systematic study of the hydrochemical characteristics of groundwater in coal mine areas and the factors controlling its formation, as well as the investigation of the composition and structure of the groundwater microbial community in coal mine areas, are the necessary means to identify the degree of pollution and its control measures. This is a much sought-after requirement to realize the exploitation and utilization of water resources and the steady development of the industry. It is worth mentioning that both have a practical significance in environmental protection.

At present, at the regional and global level, the research methods used to study the hydrochemical characteristics and control factors of groundwater mainly involve the hydrochemical type method, descriptive statistics method, and ionic proportion coefficient method. Numerous studies have been conducted to characterize groundwater hydrochemistry and controlling factors in coal mining areas. Lashari et al. [1] detected Cd in different aquifers of Thar Coal Mine, Pakistan, by using an improved liquid-liquid microextraction method; another study [14] used stable isotopes to conduct a tracer study on the pollution of Ordovician limestone water in Zhai Li coal mine in Shandong; Gui et al. [15] analyzed the main heavy metal accumulation patterns in different aquifers of Renlou mine and put forward a preliminary conception of using heavy metal morphological characteristics for water source identification. In addition, the analysis of groundwater microbial community composition using 16S rRNA gene high-throughput sequencing technology is also a current research hotspot. A recent study [16] used indoor culture and 16S rRNA sequence analysis methods to study phosphorus-solubilizing bacteria in high-fluoride groundwater and concluded that phosphorus-solubilizing bacteria could promote the dissolution rate of fluorite (CaF_2). Currently, studies on the diversity of groundwater microbial communities have mostly focused on shallow, single-aquifer groundwater [12, 17]. However, there are fewer studies on the structural characteristics of microbial communities in deeper groundwater with diversified aquifers. The geochemical behavior of groundwater is not only governed by water's physical and chemical properties but is also significantly influenced by microorganisms [18]. In the field of groundwater science, especially the research on the microbial diversity of groundwater in different aquifers, it provides a new perspective for the analysis of the root cause of groundwater pollution, the understanding of the influence mechanism of mining activities on the groundwater system and the accurate identification of water inburst sources in coal mines.

The results of this study can be used as a reference for regions around the world facing similar challenges and promote the optimization of pollution control strategies and environmental impact assessment of mining activities. At the same time, this study helps to deepen the understanding of groundwater ecosystems, promote the cross-integration of hydrogeology and environmental microbiology, and contribute to the development of the discipline.

This study sampled groundwater from different aquifers in typical mining areas of the Huaibei Coalfield in China. It systematically analyzed the aquifer hydrochemical characteristics and control factors through mathematical and statistical analysis and measured ionic ratios. The structure of the bacterial community in different aquifers in different areas and the environmental factors influencing it were assessed using high-throughput sequencing of 16S rRNA genes. This study aimed to investigate the hydrochemical formation mechanism and microbiological characteristics of different aquifers, elucidate the differences in microbiological characteristics in the study area, and determine if microbiological differences can be utilized to identify water sources in different aquifers. The results provide basic data and theoretical support for managing groundwater and preventing water hazards in mining areas.

Materials and Methods

Hydrogeological Background

The Huaibei Coalfield is located in the northwestern part of the Huaibei Plain in Anhui Province, China, and is separated from the Yangzi Plate by the Tanlu Fault in the east; it is bordered by the Xiayi Fault and the Henan Sedimentary Belt in the west, the Fengpei Rise of the Fengpei Fault in the north, and the Bengbu Rise of the Taihe-Wuhe Fracture in the south. Due to the superposition of multiple tectonic movements, the Huaibei Coalfield and its neighboring areas are crisscrossed by near-EW and NNE faults, forming a network of fault structures; these include the Fengpei, Subei, Banqiao-guzhen, and Taihe-Wuhe Faults in the near-EW direction and the Xiayi, Fengguo, and Guzhen-Changfeng Faults in the NNE direction (Fig. 1).

The area can be divided into the north and south, with the Suixiao mine in the north and the Guoyang, Linhuan, and Suxian mines in the south (Fig. 1). The area has a length of 50 km from east to west and a width of 25-45 km from north to south, covering an area of about 1,750 km^2. The area has production mines, such as the Linhuan, Haizi, Xutuan, Renlou, Tongting, and Suntuan mines [12]. According to the regional geological characteristics and the spatial distribution of groundwater storage, the groundwater in the Huaibei Coalfield is divided into four aquifers: the Cenozoic loose layer pore aquifer, the Permian coal measure sandstone fissure aquifer, the Carboniferous Taiyuan formation limestone karst fissure aquifer, and the Ordovician limestone karst fissure aquifer [12, 19]. In the Huaibei Coalfield, the groundwater of the Taiyuan limestone aquifer, Ordovician limestone fissure aquifer, and coal measure sandstone fissure aquifer are communicated

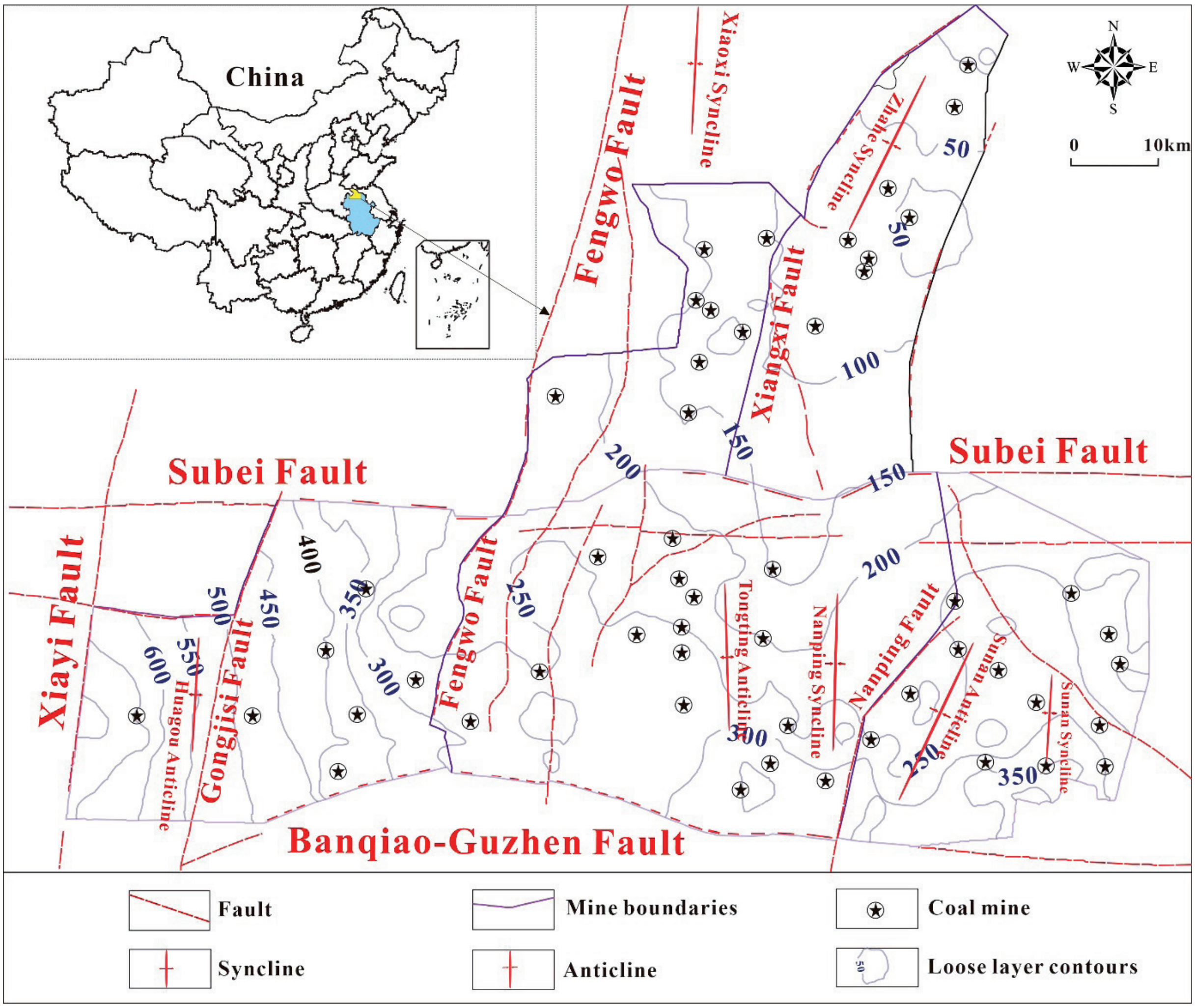

Fig. 1. Geological structure and distribution of the mining area in Huaibei coalfield.

through water-conducting fault, karst collapse column and mining fissure to form different degrees of hydraulic connection. A thick loose layer covers the southern part, and the Taiyuan limestone aquifer is connected with the Ordovician limestone karst fissure aquifer and the Cenozoic loose layer pore aquifer, forming a hydraulic connection [20].

The mineral composition of typical coal mines in the Huaibei coalfield can be summarized as follows: The metamorphic rock series is the bedrock, and the upper part is covered with a Cenozoic loose layer. The main coal-bearing strata are from the Carboniferous and Permian, and the lithology includes limestone, aluminous mudstone, sandstone, mudstone, and coal seams, reflecting the characteristics of marine and continental interaction. Among them, the coal seam is sandwiched between rock layers, and the thickness is unstable. In addition, it also contains Ordovician limestone, rich in calcite, and Jurassic, Cretaceous, and other continental sedimentary rocks. It has a long history of exploitation, which appeared in the early Tang Dynasty, began large-scale exploitation in the middle of the Ming Dynasty, and was interrupted by wars repeatedly in the late Qing Dynasty and early Republic of China. After the founding of New China, large-scale exploration and development began in the 1950s, with multiple mines, accelerated infrastructure, and frequent technological innovation, which gave birth to the prosperity of Huaibei City. At present, in the face of resource depletion, Huaibei City is actively transforming, exploring a sustainable road, committing to ecological restoration, and revitalizing the regional economy.

Sample Collection and Testing

Based on the analysis of topographic and geomorphic features as well as the hydrogeological conditions of the study area (Fig. 1), nine groups of groundwater samples from different aquifers were taken in July 2015, including four, three, and two groups from the loose layer aquifer (mainly taken from the second and the third aquifers), Taiyuan, and the Ordovician limestone aquifer, respectively (Table 1). Water samples were stored in 0.5 L sterile polyethylene bottles, preserved on dry ice for 24 h before transportation to the laboratory, and filtered through disposable syringes and 0.45 μm microporous aqueous filtration membranes into 10 mL centrifuge tubes. Samples were adjusted to pH<2 by adding superior purity HNO_3 and were then refrigerated at 4ºC as backups.

Sample collection procedures were carried out in strict accordance with the Technical Code for Environmental Monitoring of Groundwater in China (HJ/T 164-2004). Physicochemical indicators such as pH, temperature, Total Dissolved Solids (TDS), and Electric Conductivity (EC) were measured on-site using a portable analyzer (OHAUS Shanghai, China, Suite310). HCO_3^- was determined by acid-based titration. Na^+, K^+, Mg^{2+}, Ca^{2+}, F^-, Cl^-, SO_4^{2-}, NH_4^+, and NO_3^- were determined by ion chromatography (Thermo Fisher Scientific, ICS-600/ICS-900). Cr, Mn, Fe, Co, Ni, Cu, Zn, and Pb were determined by inductively coupled plasma mass spectrometry (Shimadzu, ICPMS-2030LF). The quantitative methods were based on the external standard method, and the linear correlation coefficients of the calibration curves were all higher than 0.999. The relative errors of the anion and cation balances of all water samples were less than 5%. The sample processing and analytical tests were done in the Key Laboratory of Mine Water Resource Utilization of Anhui Higher Education Institutes (Suzhou University, Anhui, P.R. China).

DNA Extraction, PCR Amplification, and Sequencing

Groundwater samples (2 L) were filtered through pre-autoclaved microporous filter membranes with a pore size of 0.22 μm to collect biomass, and the membranes were preserved on dry ice and sent to Shanghai Meiji Bio-pharmaceutical Science and

Table 1. Sample information for groundwater in different aquifers.

Samples	Coal mine	Sampling aquifer	Water level elevation (m)	Sampling location
LE-1	Liuer	third aquifer	Unknown	Off-mine water source well 80~120 m
LY-1	Liuyi	Ordovician limestone aquifer	6	North wind well water source well
LY-2	Liuyi	Second and third aquifer	15	Park water wells
LY-3	Liuyi	Taiyuan limestone aquifer	-460	46 Stone Gate Drainage Hole (Water-29)
BS-1	Baishan	third aquifer	Unknown	Water source wells (depth of formation 151)
WG-1	Wugou	Taiyuan limestone aquifer	-610	1 Mining Area Track Alley Drainage Hole
RL-1	Renlou	Ordovician limestone aquifer	Unknown	Small Cottage Water Well
RL-2	Renlou	Second and third aquifer	Unknown	Water well No. 14
RL-3	Renlou	Taiyuan limestone aquifer	-510	shaft vent

Technology Co. for 16S rRNA high-throughput sequencing. Genomic DNA was extracted using a DNA isolation kit (OMEGA-soil DNA Kit, Omega Biotek, Norcross, GA, U.S.). After completion of genomic DNA extraction, its integrity and purity were detected using 1% agarose gel electrophoresis. The V4-V5 region of the bacterial 16S rRNA gene was amplified using the universal bacterial sequencing primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) on an ABI Gene Amp® 9700, and all samples were run in accordance with the formal conditions. All samples were analyzed in accordance with the formal experimental conditions, with three replicates for each sample. PCR products of the same sample were mixed and detected by 2% agarose gel electrophoresis, gel purified using an AxyPrepDNA Gel Recovery Kit (AXYGEN). The products were eluted on Tris HCl. Referring to the preliminary quantitative electrophoresis results, the PCR products were detected and quantified using a QuantiFluorTM-ST Blue Fluorescence Quantification System (Promega); after this, they were mixed in the appropriate proportion according to the sequencing amount required for each sample and were sequenced on the Illumina Miseq sequencing platform.

Data Analysis

The processing and analysis of the data (groundwater hydrochemical data as well as factor and correlation analysis) of this experiment were completed using Excel 2016 (Microsoft) and IBM SPSS Statistics 26. CorelDRAW 2023 was used to draw the distribution map of the groundwater sampling sites. Piper trilinear, Gibbs, and ion-scaled plots were generated using Origin 2024.

Results and Discussion

Characterization of Water Chemical Composition

The hydrochemical and physicochemical statistics of groundwater in the study area are shown in Table 2. The pH of groundwater in the loose layer ranged from 7.45 to 7.88 (mean: 7.66) and was weakly alkaline; the ρ(TDS) ranged from 1260 to 1690 mg·L^{-1} (mean: 1430 mg·L^{-1}). The pH of the groundwater in the Ordovician limestone aquifer ranged from 7.20 to 7.51 (mean: 7.31) and was weakly alkaline; the ρ(TDS) ranged from 1260 to 3400 mg·L^{-1} (mean: 2136.67 mg·L^{-1}). The pH of the groundwater in the Taiyuan limestone aquifer ranged from 7.30 to 7.43 (mean: 7.37) and was weakly alkaline; the ρ(TDS) ranged from 711 to 2140 mg·L^{-1} (mean: 1425.50 mg·L^{-1}). Overall, the groundwater in the study area was weakly alkaline and hard.

The fluctuation of water chemistry in the groundwater from different aquifers was large. The anion concentrations in the groundwater of the loose and Ordovician limestone layers were, in

Table 2. Groundwater hydrochemistry indices in different aquifers.

Aquifer	project	pH	TDS	K^+	Na^+	Ca^{2+}	Mg^{2+}	NH_4^+	F^-	C^l-	NO_3^-	SO_4^{2-}	HCO_3^-
loose layer aquifer (n = 4)	Max	7.88	1690	7.20	257.31	59.18	50.30	3.48	1.64	303.37	0.00	505.30	656.76
	Min	7.45	1260	1.34	207.51	46.71	44.70	0.00	0.77	116.92	0.00	356.27	532.32
	Mean	7.66	1430	3.36	222.97	53.51	48.30	0.87	1.07	186.70	0.00	412.24	576.39
	Std	0.19	165.08	2.26	20.28	5.44	2.15	1.51	0.34	70.03	0.00	55.87	51.00
	CV	0.03	0.12	0.67	0.09	0.10	0.04	1.73	0.32	0.38	0.00	0.14	0.09
Taiyuan limestone aquifer (n = 3)	Max	7.51	3400	21.63	479.82	117.18	56.65	7.23	2.28	300.53	0.00	585.06	421.71
	Min	7.20	1260	5.57	142.59	60.32	40.99	0.00	0.54	0.00	0.00	492.60	383.34
	Mean	7.31	2136.67	12.05	296.83	88.92	49.26	3.55	1.26	130.80	0.00	534.59	403.16
	Std	0.14	915.44	6.91	139.16	23.21	6.42	2.95	0.74	125.74	0.00	38.22	15.69
	CV	0.02	0.43	0.57	0.47	0.26	0.13	0.83	0.59	0.96	0.00	0.07	0.04
Ordovician limestone aquifer (n = 2)	Max	7.43	2140	20.51	287.19	104.10	39.35	0.00	2.89	520.41	0.43	402.78	490.84
	Min	7.30	711	3.14	54.86	54.56	29.04	0.00	0.66	55.00	0.00	203.65	454.20
	Mean	7.37	1425.50	11.82	171.02	79.33	34.50	0.00	1.77	287.71	0.22	303.21	472.52
	Std	0.06	714.50	8.69	116.17	24.77	5.46	0.00	1.12	232.70	0.22	99.56	18.32
	CV	0.01	0.50	0.73	0.68	0.31	0.16	0.00	0.63	0.81	1.00	0.33	0.04

Max denotes maximum value, Min denotes minimum value, Mean denotes mean value, Std denotes standard deviation, CV denotes coefficient of variation, dimensionless; pH is dimensionless, and the rest of the components are in mg·L^{-1}.

descending order, HCO_3^- >SO_4^{2-} >Cl^- >F^- >NO_3^-; in the Taiyuan limestone aquifer, they were SO_4^{2-} >HCO_3^- >Cl^- >F^- >NO_3^-. The cation concentrations in groundwater from the loose Taiyuan limestone and Ordovician limestone aquifers were $Na^+ > Ca^{2+} > Mg^{2+} > K^+ > NH_4^+$. The coefficient of variation (CV) indicates the degree of dispersion of the data; in pollutant traceability studies, higher CV values of pollution factors usually represent greater anthropogenic perturbation. The chemical components of deep groundwater, in addition to anthropogenic perturbation factors, are more reflective of the differences in the type and degree of water-rock interactions [20, 21]. In this study, the coefficients of variation of anions and cations in different aquifers ranged from 0.00 to 1.73, indicating a high spatial variability of groundwater hydrochemistry in different aquifers. In addition, the coefficients of variation (CV) of K^+ (loose layer, Taiyuan limestone, and Ordovician limestone aquifers), Na^+ (Ordovician limestone aquifer), F^- (Taiyuan limestone and Ordovician limestone aquifers), NO_3^- (Ordovician limestone aquifer), and Cl^- (Taiyuan limestone and Ordovician limestone aquifers) in different aquifers were high ($0.5<CV\leq 1$) (Table 2), which was speculated to be related to the differences in the mineral composition of the rocks in the aquifers. In particular, the CV value of NH_4^+ in the loose bed groundwater was greater than 1, indicating that this ion has obvious spatial variability; it was hypothesized that the loose bed groundwater may have been affected by human activities, resulting in a high degree of local NH_4^+ enrichment [22], and disturbance from coal seam mining has enhanced the hydraulic connection between the aquifers [12].

Characterization of Groundwater Hydrochemical Types

The Piper three-line diagram is one of the most widely used graphics in hydrogeochemical analysis because it contains no interference from human factors; using this method, the type of groundwater hydrochemistry and its evolutionary characteristics can be visualized [23-25]. Cations were mainly distributed in the Na^+ + K^+ end member, and the anions were dominated by HCO_3^- and SO_4^{2-}. In the upper-middle rhombic region, the SO_4•Cl-Na type dominated, followed by the SO_4•Cl-Ca•Mg and HCO_3-Mg•Ca types (Fig. 2), indicating that the formation of groundwater hydrochemistry in the study area is mainly controlled by the weathering and dissolution of carbonate minerals [22]. Under the influence of factors such as mining activities and anthropogenic perturbations, groundwater chemistry types show diverse characteristics [26].

Factors Controlling Water Chemistry

Correlation Analysis

In the study of hydrogeochemistry, correlation analysis determines whether the ions in the water chemistry components result from the same origin; it is usually assumed that significantly correlated variables have a similar origin [22, 27]. The results obtained from the Pearson correlation coefficient matrix are shown in Fig. 3. TDS in the groundwater of the study area was mostly significantly positively correlated with Ca^{2+}

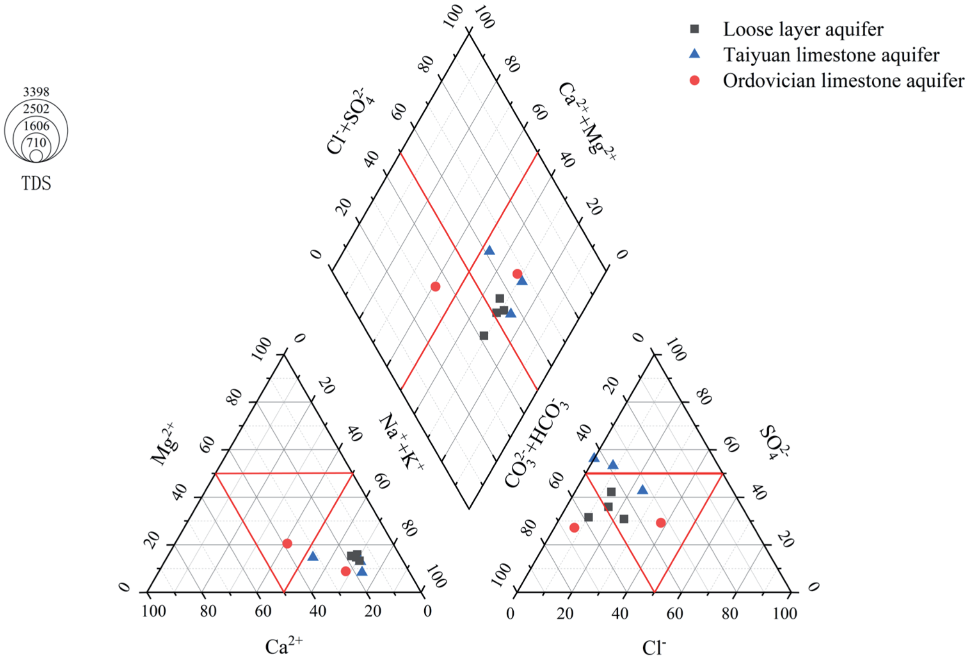

Fig. 2. Piper diagram of groundwater in different aquifers within the study area.

and Mg^{2+} ($r>0.6$, $P<0.05$), and was also significantly positively correlated with $Na^+ + K^+$ ($P<0.01$; correlation coefficient: 0.980), which indicates that these ions had a greater contribution to TDS. The correlations with all anions were not significant. There was a significant negative correlation between Ca^{2+} and HCO_3^- in the groundwater of the study area ($r = -0.687$, $P<0.05$), indicating that dissolution and weathering of carbonate and silicate minerals may have occurred in the groundwater [22, 28]. There was a significant positive correlation between Mg^{2+} and $Na^+ + K^+$ in groundwater ($P<0.05$; correlation coefficient: 0.773), which indicates that they had similar origins and may have originated from the same rock weathering process [22]. In addition, there were significant positive and negative correlations between the above two different groups of ions, which may be related to different types of water-rock interactions under different hydrogeological conditions [29].

Water-Rock Interaction Mechanisms

Groundwater chemical components change through water-rock interactions with the surrounding media; these interactions can reveal the mechanism of hydrochemical evolution, which can be classified into three types: rock weathering, atmospheric precipitation, and evaporation-concentration [23, 30-32]. As can be seen from the Gibbs diagram (Fig. 4), Gibbs I ($Na^+/(Na^++Ca^{2+})$) ranged from 0.46 to 1.00, while Gibbs II ($Cl^-/(Cl^-+HCO_3^-)$) ranged from 0.00 to 0.67.

In Gibbs I, the groundwater samples were mainly located in rock weathering and evaporation-concentration zones, which suggests that the hydrological and geochemical processes and the hydrochemical formation of the groundwater in the study area are synergistically controlled by water-rock interactions and evaporation-concentration. A small number of groundwater samples (loose and Taiyuan limestone aquifers) fall outside the control area; these sampling sites are located in the coal mining area, which is more disturbed by factors such as mining and other human activities.

In Gibbs II, most of the groundwater samples point to rock weathering end elements, indicating that water-rock interactions dominantly control the hydrochemical characteristics of groundwater in the study area; a small number of samples fall in the evaporation-concentration region, indicating that some of the groundwater sources in the study area are also affected by evaporation-concentration interactions. The groundwater samples in the study area are all far away from the precipitation control region, indicating that the contribution of atmospheric precipitation to the main ions is small. This conclusion is consistent with the findings of Liu et al. on the groundwater in the Huainan coal mine area, indicating that the ionic components of groundwater in the study area are mainly controlled by water-rock interactions [22].

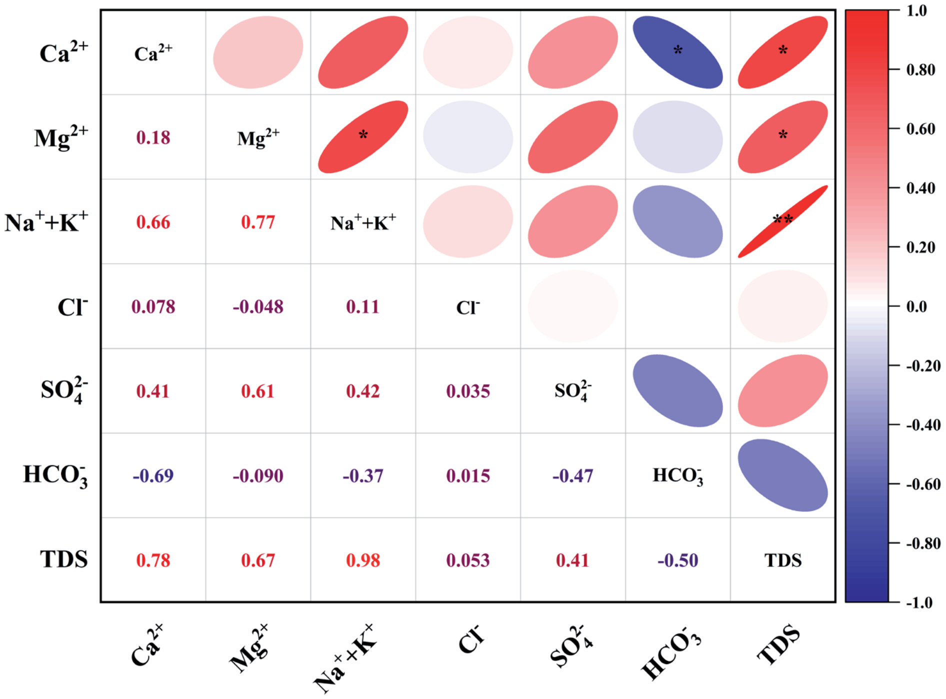

Fig. 3. Correlation of groundwater chemical components in the study area.
Note: * indicates significant at the 0.05 level, ** indicates significant at the 0.01 level; the direction of the long axis of the ellipse indicates the positive and negative correlation coefficients, with positive values in the upper-right-bottom-left direction and negative values in the upper-left-bottom-right direction, and the flatter the ellipse, the greater the correlation coefficients, and vice versa, the smaller the correlation coefficients.

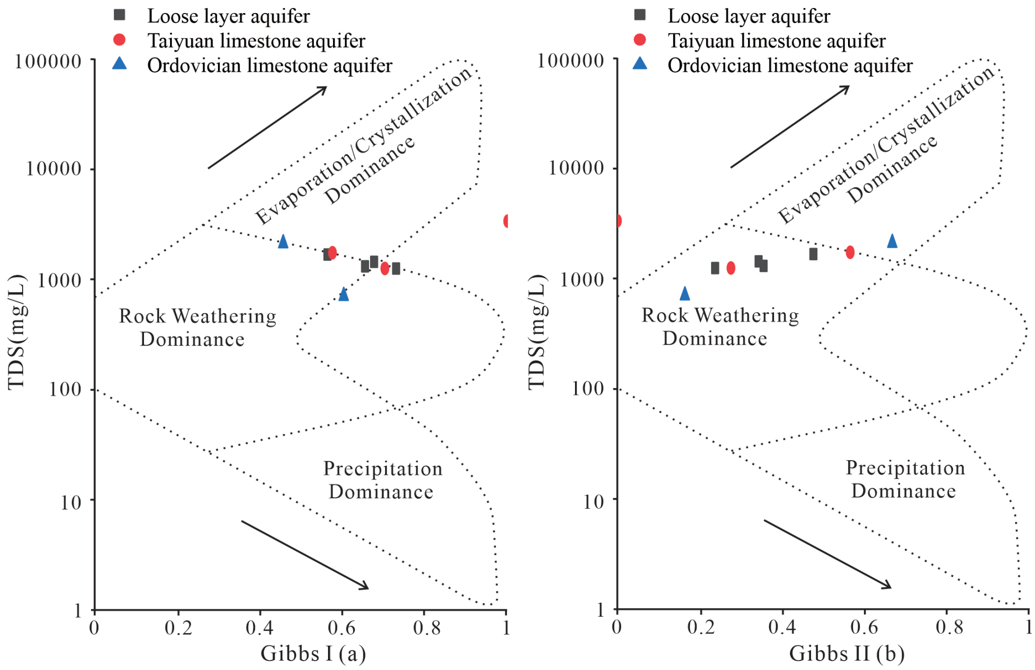

Fig. 4. Gibbs diagram for groundwater of the study area.

Ion Ratio Analysis

Groundwater systems vary significantly in their concentrations of Na^+, Ca^{2+}, and Mg^{2+} produced as a result of the weathering of carbonate, silicate, and evaporite minerals. Changes in the water chemistry components under different weathering effects can be characterized based on Mg^{2+}/Na^+, Ca^{2+}/Na^+, and HCO_3^-/Na^+ values ($meq \cdot L^{-1}$) [22, 33]. The groundwater samples in the study area are mainly located in the weathering-controlled end element of silicate rocks (Fig. 5), indicating that the formation of groundwater hydrochemistry in the study area is mainly controlled by the weathering and dissolution of silicate rocks; the samples of the loose layer were relatively concentrated, indicating that this weathering-dissolution effect is the main controlling factor. A small number of samples were in the direction of carbonate rocks, such as those of the Taiyuan limestone and Ordovician limestone aquifers; the distribution of these samples was relatively dispersed, indicating synergistic control from silicate and carbonate rocks, and that the weathering and dissolution of carbonate rocks may have some influence on the groundwater.

Microbial Community Structure

Community Diversity Analysis

The Operational Taxonomic Units (OTUs) and bacterial diversity indices of the samples are shown in Table 3. Coverage values ranged from 0.9988 to 0.9995, all above 99%, indicating that the reliability of the sequencing data was high. Abundance-based Coverage

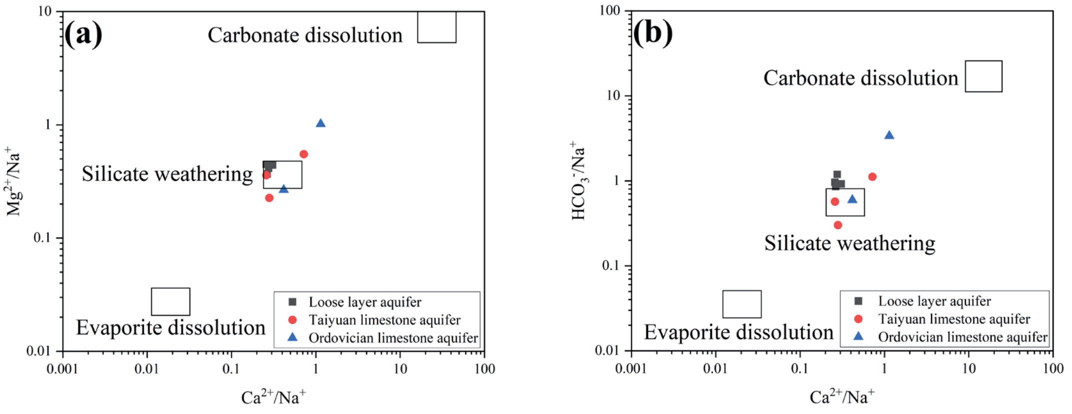

Fig. 5. Relationship between Mg^{2+}/Na^+, Ca^{2+}/Na^+, and HCO_3^-/Na^+ of groundwater.

Table 3. Bacterial OTUs and diversity indices of samples.

Sample		Coverage	Observed Species	ACE	Simpson	Shannon	Chao
loose layer aquifer	LE-1	0.9990	194	212.07	0.16	2.78	213.89
	LY-2	0.9995	226	234.19	0.18	2.65	232.65
	BS-1	0.9989	236	260.38	0.14	2.79	262.64
	RL-2	0.9988	240	261.01	0.09	3.37	261.57
	AVG	0.9991	224	241.91	0.14	2.90	242.69
Taiyuan limestone aquifer	LY-3	0.9991	212	225.40	0.15	2.83	223.50
	WG-1	0.9988	212	237.29	0.12	3.00	238.71
	RL-3	0.9990	175	198.42	0.19	2.30	195.22
	AVG	0.9990	200	220.37	0.16	2.71	219.14
Ordovician limestone aquifer	LY-1	0.9991	242	255.59	0.17	2.83	255.00
	RL-1	0.9993	198	207.88	0.17	2.69	204.33
	AVG	0.9992	220	231.74	0.17	2.76	229.67

Estimator (ACE) and Chao indices usually reflect the total number of species, while Shannon and Simpson's indices are usually used to estimate community diversity, making up the alpha diversity index; larger Shannon values indicate higher diversity, whereas larger Simpsons' values indicate lower diversity [12, 34]. From the mean values, the ACE, Shannon, and Chao values were loose layer>Ordovician limestone aquifer>Taiyuan limestone aquifer. The Simpsons' index was loose layer <Taiyuan limestone aquifer<Ordovician limestone aquifer, indicating that the bacterial community of the loose aquifer was the most diverse, followed by the Ordovician limestone and Taiyuan limestone aquifers. The average values of the observed species index in the three aquifers were loose layer (224)>Ordovician limestone aquifer (220)>Taiyuan limestone aquifer (200). The results showed that bacterial community richness was higher in the loose aquifer. In addition, Simpson and Shannon indices of Taiyuan limestone and Ordovician limestone aquifers are close, which is due to the mutual hydraulic relationship between Taiyuan limestone and Ordovician limestone aquifers, which makes the microbial community diversity structure between the two aquifers similar. [12, 19].

Analysis of Microbial Community Composition

Differences in the family-level composition of microflora with <1% abundance in the combined aquifer are shown in Fig. 6. Bacillaceae was the largest family in the groundwater of the study area, with a relative abundance of 29.93%-50.03% and a mean abundance of 39.83%. The relative and mean abundances of other major families were Streptococcaceae (29.93%-50.03%, 39.83%), Pseudomonadaceae (5.97%-10.93%, 8.71%), Burkholderiaceae (0.97%-38.56%, 9.38%), Moraxellaceae (2.82%-13.20%, 5.26%), Halothiobacillaceae (0.00%-31.76%, 4.54%), Xanthomonadaceae (1.66%-3.42%, 2.58%), and Weeksellaceae (0.64-2.35%, 1.15%). The abundance of Burkholderiaceae in BS-1 and Halothiobacillaceae in RL-3 were>30%, 38.56%, and 31.76%, respectively. It was hypothesized that anthropogenic disturbances, mining activities, and hydraulic connections between aquifers may have replenished nutrients that favor the growth and metabolism of Burkholderiaceae and Halothiobacillaceae.

The dominant bacterial families varied between aquifers at different depths. Bacillaceae remained the dominant family in the loose-layer samples, followed by Streptococcaceae, Pseudomonadaceae, and Burkholderiaceae (39.9%, 14.7%, 8.5%, and 13.5%, respectively). Bacillaceae, Streptococcaceae, Halothiobacillaceae, and Pseudomonadaceae were the top four bacterial families in the Taiyuan limestone aquifer samples (39.8%, 15.0%, 10.6%, and 9.0%, respectively). The top four families in the Ordovician limestone aquifer samples were Bacillaceae, Streptococcaceae, Pseudomonadaceae, and Moraxellaceae (46.4%, 18.6%, 10.1%, and 4.5%, respectively). The sum of the relative abundance of Bacillaceae and Streptococcaceae in each aquifer was greater than 50% at different depths, indicating that these families were the most dominant microflora in the groundwater of the study area, the microbial community structure is more balanced. The differences in aquifers at different depths may be reflected in the diversity of environmental conditions and the ecological adaptability of microorganisms, indicating the complexity and uniqueness of different groundwater microbial ecosystems [35, 36]. The relative abundance of dominant bacterial families in the groundwater in the study area varied at different depths and was loose layer>Taiyuan limestone aquifer

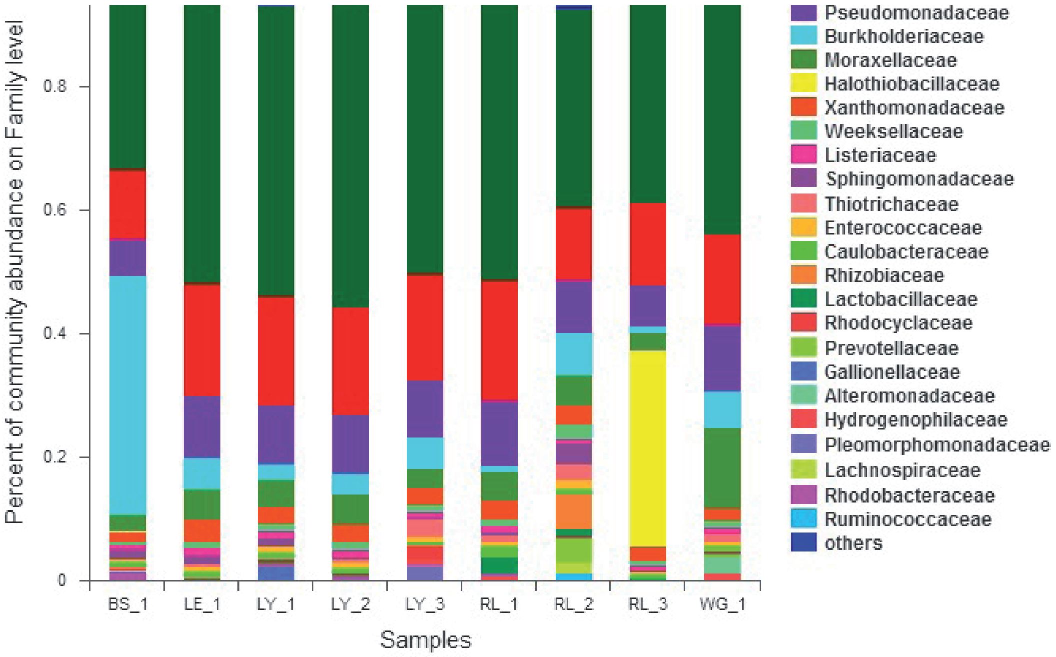

Fig. 6. Bacterial communities in groundwater from different aquifers at the family level.

>Ordovician limestone aquifer. Deeper groundwater had lower bacterial abundance, consistent with the results of Chen et al. characterizing the bacterial community in the groundwater of the Huaibei Coalfield [12].

Correlation Analysis between Microbial Communities and Environmental Factors

Autocorrelation exists between different impact factors; the closer the relationship, the greater the interference, meaning that the higher the autocorrelation, the more unfavorable the analysis of the factors impacting microbial community structure is [37]. The influence factor is considered unimportant if the Variance Inflation Factor (VIF) value exceeds ten [38]. Environmental factors leading to strong covariance (VIF>10) [17] were eliminated (Table 4).

To analyze the correlation between environmental impact factors and bacterial communities, five environmental variables (pH, F^-, Cl^-, SO_4^{2-}, HCO_3^-) were screened (Fig. 7). CCA analysis showed that the degree of impact of the factors on the bacterial communities was $HCO_3^- > F^- > Cl^- > pH > SO_4^{2-}$. HCO_3^-, F^-, and Cl^- were the three most important drivers of the groundwater bacterial community structure. The angles between HCO_3^- and pH and Cl^- F^- and SO_4^{2-} were acute and positively correlated, indicating that the mass concentration of these parameters increased together. HCO_3^- was positively correlated with BS-1 and RL-2, and pH and Cl^- were positively correlated with LE-1 and LY-2, suggesting that HCO_3^-, pH, and Cl^- had a greater effect on the loose layer. F^- and SO_4^{2-} were positively correlated with LY-1 and RL-1, as well as LY-3 and RL-3, suggesting that both F^- and SO_4^{2-} had a different degree of effect on both the Ordovician limestone and Taiyuan limestone aquifers. Studies have shown that environmental factors impact unique aquifers differently, causing environmental selection and adaptation in microbial communities [12]. The distance between the projection point and the origin on the influence factor vector indicates the strength of the correlation between the bacterial family and the influence factor (Fig. 7). The dominant bacterial families were mainly distributed along the pH vector, primarily because the pH of the groundwater in the region is weakly alkaline, which is favorable for the growth of bacteria. Zhang et al. [39] also showed that in a higher pH environment, the diversity of the microbial community would increase, which was conducive to the growth of the bacteria. Bacillaceae, Streptococcaceae, Pseudomonadaceae, Moraxellaceae, Weeksellaceae, and Xanthomonadaceae showed positive

Table 4. Groundwater chemical impact interference screening results.

Factor	Pre-screening							Post-screening				
	pH	EC	TDS	F^-	Cl^-	SO_4^{2-}	HCO_3^-	pH	F^-	Cl^-	SO_4^{2-}	HCO_3^-
VIF	4.637	18518.51	18518.52	6.03	14.339	6.565	1.739	1.601	1.776	1.404	1.327	1.666

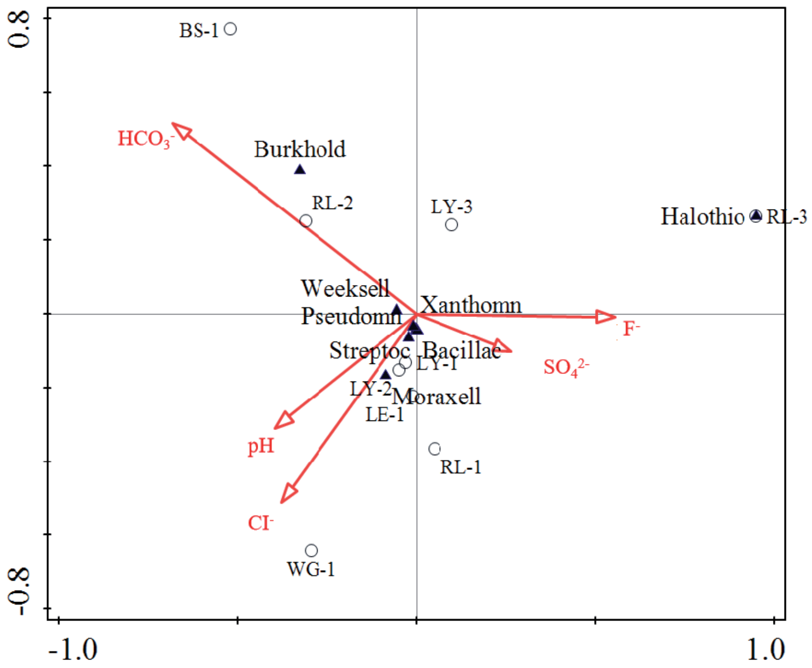

Fig. 7. Canonical correspondence analysis of samples.

correlations with HCO_3^-, F^-, and Cl^-, suggesting that a certain range of these ions favored their growth and that these bacteria may use HCO_3^- as a carbon source or participate in other metabolic processes. F^- and Cl^- may act as regulatory ions or participate in the stability of cell membranes to make them adaptable in environments rich in these elements. Previous studies have shown that Firmicutes and Proteobacteria are tolerant and adaptable in environments rich in F^- and Cl^- [39, 40].

The pollutants HCO_3^- and F^- were the most important factors influencing the composition of the groundwater bacterial community in the study area. Mining activities promote the hydraulic connection between different aquifers and accelerate the dissolution and weathering of carbonate and silicate minerals, which gradually increases HCO_3^-; their influence on the groundwater bacterial community is also prominent. Huaibei Coalfield has strong groundwater cation exchange due to the high F^- content, which decreases Ca^{2+} and increases Na^+. Lower Ca^{2+} concentration can promote the dissolution of fluorine-containing minerals such as fluorite, accelerating the release of F^- into the water column [12, 41].

Changes in contaminant concentrations lead to changes in the composition of microbial communities as microorganisms adapt to the environment by regulating their abundance and diversity [42, 43].

Different aquifers have different stratigraphic characteristics, hydraulic connections, and storage conditions that lead to water chemistry differences, affecting microbial communities' composition and metabolism. The microbial community achieves a dynamic balance through regulating nutrient transport, geochemical cycling, and pollutant degradation [44]. Examining the groundwater of different aquifers in the Huaibei Coalfield, the differences in bacterial community structure and controlling factors in different aquifers could provide a potential method for identifying water sources during mine water emergencies. However, it should be emphasized that this study has certain limitations. The research results have certain reference significance for mining areas with similar hydrogeological conditions. When there are significant differences in hydrogeological conditions in mining areas, it is necessary to reanalyze different aquifers' hydrochemical characteristics and microbial diversity.

Conclusions

(1) In summary, the groundwater in the study area is weakly alkaline, with Na^+ as the dominant cation and HCO_3^- and SO_4^{2-} as the dominant anions. The hydrochemical type is mostly SO_4•Cl-Na, followed by the SO_4•Cl-Ca•Mg and HCO_3-Mg•Ca.

(2) Groundwater hydrochemistry in the study area is primarily shaped by carbonate and silicate rock weathering and dissolution. Evaporation, concentration, and water-rock interactions play pivotal roles, while atmospheric precipitation has a lesser influence. Rock weathering and dissolution govern groundwater evolution, transitioning from silicate to carbonate rocks.

(3) The predominant bacterial families in the groundwater of various aquifers in the study area include Bacillaceae, Streptococcaceae, Pseudomonadaceae, Burkholderiaceae, Moraxellaceae, Halothiobacillaceae, Xanthomonadaceae, and Weeksellaceae. Bacillaceae

and Streptococcaceae dominated, particularly abundant in the loose-layer aquifer, followed by the Taiyuan and Ordovician limestone aquifers, respectively.

(4) The main environmental factors affecting the microbial community in groundwater in the study area were $HCO_3^->F^->Cl^->pH>SO_4^{2-}$. The characteristic groundwater pollutants, HCO_3^- and F^-, were the main factors affecting the microbial community structure.

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Conflict of Interest

The authors declare no conflict of interest.

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