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Pollutant distribution characteristics and microbial response mechanisms in surface and deep brines of the Qaidam Basin

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This research investigates the distinctions between surface and deep brines in the Salt Lake region of the Qaidam Basin, with an emphasis on their physicochemical properties, organic matter content, heavy metal concentrations, organic pollutants, and microbial community structures. Both surface and deep brine samples were subjected to analysis for total and dissolved organic carbon, heavy metals (specifically Mn, Pb, and Cd), and pollutants, including phthalate esters (PAEs), halogenated compounds, and sulfides. The microbial communities were characterized through high-throughput sequencing, and redundancy analysis (RDA) coupled with correlation heatmaps was employed to evaluate the relationships between pollutants and microbial communities. The findings revealed that surface brines contained higher levels of organic matter, whereas deep brines exhibited significantly elevated concentrations of heavy metals and pollutants. The microbial community composition also varied, with Proteobacteria being predominant in deep brines and Firmicutes in surface brines, along with notable shifts at the genus level. Statistical analyses identified pollutants, particularly Pb, Cd, PAEs, halogenated compounds, and sulfides, as major determinants of microbial community variation. The findings indicate that the accumulation of pollutants in deep brines significantly impacts microbial community structures and ecological functions. Integrating microbial response data into environmental risk assessments is crucial for the sustainable development of deep brine resources in the Qaidam Basin.

KEYWORDS

deep brine, heavy metal pollution, organic pollutants, microbial community structure, extreme environment

1 Introduction

Human activities are significantly impacting the ecological stability of natural hypersaline environments, particularly in arid and semi-arid regions. The intensification of salt mining, petroleum extraction, and industrial brine discharge has led to the increased introduction of various pollutants, both organic and inorganic, into surface brine systems (Corsellis et al., 2016; Sayed et al., 2021). These pollutants tend to accumulate in closed or

semi-closed water bodies (Rojo-Nieto et al., 2011; Wang et al., 2016), disrupting indigenous microbial communities and impairing critical ecological functions such as biogeochemical cycling and pollutant degradation (Bai et al., 2017). While pollution in freshwater and marine systems has garnered considerable attention in recent years (Huang et al., 2025; Mossotto et al., 2025), the mechanisms by which microorganisms in hypersaline ecosystems respond to pollution remain largely underexplored.

Hypersaline environments, characterized by extreme salinity, elevated osmotic pressure, and limited nutrient availability, support uniquely adapted halophilic microbial communities, comprising a diverse array of bacteria and archaea (Menéndez-Serra et al., 2020; Santini et al., 2022). These microorganisms are integral to ecosystem stability, exhibiting distinctive metabolic capabilities, that significantly contribute to the biogeochemical cycling of carbon, nitrogen, sulfur, and other essential elements (Wang and Bao, 2022; Feng et al., 2023). However, the introduction of exogenous stressors including not only organic compounds but also heavy metals such as Mn, Cd, Co and Cu can profoundly disrupt these microbial networks. Heavy metals are known to exert toxic effects on cellular processes, inhibit microbial growth and enzymatic activity, and induce alterations in community structure and functional potential (Lichter et al., 2006; Cristiano et al., 2021). Their accumulation in brine environments, particularly in regions lacking historical baseline data, complicates ecological risk assessments.

The Qaidam Basin, located in the northern Qinghai-Tibet Plateau, exemplifies a typical arid inland basin characterized by extensive distributions of salt lakes and deep brine systems (Zheng et al., 2016). This area serves as an exemplary natural setting for examining the effects of environmental pollution on hypersaline ecosystems. Its unique geological and climatic conditions give rise to two contrasting brine environments: geochemically stable deep gravel-layer brine (Bai et al., 2024; Fan et al., 2024), which remains isolated from surface influence, and surface brines that are directly impacted by anthropogenic disturbances. Due to high evaporation and poor hydrological exchange, surface brines are particularly susceptible to the accumulation of both organic and inorganic pollutants, including heavy metals and persistent organic compounds (Minella et al., 2013). Recent surveys have revealed that organic contaminants originating from industrial activities and transportation have permeated both surface and, to a lesser extent, deep brine systems (Besser and Hamed, 2019; Wang et al., 2023), thereby providing a natural "deep–surface" comparative framework for studying pollution and microbial ecological responses.

While previous research has investigated microbial diversity in salt lakes (Oren et al., 2009; Tazi et al., 2014), comprehensive comparisons of microbial community structures between surface and deep brines, particularly concerning environmental pollution, remain scarce. It remains uncertain whether deep brines preserve their indigenous microbial assemblages or have been altered by anthropogenic pollutants. Moreover, the impact of various pollutants—especially heavy metals and organic compounds—on the composition and diversity of microbial communities in hypersaline environments is not well understood. Understanding these relationships is essential for evaluating ecological risks and predicting microbial responses in extreme environments under escalating environmental pressures.

In this study, we conducted an investigation on three representative brine samples from the Qaidam Basin, comprising two deep gravel-layer brines and one surface brine. Employing 16S rRNA high-throughput sequencing, gas chromatography-mass spectrometry (GC-MS) for organic pollutant profiling, and inductively coupled plasma mass spectrometry (ICP-MS) for trace metal analysis, our objectives were to: 1) compare the microbial community composition between surface and deep brines; 2) analyze the distribution patterns of heavy metals and organic pollutants across different brine environments; and 3) explore potential microbial responses to complex pollution stress. The outcomes of this study are anticipated to provide significant insights into pollutant–microbe interactions within deep and surface hypersaline systems, thereby contributing to future environmental monitoring and pollution risk assessments in extreme saline environments.

2 Materials and methods

2.1 Hydrogeological conditions

The study area is situated in the northwestern region of the Qaidam Basin. The predominant stratigraphic units exposed in this region are the Upper Pleistocene deposits of the Quaternary System. The lithological composition primarily consists of gray to gray-white gravel and sand, with an approximate thickness of 3 m. The groundwater within this study area is part of the Mahai Basin, a subsidiary basin of the Qaidam Basin. The groundwater in the Mahai Basin is primarily sourced from atmospheric precipitation and the melting of ice and snow in the mid to high-altitude mountainous regions of the northern and eastern Qilian Mountains. It is subsequently depleted through lake water discharge and surface evaporation. The groundwater dynamics are influenced and regulated by a multitude of factors, including geological structures, landforms, lithofacies, climate, and hydrological conditions. The hilly regions serve as natural barriers to groundwater flow, while the extensive unconsolidated deposits in the piedmont alluvial fans and lacustrine plains to the east and north provide substantial capacity for groundwater storage.

The deep brine examined in this research is characterized as fracture pore water within clastic rock formations. The aquifer's lithology comprises Neogene and Paleogene sandstone, silt-fine sandstone, as well as Cretaceous and Jurassic sandstone. This highly mineralized brine frequently coexists with oil and natural gas deposits. The burial depth of the water-bearing rock formations typically ranges from 300 to 2018 m. The chemical composition of the deep brine in the study area is predominantly of the chloride type, exhibiting moderate mineralization.

2.2 Sampling locations

This study selected two deep brine samples and one surface brine sample as research subjects, based on variations in water types and spatial distribution. These samples are designated as B1, B2, and C1 (Figure 1). B1 and B2 are deep brine samples situated within the same tectonic unit in the western region of the Qaidam Basin, north of Dezong Mahai Lake. In contrast, C1 is a surface brine sample

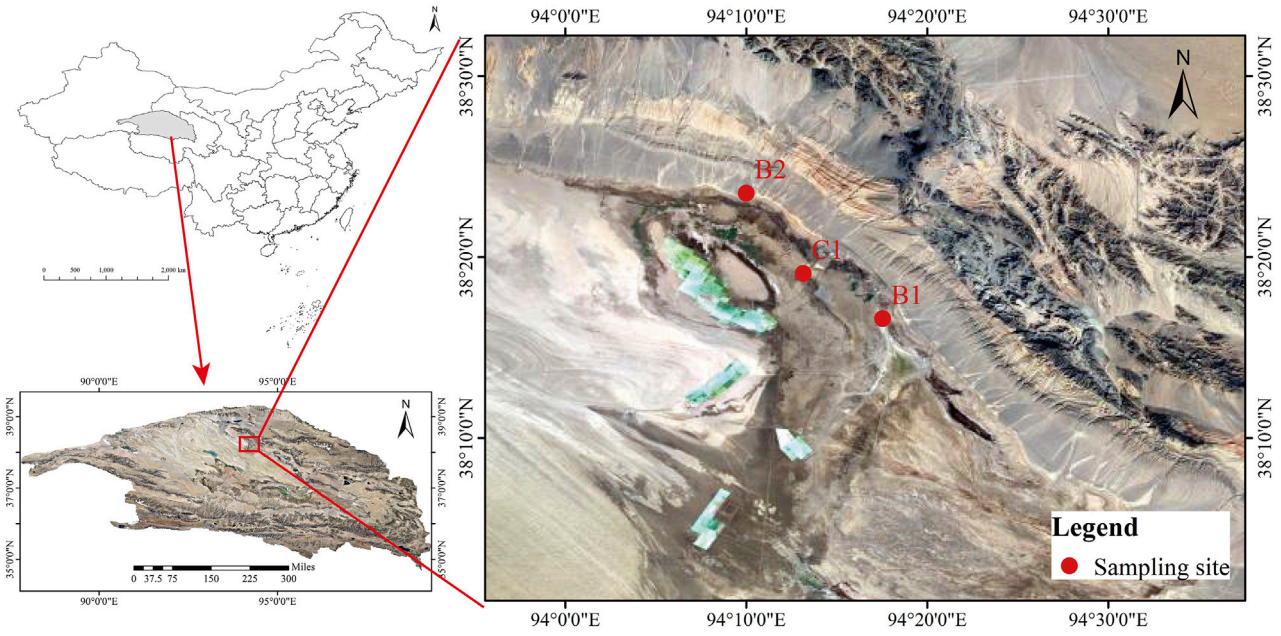

FIGURE 1
Schematic diagram of sampling point locations.

collected from the Mahai Mining Area. The sampling depths of B1 and B2 are 1092.45 m and 1122.32 m, respectively, and the horizontal distance is approximately 16 km. Despite their similar burial depths and essentially identical tectonic backgrounds, the distinct geographical locations of B1 and B2 reflect differences in spatial distribution within the deep brine system, thereby facilitating the understanding of lateral variation characteristics in this system. C1 is situated between B1 and B2 and primarily derives from DeZong Mahai Lake, representing a typical surface brine environment. The origins of the deep brines in B1 and B2 are preliminarily attributed to mountain-front leaching, which percolates underground to form these deposits.

2.3 Sample collection, transportation, and storage

In August 2024, water sampling was conducted at three locations: B1 (deep brine), B2 (deep brine), and C1 (surface brine). Five samples were collected at each sampling point, with each sample being 1L, totaling 15 samples. At each location, three samples were collected using sterile water sampling bags for 16sRNA detection, and two samples were collected using PVC bottles (rinsed three times with the water sample before collection) for heavy metal detection and GC-MS. The samples were stored at 4 °C, transported to the laboratory, and immediately subjected to microbial testing and routine ion analysis.

2.4 Heavy metal analysis

Samples were digested with $HClO_4$, HNO_3, HF, and HCl, diluted with dilute HCl, and analyzed by ICP-OES (Agilent 5110, Malaysia). For elevated Bi/Hg/Mo/Ag/W concentrations, appropriate dilutions were prepared prior to ICP-MS analysis. After correcting for spectral interferences, final results were obtained. All samples were measured in triplicate, with precision (relative deviation, RD) and accuracy (relative error, RE) controlled to <10%.

2.5 Physicochemical analysis of brines

All samples were diluted to salinity <2 g/L prior to analysis. Carbonate/bicarbonate, chloride, and magnesium were quantified by volumetric titration, while potassium, sodium, calcium, and sulfate were measured using ICP-OES (Agilent 5900, United States). Triplicate measurements yielded relative standard deviations (RSD) < 5% for all analytes.

2.6 GC-TOF-MS analysis

5 mL sample and 10 μL internal standard were added into a 20 mL Agilent headspace bottle, then placed on the sampling tray and analyzed by gas chromatograph coupled with a time-of-flight mass spectrometer (GC-TOF-MS).

GC-TOF-MS analysis was performed using an Agilent 7890B gas chromatograph coupled with a time-of-flight mass spectrometry system (Pegasus BT, Leco). A DB-WAX (30 m × 0.25 mm × 0.25 um)capillary column was used for separation. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The temperature of injection was set to 245 °C. The source temperature was 220 °C. The initial temperature was kept at 40 °C for 3 min, then raised to 105 °C at a rate of 6 °C min^{-1}, then raised to 180 °C at a rate of 4 °C min^{-1}, then raised to 245 °C at a rate of 10 °C min^{-1} and kept for 5 min at 300 °C. The energy was 70 eV in electron impact mode.

The mass spectrometry data were acquired in full-scan mode with the m/z range of 35–450 at a rate of 15 spectra per second. To assess the stability and reproducibility of the instrumental analysis, quality control (QC) samples were prepared by pooling aliquots from all individual samples and were analyzed alongside the experimental samples.

2.7 16s RNA sequencing

Firstly, the samples from the three sterile water sampling bags were filtered using a vacuum pump with a 0.22-micron organic filter membrane. After filtration, the filter membrane was placed into a cryotube and stored at −80 °C, then sent for analysis. This part of the process was completed by Zhongke New Life Co., Ltd. Then, Total genome DNA from samples were extracted using Mag-bind soil DNA kit (Omega), and tests the purity and concentration of DNA. For microbial analysis, each sample was subjected to six technical replicates to ensure the accuracy and reliability of the results. According to the selection of sequencing region, the selected V3-V4 variable region was amplified by PCR using specific primers with Barcode and high-fidelity DNA polymerase. PCR products were detected by 2% agarose gel electrophoresis, and the target fragments were cut and recovered by Quant-iT PicoGreen dsDNA Assay Kit. Referring to the preliminary quantitative results of electrophoresis, the PCR amplification recovered products were detected and quantified with the Microplate reader (BioTek, FLx800) fluorescence quantitative system, and the corresponding proportions were mixed according to the sequencing requirements of each sample. The library was constructed using TruSeq Nano DNA LT Library Prep Kit from Illumina. The constructed library is inspected by Agilent Bioanalyzer 2100 and Promega Quanti Fluor. After the library is qualified, it is sequenced.

2.8 Data analysis

Raw sequencing data were in FASTQ format. Paired-end reads were then preprocessed using cutadapt software to detect and cut off the adapter. After trimming, paired-end reads were filtering low quality sequences, denoised, merged and detect and cut off the chimera reads using DADA2 with the default parameters of QIIME2. At last, the software output the representative reads and the ASV abundance table. The representative read of each ASV was selected using QIIME 2 package. Representative sequence reads were taxonomically annotated using the SILVA database (version 138, 16S/18S rDNA) through the classify-sklearn classifier with default parameters. Alpha and beta diversity indices were calculated using QIIME2. Alpha diversity assesses microbial richness and diversity within individual samples, while beta diversity compares microbial community composition between samples.

Due to the limited environmental monitoring infrastructure and lack of reliable baseline pollution data in the study area, the screening criteria for organic pollutants detected by GC-MS were established with reference to the U.S. EPA's Integrated Risk Information System (IRIS) database and the National Institute of Standards and Technology (NIST). The correlations between dominant microorganisms and hydrochemical factors were analyzed using SPSS software (IBM SPSS Statistics 23) and represented the results through a correlation heat map. The Mantel test and redundancy analysis (RDA) were performed using R software (v 4.5.0) to determine the response relationships between microbial community diversity and hydrochemical factors.

3 Results

3.1 Physicochemical properties of brine samples

In order to gain insight into the variability of organic matter and its sources in surface and deep brines, this study examined the characteristics of the distribution of Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC), Total Nitrogen (TN), pH, and Total dissolved solids (TDS) of different samples (Table 1).

The results revealed that although B1 and B2 were collected at similar depths and within the same structural unit, they exhibited substantial differences in organic matter content. Specifically, B1 contained significantly higher organic matter (11.33%) than B2 (7.26%), whereas B2 demonstrated elevated dissolved organic carbon levels (3.63%) compared to B1 (2.19%). As shown in the table, the pH values of both surface and deep brines exhibit minimal variation, maintaining a neutral range of 6.8–6.9. According to the groundwater quality standard (GB/T 14848-2017), deep brine should be classified as a Class V water body and should not be used as a source of domestic drinking water. And TDS levels exceed the standard, possibly due to hydrogeological influences. Based on TDS classification, both surface and deep brine are categorized as brine. The TDS in surface water exceed those in deep brine, a phenomenon closely linked to evaporation processes occurring within the mining area.

Additionally, the organic matter content in surface brine is two to three times higher than that in deep brine, while the total nitrogen content remains relatively consistent between the two. The surface brine has the highest DOC concentration of 18.64 mg/L, which is 6–9 times higher than that of deep brine.

3.2 Pollutant distribution characteristics

3.2.1 Heavy metal pollutant distribution characteristics

According to the groundwater quality standard (GB/T14848-2017), analysis of Figures 2a–c reveals that both surface and deep brines are contaminated with heavy metals to varying extents. Currently, three primary heavy metal pollutants have been identified: Mn, Cd and Pb. Notably, while the concentrations of most heavy metals in deep brines exhibit minimal variation, the Mn concentration in sample B2 (average 9.70 mg/L) is significantly higher than in sample B1 (average 3.00 mg/L). Furthermore, deep brine generally exhibits higher concentrations of heavy metals compared to surface brine. Specifically, Mn concentrations in surface brine range from 0.48 to 0.52 mg/L, whereas in deep brine, they range from 2.84 to 10.10 mg/L. The Mn concentration in deep brine significantly exceeds the background value, reaching levels 2 to 7 times higher. Additionally, the

TABLE 1 Physical and chemical properties of different samples.

Sample	TOC (mg/L)	DOC (mg/L)	TN (mg/L)	pH	TDS (mg/L)	C/N
B1	11.33	2.19	11.043	6.9	2.23×10^5	1.03
B2	7.262	3.63	11.147	6.8	2.49×10^5	0.65
C1	22.193	18.64	13.062	6.9	4.98×10^5	1.70

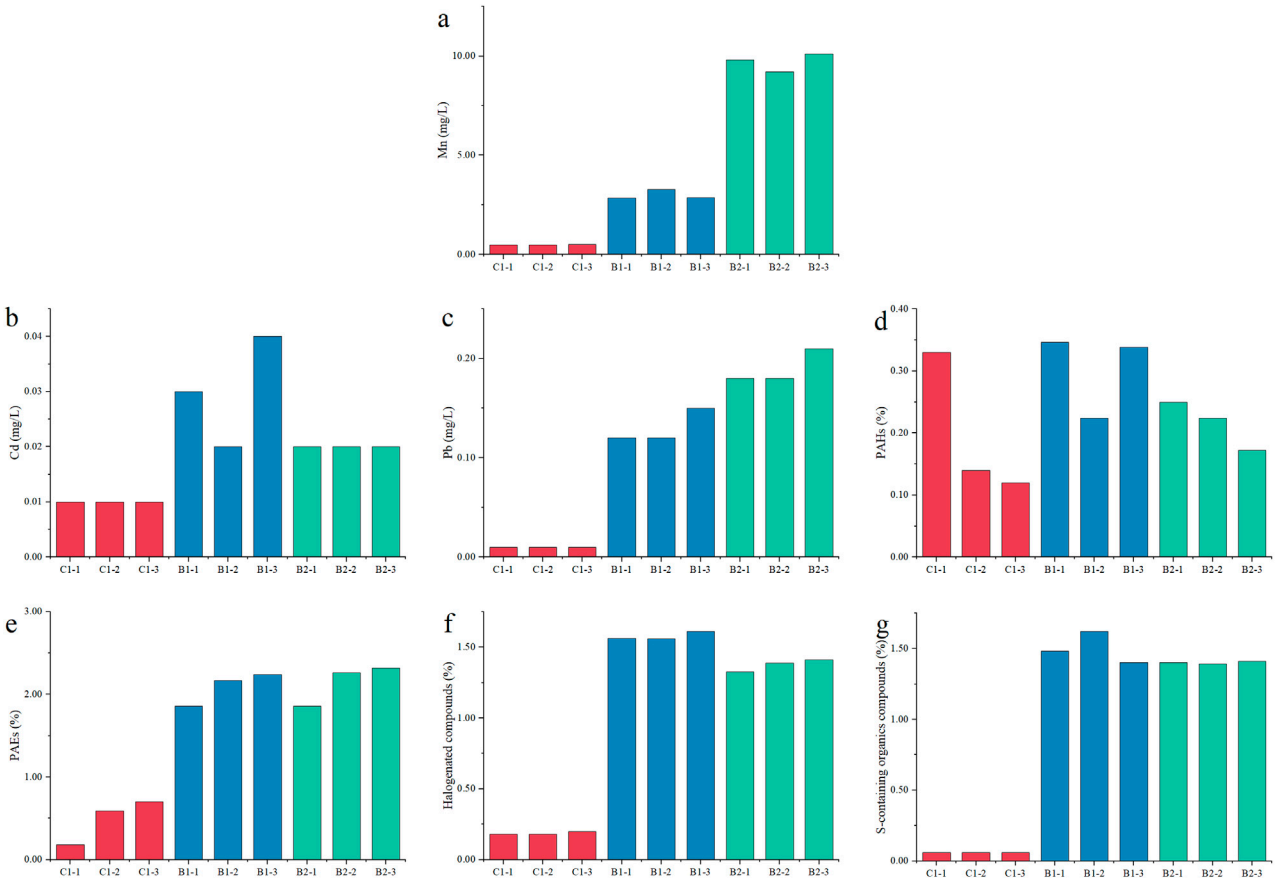

FIGURE 2
Contents of organic pollutants and heavy metal pollutants in deep brine and surface brine. **(a)**: Mn content, **(b)**: Cd content, **(c)**: Pb content, **(d)**: PAHs content, **(e)**: PAEs content, **(f)**: Halogenated compounds content, **(g)**: S-containing organics compounds content.

concentrations of Cd (0.02–0.04 mg/L) and Pb (0.12–0.18 mg/L) in deep brine are slightly elevated compared to those in surface brine (0.01–0.03 mg/L for Cd and 0.01 mg/L for Pb).

3.2.2 Volatile organic pollutant distribution characteristics

Utilizing the databases from the National Institute of Standards and Technology (NIST) and U.S. EPA Integrated Risk Information System (IRIS), we classified anthropogenic contaminants in both deep and surface brines into four categories: polycyclic aromatic hydrocarbons (PAHs), phthalate esters (PAEs), halogenated hydrocarbons, and sulfur-containing compounds. In deep brines, we identified five PAH species, compared to four in surface brines, three PAEs, compared to four, four halogenated hydrocarbons, compared to seven, and one sulfur-containing compound, compared to three (Supplementary Table S1). Although surface brines exhibited a greater diversity of organic pollutants, with a total of 15 species compared to 13 in deep brines, the concentrations of contaminants were consistently higher in deep brines (Figures 2d–g).

3.3 Microbial diversity and community structure

The Alpha diversity index values of the microbial communities in surface and deep brines are summarized in Supplementary Table S2. The coverage values of each sample were greater than 99%, indicating that most microorganisms could be explained by the applied sequencing depth. The ACE and Chao1 indexes used to estimate the total number of microbial species ranged from 319.32 to

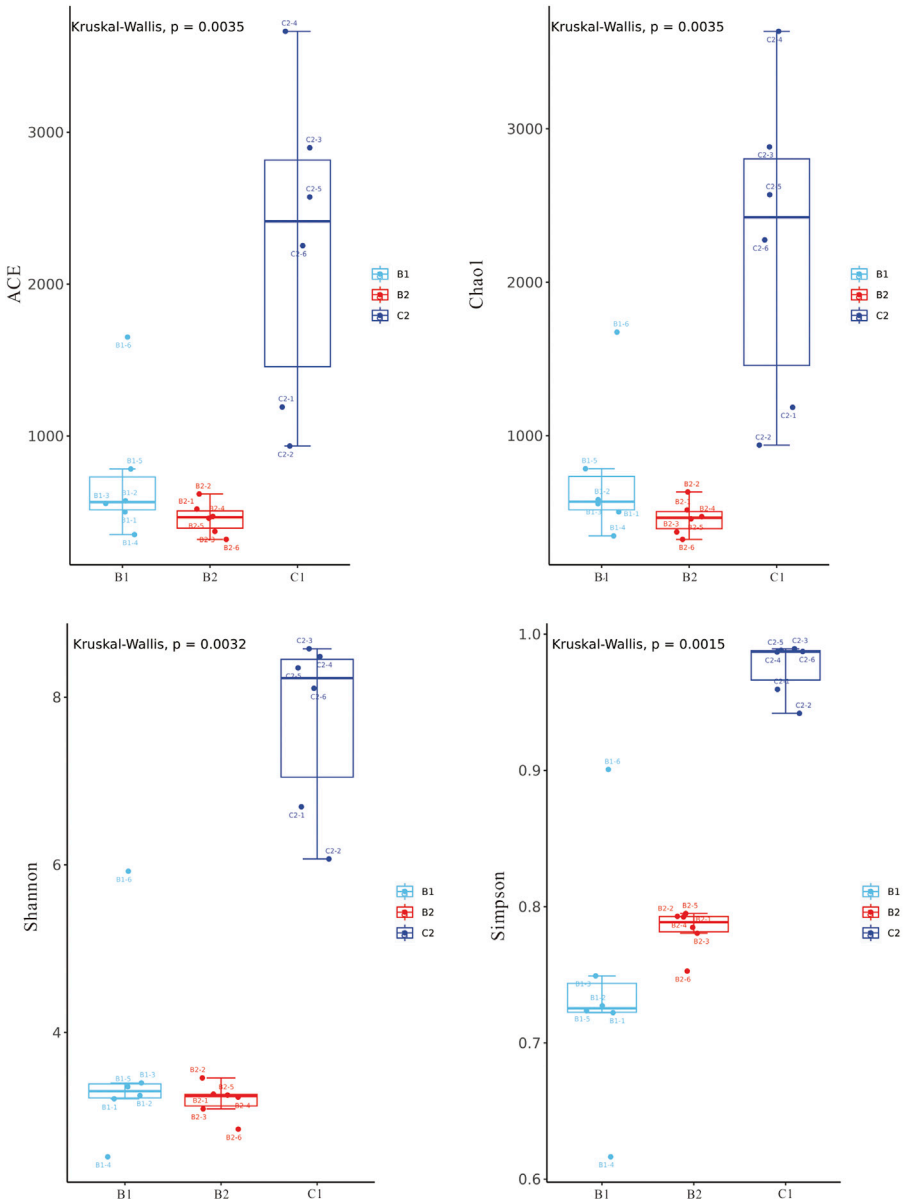

FIGURE 3
Analysis on the difference of α diversity index between deep brine and surface brine.

1651.56 and 324.25 to 1675.12 in deep brines, and from 933.92 to 3665.13 and 938.73 to 2882.30 in surface brine, respectively. The Shannon and Simpson indexes, used to estimate microbial diversity in samples, ranged from 2.52 to 5.92 and 0.62 to 0.96 in deep brines, and from 6.69 to 8.58 and 0.94 to 0.99 in surface brine (Figure 3).

The composition of microbial communities exhibited significant differences between surface and deep brines. At the phylum level, four phyla were predominant (>1%) in deep brines (Figure 4a). Among these dominant phyla, *Proteobacteria* and *Halanaerobiaeota* collectively constituted over 80%. In surface brines, four phyla were also dominant, with *Proteobacteria* and *Firmicutes* account for 74% (Figure 4a). To further elucidate the differences in microbial communities between surface and deep brines, the microbial composition was analyzed at the genus level (Figure 4b). At this level, it was observed that common genera varied considerably among samples, including those from deep brines. In sample B1, four genera were dominant (>1%), namely, including *Halovibrio*, *f_Halomonadaceae|g_uncultured*, *Halanaerobaculum* and *Halanaerobacter*, with *Halovibrio* and *Halanaerobaculum* together comprising 73% of the total. In sample B2, three genera were dominant (>1%), including *f_Halomonadaceae|g_uncultured*, *Halanaerobium* and *Paraliobacillus*, with *f_Halomonadaceae|g_uncultured* and *Halanaerobium* together accounting for 77% of the total. In sample C1, only two genera were dominant (>1%), namely, *f_uncultured|g_uncultured* and *Thiohalorhabdus*, and these two dominant genera together account for 74%.

The Venn diagram presented in Figure 4c delineates the distribution of microorganisms across various samples. In the deep

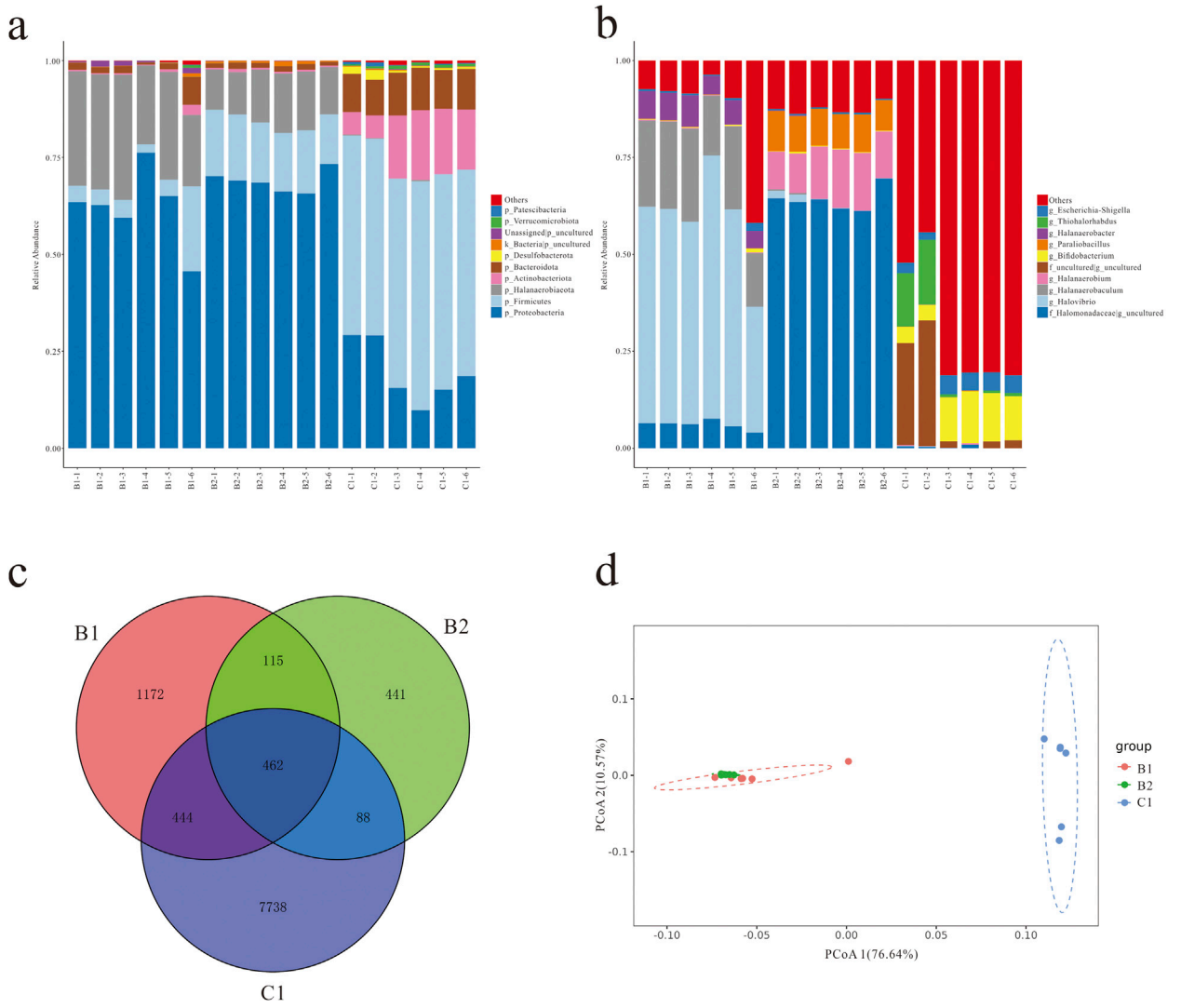

FIGURE 4
Distribution of microbial community structure in brine: **(a,b)** present the composition of the brine microbial community at the phylum and genus levels respectively, **(c)**: shows the Venn diagram of the OTU sample distribution, **(d)**: shows the PCoA analysis of the brine sample.

brine environment, sample B1 exhibits 1,172 unique Operational Taxonomic Units (OTUs), while sample B2 contains 441 unique OTUs. The surface brine sample harbors the highest number of unique OTUs, totaling of 7,738. Among the three samples, B1 and B2 share 115 OTUs, B2 and C1 share 88 OTUs, B1 and C1 share 444 OTUs, and all three samples collectively share 462 OTUs. These findings indicate significant differences in the structural composition of microbial communities between surface and deep brine environments. As depicted in the Principal Coordinate Analysis (PCoA) correlation analysis in Figure 4d, the principal components PC1 and PC2 account for 76.64% and 10.57% of the variance in microbial community composition, respectively. The PCoA results reveal that the microbial community structures within deep brine samples are similar and form distinct clusters, yet they differ markedly from those in surface brine samples.

Figure 5 illustrates the microbial taxa with LDA scores exceeding 2, indicating significant intergroup differences, alongside their phylogenetic clades. The blue nodes denote surface brine, which contains 311 significantly differentiated species primarily from the phyla *Firmicutes*, *Actinobacteriota*, and *Bacteroidota*. In contrast, the green and red nodes represent deep brine layers B2 and B1, which harbor 31 and 16 distinct microbial taxa, respectively, predominantly from the phyla *Proteobacteria* and *Halanaerobiaeota*. The results demonstrate substantially higher abundance of differential microbial communities in surface brine compared to the deep layers (311 vs. 31/16), likely due to the more open environmental conditions of the surface brine. Further investigation is necessary to explore potential correlations with environmental factors.

3.4 Relationship among microbial community structure, pollutants and environmental factors

Through a detailed analysis of the correlations among pollutants, environmental factors, and microbial communities, a

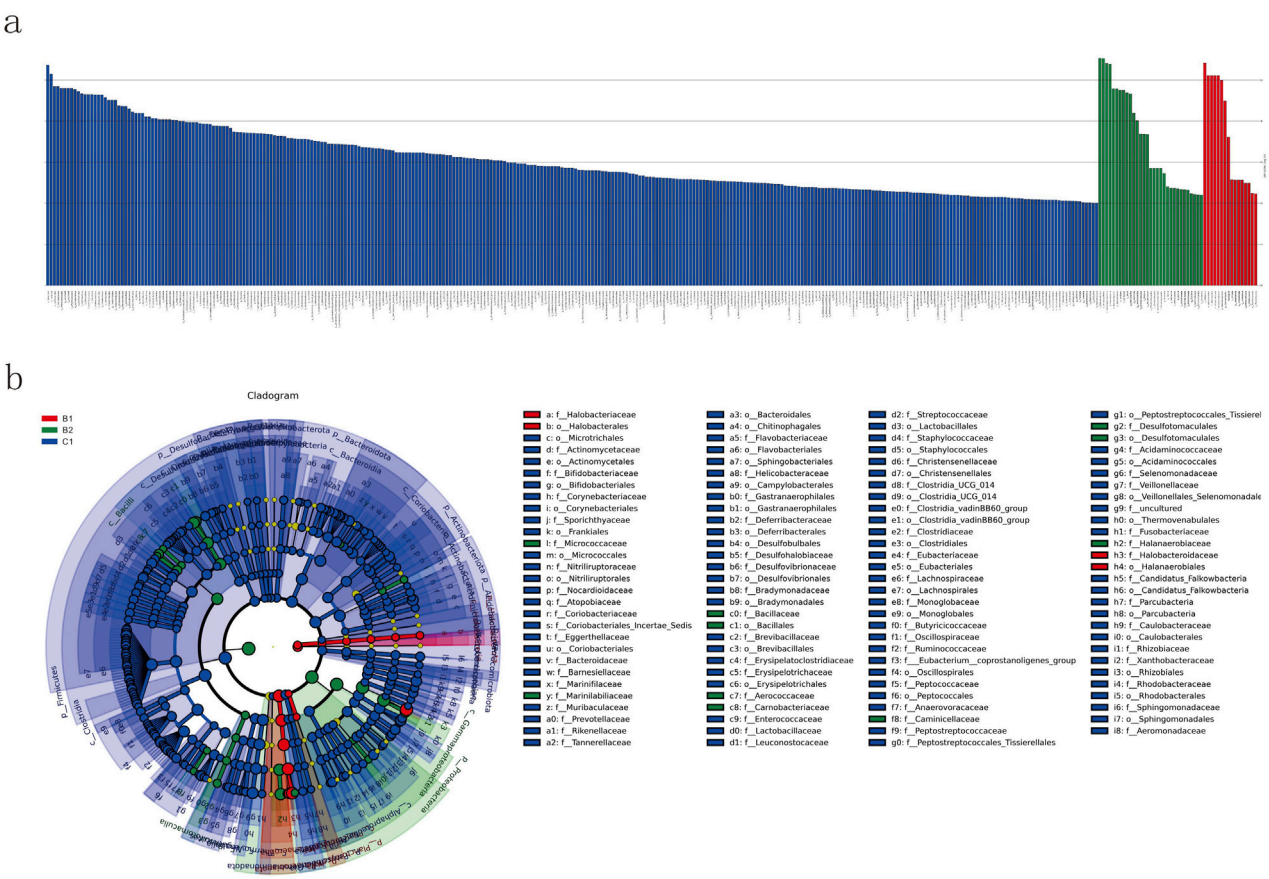

FIGURE 5
The potential biomarker was defined by LEfSe. **(a)** shows a histogram of linear discriminant analysis (LDA) scores for features rich in differences between groups. The log LDA score threshold for discriminant features is set to 2.0, **(b)** shows a cladistic plot representing significant differences in classification between deep and surface brine formations.

correlation heatmap was constructed (Figure 6), elucidating the intricate interactions between water chemistry, pollutants, and microbial composition. The findings indicated that, with the exception of PAHs and Mn, all other pollutants exerted a significant influence on the five dominant microbial groups in the brine. Although most water chemistry parameters did not exhibit strong correlations with PAHs and Mn, TC and TOC were notable exceptions, showing certain degrees of association. Specifically, Pb showed strong positive correlations with TN, TOC, TC, sulfate (SO_4^{2-}), magnesium (Mg^{2+}), TDS, and DOC. Cd was strongly correlated with TDS and potassium (K^+). PAEs exhibited significant correlations with DOC, TDS, K^+, Mg^{2+}, SO_4^{2-}, TC, and TOC. Halogenated compounds were closely related to DOC, TDS, K^+, Mg^{2+}, SO_4^{2-}, TC, TOC, and TN. Sulfides showed strong correlations with DOC, Ca^{2+}, Mg^{2+}, SO_4^{2-}, TC, TOC, and TN. Overall, the majority of pollutants were strongly associated with organic matter in the brine. Furthermore, significant correlations were identified among water chemistry parameters, as well as between water chemistry and pollutants. Consequently, further research is warranted to investigate the interactions among microbial community structure, water chemistry, and pollutants. This will enhance our understanding of the effects of pollutants on microbial composition and ecological functions.

This study conducted Redundancy Analysis (RDA) and Principal Component Analysis (PCA) to evaluate the impact of pollutants and hydrochemical factors on the microbial community structure in both deep and surface brine environments. The RDA findings demonstrated that the first two axes, RDA1 and RDA2, accounted for 70.23% and 25.41% of the variation in the native microbial community structure within high-salinity water bodies affected by organic and heavy metal pollution (Figure 7). Collectively, the influence of 20 variables explained 95.64% of the observed variation in microbial community composition. Notably, both organic and heavy metal pollutants significantly influenced the variation in microbial communities in deep brine. Importantly, both organic and heavy metal pollutants were found to significantly affect the variation in microbial communities in deep brine. Specifically, the presence of Cd, Pb, PAEs, halogenated compounds, and sulfur-containing organics exhibited strong correlations with the distribution of dominant deep-brine genera such as *Halanaerobaculum*, *Halanaerobacter*, and *Halovibrio*, as indicated by the RDA analysis. These genera exhibited distinct directional associations with the previously mentioned pollutants along the primary RDA axis (RDA1, 70.23%), indicating that fluctuations in pollutant concentrations are intricately connected to the structuring of microbial communities in hypersaline subsurface environments.

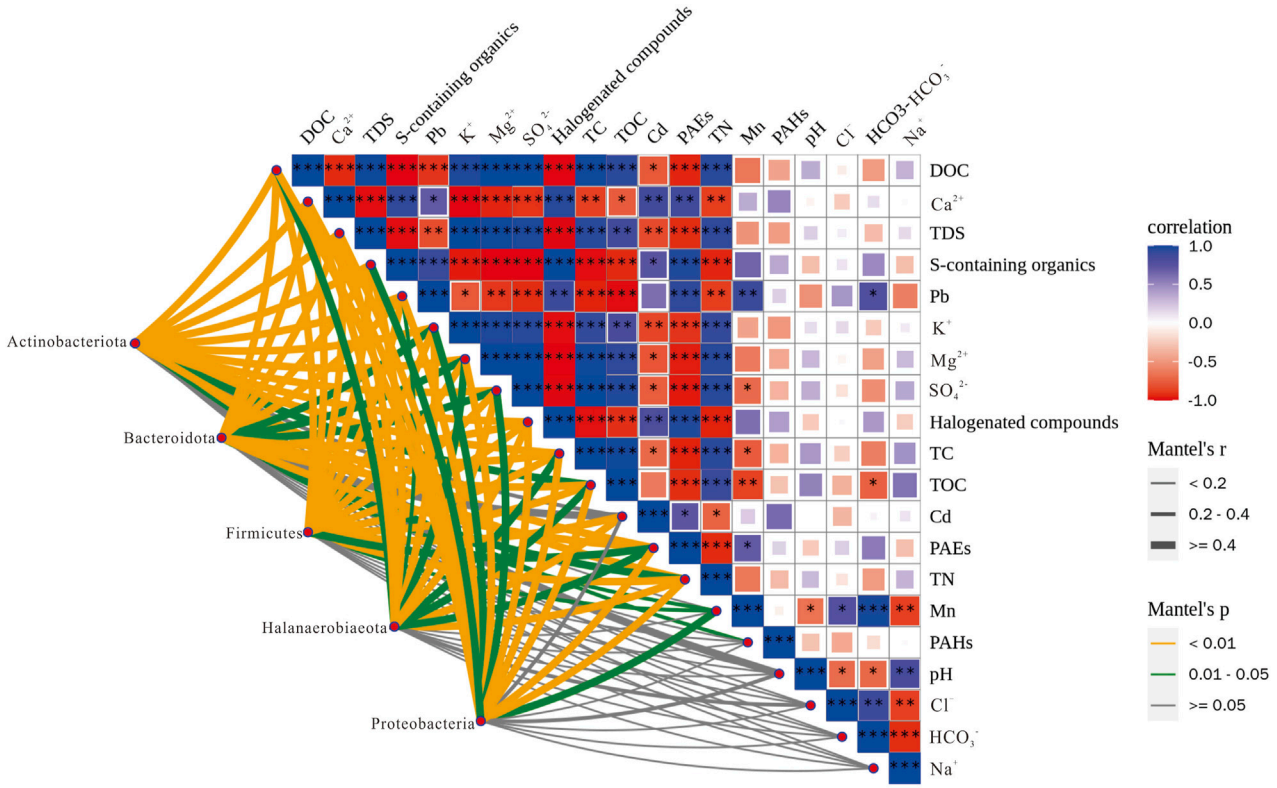

FIGURE 6
The correlation heat map depicting the relationships between different hydrochemical factors, as well as between hydrochemical factors and dominant phylum in deep brines and surface brine of the study area (All variables were normalized using Z-score transformation to enable direct comparison across different units and magnitudes).

4 Discussion

4.1 Composition and sources of organic matter in brine

Dissolved organic matter (DOM) is prevalent in high-salinity waters (Xu and Guo, 2017) and plays a crucial role in influencing nutrient cycling (Mopper et al., 1991), pH buffering capacity (Carter et al., 2012), and the solubility, toxicity, and bioavailability of metals and organic pollutants (Worms et al., 2019). In this study, we found that the TDS, TC, TOC, and DOC in surface brine were elevated compared to those in deep brine, although the total nitrogen content did not exhibit a substantial difference. Previous research has demonstrated that increasing salinity in lakes correlates with an increase in TDS, and the average molecular weight of DOM in brine tends to be larger with a higher degree of oxidation (Xu et al., 2020). Moreover, surface brine is more exposed to external influences compared to deep brine, potentially leading to greater impacts from external organic matter, such as atmospheric precipitation. Based on the analysis of the carbon-to-nitrogen (C/N) ratio, both surface and deep brines demonstrated C/N values below 2. This ratio indicates that the organic matter in these environments predominantly originates from algae (Khan et al., 2015), with a higher concentration of algae present in surface water relative to deep brine. Studies have demonstrated that microbial communities in brine, especially halophilic bacteria, are capable of efficiently utilizing organic nitrogen (Wang and Cui, 2024) and consuming organic carbon (Bonfá et al., 2013; Bonaglia et al., 2020), thereby significantly reducing the C/N ratio. This efficient utilization of organic nitrogen by microbes generally leads to an imbalance between organic carbon and nitrogen, further decreasing the C/N ratio.

Despite B1 and B2 being sampled from comparable depths within the same tectonic unit, their organic matter compositions exhibit notable differences. Specifically, B1 is characterized by a higher total organic carbon (TOC) content of 11.33%, whereas B2 contains a greater proportion of dissolved organic carbon (DOC) at 3.63%. This pattern implies that the organic matter in B2 may have undergone more extensive microbial degradation, leading to an increased presence of soluble organic fractions (Servais et al., 2020), while B1 retains a larger amount of particulate or recalcitrant organic components. This divergence is likely attributable to localized microenvironmental variations between the two boreholes. For example, increased ionic strength could lead to organic matter salting-out effects, thereby promoting the formation of colloids and particulate matter (Tóth et al., 2005). These findings underscore the existence of significant spatial heterogeneity in organic carbon composition and reactivity, even within the same deep brine system. Conversely, while microbial activity predominantly influences brine, external pollutants, notably anthropogenic contamination, may also directly contribute to organic nitrogen inputs (Valiente et al., 2022), potentially

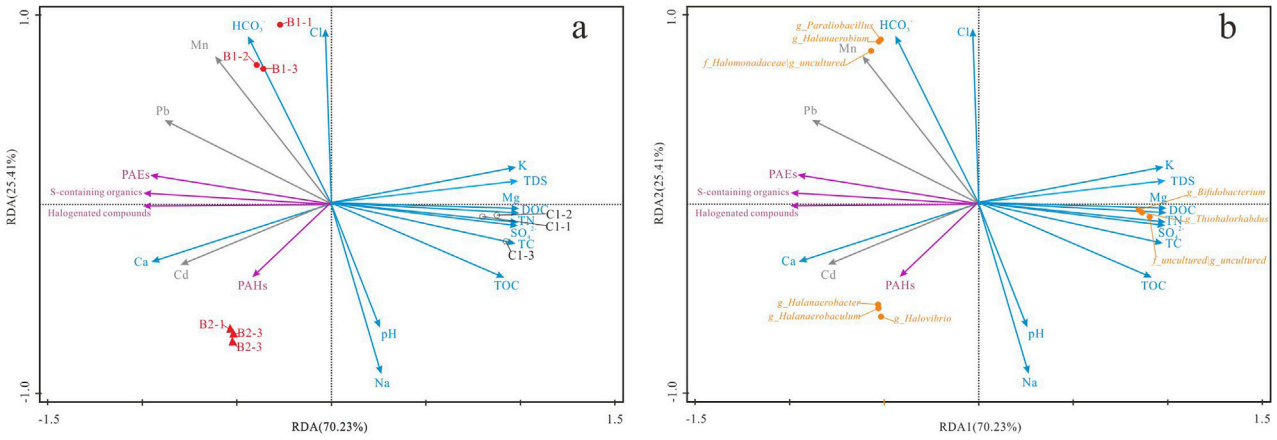

FIGURE 7
The response relationship between indigenous microflora and hydrochemical factors in deep brines and surface brine. **(a)** displays the RDA diagram, highlighting the correlation between samples and hydrochemical factors, **(b)** show cases the RDA diagram, revealing the correlation between dominant genera and hydrochemical factors in groundwater.

accounting for the exceptionally low C/N ratio observed. Thus, although microbial activity remains the principal factor, the impact of external organic nitrogen should be taken into account to offer a more comprehensive understanding of the variations in C/N ratios within brine.

4.2 Mechanisms and accumulation of heavy metals

In both surface and deep brines, we identified three heavy metals with significantly elevated concentrations: Mn, Cd, and Pb. Manganese (Mn) is recognized for its potential to induce a range of neurotoxic symptoms in clinical contexts, with chronic or acute exposure potentially resulting in severe outcomes, including cognitive and psychiatric symptoms, Parkinson's disease, motor dysfunction, and other neurodegenerative disorders (Das et al., 2015). Mn is widely distributed in geological formations, particularly with in aquifer sediments, and can leach into groundwater, thereby constituting a common chemical component (Wu et al., 2019). In natural environments, the predominant mechanism facilitating the release and migration of Mn from aquifer sediments to groundwater is its redox dissolution (Moon et al., 2020). Although Mn in groundwater primarily originates from natural sources (Zhai et al., 2019), research has also indicated that its concentration may be influenced by anthropogenic activities (Li et al., 2020). Previous research has demonstrated a positive correlation between Mn concentrations in groundwater and organic matter content (Liu et al., 2020). Additionally, the presence of organic pollutants has been shown to facilitate the release of Mn from sediments into the water (Liu et al., 2019; Zhai et al., 2021). Contrary to these findings, the present study observed that despite lower organic matter content in deep brines compared to surface brines, Mn concentrations were significantly elevated, exceeding typical industrial heavy metal pollution levels in groundwater (0.663 mg/L) as reported by Adeyemi and Ojekunle (2021). Furthermore, our data indicate substantially higher Mn concentrations in site B2 relative to site B1, which may be associated with increased levels of DOC. This suggests that DOC could potentially enhance Mn mobility through microbially-mediated and chemical reduction processes (Jiann et al., 2013).

Simultaneously, we propose the hypothesis that sediments within deep aquifers may exhibit elevated concentrations of Mn. Additionally, the relatively isolated geographical environment of deep brines may contribute to the accumulation of heavy metals to a certain degree (Stamatis et al., 2019). Pb and Cd, recognized as prevalent heavy metal contaminants, are ubiquitously found in soils (Zhou et al., 2019), rivers (Nasrabadi, 2015), and lakes (Şener et al., 2023), thereby posing substantial threats to both environmental integrity and human health. Studies have shown that Pb and Cd in soils not only affect plant growth but also presents potential health risks to animals and humans through bioaccumulation in the food chain (Chrysochoou and Dermatas, 2015). In rivers and lakes, Pb and Cd pollution predominantly arises from anthropogenic activities (Niu et al., 2021), including wastewater discharge and fertilizer application (Fei et al., 2020). In recent years, Pb and Cd contamination has also been found in high salinity environments, such as salt springs (Rezaei et al., 2019), salt lakes (Baati et al., 2022), and sediments (Rezaei et al., 2021), with primary sources attributed to industrial wastewater from factories, oilfields, and the deposition of industrial particles. This study found that the concentrations of Pb and Cd in deep brines were comparable to those found in freshwater lakes with values of 0.111 mg/L and 0.135 mg/L, respectively (Muneer et al., 2022). However, these concentrations were significantly elevated compared to those in surface brines, thereby reinforcing the hypothesis that closed environments facilitate the accumulation of heavy metals.

4.3 Types and possible sources of organic pollutants

In this study, organic pollutants characterized by their persistence and bioaccumulative properties, were identified in both surface and deep brine samples. Four major classes were

detected: PAHs, PAEs, halogenated compounds, and S-containing organics. PAHs, which consist of multiple fused aromatic rings, are well-documented for their carcinogenic potential and environmental persistence (McCarrick et al., 2019). Research indicates shown that low-molecular-weight PAHs exhibit greater water solubility, whereas high-molecular-weight PAHs are more prone to accumulation in suspended solids and sediments (Rabodonirina et al., 2015). In lakes and rivers, PAHs predominantly originate from the combustion of fossil fuels or biomass (e.g., shrubs or forests) (Wei et al., 2015), as well as from petroleum contamination (Bandowe et al., 2014). Conversely, in saline lakes, atmospheric deposition regarded as the principal source of PAHs (Idowu et al., 2020). The present study found that the PAH concentrations in surface and deep brines were comparable, measuring 0.26% and 0.20%, respectively. In light of the absence of vegetation and oil fields within the study area, it is inferred that atmospheric deposition constitutes the primary source of PAHs in surface brine. This observation further substantiates the hypothesis that deep brine originates from the infiltration of snowmelt into subsurface strata, resulting in the formation of highly mineralized brine under closed conditions (Hongpu et al., 2022). PAEs, extensively utilized as plasticizers, are frequently detected in aquatic environments and sediments. Elevated concentrations of PAEs have been reported in lakes and rivers situated near industrial zones (Gong et al., 2024). In our study, the presence of PAEs in brine is likely attributable to wastewater discharge from mining activities. The elevated levels of PAEs detected in deep brine suggest their accumulation within the confined subsurface environment over time. Halogenated hydrocarbons in saline lakes predominantly originate from biological halogenation processes, which are intensified by elevated sodium chloride concentrations (Timms, 2009).

Previous research has indicated that concentrations of halogenated hydrocarbons are elevated in neutral to alkaline saline lakes compared to acidic ones (Ruecker et al., 2015). Additionally, halophilic microorganisms and enzyme-mediated reactions are pivotal in the formation of halogens within hypersaline environments (Rhew et al., 2000). In this study, deep brine exhibited a significantly higher content of halogenated compounds (1.48%) than surface brine (0.19%), despite comparable NaCl concentrations. This suggests that microbial communities associated with halogenation processes may be considerably more abundant in deep brine. S-containing organic compounds have garnered significant attention due to their implications in atmospheric chemistry and global climate regulation (Andreae and Crutzen, 1997). In freshwater lakes, sulfide formation is generally linked to microbial activity (Hu et al., 2007) and the metabolism of sulfur-containing amino acids (Caron and Kramer, 1994). Conversely, in saline lakes, sulfide formation is more closely related to microbial degradation and sulfate reduction processes (Fritz and Bachofen, 2000; Torfstein et al., 2005).

Furthermore, salinity alterations resulting from anthropogenic activities may influence microbial community functions, thereby impacting sulfide production (Mr et al., 2017). In our study area, the deep brine, although currently undeveloped, exhibited a higher concentration of S-containing organic compounds compared to the surface brine. This suggests a greater prevalence of sulfate-reducing bacteria in the deep brine. Microbial community analysis corroborated this finding, revealing that the relative abundance of *Proteobacteria* in deep brine was more than three times greater than in surface brine, while the abundance of *Halanaerobiaeota* was over 200 times higher. Previous research has identified Proteobacteria as major sulfate-reducing species in high-salinity environments (Santos et al., 2020). Additionally, Boidi et al. (2022) reported that sulfate-reducing functional groups within the microbial mats of Laguna Negra's high-salinity environments are predominantly composed of *Halanaerobiaeota*, underscoring the significant role of sulfate reduction in deep brine.

4.4 Differences in microbial community structure and the effects of pollutants on microorganisms

As shown in Figure 4A, the microbial community in deep brine was predominantly composed of the phylum *Proteobacteria*, whereas *Firmicutes* emerged as the most abundant phylum in surface brine. Within the *Proteobacteria*, both *α-Proteobacteria* and *γ-Proteobacteria* are extensively acknowledged as pivotal microbial groups involved in the degradation of organic pollutants (Moxley and Schmidt, 2012; Cui et al., 2014; Moghadam et al., 2014). *Firmicutes*, frequently associated with petroleum degradation, flourish in eutrophic environments and plays an important role in carbon and nitrogen cycling (Hellal et al., 2021; Fang et al., 2023). In addition to Proteobacteria, the deep brine also exhibited relatively high proportions of *Firmicutes* (6.84%–15.65%) and *Halanacrobiacota* (12.95%–26.35%). Conversely, surface brine demonstrated higher relative abundances of *Actinobacteriota* (13.06%) and *Bacteroidota* (10.26%). These dominant microbial phyla have been documented in hypersaline environments and are known for their capabilities in degrading organic pollutants and heavy metals (Ye et al., 2016; Alvarez et al., 2017; Liu et al., 2023).

At the genus level (Figure 4B), several taxa exhibited high relative abundances within the brine samples, including *f_Halomonadaceae|g_uncultured*, *Halovibrio*, *Halanaerobaculum*, *Halanaerobium*, Bifidobacterium, *Paraliobacillus*, and *Thiohalorhabdus*. Notably, substantial differences were observed among the microbial communities at the genus level across samples B1, B2, and C1. Specifically, *Halovibrio* was predominant in B1, *f_Halomonadaceae|g_unculture*d was the most prevalent in B2, while Bifidobacterium was the dominant genus in C1. These observations indicate significant differences in the indigenous microbial composition between deep and surface brine, potentially attributable to variations in hydrogeological conditions, physicochemical properties, pollutant concentrations, and organic matter composition. Importantly, despite the high salinity conditions, the majority of the dominant genera identified possess known capabilities for degrading heavy metals and organic contaminants (Ottoson, 2009; Mohammadipanah et al., 2015; Cui et al., 2019; Boltyanskaya et al., 2023). Figure 4C illustrates the unique and shared OTUs between deep brine and surface brine samples. The deep brine contained the highest number of unique OTUs, totaling 7,738, indicating significantly greater microbial diversity compared to surface brine.

Additionally, PCoA demonstrated a distinct separation between the microbial communities of surface and deep brine, with a high degree of similarity observed between samples B1 and B2. Remarkably, RDA indicated that pollutants exerted a more pronounced influence on the microbial communities in deep brine compared to those in surface brine. The structure of local microbial communities was significantly shaped by most organic pollutants and heavy metals. Interestingly, PAHs exhibited a relatively minor impact on the microbial community, potentially due to their limited bioavailability in brine systems (Lindgren et al., 2014). Nonetheless, considering that the ecological effects of PAHs can vary substantially across different environmental contexts (Picariello et al., 2020), it is crucial to further investigate the conditions under which PAHs might influence microbial community dynamics in brine systems. In contrast to the long-exposed surface brine, deep brine exists in a more isolated environment. However, under high-salinity conditions, organic pollutants and heavy metals tend to accumulate and persist more readily in deep brine, exerting stronger impacts on subterranean microbial ecosystems. Consequently, greater emphasis must be placed on these factors when evaluating the future exploitation and utilization of deep brine resources.

5 Conclusion

This study conducted a systematic comparison of the surface and deep brines from the Qaidam Basin, focusing on their physicochemical properties, organic matter composition, distributions of heavy metals and organic pollutants, and microbial community structures. The results revealed that surface brine exhibited significantly higher contents of TC, TOC, and DOC, whereas deep brine showed elevated levels of Mn, Pb, and Cd. This suggests that closed subsurface environments are conducive to the accumulation of heavy metals. Furthermore, the low C/N ratios in both brine types imply a predominantly microbial origin of organic matter, potentially supplemented by exogenous organic nitrogen.

Among the pollutants identified, PAHs, PAEs, halogenated compounds, and S-containing organics were more concentrated in deep brines. This enrichment suggests that their sources are likely related to atmospheric deposition, biogenic halogenation, industrial activities, and wastewater discharge from mining operations. Microbial community analysis showed that *Proteobacteria* predominated in deep brines, whereas *Firmicutes* were more prevalent in surface brines. Variations at the genus level, OTU distributions, and PCoA results confirmed significant microbial divergence between the two environments. Furthermore, RDA and correlation heatmaps indicated that pollutants such as Cd, Pb, PAEs, halogenated compounds, and sulfides exerted substantial influences on microbial community structures, with more pronounced pollutant-driven effects observed in deep brines.

In summary, the enclosed and hypersaline environments of deep brines not only promote the accumulation and preservation of heavy metals and organic pollutants but also substantially influence the structuring of microbial communities. These findings highlight that microbial responses not only mirror the ecological stress induced by pollutants but also play a pivotal role in shaping ecological risks. Consequently, these factors should be meticulously considered in the development of future strategies for deep brine resource exploitation and pollution management.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

XL: Resources, Funding acquisition, Conceptualization, Validation, Project administration, Visualization, Writing – review and editing, Methodology, Formal Analysis, Writing – original draft. QW: Software, Writing – original draft. ZW: Formal Analysis, Writing – original draft. YM: Writing – original draft, Resources, Data curation. ZS: Writing – original draft, Investigation. DQ: Project administration, Writing – original draft, Writing – review and editing, Supervision, Validation. ZM: Writing – original draft, Project administration, Validation, Conceptualization, Writing – review and editing.

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Supplementary material

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