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The Correlation Between High-Fluoride Hot Springs and Microbial Community Structure and Diversity

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Abstract

High-fluoride hot springs serve as a natural laboratory for investigating microbial adaptation and variations in community structure under extreme environments. This study utilized water chemistry analysis and 16S rRNA gene sequencing to investigate the correlation between high-fluoride hot springs and microbial community structure and diversity. The results show that the five hot springs exhibited an average F^- content of 15.04 mg/L, with weakly alkaline pH, high total dissolved solids, and Na^+ as the dominant cation. The hydrochemical type was classified as $HCO_3 \cdot SO_4$-Na, consistent with the chemical characteristics of high-fluorine water. Microbial abundance and diversity were significantly reduced in the hot springs as compared to the surface water and groundwater samples. The dominant phyla in the study area included Pseudomonadota, Cyanobacteriota, Bacteroidota, and Actinomycetota. The genus-level composition varied significantly across samples, with no dominant genus observed universally. The specific genera present in different samples exhibit unique functional attributes, such as *Tepidimonas*, *Rhodobacter*, *Hyphomonas*, *Parvibaculum*, *Polynucleobacter* and *Limnohabitans*. Cluster analysis confirmed that dissimilarity coefficients highlight the significant influence of microbial abundance on inter-sample differences among hot springs. Redundancy analysis of the top 11 phyla by abundance in water samples revealed that the presence of F^- exerts inhibitory effects on microbial growth.

Keywords: high-fluoride hot springs; hydrochemistry; microbial community; diversity analysis

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Academic Editor: Ipek Kurtboke

Received: 11 October 2025
Revised: 3 November 2025
Accepted: 7 November 2025
Published: 8 November 2025

Citation: Gong, H.; Wang, Q.; Yang, L.; Liao, J. The Correlation Between High-Fluoride Hot Springs and Microbial Community Structure and Diversity. *Diversity* **2025**, *17*, 784. https://doi.org/10.3390/d17110784

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1. Introduction

Fluorine is an essential trace element that significantly impacts physiological systems. Groundwater fluoridation is a global concern, given the integral role of groundwater in ecosystems and as a significant source of drinking water [1]. The global distribution of fluoride contamination spans five principal endemic zones, affecting 25 nations, including 23 developing countries, commonly in arid and semi-arid regions [2–6]. The concentration of fluoride is generally higher in geothermal water than groundwater because the high temperature of geothermal water promotes dissolution of fluorine-bearing minerals and desorption of fluorine in adsorbed states [7].

Microorganisms, as essential components of groundwater systems with fundamental roles in ecosystems and biogeochemical cycle [8–10], are involved in the geochemical pro-

cesses of groundwater pollution and natural attenuation, and can serve as indicators of changes to the groundwater environment [11–14]. The microbial composition of groundwater is influenced by fluoride contamination, which can inhibit microbial growth [15]. Microbial abundance and diversity are significantly greater in groundwater with a low fluoride concentration [16] and the fluoride concentration has been significantly negatively correlated with the Chao1 and Shannon indices [17]. Fluoride and microorganisms in groundwater exhibit bidirectional interactions, as fluoride inhibits microbial growth, while specific microbial communities demonstrate fluoride removal capabilities [18]. Fluoride-resistant microorganisms mediate bioremediation through ion complexation mechanisms, thereby reducing aqueous fluoride concentrations [19]. Microorganisms exhibit distinct fluoride tolerance thresholds, as growth inhibition is greater for moderate thermophiles than mesophiles [20]. However, the metabolic activities of microorganisms can facilitate the migration and release of fluoride from sediments [21,22]. High-fluoride hot springs can be differentiated from typical groundwater by temperature, pH, and nutrient composition, which drive the unique genetic structures and functional traits of microorganisms through evolutionary processes, while preserving ancestral bacterial strains, endemic species, and new microbial resources, particularly thermophilic bacteria [23].

The aim of the present study was to assess the impact of the fluoride content of geothermal environments on microorganisms, with limited research existing on this topic. However, water temperature is a significant factor influencing the abundance and diversity of the microbial community. High-throughput 16S rRNA gene sequencing was employed to analyze microbial communities in five hot springs within Lushan Country, China, by comparing the diversity and structural composition of microbial communities in surface water and groundwater that feed hot springs, reveal the influence of the fluoride content on microbial communities, and explore the impact of other factors in geothermal water. The findings of this study provide valuable insights and references for further research on the relationship between microorganisms and the environmental response of fluoridated water.

2. Material and Methods

2.1. Study Area

The study area included five major hot spring clusters located along the Checun-Lushan Fault in Lushan County, Henan Province (from west to east: Shangtang, Zhongtang, Wentang, Xiatang, and Jianchang), which stretches over a 100-mile hot spring belt, locally known as the “Hundred Mile Hot Spring”. The hot spring water in Lushan, which exhibits exceptional quality, contains more than 10 trace elements, including boron, lithium, fluorine, radium, and radon. Li Daoyuan, a geographer from the Northern Wei Dynasty, in *Commentary on the Classic of Waterways* (Shui Jing Zhu), provides detailed descriptions of these hot springs, noting that “the hot springs emerge from North Mountain Foo, with seven sources of unique characteristics, and their heat is extraordinary” and that these hot springs “can cure all kinds of diseases.” The study area features a warm temperate continental monsoon climate characterized by four distinct seasons and abundant rainfall. The Sha River is the principal river within the study area. The predominant lithology is characterized by magmatic rocks, exhibiting distinct spatial partitioning across the Checun-Xiatang fault zone, where intrusive rock dominates the southern block, while effusive rock prevails in the northern sector. Specifically, Yanshanian granites constitute the principal lithology in Shangtang, contrasting with the andesitic porphyrite assemblages of Xiatang. Plagioclase emerges as the dominant mineral phase, with aluminosilicate as the essential chemical composition [24]. Well-developed joints within the area are closely associated with NE-trending and NW-trending minor faults.

2.2. Sample Collection and Testing

In total, 5 hot spring samples, 9 surface water samples, and 3 groundwater samples were collected along the Checun-Lushan, Luanchuan-Lushan, and Luonan-Gushi fault zones on 8 July 2024 (Figure 1). Field measurements of water temperature, pH, electrical conductivity, and total dissolved solids (TDS) were conducted with a multiparameter water quality analyzer (WTW Multi 3630 IDS; WTW Electronic GmbH, Graz, Austria). Ion chromatography was used to assess the compositions of cations (Na^+, K^+, Mg^{2+}, Ca^{2+}, Li^+) and anions (Cl^-, SO_4^{2-}, F^-). The detection limit was 0.0001 mg/L with an analytical precision of 0.001 mg/L and a charge balance error between cations and anions within ±5%. Microorganisms were enriched by filtering 10 L of each water sample through a 0.22-μm filter membrane, followed by immediate preservation on dry ice for subsequent 16S rRNA high-throughput sequencing.

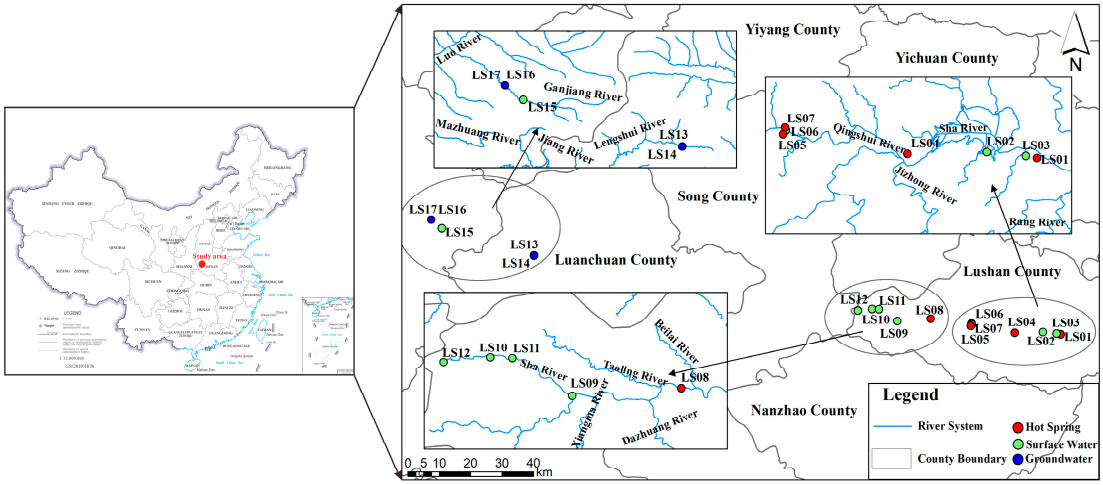


Figure 1. Distribution of the sampling sites.

2.3. Data Processing

Electrophoresis with 1% agarose gels was used to assess DNA degradation and detect impurities. DNA purity was assessed with a NanoPhotometer spectrophotometer (Implen GmbH, Munich, Germany) and the DNA concentration was measured with a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). According to the specified sequencing region, barcode-tagged specific primers or fusion primers containing mismatched bases were synthesized; PCR products were purified using the Agencourt AMPure XP nucleic acid purification kit (Beckman Coulter, Inc., Brea, CA, USA). PCR amplification of target regions was performed using 10 ng of DNA template with the primer pairs 338F/533R, 341F/805R, and 967F/1046R for the V3, V3 + V4, and V6 regions, respectively. The target fragments were amplified by polymerase chain reaction with Ex Taq™ DNA polymerase (TaKaRa Bio, Inc., Shiga, Japan). A DNA library was constructed, which involved ligation of Y-shaped adapters, bead-based size selection to remove dimerized adapters, library amplification via PCR for template enrichment, and NaOH denaturation to generate single-stranded DNA fragments.

After construction, the library was diluted to 1 ng/μL and quantified with a Qubit® 2.0 Fluorometer. The insert size of the library was then assessed using a bioanalyzer system (Agilent Technologies, Inc., Santa Clara, CA, USA). Once the insert size met expectations, the effective concentration of the library was accurately quantified using an iQ SYBR Green Supermix Kit (Bio-Rad Laboratories, Hercules, CA, USA) and a fluorescence quantitative PCR instrument (CFX 96; Bio-Rad Laboratories). For quality control, the library was

sequenced with the MiSeq platform (Illumina, Inc., San Diego, CA, USA) using the PE250 sequencing strategy.

The initial output of Illumina high-throughput sequencing is stored as raw image data files. These files are processed by the CASAVA software for base calling, converting them into raw sequencing reads. Some of these raw reads may contain adapter sequences or low-quality segments. Clean reads for subsequent analyses were acquired by eliminating low-quality reads (Q < 20), Ns reads (N > 5%), and adapter-polluted reads. The corresponding Read1 and Read2 sequence fragments obtained by sequencing in both directions from the 5′ and 3′ ends, respectively, were assembled using the sequence assembly method PEAR [25]. Subsequently, the assembled reads were analyzed using QIIME software version 1.8.0 [26–30]. The SILVA database (Release 128, http://www.arb-silva.de (accessed on 13 July 2024)) and the RDP Classifier algorithm (http://sourceforge.net/projects/rdp-classifier/ (accessed on 13 July 2024)) were employed for taxonomic classification, with a confidence threshold set at 0.7.

3. Results and Discussion

3.1. Hydrochemical Features

Hydrochemically, all five hot springs were classified as $HCO_3 \cdot SO_4$-Na (Figure 2) and the fluoride concentrations exceeded 9 mg/L, which is characteristic of high-fluoride groundwater. Surface water and groundwater samples of hydrochemical types primarily exhibited HCO_3-Ca signatures, with F^- concentrations not exceeding the standards for Class I–III thresholds established by the toxicological index requirements described in the Standard for groundwater quality (GB/T 14848–2017) [31] of ≤1.0 mg/L. Based on this criterion, the five hot spring samples with fluoride concentrations exceeding 1 mg/L were categorized into the high-fluoride (HF) group, while the twelve groundwater and surface water samples containing less than 1 mg/L fluoride were classified as the low-fluoride (LF) group.

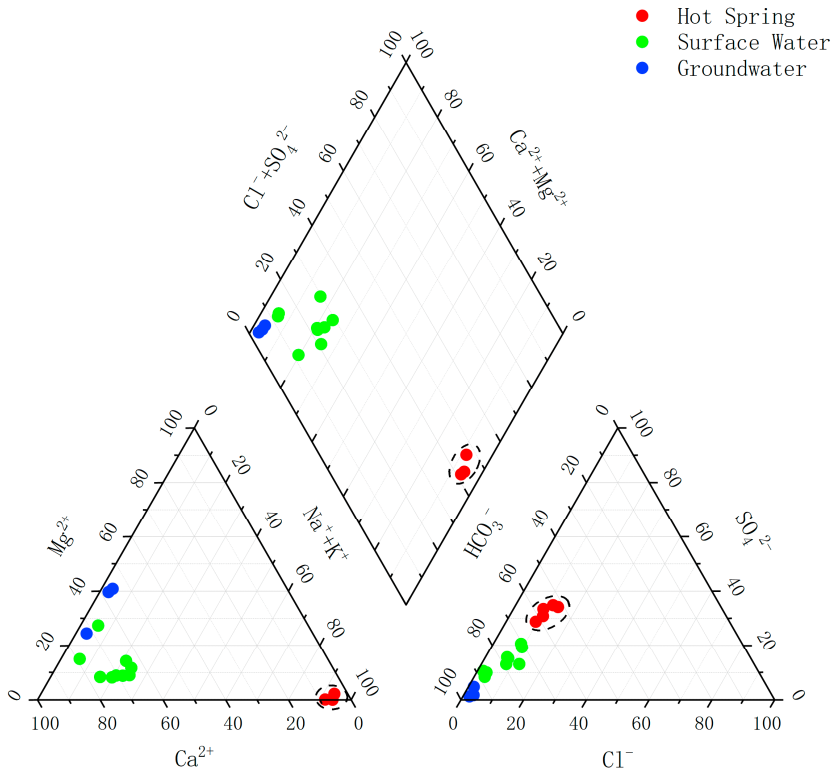


Figure 2. Piper trilinear diagram of sampling points.

The HF (hot spring) samples had an average temperature of 52.2 °C (Table 1), ranging from 47 °C (Wentang) to 60 °C (Shangtang), thereby meeting the definition in the Hot spring service—Basic terminology (GB/T 33533–2017) [32] of outlet water temperature not lower than 25 °C. The average pH value was 8.49, which is weakly alkaline, and Jianchang was the most weakly alkaline at pH 8.07. The average TDS value was 357 mg/L, with the TDS levels of the five hot springs all below 500 mg/L, as stipulated in the Class II water standard for groundwater quality as defined in the Standard for groundwater quality (GB/T 14848–2017) [31]. The F^- concentration ranged from 9.40 to 18.37 mg/L, with an average of 15.04 mg/L, which is in line with the Hot spring service-Hot spring water quality requirement (GB/T 41837–2022) [33] of >2 mg/L. Na^+ was the most dominant cation, with a concentration of 131.83–170.72 mg/L, followed by Ca^{2+}, K^+, and Mg^{2+}. The major anions were HCO_3^- and SO_4^{2-}, with concentrations of 129.67–189.16 and 70.18–123.96 mg/L, respectively. These findings were fully in line with the chemical characteristics of highly fluoridated water with weak alkalinity [34], high TDS [35], and Na^+ as the predominant cation [36].

Table 1. Physicochemical characteristics of the sampling points. TDS and ion parameters are reported in mg/L. ND: not detected.

Type	Parameters	Temp (°C)	pH	TDS	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	HCO_3^-	F^-
HF (Hot Spring)	Mean	52.2	8.49	357.0	145.93	5.54	7.93	0.73	29.85	87.29	150.11	15.04
	Max	60.0	8.73	438.0	170.72	9.20	16.14	3.31	42.49	123.96	189.16	18.37
	Min	47.0	8.07	321.0	131.83	3.70	ND	ND	23.59	70.18	129.67	9.40
LF (surface water/groundwater)	Mean	21.8	7.94	160.1	6.08	1.11	39.91	12.58	8.38	16.77	156.87	0.38
	Max	27.5	8.87	241.0	20.40	4.20	66.00	30.86	26.40	51.95	312.73	0.82
	Min	16.8	7.15	67.8	2.10	ND	16.72	2.09	3.95	4.01	38.14	ND

The LF (surface water/groundwater) samples were 21.8 °C, with little temperature variation. The average pH value was 7.94, similar to hot springs, exhibiting weak alkalinity. The Zhaopingtai reservoir surface water was the most weakly alkaline at pH 7.15. TDS ranged from 67.8 to 241 mg/L, and minimal levels were found in the Sha River's secondary tributary, which is distant from residential areas and less polluted. Ca^{2+} was the most dominant cation, with a concentration of 16.72–66.00 mg/L, followed by Mg^{2+}, Na^+, and K^+. The concentrations of the main anions HCO_3^- and SO_4^{2-} varied significantly at 38.14–312.73 and 4.01–51.95 mg/L, respectively, while the concentration of Cl^- was relatively low.

The main minerals detected in the study area were quartz, mica, plagioclase, and chlorite. Under alkaline conditions, OH^- in hot spring water readily exchanges with adsorbed F^- on minerals or soils, thereby preventing complexation of F^- with cations [17] and releasing significant amounts of F^-.

$$CaF_2 + 2OH^- = Ca(OH)_2 + 2F^- \quad (1)$$

$$CaF_2 + 2NaHCO_3 = CaCO_3 + 2Na^+ + 2F^- + CO_2 + H_2O \quad (2)$$

Surface water and groundwater in the study area were enriched with Ca^{2+}, whereas hot springs had high level of Na^+. Variation in the Na/Ca ratio promotes dissolution of F^- from mineral phases into the solution, a finding entirely consistent with prior studies [37].

3.2. Microbial Community Structure

3.2.1. Alpha Diversity Analysis

The study included 17 Illumina sequencing libraries, totaling 1,867,556 raw reads and 1,855,214 clean reads. The clean reads accounted for 99.34% of the raw reads, indicating high and reliable sequencing quality. The sequence stitching algorithm merges paired-end sequencing reads into a single sequence by identifying overlapping regions at the ends, which is then used for subsequent analysis. The PEAR program was used to merge the raw Illumina paired-end sequences derived from variable-length target fragments.

A rarefaction curve (Figure 3) revealed that as the rarefaction depth increased, the Alpha diversity index (Chao1, Observed_species, and Shannon index) curves gradually approached a distinct plateau, indicating that the acquired samples exhibited sufficient representativeness in terms of microbial abundance and species richness, while the sequencing depth adequately satisfied the requirements to reflect both the diversity and quantitative characteristics of the samples.

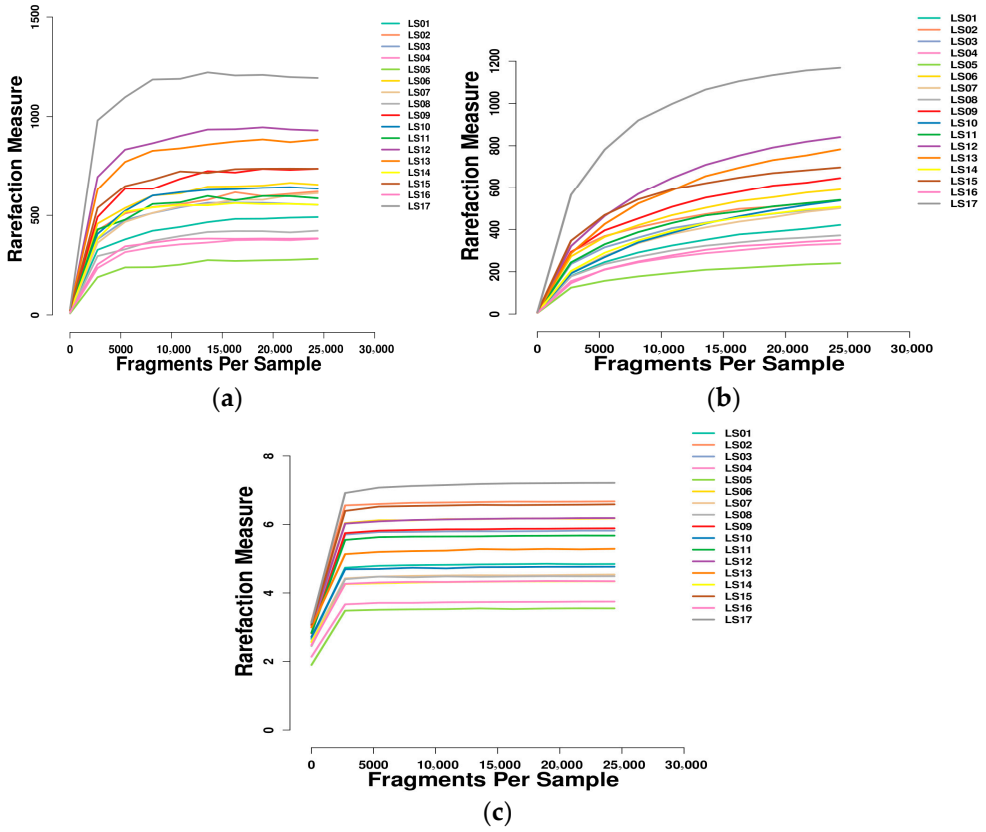


Figure 3. Rarefaction curve. (**a**) Chao1 index; (**b**) Observed_species index; (**c**) Shannon index.

A comparison of the Alpha diversity indices of the microbial communities among the HF (hot spring) samples and LF (surface water/groundwater) samples (Table 2) revealed significant variations in microbial community diversity along the fluoride concentration gradient. The Chao1 and the Observed_species indices are both metrics used to assess species richness in a community without relying on evolutionary trees. Higher values of these indices indicate greater species richness in the sample. The HF samples (Chao1: 437.82; Observed_species: 373.7) exhibited significantly lower species richness as compared to the LF samples (Chao1: 685.17; Observed_species: 642.2). The Shannon index, a diversity metric calculated based on the number and proportion of species in a sample, reflecting the diversity of microbial communities, is influenced by both species richness and evenness (distribution uniformity of species) within the sampled commu-

nity, with higher values indicating greater community diversity. Comparative analysis of the Shannon diversity indices revealed significantly lower species diversity in the HF samples (4.23) as compared to the LF samples (5.63). The HF samples demonstrated significant reductions in microbial abundance and diversity as compared to the LF samples, confirming that high-fluoride hot springs impact microbial community formation, aligning with prior research [16,18].

Table 2. Physicochemical characteristics of the sampling points. ND: not detected.

Type	Numbering	F^- (mg/L)	Chao1	Observed_Species	Shannon
HF (Hot Spring)	LS01	9.40	491.24	421.6	4.85
	LS04	18.37	382.07	332.8	3.75
	LS05	16.65	281.38	240.4	3.55
	LS07	15.43	611.94	501.3	4.54
	LS08	15.33	422.48	372.2	4.49
	Mean	15.04	437.82	373.7	4.23
LF (surface water/ groundwater)	LS02	0.56	620.24	537.7	6.67
	LS03	0.82	553.01	504.6	5.82
	LS06	0.66	655.96	591.2	6.18
	LS09	0.51	736.86	645.9	5.89
	LS10	0.61	635.16	538.2	4.77
	LS11	0.52	586.26	540.8	5.67
	LS12	0.52	929.67	841.0	6.19
	LS13	0.40	884.24	782.8	5.29
	LS14	ND	553.43	508.2	4.34
	LS15	0.02	736.77	696.6	6.59
	LS16	ND	383.86	350.4	4.34
	LS17	ND	1193.92	1169.1	7.21
	Mean	0.38	685.17	642.2	5.63

In addition, LS16 and LS17 are fissure waters from the same location within the study area. A section of pipe was installed to channel water at LS16, serving as the drinking water source for the Mo Shang Village water supply station, which involves human intervention. In contrast, LS17 is a completely natural and undisturbed fissure water source. A comparison of the Chao1, Observed_species, and Shannon indices of the two samples found that the values were significantly higher at LS17 than LS16. Both microbial richness and diversity were markedly greater at LS17 than LS16, demonstrating that human activities can substantially influence the formation of microbial communities.

3.2.2. Microbial Community Composition Analysis

The most abundant prokaryote in the samples was Pseudomonadota (Figure 4), with a relative abundance ranging from 36.09% (LS03) to 95.06% (LS14). The average abundances of Cyanobacteriota and Bacteroidota were similar, both approximately 5.33%, ranging from 0.11% (LS16) to 36.37% (LS03) and 0.47% (LS07) to 18.83% (LS15), respectively. The relative abundance of Actinomycetota ranged from 0.06% (LS05) to 24.35% (LS02). Other phyla with an average relative abundance greater than 5% included Verrucomicrobiota, Chloroflexota, Acidobacteriota, Crenarchaeota, Firmicutes, Nitrospirota, OD1, and Gemmatimonadota. At the phylum level, the microbial communities were similar in the samples (LS06, LS09, LS10, LS11, LS12) from the Sha River, which is the main source of the hot springs in the study area, while there were significant differences in the microbial communities in high-fluoride hot springs (LS01, LS04, LS05, LS07, LS08).

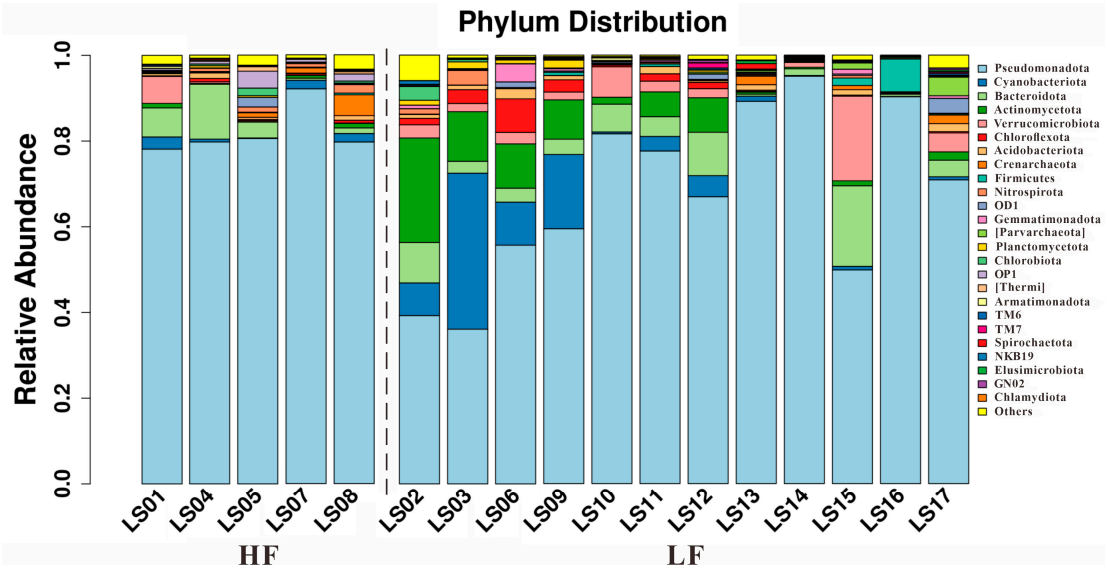


Figure 4. Phylum-level microbial community composition of the top 25 most abundant phyla.

Pseudomonadota, Cyanobacteriota, Bacteroidota, and Actinomycetota were the dominant phyla in the study area. Differences in the dominant phyla across regions suggest a certain degree of territoriality among microorganisms: Jixia (Pseudomonadota, Bacteroidota, and Cyanobacteriota) [17], Taocheng District, Hengshui City (Pseudomonadota, Bacteroidota, and Nitrospirota) [16], and the Kuitun River basin in Xinjiang (Pseudomonadota, Actinomycetota, and Bacteroidota) [18]. As the largest bacterial phylum, Pseudomonadota encompasses both pathogenic and nitrogen-fixing bacteria, including many involved in aerobic denitrification and biological removal of nitrogen from groundwater [38]. In most prior studies, the abundance of Pseudomonadota hovered around 50%, with more pronounced intergroup differences observed in hot springs. Hot springs preserve the most primitive microbial resources, and even those in close geographical proximity may harbor distinct endemic species along with novel microbial resources, particularly thermophilic strains [23]. Crenarchaeota was the most abundant archaea in the five hot springs, consistent with the dominant archaea observed in the sister springs of the Tengchong geothermal area, which are also alkaline thermal environments [39]. In contrast, Parvarchaeota and Crenarchaeota are reportedly the predominant archaeal groups in the Tagjia and Quzhuomu geothermal areas [40], while Euryarchaeota is the dominant archaea in the Eryuan hot spring [41]. The five hot springs contained Aquificota, consistent with the Jifei Hot Spring of Changning County, western Yunnan Province, China [42]. Both regions share elevated sulfur content in hot springs. Ecological studies of hot springs indicate that temperature, pH, and sulfide concentration collectively shape microbial community structures, driving the formation of unique ecological environments in hot springs across different geographical regions.

As shown in Figure 5, for clarity in visualization, genera with a mean relative abundance greater than 1% were categorized as the top taxa, with all remaining genera grouped into the 'Others' category. Among the five hot spring samples, notable differences were observed: *Tepidimonas* (a neutrophilic thermophile) was abundant in Xiatang (LS04), Wentang (LS07), and Shangtang (LS08), but scarce in Jianchang (LS01) and Zhongtang (LS05). The hot spring-specific genus *Tepidimonas* is ecologically important for maintaining ecosystem functions by enhancing the growth of Chloroflexota, thereby facilitating the targeted isolation of Chloroflexota from hot spring samples [43]. This is primarily attributed to the specific environmental conditions of hot springs, the intrinsic characteristics of the microorganisms, and the complex interactions within microbial communities. Instead, *Rhodobacter*

and *Hyphomonas* dominated in Jianchang. These genera facilitate nitrogen cycling and organic matter degradation in aquatic ecosystems [44]. Residents utilize the geothermal waters of Jianchang for large-scale fish farming, likely benefiting from these microbial-driven ecological processes. With the exception of its minimal presence in LS10 and LS17, *Parvibaculum* was detected exclusively in five hot spring samples. This genus originated from studies on marine petroleum-degrading bacteria and alkane monooxygenase gene diversity, suggesting its potential for degrading alkane hydrocarbons.

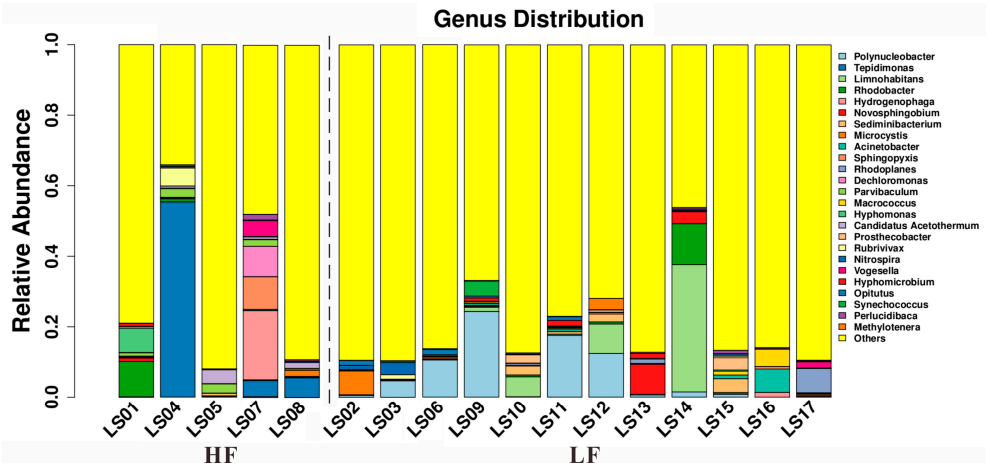


Figure 5. Genus-level microbial community composition of the top 25 most abundant genera. Only genera with a mean relative abundance >1% are represented individually; all less abundant genera are grouped as 'Others'.

The genus-level composition varied significantly across samples, with no dominant genus observed universally. In the Sha River (LS06, LS09, LS11, LS12), *Polynucleobacter* emerged as the dominant genus. *Polynucleobacter* is ubiquitous in freshwater ecosystems such as rivers and lakes, particularly in slow-flowing or stagnant waters characterized by high dissolved organic matter (DOM). It is widely regarded as one of the most abundant and globally distributed bacterial taxa in freshwater environments [45,46]. *Limnohabitans* was dominant in the first tributary of the Sha River (LS10). This discrepancy can be attributed to the substantial proliferation of reeds in the first tributary. Reeds serve as a crucial cornerstone in supporting the growth and reproduction of freshwater bacterioplankton, among which *Limnohabitans* represents a significant and prevalent group of planktonic bacteria [47].

As shown in Figure 6, significant differences were observed in the genus-level composition between the HF and LF samples. A total of 3079 bacterial genera were shared by both samples, while 354 genera were unique to the HF samples, and 1704 were unique to the LF samples. These results indicate that a considerable proportion of microbial taxa are unable to thrive in the hot springs of the HF samples. In addition to fluoride concentration, other hydrochemical characteristics may also contribute to the unsuitability of the environment for certain microbial communities.

3.2.3. Beta Diversity Analysis

In light of the substantial dispersion of microbial species at the genus level and the absence of a dominant genus in the study area, Beta diversity analysis of phylum-level species was conducted using a species matrix [48]. PC1 and PC2 accounted for 20.15% and 12.17% of the total variance, respectively (Figure 7). The HF samples were predominantly distributed along the negative axis of PC1, whereas the LF samples clustered mainly on the positive side of PC1, indicating significant differences in microbial composition between the two groups.

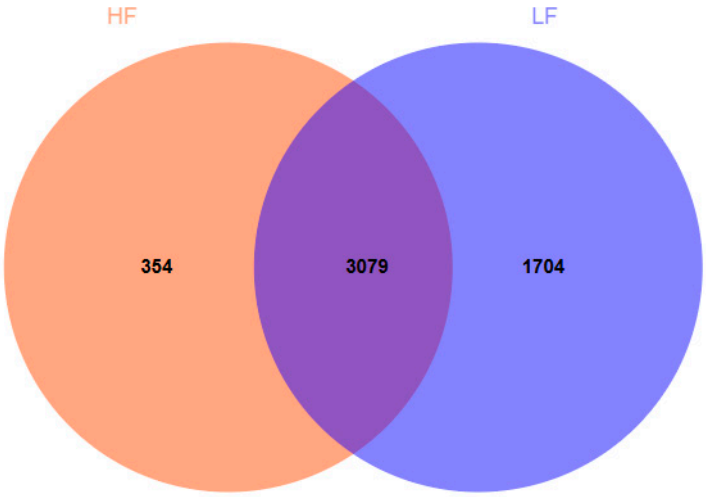


Figure 6. Venn diagram of genera.

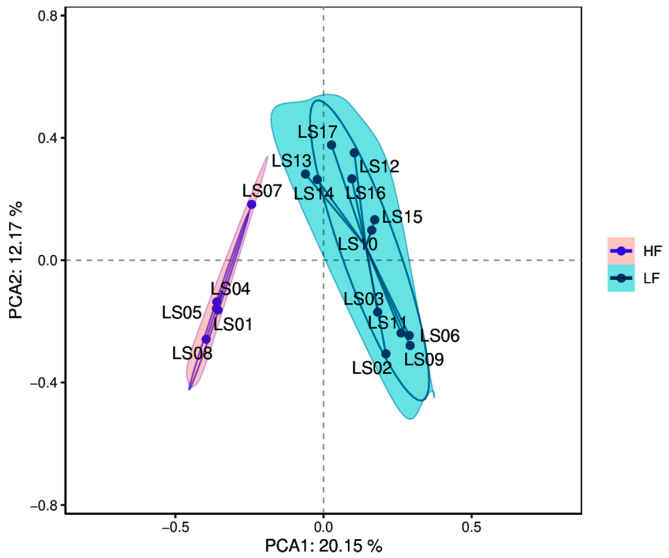
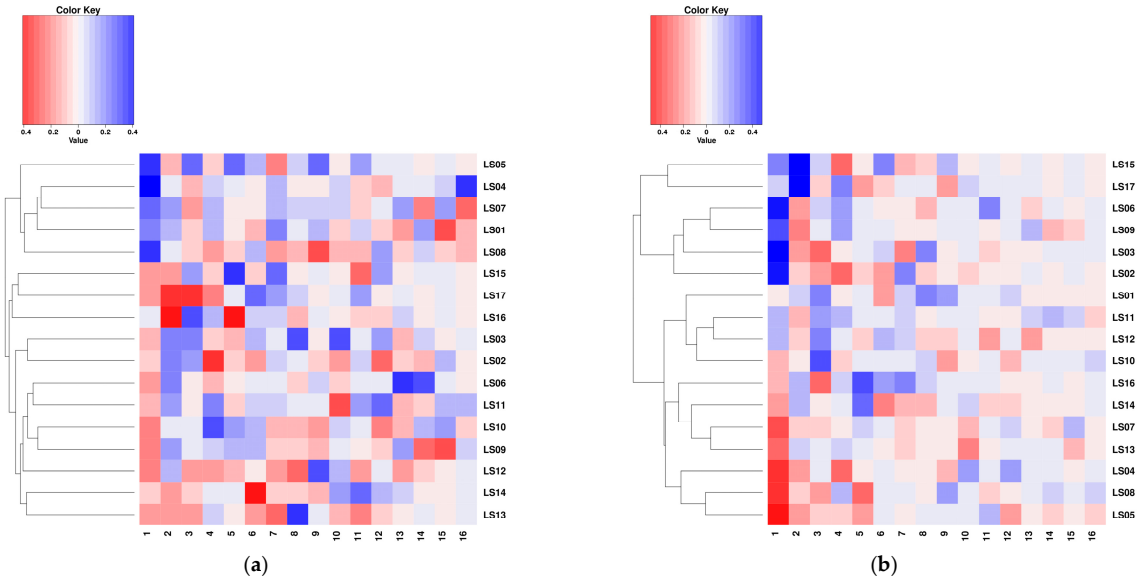


Figure 7. Principal coordinate analysis of samples. The dots represent the species composition of each sample; the x-axis represents the first principal component (PCA1) and its percentage of contribution to the sample variance; the y-axis represents the second principal component (PCA2) and its percentage of contribution to the sample variance.

Two metrics, Unweighted UniFrac and Weighted UniFrac distances, were selected to measure dissimilarity coefficients among the samples. Lower values of these metrics indicate smaller differences in species diversity between paired samples. The smallest difference in the Unweighted UniFrac distance metric (considering only microbial presence/absence, not abundance) was observed between Xiatang (LS04) and Wentang (LS07), while the largest difference occurred between Zhongtang (LS05) and Wentang. The smallest difference in the Weighted UniFrac distance metric (incorporating both phylogenetic relationships and abundance variations) occurred between Zhongtang and Wentang, whereas the largest dissimilarity shifted to Jianchang (LS01) and Shangtang (LS08). These results highlight the significant influence of microbial abundance on inter-sample differences among hot springs, and conclusions drawn from the Weighted UniFrac metric align more closely with previous studies [49,50].

Based on the aforementioned analyses, the Euclidean distance between samples was calculated, and further cluster analysis was performed using hierarchical clustering (Figure 8), which contributed to a more accurate clustering of the samples. In the un-

weighted sample clustering heatmap (Figure 8a), it is clearly observed that all HF samples (LS01, LS04, LS05, LS07, LS08) clustered within one major branch, while the LF samples grouped into other distinct branches, indicating significant differences in microbial taxa between the HF and LF samples. In the weighted sample clustering heatmap (Figure 8b), although LS04, LS05, and LS08 from the HF group clustered together in one main branch, LS01 and LS07 were located in separate branches, further demonstrating that abundance significantly influences the differences among hot spring samples.



(**a**) (**b**)

Figure 8. Sample clustering heatmap. (**a**) Unweighted; (**b**) weighted. The x-axis represents the extracted principal components, which account for over 90% of the data variability.

3.3. Correlation Analysis Between Microbial Communities and Environmental Characteristics

Redundancy analysis (RDA) of the top 11 phyla by abundance in water samples was performed using selected key environmental indicators to clearly visualize the relationships between microbial species and hydrochemical characteristics (Figure 9) [51].

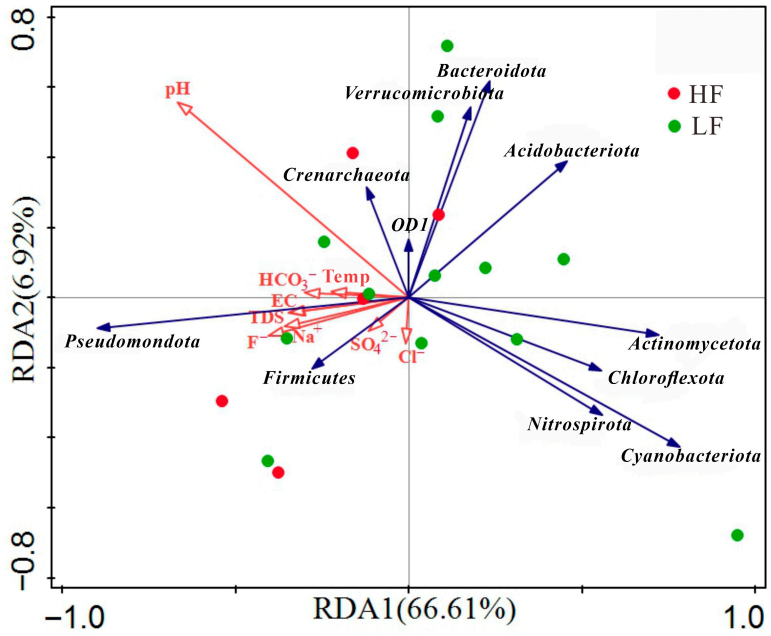


Figure 9. RDA of hydrochemical factors and bacterial phyla relationships.

The leaching process is a pivotal driver that modifies the hydrochemical characteristics of groundwater. During this process, F^- and SO_4^{2-} exhibit divergent magnitudes of variation but congruent directional trends. F^- is directly influenced by fluorite dissolution, while indirectly modulated by gypsum dissolution and the precipitation of calcite and dolomite. These processes have been shown to release substantial quantities of Ca^{2+} and SO_4^{2-} into groundwater. Additionally, anion desorption under alkaline conditions has been shown to synchronously influence SO_4^{2-} and F^- levels. Notably, the majority of species exhibit consistent correlations with both ions, indicating that most microbial communities are highly responsive to hydrochemical dynamics and maintain close associations.

Furthermore, significant associations were observed between microbial species and F^-. Among the 11 phyla analyzed, eight were negatively correlated with F^- (Nitrospirota, Cyanobacteriota, Chloroflexota, Actinomycetota, Acidobacteriota, Bacteroidota, Verrucomicrobiota, and the candidate phylum OD1), while only three were positively correlated (Crenarchaeota, Firmicutes, and Pseudomonadota). The results of this study indicate that F^- likely inhibits the growth of the majority of microbial communities, while selectively favoring specific species adapted to fluoride-rich environments. The 3 phyla showing a positive correlation with F^- concentration also exhibited significant positive correlations with pH, TDS, Na^+, SO_4^{2-}, HCO_3^-, and temperature. Based on previous hydrochemical features analysis, these results indicate their full adaptation to a typical high-fluoride hot springs environment.

4. Conclusions

The major finding of this research is that the five hot springs exhibited an average F^- content of 15.04 mg/L, with weakly alkaline pH, high TDS, and Na^+ as the dominant cation, and the hydrochemical type was $HCO_3 \cdot SO_4$-Na, consistent with the chemical characteristics of high-fluorine water. Furthermore, surface water and groundwater in the study area exhibit Ca^{2+} enrichment, whereas hot springs were enriched with Na^+. Variation to the Na/Ca ratio promotes dissolution of F^- from mineral phases into solution. Moreover, the HF samples (hot spring) demonstrated significant reductions in microbial abundance and diversity as compared to the LF samples (surface water/groundwater), confirming that high-fluoride hot springs impact microbial community formation. The dominant phyla in the study area included Pseudomonadota, Cyanobacteriota, Bacteroidota, and Actinomycetota. The genus-level composition varied significantly across samples, with no dominant genus observed universally. The specific genera present in different samples exhibit unique functional attributes. The hot spring-specific genus *Tepidimonas* is ecologically important for maintaining ecosystem functions by enhancing the growth of Chloroflexota, thereby facilitating the targeted isolation of Chloroflexota from hot spring samples. *Rhodobacter* and *Hyphomonas* facilitate nitrogen cycling and organic matter degradation in aquatic ecosystems. *Parvibaculum* originated from studies on marine petroleum-degrading bacteria and alkane monooxygenase gene diversity, suggesting its potential for degrading alkane hydrocarbons. *Polynucleobacter*, which is ubiquitous in freshwater ecosystems such as rivers and lakes, emerged as the dominant genus in the Sha River. The proliferation of reeds in the first tributary of the Sha River led to the emergence of *Limnohabitans* as the dominant genus. Significant differences were observed in the genus-level composition between the HF and LF samples. Additionally, PCA revealed significant differences in the microbial composition between the HF and LF samples. The Unweighted UniFrac and Weighted UniFrac distance metrics to measure dissimilarity coefficients highlight the significant influence of microbial abundance on inter-sample differences among hot springs. Cluster analysis further confirmed this finding. RDA of the top 11 phyla by abundance in water samples revealed that eight species were negatively correlated with F^-, whereas only three were

positively correlated. These results further demonstrate that F^- likely inhibits growth of the majority of microbial communities, while selectively favoring specific species adapted to fluoride-rich environments. The positive correlations between the 3 phyla (which showed a positive relationship with F^-) and other hydrochemical factors suggest their full adaptation to typical high-fluoride hot springs. This study investigates how elevated fluoride concentrations in geothermal waters shape microbial community diversity and structure. To establish a causal relationship and uncover the underlying molecular mechanisms, future research could employ approaches such as metagenomic, functional gene analysis, or controlled experiments.

Author Contributions: Conceptualization, H.G. and Q.W.; Data curation, L.Y. and J.L.; Formal analysis, H.G. and L.Y.; Funding acquisition, Q.W.; Investigation, H.G. and J.L.; Methodology, H.G. and Q.W.; Project administration, J.L.; Resources, Q.W.; Supervision, Q.W. and L.Y.; Validation, L.Y. and J.L.; Visualization, H.G.; Writing—original draft, H.G.; Writing—review and editing, H.G., Q.W. and L.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Key Research and Development Project of Henan Province (No. 251111322300); Henan High-level Innovative Scientific and Technological Talent Team Construction Project (No. CXTD2016053).

Data Availability Statement: The original data presented in the study are openly available in [NCBI SRA] at [PRJNA1273160].

Conflicts of Interest: Author Qi Wang was employed by the company The Second Institute of Resources and Environment Investigation of Henan Province CO., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Islam, M.S.; Mostafa, M.G. Meta-analysis and risk assessment of fluoride contamination in groundwater. *Water Environ. Res.* **2021**, *93*, 1194–1216. [CrossRef] [PubMed]
2. Fan, W.; Zhou, J.; Zheng, J.; Guo, Y.; Hu, L.; Shan, R. Hydrochemical characteristics, control factors and health risk assessment of groundwater in typical arid region Hotan Area, Chinese Xinjiang. *Environ. Pollut.* **2024**, *363*, 125301. [CrossRef] [PubMed]
3. Li, Y.; Zhang, M.; Mi, W.; Ji, L.; He, Q.; Xie, S.; Xiao, C.; Bi, Y. Spatial distribution of groundwater fluoride and arsenic and its related disease in typical drinking endemic regions. *Sci. Total Environ.* **2024**, *906*, 167716. [CrossRef] [PubMed]
4. Rajmohan, N. Assessment of human health risk and fluoride contamination in the arid coastal aquifer, Saudi Arabia. *Environ. Earth Sci.* **2022**, *81*, 241. [CrossRef]
5. Rashid, A.; Ayub, M.; Gao, X.; Khattak, S.A.; Ali, L.; Li, C.; Ahmad, A.; Khan, S.; Rinklebe, J.; Ahmad, P. Hydrogeochemical characteristics, stable isotopes, positive matrix factorization, source apportionment, and health risk of high fluoride groundwater in semiarid region. *J. Hazard. Mater.* **2024**, *469*, 134023. [CrossRef]
6. Li, J.; Dai, J.; Yang, L.; Wang, H. Enrichment mechanism and probabilistic health risk assessment of high-fluoride groundwater in Gaomi City, China. *Environ. Geochem. Health* **2025**, *47*, 26. [CrossRef]
7. Liu, J.; Ren, Z.; Yu, Q.; Qi, K.; Wu, H.; Wang, Z.; Zhang, G.; Zhang, Y.; Jia, M.; Jia, P. Hydrochemical processes and fluoride enrichment patterns in high-fluoride geothermal water in the Weihe Basin, China. *J. Hazard. Mater.* **2024**, *480*, 136468. [CrossRef]
8. Gao, X.; Naseem, A.; Luo, W.; Rashid, A.; Ma, S.; Li, C.; Zhang, X. Impact of microbial activity on fluoride release from sediments in areas with high fluoride groundwater: Mechanisms, sources and the lithology diversity. *Sci. Total Environ.* **2024**, *955*, 176940. [CrossRef]
9. Xu, Y.; He, Y.; Tang, X.; Brookes, P.C.; Xu, J. Reconstruction of microbial community structures as evidences for soil redox coupled reductive dechlorination of PCP in a mangrove soil. *Sci. Total Environ.* **2017**, *596*, 147–157. [CrossRef]
10. Wang, Y.; Weng, M.Y.; Zhong, J.W.; He, L.; Guo, D.J.; Luo, D.; Xue, J.Y. Microbial metagenomics revealed the diversity and distribution characteristics of groundwater microorganisms in the Middle and Lower Reaches of the Yangtze River Basin. *Microorganisms* **2024**, *12*, 1551. [CrossRef]
11. Griebler, C.; Lueders, T. Microbial biodiversity in groundwater ecosystems. *Freshw. Biol.* **2009**, *54*, 649–677. [CrossRef]

12. Li, J.; Yu, S.; Liu, Q.; Wang, D.; Yang, L.; Wang, J.; Zuo, R. Screening of hazardous groundwater pollutants responsible for microbial ecological consequences by integrated nontargeted analysis and high-throughput sequencing technologies. *J. Hazard. Mater.* **2023**, *445*, 130516. [CrossRef]
13. John, D.E.; Rose, J.B. Review of factors affecting microbial survival in groundwater. *Environ. Sci. Technol.* **2005**, *39*, 7345–7356. [CrossRef] [PubMed]
14. He, Z.; Ning, Z.; Huang, G.; Liu, D.; Zhang, Q.; Sun, J. The response characteristics of microbial diversity to shallow groundwater contamination in the piedmont of the Taihang Mountains using molecular biotechnologies: A case study of groundwater of Hutuo River Basin. *Geol. China* **2019**, *46*, 290–301. [CrossRef]
15. Li, N.Y.; Han, Z.Y.; Wang, S.C.; Li, L.B.; Huang, J.; Zhao, J.N. Impacts of different pollution sources on the microbial community in groundwater at municipal solid waste landfill sites. *China Environ. Sci.* **2020**, *40*, 4900–4910.
16. Zhang, H.S.; Wang, M.Y.; Cai, W.T.; Bian, C.; Liu, J.W. Characteristics of microbial community composition and environmental response in deep fluorinated groundwater. *Earth Sci.* **2021**, *48*, 3466–3479.
17. Zhang, X.; Gao, X.; Li, C.; Luo, X.; Wang, Y. Fluoride contributes to the shaping of microbial community in high fluoride groundwater in Qiji County, Yuncheng City, China. *Sci. Rep.* **2019**, *9*, 14488. [CrossRef] [PubMed]
18. Chen, T.; Qiao, L.; Su, Y.; Tao, H.; Jiang, Y.; Aihemaiti. Effects of high fluoride groundwater on microbial diversity in Kuitun River Basin. *Environ. Sci. Technol.* **2023**, *46*, 144–152.
19. Chouhan, S.; Tuteja, U.; Flora, S.J.S. Isolation, identification and characterization of fluoride resistant bacteria: Possible role in bioremediation. *Appl. Biochem. Microbiol.* **2012**, *48*, 43–50. [CrossRef]
20. Li, Q.; Ding, D.; Wang, Q.; Shi, W.; Hu, E.; Li, H.; Ma, L.; Xiao, Y.; Liu, X.; Cao, Y. Comparative study of fluoride-tolerance of five typical bioleaching microorganisms. *J. Univ. South China (Sci. Technol.)* **2013**, *27*, 16–22. [CrossRef]
21. Gao, X.; Luo, W.; Luo, X.; Li, C.; Zhang, X.; Wang, Y. Indigenous microbes induced fluoride release from aquifer sediments. *Environ. Pollut.* **2019**, *252*, 580–590. [CrossRef]
22. Zhang, Y.; Gan, Y.; Zhou, X.; Jiang, C.; Gao, X.; Li, C. Mobilization of fluoride in sediments at high fluoride area enhanced by microorganisms. *Bull. Geol. Sci. Technol.* **2022**, *41*, 228–235. [CrossRef]
23. Ma, R.; Chen, C.; Li, H.; Zhu, X.; Song, K.; Yuan, M.; Xiao, W.; Yang, X.; Wang, K. Species diversity and enzymatic activity of microorganisms in Chinese hot springs: A review. *Microbiology* **2020**, *47*, 2959–2973. [CrossRef]
24. Cao, X.W. The calculation of the composition and content of plagioclase in igneous rocks. *Geol. Explor.* **1977**, *08*, 66–72.
25. Zhang, J.; Kobert, K.; Flouri, T.; Stamatakiset, A. PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* **2014**, *30*, 614–620. [CrossRef]
26. Adler, C.J.; Dobney, K.; Weyrich, L.S.; Kaidonis, J.; Walker, A.W.; Haak, W.; Bradshaw, C.J.A.; Townsend, G.; Sołtysiak, A.; Alt, K.W.; et al. Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nat. Genet.* **2013**, *45*, 450–455. [CrossRef]
27. Ahn, J.; Sinha, R.; Pei, Z.; Dominianni, C.; Wu, J.; Shi, J.; Goedert, J.J.; Hayes, R.B.; Yang, L. Human gut microbiome and risk for colorectal cancer. *J. Natl. Cancer Inst.* **2013**, *105*, 1907–1911. [CrossRef] [PubMed]
28. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Peña, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [CrossRef]
29. Caporaso, J.G.; Bittinger, K.; Bushman, F.D.; DeSantis, T.Z.; Andersen, G.L.; Knight, R. PyNAST: A flexible tool for aligning sequences to a template alignment. *Bioinformatics* **2010**, *26*, 266–267. [CrossRef] [PubMed]
30. Vasileiadis, S.; Puglisi, E.; Arena, M.; Cappa, F.; Cocconcelli, P.S.; Trevisan, M. Soil bacterial diversity screening using single 16S rRNA gene V regions coupled with multi-million read generating sequencing technologies. *PLoS ONE* **2012**, *7*, e42671. [CrossRef]
31. *GB/T 14848-2017*; Standard for Groundwater Quality. General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China; Standardization Administration of the People's Republic of China: Beijing, China, 2017.
32. *GB/T 33533-2017*; Hot Spring Service—Basic Terminology. General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Standardization Administration of the People's Republic of China: Beijing, China, 2017.
33. *GB/T 41837-2022*; Hot Spring Service—Hot Spring Water Quality Requirement. State Administration for Market Regulation, Standardization Administration of China: Beijing, China, 2022.
34. Zhou, J.; Chen, J.; Wang, S.; Zhao, X.; Chen, D.; Dai, X. Hydrogeochemical Characterization and Enrichment Mechanisms of Fluoride-Enriched Groundwater in Shallow Aquifer Systems: A Case Study from Feng County, Jiangsu Province, China. *Earth Environ.* **2024**, *52*, 122–132.
35. Jin, Z.; Sun, C.; Kong, L.H.; Sun, S.; Xie, X.; Qian, F.; Song, Y. Chemical characteristics and high-fluoride origins of shallow groundwater around typical high fluorine reservoir in Songnen Plain. *Acta Sci. Circumstantiae* **2023**, *43*, 250–258.
36. Zhang, S.C.; Liu, K.; Wang, L.Y.; Zhu, W.; Deng, Y.F.; Yu, C.H. Identifying the hydrochemical characteristics and genetic mechanism of medium-low temperature fluoride-enriched geothermal groundwater in the Hongjiang—Qianshan Fault of Jiangxi Province. *Rock Mineral. Anal.* **2024**, *43*, 568–581.

37. Gao, X.; Wang, Y.; Li, Y.; Guo, Q. Enrichment of fluoride in groundwater under the impact of saline water intrusion at the salt lake area of Yuncheng basin, northern China. *Environ. Geol.* **2007**, *53*, 795–803. [CrossRef]
38. Ning, Z.; Cai, P.P.; Zhang, M.; Guo, C.; Shi, C.; He, Z. Abnormally low dissolved inorganic carbon in petroleum contaminated groundwater caused by microbiological geochemistry. *Acta Sci. Circumstantiae* **2019**, *39*, 1140–1147.
39. Liang, N.Y.; Liu, S.B.; Ding, J.B. Hot Springs Annals. In *Yunnan Provincial Chronicles*; Yunnan People's Publishing House: Kunming, China, 1999; Volume 25, p. 190.
40. Yuan, C.G. Study on Microbial Diversity of Two Thermal Hot Spring Areas in South of Tibetan and Screening Object Strains of Producing Cellulase and Chitinase. Master's Thesis, Yunnan University, Kunming, China, 2017.
41. Menzel, P.; Gudbergsdóttir, S.R.; Rike, A.G.; Lin, L.; Zhang, Q.; Contursi, P.; Moracci, M.; Kristjansson, J.K.; Bolduc, B.; Gavrilov, S.; et al. Comparative metagenomics of eight geographically remote terrestrial hot springs. *Microb. Ecol.* **2015**, *70*, 411–424. [CrossRef]
42. Jiang, L.; Xu, H.; Qiao, H. Biomediated precipitation of calcium carbonate in a slightly acidic hot spring, Yunnan Province. *Acta Geol. Sin.* **2017**, *91*, 145–155. [CrossRef]
43. Xian, W.D.; Salam, N.; Li, M.M.; Zhou, E.M.; Yin, Y.R.; Liu, Z.T.; Ming, Y.Z.; Zhang, X.T.; Wu, G.; Liu, L.; et al. Network-Directed Efficient Isolation of Previously Uncultivated Chloroflexi and Related Bacteria in Hot Spring Microbial Mats. *npj Biofilms Microbiomes* **2020**, *6*, 20. [CrossRef]
44. Li, Q.; Lin, F.; Yang, C.; Wang, J.; Lin, Y.; Shen, M.; Park, M.S.; Li, T.; Zhao, J. A large-scale comparative metagenomic study reveals the functional interactions in six bloom-forming Microcystis-epibiont communities. *Front. Microbiol.* **2018**, *9*, 746. [CrossRef]
45. Wang, C.; Sun, D.; Junaid, M.; Xie, S.; Xu, G.; Li, X.; Tang, H.; Zou, J.; Zhou, A. Effects of Tidal Action on the Stability of Microbiota, Antibiotic Resistance Genes, and Microplastics in the Pearl River Estuary, Guangzhou, China. *Chemosphere* **2023**, *327*, 138485. [CrossRef]
46. Donchev, D.; Ivanov, I.N.; Stoikov, I.; Ivanova, M. Metagenomic Investigation of the Short-Term Temporal and Spatial Dynamics of the Bacterial Microbiome and the Resistome Downstream of a Wastewater Treatment Plant in the Iskar River in Bulgaria. *Microorganisms* **2024**, *12*, 1250. [CrossRef] [PubMed]
47. Kasalický, V.; Jezbera, J.; Hahn, M.W.; Šimek, K. The diversity of the Limnohabitans genus, an important group of freshwater bacterioplankton, by characterization of 35 isolated strains. *PLoS ONE* **2013**, *8*, e58209. [CrossRef]
48. Zaoli, S.; Grilli, J. The stochastic logistic model with correlated carrying capacities reproduces beta-diversity metrics of microbial communities. *PLOS Comput. Biol.* **2022**, *18*, e1010043. [CrossRef]
49. Liu, L.; Zhang, K.; Li, B.J.; Li, M.C.; Chen, Q.; Zheng, X.H.; Wang, F.A.; Zhou, Y.X. Bacterial diversity analysis of five hot springs in Henan Lushan. *Microbiol. China* **2018**, *45*, 1219–1227.
50. Zhang, K.; Liu, L.; Zheng, X.H.; Wang, Y.; Zhang, J.F.; Li, B.J. Archaeal diversity analysis for five hot springs in Henan Lushan. *J. Xinyang Norm. Univ. (Nat. Sci. Ed.)* **2019**, *32*, 221–227. [CrossRef]
51. Meng, L.; Zuo, R.; Wang, J.; Yang, J.; Li, Q.; Chen, M. The Spatial Variations of Correlation between Microbial Diversity and Groundwater Quality Derived from a Riverbank Filtration Site, Northeast China. *Sci. Total Environ.* **2019**, *706*, 135855. [CrossRef] [PubMed]

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