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The Microbial Community Composition and Nitrogen Cycling Metabolic Potential of an Underground Reservoir in Rizhao, Shandong Province, China

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Abstract: Constructing underground reservoirs has emerged as a crucial strategy to address the shortage of fresh water in Rizhao, Shandong Province, China. However, the water quality, microbial community composition, and biogeochemical cycling of nutrients in underground reservoirs compared to raw water remain unknown. To unveil the characteristics of microbial community structures and their nitrogen cycling metabolic potential in coastal underground reservoirs, we utilized a functional gene array (GeoChip 5.0) in conjunction with high-throughput sequencing of 16S rRNA and 18S rRNA genes. Our findings indicate that the water quality in the underground reservoir exhibits a certain degree of eutrophication compared to raw water, with higher concentrations of TN, TP, NO_3^-N, NO_2^--N, and Chl a, but lower concentrations of DO and NH_4^+-N. The alpha diversity of bacterial and microeukaryotic communities was significantly lower in the underground reservoir. The bacterial community presented a stronger correlation with environmental factors than the microeukaryotic community. Regarding the relative abundance of bacterial communities, *Gammaproteobacteria* dominated the bacterial community in raw water, while *Gammaproteobacteria* and *Alphaproteobacteria* dominated the bacterial community in underground reservoir water. Additionally, the relative abundance of *Nitrospirae* was noticeably higher in the underground reservoir water. Moreover, we found significantly higher sequence abundance of the archaea Thaumarchaeota in the underground reservoir. Furthermore, our analysis revealed that, except for the *amoA* functional gene, which significantly increased the metabolic potential of nitrification, the metabolic potential of other microbial nitrogen functional genes was significantly reduced. This reduction may contribute to the lower concentration of NH_4^+-N in the underground reservoir. This study provides a comprehensive understanding of the microbial community characteristics and their nitrogen cycling metabolic potential in underground reservoirs. It serves as a valuable reference for water source selection, the formulation of water quality assurance measures, and the construction and management of underground reservoirs for subsequent impounding.

Keywords: underground reservoir; bacterioplankton community; microeukaryotic community; functional gene; Geochip

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1. Introduction

Population, resources, and the environment are pivotal challenges facing humanity today. Along coastlines, the ecological environment is notably fragile and highly susceptible

to man-made factors and destruction [1]. This issue significantly hampers economic and social development, as well as the enhancement of living conditions for coastal communities. Compounded by the climatic and geographical conditions specific to coastal areas, freshwater resources are exceptionally scarce. In some remote coastal regions, even the fundamental demand for basic living water supply cannot be guaranteed [2]. In Rizhao, Shandong Province, China, groundwater serves as the foundational resource supporting economic development. However, the rapid pace of economic growth has exacerbated the issues of water shortage and environmental deterioration [3]. The construction of underground reservoirs has emerged as a crucial strategy to alleviate these constraints, optimize water resource allocation, and achieve sustainable development [4]. To ensure water use safety in coastal areas and maximize water resource utilization, a construction scheme for underground reservoirs has been proposed. Underground reservoirs represent an innovative approach to the comprehensive development and utilization of water resources in coastal regions. Previous studies have revealed that underground reservoirs tend to retain large quantities of nutrients from raw water and other inflow ways [5,6]. However, there has been a lack of comprehensive studies on artificial underground reservoirs in China from the perspective of biogeochemical cycles. Addressing this knowledge gap is crucial for understanding the broader implications and potential environmental impact of underground reservoirs on water quality and ecological balance.

Microorganisms in the water play a crucial role in the aquatic ecosystem, influencing biogeochemical cycle processes such as material circulation and pollutant release through metabolic activities like assimilation and dissimilation [7–10]. They act as key drivers in material and energy cycles within ecosystems, mediating various biogeochemical processes in aquatic environments. Microorganisms are particularly significant in carbon cycling, greenhouse gas production and consumption, metal mineral transformation, and pollutant degradation in aquatic ecosystems. The study of microorganism community composition and function holds profound significance for water management and maintenance [11,12]. Additionally, microorganisms exhibit high sensitivity to changes in water environmental factors. Previous research has demonstrated that water microorganisms are influenced by complex biological and abiotic processes, including time and space distribution, pH, temperature, nitrogen, phosphorus, and other nutrient elements, as well as their circulation processes and the eutrophication state of the water [13–18]. Alterations in the nutritional status of water bodies lead to synchronous changes in the microbial community, offering a direct reflection of water body quality [19,20]. Consequently, microbial communities have garnered increasing attention due to their widespread distribution in water bodies and their significant involvement in element cycling processes. Understanding the characteristics of microbial communities in underground reservoirs serves as a vital biological index for evaluating water quality. This knowledge deepens our understanding of ecological processes and their mechanisms in water ecosystems. Despite the importance of this understanding, our current knowledge of microbial community characteristics in underground reservoirs remains limited. Further research in this area is essential for a comprehensive assessment of water quality and for refining our understanding of the ecological dynamics within water ecosystems.

Given the crucial position and intricate nature of nitrogen conversion, it is imperative to explore the nitrogen cycling state of underground reservoirs, particularly considering that the nitrogen cycling metabolic potential of such reservoirs remains unknown [21,22]. Nitrogen input into underground reservoirs undergoes a series of biological and abiotic processes, including nitrification, denitrification, and biological assimilation and absorption. These transformations, collectively known as the microbial nitrogen cycle, are mainly governed by microbial-mediated oxidation and reduction processes [23–26]. In essence, dinitrogen gas is initially fixed into ammonia nitrogen, which is then assimilated into organic nitrogen. The breakdown of organic nitrogen through ammonification can release ammonia nitrogen, subsequently oxidized to nitrite and nitrate through nitrification. Ultimately, these compounds are converted back to dinitrogen gas through denitrification

and anaerobic ammonia oxidation processes. This study delves into how microbial communities and their nitrogen cycling metabolic potential differ between raw water and underground reservoirs. The findings from this research offer valuable insights for water source selection, the formulation of water quality assurance measures, and the construction and management of underground reservoirs for subsequent impounding.

2. Materials and Methods

2.1. Information about the Studied Underground Reservoir

The study area is situated in Rizhao City, Shandong Province, China (35°25′34″ N–35°26′51″ N, 119°20′51″ E–119°21′51″ E), with the main river being the Futong River. The Futong River is a seasonal river that flows in a southeasterly direction and eventually empties into the Yellow Sea (Figure 1). The Rizhao Reservoir is positioned upstream of the Futong River, and approximately 5 km downstream of the Rizhao Reservoir lies the Futong underground reservoir. Constructed in June 2022, the Futong underground reservoir boasts a total capacity of 9,606,000 m^3. The primary water source for the Futong underground reservoir is derived from the outflow of the Rizhao Reservoir, supplemented by precipitation recharge and groundwater recharge.

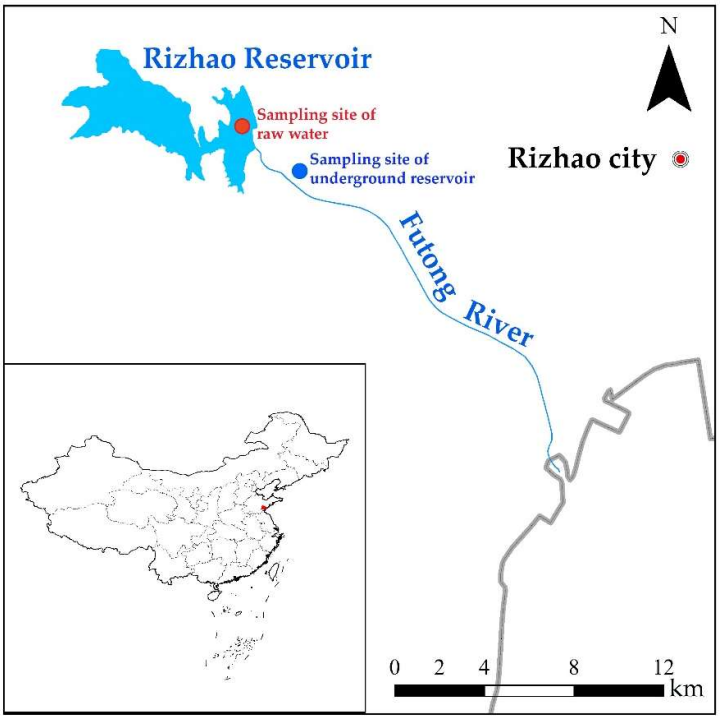

Figure 1. Sampling locations in Rizhao, Shandong Province, China.

2.2. Sample Collection and Physicochemical Analyses

In December 2022, we collected three raw water samples from the Rizhao Reservoir and three stored water samples from the underground reservoir (from the Futong underground reservoir) (Figure 1). In situ measurements of pH and dissolved oxygen (DO) were conducted using a water quality sonde (YSI 6600, Yellow Springs, OH, USA). The investigation also included the assessment of total nitrogen (TN) and total phosphorus (TP) in the water samples, following the method outlined by Rice et al. [27]. Dissolved inorganic nitrogen concentrations including ammonium (NH_4^+-N), nitrate (NO_3^--N), and nitrite (NO_2^--N) were quantified through continuous colorimetric flow analysis utilizing the Skalar SAN PLUS system (Skalar Analytical BV, Breda, The Netherlands). Chlorophyll a (Chl a) concentrations were determined spectrophotometrically, employing hot ethanol as the extraction solvent [28].

2.3. DNA Extraction, Amplification, and High-Throughput Sequencing

Microbial DNA extraction and purification were conducted following the protocol outlined by Wu et al. (2006) [29]. Subsequently, the concentration and purity of the DNA were assessed using the spectrophotometric method with a NanoDrop2000 instrument (ThermoScientific, Wilmington, DE, USA). For the analysis of 16S rRNA genes and 18S rRNA genes, PCR amplification was performed, followed by sequencing on an Illumina MiSeq platform, as previously described [30]. The sequence data for archaea were preserved throughout the study. Raw reads of the 16S rRNA genes and 18S rRNA genes underwent quality control using the Galaxy Pipeline (http://zhoulab5.rccc.ou.edu/tools.html, accessed on 23 July 2018). The primary steps employed for quality control included the elimination of: (i) sequences not perfectly matching the PCR primer at the beginning of a read; (ii) sequences with non-assigned tags; (iii) sequence reads with <200 bp after the proximal PCR primer if they terminated before reaching the distal primer; and (iv) sequences containing more than one undetermined nucleotide (N) to mitigate the impact of random sequencing errors. Only the first 250 bp after the proximal PCR primer of each sequence were included, as sequence quality tends to degrade beyond this point. Subsequently, the raw sequences were sorted and differentiated based on unique sample tags, with trimming of the tag and primers for each sample. Operational taxonomic units (OTUs) were clustered using the 'pick_otus.py' script in QIIME with the uclust method at a similarity cutoff of 97%. Taxonomic information for each OTU was retrieved by comparing with the SILVA databases using the online RDP (Ribosomal Database Project) classifier, employing a bootstrap cutoff of 80%. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database under Accession Number PRJNA1045554.

2.4. GeoChip Analysis and Data Preprocessing

The analysis of the six samples (three from raw water and three from the underground reservoir) involved the utilization of GeoChip 5.0. This version of GeoChip encompasses more than 7000 probes targeting nitrogen cycling functional genes. The functional genes include those associated with the pathways of ammonification, anammox, assimilation, assimilatory nitrogen reduction, denitrification, dissimilatory nitrogen reduction, nitrogen assimilation, nitrification, and nitrogen fixation. The GeoChip template labeling, hybridization, and imaging procedures were carried out following previously established protocols [31,32]. The raw data comprising the original signal intensity of the probes were submitted to a designated website (http://ieg.ou.edu/microarray/, accessed on 23 August 2019) and subjected to analysis using the data analysis pipeline. The data preprocessing steps included: (i) removal of probes with a signal-to-noise ratio less than 2, indicative of poor-quality probes [33]; (ii) a stipulation of a minimum of 2 positive probes for each gene in each group, with removal of probes appearing in only 1 or fewer samples among the 3 samples for each group to enhance data reliability, resulting in 5825 genes for further analysis; (iii) logarithmic transformation of the original intensity, and calculation of the normalized signal intensity for each probe by dividing the initial signal intensity of each probe by the mean intensity of the positive probes in each sample. The resulting microarray data matrix (normalized signal intensity), treated as 'species' abundance, was employed for subsequent statistical analysis. This comprehensive approach aims to elucidate the variations in nitrogen cycling functional genes between the raw water and underground reservoir samples [34].

2.5. Statistical Analysis

The pipeline-generated microarray data matrix (normalized signal intensity) and the subsampled Operational Taxonomic Unit (OTU) table were subjected to various statistical analyses. A one-way analysis of variance (one-way ANOVA), coupled with Duncan's test, was conducted to assess differences in water physicochemical properties, alpha diversity, and microbial community composition at different taxonomic levels. The statistical analysis was performed using the SPSS software (version 17). Alpha diversity was calculated

using the total OTU table [35,36]. A nonmetric multidimensional scaling (NMDS) plot was generated utilizing the Bray–Curtis dissimilarity index, and this was accomplished using the 'vegan' package in the R statistical environment (http://CRAN.R-project.org/package=vegan, accessed on 11 October 2022). Euclidean distances of all environmental variables were calculated using the 'vegdist' command in the 'vegan' package in R after standardized transformation. Mantel and partial Mantel tests were conducted to investigate if the differences in microbial communities between the two groups were correlated with environmental parameters. These analyses were performed using the 'vegan' package in R. The aim of these statistical analyses was to uncover relationships between microbial community structures, environmental parameters, and gene abundance or signal intensity.

3. Results and Discussion

3.1. Description of the Obtained Sequences and Physicochemical Index of Water Samples

In total, we obtained 1,070,494 high-quality bacterial/archaeal sequences and 1,060,867 microeukaryotic sequences from both raw water and underground reservoir water samples. The sequence distribution across each sample is as follows: 160,061, 151,204, and 155,991 for underground reservoir water bacteria/archaea, 189,459, 198,620, and 213,213 for raw water bacteria/archaea, 219,126, 207,811, and 211,211 for underground reservoir water microeukaryotes, and 139,986, 149,047, and 131,926 for raw water microeukaryotes, respectively. The sequence abundance of archaea in the underground reservoir was 84,837, 79,762, and 81,608, while in the raw water it was 31, 628, and 1120. At a 97% similarity level, the total Operational Taxonomic Unit (OTU) richness was 1297 for bacterial communities and 573 for microeukaryotic communities across all samples after being clipped by the minimum sequence number of each group. The rarefaction curves for both bacterial and microeukaryotic communities exhibited a nearly planar trend (Figure 2).

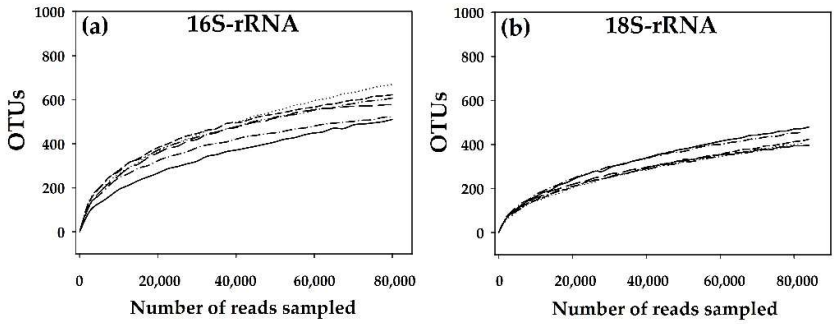

Figure 2. Rarefaction curves of the bacterial/archaeal communities (**a**) and microeukaryotic communities (**b**) in raw water and the underground reservoir.

The results of the physical and chemical indices for raw water and the underground reservoir are presented in Table 1. Overall, there were significant differences between raw water and underground reservoir samples in various environmental parameters. The underground reservoir samples exhibited significantly higher concentrations of TN, TP, NO_3^--N, NO_2^--N, and Chl a compared to the raw water. Conversely, they showed lower concentrations of DO and NH_4^+-N ($p < 0.05$, Table 1). Previous studies indicated that the water quality of underground reservoirs tends to degrade over time, potentially attributed to increased deep-layer oxygen depletion and the influx of limiting nutrients for algae growth, which could lead to a heightened risk of algal blooms and eutrophication [37,38]. The findings from this study align with these observations, indicating a certain degree of eutrophication in the underground reservoir compared to the raw water samples. A study of an underground reservoir in the vicinity of this study area has shown that agricultural nonpoint sources are the largest source of nitrate in the water of the underground reservoir, which may also have contributed to the higher concentration of TN, TP, NO_3^--N, and NO_2^--N in the underground reservoir in this study. Regarding the higher concentration of

Chl a and lower concentration of DO, we hypothesize that there may be two main reasons. On the one hand, the higher nutrient loading in the underground reservoir may have led to an outbreak of planktonic algal bloom in the water column, which in turn led to the increased Chl a content and decreased DO content [39]; on the other hand, since the underground reservoir is a low-light environment, photosynthesis was weakened in this case, and the algae need more chlorophyll to absorb and convert light energy to adapt to the low-light environment.

Table 1. Physical and chemical index results of the raw water and underground reservoir.

Sample	Raw Water	Underground Reservoir	*p*-Value (ANOVA)
pH	7.92 ± 0.19	7.69 ± 0.07	0.183
DO (mg/L)	8.67 ± 0.37	3.46 ± 0.15	<0.001
TN (mg/L)	2.05 ± 0.2	4.28 ± 0.09	<0.001
TP (mg/L)	0.022 ± 0.006	0.067 ± 0.005	<0.01
NH_4^+-N (mg/L)	0.311 ± 0.018	0.062 ± 0.004	<0.001
NO_3^--N (mg/L)	1.69 ± 0.08	3.99 ± 0.04	<0.001
NO_2^--N (mg/L)	0.001 ± 0.001	0.006 ± 0.001	<0.01
Chl a (μg/L)	0.0093 ± 0.0005	0.7758 ± 0.0085	<0.001

3.2. Succession of Microbial Community Structure in the Underground Reservoir

Alpha diversity indices were employed to thoroughly evaluate species richness, and the values for alpha diversity were determined for each individual sample. Subsequently, the average and standard deviation of alpha diversity were calculated (Table 2). Notably, both microbial community richness (OTU richness) and microbial community diversity (Simpson index) were found to be significantly lower in the underground reservoir, encompassing both bacterial and microeukaryotic communities (one-way ANOVA, $p > 0.05$) (Table 1). Despite these differences, the Shannon index exhibited comparable values between the raw water microbial communities and underground reservoir microbial communities.

Table 2. Alpha diversities of bacterial and microeukaryotic communities in the raw water and underground reservoir.

Values	Bacterial Communities			Microeukaryotic Communities		
	Raw Water	Underground Reservoir	*p*-Value (ANOVA)	Raw Water	Underground Reservoir	*p*-Value (ANOVA)
OTU richness	662 ± 31	562 ± 18	0.018	459 ± 11	411 ± 23	0.004
Shannon index	4.37 ± 0.37	4.32 ± 0.15	>0.05	5.30 ± 0.23	4.87 ± 0.14	>0.05
Simpson index	0.290 ± 0.02	0.190 ± 0.04	0.046	0.106 ± 0.016	0.040 ± 0.013	0.010

To investigate the differences in the taxonomic composition of microbial communities inhabiting the studied water samples, we conducted NMDS analyses based on the Bray–Curtis dissimilarity of sequence data. The NMDS plot illustrated that the taxonomic compositions of both bacterial and microeukaryotic communities clustered together for each water sample site (Figure 3). This clustering suggests that, whether bacteria or microeukaryotes, the communities in the same habitat exhibited greater similarity.

To assess the potential impact of environmental factors on microbial communities, a Mantel test was employed to examine the correlation between bacterial and microeukaryotic communities and the detected environmental factors (Table 3). The results indicated that both bacterial and microeukaryotic communities were significantly correlated with environmental factors ($p < 0.001$). Moreover, the bacterial community demonstrated greater sensitivity to environmental factors compared to the microeukaryotic community (bacterial community r = 0.786, microeukaryotic community r = 0.724). For bacterial communities, the correlations of DO (R = 0.437, $p = 0.018$), TN (R = 0.747, $p < 0.001$), TP (R = 0.707, $p < 0.001$), NH_4^+-N (R = 0.594, $p = 0.009$), and Chl a (R = 0.672, $p < 0.001$) were significant, while TN

(R = 0.623, $p < 0.001$), TP (R = 0.597, $p < 0.001$), NH_4^+-N (R = 0.375, $p = 0.041$), and Chl a (R = 0.528, $p = 0.005$) correlated with the microeukaryotic communities more significantly. The pH, NO_3^--N, and NO_2^--N showed no significant correlation with both bacterial and microeukaryotic communities.

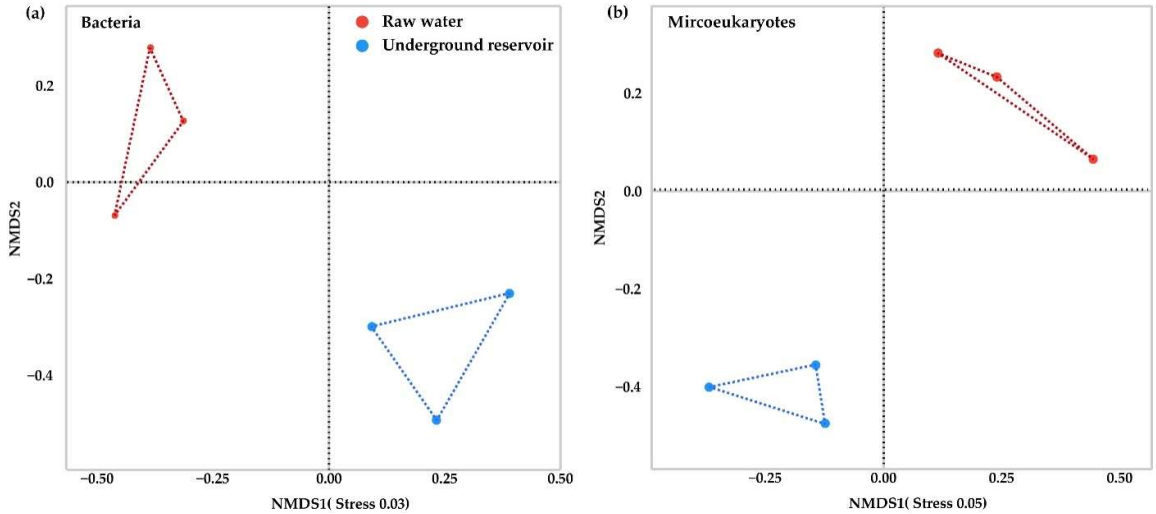

Figure 3. Non-parametric multidimensional scaling (NMDS) plots derived from the taxonomy based on the Bray–Curtis distance of the community composition of bacteria (**a**) and microeukaryotes (**b**).

Table 3. Mantel test for the correlation between community composition and environmental variables for bacteria and microeukaryotes in the raw water and underground reservoir.

Mantel Test	Bacterial Communities		Microeukaryotic Communities	
	R	*p*-Value	R	*p*-Value
pH	−0.112	NS	0.037	NS
DO (mg/L)	0.437	0.018	0.238	NS
TN (mg/L)	0.747	<0.001	0.623	<0.001
TP (mg/L)	0.707	<0.001	0.597	<0.001
NH_4^+-N (mg/L)	0.594	0.009	0.375	0.041
NO_3^--N (mg/L)	0.237	NS	0.078	NS
NO_2^--N (mg/L)	−0.220	NS	−0.150	NS
Chl a (μg/L)	0.672	<0.001	0.528	0.005
All factors	0.786	<0.001	0.724	<0.001

The significant differences observed in microbial community composition due to varying environmental variables are a well-established phenomenon in water habitats [40–42]. The impact of water nutrient levels on microbial communities is particularly noteworthy, as elevated nutrient levels can directly influence the composition and distribution of water microbial communities [43]. Furthermore, nutrient levels can affect the composition and abundance of heterotrophic flagellates and ciliates [44], as well as the biomass and distribution characteristics of algae [45], consequently influencing the overall diversity of microbial communities. Previous research has consistently demonstrated that alpha diversity and the structure of microbial communities in freshwater systems with different nutrient loads exhibit significant variations. Specifically, microbial diversity tends to decrease significantly in eutrophic waters [46,47], which is consistent with the results of this study.

Figure 4 displays the relative abundances of bacterial and microeukaryotic communities at the phylum level. For the relative abundance of bacterial communities, *Gammaproteobacteria* dominated the bacterial community in raw water, while *Gammaproteobacteria* and *Alphaproteobacteria* dominated the bacterial community in underground reservoir water. *Gammaproteobacteria* are widespread in the water environment, including many pathogenic

bacteria, such as *Escherichia coli*, *Salmonella*, *Vibrio cholerae*, *Helicobacter pylori*, and other famous species [48]. A previous study showed that the relative abundance of *Gammaproteobacteria* was high in both mesotrophic and eutrophic water environments [49], which was also observed in our investigation. In addition, the relative abundance of *Nitrospirae* was observed to be noticeably higher in the underground reservoir water, as *Nitrospirae* was identified as one of the most predominant nitrite-oxidizing bacteria in the ocean [50,51]. Regarding the average relative abundance of microeukaryotic communities, *Dinoflagellata*, *Rotifera*, and *Ochrophyta* dominated the microeukaryotic community in raw water, while *Ochrophyta* and *Choanoflagellida* dominated the microeukaryotic community in the underground reservoir. *Dinoflagellate* is an important basic part of the food chain in modern oceans, and it is also a major oxygen producer. It also widely exists in freshwater and brackish lakes and other water bodies [52,53]. *Ochrophyta* mostly live in seawater bodies along the coast of the mainland, and are rare in fresh water [54]. *Ochrophyta* were dominant species in the raw water and underground reservoir, indicating that there may be seawater intrusion in this area.

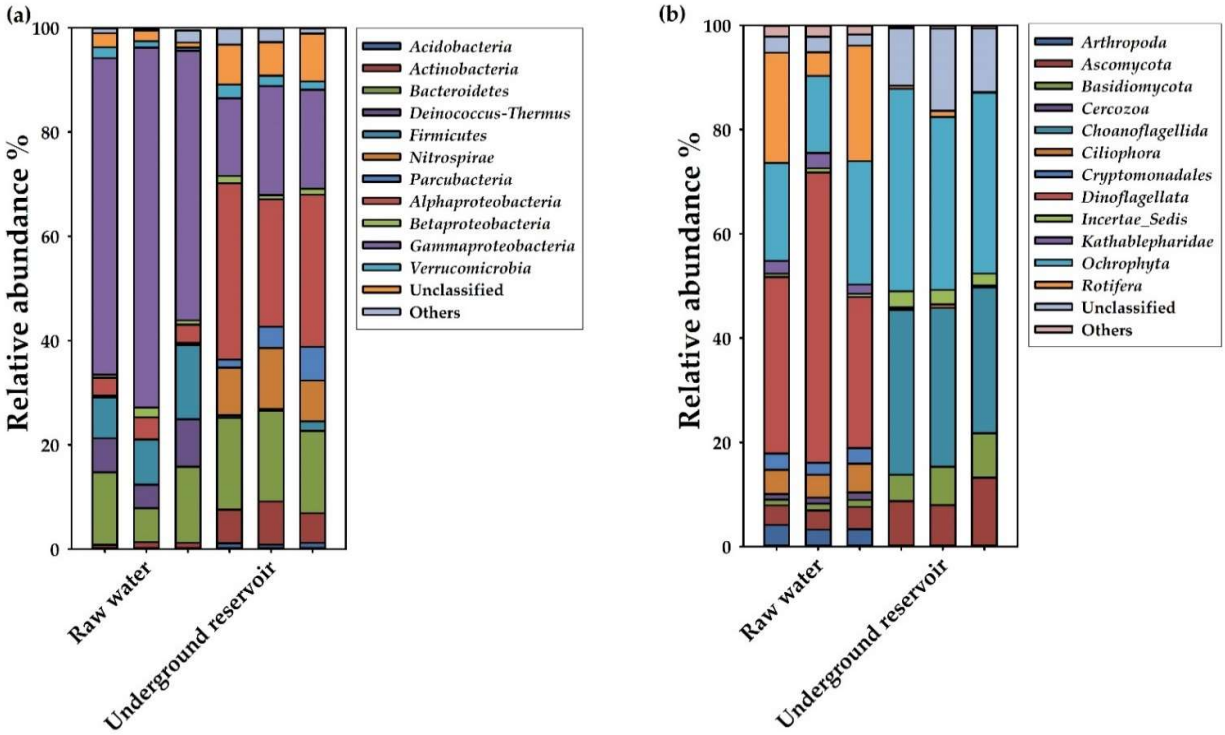

Figure 4. Relative abundance of the specific phylum(subphylum) groups in the bacterial (**a**), and microeukaryotes (**b**) of the raw water and underground reservoir. Those with a relative abundance less than 1% are classified as 'Others'.

Moreover, we found that the sequence abundance of the archaea *Thaumarchaeota* in the underground reservoir was as high as 84,811, 79,626, and 81,507, while in the raw water it was only 23, 80, and 837. It has been reported that *Thaumarchaeota* is capable of oxidizing ammonium to nitrite, and is one of the most abundant chemolithoautotrophs known in the dark ocean [55]. *Thaumarchaeota* has been found in an underground cave full of water. It metabolizes in a very special way, relying on the oxidation of ammonia in the salt water of the cave to obtain energy, and does not need sunlight at all [56]. It is believed to play an important role in the earth's nitrogen cycle by obtaining energy through the oxidation of ammonia to nitrite [57,58]. This explains to some extent why the underground reservoir is seriously eutrophicated, but the NH_4^+-N content is less.

3.3. Nitrogen Cycling Metabolic Potential of the Microbial Community in the Underground Reservior

Our study delving into bacterial and microeukaryotic community structures extended to the assessment of the metabolic potential of functional genes related to the nitrogen cycle using GeoChip 5.0. The comparison of relative signal intensities of functional genes in various pathways of the nitrogen cycle between raw water and the underground reservoir is illustrated in Figure 5.

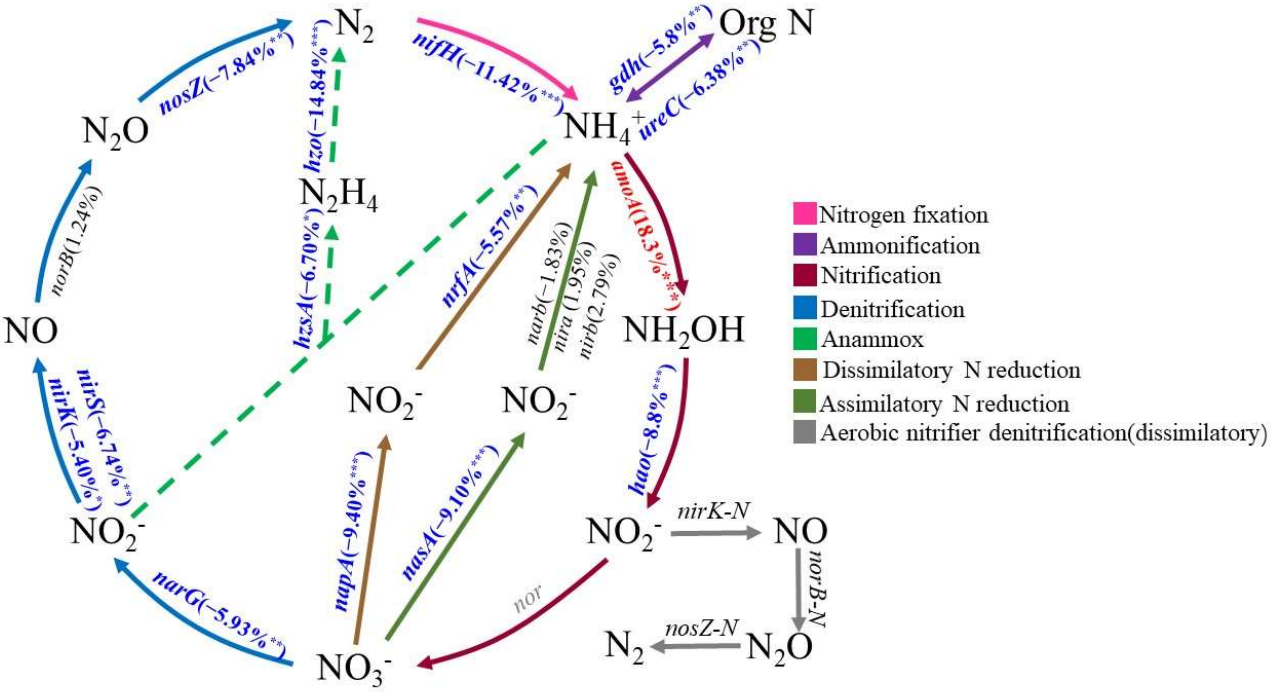

Figure 5. Nitrogen cycling metabolic potential of the microbial community in the underground reservoir compared to raw water. Red bold font indicates a significant increase in the relative signal intensity of the functional gene, while blue bold font indicates a significant decrease in the relative signal intensity of the functional gene compared to raw water. Significant differences are indicated by * (one-way ANOVA, *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$).

Our findings reveal a significant reduction in the metabolic potential of microbial nitrogen functional genes in various pathways of the nitrogen cycle, with the exception of the *amoA* functional gene. The nitrogen cycle in water systems is primarily orchestrated by microorganisms through redox processes, encompassing mineralization, nitrogen fixation, denitrification, nitrate reduction, nitrate dissimilatory reduction to ammonium, nitrogen assimilation, ammonia oxidation, nitrification, and anaerobic ammonia oxidation. Two key processes within the nitrogen cycle are nitrification and nitrate reduction. The initial stage of nitrification involves the conversion of NH_4^+-N to NO_2^--N, a crucial rate-limiting step [59,60]. Autotrophic ammonia-oxidizing bacteria and ammonia-oxidizing archaea are the primary functional microorganisms involved in this process, with the ammonia monooxygenase gene serving as a key molecular marker. The ammonia monooxygenase gene involved in catalysis consists of three subunits, *amoA*, *amoB*, and *amoC*. Quantitative studies of ammonia-oxidizing microorganisms typically use *amoA* as a molecular marker [61]. Notably, amoA, one of the subunits of the ammonia monooxygenase gene, showed a significant increase in metabolic potential, indicating heightened nitrification activity in the underground reservoir. Hou et al. [62] found that the diversity of ammonia-oxidizing bacteria in the Taihu Lake and Chaohu Lake, two large eutrophic lakes in China, increased from mesotrophic to eutrophic, and was positively correlated with the number ratio of ammonia-oxidizing bacteria and ammonia-oxidizing archaea. Our results

are consistent with higher concentrations of TN, TP, NO_3^--N, and NO_2^--N, but lower concentrations of NH_4^+-N in the underground reservoir. Furthermore, the abundance of *Thaumarchaeota* in the underground reservoir is also consistent with these findings. As *Thaumarchaeota* are known for their role in ammonia oxidation, they likely contribute to the increased nitrification potential observed in the underground reservoir.

4. Conclusions

Our study has provided valuable insights into microbial community structures and their nitrogen cycling metabolic potential in both raw water and an underground reservoir. Here are the key findings summarized. Significant differences were observed in the composition of microbial community structures, both for bacterial and microeukaryotic communities, between raw water and the underground reservoir. Alpha diversity, indicating species richness and evenness, was found to be significantly lower in the underground reservoir for both bacterial and microeukaryotic communities. The dominant species within the microbial communities showed considerable differences between raw water and the underground reservoir. In raw water, Gammaproteobacteria dominated the bacterial community at the phylum level. In the underground reservoir, Thaumarchaeota (archaea), Alphaproteobacteria, and Gammaproteobacteria dominated the bacterial community. Correlation analysis revealed that environmental factors may significantly influence both bacterial and microeukaryotic communities. The bacterial community was found to be more strongly influenced by environmental factors compared to the microeukaryotic community. The metabolic potential of microbial nitrogen functional genes exhibited a significant decline in the underground reservoir, except for the amoA functional gene. The increased potential for nitrification (amoA gene) in the underground reservoir may contribute to higher concentrations of TN, TP, NO_3^--N, and NO_2^--N, while NH_4^+-N concentrations were lower. The study provides a comprehensive understanding of microbial community characteristics and nitrogen cycling in the underground reservoir. The findings contribute to the knowledge of microbial dynamics and nitrogen cycling processes in water systems, offering practical insights for water resource management and environmental sustainability.

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Data Availability Statement: The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA1045554).

Conflicts of Interest: Author Bojun Liu was employed by the company Yellow River Engineering Consulting 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.

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