

Article

Effects of Redox Condition on Bacteria-Mediated Hydrochemical Processes and Bacterial Community During Managed Aquifer Recharge

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Abstract: During the process of managed aquifer recharge (MAR), when the aerobic surface water is recharged into the reductive aquifer, the redox environment changes along the water pathway. MAR practice can reshape the initial groundwater bacterial community, and further induce variations in the bacteria-mediated hydrochemical reactions. In this study, laboratory-scale column experiments were conducted to simulate the processes of aerobic/anaerobic recharge to aquifer. The results showed that the concentration of DO during the aerobic recharge was higher than that of the anaerobic recharge, and ORP showed a similar trend. Active nitrogen transformation was observed during the simulated MAR processes. In the early stages of both the aerobic and anaerobic recharges, nitrate reduction occurred due to denitrification and DNRA. However, in the late stages, nitrification might happen in the aerobic column, and nitrate reduction remained the major process in the anaerobic column. For the bacterial community, *Massilia*, *Ralstonia*, *Legionella*, and *Curvibacter* predominated under the aerobic recharge. Comparatively, *Cedecea*, *Cupriavidus*, and *Ralstonia* maintained high relative abundances under the anaerobic recharge. Our study provides essential information about the characteristics of bacterial-mediated hydrochemical reactions during the MAR process. The result would enhance understanding of MAR activities and provide valuable insights into the groundwater resources' sustainable development and management.

Keywords: redox condition; nitrogen transformation; bacterial community composition; high-throughput sequencing; aquifer medium; groundwater resources sustainability



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1. Introduction

The sustainable management of groundwater resources is particularly critical, due to the fact that the world's drinking water and irrigation demands highly rely on groundwater [1–4]. However, over-reliance on groundwater resources can lead to a series of environmental geological problems, posing a severe threat to the health and safety of human life [5–7]. Managed aquifer recharge (MAR), an intentional recharge of aquifers for groundwater recovery and environmental benefits, represents a valuable method for the sustainable management of water resources [8]. The MAR system can alleviate seawater intrusion, soil salinization, and land subsidence [9]. It can also improve water quality [10,11], protect the ecological environment, and control drops in water levels [12].

However, during actual MAR activities, when the aerobic surface water is recharged to subsurface aquifers, both aerobic and anaerobic conditions are alternated along with

the groundwater pathway [12]. Changes in the redox condition of aquifers have certain impacts on the removal of organic pollutants, such as phenazone, 1-Acetyl-1-methyl-2-dimethyloxamoyl-2-phenylhydrazide (AMDOPH), 1-acetyl-1-methyl-2-phenylhydrazide (AMPH), and emerging organic compounds (e.g., ibuprofen, paracetamol, and sulfamethoxazole) [13–15]. Nitrate contamination in groundwater has also become a severe environmental problem around the world [16]. Nitrogen conversion processes and the associated biogeochemical mechanisms vary depending on the redox conditions [17,18]. In the nitrogen cycle, the main processes of biological involvement in transformation are nitrification, denitrification, dissimilatory nitrate reduction to ammonium (DNRA), and anaerobic ammonium oxidation (ANAMMOX). Nitrification occurs mainly under an aerobic condition while denitrification is an anaerobic process [19,20]. DNRA and ANAMMOX also are biological transformation processes under relatively anaerobic conditions [21,22]. The changes in the redox environment of aquifers also have significant impacts on the growth and metabolism of microorganisms, thereby affecting the composition of microbial communities in aquifers [23,24]. The ecological processes behind their aggregation may have critical impacts on the quality of the groundwater and function of the ecosystem [25,26]. The combined responses of biogeochemical and microbial communities to the oxidation of aquifers and nitrogen cycling have attracted great attention in academia.

Previous studies involving MAR processes mainly focused on the aerobic condition only. However, few studies were concerned about in-depth biogeochemical reactions under different redox conditions. In this study, percolation experiments were conducted at a laboratory scale to simulate a continuous artificial recharge under aerobic/anaerobic conditions. The physicochemical properties of groundwater and features of bacterial communities were systematically characterized to explore bacteria-participated hydrogeochemical processes during the simulated MAR processes. The objectives of this study were to perform the following: (1) characterize the hydrochemical processes during the simulated aerobic/anaerobic MAR, particularly inorganic nitrogen transformation; (2) explore the differences in the bacterial community under different redox recharges; (3) clarify the correlation between functional bacteria and environmental factors. This study is of primary importance to fully characterize the bacteria-mediated hydrogeochemical reactions under both aerobic and anaerobic MAR processes. The findings would enhance our understandings about MAR activities and provide valuable insights into the sustainable management of groundwater resources.

2. Materials and Methods

2.1. Experimental Device and Design

The schematic diagram of the experimental device is shown in Figure 1a. The percolation column was made of plexiglass with a height of 20 cm and inner diameter of 5 cm. Three sampling ports were installed at distances of 3, 10, and 17 cm from the top of the column. A sealing cap was mounted at the top and connected with a constant hydraulic head device via a sterilized silicone tube. The percolation experiments were conducted to reproduce continuous artificial recharges under the aerobic and anaerobic conditions, named as A-MAR and An-MAR, respectively (Figure 1b). In the aerobic MAR, O₂-rich water (DO = 6.90 mg/L) was injected into the column. Similarly, in the anaerobic MAR, O₂-free water (DO < 2.00 mg/L) was introduced into the column. Specially, argon (Ar) was used to maintain the anaerobic environment in the An-MAR mode.

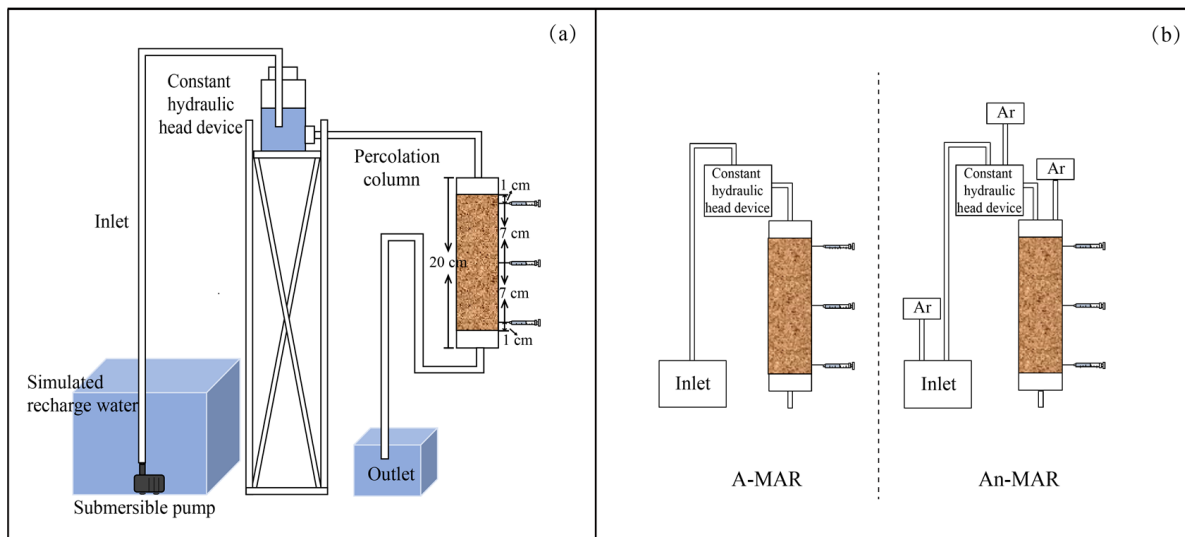


Figure 1. Schematic diagram of (a) experimental device and (b) experimental design of percolation experiments. A-MAR and An-MAR represent the simulated aerobic MAR and anaerobic MAR, respectively.

2.2. Porous Media

Standard quartz sand ($d_{50} = 0.349$ mm, $\text{SiO}_2 > 99\%$, Guangzhou Vigor Health Equipment Inc., Guangzhou, China) was used as model aquifer media to pack into the percolation column to an effective height of 16 cm. The porosity of the column was 0.425 ± 0.05 , calculated as the ratio of the water volume required for packing (mL) to the total sand volume (mL). The sand was washed repeatedly with deionized water before use. Subsequently, the sand was dried in an oven (DHG-9140A, Yihen gyiqi, Co., Ltd., Shanghai, China) at 121°C for 2 h to remove moisture and further sterilization. The particle size distribution of the sand is presented in Figure S1.

2.3. Bacterial Inoculum and Simulated Recharge Water

In this study, an in situ groundwater sample was collected from an irrigation well in Jiaozhou City, Shandong Province, China (36.372064°N , 120.169622°E). It was used for bacterial inoculation, and the characteristics of the bacterial community was described in our previous study [27]. Briefly, 5 L of groundwater was sampled and immediately transferred to the laboratory on ice and stored at 4°C before further use. For inoculation, an eight pore volume of groundwater was injected gravitationally into the sand-packed column. Then, the inlet and outlet were closed, and the column was left completely saturated for 12 h to ensure bacterial attachment.

A synthetic nutrient solution was prepared as the simulated recharge water. The nutrient solution was prepared according to the water quality of an in situ river, which was near the irrigation well. The solution contained glucose (0.045 mg/L), NaNO_3 (18.20 mg/L), NH_4Cl (3.82 mg/L), NaH_2PO_4 (11.61 mg/L), and Na_2SO_4 (295.83 mg/L). Before being injected into the column, the recharge water was sterilized at 121°C for 15 min. Then, O_2 and Ar were introduced into the recharge water to maintain DO of 6.90 and <2.00 mg/L for A-MAR and An-MAR, respectively. The physicochemical parameters of the water samples are listed in Table 1.

Table 1. Physicochemical parameters of the water samples.

Parameter ^a	Groundwater	River	Simulated Recharge Water
pH	7.1	8.1	7.2
DO ^b	2.46	9.71	A-MAR: 6.90 An-MAR: <2.00
DOC ^b	1.8	4.9	15.0
ORP ^b	−105.4	208.4	A-MAR: 192.8 An-MAR: 126.1
NO ₃ -N	0.52	1.99	3.00
NO ₂ -N	0.005	0.004	-
NH ₄ -N	1.98	0.90	1.00
SO ₄ ^{2−}	231.9	208.8	200.0
PO ₄ ^{3−}	3.0	2.5	3.0
K ⁺	176.2	217.1	-
Na ⁺	2325.5	3425.5	-
Ca ²⁺	262.5	263.9	-
Mg ²⁺	119.5	97.7	-

^a Units of the parameters are mg/L, except for ORP (mV). ^b DO, DOC, and ORP represent dissolved oxygen, dissolved organic carbon, and oxidation–reduction potential, respectively.

2.4. Percolation Experiment

During the percolation experiments, the simulated recharge water was infiltrated vertically from the top to bottom of the percolation columns under a constant hydraulic head difference of 12 cm, and then discharged through the outlet. The outflow rates of the columns were measured every 4 h (Table S1). Meanwhile, water samples were collected from the sampling ports and measured for hydrochemical parameters every 24 h. The percolation experiments were conducted for 20 d in the dark at 15 °C. Then, the sand samples in the first layer of the columns were used for microbial analysis.

2.5. Analytical Methods

2.5.1. Analysis of Water Samples for Physicochemical Parameters

The oxidation–reduction potential (ORP), DO, and pH were measured on site using a portable multiparameter water quality analyzer (HD40d, HACH, Loveland, CO, USA). Cations, such as K⁺, Ca²⁺, Na⁺, and Mg²⁺, were tested using an atomic absorption spectrophotometer (TAS-990, Beijing Puxi General Instrument Co., Ltd., Beijing, China). The NH₄⁺, NO₃[−], NO₂[−], SO₄^{2−}, and PO₄^{3−} were determined by an ultraviolet–visible spectrophotometer (UV-2802, Shanghai, China). Dissolved organic carbon (DOC) was measured using a total organic carbon analyzer (QbD1200, HACH, Loveland, CO, USA). The ORP, DO, NH₄⁺, NO₃[−], NO₂[−], and DOC were regarded as redox-sensitive indicators which revealed potential redox reactions during the percolation experiments, while the other physicochemical parameters were measured as background conditions.

2.5.2. DNA Extraction, PCR Amplification, and High-Throughput Sequencing

For microbial analysis, 0.5 g of a sand sample was collected from the first layer of each column (approximately ~1 cm) and stored at −80 °C before further analysis. Each sampling was performed in triplicate. A soil DNA kit (Omega Biotechnology, Knox, GA, USA) was used to extract DNA. The quality and concentration of DNA was determined by 1.0% agarose gel electrophoresis and a UV–Vis spectrophotometer (NanoDrop2000, Thermo Scientific, Wilmington, NC, USA), respectively.

After DNA extraction, the V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified with universal primers 338F (5′-ACTCCTACGGGGCAGG-3′) and 806R (5′-GACTACHVGGGTWTCTAAT-3′). The PCR reaction mixture included 4 µL of 5 ×

FastPfu Buffer, 2 μ L of 2.5 mM dNTPs, 0.8 μ L of each primer (5 μ M), 0.4 μ L of FastPfu Polymerase, and 10 ng of template DNA. The PCR amplification was conducted under the following conditions: initial denaturation for 3 min at 95 $^{\circ}$ C, followed by 27 cycles of 30 s at 95 $^{\circ}$ C, 30 s at 55 $^{\circ}$ C, 45 s at 72 $^{\circ}$ C, and the remaining step at 72 $^{\circ}$ C for 10 min. The PCR product was extracted from 2% agarose gel, then further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and finally quantified using QuantiFluorTM-ST (Promega, Madison, WI, USA), according to the manufacturer's protocol. Purified amplicons were pooled in equimolar amounts and paired-end sequenced (2 $\times$ 300) on the Illumina MiSeq platform (Illumina, San Diego, CA, USA), according to the standard protocols of Majorbio BioPharm Technology Co., Ltd. (Shanghai, China).

2.5.3. Bioinformatics Analysis

Raw FASTQ files were quality-filtered by Trimmomatic and merged by FLASH according to the criteria mentioned by Xia et al. [28]. Operational taxonomic units (OTUs) were clustered based on a 97% similarity cutoff using UPARSE (version 7.1, <http://drive5.com/uparse/>, accessed on 23 December 2024). The taxonomy of each 16S rRNA gene sequence was analyzed using the RDP classifier algorithm (<https://sourceforge.net/projects/rdp-classifier/>, accessed on 23 December 2024) on the Silva (SSU123) 16S rRNA database, with a confidence threshold of 70%. Based on the OTUs' data, the alpha diversity indices, including the observed OTUs, Chao1, Shannon, Evenness, and coverage, were calculated using Mothur (<http://www.mothur.org/wiki> version 1.30.1, accessed on 23 December 2024). The community composition structure and differences were analyzed by applying the R Programming Language (version 3.3.1). The redundancy analysis (RDA) was performed using Canoco 5 for investigating the relationship between the physicochemical properties of groundwater and the bacterial community structure.

2.5.4. Statistical Analysis

Changes in the bacterial community alpha diversity under different recharge conditions were analyzed using SPSS 25.0 for evaluating the correlations and significance differences ($p < 0.05$). Pearson correlation analysis was used for species and environmental factors' correlation.

3. Results

3.1. Hydrochemical Characterization of Groundwater in Simulated MAR Processes

As shown in Figure 2a, the concentration of DO fluctuated around an average value of 2.59 mg/L during the aerobic recharge, with a reduction of 62.46% compared with that of the influent (6.90 mg/L). This indicated that the concentration of DO decreases gradually with the proceeding of the aerobic artificial recharge. Comparatively, under the anaerobic condition, the average concentration of DO was 2.14 mg/L, keeping a balance with its initial value of influent (<2.00 mg/L). The ORP is considered as a direct parameter to indicate the redox environment in subsurface systems. In this study, the average ORP of groundwater was 118.3 mV during the aerobic recharge, which was higher than that in the anaerobic process (91.8 mV, Figure 2b). At the first 144 h, the ORP values showed obvious decreasing trends under both aerobic and anaerobic conditions. The carbon source is of primary importance for the growth and metabolism of microbes. Figure 2c displays the temporal variation in the concentration of DOC with the proceeding of the simulated artificial recharge. In general, DOC displayed an obviously decreasing trend, indicating that carbon was consumed in the percolation columns. Compared with the initial DOC of 15.0 mg/L in the influent, its average concentration decreased to 3.0 mg/L and 5.4 mg/L during the aerobic and anaerobic recharges, with reductions of 79.93% and 64.13%, respectively.

Particularly, a large reduction in the DOC concentration occurred within the initial 48 h, and thereafter it fluctuated at a low level. For the comparison of inorganic nitrogen species, the concentrations of nitrate, nitrite, and ammonium were reported, in $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NH}_4\text{-N}$, respectively. As shown in Figure 2d, the concentration of $\text{NO}_3\text{-N}$ rapidly decreased to 0.41 mg/L at 168 h during the aerobic recharge, with the reduction of 86.50% compared with that of the influent (3.00 mg/L). Similarly, there was even a 100% reduction of $\text{NO}_3\text{-N}$ in the anaerobic recharge (at 168 h). However, the concentration of $\text{NO}_3\text{-N}$ gradually increased after 168 h under both aerobic and anaerobic conditions. The overall concentration of $\text{NO}_2\text{-N}$ did not change significantly during the whole process (Figure 2e), with an average value of 0.318 mg/L for the aerobic recharge and 0.313 mg/L for the anaerobic recharge. For the $\text{NH}_4\text{-N}$, it showed an increase within the first 144 h, with maximum values of 1.93 and 1.76 mg/L under the aerobic and anaerobic recharges (Figure 2f). However, after 168 h, the concentration of $\text{NH}_4\text{-N}$ began to decrease and reached a minimum value of 0 mg/L under the stimulated recharges.

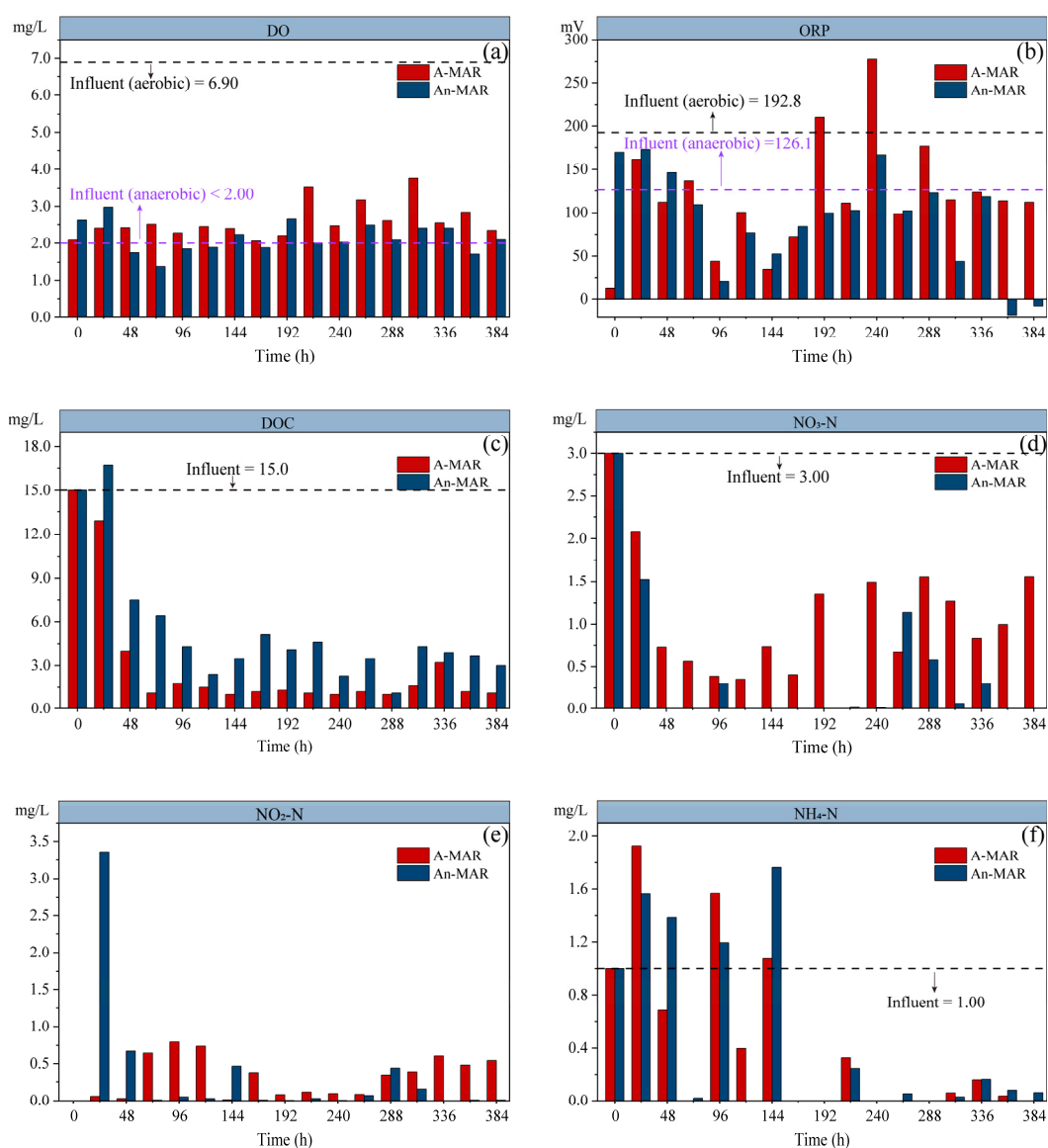


Figure 2. Hydrochemical parameters of groundwater during the simulated aerobic MAR (A-MAR) and anaerobic MAR (An-MAR) processes. (a) DO, (b) ORP, (c) DOC, (d) $\text{NO}_3\text{-N}$, (e) $\text{NO}_2\text{-N}$, (f) $\text{NH}_4\text{-N}$.

3.2. Characteristics of Bacterial Community in Simulated MAR Process

3.2.1. Bacterial Alpha Diversity

A total of 240,183 high-quality sequences were obtained from the collected samples, ranging from 36,754 to 43,014 per sample (Table 2). These sequences were clustered into OTUs based on a 97% sequence similarity. The coverage values of the samples reached 100%, suggesting enough sequencing depth that could represent information about the bacterial community in this study. The average Sobs and Chao indexes were 111 and 118 under the aerobic recharge, respectively, which were significantly higher than those in the anaerobic (61, $p < 0.01$ and 70, $p < 0.01$), implying that the richness was higher in the aerobic column than that in the anaerobic column. The average Shannon index also showed a similar trend with a higher value of 2.20 during the aerobic recharge, compared with that in the anaerobic condition (1.88, $p < 0.05$).

Table 2. Alpha diversity indexes of bacterial community under different artificial recharges. A-MAR and An-MAR refer to the simulated aerobic MAR and anaerobic MAR, respectively. A1-A3 and An1-An3 represent samples collected from the first layers of the aerobic and anaerobic columns, respectively ($n = 3$).

Groups	Samples	High Quantity Sequences	Average Length (bp ^a)	OTUs	Sobs	Chao	Shannon	Evenness	Coverage (%)
A-MAR	A1	40,855	425.43	38,274	108	117	2.29	0.49	100
	A2	38,338	425.06	36,429	111	115	2.14	0.46	100
	A3	43,014	425.80	41,387	114	124	2.17	0.46	100
	Average	40,736	425.43	38,697	111	119	2.20	0.47	100
An-MAR	An1	39,736	428.66	36,366	66	81	1.92	0.46	100
	An2	41,486	428.70	37,201	59	64	1.88	0.46	100
	An3	36,754	428.76	33,793	58	65	1.84	0.45	100
	Average	39,325	428.71	35,787	61	70	1.88	0.46	100

^a Base pair.

3.2.2. Bacterial Community Composition

The composition of the bacterial community under different recharge conditions is shown in Figure 3. On a cutoff value of 1%, a total of four phyla were obtained (Figure 3a). The most dominant phylum was *Proteobacteria* with a relative abundance of 96.53%, followed by *Bacteroidota* (2.35%), *Verrucomicrobiata* (0.71%), and *Actinobacteriota* (0.51%). Despite existing in all the samples, the compositions of bacterial communities were different in different samples. Under the aerobic recharge, the dominant phyla were *Proteobacteria*, *Bacteroidota*, *Verrucomicrobiata*, and *Actinobacteriota*, with average relative abundances of 93.12%, 4.60%, 1.06%, and 1.02%, respectively. However, in the anaerobic recharge, the bacterial community was mainly composed of *Proteobacteria*. At the class level (Figure 3b), *Gammaproteobacteria* was predominant with the average relative abundances of 80.78% and 98.83% under the aerobic and anaerobic recharges, respectively. In addition, the dominant bacterial species were *Alphaproteobacteria*, *Chlamydiae*, and *Bacteroidia* under the aerobic recharge, with average relative abundances of 12.41%, 4.62%, and 1.07%, respectively.

For further analysis of the composition of the bacterial community, the genus-level taxonomic structure was determined (Figure 3c). Under the aerobic recharge, the genera *Massilia*, *Ralstonia*, *Legionella*, and *Curvibacter* were predominant, with average relative abundances of 46.46%, 16.45%, 5.99%, and 3.51%, respectively. Comparatively, in the anaerobic recharge, *Cedecea*, *Cupriavidus*, and *Ralstonia* predominated, accounting for average relative abundances of 33.59%, 27.94%, and 15.25%, respectively. This indicated that the diversity of the bacterial community under the aerobic recharge was higher than that under

the anaerobic recharge, which was consistent with the results of the alpha diversity analysis. *Massilia*, with an average relative abundance of 46.46%, was detected as a unique bacterium in the aerobic recharge. Comparatively, *Cedecea*, which was accounting for an average relative abundance of 33.59%, was the most abundant and unique genus in the anaerobic recharge. *Cupriavidus* was the second most common genus that only existed under the anaerobic recharge. In addition, some species shared under different redox conditions, e.g., *Ralstonia*, *Curvibacter*, *Klebsiella*, and *Legionealla*, were found in both the aerobic and anaerobic columns. Their percentages also varied under different recharge conditions. Further statistical analysis demonstrated that the bacterial communities clustered into distinct groups due to different redox conditions (Figure S2), indicating obvious differences in their compositions under different recharge conditions.

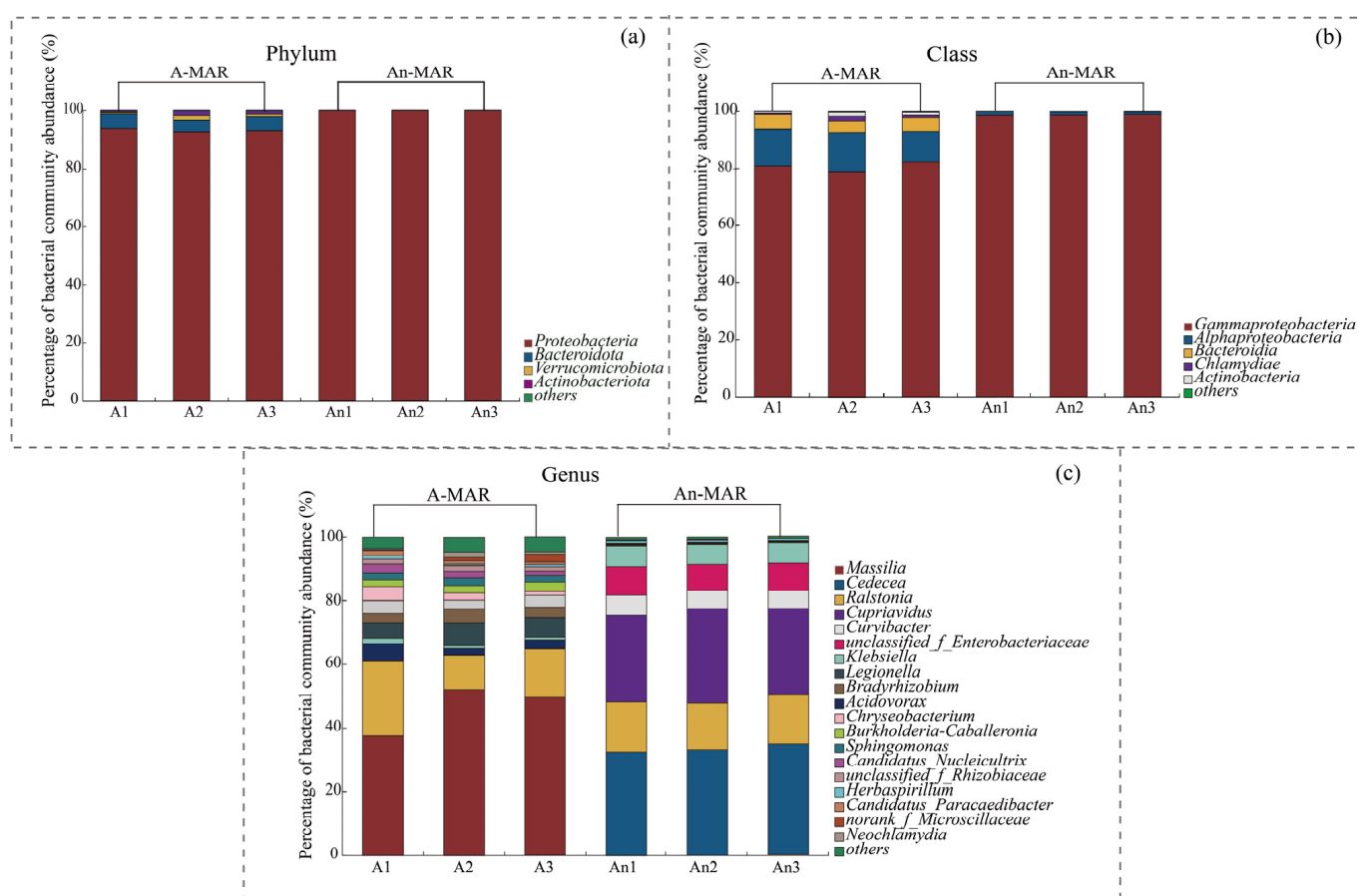


Figure 3. Bacterial community composition on (a) phylum, (b) class, and (c) genus levels, respectively. A1–A3 and An1–An3 represented samples collected from the first layers of the aerobic and anaerobic columns, receptively.

3.3. Correlation Between Groundwater Quality and Bacterial Community Under Different Recharge Conditions

The redundancy analysis (RDA) was used to establish a correlation between the bacterial community at different taxonomic levels and groundwater hydrochemical parameters. As shown in Figure 4a, a total of 90.07% variation could be explained by the five hydrochemical parameters. RDA1 and RDA2 explained variations of 73.74% and 16.33%, respectively. Among the variables considered, DO, DOC, NO₃-N, NO₂-N, and NH₄-N accounted for 67.30%, 12.00%, 8.30%, 7.70%, and 4.70% of the total variation, respectively. From the results of the RDA and heatmap of genus (Figure S2), NH₄-N and DOC were positively correlated with the dominant phylum *Proteobacteria* under both aerobic and anaerobic recharges. This was consistent with the study of Li et al. [29], who demonstrated that the

concentration of DOC had a positive relationship with the abundance of the most common phylum *Proteobacteria*. Meanwhile, during the aerobic condition, the abundance of phyla *Bacteroidota* and *Verrucomicrobiota* had negative relationships with DOC and $\text{NH}_4\text{-N}$, while positively correlated with $\text{NO}_3\text{-N}$, DO, and $\text{NO}_2\text{-N}$. On the genus level, DO, $\text{NO}_3\text{-N}$, and $\text{NO}_2\text{-N}$ had a positive correlation with the dominant genera *Massilia* and *Legionella* of the aerobic recharge. However, the environmental factors DOC ($p < 0.05$) and $\text{NH}_4\text{-N}$ were positively correlated with *Cedecea*, *Klebsiella*, and *Cupriavidus*, and negatively correlated with $\text{NO}_3\text{-N}$, DO, and $\text{NO}_2\text{-N}$ under the anaerobic recharge. Thus, these dominant species had important roles in nitrogen transformation under the aerobic and anaerobic recharge processes. The RDA results showed that the differences in certain environmental factors affected the distribution of all the bacteria.

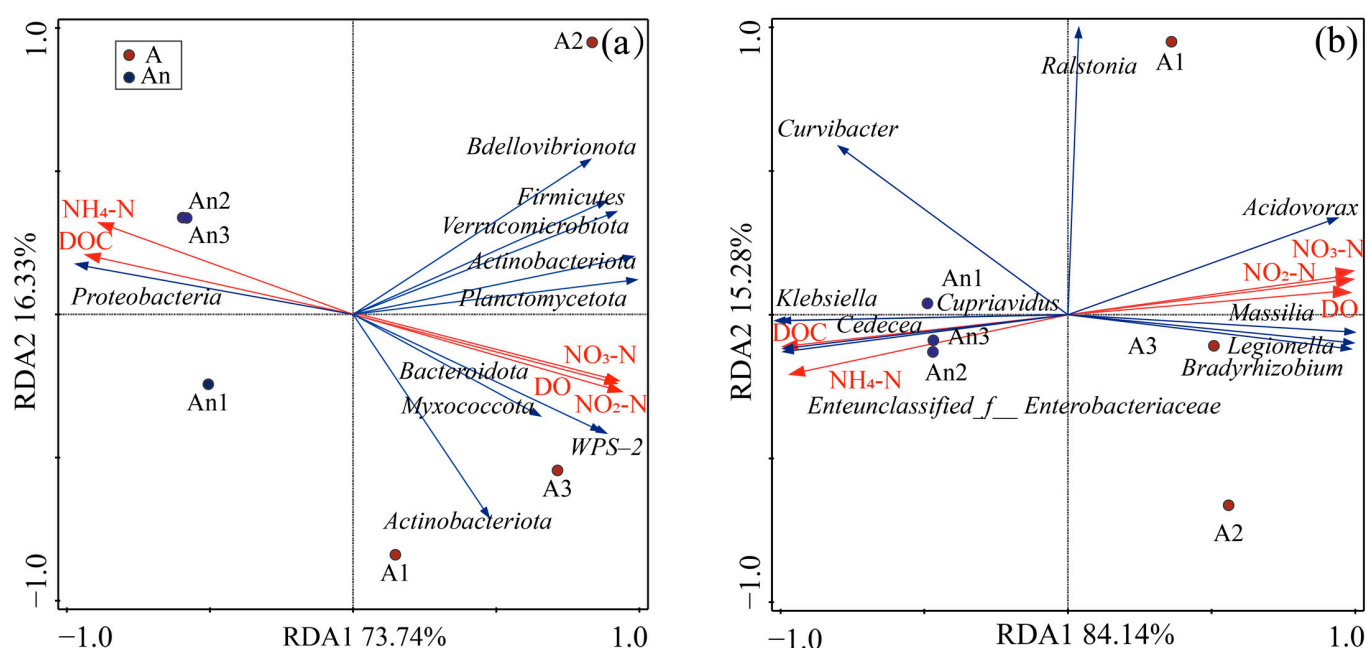


Figure 4. Redundancy analysis between groundwater hydrochemical parameters and bacterial communities on (a) phylum and (b) genus levels under different recharge conditions. A1–A3 and An1–An3 represented samples collected from the first layers of the aerobic and anaerobic columns, receptively.

The Pearson correlation analysis was also conducted to further characterize the relationships between the top 20 dominant genera and environmental factors. As shown in Figure 5, the darker the red color was, the stronger the correlation was between these environmental factors and dominant genera. Genus *Massilia*, which was unique to the aerobic recharge, was significantly positively correlated with DO, ORP, $\text{NO}_3\text{-N}$, and $\text{NO}_2\text{-N}$ ($p < 0.01$). However, in the anaerobic recharge, there was an abundance of unique genus *Cedecea* was significantly positively correlated with $\text{NH}_4\text{-N}$ and DOC ($p < 0.01$). Meanwhile, *Cupriavidus* and *Klebsiella* also had the same correlations with the environmental factors.

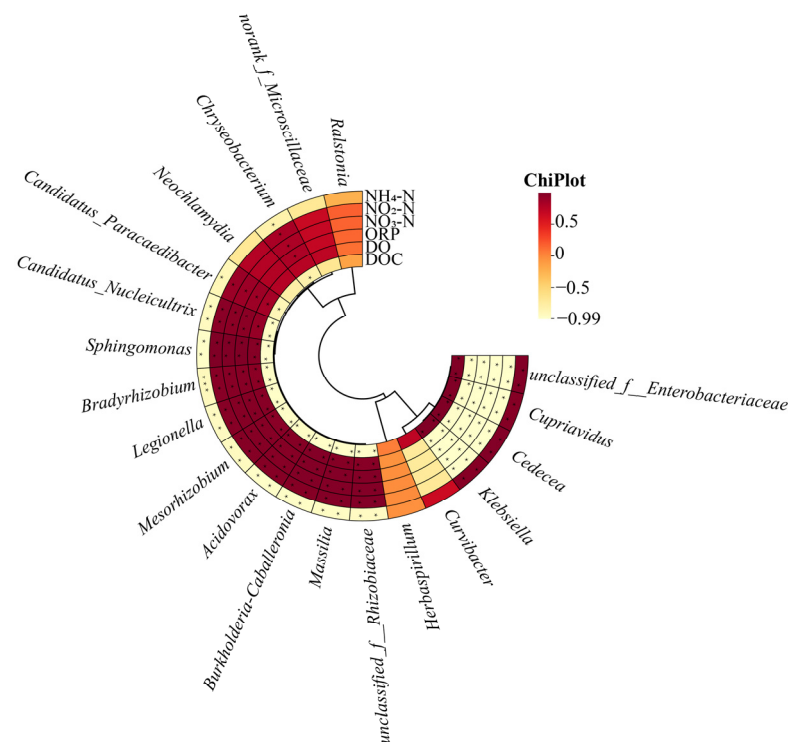


Figure 5. Pearson correlation heatmap Circos plot between environmental factors and top 20 dominant genera. Significant differences were indicated as follows: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

4. Discussion

The changes in hydrochemical indicators implied that distinct reactions occurred under different redox recharge conditions. Combining the results shown in Figure 2a,b, it was observed that although the O₂-rich recharge water (DO = 6.90 mg/L) was continuously introduced, the environment in the column was transformed to be micro-aerobic during the aerobic recharge. This might have happened due to the complex redox reactions mediated by bacteria. Meanwhile, we also observed that, compared to the aerobic recharge, the anaerobic injection caused a much more reductive environment. The decreasing concentration of NO₃-N in the initial stages of both aerobic and anaerobic recharges implied the occurrence of nitrate reduction (Figure 2d; before 168 h). Denitrification and dissimilatory nitrate reduction to ammonium (DNRA) are considered as the major nitrogen reduction pathways. Denitrification is the process of reducing NO₃-N to gaseous nitrogen compounds (e.g., NO, N₂O, and N₂), and DNRA refers to the reduction of nitrate to NH₄-N [30]. In both processes, nitrite becomes an intermediate product [31]. As detected in Figure 2b,e,f, the increasing concentrations of ORP, NO₂-N, and NH₄-N also provided evidences of possible pathways for nitrate reduction (denitrification and DNRA). In the late stage of the aerobic recharge, we deduced that nitrification might happen due to the fact that the concentration of NO₃-N increased, alongside the decreasing NH₄-N (an end-product of nitrification). Comparatively, a slight upregulation of NO₃-N was obtained in the anaerobic column at the end of the percolation experiment. It was noticed that, compared to the value of the influent (3.00 mg/L), the concentration of NO₃-N decreased during both aerobic and anaerobic recharges, implying the consumption of nitrate. In general, the variations in the concentrations of NO₃-N, NO₂-N, and NH₄-N indicated an active nitrogen cycle in the stimulated MAR processes.

The bacterial community structure and composition during different recharges were characterized using Illumina high-throughput sequencing. A total of 240,183 high-quality sequences were obtained from the collected samples, ranging from 36,754 to 43,014 per

sample (Table 2). There were obvious differences in the bacterial community under different redox conditions. On the phylum level, *Proteobacteria* was the most dominant phyla in both the aerobic and anaerobic columns, followed by *Bacteroidota* (Figure 3a). *Proteobacteria* is one of the most abundant phyla in the bacterial community in freshwater [32], playing a major role in nitrification, denitrification, and nitrogen fixation [33]. *Bacteroidota* is considered as a common bacterium in subsurface systems. It mainly exists in micro-aerobic or anoxic environments, participating in the biological denitrification process and metabolizing a variety of organic carbohydrates and proteins [34,35]. As shown in Figure 3c, *Massilia* was observed as a unique genus in the aerobic column. It is reported to be the core bacterium in the process of nitrogen degradation and plays a critical role in nitrification and aerobic denitrification [36,37]. The presence of *Massilia* provided direct evidence of the nitrification process in the aerobic column. During the anaerobic recharge, *Cedecea* and *Cupriavidus* predominated in the bacterial community. They are common denitrifiers that could reduce nitrate to nitrite [38,39]. In addition, there were also some shared species found in both columns, e.g., *Ralstonia*, *Curvibacter*, *Klebsiella*, and *Legionealla*. A previous study confirmed the existence of basic microbial communities whose main species cooperate with each other to adapt to different water environments [40]. In our study, the shared genera in both aerobic and anaerobic columns coordinated with each other in the nitrogen transformation processes. *Ralstonia* has the ability to fix nitrogen and reduce nitrate, and also to denitrify bacteria [41,42]. *Curvibacter* is a kind of microbe that can denitrify bacteria with its denitrification functional genes [43,44]. *Klebsiella* is considered as a metabolically diverse bacterium that can utilize inorganic nitrogen species (e.g., $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$), and plays a significant role in the nitrification and denitrification processes [45]. Genus *Legionealla* is a Gram-negative obligate aerobic bacterium commonly found in aquatic environments [46], which can effectively reduce nitrogen oxides [47]. By analyzing the functions of the above-mentioned bacteria in the nitrogen cycle, it deduced that active nitrogen reactions occurred during the aerobic and anaerobic recharges. The interrelationship between environmental factors and functional bacteria implied that DO, $\text{NO}_3\text{-N}$, and $\text{NO}_2\text{-N}$ promoted the growth of nitrifying bacteria, while $\text{NH}_4\text{-N}$ and DOC promoted the growth of denitrifying bacteria. It also showed that the aerobic bacteria influenced by environmental factors were related to oxygen.

5. Conclusions

In this study, bacteria-mediated hydrochemical reactions under different redox conditions during the simulated managed aquifer recharge were analyzed. The results showed that the concentration of DO during the aerobic recharge was higher than that of the anaerobic recharge, and ORP showed a similar trend. In addition, an active nitrogen cycle was found during the stimulated recharge processes. In the early stages of both aerobic and anaerobic recharges, the concentration of $\text{NO}_3\text{-N}$ rapidly decreased, alongside the increasing $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$. The dominant phyla in the aerobic recharge were *Proteobacteria*, *Bacteroidota*, *Verrucomicrobiata*, and *Actinobacteriota*. However, in the anaerobic condition, the bacterial community was mainly composed of *Proteobacteria*. On the genus level, *Massilia*, *Ralstonia*, *Legionella*, and *Curvibacter* were predominant during the aerobic recharge, while in the anaerobic recharge, *Cedecea*, *Cupriavidus*, and *Ralstonia* accounted for the main genera. The DO, $\text{NO}_3\text{-N}$, and $\text{NO}_2\text{-N}$ promoted the growth of nitrifying bacteria, while $\text{NH}_4\text{-N}$ and DOC promoted the growth of denitrifying bacteria. It also showed that the aerobic bacteria influenced by environmental factors were related to oxygen. Based on the results obtained, we deduced that in the early stages of both the aerobic and anaerobic recharges, nitrate reduction occurred due to denitrification and DNRA. However, in the late stages, nitrification might happen in the aerobic column and nitrate reduction remained the major

process in the anaerobic column. This study provides fundamental information for fully characterizing bacteria-mediated hydrochemical processes under different redox recharges.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su17010064/s1>, Figure S1: The particle size distribution curve of the sand; Figure S2: Heatmap of thirty abundant genera at the genus level. A1–A3 and An1–An3 represented samples collected from the first layers of the aerobic and anaerobic columns, respectively; Table S1: The outflow rates of percolation columns and the hydraulic residence time under the simulated aerobic MAR (A-MAR) and anaerobic MAR (An-MAR) processes.

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