Ammonium Reactive Migration Process and Functional Bacteria Response along Lateral Runoff Path under Groundwater Exploitation

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Abstract: In order to elucidate the importance of biogeochemical interactions between NH$_4^+$ and aquifer media in groundwater runoff paths, a dynamic monitoring section in the riverbank zone, which is most sensitive to environmental characteristics and perpendicular to the flow direction of the Songhua River in northeastern China, was selected for field experiments in this study. The results indicated that the NH$_4^+$ concentration decreased gradually along the groundwater runoff path under exploitation conditions. The NH$_4^+$ concentrations of J1, J2, and J3 decreased by 8%, 18%, and 22%, respectively, as compared to the starting concentration of 1.3 mg/L. Adsorption of NH$_4^+$ by aquifer media at different depths is a monolayer adsorption process in accordance with pseudo-second-order kinetic equation. The maximum reduction of NH$_4^+$ from the aquifer media from top to bottom was 76%, 67%, 56%, and 42%, respectively. The function and activity of dominant functional bacteria have characteristics of coevolution with the NH$_4^+$ transformation process. The main genera in the fluctuation zone are *Pseudomonas* (8.83%) and *Acinetobacter* (4.37%), which mainly transform NH$_4^+$ by heterotrophic nitrification–aerobic denitrification (HN–AD). The main genera in the saturated zone are *Flavobacterium* (32.60%) and *Sphingobium* (3.54%), which mainly transform NH$_4^+$ by anaerobic denitrification. The spatial variations of species and abundance for NH$_4^+$ transformation functional bacteria decrease by 2.74% and 3.47%, respectively, along groundwater runoff paths. In the vertical and horizontal directions of groundwater runoff, the percentage of adsorption in NH$_4^+$ transformation gradually decreased and the percentage of biotransformation gradually increased. The adsorption processes in the O$_2$/NO$_3^-$ reduction, Fe/Mn reduction, and SO$_4^{2-}$ reduction zones were 20.7%, 3.6%, and 1.0%, respectively. The corresponding proportions of the biotransformation process were 79.3%, 96.4%, and 99.0%. This research is critical for elucidating the bio-geochemical interaction between NH$_4^+$ and aquifer media along the course of groundwater runoff in order to offer a scientific basis for the prevention and management of groundwater nitrogen pollution.

Keywords: groundwater exploitation; ammonium; reactive migration; adsorption; functional bacteria

1. Introduction

Recent years have seen profound changes to the global nitrogen cycle as a direct result of human activity. Since the 1970s, various degrees of nitrogen pollution in surface and groundwater have been reported worldwide. Concerns about water quality have grown...
because of increased anthropic activity [1,2]. Ammonium (NH$_4^+$), one of the principal types of nitrogen, is also a typical and representative pollution component in groundwater. The pollution proportion and area are increasing year by year, which means NH$_4^+$ pollution in groundwater has been a global environmental hot issue and has been attracting wide attention [3,4]. Although NH$_4^+$ is a vital nutrient for aquatic plants, it can also lead to eutrophication and a decrease in water’s dis-solved oxygen (DO) [5,6]. NO$_3^-$ and NO$_2^-$, as the main forms of NH$_4^+$ transformation in groundwater, will lead to increased human hemoglobin denaturation and loss of oxygen transport capacity [7]. NO$_2^-$ can form stable trimer nitrosamines under the effect of nitrogenous organic compounds, which can induce digestive system diseases and pose a significant threat to human health [8]. Moreover, the accumulation of NH$_4^+$ in water may cause the water to taste unpleasant and affect the human neurological system, making it less desirable to drink [9]. Therefore, the transformation and migration of NH$_4^+$ during the extraction of groundwater for drinking water cannot be neglected.

The migration and transformation of NH$_4^+$ in groundwater is a sophisticated process that is associated with effects that are chemical, physical, and microbiological [10]. As the basic control factor of NH$_4^+$ migration and transformation in groundwater, the physical adsorption process induced by cation exchange has been recognized as an effective way to affect mobility and retention of NH$_4^+$ in aquifer media [11]. The characteristics of solid phase media, NH$_4^+$ concentration, and hydro-geochemical characteristics of the groundwater system will affect the adsorption process of NH$_4^+$ by aquifer media [12,13]. Studies have shown that porous media such as clay minerals and zeolites are effective adsorption media for NH$_4^+$ adsorption because of the great specific surface they possess and the sediment surface’s high cation exchange capacity [14]. Moreover, environmental parameters such as temperature and pH also influence the adsorption process of NH$_4^+$ by adsorption media [15,16]. However, the adsorption process and influencing factors of NH$_4^+$ by aquifer media in groundwater runoff process remain unclear, and the role of adsorption needs to be explored in NH$_4^+$ attenuation.

Subsurface transport of NH$_4^+$ can be slowed down by biological transformation and physical adsorption mechanisms [17]. On the one hand, adsorption can increase the interaction time between NH$_4^+$ and aquifer media, which is somewhat beneficial to the attenuation of NH$_4^+$. On the other hand, the migration and transformation of NH$_4^+$ are strongly dependent on microbial-mediated biogeochemical processes, and the biotransformation of NH$_4^+$ is realized through nitrification processes [18,19]. Groundwater exploitation leads to complex hydrodynamic and redox conditions, which will directly affect the groundwater runoff path, velocity, solute flux, contact time between groundwater components and aquifer media, microbial community composition, and spatial distribution [20]. The changes of the above factors have a significant influence on the bio-geochemical reaction process and, to a certain extent, on the reaction kinetics of NH$_4^+$. Ammonium nitrogen in the subsurface environment interacts with related micro-organisms. Specifically, nitrification of NH$_4^+$ is dominated by nitrifying bacteria [21,22]. At the same time, the products of processes such as nitrification and the resulting changes in the environment influence the composition and distribution of micro-organisms.

Micro-organisms, as an important medium for the biological transformation of hydrochemical components [23,24], have important symbols of the evolution process of groundwater components because of their community structure and distribution characteristics, and have become one of the hotspots of current hydrogeochemical research [25]. Studies have shown that functional core groups such as *Nitrosomonas europaea* [26], *Nitrospira* [18], *Comamonas* [27], and *Acinetobacter* [28] are gradually identified. The microbial community structure characteristics and function are of strong spatial heterogeneity in groundwater, which affects the bio-geochemical process of pollutants in groundwater [18,29]. They can illustrate the mechanism of functional groups in certain ecosystems and the characteristics of responsiveness to present and future environmental changes, which is particularly important for effectively revealing the coevolutionary relationship between the functional features
of microbial communities and the migration and transformation of NH$_4^+$. However, a new environmental shape zoning will be formed along the groundwater runoff path under the condition of groundwater exploitation. How this dynamic process affects the spatial and temporal distribution of functional micro-organisms is still unknown. Therefore, the influence mechanism of physical and chemical properties of aquifer media, redox environment, and microbial community differences on the migration and transformation of NH$_4^+$ need to be studied under the dynamic process conditions of groundwater exploitation.

This study aimed to assess the effects of adsorption and microbially driven biogeochemical processes on the reactive transport of NH$_4^+$ during groundwater exploitation. Additionally studied are the composition and distribution of functional bacteria along groundwater runoff paths. Field exploitation tests were conducted between March and April of 2018 to assess the spatial and temporal changes in concentrations of environmental factors, ammonium, and their transformation products in pore water over a 60 m experimental section. This research has centered on: (i) the spatial and temporal changes of NH$_4^+$ and its transformation products concentrations during field exploitation tests, (ii) the adsorption process and performance of aquifer media for NH$_4^+$, and (iii) the spatial distribution of functional bacteria along groundwater runoff paths and their response to NH$_4^+$ migration and the transformation process.

2. Materials and Methods

2.1. Description of the Study Site

Figure 1 depicts the location of the study site at the First Water Resource of Harbin in northeast China (45°40′ to 45°50′ N and 126°30′ to 126°40′ E). The area is most sensitive to environmental characteristics. It is perpendicular to the flow direction of Songhua River, which helps to understand the NH$_4^+$ reactive migration process and the functional bacteria response along lateral runoff paths under groundwater exploitation. The average annual temperature is 3.5 °C, while the annual average precipitation and evaporation are 505 mm and 1411 mm [30]. Figure 2b shows the result of hydro-geological drilling in the study region. Holocene Series fine- and medium-coarse sands make up the bulk of the aquifer’s lithological composition. The aquifer can be divided into 4 layers. Yellow, fine-powder sand makes up the first layer’s lithology, which extends to a depth of 1 to 6 m. The second layer is 7~15 m deep and consists primarily of gray-yellow and gray-white fine sand. The third layer is 18~35 m deep and consists of a continuous gravel bed. The fourth layer is 36~42 m thick and consists primarily of black silty and gray silty clay interspersed with gravel and coarse sand. Quaternary phreatic water is the predominant form of groundwater, with a water table ranging from 3 to 4 m.

2.2. Layout of Field Experimental Wells and Sampling Procedures

As seen in Figure 2a, at a distance of 20 m (J1), 40 m (J2), and 60 m (J3) from the Songhua River, there are three wells of 50 m in depth and 0.5 m in diameter situated. The three wells are set up in a straight line that goes along the Songhua Riverbank perpendicularly. Solid pipe was put in place 5 m below the ground. In order to reduce surface infiltration, the area between the pipe and the borehole wall is filled with bentonite or ball clay. Filter tubes were buried from 5 m to 48 m down to prevent contaminants from seeping into the groundwater. The wells were equipped with a sand-settling pipe of 2 m in length.

The field groundwater exploitation test lasted for 30 days, and the group well pumping method was used in the test. The maximum drawdown was designed to be about 12~14 m, which is 1/3 aquifer thickness. In this research, the group well yield was 1200 m$^3$/h, with an individual well output of 400 m$^3$/h. A portable water level gauge was used to measure the water level. The flow rate was measured using a vane-wheel water meter that was put at the drain. The test setting is detailed in Table 1.

A total of 23 soil samples were taken, and the unsaturated zone has a significantly different environment than the saturated zone (0~50 m depth). In the unsaturated zone (0~5 m), there were 15 sample sites with vertical spacing of 1 m, while in the saturated zone,
there were 8 sampling points with uneven spacing. The distribution of sample points is depicted in Figure 2a. Before taking soil samples, the standing plants and surface detritus were removed. Five g of soil was collected at each sampling point. Within three days, all soil samples were packaged in sterile bags and taken to the laboratory on ice. A 2 mm mesh sieving device was used to separate roots, stones, and organisms from the obtained soil samples in order to obtain representative samples with geographical and temporal features. Sieved samples were stored at $-80\,^\circ{}C$ and used for analysis of the microbial community.

Figure 1. Location of the study site.

Figure 2. (a) Hydrogeological profile diagram and in situ test well layout diagram in the study area; (b) test well structure diagram.
Table 1. Test condition setting of groundwater group wells mining.

| Experimental Wells | J1 | J2 | J3 |
|--------------------|----|----|----|
| The depth of well (m) | 49 | 49 | 49 |
| The distance off the Songhua River (m) | 20 | 40 | 60 |
| The capacity of groundwater exploration (m³/h) | 400 | 400 | 400 |
| Hydraulic conductivity (m/d) | 53.65 | | |
| Water head difference (m) | 10.04–11.91 | 8.63–10.08 | 7.91–10.08 |
| The designed drawdown (m) | 12–14 | 12–14 | 12–14 |
| The real drawdown (m) | 10.42 | 10.21 | 9.36 |
| Hydraulic gradient | 0.50–0.59 | 0.22–0.28 | 0.13–0.17 |
| Velocity (m/d) | 26.93–31.98 | 11.59–15.08 | 7.08–9.01 |

Using an automated sampler (W2BC-9600), groundwater samples were taken at the same depth (~5 m from the water table) every 24 h, and a total of 93 samples were taken. Each sampling point was sampled three times, yielding a total of 2 L of groundwater in each sample.

2.3. Analysis Methods for Groundwater Samples and Microbial Communities

In situ measurements of dissolved oxygen (DO), pH, and electrical conductivity (EC) were made with a portable water quality monitor for groundwater detection (Aquread AP-2000, Broadstairs, UK). A redox potential electrode was used to detect redox potential (ORP). An inductively coupled plasma atomic emission spectrometer (ICP-AES) was used to measure the concentrations of Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, and SO₄²⁻ ions in the water samples. By means of spectrophotometry (Perkin-Elmer Lambda 35, Arden, NC, USA), the nitrate (NO₃⁻) and ammonium (NH₄⁺) concentrations were determined. There were at least three measurements of the aquifer media and groundwater parameters for all of them. Afterwards, the average was obtained for further analysis.

We used the MoBio PowerSoil Kit (MoBio Laboratories, Carlsbad CA, USA) to extract soil DNA for microbial community analysis from 0.3 g of fresh soil samples. The kit’s manufacturer’s instructions and purity were strictly followed. A NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) was used to measure the DNA’s purity and quantity. 16S rRNA gene’s V3-V4 hypervariable region was amplified using primer pair 319F (F: TCAGCCTACGGGNGGCWGCAG) and 806R (R: GGAC TACHVGGGTATCATAATCC). Each sample was amplified using 50 L of reaction in triplicate. Table 2 details the specific reaction systems and conditions.

Table 2. Polymerase chain reaction (PCR) reaction system and conditions.

| PCR Reaction System (Kapa DNA Polymerase) | Reaction Conditions |
|------------------------------------------|---------------------|
| ddH₂O | 38.8 µL | 95 °C | 3 min |
| Buffer (10 × Taq A with Mg) | 5 µL | 95 °C | 10 s |
| dNTP | 1 µL | 50 °C | 30 s |
| DNA | 1 µL | 72 °C | 60 s |
| Primers F Mix | 2 µL | 72 °C | 30 cycles |
| Primers R Mix | 2 µL | 4 °C | 7 min |
| Polymerase | 0.2 µL | | |
| Total | 50 µL | | |

1.5% agarose gel was used to detect the PCR products after they were combined with the loading solution and electrophoresed. Further experiments used samples with a bright main strip between 400 and 450 bp [31]. The PCR mixture was purified using a GeneJET Gel Extraction Kit (Thermo Scientific, Waltham, MA, USA) by mixing the PCR products in identical density ratios. NEB Next Ultra was used to generate sequencing libraries. Following the manufacturer’s guidelines and index codes, a DNA Library Prep Kit from Illumina (NEB, Ipswich, MA, USA) was incorporated [32]. Two devices were used to evaluate library quality: the Qubit@ 2.0 Fluorometer (Thermo Scientific, Waltham, MA, USA) and
Agilent Bioanalyzer 2100 systems [33]. Illumina MiSeq Sequencers were used to produce paired-end reads of 250/300 bp from the library. The QIIME 1.8 software package organized the effective sequences into operational taxonomic units (OTUs) with 97% sequence identity. The RDP classifier was used to label each sequence’s taxonomic information using the Greengene database, with a 70% confidence level [34]. A variety of abundance-based coverage estimators (ACE), including the Chao1 alpha diversity species richness estimator, Shannon diversity index, the Simpson index, and a measure of diversity coverage, were also computed using MOTHUR for each sample in this experiment [35].

2.4. The Experiment Conditions Setting and Analysis Method for NH$_4^+$ Sorption Process

This paper mainly studies the adsorption of NH$_4^+$ by the natural soil of the aquifer due to NO$_2^-$ and NO$_3^-$ with negative charge, which was not easily adsorbed by the aquifer medium. According to the structure analysis of the aquifer in the study area, the adsorption medium was taken from the depth of 4.0~5.0 m, 9.0~10 m, 19~20 m, and 39~40 m, and the samples were labeled as L$_1$, L$_2$, L$_3$, and L$_4$, respectively. The adsorption medium used in the experiment was screened in the laboratory. Each adsorbent sample was composed of aquifer media after impurity removal and particle separation, according to the original particle gradation. The particle gradation of adsorption medium is shown in Table 3. All the experimental media were sterilized by high-pressure steam, excluding the influence of organic nitrogen mineralization, and the adsorption experiment was carried out in an anaerobic environment.

| The Depth of Aquifer Medium | Distribution of Grain Size/% |
|-----------------------------|-----------------------------|
|                            | >1 mm | 0.5~1 mm | 0.25~0.5 mm | 0.15~0.25 mm | 0.075~0.15 mm | <0.075 mm |
| L$_1$ (4~5 m)               | 0.0   | 9.3      | 14.8        | 18.6         | 32.4          | 24.9       |
| L$_2$ (9~10 m)              | 13.6  | 27.8     | 20.4        | 17.2         | 11.4          | 9.7        |
| L$_3$ (19~20 m)             | 19.3  | 31.5     | 16.3        | 16.4         | 10.0          | 6.5        |
| L$_4$ (39~40 m)             | 22.5  | 33.4     | 14.2        | 15.3         | 9.4           | 5.4        |

The adsorption capacity was evaluated as a function of contact time to analyze the kinetics of NH$_4^+$ adsorption onto an aquifer medium. The solid–liquid ratio was set at 1:10. The experimental temperature was 10 °C. NH$_4^+$ concentration was initially 10 mg/L. An oscillating water bath (TS-110 × 30, China) was used for the 72 h batch experiment. Sample measurements were taken more than 3 times, and the mean value was taken as the final measurement.

In order to determine the thermodynamic processes of NH$_4^+$ adsorption on aquifer media, the following experiments were set up. The following studies were designed to evaluate the thermodynamic mechanisms of NH$_4^+$ adsorption on aquifer media. The solid–liquid ratio was set at 1:10. The experimental temperature was 10 °C. The initial concentrations of NH$_4^+$ were, in order, 0.5, 1, 5, 10, and 30 mg/L. The solution’s pH was adjusted to neutral, and the conical flask was sealed and shaken well. Then, samples were placed in a water bath oscillator (TS-110 × 30, China) at 200 r/min for 24 h. Sample measurements were more than 3 times, and the mean value was taken as the final measurement.

The adsorption experiment was carried out in an anaerobic tank with continuous nitrogen to ensure the anaerobic environment. The kinetic and isothermal models involved in the experiments are displayed in Table 4.
Table 4. Adsorption kinetic model and isothermal adsorption model equations and parameters.

| Name                                      | Equation                                      | Key Parameters |
|-------------------------------------------|-----------------------------------------------|----------------|
| Adsorption kinetic model                  |                                              |                |
| pseudo-first-order kinetic model [36]     | \( \log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \) | \( k_1: \) constant (min\(^{-1}\)) |
|                                            |                                              | \( q_t: \) power adsorption amount (mg/g) |
|                                            |                                              | \( q_e: \) equilibrium adsorption amount (mg/g) |
|                                            |                                              | \( t: \) adsorption time (h) |
| pseudo-second-order kinetic model [36]    | \( t = \frac{1}{k_2 + q_t} + \frac{1}{q_e} \) | \( k_2: \) rate constant (g · mg\(^{-1}\) · min\(^{-1}\)) |
| Isothermal adsorption model               |                                              |                |
| Langmuir isothermal adsorption model [37] | \( \frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{K_L q_m C_e} \) | \( q_e: \) unit adsorption amount (mg/g); |
|                                            |                                              | \( C_e: \) equilibrium mass concentration (mg · L\(^{-1}\)); |
|                                            |                                              | \( q_m: \) maximum adsorption amount (mg/g); |
|                                            |                                              | \( K_L: \) Langmuir adsorption constant |
|                                            |                                              | \( K_F: \) Freundlich adsorption constant |
|                                            |                                              | \( n: \) constant |
| Freundlich isothermal adsorption model [38]| \( \log q_e = \frac{1}{n} \log C_e + \log K_F \) |                |
| Henry isothermal adsorption model [39]   | \( q_e = K_d \cdot C_e \)                     | \( k_d: \) partition coefficient |

3. Results and Discussion

3.1. Groundwater Level Variations and Hydrochemistry Analysis

The groundwater exploitation test lasted for 30 days. After establishing a steady flow net, the researchers previously found that the groundwater levels in wells J1, J2, and J3 dropped by 10.42, 10.21, and 9.36 m [40].

Due to the diverse physical, chemical, and biological features of groundwater, the redox conditions along the course of groundwater discharge would be continually changing. Within one day, river water would reach the pumping well. However, it would take longer for groundwater quality and redox conditions in the filtration zone to stabilize. Under the exploitation settings, spatial and temporal variations in ORP and DO contents (Figure 3) may provide a clear signal of general shifts in redox conditions.

The Nernst equation relates ORP to the electroactive species’ activities, suggesting that it might reflect the equilibrium between oxidized and reduced species in solution [41]. Figure 3a reveals that the raw groundwater’s ORP was low (average value 51.20 mV), indicating that reducing circumstances prevailed in the system. The ORP was significantly elevated after one day of groundwater extraction, and the conditions quickly shifted from reducing to slightly oxidizing (average value 151.20 mV). According to the three wells’ average ORPs, seepage caused a reduction in the ORP level. ORP began to vary closer to the river than farther away, demonstrating the river’s role in recharging. ORP value changed mostly as a result of changes in DO content, which were brought on by changes in the water supply from rivers and wells.

The milligram equivalent percentages (meq%) of the major ions in the groundwater samples were plotted as piper triplot, and the results are shown in Figure 4. A colored marker in the figure represents a groundwater sample. The anion of groundwater is mainly \( \text{HCO}_3^- \), and the content is 95 mg/L–423 mg/L. The second is \( \text{Cl}^- \), and the content is 20 mg/L–90 mg/L. \( \text{NO}_3^- \) content is generally 0.04 mg/L–0.05 mg/L. The cation is mainly \( \text{Ca}^{2+} \), and the content is 30 mg/L–280 mg/L; followed by \( \text{Na}^+ \) and \( \text{Mg}^{2+} \), with the contents of 11.7 mg/L–126.0 mg/L and 2.9 mg/L–60 mg/L; \( \text{NH}_4^+ \) had contents of 0.6 mg/L–2.0 mg/L. The groundwater chemical type in the study area is either \( \text{HCO}_3^-\cdot\text{Ca} \cdot \text{Na} \) or \( \text{Ca} \cdot \text{Mg} \), and the salinity is 126 mg/L–432 mg/L for low-mineralized...
freshwater. The total hardness is 43.7 mg/L–140.5 mg/L, which is medium-soft and medium-hard water. The average pH is 7.07, meaning that it is basically neutral water.

![Graph showing variations of ORP and DO in groundwater](image)

**Figure 3.** Variations of ORP (a) and DO (b) in groundwater during the pumping test.

![Piper triplot of groundwater hydrochemical type in the study area](image)

**Figure 4.** Piper triplot of groundwater hydrochemical type in the study area.

### 3.2. Temporal Variations of NH$_4^+$ and NO$_3^-$ Concentration in Groundwater during the Pumping Test

It is revealed in Figure 5a that at J1, NH$_4^+$ concentration dropped to 1.1 mg/L, a decrease of 15%, after starting the test. It took from 1 to 27 days to see an 8% decline in NH$_4^+$ concentrations from an average of 1.20 mg/L. After 28 days, NH$_4^+$ concentrations were stable at 1.0 mg/L, with a 23% decrease. Both J2 and J3 NH$_4^+$ concentrations reduced to 0.97 mg/L and 0.81 mg/L, a 25% and 38% decrease, respectively. The NH$_4^+$ concentrations ranged from 0.87 to 1.43 mg/L and from 0.81 to 1.36 mg/L, with average values of 1.13 mg/L and 1.06 mg/L, representing reductions of 13% and 18%, respectively. For 28–30 days, NH$_4^+$ concentrations remained stable at 1.06 mg/L and 1.02 mg/L before decreasing by 18% and 22%, respectively.
At the test start, concentrations in J1, J2, and J3 were 0.05, 0.13, and 0.24 mg/L. The concentration of NO₃⁻ in groundwater was less than 1 mg/L (Figure 5b). At the test start, concentrations in J1, J2, and J3 were 0.05, 0.13, and 0.24 mg/L. The concentration then ranged approximately 0.1 mg/L in the decreasing and following stable phases. J1’s NO₃⁻ concentration increased by 0.05 mg/L at the test conclusion, whereas the NO₃⁻ concentration in wells J2 and J3 declined by 0.06 and 0.19 mg/L. Overall, the average concentration of NO₃⁻ in J1, J2, and J3 were 0.13, 0.11, and 0.10 mg/L. Compared with the initial concentration, J1 increased by 160%, and J2 and J3 decreased by 15.4% and 58.3%, respectively, which indicated that the bio-geochemical process of NH₄⁺ in groundwater runoff results in the change of NO₃⁻ concentration.

3.3. Adsorption Process of Aquifer Media for NH₄⁺

An aquifer medium’s adsorption capability may also help reduce the concentration of NH₄⁺ in subsurface flow, in addition to hosting redox processes (e.g., nitrification and denitrification).

According to Figure 6, the adsorption process of NH₄⁺ in four aquifer media of different depths was conducted in three stages: rapid-reaction stage (0–0.5 h); slow-reaction stage (0.5–24 h), and adsorption equilibrium stage (after 24–48 h). At the end of the rapid-reaction stage, the unit adsorption capacities of NH₄⁺ in the aquifer medium at different depths were 0.043, 0.042, 0.021, and 0.02 mg/g, respectively, and the removal rates were 43%, 42%, 21%, and 20%, respectively. At the end of the slow-reaction stage, the unit adsorption capacity of the aquifer medium at different depths increased to 0.075, 0.065, 0.047, and 0.035 mg/g, respectively, and the removal rates were 75%, 65%, 47%, and 35%, respectively. It can be seen that the adsorption performance of different particle sizes of the aquifer medium was significantly different. The final unit adsorption capacities of
NH$_4^+$ in four aquifer media with different depths were 0.076, 0.068, 0.047, and 0.036 mg/g, respectively. The removal rates of NH$_4^+$ are 76%, 68%, 47%, and 36%, respectively. Fine sand with tiny particles and a large specific surface area dominated the fluctuation zone medium, which provided more adsorption sites for NH$_4^+$ adsorption and therefore has better adsorption performance [11]. With increasing depth, the aquifer medium gradually changed from fine sand to medium and coarse sand, and the specific surface area decreased and thus, the adsorption performance of NH$_4^+$ decreased. Therefore, the removal effect of ammonia nitrogen by different aquifers had obvious variability.

First- and second-order kinetics equations were examined to better understand the NH$_4^+$ adsorption kinetics onto an aquifer medium over time. Table 5 displays the results. As can be observed, the pseudo-second-order kinetic model’s correlation coefficient ($R^2$) was larger than the pseudo-first-order kinetic model’s. This suggested that the theoretical $q_e$ was in close agreement with the experimental $q_e$. According to the results, the aquifer medium’s NH$_4^+$ adsorption was modulated by chemisorption and the presence of functional chemical groups on the surface [36].

Table 5. Results of fitting kinetic parameters for NH$_4^+$ adsorption by aquifer medium.

| Aquifer Medium | Pseudo-First-Order Kinetic Model | Pseudo-Second-Order Kinetic Model |
|----------------|---------------------------------|----------------------------------|
|                | $q_e$(mg/g)                     | $K_1$                            | $R^2$ | $q_e$(mg/g) | $K_2$(mg/g·h) | $R^2$ |
| L$_1$(4–5 m)   | 0.065                           | 4.416                            | 0.298 | 0.068       | 105.450       | 0.879 |
| L$_2$(9–10 m)  | 0.057                           | 4.159                            | 0.479 | 0.059       | 97.710        | 0.870 |
| L$_3$(19–20 m) | 0.040                           | 1.010                            | 0.667 | 0.043       | 30.030        | 0.822 |
| L$_4$(39–40 m) | 0.031                           | 2.007                            | 0.717 | 0.033       | 23.640        | 0.867 |

The data obtained from isothermal adsorption tests were fitted to the three equations of the Langmuir model, Freundlich model, and Henry model (Figure 7), and the fitting results are shown in Table 6.

From the correlation coefficients of the fitted curves in Table 6, both the Freundlich model and the Langmuir model fit the aquifer medium in the area well ($R^2 > 0.9$). In contrast, the Langmuir model fit better than the Freundlich model for NH$_4^+$ adsorption, so it was presumed that the adsorption of NH$_4^+$ by the aquifer was via monomolecular layer adsorption. From Figure 7c, the unit adsorption capacity of media in different aquifer layers was positively correlated with NH$_4^+$ concentration. The initial concentration was 0.5 mg/L and the unit adsorption of the four aquifer mediums were 0.0029 mg/g, 0.0024, 0.0015, and 0.0012 mg/g, respectively. The removal rates of NH$_4^+$ in the four aquifer media from top to bottom were 58%, 47%, 29%, and 23%, respectively. When the starting concentration was raised to 30 mg/L, the unit adsorption increased to 0.1196, 0.0946, 0.0799, and 0.0586 mg/g, respectively, with a 40.9, 40.5, 55.2, and 50.9 times increase.
respectively. However, the removal rates decreased to 39%, 31%, 26%, and 19%, respectively. In addition, when the starting concentration was raised to 50 mg/L, the unit adsorption did not change significantly compared to 30 mg/L, indicating that the aquifer medium’s adsorption capacity was attained at this point.

First- and second-order kinetics equations were examined to better understand the NH₄⁺ adsorption kinetics onto an aquifer medium over time. Table 5 displays the results. As can be observed, the pseudo-second-order kinetic model’s correlation coefficient \( R^2 \) was larger than the pseudo-first-order kinetic model’s. This suggested that the theoretical \( q_e \) was in close agreement with the experimental \( q_e \). According to the results, the aquifer medium’s NH₄⁺ adsorption was modulated by chemisorption and the presence of functional chemical groups on the surface [36].

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To summarize, adsorption was the primary cause of NH₄⁺ decrease during exploitation of groundwater runoff, and NH₄⁺ mobility in groundwater might be slowed. As a result, a large amount of NH₄⁺ was removed from the pore water. Runoff distance slowed the migration of NH₄⁺ and reduced pollution danger.

3.4. Analysis of Microbial Community Structure and Biotransformation Process with NH₄⁺

Micro-organisms are attached to the surface of aquifer media, and groundwater has a certain degree of domestication for micro-organisms after long-term water–rock interaction. It is possible to deduce the structure and temporal–spatial distribution of microbial communities at various depths by using molecular biology techniques such as 16S rRNA gene sequencing [42]. Additionally, the correlation between the dominant bacterial species and the biotransformation process of NH₄⁺ was analyzed.
3.4.1. Analyses of Genus-Level Microbial Diversity and Community Structure

All three test wells (J1, J2, and J3) were evaluated for their microbial diversity at various depths in the fluctuation and saturation zones by utilizing the Alpha diversity index [43]. Table 7 illustrates these results. The coverage of each index was greater than 0.9, which indicated that the test outcome accurately represents the composition and spread of microbial communities in the study region [44]. To compare the differences in vertical microbial diversity in aquifer medium, the values of microbial Alpha diversity index in the aquifer medium at different depths were analyzed. Simpson average values for the three test wells were 0.98, 0.97, and 0.96 in the fluctuation zone and 0.94, 0.95, and 0.95 in the saturation zone, respectively. Both the Shannon and Simpson indexes showed a pattern of having a higher fluctuation zone than saturation zone, indicating that the species diversity of the bacterial community increased with the depth of the aquifer medium and the richer species composition [45]. According to the fluctuating patterns of the ACE, Chao, and Shannon indexes, the bacterial community in the fluctuation zone was far more diverse than that found in the saturated area.

Table 7. Alpha diversity analysis.

| Test Well | High Quality Sequence | OTUs | ACE | Simpson | Shannon | Chao1 | Dood's Coverage |
|-----------|-----------------------|------|-----|---------|---------|-------|----------------|
| Fluctuation zone (1~5 m) | | | | | | | |
| J1        | 273137                | 9000 | 24196 | 0.98    | 9.70    | 22393 | 0.91 |
| J2        | 239226                | 9025 | 22156 | 0.97    | 9.05    | 20486 | 0.92 |
| J3        | 159817                | 8862 | 18480 | 0.96    | 8.83    | 17610 | 0.94 |
| Saturation zone (5~50 m) | | | | | | | |
| J1        | 326439                | 5542 | 15843 | 0.94    | 6.57    | 13888 | 0.95 |
| J2        | 185061                | 4707 | 12280 | 0.95    | 6.92    | 10831 | 0.96 |
| J3        | 224319                | 4854 | 13228 | 0.95    | 6.69    | 11184 | 0.95 |

The average values of the Shannon indexes of micro-organisms in the aquifer medium at the sampling sites of the three test wells along the groundwater seepage direction were 9.56, 8.20, and 9.10, respectively, suggesting that the variety of micro-organisms at J1 was greater than that at J2 or J3. There were lesser values for the Chao1 and Simpson indexes in J1 than in the other two test wells, indicating a loss in the microbial community’s abundance when groundwater seepage was followed.

Figure 8 depicts the genus-level examination of the aquifer medium’s microbial community structure.

The dominant genera (relative abundance greater than 1%) in the fluctuating zone and the saturated zone were *Pseudomonas*, *Acinetobacter*, *Sphingobium*, *Sphingomonas*, *Ochrobactrum*, and *Escherichia*. The abundance of the above micro-organisms in the fluctuation zone and saturation zones was 8.83%, 4.37%, 3.48%, 3.01%, 3.58%, and 2.17%, and 5.13%, 4.53%, 3.54%, 2.42%, 2.38%, and 1.02%, respectively. There were three specific, dominant genera in the fluctuation zone bacterial community, namely *Streptomyces* (3.24%), *DA101* (1.16%), and *Streptococcus* (1.07%). The saturated zone bacterial community had eight specific dominant genera, namely, *Flavobacterium* (32.6%), *Perlucidibaca* (10.05%), *Limnobacter* (2.58%), *Aeromonas* (2.18%), *Alishevanella* (2.18%), *Cellvibrio* (1.48%), *Hydrogenophaga* (1.41%), and *Comamonas* (1.4%). It can be seen that there were more dominant genera in the saturated zone than in the fluctuation zone under genus-level analysis. However, according to the diversity analysis, bacterial communities in the fluctuation zone had a higher species richness and variety than those in the saturation zone. This indicated that although there were more species of genera in the fluctuation zone medium, the distribution of each genus was more uniform, so there were fewer dominant genera with relative abundance greater than 1%.

3.4.2. Analysis of Biotransformation of NH$_4^+$

From the results of the above analysis at the microbial genus level, it was clear that the fluctuation zone and saturated zone contained a variety of genera related to the transformation of NH$_4^+$. Different biological mechanisms involved these genera in the NH$_4^+$ transforma-
tion. Pseudomonas [46], Bacillus [47], Acinetobacter [28, 48], Paracoccus [49], Ochrobactrum [50], Vibrio [51], and Klebsiella [52] transformed NH$_4^+$ via the heterotrophic nitrification–aerobic denitrification (HN–AD). Under aerobic conditions, these heterotrophic micro-organisms were capable of simultaneous nitrification and denitrification, metabolizing various forms of nitrogen compounds. They directly transformed ammonium nitrogen to gaseous nitrogen for discharge from the system (NH$_4^+ → NH_2OH → NO_2^- → NO → N_2O → N_2$). Denitrifying bacteria of the genus Sphingobium [53], Corynebacterium [54], Novosphingobium [55], Stenotrophomonas [56], and Flavobacterium [57] transformed NH$_4^+$ through the anaerobic denitrification. Hydrogenophaga [58] removed nitrate nitrogen through the DNRA process, which reduced nitrate to ammonium nitrogen [59].

![Figure 8. Microbial community structure at the genus level in the fluctuation zone (a) and saturated (b) zone in J1, J2, and J3 (R ≥ 0.1%).](image)

From the distribution of genera involved in NH$_4^+$ transformation in Figure 9, it can be seen that the species of genera in the saturated zone and the accumulated relative abundance in each well were greater than those in the fluctuation zone. It implied that the intensity of biotransformation to NH$_4^+$ in the saturated zone will be greater than that...
in the fluctuation zone. In addition, in the fluctuation zone, the genus most involved in NH$_4^+$ transformation was *Pseudomonas*, which denitrified nitrogen by HN–AD. In the saturated zone, *Flavobacterium* was the most involved in NH$_4^+$ transformation genus, which denitrified nitrogen by anaerobic denitrification.

![Figure 9](image_url)

**Figure 9.** Distribution of microbial communities associated with NH$_4^+$ biotransformation at the genus level in fluctuation zone (a) and saturated zone (b).

The relative abundances of the microbial genera levels involved in NH$_4^+$ transformation in the test wells J1, J2, and J3 are tabulated in Table 8. The relative abundance of each genus from J1 to J2 mostly showed a decreasing trend, with the average relative abundance in wells J1, J2, and J3 being 6.84%, 4.10%, and 3.37%, respectively. This indicated that microbial biotransformation of NH$_4^+$ showed a gradual decrease in the horizontal direction of groundwater seepage. The spatial variations of species and abundance for NH$_4^+$ transformation functional bacteria decreased by 2.74% and 3.47%, respectively, along the groundwater runoff path.

The RDA analysis of microbial genera involved in NH$_4^+$ transformation with redox parameters was performed using Canoco (version 5.0), and the results are shown in Figure 10. Figure 10a,b illustrate the relationship between species of bacterial communities’ data and redox levels in fluctuation zone and saturated zone.

Figure 9a displays that the X and Y axes explained 56% and 6.42% of the variability, respectively. Microbial communities were aggregated and had positive correlations with COD, ORP, and SO$_4^{2-}$, and had negative correlations with Fe$^{2+}$, NO$_3^-$, and NH$_4^+$ concentrations. The results of the correlation analysis were consistent with the previous analysis,
which indicated that micro-organisms in the fluctuation zone mostly transformed NH$_4^+$ through the HN–AD pathway.

Table 8. Relative abundance of NH$_4^+$ transforming bacterial genera involved in test wells J1, J2, and J3.

|                | J1    | J2    | J3    |
|----------------|-------|-------|-------|
| Sphingobium    | 2.03% | 5.40% | 3.31% |
| Corynebacterium| 0.34% | 0.89% | 0.17% |
| Novosphingobium| 0.38% | 0.72% | 0.39% |
| Stenotrophomonas| 0.44% | 1.20% | 0.64% |
| Flavobacterium | 48.08%| 18.29%| 26.65%|
| Pseudomonas    | 6.64% | 11.51%| 4.98% |
| Bacillus       | 22.00%| 0.40% | 0.63% |
| Acinetobacter  | 4.63% | 5.82% | 3.02% |
| Paracoccus     | 0.33% | 0.76% | 0.08% |
| Ochrobactrum   | 2.32% | 5.62% | 1.84% |
| Klebsiella     | 0.20% | 0.58% | 0.41% |
| Hydrogenophaga | 1.53% | 1.20% | 1.44% |
| Vibrio         | 0.06% | 0.96% | 0.29% |
| Average        | 6.84% | 4.10% | 3.37% |

Figure 10. Correlation analysis of redox parameters in the fluctuation zone (a) and saturated zone (b) with micro-organisms involved in NH$_4^+$ biotransformation.

The x-axis explained 87.34% of the variance and the y-axis further explained 9.03% of the variance (Figure 10b). The microbial communities were aggregated and showed little correlation with COD, DO, and ORP, and a strong positive correlation with NO$_3^−$. This result showed that most of the micro-organisms in the saturated zone did not depend on oxidative conditions for survival and they denitrified by anaerobic denitrification.

From the previous analysis, it was clear that the direction of groundwater seepage gradually changed the subsurface redox conditions from an oxidizing to a reducing environment. That is, the redox conditions in the study site gradually changed from an oxidizing to a reducing environment with increasing offshore distance and depth. The evolution of the subsurface environment influenced the development of the bacterial community structure to some extent. Environmental differences can significantly impact microbial communities, and the microbial community structure showed some differences in composition and abundance between the fluctuation zone and saturated zone, which were mainly related to the redox conditions in the different zonation media [60]. Contaminants in groundwater can provide certain nutrients and electron acceptors for micro-organisms, which provided the necessary carbon and nitrogen sources for their growth and gradually domesticated the microbial communities [61]. The dominant genera, including Pseudomonas, Acinetobacter, Flavobacterium, and Hydrogenophaga, may be found in the fluctuation zone and saturated
zone, which were involved in NH$_4^+$ transformation through different biotransformation pathways. It indicated that the groundwater environment domesticated the microbial communities. At the same time, microbes reacted to the migration and transformation of pollutants in the groundwater.

3.5. Analysis of NH$_4^+$ Transformation Processes in the Study Site

The previous analysis showed that the redox conditions in the study site gradually shifted from oxidizing to reducing conditions in the direction of groundwater seepage, and the micro-organisms that dominated the NH$_4^+$ biotransformation also changed accordingly. The dominant micro-organisms for NH$_4^+$ transformation also evolved from Pseudomonas and Bacillus, which performed HN–AD, to Flavobacterium, which performed anaerobic denitrification. Meanwhile, the adsorption capacity of the medium for NH$_4^+$ declined gradually from top to bottom in different layers, and the percentage of the adsorption effect involved in NH$_4^+$ transformation varied among layers.

It was assumed that the decrease in groundwater NH$_4^+$ transformation in each well was caused by adsorption throughout the test. The pseudo-second-order kinetic model and the Langmuir isotherm model with a higher correlation coefficient were used to calculate the equilibrium concentration of NH$_4^+$ in each well under ideal conditions. The obtained results were compared with the actual measured concentration values to estimate the involvement of adsorption of the medium in the NH$_4^+$ transformation process during the test period in the actual case. The percentage of medium adsorption and microbial biotransformation in the total NH$_4^+$ transformation was further quantified. The results are shown in Table 9.

**Table 9.** The proportion of adsorption and biotransformation in NH$_4^+$ transformation process.

| Depth   | J1 (20 m)          | J2 (40 m)          | J3 (60 m)          |
|---------|--------------------|--------------------|--------------------|
|         | Adsorption | Biotransformation | Adsorption | Biotransformation | Adsorption | Biotransformation |
| 0–5 m   | 22.4%      | 77.6%              | 20.1%      | 79.9%              | 19.5%      | 80.5%              |
| 5–15 m  | 3.9%       | 96.1%              | 3.4%       | 96.6%              | 3.3%       | 96.7%              |
| 15–30 m | 0.5%       | 99.5%              | 0.4%       | 99.6%              | 0.4%       | 99.6%              |
| 30–49 m | 0.9%       | 99.1%              | 0.8%       | 99.2%              | 0.7%       | 99.3%              |

The percentage of adsorption tended to decrease gradually, both horizontally and vertically. In addition, biotransformation was the dominant process in all layers. There was little adsorption in the saturated zone (5–49 m), and biotransformation accounted for more than 95% of the total. When it comes to adsorption, the saturated zone medium’s increased particle size and decreased specific surface area resulted in a reduced capacity. In addition, the adsorption sites on the saturated zone medium have been occupied by other ions in the groundwater, resulting in a significant reduction in their adsorption capacity. At the same time, the saturated zone provided the necessary nitrogen and carbon sources for micro-organism growth and reproduction. In this case, the current NH$_4^+$ transformation process was formed.

In the previous research results of our research group, the underground redox zone in this area was divided. The conclusions obtained are as follows. The redox zone in the study site was divided into: O$_2$/NO$_3^-$ reduction zone from the horizontal to well J1 (20 m) and 5 m deep vertically; Fe/Mn reduction zone from the horizontal to well J2 (40 m) and 10 m deep vertically; and SO$_4^{2-}$ reduction zone from the horizontal to well J3 (60 m) and 20–40 m deep vertically [60]. On this basis, combined with previous studies, the migration and transformation process of NH$_4^+$ was refined and partitioned in this paper, as shown in Figure 11.
Figure 11. Schematic diagram of NH$_4^+$ transformation processes in the redox zone of the study site.

In the O$_2$/NO$_3^-$ reduction zone, adsorption processes accounted for 20.68% of NH$_4^+$ transformation, and biotransformation accounted for 79.32%. Pseudomonas and Bacillus were the dominant genera, transforming NH$_4^+$ through HN–AD processes. In the Fe/Mn reduction zone, the adsorption process accounted for 3.63%, and biotransformation accounted for 96.37%. Flavobacterium and Acinetobacter were the dominant genera, and HN–AD and anaerobic denitrification existed simultaneously. In the SO$_4^{2-}$ reduction zone, the adsorption process accounted for 0.98%, and biotransformation accounted for 99.02%. Flavobacterium and Hydrogenophaga were the dominant genera, and anaerobic denitrification was the main biotransformation.

4. Conclusions

In this study, the NH$_4^+$ reactive migration process and the response characteristics of functional bacteria under the groundwater exploitation condition were analyzed, and the following conclusions were obtained.

1. NH$_4^+$ concentration in test wells J1, J2, and J3 decreased by 8%, 18%, and 22%, respectively, under the groundwater exploitation. The concentration of NO$_3^-$ in J1 increased by about 0.05 mg/L and decreased by about 0.06 and 0.19 mg/L, respectively, in J2 and J3. The results indicated that biogeochemical transformation of NH$_4^+$ existed during groundwater runoff driven by exploitation.

2. According to a pseudo-second-order kinetic equation, the adsorption process was a monolayer one. The unit adsorption capacities of NH$_4^+$ in four different aquifer media with varying depths were 0.076, 0.068, 0.047, and 0.036 mg/g. The adsorption of the aquifer medium for NH$_4^+$ decreased with increasing depth in the study site.

3. The alpha diversity analysis discovered that the number of microbes in the groundwater runoff direction decreased to some degree. The main genera in the fluctuation zone were Pseudomonas (8.83%) and Acinetobacter (4.37%), which mainly transformed NH$_4^+$ by HN–AD. The main genera in the saturated zone were Flavobacterium (32.60%) and Sphingobium (3.54%), which mainly transform NH$_4^+$ by anaerobic denitrification.

4. In the vertical and horizontal groundwater runoff directions, the percentage of NH$_4^+$ adsorption gradually decreased and the percentage of biotransformation gradually increased. In the fluctuation zone and saturated zone, the difference of adsorption
was obvious. The average value of the proportion of adsorption in the fluctuation and saturated zones was 20% and 1.6%. Biotransformation was the dominant process in all layers. Especially in the saturated zone (5–49 m), the percentage was more than 95%.

5. In the $\text{O}_2/\text{NO}_3^-$ reduction zone, the proportions of adsorption and biotransformation were 20.7% and 79.3%, indicating mainly the HN–AD process to transform NH$_4^+$. In the Fe/Mn reduction zone, the proportions of adsorption and biotransformation were 3.6% and 96.4%. Both HN–AD and anaerobic denitrification were involved in the transformation of NH$_4^+$. In the SO$_4^{2-}$ reduction zone, the proportions of adsorption and biotransformation were 1.0% and 99.0%, mainly through the anaerobic denitrification process to transform NH$_4^+$.

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