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Groundwater pollution containing ammonium, iron and manganese in a riverbank filtration system: Effects of dynamic geochemical conditions and microbial responses

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Abstract

Bench-scale experiments were conducted to investigate the effect of hydraulic loadings and influent concentration on the migration and biotransformation behavior of three groundwater pollutants: ammonium (NH_4^+), iron (Fe^{2+}) and manganese (Mn^{2+}). Columns packed with aquifer media collected from a river bank filtration (RBF) site in Harbin City, NE China were introduced synthetic groundwater (SGW) or real groundwater (RGW) were at two different constant flow rates and initial contaminant concentrations to determine the impact of system conditions on the fate of the target pollutants biotransformation. The results showed that the biotransformation rate of Fe^{2+} , Mn^{2+} , and NH_4^+ decreased by 8%, 39% and 15% under high flow rate (50 L d^{-1}) compared to low flow rate (25 L d^{-1}), which was consistent with the residence-time effect. While the biotransformation rate of Fe^{2+} , Mn^{2+} , and NH_4^+ decreased by 7%, 14% and 9% under high influent concentration compared to original groundwater. The 16S rRNA analysis of the aquifer media at different depths after experiments completion demonstrated that the relative abundance of major functional microbes iron oxidizing bacteria (IOB) and manganese oxidizing bacteria (MnOB) under higher flow rate and higher influent concentration decreased 13%, 14% and 25%, 24%, respectively, whereas the ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) exhibited minimal change, compared to the lower flow rate. Above all results indicated that both high flow rate and high concentration inhibit the biotransformation of NH_4^+ , Fe^{2+} and Mn^{2+} . The biotransformation of Fe^{2+} and Mn^{2+} occurs primarily in the 0-40 cm and 20-60 cm depth intervals, respectively, whereas the NH_4^+ biotransformation appears to occur relatively uniformly throughout the whole 110cm column. The biotransformation kinetics of NH_4^+ in RGW and SGW, Mn^{2+} in RGW at different depths accords with the first order kinetics model, while Fe^{2+} in RGW and SGW, Mn^{2+} in SGW presented more complicated biotransformation process. The results

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interest.

should improve understanding of the transport and fate of common groundwater pollutants in riverbank filtration and other groundwater recharge environments.

Keywords

RBF; Groundwater; Column experiment; 16S rRNA; Microbial community response; Biotransformation kinetics model

1. INTRODUCTION

Riverbank filtration (RBF) occurs in an active biogeochemical and transitional area between surface water and groundwater, where chemicals and nutrients carried by groundwater and surface water mix and stimulate microbial activities (Fischer et al, 2005). The rapid transformation of C, N and other elements can occur in RBF system under the stimulation of high hydrodynamic loading and strong chemical gradients (Runkel et al., 2003). Iron (Fe^{2+}) and manganese (Mn^{2+}) exist widely in groundwater, especially in Northeast China (Hasan et al., 2015), which can result in aesthetic and operational problems such as undesirable color, odor, and staining of laundry when the groundwater is intended for potable use (Qin et al., 2009). In addition, ammonium (NH_4^+) can react with chlorine to form disinfection by-products, producing deteriorated taste and odor of water and damage to the human nervous system (Hasan et al., 2012). The aforementioned research results emphasize the necessity to focus on the transport and fate of NH_4^+ , Fe^{2+} and Mn^{2+} to ensure the safety and quality of drinking water. Knowledge of biotransformation processes is especially critical to the accurate prediction of contaminant transport and to conduct effective pollution remediation. However, there were few reports on simultaneous biotransformation of three target pollutants from real groundwater and still lack of study referred to the influencing factors on biotransformation performance. An understanding of the factors that influence biotransformation under the dynamic conditions associated with subsurface system was required.

In dynamic system such as RBF, solute transport and biotransformation process are coupled, and are influenced by physical properties such as hydraulic flow rate (e.g., residence time) and material heterogeneity, and by chemical factors such as initial concentration and electron-acceptor concentration (Brusseu et al., 1999; Han et al., 2013; Gouzinis et al., 1998; Hasan et al., 2015; Du et al., 2017), the delivery of electron donor and the resulting reduction/oxidation reactions are all related to groundwater concentration and flow rate. Contaminant biotransformation behavior in RBF system has been investigated in some laboratory-scale studies, which are mainly focused on the treatment technology and operating conditions to achieve efficient contaminant removal (Li et al., 2013; Tekerlekopoulou et al., 2013). These studies have also focused on single contaminants. However, the coupling of hydrodynamic transport and microbiological processes results in additional complexity for real systems wherein multiple contaminants are present. As an important process of the interaction between solute and aquifer media in water environment, adsorption affects the diffusion efflux and retention of pollutants in the aquifer, which directly or indirectly affects the biotransformation process of NH_4^+ , Fe^{2+} and Mn^{2+} and

plays an important role in the cycle of three target pollutants in the subsurface environment (Wang et al., 2000; Richard et al., 2013; Oliveira et al., 2014). The previous studies showed that the adsorption capacity of aquifer media appears to govern the rate of biologically mediated pollutants oxidation (Hou et al., 2003), and adsorption kinetic models such as first-order and second-order adsorption kinetics models were used to investigate the adsorption kinetic process. However, the adsorption mechanism of NH_4^+ , Fe^{2+} and Mn^{2+} in different regions is not completely clear due to the difference of physicochemical properties of aquifer media.

In subsurface system, the magnitude and rate of pollutant biotransformation were influenced not only by physicochemical properties but also by microbial properties (Brusseau et al., 1999; Katsoyiannis et al., 2004; Liu et al., 2017; Iii et al., 2002). The biotransformation process of three pollutants under the effect of microbial populations is one of the key issues for a long-term maintenance and stabilization of groundwater quality in situ. Characterizing the microbial community composition could improve our understanding of the underlying mechanisms of contaminant biotransformation during transport in RBF system. The biotransformation of N, Fe and Mn are tightly coupled in the subsurface environment (Chu et al., 2015; Vandenabeele, et al., 1995; Luther et al., 1997), and the principal parameters and major functional microbial species for their biotransformation have been investigated in some studies (Tekerlekopoulou et al., 2007; Ellis et al., 2000; Boano et al., 2015; Mermillod-Blondin et al., 2005; Konopka et al., 2013). Many iron oxidizing bacteria (IOB) and manganese oxidizing bacteria (MnOB) including *Pseudomonas*, *Leptothrix*, *Gallionella*, *Streptomyces*, *Toxothrix*, and *Crenothrix* have been reported (Yang et al., 2014; Tekerlekopoulou et al., 2006; Li et al., 2013; Mckee et al., 2016). Moreover, the appearance of ammonia oxidizing bacteria (AOB) (*Nitrosomonas* and *Nitrosococcus*) and nitrite oxidizing bacteria (NOB) (*Nitrospira*) demonstrated that nitrification occurred during the transformation of ammonium and nitrite, respectively (Rivett et al., 2008; Sheibley et al., 2003). However, the response of the microbial community under different hydrodynamic conditions and substrate concentration changes and the resultant impact to biotransformation of target contaminants in RBF systems remain unclear. This aspect requires more attention to be able to understand the effect of influent physicochemical characteristics on microbial communities, as well as their influence on contaminant migration and biotransformation.

In this study, a pilot-scale RBF system was established to evaluate the effect of operational factors such as varying flow rate and initial concentration. on simultaneous biotransformation of NH_4^+ , Fe^{2+} and Mn^{2+} concentration profiles along the height of RBF columns. Adsorption kinetics experiments were conducted to quantify the adsorption of the three target contaminants by the aquifer media. Based on the high throughput Illumina MiSeq sequencing analysis, phenomena about simultaneous biotransformation of NH_4^+ , Fe^{2+} and Mn^{2+} were further analyzed after approximately 34 d operation. This series of column experiments were conducted with real groundwater and aquifer media from a RBF site in the First Water Resource of Harbin without external inoculums to identify the functional microorganisms originated in the experimental system, and to systematically investigate the effect of operation factors on simultaneous biotransformation of three target pollutants.

2. MATERIALS AND METHODS

2.1 Study area

The study area is located in northwestern Harbin City, NE China, the boundary is close to the south bank of the Songhua River ($45^{\circ} 40'$ to $45^{\circ} 50'$ N and $126^{\circ} 30'$ to $126^{\circ} 40'$ E; shown in Fig.1), where the annual mean temperature is 3.5°C , and the annual average rainfall is 505 mm with annual evaporation of 1411 mm. The lithology of the aquifer is mainly fine sand and media coarse sand of the Holocene Series. There is a discontinuous silty clay aquitard layer between the upper and lower aquifers. The discontinuous nature of the aquitard allows hydraulic connection between the upper and lower aquifers, and therefore it is considered a single unified aquifer. The main type of groundwater is pores phreatic water. The depth of the aquifer ranges from 3 to 11 m, the maximum thickness is 34m, and the water level is from 3 to 4 m. The water quantity is abundant with single well inflow from 215 to 231 m^3/h , and the depth of drawdown is 1.4-4.2 m.

In order to obtain more accurate information regarding to groundwater quality and providing a reference for soil column experiment, totally 96 groundwater samples with unequal sampling interval from three wells in the study area were collected in July 2017 during pumping test. As shown in Fig.2, three pumping wells were 20, 40 and 60 m from Songhua River bank, formed a typical section perpendicular to Songhua River near the First Water Resource of Harbin. The test lasted 30 days, and groundwater samples were collected from the three wells at 0, 124, 358 min in the first day and every 24 hours for the remainder of the test. Water quality test results are shown in Fig. 3.

As can be seen from Fig.3, the concentrations of Fe^{2+} , Mn^{2+} and NH_4^+ exceeded the standard values for Class III of *Standards for groundwater quality in China* (GB/T 14848-2017), which are $\text{Fe}^{2+} \leq 0.3 \text{ mg L}^{-1}$, $\text{Mn}^{2+} \leq 0.1 \text{ mg L}^{-1}$, and $\text{NH}_4^+ \leq 1 \text{ mg L}^{-1}$. The mean concentrations of Fe^{2+} , Mn^{2+} and NH_4^+ were 5.4, 1.0, and 1.2 mg L^{-1} , respectively, consistent with the three components comprising typical regional contaminants. Thus, it is necessary to study the transformation of NH_4^+ , Fe^{2+} and Mn^{2+} in the RBF system as the main pollutants of groundwater.

Aquifer sediments were collected from three wells at a depth of 0.5~5 m below the groundwater table. The samples were subjected to physical and chemical properties analysis. The moisture content of the aquifer media was measured by weight method (CJ/T 221-2005). The pH was determined on the aqueous portion of 1:1 soil solution with an Accumet Basic AB15 pH meter (Fisher Scientific, Pittsburgh, PA, USA) using the EPA's SW-846 Method 90454D for Soil and Waste pH (USEPA, 2004). The contents of potassium, sodium, calcium, and magnesium were measured by the methods in the Chinese standard determination of total calcium, magnesium, and sodium in soil (NY/T 296-1995). Iron and manganese were identified by Tessier sequential extraction method. Nitrite, nitrate, and ammonium were measured by extraction with potassium chloride solution-spectrophotometric methods (HJ 634-2012). Sulfate was measured by gravimetric method (HJ 635-2012) and chloride was measured by silver nitrate titration method (NY/T 1121.17-2006). The results were shown in Table S1. In order to maintain the physiochemical properties of the original media and the biological activity of microbial community, the

aquifer media was stored into portable storage refrigerator filled with liquid nitrogen at -40°C immediately after collecting and transported back to the laboratory, as the filling media for subsequent column experiments.

2.2 Description of column experiments

The column experiments consisted of 3 transparent PVC columns ($L=110\text{ cm}$, $D=10\text{ cm}$), as shown in Fig. 4. To ensure that biotransformation is solely carried out by the bacterial population associated with the aquifer media, all column components were rinsed with 2% bleach and then repeatedly rinsed with high pressure deionized water. All three columns (A, B and C) were filled with aquifer media from the RBF site for 90 cm, added in increments of 4-5 cm to prevent layering in the columns. The pore volume of the packed columns was 2.5 L, bulk density was 1.4 g/cm^3 , and porosity was 0.3. Five cm of quartz sand was placed at the top of the column for better distribution of influent, and 10 cm was left void of media at the top. A 5-cm layer of gravel and quartz sand was placed at the bottom to increase air permeability and prevent clogging. Moreover, to prevent leaching of soil grains, the top and bottom of the columns were fitted with perforated PVC plates (30 holes, $d = 0.5\text{ mm}$) that were covered with filter cloth.

Regarding the setting of influencing factors, flow rate and initial pollutant concentration are the main influencing factors to be considered. It is hypothesized that altering the flow rate would impact time for sorption of target contaminants and decrease oxidation of NH_4^+ , Fe^{2+} and Mn^{2+} because these reactions are kinetically limited. Moreover, the initial target pollutants concentration would strengthen the dissolved oxygen competition and affect the transformation of NH_4^+ , Fe^{2+} and Mn^{2+} . All three columns were operated under water-saturated conditions with a constant flow from top to bottom maintained by peristaltic pumps, to simulate original aquifer conditions. The columns were fed with real groundwater (RGW) or the synthetic groundwater (SGW). RGW was extracted from the wells from a depth of 40-50 m in the study area, and the concentrations of Fe^{2+} , Mn^{2+} , NH_4^+ were 5.5-8.5, 0.9-1.3 and 0.9-1.4 mg L^{-1} , respectively. SGW was comprised by NH_4Cl , MnSO_4 and FeSO_4 . In order to synthesize high concentration experimental water under the premise of maintaining the physiochemical properties of real groundwater, 100mL deionized water was used to dissolve the weighed NH_4Cl , MnSO_4 and FeSO_4 completely and pour it into the influent tank, then test the concentration of water components to ensure the reliability of experimental results. The concentration of Fe^{2+} , Mn^{2+} and NH_4^+ was 30 mg L^{-1} , 15 mg L^{-1} and 10 mg L^{-1} , respectively. The specific components of RGW and SGW were characterized for the parameters listed in Table S2.

According to the results of the pumping test, the permeability coefficient is 65 m d^{-1} . Therefore, Column A was eluted with RGW at a constant and lower flow rate of 25 L d^{-1} based on the experiment design, and the residence time was 2.4 h. Column B was eluted with RGW at a constant and faster flow rate of 50 L d^{-1} , and the residence time was 1.2 h. Column C was eluted with SGW at a constant and lower flow rate of 25 L d^{-1} . The experiments were conducted in a temperature-controlled room ($15\pm0.5^{\circ}\text{C}$) in the dark. The experiments lasted for 34 days.

2.3 Sampling and analytical methods

There were 10 water-sampling ports located along the column at 10-cm intervals. There was a soil solution sampler immersed up to the center of the column at each sampling point. Water samples (100 mL) were collected every 24 hours from these points and from the column effluent. Fe^{2+} and Mn^{2+} were analyzed by (Pacini et al., 2005; Luther et al, 1997) inductively coupled plasma mass spectrometry (Agilent 7500ce ICP-MS, Tokyo, Japan). NO_3^- , NH_4^+ and NO_2^- (Sheibley et al., 2003) were analyzed by spectrophotometry (Perkin-Elmer Lambda 35, USA). All the determinations of the aquifer media and groundwater parameters were performed at least in triplicate, and the average values were used for further analyses. After experiment completion, sediment samples were collected along the column length at 20 cm intervals, and each layer was homogenized to obtain representative sediment samples with spatial and temporal distribution characteristic for microbial community analysis.

In order to determine the kinetics of three typical pollutants sorption process onto aquifer media, sorption capacity was investigated as a function of contact time. The ratio of solid to liquid was 1:10. The temperature was 10°C. The initial concentrations of Fe^{2+} , Mn^{2+} and NH_4^+ were 30, 15, and 10 mg L⁻¹, respectively. The batch experiment was carried out in a constant temperature water bath oscillator (TS-110X30, China) and lasted 72 h. Samples were collected at 5min, 15min, 30min, 1h, 2h, 4h, 6h, 8h, 10h, 14, 18h, 24h, 48h, and 72h. The first-order adsorption model and second-order adsorption model were as following:

$$q_t = q_e * (1 - e^{-k_1 t})$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}$$

where q_e is the equilibrium adsorption amount (mg/kg), q_t is the adsorption amount at t time (mg/kg), t is the adsorption time (h), K_1 is the first order sorption constant (L/min), K_2 is the second order sorption constant (mg/g·min).

2.4 Microbial community analysis

In order to determine the impacts of concentration change and flow rate on the spatial distribution of the microbial community, sediment samples from five depths (20cm, 40cm, 60cm, 80cm and 100cm) of three experimental columns were collected after experiments completion. Above all samples were analyzed by 16S rRNA. DNA was extracted from the column sediment using Power soil DNA Isolation Kit (MoBio Laboratories Inc, USA) (Roux et al., 2011), and the purity of DNA was examined by 1.5 % agarose gel electrophoresis and the concentration was measured with a UVeVis spectrophotometer (NanoDrop 2000, USA) (Chu et al., 2015). The V3-V4 region of 16S rRNA gene was amplified using primers 319F-806R (F: TCAGCCTACGGGNGGCWGCAG) and (R: GGACTACHVGGGTATCTAATCC) (Wang et al., 2007). The PCR amplifications and

thermal cycling were carried out for each sample using 50 μL reaction mixtures, all the specific reaction system was shown in Table S3.

The same volume of loading buffer and the PCR products were mixed, and the mixture electrophoresis was performed on 1.5% agarose gel for detection. Samples with a bright main strip between 400-450 bp were chosen for further experiments (Pruesse et al., 2012). PCR products were mixed in equidensity ratios, and the mixture of PCR products was purified using a GeneJET Gel Extraction Kit (Thermo Scientific). Sequencing libraries were generated using NEB Next Ultra. A DNA Library Prep Kit for Illumina (NEB, USA) was added following the manufacturer recommendations and index codes (Fischer et al., 2005). Library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 systems (Chu et al., 2015). Finally, the library was sequenced on an Illumina MiSeq platform and 250 bp / 300 bp paired-end reads were generated.

The effective sequences were arranged into operational taxonomic units (OTUs) at 97% sequence identity by the QIIME 1.8 software package. Representative sequences for each OUT were picked to annotate taxonomic information for each sequence using the Greengene database by RDP classifier, with a confidence threshold of 70% (Caporaso et al., 2010). Moreover, alpha diversity including the species richness estimator of Chao1, Shannon diversity index, and diversity coverage were calculated using MOTHUR for each sample.

3. RESULTS AND DISCUSSION

3.1 Adsorption kinetics result analysis

As shown in Fig.5 that the adsorption of all three pollutants exhibited typical two-stage kinetics, with approximately half of the adsorption occurring within the first hour and the remainder over 10-30 hours. The equilibrium adsorbed concentrations were 270, 87, and 76 mg/kg for Fe^{2+} , Mn^{2+} , and NH_4^+ , respectively. The respective effective K_d values are 0.41, 0.25, and 0.13 for Fe^{2+} , Mn^{2+} and NH_4^+ .

In order to clarify the adsorption kinetic characteristics of Fe^{2+} , Mn^{2+} and NH_4^+ onto aquifer media with time, first-order and second-order adsorption kinetics models were investigated, the result was shown in Table 1.

It should be noted from Table 1 that the correlation coefficients (R^2) values of second-order adsorption model were higher than first-order adsorption equation, the correlation coefficients were high, and the theoretical and experimental q_e were close to each other, which illustrated that the sorption process of three target pollutants onto aquifer media were fitted the second-order kinetic model well.

3.2 Effect of flow rate on contaminant biotransformation

The effluent concentration curves for solute transport in RGW at two flow rates, i.e., two residence times, are presented in Fig. 6a and Fig. 6b. NO_3^- , NO_2^- , NH_4^+ , Fe^{2+} and Mn^{2+} were detected in the effluent of column A and column B. The input concentration for Fe^{2+} was 6.5 mg L^{-1} for columns A and B, and the effluent concentration after one day of injection was 0.6 mg L^{-1} for column A. Thus, concentration of Fe^{2+} declined by ~90%

initially. Conversely, Mn^{2+} and NH_4^+ concentrations decreased by a factor of ~69 and ~30%, respectively. Due to the difference of redox potentials, Fe^{2+} is anticipated to undergo transformation before Mn^{2+} and NH_4^+ under the co-existence of the three pollutants (Du et al., 2017). In addition, considering the results of the adsorption experiment above, it is anticipated that adsorption of Fe^{2+} contributed to the initial decrease in concentration.

Fe^{2+} concentrations gradually increased during the first three weeks, ultimately reaching a concentration of $\sim 1.5 \text{ mg L}^{-1}$. The concentration remained relatively steady at this value for the remainder of the experiment with 77% removal rate. Similar behavior was observed for the high flow rate column B with respect to temporal changes in Fe^{2+} concentration. However, the steady-state concentration in the effluent was $\sim 2 \text{ mg L}^{-1}$, corresponding to a 69% removal rate. The higher steady-state concentration and lower removal rate observed for the higher flow-rate experiment is consistent with the shorter residence time for the higher flow-rate system. This phenomenon results from the dependency of the magnitude of biotransformation on the mean contact time between the substrate and the functional microorganism. The contact time between pollutants and microorganisms in aquifer media is shortened under the high flow rate condition, which limited the biotransformation rate and result in relatively high effluent concentration (Brusseau et al., 1999; Han et al., 2013).

The influent concentration of Mn^{2+} was 1.3 mg L^{-1} , and it decreased to 0.4 mg L^{-1} initially with 69% decrease in the effluent for column A. It increased gradually from ~ 0.4 to 1.2 mg L^{-1} during the first two weeks. It then decreased to an eventual steady value at 0.6 mg L^{-1} with 54% removal rate. Similar behavior was observed for the high flow rate column B with respect to temporal changes in Mn^{2+} concentration. The steady-state Mn^{2+} concentration from Day 30 to the experiment end was 1.1 mg L^{-1} with 15% removal rate (Fig. 4b), indicating that the removal rate of Mn^{2+} decreased by 39% due to the influence of residence time on the biotransformation of Mn^{2+} . As discussed by Berbenni et al., (2000) and Ellis et al., (2000), the attenuation of Mn^{2+} usually occurs in two ways: sorption by the sediment and oxidation by MnOB. The adsorption experiment conducted with the aquifer media showed that the K_d was 0.41, which can explain the decrease of Mn^{2+} concentration at the beginning of the experiment (Day 1-10). As the sorption capacity of aquifer media became saturated, the Mn^{2+} concentration gradually increased as observed over the first two weeks. At that point, the Mn^{2+} concentration began to decrease due to oxidation by MnOB. The rates of abiotic homogenous manganese oxidation by oxygen is very slow at pH values below 9, thus at usual pH values (6-8) encountered in groundwater, manganese cannot be oxidized. Therefore, the oxidation of Mn^{2+} is generally carried out by the MnOB (Du et al., 2017; Hasan et al., 2012).

The biotransformation phenomenon observed for Fe^{2+} and Mn^{2+} is consistent with the research results reported by McKee et al (2016). It is generally considered that microbially-mediated oxidation plays a major role in the iron and manganese biotransformation process (Katsoyiannis et al., 2004). Photographs of the aquifer media before and after the experiment illustrated that the surfaces of the aquifer media were covered by large amounts of iron and manganese oxides (Fig. S1), providing evidence of Fe and Mn oxidation.

The concentration of NH_4^+ in the influent was 1.3 mg L^{-1} , and it decreased initially to $\sim 0.9 \text{ mg L}^{-1}$ with an approximately 30% remove rate for column A after the first day of the experiment, which is substantially less than the initial decreases observed for Fe^{2+} and Mn^{2+} . The adsorption experiment showed that the K_d was 0.25, which can explain the initial decrease of NH_4^+ concentration. NH_4^+ concentrations exhibited a gradual decrease to 0.4 mg L^{-1} while NO_3^- was accumulated in the column. The increase of NO_3^- concentration (0.20 mg L^{-1}) compared with the NO_3^- concentration in the influent (0.10 mg L^{-1}) indicates that nitrification contributed to NH_4^+ transformation. The delayed biotransformation of NH_4^+ is consistent with it having the highest redox potential among the three pollutants (Gouzinis et al., 1998). The final stable effluent NH_4^+ concentration for column A 0.36 mg L^{-1} with 68% removal rate, while the stable effluent NH_4^+ concentration in column B was 0.6 mg L^{-1} with a 53% removal rate for the high flow rate experiment, consistent with the residence-time effect.

3.3 Effect of initial concentration on contaminant biotransformation

The effluent concentration curves for RGW and SGW with two initial concentrations are presented in Fig. 7a and Fig. 7b. NO_3^- , NO_2^- , NH_4^+ , Fe^{2+} and Mn^{2+} were detected in the effluent of column A and column C. The transformation of NH_4^+ , Fe^{2+} and Mn^{2+} in column A with RGW has been reported in section 3.2. For column C, the input concentration of Fe^{2+} was 30 mg L^{-1} , and the effluent concentration declined to 4 mg L^{-1} initially. The magnitude of initial decline was relatively similar to that observed for column A (~ 87 vs 90% , respectively). The concentration then increased gradually to 15 mg L^{-1} during the early stage of the experiment (0~15d). After the first two weeks, Fe^{2+} decreased during the next two weeks, ultimately attaining steady-state at 9.0 mg L^{-1} with corresponding removal rate of 70%. This removal rate is slightly lower than the 77% observed for column A with the lower pollutant concentrations in RGW.

The input concentration of Mn^{2+} was 15 mg L^{-1} for column C, and the initial effluent concentration was 13 mg L^{-1} . The initial decline in concentration for the high-input experiment was much less than observed for column A, 13% vs 69%, respectively. This may be related to the expected nonlinear adsorption of the constituents, and adsorption competition in the multi-solute system. The effluent concentration of Mn^{2+} remained steady at $\sim 13 \text{ mg L}^{-1}$ during the first three weeks, after which it decreased to $\sim 9 \text{ mg L}^{-1}$ gradually. In comparison, Mn^{2+} concentrations began to decline after two weeks for column A. This demonstrates that the Mn^{2+} biotransformation process lags significantly compared to column A. The final steady-state concentration corresponds to a removal rate of 40%, which is lower than the 54% removal rate for column A (with Mn^{2+} concentration ~ 10 times greater compared to column A). High Fe^{2+} concentration in the influent would significantly increase the oxygen consumption by the IOB, which has a negative influence on the simultaneous transformation of NH_4^+ and Mn^{2+} and result in a delay of NH_4^+ and Mn^{2+} transformation (Du et al., 2017).

The input concentration of NH_4^+ was 10 mg L^{-1} for column C, and the initial effluent concentration was 8 mg L^{-1} , equating to 20% initial removal rate. This is somewhat smaller than the 30% initial removal rate observed for the low-concentration experiment (column

A). Similar to column A, the NH_4^+ concentrations in column C gradually decreased over the course of the experiment to a steady-state 4 mg L^{-1} while NO_3^- was $1.7 \sim 2.5 \text{ mg L}^{-1}$, indicating that nitrification contributed to NH_4^+ transformation. The steady-state effluent concentration equates to a 60% removal rate, which is slightly lower than the 69% obtained for column A (with the NH_4^+ concentration increased by ~ 8 times).

Overall, the results of the transport experiments indicate that Fe^{2+} , Mn^{2+} and NH_4^+ concentrations generally exhibited relatively steady behavior by the end of the experiment. The final remove rates of NH_4^+ , Fe^{2+} and Mn^{2+} with higher influent concentration in column C were 60%, 70%, and 40%, respectively, compared to column A with 69%, 77% and 54%, illustrating that the higher initial concentration has negative influence on the simultaneous transformation of NH_4^+ , Fe^{2+} , and Mn^{2+} . According to Li et al. (2013), the theoretical oxygen demand to completely oxidize Fe^{2+} , Mn^{2+} , and NH_4^+ in raw water can be calculated as follows: $[\text{O}_2] = 0.14 [\text{Fe}^{2+}] + 0.29 [\text{Mn}^{2+}] + 4.57 [\text{NH}_4^+] = 7.98 \text{ mg L}^{-1}$. A competition mechanism for DO existed under the co-existence of the three pollutants when DO was low than 3.0 mg L^{-1} (Li et al., 2013; Luther et al., 1997; Tekerlekopoulou et al., 2006; Yang et al., 2014). The behavior of Fe^{2+} was least affected by the higher pollutant concentrations, consistent with it having the lowest redox potential. The experiment results suggest that the greater reduction in biotransformation of NH_4^+ and Mn^{2+} in column C was due to dissolved oxygen limitation. For column A experiment, the concentration of DO in the effluent was $2\sim 3 \text{ mg L}^{-1}$. Conversely, the DO concentration was consistently below 1.0 mg L^{-1} for the column C experiment, which indicates that biotransformation was constrained by insufficient DO concentration for the latter case.

3.4 Microbial Community Dynamics and Zonation

3.4.1 Analysis of vertical microbial diversity in different columns—Totally 15 aquifer media samples in three experimental columns at five different depths were defined as A1-A5, B1-B5 and C1-C5, respectively. The 16S rRNA gene libraries were constructed from pyrosequencing of A1-A5 (10228, 5357, 4080, 3280 and 7968), B1-B5 (24405, 27196, 4008, 10193 and 23037) and C1-C5 (5301, 27990, 28991, 12378 and 6750) with high quality sequence tags. By performing the alignment at a uniform length of 291 bp, the OTUs were clustered at a 3% distance (Table S4). The total number of OTUs were estimated using the Chao1 estimator, which indicated that C3 had the highest diversity (Shannon 10.9), while B4 had the lowest diversity (Shannon 6.2). The Shannon diversity indexes of the others were all above 7, except for A3, B3, and C4. These results suggest that pyrosequencing revealed bacterial communities were obviously different along the columns depths under the different experimental conditions (Roux et al., 2011).

3.4.2 Taxonomic complexity of the bacterial community—To determine the phylogenetic diversity of the bacterial communities in different depths of the three columns, qualified reads were assigned to reveal the phyla and genera (Fig.8 and Fig.9).

Proteobacteria was the dominant bacterial phylum in all three columns with relative abundance ranging from 43% $\sim$ 83% except in A1. This species was observed in prior column experiments investigating the transformation of Fe^{2+} and Mn^{2+} in groundwater

(Katsoyiannis et al., 2004). *Actinobacteria* and *Firmicutes* have been identified as MnOB (Tebo et al., 2004), and the relative abundance was 4% ~ 45% and 0.6% ~ 14%, respectively. *Acidobacteria*, as a common IOB, can take ferric iron and other high-valent metallic elements as electronic donors to participate in the biogeochemical cycle of metallic elements in the subsurface environment, and the relative abundance of 0.5% ~ 20%. *Nitrospirae*, which are involved in nitrification (Lytle et al., 2007), was found in all 15 communities with corresponding relative abundance of 0.1%-6%. *Planctomycetes* was found in all aquifer media samples with relative abundance ranging from 0.1% ~ 2%. This species participates in ammonification, nitrification and denitrification processes, which can take NO_3^- and NO_2^- as electron receptors for anaerobic ammonium oxidation (ANAMMOX) under hypoxia conditions. In addition, NO_3^- and NO_2^- can be oxidized into N_2 with organic acids decomposition by *Planctomycetes*. The remaining phylotypes were found in phyla *ODI* (0.1% ~ 2%), *TM7* (0.1% ~ 2%), *Verrucomicrobia* (0.1% ~ 6%), *Gemmatimonadetes* (0.1% ~ 4%), *Euryarchaeota* (1% ~ 6%), *Bacteroidetes* (1.5% ~ 7%), *Chloroflexi* (0.2% ~ 13%) and *Thermus* (0.1% ~ 1%).

At the genus level, the dominant population was *Pseudomonas* (Fig.9), which not only can oxidize iron, but also manganese. Other IOB were also present in columns including *Acinetobacter* (2.5%~6%), *Bacillus* (0.2% ~ 3%), *Arthrobacter* (0.1% ~ 7%) and *Gallionella* (0.2% ~ 5%). *Acinetobacter* is a typical iron and manganese oxidizing bacteria, which is closely related to the unique hydrogeological conditions of the study area. The content of iron and manganese nodules in aquifer is high in northeast China and has mineral solubility, which provides a good environment for the existence of iron and manganese oxidizing bacteria. *Bacillus* is an aerobic, endospore-forming, rod-shaped bacterium, which is commonly found in soil and water sources and can catalyze iron and manganese oxidation (Yang et al., 2014). *Arthrobacter* is an aerobic chemoheterotrophic bacterium with irregular rod-shaped structure, which can oxidize Fe^{2+} and Mn^{2+} in aerobic conditions. *Gallionella* is an obligative autotrophic bacterium with a special twisted-stalk structure and oxidizes ferrous iron to obtain energy for its growth (de Vet et al., 2011). Most of the above microorganisms were concentrated in 0-40 cm depth, which is consistent with the Fe^{2+} effluent concentration observation at 0-40 cm. Furthermore, the cumulative relative abundance of IOB in the three columns was 83%, 70% and 58%, respectively, indicating that the cumulative abundance for columns B and C decreased by 13% and 25% with respect to column A. MnOB including *Bacillus* (0.2% ~ 3%), *Arthrobacter* (0.1% ~ 7%), *Pseudomonas* (7% ~ 15%), *Corynebacterium* (0.2% ~ 1%), and *Klebsiella* (0.1% ~ 3%) were present primarily in the depth of 20-60 cm and the cumulative relative abundance in the three columns was 91%, 77% and 67%, respectively, illustrating that the cumulative abundance for columns B and C decreased by 14% and 24% with respect to column A.

The appearance of AOB (*Nitrosomonas*) and NOB (*Nitrospira*) demonstrated that nitrification and nitrification occurred in the columns to remove ammonium and nitrite, respectively (Li et al., 2013). Rivett et al. (1998) demonstrated that *Nitrospira* dominates nitrite oxidation under conditions with low NH_4^+ and NO_2^- concentrations because *Nitrospira* has both a greater affinity with ammonium. *Denitratisoma* has aerobic denitrification ability, which can directly transform NO_2^- into N_2 . Moreover, the cumulative relative abundance of AOB and NOB in the three columns was 14%, 10%, and 15%,

respectively, demonstrating that total amount of bacteria related to NH_4^+ biotransformation in all three columns exhibited minimal change. In addition, there were many other genera in the columns, such as *Sphingomonas*, *Methylophilus*, *Flavobacterium*, and *Novosphingobium* (which may oxidize organic matter), because the composition of the real groundwater was complex.

3.4.3 Response of Microbial Community—Analysis of the microbial community sequence revealed a range of taxa implicated in the biotransformation process of Fe^{2+} , Mn^{2+} and NH_4^+ . Nine species of bacteria including IOB, MnOB, AOB and NOB were characterized as shown as Fig.10. These data are used to evaluate the relative abundance of relevant bacteria as a function of depth interval.

In the surface layer (0-40 cm) of all three columns, the accumulative abundance of IOB in column A was 49.5% (*Pseudomonas* 25%, *Acinetobacter* 7%, *Bacillus* 1.5%, *Arthrobacter* 9.5% and *Gallionella* 6.5%), column B 42.5% (*Pseudomonas* 23%, *Acinetobacter* 8%, *Bacillus* 5%, *Arthrobacter* 2.5% and *Gallionella* 4%) and column C 31.5% (*Pseudomonas* 20%, *Acinetobacter* 6.5%, *Bacillus* 1%, *Arthrobacter* 3% and *Gallionella* 1%). The cumulative abundance of IOB in 0-40 cm depth (A1-A2; B1-B2; C1-C2) was obviously greater than other depths. Moreover, compared to column A, the cumulative relative abundance of IOB in column B and C decreased by 7% and 18%, respectively, indicating that high flow rate and high initial concentration inhibit the iron biotransformation.

MnOB including *Acinetobacter*, *Bacillus*, *Arthrobacter*, *Pseudomonas*, *Corynebacterium*, and *Klebsiella*. Apart from the same genus with the IOB (*Acinetobacter*, *Bacillus*, *Arthrobacter*, *Pseudomonas*), the total cumulative relative abundance of *Corynebacterium* and *Klebsiella* in three columns was 3%, 1%, and 2%, respectively, indicating manganese biotransformation potential in 0-40cm depth. NH_4^+ biotransformation mainly depended on AOB and NOB, *Nitrosomonas*, *Denitratisoma*, and *Nitrospira* were also distributed at the 0-40 cm depth. The cumulative relative abundance of the three bacteria in the three columns was 4% (*Nitrosomonas* 1%, *Denitratisoma* 1% and *Nitrospira* 2%), 4% (*Nitrosomonas* 0.5%, *Denitratisoma* 1.5% and *Nitrospira* 2%) and 6.5% (*Nitrosomonas* 1%, *Denitratisoma* 2% and *Nitrospira* 3.5%). Compared with IOB and MOB, the accumulative abundance of ammonium biotransformation bacteria was relatively low, illustrating ammonia biotransformation is not the dominant process in the 0-40 cm depth.

In the media layer (40-80 cm), the number of IOB species was 31%, 28%, and 26% in A3-A4, B3-B4, C3-C4 (*Pseudomonas* 18%, 17%, 15%; *Acinetobacter* 10%, 8%, 7%, *Arthrobacter* 0.2%, 0.3%, 2.5%; *Gallionella* 2%, 1%, 1% and *Bacillus* 0.5%, 2%, 0.5%). As can be seen from the effluent concentration that both higher influent flow rate and higher initial concentration were less conducive to iron transformation compared with the results in column A, which is consistent with the IOB relative abundance result. Compared with the depth of 0-40 cm, the cumulative relative abundance of IOB in 40-80 cm decreased 5%-20%, indicating that the iron biotransformation decreased significantly with the increase of depth. The *Corynebacterium* and *Klebsiella* have a total cumulative relative abundance of 6%, 3%, and 3% for columns A, B and C, respectively, which increased significantly compared to the 0-40cm depth. This indicated that manganese biotransformation was

focused primarily in 40-80 cm depth interval. Meanwhile, the cumulative relative abundance of *Nitrosomonas*, *Denitratisoma*, and *Nitrospira* in the three columns was 3%, 5%, and 5%, which was not significantly different from that at the 0-40cm depth, indicating that the bacteria species related to ammonium biotransformation are evenly distributed over 0-80 cm and that the biotransformation rate of NH_4^+ was relatively uniform over that interval.

In the bottom layer (80-100 cm), IOB cumulative relative abundance in the three columns was 18%, 16%, and 16%, respectively. MnOB cumulative relative abundance was 19%, 17% and 17%, respectively. The total cumulative relative abundance of AOB and NOB was 4%, 2% and 2%.

The microbial community structure, and its functional potential was related to the biotransformation of NH_4^+ , Fe^{2+} , and Mn^{2+} closely, and both the higher flow rate and high contaminant concentration inhibit the biotransformation process of three target pollutants. In turn, The microbial community influenced transformation rates and reaction products of NH_4^+ , Fe^{2+} , and Mn^{2+} . *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Gallionella*, *Corynebacterium*, *Klebsiella*, *Nitrospira*, *Nitrosomonas* and *Denitratisoma* were found in relatively significant amounts in the columns. They exhibited vertical stratification and played major roles in the biotransformation of Fe^{2+} , Mn^{2+} , and NH_4^+ .

3.5 Reaction kinetics analysis

Water samples were collected from different depths (20, 40, 60, 80, and 100 cm) for columns A and C in the last day of the experiment. The change in concentration with depth and equivalent contact time is shown in Fig. 11–13. The different sampling depths were converted into equivalent contact time according to the permeability coefficient to determine the vertical transformation rate of ammonium, iron and manganese in the stable stage under different initial concentration.

Inspection of Fig. 11a shows that the NH_4^+ concentration decreases linearly with depth. The vertical distribution of AOB and NOB including *Nitrosomonas*, *Denitratisoma*, and *Nitrospira* is relatively uniform, which is consistent with the uniform NH_4^+ biotransformation along the depth. These results indicate that NH_4^+ biotransformation occurs throughout the column length.

The contact-time data were fit with a first-order kinetics equation. The results show that $-\log(C_t/C_0)$ has a linear relationship with contact time t , and that NH_4^+ biotransformation is consistent with first-order kinetics (Fig.11b). The regression equation ($R^2 > 0.98$) for the low-concentration experiment (column A) is:

$$C_t(\text{NH}_4^+) = C_0(\text{NH}_4^+) * 10^{-0.027t} \quad (0 < t < 25\text{min})$$

The equation ($R^2 = 0.95$) for the high-concentration experiment (column C) is:

$$C_t(\text{NH}_4^+) = C_0(\text{NH}_4^+) * 10^{-0.018t} \quad (0 < t < 25\text{min})$$

The results presented in Fig. 12a show that the concentrations of Mn^{2+} declined linearly for the first 60 cm interval of the columns. However, the concentration remained constant for the deeper interval. This indicates that biotransformation of Mn^{2+} mainly occurred in the 0-60 cm interval for both influent concentration experiments. The accumulative relative abundance of MnOB including *Bacillus*, *Arthrobacter*, *Pseudomonas*, *Corynebacterium*, and *Klebsiella* was 56% and 45% in the 0-60cm depth, much greater than at other depths. This is consistent with the Mn^{2+} concentration data and suggests that they were responsible for Mn^{2+} biotransformation.

As shown in Fig. 12b, Mn^{2+} biotransformation can be represented with a first-order reaction. The regression equation ($R^2 = 0.95$) for the low-concentration experiment is as follows:

$$C_t(Mn^{2+}) = C_0(Mn^{2+}) * 10^{-0.0182t} \quad (0 < t < 25min)$$

The regression equation ($R^2 = 0.92$) for the high-concentration experiment is:

$$\log\left(\frac{C_t}{C_0}\right) = 0.0003t^2 - 0.0191t + 0.0219 \quad (0 < t < 25min)$$

The same analysis was conducted for Fe^{2+} , and the results are shown in Fig. 13. The biotransformation of Fe^{2+} occurred primarily within the 0-40 cm interval (Fig. 13a). The cumulative relative abundance of IOB including *Pseudomonas*, *Bacillus*, *Arthrobacter*, and *Gallionella* was 42% and 29% in the 0-40cm depth, much greater than at other depths. Similar to the Mn data sets, the microbial distribution results are consistent with the Fe^{2+} concentration data, indicating the IOB were responsible for the acceleration of iron biotransformation rates. It can be seen from Fig. 13b that $\log C_t/C_0$ has a polynomial relationship with time for the Fe^{2+} biotransformation, with $R^2 = 0.95$ and $R^2 = 0.98$ for columns A and C, respectively.

The regression equations for columns A and C are:

$$\log\left(\frac{C_t}{C_0}\right) = 0.0016t^2 - 0.0673t + 0.0271 \quad (0 < t < 25min)$$

$$\log\left(\frac{C_t}{C_0}\right) = 0.0018t^2 - 0.0687t - 0.0154 \quad (0 < t < 25min)$$

4. CONCLUSION

Column experiments were used to evaluate the impact of flow rate and influent concentration on the migration and biotransformation behavior of three groundwater pollutants NH_4^+ , Fe^{2+} and Mn^{2+} during transport in an aquifer media. The results indicated that the biotransformation rate of Fe^{2+} , Mn^{2+} , and NH_4^+ decreased by 8%, 39% and 15% under high flow rate (50 L d^{-1}) compared to low flow rate (25 L d^{-1}), which was consistent with the residence-time effect. While the biotransformation rate of Fe^{2+} , Mn^{2+} , and NH_4^+ decreased by 7%, 14% and 9% under high influent concentration compared to original

groundwater. The relative abundance of major functional microbes IOB and MnOB under higher flow rate and higher influent concentration decreased 13%, 14% and 25%, 24%, respectively, whereas the AOB and NOB exhibited minimal change, compared to the lower flow rate. Above all results indicated that both high flow rate and high concentration inhibit the biotransformation of NH_4^+ , Fe^{2+} and Mn^{2+} . The effects were relatively minor for Fe^{2+} compared to the other two, consistent with the former having the lowest redox potential. The spatial distribution of microbial populations along the depth of the columns revealed the stratification of the biotransformation of NH_4^+ , Fe^{2+} , and Mn^{2+} . The biotransformation kinetics of NH_4^+ in RGW and SGW, Mn^{2+} in RGW at different depths accords with the first order kinetics model, while Fe^{2+} in RGW and SGW, Mn^{2+} in SGW presented more complicated biotransformation process. The results obtained from this study could improve understanding of the coupled influence of residence time, initial concentration, and microbial properties on the biotransformation and transport of combined pollution groundwater in RBF systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCE

- Berbenni P, Pollice A, Canziani R, Stabile L, Nobili F, 2000 Removal of iron and manganese from hydrocarbon-contaminated groundwater. *Bioresource Technology*, 74(2), 109–114.
- Boano F, Harvey JW, Marion A, Packman AI, Revelli R, Ridolfi L, 2015 Hyporheic flow and transport processes: mechanisms, models, and biogeochemical implications. *Reviews of Geophysics* 52(4), 603–679.
- Brusseau ML, Hu MQ, Wang JM, Maier RM, 1999 Biodegradation during contaminant transport in porous media. 2. the influence of physicochemical factors. *Environment Science and Technology*, 33(1), 96–103.
- Brusseau ML, Xie LH, Li L, 1999 Biodegradation during contaminant transport in porous media: 1. mathematical analysis of controlling factors. *Journal of Contaminant Hydrology*, 37(3–4), 269–293.
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, 2010 QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7, 335–336. [PubMed: 20383131]
- Chu ZR, Wang K, Li XK, Zhu MT, Yang L, Zhang J, 2015 Microbial characterization of aggregates within a one-stage nitrification–anammox system using high-throughput amplicon sequencing. *Chemical Engineering Journal*, 262, 41–48.
- de Vet WW, Dinkla IJ, Rietveld LC, & van Loosdrecht MC, 2011 Biological iron oxidation by *Gallionella* spp. in drinking water production under fully aerated conditions. *Water Research*, 45(17), 5389–5398. [PubMed: 21889183]
- Du X, Liu GY, Qu FS, Li K, Shao SL, Li GB, Liang H, 2017 Removal of iron, manganese and ammonia from groundwater using a PAC-MBR system: the anti-pollution ability, microbial population and membrane fouling. *Desalination* 403, 97–106.

- Ellis D, Bouchard C, Lantagne G, 2000 Removal of iron and manganese from groundwater by oxidation and microfiltration. *Desalination* 130 (3), 255–264.
- Fischer H, Kloep F, Wilzcek S, Pusch MT, 2005 A river's liver: microbial processes within the hyporheic zone of a large lowland river. *Biogeochemistry*, 76(2), 349–371.
- Gouzinis A, Kosmidis N, Vayenas DV, Lyberatos G, 1998 Removal of Mn and simultaneous removal of NH₃, Fe from potable water using a trickling filter. *Water Research*, 32(8), 2442–2450.
- Han M, Zhao ZW, Gao W, Cui FY, 2013 Study on the factors affecting simultaneous removal of ammonia and manganese by pilot-scale biological aerated filter (BAF) for drinking water pre-treatment. *Bioresource Technology*, 145(10), 17–24. [PubMed: 23578601]
- Hasan HA, Abdullah SRS, Kamarudin SK, Kofli NT, 2015 Effective curves of completing simultaneous ammonium and manganese removal in polluted water using a biological aerated filter. *Journal of Industrial and Engineering Chemistry*, 30, 153–159.
- Hasan HA, Abdullah SRS, Kofli NT, Kamarudin SK, 2012 Effective microbes for simultaneous bio-oxidation of ammonia and manganese in biological aerated filter system. *Bioresource Technology*, 124, 355–363. [PubMed: 22995166]
- Hou LJ, Liu M, Jiang HY, Xu SY, Ou DN, Liu QM, 2003 Ammonium adsorption by tidal flat surface sediments from the Yangtze estuary. *Environmental Geology*, 45(1), 72–78.
- Iii GWL, Popp JI, 2002 Kinetics of the abiotic reduction of polymeric manganese dioxide by nitrite: an anaerobic nitrification reaction. *Aquatic Geochemistry*, 8(1), 15–36.
- Katsoyiannis IA, Zouboulis AI, 2004 Biological treatment of Mn(II) and Fe(II) containing groundwater: kinetic considerations and product characterization. *Water Research*, 38, 1922–1932. [PubMed: 15026247]
- Konopka A; Plymale AE; Carvajal DA; Lin X; McKinley JP, 2013 Environmental controls on the activity of aquifer microbial communities in the 300 Area of the Hanford Site. *Microbial Ecology*, 66 (4), 889–896. [PubMed: 24061343]
- Li XK, Chu ZR, Liu YJ, Zhu MT, Yang L, Zhang J, 2013 Molecular characterization of microbial populations in full-scale biofilters treating iron, manganese and ammonia containing groundwater in Harbin, China. *Bioresource Technology*, 147(4), 234–239. [PubMed: 23994965]
- Liu Y, Liu C, Nelson WC, Shi L, Xu F, Liu Y, 2017 Effect of water chemistry and hydrodynamics on nitrogen transformation activity and microbial community functional potential in hyporheic zone sediment columns. *Environment Science and Technology*, 51(9), 4877–4886.
- Luther GW; Sundby B; Lewis BL; Brendel PJ; Silverberg N, 1997 Interactions of manganese with the nitrogen cycle: alternative pathways to dinitrogen. *Geochim. Cosmochim. Acta*, 61 (19), 4043–4052.
- Lytle DA, Sorg TJ, Muhlen C, Wang L, Rahrig M, French K, 2007 Biological nitrification in a full-scale and pilot-scale iron removal drinking water treatment plant. *Aqua*, 56(2), 125–136.
- Mckee KP, Vance CC, Karthikeyan R, 2016 Biological manganese oxidation by *Pseudomonas putida* in trickling filters. *J. Environ. Sci. Health Part A Toxic/Hazard. Subst. Environ. Eng* 51, 523–535.
- Mermillod-Blondin F, Maucclair L, & Montuelle B, 2005 Use of slow filtration columns to assess oxygen respiration, consumption of dissolved organic carbon, nitrogen transformations, and microbial parameters in hyporheic sediments. *Water Research*, 39(9), 1687–1698. [PubMed: 15899267]
- Oliveira FMD, Marchioni C, Barros JAVDA, Lago ACD, Wisniewski Célio, Luccas PO, 2014 Assessment of cadmium and iron adsorption in sediment, employing a flow injection analysis system with on line filtration and detection by flame atomic absorption spectrometry and thermospray flame furnace atomic absorption spectrometry. *Analytica Chimica Acta*, 809, 82–87. [PubMed: 24418136]
- Pacini VA, María IA, Sanguinetti G, 2005 Removal of iron and manganese using biological roughing up flow filtration technology. *Water Research*, 39(18), 4463–4475. [PubMed: 16225901]
- Pruesse E, Peplies J, Glockner FO, 2012 SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 28 (14), 1823–1829. [PubMed: 22556368]
- Qin SY, Ma F, Huang P, Yang JX, 2009 Fe (II) and Mn (II) removal from drilled well water: a case study from a biological treatment unit in Harbin. *Desalination* 245, 183–193.

- Richard D, Bjørn Sundby, Mucci A, 2013 Kinetics of manganese adsorption, desorption, and oxidation in coastal marine sediments. *Limnology and Oceanography*, 58(3), 987–996.
- Rivett MO, Buss SR, Morgan P, Smith JW, Bemment CD, 2008 Nitrate attenuation in groundwater: a review of biogeochemical controlling processes. *Water Research*, 42(16), 4215–4232. [PubMed: 18721996]
- Roux S, Enault F, Bronner G, Debroas D, 2011 Comparison of 16S rRNA and protein-coding genes as molecular markers for assessing microbial diversity (bacteria and archaea) in ecosystems. *Fems Microbiology Ecology*, 78(3), 617–628. [PubMed: 22066608]
- Runkel RL, Mcknight DM, Rajaram H, 2003 Modeling hyporheic zone processes. *Advances in Water Resources*, 26(9), 901–905.
- Sheibley RW, Duff JH, Jackman AP, Triska FJ, 2003 Inorganic nitrogen transformations in the bed of the shingobee river, minnesota: integrating hydrologic and biological processes using sediment perfusion cores. *Limnology and Oceanography*, 48(3), 1129–1140.
- Tebo BM, Johnson HA, McCarthy JK, Templeton AS, 2005 Geomicrobiology of manganese(II) oxidation. *Trends in Microbiology*, 13(9), 421–428. [PubMed: 16054815]
- Tekerlekopoulou AG, Vayenas DV, 2007 Ammonia, iron and manganese removal from potable water using trickling filters. *Desalination*, 210(1), 225–235.
- Tekerlekopoulou AG, Pavlou S, Vayenas DV, 2013 Removal of ammonium, iron and manganese from potable water in biofiltration units: a review. *Journal of Chemical Technology and Biotechnology*, 88, 751–773.
- Tekerlekopoulou AG, Vasiliadou IA, Vayenas DV, 2006 Physico-chemical and biological iron removal from potable water. *Biochemical Engineering Journal*, 31 (1), 74–83.
- Vandenabeele J, Beer DD, Germonpré R, Sande RVD, Verstraete W, 1995 Influence of nitrate on manganese removing microbial consortia from sand filters. *Water Research*, 29(2), 579–587.
- Wang FL, Alva AK, 2000 Ammonium adsorption and desorption in sandy soils. *Soil Science Society of America Journal*, 64(5), 1669–1674.
- Wang Q, Garrity GM, Tiedje JM, Cole JR, 2007 Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, 73(16), 5261–5267. [PubMed: 17586664]
- Yang L, Li XK, Chu ZR, Ren YH, Zhang J, 2014 Distribution and genetic diversity of the microorganisms in the biofilter for the simultaneous removal of arsenic, iron and manganese from simulated groundwater. *Bioresource Technology*, 156, 384–388. [PubMed: 24507582]

HIGHLIGHTS

- The biotransformation of NH_4^+ , Fe^{2+} and Mn^{2+} has spatiotemporal characteristics.
- High flow rate and high concentration go against target pollutant biotransformation.
- Functional bacteria distribution was consistent with pollutant biotransformation.

Figure 1.
Location of the study area

Figure 2.
The schematic diagram of pumping test

Figure 3. Concentrations change of ammonium, iron and manganese in groundwater during pumping tests

Figure 4.
Schematic drawing of the column experiment system

Figure 5.

Adsorption kinetics of ammonium, iron and manganese in aquifer medium (Fe^{2+} : 30 mg L⁻¹, Mn^{2+} : mg L⁻¹ and NH_4^+ : 10 mg L⁻¹, Temperature = 10°C; the ratio of solid to liquid = 1:10; pH = 7)

Figure 6. Column experiments results at different flow rate. Column A (Fig.6a) and Column B (Fig.6b) were eluted with RGW at 25 L d⁻¹ and 50 L d⁻¹ flow rate, respectively (Influent concentrations: NH₄⁺: 1.3 mg L⁻¹, Fe²⁺: 6.5 mg L⁻¹ and Mn²⁺: 1.3 mg L⁻¹)

Figure 7.

Experimental results of column experiments at different influent concentrations. Column A (Fig.7a) was eluted with RGW at 25 L d⁻¹ (Influent concentrations: NH_4^+ : 1.3 mg L⁻¹, Fe^{2+} : 6.5 mg L⁻¹ and Mn^{2+} : 1.3 mg L⁻¹); Column C (Fig.7b) was eluted with SHGW at 25 L d⁻¹ (Influent concentrations: NH_4^+ : 10 mg L⁻¹, Fe^{2+} : 30 mg L⁻¹ and Mn^{2+} : 15 mg L⁻¹).

Figure 8. Bacterial community structure of different column aquifer medium samples at the phyla level. Phyla with low relative abundance (1%) in all fifteen libraries were classified as “others”.

Figure 9. Bacterial community structure of different column aquifer medium samples at the genus level. Genus with low relative abundance (1%) in all fifteen soil samples were classified as “others”.

Figure 10. Bacterial associated with biotransformation of NH_4^+ , Fe^{2+} , and Mn^{2+} community structure of different column soil samples at the genus level.

Figure 11.

Changes of NH_4^+ concentration at different depths (Fig. 11a) and reaction kinetics under different concentration conditions (Fig. 11b) in stable stage of experiment

Figure 12. Changes of Mn^{2+} concentration at different depths (Fig. 12a) and reaction kinetics under different concentration conditions (Fig. 12b) in stable stage of experiment

Figure 13. Changes of Fe^{2+} concentration at different depths (Fig. 13a) and reaction kinetics under different concentration conditions (Fig. 13b) in stable stage of experiment

TABLE 1.Adsorption kinetic parameters of Fe^{2+} , Mn^{2+} and NH_4^+ sorption onto aquifer media

Pollutants	First-order adsorption equation			Second-order adsorption equation		
	$q_e(\text{mg/g})$	$K_1(\text{L/min})$	R^2	$q_e(\text{mg/g})$	$K_2(\text{mg/g}\cdot\text{min})$	R^2
NH_4^+	0.065	4.416	0.298	0.068	97.456	0.879
Fe^{2+}	0.236	7.544	0.408	0.244	49.040	0.769
Mn^{2+}	0.062	1.991	0.496	0.066	42.806	0.803