Environ Earth Sci (2016) 75:84
DOI 10.1007/s12665-015-4959-5

ORIGINAL ARTICLE

Influences of microbial communities on groundwater component concentrations during managed artificial recharge

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Received: 20 April 2015 / Accepted: 24 August 2015 / Published online: 31 December 2015

Abstract Managed aquifer recharge is one of the most popular methods for dealing with local water shortage issues, and the bacterial community could be a vital factor influencing groundwater quality during this process. In this study, analysis of variations in groundwater components during artificial recharge revealed three stages at a text site in China. During stage I, total iron and dissolved organic carbon levels are stable basically, dissolved oxygen and SO_4^{2-} levels have rising trends, NO_3^- curve varied not obviously. Variation curves show increases in dissolved oxygen, NO_3^-, SO_4^{2-} and stabilization in dissolved organic carbon and total iron at stage II. During stage III, dissolved oxygen and NO_3^- have rising trends, dissolved organic carbon, total iron, and SO_4^{2-} keep stable. At 25 and 70 days the Simpson and Shannon–Wiener indices show that microbial community richness and population diversity underwent a gradual dynamic change after recharge water arrived. Correlation analysis shows that the Simpson index was mainly affected by dissolved oxygen and NO_3^-. PCR-DGGE confirmed these findings. Overall, the results revealed that the main bacterial communities reduce total nitrogen, total phosphorous, and chemical oxygen demand, which corresponded to the calculated correlation index.

Keywords Artificial recharge · Microbial diversity · Community structure · PCR-DGGE · Groundwater quality

Introduction

Over-extraction of groundwater resources has led to a range of geo-environmental problems, including cone of groundwater level depression (Qian et al. 2006; Du et al. 2013; Campos-Gaytan et al. 2014), groundwater quality deterioration (Kruawal et al. 2005; Zhai et al. 2013; Pophare et al. 2014), seawater intrusion (Vandenbohede et al. 2009; Werner et al. 2013) and land subsidence and surface fissures (Galloway and Burbey 2011; Zhang et al. 2014). Artificial recharge has become a commonly used and apparently effective method to alleviate and control groundwater-related geo-environmental problems (Du et al. 2013; Xu and Du 2014; Zhang et al. 2015). Artificial recharge also addresses the issue of water supply shortages while increasing the amount of available groundwater (Barnett et al. 2000; Bouwer 2002; Dillon 2005). However, the groundwater recharge process often results in disturbances in the hydrodynamic and hydrochemical status of groundwater, and inevitably affects microbial habitats in the recharged aquifer (Worrall and Kolpin 2004; Du 2012; Su et al. 2014a, b).

Previous studies have shown that microorganisms inhabiting aquifers are actively involved in various inorganic and organic reactions during MAR (Li et al. 2013; Alidina et al. 2014; Valhondo et al. 2015). Including the

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spatial and temporal distribution of microl community and their responses to environmental factor changes (Crump et al. 2003; Lu and Yan 2011; Xu and Huang 2011). These results demonstrate that the dominant microbial populations are more responsive to the environment they inhabit under different temperature and environmental conditions (An et al. 2012). Moreover, changes in groundwater temperature, dissolved oxygen and redox potential (Eh) strongly affect trace element water–rock interactions in the recharged aquifer during the deep groundwater recharge process (McNab et al. 2009; Shi et al. 2013; Stuyfzand 2015).

In this study, the correlations between groundwater components and microbial communities during a field scale MAR have been investigated, and polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) was used to examine microbial diversity and associated changes during artificial recharge of groundwater in the deep aquifer in. Owing to differences in the concentrations of components between recharged water and original groundwater, artificial recharge will inevitably introduce variation into the underground environment, resulting in major changes in groundwater temperature, redox conditions and water components. This will in turn influence microbial community structure and groundwater quality in the recharged aquifer.

Field conditions

The study area was at an artificial recharge test site in China that contained ten observation wells (J1–J10) and one recharge well (*R*) (Fig. 1).

The recharged aquifer, which is located in the fourth confined aquifer (Fig. 2), has weak hydraulic connections to the overlying and underlying aquifers. The aquifer is 40–50 m thick, and groundwater recharge and discharge mainly occur through lateral runoff.

In the recharged aquifer, groundwater quality reflects freshwater (salinity < 1 g/L). The recharge water is also of good quality according to the hygienic standard for drinking water, China (GB5749-2006). Because of their different environmental origins, the water quality of the recharge water and the original groundwater differs significantly, particularly their temperature, dissolved oxygen and pH. Based on the related data, the aquifer has a large burial depth, with groundwater temperature and electrical conductivity (Eh) that ranged from 20 to 23 °C and 0–110 mV, respectively, for many years. The temperature of the recharge water generally varies with atmospheric temperature, ranging from 0 to 30 °C, while the Eh ranging from 560 to 640 mV.

Materials and methods

Groundwater sample collection

Nine groundwater samples were collected and prepared for microbial analysis, including seven groundwater samples collected during the artificial recharge period, an original groundwater sample and a recharge water sample collected before artificial recharge commenced. The sampling points and collection times are shown in Table 1.

The levels of microbial biomass are low in groundwater and the detection limits of common molecular biological methods are minimal. Therefore, each groundwater sample (2.5 L) was filtered through a sterile filter membrane (pore size 0.22 μm) in the field for enrichment purposes. Filter membranes containing the enriched microbial cells were kept separately in labeled sterile centrifuge tubes or petri dishes and stored at 4 °C until analysis.

Total DNA extraction

Total genomic DNA was extracted from groundwater samples using a PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions and previously described methods (Nimnoi et al. 2010). Total DNA extracts were collected and stored at −20 °C until analysis.

The success of the DNA extraction was confirmed by agarose gel electrophoresis at 108 V for 50 min (Leite et al. 2012). Gels were post-stained, visualized and photographed using an ultraviolet transilluminator (Scion Co. Francisco USA) equipped with a Gel Smart 7.3 system (Clara Vision, Les Ulis, France).

PCR-DGGE fingerprinting

PCR amplification was conducted using an Mx3000P real-time quantitative PCR machine (Stratagene Inc., La Jolla, CA USA) with the following universal bacterial primers: forward, GC-338F (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′); reverse, 518R (5′-ATTACCGCGGCTGCTGG-3′). The PCR program was as follows: denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, elongation at 72 °C for 1 min, and then final elongation at 72 °C for 2 min. The PCR products were checked by agarose gel electrophoresis and stored at −20 °C until DGGE analysis.

Finally, the PCR products were separated by DGGE. Gels were prepared with 8 % polyacrylamide (PAGE), and the linear concentration of denaturant was 30–60 %. After

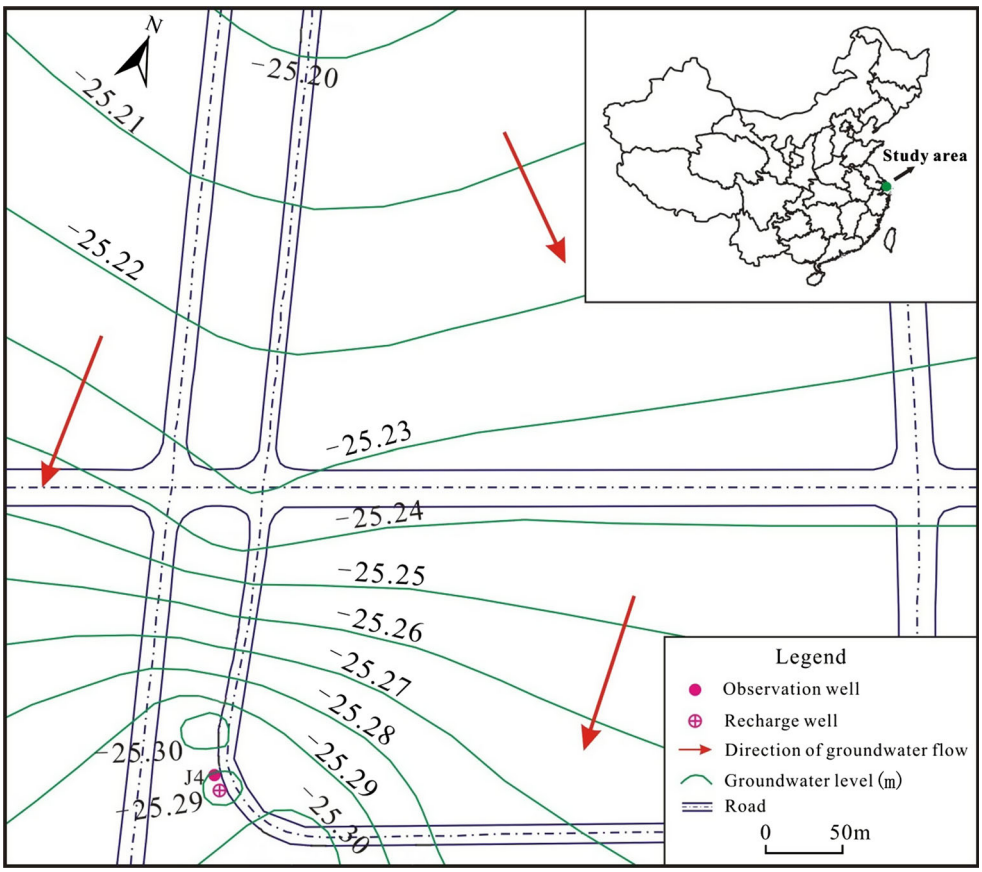

Fig. 1 Location of the recharge site and layout of drilling holes

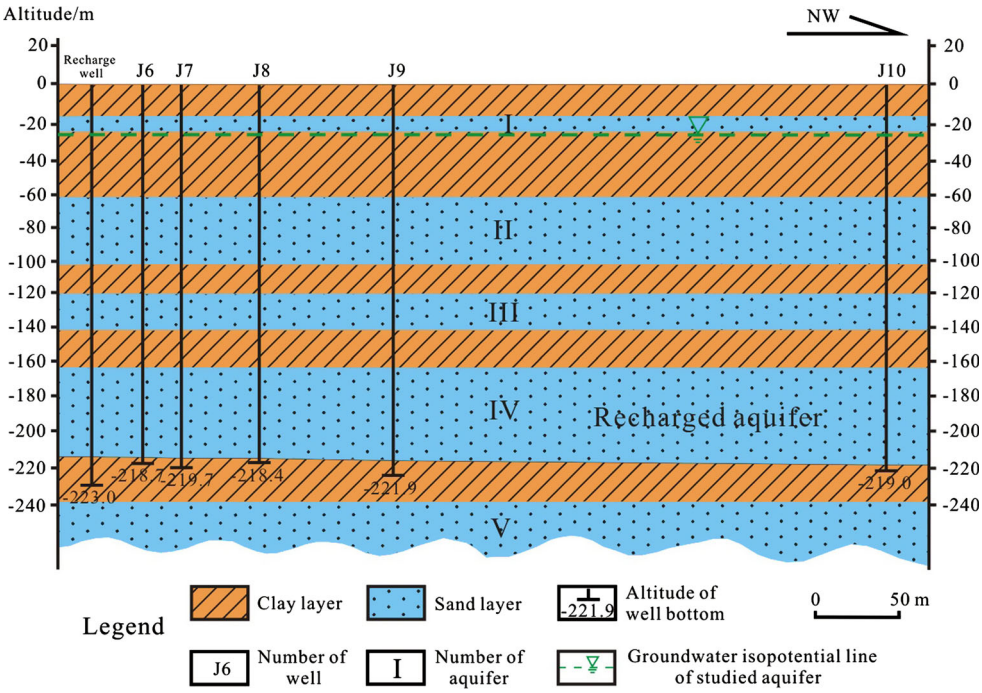

Fig. 2 Hydrogeological profile of the recharge site

complete polymerization of the PAGE gel (approx. 40 min), electrophoresis was started in a 1× Tris–acetate–EDTA buffer at a voltage of 120 V and a constant temperature of 60 °C. After 400 min of electrophoresis, DGGE fingerprints were obtained using a DCode system (BioRad Laboratories, Segrate, Italy).

Table 1 Position and time of groundwater sampling for microbial analysis

Sample No.	Position	Recharge time (h)	Recharge time (days)
S1	J4	10	0.42
S2	J4	48	2.00
S3	J4	108	4.50
S4	J4	360	15.00
S5	J4	1440	60.00
S6	J4	2928	122.00
S7	J4	4104	171.00
S8	Recharge well	384	16.00
S9	Recharge well	0	0.00

Calculation of microbial diversity indices

The number and intensity of DNA bands in the DGGE fingerprint of each sample were analyzed using Quantity One (BioRad Laboratories, USA). In addition, two α biodiversity indices, the Shannon–Wiener diversity index (H') and the Simpson dominance index (D), were calculated. The results were then used to evaluate the spatial and temporal diversity in the microbial community and associated changes in the deep confined aquifer during artificial recharge.

The H' value reflects the richness of the structure and diversity of the microbial community structure. A larger H' value indicates a richer microbial community structure and greater diversity. The D value reflects the function and status of the dominant population in the community. For the same species, a larger D value indicates higher function and status of the dominant population in the community.

The microbial diversity indices described above were calculated as follows (Shi et al. 2013):

$$H' = -\sum_{i=1}^{S} P_i \ln P_i, \quad (1)$$

$$D = 1 - \sum_{1}^{S} P_i^2, \quad (2)$$

where S is the total number of bacterial populations and P_i is the proportion of the ith bacterial population in the community.

Results and discussion

Variation in concentrations of groundwater components during artificial recharge

Typical groundwater indicators (SO_4^{2-}, NO_3^-, total iron and TOC) and an environmental factor (dissolved oxygen in this study) are presented in Fig. 3 to directly reflect the variability in groundwater components during artificial recharge. The results of observation well J4 which is 10 m away from recharge well revealed that the concentration variation could be divided into three stages.

Stage I (0–25 days after artificial recharge starts): during this stage, total iron and dissolved organic carbon levels are basically stable (<0.05, 0.6 and 1.06, 0.8 mg/L in recharge water and original groundwater, respectively). Dissolved oxygen and SO_4^{2-} levels (8.01, 50 and 1.12, 22 mg/L in recharge water and original groundwater, respectively) show increasing trends, with the same tendency as in the theoretical mixing curves. The NO_3^- curve was expected to increase, but no obvious variations were actually observed (9.5 and 0.0 mg/L in recharge water and original groundwater, respectively). This is because time is needed for the groundwater environment and oxidation capacity of different hydrochemicals in groundwater to decrease. Overall, the investigated parameters occurred in the order $O_2 > NO_3^- > Fe > SO_4^{2-}$ (Kedziorek et al. 2008; Burke et al. 2014). When the recharge water arrived with sufficient O_2, NO_3^- was not involved in oxidation–reduction reactions. Because the original groundwater environment is

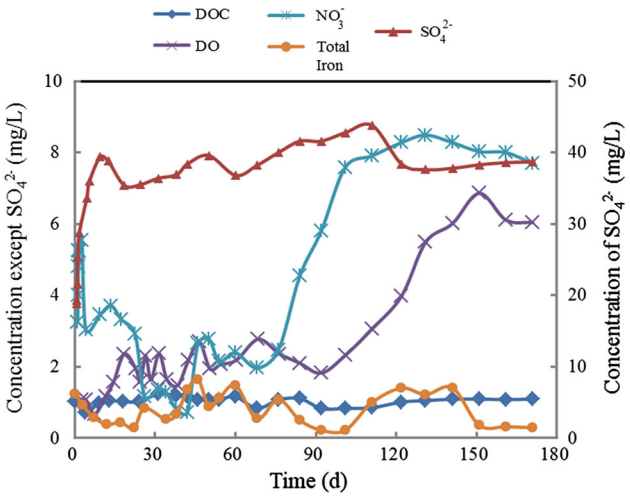

Fig. 3 Variations in groundwater hydrochemical indicators during artificial recharge in J4

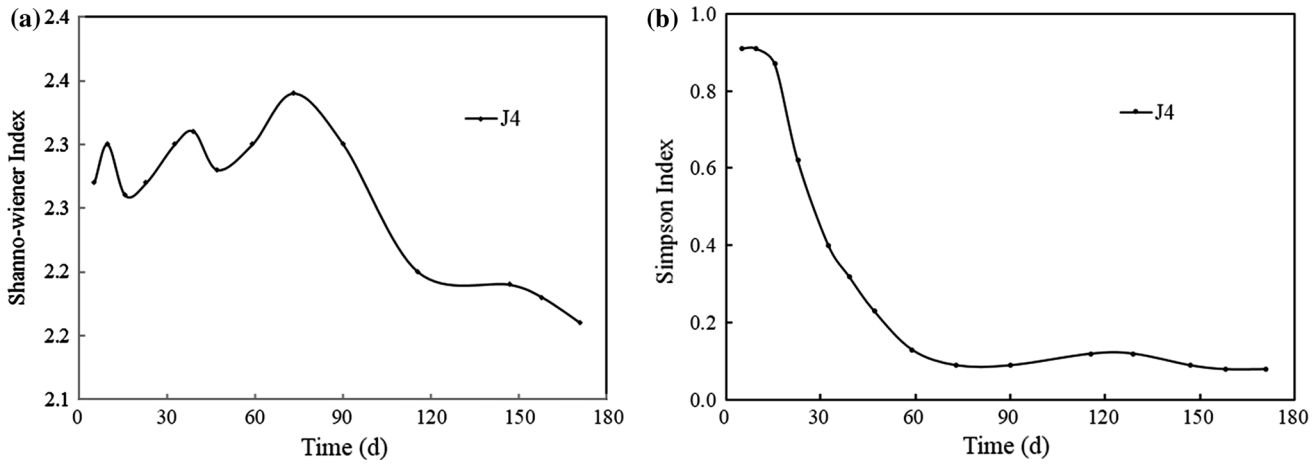

Fig. 4 Variations in the Simpson and Shannon–Wiener indices during artificial recharge

a reducing environment, changes in NO_3^- are mainly the result of mixing actions during artificial recharge.

Stage II (25–70 days after artificial recharge started): during this period, all selected indicators should remain stable when recharge water arrives at the observation wells. However, injection of recharge water causes increases in dissolved oxygen, NO_3^- and SO_4^{2-}, as well as stabilization of dissolved organic carbon and total iron. It is assumed that reactions related to dissolve organic carbon were not sufficient to induce changes in dissolved organic carbon, and that dissolved oxygen plays an active role in hydrochemical reactions, microbial reactions and microbial population changes. Research has shown that abundant dissolved oxygen actively contributes to reactions between aerobic microorganisms that consume dissolved oxygen in groundwater. Accordingly, high dissolved oxygen increases the possibility of survival of aerobic microorganisms (Terry et al. 1991). The conclusions suggest the appearance of an anaerobic microbial population. Because the dissolved oxygen level is much higher in recharge water than in groundwater (8.01 and 1.02 mg/L, respectively), dissolved oxygen tended to increase.

As explained in stage I, when there is sufficient O_2 in the environment, NO_3^- is rarely involved in oxidation–reduction reactions. Thus, changes in NO_3^- are mainly the result of mixing actions during artificial recharge. Because many hydrochemical reactions and microbial reactions that involved total iron only changed its valence, the total amount of Fe did not change. Owing to the high content of SO_4^{2-} in recharge water, injection of more recharge water led to increasing SO_4^{2-} levels and therefore more SO_4^{2-} consuming microorganisms.

Stage III (from approximately 70 days after the start of artificial recharge until the end of the present study): during this stage, dissolved organic carbon, total iron and SO_4^{2-} should remain stable, and dissolved oxygen and NO_3^- should show an increasing trend. At this stage, variation curves and theoretical curves are basically identical. However, the increasing trend of dissolved oxygen is higher in theoretical mixing curves than monitoring variation curves, and the increasing trend of NO_3^- is higher in observed variation curves than theoretical mixing curves. This is because dissolved oxygen plays an active role in the reactions, resulting in its consumption. As a result, the actual increase in dissolved oxygen is lower than the theoretical increase. Except for mixing actions, some reactions may reduce more NO_3^- than microorganisms can consume, resulting in the observed increase in NO_3^- being higher than expected.

Variation in groundwater bacterial community diversity during artificial recharge

To directly reflect the variability in groundwater microorganisms during the recharge process, microbial diversity in groundwater by calculating the H' and D values was evaluated (Fig. 4).

As the recharge time progressed, the D value in the observation wells showed an overall tendency to stabilize, decline and then re-stabilize, while the H' value did not vary obviously. Specifically, the D value varied from 0.90–0.91 to 0.09–0.10, while the H' value varied from 2.29–2.47 to 2.33–2.30, indicating that the function and status of the dominant microbial community populations in groundwater decreased after recharge water was injected, but the microbial diversities did not vary obviously. This occurred because, as the proportion of recharge water in the groundwater increased, the environment of original groundwater began to change, and microorganisms could not immediately adapt to the new environment. In addition, the previously dominant microbial community populations in groundwater decreased. However, as the recharge water was injected, microorganisms were added as well, so variations in microbial diversities were not apparent. After

Table 2 Correlation among variables during the managed aquifer recharge

	Simpson's index	Dissolved oxygen	Dissolved organic carbon	Fe	NO_3^-	SO_4^{2-}
Simpson's index	1	0.757	0.231	0.364	0.748	0.661
Dissolved oxygen		1	0.27	0.302	0.601	0.185
Dissolved organic carbon			1	0.22	0.519	0.556
Fe				1	0.269	0.016
NO_3^-					1	0.495
SO_4^{2-}						1

all the recharge water was injected into the recharged aquifer, the microbial habitat gradually stabilized, as shown by regular variations in the D and H' values.

These results indicate that, after recharge water arrived, the microbial community richness and population diversity underwent a gradual dynamic change. Plotting the variation curves of the D value as a function of time revealed three obvious stages in the entire recharge cycle (Fig. 4).

In summary, the hydrochemical composition of the original groundwater changed when the recharge water reached recharge wells, and changes in dissolved organic carbon, dissolved oxygen, NO_3^-, total iron and SO_4^{2-} influenced the groundwater quality, which was reflected in the D value. The variations in D value indicate that there are marked variations in microbial habitat. Dynamic changes in micro flora occurred after 25 days of artificial recharge for J4. After 70 days of artificial recharge for J4, the hydrochemical composition of groundwater in the recharged aquifer gradually stabilized. When the groundwater environment reached a new equilibrium, the D value also stabilized.

Correlation between groundwater components and bacterial community diversity

The principal component regression analysis function in SPSS is widely used to analyze the correlation between variable values (Liu et al. 2003). To identify the correlation between the simpson indices and elements in groundwater, principal component regression analysis was used to calculate the correlation indexes (Table 2). The results revealed that the Simpson index was highly correlated with dissolved oxygen and NO_3^-, but showed low correlation indexes with dissolved organic carbon and Fe, indicating that this index was mainly affected by dissolved oxygen and NO_3^-.

Confirmation of bacterial community by DGGE

Variations in microbial population diversity and community structure in the recharged aquifer are shown in the DGGE fingerprints of groundwater samples (Fig. 5). In the

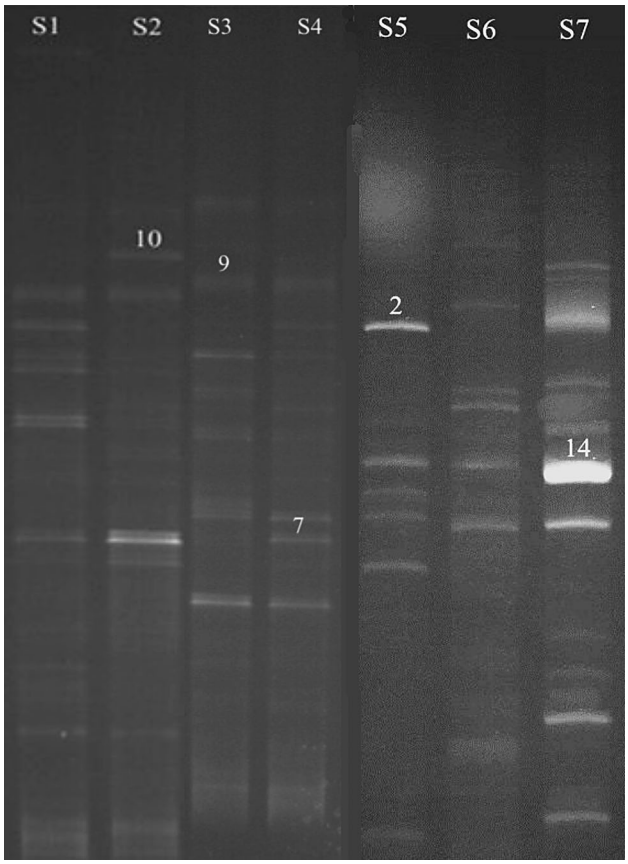

Fig. 5 PCR-DGGE fingerprints of partial bacterial 16s rDNA gene sequences

DGGE fingerprint, the number and intensity of the DNA bands reflect the number of microbial species and the abundance of bacterial species in groundwater, respectively. As the number of total DNA bands increases, the number of microbial species present in groundwater also increases, with a higher intensity of a specific DNA band indicating higher abundance of the corresponding bacterial species (Nimnoi et al. 2010; Delgado et al. 2013; Kushida 2013).

The results showed that the composition and abundance of bacterial species in groundwater varied between sampling points and between time intervals at the same sampling

Table 3 Sequencing results of selected DNA bands from the bacterial PCR-DGGE fingerprint

Bacterial species	Most closely related species in the GenBank database	Metabolic functions	Species and genus classification	Sequence identity (%)	Band No.	Detection position
a	*Acinetobacter calcoaceticus*	Reduces total nitrogen, total phosphorous, and chemical oxygen demand	I	95	1	Recharge well
b	*Rubrivivax gelatinosus*	Mainly reduces nitrate		97	2	J4
f	*Alicycliphilus denitrificans*	Primarily degrades nitrate and chlorate in the environment		96	7	J4
h	*Cyanobacterium aponinum*	Uses oxygen to produce bio-hydrogen		96	9	J4
d	*Thermoanaerobacter siderophilus*	Consumes Fe(III) and accumulates Fe(II) in the environment		98	14	J4
i	*Methylomonas methanica*	Unknown	II	92	10	J4

I indicates different bacterial species of the same genus and II indicates the same bacterial species

points. These findings demonstrate that continuous injection of recharge water leads to variations in microbial diversity and community structure of the original groundwater.

To identify the bacterial species showing the greatest homology with test sequences retrieved from groundwater, 15 DNA nucleotide sequences were selected after DGGE separation and PCR re-amplification and compared with available sequences of known bacteria deposited in the GenBank database using the BLAST tool. Generally, bacteria with 16S rDNA sequence similarities of less than 95 % are considered to be different genera, whereas those with 16S rDNA sequence similarity less than 98 % are considered to be different species (Kushida 2013; Sun et al. 2013). The 15 groups of nucleotide sequences of the 16S rDNA genes detected in groundwater were compared with closely related bacteria (Table 3). As shown in the table, the main bacterial communities reduce total nitrogen, total phosphorous and chemical oxygen demand, which corresponded to the correlation index that calculated based on the SPSS method.

Conclusions

In this study, variations in groundwater components concentrations were analyzed during artificial recharge. The results showed that the variations could be divided into three stages at 25 and 70 days. Specifically, analysis of the groundwater bacterial community diversity based on the Simpson and Shannon–Wiener indices during artificial recharge showed that, after recharge water arrived, the microbial community richness and population diversity underwent a gradual dynamic change. Correlation analysis conducted demonstrated that the Simpson index had high correlation indexes with dissolved oxygen and NO_3^-, but low correlation indexes with dissolved organic carbon and Fe. These findings indicate that the Simpson index was mainly affected by dissolved oxygen and NO_3^-, which was confirmed by PCR-DGGE. Moreover, the main bacterial communities reduce total nitrogen, total phosphorous and chemical oxygen demand, which were reflected in the calculated correlation indexes.

Acknowledgments This work was supported by the National Natural Science Foundation of China (41103045, 41472215). The authors are grateful for the support provided by the "985 Project" of Jilin University and the China Scholarship Council. We are also thankful to the staff at the Shanghai Institute of Geological Survey for their assistance in the field.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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