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Microbial Community Structure of Arsenic-Bearing Groundwater Environment in the Riverbank Filtration Zone

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Abstract: Arsenic (As) contamination of groundwater is a global public health problem. Microorganisms have a great effect on the migration and transformation of arsenic. Studying the effect of microbial community structure and function on arsenic release in the groundwater environment of the riverbank filtration zone has important theoretical and practical significance. In this paper, in-situ monitoring technology and molecular biology technology were used to study the microbial community in the process of river water infiltration in the Shenyang Huangjia water source, China. The results showed that the structure, diversity and abundance of the microbial community in groundwater were closely related to the arsenic content. Proteobacteria was the dominant phylum in the study area, and Acinetobacter, Pseudomonas, Sulfuritalea, Sphingomonas and Hydrogenophaga etc. were the main dominant bacterial genera. In addition to reducing and oxidizing arsenic, these functional microorganisms also actively participated in the biogeochemical cycle of elements such as iron, manganese, nitrogen and sulfur. There was a significant correlation between dominant bacteria and environmental factors. Fe/Mn had a significant positive correlation with As, which brought potential danger to the water supply in high iron and manganese areas.

Keywords: microbial community structure; arsenic-bearing groundwater environment; riverbank filtration zone

1. Introduction

Driven by riverside exploitation, strong interactions occur between river water and groundwater [1–3]. The riverbank filtration zone, as the key zone of interaction, affects the migration and transformation of pollutants in the process of river water infiltration [4–7]. Arsenic is a typical toxic carcinogen. Long-term drinking of arsenic-bearing groundwater will do great harm to human health, which can lead to neurasthenia, cardiovascular disease, liver cancer, kidney cancer and so on [8–10]. In view of the high risk of arsenic, the World Health Organization (WHO), the European Union and many countries (including China and the United States) stipulate that the concentration of arsenic in drinking water should not exceed 10 µg/L [11,12]. Once arsenic contamination occurs, the impact on human beings is very serious [13–16].

The formation of arsenic-bearing groundwater is the result of a series of water-rock interactions, hydrogeochemistry and geological microorganisms. The release mechanisms
are mainly as follows: desorption, reductive dissolution of arsenic-bearing minerals, oxidation of arsenic-bearing minerals and the direct reduction of arsenic [17–22]. There are mainly two forms of arsenic in nature, inorganic arsenic and organic arsenic, and its main valence states are As$^{3+}$ and As$^{5+}$. These two valence states of arsenic are the main existing forms in groundwater and soil. Arsenic mainly exists in the form of arsenite or arsenate in groundwater, and As$^{3+}$ has stronger mobility and biological toxicity than As$^{5+}$ [23–25]. Most of these studies are based on traditional hydrogeochemical or mineralogical methods. With the continuous deepening of research, scholars found that microorganisms affect the geochemical process of arsenic in groundwater [26–28]. Microorganisms with an arsenic metabolism function can directly promote the redox and methylation of arsenic, which affects the migration and transformation of arsenic [29–31]. Indigenous iron-reducing bacteria can promote the reduction of iron hydroxides and release the adsorbed arsenic [32–34]. The reduced sulfur produced by microbial reduction can form arsenic-bearing sulfide precipitation with dissolved arsenic in groundwater. If the arsenic–sulfur complex is formed in this process, it will promote the migration of arsenic in groundwater, because it is not easy to be adsorbed [35]. Microbial activities in the groundwater system, especially the microbial-mediated cycling and metabolism of elements such as iron and manganese, affect the migration and transformation of arsenic in the arsenic-bearing groundwater environment [36–39].

Previous studies have shown that arsenic enrichment during riverbank filtration is closely related to microorganisms, and most of them focus on the effect of a certain bacterial species on arsenic. However, the effects and potential mechanisms of microbial community structure and function on arsenic release in the groundwater environment of the riverbank filtration zone are still unclear. Therefore, an in-depth study on the microbial community composition and functional genes of the arsenic-containing groundwater environment is of great scientific significance for understanding the arsenic release and the formation of arsenic-bearing groundwater under the influence of microorganisms.

Shenyang Huangjia water source is located in the north of Shenyang City, China. The content of iron, manganese and arsenic in groundwater exceeds the standard seriously [40]. At present, the source and cause of arsenic in the groundwater of this water source are not completely clear. Therefore, this paper uses in-situ monitoring technology and molecular biology technology to analyze the microbial community structure characteristics of the arsenic-bearing groundwater environment in the riverbank filtration zone, and it reveals the impact of the microbial community on the biogeochemical process of arsenic in groundwater. This study can deeply understand the formation law of groundwater chemical composition and reveal the formation of arsenic-bearing groundwater under the influence of microorganisms, which is of great significance to the monitoring and protection of groundwater.

2. Materials and Methods

2.1. Study Area Description

The study area is located in the Shenyang Huangjia water source, and the Liao River flows from east to west in the north of the study area (Figure 1). Groundwater is stored in an unconsolidated phreatic Quaternary aquifer, with a buried depth of 1–4 m. The aquifer is about 50 m thick and is mainly composed of fine sand, medium coarse sand and gravel. Twelve mining wells are arranged along the river, with a total mining volume of 30,000 m$^3$/d. The groundwater receives lateral recharge from the Liao River all the year round.
2.2. Sampling and Testing

Along the groundwater flow direction, five monitoring wells (numbered W1, W2, W3, W4 and W5) were set up at 1.5 m, 4 m, 6.5 m, 12.5 m and 17 m away from the south bank of the Liao River, while these five wells were each screened to 10.0 m below the ground surface. The river water sampling point W0 was arranged about 6 m away from the south bank. We carried out sample collection in August and collected five groundwater samples and one river water sample. Before collecting groundwater samples, at least 3–5-times the volume of the monitoring well pipe was extracted, and a portable water quality analyzer (HQ40d, Hach Company, Loveland, CO, USA) was used to measure the groundwater temperature (T), pH, oxidation-reduction potential (ORP), dissolved oxygen (DO) and
other indicators on site. Samples should have been taken after the readings were stable. The river water samples were collected about 20 cm below the water surface. The collected water samples were sealed with sealing film without headspace after adding a protective agent according to the preservation requirements [41].

Fe$^{2+}$ and Mn$^{2+}$ were measured in situ by a spectrophotometer (DR2800, Hach Company, Loveland, CO, USA). Conventional anions and cations were determined by ion chromatography (881 Compact IC, Metrohm AG, Herisau, Switzerland), and HCO$_3^-$ was determined by acid–base titration. After the water samples were filtered by a GF/F membrane (1825-047 Whatman Glass Microfiber Filters, Cytiva Company, Buckinghamshire, UK), the DOC was determined by a TOC instrument (TOC-L CPH CN200, Shimadzu Corporation, Kyoto, Japan). As was determined by inductively coupled plasma mass spectrometer (7500C ICP-MS, Agilent Technologies, Santa Clara, CA, USA). The above tests were completed in the Key Laboratory of Groundwater Resources and Environment, Ministry of Education, Jilin University. The microbial community composition and arsenic functional genes were analyzed by 16S rRNA high-throughput sequencing, which was completed by Beijing Allwegene Technology Co., Ltd., Beijing, China.

3. Results and Discussion

3.1. Hydrochemical Characteristics

The pH of the river water in the study area was 8.17 (Table 1). The pH of groundwater ranged from 7.16 to 7.52, which was weakly alkaline on the whole, and it gradually decreased along the groundwater flow direction (Figure 2). The ORP and DO content of the river water were 35.02 mV and 9.34 mg/L, respectively, indicating that it was in a state of oxidation. The ORP of groundwater ranged from $-135.23$ mV to $-11.54$ mV, which decreased to $-135.23$ mV at 17 m away from the riverbank, showing strong reducibility. Along the groundwater flow direction, the ORP and DO content gradually decreased, reflecting that the infiltrated river water experienced a transition from an oxidation environment to a reduction environment. The DOC content in river water was relatively high. Along the groundwater flow direction, the DOC content in groundwater gradually decreased, reflecting the strong redox reaction between DOC and electron acceptors in groundwater.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>W0</th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
<th>W5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance to the riverbank (m)</td>
<td>$-6$</td>
<td>1.5</td>
<td>4</td>
<td>6.5</td>
<td>12.5</td>
<td>17</td>
</tr>
<tr>
<td>T ($^\circ$C)</td>
<td>22.24</td>
<td>14.71</td>
<td>13.17</td>
<td>12.21</td>
<td>10.94</td>
<td>10.49</td>
</tr>
<tr>
<td>pH</td>
<td>8.17</td>
<td>7.52</td>
<td>7.36</td>
<td>7.33</td>
<td>7.27</td>
<td>7.16</td>
</tr>
<tr>
<td>ORP (mV)</td>
<td>35.02</td>
<td>$-11.54$</td>
<td>$-69.88$</td>
<td>$-94.45$</td>
<td>$-118.79$</td>
<td>$-135.23$</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>9.34</td>
<td>7.56</td>
<td>2.07</td>
<td>2.11</td>
<td>1.93</td>
<td>1.90</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>18.76</td>
<td>9.79</td>
<td>9.34</td>
<td>8.16</td>
<td>7.27</td>
<td>6.69</td>
</tr>
<tr>
<td>K$^+$ (mg/L)</td>
<td>6.32</td>
<td>5.17</td>
<td>3.39</td>
<td>3.41</td>
<td>3.75</td>
<td>8.31</td>
</tr>
<tr>
<td>Na$^+$ (mg/L)</td>
<td>31.27</td>
<td>49.66</td>
<td>43.87</td>
<td>39.85</td>
<td>32.37</td>
<td>30.00</td>
</tr>
<tr>
<td>Ca$^{2+}$ (mg/L)</td>
<td>50.46</td>
<td>91.96</td>
<td>87.42</td>
<td>101.46</td>
<td>116.97</td>
<td>129.05</td>
</tr>
<tr>
<td>Mg$^{2+}$ (mg/L)</td>
<td>20.90</td>
<td>28.17</td>
<td>27.18</td>
<td>32.64</td>
<td>40.43</td>
<td>109.35</td>
</tr>
<tr>
<td>Cl$^-$ (mg/L)</td>
<td>41.80</td>
<td>56.56</td>
<td>57.26</td>
<td>60.11</td>
<td>69.78</td>
<td>27.57</td>
</tr>
<tr>
<td>HCO$_3^-$ (mg/L)</td>
<td>169.00</td>
<td>378.41</td>
<td>348.80</td>
<td>431.06</td>
<td>480.42</td>
<td>957.55</td>
</tr>
<tr>
<td>NO$_3^-$ (mg/L)</td>
<td>7.69</td>
<td>2.36</td>
<td>1.77</td>
<td>0.59</td>
<td>0.33</td>
<td>0.30</td>
</tr>
<tr>
<td>SO$_4^{2-}$ (mg/L)</td>
<td>75.50</td>
<td>68.63</td>
<td>68.55</td>
<td>61.01</td>
<td>62.58</td>
<td>54.79</td>
</tr>
<tr>
<td>As (µg/L)</td>
<td>2.21</td>
<td>37.04</td>
<td>40.69</td>
<td>46.95</td>
<td>48.80</td>
<td>51.34</td>
</tr>
<tr>
<td>Fe$^{2+}$ (mg/L)</td>
<td>0.11</td>
<td>13.35</td>
<td>17.02</td>
<td>19.93</td>
<td>21.89</td>
<td>25.93</td>
</tr>
<tr>
<td>Mn$^{2+}$ (mg/L)</td>
<td>0.14</td>
<td>2.00</td>
<td>5.42</td>
<td>6.14</td>
<td>7.56</td>
<td>7.42</td>
</tr>
</tbody>
</table>
The hydrochemical type of river water was a Ca-Mg-Na-HCO\textsubscript{3}-SO\textsubscript{4}-Cl type. The hydrochemical type of groundwater was mainly a Ca-Mg-HCO\textsubscript{3} type, and the SO\textsubscript{4}\textsuperscript{2-} content decreased, which was affected by reduction (Figure 3). Along the groundwater flow direction, the SO\textsubscript{4}\textsuperscript{2-} content decreased from 75.50 mg/L at the river water to 54.79 mg/L at W5, showing the consumption of SO\textsubscript{4}\textsuperscript{2-} in groundwater, indicating the gradual enhancement of SO\textsubscript{4}\textsuperscript{2-} reduction, pointing to the possible activity of sulfate-reducing bacteria in groundwater. As a sensitive redox component, the NO\textsubscript{3}\textsuperscript{-} content gradually decreased to 0.30 mg/L along the groundwater flow direction, reflecting the strong denitrification effect of NO\textsubscript{3}\textsuperscript{-} as an electron acceptor.

Figure 2. Variations in hydrochemical parameters along the groundwater flow direction.
direction, the $\text{SO}_4^{2-}$ content decreased from 75.50 mg/L at the river water to 54.79 mg/L at W5, showing the consumption of $\text{SO}_4^{2-}$ in groundwater, indicating the gradual enhancement of $\text{SO}_4^{2-}$ reduction, pointing to the possible activity of sulfate-reducing bacteria in groundwater. As a sensitive redox component, the $\text{NO}_3^-$ content gradually decreased to 0.30 mg/L along the groundwater flow direction, reflecting the strong denitrification effect of $\text{NO}_3^-$ as an electron acceptor.

![Figure 3. Piper diagram of river water and groundwater samples.](image)

The content of $\text{Fe}^{2+}$ and $\text{Mn}^{2+}$ in the river water was low, both being less than 0.2 mg/L. Along the groundwater flow direction, the content of $\text{Fe}^{2+}$ and $\text{Mn}^{2+}$ in groundwater gradually increased. At W5, the content increased to 25.93 mg/L and 7.42 mg/L, respectively, indicating that the reductive dissolution of iron and manganese minerals occurred in the gradually reducing environment, which increased the content of $\text{Fe}^{2+}$ and $\text{Mn}^{2+}$ in groundwater. Similarly, the As content in the river water was low, which was far below the 10.0 μg/L set by the WHO for water quality standards, indicating that the river water may not be polluted by arsenic. Along the groundwater flow direction, the As content in groundwater gradually increased. At W5, the As content increased to 51.34 μg/L, which was about 25-times the As content in river water. At the initial stage of river infiltration, some arsenic-bearing minerals were oxidized and dissolved to release As. With the transformation of groundwater to the reducing environment, Fe/Mn minerals underwent reductive dissolution, and As adsorbed on the mineral surface, and the lattice was released.

As was significantly positively correlated with $\text{Fe}^{2+}$ and $\text{Mn}^{2+}$ and negatively correlated with $\text{NO}_3^-$ and $\text{SO}_4^{2-}$ (Table 2). $\text{NO}_3^-$ and $\text{SO}_4^{2-}$ also had a negative correlation with $\text{Fe}^{2+}$ and $\text{Mn}^{2+}$, indicating that with the infiltration of river water, $\text{Fe}^{3+}$, $\text{Mn}^{4+}$, $\text{NO}_3^-$, and $\text{SO}_4^{2-}$ were reduced in the process of increasing the arsenic concentration. There was a significant correlation between the components, indicating that there was a close relationship between iron, manganese, nitrogen and sulfur and the release of arsenic.
Table 2. Correlations between As, Fe$^{2+}$, Mn$^{2+}$, nitrate and sulfate.

<table>
<thead>
<tr>
<th>Component</th>
<th>As</th>
<th>Fe$^{2+}$</th>
<th>Mn$^{2+}$</th>
<th>NO$_3^-$</th>
<th>SO$_4^{2-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>1.000 **</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>0.943 **</td>
<td>0.943 **</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>−1.000 **</td>
<td>−1.000 **</td>
<td>−0.943 **</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>−0.943 **</td>
<td>−0.943 **</td>
<td>−0.829 *</td>
<td>0.943 **</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Notes: ** correlation is significant at a confidence level of 0.01; * correlation is significant at a confidence level of 0.05.

3.2. Microbial Species Abundance and Diversity

Due to different physical and chemical conditions, microbial communities have different structures and compositions. The interactions among various communities and the collection of various enzymatic reactions play a significant role in the biogeochemical process of arsenic. The samples were classified by Operational Taxonomic Units (OUT) species, and a total of 2330 OTUs were obtained, belonging to 40 phyla, 106 classes and 302 genera. The microbial abundance of river water and groundwater was different, showing strong spatial heterogeneity. The species abundance of river water was relatively high, 1245 OTUs, and that of groundwater was relatively low, 157–288 OTUs (Figure 4). Along the groundwater flow direction, the OTUs decreased gradually on the whole.

The Shannon index of the river water was high, indicating that the river water had a high species diversity and rich species. Along the groundwater flow direction, the microbial diversity in groundwater generally decreased gradually, while the corresponding arsenic content in groundwater was high, reflecting that higher arsenic content had a strong inhibitory effect on microorganisms in groundwater, resulting in fewer species [42–44].

It can be seen from Figure 5 that the PC1 principal axis could explain 44.36% of the sample composition differences, and the PC2 principal axis could explain 13.79%. In the Principal Co-ordinates Analysis (PCoA) diagram, the closer the distance between two samples, the higher the similarity of species [45]. The microbial species composition of the five groundwater samples was similar, which was distributed in the upper left part of the PCoA diagram, while the river water sample was distributed in the lower right part, showing the difference in microbial species composition between river water and groundwater samples.
Figure 5. Principal Co-ordinates Analysis 3D map.

W1, which was spatially closer to the river water, had an obvious deviation from the river water (W0) in the PCoA diagram. With the increasing distance from the riverbank, the deviation between groundwater and river water decreased, indicating that W1 and river water species were less similar than other groundwater, and there were certain differences in the communities. There was a high physical and chemical gradient between W1 and the river water; the microbial activity in the groundwater was high, and the biogeochemical effect was relatively strong. With the further infiltration of river water, the species abundance decreased, and the gradient of environmental conditions decreased relatively.

3.3. Microbial Species Composition

Proteobacteria (38.4–85.4%) was the dominant bacterial phylum, followed by Bacteroidetes (4.1–18.8%) and Cyanobacteria (1.2–23.1%) (Figure 6). Proteobacteria was the most common and dominant bacterial phylum in arsenic-contaminated environments, with numerous arsenic-resistant bacteria [46], and its abundance generally increased first and then decreased along the groundwater flow. Among the Proteobacteria, Alphaproteobacteria was the dominant bacterial class, accounting for 15.2–33.7%, followed by Betaproteobacteria (8.7–34.9%), Gammaproteobacteria (1.9–30.3%) and Deltaproteobacteria (0.3–22.5%).
Figure 6. Microbial community composition: (a) phylum-level; (b) class-level; (c) genus-level (Other: the summation of the bacteria, of which the relative abundance was considerably below 2%).

**Alphaproteobacteria** included *Sphingomonas* and *Brevundimonas*, which have the ability of arsenic reduction and iron reduction [47].
Betaproteobacteria included many aerobic or facultative bacteria, usually with variable degradability. The study area mainly included Vogesella, Aquabacterium, Polaromonas, Gallionella, Hydrogenophaga, Sulfuritalea, etc. Aquabacterium is an iron-reducing bacterium. At the same time, as a facultative aerobic bacterium, Aquabacterium can use nitrate as an electron acceptor for nitrate reduction, accounting for a relatively high proportion in W1, which is related to the rapidly decreasing NO$_3^-$ content along the groundwater flow, indicating that its existence affects the change of nitrate content in groundwater. Gallionella and Hydrogenophaga are ubiquitous in groundwater and can catalyze the reduction of As(V) to As(III), reduce part of arsenite and also catalyze the reduction and dissolution of iron manganese oxide bound iron, releasing arsenic in the lattice into groundwater [48].

The species abundance of Gammaproteobacteria was significantly different. The study area mainly included Acinetobacter, Pseudomonas, Perlucidibaca, Aeromicrobium and Flavobacterium. Acinetobacter is a typical bacterium with an arsenic metabolism ability, which is widely distributed in the arsenic-contaminated environment. Some strains have significant arsenic resistance compared with other bacteria, and some strains can reduce or oxidize arsenic. In addition, some strains also show an iron reduction and sulfur metabolism capacity [49–51]. Pseudomonas is mostly facultative anaerobic bacteria, which can use nitrate as an electron acceptor for metabolism and participate in denitrification. At present, many Pseudomonas strains that have been found are arsenic-resistant bacteria, which is closely related to the release and migration of arsenic [52–54]. At the same time, Drewniak et al. found that some Pseudomonas can produce a compound bound to iron to promote the migration of arsenic from natural minerals [55,56].

Most Deltaproteobacteria can utilize various ions as electron acceptors and widely participate in sulfate reduction and iron reduction in the environment, such as sulfate-reducing bacteria and sulfur-reducing bacteria, etc. [48]. The high presence of sulfate-reducing bacteria in a certain location of the aqueous medium indicates that the source of arsenic in the groundwater may not be the reduction of iron oxides, but the pyrite provides the substrate for sulfate-reducing bacteria to metabolize, resulting in the release of arsenic.

It can be seen that the species and genetic diversity of Proteobacteria are extremely rich, covering a wide range of physiological metabolism types, and they are widely present in groundwater in the study area. Microbial bacteria show certain metabolic functions of arsenic, iron, manganese, sulfur and nitrogen; actively participate in the redox process and have a great effect on the migration and transformation of various elements.

3.4. The Impact of Environmental Factors on Microbial Communities

Environmental factors affect the composition of the microbial community in groundwater. A Redundancy Analysis (RDA) was used to analyze the microbial community and environmental factors. The length of the arrows of environmental factors indicates the degree of its impact on the microbial community. As, Fe$^{2+}$, Mn$^{2+}$, NO$_3^-$ and SO$_4^{2-}$ affect the microbial community (Figure 7). The angles between the arrows of environmental factors represent positive and negative correlations. As was positively correlated with Fe$^{2+}$ and Mn$^{2+}$ environmental factors and negatively correlated with NO$_3^-$ and SO$_4^{2-}$, which was consistent with the results of the Spearman correlation analysis. As, Fe$^{2+}$ and Mn$^{2+}$ pointed to W3, W4 and W5, indicating that they affected the microbial community structure of the groundwater sample in these three reducing-environment areas, corresponding to the high concentrations of arsenic, iron and manganese in these groundwater samples. Sphingomonas and Hydrogenophaga, which have the ability to metabolize arsenic and iron, accounted for a high proportion. Sulfuritalea is not only an arsenic dissimilatory reducing bacteria, but it is also a sulfur oxidizing bacteria and denitrifying bacteria; Pseudomonas is not only an arsenic-resistant bacteria, but it also can participate in denitrification; Acinetobacter not only has the ability of arsenic metabolism, but it also shows the ability for iron reduction and sulfur metabolism. These three bacteria had a good correlation with NO$_3^-$ and SO$_4^{2-}$. 
3.5. Arsenic Functional Genes

There were bacteria containing the As(V)-reducing gene arrA and the As(III)-oxidizing genes aioA and arxA in the river water. However, in the groundwater, only bacteria containing the As(V)-reducing gene arrA, but no As(III)-oxidizing genes, were found (Table 3).

Table 3. OTUs of river water and groundwater.

<table>
<thead>
<tr>
<th>Gene Groups</th>
<th>W0</th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
<th>W5</th>
</tr>
</thead>
<tbody>
<tr>
<td>arrA</td>
<td>1081</td>
<td>1162</td>
<td>1016</td>
<td>1082</td>
<td>1024</td>
<td>905</td>
</tr>
<tr>
<td>aioA</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>arxA</td>
<td>351</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The bacteria containing arrA were mainly Proteobacteria and Actinobacteria, including a great quantity of reducing bacteria, such as Geobacter, Sulfuritalea, Desulfuromonas, etc. (Figure 8). Dissimilatory Arsenate-Reducing Prokaryotes (DARPs) existing in anaerobic environments can use As(V) as an electron acceptor for redox reactions to obtain energy for cell growth. This reduction mechanism is called the respiratory reduction mechanism [57–59]. The functional gene arrA is a reliable marker for the reduction of As(V) by DARPs, and microorganisms containing arrA have a great effect on the biogeochemical cycle of arsenic [60–62]. DARPs can reduce As(V) in amorphous iron oxide, alumina or arsenic-containing minerals; release arsenic in sediments and increase the arsenic content, especially the As(III) content in groundwater [63–65]. Although microorganisms containing the arrA gene are mostly detected in anaerobic environments, some scholars have cloned the gene in the rhizosphere soil of rice, indicating that the expression of the arrA gene may not be strictly anaerobic, so the arrA gene was also detected in the river water in the study area. [66,67].
Figure 8. Species composition containing the As(V)-reducing gene arrA in genus-level (Other: the summation of the bacteria, of which the relative abundance was considerably below 1%).

The bacteria containing aioA were mainly Proteobacteria, including Xanthobacter and Defluviimonas (Figure 9). Bacteria containing arxA were mainly Proteobacteria and Actinobacteria, including Parvibaculum, Rhodobacter, Maricaulis and so on. Under aerobic conditions, As(III) is oxidized to As(V) under the action of microorganisms, and the oxidation of As(III) is achieved by oxidase. Currently, the As(III) oxidase is mainly divided into AioA and ArxA. The process by which microorganisms oxidize As(III) into As(V) to reduce toxicity is arsenic oxidation by microorganisms. There are mainly autotrophic arsenic-oxidizing bacteria and heterotrophic arsenic-oxidizing bacteria that can carry out this process [68–70]. These species were found only in the river water. The oxidation of As(III) occurs in the periplasm and is accompanied by electron transfer during the oxidation process [71–73].
Figure 9. Species composition containing the As(III) oxidizing gene (a) aioA and (b) arxA in the genus-level (Other: the summation of the bacteria, of which the relative abundance was considerably below 0.1%).

4. Conclusions

The river water in the study area was not polluted by arsenic. Along the groundwater flow direction, the oxidation environment gradually transitioned to the reduction environment. The content of arsenic, iron and manganese in groundwater gradually increased, while NO$_3^-$ and SO$_4^{2-}$ showed a decreasing trend, indicating that Fe$^{3+}$, Mn$^{4+}$, NO$_3^-$ and SO$_4^{2-}$ were reduced in the process of increasing arsenic concentration.

The species abundance of river water in the study area was relatively high, and that of groundwater was relatively low. There was a significant negative correlation between microbial diversity and arsenic content, and microbial diversity was higher in groundwater with a relatively low arsenic content. Proteobacteria was the dominant phylum in the groundwater, and Acinetobacter, Pseudomonas, Sulfuritalea, Sphingomonas and Hydrogenophaga
etc. were the main dominant bacterial genera. In addition to reducing and oxidizing arsenic, these functional microorganisms also actively participated in the biogeochemical cycle of elements such as iron, manganese, nitrogen and sulfur. Through the functional gene analysis, it could be seen that there were mainly DARPs in the groundwater. There was a significant correlation between dominant bacteria and environmental factors. Fe/Mn had a significant positive correlation with As, which brought potential danger to the water supply in high iron and manganese areas. It can provide reference for the site selection and construction of the water source in the future and promote the development of groundwater arsenic pollution control technology.

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