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# The microbial diversity of groundwater and manganese sand filtered water in rural water supply project

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**ABSTRACT**

Microorganism was an important part of groundwater pollution, which was related to the safety of drinking water. In rural water supply project, to identify the microbial community structure of groundwater source water and manganese sand filtered water, the structures of the microbial communities were illustrated through 16S rRNA sequencing using the Illumina MiSeq platform. The results showed that, the biodiversity of groundwater samples containing $Fe^{2+}$ and $Mn^{2+}$ was higher than those without $Fe^{2+}$ and $Mn^{2+}$. In the same water supply project, the biodiversity of source water was higher than that of manganese sand filtered water. When the abundance of genus level was more than 1%, the six water samples have high biodiversity at the genus level, which were composed of 66 genera. Bacteria phylum that may contain pathogenic microorganisms were detected in water source and manganese sand filtered water samples. Although no coliform was detected, disinfection was also necessary. The influence order of water quality factors on microbial community was DO > COD > TN > $Mn^{2+}$ > $Fe^{2+}$ (DO – Dissolved oxygen; COD – Chemical oxygen demand; TN – Total nitrogen). In the same water supply project, the correlation between water source and filtered water sample was consistent with water quality factors, that is, DO, $Mn^{2+}$ and $Fe^{2+}$ were positively correlated.

*Keywords:* Microbial diversity; Redundancy analysis (RDA); 16S rRNA genes; Groundwater source; $Fe^{2+}$ and $Mn^{2+}$

## 1. Introduction

Drinking water safety was related to public health and water environment ecological safety, and was the foundation of sustainable social development. WHO Guidelines for Drinking-Water Quality pointed out that microbes were the top priority in the safety of drinking water [1]. In rural areas of China, due to the large quantity of water supply projects, the weak disinfection of water treatment and the low level of engineering operation and management, microbial contamination of drinking water often occurs. Therefore, it was necessary to clarify the microbial community structure of water supply projects for improving the microbiological evaluation methods and optimizing the disinfection process.

The microbiological safety assessment of water supply projects has carried out a lot of fruitful work. The WHO's drinking water quality guidelines put forward 28 items of

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drinking water microbial safety evaluation criteria. In the Safe Drinking Water Act (United States) and Australian drinking water guidelines, the risk identification and evaluation method of microbiological water supply system were proposed respectively [2]. It can be used for reference in microbial risk assessment of water supply system. Most of the evaluation indexes selected common human pathogenic bacteria, such as Salmonella, Shigella, Campylobacter, Plesiomonas, Aeromonas and Vibrio and so on [3]. And most of these pathogenic bacteria did not give the corresponding exceeding limit. At present, the research mainly focuses on the bio-safety of water supply projects with surface water sources, but there were few studies on the bio-safety of water supply projects with underground water sources.

In China, groundwater was the main water supply project in rural areas, so it was very common for $Fe^{2+}$ and $Mn^{2+}$ to exceed the standard. By the end of 2020, there were 19020 rural water supply projects in Heilongjiang Province, including 17239 projects with groundwater as the water source, accounting for 90.9% of the total projects. There were 16021 water sources with excessive $Fe^{2+}$ and $Mn^{2+}$, accounting for 84.2% of the total amount of the project. Manganese sand filtration method was used to remove $Fe^{2+}$ and $Mn^{2+}$ [4,5], and ultraviolet disinfection was used [6]. Microbiological safety evaluation of water supply projects mainly focused on total coliform, thermotolerant coliform bacteria, Escherichia coli and total number of colonies in line with Standards for drinking water quality (GB5749-2006) [7]. In order to enrich the evaluation method of microbiological safety and improve the design level of disinfection process, the physical and chemical indexes of groundwater source water samples and manganese sand filtered water samples were tested in rural water supply projects in Heilongjiang Province. High throughput sequencing technology was used to analyze microbial community structure, biodiversity, sample correlation and the relationship between microbial community and environmental factors.

In this study, our aim was (i) to analyze the microbial community structure, biodiversity and sample correlation of the rural water supply project with groundwater source, and to enrich the microbial safety evaluation index system for rural water supply projects with groundwater source (ii) to explore the relationship between biological community and water quality factors, so as to provide technical guidance for disinfection design of water supply projects.

## 2. Materials and methods

### 2.1. Material

The sampling site was located in the groundwater source of rural water supply project in Heilongjiang Province, northern China. Water sample W1 was collected from the water source of rural water supply project in Wangtai village, Songbei district, Harbin city. Water sample W2 was collected from the water source of rural water supply project in Chaoyang village, Songbei district, Harbin city. Water sample W3 was collected from the water source of rural water supply project in Shuanghe village, Beilin District, Suihua City. Water sample W4 was collected from the water source of rural water supply project in Hongxing Village, Lanxi County, Suihua City. Water sample W5 was collected from the water source of rural water supply project in Weiguang village, Hulan District, Harbin city. Water sample W6 was collected from manganese sand filtered water of rural water supply project in Weiguang village, Hulan District.

### 2.2. Analysis methods

#### 2.2.1. Analysis method of physical and chemical indexes of water quality

A total of six water samples were collected at the rural water supply project using an automatic sampler (W2BC-9600) in July 2020, and the sampling interval was 24 h. Three replicates were taken at each sampling point, and 10 L groundwater was taken in each. Five groundwater samples and one filtered water sample were collected and the physiochemical properties were tested.

For groundwater detection, the water temperature was measured by mercury thermometer, chemical oxygen demand (COD) was measured by a COD detector (5B-1, Lianhua Com., China), total nitrogen (TN) and total phosphorus (TP) were determined by TP and TN analyzer(HACH NPW-160H, Hach, American). After the collected water samples were filtered by 0.45 μm filter membrane, $Fe^{2+}$ and $Mn^{2+}$ were determined by atomic absorption spectrophotometer (A3AFG, Beijing General Instruments Co., Ltd, China).

#### 2.2.2. Analysis method of microbial community structure

Water DNA was extracted from 10L fresh water samples using the E.Z.N.A. Soil DNA Kit (Omega, USA), following the manufacturer's instructions, and the purity and quantity of the DNA were determined using a Qubit 2.0 (life, USA).

The V3-V4 region of 16S rRNA gene was amplified by forward primer and reverse primer. The sequence of forward primer was CCTACGGGNGGCWGCAG, The sequence of the reverse primer was GACTACHVGGGTATCTAATCC.

Amplifications of V3-V4 16S rRNA were carried out using a thermal cycle instrument (Applied Bio-systems 9700, USA). The 30 μL temples contained 2.0 μL microbial DNA (10 ng/μL), 1.0 μL amplicon PCR forward primer (10 μM), 1.0 μL amplicon PCR reverse primer (10 μM), 15 μL 2 × KAPA, 11 μL dd water. After an initial denaturation at 98°C for 3 min, 27 cycles of touchdown PCR were carried out (re-denaturation at 98°C for 10 s, annealing at 55°C for 30 s, extension at 72°C for 45 s) and re-extension at 72°C for 10 min.

The PCR products were checked using electrophoresis in 1% (w/v) agarose gels in TBE buffer (Tris, boric acid, EDTA) stained with ethidium bromide (EB) and visualized under UV light. Illumina MiSeq pe300 (Illumina, USA) was the sequencing platform, and the main sequencing work was carried out in Shanghai Meiji biological Co., Ltd. All the original data of 16SrRNA Gene Sequencing in this study were uploaded to Ribosomal Database Project (RDP) [8]. 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

OTU were picked to annotate the taxonomic information for each sequence using the Greengene database by RDP classifier, with a confidence threshold of 70%.

#### 2.2.3. Data analysis method

The biological Alfa diversity of Shannon, Chao1, ACE and Simpson was analyzed by MOTHUR software [9], and the Venn diagram and sample correlation thermal map were also plotted by MOTHUR software. The community structure at the genus level was plotted by Circos software and R software respectively. The thermal map of the distance between samples was drawn by g plots of R. The relationship between bacterial community and environmental factors was analyzed by RDA using Canoco 4.5 software.

## 3. Results

### 3.1. Analysis of water quality characteristics

The groundwater quality data obtained from the six sampling sites was statistically analyzed, and the results are shown in Table 1.

Table 1 shows that the water samples W1-W4 met the limit requirements of the standard for drinking water quality GB5749-2006. W1 and W2 didn't contain $Fe^{2+}$ and $Mn^{2+}$, while W3 and W4 contain $Fe^{2+}$ and $Mn^{2+}$, but the concentration of $Fe^{2+}$ and $Mn^{2+}$ didn't exceed the standard. Water sample W5 and W6 were from the same rural water supply project. W5 was the source water sample and W6 was the manganese sand filtered water sample. The concentration of $Fe^{2+}$ and $Mn^{2+}$ in W5 was 0.45 and 0.14 mg/L respectively, which exceeded the limits of 0.3 and 0.1 mg/L, and other indicators met the requirements of the standard. After manganese sand filtration, the concentrations of $Fe^{2+}$ and $Mn^{2+}$ in W6 were 0.13 and 0.02 mg/L respectively, which met the requirements of the standard. The excessive contents of $Fe^{2+}$ and $Mn^{2+}$ in groundwater were mainly caused by the primary sedimentary environment [10]. According to the induced hazards map of geological groundwater in China, in Sanjiang Plain, Muling Xingkai low plain and areas along the river, there were many rocks rich in iron and manganese [11]. In natural strata the iron and manganese were usually insoluble compounds. These insoluble compounds entered into groundwater mainly through dissolution of carbonated groundwater and high valence iron manganese oxides were reduced.

### 3.2. Analysis of Alpha diversity

According to OTU cluster analysis results, OTU microbial diversity index (Shannon, Chao1, ACE and Simpson index) was analysed [12]. The greater the three index values of Shannon, Chao1, and ACE, the higher the diversity of species. The smaller the Simpson index, the higher the species diversity, and the results are shown in Table 2.

Table 2 shows that based on Shannon information, the order of microbial diversity was W3 > W5 > W4 > W2>W6>W1. Based on Chao1 and ACE information, the order of microbial diversity was W3 > W5 > W6 > W2 > W4 > W1. Based on Simpson information, the microbial diversity of water samples W3, W4 and W5 was higher than that of water samples W1 and W2. The microbial diversity of water samples W3, W4 and W5 were higher than that of W1 and W2, probably because water samples W3, W4 and W5 contained $Fe^{2+}$ and $Mn^{2+}$, but W1 and W2 didn't contain iron and manganese. After manganese sand filtration, the biodiversity of W6 was lower than that of W5. This result was similar to other previous study [13,14].

### 3.3. Analysis of Venn

In the Venn diagram, different petals represent different samples. The number of overlapping parts represents the number of OTU shared by multiple groups. The number of non overlapping parts represents the number of OTU specific to the corresponding packets [15], and the results are shown in Fig. 1.

Fig. 1 shows that the number of OTUs shared by five different water samples was less. In the same water supply project, the number of OTUs shared by source water and manganese sand filtered water was significantly higher

Table 2
Distribution of Alpha diversity in rural water supply projects

| Sample | Shannon | Chao1 | ACE | Simpson |
|---|---|---|---|---|
| W1 | 2.32 | 210.05 | 247.62 | 0.21 |
| W2 | 3.32 | 418.4 | 390.17 | 0.11 |
| W3 | 5.07 | 873.7 | 872.35 | 0.02 |
| W4 | 4.02 | 266.13 | 301.74 | 0.03 |
| W5 | 4.55 | 720.17 | 715.12 | 0.03 |
| W6 | 3.23 | 464.44 | 477.54 | 0.01 |

Table 1
Water quality characteristics of rural water supply project

| | *T* (°C) | pH | DO (mg/L) | COD (mg/L) | TN (mg/L) | TP (mg/L) | $Fe^{2+}$ (mg/L) | $Mn^{2+}$ (mg/L) |
|---|---|---|---|---|---|---|---|---|
| W1 | 4.9 | 7.65 | 7.9 | 1.42 | 1.6 | L | L | L |
| W2 | 4.5 | 7.87 | 7.5 | 1.08 | 1.7 | L | L | L |
| W3 | 4.6 | 7.59 | 8.1 | 1.79 | 3.4 | L | 0.18 | 0.07 |
| W4 | 5.0 | 7.39 | 7.4 | 1.45 | 2.2 | L | 0.21 | 0.06 |
| W5 | 4.7 | 7.76 | 8.2 | 1.04 | 1.8 | L | **0.45** | **0.14** |
| W6 | 4.8 | 7.82 | 8.0 | 0.96 | 0.7 | L | 0.13 | 0.02 |

*Note*: L means the detection result was lower than the detection limit.

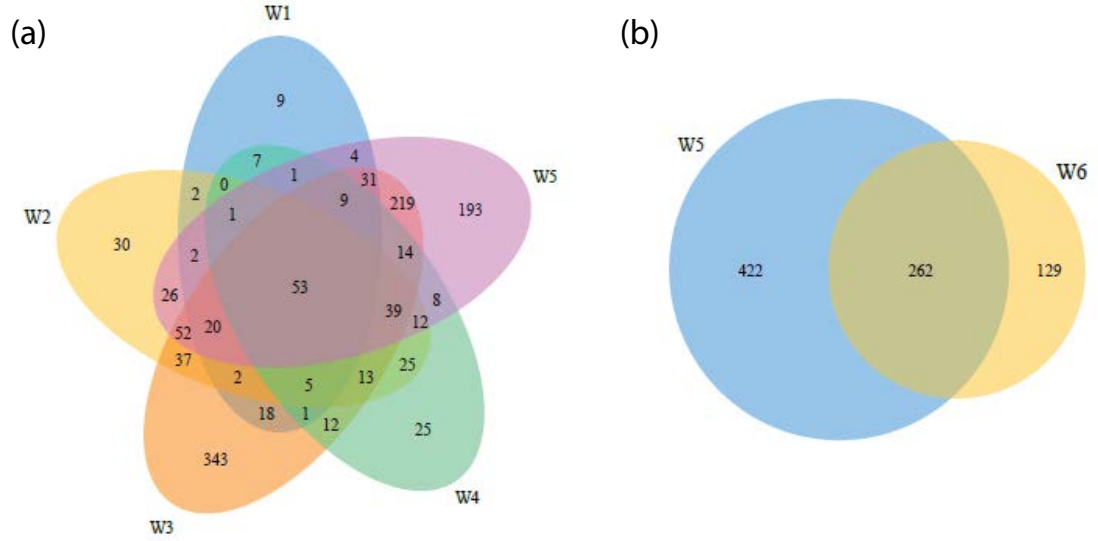


Fig. 1. Venn diagram of sample distribution. (a) Distribution of water source samples and (b) distribution of source water and filtered samples.

than that of different water source samples. In the 5 groundwater sources samples, the total OTU number was 1213, and the intersection OTU number was 53, accounting for 4.4% of the total OUT number. The OTU numbers of W1 and W2 without $Fe^{2+}$ and $Mn^{2+}$ were 165 and 319 respectively. The OTU numbers of W3, W4 and W5 with $Fe^{2+}$ and $Mn^{2+}$ were 868, 225 and 684 respectively. The OTU number of samples with $Fe^{2+}$ and $Mn^{2+}$ was obviously higher than that of samples without $Fe^{2+}$ and $Mn^{2+}$. In the same water supply project, the OTUs of W5 and W6 were 684 and 391 respectively, and the total number of OTUs was 813. After manganese sand filtration, the OTUs of water samples decreased significantly. The total number of cross OTUs was 262, accounting for 32.2% of the total.

### 3.4. Microbial community composition

When the microbial population abundance was more than 1%, the microbial composition of rural water supply project is shown in Table 3.

Table 3 shows that, when the microbial population abundance was more than 1%, the dominant bacteria include 9 phyla, 10 classes, 16 orders and 24 families. Microorganisms in water samples mainly come from soil, human and animal excreta, domestic sewage and so on. The dominant phylum in W1-W6 were Proteobacteria, Firmicutes, Nitrospirae, Actinobacteria, Bacteroidetes, Planctomycetes, Cyanobacteria, Verrucomphia. The dominant class were Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacilli, Planctomycetes, Actinobacteria, Verrucomicrobiae, Cytophagia, Nitrospira, Bacillinorank_Parcubacteria. The dominant order were Burkholderiales, Pseudomonadales, Sphingomonadales, Bacillales, Legionellales, Rhizobiales, Xanthomonadales, Planctomycetales, Flavobacteriales, Actinomycetales, Rhodobacterales, norank_Chloroplast, Caulobacterales, Enterobacteriales, Methylococcales, Nitrospirales. The dominant family were Moraxellaceae, Sphingomonadaceae, Comamonadaceae, Bacillales_Incertae_Sedis_XII, Legionellaceae, Planctomycetaceae, Xanthomonadaceae, Flavobacteriaceae, Rhodobacteraceae, Chloroplast, Microbacteriaceae, Comamonadaceae, Burkholderiales_incertae_sedis, Caulobacteraceae, Enterobacteriaceae, Burkholderiaceae, Methylococcaceae, Pseudomonadaceae, norank_Rhodospirillales, Verrucomicrobiaceae, Chitinophagaceae, Cytophagaceae, Nitrospiraceae, Nitrosomonadaceae.

Many studies have pointed out that there were a large number of opportunistic pathogens in water treatment system, such as Pseudomonas, Citrobacter, Acinetobacter, Staphylococcus, Mycobacterium, Salmonella, Streptococcus, Toxoplasma, etc. [16,17]. In this paper, the microorganism which may include pathogenic bacteria was detected. Legionella, Burkholderia and Enterobacteriaceae were found in water source or manganese sand filtered water. The results showed that even if the groundwater source and the filtered water meet GB5749-2006 standard, it was necessary to disinfect.

The distribution of microbial communities with abundance greater than 1% is shown in Fig. 2.

Fig. 2 shows that when the abundance of genus level was more than 1%, there were 66 genera. Compared with surface water, the six water samples have low biodiversity at the genus level. According to the previous research results, the groundwater temperature in Heilongjiang Province was between 4°C–8°C all the year round, and low temperature was not conducive to microbial reproduction. Some scholars have pointed out that temperature can significantly affect the matrix transfer rate and enzyme catalytic oxidation rate. The lower the temperature, the lower the microbial activity, the worse the removal of $Fe^{2+}$ and $Mn^{2+}$ [18]. Another reason may be that the growth nutrients of carbon, nitrogen and phosphorus in the groundwater were lower than that of surface water, which was not conducive to the growth and reproduction of microorganisms [19,20]. The most abundant genera in groundwater sample W1-W5

Table 3
Statistics of dominant phylum, class, order and family of microorganisms (abundance greater than 1%)

| No. | Phylum | Class | Order | Family |
|---|---|---|---|---|
| W1 | Proteobacteria, Firmicutes | Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacilli | Burkholderiales, Pseudomonadales, Sphingomonadales, Bacillales, Legionellales | Moraxellaceae, Sphingomonadaceae, Comamonadaceae, Bacillales_ Incertae_Sedis_XII, Legionellaceae |
| W2 | Proteobacteria, Bacteroidetes, Planctomycetes, Actinobacteria, Cyanobacteria_ Chloroplast | Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Planctomycetes, Actinobacteria | Burkholderiales, Sphingomonadales, Rhizobiales, Xanthomonadales, Planctomycetales, Flavobacteriales, Actinomycetales, Rhodobacterales, norank_Chloroplast | Sphingomonadaceae, Planctomycetaceae, Xanthomonadaceae, Flavobacteriaceae, Rhodobacteraceae, Chloroplast, Microbacteriaceae |
| W3 | Proteobacteria | Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria | Burkholderiales, Pseudomonadales, Sphingomonadales, Rhizobiales, Caulobacterales, Enterobacteriales, Methylococcales | Moraxellaceae, Comamonadaceae, Burkholderiales_incertae_ sedis, Caulobacteraceae, Enterobacteriaceae, Burkholderiaceae, Methylococcaceae |
| W4 | Proteobacteria, Bacteroidetes, Planctomycetes, Actinobacteria, Verrucomicrobia | Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Verrucomicrobiae, Cytophagia | Burkholderiales, Pseudomonadales | Moraxellaceae, Sphingomonadaceae, Comamonadaceae, Planctomycetaceae, Caulobacteraceae, Flavobacteriaceae, Pseudomonadaceae, norank_Rhodospirillales, Verrucomicrobiaceae, Chitinophagaceae, Cytophagaceae |
| W5 | Proteobacteria, Firmicutes, Nitrospirae, Actinobacteria | Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacilli, Nitrospira | Burkholderiales, Pseudomonadales, Sphingomonadales, Bacillales, Rhizobiales, Nitrospirales, Nitrosomonadales | Moraxellaceae, Sphingomonadaceae, Comamonadaceae, Bacillales_Incertae_ Sedis_XII, Nitrospiraceae, Nitrosomonadaceae |
| W6 | Proteobacteria, Firmicutes, Nitrospirae | Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacillinorank_ Parcubacteria | Burkholderiales, Pseudomonadales, Rhizobiales, Nitrospirales, Enterobacteriales | Moraxellaceae, Nitrospiraceae, Burkholderiales_incertae_sedis, Enterobacteriaceae |

were curvibacter, exiguobacterium, Acinetobacter, sphingorhabdus, Lysobacter, Flavobacterium, aquabacterium, Burkholderia, Pseudomonas, reyranella and sphingopyxis. The most abundant genera in filtered water sample W6 were Nitrospira and unclassified_ Moraxellaceae, unclassified_ Alphaproteobacteria and unclassified_Betaproteobacteria.

The abundance of bacteria in source water was significantly different in 5 water samples. The abundance of Sphingorhabdus in W2, W4 and W5 was 27.5%, 10.8% and 2.1% respectively, and the abundance in W1 and W3 was low, less than 1%. The abundance of curvibacter in W1 was 37.3%, while that in other water samples was low, less than 1%. The abundance of Exiguobacterium in W1, W5 and W3 was 25.6%, 6.6% and 1.5% respectively, and the abundance in W2 and W4 was low, less than 1%. The abundance of Acinetobacter in W5, W1, W3 and W4 was 11.7%, 11.4%, 5.0% and 2.7% respectively, and the abundance in W2 was low, less than 1%. The abundance of Acinetobacter in W3,

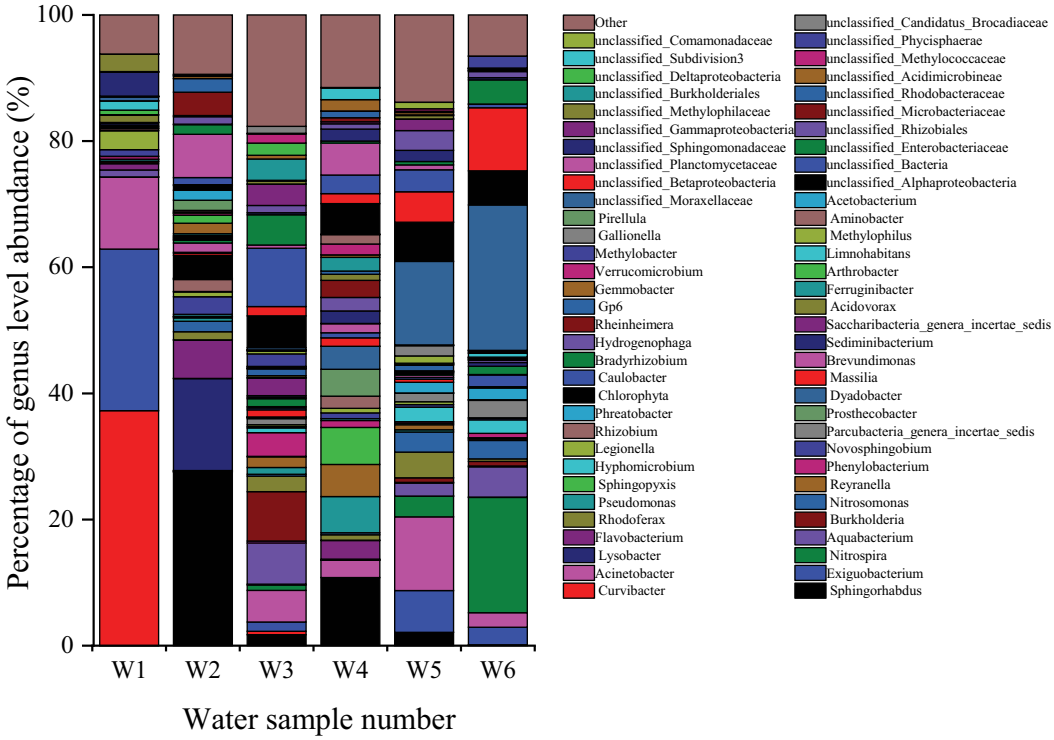


Fig. 2. Microbial community structure at genus level (abundance greater than 1%).

W5 and W1 was 6.5%, 2.1% and 1.1% respectively, and the abundance in W2 and W4 was low, less than 1%. The abundance of Burkholderia in W3 was 7.8%, and the abundance in W1, W2, W4 and W5 was low, less than 1%.

The dominant bacteria in the source water with and without $Fe^{2+}$ and $Mn^{2+}$ were obviously different. In water samples W1 and W2 without $Fe^{2+}$ and $Mn^{2+}$, Sphingorhabdus was 27.5%, Curvibacter was 37.3%, Exiguobacterium was 25.6%, Acinetobacter was 11.4%, Lysobacter was 14.6% and Flavobacterium was 1.1%. In water samples W3, w4 and W5 with iron and manganese, Sphingorhabdus was10.8%, Exiguobacterium 6.6%, Acinetobacter was5.0%, Aquabacterium was 6.5%, Burkholderia was 7.8%, Pseudomonas was 5.7, Reyranella was 5.1, Sphingopyxis was 5.9%. In the water samples containing $Fe^{2+}$ and $Mn^{2+}$, 23 manganese oxidizing bacteria related bacteria were found. They were Exiguobacterium, Acinetobacter, Nitrospira, Lysobacter, Rhodoferax, Nitrosomonas, Pseudomonas, Sphingopyxis, Hypomicrobium, Novosphingobium, Rhizobium, massilia, Chlorophyta, Caulobacter, Brevundimonas, Bradyrhizobium, Hydrogenophaga, Rheinheimera, Acidovorax, Arthrobacter, Gallionella, Aminobacter, unclassified_Alphaproteobacteria. Exiguobacterium was the largest manganese oxidizing bacteria genus. The reason for the rich species of manganese oxidizing bacteria in $Fe^{2+}$ and $Mn^{2+}$ containing water samples may be that the manganese oxidizing bacteria adsorb $Mn^{2+}$ through extracellular enzymes, and then produce oxidation reaction, convert $Mn^{2+}$ into Mn oxides(MnOx), obtain energy for themselves, and complete metabolism, growth and reproduction.

In the same water supply project, the dominant bacteria in source water and manganese sand filtered water were obviously different. In source water sample W5, Exiguobacterium was 6.6%, and Acinetobacter was 11.7%. In manganese sand filtered water sample W6, Nitrospira was 18.3%, unclassified_Moraxellaceae was 23.0%, unclassified_Alphaproteobacteria was 5.4%, unclassified_Betaproteobacteria was 10.0%. After manganese sand filtration, the abundance of most manganese oxidizing bacteria related bacteria decreased. Exiguobacterium decreased from 6.6% to 2.8%, acinetobacte decreased from 11.7% to 2.3%, Rhodoferax decreased from 4.1% to 0.4%, Nitrosomonas decreased from 3.2% to 2.9%, hypomicrobium decreased from 2.3% to 2.1%. Only Nitrospira increased from 3.3% to 18.3%. Academician Li Guibai pointed out that [21] the groundwater containing $Fe^{2+}$ and $Mn^{2+}$ enters the manganese sand filter tank after aeration, the hydroxide of high valence manganese was attached to the surface of the filter material to form a "manganese active filter membrane", under pH neutral conditions, $Mn^{2+}$ can be absorbed by the active filter membrane and then oxidized by dissolved oxygen (DO) to form a new active filter membrane to participate in the reaction. The abundance of manganese oxidizing bacteria decreased after manganese sand filtration, it may be because the biological filter membrane and manganese sand filter material intercepted some manganese oxidizing bacteria.

### *3.5. Correlation analysis of water samples*

The color blocks represent the distance between samples. The darker the color, the closer the distance between samples. And the value of each grid represents the distance between the samples corresponding to the abscissa and

ordinate, ranging from 0 to 1. The sample correlation heat map is shown in Fig. 3.

Fig. 3 shows that at genus level, the similarity of microbial population in source water was low. But in the same water supply project, the similarity of water source and manganese sand filtered water sample was high. The order of distance between water sample W1 and other samples was W2 (0.91) > W4 (0.89) > W3 (0.84) > W5 (0.73), and the order of distance between water sample W2 and other samples was W1 (0.93) > W5 (0. 83) > W3(0.82) > W4(0.64). There was no significant correlation between W1 and W2 in water samples without $Fe^{2+}$ and $Mn^{2+}$. The order of distance between water sample W3 and other samples was W1 (0.84) > W2 (0.82) > W4 (0.70) > W5 (0.56), and the order of distance between water sample W4 and other samples was W1 (0.89) > W5 (0.73) > W3 (0.70) > W2 (0.64). the order of distance between water sample W5 and other samples was W2 (0.83) > W4 (0.73) = W1 (0.73) > W3 (0.56). There was no significant correlation between sample distance W3, W4 and W5 in water samples containing $Fe^{2+}$ and $Mn^{2+}$. Some scholars pointed out that groundwater was in the state of poor nutrition, which was not conducive to the growth and reproduction of microorganisms [22]. And Sheath bacteria, Pseudomonas, Vibrio, Corynebacterium, hyphomycetes and other bacteria have been isolated from low nutrient waters [23]. This study also detected Pseudomonas and Vibrio from the lack of nutrient water samples, and achieved similar results with previous studies. The distance between W5 and W6 samples was close, and the value was 0.48. Some research found out that, in the same water supply project, the similarity between source water sample and manganese sand filtered water sample was higher [24]. This study also detected Bradyrhizobium and sphingopyxis from the source water samples and manganese sand filtered water sample.

### *3.6. Correlation analysis of microorganism and environmental factors*

At the level of 80.54% of the total contribution rate of x-axis and y-axis, the relationship between microbial community and water quality factors is shown in Fig. 4.

Fig. 4 shows that the order of environmental factors influence degree was DO > COD > TN > $Mn^{2+}$ > $Fe^{2+}$. The correlation degree between water samples and water quality

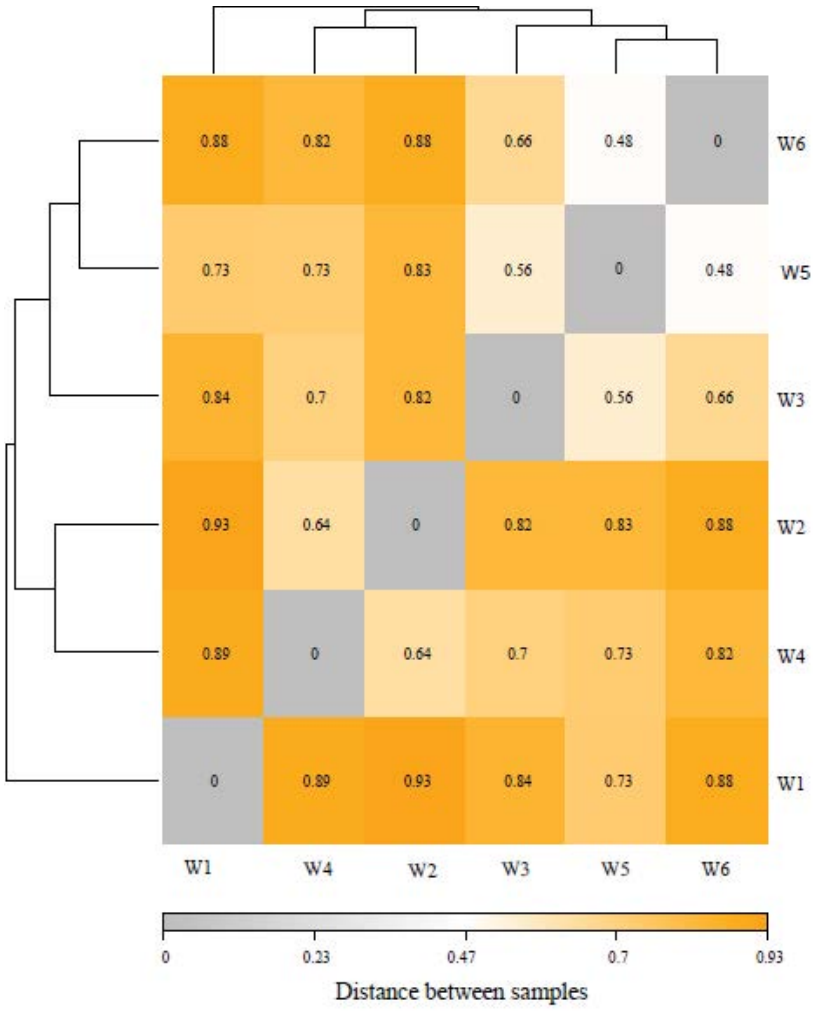


Fig. 3. Water sample correlation thermogram.

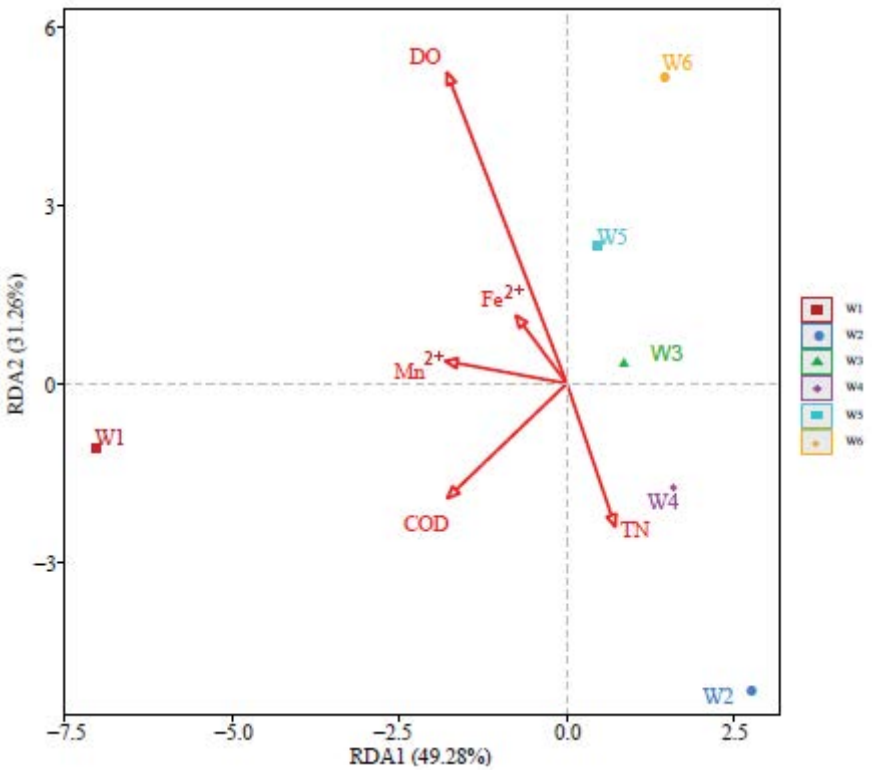


Fig. 4. RDA analysis at the generic level

factors in the same water supply project was similar. The similar results are obtained in Figs. 2 and 3. W1, W5 and W6 were positively correlated with DO. W1, W2 and W4 were positively correlated with COD. W2 and W4 were positively correlated with TN. W1, W5 and W6 were positively correlated with $Mn^{2+}$. W1, W5 and W6 were positively correlated with $Fe^{2+}$. Some studies found that $NO_3$–N and DO were the main factors affecting the bacterial community structure in the surface water [25]. The correlation between DO, COD, TN, $Mn^{2+}$ and $Fe^{2+}$ in the five source water samples was not obvious, which may be due to the low temperature, poor nutrition and low pollution of the confined water. In the same water supply project, W5 and W6 were consistent with water quality factors, and positively correlated with DO, $Mn^{2+}$ and $Fe^{2+}$. This study analyzed the correlation between the microorganism and water quality factors of 6 samples. The number of samples was small and the range of water quality parameters was small too. In order to get more reliable correlation between groundwater source and water quality factors, it was necessary to carry out research on the basis of enlarging the quantity of samples and accumulating water quality data.

## 4. Discussion

The study of microbial diversity can enrich the microbial safety evaluation index system of rural water supply project. According to GB5749-2006, four sub indicators of total coliform, heat-resistant coliform, Escherichia coli and the total number of colonies were selected to construct the microbial safety evaluation index system for rural water supply projects in China [26,27]. In this study, Legionella, Burkholderia, Enterobacteriaceae and other pathogenic bacteria were found in the source water and manganese sand filtered water (Table 3). It can be seen that it was not comprehensive to only use the four microbial indicators specified in GB5749-2006 as evaluation indicators. Therefore, in order to enrich the microbial safety evaluation index of rural water supply projects, it is necessary to accumulate a large number of microbial community structure data for water supply projects and summarize the characteristic pathogenic microorganisms.

Research on the relationship between microorganisms and water quality can provide technical guidance for disinfection design of rural water supply projects. At present, ultraviolet disinfection was the main choice of rural water supply projects in Heilongjiang Province. The suitable disinfection conditions for UV were water temperature 20°C–30°C, chroma ≤ 15°C, turbidity ≤ 5 NTU, Fe ≤ 0.5 mg/L, Mn ≤ 0.3 mg/L, hardness ≤ 120 mg/L, total coliform group ≤ 1000 MPN/100 mL and total bacterial count ≤ 2,000 CFU/mL [28,29]. The water temperature of underground water source was between 4°C–8°C all the year round, and the actual water temperature was not within the temperature range suitable for ultraviolet disinfection. It was very common for groundwater to exceed the standard of iron and manganese, which was not suitable for ultraviolet disinfection. Therefore, it was very important to summarize the correlation between microorganisms and water quality factors, which can provide technical guidance for disinfection process design of rural water supply projects.

## 5. Conclusions

Microbial safety was the basis for water source protection, and was also the basis for early warning of water treatment process. In this paper, the microbial community structure, biodiversity, sample correlation and the relationship between microbial community and environmental factors were analyzed. The results showed that the biodiversity of groundwater samples containing $Fe^{2+}$ and $Mn^{2+}$ was higher than that of water samples without $Fe^{2+}$ and $Mn^{2+}$, in the same water supply project, the biodiversity of source water was higher than that of manganese sand filtered water. When the abundance of genus level was more than 1%, the six water samples have high biodiversity at the genus level, which were composed of 66 genera. Bacteria phylum that may contain pathogenic microorganisms were detected in water source and manganese sand filtered water samples. Although no coliform was detected, disinfection was also necessary. There was no significant correlation among the five water source samples, and the correlation between the same water source and manganese sand filtered samples was higher than that of other water sources samples. The influence order of water quality factors on microbial community was DO > COD > TN > $Mn^{2+}$ > $Fe^{2+}$. There was no significant relationship between water quality factors and source water samples. In the same water supply project, the correlation between source water, filtered water and water quality was basically the same, and was positively correlated with DO, $Mn^{2+}$ and $Fe^{2+}$.

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