Impact of the South-to-North water diversion on the soil microbial community structure in the Miyun Reservoir riparian zone

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Abstract. In order to clarify the effect of the South-to-North Water Diversion Project on the microbial community structure in the reservoir riparian zone, the differences of microbial community structure and diversity before and after water transfer were investigated using the Miseq high-throughput sequencing methods. The results showed that pH and TN content increased after water transfer, but not significantly (p> 0.05). However, TP and TOC contents were significantly lower than those before water transfer (p <0.05). Proteobacteria, Chloroflexi, Acidobacteria, Nitrospirae and Bacteroidetes were the dominant soil microbes before water transfer. The main phylum species after water diversion were Proteobacteria, Bacteroidestes, Chloroflexi, Verrucomicrobia and Actinobacteria. The relative abundance of Proteobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia, Cyanobacteria and Chlorobi were significantly increased after water diversion (p <0.05), indicating that water diversion had a significant effect on microbial community structure. The communities Cyanobacteria, Verrucomicrobia, Actinobacteria and Bacteroidetes have a strong positive correlation with pH and TN. Planctomycetes and Nitrospirae are closely related to TOC. Our results showed that the TOC content decreased obviously after water diversion (p <0.05), which caused the soil nitrification bacteria abundance decreased. This indicates that impoundment had a significant impact on the eco-physiological process of soil nitrifying microorganisms in the early stage of water diversion.

1. Introduction

The South-to-North water diversion project is a great project to solve the water shortage in North China, but the impoundment submerged the reservoir original riparian zone that may lead to drastic changes in the physicochemical and biological properties of soil [1]. Mayan Reservoir is the final water receiving area of the South-to-North water diversion project and most of reservoir riparian zone belongs to farmland ecosystem[2]. Before the impoundment, the farmland ecosystem under the extensive high-intensity agricultural management practices such as the application of chemical and organic fertilizers[3]. Urea and ammonium nitrogen fertilizers were converted into nitrates by nitrifying microorganisms and accumulated in soil. However, soluble ammonium nitrogen and nitrate

nitrogen accumulated in soil entry to water body, which may have some impacts on the water quality[4]. In addition, the impoundment changed the condition of oxygen, pH, temperature and organic matter in the soil, which may effect on the microbial community structure [5].

Microbes are the most important biological and organic components in soil [6].As a complex ecological environment, the microbial composition and structure in the soil have great impact on the balance of nutrients (such as phosphorus cycle) in the water environment[7]. At present, microbial diversity of soil and sediment in the ocean [8], freshwater lake [9] and the marsh [10]were well investigated, but few studies associated with project impacts on soil ecosystem. Therefore, the research on the ecological impact of water diversion has important practical significance.

Mayan Reservoir is the only surface drinking water source in Beijing. After the water transfer, the water level in the reservoir will increase from133.7m to 148m. 60 thousand acres of farmland in the reservoir riparian zone will be submerged. Therefore, we compare the differences of soil microbial community structure and diversity before and after water transfer, respectively. The aim of the study is to understand the water diversion impacts on soil microbial diversity for providing a basic theory of restoration and reconstruction in the reservoir riparian zone.

2. Materials and Methods

2.1. Sites and Sampling
The Mayan reservoir is the largest drinking water supply reservoir in Beijing. At the end of October 2015, South-to-North water diversion project started. By the end of 2016, the storage capacity of Mayan reservoir reached 1.65 billion cubic meters from 0.8 billion cubic meters in 2014. Four typical sites (HTG, DGZ, WY and FHS) were chosen for sampling at October 4, 2014 and October 15, 2016 represented for before and after water transfer samples, respectively. Triplicate samples were collected at each sampling sites. After removing stones, roots and other sundry, samples divided into three parts. One part was immediately stored at - 80 ° C in the refrigerator for DNA extraction. The other part was used for detecting total organic carbon content. The left part is dried for measuring soil properties (such as pH, total nitrogen and total phosphorus).

2.2. DNA Extraction, PCR Amplification and Sequencing
DNA isolation according to the protocol reported previously[11]. PCR amplification and IIumina Miser PE 250 sequencing were carried out in Shanghai Major Bio Bio-pharm Technology C., Ltd. The OTUs (operational taxonomic units) were calculated in Mother. The coverage of Goods, the calculation of Chao1 and Shannon's diversity indices were calculated using the QIIME platform and the taxonomic of OTU representative sequences was identified by the RDP Classifier.

2.3. Soil characteristics
Total nitrogen, total phosphorous and TOC in soil were measured according to National standard methods (HJ 717-2014, NY/T 88-1988 and HJ 615-2011), respectively.

2.4. Data statistics
All data was processed by Excel 2013 and analyzed by ANOVA using SPSS 20.0. The relationship between environmental factors and the bacterial populations was calculated using the R vegan package.

3. Results

3.1. Effect of water transfer on physical and chemical properties of soil
After water transfer, the physicochemical properties of submerged soil changed a lot. As shown in Table.1, pH value increased from 7.24 ± 0.04 to 7.98 ± 0.03. TN content also increased after water transfer, but no significant difference ($p > 0.05$). However, the contents of TP and TOC in soil were significantly lower than those before water diversion ($p < 0.05$).

Table 1. Basic characteristics in soil

Sampling sites	pH	TP g/Kg	TN g/Kg	TOC g/Kg
DGZ-B	7.18	0.48	2.30	10.90
FHS-B	7.34	0.05	1.06	11.74
WY-B	7.26	0.41	1.48	8.00
HTG-B	7.16	0.12	2.84	12.70
DGZ-A	7.94	0.07	2.30	1.37
FHS-A	7.95	0.08	2.21	1.22
WY-A	8.05	0.03	2.67	0.85
HTG-A	8.00	0.02	2.69	0.90
Before	7.24±0.04	0.27±0.11	1.92±0.40	10.84±1.01
After	7.98±0.03	0.05±0.01	2.46±0.12	1.09±0.13

3.2. Impacts of water transfer on microbial diversity

As shown in Table 2, the number of sequence reads obtained from each sample was from 20690 and 52430. We used the 97% similarity to calculate richness and biodiversity, which estimates based on the indices of Chao1, Ace and Shannon (Table.2). The results showed that after water diversion microbial diversity is more abundant ($p<0.05$).

Table 2. Biodiversity indices relative to each sample

Sites	Reads	OTU	Ace	Chao1	Shannon	Coverage
DGZ-B	36085	823	1309	1144	4.8	0.9928
FHS-B	20690	2421	3253	3310	6.38	0.9597
WY-B	42732	761	979	1010	3.95	0.9950
HTG-B	32456	2710	3358	3416	6.54	0.9768
DGZ-A	47833	8122	21037	18982	9.15	0.8899
FHS-A	48882	7374	20532	18038	8.94	0.9007
WY-A	50967	5435	14107	12274	9.14	0.9346
HTG-A	52430	4552	12910	11485	8.42	0.9453

3.3. Impacts on microbial community structure and composition

High throughput sequencing found that before the water diversion (Fig.1 (A)), Proteobacteria, Chloroflexi, Acidobacteria, Nitrospirae and Bacteroidetes were the dominate phylum bacteria in soil. The bacteria Proteobacteria, Bacteroidetes, Chloroflexi, Verrucomicrobia and Actinobacteria were the most phylum in the after water transfer samples (Fig .1(B)). By comparing the ANOVA variance of the top ten relative abundances, the relative abundance of Proteobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia, Cyanobacteria and Chlorobi in emerged soil increased significantly after water diversion ($p<0.05$), but relative abundance of the Chloroflexi, Acidobacteria, Planctomycetes and Nitrospirae showed no significant difference ($p >0.05$) in before and after samples.
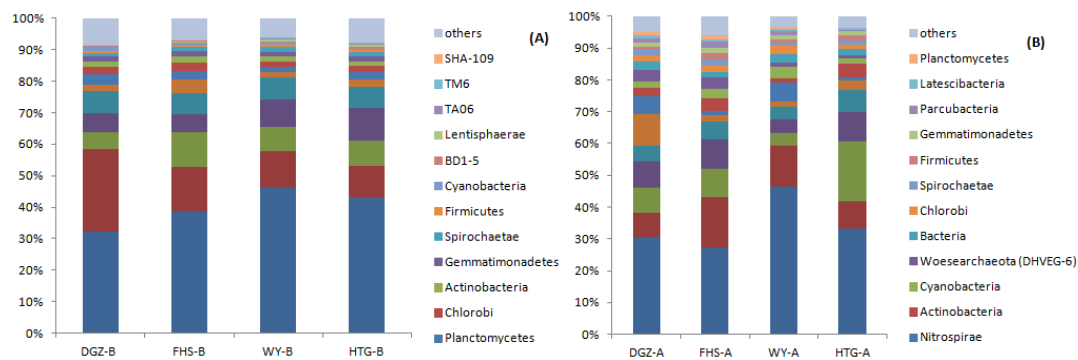
Figure 1. Microbial community composition at phylum level in the Mayan reservoir

3.4. Environmental factors impact on microbial community structure

The RDA analysis was used to explore the environmental factors impact on microbial community structure (Figure 2). The first two axes (RDA1 and RDA2) explain 67.08% and 0.07% of the total variation, respectively. Before water transfer, pH and TN were the main influential factors affecting the distribution of the microbial community. However, the microbial community in each sampling sites was more obviously influenced by TP and TOC after water diversion. The phylum Cyanobacteria, Verrucomicrobia, Actinobacteria and Bacteroidetes had strong positive correlation with pH and TN. Planctomycetes and Nitrospirae are closely related to TOC. It indicates that the soil microbial community in the reservoir riparian zone is greatly affected by the change of soil properties after water diversion.
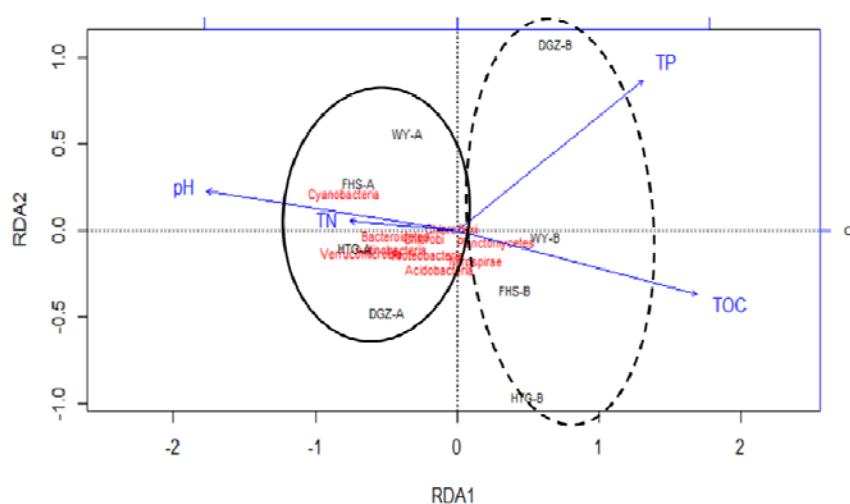

Figure 2. Environmental factors impact on microbial community structure

4. Discussion

The South-to-North water diversion has been started, which made the water storage of the Miyun Reservoir increase from 800 million cubic meters in 2014 to 1.65 billion cubic meters in 2016. The environmental factors and microbial community structure in the reservoir riparian zone soil have changed greatly after water transfer.

As showed in Table 1, the concentrations of the TP and TOC had decreased significantly ($p < 0.05$) after the impoundment. Phosphorus release in emerged soil influenced by many factors, such as

temperature, dissolved oxygen, oxidation-reduction reaction and phosphorus morphology [12]. Some of factors were indirectly regulated by the microbial activity in the soil [13]. Due to the disturbance of impoundment, the surface emerged soil suspension directly entered the upper water body, increasing the reaction interface of the sediment particles and accelerating the phosphorus diffusion[14]. The release of organic matter from the emerged soil to the overlying water is an important factor affecting the organic matter content of the water body [15], while the microorganisms are also important influencing factors during the release of organic matter from the sediment and its role is irreplaceable[16]. In addition, pH, as a common chemical indicator of water, can change the blood of aquatic animals through the permeation and absorption of hydrogen ions affecting the life activities of aquatic animals [17]. For aquatic plants, the change of pH will affect their absorption of nutrient elements [18], which may indirectly affect the growth of aquatic plants during reservoir riparian zone reconstruction.

In this study, high-throughput sequencing of microorganisms in the soil of the reservoir riparian zone before and after the South-to-North Water Diversion Project was conducted. Our results showed that the dominate bacteria in soil were similar to the other freshwater environment, indicating the widespread distribution of microorganisms in such similar environment. The microbial composition indicated that Proteobacteria is the most important phylum. Previous study found that Proteobacteria is the main group of alkaline soil [19]. In this study, the slightly higher pH value in soil was also proved that Proteobacteria is more abundant in the alkaline soil. After water transfer, the main group Actinobacteria relative abundance was significantly higher than those before water transfer (P <0.05). This may be due to the accumulation of a large amount of litter on the surface of the soil, which may affect soil nutrient cycling and biodiversity. The RDA results showed that pH and TN were the main influential factors affecting the distribution of microbial communities before water transfer. The phylum Cyanobacteria, Verrucomicrobia, Actinobacteria and Bacteroidetes had strong positive correlation with pH and TN (Fig.2). Soil pH is one of the important factors affecting soil nitrification. It not only affects the process of soil nitrification, but also affects the quantity and community structure of nitrifying microorganisms. In addition, the soil TOC contents were significantly decreased (P <0.05), and Planctomycetes and Nitrospirae were closely related to TOC (Fig.2). Previous study have shown that soil organic carbon concentration is the main reason driving the distribution of soil microbial biogeography [20].However, the content of TOC decreased significantly after the water transfer may cause the soil nitrification decreased. Nitrospirae is often found in the natural environment and has the higher affinity to nitrite [21]. The results showed that Nitrospirae was the dominate ammonia-oxidizing bacteria before the water transfer, but not the main group in the after water transfer samples. Our results suggested that the initial stage of water diversion had a significant impact on the eco-physiological process of soil nitrifying microorganisms in the Miyun Reservoir riparian zone.

Microbial community are not only as a sensitive indicator of soil quality changes [22], but also as a biological indicator of monitoring changes in regional or historical environment [23].The impact of South-to-North water diversion on the reservoir ecological environment is complex due to human regulation and disturbance. Miyun Reservoir, as a typical representative of the water receiving areas in the South-to-North water diversion project, has significant variability in the ecological system of water storage. This study not only provides an insight into the microbial community in the Miyun Reservoir riparian zone, but also enhances our understanding on the effects of impoundment on microbial community diversity and structure.

5. Conclusion

Miseq high-throughput sequencing results can comprehensively reflect the composition and structure of microbial communities in soil. The diversity index indicates that the biodiversity of emerged soil after water diversion is higher than that before impoundment.

Proteobacteria, Chloroflexi, Acidobacteria, Nitrospirae and Bacteroidetes were the dominant soil microbes before water diversion. The main phylum after water diversion were Proteobacteria, Bacteroidestes, Chloroflexi, Verrucomicrobia and Actinobacteria.

Before water transfer, pH and TN were the main factors affecting the distribution of soil microbial community in the reservoir riparian zone. The microbial community was more obviously influenced by TP and TOC after water diversion.

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