The effect of thermal stratification on microbial community diversity and structure in a temperate reservoir

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Abstract. Miyun reservoir is a typical temperate deep reservoir located in the northeast of Beijing, China. In order to explore the effect of thermal stratification on microbial community diversity, structure and its influencing environmental factors, stratified sampling at three sites was conducted during the summer period. Field observations indicate that the water temperature and dissolved oxygen concentrations dropped to 11.9 °C and 1.57 mg/L, respectively, leading to the development of anoxia in the hypolimnetic layer. The Illumina Miseq sequencing results showed that microbial communities from different thermal stratification showed obvious differences, the highest microbial diversity and richness in the hypolimnion samples. RDA ordination analysis suggested that the microbial communities in the epilimnion and metalimnion were mainly affected by water temperature, pH and dissolved oxygen, while total nitrogen was the key environmental factor which shaped the microbial structure in hypolimnion.

1. Introduction

In recent years, with the limitation of urban groundwater exploitation in China, reservoirs have become the main drinking water source in many cities. A drinking water reservoir is a semi artificial -natural water body between rivers and lakes, and its ecosystem is directly regulated by human activities and has unique ecological characteristics such as volume, surface and depth [1]. Many rivers have become artificial reservoirs through the construction of dams. However, the construction and use of the reservoir has profoundly changed the natural water succession characteristics [2-3].The seasonal thermal stratification, which divided water column into the epilimnion, metalimnion, and hypolimnion, is a common phenomenon in these reservoirs [4]. Stable thermal stratification hinders flow of energy and cycling of matter of the upper and lower water bodies, and has a significant effect on the distribution of dissolved oxygen, nutrients and aquatic microorganisms in reservoirs [5-7].

Microbial community are the most important component in aquatic environment, which play an crucial role in the energy flow, material circulation and information transmission of the whole ecosystem[8-9].In addition, some living bacteria participate in the role of carbon cycling in aquatic ecology, transfer the fixed carbon in the atmosphere, enter the traditional food chain through the micro-food network, and promote the carbon cycle in the aquatic ecosystem [10].Moreover, microbial community are very sensitive to changes in the external environment and its composition and diversity dynamic are strong associated with water quality changes[11-13].
In summer, water stratification occurs in temperate deep reservoirs. However, the effect of water thermal stratification on the microbial community diversity and structure is still lack of investigation. In this study, we sought to determine the microbial community composition and relative different vertical distribution characteristics to the thermal stratification environment. Our result will provide more information about microbial diversity and structural changes during thermal stratification conditions and assist in the assessment and management of the drinking water reservoir.

2. Materials and methods

2.1. Sampling sites
The Miyun reservoir is the largest drinking water supply reservoir in the capital of China and covers 1.1 billion cubic meters with a catchment area of 15788 km². Three sampling sites (BHB, CHB and KZX) were chosen at the deepest area of the Miyun reservoir during water thermal stratification on 22 August 2016 (Figure 1.).

![Figure 1. Location of sampling sites in Miyun reservoir.](image)

2.2. Water Quality Monitoring
Water samples were collected at three sampling sites and stored at 500 ml sterile glass bottles. The water quality parameters including water temperature (T), pH and dissolved oxygen (DO) were tested in the field with a water quality analysis machine (AP-7000, USA). The other parameters such as TN and TP were tested in the lab according to the standard methods [14]. All data were processed using Microsoft Excel 2007.

2.3. DNA Extraction and PCR Amplifications
The DNA isolation according to the protocol reported previously [15]. PCR amplification was carried out as described by Fan et al. (2014) [16].

2.4. Illumina Miseq sequencings
The sequencing were performed by Shanghai Major Bio-pharam Technology Co., Ltd using Illumina Miseq PE 250 strategy. The OTUs (operational taxonomic units), Rarefaction analysis, ACE (abundance-based coverage estimator) and Chao estimator were all calculated in Mothur. Bioinformation analysis was performed using R package software.
3. Results

3.1. Water quality variables
As shown in Table 1, there was a significant difference in temperature and dissolved oxygen variables between epilimnion, metalimnion, and hypolimnion (ANOVA test, \( p < 0.05 \)). The temperature was decreased steadily from the surface to the bottom. The average temperature in epilimnion, metalimnion and hypolimnion were 22.17 °C, 17.44 °C, and 11.9 °C, respectively. The pH decreased slightly with the increase of water depth. The dissolved oxygen (DO) did show important differences between water layers. The DO levels decreased sharply below 20 m and were consistently low in the deepest parts of hypolimnion. Total nitrogen, ammonia nitrogen and nitrate nitrogen showed a slight tendency to increase first and then decrease. Total nitrogen changed among these three stratified layers from 1.9 to 2.62 mg/L and nitrate nitrogen changed from 0.04 to 0.1 mg/L. The concentration of nitrite nitrogen was very low. Therefore, our results indicated that thermal stratification had a dramatic direct influence on the water quality variables.

Table 1. Water quality variables (mean ± SD) of Miyun reservoir in this study.

| Sampling sites | T °C | pH | DO mg/L | TP mg/L | TN mg/L | NH4+-N mg/L | NO3-N mg/L | NO2-N mg/L |
|---------------|-----|----|---------|--------|---------|-------------|------------|------------|
| BHB           | 17.37 ± 5.36 | 7.94 ± 0.2 | 5.69 ± 3.6 | 0.06 ± 0.02 | 1.11 ± 0.38 | 0.09 ± 0.04 | 0.2 ± 0.06 | 0.02 ± 0.00 |
| CHB           | 17.44 ± 4.46 | 7.71 ± 0.46 | 5.92 ± 4.3 | 0.07 ± 0.01 | 1.58 ± 0.3 | 0.07 ± 0.04 | 0.55 ± 0.18 | 0.013 ± 0.00 |
| KZX           | 16.7 ± 5.64 | 7.38 ± 0.59 | 6.16 ± 3.93 | 0.05 ± 0.03 | 3.7 ± 0.8 | 0.04 ± 0.03 | 0.37 ± 0.3 | 0.01 ± 0.00 |

Epilimnion: 22.17 ± 0.31 | 8.09 ± 0.05 | 9.23 ± 0.31 | 0.04 ± 0.02 | 1.9 ± 1.59 | 0.06 ± 0.03 | 0.39 ± 0.22 | 0.02 ± 0.00 |
Metalimnion: 17.44 ± 0.39 | 7.64 ± 0.45 | 6.96 ± 0.54 | 0.06 ± 0.01 | 2.62 ± 1.65 | 0.1 ± 0.03 | 0.52 ± 0.22 | 0.02 ± 0.00 |
Hypolimnion: 11.9 ± 1.25 | 7.31 ± 0.4 | 1.57 ± 0.36 | 0.08 ± 0.01 | 1.88 ± 0.9 | 0.04 ± 0.01 | 0.18 ± 0.17 | 0.01 ± 0.00 |

3.2. Microbial community diversity

Table 2. Microbial community diversity of Miyun reservoir during thermal stratification.

| Layers   | Sampling sites | OTUs | reads | Chao  | Shannon |
|----------|----------------|------|-------|-------|---------|
| Epilimnion | BHB (EPI1) | 566 | 42822 | 649 | 4.48 |
| KZX (EPI2) | 696 | 40738 | 788 | 5.05 |
| CHB (EPI3) | 705 | 40713 | 779 | 4.84 |
| Mean     | 656 | 41424 | 739 | 4.79 |
| Metalimnion | BHB (MET1) | 522 | 43971 | 565 | 4.41 |
| KZX (MET2) | 781 | 32638 | 931 | 5.03 |
| CHB (MET3) | 694 | 36515 | 868 | 4.84 |
| Mean     | 667 | 37708 | 788 | 4.76 |
| Hypolimnion | BHB (HYP1) | 721 | 32386 | 789 | 4.81 |
| KZX (HYP2) | 689 | 29707 | 768 | 4.85 |
| CHB (HYP3) | 705 | 32622 | 823 | 4.99 |
| Mean     | 705 | 31572 | 793 | 4.88 |
After quality filtering and chimera removal, a total of 332,112 reads were produced from nine water samples by Illumina Miseq sequencing. The Good’s coverage was more than 99.58%, and it suggested that these libraries represented the majority of microbial 16S rRNA reads. OTUs were used to calculate Alpha-diversity indexes, and these data were shown in Table 2. The diversity of indexes Shannon diversity and Chao values showed that the highest diversity was found in hypolimnetic samples.

3.3. Microbial community composition

Across all samples, the microbial community composition at the phylum and genus levels in different stratified layers was shown in Figure 2. In phylum, the most classes were Proteobacteria(22%), Firmicutes(19%), Cyanobacteria(17%), Planctomycetes(14%), Verrucomicrobia(10%), Actinobacteria(11%), Chloroflexi(4%), Chlorobi(2%), and Acidobacteria(1%), respectively. In terms of genus, CL500-3, Enterococcus and hgcl-clade were the most abundant genus. The results revealed that the most genera CL500-3 percentage decreased from epilimnion to hypolimnetic layer, but another most group Enterococcus percentage increased from surface to bottom of water column.

3.4. Structure variations

The RDA analysis was used to explore the relationship between microbial structure and water quality variables in different stratified layers (Figure 3). The first two axes (RDA1 and RDA2) explain 49.68% and 8.84% of the total variation, respectively. Except TP, TN and NO3-N, the first axis was negative correlated with most other variables. But the concentration of TN has shown a strong positive relationship with hypolimnetic layer and the classes Chlorobi, Acidobacteria, Planctomycetes and
Cyanobacteria. It was shown that the TN was shown to play the most significant roles in water stratified and microbial composition. The second axis showed that the TN, T, pH and DO were the most important factors to shape the microbial communities structure living in the epilimnion and metalimnion.

Figure 3. RDA ordination biplot showed factors that driving the variation of microbial communities among different thermal stratification.

4. Discussion
The drinking water reservoir Miyun is located in the region of temperate monsoon climate, and the thermal stratification is closely related with water quality seasonal changes [17]. In summer, due to the increase of radiant heat and climate temperature, the surface water temperature of the reservoir rises rapidly and is close to the air temperature, but the bottom layer temperature increase only by the water transmission that caused the stable thermal stratification in the deep reservoir [18]. In our study, there is a clearly difference in physical and chemical during the stratification. The difference of water temperature between the epilimnion and hypolimnion was 10°C. Especially, the dissolved oxygen concentration in the epilimnion decreased quickly from aerobic environment to hypoxic environment in the hypolimnion (Table 1). Thus, thermal stratification limits the vertical exchange of and block the oxygen consumption of the upper water body to the underlying water, which may affect the hydrochemical and geochemical cycles [19]. The seasonal thermal stratification plays a key role in controlling or influencing the cycle of nitrogen and phosphorus in reservoirs [20]. Our result showed that the content of total nitrogen and phosphorus increased with the water depth. However, the content of nitric nitrogen and ammonia nitrogen was higher in metalimnion and decreased significantly (p<0.05) in the hypoxia area, it suggested there may be a strong denitrification cycling and nitrate nitrogen reduction process in hypolimnion [21].

Our study provides a detailed field observations and statistical comparison of microbial communities in different thermal stratification. Within our experimental samples, the hypolimnion samples showed the highest microbial diversity and richness, while the epilimnion samples had the lowest number of taxa. These data were consistent with recent studies results showing that bacterial communities in the hypolimnion had significantly higher indices in functional diversity than those in the epilimnion [22]. In the present study, microbial communities from different water layers showed obvious differences, especially in the most abundant phylum and genus taxa (Figure 1). It is suggested that different water physicochemical properties may affect the microbial community structure. The RDA results showed the correlations between microbial communities and environmental factors, a
positive correlation between TN and Chlorobi, Acidobacteria, Planctomycetes and Cyanobacteria in hypolimnetic layer. The result indicates the TN content has directly affected the microbial communities of this layer. It is reasonable to explain that the ammoxidation gene of microbial community can convert NH$^+$ to NO$^-$ under aerobic condition, while NO$^-$ can be reduced to N2 by the microbes with denitrification gene under hypoxic conditions [23]. These processes may directly or indirectly affect the presence and transformation of nitrogen elements in water bodies as well as the microbial community distribution.

At present, urban drinking water is more dependent on small or medium-sized reservoirs. The current water resources management problem is water quality monitoring and analysis lags behind water quality management needs. Therefore, we should strengthen the water quality monitoring capacity to meet the local social needs.

5. Conclusions

Miyun Reservoir is a large-scale deep-water reservoir. In summer, the thermal stratification directly hinders the exchange of water, and changes the physical and chemical properties of water quality and microbial community diversity. The RDA analysis showed that the microbial communities in the epilimnion and metalimnion were mainly affected by TN, T, pH and DO, while TN is the key environmental factor which shapes the microbial structure in hypolimnion. Therefore, the reservoir management department should pay attention to the endogenous pollution and strengthen the water quality monitoring capacity.

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