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Microbial community functional diversity and enzymatic activity in the sediments of drinking water reservoirs, Northwest China

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ABSTRACT

Sediment microbial communities act as an important role in the aquatic environmental conditions by influencing nutrient cycling, organic matter metabolism, heavy metal transportation, and organic pollutant transformation. In this study, microbial community functional diversity and enzyme activity in the sediments of two drinking water reservoirs were examined. Sediment bacterial and fungal communities' functional diversity were determined using substrate utilization profiling (BIOLOG) method. Sucrase, dehydrogenase, urease, alkaline phosphatase activities were measured spectrophotometrically. The results showed that sucrase activity in Tang Yu (TY) reservoir was 329.77 mg glucose/g 24 h, which is significant higher than that of Shi Bian Yu (SBY) ($p < 0.01$). Dehydrogenase activities in SBY and TY reservoirs were 63.21 and 104.78 μg TF/g 24 h, respectively. However, there were no significant differences in the alkaline phosphatase activity between SBY and TY reservoirs ($p > 0.05$). Average well color development of bacterial and fungal communities in sediment of TY was higher than that of SBY. The principle component analyses revealed that principle component analyses one and principle component analyses two explained 35.92 and 22.63% of the total variation, respectively. There is a significant different carbon source utilization pattern of bacterial and fungal communities harbored in sediments of SBY and TY reservoirs in northwest China.

Keywords: BIOLOG; Drinking water reservoir; Enzyme activity; Microbial community; Sediment

1. Introduction

Sediment microbial communities act as an important role in the aquatic ecosystem by influencing nutrient cycling, organic matter metabolism, heavy metal transportation, and organic pollutant transformation [1,2]. Previous publications have recorded sediment microbial communities from the sea [3], lake [4–9], river [10,11], wetland [12], freshwater pond [13], and drinking water reservoirs [2,14–17].

Drinking water reservoir, either man made or natural, is the main source of drinking sources water

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supply for the cities in the northwest areas with low groundwater stocks, China [18]. During the past few decades, to ensure the safety of urban water supply, several man made drinking water reservoirs have been built by the Chinese government. The raw water quality of drinking water reservoir is regulated by the complex interactions between surface sediment and overlying water [1]. To monitor the raw water quality of reservoir, much attention has been paid to phosphorus release [1,19], heavy metals and organic contaminants [20] and cyanobacterial water blooms [21]. Wei et al. [22] reported that denitrifying and ammonia oxidizing bacteria played a major role in the process of nitrogen release from the sediments of drinking water reservoir through static simulation experiment. However, little is known about the microbial community functional diversity living in the sediments of drinking water reservoirs.

Sediment microbial community diversity is also closely related to microbial enzyme activities [14]. The mineralization of organic material by microbial communities is initiated by the activities of enzymes. Enzymes are inherently more sensitive to environmental conditions and employed as biological parameter for sediment fingerprinting [23]. But only a few studies of enzymatic activity from the sediment of the reservoir were investigated [14,24]. In this research, therefore, we reported sediment microbial community functional diversity determined using substrate utilization profiling (BIOLOG) method and the activities of enzymes involved in phosphorus (alkaline phosphatase) and nitrogen (urease) cycling from the two drinking water reservoirs in northwest China.

2. Materials and methods

2.1. Samplings program

In this work, two drinking water reservoirs were selected to evaluate the microbial community functional diversity and enzyme activities. Sediment cores were collected from Shi Bian Yu (SBY) and Tang Yu (TY) reservoirs, located in Changan district and Lantian County of Xi'an city, Shaanxi, China. The detail information of the reservoirs was described by Huang et al. [19]. SBY and TY reservoirs play an important role in Xi'an urban water supply. In November 2011, surface sediments (0–30 cm) were collected using a Peterson sampler and transported to the laboratory within 4 h. The sediments were taken in three replicates and then subdivided for BIOLOG and enzyme activity determination. In this work, BIOLOG and enzyme activities were examined within 24 h after sampling.

2.2. Enzyme activity determination

To explore the sediment enzyme activity profiles, four enzyme activities, including sucrase activity, urease activity, dehydrogenase activity, and alkaline phosphatase activity, were determined. The sediment enzyme activities were measured by the method developed by Guan Songyin [25] and little modification. Sucrase activity was examined using sucrose as the substrate. Urease activity was examined using urea as the substrate and measured spectrophotometrically at 578 nm. The sediment dehydrogenase activity was measured by 2,3,5 triphenyl tetrazolium chloride reduction, and the results were expressed as TF μg/(g 24 h). Alkaline phosphatase activity was examined as the amount of phenol, and the activity was expressed as phenol mg/(g 24 h). Sediment enzyme activities were examined with three replicates. Blank assays without sediment suspension and without substrate were also examined at the same time acted as control. The results of sediment enzyme activities were determined on the dry weight (d.w.).

2.3. Microbial community functional diversity determination

To explore the microbial community functional diversity profiles, bacterial and fungal community were measured using BIOLOG ECO and FF microplates (BIOLOG Inc., CA, USA), respectively. According to the methods described by Zak et al. [26] and Zhang et al. [27], sediment samples (10.0 g d.w.) were shaken in 90 mL of sterile 0.85% sodium chloride for 30 min at 120 rpm. The sediment suspending was diluted to 10^{-3}, and 150 μL sediment suspending was added into every well in ECO and FF plates using 8 way electronic pipettes (Bio-Rad, USA). BIOLOG ECO plate contains 31 different carbon sources: ten carbohydrates, two phenolic compounds, four polymers, seven carboxylic acids, two amines, and six amino acids (Table 1). FF plate contains 95 different carbon sources.

The absorbance at 590 nm (ECO) and 750 nm (FF) was recorded every 12-h interval, respectively. Microbial activity in BIOLOG ECO and FF plate was expressed as average well color development (*AWCD*). *AWCD* was assessed using the formula [26]:

$$AWCD = \Sigma(Y - Y_0)/31 \text{ or } 95 \quad (1)$$

Table 1
31 different carbon sources located in BIOLOG ECO plate used in this study

Carbohydrates	Carboxylic acids	Amino acids	Polymers	Phenolic compounds	Amines
D,L-a-Glycerol	Pyruvic acid methyl ester	Arginine	α-Cyclodextrin	4-Hydroxy benzoic acid	Phenyl ethylamine
α-D-L-lactose	γ-Hydroxy butyric acid	Threonine	Glycogen	2-Hydroxy benzoic acid	Putrescine
β-Methyl-D-glucoside	D-Galacturonic acid	Serine	Tween40		
Phosphate	α-Ketobutyric acid	Phenylalanine	Tween80		
i-Erythritol	D-Glucosaminic acid	Asparagine			
D-Cellobiose	D-Malic acid	Glycyl-L-glutamic acid			
D-Mannitol	Itaconic acid				
D-Xylose					
Glucose-1-phosphate					
N-Acetyl-D-glucosamine					
D-Galactonic acid lactone					

Community functional diversity was expressed as species richness (R) and Shannon's diversity (H). R was the number of oxidized carbon substrates in the ECO or FF plates. H was calculated as formula:

$$H' = -\sum_{i=1}^{s} Pi \ln Pi = -\Sigma_{i=1}^{s} (Ni/N) \ln(Ni/N) \quad (2)$$

where Pi was proportional color development of the ith well over total color development of all wells of a plate. According to the $AWCD$ curve, 96-h data were used for principle component analyses (PCA) of bacterial and fungal community diversity.

2.4. Statistical analyses

The data were expressed by the mean and standard errors (SE). Means were compared by one-way analysis of variance and Tukey–Kramer HSD test at the 5% level of significance ($p < 0.05$). PCA was carried out with SPSS (Version 17.0) software for Windows.

3. Results and discussion

3.1. Enzyme activities in the sediments

As shown in Fig. 1(A), sucrase activity in TY reservoir was 329.77 mg glucose/g 24 h, which is significantly higher than that of SBY ($F = 31.85$, $p = 0.0049$). Dehydrogenase activities in SBY and TY reservoirs were 63.21 and 104.78 μg TF/g 24 h, respectively. (Fig. 1(B)) The higher urease activity, 1.024 mg NH_2–N/g 24 h, was recorded with SBY reservoir, which was 5.72% higher than that of TY reservoir, 0.968 mg NH_2–N/g 24 h ($p < 0.05$). However, there were no significant differences in the alkaline phosphatase activity between SBY and TY reservoirs ($F = 0.285$, $p = 0.622$) (Fig. 1(D)).

3.2. Microbial community functional diversity in the sediments

As shown in Fig. 2, $AWCD$ was recorded. $AWCD$ of bacterial community ($AWCD_{590\,nm}$) in sediment of TY was higher than that of SBY (Fig. 2(A)). $AWCD_{590\,nm}$ was lower before early incubation process (0–12 h), $AWCD_{590\,nm}$ was increased rapidly during 24–72 h and steadied after 96 h with 1.75 and 1.58 in TY and SBY reservoirs, respectively. The total $AWCD_{590\,nm}$ in TY was 32.25, 8.55% higher than that of SBY reservoir. No significant different was found in species richness (R) ($p > 0.05$) (Table 2). When fungal community was expressed as $AWCD_{750\,nm}$, the results showed that the total $AWCD_{750\,nm}$ were 0.32 and 0.37 in SBY and TY reservoirs ($p < 0.05$), respectively. The species richness and Shannon's diversity in TY were also higher than that of SBY ($p < 0.05$) (Table 2).

The PCA revealed that PC1 and PC2 explained 35.92 and 22.63% of the total variation for bacterial community, 31.27 and 23.62% for fungal community, respectively (Table 3).

There was a significantly different carbon source utilization pattern in bacterial and fungal communities harbored in sediments of SBY and TY reservoirs

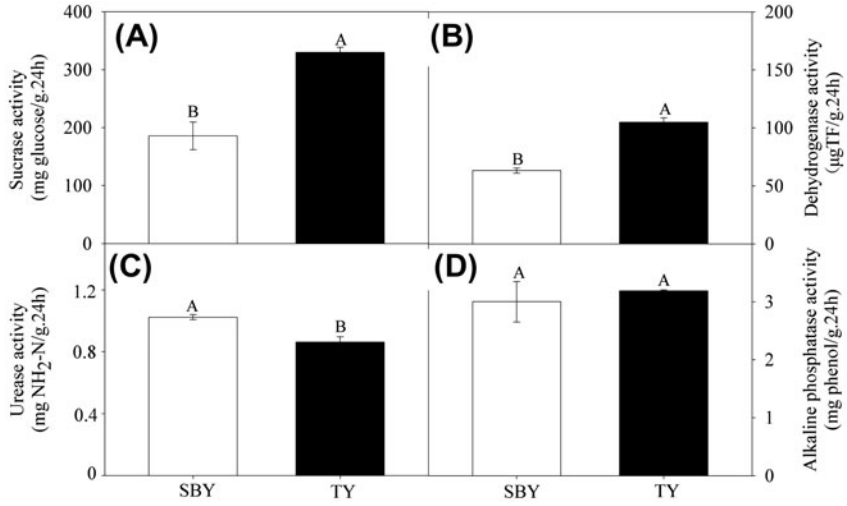

Fig. 1. (A) Sucrase activity, (B) Dehydrogenase activity, (C) Urease activity, (D) Alkaline phosphatase activity in the sediments of SBY and TY reservoirs, respectively. The data shown are the means and standard error ($n = 3$). The same letter indicates no significant difference by Tukey–Kramer HSD ($p < 0.05$).

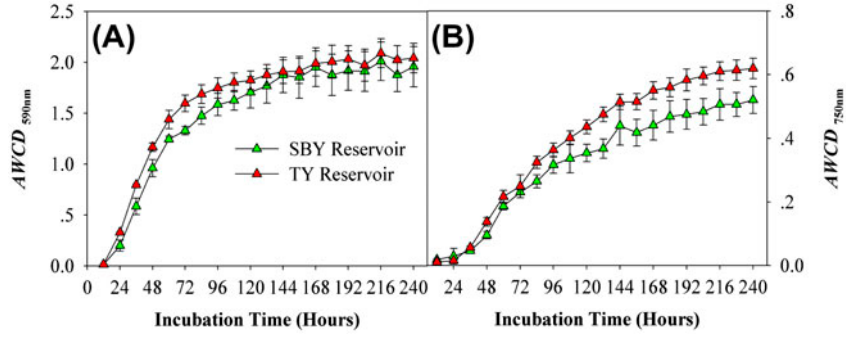

Fig. 2. *AWCD* of (A) bacterial community ($AWCD_{590\,nm}$) and (B) fungal community ($AWCD_{750\,nm}$) in the sediments of SBY and TY reservoirs, respectively. The data shown are the means and standard error ($n = 3$).

Table 2
The $AWCD_{(590\,nm,\ 750\,nm)}$, Species richness ($R$), and Shannon's diversity (H) index of sediment microbial communities in the sediments of SBY and TY reservoirs, respectively

Sediment microbe	Reservoirs	$AWCD_{(590\,nm,\ 750\,nm)}$	Species richness (R)	Shannon's diversity (H)
Bacterial community	SBY	1.58 ± 0.06 B	28 ± 1 A	3.21 ± 0.03 B
	TY	1.75 ± 0.05 A	29 ± 1 A	3.36 ± 0.01 A
Fungal community	SBY	0.32 ± 0.01 B	41 ± 2.31 B	4.11 ± 0.02 B
	TY	0.37 ± 0.01 A	51 ± 1.53 A	4.36 ± 0.03 A

Note: The data shown are the means and standard error ($n = 3$). The same letter indicates no significant difference by Tukey–Kramer HSD ($p < 0.05$).

(Fig. 3). Meanwhile, numbers of carbon substance with loadings $\geqslant 0.50$ grouped by the sediment bacterial and fungal community for principle component analyses one (PC1) and principle component analyses two (PC2) were listed in Table 4.

In this work, the bacterial and fungal community diversity and enzyme activities revealed specific patterns of metabolic potentials in the sediments of SBY and TY reservoirs were evaluated. This study showed that sucrase activity in TY reservoir was

Table 3
The PCA of latent root, percent of variance and percent of total variance of sediment microbial communities in the sediments of SBY and TY reservoirs, respectively

Sediment microbe	Item	Latent root	Percent of variance	Percent of total variance
Bacterial community	PC1	11.134	35.916	35.916
	PC2	7.016	22.631	58.547
	PC3	5.958	19.220	77.767
	PC4	4.378	14.123	91.890
	PC5	2.514	8.111	100
Fungal community	PC1	29.398	31.274	31.274
	PC2	22.198	23.615	54.889
	PC3	16.526	17.581	72.470
	PC4	13.610	14.479	86.949
	PC5	12.268	13.051	100

Note: PCA represents principle component analyses.

329.77 mg glucose/g 24 h, which was significant higher than that of SBY. Dehydrogenase activities in SBY and TY reservoirs were 63.21and 104.78 μg TF/g 24 h, respectively. However, there were no significant differences in the alkaline phosphatase activity between SBY and TY reservoirs. There was a significant different carbon source utilization pattern in bacterial and fungal communities harbored in sediments of SBY and TY reservoirs. The following questions should be discussed.

In aquatic ecosystems, sediment enzymes play an important role in organic matter decomposition and the nutrition biogeochemical cycles, e.g. nitrogen and phosphorus cycling [28]. The esterases, phosphatases, glucosidases, and aminopeptidases activities in sediments of the four reservoirs in Saxony (Germany) were reported by Wobus et al. [14] and suggested that different trophic state and catchment have different sediment enzyme activity. Meanwhile, Curticapean and Dragan-Bularda [24] reported the enzymatic activities of phosphatase, actual and potential dehydrogenase, catalase, urease, and protease from the sediment of the Gilau reservoir were measured and revealed that the highest activities were observed at the tail of the reservoir. Enzyme activity can be, therefore, used as biological characteristics of sediment in reservoir ecosystems.

Microbial community in the sediments of aquatic ecosystem also plays a vital role in monitoring the history of pollution of sea, river, lake, and reservoir [3,4,10,14]. However, compare with the massive literatures on the lakes, the microbial community function diversity in the sediments of reservoir is poorly understood. In this study, we used BIOLOG method to examine the bacterial and fungal communities' functional diversity and found that there was a significantly different carbon source utilization pattern in bacterial and fungal communities harbored in sediments of SBY and TY reservoirs. Notwithstanding its limitation, this study suggests the microbial community functional diversity; the microbial community genetic diversity should be evaluated in the future. In the previous studies, molecular methods were employed to explore the microbial compositions in sediment from aquatic ecosystem. Based on polymerase chain reaction-denaturing gradient gel electrophoresis method, Qu et al. [16] also determined the bacterial diversity in the sediment of Guanting reservoir, China; the results showed that the communities of bacterial types were much different in different

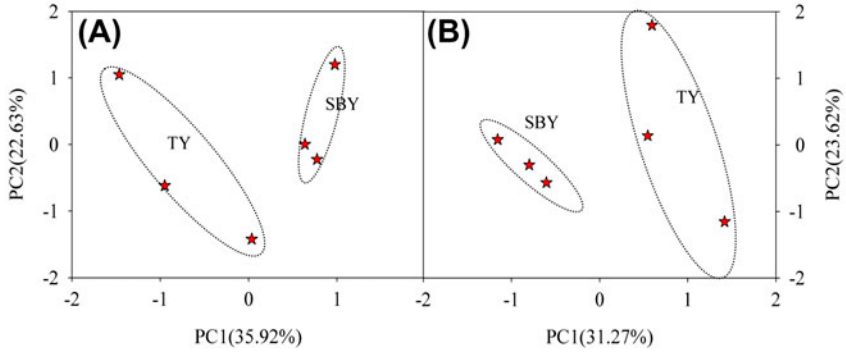

Fig. 3. PCA of (A) bacterial community functional diversity and (B) fungal community functional diversity in the sediments of SBY and TY reservoirs, respectively. (A) Data were calculated based on substrate utilization pattern using BIOLOG ECO micro plates after incubation of 96 h. PC1 explained 35.92% of the total variance, while PC2 explained 22.63%. (B) Data were calculated based on substrate utilization pattern using BIOLOG micro FF plates after incubation of 96 h. PC1 explained 31.27% of the total variance, while PC2 explained 23.62%.

Table 4
Numbers of substance with loadings ⩾0.50 grouped by the sediment bacterial and fungal community for PC1 and PC2

Sediment microbial communities	Carbon sources	Principle component analyses one (PC1)	Principle component analyses two (PC2)
Bacterial community	Carbohydrates	5	3
	Carboxylic acids	5	2
	Amino acids	4	2
	Polymers	3	1
	Phenolic compounds	1	0
	Amines	2	0
Fungal community	Carbohydrates	17	8
	Carboxylic acids	10	6
	Amino acids	11	4
	Polymers	1	0
	Phenolic compounds	0	0
	Amines	1	0

layers of sediments. However, the structure compositions of specific functional microbial communities such as anaerobic ammonia oxidation bacteria, aerobic denitrifying bacteria, and methanobacteria should be also determined in the future.

4. Conclusions

The present work showed that sucrase activity in TY reservoir was 329.77 mg glucose/g 24 h, which is significant higher than that of SBY. Dehydrogenase activities in SBY and TY reservoirs were 63.21 and 104.78 μg TF/g 24 h, respectively. However, there were no significant differences in the alkaline phosphatase activity between SBY and TY reservoirs. *AWCD* of bacterial and fungal communities in sediment of TY was higher than that of SBY. The PCA revealed that PC1 and PC2 explained 35.92 and 22.63% of the total variation, respectively. There is a significant different carbon source utilization pattern of bacterial and fungal communities harbored in sediments of SBY and TY reservoirs in northwest China.

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