

MICROBIAL COMMUNITY STRUCTURE DURING THE RESTORATION OF WULIANGSUHAI SEDIMENT

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Abstract

In this study, the sediment in the process of in-situ restoration was taken as the research object. Based on high-throughput sequencing, microbial community structure in sediment at 2 and 6 months was analyzed. The results showed that the dominant bacterial phyla in the repair process included Proteobacteria, Bacteroidota, Chloroflexi and Desulfobacterota. The dominant phyla of fungi changed from Ascomycota, Basidiomycota and Mortierellomycota to Ascomycota. *Thiobacillus* is the dominant bacterium in the genus. The dominant strain of fungi has changed from *Solicoccozyma* and *Mortierella* to *Exophiala*. Redundancy analysis showed that COD was the main environmental factor affecting bacterial communities (Proteobacteria and Bacteroidota). TP is the main environmental factor affecting the sediment fungal community. This study provides a theoretical basis for the development of in-situ remediation technology of Wuliangsuhai sediment.

Introduction

Wuliangsuhai is located in Bayannaoer City, Inner Mongolia. It is the largest lake wetland in the Yellow River basin. It is an important part of the irrigation and drainage system of Hetao Irrigation Area in Inner Mongolia and it is also a barrier to protect the ecological security in northern China (Mao *et al.* 2020, Shi *et al.* 2020). The water area of the Wuliangsu Sea is 293 km². Then plankton increase, aquatic plants and floating algae debris increase, sediment accumulation. As a result, the eutrophication of Wuliangsuhai becomes more diversified and the water environment continues to deteriorate (Shi *et al.* 2023, Liu *et al.* 2024).

In situ remediation and ectopic remediation are the main techniques for treating sediment in polluted water bodies. Sediment dredging in ectopic treatment technology can remove sediment quickly and improve water quality. However, there are some problems such as high cost and difficult transportation, and limited by time and space. There is also a risk of secondary pollution (Fan *et al.* 2020). It is common to add chemical reagents in in-situ treatment technology, but secondary pollution may be introduced to increase the toxicity of water (Zhang *et al.* 2021). The ecosystem of Wuliangsuhai is fragile and direct dredging will destroy the animals, plants and microorganisms in the lake, and add uncertainty and management costs to re-architecting ecosystems. Microbial in situ remediation is ideal for water purification and water quality improvement. Microbial community is an important component of aquatic ecological environment in the process of in-situ sediment restoration. Microorganisms play an important role in removing refractory pollutants and reducing nitrogen and phosphorus nutrients (Wu *et al.* 2018, Liu *et al.* 2020,). It is the key to self-purification of water body. The water environment also affects the microbial community structure or abundance to some extent. It is important to understand the distribution characteristics of bacterial and fungal communities in the process of sediment restoration for water environment restoration.

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The in-situ restoration process of the Wuliangsuohai submarine mud consists of three stages such as "Microbial repair" and "benthic animal repair", "Fish repair". In view of the internal pollution of Wuliangsuohai, this study introduced complex enzyme technology in the "microbial remediation" stage. The complex enzyme was fermented by microbial dynamic cycle in lake area. Microorganisms use metabolites and secretions as the growth substrate between each other. After symbiosis, a diverse microbial community with stable structure and extensive energy resources is formed. Activation of beneficial microorganisms in sludge environment by catalytic action of the enzymes associated with the bacterial community and promote the circulation of substances in the lake area.

This research work mainly focuses on the status quo of water environment, pollution characteristics and comprehensive treatment. The community composition and diversity of bacteria and fungi in the sediment in this area will be analyzed to provide reference for microbial remediation technology of polluted water and sediment of Wuliangsuohai.

Materials and Methods

A relatively closed water ecosystem was built in the heavily polluted area of the submarine mud in Wuliangsuohai. The test area is 1000 m² of water. *In situ* remediation of polluted water and sediment was carried out in the system by using complex enzyme technology. In this study, four sampling sites (DN1, DN2, DN3 and DN4) were selected in the test area. Reference points (CK1, CK2 and CK3) that did not use the complex enzyme technique were selected outside the test area. Collect the bottom mud and remove the debris (stones, rotten leaves, etc.). Then these were sealed and refrigerated.

There are four main physical and chemical indexes in the sediment: pH, COD, total phosphorus and total nitrogen. Microbial purification systems were tested at two and six months of operation. pH was measured with PHS-3E pH meter. COD was determined by potassium dichromate method. Total phosphorus was determined by ammonium molybdate spectrophotometer. Total nitrogen was determined by ultraviolet spectrophotometer with potassium sulfate digestion.

High-throughput sequencing of sediment microbial communities was conducted after 2 and 6 months of operation of the microbial purification system. DNA extraction, amplification, and sequencing of bacterial 16S rRNA and fungal ITS genes were performed by Nanjing Jisi Huiyuan Biotechnology Co., Ltd. (Nanjing, China). Sequence data were processed using the QIIME2 (version 2021.11) platform with the DADA2 pipeline. The raw sequences underwent quality control steps including primer trimming, quality filtering, denoising, sequence merging, and chimera removal, resulting in high-quality amplicon sequence variants (ASVs). For bacterial community analysis, the SILVA 138 rRNA database was used for taxonomic assignment of ASV representative sequences. For fungal community analysis, the UNITE database was employed for ITS sequence annotation. Taxonomic classification was performed at the phylum, class, order, family, genus, and species levels. Based on the relative abundance and annotation of ASVs, the composition and abundance of bacterial and fungal taxa were calculated, and species abundance histograms were generated at both the phylum and genus levels. Alpha diversity indices, including Ace, Chao1, Shannon, and Simpson, were calculated using QIIME2 to assess the bacterial and fungal community diversity within samples.

Results and Discussion

The results of bacterial sequencing were classified and analyzed at the gate level. After 2 months of operation of the system (Fig. 1a), the dominant bacteria in the sediment was

Proteobacteria, Bacteroidota, Chloroflexi and Desulfobacterota. Rounding out the top 10 are Proteobacteria (26.09%), Bacteroidota (14.04%), Chloroflexi (11.52%), Desulfobacterota (11.28%), Acidobacteriota (5.07%), Planctomycetota (5.01%), Verrucomicrobiota (2.72%), Latescibacterota (2.38%), Firmicutes (2.36%) and Spirochaetota (2.26%). After 6 months of operation of the system (Fig. 1b), the species of dominant bacteria in the sediment did not change, but their proportion changed. Rounding out the top 10 are Proteobacteria (31.58%), Bacteroidota (14.45%), Chloroflexi (11.59%), Desulfobacterota (8.70%), Acidobacteriota (5.72%), Planctomycetota (3.78%), Firmicutes (2.48%), Latescibacterota (2.22%), Spirochaetota (1.59%) and Sva0485 (1.37%).

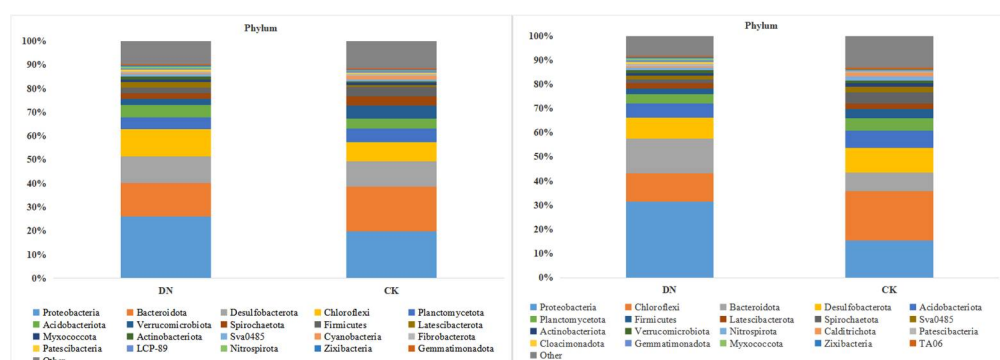


Fig. 1. Horizontal bacterial species abundance histogram.
a : 2 months b : 6 months

Gate level classification analysis was performed on the fungal sequencing results. After the system was run for 2 months (Fig. 2a), the dominant bacteria in the sediment were Ascomycota, Basidiomycota, and Mortierellomycota. Rounding out the top five are Ascomycota (39.04%), Basidiomycota (30.74%), Mortierellomycota (20.96%), Rozellomycota (1.96%) and Glomeromycota (1.36%). After 6 months of operation of the system (Fig. 2b), the dominant phyla became Ascomycota and Rozellomycota, and their proportions changed greatly. Rounding out the top five are Ascomycota (62.62%), Rozellomycota (7.65%), Mortierellomycota (2.13%), Basidiomycota (0.97%) and Aphelidiomycota (0.69%).

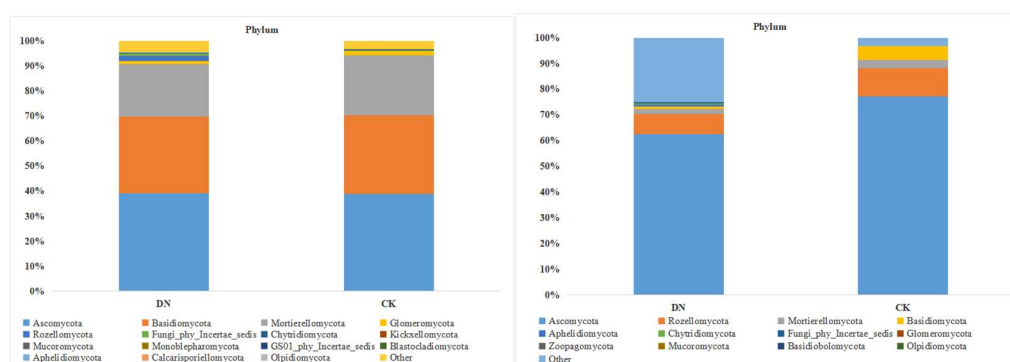


Fig. 2. Horizontal fungal species abundance histogram.

Compared with 2 months, Ascomycota and Rozellomycota significantly increased, while Basidiomycota and Mortierellomycota significantly decreased. The bacterial sequencing results were classified and analyzed at the genus level. After 2 months of system operation (Fig. 3a), the dominant bacterium in the sediment was *Thiobacillus*. The top five bacterial species were *Thiobacillus* (8.88%), *Bacteroidetes_vadinHA17* (3.39%), *Aminicenanales* (2.27%), *Sva0081_sediment_group* (2.15%) and *JTB255_marine_benthic_group* (1.75%). After 6 months of operation of the system (Fig. 3b), the species of dominant bacteria in the sediment did not change, but their proportion changed slightly. The top five bacterial species were *Thiobacillus* (9.44%), *Aminicenanales* (2.84%), *Bacteroidetes_vadinHA17* (2.65%), *JTB255_marine_benthic_group* (2.01%) and *Desulfatiglans* (1.61%).

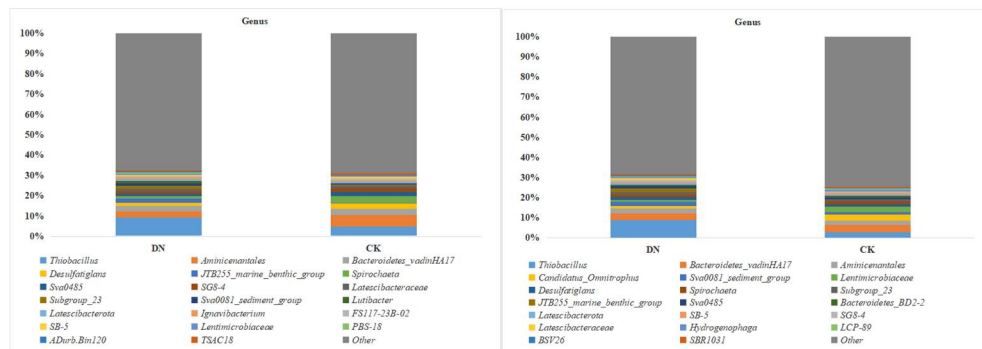


Fig. 3. Histogram of species abundance of bacteria at genus level.

Genus level classification analysis was carried out on the fungal sequencing results. After two months of system operation (Fig. 4a), the dominant bacteria in the sediment were *Solicoccozyma* and *Mortierella*. The top five strains were *Solicoccozyma* (28.40%), *Mortierella* (13.61%), *Fusarium* (3.70%), *Metarhizium* (3.12%) and *Pyrenochaetopsis* (2.47%). After 6 months of operation of the system (Fig. 4b), the dominant bacteria in the sediment became *Exophiala* and *Rozellomycota_gen_Incertae_sedis*. The top five strains were *Exophiala* (40.28%), *Rozellomycota_gen_Incertae_sedis* (7.54%), *Epicoccum* (4.15%), *Mortierella* (2.05%) and *Abrothallus* (1.91%). Compared with 2 months, the species and proportion of dominant bacteria changed greatly. *Exophiala* and *Rozellomycota_gen_Incertae_sedis* increased dramatically, replacing *Solicoccozyma* and *Mortierella* as dominant bacteria.

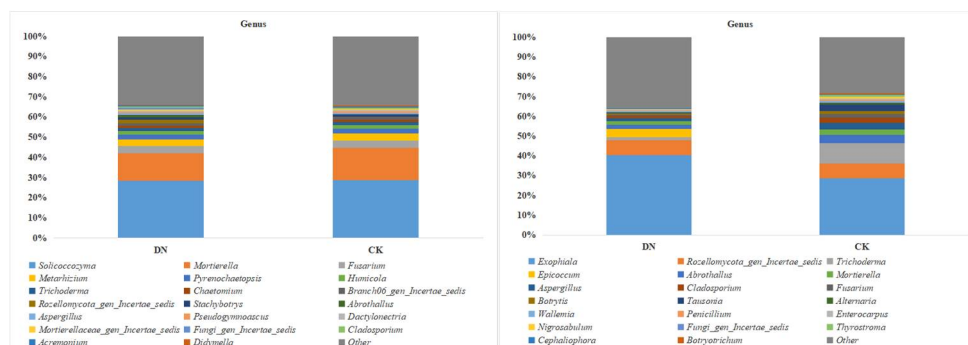


Fig. 4. Histogram of species abundance of horizontal fungi in genera.

In this study, microbial diversity index is still used as an important index to reflect the characteristics of microbial community structure. The Alpha diversity index of the sediment bacterial community was analyzed, and the statistical results were shown in Table 1. Goods coverage index is close to 1. The depth of sequencing basically covered all the species in the sample. From the higher Ace index and chao1 index, it can be seen that the richness and quantity of bacterial communities in the sediment are relatively high. It was found that the richness and quantity of the system decreased after 6 months compared with 2 months. Shannon index and Simpson index can reflect community diversity. In Table 1, the Shannon and Simpson indexes are close to each other and maintain a high level.

Table 1. Statistical analysis of bacterial Alpha diversity in sediment.

Month	Sample name	Ace index	Chao1 index	Simpson index	Shannon index
2	DN1	2471.15	2470.75	0.9973	10.09
	DN2	2036.32	2032.71	0.9968	9.82
	DN3	2972.06	2967.18	0.9991	10.72
	DN4	2954.20	2951.72	0.9991	10.74
	CK1	2913.16	2907.98	0.9991	10.65
	CK2	3010.40	3002.81	0.9991	10.69
	CK3	2546.00	2546.00	0.9989	10.40
6	DN1	826.94	826.22	0.9941	8.46
	DN2	899.05	898.48	0.9932	8.54
	DN3	977.32	976.88	0.9970	9.03
	DN4	902.78	901.85	0.9963	8.85
	CK1	827.44	827.04	0.9963	8.73
	CK2	1077.20	1073.76	0.9974	9.16
	CK3	933.52	932.43	0.9967	8.92

Table 2. Statistical analysis of fungal Alpha diversity in sediment.

Month	Sample name	Ace index	Chao1 index	Simpson index	Shannon index
2	DN1	433.34	433.09	0.8959	5.18
	DN2	478.00	478.00	0.9223	5.77
	DN3	447.21	447.36	0.8941	5.21
	DN4	454.47	456.50	0.8911	5.16
	CK1	431.63	431.25	0.8903	5.14
	CK2	422.49	422.06	0.8937	5.17
	CK3	405.21	405.00	0.9037	5.17
6	DN1	369.38	369.33	0.9237	5.58
	DN2	420.00	420.00	0.9334	5.86
	DN3	452.27	452.09	0.7967	4.29
	DN4	6.33	7.00	0.0701	0.23
	CK1	8.00	6.33	0.2435	0.68
	CK2	406.15	406.00	0.8953	4.99
	CK3	545.14	545.00	0.9267	5.78

Similarly, Alpha diversity analysis can reflect the richness and diversity of fungal communities. The statistical results of fungal Alpha diversity in sediment are shown in Table 2. Goods coverage index is close to 1. It reflects that the sequencing depth basically covers all the

species in the sample. In the process of system operation, the Ace and chao1 indices are higher, which indicate that the abundance and quantity of fungal communities in the sediment are higher. In Table 2, the shannon and simpson indexes of each point are close to each other and maintain a high level.

The results of the main physical and chemical indexes of the sediment (Table 3) show that the sediment is weakly alkaline. During the operation of the system, COD decreases obviously, while TP and TN decrease slightly. Variance analysis of the data showed that COD of sediment was significantly different ($p < 0.05$), while pH, TP and TN were not significant ($p > 0.05$).

Table 3. Main physical and chemical indexes of sediment.

	pH	COD	TP	TN
DN	$8.78 \pm 0.087a$	$63.25 \pm 35.03b$	$0.423 \pm 0.053a$	$2.73 \pm 1.61a$
CK	$8.50 \pm 0.43a$	$235.00 \pm 23.65a$	$0.457 \pm 0.3a$	$3.06 \pm 0.96a$
p	0.26	0.001	0.827	0.765

Different lowercase letters indicate significant difference ($p < 0.05$).

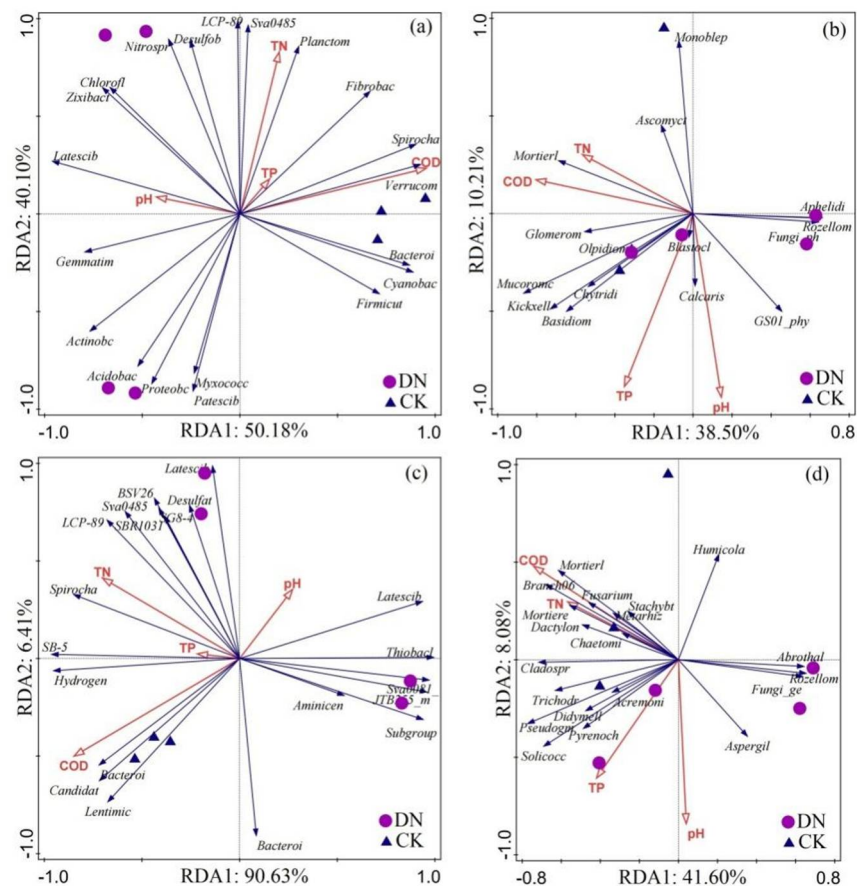


Fig. 5. RDA redundancy analysis of microbial community composition and environmental factors after 2 months: (a) Bacteriophyla level, (b) Mycophyla level, (c) Bacterial level and (d) Fungal level.

COD, pH, TP and TN were selected for redundancy analysis (RDA) at gate level and genus level. The RDA analysis results after 2 months of system operation are shown in Fig. 5. The eigenvalues of the first and second axis were 50.18 and 40.10%, respectively (Fig. 5a). The cumulative explanatory variables account for 90.28% of the total eigenvalues. Among the groups with the highest horizontal abundance of phyla, Proteobacteria was positively correlated with pH. Bacteroidota showed significant positive correlation with COD and TP. There was a positive correlation between Chloroflexi and TN. There was a significant positive correlation between Desulfobacterota and TN and TP. The eigenvalues of the first and second axes of the genus were 90.63 and 6.41%, respectively. The cumulative explanatory variables account for 97.04% of the total eigenvalues. *Thiobacillus* was significantly positively correlated with pH in bacteria with high horizontal abundance. *Bacteroidetes vadinHA17* had a significant positive correlation with COD and TP. *Aminicenantales* and *Sva0081* sediment group were positively correlated with pH. The eigenvalues of the first and second axes were 38.50 and 10.21%, respectively. The cumulative explanatory variables account for 48.71% of the total eigenvalues. Ascomycota and Mortierellomycota showed significant positive correlation with COD and TN among the most abundant fungi. Basidiomycota were positively correlated with pH, COD and TP. The first and second rank of fungi were 41.60 and 8.08% respectively. The cumulative explanatory variables accounted for 49.68% of the total eigenvalues. In the most abundant fungi, *Solicozozyma* was positively correlated with pH and TP, as well as COD and TN. *Mortierella* showed significant positive correlation with COD and TN.

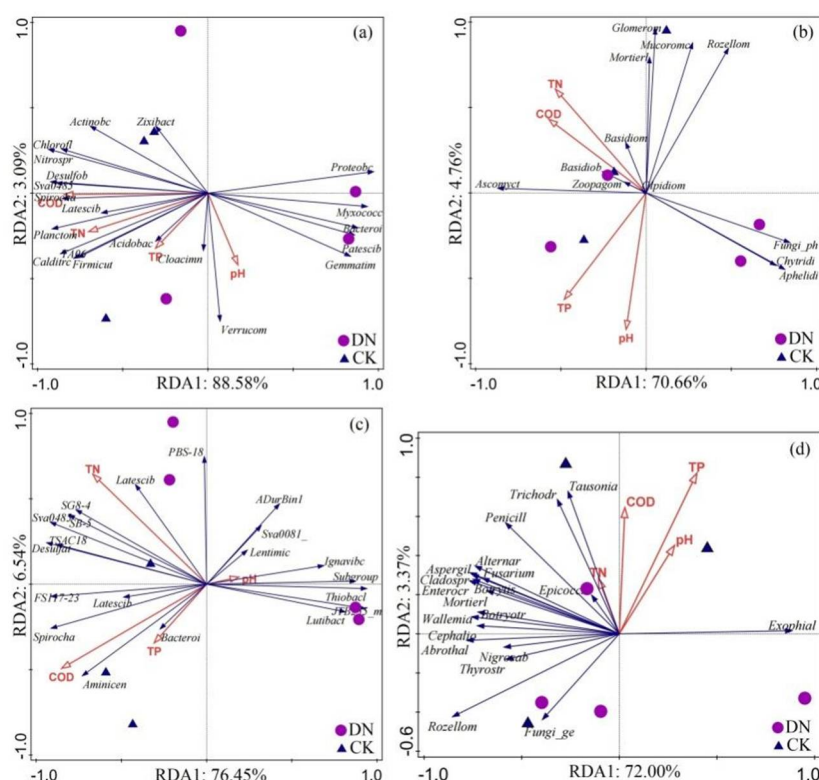


Fig. 6. RDA redundancy analysis of microbial community composition and environmental factors after 6 months: (a) Bacteriophyla level, (b) Mycophyla level, (c) Bacterial level and (d) Fungal level.

The RDA analysis results after 6 months of system operation are shown in Fig. 6. The eigenvalues of the first and second axes were 88.58 and 3.09%, respectively. The cumulative explanatory variables account for 91.67% of the total eigenvalues. Among the groups with the highest horizontal abundance, Proteobacteria and Bacteroidota were positively correlated with pH. Both Chloroflexi and Desulfobacterota were positively correlated with COD and TN. The eigenvalues of the first and second ordering axes were 76.45 and 6.54%, respectively. The cumulative explanatory variables account for 82.99% of the total eigenvalues. In bacteria with high horizontal abundance, *Thiobacillus* was positively correlated with pH. *Bacteroidetes vadinHA17* and *Aminicenantales* had significant positive correlation with COD and TP. *Sva0081 sediment group* is positively correlated with pH. The eigenvalues of the first and second axes were 70.66 and 4.76%, respectively. The cumulative explanatory variables account for 75.42% of the total eigenvalues. Ascomycota had a significant positive correlation with COD and TN, and a positive correlation with pH and TP. There was a positive correlation between Rozellomycota and TN. The eigenvalues of the first and second axes of fungi were 72.00 and 3.37%, respectively. The cumulative explanatory variables account for 75.37% of the total eigenvalues. *Exophiala* was positively correlated with COD, pH and TP among the most abundant fungi.

In the process of sediment restoration, the dominant phylum genus of bacteria in the bacterial community include Proteobacteria, Bacteroidota, Chloroflexi and Desulfobacterota, and the dominant bacteria are *Thiobacillus*. Proteobacteria is the best category in most sediment studies. It is also a widely existing category in current studies (Gao *et al.* 2017, Cai *et al.* 2020, Zheng *et al.* 2024). The research results of Chen *et al.* (2024) show that Proteobacteria always occupy a dominant position in the process of sewage purification. Li *et al.* (2024) found that Proteobacteria, Chloroflexi and Desulfobacterota were dominant bacteria in the bottom mud of Jinyinhu Lake in Wuhan City. The results are similar to the above results for the main bacterial communities in the submarine mud of Wuliangshuai. Liu (2017) analyzed the microbial community changes in the anaerobic dechlorination process of PCBS in the sediment of Taihu Lake. It was found that Proteobacteria, Bacteroidota, Chloroflexi and Firmicutes were the main microorganisms in the process. It is consistent with the dominant bacteria in the restoration process of Wuliangshuai submarine mud.

Ascomycota always existed as the dominant phyla of the fungal community in the process of sediment restoration and the proportion of sediment in the sediment is increasing over time. After 6 months, the dominant bacteria gradually changed from *Solicoccozyma* of Ascomycota to *Exophiala* of this phylum. Wei *et al.* (2022) studied the community structure of sediment fungi in Qianwei Zhihui National Wetland Park. The dominant bacterial groups were Ascomycota and Basidiomycota. It is partially consistent with the dominant bacteria in this study.

Proteobacteria, mainly Gram-negative bacteria, is the main bacteria in the biological nitrogen and phosphorus removal process of sewage treatment. This phylum contains a large number of bacteria involved in the C and N cycles (Nguyen *et al.* 2011, Wang *et al.* 2019). Both Proteobacteria and Chloroflexi contain a large number of bacteria that can complete the nitrification and denitrification processes (Emily *et al.* 2014). They play an important role in nitrogen metabolism of sediment. Chloroflexi can perform photosynthesis and are bacteria capable of producing energy (Xian *et al.* 2020). In the classification of bacterial genera, the genus with the highest proportion is *Thiobacillus*. It has vulcanization and de-vulcanization. As an important component of soil microorganisms, most fungi live saprophytically. Fungi are important decomposers of organic matter and play an important role in the material cycle. Ascomycota in the fungal group are mainly composed of saprophytic bacteria. However, saprophytic bacteria are the main groups of bacteria that degrade complex organic matter and participate in nutrient cycling (Wang *et al.* 2024). The variance analysis of physical and chemical indexes of sediment showed

that COD of sediment was significantly different. This indicates that sediment bacteria and fungi actively participate in the physiological and metabolic activities of sediment ecosystem. Due to these metabolic activities of the microbial community, water purification is promoted. The COD of the sediment decreased significantly and the TP and TN also decreased.

Previous studies have shown that environmental factors such as pH, nutrient content, physical and chemical properties of sediment affect the composition and diversity of microbial communities. Microbial community structure in sediment can be regulated. During the operation of this research system, COD, TP and TN are reduced to different degrees. The combination of Ace index and chao1 index also showed a downward trend. The results indicated that the physical and chemical properties of sediment affected the richness of microbial community.

The horizontal redundancy analysis showed that Proteobacteria was negatively correlated with COD, TP and TN. During the operation of this research system, the abundance value of Proteobacteria increased with the decrease of COD, TP and TN. The reason is that Proteobacteria contain a large number of soil bacteria that can participate in the C and N cycles. Increased abundance speeds up nutrient consumption in the sediment and intensifies competition between bacterial communities. Bacterial diversity showed a downward trend. There was always a positive correlation between pH and Proteobacteria. Zhang *et al.* (2024) studied the relationship between phosphorus form release and microbial community structure in Nansi Lake. Proteobacteria were also found to be positively correlated with TP. Bacteroidota were positively correlated with COD at 2 months and negatively correlated with COD at 6 months. It can be seen that Bacteroidota are greatly affected by COD. Bacteroidota can participate in denitrification process and plays an important role in nitrogen metabolism. The TN content in the sediment decreased but changed little during the experiment, which may be caused by the environmental influence of Bacteroidota. The results of redundancy analysis of bacterial genera showed that *Thiobacillus* was positively correlated with pH and negatively correlated with COD, TP and TN. The increment of *Thiobacillus* abundance was small in the process of sediment restoration. It shows that the sediment environment is not conducive to its growth. However, this bacterium can oxidize sulfide, hydrogen sulfide and other sulfur-containing compounds to produce sulfuric acid and other substances. It increases the acidity of the sediment and plays an important role in the removal of organic matter.

The horizontal redundancy analysis of phyla showed that TP was an important environmental factor affecting the sediment fungal community. For Ascomycota, there was a negative correlation with TP and pH at 2 months and a positive correlation with TP and pH at 6 months. Ascomycota abundance increased significantly from 39.04 to 62.62%. For Basidiomycota, there was a significant positive correlation with TP at 2 months and a negative correlation with TP at 6 months. Its abundance value also decreased from 30.74 to 0.97%. It is suggested that the phosphorus content of the sediment is very important for the reproduction of Ascomycota and Basidiomycota. In addition, the correlation between fungal community and COD was significant during sediment restoration. Ascomycota, Basidiomycota and Mortierellomycota were positively correlated with COD. Because the saprophytic fungi of Ascomycota can effectively degrade complex organic matter in the sediment, the COD of the sediment decreases significantly after 6 months. The results of fungal level redundancy analysis also confirmed that TP is an important environmental factor affecting the sediment fungal community. *Solicozozyma* as the dominant genus, was significantly positively correlated with TP at 2 months, but not detected at 6 months. *Exophiala* was not detected at 2 months, while COD, pH and TP were positively correlated at 6 months and the abundance value increased to 40.28%.

In this study, high-throughput sequencing was used to study the structure of bacterial and fungal flora in the restoration process of Wuliangshuai submarine mud. The results of species

abundance showed that the dominant bacterial groups at the phyla level included Proteobacteria Bacteroidota, Chloroflexi and Desulfobacterota. The dominant phyla of fungi changed from Ascomycota, Basidiomycota and Mortierellomycota to Ascomycota. At the generic level, *Thiobacillus* dominated the bacteria. The fungi gradually changed from *Solicoccozyma* and *Mortierella* to *Exophiala*. There is an interaction between the microbial community and the sediment environment. The variance analysis of main physical and chemical indexes of sediment showed that COD of sediment was significantly different, while pH, TP and TN were not significant. RDA redundancy analysis showed that different physical and chemical properties of sediment had significantly different effects on different microbial communities. pH, COD, TP and TN are important environmental driving factors affecting microbial community changes in water bottom mud. COD is the main environmental factor affecting bacterial communities (Proteobacteria and Bacteroidota). TP is the main environmental factor affecting the sediment fungal community. During the operation of this research system, the metabolism of the sediment bacterial community and fungal community was vigorous. These metabolic activities promote the metabolism of water ecosystem and play a positive role in restoring water sediment.

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