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To cite this article: Ming Sui *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **252** 052152

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Response Mechanism of Microbial Communities in River Sediments under Different Hydrodynamic Conditions

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Abstract. This paper studies the response of sedimentary microbial communities to different hydrodynamic conditions through laboratory experiments. The hydrodynamic conditions and flow field distribution in the experimental tank were measured and simulated by acoustic Doppler flowmeter and FLUENT software. The sediment DNA of different culture time and different flow velocity points was extracted and amplified by PCR amplification and high-throughput sequencing. The OTU sequence of the microbial organism.

1. Introduction

The river bottom microbial community is an important part of the river ecosystem, which will affect the biogeochemical cycle of the entire river, and it is sensitive to changes in environmental conditions, changes in organic matter concentration, pH, temperature and hydrodynamic conditions in the river. It will have an impact on the composition of the microbial community. [1-4] Among them, hydrodynamic conditions can reflect the characteristics of rivers, and the construction of water conservancy projects and the application of water diversion and pollution control technologies will have a greater impact on the hydrodynamic conditions of rivers. Therefore, the response of river bottom microbial communities to hydrodynamic conditions can be studied. Provide scientific guidance and basic data for water conservancy construction and river ecological management. [5-9]

2. Experimental method

The bottom of the experiment was made of natural river bottoms. Use the grab mud to obtain the upper sediment, remove the branches, leaves, stones and other impurities in the sediment and evenly lay them in the second and third sections of the tank. The thickness of the sediment is 3cm.

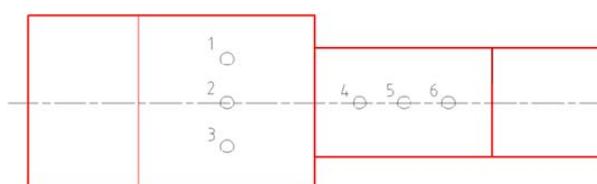
The overlying water is also taken from the Hanzhong section of the Qinhuai River, and the sampling point is the same as the sampling point of the sediment. There are two purposes for using natural river water: a). Microorganisms present in river water are important sources of inoculation for sediment microorganisms; b). Natural river water can provide certain nutrients for the growth of microorganisms. However, it has been found through pre-experiment that only the nutrients in the river water can be used, and the sediment will rapidly become sandy. After reviewing the data, it may be that the nutrients in the river water are relatively poor, and the experiment uses circulating water, so the water Nutrients are quickly depleted and the underlying microbes begin to be caused by endogenous respiration. Therefore, this experiment uses the method of regularly adding nutrient solution to ensure sufficient nutrients in the system. The specific composition of nutrient solution is shown in Table 1. The experimental design has a water depth of 13 cm.



Table 1. Nutrient ratio

Reagent	glucose	KH ₂ PO ₄	NaCl	MgSO ₄	NH ₄ Cl	CaCl ₂
concentration	50mg/L	500mg/L	500mg/L	50mg/L	100mg/L	15mg/L

The experiment added a nutrient solution every three days, and each time 100 ml was added to ensure sufficient nutrients in the system. The overlying water is changed once a week. Samples were taken at different points in the trough every half month, and 1.5 g each was taken for subsequent extraction of DNA from the sediment. The sampling points are shown in Figure 1. Fang et al. showed that the biomass of the substrate microorganisms peaked after 30-42 days of culture, so the experiment lasted for 30 days.

**Figure 1.** Sink sampling point schematic

3. Flow rate and related hydrodynamic condition distribution

The flow rate in the water tank was measured by a three-dimensional ultrasonic Doppler velocimeter (FlowTracker2) produced by SonTek, USA. The measurement position was 1 cm above the surface of the substrate. The sampling time for setting ADV for each point was 40 s, and the sampling frequency was 50 Hz. The signal strength is kept above 15dB. In order to obtain more flow gradients, two experimental water tanks were set up in parallel, and two submersible pumps with different flow rates were used to circulate water. The pump flow rates were 16000 L/h and 9000 L/h, respectively. The widths of the second and third sections of the tank are different, so the flow rate is also different. The second segment has a smaller width and a corresponding flow velocity; the third segment has a larger width and a corresponding flow rate is smaller. Each sampling tank has 6 sampling points, 3 in each section. The specific flow rate of the sampling point is measured by ADV, and the sampling point number is shown in Fig. 1.

Through the relevant calculation methods, the specific conditions of the Reynolds number, turbulence intensity and turbulent flow energy corresponding to each sampling point of the water tank are shown in Table 2 and Table 3.

Table 2. Hydrodynamic distribution of sampling point of 16000L/h water flow

	1	2	3	4	5	6
Re	4605.310074	7848.62	4539.833	10338.35	11984.52	10489.7
I	0.05574586	0.052152	0.060051	0.050386	0.049464	0.050295
TKE	2.26E-05	5.73E-05	7.96E-06	1.65E-04	2.14E-04	1.69E-04

It can be seen from the existing hydrodynamic conditions that the three-dimensional flow velocity generally shows that the second segment is larger than the third segment, mainly due to the difference in the width of the two segments, so the actual hydrodynamic conditions are consistent with expectations. In the third paragraph, the flow velocity of the sampling point No. 2 is obviously larger than that of the No.1 and No.3 points. This is mainly because the flow of water from the second segment into the third segment has more streamlines passing through the No. 2 point, so the hydrodynamic conditions are also better.

4. Cluster analysis of microbial communities in different hydrodynamic conditions

In order to more intuitively see the relationship between the substrate microbial community and the flow rate, this experiment has clustered the OTU composition of the substrate microorganisms. Four clustering analyses were performed on the existing experimental data, that is, the cluster composition of the large and small flow troughs on the 15th and 30th day were clustered.

This paper further explores the trace distribution map of the water tank. From the simulation diagram of the flow field, it is easy to see that there are several traces passing through the 2, 4, and 5 points at the same time, but since the 6th point is closer to the inlet section, Therefore, the flow field at this point is more disordered, so the consistency with other points is not good. Therefore, the distribution law of the traces is in good agreement with the results of the cluster analysis. Therefore, it can be easily inferred that the flow field or the distribution of the traces is an important factor affecting the microbial community.

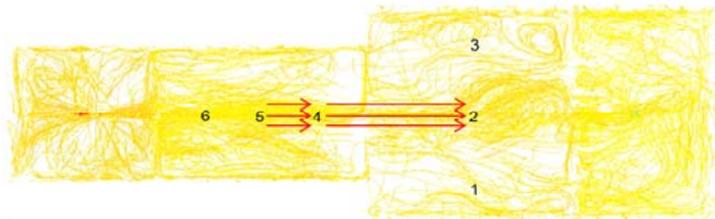


Figure 2. Trace distribution of 2, 4, and 5 points of flow tank 9000L/h

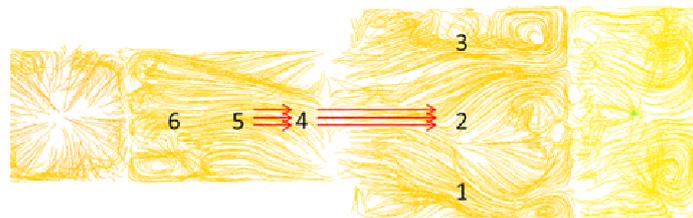


Figure 3. Trace distribution of 2, 4, and 5 points of flow rate 16000L/h

In addition to the cluster analysis results of points 2, 4, and 5, there is another phenomenon that can support the conclusion that the flow field affects the distribution of microbial communities. The No. 1 and No. 6 points of the large flow tank showed high similarity in both the 15 and 30 day samplings. However, the 1st and 6th points are the two points with the longest spatial distance among the 6 sampling points, and the hydrodynamic conditions such as the flow velocity of these two points are also quite different, so the two points according to the previous assumptions The similarity between bits should be poor. However, it can be seen from the analysis of the flow field of the water tank that several strands are connected to the No. 1 and No. 6 points. Therefore, due to the action of the water flow, the microbial community at the No. 1 point migrates to the No. 6 point. The possibility of position is very large, which may also be the reason for the high similarity of microbial community composition at these two points.

5. Phenotypic analysis of microbial community in different hydrodynamic conditions

Phenotypes refers to the characteristics and characteristics exhibited by an individual, including shape, development, physical and chemical properties, behavior and behavioral products. The difference in characteristics caused by gene expression is called the gene phenotype. In the BugBase software, microbial abundance alignment data can be used to predict microbial phenotypes in different samples.

We used BugBase software to perform phenotypic analysis of the substrate microbial communities at different flow rates, and obtained aerobic, anaerobic, facultative anaerobic, biofilm formation, mobile artifacts, Gram-negative, Gram Differences in characteristics such as positive, potential pathogenicity, and stress tolerance. The specific results are shown in Figure 4.

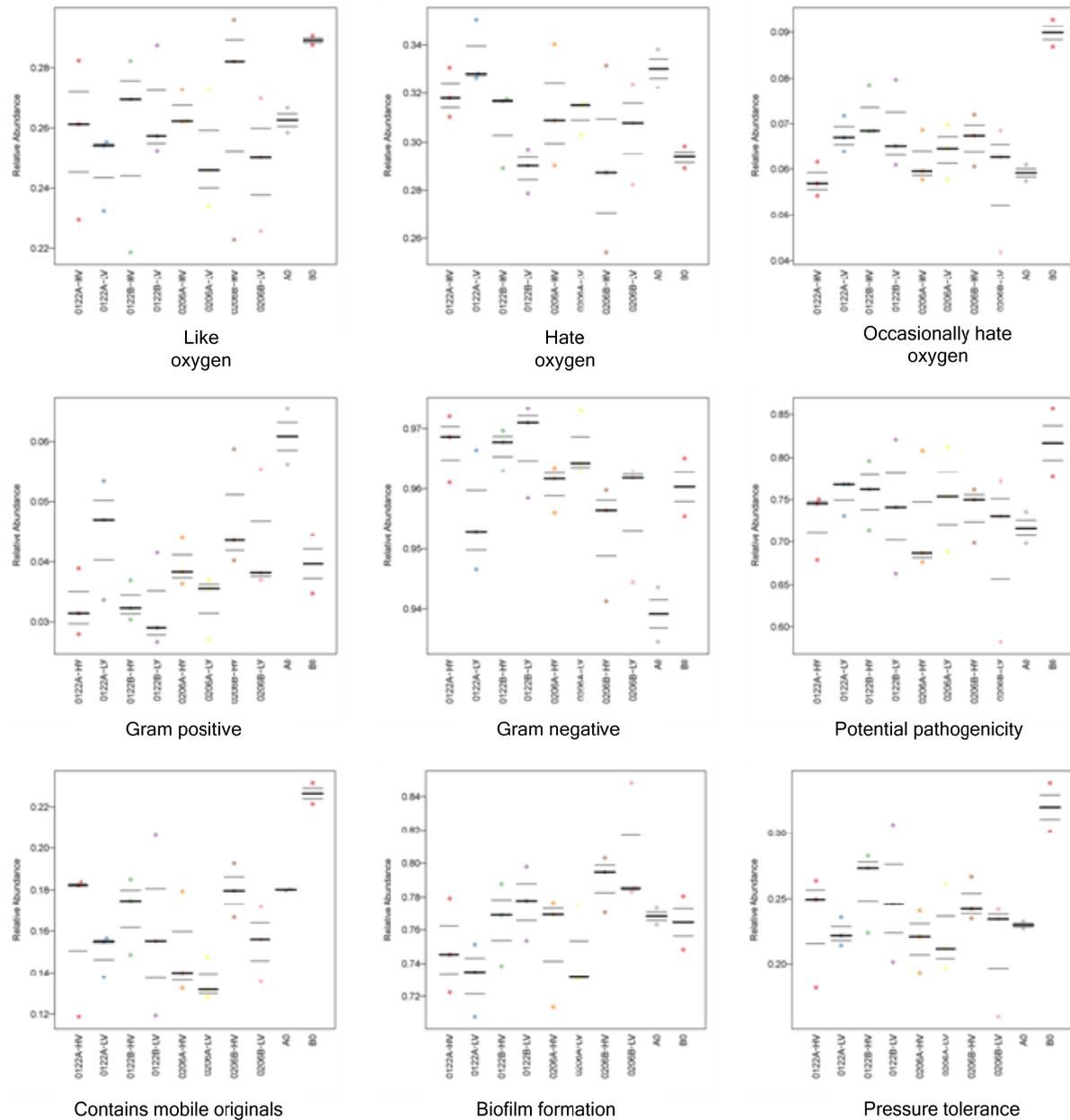


Figure 4. Phenotypic analysis of substrate microbial community

It can be seen from Fig. 4 that the abundance of the aerobic phenotype shows a trend of high flow rate higher than the low flow rate, and the abundance of the anaerobic phenotype generally shows a trend of lower flow rate than high flow rate, which can also explain the high flow rate to some extent. The dissolved oxygen concentration of the substrate is less than the dissolved oxygen of the low flow rate substrate. The relative abundance of the facultative anaerobic phenotype is generally 15 days, the high flow rate is lower than the low flow rate, and the high flow rate is higher than the low flow rate

when the culture is 30 days, which means that the time is increased, the high flow rate conditions are under the bottom. The rate of growth of the relative abundance of facultative anaerobics in the microbial community also increases. The relative abundance of Gram-negative and Gram-positive phenotypes is less regular. However, the relative abundance of latent pathogens also shows that the high flow rate is lower than the low flow rate in 15 days and the high flow rate is higher than the low flow rate in the 30 days of culture. It also means that the substrate grows under high flow conditions with increasing time. The possibility of germs is greater than the low flow rate. The relative abundance of the moving originals and the pressure-tolerant phenotypes is higher than the low flow rate under high flow conditions, which is consistent with the law that the water flow shear force is higher than the low flow rate under high flow conditions. At low flow rates, the relative abundance of the biofilm phenotype increases much faster than the high flow rate, which means that the substrate is more likely to form biofilms at low flow rates over time.

6. Conclusion

This study uses laboratory experiments to design experimental water tanks that can simulate the hydrodynamic conditions of natural rivers, collect natural river water and sediment for indoor cultivation, and regularly collect sediments at different flow points for DNA extraction, PCR amplification and high-throughput sequencing. The QIMME and R language packages were used to further analyze the high-throughput sequencing results, and the differences in the diversity, composition, indicator species and phenotype of the substrate microbial community under different hydrodynamic conditions were obtained.

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