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# Analysis of the Impact of Acidity and Alkalinity on the Microbial Community Structure in Urban Rivers

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**Abstract:** To address the impact of low-temperature pH on the microbial community structure of river sediment in urban rivers during winter, the sediment of the Pi River was sampled to investigate. Four experimental samples were designed by varying the pH to 5.0, 7.0, and 8.5 based on the original sample (pH = 5.5) and the characteristics of the microbial community were analyzed using the high-throughput sequencing. The results showed that the samples contained 23 phyla, 68 classes, and 100 genera of bacteria, of which *Sphingomonales* and *Burkholderiales* were the dominant genera. The highest abundance of *Sphingomonales* was 20%, whereas the highest abundance of *Burkholderiales* was up to 15% in the acid samples. The abundance of *Proteobacteria* was the highest in the original and alkaline samples, followed by *Bacteroides* and the number of other genera in alkaline and neutral samples is more than that in acidic samples. In addition, the abundance of *Nitrosomonadales* was more than 5% in the original, alkaline, and neutral samples and less than 1% in acidic conditions. The microbial community of the acid sample was quite different from the other three samples, with less richness and uniformity, which indicated that the acid pH had a great impact on the microbial community and the denitrifying bacteria were less active at the low-temperature condition in winter. This study provides a reference for the microbial community structure of the Pi River and a scientific basis for effectively addressing urban pollution in river management.

**Keywords:** pH, High-Throughput, Microbial Community, Sediment

## Introduction

Rivers are the main water sources for human industry, agriculture, and domestic use, as well as the main receivers of solid waste, rainwater, and domestic sewage. With the acceleration of urbanization, the discharge of pollutants is increasing and the water quality of many rivers, especially urban rivers, has begun to decline. Rivers face many environmental issues, such as eutrophication (Shi *et al.*, 2020), heavy metal pollution (Jiao *et al.*, 2020; Wang *et al.*, 2020; Han *et al.*, 2022), and organic pollution (Li *et al.*, 2022; Oyejobi *et al.*, 2022; Teixeira *et al.*, 2022). Sediment is the enrichment place of river pollutants. Through different ways, various pollutants quickly change from dissolved state in water to solid state by adsorption, complexation, sedimentation, and other actions and deposit in the sediment, further affecting aquatic organisms and human health, becoming a potential ecological. A large number of pollutants deposited can alter the microbial community structure and affect the stability of the water ecosystem in the sediment.

The input of bacteria caused by human activities and the water chemicals in sewage significantly affect the river bacterial community. For example, the bacterial community of the Yenisei River in Russia is influenced by different vegetation and the intensity of human activities along the coast (Kolmakova *et al.*, 2014). Wang *et al.* found that the distribution of *Proteobacteria*, *Cyanobacteria*, *Actinomycetes*, *Bacteroides*, and *Acidobacteria* in the surface water of the Jialing River was significantly different along the river and the bacterial diversity was significantly higher than that of other water bodies. In addition, the construction of a hydrochloric power station along the river led to a change in the abundance of *Cyanobacteria* (Wang *et al.*, 2018). Wang *et al.* measured the microbial community of Chaobai River at different groundwater depths and examined related environmental factors and found that the aquifer depth, water temperature, electrical conductivity, and coexisting anions were closely related to the distribution of endocrine disruptors in groundwater (Wang *et al.*, 2019). Zhang *et al.* studied the effects of seasonal variation and human disturbance on sediments in different areas of the Nanfeihe River (including urban, urban-rural, and rural areas) and found that chemical parameters exhibited the most significant regional heterogeneity; seasonal variation had a greater impact on the structure of the entire microbial community than regional heterogeneity. Additionally, the abundances of *Firmicutes* and *Bacteroides* were more sensitive to seasonal variations (Zhang *et al*., 2019).

Studies have shown that changing the basic parameters of organic and inorganic pollutants, such as pH, temperature, dissolved oxygen content, and light penetration, may alter the structure of bacterial communities (Mark Ibekwe *et al.*, 2012; García-Armisen *et al*., 2014). At present, most studies evaluating the impacts of urban human activities on surface water and sediment focus on physicochemical indicators, such as light penetration, antibiotics (Li *et al*., 2022; Oyejobi *et al*., 2022; Teixeira *et al*., 2022), phenolic compounds (Wu *et al*., 2016), nutrients (Pessi *et al*., 2016) and algal characteristics (Mohit *et al*., 2014; Zhang *et al*., 2015). However, most of them are concentrated in lakes, reservoirs, and rivers far from cities and little is known about the microbial communities in urban rivers, especially the diversity of microorganisms affected by low winter temperatures.

In winter, rivers have low rainfall, long dry periods, and slow water flow rates. However, the pollutants entering the river have not decreased and the amount of sediment in the sediment has increased. Due to the low water temperature, the microbial activity in the sediment has decreased, leading to more severe water pollution. Therefore, the study of winter sediment microorganisms in urban rivers is very typical and not only has important biological significance but also has potential application prospects in urban river ecological management and environmental protection.

As a second-generation sequencing technology, high-throughput sequencing has low cost, high throughput, and process automation and can perform the rapid and accurate processing of large-scale samples to determine the microbial community composition (Xiao *et al*., 2023). High-throughput sequencing has been widely applied in the investigation of bacterial community structure in water bodies, including rivers (Sun *et al*., 2017; Song *et al*., 2023), reservoirs (Chen *et al*., 2017; Peng *et al*., 2022), lakes (Zhou *et al*., 2022) and wetland (Wei *et al*., 2022). Therefore, it is necessary to study the microbial community structure of cold urban rivers in winter through high-throughput sequencing.

To this end, the microbial community of the Pi River (located in Lu' an city, west Anhui province, China) was taken as the research object in this study. Pi River originates from the north foot of Dabie Mountain and converges in the urban area of Lu' an city by the East and West Rivers, passing through many cities and towns along the way. The tailwater is accepted, which comes from the sewage treatment plant of Chengbei, Dongcheng, and Fenghuangqiao. In addition, untreated domestic sewage in the urban area flows into the Pi River, along with the heavily polluted water bodies of the Jiangjiagou and Jun Rivers, which affects water quality. According to the national assessment section water quality standards released by the Anhui network on May 20, 2020, from January to March 2018, the water quality at the Xin' an Ferry on the Pi River exceeded the standard for 3 consecutive months. At present, there are no research reports on the diversity and differences of microbial community structure in the Pi River water body and sediment and the impact of environmental factors on microbial communities is still unclear. This study aimed to achieve two key goals by throughput sequencing technology: (1) To analyze the characteristics of the bacterial community in the Pi River in winter and (2) To discuss the influence of pH on the microbial community in sediments of the Pi River.

## Materials and Methods

The sampling point is located on West Yueliang Island in the Pi River, as shown in (Fig. 1). The island is located in the main urban area of Lu'an City. There is a university and a community on the island, with nearly 25,000 residents. The island's stormwater and sewage are not completely segregated and the oily sewage from domestic kitchens enters the river. The east and west banks receive sewage from the living areas in the city center. In this study, according to methods described in the literature (Yu *et al*., 2021), Sediment from the riverbed was collected in November 2018 during the dry season. In addition, the four plexiglass cylindrical sampling columns with a diameter of 10 cm were used to collect samples with a thickness of no less than 20 cm from the sediment surface, and the method roadmap is shown in (Fig. 2). The upper end of the sampling column was be covered with water, both ends were plugged with rubber stoppers and the column was placed vertically and brought back to the laboratory.

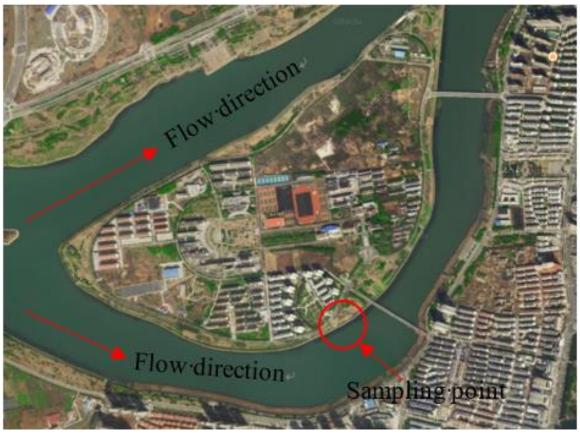

**Fig. 1:** Schematic diagram of sampling points

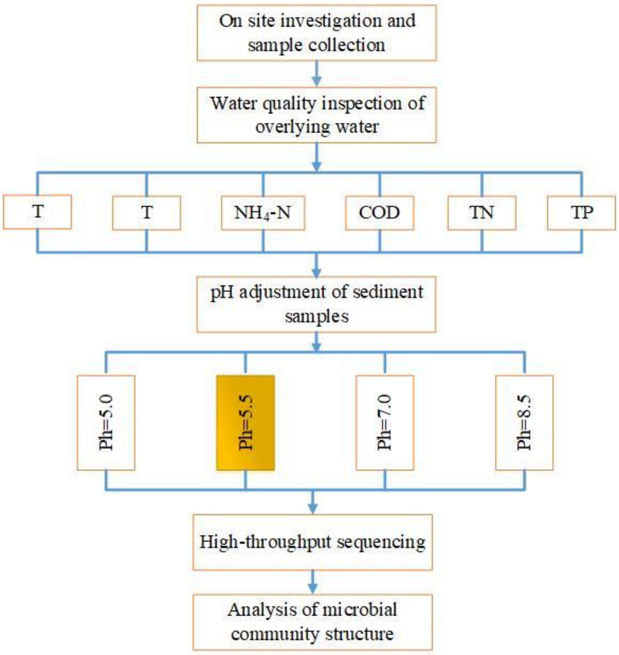

**Fig. 2:** Method roadmap

Stones and weeds were removed and the samples were then placed in the same container, mixed evenly, and divided into four parts for a pH adjustment experiment. As is known, pH not only has a significant effect on nitrogen and phosphorus release under the action of microorganisms but also significantly affects the total phosphorus concentration under physical/chemical action. To this end, the above uniformly mixed sediment was spread on the bottom of four containers to a thickness of approximately 10 cm. and. Low-concentration NaOH and HCl solutions were slowly added along the siphon wall to adjust to the original pH of 5.5 to pH = 5.0, 8.5, 7.0 (Yu *et al*., 2021). In the original sample, acidic, alkaline, and neutral pH, the serial numbers were named Y1, S2, J3, and Z4, respectively. Finally, the four experimental samples were sealed with sterile plastic bottles and sent to Beijing Biomark Biotechnology Co., Ltd., Beijing for high-throughput testing based on the Illumina HiSeq sequencing platform. The tested results could be analyzed in the sequencing data, Operational Taxonomic Units (OTU), species annotation, taxonomic, diversity and the significance of inter-group differences, and the detailed experimental flowchart was shown in (Fig. 3). Furthermore, a simple experiment was conducted, in which the overlying water at the upper end of the sampling column was absorbed by the siphon method and the physical and chemical indexes of the water body were measured according to reference (Yu *et al*., 2021). The water parameters were shown in Table 1.

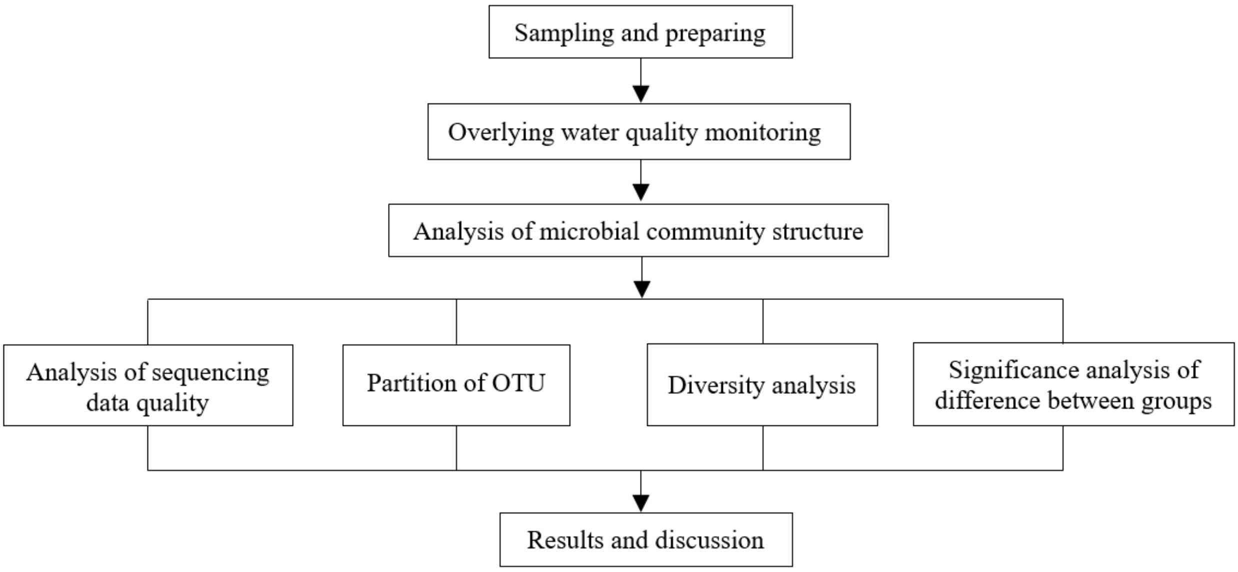

**Fig. 3:** Flow chart of the experiment

**Table 1:** The quality parameters of the overlying water

| Index | T (°C) | DO (mg/L) | COD (mg/L) | $NH_4$-N (mg/L) | TN (mg/L) | TP (mg/L) |
|---|---|---|---|---|---|---|
| Value | 8 | 8.5 | 37 | 0.35 | 2.7 | 0.16 |

## Results and Discussion

### *Evaluation and Analysis of Sequencing Data Quality*

After extracting the total DNA from each sample, primers were designed according to the conservative region. Sequencing connectors were added to the end of the primers for PCR amplification and purification and the quantification and homogenization of the products were conducted to assemble a sequencing library. The original image data files obtained by high-throughput sequencing were transformed into original sequencing sequences by base recognition analysis. The original sequencing results were filtered and spliced to obtain the optimized sequences and the OTUs were divided. Then the samples were classified and analyzed at various classification levels. Table 2, a total of 320,155 pairs of Reads were obtained from the sequencing of four samples. After splicing and filtering of double-ended Reads, a total of 219,058 Clean tags were generated. Each sample produced at least 53,305 Clean tags, with an average of 54,765 Clean tags. These results imply that the bacterial population in the bottom mud of the Pi River is species richness.

In total, 23 phyla, 68 classes, and 100 genera of bacteria were identified in the four samples. (Fig. 4), *Proteobacteria* and *Bacteroidetes* were the dominant phyla among the four enriched cultures (Yu *et al*., 2021). Among them, *Proteobacteria* has the highest relative abundance, at more than 57% in the S2 sample; while J3 had the lowest abundance of *Proteobacteria* at over 43%. The relative abundance of *Bacteroidete*in in each sample could be ranked as S2 > Z4 > Y1 > J3, which was consistent with the trend found in *Proteobacteria*. The relative abundance of *Acidobacteria* was the lowest in S2 compared with those of Z4, Y1, and J3, while *Proteobacteria* and *Bacteroidetes* had the highest relative abundances in S2. This is because acid bacteria tend to grow in the sediment of slightly eutrophic lakes with low organic matter content and better adapt to this environment (Naether *et al*., 2012).

### *OTU Analysis*

The coverage indexes of the four samples were higher than 0.99 in this sequencing, which indicated that the testing could truly reflect the microbial community of sediment samples. Figure 5, the OTU number of each sample was obtained by clustering and the numbers of OTUs were 1164 (Y1), 923 (S2), 1154 (J3), and 1135 (Z4). Among them, S2 had the fewest OUTs, far fewer than Y1, while the number of OUTs in J3 was very close to that in Y1, which had the highest OTU number. These results demonstrated that the acidic environment had a significant impact on microorganisms, whereas the alkaline environment had little impact on the original microorganisms.

### *Species Annotation and Taxonomic Analysis*

The OTUs were filtered to remove the low content and the final OTU list was obtained. The number of tags annotated to the species of each grade in each sample was counted. The results are shown in Table 3.

**Table 2:** Statistics of sample sequencing results

| Sample ID | PE reads | Raw tags | Clean tags | Effective tags | AvgLen (bp) | GC (%) | Q20 (%) | Q30 (%) | Effective (%) |
|---|---|---|---|---|---|---|---|---|---|
| J3 | 80,499 | 69,700 | 55,773 | 52,837 | 417 | 55.14 | 96.22 | 92.78 | 65.64 |
| S2 | 79,917 | 69,177 | 55,815 | 49,230 | 421 | 53.93 | 96.12 | 92.70 | 61.60 |
| Y1 | 79,933 | 67,759 | 54,165 | 51,089 | 418 | 55.41 | 96.16 | 92.69 | 63.91 |
| Z4 | 79,806 | 67,436 | 53,305 | 50,394 | 419 | 55.05 | 95.95 | 92.28 | 63.15 |

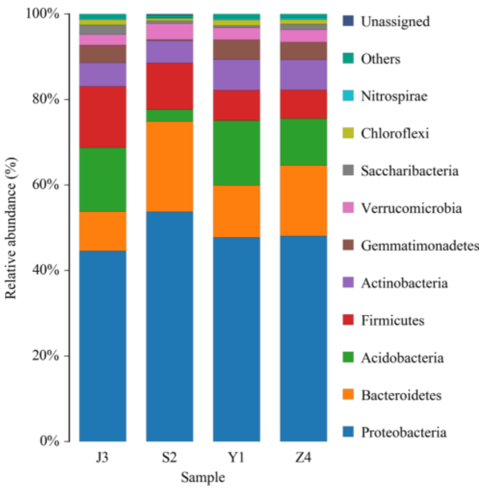

**Fig. 4:** Phylogenetic analysis of the experimental samples

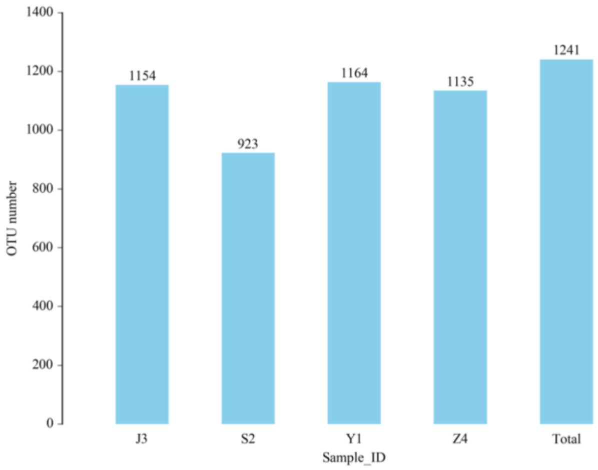

**Fig. 5:** Number of OTUs of the experimental samples

**Table 3:** Tags of the grade of the sample

| Sample | Kindom | Phylum | Class | Order | Family | Genus | Species |
|---|---|---|---|---|---|---|---|
| J3 | 36,605 | 36,605 | 35,832 | 34,932 | 33,441 | 33,018 | 20,254 |
| S2 | 38,491 | 38,491 | 38,336 | 38,017 | 37,751 | 36,699 | 13,449 |
| Y1 | 33,480 | 33,480 | 33,330 | 32,559 | 30,976 | 29,829 | 18,075 |
| Z4 | 35,743 | 35,743 | 35,382 | 34,673 | 33,605 | 32,865 | 18,533 |

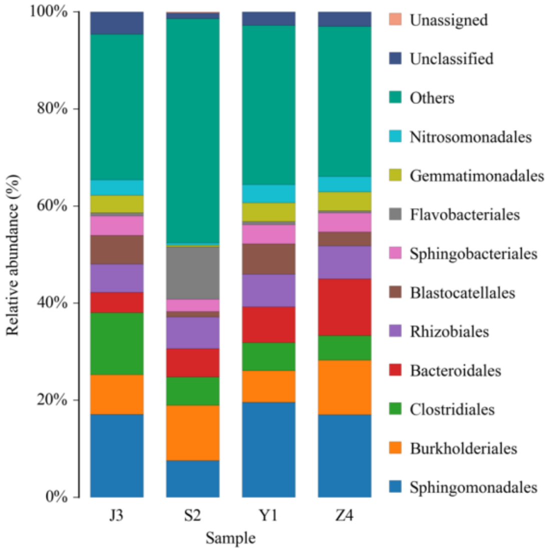

**Fig. 6:** Microbial community structure of different sediment enrichment cultures at the genus level

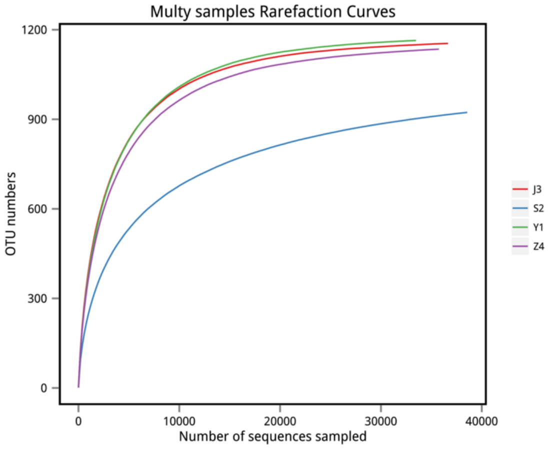

**Fig. 7:** Dilution curve for the experimental samples

As can be seen from Table 3, compared with the original Y1 sample, the Kingdom, Phylum, Class, Order, Family, Gene, and Species identified in samples S2, Z4, and J3 were improved after adjusting the pH. S2 had the most abundant and Z4 was the least. The pH of Y1 and S2 were the closest, whereas the difference in the number of species is the largest, which indicated that acidity had the greatest impact on the bacterial community of sediment. Additionally, the results of the study showed that the microorganisms involved in biological sewage treatment generally had an optimal pH between 6.5 and 8.5.

The microorganisms included sediment samples with four different pH levels representing 23 phyla, 68 classes, and 100 genera. The composition of flora at the level of the dominant genera in each sample is shown in (Fig. 6). *Sphingomonales* and *Burkholderiales* were the dominant genera among the four enriched cultures, of which *Sphingomonales* had the highest relative abundance (Yu *et al*., 2021). The relative abundance of *Burkholderiales* in the S2 sample was the highest, reaching over 15%, while the relative abundance of *Burkholderiales* in J3 was the lowest at more than 5%. In addition, the microbial community of S2 was quite different from those of the other three samples. For example, the relative abundance of *Sphingomonadales* in S2 was the lowest compared with Z4, Y1, and J3, while *Burkholderiales* had the highest relative abundance. The abundance of *Nitrosomonadales* in the original sample, alkaline, and neutral samples is more than 5% and less than 1% at acidic conditions because *Nitrosomonadales* like a slightly alkaline environment. Another reason is that the optimum temperature of *Nitrosomonadales* is 24-28°C and the temperature of this experiment is approximately 11°C, which is far lower than the optimum temperature of *Nitrosomonadales* (Sudarno *et al*., 2011).

## *Diversity Analysis*

The dilution curve can directly reflect the rationality of the amount of sequencing data and indirectly reflect the richness of species in the sample. Figure 7 the number of OTUs entered the platform stage after the number of sequences reached 20000; it was observed that the number of OTUs exceeded 600 when the number of sequences reached 10000. This showed that the number of sequence bars could well reflect the bacterial diversity of the samples. The sequencing quantity obtained from each sample could reflect the overall species classification and the sequencing quantity was great enough to meet the requirements of analysis. In order to mine deeper microbial information, the Shannon index was used to analyze the diversity index. As shown in (Fig. 8), the number of detection reached a stable level when the number of sequencing was 500. The species richness and uniformity of Y1, J3, and Z4 were higher, while the richness and uniformity of the S2 samples were less. which is consistent with the research results of Lindström *et al*. (2005).

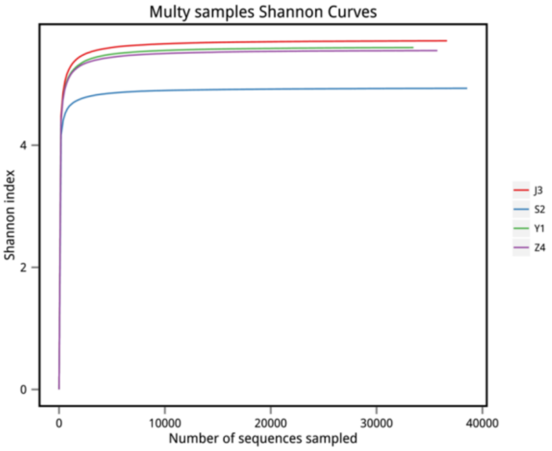

**Fig. 8:** Shannon Index of the experimental samples

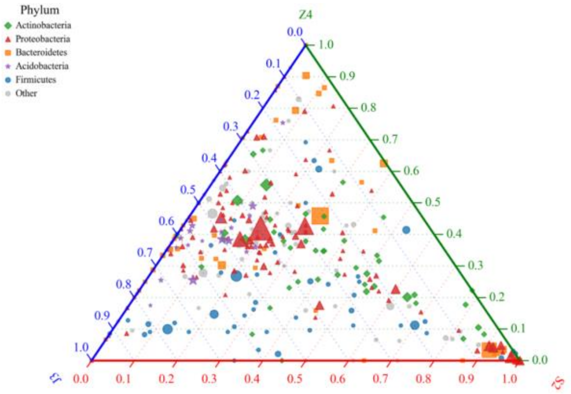

**Fig. 9:** Analysis of phase diagram of S2, J3, and Z4

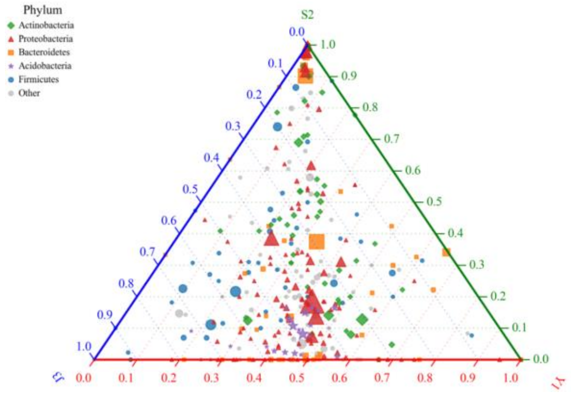

**Fig. 10:** Analysis of phase diagram of S2, J3, and Y1

### *Significance Analysis of Difference Between Groups*

To better analyze the effect of pH on the sediment microbial community, a ternary phase diagram was used to analyze the differences between the three samples, which could intuitively illustrate the proportion and relationships between different species in the samples, as shown in (Fig. 9). Among the three samples of S2, J3 and Z4, the abundance of *Proteobacteria* in J3 was the highest, followed by *Bacteroides* and the abundance of both phyla was also high than in S2. There are a few other species detected in S2 and Z4. Figure 10 shows the ternary phase diagram of all samples. Among the three samples S2, J3 and Y1, the abundance of *Proteobacteria* in Y1 was the highest, followed by *Bacteroides*, whereas the abundances of *Proteobacteria* in J3 and Y1 were more than those in S2, indicating that the acidic environment had a great impact on the species.

## Conclusion

In this study, the sediment of the Pi River was investigated by adjusting the pH, and levels of the sediment samples and then testing the samples using high-throughput sequencing. The characteristics of the bacterial community were obtained after the samples were tested. According to the results, the following conclusions were drawn:

(1) The coverage of the four samples was higher than 0.99 in this sequencing and the microorganisms contained 23 phyla, 68 classes, and 100 genera; thus, this experiment could truly affect the microbial community characteristics of sediment samples and fully reflect the bacterial diversity contained in the community samples
(2) The species richness and evenness of the original, alkaline, and neutral samples were greater than those of the acidic samples. The abundance of *Proteobacteria* was the highest in the original and alkaline samples, followed by *Bacteroides* and other species were more than in acidic samples, thus, the acidic environment had a great impact on species and pH was an important environmental factor affecting the distribution of microbial communities
(3) *Sphingomonadales* and *Burkholderiales* were the dominant genera in all four samples. The highest abundance of *Sphingomonadales* was 20%, the highest abundance of *Burkholderiales* was over 15% and the lowest relative abundance of *Bacteroidales* was over 5%. The abundance of *Nitrosomonadales* in the original sample, alkaline, and neutral samples did not exceed 5% and were less than 1% in acidic conditions, which was related to the lack of activity of denitrifying bacteria under low temperatures in winter

The influence of pH on the Pi River microbial community was investigated in this study. Moreover, some factors were not considered in this experiment, such as temperature variation and dissolved oxygen, and the location of the sampling points. In the future, these limitations will be conducted in the experiments.

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## Author's Contributions

**Dongmei Shen:** Experiment design, execution, and paper written.

**Cairui Yu:** Guide the research direction.

**Xinwei Song:** Support experiment design and data analysis.

**Yulan Gao:** Participated to collect the materials related to the experiment.

**Rusheng Jia and Luxiu Chai:** Support the data statistical analysis.

## Ethics:

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

### *Acronyms*

| | |
|---|---|
| pH: | Potential of Hydrogen |
| DO: | Dissolved Oxygen |
| COD: | Chemical Oxygen Demand |
| TN: | Total Nitrogen |
| TP: | Total Phosphorus |
| OUT: | Operational Taxonomic Units |
| PE: | Paired-End |

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