



# Influence of aquaculture practices on microbiota composition and pathogen abundance in pond ecosystems in South China

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## ABSTRACT

Pond microbiota play a crucial role in maintaining water quality and the health of aquaculture species. This study aimed to explore the relationship between pond water and sediment microbiota (especially potential pathogens) and physicochemical parameters under different aquaculture conditions. Samples of pond water and sediment were collected from 21 monitoring sites across eastern, western, and northern Guangdong, and the Pearl River Delta in November 2021, March 2022, and July 2022. Microbial structures were analyzed using high-throughput sequencing of the 16S rRNA gene. The results indicated that sediment microbiota distribution was more uniform than that of water microbiota. Additionally, sampling time significantly influenced the uniformity of water microbiota distribution more than that sediment microbiota. Factors such as aquaculture species, culture pattern,  $\text{NH}_4^+\text{-N}$ , longitude, latitude, total nitrogen (TN),  $\text{NO}_3^-\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ , and total phosphorus (TP) were significantly correlated with water microbiota structure, and TN, TP, and organic carbon were significantly correlated with sediment microbiota structure. Furthermore, an increase in the  $\text{NH}_4^+\text{-N}$  concentration in the pond water significantly increased the variety of pathogenic bacteria. Higher nitrogen levels also increased the relative abundance of *Mycobacterium* in pond water, whereas the culture pattern (freshwater, seawater, brackish, modern captive culture, freshwater factory container aquaculture, or seawater factory culture) and species significantly influenced the relative abundances of *Vibrio*, *Tenacibaculum*, *Pseudoalteromonas*, and *Francisella*. Additionally, the relative abundance of pathogenic bacteria in the sediment microbiota was significantly higher than that in the water microbiota. Our results suggest that the culture patterns, species, and nitrogen concentrations should be considered when preventing pathogenic bacteria growth in aquaculture waters.

## 1. Introduction

In 2020, fishery and aquaculture production reached 214 million tons, and aquatic foods constituted approximately 17 % of global animal protein (FAO, 2022). As fishery resources continue to decline owing to overfishing, pollution, and other factors and intensive aquaculture technology is being promoted, aquaculture has become an important method for producing aquatic products. Although intensive aquaculture technology has driven the rapid development, especially in Asia and mainland China, its high density and feed characteristics result in significant amounts of undigested feed entering aquaculture water,

resulting in high nitrogen and phosphorus contents (Li et al., 2023). Untreated effluents from aquaculture, industrial wastewater, and sewage damages the ecology of culture areas, causing pathogenic microorganisms to proliferate and spread quickly (Zhou et al., 2009). Therefore, a systematic assessment of the compositions of pollutants and microbiota in aquaculture wastewater is important for assessing their impact on the surrounding aquatic environment and formulating reasonable treatment plans.

Microorganisms play a crucial role in the transformation and cycling of most of the biologically active elements in aquaculture ecosystems (Newton et al., 2011; Robles-Porchas et al., 2020; Zhao et al., 2023).

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This key position does not only originate from the role of bacteria as the principal degraders and mineralizers of organic compounds to their inorganic constituents, but also from their biomass production and trophic coupling to eukaryotic predators, which, by supporting the food web, profoundly impact elemental fluxes and water quality in an ecosystem (Newton et al., 2011). Liang et al. (2015) reported that supplementation with *Bacillus licheniformis* BSK-4 significantly decreased the levels of  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N, and total nitrogen (TN) and altered the microbiota in grass carp culture water. Zhang et al. (2022a) studied a novel *Bacillus* species capable of simultaneous heterotrophic nitrification and aerobic denitrification from aquaculture pond sediments and shrimp guts and demonstrated that *Bacillus amyloliquefaciens* effectively removed  $\text{NO}_2^-$ -N and  $\text{NH}_4^+$ -N in recirculating aquaculture water. Our previous study demonstrated that the aerobic denitrifying *Pseudomonas furukawaii* ZS1 isolated from a grass carp culture pond could effectively remove nitrogen (Zhu et al., 2020) and regularly regulate the water microbiota structure in grass carp culture ponds (Gong et al., 2021).

Additionally, microbiota in aquaculture systems play a crucial role in the health and growth of aquaculture organisms (Zhang et al., 2022b; Lorgen-Ritchie et al., 2023). Previous studies have shown that low microbial diversity may increase risk of invasion by disease-causing species, which in turn adversely affects aquaculture (De Schryver and Vadstein, 2014). Murphy et al. (2022) reported that aquaculture production of Hawaiian bobtail squid (*Euprymna scolopes*) hatchlings is negatively affected by low environmental microbiome diversity. Lian et al. (2023) reported that many opportunistic pathogens could be detected in the microbiota in the water of grass carp aquaculture ponds.

Considering the important role of microbiota in nutrient cycling and health of aquaculture organisms, exploring factors influencing microbiota structure in aquaculture and mechanisms for maintaining diversity has important theoretical and practical value (Tarnecki and Guttman, 2023). Microbiota in aquaculture water is easily influenced by aquaculture pattern and water treatment methods, while the sediment microbiota is more affected by regional limitations and seasons factors.

We hypothesize that aquaculture pattern and water treatment methods primarily influence water microbiota, while sediment microbial community show regional differences. Furthermore, compositional changes in the microbiota would affect the relative abundance of pathogenic bacteria. To verify these hypothesis, we collected water and sediment samples from aquaculture pond systems in different regions of Guangdong Province, China, and used high-throughput sequencing technology to analyze the microbial community structures, combining the sampling season, region, and aquaculture methods to analyze the changes in and factors influencing the water and sediment microbiota structure in the system.

## 2. Results

### 2.1. Variations in physicochemical characteristics of water and sediment in aquaculture ponds

The physicochemical characteristics of the pond water exhibited significant regional differences in July, whereas only a few characteristics exhibited significant regional differences in March and November ( $P < 0.05$ ; Fig. 1). In July, the COD concentration of the pond water in the eastern Guangdong region was significantly higher than those in the Pearl River Delta and western Guangdong regions ( $P < 0.05$ ; Fig. 1A). Similarly, the TN concentration in the pond water in the eastern Guangdong region was significantly higher than those in the other three regions ( $P < 0.05$ ; Fig. 1B).  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N concentrations were significantly higher in eastern Guangdong and the Pearl River Delta than in western and northern Guangdong ( $P < 0.05$ ; Fig. 1C and 1D). The  $\text{NH}_4^+$ -N concentration in the pond water in northern Guangdong was significantly lower than those in the other three regions ( $P < 0.05$ ; Fig. 1E). In contrast, TP concentration in pond water in the Pearl River Delta was significantly higher than that in the northern Guangdong region ( $P < 0.05$ ; Fig. 1F).

In March, the COD, TN,  $\text{NO}_2^-$ -N, and TP concentrations of pond water

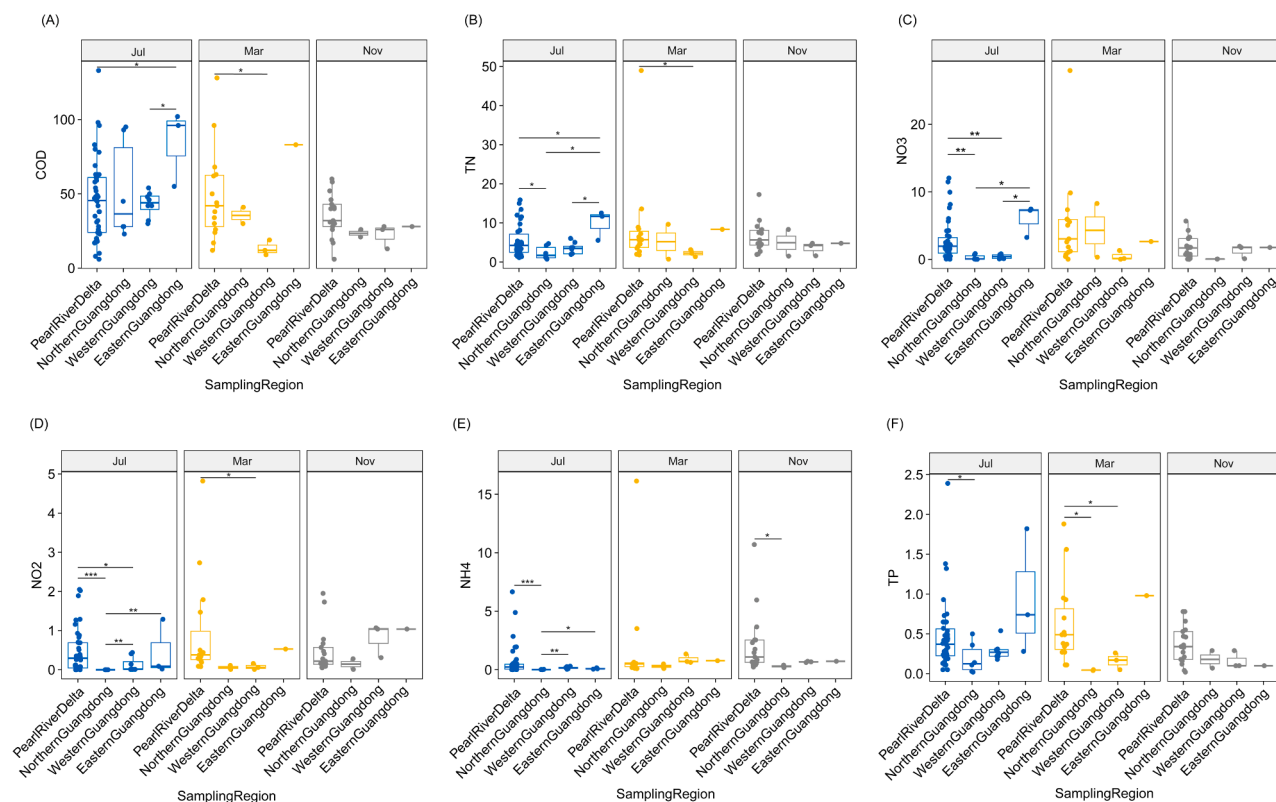


Fig. 1. Differences in water physicochemical indicators of aquaculture ponds in different seasons and regions.

in the Pearl River Delta were significantly higher than those in the western Guangdong region ( $P < 0.05$ ; Fig. 1A and 1B). Seasonal comparisons revealed that in western Guangdong, COD concentrations in July were significantly higher than in March and November ( $P < 0.05$ ; Fig. S2A), while  $\text{NO}_2\text{-N}$  concentration in November was significantly higher than those in March and July in northern Guangdong ( $P < 0.05$ ; Fig. S2D). Similarly,  $\text{NH}_4^+\text{-N}$  concentration in July were significantly lower than those in March and November in both the Pearl River Delta and western Guangdong regions ( $P < 0.05$ ; Fig. S2E).

In pond sediments, OC, TN, and TP concentrations in eastern Guangdong were significantly higher in November compared to March and July, while the highest values for these parameters were recorded in northern Guangdong and the Pearl River Delta in July (Fig. S3A-C). In saltwater aquaculture systems, the concentrations of OC, TN, and TP in sediments were the highest in November, while in freshwater systems, they were highest in July (Fig. S3D-F).

## 2.2. Geographical differences in water and sediment microbiota structures in aquaculture ponds

A total of 7174,281 high-quality 16S rRNA gene (V4–V5 region) were obtained from 175 samples (103 water and 72 sediment samples). Based on a 97 % sequence similarity threshold, 367,438 operational taxonomic units (OTUs) were identified, of which 253,730 were singletons. The rank-abundance curve indicated that the sediment microbiota were more evenly distributed than the water microbiota, and that sampling time had a greater impact on the distribution of the water microbiota compared to sediment microbiota (Fig. 2A). The  $\alpha$ -diversity indices (OTU richness, Chao1, and Shannon) were significantly higher for sediment microbiota than for water microbiota ( $p < 0.01$ ; Fig. 2B–D), resulting in a lower Good's coverage for sediment samples (Fig. 2E). Principal coordinates analysis (PCoA) revealed substantial compositional differences between water and sediment microbiota, with water microbiota showing greater variability over time (Fig. 2F).

Microbiota sequences were classified into 78 phyla (3 archaea, 75 bacterial), with dominant groups including Crenarchaeota, Euryarchaeota, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, and Proteobacteria (Fig. 2G). Although the relative abundances of these phyla remained relatively stable over time, significant differences were observed between water and sediment microbiota ( $p < 0.05$ ; Fig. S4). Heatmap analysis of 109 dominant genera showed clear separation between water and sediment microbiota, with significant differences detected at all sampling time points (Fig. S5 and S7). Linear discriminant analysis effect size (LEfSe) further supported that most differences were attributed to water and sediment microbiota, rather than temporal variation (Fig. S6).

Distance-based redundancy analysis (db-RDA) indicated that water microbiota structure was significantly influenced by aquaculture species, culture pattern (freshwater, seawater, brackish, modern captive culture, freshwater factory container aquaculture, or seawater factory culture),  $\text{NH}_4^+\text{-N}$ , longitude, latitude, TN,  $\text{NO}_3^-\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ , and TP ( $p < 0.05$ ; Fig. 3A), while sediment microbiota were primarily affected by TN, TP, and organic carbon (OC) ( $p < 0.001$ ; Fig. 3B). Spearman correlation analysis showed that the relative abundances of key genera (e.g., *Synechococcus*, *Polynucleobacter*, *Mycobacterium*) were significantly correlated with nitrogen and phosphorus levels in the pond water ( $p < 0.05$ ; Fig. 3C). In sediment, genera such as *Thiobacillus*, *Gallionella*, and *Crenothrix* showed significant correlations with OC, TN, and TP concentrations ( $p < 0.05$ ; Fig. 3D).

## 2.3. Correlation between geographical distance and microbiota dissimilarity

The linear regression models for water microbiota dissimilarity shown positive slopes ( $p < 0.001$ ), suggesting that as geographical distance increases, microbiota composition becomes more dissimilar

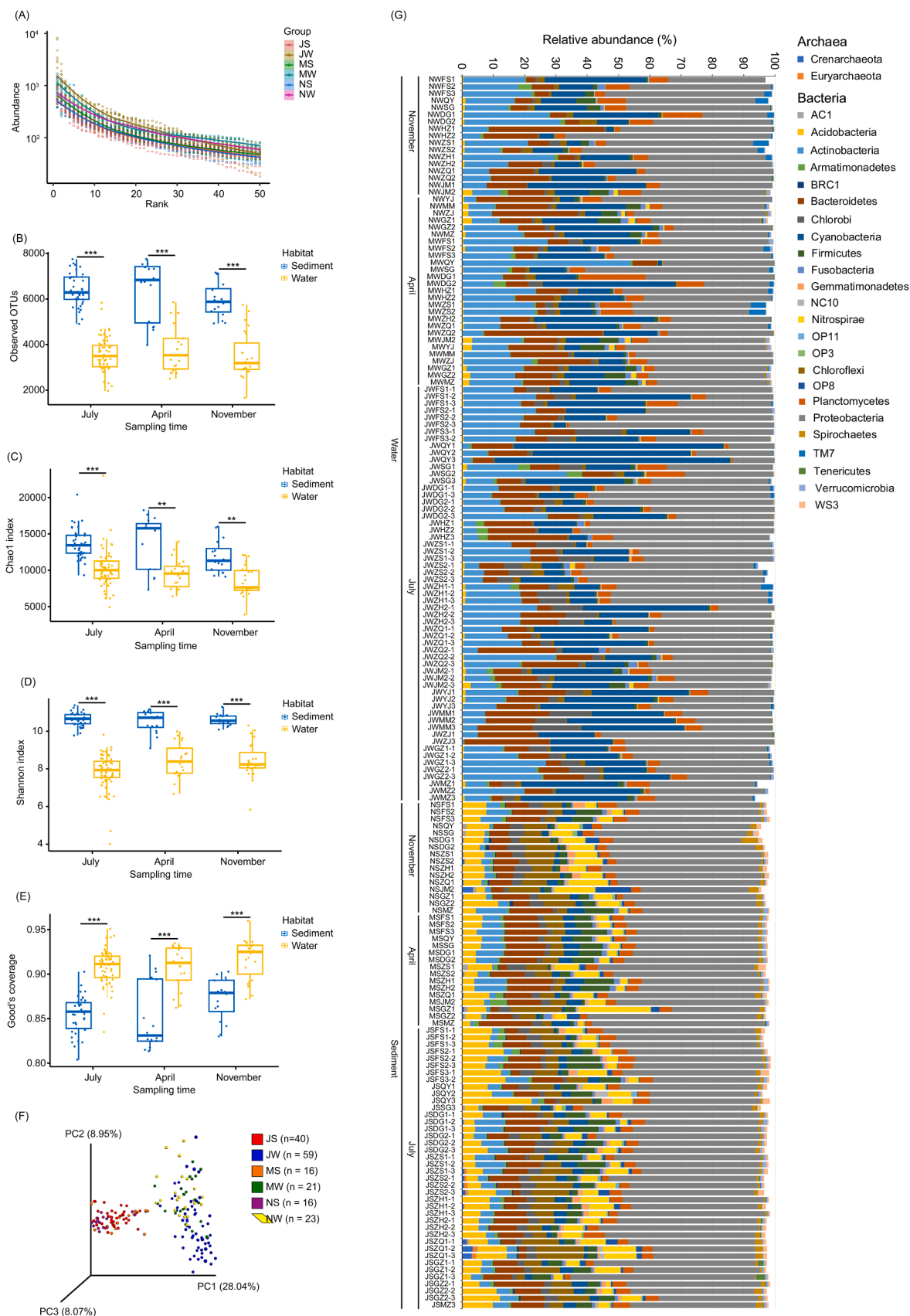
(Fig. 4A–C). Similarly, sediment microbiota dissimilarity also exhibited positive correlations with geographical distance ( $p < 0.001$ ; Fig. 4D–F). However, the limited strength of the correlation between distance and microbiota composition highlights the need for a more comprehensive set of variables to explain the observed dissimilarities.

## 2.4. Temporal variation in pathogenic bacterial communities

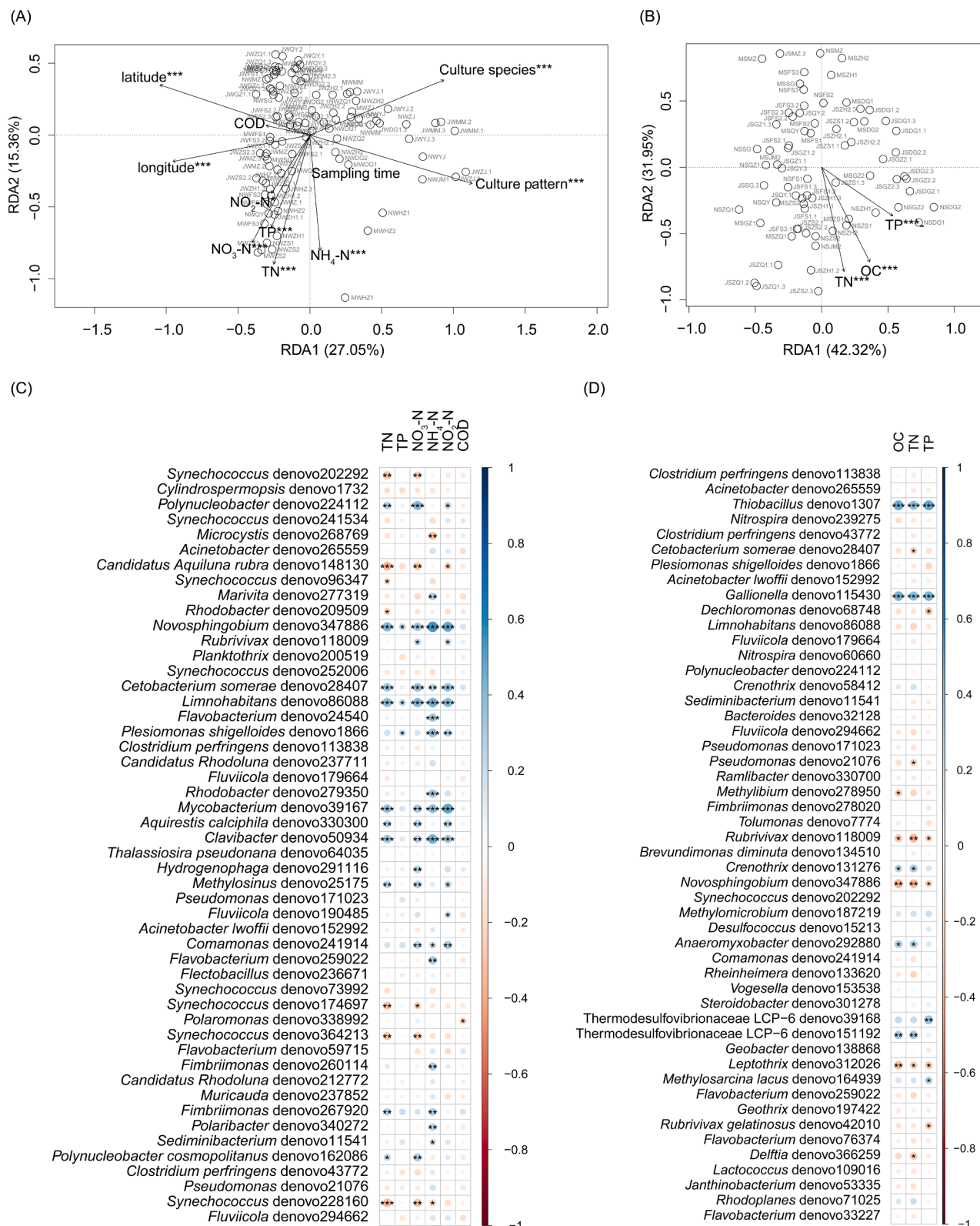
A total of 65 pathogenic genera were identified from the aquaculture bacterial pathogen database described by Lo et al. (2023) (Table S2). The most abundant pathogenic genera included *Clostridium*, *Acinetobacter*, and *Flavobacterium* (Fig. S8A). Temporal analysis revealed that in November and July, the relative abundance of pathogenic bacteria in sediment was significantly higher than in water ( $p < 0.01$ ; Fig. S8B–D). In July, the relative abundance of pathogenic bacteria in water was significantly lower compared to March and November ( $p < 0.01$ ), showing a downward trend over time (Fig. S8E). In sediment, the abundance of pathogenic bacteria in July was significantly lower than in March ( $p < 0.05$ ) but not significantly different from November (Fig. S8F).

The db-RDA results showed that aquaculture species, culture pattern (freshwater, seawater, brackish, modern captive culture, freshwater factory container aquaculture, or seawater factory culture), and site geography (longitude, latitude) significantly influenced water and sediment pathogenic ( $p < 0.05$ ; Fig. 5A and B). Among physicochemical parameters,  $\text{NO}_3^-\text{-N}$  concentration was significantly correlated with water pathogenic bacteria ( $p < 0.05$ ; Fig. 5A). In water, *Citrobacter* and *Klebsiella* were positively correlated with sampling time ( $p < 0.05$ ), while *Stenotrophomonas* was negatively correlated ( $p < 0.05$ ). Culture patterns were positively correlated with pathogenic genera such as *Francisella*, *Pseudoalteromonas*, *Tenacibaculum*, and *Vibrio*, while negatively correlated with *Edwardsiella* ( $p < 0.05$ ). Nitrogen compounds (TN,  $\text{NO}_3^-\text{-N}$ ,  $\text{NH}_4^+\text{-N}$ , and  $\text{NO}_2^-\text{-N}$ ) were positively correlated with *Mycobacterium*, and  $\text{NO}_3^-\text{-N}$  was negatively correlated with *Vibrio* ( $p < 0.05$ ; Fig. 5C). In sediment, genera such as *Acinetobacter*, *Clostridium*, *Delftia*, and *Stenotrophomonas* were significantly negatively correlated with sampling time, while only *Myxococcus* was positively correlated with culture patterns ( $p < 0.05$ ; Fig. 5D). Nitrogen and phosphorus concentrations were significantly correlated with pathogenic genera such as *Aeromonas*, *Serratia*, and *Vibrio*, showing negative correlations with TN and TP ( $p < 0.05$ ). These findings indicate that elevated  $\text{NH}_4^+\text{-N}$  levels in pond water significantly increased the diversity of pathogenic bacteria, while nitrogen levels positively correlated with the abundance of *Mycobacterium*. Culture patterns and species also influenced the relative abundance of key pathogens, including *Vibrio*, *Tenacibaculum*, *Pseudoalteromonas*, and *Francisella*. Furthermore, culture pattern mainly caused the relative abundance differences of *Tenacibaculum*, *Aeromonas*, *Microbacterium*, *Eubacterium*, *Plesiomonas*, *Flavobacterium*, *Pseudoalteromonas*, and *Francisella* in water microbiota, and *Myxococcus* in sediment microbiota (Fig. S9). Moreover, culture species mainly caused the relative abundance differences of *Plesiomonas*, *Flavobacterium*, and *Mycobacterium* in water microbiota, and *Mycoplasma*, *Acinetobacter*, and *Enterobacter* in sediment microbiota (Fig. S9).

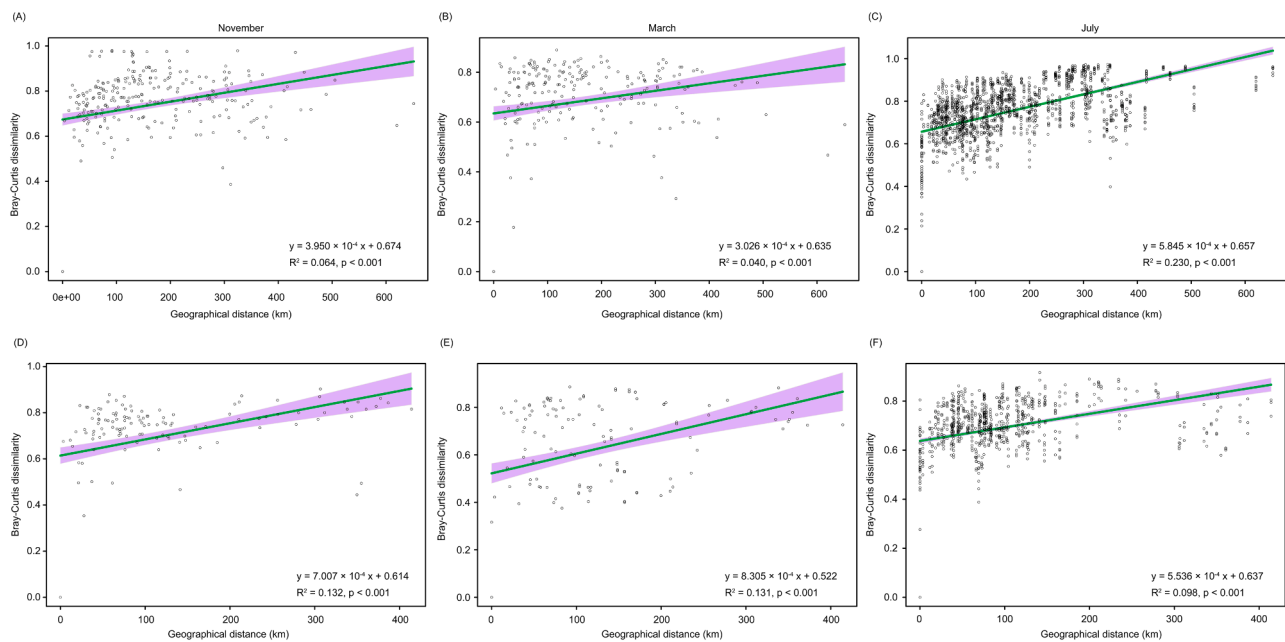
Co-occurrence networks based on the spearman correlation coefficient of genera indicated that the water microbiota exhibited the highest co-occurrence in March, the lowest co-occurrence in July, and that the average clustering coefficient increased from November to July (Fig. 6A–C). The sediment microbiota exhibited the highest co-occurrence in November, whereas they exhibited the lowest co-occurrence in July, and the average clustering coefficient increased from November to July (Fig. 6D–F). Although only three pathogenic genera showed significant co-occurrence with other genera in the water microbiota in July, more pathogenic genera showed significant co-occurrence with other genera in the sediment microbiota (Fig. 6C and 6F).



**Fig. 2.** Water and sediment microbiota structures in aquaculture pond systems. (A) Rank–abundance curve; (B) OTU richness; (C) Chao1 index; (D) Shannon index; (E) Good's coverage; (F) PCoA based on weighted UniFrac distances of water and sediment microbiota; (G) dominant phyla of water and sediment microbiota. \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .



**Fig. 3.** Correlations between physicochemical indicators and microbiota in aquaculture water and sediments. (A) Distance-based redundancy analysis (db-RDA) profile indicating correlations between water physicochemical characteristics and microbiota in aquaculture pond systems; (B) db-RDA profile indicating correlations between sediment physicochemical characteristics and microbiota in aquaculture pond systems; (C) correlations between water physicochemical characteristics and top 50 microbial OTUs in aquaculture pond systems; (D) correlations between sediment physicochemical characteristics and top 50 microbial OTUs in aquaculture pond systems. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .



**Fig. 4.** Correlation between geographical distance and Bray–Curtis dissimilarity. (A), (B), and (C) Water microbiota. (D), (E), and (F) Sediment microbiota. The light-purple background represents the 95 % confidence interval.

### 3. Discussion

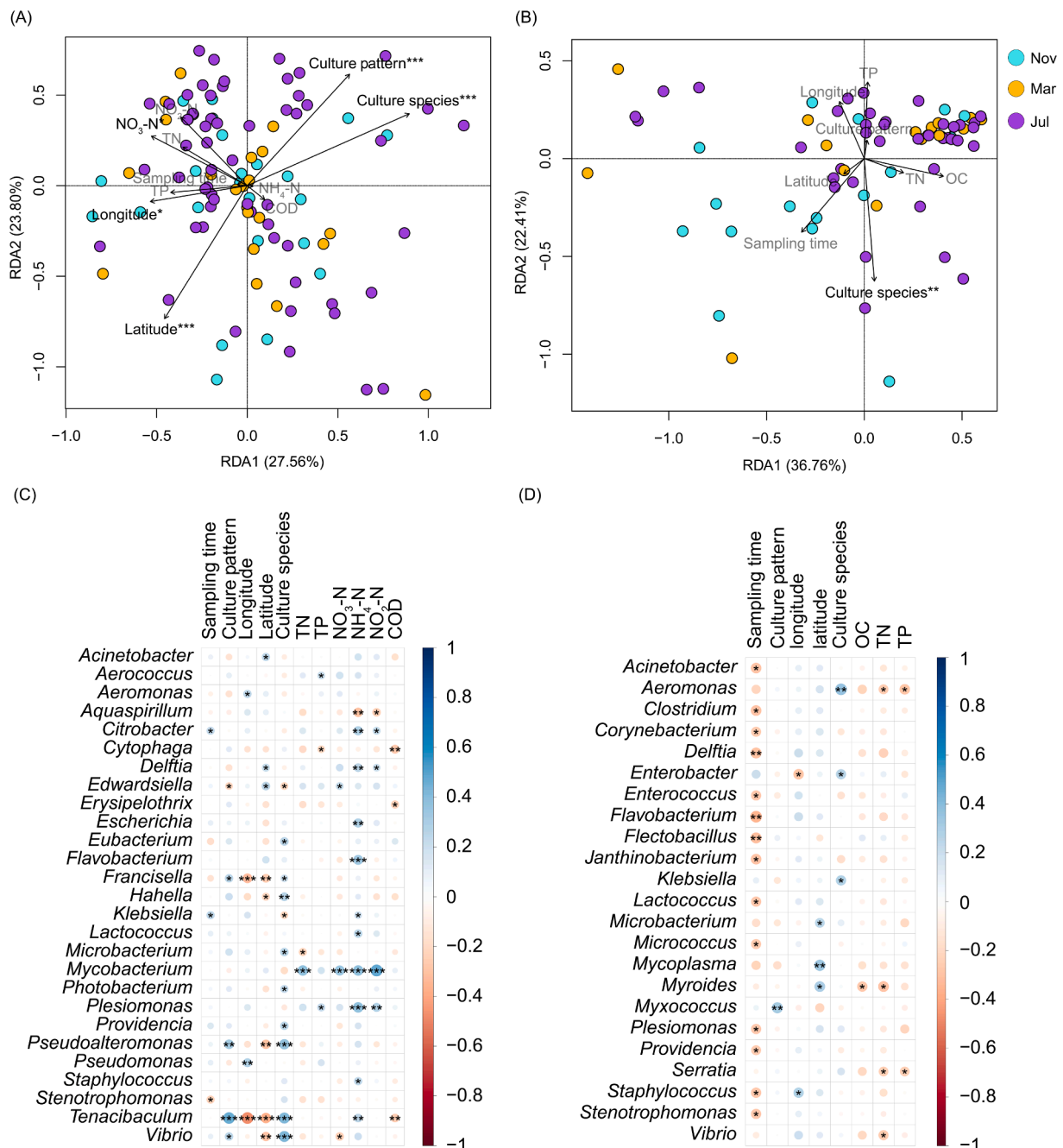
Microbes communities play a key role in aquaculture systems by influencing water quality, animal health, and disease dynamics (Perry et al., 2020). Our results confirm that microbial diversity, particularly in sediments, is crucial for the stability of pond ecosystems. High microbial diversity is known to confer resilience to ecological disturbances and reduce the risk of pathogenic invasions (De Schryver and Vadstein, 2014). The higher diversity in sediments may contribute to the resilience of these systems, buffering against pathogen invasions and environmental disturbances. In contrast, water microbiota exhibited lower diversity, which could make water more vulnerable to opportunistic pathogens (Liu et al., 2020). Our results indicated that the culture pattern and species significantly influenced the relative abundances of pathogenic genera such as *Vibrio*, *Tenacibaculum*, *Pseudoalteromonas*, and *Francisella* in pond water. Intensive aquaculture systems, with their nutrient inputs, often lead to stress in organisms and may promote pathogen proliferation (Tarnecki and Guttman, 2023). Our findings highlight that high microbial diversity in aquaculture systems is essential for disease prevention and ecosystem stability, reinforcing the idea that biodiversity contributes to ecosystem health.

Similar to open water systems, aquaculture ponds are subject to fluctuating environmental parameters, but their small size and limited water exchange result in rapid shifts in physicochemical and biotic conditions. These fluctuations can significantly influence the composition of microbial communities, especially when compared to more stable open water environments (Lorgen-Ritchie et al., 2023). Our study demonstrated that geographic distance was positively correlated with microbiota dissimilarity in both water and sediment samples, supporting the hypothesis of a distance-decay relationships in microbial biogeography (Huang et al., 2016). However, the correlation was weaker for water microbiota, suggesting that local environmental factors such as aquaculture practices and nutrient concentrations may play a more significant role than spatial distance alone in shaping water microbiota. The relative isolation of pond environments, often coupled with limited water exchange, could exacerbate this pattern by creating localized microbial communities. During the summer months, where sedimentary microbial communities have shown stronger spatial structuring due to the more stable nature of sediment environments (Sriyasa

et al., 2015; Dien et al., 2019). Seasonal shifts in microbial communities and pathogen abundance were evident in both water and sediment microbiota. Temperature, nutrient levels, and other physicochemical factors likely contribute to these temporal dynamics (Resende et al., 2015). These seasonal patterns in microbial interactions suggest that microbial networks become more fragmented in the warmer months, potentially reducing the ability of the microbiome to suppress pathogens during this period. This is particularly concerning in the summer, when environmental factors such as high temperatures and eutrophication may further exacerbate the risk of disease outbreaks (Dien et al., 2019).

*Bacillus* species are commonly used as probiotics in aquaculture to control nitrogenous pollutants (Hlodzi et al., 2020; Ren et al., 2021). However, we observed a very low relative abundance of *Bacillus* in the pond water and sediment microbiota. Diseases in cultured animals cause serious losses in aquaculture and mariculture (Zhang et al., 2022a). This finding points to the need for a more integrated approach to microbial management in aquaculture, where probiotic use is combined with broader practices such as controlling nutrient inputs, monitoring water chemistry, and optimizing pond design to promote the growth of beneficial microbes. An ecosystem-based approach that takes into account the complex interactions between the microbiome, environmental factors, and host organisms is essential for ensuring the sustainability of aquaculture practices (Lorgen-Ritchie et al., 2023).

According to incomplete statistics in China, the culture area with medium disease prevalence accounted for 15–20 % of the total culture area, with a loss of over 1 million tons a year (Zhou et al., 2009). Clois-Fuentes et al. (2023) investigated water bacterial communities at three locations within recirculating aquaculture systems for African catfish (*Claria gariepinus*) at different stocking densities and detected potentially pathogenic genera with their total abundance increasing with fish biomass. The monitoring and screening of pathogens in culture water are considered important ways to prevent the occurrence of aquaculture diseases (Lo et al., 2023). Another approach is to analyze and control the factors that affect pathogenic bacteria. Our results suggest that culture practices such as stocking density and water exchange frequency might play a key role in influencing the abundance of these pathogenic genera and most pathogenic genera were detected in the pond water and sediment microbiota, with sediment showing higher relative abundances of pathogenic bacteria. The increase in the  $\text{NH}_4^+\text{-N}$

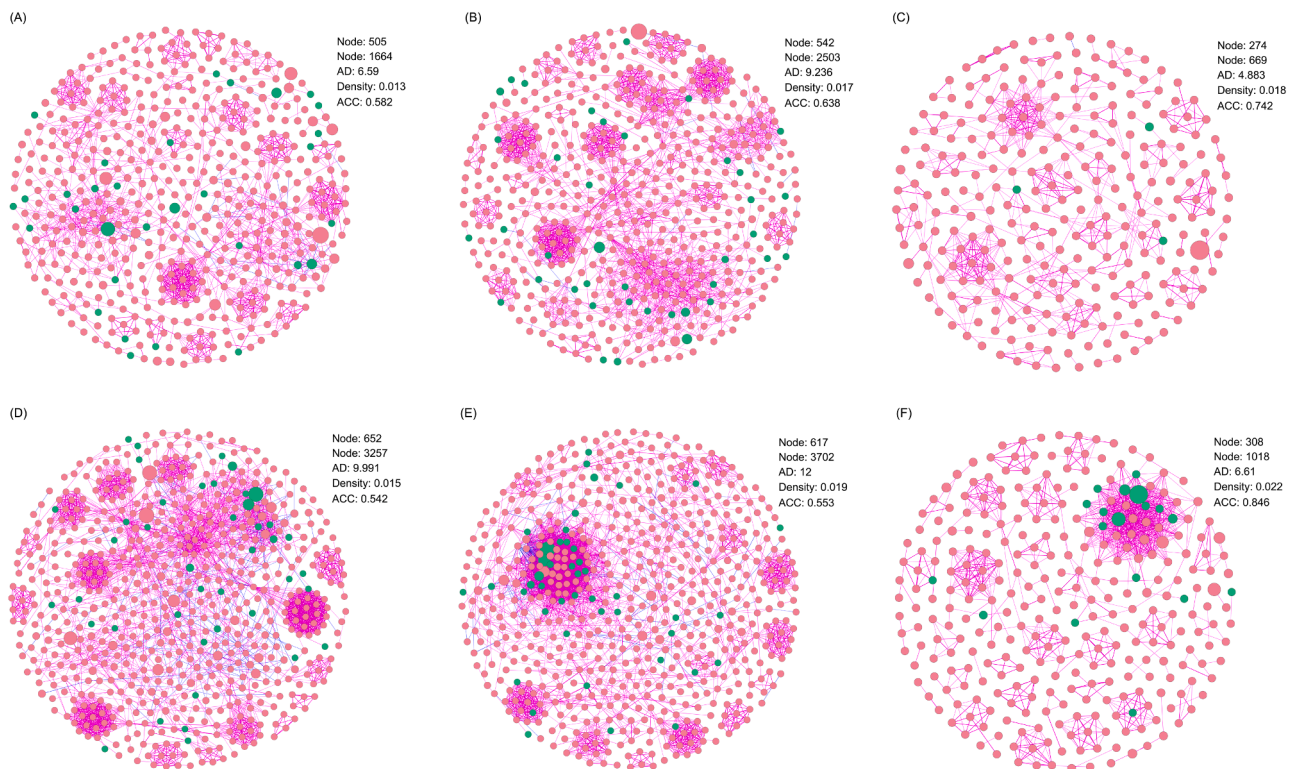


**Fig. 5.** Correlations between pathogenic bacteria and environmental factors in pond water and sediment. (A) db-RDA profile indicating correlations between water physicochemical characteristics and pathogenic bacteria in aquaculture pond systems; (B) db-RDA profile indicating correlations between sediment physicochemical characteristics and pathogenic bacteria in aquaculture pond systems; (C) correlations between water physicochemical characteristics and pathogenic bacteria in aquaculture pond systems; (D) correlations between sediment physicochemical characteristics and pathogenic bacteria in aquaculture pond systems. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

concentration in the pond water significantly increased the variety of pathogenic bacteria. *Vibrio* plays an important role in the metabolism of nitrogen, carbon, and sulfur in aquaculture environments, and *Vibrio* and *Pseudoalteromonas* have been proven to be closely associated with seawater eutrophication (Liu et al., 2024). However, sediment pathogens were most affected by sampling time. Resende et al. (2015) also reported significant seasonal changes in the abundances of several pathogenic bacteria in Nile tilapia aquaculture systems. These results suggest that the importance of culture practices such as aquaculture pattern, species selection, and nutrient management in pond water, should be considered when preventing the growth of pathogenic

bacteria in aquaculture water.

As aquaculture continues to expand, it would be beneficial to employ an ecosystem-based approach when designing and optimizing aquaculture systems to integrate interactions between the physical environment, host, and microbiome in order to promote the establishment of desirable microbiomes for productivity and host health (Lorgen-Ritchie et al., 2023). This suggests that aquaculture managers should consider adjusting farming practices (e.g., water exchange rate, stocking density, and feeding practices) to optimize microbiome health and prevent pathogen proliferation. Several studies have highlighted that integrated approaches, combining probiotic use with the optimization of water



**Fig. 6.** Co-occurrence networks based on the Spearman correlation coefficient of water and sediment genera showing the co-occurrence at different sampling times. (A) Water genera in November; (B) water genera in March; (C) water genera in July; (D) sediment genera in November; (E) sediment genera in March; (F) water genera in July. AD, average density; ACC, average clustering coefficient. Pink and blue edges indicate significant positive (Spearman correlation coefficient  $> 0.7$  and  $P < 0.05$ ) and significant negative (Spearman correlation coefficient  $< -0.7$  and  $P < 0.05$ ) correlations, respectively. The green nodes indicate the pathogenic genera.

management strategies, significantly improve the microbiome stability in aquaculture environments (Hlordzi et al., 2020; Li et al., 2023).

In low-diversity microbial environments with an input of organic material, fast-growing opportunistic R-strategists, which are markers of instability, can outcompete slow-growing K-strategists, which are markers of stability. However, if the water retention time in the system is short, opportunists are less able to replicate, and the stability of the system is more likely to be maintained (Attramadal et al., 2012; Vadstein et al., 2018). A key end goal of system optimization in aquaculture is to define environmental parameters that are conducive to desirable microbiomes in terms of sustainability and productivity, and to monitor and control these parameters in order to ensure optimal performance. However, owing to the wide range of aquaculture system types (categorized as flow-through, open, or recirculating) and their associated temporal dynamics, adopting a flexible, adaptive management strategy that accounts for specific system needs and farming conditions is essential (Lorgen-Ritchie et al., 2023). Instead, aquaculture managers must adopt flexible, adaptive management strategies that consider the specific needs and conditions of each system. These strategies could include regular microbiome monitoring, nutrient management to control eutrophication, and the use of beneficial microbial consortia that enhance system stability and productivity.

## 4. Materials and methods

### 4.1. Study area and sample collection

Pond water and sediment samples were collected from 21 monitoring sites in eastern, western, and northern Guangdong and the Pearl River Delta in November 2021, March 2022, and July 2022 (Fig. S1 and Table S1). The main aquaculture patterns in the investigated area are: freshwater pond, seawater pond, seawater intensive aquaculture,

freshwater factory aquaculture, and higher-place pond aquaculture. The high level pond is usually a cement pond or an earth pond with mulch. Compared with the common pond, the higher-place ponds has a perfect inlet and drainage system, and its bottom level is higher.

Approximately 500-mL water sample was collected using the five-point sampling method from 0.5 m below the water's surface at each pond using an organic glass water sampler. The water samples were then stored in ice bags and transported to the laboratory, where their physicochemical characteristics were measured within 24 h. To analyze the microbiota composition, microorganisms were collected by filtering approximately 500 mL of water through a polycarbonate membrane with an aperture of 0.22  $\mu\text{m}$  (47-mm diameter, Whatman, Maidstone, UK). All filter membranes were stored at  $-80\text{ }^{\circ}\text{C}$  until DNA extraction. Sediment samples were collected using the five-point sampling method from 0 to 5 cm using a cylindrical sampler and transported to the laboratory in ice bags and stored at  $-20\text{ }^{\circ}\text{C}$ . In the laboratory, each sample was divided into two sub-samples, one for physicochemical analysis and the other kept at  $-80\text{ }^{\circ}\text{C}$  for microbiota composition analysis.

### 4.2. Determination of physicochemical characteristics

The chemical oxygen demand (COD), TN, total phosphorus (TP),  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and  $\text{PO}_4^{3-}\text{-P}$  of the water were determined according to our previously described methods (Li et al., 2023). The sediment organic carbon (OC), TN, and TP were determined according to methods described by Lu et al. (2023).

### 4.3. Analysis of microbiota structure

Approximately 500 mL of water was filtered through a glass fiber filter with an aperture of 0.22  $\mu\text{m}$  (Whatman, Metstone, UK) to separate the microbiota. The filters were cut into small pieces (Ni et al., 2010)



and microbial DNA was extracted using the FastDNA Spin kit for soil (MP Biomedicals, Solon, OH, USA). The sediment microbial DNA was extracted from approximately 0.3 g of sediment using the same kit. DNA concentration and purity were evaluated using a Nanodrop 2000 spectrophotometer (Thermo Scientific, USA) and DNA integrity was evaluated using 1 % agarose gels.

The V4–V5 hypervariable region of the 16S rRNA gene was amplified using the universal primer pair 515F and 907R (Cai et al., 2016; Huang et al., 2021) with a 12-nucleotide sample-specific barcode included at the 5'-end of the 515F sequence to allow the samples to be distinguished (Fu et al., 2023). Polymerase chain reactions (PCRs) and sequencing were conducted at Guangdong Meilikang Bio-Science Ltd. (Foshan, Guangdong, China) as previously described (Xiang et al., 2018).

Raw sequence data were merged using FLASH 1.2.8 (Magoc and Salzberg, 2011) and processed using QIIME 1.9.0 (Caporaso et al., 2010), as previously described (Ni et al., 2017; Xiang et al., 2018). To detect the changes in conditional pathogenic bacteria in water and sediment microbiota during the culture process, we acquired the pathogenic genera from the database described by Lo et al. (2023), confirmed the genera according to Irshath et al. (2023), and then analyzed the changes in the relative abundances of these genera.

#### 4.4. Data analysis

Data are presented as means  $\pm$  standard error (SE) for each group. PCoA was performed using QIIME 1.9.0. A non-parametric multivariate analysis of variance (PERMANOVA) (Anderson, 2001) was conducted using the R vegan package (Dixon, 2003). Results with  $P < 0.05$  were considered as statistically significant.

## 5. Conclusions

Although the microbiota were significantly affected by physical and chemical parameters, the relative abundance of pathogenic bacteria in the pond water microbiota was mainly affected by aquaculture pattern and species. Overall, the relative abundances of pathogenic bacteria in the sediment were higher than those in the water. In the pond water, an increase in the  $\text{NH}_4^+$ -N concentration of the pond increased the variety of pathogenic bacteria, an increase in nitrogen significantly increased the relative abundance of *Mycobacterium*, and the culture pattern and species significantly affected the relative abundances of *Vibrio*, *Tenacibaculum*, *Pseudoalteromonas*, and *Francisella*. However, sediment pathogens were most strongly affected by sampling time. These results suggest that the culture pattern, culture species, and nitrogen concentration should be considered fully when preventing the growth of pathogenic bacteria in aquaculture water.

## Ethics statement

This study has no animal or human experiments. There are no ethical issues involved.

## CRedit authorship contribution statement

**Shuhui Niu:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Data curation. **Chuanlong Li:** Validation, Data curation. **Jun Xie:** Resources, Project administration, Funding acquisition. **Zhifei Li:** Validation, Methodology. **Kai Zhang:** Validation, Methodology. **Guangjun Wang:** Visualization, Validation, Data curation. **Yun Xia:** Validation. **Jingjing Tian:** Validation. **Hongyan Li:** Validation. **Wenping Xie:** Validation. **Wangbao Gong:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.wroa.2025.100302.

## Data availability

The data analysed in the study was deposited in the NCBI sequence reads archive database under BioProject number PRJNA1135658.

## References

- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral. Ecol.* 26, 32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x>.
- Attramadad, K.J.K., Salvesen, I., Xue, R., Öie, G., Størseth, T.R., Vadstein, O., et al., 2012. Recirculation as a possible microbial control strategy in the production of marine larvae. *Aquac. Eng.* 46, 27–39. <https://doi.org/10.1016/j.aquaeng.2011.10.003>.
- Cai, Y., Zheng, Y., Bodelier, P.L.E., Conrad, R., Jia, Z., 2016. Conventional methanotrophs are responsible for atmospheric methane oxidation in paddy soils. *Nat. Commun.* 7, 11728. <https://doi.org/10.1038/ncomms11728>.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7, 335–336. <https://doi.org/10.1038/nmeth.f.303>.
- Clois-Fuentes, J., Nguinkal, J.A., Unger, P., Kreikemeyer, B., Palm, H.W., 2023. Bacterial community in African catfish (*Clarias gariepinus*) recirculating aquaculture systems under different stocking densities. *Front. Mar. Sci.* 10, 1073250. <https://doi.org/10.3389/fmars.2023.1073250>.
- De Schryver, P., Vadstein, O., 2014. Ecological theory as a foundation to control pathogenic invasion in aquaculture. *ISME J.* 8, 2360–2368. <https://doi.org/10.1038/ismej.2014.84>.
- Dien, L.D., Hiep, L.H., Faggotter, S.J., Chen, C., Sammut, J., Burford, M.A., 2019. Factors driving low oxygen conditions in integrated rice-shrimp ponds. *Aquaculture* 512, 734315. <https://doi.org/10.1016/j.aquaculture.2019.734315>.
- Dixon, P., 2003. VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* 14, 927–930. <https://doi.org/10.1111/j.1654-1103.2003.tb02228.x>.
- FAO, 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. FAO, Rome. <https://doi.org/10.4060/cc0461en>.
- Fu, C., Ni, J., Huang, R., Gao, Y., Li, S., Li, Y., Li, J., Zhong, K., Zhang, P., 2023. Sex different effect of antibiotic and probiotic treatment on intestinal microbiota composition in chemically induced liver injury rats. *Genomics* 115, 110647. <https://doi.org/10.1016/j.ygeno.2023.110647>.
- Gong, W., Gao, S., Zhu, Y., Wang, G., Zhang, K., Li, Z., Yu, E., Tian, J., Xia, Y., Xie, J., Ni, J., 2021. Effect of the aerobic denitrifying bacterium *Pseudomonas furukawaii* ZS1 on microbiota compositions in grass carp culture water. *Water (Basel)* 13, 1329. <https://doi.org/10.3390/w13101329>.
- Hlordzi, V., Kuebutornye, F.K.A., Afriyie, G., Abarike, E.D., Lu, Y., Chi, S., Anokyewaa, M.A., 2020. The use of *Bacillus* species in maintenance of water quality in aquaculture: a review. *Aquac. Reports* 18, 100503. <https://doi.org/10.1016/j.aqrep.2020.100503>.
- Huang, S., Wu, M., Zang, C., Du, S., Domagalski, J., Gajewska, M., et al., 2016. Dynamics of algae growth and nutrients in experimental enclosures culturing bighead carp and common carp: phosphorus dynamics. *Int. J. Sediment Res.* 31, 173–180. <https://doi.org/10.1016/j.ijsrsc.2016.01.003>.
- Huang, R., Crowther, T.W., Sui, Y., Sun, B., Liang, Y., 2021. High stability and metabolic capacity of bacterial community promote the rapid reduction of easily decomposing carbon in soil. *Commun. Biol.* 4, 1376. <https://doi.org/10.1038/s42003-021-02907-3>.
- Irshath, A.A., Rajan, A.P., Vimal, S., Prabhakaran, V.-S., Ganesan, R., 2023. Bacterial pathogenesis in various fish diseases: recent advances and specific challenges in

- vaccine development. *Vaccines* (Basel) 11, 470. <https://doi.org/10.3390/vaccines11020470>.
- Li, Z., Gao, Y., Lu, Z., Xie, J., Liu, Y., Wang, G., Cheng, X., 2023. Strengthening and microbial regulation mechanism of *Bacillus* on purification device for grass carp culture wastewater. *Frontiers Environ. Sci.* 11, 1128329. <https://doi.org/10.3389/fenvs.2023.1128329>.
- Lian, Y., Zheng, X., Xie, S., A. D., Wang, J., Tang, J., Zhu, X., Shi, B., 2023. Microbiota composition and correlations with environmental factors in grass carp (*Ctenopharyngodon idella*) culture ponds in South China. *PeerJ* 11, e15892. <https://doi.org/10.7717/peerj.15892>.
- Liang, Q., Zhang, X., Lee, K.H., Wang, Y., Yu, K., Shen, W., Fu, L., Shu, M., Li, W., 2015. Nitrogen removal and water microbiota in grass carp culture following supplementation with *Bacillus licheniformis* BSK-4. *World J. Microbiol. Biotechnol.* 31, 1711–1718. <https://doi.org/10.1007/s11274-015-1921-3>.
- Liu, Z.G., Lqbal, M., Zeng, Z.B., Lian, Y.X., Zheng, A.F., Zhao, M.M., Li, Z.X., Wang, G.J., Li, Z.F., Xie, J., 2020. Comparative analysis of microbial community structure in the ponds with different aquaculture model and fish by high-throughput sequencing. *Microb. Pathogenesis* 142, 104101. <https://doi.org/10.1016/j.micpath.2020.104101>.
- Liu, Z.T., Wang, P.D., Li, J.L., Luo, X.Q., Zhang, Y., Huang, X.H., Zhang, X., Li, W.J., Qin, Q.W., 2024. Comparative metagenomic analysis of microbial community compositions and functions in cage aquaculture and its nearby non-aquaculture environments. *Front. Microbiol.* 15, 1398005. <https://doi.org/10.3389/fmicb.2024.1398005>.
- Lo, L.S.H., Liu, X., Liu, H., Shao, M., Qian, P.-Y., Cheng, J., 2023. Aquaculture bacterial pathogen database: pathogen monitoring and screening in coastal waters using environmental DNA. *Water Res. X* 20, 100194. <https://doi.org/10.1016/j.wroa.2023.100194>.
- Lorgen-Ritchie, M., Webster, T.U., McMurtrie, J., Bass, D., Tyler, C.R., Rowley, A., Martin, S.A.M., 2023. Microbiomes in the context of developing sustainable intensified aquaculture. *Front. Microbiol.* 14, 1200997. <https://doi.org/10.3389/fmicb.2023.1200997>.
- Lu, J., Feng, S., Wang, S., Zhang, B., Ning, Z., Wang, R., Chen, X., Yu, L., Zhao, H., Lan, D., Zhao, X., 2023. Patterns and driving mechanism of soil organic carbon, nitrogen, and phosphorus stoichiometry across northern China's desert-grassland transition zone. *Catena* 220 (Part A), 106695. <https://doi.org/10.1016/j.catena.2022.106695>.
- Magoc, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27, 2957–2963. <https://doi.org/10.1093/bioinformatics/btr507>.
- Murphy, T.R., Xiao, R., Brooks, M.L., Rader, B.A., Hamilton-Brehm, S., 2022. Aquaculture production of hatchling Hawaiian bobtail squid (*Euprymna scolopes*) is negatively impacted by decreasing environmental microbiome diversity. *J. Appl. Microbiol.* 132, 1724–1737. <https://doi.org/10.1111/jam.15350>.
- Newton, R.J., Jones, S.E., Eiler, A., McMahon, K.D., Bertilsson, S., 2011. A guide to the natural history of freshwater lake bacteria. *Microbiol. Mol. Biol. Rev.* 75 (1), 14–49. <https://doi.org/10.1128/MMBR.00028-10>.
- Ni, J., Yu, Y., Feng, W., Yan, Q., Pan, G., Yang, B., Zhang, X., Li, X., 2010. Impacts of algal blooms removal by chitosan-modified soils on zooplankton community in Taihu Lake. *China. J. Environ. Sci.* 22 (10), 1500–1507. [https://doi.org/10.1016/S1001-0742\(09\)60270-9](https://doi.org/10.1016/S1001-0742(09)60270-9).
- Ni, J., Li, X., He, Z., Xu, M., 2017. A novel method to determine the minimum number of sequences required for reliable microbial community analysis. *J. Microbiol. Methods* 139, 196–201. <https://doi.org/10.1016/j.mimet.2017.06.006>.
- Perry, W.B., Lindsay, E., Payne, C.J., Brodie, C., Kazlauskaitė, R., 2020. The role of the gut microbiome in sustainable teleost aquaculture. *Proc. R Soc. B* 287 (1926), 20200184. <https://doi.org/10.1098/rspb.2020.0184>.
- Ren, W., Wu, H., Guo, C., Xue, B., Long, H., Zhang, X., Cai, X., Huang, A., Xie, Z., 2021. Multi-strain tropical *Bacillus* spp. as a potential probiotic biocontrol agent for large-scale enhancement of mariculture water quality. *Front. Microbiol.* 12, 699378. <https://doi.org/10.3389/fmicb.2021.699378>.
- Resende, J.A., Silva, V.L., Cesar, D.E., Del'Duca, A., Fontes, C.O., Diniz, C.G., 2015. Seasonal dynamics of microbial community in an aquaculture system for Nile tilapia (*Oreochromis niloticus*). *Aquac. Res.* 46, 1233–1240. <https://doi.org/10.1111/are.12281>.
- Robles-Porchas, G.R., Gollas-Galván, T., Martínez-Porchas, M., Martínez-Cordova, L.R., Miranda-Baeza, A., Vargas-Albores, F., 2020. The nitrification process for nitrogen removal in biofloc system aquaculture. *Rev. Aquac.* 12 (4), 2228–2249. <https://doi.org/10.1111/raq.12431>.
- Sriyasa, P., Chitmanat, C., Whangchai, N., Promya, J., Lebel, L., 2015. Effect of water de-stratification on dissolved oxygen and ammonia in tilapia ponds in northern Thailand. *Int. Aquat. Res.* 7, 287–299. <https://doi.org/10.1007/s40071-015-0113-y>.
- Tarnecki, A.M., Guttman, L., 2023. Editorial: microbial diversity as a prerequisite for resilience and resistance in sustainable aquaculture. *Front. Mar. Sci.* 10, 1227795. <https://doi.org/10.3389/fmars.2023.1227795>.
- Vadstein, O., Attramadal, K.J.K., Bakke, I., Olsen, Y., 2018. K-selection as microbial community management strategy: a method for improved viability of larvae in aquaculture. *Front. Microbiol.* 9, 2730. <https://doi.org/10.3389/fmicb.2018.02730>.
- Xiang, J., He, T., Wang, P., Xie, M., Xiang, J., Ni, J., 2018. Opportunistic pathogens are abundant in the gut of cultured giant spiny frog (*Paa spinosa*). *Aquac. Res.* 49, 2033–2041. <https://doi.org/10.1111/are.13660>.
- Zhang, F., Xie, F., Zhou, K., Zhang, Y., Zhao, Q., Song, Z., Cui, H., 2022. Nitrogen removal performance of novel isolated *Bacillus* sp. capable of simultaneous heterotrophic nitrification and aerobic denitrification. *Appl. Biochem. Biotechnol.* 194, 3196–3211. <https://doi.org/10.1007/s12010-022-03877-w>.
- Zhang, X., You, Y., Peng, F., Tang, X., Zhou, Y., Liu, J., Lin, D., Zhou, Y., 2022. Interaction of microbiota between fish and the environment of an in-pond raceway system in a lake. *Microorganisms* 10 (6), 1143. <https://doi.org/10.3390/microorganisms10061143>.
- Zhao, A., Lu, Y., Li, Q., Li, T., Zhao, J., 2023. Metagenomics reveals the diversity and role of surface-water microbes in biogeochemical cycles in lakes at different terrain ladders. *Frontiers Environ. Sci.* 11, 1121775. <https://doi.org/10.3389/fenvs.2023.1121775>.
- Zhou, Q., Li, K., Jun, X., Bo, L., 2009. Role and functions of beneficial microorganisms in sustainable aquaculture. *Bioresour. Technol.* 100, 3780–3786. <https://doi.org/10.1016/j.biortech.2008.12.037>.
- Zhu, Y., Gong, W., Xie, J., Wang, G., Yu, D., Li, Z., Zhang, K., Tian, J., 2020. Identification and denitrification characteristics of the aerobic denitrifying bacteria *Pseudomonas furukawii* ZS1. *Acta. Hydrobiol. Sin.* 44, 895–903.