MICROBIAL COMMUNITY STRUCTURE AND ITS DRIVING FACTORS IN THE WHITELEG SHRIMP (*LITOPENAEUS VANNAMEI*) AQUACULTURE SYSTEM

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Abstract. Microbial community is a vital component of aquaculture. In this study, microbial community structure and function in whiteleg shrimp (*Litopenaeus vannamei*) aquaculture were examined through 16S rRNA gene high-throughput sequencing. Proteobacteria, Bacteroidota and Actinobacteriota were observed as the dominant microbial phyla during whiteleg shrimp aquaculture at an industrial scale. With time, the relative abundance of Proteobacteria initially increased and then showed a declining trend. On the contrary, relative abundance of Bacteroidota and Actinobacteriota initially decreased and then increased in the flowing water samples. However, in aquaculture water samples, these dominant bacterial phyla showed an opposite trend. Compared to flowing water, microbial richness was significantly lower in the aquaculture water samples. Furthermore, PICRUSt2 analysis and Welch's t-test revealed significant variations between the predicted functions of microbial communities in flowing water and aquaculture water samples. In the samples of aquaculture water, carbon metabolism, glycine, serine and threonine metabolism in the microbial community increased significantly, as compared to the flowing waters. *Marivita*, *Candidatus Aquiluna*, *Donghicola* and *Polaribacter* played important roles in sequestration of carbon and reduction of nitrogen. Redundancy analysis revealed that nitrate-N, nitrite-N, ammonia-N, pH, salinity and temperature were the key factors influencing the microbial communities during the aquaculture of whiteleg shrimp at an industrial scale.
Keywords: *aquaculture, shrimp, bacterial community, high-throughput sequencing, seawater*

Introduction

With the modernization of fishery, industrial aquaculture has developed rapidly in China (Wang, 2015; Abakari et al., 2022). In 2022, industrial mariculture occupied an area of approximately 4.32 million m^3, with an annual yield of 389,583 tons (CFSY, 2023). Industrial aquaculture has the advantages of land and manpower saving and high productivity, and its impacts on climate and environment are limited. Thus, industrial aquaculture can be helpful in achieving the goals of energy saving, emission reduction, and transformation of the economic development model (Tang, 2017). Whiteleg shrimp (*Litopenaeus vannamei*) is an important species cultivated globally through aquaculture. In 2022, the total yield of whiteleg shrimp was 1,340,280 tons, which was 5.23% higher than that in 2021 (CFSY, 2023). With the increase in aquaculture yield, the decreased water quality has attracted more and more attention among researchers (Jana and Sarkar, 2005; Ritonga, 2021). In shrimp culture, only 10-

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20% of phosphorus and 20-30% of nitrogen in the feeds were consumed by shrimps, while over 50% of nutrients remained in the aquaculture waters (Thakur and Lin, 2003; Zang et al., 2009; Sahu et al., 2013; Chen et al., 2018; Abakari et al., 2022). The increased levels of nutrients in aquaculture waters induced the decrease in dissolved oxygen and increase in nitrogen, phosphorus and organic matters, and even cause the outbreak of harmful algal blooms (Yusoff et al., 2002), which further affected the growth and yield of shrimps.

Microbial community is a vital component of the aquaculture environment and plays a crucial role in material cycling, energy flow, water quality control, pathogen defense and host health (Abraham et al., 2004; Rungrassamee et al., 2016; Fan et al., 2019; John et al., 2020; Zhao et al., 2022). Certain microbes may have a positive effect on the health of farmed animals, while others may cause diseases and even death of cultured species (Crab et al., 2012; De Schryver et al., 2014; Abakari et al., 2022). Therefore, a complete understanding of the characteristics and ecological functions of microbial community in an aquaculture ecosystem is essential to establish effective microbial ecological strategies for sustainable management of shrimp aquaculture. With the advancements in molecular biology techniques, high-throughput sequencing (HTS) of microbial DNA (obtained from environmental samples) has been extensively applied to analyze microbial diversity and community structure in aquaculture ecosystems (Herlambang et al., 2021; Kolda et al., 2020; Zhou et al., 2021; Dahle et al., 2022). Compared to traditional techniques, HTS is more accurate, highly efficient, easy to operate, and suitable for large-scale spatial and temporal investigation. HTS has a good application prospect in the identification of new microbes as well as in assessing the diversity of microbial communities in the ecosystems (Reuter et al., 2015).

In this study, diversity and structure of microbial community were investigated in whiteleg shrimp aquaculture system at industrial scale, using 16S rRNA gene high-throughput sequencing. The objectives were to (1) assess the microbial community dynamics in the aquaculture water; (2) identify the key environmental factors affecting the succession of microbial community.

Materials and methods

Sample collection

Experiments were carried out in Haiyang Yellow Sea Aquatic Products Co., Ltd. (36°40′23″N; 121°09′00″E), located in Shandong Province of China. Shrimps were cultured in the cement tanks with an area of 80 square meters and a height of 1.5 meters. Water depth of was about 1 m. The water supplied for shrimp farming was the mixture of seawater and underground brine in the early phase, and then changed to seawater in the middle and later phases. The stocking density of was 400 shrimps per square meter. During the experiment, the shrimps were fed with commercial feed (Chia Tai Group, Qingdao) four times a day. The water was changed four times a day, and the quantity accounted for about 20-40% of the total volume. Organic matters accumulated at the bottom of ponds were usually was thrown away by using siphon technology or through outlets. During the process of shrimp cultivation, some water purification agents including magnesium hydroxide, calcium hydroxide, silicon dioxide, 12 trace elements and some probiotics including photosynthetic bacteria, lactic acid bacteria, yeast, other active bacteria were used to purify water and promote the healthy growth of shrimps.

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Samples of flowing water (FW) and aquaculture water (AW) in four tanks whiteleg shrimp aquaculture were collected in every ten days from February to May in 2019. The sampling period covered the early stage (27th Feb to 9th Mar), the middle stage (19th Mar to 8th Apr), the later stage (18th Apr to 18th May), and the final stage (28th May) of whiteleg shrimp cultivation. Dissolved oxygen (DO) salinity (S), temperature (T), and pH of water samples were detected in situ by Aqua TROLL®600 (In-Situ Inc., USA). Collected water samples were placed in cool boxes and transported to laboratory for further analysis. 100 mL of each sample was allowed to pass through 0.22 μm Millipore membrane, and then the used membrane was utilized for DNA analysis. Simultaneously, 500 mL of each water sample was passed through 0.45 μm Millipore membrane, and the filtered water was used to determine the nitrite-N, nitrate-N, ammonia-N, and orthophosphate-P contents.

DNA extraction and amplicon sequencing

Genomic DNA was extracted from the collected water samples using the FastDNA spin kit for soil (MP Biomedicals, OH, USA), as instructed by the manufacturer. Quality and concentration of DNA were assessed by a Nanodrop spectrophotometer (NanoDrop Technologies, USA), while DNA purity was analyzed through 1% agarose gel electrophoresis. In polymerase chain reaction (PCR), V3-V4 region of 16S rRNA genes was targeted using 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers (Mori et al., 2013). All experiments were performed on three replicates of each sample. PCR amplicons of the same sample were mixed and separated by 2% agarose gel electrophoresis. Furthermore, the amplicons were purified using the AxyPrepDNA Gel Extraction Kit (Axygen Biosciences, USA), and analyzed by QuantiFluor™-ST (Promega, USA). Subsequently, purified amplicons were pooled in equimolar and the sequence library was prepared using a TruSeqTM DNA Sample Prep Kit. The paired-end (PE300) sequencing was performed on MiSeq platform (Illumina, USA) at Majorbio Bio-Pharm Technology Co. Ltd (Shanghai, China). Obtained raw sequences were deposited in National Center for Biotechnology Information (NCBI), with accession number: SRP349336.

Bioinformatics analysis and statistical analysis

QIIME (version 1.9.1) was used to demultiplex and filter the raw sequences after assessing their quality. Using UPARSE (version 11), filtered sequences were clustered in same operational taxonomic unit (OTU), based on the 97% similarity level. The taxonomic assignment of each OTU was done using ribosomal database project (RDP) classifier (version 2.13), based on the Silva16S rRNA database (version 138). Alpha and beta diversity indices of microbes were determined using Mothur (version 1.30.2) and QIIME (version 1.9.1), respectively. The Chao1 and Shannon indices were used for alpha diversity analysis (Chao, 1984; Lemos et al., 2011), while non-metric multidimensional scaling (NMDS) was employed for beta diversity assessment. Variance inflation factor (VIF) analysis was used to determine the collinearity among the different water quality parameters. The relationships between the water quality parameters and the microbial community were determined by the redundancy analysis (RDA) based on linear model and the Spearman's rho correlation analysis. To predict the functional composition of microbial community, phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt 2, version 1.1.0) was

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applied based on 16S rRNA sequences. Welch's t-test ($p < 0.05$) was conducted in statistical analysis of metagenomic profiles (STAMP) software to determine the statistically significant variations in the predicted functions and structures of microbial communities between FW and AW samples.

Results

Water quality parameters

The water quality parameters in the FW and AW are summarized in *Table A1*. During the study period, the water temperature in the FW was lower than those in the AW. The salinity in the FW and AW had no obvious difference, showing increased firstly and then maintained stable. DO concentrations increased firstly and then decreased in the FW, but showed the opposite tendency of changes in the AW. The average concentration of DO in the FW was higher than those in the AW. pH values increased gradually in the FW, but decreased firstly and then increased in the AW. The average concentrations of ammonia-N, nitrate-N, nitrite-N and orthophosphate-P in the FW were 0.03 mg/L, 0.56 mg/L, 0.03 mg/L and 0.06 mg/L, respectively. The average concentrations of ammonia-N, nitrate-N, nitrite-N and orthophosphate-P in the AW were 1.48 mg/L, 1.28 mg/L, 2.16 mg/L and 0.29 mg/L, respectively. The concentrations of these nutrients were lower in the FW than those in the AW.

Microbial community composition

The composition of microbial community and distribution of microbial phyla in the FW and SW samples have been shown in *Figure 1*. In FW samples (*Fig. 1a*), Bacteroidota was the most abundant phylum in the early stage of cultivation, with a relative abundance ranging from 41.11% to 56.63%, followed by Proteobacteria (34.09% - 40.42%). In the middle stage of cultivation, abundance of Bacteroidota declined, and Proteobacteria became the most dominant phylum. In the later and final stages of cultivation, a decrease in the relative abundance of Proteobacteria was observed, along with the increase in the abundance of Bacteroidota and Actinobacteriota; however, Proteobacteria was still more dominant than the other phyla. In the AW samples (*Fig. 1b*), Proteobacteria, Bacteroidota and Actinobacteriota were the dominant phyla. The relative abundance of Proteobacteria initially decreased decreasing, and then increased as the cultivation progressed. On the contrary, relative abundance of Bacteroidota and Actinobacteriota initially increased and then decreased.

Diversity of the microbial community

Microbial community diversity and richness were evaluated using the Shannon index and Chao1 index (*Fig. 2a, b*), respectively. The findings indicated that there was no noteworthy difference between the species diversities in AW and FW samples, but the species richness in AW samples was significantly lesser than that in FW samples ($p < 0.01$). NMDS analysis revealed that the distances of samples in FW varied significantly compared to AW (*Fig. 3*). Samples of FW and AW were clustered discretely with significant distances between the clusters, indicating that the microbial community in FW was significantly different than the microbial community in AW samples (*Fig. 3*).

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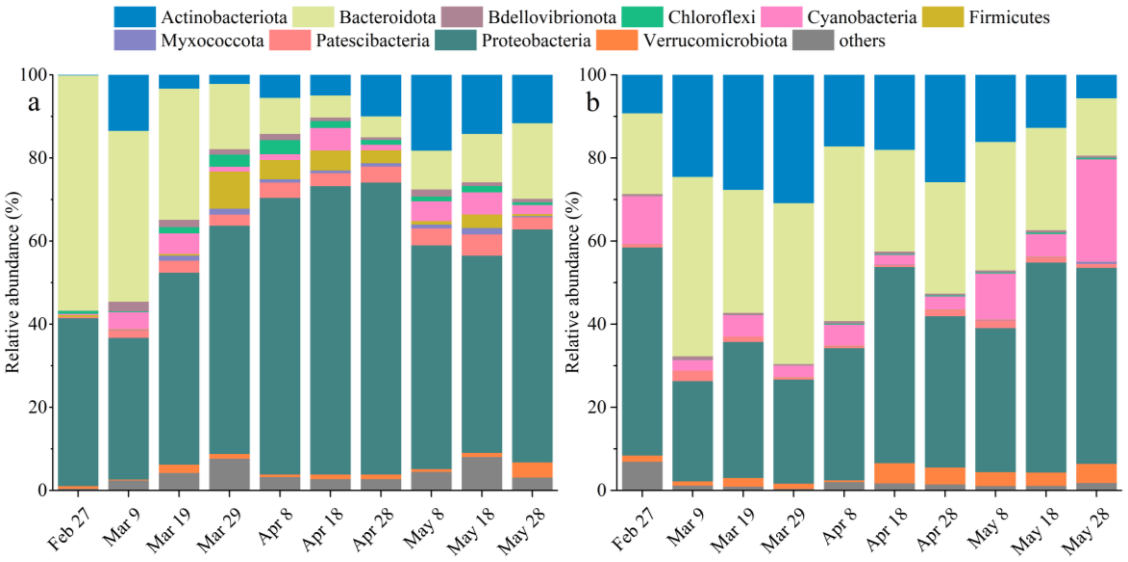

Figure 1. *Microbial community composition at phylum level in the FW (a) and AW (b) samples obtained from the whiteleg shrimp aquaculture tank*

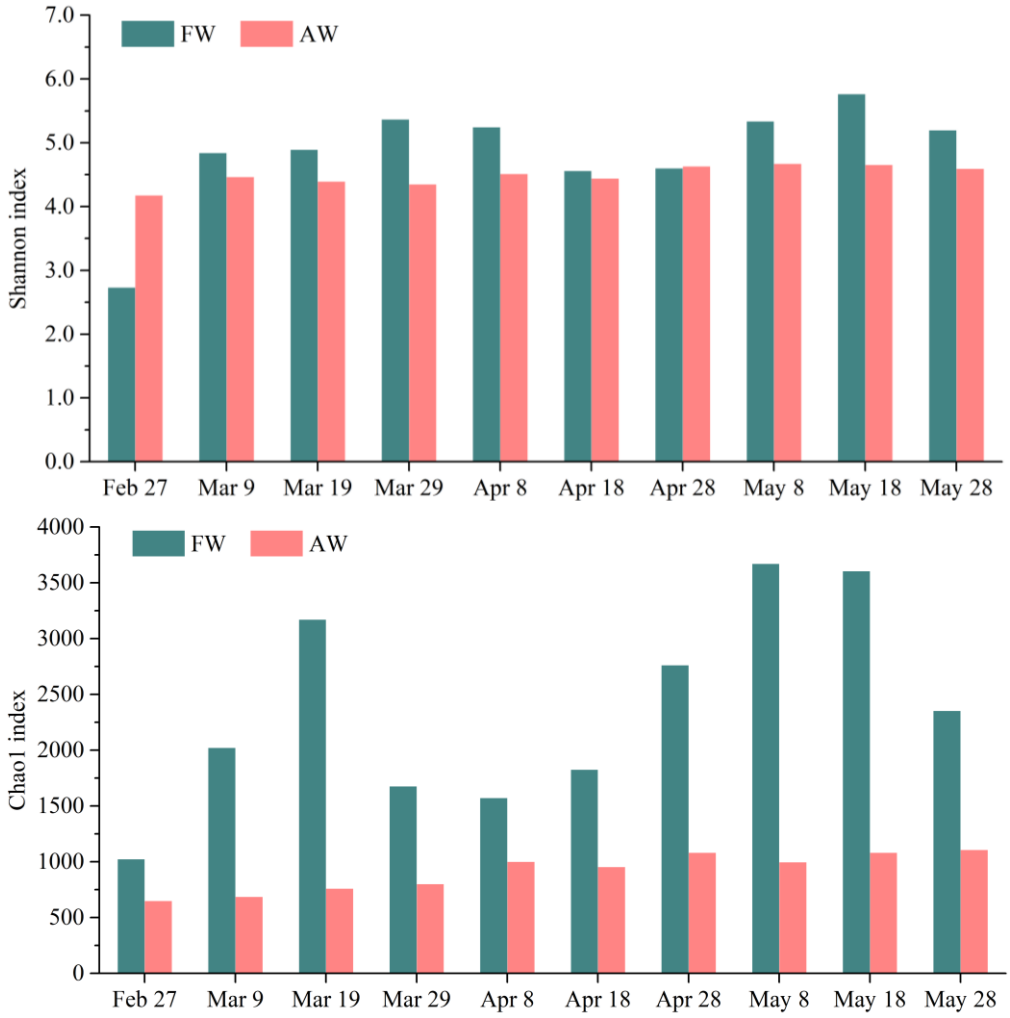

Figure 2. *Shannon (a) and Chao1 (b) indices of microbial communities at the OTU level*

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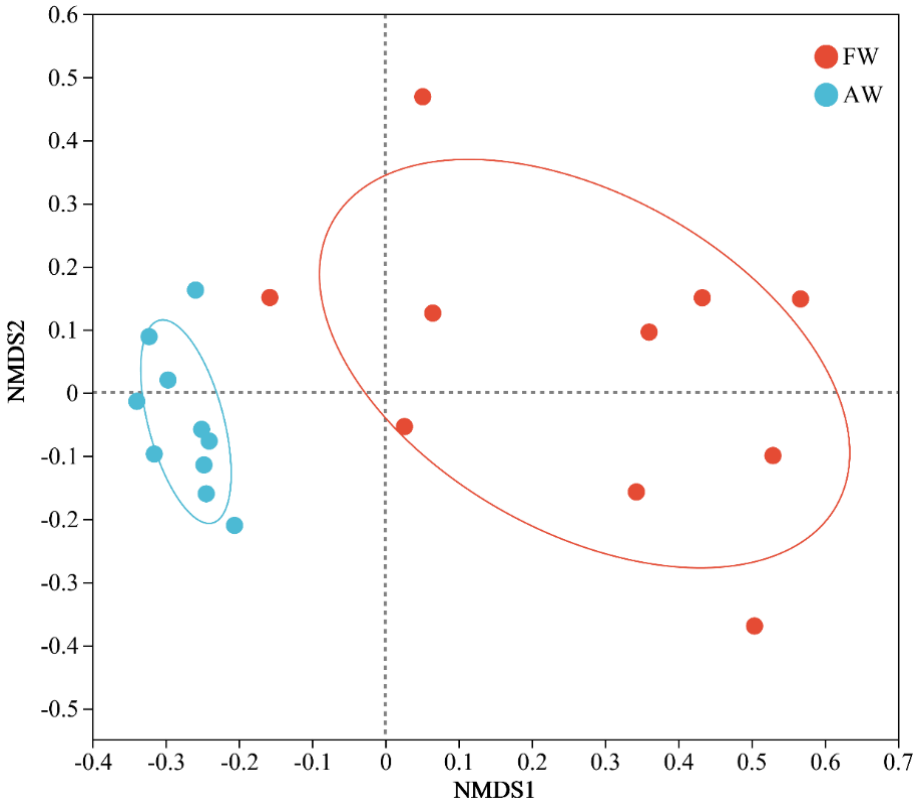

Figure 3. *Results of the NMDS analysis of microbial communities*

Connections between water quality indicators and microbial community

A total of 6 water quality parameters (T, S, pH, nitrate-N, ammonia-N, and nitrite-N) were used for RDA after VIF analysis. Results of RDA showing the relationships between water quality parameters and microbial communities (genus level) in the FW and AW samples have been presented in *Figure 4*. The first axis explained 23.94% variations, while the second axis was able to explain 16.19% of the total variations between the FW and AW samples. Temperature, salinity, nitrate-N, nitrite-N, ammonia-N, and pH were the major factors affecting the microbial communities ($p < 0.05$). Temperature, nitrate-N, nitrite-N and ammonia-N were found to be positively related with microbial community in the AW samples, but negatively connected to the microbial community in FW. On the contrary, pH showed a negative relationship with microbial community in the AW samples, but a positive connection with microbial community in FW.

The correlation heatmap demonstrating the relationships between water quality parameters and the dominant genera (average relative abundance over 0.1%) in FW and AW samples has been shown in *Figure 5*. In FW samples, *Pseudomonas* showed significant positive relationship with orthophosphate-P, DO and salinity ($p < 0.05$), and significant negative connection to temperature ($p < 0.01$). *Mycobacterium* exhibited substantial positive correlation with pH ($p < 0.01$) and significant negative correlation with nitrate-N ($p < 0.01$) and ammonia-N ($p < 0.05$). NS3a marine group showed significantly positive relationship with ammonia-N ($p < 0.01$) and negative relationship with pH ($p < 0.05$). *Erythrobacter* exhibited strong positive relationship with salinity ($p < 0.05$). In AW samples, *Vibrio* and *Pseudomonas* showed strong positive

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connections to nitrite-N and orthophosphate-P ($p < 0.01$), and strong negative relationships with pH ($p < 0.01$) and temperature ($p < 0.05$). *Pseudoalteromonas* showed significant negative association with temperature. *Phaeocystidibacter* exhibited noteworthy negative relationship with DO ($p < 0.05$), while *Erythrobacter* was strongly and negative connected to salinity ($p < 0.05$).

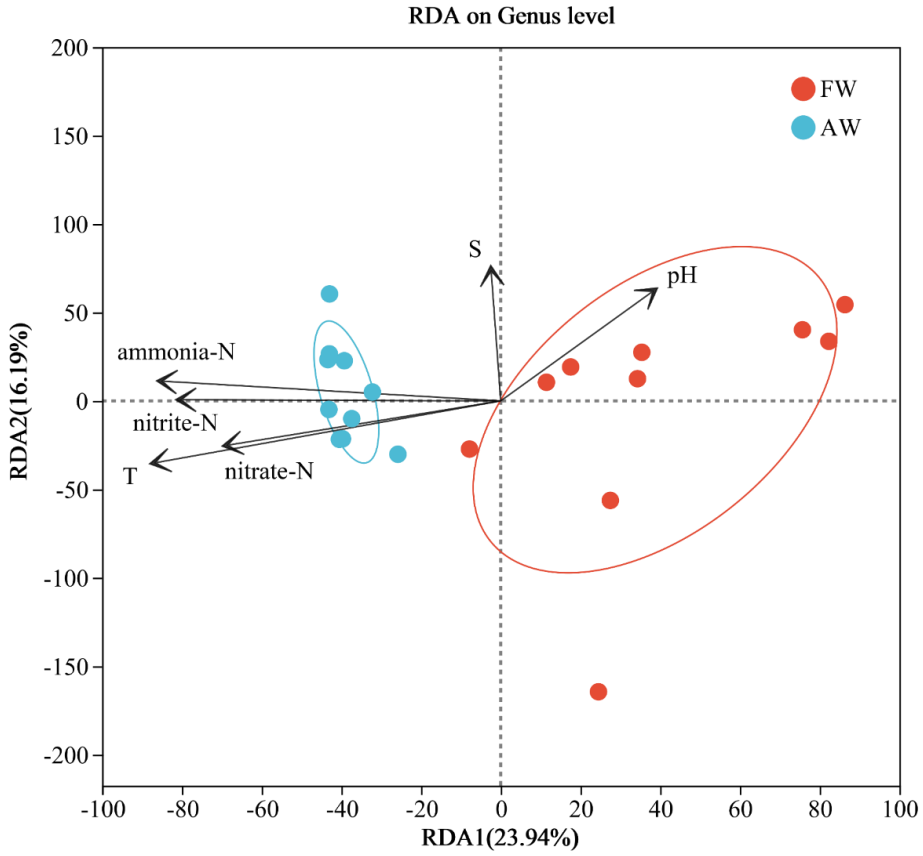

Figure 4. *Results of RDA conducted on the microbial communities in FW and AW samples*

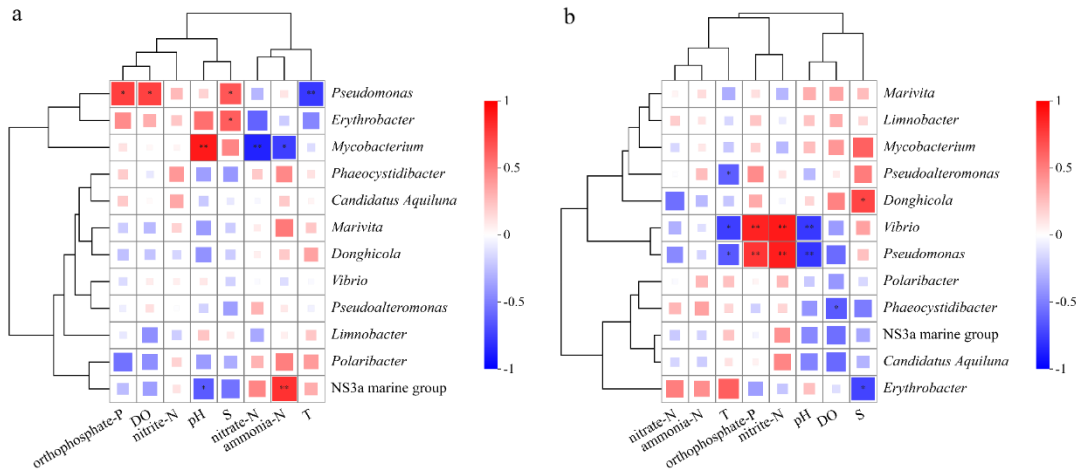

Figure 5. *The correlation heatmap of dominant genera and water quality parameters in FW (a) and AW samples (b). *Represents significant correlation at the 0.05 level, ** represents significant correlation at the 0.01 level*

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Predicted function of microbial community

A total of 6 KEGG pathways at level 1 were identified by PICRUSt2 analysis. Metabolism was found to be the most abundant function in the communities of AW and FW samples, followed by genetic information processing, environmental information processing, cellular processes, human diseases and organismal Systems. Among these, 3 pathways significantly varied between FW and AW samples ($p < 0.05$). Metabolism function was enriched in the AW samples, while cellular processes and organismal systems were enriched in the FW samples (*Fig. 6a*). A total of 46 KEGG pathways at level 2 were also identified. Global and overview maps were found to be the most dominant pathways. Among these, 4 pathways of FW (with average relative abundance greater than 1%) were significantly different than those in AW samples ($p < 0.05$). Global and overview maps were more abundant in the AW than in the FW, whereas xenobiotics biodegradation and metabolism, signal transduction, and lipid metabolism were significantly enriched in the FW samples (*Fig. 6b*). A total of 400 KEGG pathways at level 3 were identified in this study. Among these, 7 pathways with average relative abundance greater than 1% varied significantly between FW and AW samples ($p < 0.05$). ABC transporters, quorum sensing, carbon metabolism, and metabolism of glycine, serine and threonine were more abundant in the AW than in the FW, whereas two-component system, metabolic pathways and pyruvate metabolism were more enriched in the FW (*Fig. 6c*).

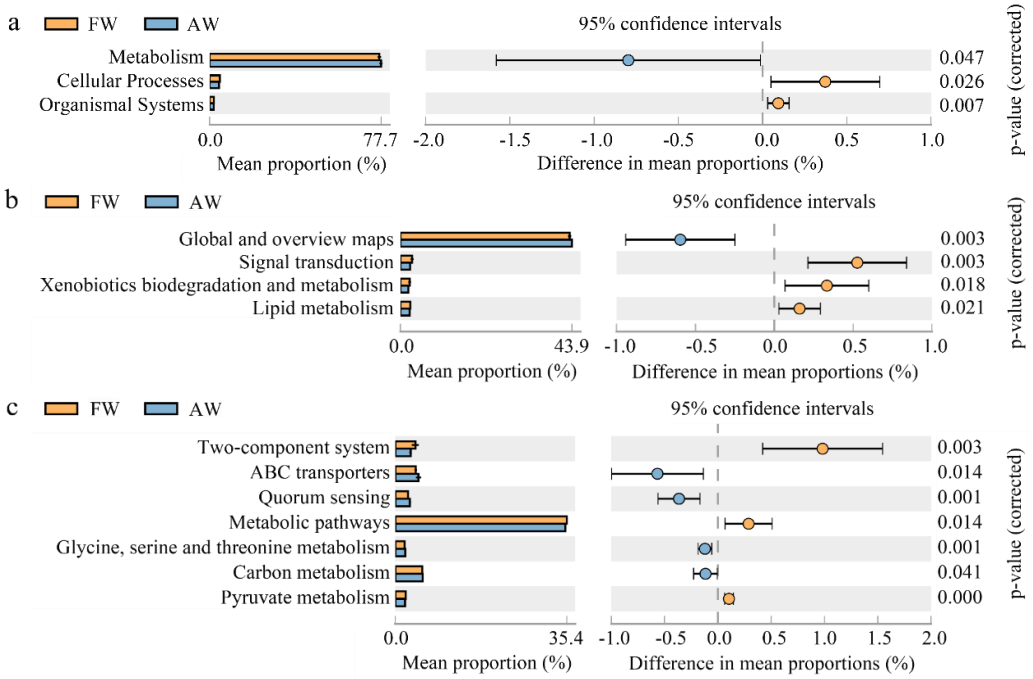

Figure 6. *Extended error bar plots revealing the predicted potential functions of microbial communities based on the KEGG annotation at level 1 (a), level 2 (b) and level 3 (c) differing significantly between FW and AW samples*

Discussion

Differences of microbial communities and functions between FW and AW samples

In this study, microbial diversity and richness in AW samples were lower than that in FW samples (*Fig. 2*). This indicated that aquaculture decreased the diversity and

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richness of microbial communities in pond water. Aquaculture changed the physicochemical environment of waters and sediments (Yusoff et al., 2002). Specifically, aquaculture activities increased the contents of nutrients and organic matters in the waters, which was conducive to the growth and reproduction of microbes that prefer such an environment, while inhibited the growth of environmentally sensitive species, thus affecting the microbial community composition and diversity (Sun et al., 2021). As the process of aquaculture progressed, the relative abundance of Proteobacteria initially increased and then showed a declining trend, while the relative abundance of Bacteroidota and Actinobacteriota in FW samples showed an initial decline, followed by an increasing trend (*Fig. 1a*). In AW samples, an opposite trend was observed, with an initial decline in the relative abundance of Proteobacteria, which later increased; whereas the relative abundance of Bacteroidota and Actinobacteriota initially increased and then began to decline (*Fig. 1b*). Proteobacteria was more abundant in the FW samples, while Bacteroidota and Actinobacteriota were more dominant in the AW samples (*Fig. 1*). According to the previous studies, some members of Bacteroidota are useful decomposers of organic matters (Thomas et al., 2011), while Actinobacteriota are well-known producers of bioactive natural products (Van Keulen et al., 2014; Bernal et al., 2015). Therefore, the higher relative abundance of Bacteroidota and Actinobacteriota in FW was probably due to the rich nutrients available in the environment. Further comparisons and analyses of microbial communities revealed that 8 dominant phyla differed significantly between AW and FW samples ($p < 0.05$). Actinobacteriota and Verrucomicrobiota were more abundant in AW samples, while Proteobacteria, Firmicutes, Patescibacteria, Chloroflexi, Myxococcota and Bdellovibrionota were more enriched in the FW samples (*Fig. 7a*). A total of 8 dominant genera showed significant variations between FW and AW samples ($p < 0.05$). *Marivita*, *Candidatus Aquiluna*, *Donghicola* and *Polaribacter* were more abundant in AW samples, while *Pseudomonas*, *Erythrobacter*, *Limnobacter* and *Pseudoalteromonas* were enriched in the FW samples (*Fig. 7b*).

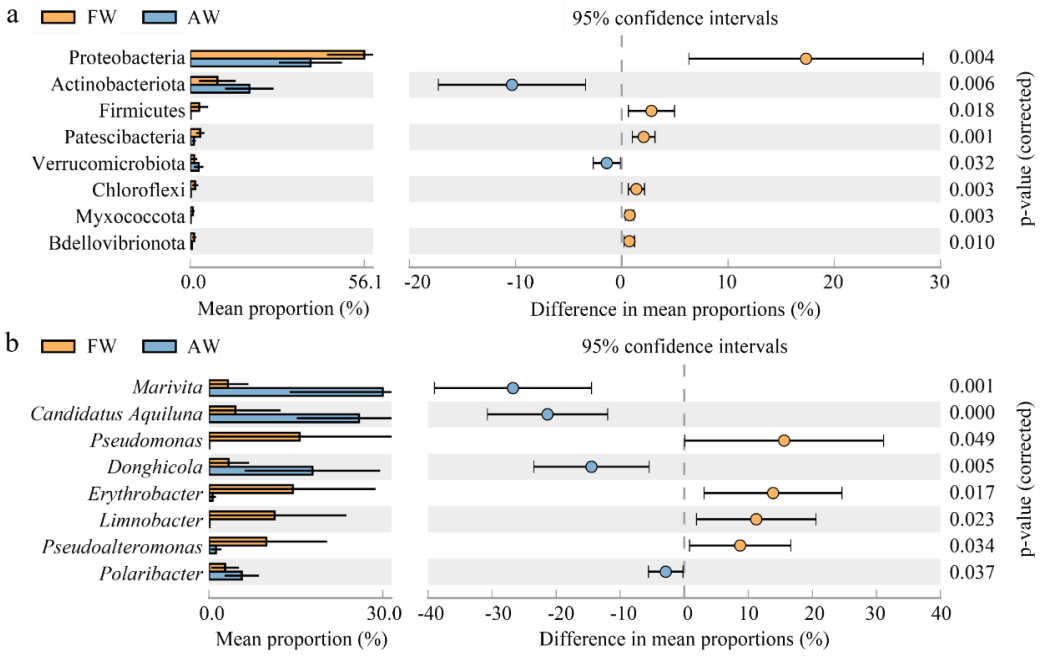

Figure 7. *Dominant microbial phyla (a) and genera (b) in FW and AW samples*

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Bacteria of *Marivita* genus are ubiquitous in marine environments, and can participate in the absorption and metabolism of inorganic and organic compounds (containing carbon, nitrogen and phosphorus) in the marine ecosystems (Hwang et al., 2009; Zheng et al., 2019; Mesquita et al., 2022; Wei et al., 2023). In previous investigations, *Marivita* was often found to be dominant in marine or saline environments associated with phytoplankton blooms or high organic matter content (Slightom and Buchan, 2009; Wei et al., 2023). The genome of *Marivita* comprises a complete cluster of photosynthetic genes, suggesting that the bacteria of *Marivita* genus can perform photoheterotrophic metabolism (Zheng et al., 2019). When *Marivita* enter the late-exponential growth phase, it can even compete with phytoplankton for inorganic nitrogen (Zheng et al., 2019). The genome of *Marivita* shows diverse metabolic functions (e.g., assimilatory nitrate reduction, CO oxidation, poly-β-hydroxybutyrate metabolism, numerous transporters, mixotrophy, and sulfur metabolism), which enables these microbes to inhabit different marine environments (Zheng et al., 2019). *Candidatus Aquiluna* has been reported as an important genus of bacteria in aquaculture. It is a photoheterotroph carrying actinorhodopsin and has the ability to fix carbon (Kang et al., 2012). Bacteria of *Donghicola* genus are oxidase- and catalase-positive, and can reduce nitrate to nitrite (Tan et al., 2009), thus playing a crucial role in the nitrogen cycling in aquaculture system and promoting the growth of shrimps. Polaribacter is a heterotrophic bacterium that is widely distributed in various marine ecosystems (Gosink et al., 1998; Nedashkovskaya et al., 2005, 2013, 2018; Yoon et al., 2006). Previous studies have reported that the abundance of Polaribacter is usually connected positively with the concentration of chlorophyll a (Williams et al., 2013). Moreover, genes encoding polysaccharide hydrolase and protease are abundant in the genome of Polaribacter, which enable it to degrade polymer compounds (Gómez-Pereira et al., 2010, 2012; Williams et al., 2013). Therefore, Polaribacter also plays an important role in the marine carbon cycle. Overall, these bacteria can transform and metabolize nutrients, and due to their high abundance in the AW samples, carbon metabolism and the metabolism of glycine, serine and threonine increased significantly in the AW samples, as compared to FW samples (*Fig. 6c*).

Environmental factors driving the shifts in microbial community structure

Previous studies have reported that only 20-30% of N and 10-20% of P in the feeds were assimilated and absorbed by shrimps, and the rest of the nutrients were retained in the AW in multiple forms during marine shrimp culture (Thakur and Lin, 2003; Zang et al., 2009; Sahu et al., 2013; Chen et al., 2018; Abakari et al., 2022). The remaining nutrients stimulated variations in the physicochemical properties of AW, thus changing the microbial community and diversity (Herlambang et al., 2021; Li et al., 2021; Sun et al., 2021; Zhou et al., 2021; Dahle et al., 2022). In this study, temperature, salinity, nitrate-N, nitrite-N, ammonia-N, and pH were the major factors affecting the microbial communities in FW and AW samples (*Fig. 4*). This finding which was in agreement with the former studies (Zhang et al., 2014; Sun et al., 2021). The relatively rich inorganic nitrogen in AW samples induced rapid propagation of some bacteria that prefer the eutrophic environment, and inhibited the growth of environmentally sensitive bacteria (Chrzanowski et al., 1995; Wang et al., 2014). This led to decreases diversity and richness of microbial community, as observed in a previous study (Wang et al., 2014; Sun et al., 2021). This also explains the lower microbial richness in AW samples, as compared to FW (*Fig. 2*).

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The correlation analysis between dominant genera and water quality parameters revealed that *Vibrio* and *Pseudomonas* in the AS samples showed significantly positive relationships with orthophosphate-P and nitrite-N, and strong negative connections with pH and temperature (*Fig. 5*). *Vibrio* and *Pseudomonas* are generally recognized as opportunistic pathogens in aquaculture. Vibriosis caused by *Vibrio* species has emerged as a serious concern in shrimp aquaculture due to the sudden outbreak and rapid spread of the infection, and lack of safe and efficient controlling agents (Lightner, 2005; Ananda Raja et al., 2017). Among the *Vibrio* species, *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* have been reported as the most harmful species to aquaculture organisms, causing huge economic losses in shrimp farming industry (Kumar et al., 2014; Cao et al., 2015; Zhang et al., 2016). Most *Vibrio* species are oxidase-, indole-, and citrate-positive, and can reduce nitrate to nitrite. Furthermore, these *Vibrio* species can also hydrolyze carbohydrates, lipids, and proteins, and degrade starch, gelatin, lignin, chitin, and alginate through extracellular enzymes (Farmer and Hickman-Brenner, 2006; Li et al., 2017; Grimes, 2020; Sampaio et la., 2022; Noorianet al., 2023). Therefore, *Vibrio* species play a crucial role in nitrogen and carbon cycling in the aquatic environments. In addition to carbon and nitrogen, phosphorus has also been reported as a main factor affecting the distribution of Vibrio communities (Gregoracci et al., 2012). Pseudomonas, as a denitrifying bacterium, is important for nitrogen removal (Huang et al., 2015; Zhao et al., 2018; Gao et a., 2019). According to previous studies. some species of Pseudomonas can directly reduce nitrate to gaseous nitrogen without accumulating nitrite, while some species possess a two-phase denitrification process; for others species of Pseudomonas, low nitrite accumulation has been reported (Su et al., 2001; Sun et al., 2017). Nitrite is one of the key factors affecting the water quality in aquaculture. At a concentration of more than 6.67 mg/L, nitrite can be detrimental to shrimps (Huang et al., 2020). A high level of nitrite has been reported to affect the growth, moulting, feed intake, oxygen consumption, ammonia excretion, nutritional modulation, energy metabolism, antioxidant capacity and disease resistance in shrimps (Chen and Chen, 1992; Cheng and Chen, 1998; Tseng and Chen, 2004; Wang et al., 2015; Guo et al., 2016; Li et al., 2019; Xiao et al., 2019). In this study, the nitrite concentration was 0.01-3.58 mg/L, and no disease was observed in whiteleg shrimp, which might be related to the effective microbial nitrogen-cycling network.

Conclusions

Overall, high-throughput sequencing revealed that Proteobacteria, Bacteroidota and Actinobacteriota were the dominant phyla in the whiteleg shrimp aquaculture system. As aquaculture proceeded, the relative abundance of Proteobacteria in FW initially increased and then started to decline, whereas abundance of Bacteroidota and Actinobacteriota initially declined and then increased. In AW samples, Proteobacteria, Bacteroidota and Actinobacteriota showed opposite trends. In addition, microbial richness in AW samples was significantly lower than that in FW. Furthermore, noteworthy differences were observed between the predicted functions of microbial communities in FW and AW samples, with carbon metabolism as well as metabolism of glycine, serine and threonine increasing significantly in the AW samples. *Marivita*, *Candidatus Aquiluna*, *Donghicola* and *Polaribacter* played important roles in sequestration of carbon and reduction of nitrogen. Temperature, nitrite-N, ammonia-N, salinity, nitrate-N, and pH were found to be the key factors affecting the microbial communities.

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APPENDIX

Table A1. *Water quality parameters in the FW and AW*

Samples	Date	T (℃)	S (g/L)	DO (mg/L)	pH	Ammonia-N (mg/L)	Nitrate-N (mg/L)	Nitrite-N (mg/L)	Orthophosphate-P (mg/L)
FW	Feb 27	28.92	29.12	6.35	8.10	0.06	1.07	0.01	0.00
	Mar 9	28.78	30.38	6.75	8.12	0.03	1.06	0.02	0.04
	Mar 19	20.43	31.94	7.59	8.14	0.07	1.00	0.08	0.10
	Mar 29	24.66	31.66	6.94	8.28	0.01	0.63	0.01	0.06
	Apr 8	21.57	32.71	7.41	8.25	0.03	0.33	0.01	0.06
	Apr 18	21.13	32.87	7.33	8.40	0.02	0.49	0.02	0.06
	Apr 28	24.31	31.42	6.80	8.28	0.03	0.44	0.08	0.07
	May 8	22.55	32.67	7.11	8.42	0.01	0.17	0.02	0.06
	May 18	23.71	32.03	6.89	8.45	0.01	0.25	0.01	0.05
	May 28	25.00	32.44	6.60	8.42	0.02	0.15	0.02	0.05
	Average	**24.11**	**31.72**	**6.98**	**8.29**	**0.03**	**0.56**	**0.03**	**0.06**
AW	Feb 27	29.69	29.91	6.57	8.42	1.39	2.00	0.19	0.10
	Mar 9	30.56	29.94	5.95	8.23	1.99	1.49	1.82	0.17
	Mar 19	28.48	31.16	6.57	8.22	0.83	1.92	2.37	0.23
	Mar 29	28.92	32.22	6.17	8.10	1.37	0.88	2.50	0.26
	Apr 8	27.32	32.58	5.77	7.98	2.34	1.52	2.91	0.40
	Apr 18	27.79	33.23	6.23	8.14	1.18	0.54	3.58	0.43
	Apr 28	27.85	32.68	6.16	8.06	1.27	1.16	2.97	0.41
	May 8	28.01	33.25	6.48	8.26	1.24	0.95	1.64	0.22
	May 18	28.01	33.03	6.27	8.20	1.59	0.78	1.73	0.32
	May 28	28.04	32.91	6.60	8.32	1.56	1.55	1.95	0.36
	Average	**28.47**	**32.09**	**6.28**	**8.19**	**1.48**	**1.28**	**2.16**	**0.29**

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