# Microbiota composition and correlations with environmental factors in grass carp (*Ctenopharyngodon idella*) culture ponds in South China

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## Abstract

To maintain the health of aquaculture fish, it is critical to understand the composition of microorganisms in aquaculture water and sediment and the factors affecting them. This study examined the water and sediment microbiota compositions of four different types of ponds in South China that were used to culture grass carp (*Ctenopharyngodon idella*) of different sizes through high-throughput sequencing of the 16S rRNA gene, and analyzed their correlations with environmental factors. The results showed that ponds with cultured grass carp of different sizes exhibited significant differences in terms of water physicochemical properties and composition of water and sediment microbiota. Furthermore, the exchange of microorganisms between water and sediment microbiota was lowest in ponds with the smallest grass carp and highest in ponds with the largest grass carp. All detected environmental factors except water temperature were significantly correlated with the water microbiota, and all detected environmental factors in the sediment were correlated with sediment microbiota. Moreover, *Aeromonas* and *Vibrio* were significantly increased in the water microbiota, especially in ponds with small juvenile grass carp,

implying an increased risk of *Aeromonas* and *Vibrio* infections in these environments. Our results provide useful information for the management of grass carp aquaculture ponds.

**Keywords** Microbiota, Aquaculture pond, Grass carp, Bacteria-environment interaction

## Introduction

Add your introduction here.Aquaculture is an important source of high-quality protein for humans, providing 15-20% of the animal protein consumed by > 4 million people worldwide (*Tezzo et al., 2021*). Currently, intensive aquaculture is the main method of aquaculture in China because of its advantages in boosting the output of aquatic products and profits (*Dai et al., 2014; Edwards et al., 2015; Zou et al., 2015*). However, intensive aquaculture, characterized by high density and high feed loading, can deteriorate aquaculture water quality, especially during the later stages of cultivation (*Dauda et al., 2019*). This not only affects the growth of cultured fish directly (*Colt et al., 1981; Alcaraz et al., 1997; Cheng et al., 2015; Wang et al., 2016*), but may also cause excessive growth of conditional pathogens, eventually posing a threat to the health of cultured fish (*Tengs &Rimstad, 2017; Li et al., 2019* ).

Various microorganisms live in aquaculture ponds and participate in the metabolism of nutrients in aquaculture water and sediments, which play an important role in maintaining water quality (*Ni et al., 2018; Li et al., 2020b*). Simultaneously, microorganisms also interact with aquatic life and potentially lead to bacterial diseases in farmed fish (*Zeng et al., 2020; Liu et al., 2021; Jing et al., 2021; Zhang et al., 2022*). To ensure the health of aquaculture fish, it is necessary to clarify the composition of microorganisms in aquaculture water and sediment, and the factors affecting them. Moreover, microorganism exchanges between the pond water and sediment microbiota (*Liu et al., 2021; Zheng et al., 2021*) probably affects the distribution of microorganisms in pond systems and the health of aquatic organisms *(Wu et al., 2012)*. However, the effects of such exchanges on microbiota metabolism and aquatic organisms have not been widely studied.

Therefore, in this study, we investigated water and sediment microbiota compositions and their correlation with environmental factors in grass carp (*Ctenopharyngodon idella*) culture ponds in South China. Our findings provide valuable insights that can be used as references for effective aquaculture pond management.

## Materials & Methods

### Aquaculture ponds and sample collection

Water and sediment samples were collected from grass carp aquaculture ponds in the Nansha district (22.61°N, 113°E), Guangdong Province, China, on May 31, 2018. Four ponds cultured with different sizes of grass carp, that is, larval fish (LF), small juvenile fish (SJ), middle juvenile fish (MJ), and large juvenile fish (LJ) were sampled. Each pond is 1.5 km2 in area and approximately 2.0 m in depth without water exchange. Clay was removed from all ponds, and the

sediments were further disinfected with quicklime before culturing the grass carp. The ponds used for culture had lasted for nearly one year before sampling. During the one year of culturing, each pond was only used for culturing one size of fish, and the fish size grew large enough to be transferred to another corresponding type of pond. The larval fish had a body weight of approximately 1.0 g and a culture density of 750 individuals per $m^2$. The small juvenile fish weighed approximately 200.0 g of body weight and their culture density was 30 individuals per m2. The middle juvenile fish were approximately 310.0 g in body weight, and their culture density was 10 individuals per m2. The large juvenile fish had a body weight of approximately 580.0 g and a culture density of 5 individuals per m2. Larval and juvenile fish were fed commercial crumbled and pelleted formulated feeds at a ratio of 5% body weight per day. Three surface waters approximately 50 cm below the water level (approximately 1 L) and three upper (0-8 cm) sediment (approximately 500 g) samples were collected from the left, center, and right of each pond using a 5 L hydrophore sampler and Van Veen Grab sampler, respectively (*Zhang et al., 2020*). All samples were stored in an ice box and brought back to the laboratory. A subset of approximately 50 g of each sediment sample was separated and frozen at -80°C for further DNA extraction. Water samples (approximately 500 mL) were filtered using glass fiber (GF/C) with a 0.22 μm aperture for extracting microbial genomic DNA (*Chen et al., 2021*). The remaining sediment and water samples were used for physicochemical analysis.

**Physicochemical analysis**

Dissolved oxygen (DO), pH, and water temperature were measured in situ using a multi-parameter water quality probe YSI EXO2 (Yellow Springs Instruments, USA). The transparency of the ponds was measured using a Secchi disk. Ammonia, nitrite, nitrate, total nitrogen (TN), phosphate, and total phosphorus (TP) were measured, as described by *Zheng et al.* (*2017a; 2017b*). Chlorophyll-a (Chla) content was measured using a spectrophotometer and calculated as described by Lichtenthaler (*Lichtenthaler et al., 1987*). Dissolved organic carbon (DOC) was analyzed using a Formacs total organic carbon analyzer (Skalar, Netherlands). Particle-organic carbon (POC), total suspended solids (TSS), and total organic carbon (TOC) were calculated according to *Zheng et al.* (*2021*).

Sediment physicochemical variables were analyzed according to *Zhang et al.* (*2020*). Briefly, 10 g of each sediment sample was separated to measure the TOC, TN, and total sulfur (TS) using a PRIMACS TOC analyzer (Skalar, Netherlands) and a CHNS/O elemental analyzer (Vario EL cube, Germany) for TNS and TS, respectively. TP in the dried sediment was measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES), as previously described by *Murray et al.* (2000).

**DNA extraction and high-throughput sequencing of 16S rRNA gene amplicon**

Water filtration membranes were cut into small pieces with sterilized scissors before DNA extraction (*Ni et al, 2010*). Subsequently, total microbial DNA in the water and sediment was extracted using a PowerSoil DNA isolation kit (Mo Bio, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration and purity of the DNA were determined using a

NanoDrop One spectrophotometer (Thermo Fisher Scientific, MA, USA). The DNA was diluted to 10 ng/μL and stored at -80°C for further analyses.

The V4 region of the 16S rRNA gene was amplified using the primer pair 515F (5′ -GTGYCAGCMGCCGCGGTAA-3′ ) and 806R (5′ -GGACTACNVGGGTWTCTAAT-3′ ), as previously described (*Zhang et al., 2020*). The amplified DNA fragments were then high-throughput sequenced on Illumina HiSeq platforms with paired-end sequencing at the Biomarker Technologies Corporation (Beijing, China).

Raw paired-end reads were trimmed using Trimmomatic v.0.36 (*Bolger et al., 2014*) to remove "N" bases, adaptor sequences, and bases with Q-values < 20 to obtain high-quality fragments. The high-quality fragments were merged using FLASH 1.2.8 software (*Magoc & Salzberg, 2014*) and processed using Quantitative Insights into Microbial Ecology (QIIME) 1.9.1 (*Caporaso et al., 2010*). Chimeric sequences were identified and removed using the Uchime algorithm (*Edgar et al., 2011*) before operational taxonomic unit (OTUs) clustering. The OTUs were then clustered using USEARCH v11 (*Edgar, 2013*) based on the Ribosomal Database Project (RDP) database (*Maidak et al., 1996*), and an OTU table was generated using the UNOISE2 method with a 97% cut-off (*Edar, 2016*). The taxonomy of each OTU was annotated using the RDP classifier (*Wang et al., 2007*) based on Silva database release 132.

**Data analysis**

Microbial community dissimilarities were visualized by principal component analysis (PCA) using the R vegan package (*Dixon, 2003*). Permutational multivariate analysis of variance (PERMANOVA) was conducted using the R vegan package to detect differences among the microbiota of different groups. Source tracking of the microbiota was conducted using SourceTracker (*Knights et al., 2011*). Pearson's correlation coefficient was used to determine the correlation between environmental factors, and between environmental factors and dominant microorganisms. A correlation between microbial communities and environmental factors was determined using Mantel tests and distance-based redundancy analysis (db-RDA) using the R vegan package (*Borcard et al, 2011*). Statistical significance of the RDA model was tested using Monte Carlo permutation tests with 999 permutations. Wilcoxon rank-sum exact test and Kruskal-Wallis rank-sum test with Dunn's post-hoc test were conducted using R with the FSA v.0.9.3 package to detect significance of data differences between groups. $P < 0.05$ was considered as statistically significant.

## Results

**Physicochemical indices of water and sediment in ponds cultured with different sizes of grass carp**

Differences in water physicochemical indices between ponds cultured with grass carp of different sizes were more pronounced than those in the sediment (Fig. 1). Water temperature in the SJ ponds was significantly lower than that in the MF and LJ ponds (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P < 0.05$; Fig. 1A). DO and pH of pond water in LJ ponds were significantly lower than those in LF and MJ ponds (Kruskal-Wallis rank sum test with Dunn's

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post-hoc test, $P < 0.05$; Fig. 1B and 1C). TOC concentrations in the MJ and LJ waters were significantly lower than those in the LF and SJ waters (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P < 0.05$; Fig. 1D). Water DOC concentrations in the MJ and LJ ponds were significantly lower than those in the LF ponds (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P < 0.05$; Fig. 1E). The water POC concentration in the MJ ponds was significantly lower than that in the SJ ponds (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P < 0.05$; Fig. 1F). Water $NH_4^+$-N concentrations in the SJ and MJ ponds were significantly lower than those in the LJ ponds (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P < 0.05$; Fig. 1G). The water $NO_2^-$-N concentration in the LF ponds was significantly lower than that in the SJ and LJ ponds (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P < 0.05$; Fig. 1H). Water $NO_3^-$-N, TN, and TSS concentrations in the LF ponds were significantly lower than those in the MJ and LJ ponds (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P < 0.05$; Fig. 1I, J, and M). Water $PO_4^{3-}$-P and TP concentrations in the LF ponds were significantly higher than those in the other kinds of ponds (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P < 0.05$; Fig. 1K and L). The water Chla content in the MJ ponds was significantly lower than that in the LF and SJ ponds (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P < 0.05$; Fig. 1N). The above results indicate that the management modes of different kinds of ponds cultured with different sizes of grass carp were different, leading to significant differences in water physicochemical indices. Moreover, the water $NH_4^+$-N, $NO_2^-$-N, $NO_3^-$-N and TN in the ponds exhibited an increasing trend with the size of the cultured fish, whereas the water $PO_4^{3-}$-P and TP exhibited a decreasing trend.

Among the five physicochemical indices measured in sediment, the TS content in the LF ponds was significantly higher than that in the MJ and LJ ponds (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P < 0.05$; Fig. 1P), and no significant differences were found in terms of the other physicochemical indices (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P \geq 0.05$; Fig. 1O, Q-S). This implies that the pond management mode has a relatively smaller impact on the physicochemical indices of sediment than on those of water. Moreover, the sediment TS exhibited a decreasing trend with the size of the cultured fish.

**Microbiota structure of water and sediment in ponds cultured with different sizes of grass carp**

The richness and abundance-based coverage estimator (ACE) indices of sediment microbiota were significantly higher than those of the water microbiota (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P < 0.05$; Fig. 2A and 2C), whereas only the Shannon index of the sediment microbiota of the LF and MJ ponds was significantly higher than that of the water microbiota in the same kind of pond (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P < 0.05$; Fig. 2B). Richness, Shannon, and ACE indices of water or sediment microbiota in different kinds of ponds were not significantly different, although these α-diversity indices exhibited an increasing trend with the size of the cultured fish (Kruskal-Wallis rank sum test with Dunn's post-hoc test, $P \geq 0.05$; Fig. 2A-C). However, db-PCA with PERMANOVA showed that not only did the microbiota compositions differ significantly between water and sediment, but also that the

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microbiota compositions in water and sediment were significantly different between different kinds of ponds cultured with different sizes of grass carp (PERMANOVA, $P < 0.05$; Fig. 2D).

Acidobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Cyanobacteria, Firmicutes, Fusobacteria, Gemmatimonadetes, Patescibacteria, Planctomycetes, Proteobacteria, and Verrucomicrobia dominated the pond water microbiota (Fig. 3A), whereas Euryarchaeota, Nanoarchaeaeota, Acetothemia, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Epsilonbacteraeota, Firmicutes, Fusobacteria, Gemmatimonadetes, Kiritimatiellaeota, Nitrospirae, Patescibacteria, Planctomycetes, Proteobacteria, Spirochaetes, and Verrucomicrobia dominated pond sediment microbiota (Fig. 3B). Although significant differences were found in the most dominant phyla in the water among the four types of ponds, only the relative abundances of Chlamydiae, Firmicutes, Fusobacteria, and Patescibacteria increased with the size of cultured grass carp (Fig. 3C). Similarly, the relative abundances of Bacteroidetes, Firmicutes, Fusobacteria, and Patescibacteria in sediment increased with the size of the cultured grass carp, whereas the relative abundances of Acidobacteria, Kiritimatiellaeota, Nitrospirae, and Planctomycetes decreased with the size of the cultured fish (Fig. 3D).

A heatmap with hierarchical clustering based on dominant OTUs showed that water and sediment microbiota were first completely clustered into different groups, and then the water microbiota were completely clustered according to the types of ponds cultured with grass carp of different sizes. Except for the three samples of LJP2S3S, LJP3S3S, and LJP2S1S, the other sediment microbiota were clustered according to the type of pond (Fig. 4). Furthermore, water microbiota in different ponds with the same size of grass carp were also completely clustered according to ponds, whereas sediment microbiota were not clustered according to ponds (Fig. 4). These findings suggest that the management practices of ponds stocked with grass carp of varying sizes significantly influenced the dominant OTUs in both water and sediment microbiota, with a stronger impact on water microbiota than on sediment microbiota. The dominant OTUs in the water microbiota differed notably among the ponds, whereas the dominant OTUs in sediment microbiota showed less significant differences across the ponds (Fig. 4).

The linear discriminant analysis of effect size (LEfSe) results showed that many kinds of bacteria in Bacteroidetes, Cyanobacteria, Actinobacteria, Planctomycetes, and Verrucomicrobia were significantly enriched in the water microbiota, whereas many kinds of bacteria in Euryarchaeota, Acidobacteria, Firmicutes, and Fusobacteria were significantly enriched in sediment microbiota (linear discriminant analysis (LDA) score > 2; Fig. S1). Different bacteria in Chloroflexi and Proteobacteria were significantly enriched in the water and sediment microbiota (LDA score > 2; Fig. S1). In particular, *Methanosaeta*, *Actibacter*, *Ignavibacterium*, RBG_16_58_14, *Clostridium sensu stricto* 1, *Hydrogenispora*, *Cetobacterium*, *Desulfobacterium*, Sva0081 sediment group, *Geobacter*, *Geothermobacter*, *Limnohabitans*, *Ottowia*, *Thiobacillus*, *Dechloromonas*, *Uliginosibacterium*, and Candidatus *Competibacter* were significantly enhanced in sediment compared to those in water, whereas the CL500_29 marine group, *Mycobacterium*, Candidatus *Aquiluna*, Candidatus *Limnoluna*, *Conexibacter*, *Sediminibacterium*, Candidatus *Aquirestis*, *Lewinella*, *Phaeodactylibacter*, *Algoriphagus*,

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*Fluviicola*, *Wandonia*, Candidatus *Chloroploca*, and *Discoplastis sp*. Banmun 010910B, *Lepocinclisacus* var. major, *Lepocinclis* sp. *Psurononuma*100609I, *Trachydiscus*, *Microcystis* PCC_7914, *Planktothrix* NIVA_CYA15, *Nodosilinea* PCC_7104, *Cyanobium* PCC_6307, *Pirellula*, *Planctomyces*, *Rhodopirellula*, *Roseomonas*, *Methylocystis*, *Alsobacter*, Candidatus *Megaira*, *Novosphingobium*, *Aeromonas*, *Rheinheimera*, *Hydrogenophaga*, *Kerstersia*, *Limnobacter*, MWH_Uni P1 aquatic group, *Massilia*, *Polaromonas*, *Polynucleobacter*, Candidatus *Methylopumilus*, *Pseudomonas*, *Vibrio*, and *Silanimonas* were significantly enhanced in the water microbiota compared to sediment microbiota (LDA score > 2; Fig. S1).

In water microbiota, CL500_29 marine group, *Mycobacterium*, Candidatus *Aquiluna*, Candidatus *Limnoluna*, *Conexibacter*, *Sediminibacterium*, *Lewinella*, *Wandonia*, *Flavobacterium*, *Chryseobacterium*, *Microcystis* PCC_7914, *Planktothricoides* SR001, *Cyanobium* PCC_6307, *Pirellula*, *Rhodopirellula*, *Roseomonas*, *Alsobacter*, *Tabrizicola*, *Desulfobacterium*, *Hydrogenophaga*, *Kerstersia*, *Ottowia*, *Methyloparacoccus*, *Silanimonas*, and *Luteolibacter* were enhanced in the LF ponds; Candidatus *Aquirestis*, *Lacihabitans*, *Actibacter*, *Planktothrix* NIVA_CYA15, *Cetobacterium*, *Planctomyces*, Candidatus *Megaira*, *Aeromonas*, *Massilia*, *Pseudomonas*, and *Vibrio* were enhanced in the SJ ponds; *Methanosaeta*, *Fluviicola*, *Nodosilinea* PCC_7104, *Geothermobacter*, *Limnobacter*, *Polaromonas*, Candidatus *Methylopumilus*, and *Legionella* were enhanced in the MJ ponds; and *Aurantimicrobium*, *Discoplastis* sp. *Banmun*010910B, *Lepocinclisacus* var. major, *Lepocinclis* sp. *Psurononuma*100609I, Clostridium sensu stricto 1, *Rhodobacter*, *Novosphingobium*, *Geobacter*, *Rheinheimera*, MWH_UniP1 aquatic group, *Thiobacillus*, *Dechloromonas*, and *Uliginosibacterium* were enhanced in LJ ponds (LDA score > 2; Fig. 5A).

In sediment microbiota, Candidatus Aquiluna, *Flavobacterium*, *Ignavibacterium*, R8G_16_58_14, *Microcystis* PCC_7914, *Cyanobium* PCC_6307, *Pirellula*, *Rhodopirellula*, *Roseomonas*, *Desulfobacterium*, Sva0081 sediment group, *Hydrogenophaga*, *Methyloparacoccus*, and *Luteolibacter* were enhanced in the LF ponds; *Mycobacterium*, *Actibacter*, *Nannochloropsis*, *Planktothrix* NIVA_CYA15, *Nodosilinea* PCC_7104, *Planctomyces*, *Ottowia*, and *Legionella* were enhanced in the SJ ponds; *Methanosaeta*, *Aurantimicrobium*, *Conexibacter*, Candidatus *Aquirestis*, *Clostridium* sensu stricto 1, *Hydrogenispora*, *Cetobacterium*, and *Geothermobacter* were enhanced in the MJ ponds; and *Lewinella*, Candidatus *Chloroploca*, *Discoplastis* sp. Banmun010910B, *Trachydiscus*, *Rhodobacter*, *Novosphingobium*, *Geobacter*, *Thiobacillus*, *Dechloromonas*, *Uliginosibacterium*, and Candidatus *Competibacter* were enhanced in LJ ponds (LDA score > 2; Fig. 5B).

Source tracking results showed that the exchange proportions of microorganisms in the water and sediment microbiota were lowest in the LF ponds and highest in the LJ ponds (Fig. 6). Simultaneously, there was no significant difference between the proportions of microorganisms from sediment to water microbiota and the proportions of microorganisms from water to sediment microbiota in all kinds of ponds (Wilcoxon rank sum exact test, $P < 0.05$; Fig. 6).

**Correlation between microbiota composition and physicochemical indices in water and sediment**

RDA with Monte Carlo test results showed that all water physicochemical indices except temperature were significantly correlated with water microbiota (Monte Carlo test, $P < 0.05$; Fig. 7A), and all sediment physicochemical indices were significantly correlated with sediment microbiota (Monte Carlo test, $P < 0.05$; Fig. 7B). Co-occurrence network analysis based on the Pearson correlation coefficients of the dominant OTUs and physicochemical indices showed that water physicochemical indices were more significantly correlated with dominant OTUs than sediment physicochemical indices (Pearson correlation coefficient > 0.6 and $P < 0.05$; Fig. 7C). There was no significant correlation between sediment physicochemical indices and dominant OTUs (Fig. 7D), suggesting that these physicochemical indices may affect the microbiota structure through non-dominant OTUs.

Pearson correlation analysis also showed that water $NH_4^+$-N concentration was significantly positively correlated with the relative abundances of *Aurantimicrobium* sp., Candidatus *Aquiluna* sp., Candidatus *Competibacter* sp., *Cetobacterium* sp., *Chryseobacterium* sp., *Conexibacter* sp., *Dechloromonas* sp., *Discoplastis* sp. Banmun010910B, *Geobacter* sp., *Kerstersia* sp., *Lepocinclis acus* var. major, *Lepocinclis* sp. *Psurononuma*100609I, *Limnobacter* sp., *Mycobacterium* sp., *Novosphingobium* sp., *Polynucleobacter* sp., *Rhodobacter* sp., *Sediminibacterium* sp., *Trachydiscus* minutus, and *Uliginosibacterium* sp., and significantly negatively correlated with the relative abundances of *Cyanobium* PCC-6307, *Fluviicola* sp., *Pirellula* sp., *Planctomyces* sp., and *Rhodopirellula* sp. ($P < 0.05$; Fig. S2A). Water $NO_2^-$-N concentration was significantly positively correlated with the relative abundances of Candidatus *Aquirestis* sp., Candidatus *Competibacter* sp., *Cetobacterium* sp., *Clostridium perfringens*, *Cyanobium* PCC-6307, *Dechloromonas* sp., *Discoplastis* sp. Banmun010910B, *Geobacter* sp., *Geothermobacter* sp., *Ignavibacterium* sp., *Lacihabitans* sp., *Legionella* sp., *Lepocinclis acus* var. major, *Lepocinclis* sp. *Psurononuma*100609I, *Limnobacter* sp., *Mycobacterium* sp., *Novosphingobium* sp., *Rhodobacter* sp., *Thiobacillus* sp., *Trachydiscus* minutus, and *Uliginosibacterium* sp., and significantly negatively correlated with the relative abundances of *Algoriphagus* sp., *Alsobacter* sp., Candidatus *Aquiluna* sp., Candidatus *Limnoluna* sp., Candidatus *Methylopumilus* sp., *Cyanobium* PCC-6307, *Desulfobacterium* sp., *Flavobacterium* sp., *Hydrogenophaga* sp., *Lewinella* sp., *Luteolibacter* sp., *Methyloparacoccus* sp., *Microcystis aeruginosa* DIANCHI905, *Mycobacterium* sp., Ottowia sp., *Phaeodactylibacter* sp., *Pirellula* sp., *Rhodobacter* sp., *Rhodopirellula* sp., *Roseiflexus* sp., *Roseomonas* sp., *Silanimonas* sp., *Sunechococcus* sp., *Tabrizicola* sp., and *Tabrizicola* sp. ($P < 0.05$; Fig. S2A). The water microorganisms that were significantly related to water $NO_3^-$-N and TN were similar. Water $NO_3^-$-N and TN concentrations were significantly positively correlated with the relative abundances of Candidatus *Competibacter* sp., *Cetobacterium* sp., *Clostridium perfringens*, *Cyanobium* PCC-6307, *Dechloromonas* sp., *Discoplastis* sp. Banmun010910B, *Geobacter* sp., *Geothermobacter* sp., *Ignavibacterium* sp., *Legionella* sp., *Lepocinclis* sp. *Psurononuma*100609I, *Mycobacterium* sp., *Novosphingobium* sp., *Rhodobacter* sp., *Thiobacillus* sp., *Trachydiscus* minutus, and *Uliginosibacterium* sp., and significantly negatively correlated with the relative abundances of *Algoriphagus* sp., Candidatus *Aquiluna* sp., Candidatus *Limnoluna* sp., *Desulfobacterium* sp.,

*Flavobacterium* sp., *Hydrogenophaga* sp., *Kerstersia* sp., *Lewinella* sp., *Luteolibacter* sp., *Methyloparacoccus* sp., *Mycobacterium* sp., *Phaeodactylibacter* sp., *Pirellula* sp., *Planktothrix* agardhii NIVA-CYA_126/8, *Rhodobacter* sp., *Rhodopirellula* sp., *Roseiflexus* sp., *Sediminibacterium* sp., and *Tabrizicola* sp. ($P < 0.05$; Fig. S2A). The correlation between water $PO_4^{3-}$-P and TP concentration and water microorganisms exhibited an opposite trend to the correlation between water $NO_3^-$-N and TN concentrations and water microorganisms (Fig. S2A). The correlation between Chla, DOC, TOC, and water microorganisms was more similar to that of $PO_4^{3-}$-P and TP compared with those of $NO_3^-$-N and TN (Fig. S2A). Fewer water-dominant microorganisms were significantly correlated with water temperature, which is probably caused by small differences in water temperature (Fig. S2A). Less sediment-dominant microorganisms were significantly related to sediment physicochemical indices comparing with water-dominant microorganisms (Fig. S2B). These findings suggest that water microbiota in grass carp ponds is more susceptible to environmental parameters than sediment microbiota.

## Discussion

Biodiversity is the basis of ecosystem structure and functional maintenance (*Tilman &Downing, 1994; Hulot et al., 2000*). Although the role of microorganisms in nutrient metabolism and circulation in aquaculture ponds has been widely confirmed (*Ni et al., 2018;Thurlow et al., 2019; Gong et al., 2021*), the composition and function of different types of pond microbial communities and their influencing factors have also been extensively investigated (*Moriarty, 1997; Mao, 2022*), the impact of different types of ponds cultured with different sizes of fish on pond water and sediment microbiota has not been fully elucidated. Our results indicated that water physicochemical indices of ponds cultured with different sizes of grass carp were more susceptible to the influence of the size of the fish than the sediment physicochemical indices, and the structures of the water and sediment microbiota were also different because of the size of grass carp. This is probably due to the different cultivation and pond management modes of grass carp culture at different stages, which changes the physicochemical indices of the pond and affects the water and sediment microbiota. Moreover, fish microbiota is one of the primary sources of sediment microbiota, and > 15% of the sediment microbiota is derived from fish (*Zhang et al., 2022*). Therefore, the differences in water and sediment microbiota in different ponds were probably caused by differences in the gut microbiota of grass carp of different sizes.

Habitat microbiota is considered to be an important factor affecting the microbiota structure of aquaculture organisms(*Liu et al., 2021; Zhang et al., 2022; Giatsis, 2015*), and is closely related to the health of aquaculture organisms(*De Schryver et al., 2021; Chen et al., 2017; Kaktcham et al., 2017; Huang et al., 2018*). *Aeromonas hydrophila*(*Song et al., 2017*) , *Citrobacter* spp. (*Lü et al., 2011*), *Aeromonas veronii* (*He et al., 2018*), *Aeromonas sobria* (*Zou et al., 2019*), *Aeromonas allosaccharophila* (*Zou et al., 2019*), *Aeromonas punctata* (Xu et al., 1987), *Plesiomonas shigelloides* (*Zou et al., 2019*), *Lactobacillus gasseri* (*Zou et al., 2019*), *Fiexibacter coiumnaris* (*Xu et al., 2007*), *Vibrio mimicus* (*Li et al., 2020a*), *Vibrio vulnificus* (Liu et al., 2019), and *Myxococcus piscicola* (*Lu et al., 1975; Huang et al., 1983*) are commonly reported as bacterial

pathogens of grass carp. Our results showed that *Aeromonas* and *Vibrio* were significantly enhanced in terms of water microbiota, especially in the SJ water microbiota. This result implied that grass carp are at an increased risk of infection by *Aeromonas* and *Vibrio* in SJ ponds.

The microbiota plays a crucial role in facilitating the conversion of various forms of nitrogen and phosphorus in pond ecosystems (*Ni et al., 2018; Gong et al., 2021; Wang et al., 2022*). Members of Dechloromonas were determined as denitrifying polyphosphate-accumulating organisms (*Dai et al., 2017*). *Limnohabitans* spp. participate in nitrogen and phosphorus metabolism as photoautotrophs and ammonia oxidizers (Zeng et al., 2012)[61]. Candidatus *Aquiluna*, a photoheterotroph (*Kang et al., 2012*), was reportedly positively correlated with the microbial metabolic activity of organic nitrogen (*Lukwambea., 2020*). *Pseudomonas furukawaii* ZS1, *Acidovorax facilis*, *Citrobacter diversus*, and certain *Thauera* species also participate in nitrogen removal in aquaculture ponds (*Mai et al., 2021; Niu et al., 2022*). Our results showed that *Dechloromonas*, *Limnohabitans*, Candidatus *Aquiluna*, and *Pseudomonas* were detected in the pond water and sediment microbiota. In addition, *Dechloromonas* sp. OTU was significantly positively correlated with water $NH_4^+$-N, $NO_2^-$-N, $NO_3^-$-N, and TN, and was enhanced in LJ pond water and sediment; and Candidatus Aquiluna OTU was significantly negatively correlated with water $NO_2^-$-N, $NO_3^-$-N, and TN, and significantly positively correlated with water $NH_4^+$-N (Fig. S2), and was enhanced in LF pond water and sediment. Moreover, our results indicated that water $NO_2^-$-N, $NO_3^-$-N, and TN concentrations were significantly positively correlated with the relative abundance of *Cyanobium* PCC-6307 OTUs, whereas $NH_4^+$-N concentration was significantly negatively, implying that these OTUs may play important roles in nitrogen metabolism of pond water, although further verification is needed. Moreover, *Cyanobium* PCC-6307 was enhanced in LF pond water and sediment. These results implied that different bacterial species participate in nitrogen metabolism in the ponds cultured with grass carp of different sizes. Moreover, the enhancement of *Cyanobium* PCC-6307 in LF pond implied that LF pond was more prone to cyanobacteria bloom.

The impact of environmental factors on the aquatic microbiota community structure has been extensively studied, and water temperature, DO, pH, and nutrients have been found to significantly affect microbiota structure (*Ni et al., 2018; Guan et al., 2019*). Because water environmental factors are more susceptible to changes between day and night than sediment environmental factors, differences in water temperature between the ponds in this study were likely to be caused by the different sampling times, which led to more frequent changes in the water microbiota than in the sediment microbiota, and ultimately led to the sediment microbiota being more stable than water microbiota, consistent with previous research (*Zheng et al., 2021*).

## Conclusions

Different types of ponds cultured with grass carp of different sizes exhibited significant differences in water physicochemical indices and composition of the water and sediment microbiota. The exchange of microorganisms between the water and sediment microbiota was lowest in ponds with small grass carp and highest in ponds with large grass carp. Moreover,

*Aeromonas* and *Vibrio* were significantly increased in the water microbiota, especially in ponds with small juvenile grass carp, implying an increased risk of *Aeromonas* and *Vibrio* infections in these environments. Additionally, there were significant correlations between water parameters including POC, DOC, Chla, pH, TP, $PO_4^{3-}$-P, DO, $NH_4^+$-N, $NO_2^-$-N, $NO_3^-$-N, TN, and TSS, and the water microbiota. All detected sediment parameters including TS, TNS, TCS, TOCS, and TPS showed correlations with the sediment microbiome.

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