



Microbial community dynamics and functional shifts in aquaponic systems: Implications for sustainable aquaculture

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ABSTRACT

Aquaponics presents an innovative and sustainable approach to integrated agriculture and aquaculture. However, studies on bacterial communities and their interactions within aquaponics systems, particularly concerning farmed fish, remain limited. This study explores the microbial dynamics within an aquaponics system through 16S rRNA gene amplicon sequencing of the V3-V4 regions over 60-days. We assessed microbial diversity at the genus level across nine samples between control and treatment groups. Both species richness and Shannon index analyses revealed no significant differences in overall microbial diversity between the two groups. However, SIMPER analysis identified 16 genera that contributed to the observed differences between the control and treatment groups, highlighting specific shifts in microbial composition despite the overall similarity in diversity metrics. The *Candidatus Planktophila* (hgcI clade), which increased after day 30 in the treatment group, is thought to have improved water quality through nutrient cycling and organic carbon degradation, positively impacting both fish and plants. Functional analysis also revealed key shifts, including an increase in phototransduction, vascular smooth muscle contraction, and flavonoid biosynthesis. Notably, the increased presence of flavonoid biosynthesis pathways may contribute to immune function and stress reduction in fish, while supporting the development and resilience of plants. These results suggest that aquaponics supports beneficial microorganisms without drastically altering microbial composition, while creating a stable, symbiotic environment that benefits both fish and plants, highlighting it as an environmentally sustainable method for both aquaculture and agriculture.

1. Introduction

Among various human activities contributing to the rapid degradation of our environment, traditional aquaculture is identified as a significant factor. Traditional aquaculture, typically conducted near coastal areas, contributes significantly to this issue. The byproducts from these activities, including excreta, uneaten feed, and deceased organisms, are frequently discharged into coastal waters (Bergheim et al., 2019). The effluent from aquaculture activities, rich in organic matter,

detrimentally affects marine ecosystems by triggering harmful algal blooms and creating hypoxic zones (Anderson et al., 2012; Richlen et al., 2010). On land, agriculture poses similar environmental threats. To cultivate healthy crops, farmers heavily rely on fertilizers and pesticides (Sun et al., 2018). Particularly, the application of chemical fertilizers leads to the production of methane and nitrous oxide, potent greenhouse gases that significantly contribute to global warming (Tian et al., 2020). To mitigate these environmental impacts, hydroponic systems, which cultivate plants in water-based nutrient solutions, have been introduced.

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However, these systems are not entirely devoid of environmental pollution, as the disposal of nutrient-rich effluents still poses a concern (Resh, 2022).

Aquaponics systems have emerged as a revolutionary solution that overcomes the limitations of both traditional aquaculture and agriculture. An aquaponics system operated without soil synergistically integrates advantages of aquaculture (mostly fish), hydroponics and microorganisms (Love et al., 2015). In this system, fish produce feces, while plants use this waste as a fertilizer and return clean water to the fish (Yep and Zheng, 2019). However, the feces from the fish are not directly absorbed by the plants. In order for plants to absorb the nutrients from the feces, they rely on the assistance of microorganisms that convert it into ammonium and nitrate through a process known as nitrification (Wongkiew et al., 2017). Also, this system does not require pesticides, antibiotics or chemical fertilizer which contribute to water pollution and can be harmful to marine ecosystems when discarded (Masabni and Niu, 2022; Taha et al., 2022). Since water is recycled and only needs to be replenished due to evaporation, aquaponics systems employ approximately 90 % less water compared to the traditional farming methods (Abraham et al., 2017). By minimizing wastewater and reducing the reliance on chemical inputs, this system presents a more sustainable approach to food production. Consequently, aquaponics has emerged as an economically efficient and environmentally sustainable method of farming (Love et al., 2015).

Currently, the world is experiencing a rapid increase in consumption of both fish and plants. As a result, aquaponics is increasingly considered the most promising alternative for addressing food scarcity and mitigating water shortages (Ibrahim et al., 2023). In some studies, aquaponics systems were shown to improve both plant and animal growth by improving photosynthetic availability in plant and lowering stresses in fish (Mao et al., 2023). Compared to the conventional production systems, aquaponics system showed 1.65 times higher in growth rate in catfish (Oladimeji et al., 2020). Therefore, aquaponics is considered as one of the most efficient and environmentally sustainable techniques of the twenty-first century (Oladimeji et al., 2020).

Eels are an ideal species for aquaculture due to their resilience to diverse environmental conditions and their ability to thrive in high-density farming systems (Banmali and Tam, 2021). While aquarium tanks may offer lower yields and profitability due to their limited space compared to larger ponds, their potential for farming eels remains considerable (Li et al., 2024). The controlled environment of tanks, coupled with the resilience of eels in high-density conditions, suggests that they could still be highly effective for eel aquaculture, despite the spatial limitations. Recently, *Anguilla marmorata* has become a preferred species in South Korean aquaculture due to lower costs, stronger disease resistance, adaptability to intensive systems, and reduced reliance on expensive eel larvae. Despite their suitability for aquaculture, there has been no in-depth study on the metagenomics of aquaponics systems utilizing eels, which led to their selection for this research. Addressing this research gap could provide critical insights that enhance the efficiency and productivity of eel farming, offering substantial benefits to the industry. Aquaponics is an emerging approach that integrates aquaculture and agriculture into a single system, aiming to enhance resource efficiency and sustainability (Tschirner and Kloas, 2017). Despite its growing recognition as an innovative system, research on aquaponics remains limited when compared to traditional agricultural and aquacultural fields.

Since microorganisms connect aquaculture and hydroponics systems, understanding their community dynamics is important to determine their functional role in the symbiotic ecosystem (Munguia-Fragozo et al., 2015; Schmutz et al., 2017). Several studies have utilized metagenomic analysis to investigate the dynamics between microorganisms and their environments. However, these studies have primarily focused on species such as tilapia (Fan et al., 2025), carp (Wang et al., 2022), and catfish (Yamane et al., 2021). To date, no metagenomic research have been conducted on anguillid eels. This study aims to assess the

effectiveness of aquaponics systems by examining microbial community structures obtained from the water.

2. Materials and methods

2.1. Aquaponics design and sampling

The study was conducted for 60 days at the College of Fisheries and Ocean Sciences, Chonnam National University, Yeosu, Jeollanam-do, South Korea under indoor conditions. Two experimental groups each with three replications were included in this experiment: a treatment group in which Chinese cabbage (*Brassica rapa* var. *pekinensis*) was planted, and a control group without Chinese cabbage. An indoor media-based aquaponics system was used for this experiment, where 10 % of tank water from the control group was changed at 7-day interval and throughout the whole trial, the treatment group's water was kept constant only by adding more to replace the water which was lost due to evaporation. Red clay ball (ϕ : 1.5 cm) was used for this study as media for bacterial growth and vegetable cultivation. In this experiment, each sub-system was made up of an eel holding cylindrical tank (0.38 m^3) filled with 240 L water, an oval shaped vegetable growing bed (0.54 m^2), siphoning tube, an air stone (length: 12 cm, and ϕ : 2.5 cm) for supplying proper oxygenation, a 15.8 W submersible water pump (ϕ : 1.6 cm; voltage: 220 V; and water flow rate: 8 L min^{-1}) (PhilGreen POWER HEAD PUMP BT-20, China) for draining the water and effluents from the eel's tank water to the vegetable growing bed, a water heater (300 W) to maintain a constant water temperature and LED lights (10 h day^{-1}) for offering the right spectrum of light for plant photosynthesis during the whole experiment. Each vegetable growing bed had a hole cut beneath it to serve as an exit for a PVC line (ϕ : 3.1 cm) that drains water from the vegetable bed into the fish tank. Moreover, in this media-based aquaponic experiment, the red clay balls were used as the growth medium, providing support for plant roots and extensive surface area for microbial colonization, thereby enhancing nitrification (Rakocy et al., 2006; Somerville et al., 2014). No separate biofilter was included, as the

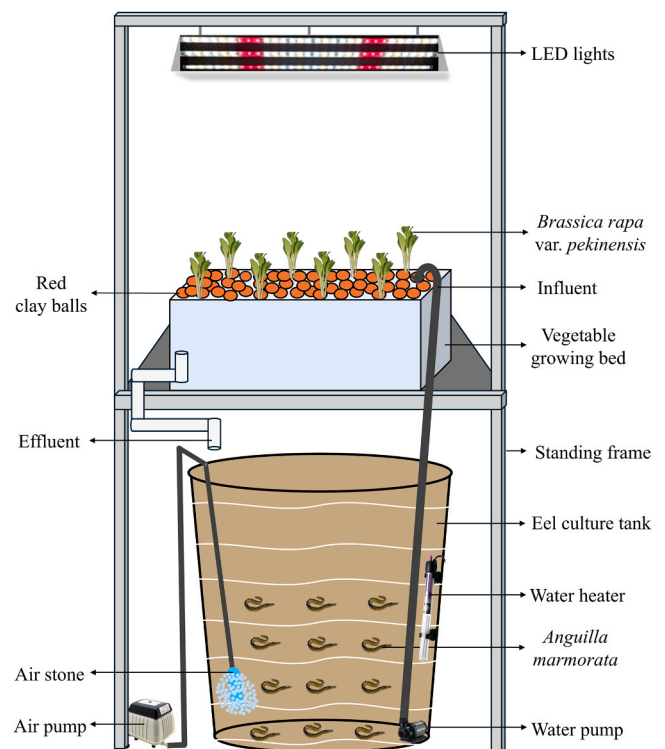


Fig. 1. Schematic representation of the indoor media-based aquaponic system utilized during the experiment.

system was designed to evaluate plant–fish interactions under these conditions. The layout of the aquaponic system of this study is illustrated in Fig. 1, indicating its coupled design with fish tanks, plant beds and water flow.

The Dongwoo Fisheries Farm, located in 640 Jungcheonpo-ro, Hakgyo-myeon, Hampyeong-gun, Jeollanam-do, Republic of Korea, is where the *Anguilla marmorata* was collected. Prior to use, the eel culture tank's water was aerated for a week to dissolve the oxygen. A constant density of 12 eels (average weight: 192.43 ± 3.80 g; and average length: 43.17 ± 0.29 cm) per eel culture tank was maintained. Each eel tank was comprised of a 15.8 W submersible water pump to ensure constant water circulation between the eel tanks and plant beds, a 300 W water heater to maintain the ideal temperature, and an air stone (length: 12 cm; diameter: 2.5 cm) to maintain the dissolved oxygen. The eels were kept in a dark environment and fed with commercial moist feed once a day (Moisture: 6.0 %, crude protein: 48.0 %, crude lipid: 4.0 %, crude fiber: 3.0 %, ash: 13.0 %, calcium: 2.0 %, and phosphorous: 2.7 %) at a rate of 1.5 % body weight. Before feeding, 1.5 % of the dry weight of the feed was mixed with tap water and a ball of dough was formed. To reduce the formation of algae and fungi, the uneaten feed was removed from the tank water after 30 min of feeding using a siphoning pipe.

A total of eight Chinese cabbage seedlings (average shoot length: 11.55 ± 0.60 cm; and average total seedlings weight: 2.89 ± 0.12 g) were planted in the vegetable growing bed in the treatment groups with planting distance of 25 cm. Throughout the experiment, four sets of LED grow lights (10,000 lux, NINE S Co., Korea) were placed above each plant bed and run for 10 h every day to provide the ideal conditions for appropriate photosynthesis. As the nutrients relied solely on eel culture waste, no additional nutrients were introduced in the sub-systems during this cultivation period. Our experimental period was designed to align with the harvest period of the Chinese cabbage used in this study, which generally occurs around 60 days after planting. Additionally, in this experiment, the fish-to-plant ratio was determined based on established aquaponic design principles to maintain system balance in the absence of species-specific guidelines for *A. marmorata*. A moderate fish biomass (12 individuals per tank) and a low-density planting of leafy greens (8 seedlings per bed) were chosen to maintain water quality, prevent nutrient surpluses, and ensure overall system stability, while avoiding nutrient limitations for plants and excessive TAN and NH_3 loading for fish, consistent with coupled, media-based aquaponic systems (Rakocy et al., 2006; Somerville et al., 2014).

Water temperature, pH, and dissolved oxygen (DO) were consistently measured during the experiment with a YSI meter (ProDSS, USA). Electrical conductivity (EC) and total dissolved solids (TDS) were assessed every 15 days using an E-1 portable meter (COMS, ID944). Additionally, the concentrations of ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), and phosphate (PO_4) were tested at 15-day intervals at the Sienna Marine Research Institute in Yeosu-si, South Korea.

For bacterial analysis, a total of 2 L water was collected randomly from both eel culture control ($n = 3$) and treatment tanks ($n = 3$) using a sterile 5 L plastic beaker and pooled to create representative samples every 15 days. From the 2 L of collected water of control tanks, a total of 120 mL of pooled water was then filtered through a sterile single-use syringe (JUNG RIM MEDICAL INDUSTRIAL CO., LTD. Korea), with a $0.22 \mu\text{m}$ pore size sterile cellulose acetate membrane filter which was 47 mm in diameter (MCE02047A, HYUNDAI MICRO, Korea) contained in a Whatman 47 mm plastic filter holder (TARSONS PRODUCTS LTD., KOLKATA, India). After filtration, the filters were placed into a sterile 2 mL microtube and stored at -80°C until DNA extraction. The same procedures were repeated for the collected 2 L water of eel culture treatment tanks.

2.2. DNA extraction to sequencing

The genomic DNA (gDNA) was extracted from the water filters using

the Qiagen DNeasy Power Soil Pro Kit (Qiagen Inc., Hilden, Germany). Then, the quality of the gDNA was evaluated using the dsDNA HS Assay Kit (Thermo Fisher Scientific, USA). It is important that the DNA remains as intact as possible, exhibiting an OD₂₆₀/280 ratio within the range of 1.8–2.0. We prepared gene-specific sequences targeting the hypervariable regions (16S V3–V4) using the 16S Metagenomic Sequencing Library Preparation protocol. The primer sequences targeting these regions are as follows: 341 F (5'-TCGTCGGCAGCGTCAGATGTGTA-TAAGAGACAGCCTACGGGNGGCWGCAG-3'), 805 R (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGA-CAGGACTACHVGGGTATCTAATCC-3'). The amplified library was purified with AMPure XP beads, after which the multiplex index and Illumina sequencing adapter were added through an Index PCR. We used automated electrophoresis (Tapestation; Agilent, USA) to purify and check the quality of these indexed libraries. Next, 2×300 bp paired-end metagenomic sequencing was conducted on the Illumina MiSeq platform according to the manufacturer's protocol.

2.3. Metagenome analysis

We employed QIIME2-amplicon (v2024.5) software to carry out all bioinformatic analyses (Bokulich et al., 2018). First, a summary of the demultiplexed sequence was generated to assess the quality of the reads using the q2-demux plugin, followed by the removal of adapters and primers using the q2-cutadapt plugin (Martin, 2011). The trimmed sequences were then denoised with the q2-dada2 plugin, which performs error correction, chimera removal, and merging of paired-end reads, resulting in the production of amplicon sequence variants (ASVs) (Callahan et al., 2016). The parameters were set to truncate sequences at 240 bp for both forward and reverse reads to ensure consistent read length and avoid potential biases.

The taxonomy of the representative sequences was assigned using a Naïve Bayes classifier with the “fit-classifier-naive-bayes” command, based on SILVA 138 SSURef NR99 full-length sequences (MD5: de8886bb2c059b1e8752255d271f3010) and SILVA 138 SSURef NR99 full-length taxonomy (MD5: f12d5b78bf4b1519721fe52803581c3d), specific to the V3–V4 region. The classifier was trained using sequences with the primers used in this study. Taxonomy identification was performed using the “classify-sklearn” command within the q2-feature-classifier plugin (Bokulich et al., 2018). Finally, taxonomic filtering was carried out to remove specific taxa and unassigned sequences from the dataset. The filtering was achieved using the “filter-table” command within the q2-taxa plugin. This process excluded sequences classified as “Unassigned”, “Archaea”, “Chloroplast”, or “Mitochondria” from the feature table.

The representative sequences and feature table were exported from QIIME2. Functional predictions of microbial communities were made using PICRUSt2 (v2.5.2) (Douglas et al., 2020), followed by subsequent analysis conducted using the ggpircrust2 package (v1.7.3) (Yang et al., 2023) in R (v4.4.0). PICRUSt2 is a tool that utilizes 16S rRNA sequencing data to predict the functions of whole genomes, yielding results similar to those obtained from shotgun metagenome analysis (Douglas et al., 2020). Functional annotation was based on the KEGG (Kyoto Encyclopedia of Genes and Genomes) database (Kanehisa and Goto, 2000). Significant differences in functional profiles between control and treated groups were identified using edgeR (v4.2.0) (Robinson et al., 2010), with cut-off of $q < 0.05$.

2.4. Statistical analysis

The statistical analysis was performed using the phyloseq, vegan, and MicrobiotaProcess R packages (McMurdie and Holmes, 2013; Oksanen et al., 2013; Xu et al., 2022). All samples were rarefied to a minimum read depth of 644,539. To analyze α -diversity, disparities among the periods were evaluated using the Kruskal–Wallis H-test. Additionally, pairwise differences between period groups were determined using

pairwise Kruskal-Wallis H-tests.

For β -diversity analysis, frequency data were standardized using the Hellinger method, and the Bray-Curtis distances were calculated. The homogeneous dispersion of the samples was evaluated using the beta-disper test (Anderson et al., 2006), and differences between groups were investigated through Permutational Multivariate Analysis of Variance (PERMANOVA) using the distance matrices, with pairwise comparisons between groups assessed using pairwise PERMANOVAs. A Similarity Percentage (SIMPER) analysis was conducted to assess the contribution of specific microbiota taxa to the dissimilarities observed between the control and treated groups. A t -test was also used for analyzing the significant differences between the control and treatment groups, and GraphPad Prism (v8.0.2) was used to create the graphs. The experimental workflow are illustrated in Fig. 2.

3. Results

3.1. Overall results of 16S amplicon sequencing for all samples

During the experiment, there was no observed mortality for both eels and cabbages. A total of nine samples (five controls and four treatments) were collected at 15-day intervals for analysis. In the analysis process, a total of 12,084,146 paired-end reads were used (Table 1). After removing adapter sequences and low-quality reads, a total of 9596,771 (79.42 %) clean reads were used in this study. Of these, 3035 ASVs were identified, including sequences from the Archaea domain and unassigned sequences. Subsequently, we filtered the data to obtain domain sequences from domain Bacteria. Following rarefaction, 2961 ASVs were identified, with a total frequency of 5800,851. After taxonomy assignment, 28 bacterial phyla were identified, 722 taxa at the genus level, and 1244 at the species level. However, microorganisms that were identified as “uncultured”, were excluded, leading to the identification of 27 phyla, 577 genera, and 387 species. Additionally, species identified as “s_metagenome” were excluded from this study, resulting in a total of 266 identified species. A total of 68.8 % and 61.7 % of the taxa were shared between the control and treatment groups at the genus and species levels, respectively (Fig. S1).

3.2. Microbial community composition changes in the aquaponic system

We analyzed the relative abundance of the microbiome at both the phylum (top 10 taxa) and the species levels (top 20 taxa) across nine samples (Fig. 3). At the phylum level, most samples were predominantly composed of Proteobacteria (mean of 37.27 %), with Bacteroidota (32.03 %) following in relative abundance (Fig. 3a). The water-con-01 sample, representing the first day of the experiment, showed a higher proportion of Proteobacteria (83.20 %) compared to Bacteroidota

(14.11 %). However, in the control group samples from days 15, 30, and 45, the proportions of these two phyla were relatively similar. By day 60, the composition reverted, with Proteobacteria becoming more abundant than Bacteroidota (Fig. 3a). In the treatment group, all samples, except for day 30 and 60, exhibited a predominance of Proteobacteria (25.34 %) and Bacteroidota (57.11 %), similar to the control group. In the water-treat-15 sample, Bacteroidota (68.48 %) was the most abundant. Notably, the day 30 sample showed a distinct predominance of Actinobacteriota (78.76 %). Furthermore, the day 60 sample, Bacteroidota (35.94 %) and Verrucomicrobiota (34.57 %) exhibited a similar dominance.

At the species level, the composition plot was constructed for the top 20 taxa representing 89.21 % of the total relative abundance (Fig. 3b). Interestingly, in the treatment group, the samples from days 15, 30, and 45 showed that *Flectobacillus* sp., *Candidatus Planktophila* and *Flavobacterium indicum* each predominated at 74.17 %, 86.29 %, and 68.50 %. Additionally, on day 60, *Rurimicrobium arvi* accounted for more than 50 % relative abundance along with *Candidatus Planktophila*. On the other hand, in the control group, the number of dominant species was lower, and the community was composed of more diverse species compared to the treatment group. The water-con-15 sample exhibited a predominance of *Flectobacillus* sp., similar to the same period of treatment samples, but had a relatively lower proportion (51.28 % vs. 74.17 %). Furthermore, *Curvibacter lanceolatus* accounted for a substantial portion at 28.15 %. On day 30, the samples displayed a diverse distribution of species without any over-representative taxa. By day 45, *Coralimargarita* sp. was dominant (53.89 %), and by day 60, both *Peredibacter starrii* (47.74 %) and *Coralimargarita* sp. (26.08 %) became the dominant taxa.

3.3. Microbial diversity and community structure analysis in aquaponics systems

We assessed microbial diversity between control and treatment groups at genus level across nine samples. Both species richness and the Shannon index showed no significant differences in microbial diversity between two groups ($p = 0.92$ and $p = 0.6$, respectively) (Fig. 4a). The observed taxa for the control group were 251.0 ± 57.32 , while the treatment group had 241.4 ± 42.26 , indicating a relatively higher mean and standard deviation in the control group. For the Shannon index, the control group had a mean value of 2.80 ± 0.41 , with the treatment group showing greater variability as 2.52 ± 0.61 . Although species richness from both groups showed a gradual increase over time, no significant difference was observed (Figs. 4b and 5). Interestingly, the Shannon index decreased for both groups on day 15 compared to day 1, but by day 30, the control group recovered earlier. Afterward, both groups gradually increased in a similar trend. This suggests that greater

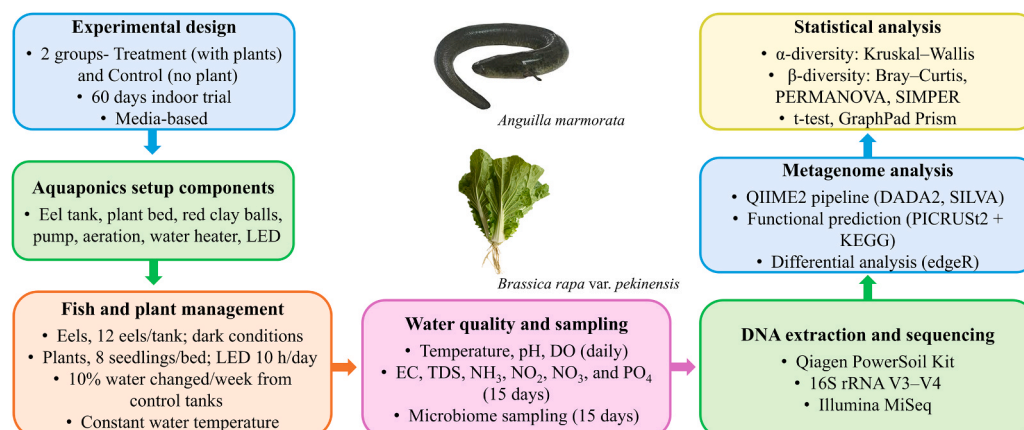


Fig. 2. Flowchart of the proposed methodology used in this experiment.

Table 1
General information on sequencing data.

Sample information		Number of reads			Number of ASVs				
Sample ID	Raw sequences	After cutadapt (%)	After DADA2 (%)	Bacteria	Arachea	Unassigned	Total ASVs	Total ASVs after filtering and rarefying	
water-con-01	1541,725	1531,653 (99.35)	1234,484 (80.07)	1234,484	0	0	646	638	
water-con-15	1249,141	1240,503 (99.31)	962,802 (77.08)	962,802	0	0	546	543	
water-con-30	795,756	789,835 (99.26)	644,553 (81.00)	644,553	0	0	621	619	
water-con-45	1013,004	1005,272 (99.24)	811,102 (80.07)	811,024	78	0	827	815	
water-con-60	1460,360	1448,089 (99.16)	1129,289 (77.33)	1129,035	254	0	959	942	
water-treat-15	1943,839	1930,725 (99.33)	1554,189 (79.95)	1554,160	0	29	672	652	
water-treat-30	1300,244	1292,303 (99.39)	989,311 (76.09)	989,311	0	0	606	596	
water-treat-45	1313,619	1304,790 (99.33)	1079,821 (82.20)	1079,821	0	0	765	744	
water-treat-60	1466,458	1455,401 (99.25)	1191,220 (81.23)	1191,216	4	0	894	878	
Total	12,084,146	11,998,571 (99.29)	9596,771 (79.42)	9596,406	336	29	-	-	

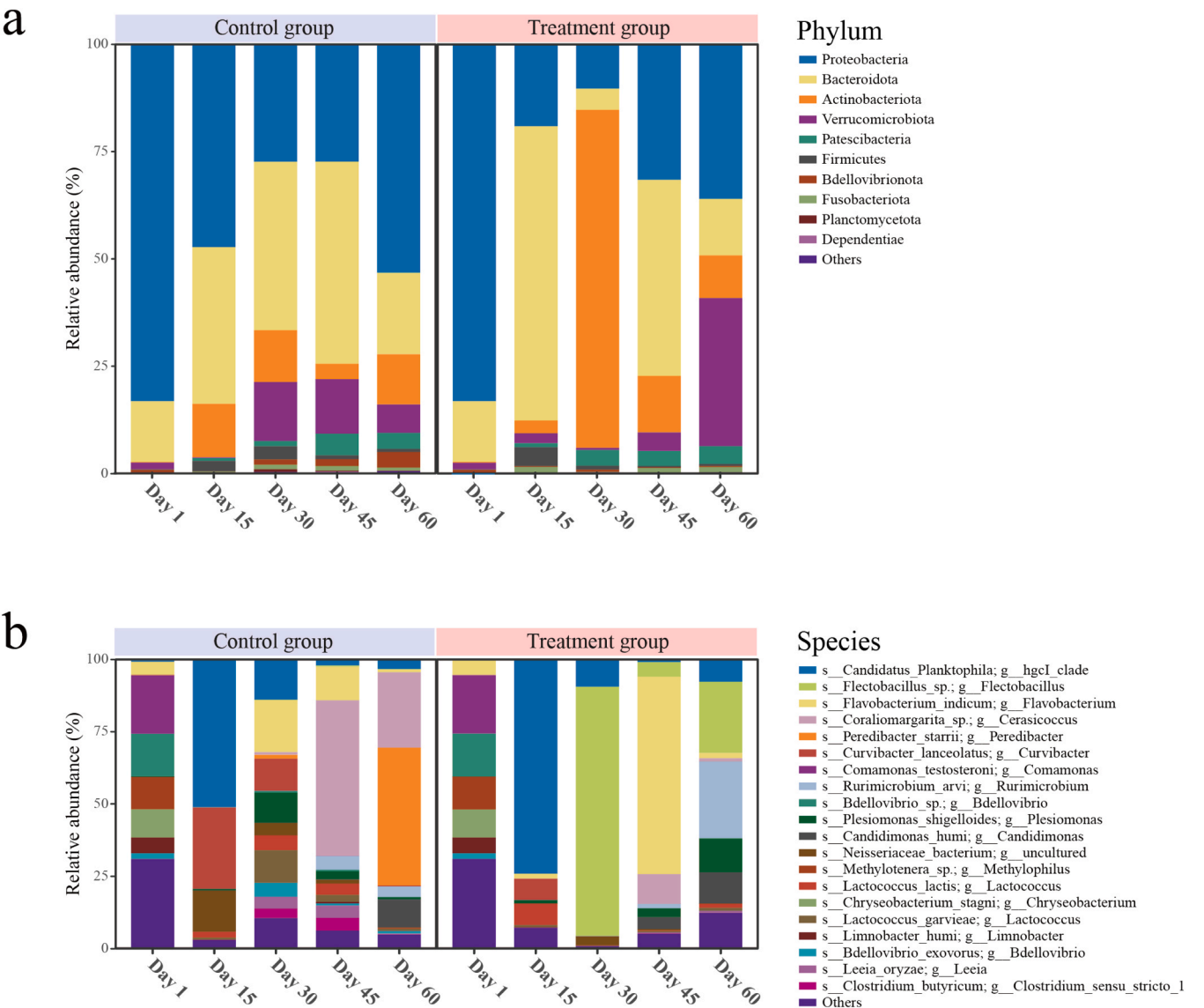


Fig. 3. Relative abundance of dominant microbial taxa in the waters aquaponics system. **(a)** At the phylum level (top 10). **(b)** At the species level (top 20). The x-axis represents the samples, and the height of each bar indicates the relative abundance of each taxonomic group. The Day 1 sample from control group corresponds to the same sample as Day 1 sample in the treatment group.

variability in microbial community structure occurred on day 30, influenced by the application of the aquaponics system (Fig. 3, Figs. 4b, and 5).

Additionally, we investigated differences in microbiome community

structure across all samples (Fig. 6). Results from Betadisper and PERMANOVA revealed no significant differences in community structure between groups (Table S1). However, when conducting SIMPER analysis, we identified 16 genera contributing to the differences between the

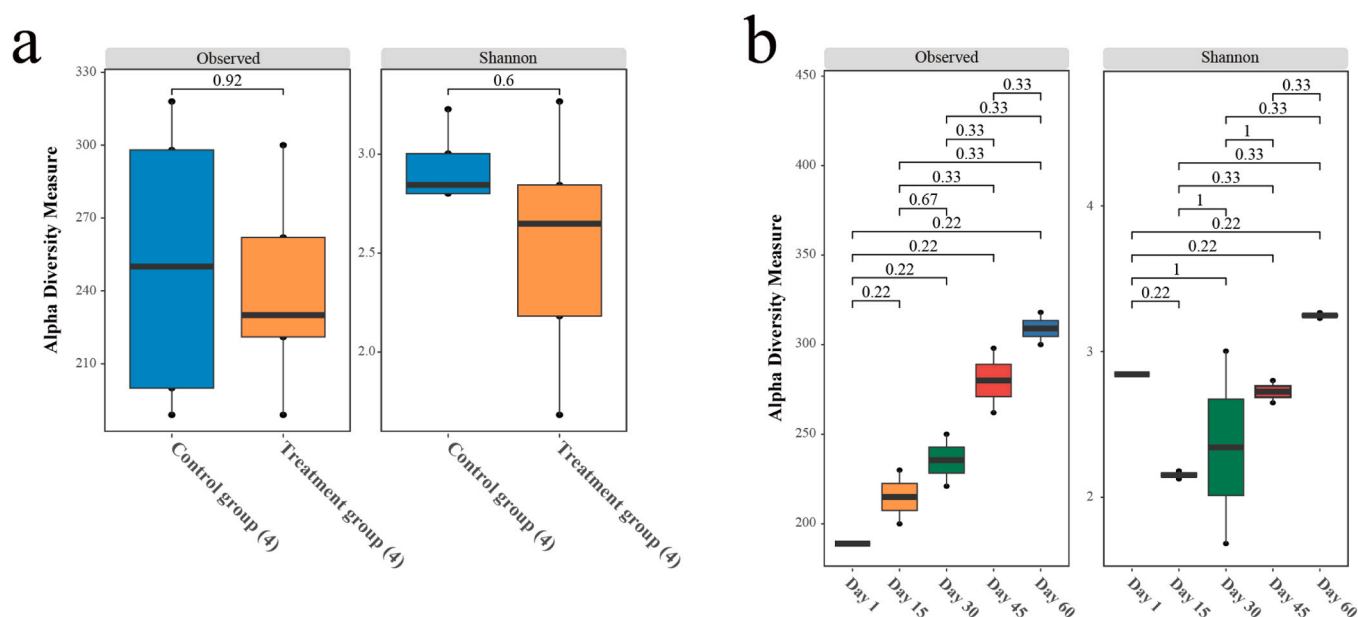


Fig. 4. The α -diversity result at the genus level. (a) Comparison between control and treatment groups. (b) α -diversity over time in treatment groups using two indices. The left panel represents Observed Species Richness, and the right panel represents Shannon diversity indices. Statistical differences between groups were assessed using the Kruskal-Wallis H-test, with pairwise comparisons between two groups. The Day 1 sample from control group corresponds to the same sample as Day 1 sample in the treatment group.

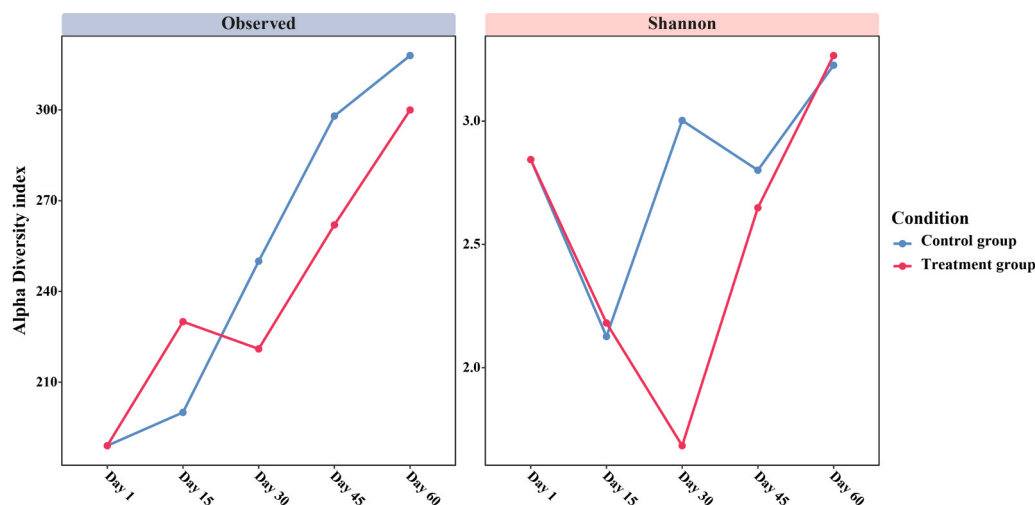


Fig. 5. Changes in α -diversity indices overtime at the genus level. (a) Observed species. (b) Shannon index. The control and treatment groups were represented by green and red, respectively.

control and treatment groups ($p \leq 0.001$) (Table 2). Notably, *hgcI* clade (*f_Sporichthyaceae*), which contributed the most to the compositional differences, was highly predominant in the treatment group with an average abundance of 63,141.25 compared to 53.25 in the control group. This genus was predominant on day 30 sample of the treatment group (Fig. 3b). Additionally, other genera such as *Ramlibacter* (*f_Comamonadaceae*) and *Candidatus_Adlerbacteria* (*f_Candidatus_Adlerbacteria*) were more prevalent in the treatment group with average abundances of 246.5 and 88.25, respectively, compared to their lower values in the control group (8.5 and 2.5, respectively). On the other hand, some genera were more abundant in the control group. For instance, *cvE6* (*f_cvE6*) showed the highest average abundance in the control group, with a value of 242 compared to only 3.5 in the treatment group. *Gemmata* (*f_Gemmataceae*) also exhibited an average abundance of 39.5 in the control group but was nearly absent in the treatment group (0.25). Similarly, *Mesorhizobium* (*f_Rhizobiaceae*) had an average

abundance of 35 in the control group and 5 in the treatment group.

3.4. Predicted microbial functional changes in aquaponics system application

We utilized PICRUSt2 to predict the functional potential of microbial communities that were altered by the aquaponics system. Since the day 1 sample was used in both the control and treatment groups we excluded this sample from the functional differences analysis. Therefore, we used eight out of the nine samples (four from the control group and four from the treatment group) to explore functional differences ($q \leq 0.05$) (Figs. 7 and S2). As a result, three key pathways were identified as significantly enriched in the treatment group compared to the control group: phototransduction, vascular smooth muscle contraction, and flavone and flavonol biosynthesis. Conversely, 22 pathways (e.g., basal transcription factor, cell cycle, mTOR signaling pathway) were found to

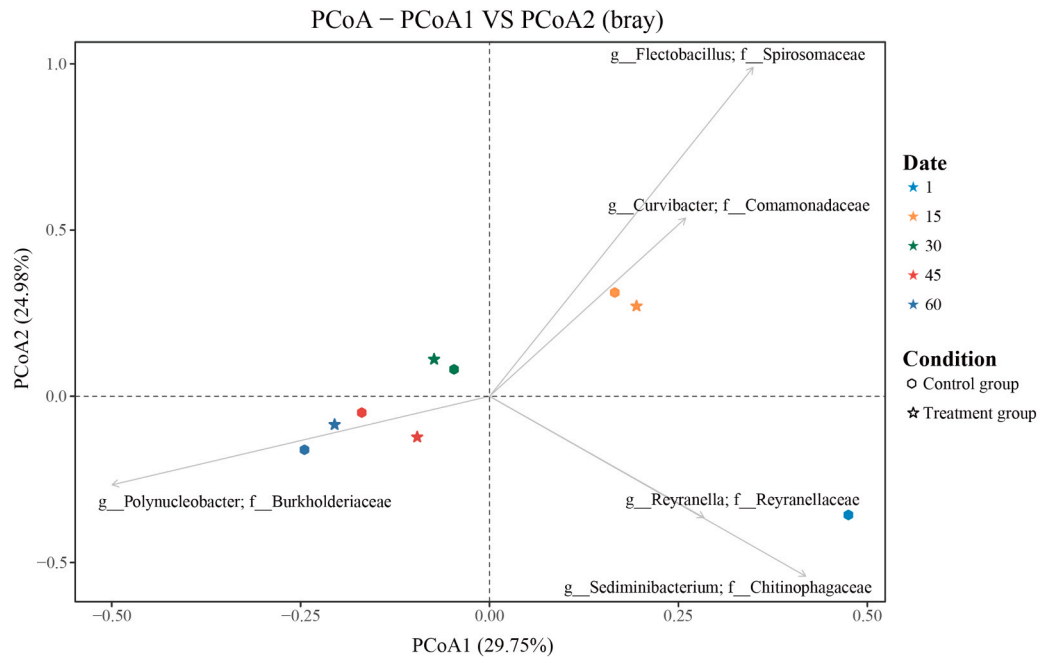


Fig. 6. The β -diversity result at genus level. The circle and star represent control and treatment samples, respectively. Colors represent the sampling dates. The arrows indicate the genus that had the most significant impact on the distribution of the group.

Table 2
Result from SIMPER analysis at genus level.

Family	Genus	average	sd	ratio	ava (control)	avb (treated)	cumsum	p-value
Sporichthyaceae	hgcI_clade	0.077	0.122	0.627	53.25	63141.25	0.218	0.001
Comamonadaceae	Ramlibacter	0	0	0.848	8.5	246.5	0.981	0.001
cvE6	cvE6	0	0	0.742	242	3.5	0.981	0.001
Candidatus_Adlerbacteria	Candidatus_Adlerbacteria	0	0	1.313	2.5	88.25	0.993	0.001
Gemmataceae	Gemmata	0	0	0.869	39.5	0.25	0.996	0.001
Rhizobiaceae	Mesorhizobium	0	0	0.82	35	5	0.997	0.001
Simkaniaceae	Candidatus_Fritschea	0	0	0.878	16.75	0	0.998	0.001
Lachnospiraceae	Lachnospiraceae_NK4A136_group	0	0	1.172	21	6	0.998	0.001
Candidatus_Uhrbacteria	Candidatus_Uhrbacteria	0	0	0.86	0	9	0.999	0.001
Tepidisphaeraceae	Tepidisphaera	0	0	0.553	0.25	0	1	0.001
Rhodobacteraceae	Nereida	0	0	0.553	0.25	0	1	0.001
Desulfotobacteriaceae	Desulfosporosinus	0	0	0.553	0.25	0	1	0.001
Rikenellaceae	dgA-11_gut_group	0	0	0.553	0.25	0	1	0.001
Lachnospiraceae	Lachnospiraceae_UCG-010	0	0	0.553	0.25	0	1	0.001
JG30-KF-CM66	JG30-KF-CM66	0	0	0.553	0.25	0	1	0.001
Beijerinckiaceae	Camelimonas	0	0	0.554	0	0.25	1	0.001

be significantly reduced in the treatment group.

4. Discussion

The application of aquaponics systems has the potential to revolutionize traditional aquaculture and agriculture by creating a more sustainable and eco-friendly food production model. In this study, we explored microbial community dynamics within an aquaponics system, assessing both composition and functional capabilities to understand its implications for fish health and overall ecosystem stability.

In terms of microbial community dynamics, specific taxa responded distinctly to the aquaponics system. Notably, *Candidatus planktophila* (f_Sporichthyaceae, g_hgcI_clade) emerged as a dominant species in the treatment group, particularly from day 30 onwards (Fig. 3b). This microorganism, known for its carbohydrate metabolism pathways, is associated with nutrient cycling (Neuenschwander et al., 2018). Additionally, this species is involved in the degradation and recycling of organic carbon, potentially contributing to nutrient cycling and improving water quality within the aquaculture system (Kasozi et al.,

2021; Menezes-Blackburn et al., 2021; Schmutz et al., 2021). The increased abundance of this microorganism may enhance nutrient balance in the aquaponics system, potentially benefiting both fish and plants.

Despite the application of the aquaponics system, overall α - (e.g., Shannon index) and β - (e.g., Bray-Curtis dissimilarity) diversity analysis results showed no significant differences between two groups (Figs. 4, 5 and Table S1). This aligns with previous research indicating that while each compartment of an aquaponics system harbors distinct microbial communities, the overall pattern remains consistent (Schmutz et al., 2017). Furthermore, compared to studies involving single time-point sampling, our data may provide more in-depth insights, as our study explored microbial community over a 60-day period (Eck et al., 2019). Since α - and β - diversity of the microbiome did not change significantly when compared between groups or over time (Figs. 3 and 4), this suggests that the application of an aquaponics system does not substantially alter the overall structure or diversity of the microbial community. However, the system may subtly influence the aquatic environment by affecting a small number of specific microbial taxa, which could play a

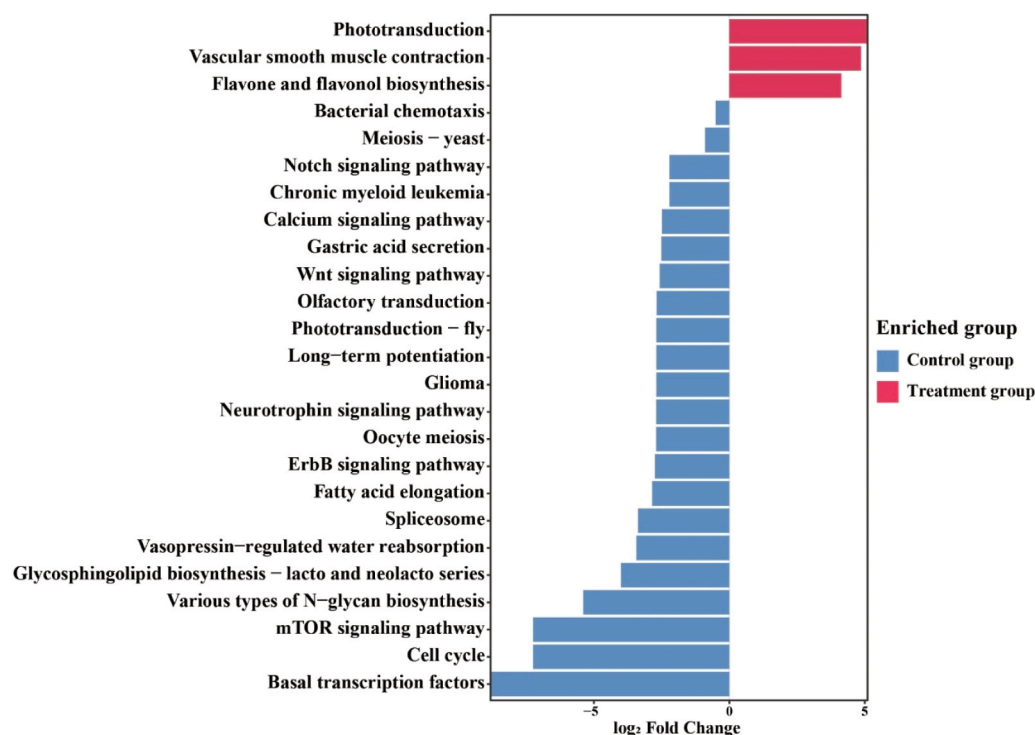


Fig. 7. Functional pathways analysis using PiCrust2 on four control and treatment samples. The x-axis represents the $\log_2$ FC, and the y-axis represents the KEGG pathways.

key role in supporting fish health and function without inducing large-scale changes in microbial composition. This stability may be considered as positive outcome, as the aquaponics system appears to preserve the existing microbial ecosystem without causing significant structural disturbances. Additionally, this stability, combined with the emergence of beneficial taxa, may contribute to the long-term sustainability and efficiency of aquaponics.

Next, we explored the functional changes induced by the microorganisms to understand their impact on the aquaponics system. In addition to the stability of the microbial community, the functional changes between two groups provided insights into the potential impacts of the aquaponics system on the overall ecosystem. Our study revealed an increase in pathways related to flavone and flavonol biosynthesis. These flavonoid components could potentially enhance the immune status in fish (Tan et al., 2020). Moreover, the immune-enhancing properties of flavonoids could lead to reduced disease prevalence, thereby improving the overall health and productivity of fish within aquaponic systems. The anti-inflammatory characteristics of flavonoids can help regulate inflammatory responses (Zhang et al., 2020), while also stimulating non-specific immune responses, thereby strengthening the overall immune system in fish (Zhang et al., 2022). Furthermore, flavonols are plant-specific metabolites that play crucial roles in plant development and growth (Daryanavard et al., 2023). However, as the roots of Chinese cabbage may serve as the initial source of flavonoid synthesis-related microorganisms introduced into the aquaponics system, our study is limited in its ability to identify the exact origin of these beneficial microorganisms, given that we did not perform metagenomic analysis on the cabbage roots.

Infact, *Flavobacterium* spp. has several beneficial effect on plant growth and protection (Liu et al., 2012; Kolton et al., 2016) as well as pathogen defense (Munguia-Fragozo et al., 2015). This genus has been reported to be commonly found in aquaponics systems in several studies (Itoi et al., 2007; Munguia-Fragozo et al., 2015; Schmautz et al., 2017). Our results also revealed an enhanced presence of these pathways and dominance of *Flavobacterium indicum*, suggesting that the aquaponics

system may promote the production of compounds that could play a crucial role in supporting both fish health and disease resistance, as well as plants development.

The alterations in microbial community composition and predicted functions align with the goals of aquaponics to create a symbiotic environment that benefits both fish and plants while minimizing waste and resource use (Yep and Zheng, 2019). In this context, the application of aquaponics resulted in a 1.65-fold increase in the growth rate of catfish (Oladimeji, S.A. et al., 2020). The treatment group showed significantly greater final body weight, weight gain, specific growth rate, and daily weight gain than the control, reflecting enhanced growth performances (Table S2). Improved feed efficiency, a higher protein efficiency ratio, and a reduced feed conversion ratio indicate more effective nutrient utilization. These results align with those of (Lee and Kim, 2021), who observed similar improvements in eel growth parameters over the feeding period. The observed enhancements may be attributed to the nutritional or microbial benefits provided by the treatment. Furthermore, the lack of significant differences in survival rate and hepatosomatic index suggests that the treatment had no adverse effects on fish health.

Evaluating water quality is fundamental to sustaining an integrated and balanced aquaponic ecosystem that supports the health and productivity of aquatic animals, cultivated plants, and beneficial microbial communities (FAO Food and Agriculture Organization, 2014). Proper water management not only optimizes biological performance but also enhances overall system efficiency and long-term sustainability. Previous studies (Effendi et al., 2017; Estim et al., 2019) demonstrated significant improvements in water parameters within aquaponic systems, emphasizing the role of plant-microbe-fish interactions in regulating nutrient cycling. In the present study, the integration of *B. rapa* var. *pekinensis* significantly improved water quality by reducing concentrations of key nitrogenous and phosphate compounds. Specifically, at the end of the experiment, lower concentrations of NH_3 , and higher concentrations of NO_2 , NO_3 , and PO_4 were recorded in the treatment group compared to the control group (Fig. S3). These improvements can be

attributed to the active uptake of nutrients by plant roots and enhanced microbial activity in the rhizosphere, which together reduce the accumulation of metabolic waste products in the water. Furthermore, the slightly elevated NO₂ may reflect a transient accumulation during active nitrification in the media, consistent with previous observations (Rakocy et al., 2006; Somerville et al., 2014) in media-based aquaponic systems. However, a reduction in DO and pH was observed in the treatment group, likely due to increased microbial respiration and organic acid production in the root zone (Fig. S4). Moreover, throughout the experiment, the control groups (without plants) exhibited higher pH than the treatment groups (with plants). This difference can be attributed to several factors: in the control tanks, the absence of plant nutrient uptake and reduced rhizosphere microbial activity resulted in less hydrogen ion release, leading to higher pH; the weekly 10 % water exchange may have introduced slightly alkaline water; and variations in CO₂ dynamics and localized root-zone processes further contributed to the observed differences. Despite the lack of active parameter control, these inherent biological and chemical processes stabilized water quality in the treatment tanks and produced meaningful results. These observations are consistent with previous studies showing that media-based aquaponic systems can self-regulate water quality, and that plant presence naturally stabilizes and slightly acidifies pH (Rakocy et al., 2006; Somerville et al., 2014). In contrast, EC and TDS increased, possibly because of ongoing mineralization and ion release from fish feed and waste, exceeding the rate of nutrient uptake by plants. Such changes reflect the dynamic nature of nutrient cycling in aquaponic systems and highlight the importance of balancing plant absorption with microbial and aquatic animal activity to maintain optimal water quality for *A. marmorata*.

While this study provides valuable insights into the microbial dynamics of aquaponics systems, it has limitations. The use of predicted functional pathways, while informative, should be validated through metagenomic or metatranscriptomic analyses in future studies. While 16S rRNA sequencing offers valuable insights into the taxonomic composition of microbial communities, it does not provide direct information about their functional capabilities. The application of PICRUSt2 enables functional predictions based on the taxonomic profiles derived, but such predictions are speculative, relying on the assumption that the functional potential of microorganisms in our system corresponds to the general patterns observed in other environments. Nevertheless, the identification of species associated with pathways like flavonoid biosynthesis suggests the potential for these species to engage in related functions within the aquaponics system (Zhu et al., 2021). Furthermore, research utilizing metagenome-assembled genomes (MAGs) can provide a more detailed understanding of the functions of microbial communities in the aquaponics system. Also, future research is needed to focus on validating these predicted pathways, particularly examining the functional roles of dominant taxa in nutrient cycling and disease resistance.

5. Conclusion

In conclusion, our study elucidates that aquaponics systems can promote a stable yet functionally altered microbial community, which potentially enhances the health and growth of both fish and plants. When applying the aquaponics system, one of the main advantages is its ability to create a beneficial environment that supports both plant and fish health by promoting beneficial microbial communities without significantly changing the overall microorganism composition. Additionally, the system's design minimizes waste and resource use, contributing to a more sustainable and efficient method of food production. Overall, this study strengthens the growing evidence that aquaponics systems are not only sustainable but also capable of maintaining ecosystem stability while supporting both aquaculture and plant growth, making them a promising solution for integrated food production.

CRedit authorship contribution statement

Hyeonwoo Choi: Writing – original draft, Validation, Software, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Prosun Roy:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Haeyoung Park:** Visualization, Formal analysis. **Jihye Yang:** Visualization, Formal analysis. **Seung Ju Oh:** Visualization, Formal analysis. **Hyeongju Choi:** Methodology, Visualization, Formal analysis. **Daehwan Lee:** Methodology. **SungHun Lee:** Data curation, Formal analysis, Methodology, Software. **Jeonghui Hong:** Writing – review & editing, Validation, Data curation, Supervision, Conceptualization, Project administration. **Seong-il Eyun:** Writing – review & editing, Supervision, Conceptualization, Resources, Project administration, Funding acquisition.

Ethical approval

The experimental protocols received approval from the Institutional Animal Care and Use Committee (IACUC) at Chonnam National University (CNU IACUC-YS-2023–9).

Declaration of Competing Interest

The authors report no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2025.103248.

Data availability

Data will be made available on request. The raw data were deposited in GenBank SRA under PRJNA1167674.

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