Aquaculture Reports 47 (2026) 103475

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Gut and blood microbiota of Nile tilapia (*Oreochromis niloticus*) reared with lettuce (*Lactuca sativa*) and challenged with *Escherichia coli* and *Vibrio cholerae* in aquaponic systems

Angélica Adiação Jossefa [a,b,c,*,1], Leonildo dos Anjo Viagem [a,b,c,2], Brunno da Silva Cerozi [c], Sebastian Wilson Chenyambuga [a]

[a] Department of Animal, Aquaculture and Range Sciences, Sokoine University of Agriculture, PO Box 3004, Morogoro, Tanzania
[b] SACIDS Africa Centre of Excellence for Infectious Diseases, SACIDS Foundation for One Health, Sokoine University of Agriculture, Morogoro, Tanzania
[c] Department of Animal Science, College of Agriculture, University of São Paulo, Avenue Padua Dias, 11, PO Box 9, Piracicaba, São Paulo 13418-900, Brazil

ARTICLE INFO

Keywords:
Pathogenic bacteria
Foodborne
Public health
Microbiota diversity

ABSTRACT

Aquaponics, a bio-integrated system that combines fish farming and vegetable cultivation, has gained significant attention due to its rapid expansion and potential for sustainable food production. In aquaponics, along with plants and fish, both beneficial and harmful microorganisms are also present. This study was conducted to assess the microbiota diversity of Nile tilapia reared with lettuce in aquaponic systems, with a focus on food safety implications. A total of 144 Nile tilapia juveniles with an average initial weight of 30.7 ± 0.03 g were randomly stocked and allocated to three treatments, each with three replicates. In treatment one, the fish were not challenged with any pathogenic bacteria, while in treatments two and three, they were challenged with *Escherichia coli* (ATCC 8739) and *Vibrio cholerae* (ATCC 9458), respectively. Faecal and blood samples were collected before and after challenging Nile tilapia. The diversity of microbial communities was assessed by sequencing the V1-V3 regions of the 16S rRNA gene using Illumina NextSeq2000. In total, 216 amplicon sequence variants were identified in faecal samples and 162 in blood samples, all belonging to the bacteria kingdom. Taxonomic analysis demonstrates that multiple detected genera encompass species with known pathogenicity to humans and documented association with foodborne illnesses and mortality. These included Agrobacterium, Streptococcus, Klebsiella, Clostridium, Brucella, Plesiomanas, Edwardsiella, Aeromonas, and Bacillus. The findings highlight the importance of incorporating public health considerations within an aquaponic system in which vegetables are grown together with fish, in order to reduce the risks of contamination and enhance food safety.

1. Introduction

Aquatic food culture systems are diverse and offer environmental, economic, and social benefits and services for humans. These systems are increasingly recognized worldwide for their valuable ecosystem services, which sustain aquatic biodiversity and preserve the environment FAO (2024). Aquaponics is one such system, a bio-integrated, sustainable, and closed recirculation system that combines soilless cultivation of edible plants with fish rearing by recycling nutrients from fish waste for vegetable production through the nitrifying bacteria, and the vegetables absorb the nutrients from the water, thus purifying water for the fish, creating mutually beneficial ecosystems for both organisms (Love et al., 2014; Prastowo et al., 2024; Rakocy et al., 2006). Aquaponic practice is growing rapidly due to its environmental benefits and economic sustainability. It reduces pollution, fertilizer costs, and water exchange, whose availability is limited. However, several challenges, such as system design, hydroponic components, optimal fish density, management techniques, potential water pollution, and microbial contamination within the systems, are also increasing, highlighting the need for research in various components of the system (Prastowo et al., 2024; Atique, 2023; Yep and Zheng, 2019). Previous studies conducted in aquaponics systems recommended differentiating between beneficial

* Corresponding author at: Department of Animal, Aquaculture and Range Sciences, Sokoine University of Agriculture, PO Box 3004, Morogoro, Tanzania.
E-mail address: angelica.jossefa@sacids.org (A.A. Jossefa).
[1] Department of Agrarian Production, Higher School of Rural Development, Eduardo Mondlane University, Inhambane, Mozambique.
[2] Institute of Natural Resources and Environment, Rovuma University, Cabo Delgado, Mozambique.

https://doi.org/10.1016/j.aqrep.2026.103475
Received 16 October 2025; Received in revised form 7 January 2026; Accepted 17 February 2026
Available online 19 February 2026

microorganisms and those that can cause diseases in humans Hollyer et al. (2009). Understanding the bacterial community in the various components of the aquaponics system is crucial, as it sustains a healthy and sustainable environment for fish and plants (Prastowo et al., 2024; Sawyer, 2021). Factors such as rearing system, bacterial interactions, fish phases, water quality parameters, season, nutrient availability, stress, hygiene levels, antibiotics, diet, and surrounding water significantly influence and modulate the composition and development of the bacterial community in different components of the systems (Atique, 2023; Bereded et al., 2022; Deng et al., 2021; Giatsis et al., 2015; Kelly, 2010; Ringø et al., 2003; Schmidt et al., 2011; Song et al., 2016; Talwar et al., 2018; Wong and Rawls, 2012).

A variety of fish can be grown in aquaponics system, including tilapia, which is an African fish that has been introduced in many countries for aquaculture. It significantly contributes to the livelihoods and economies of rural societies in many developing countries El-Sayed and Fitzsimmons (2023). Seventy-five percent of aquaponics systems use Nile tilapia due to its resilience, rapid growth, and ability to thrive in various climatic and ecological regions (Abd El-Hack et al., 2022; Grammer et al., 2012; Hussain and Brown, 2024; Tiwari et al., 2025). Nile tilapia contains microbiota that protect the fish against pathogenic microorganisms and assist with nutrient digestion, adaptation to extreme environmental conditions, metabolic processes, and overall integrity (Diwan et al., 2024; Merrifield and Rodiles, 2015). However, Nile tilapia also harbor pathogenic microorganisms that may cause foodborne infections in humans (Sawyer, 2021; Bereded et al., 2022; Elumalai et al., 2017). A literature review conducted by (Novotny et al., 2004 reported that Nile tilapia hosts human bacterial pathogens such as Vibrio, Clostridium, Mycobacterium, Aeromonas, Legionella, and Plesiomonas.

In aquaponics, microorganisms such as Aeromonas, Pseudomonas, Flavobacterium, Fusibacter, Planctomycetes, Parcubacteria, Lentisphaerae, Ignavibacteria, Hydrogenedentes, Gammatimonadetes, Fusobacteria, Cyanobacteria/Chloroplast, Cloacimonetes, Chloroflexi, Candidatus-Saccharibacteria, Bacteroidetes, and Armatimonadetes have already been identified (Prastowo et al., 2024; Tiwari et al., 2025; Eck et al., 2021; Ruiz et al., 2023; Sanchez et al., 2019; Schmautz et al., 2017; Yamane et al., 2021). The presence of these bacteria can promote plant growth (Tiwari et al., 2025; Day et al., 2021) and may also pose risks to humans through consumption of raw or undercooked food (Sawyer, 2021; Bereded et al., 2022). Pathogens such as *Vibrio cholerae* and *Escherichia coli* have been reported as part of tilapia microbiota, raising concerns about food safety (Banerjee and Ray, 2017; Bereded et al., 2020; Hamom et al., 2020; Wang et al., 2017). These pathogens can multiply in aquatic environments and colonize tilapia (Wang et al., 2017; Ali et al., 2023; Elewasy et al., 2024; Elgendy et al., 2022; Hounmanou et al., 2019; Tey et al., 2015). In aquaponics, many studies have examined microbial communities in biofilters, fish tanks, water, plant roots, sedimentation tanks, sumps, and hydroponic units. Studies focusing on the faecal and blood microbiota of Nile tilapia from the perspective of food safety are scarce. Therefore, this study was conducted to characterize the faecal and blood microbiota of Nile tilapia challenged with *E. coli* and *V. cholerae* in aquaponics systems with lettuce, in the context of food safety. These bacteria were selected because, in addition to being recognized in the CZ ID 2024 List of Pathogens, the WHO 2024 Priority List, and the NCBI Pathogen Detection Organisms Group as causative agents of infectious diseases in humans associated with the consumption of raw or undercooked fish or vegetables, they have also been isolated from tilapia and lettuce (Banerjee and Ray, 2017; Bereded et al., 2020).

2. Material and methods

2.1. Study location

This study was carried out from April to May 2025 at the fish farming center of the Department of Animal Science, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, SP, Brazil, located at 22°42'30" S latitude and 47°38'01" W longitude. The area has a humid subtropical to tropical climate, with summer rain (November-March) and a drier winter (May-August). The annual average rainfall and temperature are around 1280 mm to 1500 mm and 20–21 °C, respectively.

2.2. Statement of ethical clearance

The procedures for the care and use of animals were adhered to. This study was approved by the Ethics Committee on Animal Use of Luiz de Queiroz, College of Agriculture-ESALQ (CEUA/ESALQ), with protocol number CEUA 1853260225 and ID 000612.

2.3. Experimental design and treatments

The experiment was conducted in an aquaponic system comprised of nine fish culture tanks, each holding 70 L of water and eighteen hydroponic units. The fish tanks were randomly assigned to three treatments, each with three replicates in a completely randomized design. The treatments involved challenging the fish with pathogenic bacteria as follows: treatment one served as the control, whereby the fish were not challenged with any pathogenic bacteria; in treatment two, the fish were challenged with *Escherichia coli* (ATCC 8739), and in treatment three, the fish were challenged with *Vibrio cholerae* (ATCC 9458). Each treatment was randomly allocated to three fish culture tanks.

2.4. Aquaponic system setup

The setup of the aquaponic system (19 m x 15 m) is shown in Fig. 1. Fish were cultured in tanks, each tank having a submersible pump connected to a biofilter (Janeca, IPF-5990, China) housing bio-balls (K2–44MM, Lana Produtos, Brazil) for nitrifying bacteria, along with an air stone for aeration. Nitrifying bacteria colonization and stabilization of the nitrification process occurred over 15 days before lettuce seedlings were introduced into the system. Each fish tank was linked to two lines of hydroponic units (30 cm×15 cm x 4 cm), each with nine plants. Fish tanks were filled with deionized water, purified via reverse osmosis (Biothec, CM-230, Brazil), sterilized using a UV filter (OceanTech Professional Aquarium, UV-567, 8000 L/min), and tested negative for bacterial contamination on Eosin Methylene Blue (EMB) agar for *E. coli*, Thiosulfate Citrate Bile Sucrose (TCBS) agar for *V. cholerae,* and Tryptic Soy Agar (TSA), a non-selective medium. Water was pumped from the fish tanks to the hydroponic units through a submersible pump (Jeneca, IPF-5990–10V, China) with a capacity of 4000 L/h, and returned to the fish tanks by gravity. The hydroponic units were equipped with LAD lamps (ES-600Q-72A, SawGlant, China) providing a photoperiod of 18 and 6 h during the day and night, respectively.

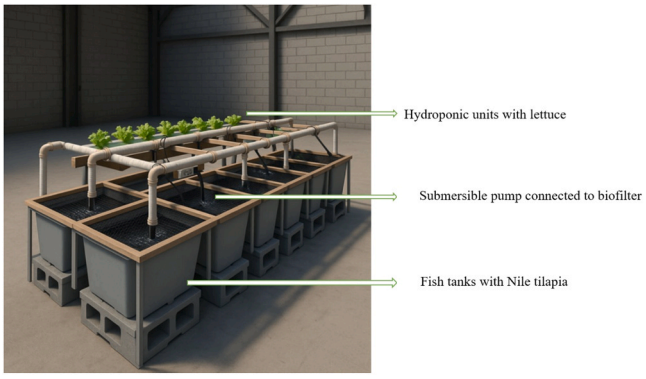

Fig. 1. Aquaponic system used during the study to raise Nile tilapia and grow lettuce.

2.5. Lettuce seedling production and planting

To produce lettuce seedlings, pellet seeds of *Lactuca sativa* var. crispa L. were purchased from an accredited company (Blueseeds, BS AC0055, Brazil). The seeds were germinated in seedling growing trays for hydroponic (MINGOTI, 53 cm×27 cm x 4 cm), containing substrate for plant growth (cod 75, rice straw, Carolina Soil, Brazil). Before and after germination, the seedlings were watered twice a day with water obtained from a previously used aquaponic system from the fish farming center. The seedlings were transplanted in the aquaponic system 28 days after germination, when the root system was capable of accessing nutrients from the fish tank.

2.6. Stocking of Nile tilapia juveniles

Nile tilapia juveniles with a mean weight of 30.7 ± 0.03 g were collected from the fish culture tanks at the fish farming center of the Department of Animal Science, Luiz de Queiroz College of Agriculture, University of São Paulo, Brazil. The juveniles were quarantined for 15 days in two tanks with good water quality (well water) before being stocked in the fish tanks at a density of 16 fish per tank. They were fed *ad libitum* twice a day (at 9:00 and 17:00 h) with a feed produced by the Department of Animal Science. The information about the ingredients used to formulate the food is listed in Table 1 below.

2.7. Challenging Nile tilapia with pathogenic bacteria

The Nile tilapia cultured in fish tanks were challenged for five days with two bacterial species (*Escherichia coli* ATCC 8739 and *Vibrio cholerae* ATCC 9458). *Escherichia coli* ATCC 8739 was provided by the fish microbiology laboratory, Department of Food Science and Technology, University of São Paulo, Brazil, with 10^9 UFC/ml. *Vibrio cholerae* ATCC 9458 was provided by the Tropical Cultures Collection-André Tosello Foundation, Brazil, with 10^9 UFC/ml. The identity and purity of the bacterial strains were confirmed by cultivation in EMB agar for *E. coli* and TCBS agar for *V. cholerae*. Colony-forming units (CFUs) were estimated by spectrophotometer analysis using a 600 Plμs spectrophotometer (FEMTO Ind. Com. Instruments, Ltd., Brazil). Nile tilapia juveniles were reared for 30 days in aquaponics systems, then the fish were challenged by injecting 0.5 ml of 1×10^4 UFC/ml of each pathogenic bacteria, via the peritoneal vein using sterile hypodermic needles (SR-Products LA Salud SA, Paraguay).

2.8. Water quality monitoring in the aquaponic system

To assess the water quality within the systems, temperature and dissolved oxygen (DO) were measured twice a day (at 8:00 h and 16:00 h) using an oximeter (AT160, Alfakit, Brazil). pH was measured once a day at 16:00 h using a pH-meter (SL110, SOLAR instrumentation ltd, Brazil) while electrical conductivity (EC) was measured once a week using condutivimeter mCA-150, MS TecnoPon Special Equipment Ltd., Brazil. Furthermore, a chemical kit from ProDac Test Brazil was used to confirm the presence of nitrate within the systems.

Table 1
Ingredients used to formulate the diet used for feeding fish during the experiment.

Ingredients	%
Fish meal	40
Wheat gluten	10
Maize bran	24
Rice bran	25
Vitamin and mineral Premix*	1

* 1 kg of mixture contains folic acid: 250 mg; pantothenic acid: 5000 mg; mixed herb flavouring: 50 mg; BHT: 15 g; biotin: 50 mg; copper: 1995 mg; choline: 50 g; onion extract: 87 mg; grape seed extract: 15.6 mg; Iron: 13. 47 g; iodine: 75 mg; manganese: 3733 mg; niacin: 5000 mg; calcium propionate: 200 mg; selenium: 75 mg; vitamin A: 1000000UI; vitamin B1: 500 mg; vitamin B12: 3750 mg; vitamin B2: 1750 mg; vitamin B6: 2488 mg; vitamin C: 25 g; vitamin D3: 500000UI; vitamin E: 18750UI; vitamin K3: 500 mg; zinc: 20 g.

2.9. Data collection

For microbiota analysis, before and after Nile tilapia being challenged with pathogenic bacteria (*Escherichia coli* and *Vibrio cholerae*), three fish from each treatment were collected after a 48-h fast. The fish were anaesthetized by being put on ice, and then blood was collected from the caudal vein using sterile hypodermic needles (SR-Products LA Salud SA, Paraguay), after disinfecting the fish surface with a gauze soaked in 70 % ethanol. The blood was put into a 2 ml sterile centrifuge tube kept on a polypropylene microcentrifuge tube rack placed on ice. After collecting the blood, the fish were sacrificed by exposure to the anesthetic benzocaine (1 g/10 L of water) to collect the intestine. Intestines were removed from the fish and placed in a petri dish containing PBS solution to remove external residues, then the feces were collected by squeezing the intestine and put into a 2 ml sterile centrifuge tube, also kept on a polypropylene microcentrifuge tube rack placed on ice. Both blood and faecal samples were sent to the Animal Biotechnology Laboratory of the College of Agriculture, Luiz de Queiroz, University of São Paulo, Brazil, 30 min after collection, for DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing.

2.10. DNA extraction, PCR, and sequencing

The DNA extraction from both blood and faecal samples was performed using the Extracta 96 equipment (Loccus), with the MagMax Core Mechanical Lysis Module and the MegMax Core Nucleic Acid Purification Kit (Thermo Fisher Scientific), following the manufacturer's protocol. A specific amount was used for each sample type (i.e., 100 μL of blood and 200 μL of faecal supernatant). The region V1-V3 of the 16S rRNA gene was amplified. For amplification, the PCR was performed using specific primers (27_F AGAGTTTGATCCCTCAG and 1492_R GGTTACCTTGTTACGACTT) at 10 μM. The PCR reactions were carried out in a final volume of 25 μL, containing 1 μL of genomic DNA, 10 μL of PCRBIO Ultra Mix, 0.5 μL of each primer, and 13 μL ultrapure water. The temperature cycling protocol consisted of an initial denaturation at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 30 s, and extension at 72 °C for 30 s. A final extension was performed at 72 °C for 5 min, followed by a hold at 4 °C. The PCR products were assessed by gel electrophoresis using a 1.5 % agarose gel and subsequently purified with AMPure XP Beads (Beckman Coulter, Life Sciences). After purification, the PCR products were used in another PCR reaction using the following primer pair: 26SV1V3 (16SV1_27F AGAGTTTGATCMTGGCTCAG and 16SV3_534R ATTACCGCGGCTGCTGG) at 10 μM. The PCR reaction followed the same conditions described above. The amplified products were again visualized on a 1.5 % agarose gel and purified with AMPure XP Beads. Subsequently, Illumina adapters were attached in a PCR reaction using Nextera XT Index Primer 1 (N7xx) and Nextera XT Index Primer 2 (S5xx) with the 16SV1V3 PCR products. These PCR products were purified with AMPure XP Beads and visualized on a 1.5 % agarose gel. The PCR products were quantified using a NanoDrop and then normalized to the same concentration. An equimolar pool of all samples was prepared and further quantified by qPCR to confirm and determine the final pool concentration in Nm, using the KAPA Library Quantification kit for Illumina. Sequencing was performed on the Illumina NextSeq2000 platform with 2 × 300 bp reads.

2.11. Bioinformatics and statistical analyses

The data were analyzed as described previously by Callahan et al. (2016a) using a suite of packages implemented in the R programming language, available through the Bioconductor project (Gentleman et al., 2004; Huber et al., 2015). Bioconductor workflow for Microbiome Data Analysis from raw reads to community analyses (https://f1000research.com/articles/5–1492).

The multiplexed reads were assigned to biological samples. The DADA2 program Callahan et al. (2016b), an open-source package in R, was used for amplicon error modelling and correction without OUT (Operational Taxonomic Units) construction. Fastq files were filtered to trim PCR primer sequences and remove the 3' ends and chimeras, while maintaining overlap between reads for subsequent merging and reconstruction of the amplified region.

After sequence data processing with DADA2, taxonomies were assigned to each ASV (Amplicon Sequence Variant) using a naive Bayesian classifier implemented in DADA2 Wang et al., (2007). The SILVA database was used as the taxonomic reference Glöckner et al. (2017), and all ASVs with less than 1 % of reliable alignment with the database were not classified. Taxonomic classification generated by DADA2 and their abundances were imported into the phyloseq package McMurdie and Holmes (2013) in R.

The alpha diversity for each treatment before and after the challenge was calculated using the Shannon index and the Gini-Simpson index. To compare the communities between the treatments before and after the challenge, beta diversity was assessed using the generated OTUs and visualized with MDS. The normal distribution of alpha diversity was tested with the Shapiro-Wilk test, while the homogeneity of the data was examined using the Bartlett test. The statistical significance of alpha diversity was evaluated using ANOVA.

3. Results

3.1. Water quality parameters

Water quality parameters measured during the experiment are shown in Table 2. The water temperature in the system remained stable across treatments, ranging from 25.75 ± 2.99 °C to 25.88 ± 2.71 °C. Dissolved oxygen (DO) had average values between 6.41 ± 1.05 mg/L and 6.81 ± 1.02 mg/L, and the pH stayed near neutrality, with values from 7.08 ± 0.53–7.18 ± 0.58. The electrical conductivity (EC) of the water ranged from 167.83 ± 39.02–176.66 ± 38.42 μS/cm. Ammonia and nitrite concentrations were similar across all treatments, averaging about 0.38 mg/L. Nitrate values were also stable between treatments, with an average of 91.7 ± 19.46 mg/L. Statistical analyses showed no significant differences among the treatments ($P > 0.05$).

Table 2
Water quality parameters during the study.

Water quality parameters	Treatments			P - value
	T_1	T_2	T_3	
Temperature(°C)	25.86 ± 2.86	25.75 ± 2.99	25.88 ± 2.71	0.8743
DO (mg/l)	6.81 ± 1.02	6.63 ± 0.98	6.41 ± 1.05	0.0974
pH	7.18 ± 0.58	7.11 ± 0.61	7.08 ± 0.53	0.2761
EC (μS/cm)	168.33 ± 41.93	176.66 ± 38.42	167.83 ± 39.02	0.8086
Ammonia (mg/l)	0.39 ± 0.12	0.38 ± 0.12	0.39 ± 0.12	0.8964
Nitrite (mg/l)	0.38 ± 0.13	0.38 ± 0.13	0.38 ± 0.13	1
Nitrate (mg/l)	91.7 ± 19.46	91.7 ± 19.46	91.7 ± 19.46	1

3.2. Identified Nile tilapia microbiota

The Venn diagram in Fig. 2 illustrates the distribution of ASVs in the faecal and blood samples. It reveals that each environment is unique, with each sample having a distinct microbiota community. A total of 125 ASVs for faecal samples and 71 SVs for blood samples were observed. However, some bacteria species were present in both samples (Todd, 2014), indicating commonalities in the microbial community.

3.3. Nile tilapia microbiota and food safety

Taxonomic analysis of both faecal and blood samples revealed the presence of 378 ASVs, of which 257 were possible to classify at the genus level. About 27 of those genera are pathogenic to humans and are associated with the consumption of raw or undercooked fish or vegetables. Aeromonas, Streptococcus, Klebsiella, Agrobacterium, Bacillus, Brucella, Clostridium, Edwardsiella, Mycobacterium, Staphylococcus, Stenotrophomonas, Ralstonia, Legionella, Plesiomonas, and Citrobacter are some of the genera identified in this study that include at least one human pathogenic species linked to the consumption of contaminated fish or vegetables.

3.4. Diversity of gut microbiota

A total of 216 ASVs in faecal samples were analyzed, all of them belonged to the kingdom Bacteria and were classified to the genus level. However, some ASVs were not taxonomically classified beyond the genus level. Fig. 3 shows the genus-level abundance of faecal microbiota before challenge (FA) and after challenge (FD). The FA group showed high and balanced microbial diversity, with multiple genera represented in variable proportions across the treatments, with *Bacillus* and *Cetobacterium* being the dominant genera. The FD group showed a decrease in microbiota diversity only in treatment 3; however, *Cetobacterium* remained the dominant genus in all treatments. Before the challenge, treatment 1 was richer in microbiota composition, and the lowest microbiota composition was observed in treatment 2. After the challenge, treatment 1 (control) and treatment 2 (challenged with *E. coli*) showed a significant increase in microbiota composition, more than double the diversity, while in treatment 3, in which the fish were challenged with *V. cholerae,* the microbiota composition was reduced. This pattern reflects a state of dysbiosis in microbial composition related to the challenge imposed on Nile tilapia.

The Beta diversity visualized by Multidimensional Scaling (MDS) plots was used to compare the microbial community structures in faecal samples across different treatments (Fig. 4). The results showed that in the faecal samples, Axis 1 explained 40.2 % of the variance, while Axis 2 explained 19.9 %. Samples were separated mainly along Axis 1, suggesting that this axis represents the primary differences between groups.

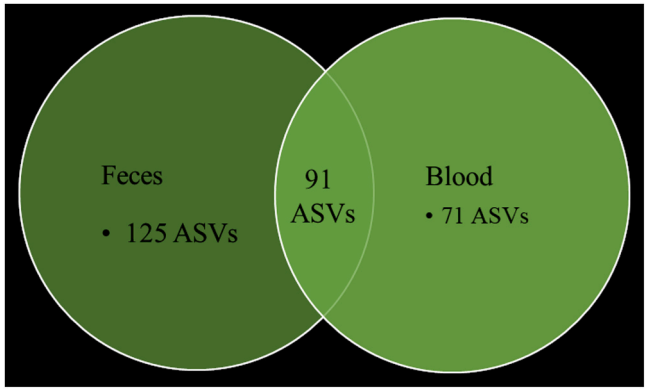

Fig. 2. Venn diagram showing the unique and shared ASVs between faecal and blood samples.

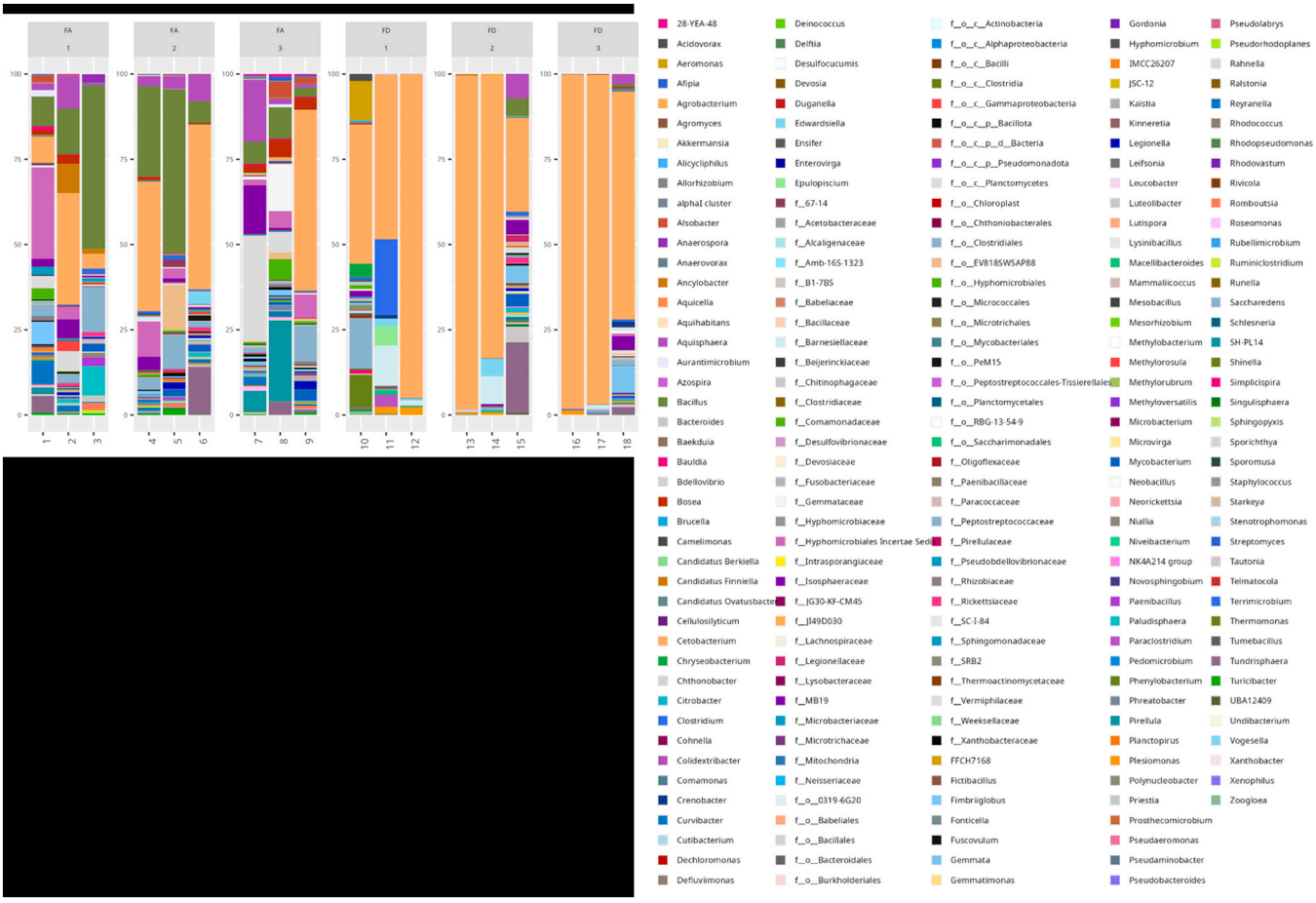

Fig. 3. Bar charts representing the relative abundance of the bacterial genera in faecal samples for each treatment before (FA) and after (FD) the challenge.

Treatments were found to be partially overlapping, but there was a visible separation between the FA and FD groups, indicating that the FA group represents communities that are more similar to each other, while the FD group shows a greater variability in microbial composition.

The alpha diversity index analysis in faecal samples (Fig. 5) revealed that the FA group showed relatively high diversity (around 2.12–2.96) for the Shannon index (Fig. 5A). In contrast, the FD group exhibits a wider spread, with some points dropping below 0.12. The Simpson index (Fig. 5B) in the FA samples again showed higher diversity than the FD group, which indicated a marked decline in diversity. Therefore, the FA group maintained consistently higher alpha diversity than the FD group, which shows reduced diversity.

3.5. *Diversity of blood microbiota*

A total of 162 ASVs were analyzed in the blood samples of Nile tilapia. The results of the diversity analysis of blood samples are represented in Fig. 6. The SA group (before the challenge) showed higher diversity with treatment 2 having the highest microbiota composition and treatment 1 the lowest. with The unclassified bacteria (f_o_c_p_d_Bacteria) were dominant across all treatments. Notably, Nile tilapia in treatments 1 (control) and 2 (challenged with *E. coli*) exhibited a reduction in microbiota diversity after the challenge, whereas fish in treatment 3, challenged with *V. cholerae*, showed a significant increase in microbial diversity. However, in both treatments, the unclassified bacteria (f_o_c_p_d_Bacteria) remained as dominant. Interestingly, these results contrast with those observed in faecal samples, whereby in treatment 2, the injection of *E. coli* doubled the microbial diversity, while in treatment 3, the injection of *Vibrio* reduced it.

The Beta diversity visualized by Multidimensional Scaling (MDS) analysis showed that axis 1 and axis 2 explained 33.8 % and 2 19 %, respectively, of the variance in blood samples (Fig. 7), showing a more dispersed distribution, with weaker clustering among treatments. However, a moderate distinction between the SA and SD groups was still observed, especially along axis 1, suggesting the effects of challenging the fish on blood-associated microbial profiles. These findings indicate that challenging the fish with pathogenic bacteria can influence the microbial community composition in blood samples.

The result of alpha diversity analysis (Fig. 8) revealed that the SA groups in treatments 1 and 2 showed consistently higher Shannon diversity (0.12 – 1.81) while those in treatment 3 exhibited more variability, including lower values (lower than 0.21). For the SD groups, those in treatment 3 tended to show the highest diversity (above 2.14), while those in treatment 1 exhibited the lowest (below 0.02 in some cases). Overall, Shannon diversity was higher in the SA group than in the SD group, except for treatment 3, which showed a slight increase in the SD group (Fig. 8A). The Simpson index (Fig. 8B) also revealed higher diversity in SA group than in SD group across the treatments. The fish in treatment 2 consistently showed high diversity in both groups, while those in treatment 3 again showed variable results, particularly for the SA group. The fish in treatment 1 generally had the lowest Simpson diversity, particularly in the SD group. Therefore, treatment 2 maintained the most stable and highest microbial diversity, while treatment 1 was associated with lower diversity, particularly in the SD group.

4. Discussion

4.1. *Water quality parameters*

During the experiment, key water quality parameters, including

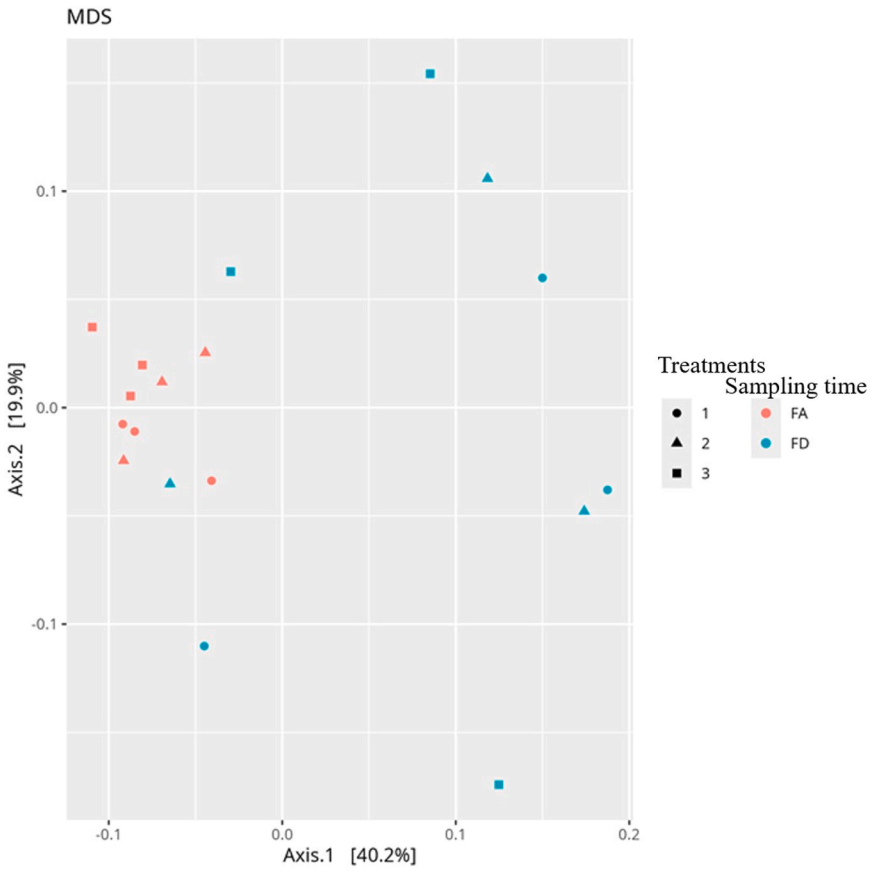

Fig. 4. Beta diversity plot representing faecal microbial community variation among the treatments. Note: FA = faecal samples collected before the challenge, FD = faecal samples collected after the challenge.

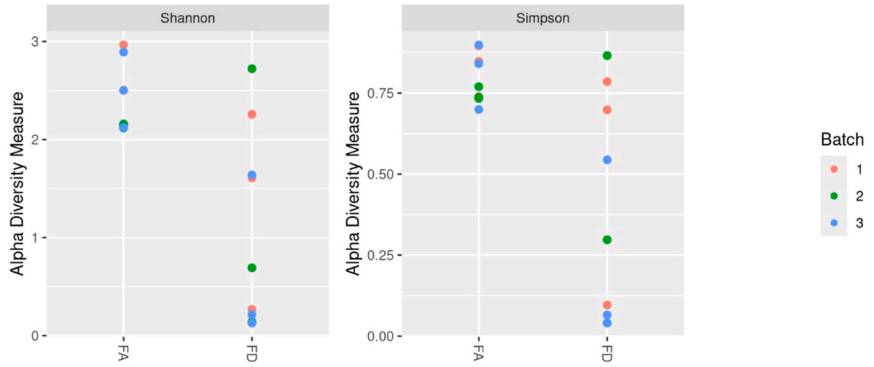

Fig. 5. Alpha Diversity of faecal sample microbiota composition across treatments. Note: Batch = treatment. FA = faecal samples before the challenge. FD = faecal samples after the challenge.

temperature, pH, dissolved oxygen, electrical conductivity, ammonia, nitrite, and nitrate, were continuously monitored and remained within the recommended ranges for optimal Nile tilapia growth and survival (Prastowo et al., 2024; Krastanova et al., 2022; Tyson and Simonne, 2014; Estim et al., 2019; Ujjania et al., 2021). These parameters were stable throughout the experimental period and showed no significant differences among treatments, indicating that well-controlled and suitable water quality conditions were successfully maintained. This stability was likely supported by the presence of a biofilter, which promoted the continuous conversion of ammonia to nitrite and subsequently to nitrate, thereby preventing the accumulation of toxic compounds and contributing to pH regulation. In addition, nutrient uptake by lettuce and the constant water circulation within the system likely enhanced nutrient removal and water homogenization, minimizing abrupt fluctuations in temperature, dissolved oxygen, and electrical conductivity. Maintaining optimal water quality is critical, as physicochemical parameters strongly influence microbial community structure and interactions in aquaculture systems (Bruno et al., 2023; Ortiz et al., 2023; Sylvain et al., 2016). Previous studies have demonstrated that variations in pH, temperature, ammonia, and nitrite can markedly alter microbial composition and diversity in fish and their surrounding environment (Bruno et al., 2023; Ortiz et al., 2023; Sylvain et al., 2016; Chen et al., 2025; Wei et al., 2025; Han et al., 2025). Therefore, the observed stability of water quality suggests that the microbial changes detected in this study were not driven by fluctuations in water quality parameters but were more likely associated with the applied

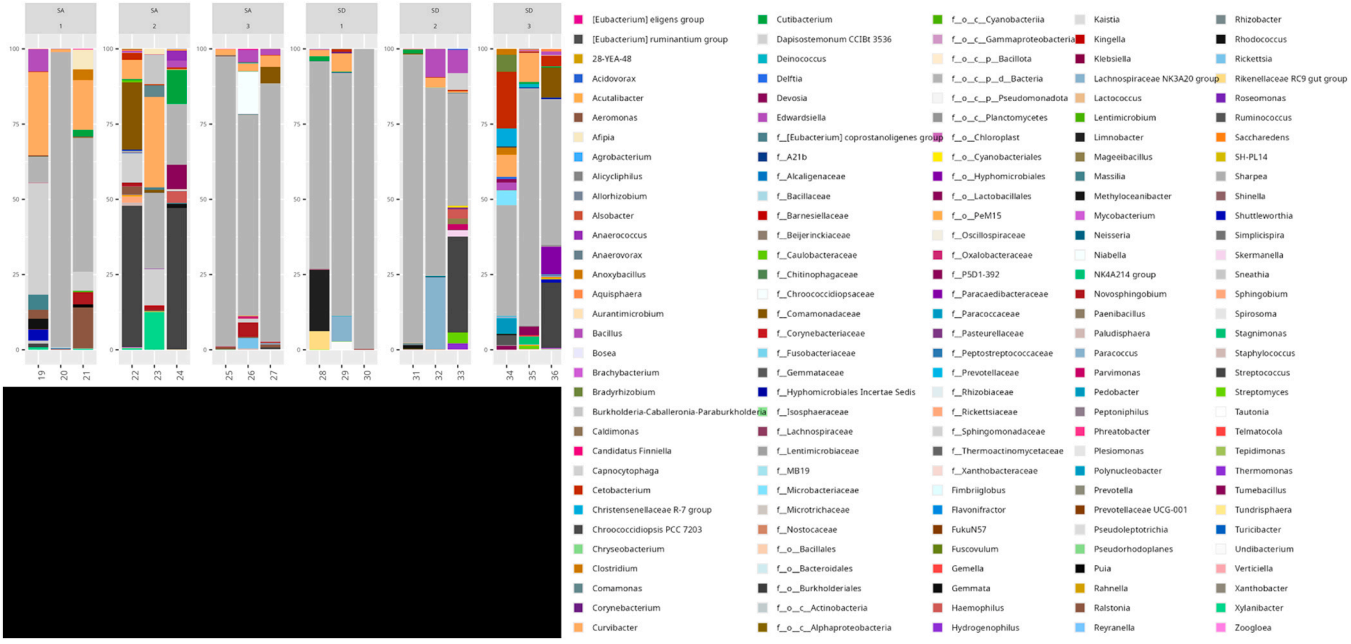

Fig. 6. Bar charts representing the relative abundance of the bacteria genera in blood samples for each treatment before (SA) and after (SD) the challenge.

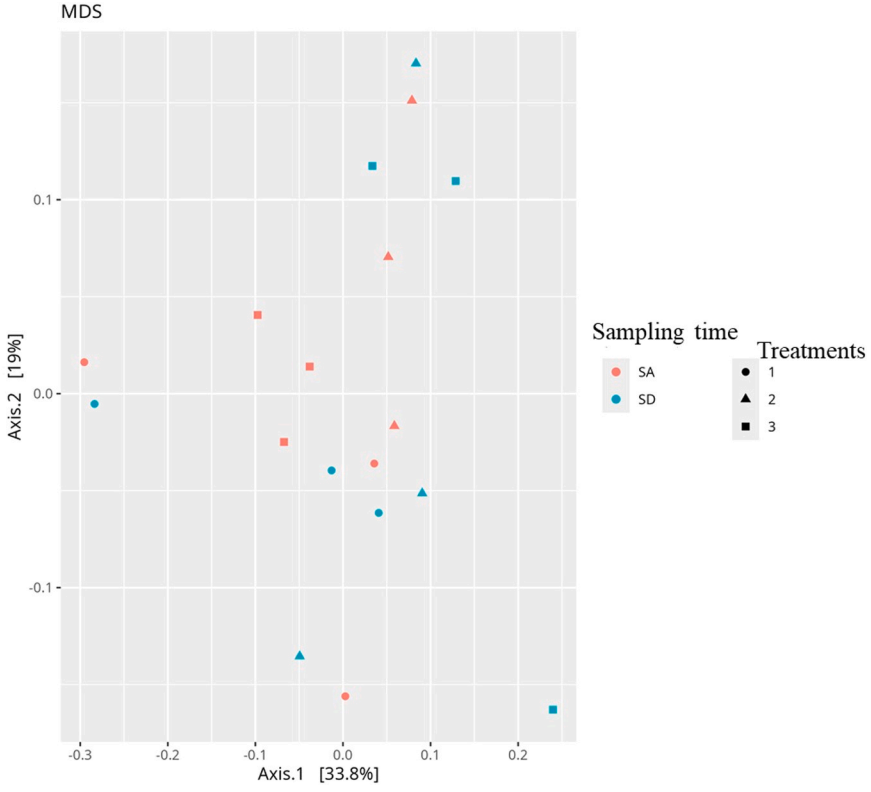

Fig. 7. Beta diversity plot representing the blood microbial community variation among the treatments. Note: SA = blood samples collected before the challenge. SD = blood samples collected after the challenge.

experimental treatments.

4.2. *Nile tilapia microbiota composition*

Bacterial communities in aquaponics vary across different compartments, and the injection of pathogens can impact the microbiota communities in fish (Prastowo et al., 2024; Luan et al., 2023). In this study, we evaluated the microbiota composition in faecal and blood of Nile tilapia before and after being challenged with *E. coli* (ATCC 8739) and *V. cholerae* (ATCC 9458). The findings revealed that faecal samples are richer in microbial community than blood samples, although they share some bacteria communities. This may be related to the connectivity between the fish gut and the environment. The ingestion of water and feed may have allowed the migration of microorganisms from water to

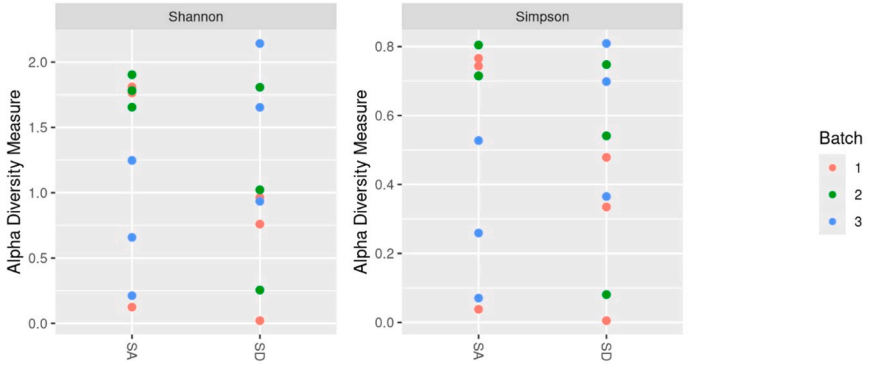

Fig. 8. Alpha Diversity of blood sample microbiota composition across treatments. Note: Batch = treatments. SA = blood samples collected before the challenge. SD = blood samples collected after the challenge.

the gut, thus increasing its composition. The microbial composition of the fish gut depends on diet and environmental factors. It plays an essential role in the host's metabolism and immune modulation Luan et al. (2023). Some studies have observed that a greater proportion of the OTUs detected in fish intestines were shared with the aquatic environment, indicating that many gut microorganisms originated from the external environment (Yang et al., 2022; Kim et al., 2021). observed that the microbial community in fish is strongly shaped by the host habitat; they found that freshwater and marine fish had significantly different microbial communities. Freshwater fish have a high level of connectivity with their habitat, which allows microorganisms from the environment to interact with the fish's intestine, and this increases the diversity of microorganisms, particularly in subtropical regions, such as southern Brazil (Kim et al., 2021; Kanika et al., 2024). A study conducted with *Lutjanus campechanus* indicated that microbiota diversity in faecal samples is more abundant than in blood samples Tarnecki et al. (2016).

Blood may have prevented the multiplication of some bacterial communities. The study carried out with Rainbow Trout plasma revealed that the plasma has high antimicrobial activity and contains specific proteins with different biological activities, which include antibacterial properties, such as immunoglobulins and lectins Mizaeva et al. (2023). Reinforce the idea that the blood of healthy animals is a sterile environment by observing that blood cultures were positive for bacterial growth in a few elasmobranchs.

The transfer of microorganisms from the intestine to the blood may explain the presence of shared microorganisms between the faecal and blood samples. According to (Fronton et al., 2025; Khan et al., 2022), bacteria can move to the blood from niches closely associated with the blood, such as the intestine, oral mucosa, and skin.

The findings of this study also revealed significant differences in microbial composition among the treatments and between the period before and after the challenge with pathogenic bacteria, indicating that the challenge impacted the microbiota composition in both samples. In faecal samples, before the challenge, the fish in treatment 1 had the highest microbiota composition, while those in treatment 3 had the lowest. After challenging, the Nile tilapia in treatment 2 challenged with *E. coli* increased their microbiota composition, while those in treatment 3 challenged with *V. cholerae* reduced. In blood samples before the challenge, the fish in treatment 2 showed high microbiota composition, while those in treatment 1 had the lowest microbiota composition. After the challenge, the fish in treatment 2 indicated a reduction in microbiota composition, while those in treatment 3 showed a significant increase in microbiota composition. These findings suggest that the environment in faecal samples before the challenge provided better conditions for the growth of various bacterial communities in treatment 3, while the environment after the challenge restricted the growth of many bacterial communities in the same treatment and was favorable for treatment 2. However, in blood samples, although the environment before the challenge favored the growth of some microbial communities in treatment 2, after the challenge, the environment was favorable only in treatment 3. According to Banerjee and Ray (2017), the microbiota composition may vary based on the environment and the conditions of the host. Different microbiota communities can establish themselves in the gut of the fish and, over time, become more resilient to changes in the health status of the fish (Deng et al., 2021; Eck et al., 2021). In tilapia, microbiota composition is strongly influenced by the health of the fish, and its immunity is impacted by co-infection (Deng et al., 2021; Cai et al., 2023; Kotob et al., 2016; Ofek et al., 2022).

Numerous studies (Ruiz et al., 2023; Schmautz et al., 2017), have been conducted on the microbiota of various compartments within aquaponic systems. Some bacterial phyla present in these compartments were also detected in the gut and blood of tilapia used in this study, suggesting that the fish microbiota may originate from these compartments through water intake.

In faecal samples, Nile tilapia challenged with *E. coli* in treatment 2 increased the microbiota composition, while in blood samples, the fish in the same treatment showed a reduction in microbiota composition. Treatment 3 challenged with *V. cholerae* showed a significant increase in microbiota composition in blood samples; however, in faecal samples, the fish in the same treatment experienced a decrease in microbiota composition. The injection of *E. coli* into the intestine may have created inflammation that favored the development of new microbial communities. In pigs, intravenous injection of *E. coli* activated a strong pro-inflammatory response, characterized by a significant increase in the expression of tumor necrosis factor alpha (TNF-α) soon after injection, which helped to control the multiplication of pathogenic microorganisms Spyropoulos et al. (2020). The presence of inflammatory bacteria in the intestine can increase intestinal permeability, causing disorders that trigger an increase in the intestinal microbiota Kim et al. (2020). However, the injection of *V. cholerae* led to the reduction of the microbial community in the feacal samples. *Vibrio spp* injected in fish (*Cynoglossus semilaevis* and *Danio rerio*) and shrimp (*Litopenaeus vannamei*) significantly altered the intestinal microbiota, leading to the reduction or even elimination of microbial community by overcompeting the normal flora of the intestine (Breen et al., 2021; Hao et al., 2023; Liao et al., 2022).

Blood is considered a sterile environment by some studies, such as that of Mylniczenko et al. (2007). The injection of microorganisms may have activated the defense, allowing the reduction of bacterial communities, but also, it may have created conditions for the entry of microorganisms from the intestine. Previous studies have shown that, depending on the type of sample, injection of Vibrio can increase the microbiota diversity. *Dicentrarchus labrax* infected with *Vibrio harveyi* have been shown to have a higher Shannon index compared with the healthy fish Cámara-ruiz et al. (2021). Factors such as the aquatic environment, the presence of other microorganisms, and the health status of the fish shape the relationship between *E. coli* and microbial diversity in aquatic environments. Environments rich in *E. coli* allow the

formation of pathogenic bacterial biofilms, and the co-presence of *E. coli* with pathogenic *Bacillus spp*. such as *B. cereus,* creates conditions for the formation of pathogenic biofilms. However, sanitized environments reduce biofilm formation Kuan et al., (2025).

The dominance of the *Cetobacterium* genus observed in the faecal samples across all treatments, mainly after the challenge, may be due to the fact that tilapia is a freshwater fish, in which this genus is often found at high concentrations. Alternatively, its presence may also be linked to the fish's self-defense against pathogenic species. *Cetobacterium* genus is a fish-dominant commensal bacterium present in the fish intestine (Ofek et al., 2022; Dou et al., 2023; Eck et al., 2019; Larsen et al., 2014; Zhang et al., 2022). Its dominance is associated with a faecal origin in cultured tilapia, and its presence in the fish intestine is not influenced by the age of the fish (Deng et al., 2021; Sanchez et al., 2019; Schmautz et al., 2017). As the fish grows, the proliferation of the genus Cetobacterium increases in the intestine (Cai et al., 2023; Ramírez et al., 2018). The genus Cetobacterium reduces the composition of pathogenic bacteria and viruses in the intestines of tilapia, but supports beneficial genera, thus improving the health of the tilapia intestine and its resistance against pathogenic bacteria (Zhang et al., 2022; Xie et al., 2022; Zhao et al., 2023; Zhou et al., 2022).

In this study, blood samples were dominated by *f-o-c-p-d-bacteria*, an unclassified bacterium whose ASVs were only classified at the kingdom level. This may be due to the lack of precise sequence correspondence in the SILVA database used for taxonomic assignment of the DNA sequence reads, or the sequences are probably new or have not been previously detected in the blood of Nile tilapia.

4.3. Nile tilapia microbiota and food safety

In this study, several bacterial genera detected in faecal and blood samples include species known to cause foodborne diseases in humans. Although 16S rRNA analysis did not allow for species-level identification, the identified genera (e.g., *Aeromonas, Streptococcus, Klebsiella, Bacillus, Clostridium, Edwardsiella, Mycobacterium, Staphylococcus, Plesiomonas,* and *Citrobacter*) comprise well-known human pathogens associated with contaminated food consumption. Pathogenic species such as *Aeromonas veronii*, *Aeromonas hydrophila* (D'Sa and Harrison, 2010; Bhowmick and Bhattacharjee, 2018; Janda and Abbot, 1999), *Bacillus cereus* (Hou et al., 2013; Tewari and Abdullah, 2015; Todd, 2014), *Clostridium perfringens* Todd (2014), *Streptococcus agalactiae*, *S. iniae* (D'Sa and Harrison, 2010; Baiano and Barnes, 2009), *Klebsiella oxytoca*, *K. aerogenes*, *K. pneumoniae* (Yang et al., 2022; Cortés-Sánchez et al., 2025; Elsafi et al., 2024; Ni et al., 2021), *Plesiomonas shigelloides* (D'Sa and Harrison, 2010; Janda and Abbot, 1999), *Edwardsiella tarda* (Janda and Abbot, 1999; Hirai et al., 2015), *Mycobacterium avium* (D'Sa and Harrison, 2010, *Agrobacterium radiobacter* (Paphitou and Rolston, 2003), *Staphylococcus aureus* Todd (2014), and *Citrobacter freundii* (Cortés-Sánchez et al., 2025; Jabeen et al., 2023) have been associated with illness and mortality in humans. These pathogens have previously been isolated from tilapia (Fadel et al., 2025) and are listed as human pathogens in the CZ ID Pathogen List 2024 (https://czid.org/pathogen_list), the WHO Priority List 2024 (https://www.who.int/publications/i/item/9789240093461), and the NCBI Pathogen Detection Organisms group (https://www.ncbi.nlm.nih.gov/pathogens/organisms/). They are associated with a wide range of clinical conditions, including gastroenteritis, septicemia, diarrhea, food poisoning, urinary tract infections, meningitis, and respiratory infections (D'Sa and Harrison, 2010; Bhowmick and Bhattacharjee, 2018; Janda and Abbot, 1999; Hou et al., 2013; Tewari and Abdullah, 2015; Todd, 2014; Baiano and Barnes, 2009; Cortés-Sánchez et al., 2025; Elsafi et al., 2024; Ni et al., 2021; Hirai et al., 2015; Paphitou and Rolston, 2003; Jabeen et al., 2023). Notably, several of these bacteria have been isolated from both fish and vegetables, including lettuce (Yang et al., 2022; Elsafi et al., 2024; Ni et al., 2021; Fadel et al., 2025). Therefore, the cultivation of Nile tilapia in aquaponic systems with lettuce may pose a public health risk, as pathogenic bacteria can persist in water, circulate within the system, and contaminate or internalize in vegetables (Elumalai et al., 2017; Hou et al., 2013; Sobiczewski and Iakimova, 2022). In aquaponics, water acts as a key transmission route, facilitating cross-contamination and the accumulation of biological hazards (Sobiczewski and Iakimova, 2022; Dong and Feng, 2022). Consequently, fresh produce grown in aquaponic systems may present food safety risks due to contamination originating from fish (Nissen et al., 2021; Wang et al., 2021, 2020).

The presence of potentially pathogenic bacteria for humans in aquaponics systems has implications for the health of the operators of these systems, because they may be contaminated through direct contact with the system water, fish, biofilters, and plant roots. Individuals with skin wounds, compromised immune systems, or who do not use adequate protective equipment are more prone to opportunistic infections. Thus, the presence of these microorganisms reinforces the importance of adopting good biosecurity practices, including the use of gloves, hand hygiene, proper cleaning of equipment, and regular monitoring of the microbiological quality of the system. Due to these risks for human infection, methods such as UV light and ozone have been tested to control pathogens in aquaponic systems. However, these methods have proved not to be effective due to the existence of some resistant bacteria species and the possibility of these treatments harming beneficial microorganisms through DNA damage (Eck et al., 2019). To overcome this problem, the use of probiotics may be the best solution because they do not pose a risk to the health of cultured fish and planted vegetables or to humans (Prastowo et al., 2024; Sirakov et al., 2016; Rivas-García et al., 2020).

5. Conclusion

This study assessed the diversity of microbiota in Nile tilapia cultured with lettuce in aquaponic systems, with a focus on food safety implications. The findings demonstrated that exposing Nile tilapia to *E. coli* and *Vibrio* altered the diversity and abundance of microbiota in both the faecal and blood samples. In faecal samples, injection of *E. coli* increased the microbiota composition, while the injection of *V. cholerae* reduced the microbiota composition. In blood samples, *E. coli* reduced the microbiota composition, while *V. cholerae* increased the microbiota composition. These findings revealed a dysbiosis of microbiota structure impacted by the challenge. Analysis of microbiota composition in faecal and blood samples revealed the presence of microorganisms such as Streptococcus, Klebsiella, Clostridium, Brucella, Edwardsiella, Staphylococcus, Mycobacterium, Legionella, and Plesiomonas, which are linked to illness such as vomiting, fever, diarrhea, meningitis, abdominal cramps, urinary tract infections and septicemia resulting from the consumption of raw and undercooked fish or vegetables. This highlights the need for appropriate sanitary measures to prevent their transmission to vegetables grown in aquaponics, such as the use of probiotics to control human pathogens in the systems before the introduction of lettuce seedlings. We recommend further study for identifying microorganisms at the species level in fish, especially those with pathogenic potential for humans, and associated with the consumption of contaminated food. In addition, further studies are also recommended to evaluate whether human pathogenic bacteria present in fish can contaminate water and vegetable roots and subsequently be internalized into the edible parts of the plants.

CRediT authorship contribution statement

Sebastian Wilson Chenyambuga: Writing – review & editing, Supervision, Methodology. **Jossefa Angelica Adiacao:** Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Cerozi Brunno Da Silva:** Writing – review & editing, Supervision. **Viagem Leonildo Dos Anjo:** Writing – review & editing, Validation, Methodology, Funding

acquisition, Formal analysis, Data curation, Conceptualization.

Ethical statements

This study was approved by the Ethics Committee on Animal Use of Luiz de Queiroz, College of Agriculture-ESALQ (CEUA/ESALQ) with protocol number CEUA 1853260225 and ID 000612.

Funding

This work was funded by the Partnership for Applied Skills in Sciences, Engineering and Technology-Regional Scholarship and Innovation Fund (PASET-Rsif) and Carnegie Corporation of New York, awarded to AAJ (B850JN10016) and LAV (B850IG30223) for Ph.D. studies at the SACIDS Africa Centre of Excellence for Infectious Diseases, the SACIDS Foundation for One Health, Sokoine University of Agriculture, Morogoro, Tanzania.

Declaration of Competing Interest

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

Acknowledgments

The authors thank the Partnership for Skills in Applied Sciences, Engineering and Technology (PASET) through the Regional Scholarship and Innovation Fund (Rsif) for funding this study as part of the PhD program. Our acknowledgment also goes to the invaluable financial contribution from Mawazo Institute and its partners in supporting this research, which has been instrumental in enabling us to accomplish the study. Our sincere gratitude is extended to Mr. Jorge Luis Ferreira de Andrade from the Animal Biotechnology Laboratory at the University of São Paulo for designing the layout of the aquaponic systems. We are also grateful to technicians Ismael Baldessin Junior and Sérgio Vanderlei Pena from the aquaculture center of the Department of Animal Science at the University of São Paulo for assembling the aquaponics systems.

Data availability

Data will be made available on request.

References

Abd El-Hack, M.E., El-Saadony, M.T., Nader, M.M., Salem, H.M., El-Tahan, A.M., Soliman, S.M., Khafaga, A.F., 2022. Effect of environmental factors on growth performance of Nile tilapia (*Oreochromis niloticus*). Int. J. Biometeorol. 66, 2183–2194. https://doi.org/10.1007/s00484-022-02347-6.

Ali, E.A.E., Abd Al-azeem, M.W., Younis, W., 2023. Microbiological and molecular characterization of *Vibrio cholera* and *Vibrio parahaemolyticus* isolated from Tilapia fish (*Oreochromis niloticus*). SVUInt. J. Vet. Sci. 6, 1–16. https://doi.org/10.21608/svu.2023.203762.1266.

Atique, F. The Effect of Plants on Microbes, Water Quality, and Fish Performance in an Aquaponic System. dissertation. University of Jyväskylä, 2023.

Baiano, J.C.F., Barnes, A.C., 2009. Towards control of *Streptococcus iniae*. Emerg. Infect. Dis. 15, 1891–1896. https://doi.org/10.3201/eid1512.090232.

Banerjee, G., Ray, A.K., 2017. Bacterial symbiosis in the fish gut and its role in health and metabolism. Symbiosis 72, 1–11. https://doi.org/10.1007/s13199-016-0441-8.

Bereded, N.K., Beneberu, G., Dar, A.B., Workneh, S., Bahir, F., Curto, M., Meimberg, H., Domig, K.J., Abebe, G.B., Fanta, S.W., Curto, M., Waidbacher, H., Meimberg, H., Domig, K.J., 2022. The gut bacterial microbiome of Nile tilapia (*Oreochromis niloticus*) from lakes across an altitudinal gradient. BMC Microbiol. 22, 1–14. https://doi.org/10.1186/s12866-022-02496-z.

Bereded, N.K., Curto, M., Domig, K.J., Abebe, G.B., Fanta, S.W., Waidbacher, H., Meimberg, H., 2020. Metabarcoding analyses of gut microbiota of Nile tilapia (*Oreochromis niloticus*) from Lake Awassa and Lake Chamo, Ethiopia. Microorganisms 8, 1–19. https://doi.org/10.3390/microorganisms8071040.

Bhowmick, U.D., Bhattacharjee, S., 2018. Bacteriological, clinical, and virulence aspects of aeromonas-associated diseases in humans. Pol. J. Microbiol. 67, 137–149. https://doi.org/10.21307/pjm-2018-020.

Breen, P., Winters, A.D., Theis, K.R., Withey, J.H., 2021. *Vibrio cholerae* infection induces strain-specific modulation of the zebrafish intestinal microbiome. Infect. Immun. 89, 1–19. https://doi.org/10.1128/IAI.00157-21.

Bruno, A., Sandionigi, A., Panio, A., Rimoldi, S., Orizio, F., Agostinetto, G., Hasan, I., Gasco, L., Terova, G., Labra, M., 2023. Aquaculture ecosystem microbiome at the water-fish interface: the case-study of rainbow trout fed with *Tenebrio molitor* novel diets. BMC Microbiol 23, 1–17. https://doi.org/10.1186/s12866-023-02990-y.

Cai, M., Deng, H., Sun, H., Si, W., Li, X., Hu, J., Huang, M., Fan, W., 2023. Changes of intestinal microbiota in the giant salamander (*Andrias davidianus*) during growth based on high-throughput sequencing. Front Microbiol 14, 1–13. https://doi.org/10.3389/fmicb.2023.1052824.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016b. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Callahan, B.J., Sankaran, K., Fukuyama, J.A., McMurdie, P.J., Holmes, S.P., 2016a. Bioconductor workflow for microbiome data analysis: From raw reads to community analyses. F1000Research 5, 1–48. https://doi.org/10.12688/F1000RESEARCH.8986.1.

Cámara-ruiz, M., Cerezo, I.M., Guardiola, F.A., García-beltrán, J.M., Balebona, M.C., Moriñigo, M.Á., Esteban, M.Á., 2021. Alteration of the immune response and the microbiota of the skin during a natural infection by *Vibrio harveyi* in European seabass (*Dicentrarchus labrax*). Microorganisms 9, 1–18. https://doi.org/10.3390/microorganisms9050964.

Chen, C.C., Chen, Y.P., Yang, H.T., Chen, Y.L., Wu, C.W., Gong, H.Y., Ho, Y.S., Ho, Y.N., 2025. Temperature-dependent shifts in gut microbiota and metabolome of olive flounder (*Paralichthys olivaceus*): implications for cold-water aquaculture expansion and probiotic applications. Anim. Micro 7, 1–19. https://doi.org/10.1186/s42523-025-00417-9.

Cortés-Sánchez, A.D.J., Diaz-Ramírez, M., Rayas-Amor, A.A., Espinosa-Chaurand, L.D., Torres-Ochoa, E., Salgado-Cruz, M.D. la, 2025. P. Microbiological Hazards in the Food Chain of Fish and Products: A Focus on Klebsiella spp. Vet. Sci. 12, 1–18. https://doi.org/10.3390/vetsci12020133.

D'Sa, E.M. Harrison, M.A. Other Bacterial Pathogens: *Aeromonas, Arcobacter,Helicobacter, Mycobacterium, Plesiomonas,* and *Streptococcus* In: Pathogens and Toxins in Foods: Challenges and Interventions. Editor(s) Vijay K. Juneja, John N. Sofos. Washington, DC, 2010. pp. 181–194. https://doi.org/10.1128/9781555815936.ch12.

Day, J.A., Diener, C., Otwell, A.E., Tams, K.E., Bebout, B., Detweiler, A.M., Lee, M.D., Scott, M.T., Ta, W., Ha, M., Carreon, S.A., Tong, K., Ali, A.A., Gibbons, S.M., Baliga, N.S., 2021. Lettuce (*Lactuca sativa*) productivity influenced by microbial inocula under nitrogen-limited conditions in aquaponics. PLoS One 16, 1–14. https://doi.org/10.1371/journal.pone.0247534.

Deng, Y., Kokou, F., Eding, E.H., Verdegem, M.C.J., 2021. Impact of early-life rearing history on gut microbiome succession and performance of Nile tilapia. Anim. Micro 3, 1–19. https://doi.org/10.1186/s42523-021-00145-w.

Diwan, A.D., Harke, S.N., Panche, A.N., 2024. Studies on exploring the potentials of gut microbiomes to mitigate the bacterial and viral diseases of fish and shellfish in aquaculture farming. Microbe 2, 1–15. https://doi.org/10.1016/j.microb.2023.100031.

Dong, M., Feng, H., 2022. Microbial community analysis and food safety practices survey-based hazard identification and risk assessment for controlled environment for hydroponic/aquaponic farming systems. Front. Microbiol. 13, 1–15. https://doi.org/10.3389/fmicb.2022.879260.

Dou, Y., Wen, M., Shen, H., Zhang, S., Jiang, G., Qiao, Y., Cheng, J., Cao, X., Wan, X., Sun, X., 2023. Intestinal microbiota differences in *Litopenaeus vannamei* shrimp between greenhouse and aquaponic rearing. Life 13, 1–13. https://doi.org/10.3390/life13020525.

Eck, M., Sare, A.R., Massart, S., Schmautz, Z., Junge, R., Smits, T.H.M., Jijakli, M.H., 2019. Exploring bacterial communities in aquaponic systems. Water 11, 1–16. https://doi.org/10.3390/w11020260.

Eck, M., Szekely, I., Massart, S., Jijakli, M.H., 2021. Ecological study of aquaponics bacterial microbiota over the course of a lettuce growth cycle. Water 13, 1–21. https://doi.org/10.3390/w13152089.

Elewasy, O.A., Elrafie, A.S., Rasheed, N.A., Adli, S.H., Younis, E.M., Abdelwarith, A.A., Davies, S.J., Ibrahim, R.E., 2024. The alleviative effect of *Bacillus subtilis*-supplemented diet against *Vibrio cholerae* infection in Nile tilapia (*Oreochromis niloticus*). Vet. Res Commun. 48, 2513–2525. https://doi.org/10.1007/s11259-024-10418-9.

Elgendy, M.Y., Abdelsalam, M., Kenawy, A.M., Ali, S.E., 2022. Vibriosis outbreaks in farmed Nile tilapia (*Oreochromis niloticus*) caused by *Vibrio mimicus* and *V. cholerae*. Aquac. Int. 30, 2661–2677. https://doi.org/10.1007/s10499-022-00921-8.

Elsafi, S.H., Al, Z.E.M., Zaid, R.F., Alshagifi, S.A., Farghal, T.A., Alshamuse, K.B., Albalawi, A.S., Alkhalaf, F., Sumaily, A.A., Almusabi, S., George, S.K., 2024. Antibiotic-resistant bacteria contaminating leafy vegetables in Saudi Arabia's eastern region. BMC Microbiol 24, 1–8. https://doi.org/10.1186/s12866-024-03456-5.

El-Sayed, A.F.M., Fitzsimmons, K., 2023. From Africa to the world. Rev. Aquac. 15, 6–21. https://doi.org/10.1111/raq.12738.

Elumalai, S.D., Shaw, A.M., Pattillo, D.A., Currey, C.J., Rosentrater, K.A., Xie, K., 2017. Influence of UV Treatment on the Food Safety Status of a Model Aquaponic System. Water 9, 1–9. https://doi.org/10.3390/w9010027.

Estim, A., Saufie, S., Mustafa, S., 2019. Water quality remediation using aquaponics subsystems as biological and mechanical filters in aquaculture. J. Water Process Eng. 30, 1–10. https://doi.org/10.1016/j.jwpe.2018.02.001.

Fadel, A., Mahmound, M.A., Abdelsalam, M., Eissa, El-S.H., Sherif, A.H., 2025. *Aeromonas veronii* infection in cultured *Oreochromis niloticus*: prevalence, molecular and histopathological characterization correlated to water physicochemical

characteristics, with the protective autochthonous probiotic. Aquac. Int. 33, 1–29. https://doi.org/10.1007/s10499-025-01960-7.
FAO. The State of World Fisheries and Aquaculture 2024. Blue Transformation. Roma, 2024. https://doi.org/10.4060/cd0683en.
Fronton, F., Gandin, A., Deslauriers, D., Small, D., Robert, D., St-pierre, Y., 2025. Expanding the use of circulating microbiome in fish: contrast between the gut and blood microbiome of *Sebastes fasciatus*. Communications 5, 1–15. https://doi.org/10.1093/ismeco/ycaf116.
Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J., Hornik, K., Hothorn, T., Huber, W., Iacus, S., Irizarry, R., Leisch, F., Li, C., Maechler, M., Rossini, A.J., Zhang, J., 2004. Open Access Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 5, 1–16. ⟨http://genomebiology.com/2004/5/10/http://genomebiology.com/2004/5/10/R80⟩.
Giatsis, C., Sipkema, D., Smidt, H., Heilig, H., Benvenuti, G., Verreth, J., Verdegem, M., 2015. The impact of rearing environment on the development of gut microbiota in tilapia larvae. Sci. Rep. 11, 1–15. https://doi.org/10.1038/srep18206.
Glöckner, F.O., Yilmaz, P., Quast, C., Gerken, J., Beccati, A., Ciuprina, A., Bruns, G., Yarza, P., Peplies, J., Westram, R., Ludwig, W., 2017. 25 years of serving the community with ribosomal RNA gene reference databases and tools. J. Biotechnol. 261, 169–176. https://doi.org/10.1016/j.jbiotec.2017.06.1198.
Grammer, G.L., Slack, W.T., Peterson, M.S., Dugo, M.A., 2012. Nile tilapia Oreochromis niloticus (Linnaeus, 1758) establishment in temperate Mississippi, 7. Multi-year survival confirmed by otolith ages, Aquat Invasions, USA, pp. 367–376. https://doi.org/10.3391/ai.2012.7.3.008.
Hamom, A., Alam, M.M.M., Mahbub, I.M., Ibrahim, K.S.M., Parven, M., Sumon, T.A., Al Mamun, M.A., 2020. Identification of pathogenic bacteria from diseased Nile tilapia *Oreochromis niloticus* with their Sensitivity to antibiotics. Int J. Curr. Microbiol Appl. Sci. 9, 1716–1738. https://doi.org/10.20546/ijcmas.2020.903.200.
Han, Y., Li, X., Li, X., Wang, S., Zhang, M., Li, M., 2025. Effects of acute and chronic ammonia exposure on survival, growth, and intestinal microbiota composition of hybrid carp. Aquac. Rep. 42, 1–14. https://doi.org/10.1016/j.aqrep.2025.102834.
Hao, Y., Zhao, Y., Zhang, Y., Liu, Y., Wang, G., He, Z., Cao, W., Han, T., Zhang, X., Zhang, Z., Wang, Y., Gong, C., Hou, J., 2023. Population response of intestinal microbiota to acute *Vibrio alginolyticus* infection in half-smooth tongue sole (*Cynoglossus semilaevis*). Front Microbiol 14, 1–14. https://doi.org/10.3389/fmicb.2023.1178575.
Hirai, Y., Asahata-Tago, S., Ainoda, Y., Fujita, T., Kikuchi, K., 2015. *Edwardsiella tarda* bacteremia. A rare but fatal water- and foodborne infection: review of the literature and clinical cases from a single centre. Can. J. Infect. Dis. Med. Microbiol 26, 313–318. https://doi.org/10.1155/2015/702615.
Hollyer, J., Tamaru, C., Riggs, A., Klinger-Bowen, R., Howerton, R., Okimoto, D., Castro, L., Troegner, V., Martinez, G., 2009. On-Farm food safety: aquaponics. Food Saf. Technol. 38, 1–8.
Hou, Z., Fink, R.C., Radtke, C., Sadowsky, M.J., Diez-Gonzalez, F., 2013. Incidence of naturally internalized bacteria in lettuce leaves. Int J. Food Microbiol. 162, 260–265. https://doi.org/10.1016/j.ijfoodmicro.2013.01.027.
Hounmanou, Gildas, Mdegela, Y.M., Dougnon, R.H., Madsen, T.V., Withey, H., Olsen, J. H., Dalsgaard, J.E., 2019. Tilapia (*Oreochromis niloticus*) as a putative reservoir host for survival and transmission of *Vibrio cholerae* O1 Biotype El Tor in the aquatic environment. Front Microbiol. 10, 1–14. https://doi.org/10.3389/fmicb.2019.01215.
Huber, W., Carey, V.J., Gentleman, R., Anders, S., Carlson, M., Carvalho, B.S., Bravo, H. C., Davis, S., Gatto, L., Girke, T., Gottardo, R., Hahne, F., Hansen, K.D., Irizarry, R.A., Lawrence, M., Love, M.I., MaCdonald, J., Obenchain, V., Oleś, A.K., Morgan, M., 2015. Orchestrating high-throughput genomic analysis with Bioconductor. Nat. Methods 12, 115–121. https://doi.org/10.1038/nmeth.3252.
Hussain, A.S., Brown, P.B., 2024. A Literature Review of Tilapia/Lettuce Aquaponics: Production Status, Varieties, and Research Gaps. Aquac. Res 2024, 1–16. https://doi.org/10.1155/2024/2642434.
Jabeen, I., Islam, S., Hassan, A.K.M.I., Tasnim, Z., Shuvo, S.R., 2023. A brief insight into Citrobacter species - a growing threat to public health. Front Antibiot. 2, 1–13. https://doi.org/10.3389/frabi.2023.1276982.
Janda, J.M., Abbot, S.L., 1999. Unusual food-borne. Pathog. List. monocytogenes Aeromonas Plesiomonas Edw. Species FoodBorne Dis. 19, 553–558.
Kanika, N.H., Liaqat, N., Chen, H., Ke, J., Lu, G., Wang, J., Wang, C., 2024. Fish gut microbiome and its application in aquaculture and biological conservation. Front Microbiol 15, 1–3. https://doi.org/10.3389/fmicb.2024.1521048.
Kelly, P., 2010. Gut function: Effects on over- and undernutrition Nutrition, intestinal defence and the microbiome. Proc. Nutr. Soc. 69, 261–268. https://doi.org/10.1017/S0029665110000108.
Khan, I., Khan, I., Jianye, Z., Xiaohua, Z., Khan, M., Hilal, M.G., Kakakhel, M.A., Mehmood, A., Lizhe, A., Zhiqiang, L., 2022. Exploring blood microbial communities and their influence on human cardiovascular disease. J. Clin. Lab Anal. 36, 1–11. https://doi.org/10.1002/jcla.24354.
Kim, J.K., Lee, K.E., Lee, S.A., Jang, H.M., Kim, D.H., 2020. Interplay Between Human Gut Bacteria *Escherichia coli* and *Lactobacillus mucosae* in the Occurrence of Neuropsychiatric Disorders in Mice. Front Immunol. 11, 1–13. https://doi.org/10.3389/fimmu.2020.00273.
Kim, P.S., Shin, N.R., Lee, J.B., Kim, M.S., Whon, T.W., Hyun, D.W., Yun, J.H., Jung, M. J., Kim, J.Y., Bae, J.W., 2021. Host habitat is the major determinant of the gut microbiome of fish. Microbiome 9, 1–16. https://doi.org/10.1186/s40168-021-01113-x.
Kotob, M.H., Menanteau-Ledouble, S., Kumar, G., Abdelzaher, M., El-Matbouli, M., 2016. The impact of co-infections on fish: a review. Vet. Res 47, 1–12. https://doi.org/10.1186/s13567-016-0383-4.
Krastanova, M., Sirakov, I., Ivanova-Kirilova, S., Yarkov, D., Orozova, P., 2022. Aquaponic systems: biological and technological parameters. Biotechnol. Biotechnol. Equip. 36, 305–316. https://doi.org/10.1080/13102818.2022.2074892.
Kuan, S., Chin, N.L., Tee, T.P., Hasnan, N.Z.N., 2025. Microbiome Diversity in Seafood Factories via Next-Generation Sequencing for Food Safety Management System (FSMS) Certifications in Malaysia. Foods 14, 1–23. https://doi.org/10.3390/foods14091517.
Larsen, A.M., Mohammed, H.H., Arias, C.R., 2014. Characterization of the gut microbiota of three commercially valuable warmwater fish species. J. Appl. Microbiol 116, 1396–1404. https://doi.org/10.1111/jam.12475.
Liao, G., Wu, Q., Mo, B., Zhou, J., Li, J., Zou, J., Fan, L., 2022. Intestinal morphology and microflora to *Vibrio alginolyticus* in Pacific white shrimp (*Litopenaeus vannamei*). Fish. Shellfish Immunol. 121, 437–445. https://doi.org/10.1016/j.fsi.2022.01.026.
Love, D.C., Fry, J.P., Genello, L., Hill, E.S., Frederick, J.A., Li, X., Semmens, K., 2014. An international survey of aquaponics practitioners. Plos ONE 9, 1–10. https://doi.org/10.1371/journal.pone.0102662.
Luan, Y., Li, M., Zhou, W., Yao, Y., Yang, Y., Zhang, Z., Ringø, E., Erik, O.R., Liu, C.J., Xie, S., Mai, K., Ran, C., Zhou, Z., 2023. The fish microbiota: research progress and potential applications. Engineering 29, 137–146. https://doi.org/10.1016/j.eng.2022.12.011.
McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8, 1–11. https://doi.org/10.1371/journal.pone.0061217.
Merrifield, D.L., Rodiles, A., 2015. The fish microbiome and its interactions with mucosal tissues. Mucosal Health Aquac. E. Peatman San. Diego 273–296.
Mizaeva, T., Alieva, K., Zulkarneev, E., Kurpe, S., Isakova, K., Matrosova, S., Borvinskaya, E., Sukhovskaya, I., 2023. Antibacterial activity of rainbow trout plasma: in vitro assays and proteomic analysis. Animals 13, 1–21. https://doi.org/10.3390/ani13223565.
Mylniczenko, N.D., Harris, B., Wilborn, R.E., Young, F.A., 2007. Blood culture results from healthy captive and free-ranging elasmobranchs. J. Aquat. Anim. Health 19, 159–167. https://doi.org/10.1577/H06-039.1.
Ni, L., Xu, Y., Chen, L., 2021. First experimental evidence for the presence of potentially virulent *klebsiella oxytoca* in 14 species of commonly consumed aquatic animals, and phenotyping and genotyping of *k. Oxytoca* isolates. Antibiotics 10, 1–15. https://doi.org/10.3390/antibiotics10101235.
Nissen, L., Casciano, F., Gianotti, A., 2021. Plant volatiles of lettuce and chicory cultivated in aquaponics are associated to their microbial community. Microorganisms 9, 1–14. https://doi.org/10.3390/microorganisms9030580.
Novotny, L., Dvorska, L., Lorencova, A., Beran, V., Pavlik, I., 2004. Fish: a potential source of bacterial pathogens for human beings. Vet. Med 49, 343–358. https://doi.org/10.17221/5715-VETMED.
Ofek, T., Lalzar, M., Izhaki, I., Halpern, M., 2022. Intestine and spleen microbiota composition in healthy and diseased tilapia. Anim. Micro 4, 1–10. https://doi.org/10.1186/s42523-022-00201-z.
Ortiz, W.E., Carlos-Shanley, C., Huertas, M., 2023. Impact of sublethal concentrations of nitrite on goldfish (*Carassius auratus*) microbiomes. Micro Ecol. 86, 1331–1342. https://doi.org/10.1007/s00248-022-02123-4.
Paphitou, N.I., Rolston, K.V.I., 2003. Catheter-related bacteremia caused by agrobacterium radiobacter in a cancer patient: case report and literature review. Infection 31, 421–424. https://doi.org/10.1007/s15010-003-3175-5.
Prastowo, B.W., Lestari, I.P., Agustini, N.W.S., Priadi, D., Haryati, Y., Jufri, A., Deswina, P., Adi, E.B.M., Zulkarnaen, I., 2024. Bacterial communities in aquaponic systems: insights from red onion hydroponics and koi biological filters. Case Study Chem. Environ. Eng. 10, 1–13. https://doi.org/10.1016/j.cscee.2024.100968.
Rakocy, J.E., Masser, M.P., Losordo, T.M., 2006. Recirculating aquaculture tank production systems: aquaponics-integrating fish and plant culture. South. Reg. Aquac. Cent. 454 (2006), 1–16.
Ramírez, C., Coronado, J., Silva, A., Romero, J., 2018. Cetobacterium is a major component of the microbiome of giant amazonian fish (*Arapaima gigas*) in Ecuador. Animals 8, 1–6. https://doi.org/10.3390/ani8110189.
Ringø, E., Olsen, R.E., Mayhew, T.M., Myklebust, R., 2003. Electron microscopy of the intestinal microflora of fish. Aquaculture 227, 395–415. https://doi.org/10.1016/j.aquaculture.2003.05.001.
Rivas-García, T., González-Estrada, R.R., Chiquito-Contreras, R.G., Reyes-Pérez, J.J., González-Sales, U., Hernández-Montiel, L.G., Murillo-Amador, B., 2020. Biocontrol of phytopathogens under aquaponics systems. Water 12, 1–15. https://doi.org/10.3390/w12072061.
Ruiz, A., Scicchitano, D., Palladino, G., Nanetti, E., Candela, M., Furones, D., Sanahuja, I., Carbó, R., Gisbert, E., Andree, K.B., 2023. Microbiome study of a coupled aquaponic system: unveiling the independence of bacterial communities and their beneficial influences among different compartments. Sci. Rep. 13, 1–17. https://doi.org/10.1038/s41598-023-47081-0.
Sanchez, F.A., Vivian-Rogers, V.R., Urakawa, H., 2019. Tilapia recirculating aquaculture systems as a source of plant growth-promoting bacteria. Aquac. Res 50, 2054–2065. https://doi.org/10.1111/are.14072.
Sawyer, T., 2021. Food safety and *E. coli* in aquaponic and hydroponic systems. Horticulturae 7, 1–5. https://doi.org/10.3390/horticulturae7030036.
Schmautz, Z., Graber, A., Jaenicke, S., Goesmann, A., Junge, R., Smits, T.H.M., 2017. Microbial diversity in different compartments of an aquaponics system. Arch. Microbiol 199, 613–620. https://doi.org/10.1007/s00203-016-1334-1.
Schmidt, B., Mulder, I.E., Musk, C.C., Aminov, R.I., Lewis, M., Stokes, C.R., Bailey, M., Prosser, J.I., Gill, B.P., Pluske, J.R., Kelly, D., 2011. Establishment of normal gut microbiota is compromised under excessive hygiene conditions. PLoS One 6, 1–10. https://doi.org/10.1371/journal.pone.0028284.

Sirakov, I., Lutz, M., Graber, A., Mathis, A., Staykov, Y., Smits, T.H.M., Junge, R., 2016. Potential for combined biocontrol activity against fungal fish and plant pathogens by bacterial isolates from a model aquaponic system. Water 8, 1–7. https://doi.org/10.3390/w8110518.

Sobiczewski, P., Iakimova, E.T., 2022. Plant and human pathogenic bacteria exchanging their primary host environments: A review. J. Hortic. Res. 30, 11–30 https://doi10.2478/johr-2022-0009.

Song, W., Li, L., Huang, H., Jiang, K., Zhang, F., Chen, X., Zhao, M., Ma, L., 2016. The gut microbial community of antarctic fish detected by 16S rRNA gene sequence analysis. Biomed. Res Int 2016, 1–8. https://doi.org/10.1155/2016/3241529.

Spyropoulos, V., Chalkias, A., Georgiou, G., Papalois, A., Kouskouni, E., Baka, S., Xanthos, T., 2020. Initial immune response in *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* bacteremia. Inflammation 43, 179–190 https://doi10.1007/s10753-019-01108-9.

Sylvain, F.É., Cheaib, B., Llewellyn, M., Gabriel, C.T., Barros, F.D., Luis, V.A., Derome, N., 2016. PH drop impacts differentially skin and gut microbiota of the Amazonian fish tambaqui (*Colossoma macropomum*). Sci. Rep. 6, 1–10. https://doi.org/10.1038/srep32032.

Talwar, C., Nagar, S., Lal, R., Negi, R.K., 2018. Fish gut microbiome: current approaches and future perspectives. Indian J. Microbiol. 58, 397–414. https://doi.org/10.1007/s12088-018-0760-y.

Tarnecki, A.M., Patterson, W.F., Arias, C.R., 2016. Microbiota of wild-caught Red Snapper Lutjanus campechanus. BMC Microbiol 16, 1–10. https://doi.org/10.1186/s12866-016-0864-7.

Tewari, A., Abdullah, S., 2015. *Bacillus cereus* food poisoning: international and Indian perspective. J. Food Sci. Technol. 52, 2500–2511. https://doi.org/10.1007/s13197-014-1344-4.

Tey, Y.H., Jong, K.J., Fen, S.Y., Wong, H.C., 2015. Occurrence of *Vibrio parahaemolyticus*, *Vibrio cholerae*, and *Vibrio vulnificus* in the aquacultural environments of Taiwan. J. Food Prot. 78, 969–976. https://doi.org/10.4315/0362-028X.JFP-14-405.

Tiwari, S.P., Suhr, M., Bang, C., Schulz, C., Hornburg, S.C., 2025. Exploring the potential of microalgae cocultivation on bacterial community dynamics in a Nile tilapia and garlic aquaponic system. Aquac. Res 2025, 1–17. https://doi.org/10.1155/are/6365949.

Todd, E.C.D., 2014. Foodborne diseases: overview of biological hazards and foodborne diseases. Encycl. Food Saf. 1, 221–242. https://doi.org/10.1016/B978-0-12-378612-8.00071-8.

Tyson, R.V., Simonne, E.H.A., 2014. practical guide for aquaponics as an alternative enterprise. Hortic. Sci. Dep. Univ. Fla. HS1252 1–11.

Ujjania, V.K., Sharma, B.K., Sharma, S.K., Upadhyay, B., 2021. Determination of water quality parameters with reference to management of aquaponics system. Environ. Ecol. 39, 1289–1294. https://doi.org/10.13140/RG.2.2.22603.46887.

Wang, Y.J.J., Deering, A.J., Kim, H.J.J., 2020. The occurrence of *Shiga Toxin-Producing E. coli* in aquaonic and hydroponic systems. Horticulturae 6, 1–14. https://doi.org/10.3390/horticulturae6010001.

Wang, Y.J., Deering, A.J., Kim, H.J., 2021. Effects of plant age and root damage on internalization of *Shiga Toxin-Producing Escherichia coli* in leafy vegetables and herbs. Horticulturae 7, 1–12. https://doi.org/10.3390/horticulturae7040068.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol 73, 5261–5267. https://doi.org/10.1128/AEM.00062-07.

Wang, J., Yan, M., Gao, H., Lu, X., Kan, B., 2017. *Vibrio cholerae* colonization of soft-shelled turtles. Apply Environ. Microbiol. 83, 1–9. https://doi.org/10.1128/AEM.

Wei, D., Guo, Z., Chen, F., Su, H., Wang, Y., Deng, L., Huang, J., Huang, L., Ling, F., Wang, G., Li, Y., Lu, C., Yu, Q., Li, P., 2025. Gut microbiota of largemouth bass in response to temperature stress. Aquac. Rep. 43, 1–11. https://doi.org/10.1016/j.aqrep.2025.102912.

Wong, S., Rawls, J.F., 2012. Intestinal microbiota composition in fishes is influenced by host ecology and environment. Mol. Ecol. 21, 3100–3102. https://doi.org/10.1111/j.1365-294X.2012.05646.x.

Xie, M., Xie, Y., Li, Y., Zhou, W., Zhang, Z., Yang, Y., Olsen, R.E., Ringø, E., Ran, C., Zhou, Z., 2022. Stabilized fermentation product of *Cetobacterium somerae* improves gut and liver health and antiviral immunity of zebrafish. Fish. Shellfish Immunol. 120, 56–66. https://doi.org/10.1016/j.fsi.2021.11.017.

Yamane, K., Kimura, Y., Takahashi, K., Maeda, I., Iigo, M., Ikeguchi, A., Kim, H.J., 2021. The growth of leaf lettuce and bacterial communities in a closed aquaponics system with catfish. Horticulturae 7, 1–12. https://doi.org/10.3390/horticulturae7080222.

Yang, J., Long, H., Hu, Y., Feng, Y., McNally, A., Zong, Z., 2022. *Klebsiella oxytoca* complex: update on taxonomy, antimicrobial resistance, and virulence. Clin. Microbiol. Rev. 35, 1–39. https://doi.org/10.1128/CMR.00006-21.

Yep, B., Zheng, Y., 2019. Aquaponic trends and challenges – a review. J. Clean. Prod. 228, 1586–1599. https://doi.org/10.1016/j.jclepro.2019.04.290.

Zhang, Z., Fan, Z., Yi, M., Liu, Z., Ke, X., Gao, F., Cao, J., Wanh, M., Chen, G., Lu, M., 2022. Characterization of the core gut microbiota of Nile tilapia (*Oreochromis niloticus*): indication of a putative novel Cetobacterium species and analysis of its potential function on nutrition. Arch. Microbiol 204, 1–12. https://doi.org/10.1007/s00203-022-03301-1.

Zhao, Y., Li, S., Lessing, D.J., Guo, L., Chu, W., 2023. Characterization of *Cetobacterium somerae* CPU-CS01 isolated from the intestine of healthy crucian carp (*Carassius auratus*) as potential probiotics against Aeromonas hydrophila infection. Micro Pathog. 180, 1–6. https://doi.org/10.1016/j.micpath.2023.106148.

Zhou, W., Xie, M., Xie, Y., Liang, H., Li, M., Ran, C., Zhou, Z., 2022. Effect of dietary supplementation of *Cetobacterium somerae* XMX-1 fermentation product on gut and liver health and resistance against bacterial infection of the genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*). Fish. Shellfish Immunol. 124, 332–342. https://doi.org/10.1016/j.fsi.2022.04.019.