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Establishment and evaluation of an indoor aquaponics system for culturing giant mottled eel (*Anguilla marmorata*) with apple mint (*Mentha suaveolens*)

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ABSTRACT

Aquaponics integrates fish and plant cultivation in a closed-loop system in which nitrifying bacteria convert fish waste into plant-available nutrients, conserving water and reducing environmental impact. This study evaluated the effects of indoor aquaponic effluent, with and without nitrifying bacteria, on the growth of apple mint (*Mentha suaveolens*) and giant mottled eels (*Anguilla marmorata*), including eel growth performance, blood biochemistry, intestinal histology, proximate composition, and microbial community structure in eel intestines and water, using metagenomics. Four experimental groups (T_0: water-apple mint; T_1: water-eel; T_2: eel-apple mint; and T_3: eel-apple mint-bacteria: *Nitrosomonas* sp. and *Nitrobacter* sp.) were conducted in triplicate for 8 weeks. T_3 yielded the highest eel weight (330.43 ± 9.65 g), surpassing T_2 and T_1, while groups demonstrated 100% survival. Growth performance parameters, hepatosomatic index, and eel proximate composition (moisture, crude protein, crude lipid, and ash) differed significantly among groups ($P < 0.05$), with T_3 showing the most favorable outcomes. These improvements in T_3 were accompanied by superior water quality, bacterial abundance, and intestinal histomorphology. Apple mint growth varied significantly, with T_3 yielding the highest ($P \leq 0.01$; 567.92 ± 21.31 g m^{-2}). Metagenomics revealed distinct microbial communities in T_3, predominated by Firmicutes and beneficial genera such as *Romboutsia* and *Cetobacterium*, and principal component analysis confirmed clear clustering by treatment. These findings support the successful establishment of the first indoor aquaponics system integrating giant mottled eel, apple mint, and nitrifying bacteria, highlighting the potential of nitrifying bacteria to enhance water quality, modulate microbiota, improve eel growth, gut health, and proximate composition, and increase plant productivity.

1. Introduction

The demand for aquaponics is increasing globally as a bio-integrated aquaculture system, valued for its soil-free cultivation, eco-friendly approach, organic benefits, and sustainability (Love et al., 2015; Junge et al., 2017). Aquaponics is an innovative agricultural technology that integrates recirculating aquaculture systems with hydroponics, enhancing productivity and water efficiency while maximizing yield without increasing water consumption (Greenfeld et al., 2019). This system minimizes water use by enabling the cultivation of fish and vegetables through a mutually beneficial water-reuse process (Simeonidou et al., 2012). It utilizes fish waste, converted by microorganisms into nutrients for plants, which in turn purify the water recirculated to the fish (Lee and Kim, 2021). This approach offers advantages

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such as reducing ecological risks, facilitating nitrogen transformations, and maintaining optimal water quality within the system (Wongkiew et al., 2017).

The giant mottled eel (*Anguilla marmorata*) is a tropical freshwater species capable of reaching lengths of up to 2 m and weights of up to 21 kg (Castle, 1984). This species has the most extensive range among all 19 freshwater eel species and subspecies, spanning the east coast of Africa to the Marquesas Islands in the southeast Pacific and extending as far north as southern Japan (Ege, 1939). On Yakushima Island, Japan, *A. marmorata* inhabits rivers and streams with rocky substrates, including large boulders and cobbles (Kumai et al., 2020), and often shelters in crevices within its natural habitat (Valdez and Castillo, 2016). In recent years, *A. marmorata* has gained popularity in South Korean aquaculture due to its lower production costs compared to *A. japonica* and its superior disease resistance. These traits, combined with its adaptability to intensive aquaculture systems and reduced reliance on high-cost eel larvae production, have significantly contributed to its growing demand.

Mentha, a genus within the Lamiaceae family, comprises approximately 31 species and 13 natural hybrids. These perennial herbs are commonly found in damp or wet habitats across Europe, Asia, Africa, Australia, and North America (Kumar et al., 2011). Widely cultivated varieties include peppermint (*Mentha piperita*), spearmint (*Mentha spicata*), and apple mint (*Mentha suaveolens*) (Chauhan and Agarwal, 2013). Although it is known for its low nutrient requirements and robust growth potential (Somerville et al., 2014), research on *Mentha* species within aquaponic systems remains relatively limited. However, existing studies suggest that *Mentha* spp. can be effectively cultivated in small-scale aquaponic systems, demonstrating its potential for integration into sustainable agricultural practices (Wahap et al., 2010; Shete et al., 2016, 2017). In addition, plants of the genus *Mentha* are fast-growing and invasive, tolerant of a wide range of agro-climatic conditions (Božović et al., 2015). Notably, *M. suaveolens*, an aromatic herb with a spearmint flavor, is widely used in traditional Mediterranean medicine (Metin et al., 2021). Furthermore, the essential oil extracted from mint leaves holds significant economic value and is widely utilized in the food industry, cosmetics, and confectionery (Lawrence, 2006). The biological properties of *Mentha* species are diverse, including cytotoxic, antibacterial, antioxidant, anti-inflammatory, hypotensive, analgesic, sedative, and insecticidal effects (Moreno et al., 2002; El-Kashoury et al., 2014; Božović et al., 2015).

Fish, plants, and microbes are essential components of aquaponic systems, while nitrogen is a vital nutrient for all three. In aquaponic systems, nitrogen is supplied exclusively through fish feed (Ajijah et al., 2020). However, ammonia (NH_3), the most toxic form of nitrogen dissolved in water, poses a significant threat to tropical fish species, which are particularly sensitive to its adverse effects (Effendi et al., 2015; Wang and Leung, 2015). NH_3 can impair fish growth by reducing appetite and feed intake (Mondal et al., 2025). Although the accumulation of NH_3 from decomposed fish feed and waste is toxic, nitrifying bacteria convert NH_3 into NO_3, a nitrogen source essential for plant growth (Goddek et al., 2015). The efficient function of an aquaponic system relies on integrating hydroponics, aquaculture, and a balanced nutrient and microbial ecosystem. Bacteria facilitate the conversion of fish waste into nutrients for plants, whereas aquaculture effluents purify water and provide essential nutrients to hydroponically grown plants (Wong et al., 2020; Ani et al., 2022). This synergy among fish, plants, and beneficial microbes, such as nitrifying bacteria, supports the development of microbial communities and produces by-products that enhance water quality and plant growth (Wielgosz et al., 2017). The nitrifying autotrophic bacterial consortium includes nitroso-bacteria (e.g., *Nitrosomonas* sp.) and nitro-bacteria (e.g., *Nitrospira* sp. and *Nitrobacter* sp.), which are integral to nitrogen transformation (Goddek et al., 2019). Nitroso-bacteria convert NH_3 to NO_2, while nitro-bacteria further convert NO_2 to NO_3, which is then utilized by plants for growth (Goddek et al., 2015). As microorganisms serve as a link between aquaculture and hydroponic systems, analyzing their community dynamics is essential for elucidating their functional roles within the symbiotic ecosystem (Munguia-Fragozo et al., 2015; Schmautz et al., 2017). Numerous studies have employed metagenomic techniques to explore interactions between microbial communities and their surrounding environments. The microbial community is an essential biological component that connects aquaculture and hydroponics and determines the productivity and stability of the system by driving the mineralization and nutrient cycling of organic matter (Kasozi et al., 2021; Schmautz et al., 2022). While early studies focused on the presence or simple classification of microorganisms, recent study has focused on identifying complex interactions between the functional roles of microbial communities and environmental factors (Rajeev et al., 2024). Metagenomic analysis of the *A. marmorata* has not yet been explored.

According to available information, no previous studies have combined *A. marmorata* and *M. suaveolens* with nitrifying bacteria, nor have any experiments involving *A. marmorata* and *M. suaveolens* individually been conducted in aquaponic systems. This establishes the present study as the first to explore their integrated cultivation within this context. Choi and Chiang (2013) conducted experiments on *M. suaveolens* in hydroponic systems, while Tan et al. (2018) performed density-based experiments on *A. marmorata* in a recirculating aquaculture system. The present study employed a novel approach, integrating *A. marmorata*, *M. suaveolens*, and nitrifying bacteria to evaluate their combined effects in an aquaponic system. In this study, an indoor aquaponics system was designed to combine *A. marmorata* and *M. suaveolens*, with the addition of nitrifying bacteria (*Nitrosomonas* sp. and *Nitrobacter* sp.), to examine nutrient cycling efficiency, water quality improvement, bacterial abundance, blood biochemical parameters, and intestinal histomorphological analysis of *A. marmorata*. Furthermore, the study assessed the growth performance of both the plant and fish, along with metagenomic profiling of water and eel intestine to characterize microbial community composition and functional dynamics within the system.

2. Materials and methods

2.1. Ethical statement

The Animal Care and Use Committee of Chonnam National University approved experimental methods (CNU IACUC-YS−2023–9). The experiment was conducted in accordance with the National Institutes of Health's Guidelines for the Care and Use of Laboratory Animals.

2.2. Experimental design

The study was conducted for 8 weeks from December 2023 to February 2024 at the College of Fisheries and Ocean Sciences, Chonnam National University, Yeosu-si, Jeollanam-do, South Korea (126.581316E, 34.9860324 N). An indoor deep-water aquaponics system was employed, and four experimental groups were examined (T_0: water-apple mint, T_1: water-eel, T_2: eel-apple mint, and T_3: eel-apple mint-bacteria), each with three replicates. Each subsystem consisted of an eel culture tank (81 × 61 × 76 cm) with 310 L water holding capacity (in where 250 L water was used), an apple mint growing bed (90 × 62 × 32 cm), a siphoning tube, an external biofilter (26 × 26 × 47 cm) for filtering eel tank water before irrigating to the apple mint growing bed, a water heater (300 W) to maintain the water temperature, and four sets of LED plant growth lights (10,000 lux) to provide adequate light intensity for photosynthesis (Fig. 1). The experiment was conducted in winter to evaluate the performance and stability of an indoor deep-water aquaponics system under increased thermal regulation demands. Despite indoor operation, maintaining plant-suitable temperatures required active environmental control, allowing assessment of system robustness and operational stability under seasonally constrained conditions

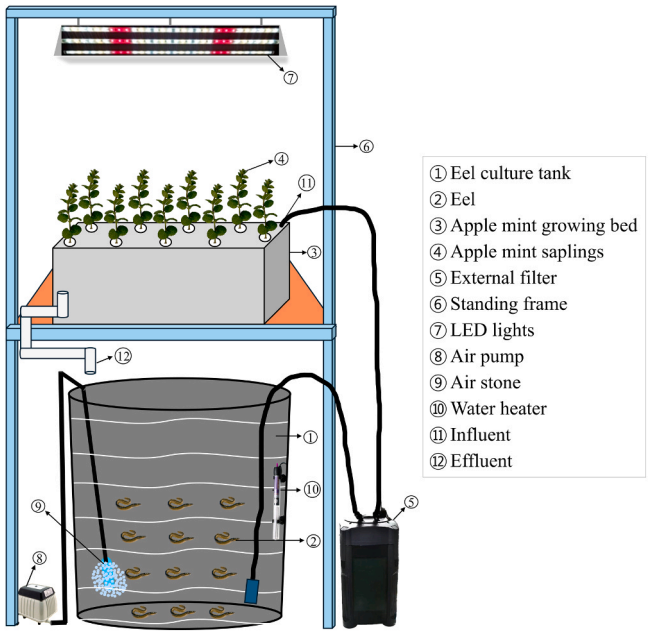

Fig. 1. Schematic diagram of the typical deep-water-based indoor aquaponics set-up used in this experiment.

relevant to practical aquaponic applications.

2.3. Stocking and rearing eel

Water circulation in each sub-system was initiated with aeration and heating one week prior to stocking to stabilize dissolved oxygen (DO) levels and temperature. *A. marmorata* (average length: 46.57 ± 0.63 cm; average weight: 233.28 ± 7.19 g) were obtained from Dongwoo Fisheries Farm, Hampyeong-gun, Jeollanam-do, South Korea. The eels were acclimated for one week and subsequently stocked at 12 individuals per tank. The stocking density of 12 *A. marmorata* was selected to optimize water quality, prevent nutrient imbalances, and maintain system stability, while ensuring sufficient nutrients for the plants and minimizing NH_3 and TAN accumulation for the fish, in line with the principles of a coupled, deep-water aquaponics system (Rakocy et al., 2006; Somerville et al., 2014). The eels were maintained under 24 h darkness using a black shading net and fed a commercial floating feed (moisture: ≤ 12.0%, crude protein: ≥ 52.0%, crude lipid: ≥ 12.0%, crude fiber: ≤ 6.0%, ash: ≤ 18.0%, calcium Ca: ≥ 1.0%, and phosphate: ≤ 2.7%; F-GR, Purina, Seongnam, Korea) once daily at 1.75% of their body weight. This feeding rate was selected based on preliminary studies and the metabolic needs of *A. marmorata* at the beginning of the experiment. This rate was chosen to maintain water quality and prevent overfeeding in this indoor aquaponics system. Moreover, the use of floating feed was essential to avoid water quality degradation associated with moist feed, as the aquaponics system did not allow for water exchange during the experiment. Each eel tank was equipped with an air stone (length: 12 cm; diameter: 2.5 cm), a 300 W water heater (PhilGreen High Class Aquarium Heater PH−300, Chuangxing., Co., Hong Kong, China) to maintain the temperature at approximately 28 °C, as higher temperature may adversely affect plant growth in the aquaponics system, and a 30 W external biofilter (220 V ~ 60 Hz; PhilGreen External Filter CF−30, Chuangxing., Co., Hong Kong, China) containing red clay balls (diameter: 1.5 cm) and sera siporax as the filtration medium, ensuring continuous water circulation between the eel tanks and plant beds. Aeration, heating, and filtration systems were operated continuously throughout the experimental period. Moreover, no additional water was added to the fish tank until the water volume decreased due to evaporation.

2.4. Collection of apple mint saplings and preparation of growing beds

Apple mint (*M. suaveolens*) saplings were sourced from a reputable plant shop in South Korea. Nine oval-shaped rubber containers (90 × 62 × 32 cm) were used as plant beds, each equipped with a drainage outlet (PVC, 3.1 cm in diameter) connected to the eel tank. Each plant bed contained a floating foam board (66 × 53 × 0.5 cm) with 15 holes (2.6 cm in diameter) spaced 15 cm apart. Fifteen saplings (average initial length: 21.37 ± 0.89 cm; shoot length: 7.26 ± 0.59 cm) were planted in hydroponic pots with sponges (2.5 cm in diameter). Four sets of LED grow lights (Plant LED Bar, NINE S Co., Chuncheon-si, Korea) were installed above each plant bed and operated for 11 h per day to ensure optimal photosynthesis throughout the experiment.

2.5. Initiation of aquaponics

The experiment commenced after the eel tanks and apple mint beds were set up. An initial "lag phase", as described by Roy et al. (2021) and Nadia et al. (2023), was observed to allow the development of nitrifying bacteria in the plant beds, thereby enhancing plant growth. Regular pruning and weeding were performed throughout the experimental period to maintain system efficiency.

2.6. Introduction of nitrifying bacteria

As per the manufacturer's (ABC Animal Bio Care, Korea) instructions, 5 mL of commercial nitrifying bacteria (comprising *Nitrosomonas* sp. and *Nitrobacter* sp., 8.70×10^9 CFU mL^{-1}) was introduced once daily to the T_3 system (total water volume: 250 L) to enhance the nitrification process. This dosing corresponds to a final applied concentration of approximately 1.74×10^8 CFU L^{-1} in the system. Moreover, the nitrifying bacteria inoculation was intended to accelerate the breakdown of organic substances, reduce toxic NH_3 or TAN levels for the fish, and improve nutrient availability for the plants (Effendi et al., 2017).

2.7. Growth performance and yield of apple mint

Apple mint shoot length was measured weekly from the foam board surface to the main stem tip using a plastic ruler. Each week, the number of leaves and chlorophyll content (SPAD−502PLUS, Konica Minolta, Japan) were also recorded. Leaf area (cm^2) was calculated biweekly by multiplying leaf length and width. At the end of the experiment, all plants were removed from the beds for measurement of total plant length, fresh shoot weight, and root weight. Leaves from each apple mint plant were separated and weighed using a digital scale (XIN YUAN XY−8006, China) to calculate total leaf production. The root-shoot ratio was determined by dividing root weight by shoot weight.

2.8. Sampling and harvesting of eel

Eel measurements were conducted at the beginning and end of the 8-week experiment. After a 24 h fasting period, eels were collected from the tanks using a scoop net and transferred to a 50-L bucket. The eels were anesthetized with 2-phenoxyethanol (0.1 mL L^{-1}), according to the protocol outlined by Tan et al. (2018). Total body length and weight were measured using a measuring scale and an electronic balance (PAG 2102, OHAUS Co., Parsippany, NJ, USA), respectively. Growth parameters, including length gain, percent length gain (%), weight gain (WG), percent weight gain (%), specific growth rate (SGR, % day^{-1}), food conversion ratio (FCR), survival rate (SR, %), and production (kg m^{-3}), were evaluated based on the method described by Salama et al. (2021). Additional metrics, including daily weight gain (DWG) and protein efficiency ratio (PER), were measured following the method described by Tan et al. (2018); feed efficiency (FE) and hepatosomatic index (HSI) as per Shahkar et al. (2016); feeding rate (FR) based on Luo et al. (2013);

and feed intake (FI) (g fish^{-1}) according to Aya et al. (2023).

2.9. Water sampling of the eel tank

Water temperature, pH, and dissolved oxygen (DO) were regularly monitored throughout the experiment using a YSI ProDSS meter (USA). Electrical conductivity (EC) and total dissolved solids (TDS) were measured weekly with an E–1 portable meter (COMS, ID944). Nitrogenous compounds, including total ammonia nitrogen (TAN), nitrite (NO_2), and nitrate (NO_3), were also monitored weekly with API freshwater test kits (Mars Fishcare, USA), allowing assessment of system stability and temporal trends. At the end of the experiment, concentrations of potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) were analyzed at the Sienna Marine Research Institute, Yeosu-si, South Korea.

2.10. Analysis of water bacterial abundance

A total of 2 L of tank water was collected from each experimental tank on the initial and final days of the experiment and filtered through a 0.45 μm MCE membrane filter (MF-Millipore). To prepare the sample, a 10-fold dilution was prepared by mixing 100 μL of filtered water with 900 μL of PBS (Phosphate-buffered saline). The diluted samples were evenly plated on BHIA agar (Brain Heart Infusion Agar, BD Difco™) using a spreader (Spreader, SPL) and incubated at room temperature for 3 days to allow bacterial colonies to grow. The bacterial colonies were then counted using total plate counting (TPC) to quantify total culturable bacterial abundance across the experimental groups, not to distinguish between introduced nitrifying bacteria and the background microbial community.

2.11. Proximate composition analysis of A. marmorata

At the end of the experiment, three eels were randomly selected from each experimental group for analyzing the moisture, ash, crude protein, and crude lipid contents of *A. marmorata* according to the (AOAC (Association of Official Analytical Chemists), 2005) methods. Moisture content was determined by desiccation at 105 °C, ash content by incineration at 550 °C, and crude lipid by Soxhlet extraction using an Automatic Solvent Extractor (SER 158/6). Moreover, crude protein was measured using an elemental analyzer (DKSH, vario MAX cube) and calculated as total nitrogen multiplied by 6.25.

2.12. Eel blood biochemical parameters analysis

At the end of the experiment, five eels from each experimental group were fasted for 24 h and, after being kept in a 30-L bucket, the eels were sedated with 2-phenoxyethanol (2 mL L^{-1} for 5 min) for blood collection, as described by Aya and Garcia (2022). Blood samples were drawn from the caudal vein using a 3 mL single-use sterile hypodermic syringe, then centrifuged at 4000 rpm for 20 min at 4 °C. Following the method outlined by Saseendran et al. (2021), the blood serum was separated and stored in a 2 mL non-heparinized Eppendorf tube at −80 °C for further analysis. The following serum parameters were analyzed: Comprehensive [albumin (ALB); total protein (TP); globulin (GLOB); albumin/globulin ratio (A/G); total bilirubin (TB); gamma-glutamyl transferase (GGT); aspartate aminotransferase (AST); alanine transaminase (ALT); alkaline phosphate (ALP); amylase (AMY); creatine (CREA); uric acid (UA); blood urea nitrogen (BUN); glucose (GLU); total cholesterol (TC); and triglyceride (TG)] and electrolytic profiles [Potassium (K^+); sodium (Na^+); chlorine (Cl^-); calcium (Ca^{2+}); magnesium (Mg^{2+}); phosphorous (PHOS); and total carbon di-oxide (TCO_2)]. These were analyzed using the ExigoTM C200 Clinical Chemistry Analyzer (Boule Medical AB, Sweden), with results obtained in less than 12 min.

2.13. Eel histological tissue specimens

Following the procedure of Aya and Garcia (2022), four *A. marmorata* from each experimental group were randomly selected at the end of the experiment and anesthetized with 2-phenoxyethanol (2 mL L^{-1} for 5 min). Middle intestinal samples were collected using sterile instruments, cleaned, and fixed in 10% PBS for 48 h. The fixed tissues were preserved in 70% ethanol at 4 °C until histological preparation. After ethanol series grading, samples were embedded in molten wax, trimmed, and sectioned at 4 μm thickness using a rotary microtome (Leica Biosystems, HistoCore MULTICUT R, China). Samples were dehydrated with 70–100% ethanol, stained with xylene and hematoxylin–eosin, and the tissue sections were examined under a microscope (Olympus BX51, Japan), and histological parameters were measured using the NIS-Elements AR software (Version 5.10).

2.14. DNA sequencing

Library preparation was followed by sequencing on the Illumina MiSeq platform using the MiSeq Reagent Kit v3 (2 × 300 bp), generating approximately 13.2–15 Gb of data per run. The following primers were used: 341 F (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG−3′) and 805 R (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC−3′).

2.15. Metagenome analysis

A total of seven samples were collected during the 8th week of the incubation period for subsequent microbial community analysis. The main purpose of this study is to investigate the structural differences in the final microbial community formed after each treatment condition was applied to the system over a long period. Therefore, the sampling time point was selected at the end of the experiment (week 8), when the microbial community in the system was sufficiently stabilized following adaptation to the treatment conditions. Metagenome analyses were conducted using the QIIME2-amplicon (v2024.10) (Bokulich et al., 2018). Initially, demultiplexed sequences were summarized with the q2-demux plugin to evaluate read quality. Adapters and primer sequences were subsequently removed using the q2-cutadapt plugin (Martin, 2011). Denoising was performed with the q2-dada2 plugin, which includes error correction, chimera filtering, and the merging of paired-end reads, ultimately yielding high-resolution amplicon sequence variants (ASVs) (Callahan et al., 2016). To optimize reading quality and minimize bias, truncation parameters were set to 240 bp for forward reads and 200 bp for reverse reads.

Taxonomic classification of representative sequences was performed using a Naïve Bayes classifier trained on the SILVA 138 SSURef NR99 full-length sequence dataset (MD5: de8886bb2c059b1e8752255d271f3010) and corresponding taxonomy file (MD5: f12d5b78bf4b1519721fe52803581c3d), both tailored to the V3–V4 region. The classifier was trained using the same primer sequences applied in this study, employing the "fit-classifier-naive-bayes" command. Taxonomic assignments were conducted via the "classify-sklearn" method implemented in the q2-feature-classifier plugin (Bokulich et al., 2018). To refine the dataset, taxonomic filtering was applied using the "filter-table" function in the q2-taxa plugin, excluding features annotated as "Unassigned", "Archaea", "Chloroplast", or "Mitochondria".

2.16. Statistical analysis

Data were entered into Microsoft Excel 2016 and analyzed using descriptive statistics (mean ± SD) for three replicates per experimental group. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences, version 27.00), with one-way ANOVA and Duncan's Multiple Range Test (DMRT) at a significance level of 0.05 for mean comparisons (Duncan, 1995). Graphs were created using

GraphPad Prism (version 8.0.2). For the metagenome, statistical analyses were carried out using the R packages phyloseq, vegan, and MicrobiotaProcess (McMurdie and Holmes, 2013; Oksanen et al., 2013; Xu et al., 2023). Prior to analysis, each sample was rarefied to a uniform sequencing level of 353,972 reads to minimize sampling bias. Rarefaction is an approach used to account for variations in library sizes across samples, thereby facilitating a more accurate comparison of diversity. First proposed by Sanders (1968), rarefaction involves selecting a specified number of samples equal to or lower than the smallest sample and then randomly discarding reads from larger samples until the number of remaining samples equals this threshold (Willis, 2019). Based on these equally sized subsamples, diversity metrics can be calculated that contrast ecosystems fairly, independent of differences in sample sizes (Weiss et al., 2017).

3. Results

3.1. Water quality parameters

Water temperature in the eel tanks remained stable throughout the experiment, ranging from 27.47 to 28.13 °C, with no significant differences among groups ($P > 0.05$; Fig. 2a). DO levels ranged from 7.94 to 8.91 mg L^{-1} in T_0, and from 6.45 to 7.54 mg L^{-1} in T_1 to T_3, with the highest mean DO observed in T_0 (8.61 ± 0.33 mg L^{-1}) and the lowest in T_2 (6.71 ± 0.24 mg L^{-1}) (Fig. 2b). pH ranged from 7.25 to 7.73 in T_0, and from 5.55 to 6.49 across T_1 to T_3 throughout the study period (Fig. 2c).

Mean TAN concentration in T_1 was 27%, 28%, and 100% higher than in T_3, T_2, and T_0, respectively, with highly significant differences among groups ($P < 0.01$; Fig. 2d). The highest NO_2 and NO_3 concentrations were in T_3 (3.69 ± 1.76 and 24.07 ± 17.24 mg L^{-1}, respectively), with highly significant differences among experimental groups ($P < 0.01$; Fig. 2e and f).

The highest and lowest mean EC values were 1527.04 ± 753.65 μs cm^{-1} in T_3 and 118.76 ± 23.30 μs cm^{-1} in T_0, respectively. TDS was also highest in T_3 (763.44 ± 380.69 mg L^{-1}), followed by T_2, T_1, and T_0. Significant differences among experimental groups were observed for pH, DO, EC, and TDS ($P < 0.01$; Fig. 2g and h).

T_3 exhibited the highest concentrations of K (0.26 ± 0.05 μg L^{-1}), Ca (0.70 ± 0.01 μg L^{-1}), Mg (0.06 ± 0.01 μg L^{-1}), and S (1.16 ± 0.00 μg L^{-1}), whereas the lowest concentrations were observed in T_1 (excluding T_0). Additionally, T_1 showed the lowest values for K (0.11 ± 0.01 μg L^{-1}), Ca (0.36 ± 0.01 μg L^{-1}), Mg (0.03 ± 0.01 μg L^{-1}), and S (0.68 ± 0.02 μg L^{-1}) (Table 1). Significant differences among experimental groups were observed for K, Ca, Mg, and S among experimental groups ($P < 0.01$).

3.2. Bacterial abundance

Bacterial abundance significantly differed ($P < 0.01$) between experimental groups at both the start and end of the experiment. T_3 exhibited the highest bacterial abundance at both sampling periods. At the end of the experiment, bacterial abundance was the highest in T_3 (76950 ± 5727 CFU mL^{-1}), followed by T_2 (38450 ± 5020 CFU mL^{-1}), T_1 (29450 ± 8838 CFU mL^{-1}), and T_0 (2445 ± 1463 CFU mL^{-1}) (Fig. 3).

During sequencing, 12,084,146 paired-end reads were initially obtained, of which 4106,307 were retained for downstream analysis (Table 2). After removing adaptor sequences and low-quality reads, 3265,333 high-quality clean reads (79.52%) were selected for further processing. To focus on bacterial communities, sequences were filtered to include only those affiliated with the domain bacteria, yielding 2029 ASVs with a total read frequency of 3265,333 based on rarefaction analysis (Fig. 4). Taxonomic classification revealed 30 bacterial phyla and 327 genera in water samples, and 22 phyla and 334 genera in eel samples. After excluding taxa as “uncultured”, the final dataset comprised 30 phyla and 327 genera in water samples, and 22 phyla and 334 genera in eel samples.

The relative abundance of the microbiome was analyzed at both the phylum (top 10 taxa) and genus (top 10 taxa) levels in water (Fig. 5) and eel samples (Fig. 6). At the phylum level, water samples were primarily dominated by Bacteroidota (mean of 51.6%), followed by Verrucomicrobiota (mean of 20.2%). The T_2 and T_3 water samples exhibited comparable microbial community structures (Fig. 5a). At the genus level, the T_2 water samples mainly exhibited *Cephaloticoccus* (21.3%) and *Kapabacteriales* (12.3%). In the T_3 water sample, this order was reversed, with *Kapabacteriales* becoming the more dominant genus at 41.6%, and *Cephaloticoccus* decreasing to 10.3% (Fig. 5b).

In contrast to the water samples, which showed minimal variation in genus composition, the eel samples exhibited more pronounced differences (Fig. 6). At the phylum level, Proteobacteria dominated both the T_1 and T_2 eel samples (45.0% and 64.6%, respectively), whereas the T_3 eel sample showed a distinct compositional shift, with Firmicutes accounting for 61.0% of the microbiome (Fig. 6a). At the genus level, the T_1 eel sample was dominated by *Romboutsia* (17.4%), the T_2 sample by *Citrobacter* (68.4%), and the T_3 sample by both *Cetobacterium* (27.7%) and *Romboutsia* (27.0%), which occurred at comparable relative abundances (Fig. 6b).

The Venn diagrams (Figs. 7 and 8) display different colored circles, each representing water and eel samples subjected to different treatments. The numbers within the circles indicate the counts of ASVs that are either shared among the samples or unique to each sample. Values in the overlapping regions correspond to shared ASVs between samples, whereas those in the non-overlapping regions indicate ASVs exclusive to each sample.

At the phylum level, three ASVs were uniquely observed in the T_2 water sample and were taxonomically classified as Desulfobacterota, Halanaerobiaeota, and Thermotogota. Similarly, the T_3 water sample also contained three unique ASVs affiliated with Elusimicrobiota, Synergistota, and Nitrospirota (Fig. 7a).

In eel samples, a total of 22 ASVs were shared across T_1, T_2, and T_3, with the T_1 sample encompassing all ASVs present in both T_2 and T_3 samples. When comparing T_2 and T_3 specifically, one ASV affiliated with the phylum Bdellovibrionota was uniquely assigned to the samples, whereas the T_3 samples were also assigned with three unique ASVs, corresponding to the phyla Chloroflexi, Cyanobacteria, and Planctomycetota (Fig. 7b).

At the genus level, across the two water samples, a total of 327 ASVs were detected, of which 212 were shared by both samples. A total of 53 ASVs were found to be exclusively present in the T_2 water samples and were taxonomically assigned to a diverse range of genera, including *Shewanella*. A total of 62 ASVs were uniquely identified in the T_3 water samples and were taxonomically affiliated with a wide variety of genera, including *Rhizobacter* (Fig. 8a). A total of 334 ASVs were identified across the three eel samples, among which 38 ASVs were shared by all samples. The eel samples collected from T_1 harbored the highest number of unique ASVs (227), while T_2 and T_3 contained 15 and approximately 16 unique ASVs, respectively. A total of 15 ASVs were detected exclusively in the T_2 water samples and assigned to various genera, including *Dysgonomonas*, affiliated with four different phyla: Firmicutes (7 genera), Proteobacteria (5 genera), Bacteroidota (2 genera), and Verrucomicrobiota (1 genus). And T_3 water samples’ ASVs were taxonomically affiliated with a range of bacterial genera, including *Lachnoclostridium*, distributed across six different phyla: Firmicutes (7 genera), Proteobacteria (5 genera), Actinobacteriota (2 genera), Bacteroidota (2 genera), Verrucomicrobiota (2 genera), and Planctomycetota (1 genus). Notably, the highest number of shared ASVs was observed between the T_1 and T_3 samples, whereas the T_2 and T_3 samples exhibited the lowest overlap, sharing only 4 ASVs (Fig. 8b).

Principal component analysis (PCA) of the eel samples revealed that PC1 and PC2 accounted for the majority of the variance, with their combined eigenvalues explaining 100% of the total variability. Notably, PC1 alone explained 74.3% of the total variation at the phylum level and

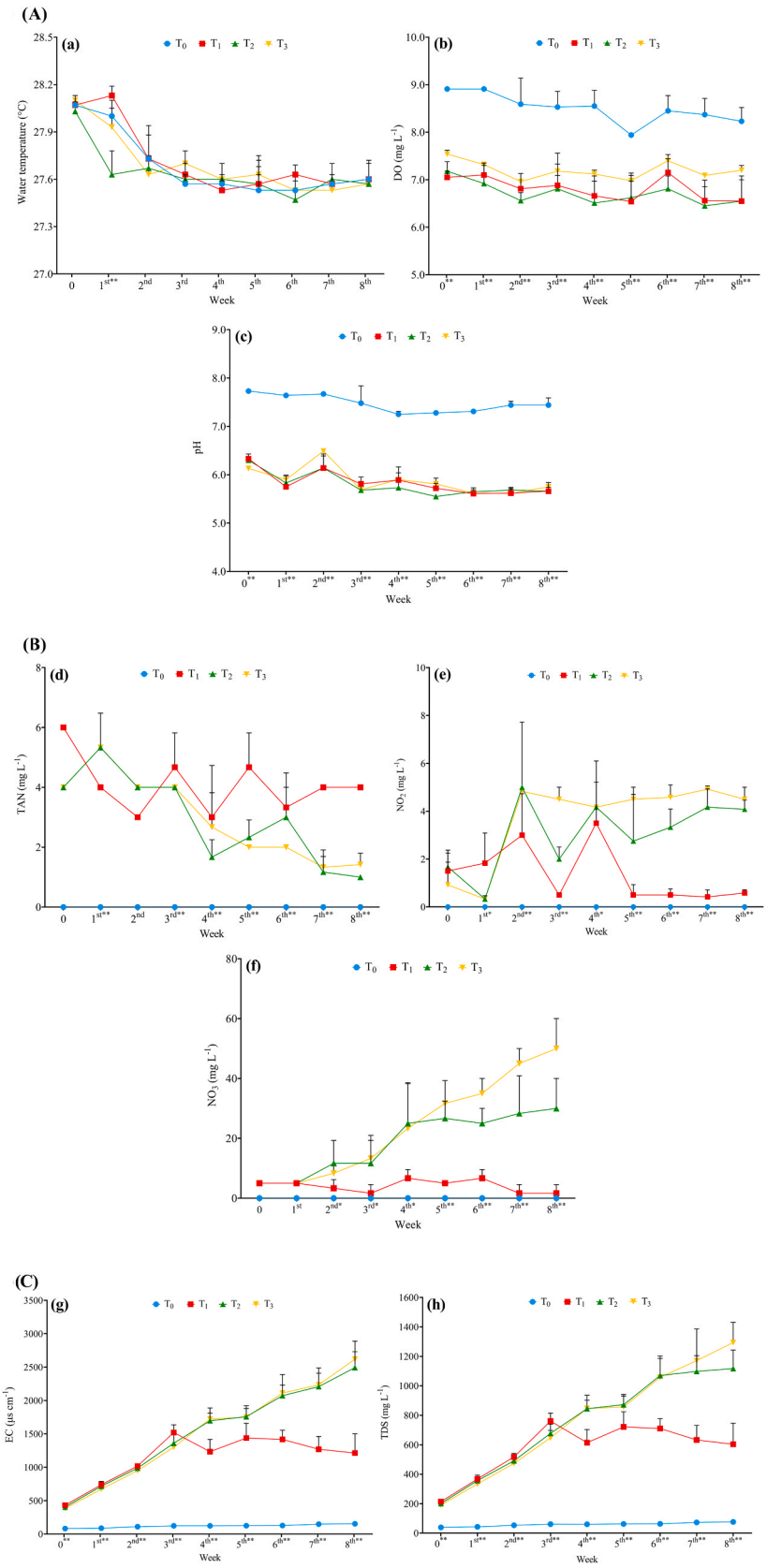

Fig. 2. Changes of water quality parameters: **2 A**, **(a)** Water temperature, **(b)** DO (dissolved oxygen), **(c)** pH; **2 B (d)** TAN (total ammonia nitrogen), **(e)** NO_2 (nitrite), **(f)** NO_3 (nitrate); **2 C**, **(g)** EC (electrical conductivity), and **(h)** TDS (total dissolved solids) in T_0 (water-apple mint), T_1 (water-eel), T_2 (eel-apple mint), and T_3 (eel-apple mint-bacteria) throughout the experimental period. Different symbols represent measured values (mean ± SD) on different sampling weeks. * and ** indicate statistically significant differences among experimental groups at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 1
Mean concentration (μg L^{-1}) of minerals (K, Ca, Mg, and S) in the tank water at the end of the experiment.

Parameters	Experimental groups				Sig.
	T_0	T_1	T_2	T_3	
K (μg L^{-1})	0.03 ± 0.01^c	0.11 ± 0.01^b	0.23 ± 0.00^a	0.26 ± 0.05^a	**
Ca (μg L^{-1})	0.08 ± 0.00^c	0.36 ± 0.01^b	0.69 ± 0.02^a	0.70 ± 0.01^a	**
Mg (μg L^{-1})	0.02 ± 0.01^c	0.03 ± 0.01^b	0.06 ± 0.01^a	0.06 ± 0.01^a	**
S (μg L^{-1})	0.49 ± 0.01^d	0.68 ± 0.02^c	1.02 ± 0.01^b	1.16 ± 0.00^a	**

Note: Values are presented as mean ± SD for three replicates. T_0 = water-apple mint; T_1 = water-eel; T_2 = eel-apple mint; T_3 = eel-apple mint-bacteria; K = potassium; Ca = calcium; Mg = magnesium; S = sulfur. In the column, "Sig." indicates significance. In the rows, means followed by different letters denote significant differences among the experimental groups. ** Indicates significance at $P \leq 0.01$.

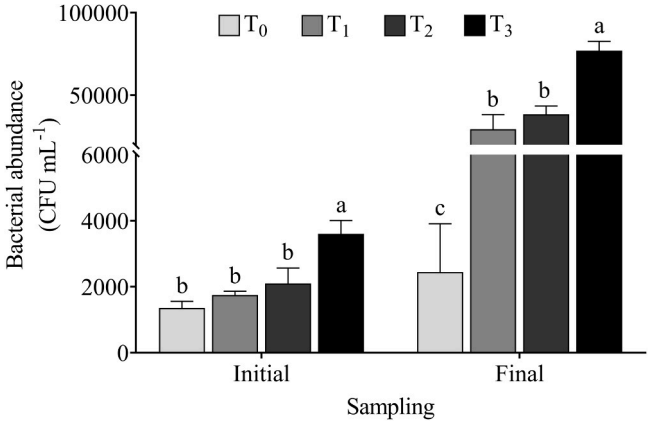

Fig. 3. Mean initial and final bacterial abundance (CFU mL^{-1}) among the experimental groups in an indoor aquaponics system. The mean (± SD) values were calculated from three replications of each experimental group where T_0 = water-apple mint; T_1 = water-eel; T_2 = eel-apple mint; and T_3 = eel-apple mint-bacteria. Bars with different letters indicate significant differences at $P \leq 0.05$ based on DMRT.

69.52% at the genus level (Fig. 9) and revealed clear distinctions in the microbial communities of eel samples across treatment groups. At the phylum level, T_1 samples were associated with Actinobacteriota and Bdellovibrionota, while T_2 samples showed strong associations with Proteobacteria and Fusobacteriota. In contrast, T_3 samples were primarily related to Firmicutes and showed associations with Fusobacteriota (Fig. 9a). At the genus level, *Polynucleobacter* and *Novosphingobium* were dominant in T_1 samples, while T_2 samples were strongly associated with *Citrobacter*. On the other hand, T_3 samples showed close associations with *Cetobacterium* and *Romboutsia* (Fig. 9b).

3.3. Growth performances of A. marmorata

At the end of the experiment, *A. marmorata* in T_3 exhibited the highest final body length (50.83 ± 0.42 cm) and weight (330.43 ± 9.65 g), with significant differences ($P < 0.05$) compared to the other groups (Fig. 10a and b). WG and percent WG were significantly higher ($P < 0.01$) in T_3 (96.11 ± 2.24 g; 41.03 ± 0.66%) compared to T_1, which had the lowest values (78.29 ± 1.70 g; 34.00 ± 1.21%). SGR and DWG were significantly higher ($P < 0.01$) in T_3, with DWG in T_3 exceeding T_1 by 19% and T_2 by 9% (Table 3). The FCR improved progressively from T_1 (2.78 ± 0.10) to T_3 (2.30 ± 0.04), with T_3 achieving the highest FE (43.41 ± 0.70%) and PER (6.96 ± 0.11%). FI was highest in T_2 (222.19 ± 9.15 g $fish^{-1}$), with no significant differences among groups ($P > 0.05$). HSI was significantly higher ($P < 0.05$) in T_3 (1.86 ± 0.23%). Survival rates were consistently 100% across all groups. However, eel production was significantly higher ($P < 0.05$) in T_3 (15.86 ± 0.46 kg m^{-3}) compared to T_2 (15.47 ± 0.41 kg m^{-3}) and T_1 (14.82 ± 0.30 kg m^{-3}) (Table 3).

3.4. Growth performances of apple mint

At the start of the experiment, the shoot lengths of apple mint saplings were similar across all groups ($P > 0.05$) (Fig. 11a). By the end of the experiment, T_3 exhibited the highest shoot length (49.76 ± 4.68 cm) with significant differences ($P < 0.01$). Initial plant weights showed no significant differences ($P > 0.05$); however, final weights differed significantly ($P < 0.01$). Root lengths increased significantly ($P < 0.01$), and the fresh root weight in T_3 was 23.66% and 22.65% higher than in T_0 and T_2, respectively (Table 4). Additionally, T_3 had the highest fresh shoot weight (20.04 ± 1.44 g; $P < 0.01$). The root-shoot ratio was highest in T_0 (0.46 ± 0.06), with significant differences among groups ($P < 0.01$).

The mean values for leaf number (246.52 ± 27.71), area (9.63 ± 0.89 cm^2), length (3.61 ± 0.11 cm), width (2.65 ± 0.16 cm), and chlorophyll content (33.98 ± 4.51) were significantly higher ($P < 0.01$) in T_3 compared to the other groups (Fig. 11b, c, and d). Additionally, final leaf production in T_3 (567.92 ± 21.31 g m^{-2}) was 30.30% and 64.57% higher than in T_2 and T_0, respectively, with highly significant differences ($P < 0.01$) among the experimental groups (Table 4).

3.5. Proximate composition of A. marmorata

The moisture content of *A. marmorata* was highest in T_3 (69.02 ± 0.80%) and lowest in T_1 (65.47 ± 0.53%). Crude protein contents were 17.79 ± 0.45, 17.36 ± 0.08, and 16.78 ± 0.18% in T_1, T_2, and T_3, respectively. Moreover, the crude lipid and ash contents were highest in T_1 (8.91 ± 0.57 and 2.33 ± 0.03%) and lowest in T_3 (7.41 ± 0.17 and 1.96 ± 0.02%), respectively. Significant differences ($P < 0.01$) were observed across all measured proximate parameters, including moisture,

Table 2
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
eel_T_1_8th_week	626,112	623,676 (99.61)	495,938 (79.12)	495,365	0	0	835	829
eel_T_2_8th_week	675,706	667,983 (98.86)	526,223 (77.85)	525,983	0	0	177	164
eel_T_3_8th_week	593,581	590,178 (99.43)	462,354 (77.88)	462,294	0	0	186	179
water_T_2_4th_week	597,758	593,619 (99.31)	484,463 (81.01)	484,294	0	0	722	716
water_T_2_8th_weel	538,341	531,852 (98.80)	434,749 (80.65)	434,217	0	0	715	706
water_T_3_4th_week	442,679	439,840 (99.36)	354,190 (79.99)	353,972	0	0	602	598
water_T_3_8th_week	632,130	627,921 (99.33)	507,416 (79.93)	505,322	0	0	803	797
Total	4106,307	4075,069 (99.24)	3265,333 (79.52)	3261,447	0	0	-	-

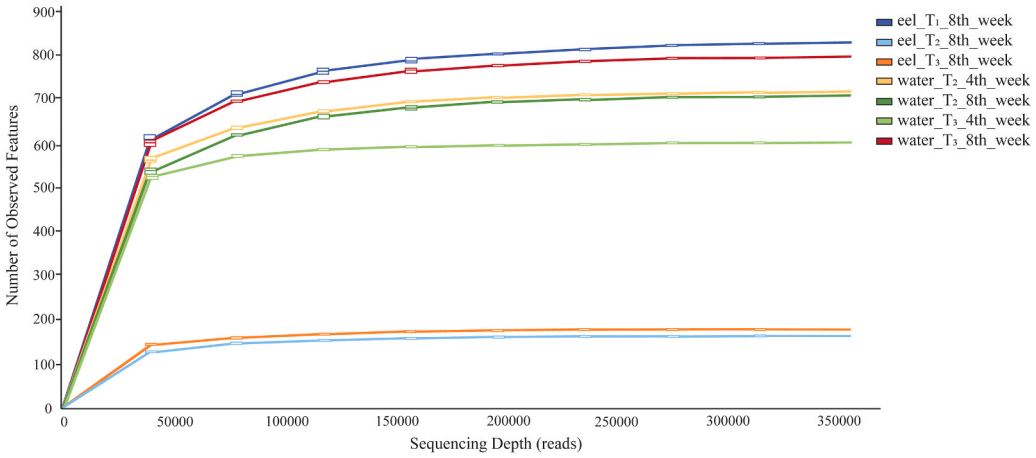

Fig. 4. Rarefaction curves of samples in aquaponics system.

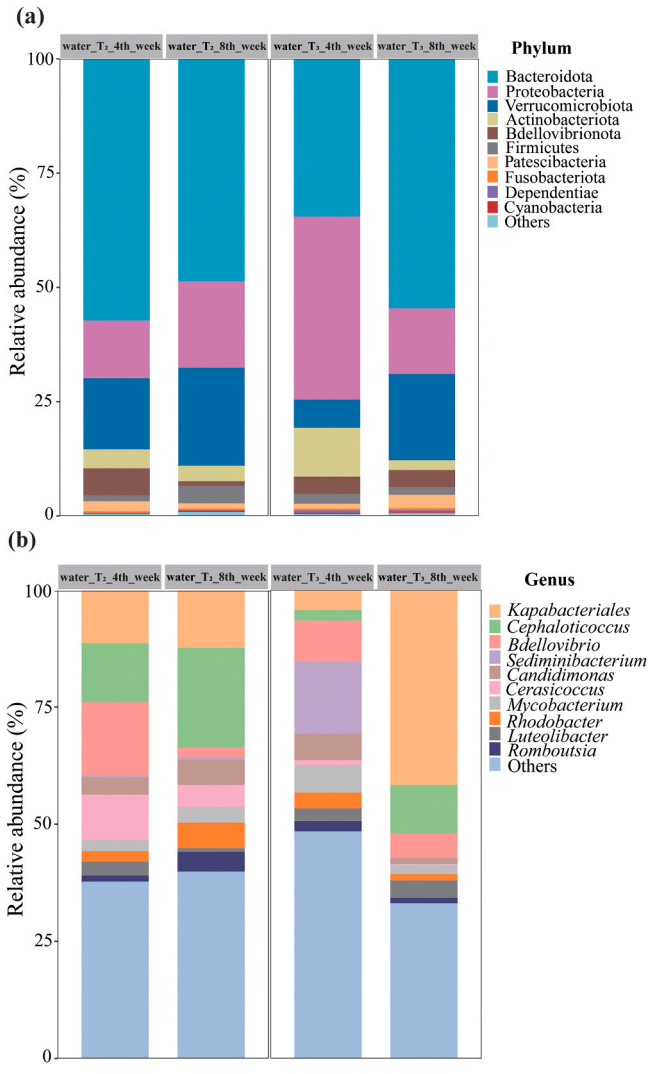

Fig. 5. Relative abundances of dominant microbial taxa in water samples from the aquaponics system at **(a)** the phylum level and **(b)** the genus level across the three experimental groups (T_1 = water-eel; T_2 = eel-apple mint; and T_3 = eel-apple mint-bacteria).

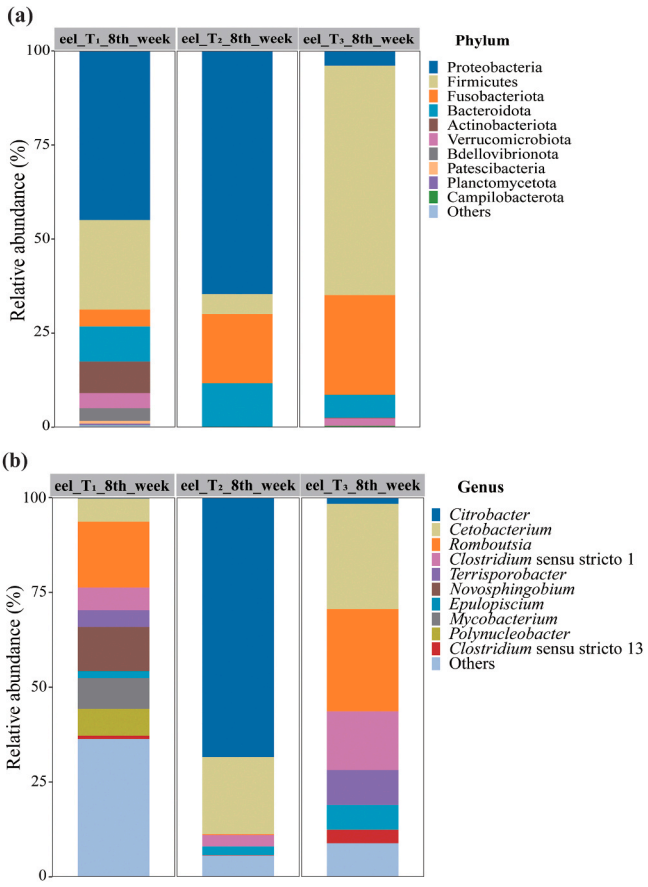

Fig. 6. Relative abundances of dominant microbial taxa in *A. marmorata* from the aquaponics system at **(a)** the phylum level and **(b)** the genus level across the three experimental groups (T_1 = water-eel; T_2 = eel-apple mint; and T_3 = eel-apple mint-bacteria).

crude protein, crude lipid, and ash content (Table 5).

3.6. Eel blood biochemical parameters

At the end of the experiment, no significant differences ($P > 0.05$) were observed in electrolyte parameters among the groups. The highest and lowest concentrations of K^+, Na^+, Cl^-, Ca^{2+}, and TCO_2 were observed in T_3 and T_1, respectively. Mg^{2+} levels were highest in T_1 (2.51 ± 1.98 mmol L^{-1}) and lowest in T_3 (1.63 ± 0.33 mmol L^{-1}),

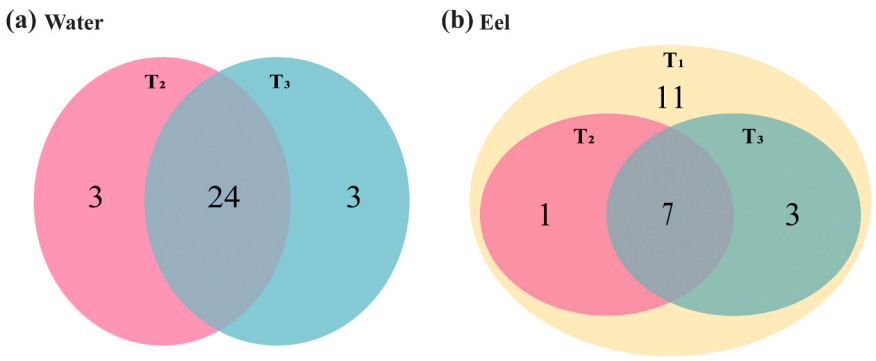

Fig. 7. Venn diagrams illustrating the number of ASVs assigned to each phylum in aquaponics system samples: **(a)** water and **(b)** *A. marmorata*.

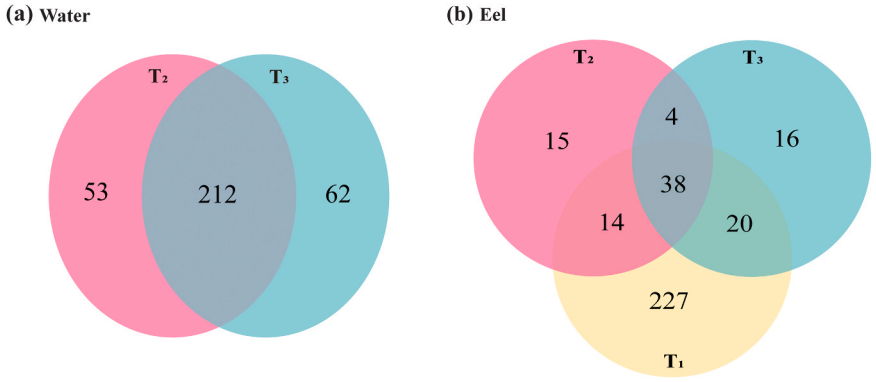

Fig. 8. Venn diagrams illustrating the number of ASVs assigned to each genus in aquaponics system samples: **(a)** water and **(b)** *A. marmorata*.

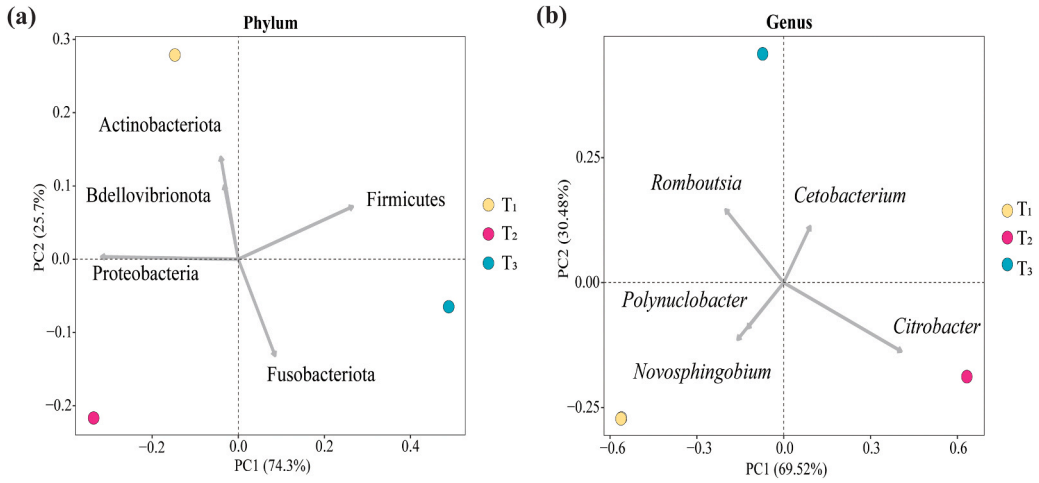

Fig. 9. Principal component analysis (PCA) of microbial communities in *A. marmorata* samples from the aquaponics system across the three experimental groups (T_1 = water-eel; T_2 = eel-apple mint; and T_3 = eel-apple mint-bacteria), shown at **(a)** the phylum level and **(b)** the genus level. PC1 and PC2 represent the principal axes capturing the majority of variation in microbial composition.

whereas PHOS levels were highest in T_2 and lowest in T_1 (Table 6).

Among the comprehensive parameters, TP, GLOB, TB, GGT, AST, ALT, ALP, BUN, GLU, and TG were highest in T_1 and lowest in T_3. In contrast, ALB, A/G, UA, ALP, and Crea levels were highest in T_3, and AMY and TC were highest in T_2 (Table 6).

3.7. Eel intestinal morphological and histological parameters

Administration of nitrifying bacteria significantly ($P < 0.05$) increased the villi length, villi width, villi area, crypt depth, wall thickness, and muscle thickness in T_3 compared to T_1 and T_2 (Figs. 12 and 13).

Additionally, compared to T_1 and T_2, T_3 showed a substantial ($P < 0.05$) increase in intestinal mucosal fold (54.92 ± 3.05 μm), lamina propria width (76.65 ± 5.21 μm), and enterocyte height (253.48 ± 4.47 μm) (Figs. 12 and 13).

4. Discussion

This study represents the first attempt to establish an indoor

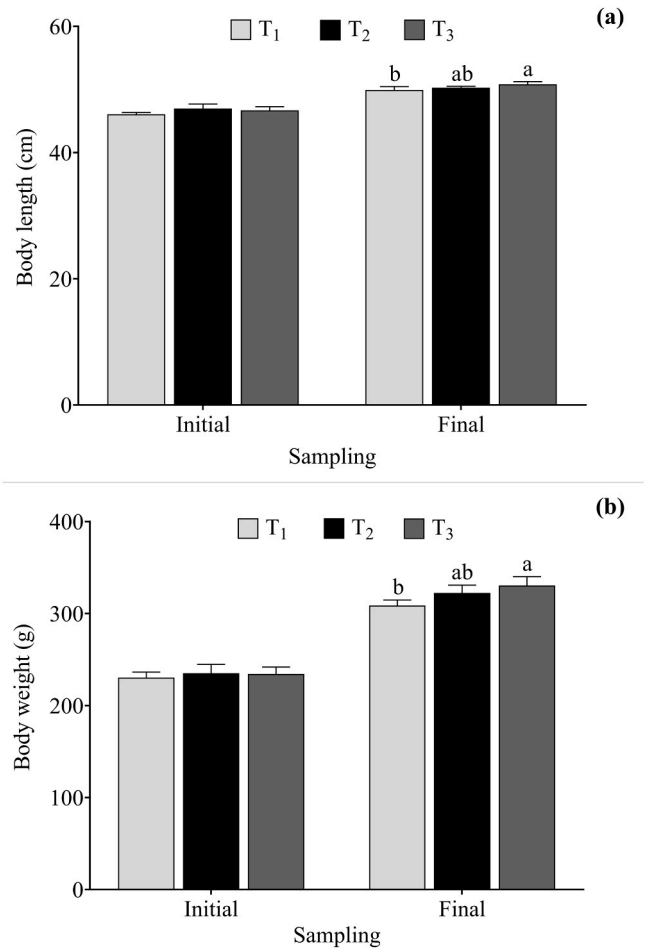

Fig. 10. **(a)** Total body length and **(b)** body weight of *A. marmorata* during the initial and final sampling periods. The mean (± SD) values were calculated based on three replications of each experimental group where T_1 = water-eel; T_2 = eel-apple mint; and T_3 = eel-apple mint-bacteria. Bars with different letters indicate significant differences at $P \leq 0.05$ based on DMRT. The absence of letters denotes no significant differences ($P > 0.05$).

Table 3
Mean growth performance and production of *A. marmorata* used in an indoor aquaponics system.

Traits	Experimental groups			Sig.
	T_1	T_2	T_3	
Initial body length (cm)	46.06 ± 0.29	46.97 ± 0.73	46.70 ± 0.58	NS
Final body length (cm)	49.93 ± 0.54^a	50.28 ± 0.22^a	50.83 ± 0.42^a	*
Length gain (cm)	3.87 ± 0.69	3.32 ± 0.59	4.14 ± 0.28	NS
Length gain (%)	8.41 ± 1.52	7.08 ± 1.36	8.87 ± 0.70	NS
Initial body weight (g)	230.39 ± 6.01	235.13 ± 9.68	234.32 ± 7.61	NS
Final body weight (g)	308.68 ± 6.07^b	322.35 ± 8.48^{ab}	330.43 ± 9.65^a	*
Weight gain (g)	78.29 ± 1.70^c	87.23 ± 1.26^b	96.11 ± 2.24^a	**
Weight gain (%)	34.00 ± 1.21^c	37.16 ± 2.06^b	41.03 ± 0.66^a	**
Specific growth rate (% day^{-1})	0.52 ± 0.02^c	0.56 ± 0.03^b	0.61 ± 0.01^a	**
Daily weight gain (g day^{-1})	1.40 ± 0.03^c	1.56 ± 0.03^b	1.72 ± 0.04^a	**
Feed conversion ratio	2.78 ± 0.10^a	2.55 ± 0.14^b	2.30 ± 0.04^c	**
Feed efficiency (%)	35.97 ± 1.28^c	39.32 ± 2.18^b	43.41 ± 0.70^a	**
Protein efficiency ratio (%)	5.76 ± 0.21^c	6.30 ± 0.35^b	6.96 ± 0.11^a	**
Feeding rate (% day^{-1})	1.44 ± 0.01^a	1.42 ± 0.01^b	1.40 ± 0.00^c	**
Feed intake (g fish^{-1})	217.70 ± 5.69	222.19 ± 9.15	221.43 ± 7.19	NS
Hepatosomatic index (%)	1.46 ± 0.24^b	1.62 ± 0.19^{ab}	1.86 ± 0.23^a	*
Survival rate (%)	100	100	100	-
Eel production (kg m^{-3})	14.82 ± 0.30^b	15.47 ± 0.41^{ab}	15.86 ± 0.46^a	*

Note: Values are expressed as mean ± SD from three replicates. Here, T_1 = water-eel; T_2 = eel-apple mint; and T_3 = eel-apple mint-bacteria. In the column, "Sig." indicates significance. In the rows, means followed by different letters denote significant differences among experimental groups. The absence of letters indicates no significant differences. * indicates significance at $P \leq 0.05$; ** indicates significance at $P \leq 0.01$; and NS indicates non-significance ($P > 0.05$).

aquaponics system integrating giant mottled eel (*Anguilla marmorata*) with apple mint (*Mentha suaveolens*) and to evaluate the impact of nitrifier inoculation (*Nitrosomonas* sp. and *Nitrobacter* sp.) on system performance. The addition of nitrifying bacteria leads to significant improvements in fish growth, feed efficiency, intestinal health, and overall productivity, as well as enhanced plant growth and nutrient cycling. Incorporating nitrifying bacteria optimizes microbial communities, enhancing water quality, which is most important for maintaining a balanced and sustainable aquaponic environment. Metagenomic analysis of water and eel intestinal samples confirmed shifts in microbial composition and functional pathways associated with nitrogen cycling and gut health, underscoring the microbial mechanisms underlying system improvements. These results align with previous research suggesting that microbial optimization enhances resource utilization and promotes environmental sustainability in aquaponics. This discussion interprets the implications of these findings, compares them with existing literature, and explores the potential to scale and improve aquaponic systems to broaden applications in sustainable food production. A limitation of this study is that it was not possible to track in detail the transition process and the dynamics of microbial communities during the experimental period due to single-point analysis. Although the results in week 8 showed clear microbiological differences between the treatment zones, future studies should investigate the temporal dynamics of microbial community formation in more depth by sampling across early, mid, and late experimental stages.

4.1. Water quality parameters

The water temperature ranged from 27.47 °C to 28.13 °C, with no significant differences among the experimental groups, indicating that bacterial activity did not affect this parameter. The optimal temperature range for warm-water fish is 22–32 °C (Yildiz et al., 2017), and *A. marmorata* growth is optimal between 27–33 °C (Luo et al., 2013). These temperature ranges were consistently maintained throughout this experiment. Minor fluctuations in temperature were likely caused by water circulation through the water inlet and outlet systems and by variations in room temperature (Ajijah et al., 2020). The stability of water temperature ensured that environmental factors did not confound the results, enabling a clear assessment of the effects of bacterial supplementation.

DO is a crucial factor in aquaponic systems, supporting both NH_3 oxidation and fish survivability (Effendi et al., 2017). Low DO levels are known to hinder fish growth, feed efficiency, and swimming ability (Yildiz et al., 2017). Maintaining DO concentrations above 5 mg L^{-1} is essential for optimum fish growth performance (Colt, 2006), whereas DO levels below 2 mg L^{-1} can hinder nitrifying bacteria responsible for NH_3 and NO_2 oxidation (Hargreaves, 2006). In this study, DO levels in T_0 ranged from 7.94 to 8.91 mg L^{-1}, and in T_1–T_3, from 6.45 to 7.54 mg L^{-1}, which were within the suitable range throughout the

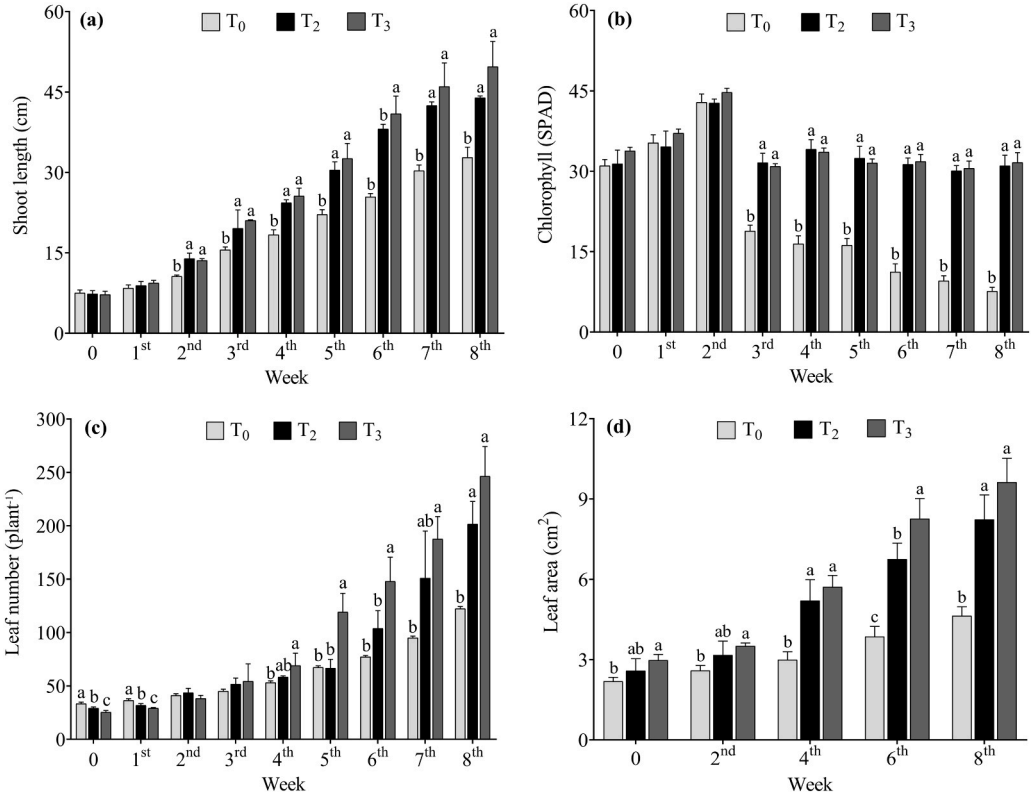

Fig. 11. Recorded **(a)** shoot length, **(b)** chlorophyll content (SPAD), **(c)** leaf number (plant^{-1}) and **(d)** leaf area (cm^2) of apple mint at different sampling weeks throughout the experimental period. The mean (± SD) values were calculated based on three replications for each experimental group, where T_0 = water-apple mint; T_2 = eel-apple mint; and T_3 = eel-apple mint-bacteria. Bars with different letters indicate significant differences at $P \leq 0.05$ based on DMRT. The absence of letters denotes no significant differences ($P > 0.05$).

experiment.

pH is another crucial parameter in aquaponic systems, influencing the health and functionality of fish, plants, and bacteria. The pH gradually decreased over time, likely due to fish respiration and bacterial CO_2 production, which increased H^+ ion concentration and consequently lowered pH levels (Effendi et al., 2017). Additionally, NH_3 oxidation in high-input environments may further contribute to pH reduction (Huynh et al., 2024). As different fish species used in aquaponic systems exhibit varying pH tolerance ranges (Wang et al., 2023), maintaining pH stability is essential to ensure the system's resilience and sustainability. In this experiment, pH values for T_1–T_3 ranged from 5.55 to 6.49, which was close to the optimal range (5.8–6.5) for plant growth (Somerville et al., 2014).

Significant differences in TAN, NO_2, and NO_3 concentrations were observed among all the experimental groups, demonstrating the efficacy of bacterial supplementation in T_3 in influencing nutrient dynamics. Initially, a lag phase was observed in this experiment, during which changes in nitrogen compounds were minimal. This was likely due to the time required for nitrifying bacteria to establish and become active in the system. However, as the experiment evolved, the introduction of *Nitrosomonas* sp. and *Nitrobacter* sp. in T_3 enhanced the nitrogen cycle, leading to improved NH_3 or TAN oxidation and NO_3 production. As a result, TAN levels decreased, while NO_2 and NO_3 concentrations increased due to accelerated microbial activity and nutrient conversion. In aquaponic systems, ammonia-oxidizing bacteria such as *Nitrosomonas* sp. reduce NH_3 or TAN levels, while nitrite-oxidizing bacteria such as *Nitrobacter* sp. convert the unstable intermediate NO_2 to NO_3 under sufficient DO conditions (Effendi et al., 2017; Dwiardani et al., 2021). Despite higher NO_2 levels in T_1, they remained within safe limits for *A. marmorata* (Losordo et al., 1998), underscoring the importance of maintaining oxygen levels for effective nutrient cycling.

NH_4 can be directly utilized by plants, whereas NO_3 must be converted into a usable form prior to uptake (Effendi et al., 2017). Maintaining NO_3 levels below 100 mg L^{-1} is recommended for aquaponic systems (Watson and Hill, 2006), a condition achieved throughout this study. The absence of apple mint in T_1 led to significant decreases in NO_3 levels, as NO_3 was absorbed by microorganisms in the absence of plants (Ajijah et al., 2020). In contrast, the presence of apple mint in T_3 further facilitated NO_3 uptake, thereby enhancing nutrient absorption. The introduction of *Nitrosomonas* sp. and *Nitrobacter* sp. in T_3 facilitated the conversion of TAN to NO_3, thereby enhancing nutrient availability for both plants and fish (Effendi et al., 2015, 2017; Ajijah et al., 2020). Concentrations of key nitrogenous compounds (TAN, NO_2, and NO_3) in this experiment were measured using API freshwater test kits to monitor temporal trends and ensure operational stability of the zero-water-exchange system. Similar types of test kits are commonly used for routine monitoring in aquaculture and aquaponics research (Nadia et al., 2023; Akter et al., 2024; Emerenciano et al., 2025). However, these kits have limitations in precision and accuracy compared with advanced analytical techniques such as spectrophotometry or ion chromatography. As a result, nitrogen-related results in this study are interpreted in terms of relative changes and general trends, rather than absolute concentration. Given the importance of accurate nitrogen quantification in zero-water-exchange aquaponic systems, future studies should use standardized analytical methods (e.g., APHA-based spectrophotometric assays or ion chromatography) to improve measurement accuracy and strengthen mechanistic interpretation.

EC is another crucial parameter for aquaponics plant growth (Yuhasari et al., 2021) and is closely related to TDS, which measures dissolved mineral salt concentration (Channa et al., 2024). In this study, the EC values remained within the optimal range (<1500 μs cm^{-1}) for

Table 4
Mean growth performance of apple mint plant and leaf production in an indoor aquaponics system.

Traits	Experimental groups			Sig.
	T_0	T_2	T_3	
Initial plant length (cm)	20.89 ± 0.39	21.69 ± 1.51	21.54 ± 0.40	NS
Final plant length (cm)	50.89 ± 0.67^{c}	65.67 ± 1.92^{b}	73.87 ± 3.87^{a}	**
Initial plant weight (g)	2.64 ± 0.19	2.67 ± 0.24	2.42 ± 0.28	NS
Final plant weight (g)	11.04 ± 1.33^{b}	19.24 ± 2.61^{a}	19.91 ± 1.96^{a}	**
Initial shoot length (cm)	7.18 ± 0.81	7.35 ± 0.60	7.24 ± 0.59	NS
Final shoot length (cm)	32.89 ± 0.94^{c}	43.94 ± 0.34^{b}	49.76 ± 4.68^{a}	**
Root-shoot ratio	0.46 ± 0.06^{a}	0.19 ± 0.02^{b}	0.20 ± 0.02^{b}	**
Initial root length (cm)	12.05 ± 1.57	14.35 ± 0.92	14.30 ± 0.98	NS
Final root length (cm)	17.49 ± 1.27^{b}	22.36 ± 2.45^{a}	24.10 ± 1.43^{a}	**
Fresh root weight (g)	3.00 ± 0.46^{b}	3.04 ± 0.42^{b}	3.93 ± 0.27^{a}	*
Fresh shoot weight (g)	6.41 ± 0.40^{c}	16.18 ± 1.06^{b}	20.04 ± 1.44^{a}	**
Leaf length (cm)	2.37 ± 0.07^{c}	3.28 ± 0.17^{b}	3.61 ± 0.11^{a}	**
Leaf width (cm)	1.95 ± 0.09^{b}	2.45 ± 0.17^{a}	2.65 ± 0.16^{a}	**
Leaf production (g m^{-2})	201.21 ± 16.52^{c}	395.83 ± 31.55^{b}	567.92 ± 21.31^{a}	**

Note: Values are expressed as mean ± SD from three replicates. Here, T_0 = water-apple mint; T_2 = eel-apple mint; and T_3 = eel-apple mint-bacteria. In the column, "Sig." indicates significance. In the rows, means followed by different letters denote significant differences among experimental groups. The absence of letters indicates no significant differences. * indicates significance at $P < 0.05$; ** indicates significance at $P \leq 0.01$; and NS indicates non-significance ($P > 0.05$).

Table 5
Moisture, crude protein, crude lipid, and ash contents of *A. marmorata* at the end of the experiment in an indoor aquaponics system.

Nutrient components	Experimental groups			Sig.
	T_1	T_2	T_3	
Moisture (%)	65.47 ± 0.53^{b}	68.19 ± 0.33^{a}	69.02 ± 0.80^{a}	**
Crude protein (%)	16.78 ± 0.18^{b}	17.36 ± 0.08^{a}	17.79 ± 0.45^{a}	**
Crude lipid (%)	8.91 ± 0.57^{a}	8.84 ± 0.10^{a}	7.41 ± 0.17^{b}	**
Ash (%)	2.33 ± 0.03^{a}	2.23 ± 0.03^{b}	1.96 ± 0.02^{c}	**

Note: Values are presented as mean ± SD from three replicates per experimental group (n = 3). Here, T_1 = water-eel; T_2 = eel-apple mint; and T_3 = eel-apple mint-bacteria. In the column, "Sig." indicates significance. In the rows, means followed by different letters denote significant differences among the experimental groups. ** indicates significance $P \leq 0.01$.

aquaponics (Somerville et al., 2014), and the highest TDS levels observed in T_3 were also within the suitable range (<1000 mg L^{-1}) (Setiadi et al., 2019).

Variations in K, Ca, Mg, and S concentrations were observed among the experimental groups, with the highest levels recorded in T_3. T_2 and T_3 show a similar trend, suggesting that apple mint influences K, Ca, Mg, and S concentrations, while the effect of bacteria is limited. Khanjani and Sharifinia (2022) observed that increased nutrient availability often correlates with higher microbial consumption, highlighting the importance of balanced nutrient supplementation strategies. This study demonstrates the potential of bacterial supplementation to enhance nutrient cycling and overall system performance in aquaponics. Future research should aim to optimize bacterial formulations for specific fish-plant systems and assess their long-term commercial scalability.

Table 6
Electrolyte and comprehensive parameters of *A. marmorata* used in an indoor aquaponics system.

Parameters	Experimental groups			Sig.
	T_1	T_2	T_3	
Electrolyte parameters				
K^+ (mmol L^{-1})	2.40 ± 0.53	2.59 ± 1.21	3.48 ± 1.26	NS
Na^+ (mmol L^{-1})	126.74 ± 24.18	140.68 ± 5.59	144.60 ± 3.29	NS
Cl^- (mmol L^{-1})	92.96 ± 36.00	113.44 ± 4.39	115.25 ± 5.20	NS
Ca^{2+} (mmol L^{-1})	2.18 ± 1.04	2.38 ± 0.08	2.43 ± 0.07	NS
Mg^{2+} (mmol L^{-1})	2.51 ± 1.98	2.10 ± 0.62	1.63 ± 0.33	NS
PHOS (mmol L^{-1})	3.29 ± 1.78	4.38 ± 0.95	3.64 ± 0.51	NS
TCO_2 (mmol L^{-1})	7.62 ± 2.73	9.58 ± 1.40	9.80 ± 0.35	NS
Comprehensive parameters				
ALB (g L^{-1})	9.58 ± 3.68	11.36 ± 2.63	11.66 ± 2.63	NS
TP (g L^{-1})	64.76 ± 33.60	47.58 ± 11.08	38.76 ± 8.80	NS
GLOB (g L^{-1})	38.44 ± 23.05	36.24 ± 10.66	27.08 ± 6.52	NS
A/G	0.28 ± 0.12	0.33 ± 0.10	0.43 ± 0.07	NS
TB (umol L^{-1})	5.18 ± 4.26	2.44 ± 2.55	2.30 ± 2.48	NS
GGT (U L^{-1})	29.80 ± 16.89	26.20 ± 11.97	22.60 ± 13.03	NS
AST (U L^{-1})	266.00 ± 220.91	210.00 ± 248.36	205.20 ± 253.45	NS
ALT (U L^{-1})	37.60 ± 35.37	14.20 ± 13.59	8.80 ± 2.59	NS
ALP (U L^{-1})	469.60 ± 468.45	204.40 ± 83.14	298.20 ± 63.26	NS
AMY (U L^{-1})	850.80 ± 335.73	1140.40 ± 522.22	1033.60 ± 853.38	NS
Crea (umol L^{-1})	46.84 ± 64.02	13.64 ± 9.60	19.12 ± 19.80	NS
UA (umol L^{-1})	9.72 ± 0.19	9.72 ± 0.19	14.42 ± 10.27	NS
BUN (mmol L^{-1})	4.32 ± 3.42	3.56 ± 0.66	2.94 ± 0.37	NS
GLU (mmol L^{-1})	20.43 ± 7.09	19.85 ± 11.01	19.43 ± 1.97	NS
TC (mmol L^{-1})	12.53 ± 0.41	12.64 ± 0.24	12.33 ± 0.89	NS
TG (mmol L^{-1})	10.64 ± 0.17	10.52 ± 0.48	9.68 ± 1.43	NS

Note: Values are presented as mean ± SD from three replicates per experimental group (n = 5). Here, T_1 = water-eel; T_2 = eel-apple mint; T_3 = eel-apple mint-bacteria; K^+ = potassium; Na^+ = sodium; Cl^- = chlorine; Ca^{2+} = calcium; Mg^{2+} = magnesium; PHOS = phosphorous; TCO_2 = total carbon dioxide; ALB = albumin; TP = total protein; GLOB = globulin; A/G = albumin/globulin ratio; TB = total bilirubin; GGT = gamma-glutamyl transferase; AST = aspartate aminotransferase; ALT = alanine transaminase; ALP = alkaline phosphate; AMY = amylase; Crea = creatine; UA = uric acid; BUN = blood urea nitrogen; GLU = glucose; TC = total cholesterol; and TG = triglyceride. In the column, "Sig" denotes significance. In the rows, means followed by the absence of letters denote no significant differences ($P > 0.05$).

4.2. Bacterial abundance

Bacterial abundance plays a pivotal role in aquaponic systems by driving nutrient cycling and maintaining water quality, both of which are essential for system efficiency. In this study, at the end of the experiment, the highest bacterial abundance (76950 ± 5727 CFU mL^{-1}) was observed in T_3. These results exceed bacterial densities reported in previous studies on aquaponics with nitrifying bacteria (Ajijah et al., 2020: 5.3×10^4 CFU mL^{-1} on day 28) but remain lower than those observed by Wahyuningsih et al. (2015) (10^6 CFU mL^{-1}) and Effendi et al. (2017) (1.15×10^6 CFU mL^{-1} on day 35). Differences in bacterial abundance are likely influenced by factors such as nutrient availability, initial bacterial density, fish species, inoculation frequency, and the deliberate introduction of bacteria to T_3, which likely contributed to the higher bacterial count observed in this treatment. It is reasonable to assume that the introduced bacteria are thriving in the tank and effectively improving water quality. Research by Tyson et al. (2008) and Effendi et al. (2017) underscores that maintaining optimal bio-filtration conditions, particularly pH balance, is more crucial for system performance than achieving high bacterial densities alone. In this experiment, the TPC method was used to measure total culturable bacterial abundance across groups, but it did not differentiate between introduced

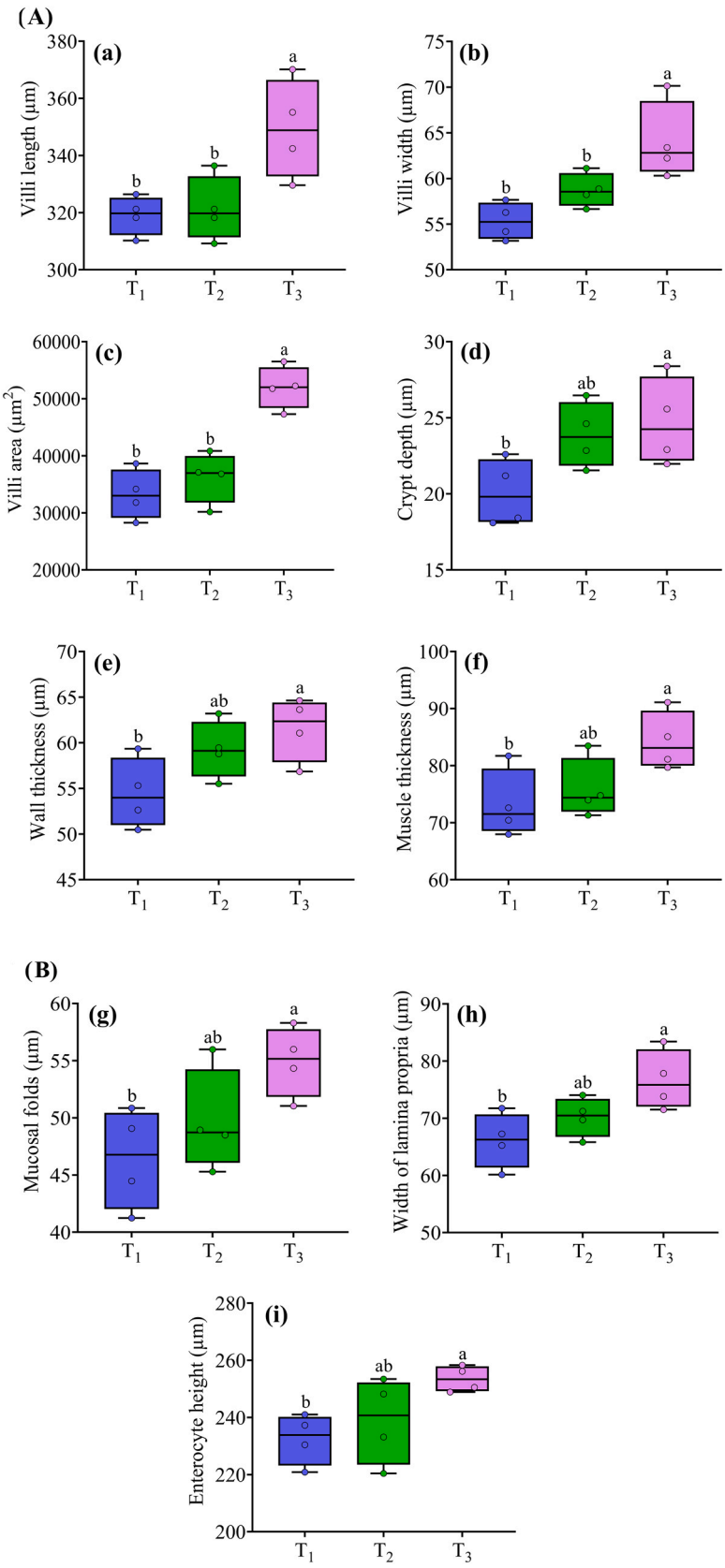

Fig. 12. **(A)** Intestinal morphology and **(B)** histology parameters reflecting both digestive and immune response of *A. marmorata* among the different experimental groups in an indoor aquaponics system. In the box, different letters denote significant differences among experimental groups at $P < 0.05$.

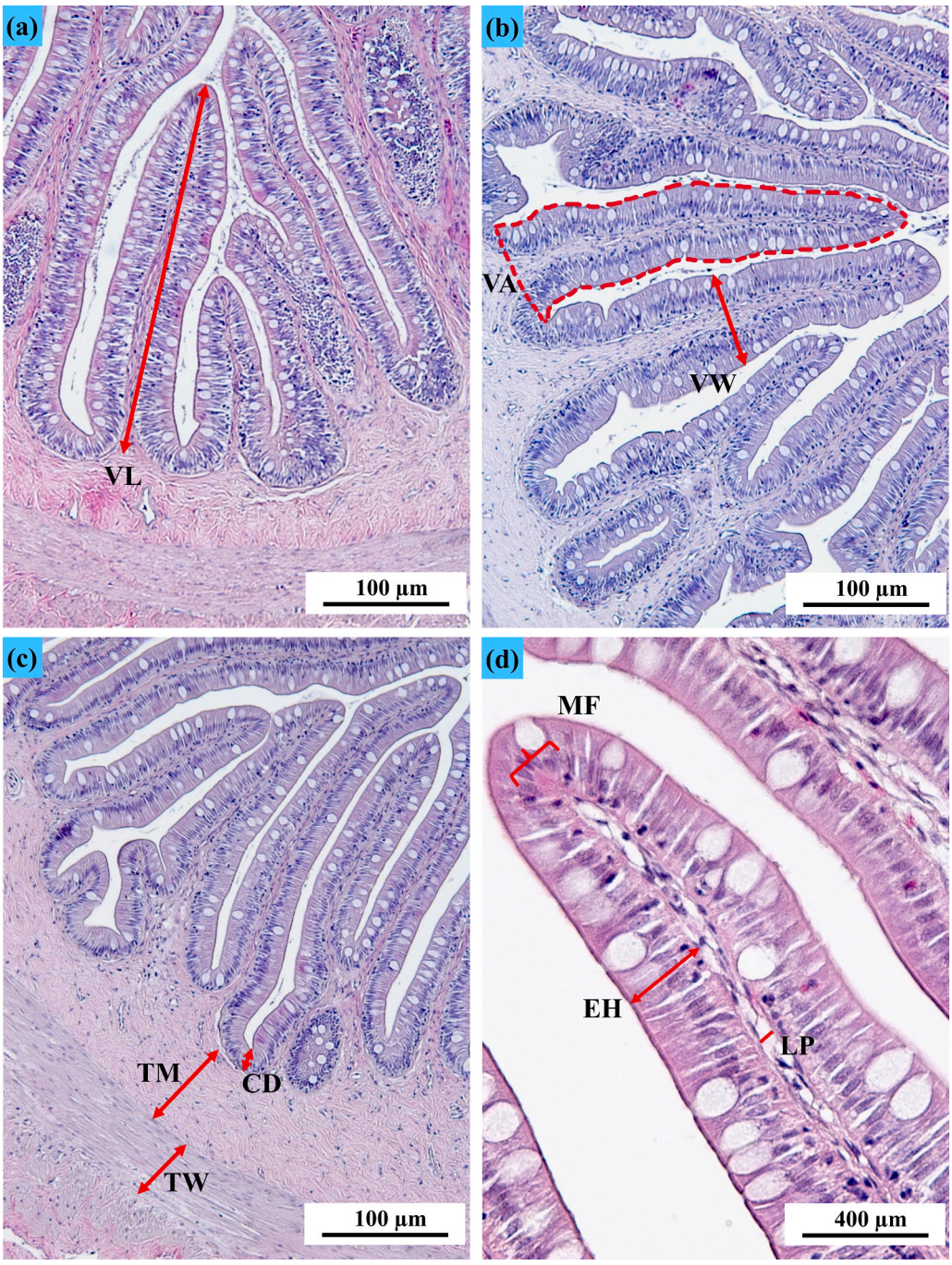

Fig. 13. Histological changes (**a-c**) of the intestine of *A. marmorata* and digestive and immune response indicators (d) of intestinal histology of *A. marmorata* were used in this study for 8 weeks. **(a)** = T_1 (water-eel); **(b)** = T_2 (eel-apple mint); and **(c)** = T_3 (eel-apple mint-bacteria); VL = villus length; VW = villus width; VA = villus area; CD = crypt depth; TM = muscle thickness; TW = wall thickness; MF = fattening of mucosal folds; LP = width of lamina propria; and EH = enterocyte height.

nitrifying bacteria and the background microbial community. Therefore, the presence and activity of the added nitrifying bacteria were inferred indirectly from nitrogen transformation dynamics (TAN, NO_2, and NO_3), rather than by strain-specific identification. Additionally, this method has limitations, including the inability to detect non-culturable microorganisms and reliance on microbial substrate utilization (Rojas-Tirado et al., 2017). Hence, advanced molecular methods, such as metaproteomics (Jose et al., 2020), metabolomics (Roques et al., 2020), and metagenomics (Tepaamorndech et al., 2020) may offer more comprehensive insights into microbial dynamics and their roles in aquaponic systems (Lorgen-Ritchie et al., 2023).

Overall, the water samples exhibited relatively stable species compositions across treatments, whereas the eel samples showed distinct compositional differences depending on treatment (Fig. 8). These findings suggest that subtle environmental differences may significantly influence eel gut microbial diversity. The diverse microbial community that colonizes the GIT (gut microbiota) plays a critical role in modulating the host's physiology (Feng et al., 2018; Butt and Volkoff, 2019). Biotic (e.g., genotype, physiological status, pathobiology, lifestyle) and abiotic (e.g., environmental) factors may affect the fish gut microbiota and influence its composition and diversity, as well as its function and metabolic activity, thus affecting feeding, growth, energy storage, and

health of the fish (Luan et al., 2023).

Citrobacter exhibited a strong dominance in the T_2 group (Fig. 6b). *Citrobacter* species are known to cause infections in various fish species, including *Anguilla japonica* (Jeong et al., 2025) and are opportunistic bacterial pathogens (Huang et al., 2022). However, under T_3 conditions, the relative abundance of *Citrobacter* was reduced (Fig. 6b). This suggests that the biological enhancement of nitrifying bacteria in T_3 may have contributed to a safer and healthier aquaponics environment by inhibiting the growth of opportunistic pathogens, such as *Citrobacter*, through competitive exclusion.

As a result of PCA analysis, the structure of the intestinal microbial community showed distinct differences across treatment conditions. This suggests that changes in the aqueous environment, such as whether nitrifying bacteria are added, directly affect the composition of the eel's intestinal microorganisms (Fig. 9b). The T_1 group showed a strong association with *Polynucleobacter* and *Novosphingobium*. *Polynucleobacter* is the most representative Planktonic bacterium of the world's freshwater environment (Hahn, 2006), and the genus *Novosphingobium* is known to have been isolated from a wide range of environmental matrices, including soil, freshwater, seawater, and sediments (Belmok et al., 2023). The dominance of these strains in T_1 suggests that, in the absence of separate treatment, the eel gut microbiota tends to reflect the microbiological composition of the external feedwater. The T_2 group showed a strong association with *Citrobacter*, a known opportunistic pathogen (Huang et al., 2022). This indicates, as mentioned above, that intestinal microbiosis may occur in the absence of adequate biological control (T_3), thereby creating an environment in which potential pathogens are prone to proliferation. On the other hand, in the T_3 group, *Cetobacterium* and *Romboutsia* were dominant. In particular, *Cetobacterium* is a dominant bacterium in the intestines of several freshwater fish, well known for producing vitamin B_{12} through protein fermentation and for supporting the metabolic health of fish (Ramírez et al., 2018). *Romboutsia* is also a symbiotic bacterium that helps maintain intestinal homeostasis (Bu et al., 2023). Therefore, the T_3 condition provides the most suitable microbiological environment for eels, thereby excluding pathogens (*Citrobacter*) and promoting the settlement of beneficial key strains (*Cetobacterium*).

4.3. Growth performances of A. marmorata

Growth is a key physiological indicator commonly used to evaluate social stress due to its ease of measurement (Tan et al., 2018). In this study, SGR, DWG, weight gain, and percent weight gain (%) showed highly significant differences among the experimental groups. *A. marmorata* production (kg m^{-3}) was highest in T_3, followed by T_2 and T_1, reflecting variations in TAN concentrations. Elevated TAN levels in T_1 adversely affected appetite, energy utilization, and growth. These findings align with those of Losordo et al. (1998) and Effendi et al. (2017), who documented the detrimental effects of NH_3 on growth performance, liver function, and gill health. Prolonged exposure to elevated NH_3 concentrations further resulted in reduced appetite, impaired digestion, depletion of liver glycogen, and increased blood acidity, thereby heightening susceptibility to hypoxia.

The FCR, FE, and FI were partially consistent with the results reported by Tan et al. (2018) for *A. marmorata* in recirculatory aquaculture systems. Additionally, FE and HSI values corresponded with those reported by Shahkar et al. (2016) for *A. japonica*, reflecting shared physiological responses under aquaculture conditions. Enhanced enzyme activity, improved nutrient digestibility, and efficient absorption, facilitated by bacterial inoculation, contributed to improved growth performance (Gaffar et al., 2023). The inoculated bacteria, which also act as probiotics, served as growth promoters by stimulating appetite, improving metabolic processes, and ensuring efficient digestion and feed utilization (Rahayu et al., 2024). Consequently, bacterial inoculation in T_3 significantly improved *A. marmorata* growth performance compared to T_1 and T_2. This study underscores the pivotal role of bacterial inoculation in enhancing growth performance by mitigating the negative impacts of TAN and improving digestion and nutrient uptake. Further research should focus on optimizing bacterial formulations and investigating their long-term effects on fish health and production efficiency in various aquaculture systems.

Moreover, *Cetobacterium* showed an increasing trend in both T_2 and T_3, with the highest proportion observed in the T_3 group (Fig. 6b). *Cetobacterium* is an anaerobic indigenous bacterium present in the gut of most freshwater fish (Tsuchiya et al., 2008). In normally growing eels, *Cetobacterium* was identified as the predominant genus in the gut microbiota, with *C. somerae* accounting for more than 98.7% of the sequences within this genus. Interestingly, this taxon was largely absent in stunted-growth eels and is rarely found in culture-dependent studies of fish, although it has been detected in various other freshwater species (Silva et al., 2011; Di Maiuta et al., 2013; Larsen et al., 2014; Bledsoe et al., 2016). These suggested that gut indigenous anaerobic bacteria and their metabolites played a non-negligible role in maintaining the host's health. This study also found that *Cetobacterium* was a dominant member of the gut microbiota of healthy fish, while its levels were significantly reduced in the gut of infected fish, suggesting that *Cetobacterium* served as a sensor of health, especially in fish infected with pathogenic bacteria (Qi et al., 2023). In addition, *Cetobacterium* can synthesize fats, proteins, and carbohydrates used by the host and plays an important role in growth and development (Hsu et al., 2018). Previous studies have reported that *C. somerae* produces substantial amounts of vitamin B_{12} and acetic acid, which are beneficial to the host by supporting protein synthesis and enhancing carbohydrate and lipid metabolism in conjunction with coenzyme A (Finegold et al., 2003; Tsuchiya et al., 2008). *Cetobacterium* has the ability to produce vitamin B_{12}. Supplementation of vitamin B_{12} reduces the redox potential in the gut, alters gut microbiome structure and function, and improves microbial interactions and enhances the stability of the gut microbiota network (Qi et al., 2023). Thus, this bacterium, which is present in the gut of normal-growth farmed *A. marmorata* at high abundance, may play an important role in the healthy growth of cultured marbled eels (Lin et al., 2019).

In the T_3 group of eel samples, *Romboutsia* was the second most dominant genus after *Cetobacterium*. *Romboutsia* possesses diverse metabolic capabilities, facilitating carbohydrate fermentation and aiding amino acid utilization (Therdtatha et al., 2021). *Romboutsia sedimentorum* can ferment glucose as the sole carbon source, producing short-chain fatty acids such as acetic acid, isobutyric acid, and isovaleric acid (Wang et al., 2015).

4.4. Growth performances of apple mint

The mean length of apple mint plants at the end of the experiment (50.89 ± 0.67 cm to 73.87 ± 3.87 cm) exceeded the results reported by Choi and Chiang (2013) in hydroponic systems and Baek et al. (2016) in traditional systems. Additionally, the fresh shoot and root weights were higher, whereas the leaf length and width were lower compared to those reported in the aforementioned studies. These differences are potentially attributable to the aquaponics system and the introduction of nitrifying bacteria in this study.

Apple mint growth was significantly enhanced in both the T_2 and T_3 groups compared to the water-apple mint group (T_0). Notably, in the T_0 group, the chlorophyll concentration dropped sharply after just 3 weeks. Additionally, chlorophyll concentration, as indicated by SPAD values, was significantly higher in T_3 than in T_2 and T_0, from the 3rd week through the 8th week, reflecting improved nutritional status and photosynthetic capacity due to bacterial inoculation (Taha et al., 2024). Furthermore, the T_3 group outperformed T_2, with statistically significant improvements in shoot length, leaf number, and leaf area observed at 6th weeks, likely attributable to enhanced nutrient availability from aquaculture effluent and bacterial activity (Nozzi et al., 2018; Ajijah et al., 2020). Nitrifying bacteria contribute to nutrient cycling by

converting NH_4^+ and NH_3 into NO_3, which are readily absorbed by plants without harmful effects (Goddek et al., 2019). Apple mint leaf production at the end of the experiment in T_3 was 64.57% and 30.30% higher than in T_2 and T_0, respectively (Table 4), highlighting the beneficial impact of nitrifying bacteria on plant production in aquaponic systems. This finding aligns with the results reported by Wahyuningsih et al. (2015), who observed enhanced growth of lettuce and vetiver grass in similar systems with bacterial supplementation. Enhanced nutrient availability from fish feed and tank inputs, such as minerals and nitrogenous compounds, likely contributed to this outcome (Pinho et al., 2017). Thus, under these experimental conditions, the addition of nitrifying bacteria did have a positive impact on apple mint growth performance. Additionally, bacterial inoculation aids in the breakdown of organic matter, releasing essential nutrients and reducing harmful pathogens. This process leads to improved nutrient absorption and higher agricultural yields (Nadia et al., 2023; Kasozi et al., 2023).

4.5. Proximate composition of *A. marmorata*

To the best of our knowledge, no previous studies have examined the body composition of *A. marmorata* in an aquaponics system. Notably, fish body composition is a crucial indicator of health and quality (Xie et al., 2025). In this study, the proximate composition of *A. marmorata* aligns with the findings of Tan et al. (2018) in a recirculatory aquaculture system. Furthermore, inoculating nitrifying bacteria significantly improved the body composition in T_3, enhancing eel digestibility and feed protein utilization. Consequently, this led to a reduction in lipid content and an increase in protein content. These findings are further supported by Nadia et al. (2023), who also observed similar improvements in fish composition with probiotic supplementation in aquaponics systems.

4.6. Eel blood biochemical parameters

Blood biochemical parameters are commonly used to assess fish health, physiological status, and stress levels (Xie et al., 2025). In this experiment, no significant differences in blood biochemical parameters were observed among treatments with or without the addition of nitrifying bacteria. The stability of these parameters suggests that eels maintained physiological homeostasis despite the introduction of the different treatments. Fish often exhibit robust internal regulatory mechanisms that buffer mild environmental changes, especially under stable water-quality conditions (Bojarski et al., 2025). Consistently, other studies have reported that not all biochemical variables show significant changes unless fish are exposed to severe stressors or toxic levels of water quality parameters (Bojarski et al., 2025; Fortunato et al., 2025). In this experiment, TAN, NO_2, and NO_3 remained within compatible ranges for eel culture, likely preventing physiological stress. Therefore, the aquaponics system maintained water quality within a range that avoided significant stress or physiological disruption in eels. Future studies should use additional biomarkers or longer exposure periods to improve the detection of stress response.

4.7. Eel intestinal morphological and histological parameters

The fish intestine primarily functions to digest and absorb nutrients, while also serving as a barrier against infections (Firdaus-Nawi et al., 2013). This study demonstrated that administering nitrifying bacteria significantly enhanced several key intestinal morphological parameters in eels compared with T_1 and T_2. These improvements may be attributed to the beneficial effects of nitrifying bacteria on the intestinal environment, including the modulation of microbial communities, reduction of harmful metabolites, and enhancement of nutrient availability. These findings align with similar studies, such as Asha et al. (2024), who found that adding bacteria with enzymes to the water improved villus length,

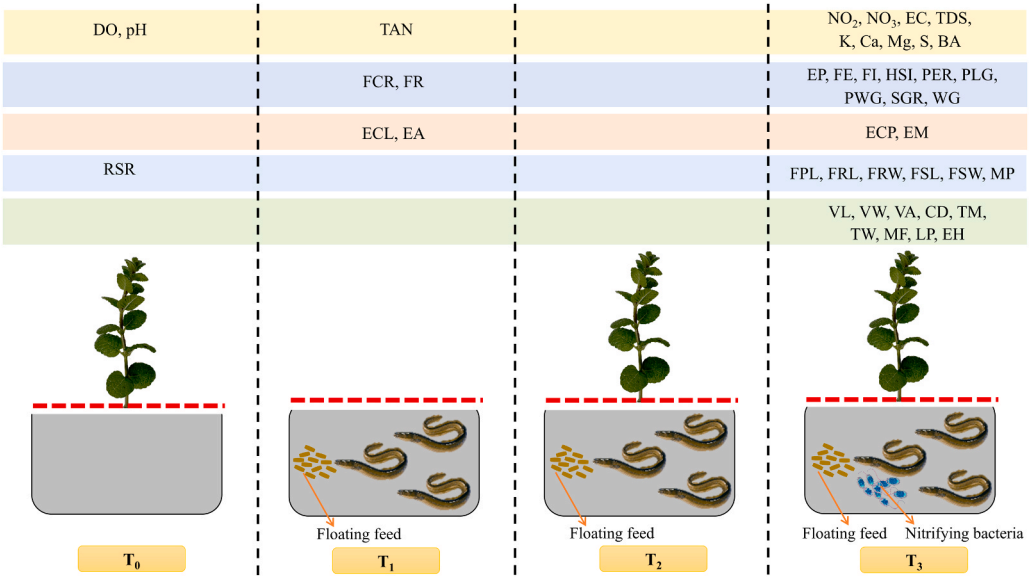

Fig. 14. Conceptual framework of the experiment. In the diagram, the four experimental groups (T_0 = water-apple mint; T_1 = water-eel; T_2 = eel-apple mint; and T_3 = eel-apple mint-bacteria) are delineated. Additionally, the different colors of horizontal stripes on the top of this figure (1st stripe = water quality parameters; 2nd stripe = eel growth; 3rd stripe = proximate composition; 4th stripe = plant growth; and 5th stripe = intestinal histo-morphological parameters, respectively) indicated the highest significant outcomes of the most important parameters of this experiment. The following abbreviations are used: BA = bacterial abundance; Ca = calcium; CD = crypt depth; DO = dissolved oxygen; EA = eel ash content; EC = electrical conductivity; ECL = eel crude lipid; ECP = eel crude protein; EH = enterocyte height; EM = eel moisture content; EP = eel production; FCR = feed conversion ratio; FE = feed efficiency; FI = feed Intake; FPL = final plant length; FR = feeding rate; FRL = fresh root length; FRW = fresh root weight; FSL = fresh shoot length; FSW = fresh shoot weight; HSI = hepatosomatic index; K = potassium; LP = width of lamina propria; MF = fattening of mucosal folds; Mg = magnesium; MP = mint production; NO_2 = nitrite; NO_3 = nitrate; PER = protein efficiency ratio; PLG = percent length gain of *A. marmorata*; PWG = percent weight gain of *A. marmorata*; RSR = root-shoot ratio; S = sulfur; SGR = specific growth rate; TAN= total ammonia nitrogen; TDS = total dissolved solids; TM = muscle thickness; TW = wall thickness; VA = villus area; VL = villus length; VW = villus width; and WG = weight gain of *A. marmorata*.

crypt depth, muscle thickness, and intestinal thickness in Nile Tilapia using biofloc technology. Villus height and crypt depth are fundamental indicators of intestinal function and health, with increased villus height contributing to a larger surface area, thereby enhancing nutrient absorption (Wei et al., 2020). This is supported by Bieczynski et al. (2021), who showed that greater intestinal surface area directly correlates with improved intestinal performance. The thickness of the intestinal muscle layer is an important indicator of peristalsis and contraction capabilities, which are essential for effective feed digestion and the reduction of chyme circulation (Bian et al., 2021; Jiao et al., 2023). Furthermore, the thickness of the lamina propria, rich in macrophages, neutrophils, and lymphoid cells, is a crucial element in the immune response, with its concentration correlating to lymphoid cell activity (Zhang et al., 2019). Mucosal immunity, characterized by the presence of fatty protrusions in the intestinal tract, indicates enhanced digestion, metabolism, and nutrient absorption (Asha et al., 2024). This study found significant improvements in mucosal fold and lamina propria, likely due to the role of nitrifying bacteria in promoting a more stable and beneficial gut microbiota, reducing oxidative stress, and enhancing immune function. Notably, the significant increase in enterocyte width in the nitrifying bacteria-treated group suggests improved nutrient absorption capacity, as wider enterocytes can enhance nutrient transport and uptake across the intestinal wall. This could result from nitrifying bacteria facilitating more efficient nitrogen cycling and microbial homeostasis, thereby improving the overall gut environment. Hence, the administration of nitrifying bacteria in aquaponic systems significantly improves key intestinal morphological and histological parameters, enhancing digestion, immune defense, and overall fish health.

Additionally, at the end of the experiment, the highest significant outcomes of the most important parameters are presented in Fig. 14. Future research should specifically focus on investigating the long-term effects of nitrifying bacteria administration, analyzing the mechanisms underlying interactions between microbial communities and fish physiology, and determining optimal bacterial strains and dosages to maximize system efficiency and fish welfare.

5. Conclusions

This study successfully established and evaluated a novel indoor aquaponics system integrating giant mottled eel (*A. marmorata*) with apple mint (*M. suaveolens*). Nitrifier inoculation (*Nitrosomonas* sp. and *Nitrobacter* sp.) in T_3 enhanced eel growth performances (lower FCR and FR; higher SGR, DGR, and PER), improved intestinal health and proximate composition, boosted plant biomass accumulation, and stabilized water quality. Metagenomic analysis confirmed shifts in microbial composition and functional pathways linked to nitrogen cycling and gut health, underscoring the pivotal role of nitrifying bacteria in system efficiency.

Future research should focus on exploring the long-term effects of various bacterial species on aquaponics system stability, investigating microbial community dynamics, and evaluating the economic feasibility of such systems on a larger scale. Additionally, further research should explore the applicability of this approach to a wider range of plant and fish species to support broader implementation in sustainable food production systems.

CRediT authorship contribution statement

Prosun Roy: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hyeongwoo Choi:** Writing – review & editing, Visualization, Validation, Methodology, Formal analysis. **Haeyong Park:** Software, Methodology, Formal analysis, Data curation. **Chi-une Song:** Software, Data curation. **Daehwan Lee:** Methodology. **Sungmin Choi:** Methodology, Data curation. **Sung-Ju Jung:** Writing – review & editing, Validation, Resources, Project administration, Funding acquisition, Data curation, Investigation. Gyeong Jae Kim: Methodology. **Jeongwhui Hong:** Writing – review & editing. **Seong-il Eyun:** Writing – review & editing, Validation, Resources, Project administration, Funding acquisition, Data curation. **Sang Duk Choi:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Consent for publication

Not applicable.

Declaration of Competing Interest

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

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Data availability

Data will be made available on request.

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