



The functional diversity of microbial communities in the multi-compartment biofilters of shrimp mariculture effluents using 16S rRNA metabarcoding

Betina Lukwambe¹ · Regan Nicholous² · Zhongming Zheng³

Received: 15 February 2023 / Accepted: 30 June 2023

© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2023

Abstract

Microbes play important roles in mariculture biofilter systems, such as biogeochemical cycling of nutrients and organic matter degradation. However, their functional diversity for nutrient removal in shrimp mariculture effluents influenced by multi-compartment biofilters remained elusive. This study explored the functional diversity of microbial communities for nutrient removal in the multi-compartment biofilters of shrimp mariculture effluents. We explored the bacterial taxonomy using Illumina sequencing of the 16S rRNA gene, and the ecological functional diversity of the bacteria was assigned using Functional-Annotation-of-Prokaryotic-Taxa analysis (FAPROTAX). According to the Illumina dataset, there was a high heterogeneity, with phase two (P2) treatment showing significant reversible shifts in microbial communities' population and biogeochemical dynamics relative to phase one (P1). The genera *Candidatus aquiluna*, *Marivita*, *HTCC*, *Anaerospira*, and *Arcobacter* were abundant in both stages and may have the functional capability of biodegrading inorganic matter (OM). FAPROTAX results showed that the predominant functional groups (P1 and P2) for nutrient removal were related to chemo-heterotrophy. Furthermore, the nitrate-reduction and nitrate-ammonification were highly significant ($P < 0.05$) at P2, resulting in an average removal efficiency of 81.39% for total nitrogen (TN) and 80.63% for total phosphorus (TP). The results suggested that incorporating multi-compartment biofilters in the system provides a suitable substrate environment for the potential proliferation of Proteobacteria, Nitrospirae, and Bacteroidetes. Overall, this study enlightens the potential roles of the microbial communities under the influence of biofilters in promoting the feasible and most efficient bioremediation approaches for mariculture effluents.

Keywords Functional diversity · Mariculture effluents · Metabarcoding · Microbial communities · Multi-compartment biofilters

Handling editor: Daniel Merrifield

Extended author information available on the last page of the article

Introduction

Intensive shrimp mariculture production plays a vital role in promoting global economies, contributing billions of dollars annually and providing livelihoods for numerous communities. However, the rapid expansion of its production has been accompanied by stronger controversies on the environmental pollution of surrounding water bodies (Lukwambe et al. 2015; Chaikaew et al. 2019; Jayasinghe et al. 2019). Generally, the effluent from mariculture contains higher levels of organic matter (OM) and dissolved inorganic nutrients such as nitrogen (N) and phosphorus (P), which may cause eutrophication of the surrounding water bodies. Furthermore, the nutrients derived from the metabolic waste of shrimp can be toxic at high concentrations and threaten the shrimp's health status (Chaikaew et al. 2019). Therefore, achieving sustainable shrimp mariculture production depends on successful approaches that lower the concentrations of undesirable nutrients.

Studying microbial communities and their interactions within the ecosystem provides valuable knowledge that can be harnessed for the development of natural and eco-friendly solutions aimed at effectively maintaining low levels of dissolved inorganic N and P (Ramanan et al. 2016; Tang et al. 2018; Fan et al. 2021) in the ecosystem. A cheap and effective method for removing high organic matter (OM) loads and other contaminants is wastewater purification via natural biofilters (Nicholaus et al. 2020a, b; Lukwambe et al. 2020). Microorganisms have been widely used in the bioremediation of nutrient-rich effluents (Van Den Hendel et al. 2011; Natrah et al. 2014; Tang et al. 2018; Fan et al. 2021). Despite the widely accepted beneficial applications of microorganisms, the mechanisms of these communities in the multi-compartment biofilters in mariculture remain unknown.

The treatment system within a typical shrimp mariculture consists of different biofilter compartments such as sedimentation, biofilm, shellfish culture, and constructed wetlands that have different roles in water purification. For example, the sedimentation sub-compartment acts as a pre-treatment that allows suspended material such as clay, silt, or other particles to settle down by gravity. The biofilm sub-compartment, with artificial bio-cords, provides a favorable condition for microbial growth (Sanz-Lázaro et al. 2011; Battin et al. 2016; Peng et al. 2018). At the same time, the biofilm attracts microalgae, which exude dissolved organic carbon that microbes could assimilate (Yao et al. 2019). In exchange, the degraded inorganic nutrients such as N and P influence the microbial metabolism, which may promote further growth of microalgae (Nicholaus et al. 2020a, b). The treatment system also involves shellfish organisms such as extractive species for absorbing suspended organic particles through grazing (Nicholaus et al. 2020a, b; Lukwambe et al. 2020) and bioturbation at the sediment–water interface (Nicholaus et al. 2020a, b). Subsequently, the system incorporates a constructed wetlands sub-compartment for assimilating and filtering inorganic matter (Vymazal 2007; Søndergaard et al. 2010; Kim et al. 2016). The constructed wetlands provide optimum conditions and surface area for microbes responsible for the degradation of organic nutrients in the water column.

Ecological studies have shown that microbial communities are highly complex, dynamic in their interactions (Natrah et al. 2014; Ramanan et al. 2016), and can be species-specific between different areas. Moreover, the interaction and assembly processes of microbial communities are rarely elucidated in aquaculture environments. The microbial relationships can be either mutualistic (symbiotic) or competitive (antagonistic) (Fuentes et al. 2016), thereby affecting the system's performance. These relationships change depending on certain conditions in the aquatic environment. For instance, a symbiotic relationship between microbial communities may be changed to a competitive relationship depending

on the P and C source's availability (Liu et al. 2012). Some interactions may vary depending on the bacterial metabolism activity that can alter the environment, affect microalgae growth (Lam et al. 2018), and cause the lysing of aquatic bacteria. Therefore, for developing an effective Mariculture Effluent Treatment System (METS), there is a need to understand the roles, interactions, and distribution of microbial communities.

Functional Annotation of Prokaryotic Taxa (FAPROTAX) is among the methods for assigning the ecological functions of bacteria (Louca et al. 2016; Sansupa et al. 2021) and biogeochemical cycling. This method provides an opportunity for understanding the differences in the structural and functional groups of microbes (Yan et al. 2019). The FAPROTAX analysis provides multiple functions of microorganisms; in particular, all taxa related to nitrate denitrification may also associate with nitrate respiration and nitrate reduction (Ge et al. 2018). Despite its importance, information on functional groups of microorganisms based on FAPROTAX analysis is rarely available.

Several studies focused on microbial/bacterioplankton community composition in the aquatic ecosystem (e.g., Wan et al. 2011; Hu et al. 2012; Nicholaus et al. 2019; Lukwambe et al. 2020; Zheng et al. 2023). However, studying the microbial communities associated with biological filters in the degradation of organic matter (OM) in the bioremediation of shrimp mariculture effluents is still unclear. In this study, we performed two phases-time-series experiments aimed to (i) investigate the distribution, variation, and interaction of microbial communities, (ii) examine the functional couplings of microbial communities in the biodegradation of OM, and (iii) assess the relationship between the microbial communities and environmental change. This study will enlighten the potential roles of the microbial communities under the influence of multi-compartment biofilters in promoting the feasible and most efficient bioremediation approaches for mariculture effluents.

Materials and methods

Site description and sample collection

A comprehensive field study in a full-scale Mariculture Effluent Treatment System (METS) was performed at Xiangshan Bay, Ningbo City, Zhenjiang Province, China (29°32'N, 121°31'E). There were a total of 30 ponds on this shrimp farm; these ponds were within the greenhouse to maintain a relatively stable temperature during the cool season. The ponds, approximately 2000 m² in size, were managed identically in terms of sea water input, whereby a daily water exchange rate of 5% and a depth of 1.5 m were maintained. To maintain a suitable level of dissolved oxygen, bottom aeration was used. Shrimp post-larvae (*Litopenaeus vannamei*) with a body weight of 3 g and a length of 6 cm were introduced into the ponds at a stocking density of 360,000 ind./pond. During the experiment, shrimps were fed commercial feed (40.39% crude protein, 3.64% crude fat) two to five times per day at an 8% daily feeding rate. Juvenile shrimp were introduced to the ponds in April 2019, and the total harvest was about 5500 kg/pond. A full-scale mariculture effluent treatment system (4000 sq. m of the total system area) was in continuous operation, receiving wastewater from intensive shrimp ponds (Fig. 1). A system consists of six sub-compartments operating under two distinct phases [phase one (P1) and phase two (P2)] of shrimp production. P1: treatment from April to June, and P2 from June to August. The six treatment compartments were as follows: (1) a sedimentation system

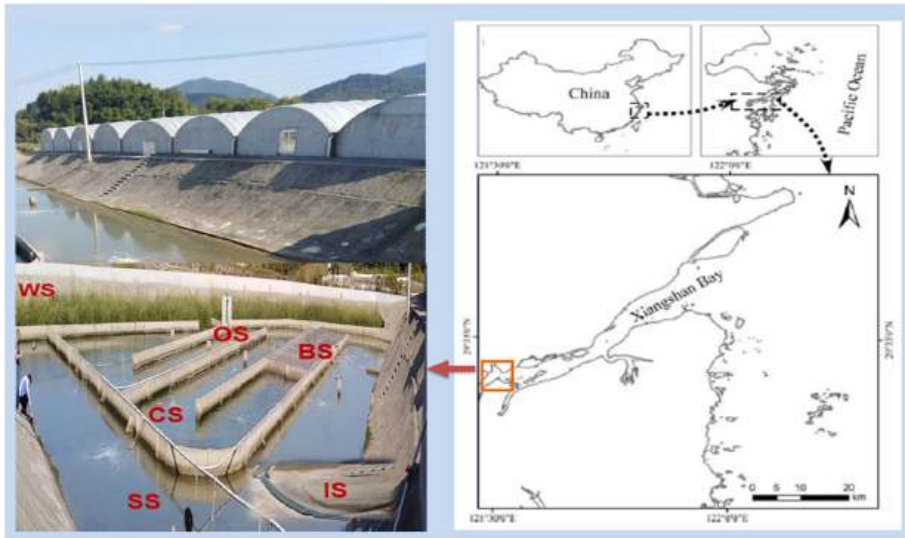


Fig. 1 A shrimp-mariculture effluent treatment system and sampling sites in each sub-compartment (IS, inlet sub-compartment; SS, sedimentation sub-compartment; BS, biofilm sub-compartment; CS, clam-shellfish sub-compartment; WS, wetland sub-compartment; OS, outlet sub-compartment), located at Xiangshan Bay, China

(SS) (for pre-treatment screening, grit removal, and primary clarification); (2) a biofilm with artificial bio-carriers made of polypropylene fine fiber with an inbuilt aerator system (BS) was placed in the biofilm sub-compartment; (3) a constructed wetland composed of emergent macrophytes (*Spartina anglica*) with an aerator (WS); (4) two sub-compartments for clam-shellfish (*Scapharca subcrenata*) with aerator pumps (CS); (5) the inlet sub-compartment (IS) as control; and (6) the outlet sub-compartment (OS). The effluents flow through the perforated holes (inlet) chamber to the sedimentation, then to the biofilm biofilter, to the shellfish headed to a wetland compartment, respectively, and finally to the outlet sub-compartment (Fig. 1).

The microbial communities at different phases in sub-compartments were investigated to reveal the efficient and important roles of the long-time operation of the METS. A total of three water samples (three replicates each) were collected for microbial community and environmental water quality data. Water samples were collected three times a day: in the morning from 9:00 to 10:00 a.m., in the afternoon from 13:00 to 14:00 p.m., and in the evening from 17:00 to 18:00 p.m., for four consecutive days at each phase. At each sampling time point, three replicate samples were sampled. For the microbial analysis, a sieve 0.2 μm pore-sized polycarbonate filter (47 mm diameter, Millipore, Boston, MA, USA) was used to filter 1 L of water samples. The filtered membranes were stored at a temperature of $-80\text{ }^{\circ}\text{C}$ until further analysis.

Determination of physical and chemical water parameters

We used IL “polyethylene” plastic bottles to collect water samples (0.5 m from the surface) to measure the physical and chemical water variables. For physical water quality

parameters, we collected in situ data regarding dissolved oxygen (DO), temperature (T), salinity, and pH using a handheld automated YSI 6000 multi-parameter probe (USA). The water samples for determining the concentration of nutrients such as nitrite nitrogen (NO_2^- -N), nitrate nitrogen (NO_3^- -N), chemical oxygen demand (COD), biochemical oxygen demand (BOD), total nitrogen (TN), total phosphate (TP), and ammonium nitrogen (NH_4^+ -N) were collected from the inflow and outflow of each sub-compartment. Additionally, water samples were collected and transported back to the Ningbo Meishan laboratory, where total nitrogen (TN) and total phosphorus (TP) were determined by using the potassium persulfate oxidation method. Phosphate (PO_4^{3-} -P) was analyzed by using the ascorbic acid reduced phosphorus-molybdenum blue method. COD and BOD were measured using national standard methods (APHA 2012). The nutrient concentrations of NH_4^+ -N, NO_2^- -N, and NO_3^- -N were measured using a discrete automated colorimetric analyzer (Westco, Smart-Chem analyzer 200, USA).

Microbial DNA extraction, PCR amplification, and MiSeq sequencing

Total environmental microbial DNA water samples from each sub-compartment of the METS were collected and extracted using a PowerSoil DNA Isolation Kit (MO BIO, USA). The quantity and quality of DNA were measured using the NanoDrop One (Thermo Fisher Scientific, MA, USA). The extracted genomic DNA was stored at $-80\text{ }^\circ\text{C}$ until needed for amplification. The PCR amplification conditions were set as follows: initial denaturation at $95\text{ }^\circ\text{C}$ for 3 min; 27 cycles at $95\text{ }^\circ\text{C}$ for 30 s; annealing at $55\text{ }^\circ\text{C}$ for 30 s; extension at $72\text{ }^\circ\text{C}$ for 45 s; and a final extension of 10 min at $72\text{ }^\circ\text{C}$. The V3 and V4 regions of the microbial 16S rRNA were amplified by using the two universal barcoded PCR primers: a forward primer 341F (5'-CCTAYGGGRBGCASCAG-3') and a reverse primer 806R (5'-GGACTACNNGGGTATCTAAT-3'). The amplified products of each sample were pooled, purified, equilibrated, and sequenced using an Illumina MiSeq high-throughput sequencing platform (MajorBio Co. Ltd., Beijing).

Bioinformatics analysis

Bioinformatics processing of the sequencing data was performed on USEARCH (v11.0.667_i8). A zero-radius operational taxonomic units (ZOTUs) table for classifying and clustering similar sequences into operation taxonomic units was generated using the UPARSE pipeline (Edgar 2016), and the UNOISE3 (unoise_alpha=2 and minsize=4 as per default settings) algorithm was used to denoise, correct errors, and remove chimeras (Edgar 2016). The SILVA database (release v123) was used to assign the representative sequences for each ZOTU at 99% similarity using the RDP classifier. The raw nucleotide sequences data generated in this study were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under the accession number PRJNA985065.

Statistical analysis

The R (version, 3.6.1, Foundation for Statistical Computing, Vienna, Austria) and SPSS version 19 (IBM Corporation, Armonk, NY, USA) were used to perform all statistical analyses unless otherwise indicated. To meet normality and homoscedasticity, microbial

community data were Hellinger transformed, and the environmental variables data were normalized using the “vegan” package’s function `decostand/p-p` plot. The beta-diversity of the community composition across the treatments was analyzed using non-parametric test permutational multivariate analysis of variance (PERMANOVA) with `Adonis` function.

The nonmetric multidimensional scaling (NMDS) ordination in the R-vegan was used to evaluate the microbial community composition based on Bray–Curtis dissimilarities (Anderson 2001). Canonical correspondence analysis (CCA) in the “vegan” package was used to analyze the correlations between community compositions and environmental variables. We used the paired *t*-test and one-way ANOVA in SPSS for group comparisons. The non-parametric Kruskal–Wallis test was used to compare the taxonomic distributions of the samples. The α -diversity indexes such as Evenness, Shannon–Wiener, and Faith’s Phylogenetic Diversity (PD) used to characterize the diversity of the microbial community were calculated based on ZOTUs using the QIIME workflow-alpha diversity scripts. The annotated bacterial ZOTUs and predicted functional groups were analyzed through FAPROTAX (<http://www.ehbio.com/ImageGP/>). The functional components and abundance of associated metabolic pathways were characterized using the annotation results. Venn diagrams were created to show the taxa that were shared and unique among samples, regardless of their relative abundance. The results of the statistical analysis were deemed significant at a $P \leq 0.05$. R-program and OriginPro 8.0 software were used to make the figures.

Results

Microbial community composition and structure

The microbial community compositions varied among the sedimentation (SS), wetland (WS), and outlet (OS) compartments (OS > WS > SS) and between the two phases (Fig. 2). A total of 7161 (grouped into 47 phyla) and 9358 (grouped into 52 phyla) ZOTUs were identified at phases one and two, respectively. At both phases, the microbial communities were mainly composed of the phylum Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Cyanobacteria, and Candidate Phylum OD1 bacteria as Parcubacteria (Fig. 2).

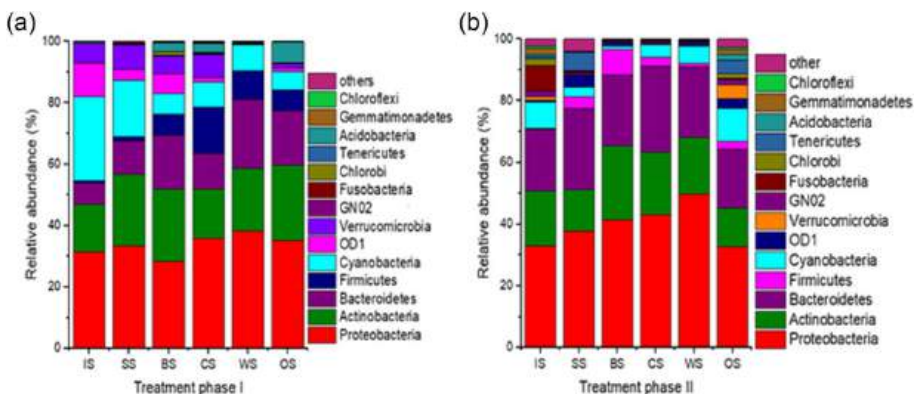


Fig. 2 The percentage composition (%) of microbial communities at phylum level in the METS **a** at phase one **b** at phase two (IS, inlet sub-compartment; SS, sediment sub-compartment; BS, biofilm sub-compartment; CS, clam-shellfish sub-compartment; WS, wetland sub-compartment; OS, outlet sub-compartment)

Proteobacteria were the most dominant community in all sub-compartments, followed by Actinobacteria (Fig. 2). The top forty genera representing the highest percentage composition of all bacterial ZOTUs were selected after a heatmap analysis. Out of the forty genera, *Candidatus aquiluna*, *Marivita*, *Anaerospora*, *HTCC*, and *Arcobacter* were the most highly distributed taxa at both phases (Fig. 3). Moreover, the ANOSIM (analysis of similarities) results at P2 ($R^2=0.02286$ at significance $P=0.001$) indicated a high correspondence against P1 ($R^2=0.06576$ at significance $P=0.006$).

The functional profiles of microbial communities in the system

A total of 31 Functional-Annotations of Microbial Taxa from FAPROTAX were identified as drivers for nutrient removal (Fig. 4). The analysis of microbial functional groups revealed that chemoheterotrophy, aerobic chemoheterotrophy fermentation bacteria, and intercellular parasites were dominant at P1, while chemoheterotrophy, nitrate reduction, and oxygenic photoautotrophs were dominant at P2 (Fig. 4). The functional profile composition of microbial communities revealed by FAPROTAX at phase two was significantly different (ANOSIM, $R^2=0.1023$; $P=0.005$) relative to P1. Overall, chemoheterotrophy was the main metabolic functional group in all sub-compartments of the METS. Other functional groups, such as aerobic-chemoheterotrophy, fermentation, and nitrite reduction bacteria, varied within sub-compartments.

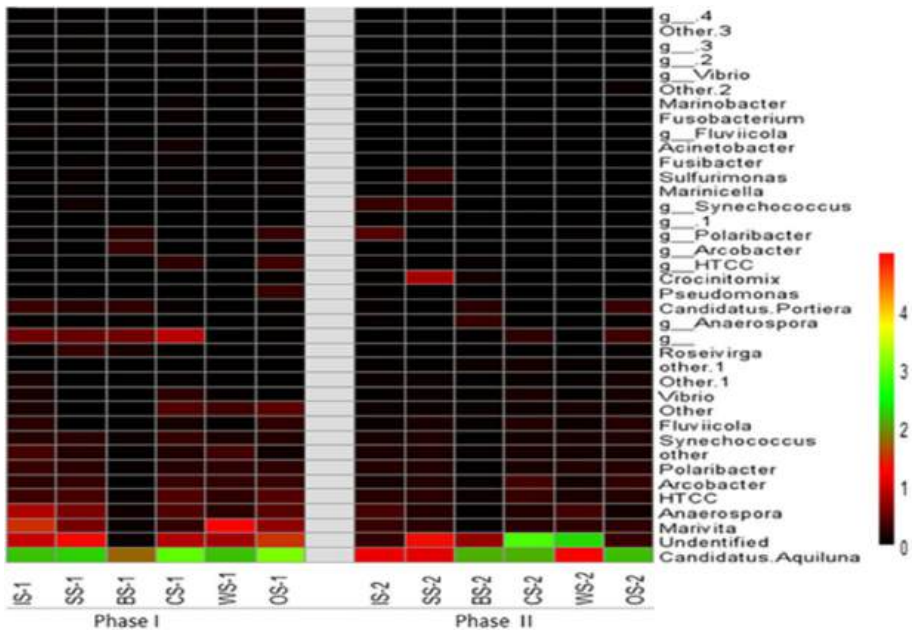


Fig. 3 The heatmap represent the highest to lowest percentage of genera composition of all ZOTUs based on 16S bacterial rRNA at phase one and phase two (IS, inlet sub-compartment; SS, sediment sub-compartment; BS, biofilm sub-compartment; CS, clam-shellfish’s sub-compartments; WS, wetland sub-compartment; OS, outlet sub-compartment)

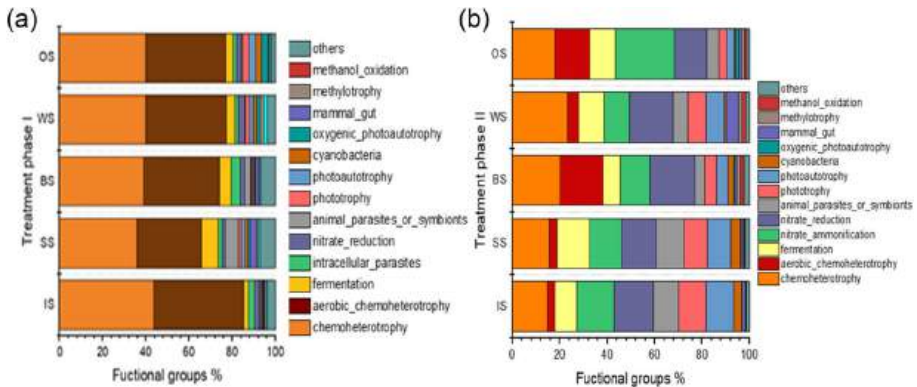


Fig. 4 The relative abundance of the functional groups in the system at **a** phase one **b** phase two (IS, inlet sub-compartment; SS, sediment sub-compartment; BS, biofilm sub-compartment; CS, clam-shellfish's sub-compartments; WS, wetland sub-compartment; OS, outlet sub-compartment)

Table 1 Diversity of the microbial community in the biofilters system

Sub-unit	Shannon	Chao1	PD whole tree	Number of taxa	Simpson
IS-P1	4.73 ± 1.00	398.01 ± 108.34 ^a	14.57 ± 2.14 ^a	174.00 ± 13.89 ^a	0.85 ± 0.11
IS-P2	4.89 ± 1.14	1326.68 ± 366.10 ^b	35.85 ± 7.93 ^b	624.67 ± 84.04 ^b	0.84 ± 0.13
SS-P1	4.60 ± 0.52	443.53 ± 116.52 ^a	21.75 ± 2.80 ^a	231.67 ± 33.65 ^a	0.93 ± 0.03
SS-P2	5.07 ± 0.49	1655.46 ± 153.66 ^b	45.43 ± 4.60 ^b	804.78 ± 93.76 ^b	0.93 ± 0.03
BS-P1	5.33 ± 0.59	593.44 ± 178.87 ^a	19.26 ± 3.51 ^a	211.44 ± 26.30 ^a	0.92 ± 0.05
BS-P2	5.70 ± 0.58	1794.13 ± 178.12 ^b	52.71 ± 6.60 ^b	862.22 ± 92.85 ^b	0.92 ± 0.05
CS-P1	5.74 ± 0.56	614.27 ± 183.53 ^a	21.18 ± 5.10 ^a	230.78 ± 59.85 ^a	0.94 ± 0.02
CS-P2	6.11 ± 0.64	1805.80 ± 290.03 ^b	54.75 ± 11.31 ^b	911.89 ± 214.30 ^b	0.94 ± 0.02
WS-P1	5.60 ± 0.33	669.03 ± 163.10 ^a	18.71 ± 2.46 ^a	207.22 ± 31.04 ^a	0.94 ± 0.03
WS-P2	6.99 ± 0.35	1834.26 ± 445.05 ^b	54.03 ± 12.30 ^b	869.67 ± 218.29 ^b	0.95 ± 0.01
OS-P1	5.68 ± 0.36	664.48 ± 163.11 ^a	19.94 ± 3.62 ^a	215.67 ± 44.28 ^a	0.95 ± 0.01
OS-P2	6.94 ± 0.33	1871.45 ± 354.19 ^b	56.04 ± 12.33 ^b	849.11 ± 166.44 ^b	0.94 ± 0.04

Different letters specify the significant between sub-compartment of phase one and two ($P < 0.05$); no letters represent no significant difference. Results are mean ± standard deviation. (IS inlet sub-compartment, SS sediment sub-compartment, BS biofilm sub-compartment, CS clam-shellfish's sub-compartment, WS wetland sub-compartment, OS outlet sub-compartment, P1 phase one, P2 phase two)

The diversity index of the microbial community

The alpha diversity metrics, including the number of observed ZOTUs, Chao1 index, Shannon diversity, and Inverse Simpson) of P1 and P2 of the METS are represented in Table 1. The biofilm, clam, outlet, and wetland sub-compartments had a higher alpha diversity and phylogenetic diversity (PD) whole tree ($P < 0.05$) than the inlet (IS) and sedimentation (SS) sub-compartments at both phases (Table 1), suggesting that clam-shellfish (CS), wetland (WS), and biofilm (BS) formations compartments harbor more diverse microorganisms than the IS and SS sub-compartments. The number of taxa

obtained was significantly higher ($P < 0.05$) in P2 than P1. The outlet and wetland sub-compartments of phase two had significantly higher Chao1 values than phase one ($P < 0.05$). There is no statistically significant difference ($P > 0.05$) in Simpson diversity and Shannon indexes between P1 and P2 of the METS. Alpha diversities (Chao1, PD whole tree, the number of taxa) decreased monotonically with the increased level of nutrients (N, P) in the inlet and sedimentation sub-compartments in P1 relative to P2.

Succession changes in microbial communities

To examine the overall taxonomic and structural changes of microbial assemblages, NMDS using Bray–Curtis’s dissimilarities, cluster analysis, and PERMANOVA were used. The differentiation of microbial community composition by NMDS ordination, showed differences in assemblages of microbial community between the sampling sites (Fig. 4). The NMDS plot with lower stress values indicated a measure of goodness of fit (stress values < 0.1 , Fig. 5a, b) and showed the variation of microbial community structure among the two phases. The NMDS showed samples were grouped separately in each sub-compartment. However, some microbial communities showed overlapping among the treated sub-compartments of the biofilter system (Fig. 5a, b). The PERMANOVA showed there is a significant difference in the composition of the microbial communities between two phases (PERMANOVA, $R^2 = 0.101$; $P = 0.001$) at P1, and (PERMANOVA, $R^2 = 0.102$; $P = 0.005$), at P2. We further analyzed the functional groups by using a Venn diagram to understand the distribution of the microbial community (Fig. 5c, d). In phase one, we

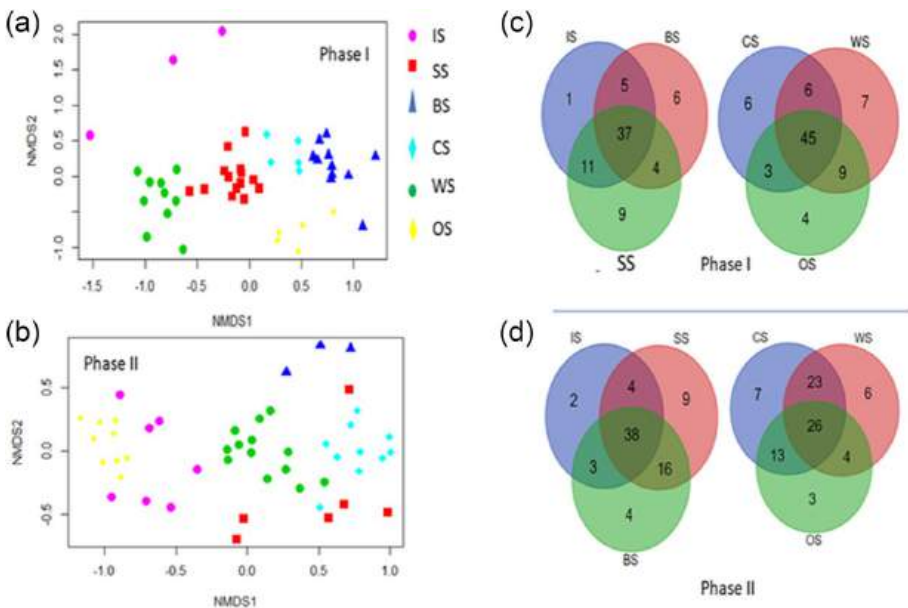


Fig. 5 Bray–Curtis nonmetric multidimensional scaling (NMDS) for microbial dissimilarities in the multi-compartment biofilters system at **a** phase one, **b** phase two; Venn diagrams were created to show the shared and unique taxa among functional groupings in the system at **c** phase one, **d** at phase two

found 37 common functional groups shared by three sub-compartments (inlet, sedimentation, and biofilm) and 45 functional couplings at the clam-shellfish, wetland, and outlet sub-compartments. Moreover, we found high variability in the unique number of strict indicator taxa over time (33 during phase one and a total of 31 unique taxa during phase two) (Fig. 5c, d).

Physical and chemical water parameters

Physical variables (water temperature (WT), dissolved oxygen (DO) and pH) were directly measured in the system, and values fluctuated from 5.20 to 8.11, 21.02 to 28.02 °C, and 3.41 to 8.01 mg/L, respectively. The chemical water parameters (NO_2^- -N, NO_3^- -N, COD, TN, and TP) were minimal at WS and OS compared to other sub-compartments. The BS showed the irregular variations of the environmental water parameters for both phases. In P1, the concentration of NH_4^+ -N effluent showed a significant decreasing trend (Table 2) and finally stabilized at the OS subunit. The effluent NO_2^- -N and TP were high in P1 at IS and SS compared to P2. Overall, the dissolved inorganic nutrients showed a trend variation among the sub-compartments. The NH_4^+ -N, NO_3^- -N, TP, and TN in BS, CS, WS, and OS were substantially different ($P < 0.05$). The nitrate-reduction and nitrate-ammonification were highly significant at P2, resulting in an average removal efficiency of 81.39% for total nitrogen (TN) and 80.63% for total phosphorus (TP).

Relationship between environmental variables and the microbial community

To explore the association between the populations and environmental variables, a CCA analysis was performed. Canonical correspondence analysis indicated a substantial correlation between extrinsic and intrinsic factors. Generally, nine environmental variables were

Table 2 The environmental water quality parameters of the mariculture effluents treatment system

Sub-unit	Nutrients (mg/L)						
	NO_2^- -N	NH_4^+ -N	PO_4^{3-} -P	NO_3^- -N	TP	TN	COD
IS-P1	1.87 ± 0.48	1.34 ± 0.36	1.44 ± 0.181	1.82 ± 0.83	0.70 ± 0.22	3.97 ± 1.67	15.54 ± 4.12
IS-P2	1.67 ± 0.44	1.19 ± 0.32	1.40 ± 0.13	1.84 ± 0.60	0.52 ± 0.23	3.87 ± 1.39	16.14 ± 3.95
SS-P1	0.97 ± 0.44	1.19 ± 0.32	1.39 ± 0.130	1.34 ± 0.60	0.62 ± 0.23	3.87 ± 1.39	16.14 ± 3.40
SS-P2	0.82 ± 0.36	1.12 ± 0.48	1.35 ± 0.11	1.17 ± 0.40	0.50 ± 0.15	3.26 ± 0.87	12.55 ± 3.82
BS-P1	0.72 ± 0.36	1.32 ± 0.48 ^a	0.65 ± 0.11 ^a	1.17 ± 0.40	0.50 ± 0.15	3.26 ± 0.87	12.55 ± 3.82
BS-P2	0.71 ± 0.59	0.86 ± 0.28 ^b	0.27 ± 0.09 ^b	1.00 ± 0.43	0.37 ± 0.13	2.22 ± 0.60	8.83 ± 3.36
CS-P1	0.71 ± 0.59 ^a	1.86 ± 0.28 ^a	0.27 ± 0.10	1.10 ± 0.43 ^a	0.38 ± 0.16	2.22 ± 0.60	8.83 ± 3.36
CS-P2	0.44 ± 0.48 ^b	0.48 ± 0.20 ^b	0.19 ± 0.08	0.46 ± 0.16 ^b	0.28 ± 0.10	1.83 ± 0.73	6.14 ± 2.78
WS-P1	0.73 ± 0.25 ^a	1.34 ± 0.23 ^a	0.21 ± 0.08	0.80 ± 0.49 ^a	0.40 ± 0.15 ^a	2.17 ± 0.60 ^a	16.75 ± 3.00 ^a
WS-P2	0.27 ± 0.48 ^b	0.12 ± 0.46 ^b	0.44 ± 0.18	0.32 ± 0.13 ^b	0.01 ± 0.02 ^b	0.08 ± 0.68 ^b	5.54 ± 4.12 ^b
OS-P1	0.64 ± 0.28 ^a	0.58 ± 0.20 ^a	0.69 ± 0.08 ^a	0.76 ± 0.16 ^a	0.48 ± 0.10 ^a	2.83 ± 0.73 ^a	6.14 ± 2.76 ^a
OS-P2	0.23 ± 0.45 ^b	0.22 ± 0.07 ^b	0.21 ± 0.08 ^b	0.20 ± 0.49 ^b	0.10 ± 0.05 ^b	0.72 ± 0.12 ^b	2.75 ± 3.00 ^b

The significance between phase one and phase two ($P < 0.05$); is denoted by different letters. No letters indicate that there is no significant difference. Results are mean ± standard deviation ($n = 3$). (IS inlet sub-compartment, SS sediment sub-compartment, BS biofilm sub-compartment, (3) CS clam-shellfish's sub-compartment, WS: wetland sub-compartment, OS: outlet sub-compartment)

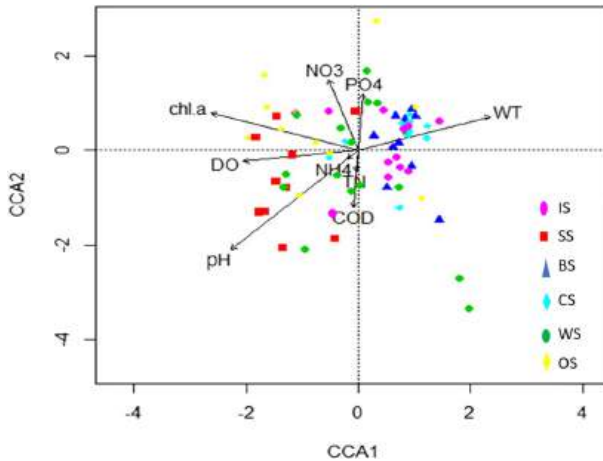


Fig. 6 Canonical correspondence analysis (CCA) of microbial communities signifying the relationship between microbial communities and abiotic and biotic factors in the system. The abbreviations indicate DO, dissolved oxygen; WT, water temperature; TN, total nitrogen; chl.a, chlorophyll α ; NO₃, nitrate; COD, chemical oxygen demand; TN, total nitrogen; PO₄, phosphate; NH₄, ammonium (IS, inlet sub-compartment; SS, sediment sub-compartment; BS, Biofilm sub-compartment; CS, clam-shellfish's sub-compartments; WS, wetland sub-compartment; OS, outlet sub-compartment)

significantly associated to the microbial community among the treatments (Fig. 6). The results showed a positive correlation between DO ($R=0.013$), WT ($R=0.011$), NO₂⁻-N ($R=0.018$), and TP ($R=0.016$), parameters with the microbial community (Fig. 6). To lessen the collinearity of many parameters, the (biochemical oxygen demand (BOD), Salinity (SAL), and NO₂⁻-N were excluded from the group of important variables based on the variance inflation factor ($VIF > 20$). The results showed that NO₂⁻-N, WT, pH, DO, and WT were the major environmental factors that influenced the microbial communities. Specifically, the microbial communities in the IS and CS were distinct from mainly by the axes of DO, and pH and chlorophyll α .

Discussion

Distribution and composition of microbial community in the biofilter's system

The microbial community in the aquatic system may experience succession dynamics or temporal variations. In this study, the presence of biofilters strongly influenced the variation and distribution of microbial communities at the overlying water interface (Fig. 2). Proteobacteria, Bacteroidetes, and Actinobacteria were the dominant phyla in the two phases of the treatment system. Previous studies have shown that the microbial community of the above-mentioned phyla is responsible for the mineralization of organic matter in various ecosystems (Pierce et al. 2016; Wei et al. 2018; Banker and Vermeij 2018; Nicholaus et al. 2020a, b). These results suggested that incorporating biofilters (shellfish, biofilm, and wetland) in the system for a certain period provided a suitable substrate environment for the proliferation of potential bacteria such as Proteobacteria, Nitrospirae, and

Bacteroidetes. Furthermore, the distribution of denitrifying microbes capable of converting nitrate to nitrite (Chen et al. 2012) in phase two of the wetland, shellfish, and biofilm sub-compartments was very diverse. Subsequently, the genera *Marivita*, *Candidatus aquiluna*, and *Anaespora* that might have the functional capability of degrading inorganic matter were highly distributed in phase two relative to phase one (Fig. 3). The composition of microbes in the biofilm, shellfish culture, and wetland biofiltration units increased significantly at phase two of the multi-compartment biofilter system (Fig. 2). This suggests that each sub-compartment had a unique community composition compared to other sub-compartments of the biofilter system (Fig. 5). The abundance and succession of the microbial community have gained a lot of interest and are showing promising results to many researchers (Lukwambe et al. 2019; Yang et al. 2020a, b; Wei et al. 2022). The abundance of the microbial community fluctuated greatly in the six treatment units, indicating that in the treatment system, the water environment is constantly changing, and the microbial community composition also varies from one sub-component to another (Fig. 2) and from phase one to phase two. These suggest that the microbial communities in the phase two (P2) treatment exhibited significant reversible shifts in microbial community population and biogeochemical dynamics compared to phase one (P1). Moreover, in the clam-shellfish and wetland sub-compartments, the diversity of potential microbial communities increased, possibly due to filter-feeding shellfish and the effect of cordgrass *Spartina anglica* in the constructed wetland. Generally, phase two of the treatment system affects each biofiltration unit and increases the biomass of potential microbes (Fig. 2). The early microbial colonizers appeared to be “sedimentation-biofilm-specific,” while later the wetland and outlet sub-compartments became dominant (Fig. 2). Overall, phase two of the biofilter system influenced the functional community and decreased the relative abundance of those opportunistic microbial communities with competitive growth (Fig. 4). The prevalence of Cyanobacteria and Gammaproteobacteria in phase one is possibly associated with the lower richness of beneficial bacteria. Generally, our findings suggest that the treatment process in phase two maintained the potential microbial community and constrained the dominance of opportunistic bacteria such as Gammaproteobacteria and Cyanobacteria in the system. Overall, the sustainability of the aquaculture treatment system is dependent on the presence of viable and stable assemblages free of antagonistic and dangerous microorganisms (Lukwambe et al. 2020).

The functional profiles of microbial communities in the system

The functional profiles of microbial communities are vital aspects that help to understand the interaction of communities in mariculture effluent treatment systems. In the present study, FAPROTAX showed that the predominant functional groups (at P1 and P2) for nutrient removal were related to chemo-heterotrophy that might have the functional capability of biodegrading inorganic matter in the system. Moreover, several functional groups of the microbial community for biogeochemical nutrients cycling and degradation of organic matter were discovered (Fig. 3). The predominant functional groups across all samples belonged to chemoheterotrophy, followed by aerobic chemoheterotrophy (Fig. 3). Chemoheterotrophy is essential in the decomposition of organic matter and the recycling of essential nutrients, such as carbon (C), nitrogen (N), and phosphorus (P). However, when we focused on more specific functions, the result showed differences in dominant functions involved in biogeochemical cycling derived from each biofiltration system. The removal of nutrients is dependent on the actions of chemoheterotrophic microorganisms that served as

the principal decomposers of organic matter (Hayatsu et al. 2008; Peng et al. 2018; Jiang et al. 2019). The functional groups of chemoheterotrophy were significantly higher in both phases of the system, while the aerobic-chemoheterotrophy and nitrate reduction were highly significant in the wetland subunit than the sedimentation area. This indicated that there was a high level of interaction among the constructed wetland, microbes, substrates, and pollutants as a result of the complex rhizosphere system (Carvalho et al. 2013) of the cord grass, *Spartina anglica*. Besides, the roots of the plants in the constructed-wetland harbor/store useful nitrifying and denitrifying bacteria (Chen et al. 2020; Lukwambe et al. 2019). *Spartina anglica* acts as a key carbon source to support microbial communities. This is evident especially in the rhizosphere of emergent aquatic plants in the wetland sub-compartment, where the plant roots create a conducive environment for the growth of numerous functional bacteria responsible for nitrogen content transformation (Zhang et al. 2013; Zou et al. 2013). Furthermore, the denitrification process in wetland and clam-shellfish biofilter systems could be attributed to the functional redundancy of diverse organisms that can execute the same ecological function of denitrification removal (Lukwambe et al. 2018). More importantly, we confirmed the presence of high functional groups of bacterial taxa found in wetland, shellfish and biofilm systems. In general, the biofilm and wetland biofilter areas offered suitable aerobic conditions for nitrification, while the wetland was ideal for both anoxic/anaerobic denitrification and nitrification in the system (Vymazal 2007; Lukwambe et al. 2018).

Effect of Physicochemical variables on microbial communities in the system

Microbial communities play a significant role in regulating biogeochemical cycles, whereas environmental parameters regulate their diversity, distribution, and physiology in most marine ecosystems (Thajudeen et al. 2017). The changes in surrounding environments might affect the microbial interaction, which may disturb the ecosystem community. In the present study, physical and chemical factors of the environmental water quality parameters changed after the treatment process (Table 2). The nutrient concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{PO}_4^{3-}\text{-P}$ and COD varied significantly among the treatments (Table 2). Furthermore, the system at phase two showed decreases in nutrient content (nitrogen, phosphorus, and COD) from one unit to another (Table 2). The filter feeders (shellfish) and emerging macrophytes (wetland) increased the nutrient removal rate in the system (Table 1). Macrophytes enhanced the nitrification process by releasing oxygen through their stems, roots, and rhizomes (Rehman et al. 2017). The advent of high nutrient removal in phase two of the wetland and clam-shellfish sub-compartments not only improved the efficiency of the process but also enhanced sustainable aquaculture effluent treatment (Craggs et al. 2011; Kang et al. 2015; Lukwambe et al. 2020). The removal of ammonia and nitrite is mainly mediated through the nitrification process by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) (Hayatsu et al. 2008; Peng et al. 2018; Jiang et al. 2019; Yoon et al. 2015). Additionally, CCA indicated strong relationships between the bacterial communities and physicochemical factors (Fig. 6). Results elucidated that $\text{NO}_3^-\text{-N}$, DO, WT, and TN were the main environmental factors, that altered the microbial communities either directly or indirectly. Several studies showed that the microbial communities are indispensable components of the water environment and play central roles by affecting the ecological functions of the ecosystem (Ramanan et al. 2016; Natrah et al. 2014; Zheng et al. 2023). In the present study, DO was the most abiotic factor that affected the ecosystem's microbial community structure (Jiang et al. 2019). DO

was highest and correlated with the microbial assemblage in the biofilm, shellfish culture, and ecological wetland sub-compartments (Fig. 6). The optimum DO could help maintain water quality (Lukwambe et al. 2020; Jiang et al. 2019), which leads to the reduction of harmful algae. Many studies have found that water temperature is a key element in determining community composition (Crump and Hobbie 2005; Kan et al. 2007; Zhu et al. 2019). Moreover, the high nutrient concentrations in sedimentation and inlet sub-compartment (Table 2) may have been caused by the elevated water temperature (Paerl and Paul 2012; Ponce-Palafox et al. 2019; Lai et al. 2021). The elevated water temperature can increase metabolic rates in aquatic organisms, leading to increased nutrient uptake and utilization. This increased metabolic activity, coupled with potential changes in nutrient release from sediments or other sources, contributes to higher nutrient concentrations in the water. Additionally, a substantial correlation between extrinsic and intrinsic factors was most manifested at the wetland sub-compartment during phase two of the system (Table 1, Fig. 6). This distribution trend was likely due to an increase in microbial community activities associated with mineralization in the wetland. Overall, mineralization in wetlands is driven by microbial communities that break down organic matter, releasing nutrients and fueling the nutrient cycling process. Generally, the constructed-wetland and biofilm had more affiliated taxa linked with physicochemical variables relative to the inlet and sedimentation sub-compartments, indicating a greater association between the environmental parameters and microbial functional groups. Since the microbial community was compelled significantly by the extrinsic factors (surrounding environment) (DO, pH, etc.), it is plausible to prevent the microbial community from shifting and crashing by controlling the environmental parameters, including minimizing nutrient loads (Yang et al. 2020a, b). However, techniques for controlling the extrinsic factors merit further research.

The alpha diversity of the microbial community

The alpha diversity parameters were higher in phase two relative to phase one, suggesting that the presence of biofilters influenced the potential bacteria required for bioremediation in the system (Table 2). The dominance of Proteobacteria, Bacteroidetes, and Actinobacteria increased the richness, evenness, and number of observed ZOTUs in phase two. The Simpson's diversity index and evenness index were lower in phase one than phase two (Table 1), possibly related to the low ratio of beneficial microorganisms influenced by high nutrient enrichment (Hu et al. 2012; Wan et al. 2011). In the biofilter system, the biofilm sub-compartment played a key role in maintaining the community diversity of the system at phase one. Unstable communities could cause the algal collapse leading to anoxia and the substantial release of sulfides and toxins (Cremen et al. 2007; Lukwambe et al. 2015). Moreover, high organic matter concentrations exudate from phytoplankton or the lysing of senescent and dead ones may affect the assembly of microbial communities (Landa et al. 2016; Logue et al. 2016; Lucas et al. 2010). In the present study, the bio-carriers provide a surface area and structure that facilitate the attachment and growth of diverse microbial communities. This favorable environment created by the bio-carriers can support the colonization and proliferation of different microbial community, leading to an increase in system diversity. The composition between the sedimentation area and the attached biofilm area was significantly different, indicating that the biofilm bio-carriers had improved the system's diversity (Sanz-Lázaro et al. 2011; Battin et al. 2016; Peng et al. 2018). We hypothesize that, an explicit internal circulation (aeration) in the biofilm-bio-carriers, was

the main reason for the rapid formation of the stable microbial community. This is a fascinating discovery as well as a novel strategy to promoting rapid biomass in the system. Moreover, incorporating a constructed wetland in the study provide oxic-habitats/niches created by the roots' aeration rhizospheres that affect the diversity of the wetland's heterotrophic biota (Bodelier 2003). Understanding the mechanisms underlying microbial interactions in response to biological filters will facilitate the development of efficient, productive, and sustainable aquaculture biotechnology processes.

Conclusions

The study utilized FAPROTAX, as a tool for predicting the functional profile of microbial communities for nutrient removal in multi-compartment biofilters of mariculture effluent. The high-throughput sequencing (HTS) of 16S rRNA gene metabarcoding revealed a highly dynamic pattern of community composition with high heterogeneity. Consequently, there was a significant increase in the functional groups of chemoheterotrophic and nitrate reduction in phase two of the wetland sub-compartment of the biofilter system. The study revealed that the co-existence of multi-compartment biofilters significantly altered the functional profiles of the microbial community and increased its diversity in phase two. Our study's results suggest that incorporating multi-compartment biofilters in mariculture shrimp effluent treatment systems enhances our understanding of the ecological functioning of microbial communities involved in organic matter degradation. However, more studies are needed to elucidate the functional roles of specific microbial taxa and investigate the long-term stability and performance of multi-compartment biofilter systems. Moreover, future studies should consider higher-resolution sequencing methods to obtain more specific information on species identification and functional services.

Acknowledgements This study was supported by the National Key R & D Program of China (2020YFD0900201), the Zhejiang Public Welfare Technology Research Program of China (ZPWTP) (LGN18C190008) and the K.C. Wong Magna Fund in Ningbo University.

Author contribution ZZ supervised the study. BL conceptualized the concept and the methodology. BL and RN wrote the first draft of the manuscript. And data analysis, interpretation, and final revision of the manuscript were done by BL and RN.

Funding This study was funded by the National Key R & D Program of China (2020YFD0900201), the Zhejiang Public Welfare Technology Research Program of China (ZPWTP) (LGN18C190008) and the K.C. Wong Magna Fund in Ningbo University.

Data availability All the data is provided.

Code availability Not applicable.

Declarations

Ethics approval Not relevant/applicable to the study.

Consent to participate All the authors have participated in the investigation and writing of the manuscript.

Competing interests The authors declare no competing interests.

References

- Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 26:32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070.ppx>
- APHA (2012) Standard methods for examination of water and wastewater, twenty second.ed. American Public Health Association, American Water Works Association, Washington DC
- Banker R, Vermeij GJ (2018) Oyster communities and implications for chalky deposit formation. *Hydrobiologia* 816:121–135. <https://doi.org/10.1007/s10750-018-3569-0>
- Battin TJ, Besemer K, Bengtsson MM, Romani AM, Packmann AI (2016) The ecology and biogeochemistry of stream biofilms. *Nat Rev Microbiol* 14:251. <https://doi.org/10.1038/nrmicro201615>
- Bodelier PE (2003) Interactions between oxygen-releasing roots and processes in flooded soils and sediments. In *Root ecology* (pp 331–362) Springer Berlin Heidelberg https://doi.org/10.1007/978-3-662-09784-7_13
- Carvalho PN, Araújo JL, Mucha AP, Basto MCP, Almeida CMR (2013) Potential of constructed wetlands microcosms for the removal of veterinary pharmaceuticals from livestock wastewater. *Bioresour Technol* 134:412–416. <https://doi.org/10.1016/j.biortech.2013.02.027>
- Chaikaew P, Rungkarn N, Pongpipatwattana V, Kanokkantapong (2019) Enhancing ecological-economic efficiency of intensive shrimp farm through in-out nutrient budget and feed conversion ratio Sustainable. *Environ Res* 29:28. <https://doi.org/10.1186/s42834-019-0029-0>
- Chen Y, Wang D, Zhu X, Zheng X, Feng L (2012) Long-term effects of copper nanoparticles on wastewater biological nutrient removal and N₂O generation in the activated sludge process. *Environ Sci Technol* 46:12452–12458. <https://doi.org/10.1021/es302646q>
- Chen ZJ, Shao Y, Li YJ, Lin LA, Chen Y, Tian W, Li BL, Li YY (2020) Rhizosphere bacterial community structure and predicted functional analysis in the water-level fluctuation zone of the Danjiangkou reservoir in China during the dry period. *Int J Environ Res Public Health* 17:1266. <https://doi.org/10.3390/ijerph17041266>
- Craggs RJ, Heubeck S, Lundquist TJ, Benemann JR (2011) Algal biofuels from wastewater treatment high-rate algal ponds. *Water Sci Technol* 63:660–665. <https://doi.org/10.1016/j.biortech.2010.06.158>
- Cremen MCM, Martínez-Goss MR, Corre VL, Azanza RV (2007) Phytoplankton bloom in commercial shrimp ponds using green-water technology. *J Appl Phycol* 19:615–624. <https://doi.org/10.1007/s10811-007-9210-7>
- Crump BC, Hobbie JE (2005) Synchrony and seasonality in bacterioplankton cyanobacteria. *Water Res* 46:1349–1363. <https://doi.org/10.1038/ismej201758>
- Edgar RC (2016) UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *BioRxiv* p081257 <https://doi.org/10.1101/081257>
- Fan S, Ji B, Hasan HA, Fan J, Guo S, Wang J, Yuan J (2021). Microalgal–bacterial granular sludge process for non-aerated aquaculture wastewater treatment. *Bioprocess Biosyst Eng* 1–7. <https://doi.org/10.1007/s00449-021-02556-0>
- Fuentes JL, Garbayo I, Cuaresma M, Montero Z, González-del-Valle M, Vilchez C (2016). Impact of microalgae–bacteria interactions on the production of algal biomass and associated compounds. *Mar Drugs* 14. <https://doi.org/10.3390/md14050100>
- Ge Y, Shen C, Wang Y, Sun YQ, Schimel JP, Gardea-Torresdey JL, Holden PA (2018) Carbonaceous nanomaterials have higher effects on soybean rhizosphere microbial communities during the reproductive growth phase than during vegetative growth. *Environ Sci Technol* 52:6636–6646. <https://doi.org/10.1021/acest8b00937>
- Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification *Soil. Sci Plant Nutr* 54:33–45. <https://doi.org/10.1016/j.chemosphere.2005.09.014>
- Hu M, Wang X, Wen X, Xia Y (2012). Community structures in different wastewater treatment plants as revealed by 454-pyrosequencing analysis. *Bioresour Technol* 117:72–79. <https://doi.org/10.1016/j.biortech.2012.04.061>
- Jayasinghe J, Gamage D, Jayasinghe J (2019). Combating climate change impacts for shrimp aquaculture through adaptations: Sri Lankan perspective In: *Sustainable Solutions for Food Security* 287–309. https://doi.org/10.1007/978-3-319-77878-5_15
- Jiang W, Tian X, Li L, Dong S, Zhao K, Li H, Cai Y (2019) Temporal bacterial community succession during the start-up process of biofilters in a cold-freshwater recirculating aquaculture system. *Bioresour Technol* 287:121441. <https://doi.org/10.1016/j.biortech.2019.121441>
- Kan J, Suzuki MT, Wang K, Evans SE, Chen F (2007) High temporal but low spatial heterogeneity of bacterioplankton in the Chesapeake Bay. *Appl Environ Microbiol* 73:6776–6789

- Kang Z, Kim BH, Ramanan R, Choi JE, Yang JW, Oh HM, Kim HS (2015) A cost analysis of microalgal biomass and biodiesel production in open raceways treating municipal wastewater and under optimum light wavelength. *J Microbiol Biotechnol* 25:109–118
- Kim SH, Cho JS, Park JH, Heo JS, Ok YS, Delaune RD, Seo DC (2016) Long-term performance of vertical-flow and horizontal-flow constructed wetlands as affected by season N load and operating stage for treating nitrogen from domestic sewage. *Environ Sci Pollut Res* 23:1108–1119
- Lai H, Zhao L, Yang W, Nicholas R, Lukwambe B, Zhu J, Zheng Z (2021) Eukaryotic distribution pattern and its potential effects on fisheries in the fish reserves of Qiantang River in breeding season. *J Oceanol Limnol* 39:566–581. <https://doi.org/10.1007/s00343-020-9331-2>
- Lam TP, Lee TM, Chen CY, Chang JS (2018) Strategies to control biological contaminants during microalgal cultivation in open ponds. *Bioresour Technol* 252:180–187
- Landa M, Blain S, Christaki U, Monchy S, Obernosterer I (2016) Shifts in bacterial community composition associated with increased carbon cycling in a mosaic of phytoplankton blooms. *Isme J* 10:39–50
- Liu H, Zhou Y, Xiao W, Ji L, Cao X, Song C (2012) Shifting nutrient-mediated interactions between algae and bacteria in a microcosm: evidence from alkaline phosphatase assay. *Microbiol Res* 167:292–298
- Logue JB, Stedmon CA, Kellerman AM, Nielsen NJ, Andersson AF, Laudon H, Lindstrom ES, Krutzberg ES (2016) Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. *Isme J* 10:533–545
- Louca S, Parfrey LW, Doebeli M (2016) Decoupling function and taxonomy in the global ocean microbiome. *Science* 353:1272–1277
- Lucas R, Courties C, Herbland A, Gouletquer P, Marteau AL, Lemonnier H (2010) Eutrophication in a tropical pond: understanding the bacterioplankton and phytoplankton dynamics during a vibriosis outbreak using flow cytometric analyses. *Aquaculture* 310:112–121
- Lukwambe B, Nicholas R, Zhao L, Yang W, Zhu J, Zheng Z (2020) Microbial community and interspecies interaction during grazing of ark shell bivalve (*Scapharca subcrenata*) in a full-scale bioremediation system of mariculture effluents. *Mar Environ Res* 158:104956
- Lukwambe B, Qiuqian L, Wu J, Zhang D, Wang K, Zheng Z (2015) The effects of commercial agents (probiotics) on phytoplankton community structure in intensive white shrimp (*Litopenaeus vannamei*) aquaculture ponds. *Aquacult Int* 23:1443–1455. <https://doi.org/10.1007/s10499-015-9895-6>
- Lukwambe B, Yang W, Zheng Y, Zhu J, Zheng Z (2018) Bioturbation by the razor bivalve (*Sinonovacula constricta*) on the community and enzymatic activities in the sediment of an ecological aquaculture wastewater treatment system. *Sci Total Environ* 643:1098–1107. <https://doi.org/10.1016/j.scitotenv.2018.06.251>
- Lukwambe B, Nicholas R, Zhang D, Yang W, Zhu J, Zheng Z (2019) Successional changes of microalgae community in response to commercial probiotics in the intensive shrimp (*Litopenaeus vannamei* Boone) culture systems. *Aquaculture* 734257. <https://doi.org/10.1016/j.aquaculture.2019.734257>
- Natrah FM, Bossier P, Sorgeloos P, Yusoff FM, Defoirdt T (2014) Significance of microalgal–bacterial interactions for aquaculture. *Rev Aquac* 6:48–61
- Nicholaus R, Lukwambe B, Mwakalapa E, Yang W, Zhu J, Zheng Z (2020a) Impacts of bioturbation by Venus clam *Cyclina sinensis* (Gmelin 1791) on benthic metabolism and sediment nutrient dynamics in a shrimp–clam polyculture pond. *Indian J Fisheries* 67(3). <https://doi.org/10.21077/ijf202067379036-04>
- Nicholaus R, Lukwambe B, Yang W, Zhu J, Zheng Z (2020b) In situ assemblies of bacteria and nutrient dynamics in response to an ecosystem engineer marine clam *Scapharca subcrenata* in the sediment of an aquaculture bioremediation system. *J Ocean Univ China* 19:1447–1460
- Nicholaus R, Lukwambe B, Zhao L, Yang W, Zhu J, Zheng Z (2019) Bioturbation of blood bivalve *Tegillarca granosa* on benthic nutrient fluxes and community in an aquaculture wastewater treatment system. *Int Biodeter Biodegr* 142:73–82. <https://doi.org/10.1016/j.ibiod.2019.05.004>
- Paerl HW, Paul VJ (2012) Climate change: links to global expansion of harmful cyanobacteria. *Water Res* 46:1349–1363
- Peng Y, Li J, Lu J, Xiao L, Yang L (2018) Characteristics of community involved in early biofilms formation under the influence of wastewater treatment plant effluent. *J Environ Sci* 66:113–124
- Pierce ML, Ward JE, Holohan BA, Zhao X, Hicks RE (2016) The influence of site and season on the gut and pallial fluid communities of the eastern oyster *Crassostrea virginica* (Bivalvia Ostreidae): community-level physiological profiling and genetic structure. *Hydrobiologia* 765:97–113. <https://doi.org/10.1007/s10750-015-2405-z>
- Ponce-Palafox JT, Pavia ÁA, López DM et al (2019) Response surface analysis of temperature–salinity interaction effects on water quality growth and survival of shrimp *Penaeus vannamei* postlarvae raised in biofloc intensive nursery production. *Aquaculture* 503:312–321


- Ramanan R, Kim BH, Cho DH, Oh HM, Kim HS (2016) Algae–bacteria interactions: evolution ecology and emerging applications. *Biotechnol Adv* 34:14–29
- Rehman F, Pervez A, Khattak BN, Ahmad R (2017) Constructed wetlands: perspectives of the oxygen released in the rhizosphere of macrophytes. *Clean Soil Air Water* 45:1. <https://doi.org/10.1002/clen.201600054>
- Sansupa C, Wahdan SFM, Hossen S, Disayathanoowat T, Wubet T, Purahong W (2021) Can we use functional annotation of microbial taxa (FAPROTAX) to assign the ecological functions of soil bacteria. *Appl Sci* 11:688. <https://doi.org/10.3390/app11020688>
- Sanz-Lázaro C, Navarrete-Mier F, Marín A (2011) Biofilm responses to marine fish farm wastes. *Environ Pollut* 159:825–832
- Søndergaard M, Johansson LS, Lauridsen TL, Jørgensen TB, Liboriussen L, Jeppesen E (2010) Submerged macrophytes as indicators of the ecological quality of lakes. *Freshw Biol* 55:893e908. <https://doi.org/10.1111/j.1365-2427.2009.02331x>
- Tang CC, Tian Y, He ZW, Zuo W, Zhang J (2018) Performance and mechanism of a novel algal-bacterial symbiosis system based on sequencing batch suspended biofilm reactor treating domestic wastewater. *Bioresour Technol* 265:422–431
- Thajudeen J, Yousuf J, Veetil VP, Varghese S, Singh A, Abdulla MH (2017) Nitrogen fixing bacterial diversity in a tropical estuarine sediment. *World J Microbiol Biotechnol* 33:41
- Van Den Hende S, Vervaeren H, Saveyn H, Maes G, Boon N (2011) Microalgal bacterial floc properties are improved by a balanced inorganic/organic carbon ratio. *Biotechnol Bioeng* 108:549–558
- Vymazal J (2007) Removal of nutrients in various types of constructed wetlands. *Sci Total Environ* 380:48–65. <https://doi.org/10.1016/j.scitotenv.2006.09.014>
- Wan CY, De Wever H, Diels L et al (2011) Biodiversity and population dynamics of microorganisms in a full-scale membrane bioreactor for municipal wastewater treatment. *Water Res* 45:1129–1138. <https://doi.org/10.1016/j.watres.2010.11.008>
- Wei Y, Shen D, Lukwambe B, Wang Y, Yang W, Zhu J, Nicholas R, Zheng Z (2022) The exogenous compound bacteria alter microbial community and nutrients removal performance in the biofilm unit of the integrated aquaculture wastewater bioremediation systems. *Aquacult Rep* 27:101414. <https://doi.org/10.1016/j.aqrep.2022.101414>
- Wei Z, Liu Y, Feng K et al (2018) The divergence between fungal and bacterial communities in seasonal and spatial variations of wastewater treatment plants. *Sci Total Environ* 629:969–978. <https://doi.org/10.1016/j.scitotenv.2018.02.003>
- Yan DB, Xia PH, Song X, Lin T, Cao HP (2019) Community structure and functional diversity of epiphytic bacteria and planktonic bacteria on submerged macrophytes in Caohai Lake southwest of China. *Ann Microbiol* 69:933–944. <https://doi.org/10.1007/s13213-019-01485>
- Yang W, Zheng SZ, Zhou SH, Zhao L, Zhu JY, Lukwambe B, Nicholas R, Li CH, Zheng ZM (2020a) Structure and functional diversity of surface bacterioplankton communities in an overwintering habitat for large yellow croaker *pseudosciaena crocea* of the Southern East China Sea. *Front Mar Sci* 7:472. <https://doi.org/10.3389/fmars.2020.00472>
- Yang W, Zhu J, Zheng C, Lukwambe B, Nicholas R, Lu K, Zheng Z (2020) Succession of phytoplankton community during intensive shrimp (*Litopenaeus vannamei*) cultivation and its effects on cultivation systems. *Aquaculture* 520:734733. <https://doi.org/10.1016/j.aquaculture.2019.734733>
- Yao S, Lyu S, An Y, Lu J, Gjermansen C, Schramm A (2019) Microalgal–bacteria symbiosis in microalgal growth and biofuel production: a review. *J Appl Microbiol* 126:359–368. <https://doi.org/10.1111/jam.14095>
- Yoon S, Cruz-García C, Sanford R, Ritalahti KM, Löffler FE (2015) Denitrification versus respiratory ammonification: environmental controls of two competing dissimilatory NO₃(-)/NO₂(-) reduction pathways in *Shewanella loihica* strain PV-4. *ISME J* 9:1093–1104. <https://doi.org/10.1038/ismej.2014.201>
- Zhang XY, Wang ZZ, Liu XY, Hu X (2013) Degradation of diesel pollutants in Huangpu-Yangtze River estuary wetland using plant-microbe systems. *Int Biodeterior Biodegrad* 76:71e75. <https://doi.org/10.1016/j.proenv.2012.10.090>
- Zheng S, Zhou S, Yang W, Lukwambe B, Nicholas R, Zhu J, Zheng Z (2023). Spatial-temporal dynamics of bacterioplankton communities in the breeding area of large yellow croaker *Larimichthys crocea* in Sansha Bay, China. *J Oceanol Limnol* 24:1–2. <https://doi.org/10.1007/s00343-022-1431-8>
- Zhu C, Zhang J, Nawaz MZ, Mahboob S, Al-Ghanim KA, Khan IA, Lu Z, Chen T (2019) Seasonal succession and spatial distribution of bacterial community structure in a eutrophic freshwater Lake Taihu. *Sci Total Environ* 669:29–40. <https://doi.org/10.1016/j.scitotenv.2019.03.087>

Zou J, Liu X, He C, Zhang X, Zhong C, Wang C, Wei J (2013) Effect of *Scripus triquetus* of its rhizosphere and root exudates on community structure of simulated diesel-spiked wetland. *Int Biodeterior Biodegrad* 82:110–116. <https://doi.org/10.1016/j.ibiod.2013.03.006>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

Authors and Affiliations

Betina Lukwambe¹  · Regan Nicholous² · Zhongming Zheng³

✉ Betina Lukwambe
blukwambe@yahoo.com; blukwambe@udsm.ac.tz

¹ School of Aquatic Sciences and Fisheries Technology, University of Dar Es Salaam, Box 60091, Dar Es Salaam, Tanzania

² Department of Natural Sciences, Mbeya University of Science and Technology, Mbeya, Tanzania

³ School of Marine Sciences, Ningbo University, Ningbo, China