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The impact of mariculture biofilters on the distribution of benthic nutrient fluxes, organic matters and bacterial community in a mariculture wastewater treatment system

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ABSTRACT

Constructed-wetlands, biofilms, and sedimentation are among biological filters used in mariculture wastewater treatments, however, their impacts on the distribution of benthic microbial community and inorganic-nutrient fluxes have not been fully explored. This study applied 16 S rRNA high-throughput sequencing technology to investigate the microbial community distribution and their impacts on nutrient fluxes in mariculture biofilters system. Results showed that bacterial community compositions were significantly different in the constructed-wetland and biofilm treatments ($p < 0.05$) relative to sedimentation. The composition of the 16 S rRNA genes among the treatments were enriched with *Proteobacteria* (73%), *Bacteroidetes* (69%), *Firmicutes* (62%), and *Flavobacteria* (61%) in Biofilm compared to *Proteobacteria* (53%), *Bacteroidetes* (39%), *Firmicutes* (32%), and *Flavobacteria* (21%) in constructed wetlands. NMDS analysis showed that bacterial composition in constructed-wetland and biofilms clustered separately compared to sedimentation treatment. Functional-Annotation-of-Prokaryotic-Taxa analysis indicated that the proportions of sediment-microbial-functional groups (aerobic-chemoheterotrophy, chemoheterotrophy, and nitrate-ammonification) were 47% in the constructed-wetland, 32% in biofilm and 13% in sedimentation system. Benthic-nutrient fluxes for phosphate, ammonium, nitrite, nitrate and sediment oxygen consumption differed markedly among the treatments ($p < 0.05$). Canonical correspondence analysis indicated constructed-wetland had the strongest association between biogeochemical contents and the bacterial community relative to other treatments. This study suggests that the mariculture wastewater biofilters promoted microbial community distributions, sediment bacterial functional-groups including chemoheterotrophy, aerobic-chemoheterotrophy, denitrification, and nitrification and interactions with nutrient fluxes which was more pronounced in the constructed-wetland system.

1. Introduction

Intensive mariculture farming practices have led to significant environmental impacts, including, the accumulation of allochthonous organic matter (OM), excreta, food-wastes, and nutrients polution (e.g., excess nitrogen and phosphorous) (Roeselers et al., 2007; Dean et al., 2007; Basaran et al., 2010; Martinez-Porchaz and Martinez-Cordova, 2012; Aubert et al., 2020). This has increased the concern for the adoption of aquaculture effluent treatment systems. Constructed wetland and biofilm and physical treatments like sedimentation are increasingly used in treating aquaculture effluents (Brito et al., 2018; Nicholaus et al., 2019a,b; Masoud et al., 2022).

Constructed-wetlands are well-established, cost-effective, and viable methods useful in wastewaters treatment. (Kivaisi, 2001; Webb et al., 2012). Wetland plants perform photosynthesis activities by using their aboveground organs, while their roots and rhizospheres (oxic-habitats or niches created by the roots' aeration) interact with the belowground-sediment to drive the productivity of the heterotrophic soil biota (Bonkowski et al., 2009; Neori, Agami, 2016). Wetlands can influence water and/ or sediment physicochemical properties through different mechanisms including microbial OM mineralization, sedimentation and substrate-adsorption processes (Kadlec, Knight, 1996;

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Lukwambe et al., 2019).

Biofilms, which are auto-aggregate forms of heterogeneous microbial communities, contribute significantly to nutrient cycling, OM degradation, and community enrichment through bacteria mineralization (Baldwin et al., 2006; Sanz-Lázaro et al., 2011; Nicholaus et al., 2019a; Lukwambe et al., 2019). Biofilm communities consist of bacteria and microalgae that secrete an extracellular polymeric substance matrix (polysaccharides), which facilitates the adhesion of the community to other substrates. The physical nature of biofilm exopolymers has a great adsorptive capacity with a super binding affinity for nutrients (Sanz-Lázaro et al., 2011).

Sedimentation, the physical process of suspended materials settling by gravity, can form microbial communities and nutrient-rich ecosystems. Sediment microorganisms, such as heterotrophic marine bacteria, are crucial in nutrient cycling and OM processing. (Freel et al., 2012). Provoost et al. (2013) reported that sediment can harbor up to 30% of the pelagically produced organic matter. Various pollutants associated with fish farming in the water body are gradually deposited onto the sediments (Nicholaus et al., 2020a,b) and through microbial waste degradation processes such as bioremediation undergo biological transformations resulting in increased nutrient cycling, pollution reduction, and bacterial diversity (Abatenh et al., 2017; Nicholaus et al., 2020a,b; Vega et al., 2009; Soares-Castro, 2019). Sediment microorganisms like heterotrophic marine bacteria are very crucial in nutrient cycling and OM processing (Soares-Cas et al., 2019).

The strength and efficacy of the constructed wetland and biofilm treatments are supported by the consortium of bacterial communities (Akyon et al., 2015; Lukwambe et al., 2019). Several studies have been conducted focusing on microbial community composition and distribution (Lukwambe et al., 2018; Nicholaus et al., 2019a; Lukwambe et al., 2019). However, the impacts of constructed wetland, biofilm, and sedimentation on benthic properties, nutrient fluxes, and bacterial community distribution in bioremediation of mariculture wastewater remain unclear. This study aims to evaluate the impacts of constructed wetland, biofilm, and sedimentation biosystem filters on benthic OM contents, nutrient fluxes, and bacterial community distribution in mariculture wastewater bioremediation system.

2. Materials and methods

2.1. Study area, experimental design and sampling

The experiment was carried out at a land-based mariculture biofilters system (Fig. 1) built in Ningbo Xiangshan Bay, Zhejiang Province, China. This system was primarily used to restore effluents resulting from an intensive commercial Vannamei shrimp (*Litopenaeus vannamei*) production farm. A comprehensive mariculture biofilters system composed of subsystems: constructed wetland, biofilm, and sedimentation were studied (Lukwambe et al., 2019, Fig. 1). The constructed wetland subsystem was composed of emergent macrophytes(*Spartina anglica)*, occupying 400 sq. m of the total system area. The planting densities of *S. anglica* were 50% of the total wetland cover. These plants grew rapidly

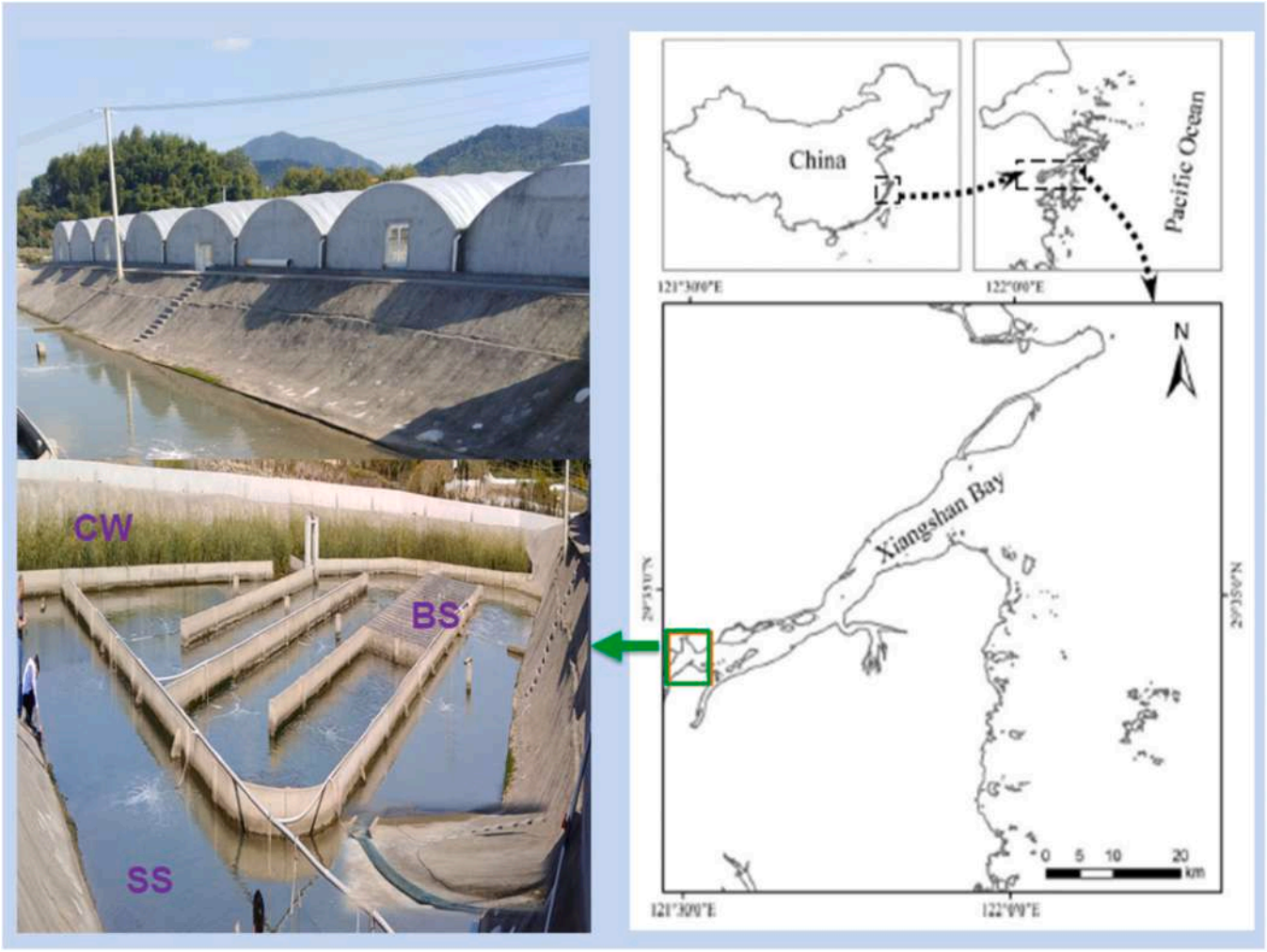

Fig. 1. The study area indicating the mariculture treatment subsystems and the sampling sites.

to colonize the wetland and they were not harvested during this study. The biofilm system was deployed with suitable aeration facilities and suspended carriers in the form of fiber threads (adhesive matrix of extracellular polymeric substances) for enhancing the surface area for microorganism attachment. The physical sedimentation consisted of bare sediment surface, overlying mariculture effluent water, and aeration. This system was involved in filtering and settling large particles of the incoming effluent water from the production center.

Sampling started one year after the ecological succession have been fully develop. To ensure a representative sampling strategy, data was collected three times consecutively, between April to July. Four different sampling points from each system were identified and sampled (Fig. 1). 0.5 L of the overlying water was collected from each system for water quality analysis. Using a handheld sediment corer four undisturbed sediment cores (8 cm height) from each system were gently collected in cylindrical plastic tubes (i.d. 6.4 cm, height 19.4 cm). The sediment cores and water samples were immediately brought back to the laboratory for physicochemical analysis and incubation experiment. Water samples were stored at 4 °C, whereas the sediment cores were kept ready for the incubation experiment.

The incubation experiment was done as previously described (Nicholaus and Zheng, 2014). Water samples for the determination of benthic flux rates for the total ammonia nitrogen (TAN), nitrate (NO_3^--N) and nitrite (NO_2^--N), and soluble reactive phosphate (SRP) were collected, filtered in 0.45 GF/F and stored under − 20 °C until analysis. After the incubation experiment, using a clean stainless steel microspatula, the sediment cores were sliced into three sub-sampling points (surface 0–2 cm, middle 2–4 cm, and bottom 4–8 cm). These subsamples were thoroughly homogenized and divided into two portions. One potion was freeze-dried for physicochemical contents analysis and the other potion was stored in clean polypropylene tubes at − 20 °C for the 16 S rRNA extraction.

2.2. Analysis of physical properties and nutrient flux rates

The water parameters (dissolved oxygen, temperature, and salinity) were measured in situ during sampling using a handheld automated YSI 6000 multi-parameter probe (USA). All water samples were analyzed using standard methods (APHA, 2012), where TAN was treated with indophenol blue, NO_2^--N/NO_3^--N with the cadmium-copper reduction and the SRP were treated with the ammonium molybdate/ascorbic acid method. All the concentrations of inorganic nutrients were measured using a WESTCO SmartChem discrete analyzer 200 USA. Nutrient flux rates (μmol $m^{-2}h^{-1}$) and SOC were calculated from slopes of a linear regression concentration against time using the equation previously described (Nicholaus and Zheng, 2014).

$$Flux = \frac{\Delta C \cdot V}{A \cdot t}$$

Where: Flux is the nutrients or sediment oxygen fluxes (mmol $m^{-2}h^{-1}$); ΔC (mgL^{-1}) is the change in concentration of oxygen/nutrients (prior and after incubation); V (m^3) the volume of overlying water; A (m^2), is the cross-sectional area of the incubation chamber; t (h) is the duration of incubation.

The sediment grain size distribution was determined using sieves with different mesh sizes. Briefly, grain-size parameters were conducted mechanically from oven-dried subsamples using standard sieving methods for the sand content (500–63 μm) and sedigraph techniques for the silt/clay fraction (<63 μm). Particles sizes (clay: <0.002, silt: <0.02, fine sand: <0.2, sand: <2) were determined. Sediment OM was determined using the loss on ignition method (LOI) (Heiri et al., 2001). The sediment samples were freeze-dried, pulverized, and pre-weighed before being placed in a muffle furnace at 475 °C for 4 h. Then the samples were reweighed with the difference equals to the %OM content. TOC (total organic carbon), TON (total organic nitrogen), and C/N (carbon/nitrogen) ratio were analyzed commercially by using Carbon Elemental Analyzer. Briefly, during pretreatment, 5 g of the post-freeze-dried wet-sediment were ground using a mortar into powder to pass through a 1-mm mesh sieve. Before analysis, further pretreatment procedures necessary especially for TOC were done to remove the carbon dioxide by adding 1:1 HCl and oven-dried at 80 °C, overnight to a constant weight.

2.3. Extraction, amplification and miseq sequencing of the benthic bacterial DNA

The total genomic sediment DNA extraction was performed from ~0.5 g of homogenized sediment samples using the PowerSoil™ DNA isolation kit (MoBio Laboratories, Inc., USA) according to the manufacturer's recommendations. The extracted genomic DNA was stored at − 80 °C until amplification. The PCR amplification conditions were according to Lukwambe et al. (2018) whereby the total genomic DNA was dissolved in 100 μl of DES, supplied with the kit. The DNA quality and/or quantity of the samples were measured using a spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) at the A260/A280 ratio. A combination of reverse primer (5′-GGACTACHVGGGTWTCTAAT-3′) and a forward primer (5′-CCTACGGGAGGCAGCAG-3′) for the hypervariable V3-V4 regions of the 16 S rRNA gene was used. 5 μl of the total DNA template was used and amplified in a 50-μl reaction system. Then the amplification process followed 30 cycles of 95 °C denaturation for 30 s, annealing (55 °C, 30 s), and extension (72 °C, 45 s) and a final extension for 5 min at 72 °C. Successful amplification product and size of the PCR was electrophoresed in 1% agarose gel. The triplicate amplified products of each sample were pooled, purified, equilibrated, and sequenced in an Illumina MiSeq high-throughput sequencing platform.

2.4. Bioinformatics analysis

The sequencing process of the paired reads was initially joined with FLASH using default settings (Magoč and Salzberg, 2011), then, the Raw FASTQ files were processed using Quantitative-Insights-Into-Microbial-Ecology (QIIME version 1.8.0, (Caporaso et al., 2010). The operational taxonomic units (OTUs) assigned at a 97% similarity cut-off point in all samples were clustered using USEARCH (version 7.1; http://drive5.com/uparse/). The sequences were quality filtered based on sequence length, quality score, chimera, and primer mismatch thresholds. In a nutshell, homopolymer runs exceeding 6 bp were screened-out by PyroNoise. Sequences with the same barcodes were assigned to the same sample. The phylotypes were performed using the UCLUST algorithm (Edgar, 2013). The most abundant sequences of each phytotype were selected as the clean sequence and were taxonomically assigned (Greengenes database, release 13.8) using PyNAST (DeSantis et al., 2006). Diversity indices (Shannon index, Simpson, Chao1, and observed OTUs) were performed using the phylogenetic tree (QIIME pipeline).

2.5. Statistical analyses

The variations of the different physicochemical variables were analyzed by a one-way or two-way repeated ANOVA. Post Hoc tests were performed to determine the significant groups. The normal distribution and homogeneity of variances among treatments were verified before the ANOVA test. All the data were Hellinger transformed post statistical analyses, and then normalized by using the function decostand/p-p plot in the "vegan" package to improve normality and homoscedasticity. Permutational multivariate analysis of variance-PERMANOVA (Bray-Curtis dissimilarity matrices) (Anderson, 2001), phyloseq v1.22.3 and Nonmetric Multidimensional Scaling (NMDS) was performed to analyze the microbial community composition among the treatments. One-way analysis of similarity (ANOSIM) was used to verify whether the distribution of different samples visualized in the NMDS

plot was significant (Legendre and Legendre, 1998). Canonical correspondence analysis (CCA) was used to analyze the correlations between bacterial community compositions and environmental variables.

The sediment microbial functional groups were predicted by using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database. According to Louca et al. (2017), the annotated bacterial OTU table from the Silva database was read, and the data was matched with the species information in the database using a python program. The predicted functions were outputted through FAPROTAX (http://www.ehbio.com/ImageGP/). The annotation results were used to describe the microbial functional compositions and abundance of related metabolic pathways involved in ammonification, denitrification, carbohydrate metabolism, aromatics degradation, and nitrogen fixation. The relative abundances of the functional groups were calculated as the cumulative abundance of OTUs assigned to each functional group, which was obtained by standardizing the cumulative abundance of OTUs correlated with at least one function. All statistical analyses were performed with R, version, 3.6.1, (R Core Team, 2019) and the results of the statistical tests were considered to be significant at $p \leq 0.05$. The figures were drawn with R and OriginPro 8.0 software.

2.6. Data deposition

The sequences used in this study have been deposited in the GeneBank of NCBI with the BioProject database ID PRJNA593691 (https://www.ncbi.nlm.nih.gov/sra/PRJNA593691) and SRA accession numbers ranging from SAMN13483434 to SAMN13483469.

3. Results

3.1. Sediment physical and chemical contents

The sediment physical and chemical contents are described in Table 1. The results indicate distinct differences in sediment organic and inorganic contents among the systems. The surface sediments of the constructed wetland consisted of 79% medium sand, 17% very fine sand, and 4% silt/clay whereases the compositional contents in the biofilm were 68% medium sand, 25% very fine sand, and 7% silt. The sedimentation system was dominated by 84% (medium sand), 11% (very fine sand), and 5% (silt). Cores from the sedimentation system had significantly higher contents of OM, TN, TP, TOC, and C/N ratio at all three depths (0–2 cm, 2–4 cm, and 4–8 cm), relative to the others. C/N ratio were 8.17 ± 1.5 (biofilm), 7.7 ± 1.6, (constructed wetland) and 12.32 ± 3.1 (sedimentation) on average. Total OM was much lower in biofilm (depth 0–2 cm) compared to constructed wetland and sedimentation. All sediment organic contents varied differently between the systems however no stable variational trends were observed within different depths of the same treatment (Table 1, $p > 0.05$).

Table 1
Sediment organic contents in the treatments (Constructed-wetland, Biofilms, and Sedimentation). OM represents organic matter; TN: Total nitrogen; TP: Total phosphorus; TOC: Total organic carbon; and C/N: Carbon to nitrogen ratio. All data are presented as Mean (± SD).

System	Depth (cm)	OM%	TN%	TP%	TOC%	C/N
CW	Surface	3.13 ± 0.14	0.48 ± 0.05^{a}	0.031 ± 0.061	2.94 ± 0.06^{a}	6.12 ± 1.31^{a}
	Middle	3.23 ± 0.11	0.38 ± 0.02^{b}	0.029 ± 0.053	3.13 ± 0.03^{a}	8.24 ± 1.05^{b}
	Bottom	3.07 ± 0.08	0.30 ± 0.12^{b}	0.021 ± 0.006	2.63 ± 0.04^{b}	8.77 ± 2.09^{b}
BF	Surface	1.57 ± 0.05^{a}	0.43 ± 0.03^{a}	0.086 ± 0.04^{a}	3.10 ± 0.06a	7.21 ± 0.81^{a}
	Middle	2.55 ± 0.04^{b}	0.41 ± 0.01^{a}	0.081 ± 0.03^{a}	3.07 ± 0.02a	7.49 ± 3.32^{a}
	Bottom	3.47 ± 1.02^{c}	0.22 ± 0.01^{b}	0.952 ± 0.02^{b}	2.16 ± 0.08^{b}	9.82 ± 3.74^{b}
SD	Surface	4.79 ± 0.26a	0.16 ± 0.01	0.263 ± 0.01	2.31 ± 0.06	14.43 ± 5.64^{a}
	Middle	4.12 ± 0.24^{a}	0.21 ± 0.01	0.289 ± 0.041	2.33 ± 0.25	11.09 ± 4.81^{b}
	Bottom	3.98 ± 0.31^{b}	0.26 ± 0.12	0.204 ± 0.025	2.98 ± 0.22	11.46 ± 6.06^{b}

3.2. Nutrients flux rates among the treatments

All dissolved inorganic nutrient flux rates showed an efflux trend among the treatments. The mean release rates of TAN, NO_3^--N, NO_2^--N, and SRP fluxes between biofilm, constructed wetland, and sedimentation cores were significantly different (2-way ANOVA, $p < 0.05$). NO_2^--N and TAN accounted for more than 87.51% (constructed wetland) and 71.14% (biofilm) net flux rate relative to 37.43% (sedimentation) (Fig. 2). The NO_3^--N flux rates were 396.15 ± 61.09 μmol $m^{-2}h^{-1}$ (constructed wetland), 249.83 ± 71.12 μmol $m^{-2}h^{-1}$ (biofilm), 173.7 ± 33.01 μmol $m^{-2}h^{-1}$, (sedimentation) (Fig. 1B). The constructed wetland had the highest exchange rate of NO_3^--N, and NO_2^--N relative to other systems (biofilm and sedimentation). The SRP had the highest mean flux rate in biofilm. The release rate of TAN into the overlying water (constructed wetland) was approximately twice higher in both biofilm and sedimentation, indicating sedimentary remineralization of ammonia and nitrate. SOC were 4.91 ± 0.75 mmol $m^{-2}h^{-1}$ (biofilm), 3.82 ± 0.37 mmol $m^{-2}h^{-1}$ (constructed wetland), 1.89 ± 0.31 mmol $m^{-2}h^{-1}$ (sedimentation) (Fig. 2A). Oxygen level in sedimentation subsystem was the lowest followed by constructed wetland and finally biofilm. Generally, the mean release rates of all nutrient groups including soc followed the order: biofilm>constructed wetland>sedimentation.

3.3. Microbial community composition and structure

The bacterial community compositions varied among depths and between the treatment systems (Fig. 3). A total of 519, 692, and 837 OTUs were identified for sedimentation, constructed wetland, and biofilm treatments respectively. Jointly the OTUs represent 54 phyla, 85 classes, 152 families, and 471 genera among all treatments. The relative content of the microbial community (>0.3% relative abundance) at phylum, class, and family level is illustrated (Fig. 3A-C). At the phylum level, *Proteobacteria* were the most dominant community in all three systems accounting for 32.17 ± 7.51%, followed by *Bacteroidetes* (29.32 ± 7.04%), *Chloroflexi* (20.65 ± 6.21%), *Actinobacteria* (19.44 ± 5.92%), *Firmicutes* (13.92 ± 4.09%), *Acinetobacter* (11.85 ± 3.71%) and *Planctomycetes* (8.83 ± 2.54%) (Fig. 3A). The phylum *Firmicutes* and *Proteobacteria* were most dominant in constructed wetland and biofilm, while the sedimentation community was mainly dispersed by *Firmicutes, Proteobacteria,* and *Bacteroidetes*. *Gamma-, Delta-,* and *Alpha-proteobacteria* were dominant classes in all systems, followed by *Anaerolineae*, *Actinobacteria*, *Cytophagia*, *and Flavobacteriia* (Fig. 3B). Further, at the family level, several predominantly expressed bacterial taxa ((*Clostridiaceae* and *Acidaminobacteraceae,* (Order-*Clostridiales*), *Rhodobacteraceae* (Order-*Rhodobacterales*) *Chloroflexi, Anaerolineaceae,* [*Thermodesulfovibrionaceae*] (order-*Nitrospirales*) were predominant in all three treatments (Fig. 3C). The family *Flavobacteriaceae* was highly distributed in biofilm (15 to 65-fold) relative to constructed-wetland (9–31-fold) and sedimentation (7–17-fold). Other families were *Nitrospiraceae* and *Planctomycetaceae* with a 20–50-fold higher (biofilm) relative to constructed-wetland and sedimentation (jointly 6–12-fold). The distribution of the most dominant bacterial community (at the genera level) among the treatments is represented by heatmap (Fig. 4). The heatmap includes the top thirty genera, which represent 94.2–97.5% of all 16 S rRNA bacterial genes reads. The *Disulvococcus, Novosphingomium, Fusibacteria, Kordia, Clostridium,* and *Lysobacter* were the genera highly distributed among the treatments (Fig. 4; 0–4 cm depth). Vertically, the proportions of *Proteobacteria, Acidobacteria,* and

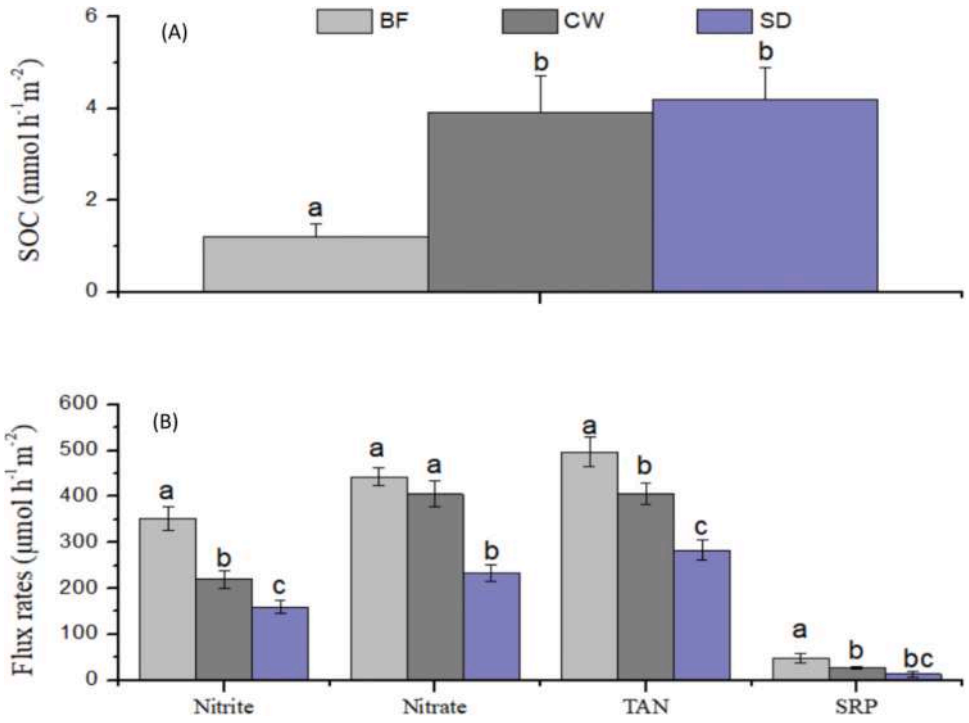

Fig. 2. Benthic inorganic nutrient flux rates. (A) Sediment oxygen consumption- SOC and (B) (Total ammonia nitrogen- TAN, soluble reactive phosphate- SRP, nitrate, and nitrite) estimated during laboratory incubations (Means ± SD, n = 3) in each treatment system.

Bacteroidetes were high in surface sediments, whereas *Chloroflexi* and *Firmicutes* tended to be enriched in deep layers.

3.4. Diversity of bacterial community among the systems

The alpha diversity metrics (OTUs, Chao1, Shannon diversity, and Inverse Simpson) differed significantly among the treatments (ANOSIM, $p = 0.031$). The microbial community richness estimate (Chao1) ranged from 7321 to 9531 sequences (biofilm), 4637 to 9017 (constructed wetland), 6214 to 8973 (sedimentation). Biofilm had significantly Chao1 values ($p < 0.05$) and constructed wetland had the highest bacterial diversity (Shannon index values). Bacterial richness estimates were highest and most diverse at the surface sediments (0–2 cm) and dropped with a depth increase (Fig. 4). The Shannon index ranged from 6.79 (surface), 6.03 (middle) to 5.81 (bottom) in biofilm samples and 6.21 (surface), 6.05 (middle), 4.93 (bottom) in constructed-wetland samples whereas sedimentation ranged from 5.06 (surface), 3.5 (middle), 2.53 (bottom). The diversity trend order was constructed-wetland>biofilm>sedimentation. Moreover, the NMDS ordinations assessment revealed marked differences in community composition grouping patterns between the systems, and between depths (Fig. 5). The samples were grouped separately within depths and between treatments. The microbial community in constructed wetland treatment samples was more clearly separated suggesting the highest species dissimilarity compared to other treatments. PERMANOVA further confirms that bacterial community composition between the treatments and within depth groups was significantly different (Table 2: $p < 0.05$).

3.5. Microbial community and benthic organic contents

To explore the relationship between the sediment-microbial community and benthic organic contents, a correlation analysis was performed based on CCA. The analysis showed significant correlations between microbial community composition (genus level) and the environmental factors (Mantel test, $p < 0.05$). The CCA showed the two components of the graph jointly explained 78.89% (axis 1: 41.37% and axis 2: 29.52%) of the total sediment microbial community variance, implying that physical and chemical factors and bacterial community composition had a substantive influence to each other. Generally, nine environmental variables were significantly associated with the bacterial community among the treatments (Fig. 5). The weakest correlation was observed between SRP and NO_2^--N and the communities (sedimentation). A significant correlation between *Desulphobacterales, Nitrospira,* and *Clostridia* taxa and the variables TN, TOC, and TP were evident. Significant correlations between *Nitrospira* and TOC, TN, and SRP (biofilm) and TAN in the constructed-wetland samples were observed. Furthermore, significant correlations between *Desulfomicrobium, Cytophagales,* and *Planctomyces* and NO_2^--N, and TP in the sedimentation samples were found. Whilst *Ferimonas* and *Verrucomicrobium* were positively correlated with TOC, *Burkholderiales* positively correlated with TAN, TP, and SRP especially in the constructed-wetland samples (Fig. 6).

3.6. Sediment microbial functional groups distributions

Using FAPROTAX the analysis revealed a comparative number of various specific metabolic functional groups/pathways (e.g., chemoheterotrophy, nitrate-ammonification, nitrification, denitrification, Fig. 7) associated with the 16 S rRNA genes. Different functional groups involved in nitrogen transformation pathways especially in the surface (0–2 cm) and middle (2–4 cm) cores were predicted suggesting elevated nitrogen mineralization activities. The relative abundance of genes mediating denitrification and dissimilatory reduction of nitrate to ammonia were mostly higher in the bottom layers (4–8 cm) in all systems. Chemoheterotrophy (29.73 ± 0.11%) was the main metabolic functional group, followed by denitrification (23.51 ± 0.03%), and complete nitrification (15.05 ± 0.81%). Other promoted functional groups/pathways included aerobic-chemoheterotrophy, sulfate_respiration, nitrate_ammonification, and nitrite_ammonification, especially in biofilm cores. Of all functional groups, groups related to nitrogen-cycling were highly predicted in the constructed wetland relative to biofilm and sedimentation samples.

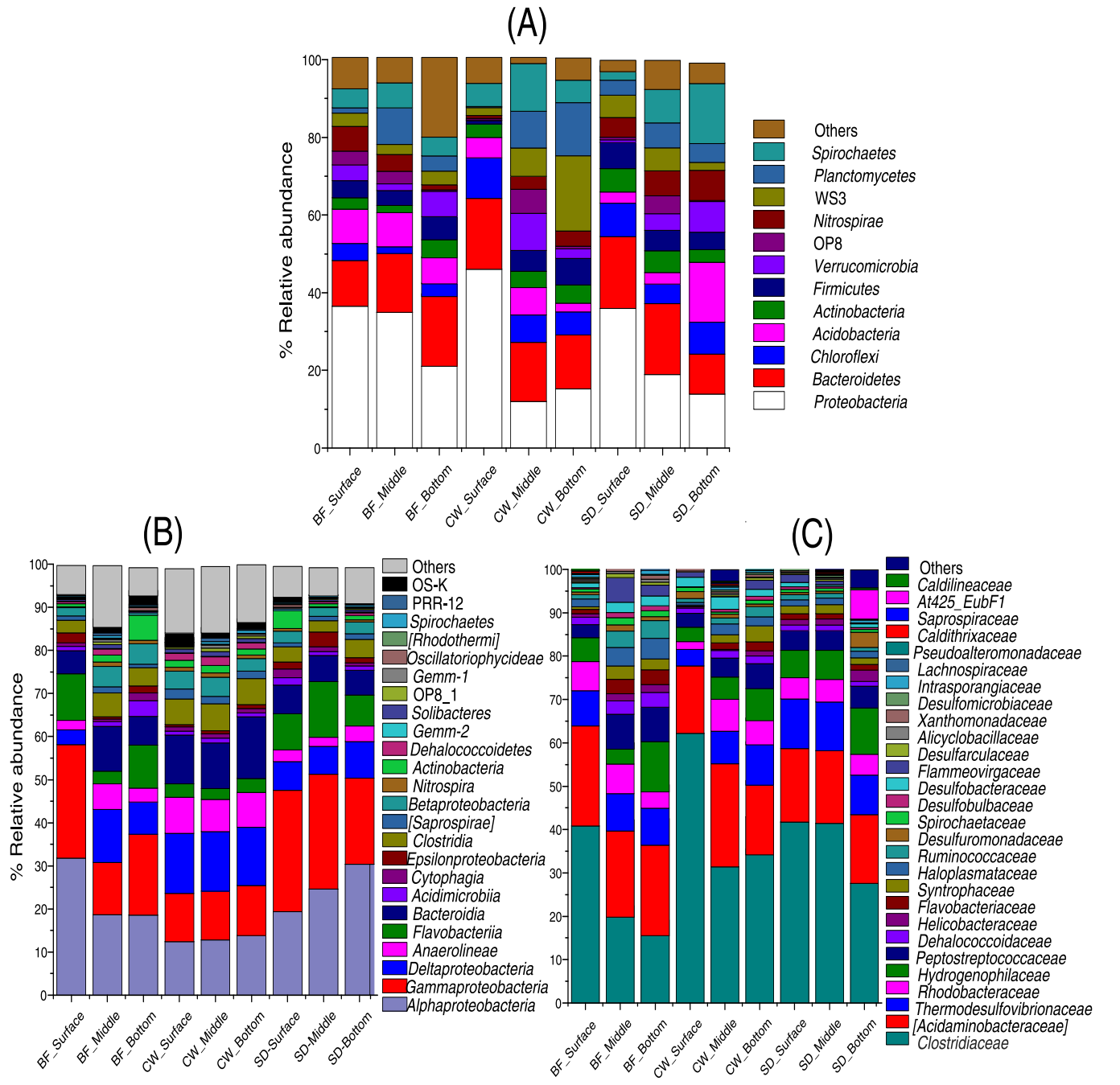

Fig. 3. The relative abundance of the vertical sediment microbial community composition and diversity among the treatments (Biofilm, Constructed-wetland, Sedimentation) at the phylum (A), class (B) and family (C) levels revealed by 16 S rRNA genes sequencing. Taxa making up less than 0.03% of total composition in all libraries were classified as 'others'.

4. Discussion

Biofilm, constructed wetland, and sedimentation are potential biofilters for improving wastewater and sediment quality (Brito et al., 2018; Nicholaus et al., 2019a,b; Lukwambe et al., 2019). This biofilters can improve the ecosystem processes including sediment microbial community activities, distribution, structure, abundance, and successions. The availability and distribution of bacterial communities in the sediment can help to improve and optimize the bioremediation process (Shen et al., 2017; Nicholaus et al., 2019a,b; Bharagava et al., 2019). This study indicates that a couple of ecological activities including microorganism distribution patterns, nutrient dynamics, and contents of the OM were significantly influenced by the treatments.

4.1. Microbial community composition and distribution

The sedimentary bacterial community compositions and structure among the treatments were differently distributed (Figs. 4, 5; Table 2). Biofilm and constructed wetland treatments supported more abundant and distinct microbial communities especially at the surface layers (Fig. 3). Particularly, phyla such as Proteobacteria and Acidobacteria were most abundantly distributed across all samples, with the highest relative abundance been recorded in biofilm samples relative to constructed wetland and sedimentation suggesting an elevated mineralization hence higher nutrient fluxes (Fig. 2). This suggests that the treatments probably created varying ecological conditions that accelerate microbial activities and their multiplication. The *Proteobacteria,*

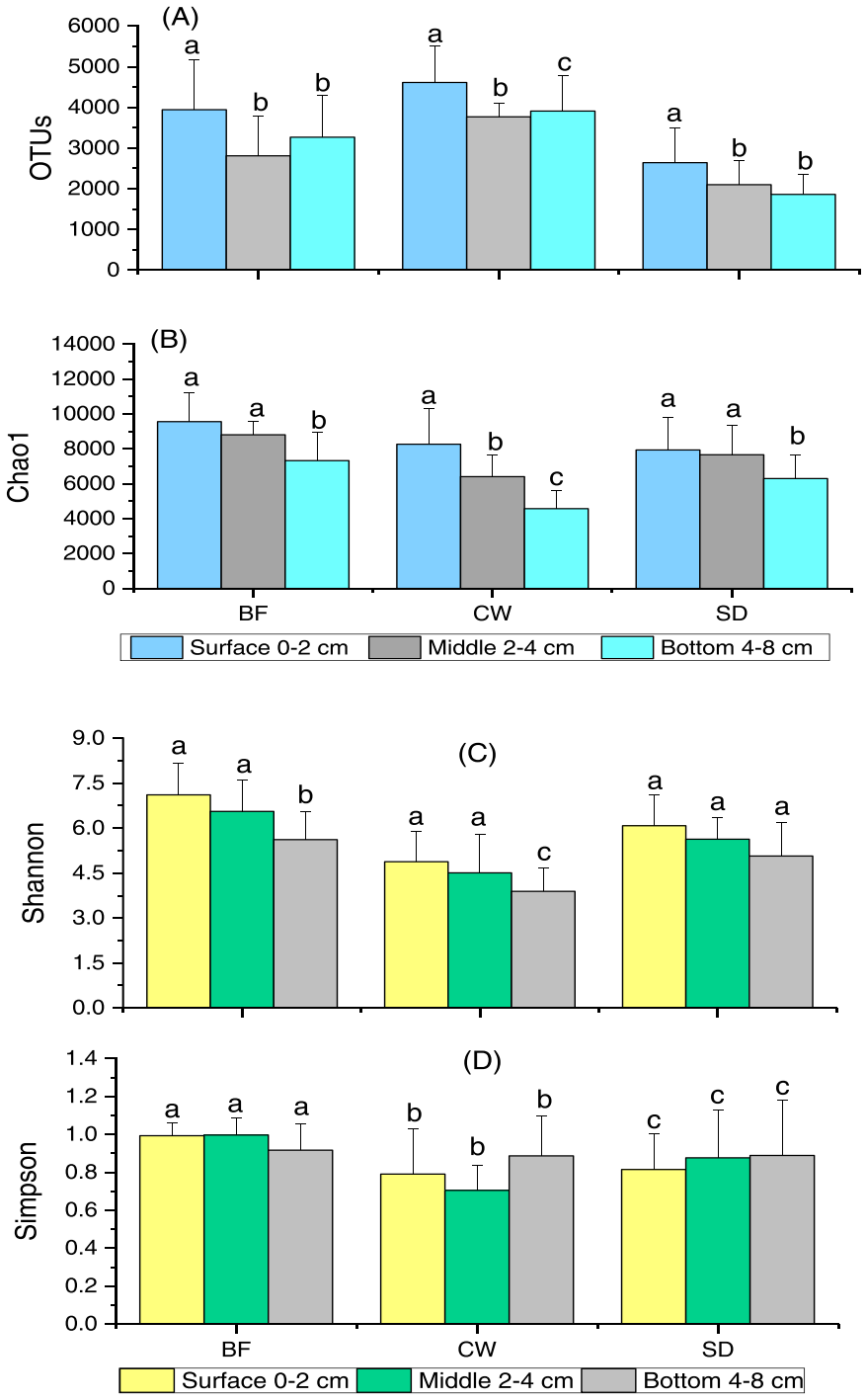

Fig. 4. Alpha diversity estimates of each treatment at different sampling depths obtained by 16 S rRNA genes high-throughput sequencing. (A) Number of OTUs, (B) Chao1 richness estimate index, (C) Shannon index and (D) Simpson.

Bacteroidetes, Acidobacteria, Deltaproteobacteria, Clostridia, Firmicutes Gammaproteobacteria, and *Bacilli* were among the most dominant phyla with approximately 37% and 43% bacterial composition in constructed wetland and biofilm respectively compared sedimentation (19%) (Fig. 5). Some studies suggest that soil microbial distribution can be regulated by the different vegetation types (Deng et al., 2018; Deng et al., 2019). In this study, microbial diversity was highly distributed especially within the constructed wetland subsystem (Figs. 3A-C, 4) implying elevated mineralization. This is supported by a previous study by Carvalho et al. (2013) which reported a substantial interaction among the constructed wetland plants, microorganisms, and contaminants that were supported the plants complex rhizosphere system). Furthermore, studies by Bodelier (2003) and Lukwambe et al. (2019) explained that wetland rhizospheres are oxic-habitats created by the roots' aeration and can markedly affect the diversity of the wetland's heterotrophic biota and activate nutrient fluxes. Besides, the plants roots forming the constructed-wetland harbor/store useful nitrifying-denitrifying bacteria (Chen et al., 2020). This is evident especially with the rhizosphere of emergent aquatic plants, where the plant roots provide a favorable habitat and exudate the growth of various microbes responsible for sediment reworking including sediment nitrogen content transformation (Zhang et al., 2013; Zou et al.,

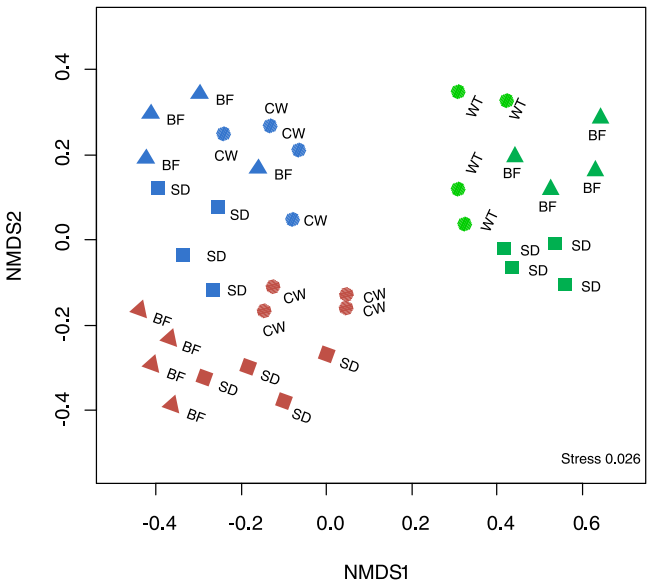

Fig. 5. Non-metric multidimensional scaling plot based on the Bray-Curtis dissimilarity showing the relationship between the samples in each treatment. Shapes in triangle, circles, and squares represent biofilm, constructed-wetland, and sedimentation treatments respectively. Colors in brown, blue and green represent samples at the surface, middle and bottom sediment depth in each system respectively.

Table 2
16 S rRNA sediment microbial community distributions, structure, and composition determined by a permutational multivariate analysis of variance (PERMANOVA, Adonis function) among the treatments.

	Sums of Sqs	Means Sqs	F. Model	R^2	P
Groups	3.0131	1.73516	7.7641	0.29737	0.000*
Depth	0.9362	0.58454	4.5153	0.34574	0.003*
Groups*Depth	0.1795	0.30134	1.9438	0.03952	0.042*

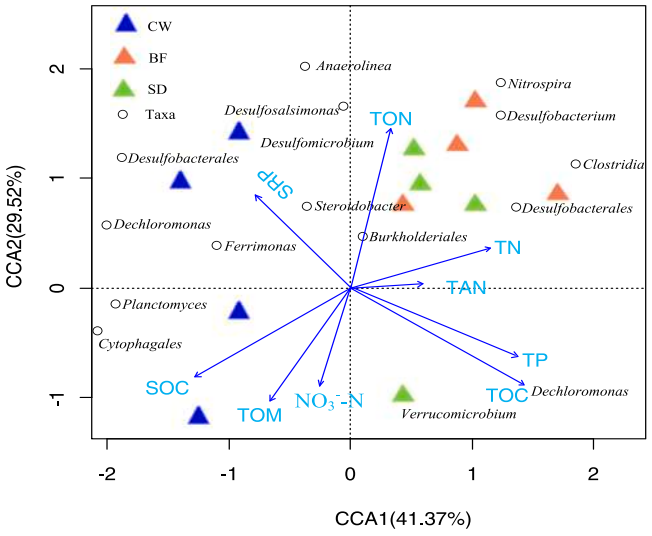

Fig. 6. Canonical Correspondence Analysis ordination plot showing the relationships between bacterial community and physicochemical variables in the three treatments of the mariculture wastewater treatment system. Samples collected from the surface, middle, and bottom of each system are designated with blue, orange, and green triangles, respectively. The abbreviations indicate sediment oxygen consumption (SOC), total organic matter (TOM), organic nitrogen (TON), ammonia nitrogen (TAN), phosphate (TP), nitrate (NO_3-N), nitrite (NO_2-N) and sediment reactive phosphate (SRP).

2013).

A profound number of *Proteobacteria, Chloroflexi,* and *Acidobacteria,* were observed within the constructed wetland and biofilm, suggesting the presence of enhanced mineralization activities which affect the succession and stability of the waste processing bacterial community (Thomas et al., 2012; Nguyen et al., 2016; Zhang et al., 2020). Bacterial community distributions may be enhanced by a biofilm environment (Song et al., 2019). Based on this observation, we can conclude that both biofilms and constructed wetlands supported a larger number of bacterial communities related to organic wastes degradation relative to sedimentation systems.

Also, remediation measures by using constructed wetland, biofilm, and sedimentation are known to influence the aquatic ecosystem biodiversity due to improved sediment conditions (Nicholaus and Zheng, 2014). The sedimentary ecological niches created by each biofilter system differently affect the biotic and abiotic characteristics, thereby resulting in the change of the aquatic microbial diversity and functional diversity.

4.2. *Biogeochemical fluxes and functional microbial community*

In this study, constructed wetland showed a higher SRP, NO_2-N, NO_3-N, and TAN flux rates relative to other treatments (Fig. 2). This release pattern is ascribed to promoted physicochemical-microbial mediated activities such as mineralization, nitrification-denitrification, and redox reaction (Baldwin et al., 2006; Brito et al., 2018; Nicholaus et at., 2019a,b). Literatures show that the root system of the constructed wetland plants has rhizomes that aerate the sediment potentially resulting in increased dissolved oxygen which promotes microbial assemblages and nitrification-denitrification activities (Faulwetter et al., 2009; Ki et al., 2018; Lukwambe et al., 2019). In this study, we found several bacterial taxa associated with ammonium oxidizing bacterial (AOB, e.g., *Nitrospira*) and nitrite-oxidizing bacteria (NOB, *Nitrospina, Nitrosomonas*) and sulfate-reducing bacteria (SRB, *Desulfatibacillum* and *Desulfobacterium*) (Fig. 6 &7) which are reported to contribute to effluent degradation and material transformation (Ki et al., 2018; Soares-Castro et al., 2019). These species were enriched in both constructed wetland and biofilm indicating that the elevated nutrient fluxes were probably due to enhanced bacterial activities such as OM mineralization. On the other hand, putatively performing dissimilatory nitrate reduction to ammonia taxa were about 2.5- to 3-fold more in biofilm and constructed wetland suggesting an increased mineralization activity including nitrification. Normally nitrification process is facilitated by both AOB and NOB bacterial (Mosier and Francis, 2008). Under the presence of oxygen, microbial nitrogen transformation is supported. Macrophyte species with high root-oxygen release capacity may enhance the diversity and activity of ammonia oxidizers leading to increased nitrogen content transformation (Vila-Costa et al., 2015).

Lower TAN fluxes were observed in the biofilm treatment system suggesting increased ammonia utilization by nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter*. These group of bacterial are reported to reduce excess nitrogenous content in the sediment (Baldwin et al., 2006; Nicholaus et al., 2019a,b). In our study, several bacterial functional groups related to biogeochemical nutrient metabolism, cycling, and degradation were discovered (Fig. 7). The expression of chemoheterotrophy, aerobic-chemoheterotrophy, and denitrification microbial functional groups was significantly higher in the constructed wetland than biofilm and sedimentation. This implied that constructed wetland best enhanced the activities related to effluent degradation that led to increased nutrient transformation and fluxes. This as well suggests that the genes associated with different biogeochemical functions were favored and enhanced. Sediment nitrogen fixation, nitrification, denitrification, ammonification, and other major nitrogen transformation processes are mediated by soil bacteria (Yoon et al., 2015; Ki et al., 2018). The sedimentary nitrogen cycle can be improved by biological nitrification and denitrification pathways leading to healthy

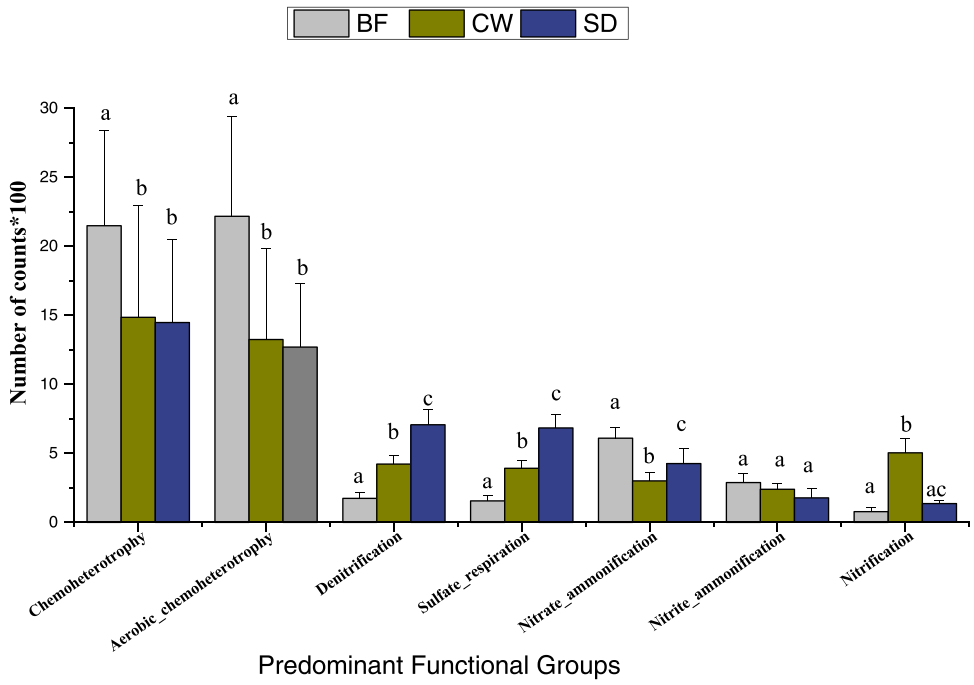

Fig. 7. Bar plot of the relative abundance distributions of the predicted predominant functional groups among the treatments as annotated by the FAPROTAX database.

environmental ecosystems. For example, we observed an increase in the absolute content of functional group related *Acinetobacter* (*Moraxellaceae*), which is responsible for detoxification of different pollutants, such as degradation of aromatic compounds (Felföldi et al., 2010). The increased SRP flux rate from the sediment into the water (biofilm, Fig. 1B) indicates OM transformation could have been promoted by the bacterial community. Ki et al. (2018)) indicated that organic wastes can be decomposed into soluble reactive phosphate by the SRB bacteria, such as *Desulfobacterium, Desulfatibacillum, Desulfomicrobium,* and *Desulfosalsimonas*, which were most evident in both biofilm and constructed-wetland.

4.3. Bacterial community and sediment organic contents among the biofilters

The contents of TON, TOC, TP, and TOM varied significantly among the treatments (Table 1). The observed distribution trend was likely due to improved bacterial community activities associated with mineralization, such as nitrification-denitrification. Sediment nitrogen fixation, nitrification, denitrification, ammonification, and other major nitrogen transformation processes are mainly mediated by soil bacteria (Yoon et al., 2015). The expression of *Dechloromonas, Steroidobacter,* and *Novosphingobium* among the treatments are likely to support the denitrification process and strengthen the physicochemical-microbial interactions. For instance, *Dechloromonas* has been described as denitrifers that produce nitrogen gas as a reduced nitrogen product (Weber et al., 2006). Denitrification can also be fueled by the presence of *Steroidobacter* in the sediments (Fabian et al., 2003). Generally, the lower TN and OM in biofilm and constructed wetland over the sedimentation is probably due to the TAN transformation through microbial oxidation to NO_3^--N and NO_3^--N. The majority of nitrogen content reduction in wetlands is believed to result from the microbial coupled nitrification-denitrification interactions and uptake by the wetland plants (Yeh et al., 2010). The 16 S rRNA sequencing result showed that taxa such as *Firmicutes* and *Nitrospinae* were differentially enriched among the treatments a phenomenon that may have attributed to the reduced level of the organic contents.

Additionally, CCA indicated strong relationships between the bacterial communities and physicochemical factors (Fig. 6). Among the treatments, nine benthic variables (TAN, NO_2^--N, NO_3^--N, SRP, TN, TP, TOC, OM and, SOC) correlated more closely with microbial community groups. The correlations between bacterial communities and nutrient fluxes and organics were moderately high (ordination axis 1 = 58.1%, axis 2 = 42.7% of the total variation). The constructed wetland and biofilm had more affiliated taxa linked with physicochemical variables relative to sedimentation indicating a greater association between the functional genera among the two treatments. This result is similar to Wu et al. (2008), Lukwambe et al. (2019) and Ki et al. (2018) who reported a substantial correlation among the bacteria and nitrogen transformation. In similar patterns, CCA results revealed that TAN, TP, TOC, and TN contents were factors that strongly correlated with *Desulfomicrobium* (surface sediment) and *Chloroflex* (deeper sediment, biofilm) while SOC, TP, and SRP mostly correlated with *Ferrimonas, Burkholderia, Dechloromonas,* and *Desulfomicrobium,* especially in constructed wetland. This can be supported by a couple of previous studies (Sinkko et al., 2011; Sinkko et al., 2013; Ki et al., 2018) which indicated *Desulfomicrobiuim* and *Methylobacter* had strong association with TAN resulting in reduced organic contents.

5. Conclusions

This study investigates the impact of constructed wetland, sedimentation and biofilm biofilters on the distributions of the bacterial community, benthic nutrient fluxes, TP, TON, SOC and organic matter in a comprehensive mariculture wastewater treatment system. The study showed that the treatments improved the sediment bacterial dynamics (community structure, diversity, and composition), elevated nutrient dynamics across the sediment water-interface, and reduced organic matter contents. Microbial groups associated with AOB, NOB, SRB were enriched in the constructed wetland and biofilm mostly within the 0–4 cm sediment depth. The chemoheterotrophy, aerobic-chemoheterotrophy, denitrification, and nitrification were the most dominant functional groups in the mariculture treatments systems. The TAN, NO_2^--N, and NO_3^--N flux rates across the sediment-water interface

were higher in constructed wetland compared to biofilm and sedimentation subsystems. Also, the constructed-wetland and biofilm had reduced organic effluents and improved sediment properties relative to sedimentation. This study suggests that constructed wetland exceedingly enhanced the bacterial community composition and nutrient fluxes and reduced organic matter contents relative to the biofilm and sedimentation biofilters.

Consent to participate

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

Consent for publication

All the authors have consented to the publication.

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CRediT authorship contribution statement

ZZ supervised the study. RN 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 was done by BL and RN.

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.

Data Availability

Data will be made available on request.

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