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RESEARCH

Nitrogen and phosphorus-related functional genes enhance nutrient removal in the integrated aquaculture wastewater bioremediation system in the presence of photosynthetic bacteria

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Abstract

Integrated Aquaculture Wastewater Bioremediation Systems (IAWBSs) are crucial for treating nutrient-rich mariculture water. However, there is a lack of detailed information about the functional mechanisms between nitrogen (N) and phosphorus (P) functional genes and the bacterioplankton community in the presence of photosynthetic bacteria (PSB). This study evaluated the connections between N and P functional genes in IAWBSs under the influence of PSB. The results showed significant improvements in effluent quality, with removal efficiencies of 79, 74, 83, 90, and 71% for $NO_2^{-}{-}N$, $NO_3^{-}{-}N$, PO_4^{3-}-P, $NH_4^{+}{-}N$, and COD_{Mn}, respectively. Adding PSB enhanced and altered microbial diversity within the system, promoting the abundance of functional genes related to N and P cycling. Notably, genes associated in denitrification (*nirK* and *nirS*), ammonification (*ureC*) and *amoB*, involved in nitrification, were significantly increased after PSB was added. Furthermore, genes such as *phnK*, *phoD*, and *phoX*, which are involved in P transformation, also showed increased abundance levels. These genes were closely linked to the microbial community distribution, species diversity, and nutrient cycling. Microbial community changes can result in changes in functional gene abundance. This study provides important and novel insights for developing bioremediation strategies for polluted sites. This demonstrates the fundamental relationships between the IAWBSs' functional units and the distribution of microbial communities under the influence of PSB.

Keywords Functional genes · Nitrogen · Phosphorus · Bioremediation · IAWBS

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Introduction

In recent decades, intensive aquaculture activities have released many nutrients, like nitrogen and phosphorus, into surrounding waters, which leads to overstimulated algal growth and disrupts aquatic ecosystems (Yan and Zhong. 2022; Fernandes et al.2001). Several methods have been developed to restore ecosystems through aquaculture practices that integrate natural processes. For managing the eutrophication in aquaculture wastewater systems, combined biological filters, including clams, biofilms, and macrophytes, are increasingly being used as ecologically benign bioremediation methods (Li et al. 2010; Qin et al. 2017; Lukwambe et al. 2019). One way to improve the balance of nitrogen cycling and decrease aquaculture effluents is to adopt an integrated approach (Brito et al. 2018a, b; Wang et al. 2018a, b; Lukwambe et al. 2018; Strand et al. 2019). Enhancing the compatibility between substrates and microorganisms is crucial for successfully implementing Integrated Aquaculture Wastewater Bioremediation Systems (IAWBSs).

Bioremediation utilizes geologically existing or intentionally proposed microbes to consume and decompose organic wastes in the aquatic environment (Gifford et al. 2006). IAWBS technology has gained significance as a cleaning-up method in environmental attribute programs because of its dependable performance, adaptability, cost-effectiveness, and eco-friendliness. The efficiency of the IAWBS method can be enhanced by using microorganisms that can remove complex contaminants. One such group consists of photosynthetic bacteria, which are prokaryotic organisms that can perform the process of photosynthesis. Lakes, paddy fields, soils, rivers, activated sludge, and oceans are just a few environments where these bacteria commonly thrive and propagate (Koblízek et al. 2006; Okubo et al. 2006). Photosynthetic bacteria are generally categorized into two groups: (1) oxygenic bacteria, which include prochlorophytes and cyanobacteria. These bacteria utilize water as an electron donor during photosynthesis, producing oxygen (O_2) as a byproduct, and (2) anoxygenic bacteria, which cannot use water as an electron donor and, as a result, do not produce O_2 during photosynthesis. Instead, species like Rhodobacter and Rhodopseudomonas can assimilate carbon dioxide (CO_2), nitrogen, and various organic or inorganic substances (He et al. 2010; Koblızek et al. 2006; Okubo et al.2006). Due to their rapid growth rates and ability to metabolize a wide range of organic materials as substrates, photosynthetic bacteria offer significant advantages for bioremediation. Furthermore, certain species possess cellular components such as ubiquinone, carotenoids, and vitamin B12 that play a role in denitrification (Idi et al. 2015). Denitrification is a microbiological method where dissolved nitrogen molecules are alternate electron acceptors for power generation. The primary biological process responsible for recovering fixed nitrogen into the atmosphere. PSB microbes in these systems help remove nitrogenous compounds from wastewater and are strongly linked to the nitrogen-phosphorus cycle (Deng et al. 2021). Genes associated with nitrogen (N) and phosphorus (P) have vital functions in bio-remediation of wastewater. In the nitrogen cycle, denitrifying genes play an important role and serve as an excellent model for understanding the evolutionary connections among functional genes. This is because many bacteria can do the process of denitrification, resulting in significant diversity. The nutrient cycle is intricately linked to microorganisms, specifically focusing on loading and converting nitrogen (N) and phosphorus (P). The nitrogen cycle involves the presence of nitrite (NO_2^--N), nitrate (NO_3^--N), ammonium (NH_4^+-N), and nitrogen gas (N_2). Microbial metabolism is typically linked to ammonia oxidation, nitrification, and denitrification during wastewater treatment (Kleindienst et al. 2014; Raza et al. 2024c).

Research has demonstrated the existence and prevalence of genes related to phosphorus (P) from wastewater (Wang et al. 2020a, b). These genes are linked to phosphorus-accumulating organisms (PAOs) and denitrifying phosphorus-accumulating organisms (DPAOs) such as *Thauera* and *Dechloromonas*. David L (2016) found that when phosphonic acids served as the sole phosphorus source, the *phnK* and *ppk1* genes were essential for the growth and reproduction of *E. coli*. Since microorganisms can only utilize KH_2PO_4, the *phnK* genes were highly prevalent among phosphorus-cycling genes, accounting for 41–49% of the total. (Wang et al. 2020a, b). Therefore, utilization of functional genes in molecular approaches is crucial for denitrification. Combining functional genes with microalgae-based technologies can greatly improve the effectiveness of bioremediation processes for (N) and (P) in wastewater. This promotes sustainable environmental practices (Nirmala 2023).

Photosynthetic bacteria (PSB), including *Rhodopseudomonas*, *Rhodobacter*, and *Rhodococcus*, have been extensively utilized for pollutant treatment. *Rhodopseudomonas* plays a pivotal role in wastewater management by efficiently mineralizing organic waste, removing hydrogen sulfide, and participating in denitrification processes (Wu et al. 2021). It has been demonstrated to promote the growth of purple non-sulfur bacterial biomass in various wastewater sources, thereby reducing chemical oxygen demand (COD) and lowering concentrations of various ions and compounds (Tarabas et al. 2019). Furthermore, strains of *Rhodopseudomonas* have been shown to enhance aquaculture water quality, boost disease resistance, and improve the growth and yield of fish species (Xu et al. 2012). *Rhodococcus*, on the other hand, plays a critical role in pollutant treatment due to its exceptional biodegradation capabilities and diverse metabolic pathways (Ivshina et al. 2022; Hosseini et al. 2023; Wang et al. 2020a, b). However, the treatment efficacy of single bacterial strains can be limited in practical applications. Composite strains offer significant advantages, particularly in addressing complex contaminants (Aparicio et al. 2018). For instance, a reagent composed of *Rhodobacter blasticus* and *Rhodobacter capsulatus* in a 1:1 ratio was shown to reduce COD in anaerobically digested swine wastewater by 83.3%, which was 19.3% higher than using *Rhodobacter blasticus* alone and 10.6% higher than using *Rhodobacter capsulatus* individually (Wen et al. 2016). Additionally, a compound of *Bacillus cereus*, *Bacillus amyloliquefaciens*, and *Pseudomonas stutzeri* proved effective in reducing ammonium nitrogen (NH_4^+-N), nitrate (NO_3^--N), and nitrite (NO_2^--N) in aquaculture wastewater (Jones et al. 2001). These findings collectively underscore the significant potential of PSB in bioremediation strategies to address environmental pollution challenges.

However, IBSAW technologies are still immature. There is still room for improvement in the system's operation and nutrient removal capabilities (Aquilino et al. 2020; Tom et al. 2021). However, in this experiment, we used a photosynthetic bacterium (*Rhodopseudomonas palustris, Rhodobacter capsulatus,* and *Rhodospirillum rubrum*). Notable contributions of the present study to the existing scientific literature are as follows: (1) the assessment of the performance efficiency of effluent removal in the IAWBS system with the addition of photosynthetic bacteria (PSB), (2) assess the influence of PSB on the microbial community and functional genes related to phosphorus and nitrogen cycling, and (3) to investigate the relationship between bacterial community and functional genes related to nitrogen and phosphorus.

Materials and methods

Experimental design

This study was conducted at Ningbo University's Pilot Test Base in Meishan, where an IAWBS with four treatment units (sedimentation, biofilm, filter-feeding shellfish, and macroalgae) was established. Each unit was housed in a white polyethylene (PE) barrel with a 70 L capacity, through which the effluent flowed. The biofilm unit was equipped with polyethylene brushes (0.2 m diameter, 0.5 m length) at a density of four brushes per barrel. To prevent the brushes from floating above the water surface, one end was anchored at the bottom, and the other was hanging perpendicularly in the middle of the barrel. *Tegillarca granosa* clams were added to the shellfish unit at a density of 70 individuals per square meter (Nicholaus et al. 2019a, b), whereas *Gracilaria lemaneiformis* was added to the macroalgae unit at a density of 0.2 g/L. The wastewater used in the experiment was obtained from a high-density greenhouse shrimp farm (Haohai Aquaculture Farm) in the Yinzhou District, Ningbo City, Zhejiang Province, China. To imitate the wastewater treatment process, every 2 days, 20 L of wastewater were sent to the subsequent treatment unit in the following sequence: sedimentation, biofilm, shellfish, and macroalgae.

Bacterial consortium *(Rhodopseudomonas palustris, Rhodobacter capsulatus,* and *Rhodospirillum rubrum*), herein referred to as 3Rs, were incubated in a light incubator at a temperature of 30 degrees Celsius following the activation of the culture under light conditions of 3000–4000 LX. In the treatment group, 1‰ photosynthetic bacteria (PSB), specifically 3Rs, was introduced into the system at regular intervals every 4 days. The concentrations of the 3Rs were organized in a ratio of 2:2:3, as provided by the BeNa culture collection facility. The trial was conducted over 1 month.

Sample collection and water quality index analysis

Following comprehensive mixing of the entire water column, samples of water and microorganisms were obtained before and after each water change. The water samples were filtered using a 0.45 μm polycarbonate membrane to quantify the levels of ammonia nitrogen (NH_4^+-N), nitrate (NO_3^--N), nitrite (NO_2^--N), and orthophosphate (PO_4^{3-}-P). Unfiltered water samples were utilized to find the levels of total nitrogen (TN), total phosphorus (TP), and chemical oxygen demand (COD_{Mn}). The samples were stored at – 20 °C until analysis. The microbial samples were passed through 0.22 μm polycarbonate membranes using a vacuum pump and stored at – 80 °C for subsequent DNA extraction and sequencing.

The concentrations of NH_4^+-N, NO_3^--N, NO_2^--N, PO_4^{3-}-P, TN, and TP were measured using an automated batch chemical analyzer (Smart-Chem 200). The following parameters were measured using the specified methods: NH_4^+-N, TN, TP, NO_3^--N, NO_2^--N, PO_4^{3-}-P, and COD_{Mn} were determined using hypobromite oxidation, potassium persulfate oxidation, cadmium column reduction, naphthalene ethylenediamine spectrophotometry, phosphomolybdenum blue spectrophotometry, and alkaline potassium permanganate methods.

DNA extraction and sequencing

The Thermo Fisher Scientific spectrophotometer (Nanodrop One) was used for additional purification and quantification following bacterial DNA extraction with the Minkgene Water

DNA Kit. The universal primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the V3–V4 regions of the 16S rRNA gene. Sequencing was conducted on the Illumina HiSeq 2500 platform by Guangdong Magigene Biotechnology Co., Ltd. (Guangzhou, China), producing 250 bp paired end reads. The sequence data, including the submission ID (SUB14924924) and BioProject accession number (PRJNA1197180), have been submitted to the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov). The sequencing data were processed using USEARCH (v11.0.667_I8) for bioinformatic analysis (Edgar 2010). The UNOISE3 algorithm, with parameters set to `unoise_alpha=2`, `minsize=8`, and default settings, was applied to refine the sequences. This process included generating zero-radius operational taxonomic units (ZOTUs), removing chimeras, and correcting sequencing errors. Taxonomic classification and quantification of the ZOTUs were performed using the SILVA database.

Detection of nitrogen and phosphorus, functional gene chip

After acquiring the initial experimental sample, microbial DNA was extracted and assessed for its quantity and purity. Following this evaluation, DNA samples and the reagents required for quantitative polymerase chain reaction (qPCR) were loaded into a 384-well plate called the "Sample Plate." The Assay Source Plate was prepared by combining the source plate, primers, and qPCR reagents in another 384-well plate. Both the sample plate and primer reagents were transferred into the nanopores of a high-throughput qPCR GeoChip using an automated high-throughput microsampler. The qPCR and fluorescence signal detection were done using the SmartChip Real-Time PCR System, which automatically generated amplification and melting curves (Nolan et al. 2006). The *Canco* program determined the detection status and (Ct) value for each gene in the sample. Relative quantification of each gene was achieved by normalizing the results using the 16S rRNA gene as an internal reference. Absolute quantification of the 16S rRNA gene was performed using the Roche detection method, while the quantities of other genes were determined through data conversion, as described by (Mardis and McCombie. 2017).

Statistical analysis

Nutrient contents were examined using one-way analysis of variance (ANOVA) with Tukey's post hoc multiple comparisons. Genetic data were analyzed using the SPSS statistical tool and visualized using GraphPad Prism 9 and R 4.0.4. Functional gene quantification was performed using Excel software. SR plot software was used to visualize the variation in the relative abundance of genes through balloon analysis. The Kruskal–Wallis rank test was used to differentiate specific differences across groups (Chang et al. 2022). The "Hmisc" package was utilized to calculate the correlation coefficient between the abundance of functional genes and the microbial abundance. The network was then generated using the "igraph" program and viewed using Gephi 0.10.1.

Results

Abundance of bacterioplankton community and functional genes related to nitrogen and phosphorus

The dominant bacterioplankton phyla identified in the system included *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* (Fig. 1a). Among these, *Proteobacteria* and *Bacteroidetes* were the most abundant in the sedimentation and biofilm areas, respectively. In the shellfish area, the relative abundance of *Proteobacteria* increased in both the control (CS) and treatment (TS) groups, whereas *Bacteroidetes* decreased in both groups. In the CS group, *Proteobacteria* and *Actinobacteria* were more abundant than the TS group. However, the addition of photosynthetic bacteria (PSB) resulted in an increased abundance of *Actinobacteria* in the TS group. In the macroalgal area, the relative abundance of *Proteobacteria* slightly decreased from the control (CM) to the treatment (TM) group, while *Bacteroidetes* exhibited a similar slight decline (Fig. 1a). At the class level, the most abundant classes in the sedimentation area were *Alphaproteobacteria*, *Bacteroidia*, and *Gammaproteobacteria*, with *Bacteroidia* being the most dominant. However, the relative abundance of *Bacteroidia* decreased in the biofilm area, whereas *Alphaproteobacteria* and *Gammaproteobacteria* showed an increase.

In the shellfish area, *Alphaproteobacteria* exhibited the highest abundance in the CS group but declined in the TS group. Conversely, the abundance of *Gammaproteobacteria* increased in the TS group. In the macroalgal area, *Alphaproteobacteria* increased in the CM group, while *Actinobacteria* showed a marked increase in the TM group (Fig. 1b). Overall, analysis at the class level identified four prominent bacterial classes: *Alphaproteobacteria*, *Bacteroidia*, *Gammaproteobacteria*, and *Actinobacteria*. While *Alphaproteobacteria* and *Bacteroidia* decreased in the treatment areas, *Gammaproteobacteria* showed a slight increase. Notably, *Actinobacteria* abundance significantly increased in the TM.

The assimilatory and dissimilatory nitrate reduction pathways, organic phosphorus mineralization, nitrification, denitrification, anammox, nitrogen fixation, and other phosphorus cycling processes are among the important functional genes associated with nitrogen and phosphorus metabolism pathways that have been identified. The GeoChip technique was used to identify these genes, yielding 31 genes: 22 related to N and 9 related to P. As

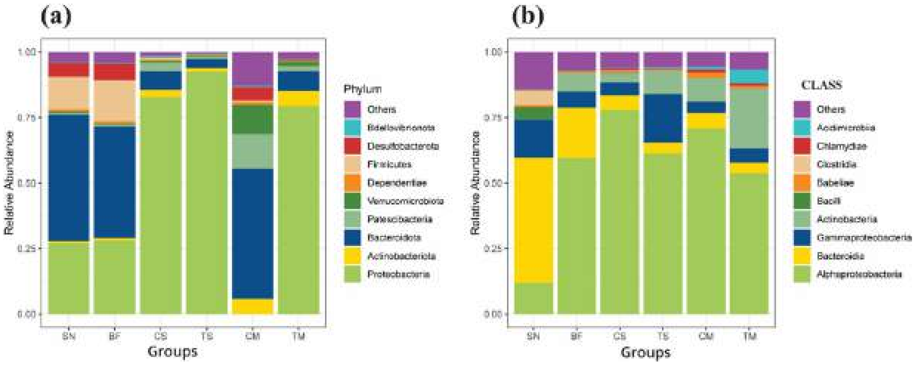

Fig. 1 Relative abundance of bacteria at the phylum level (**a**) and class level (**b**). *SN* sedimentation area, *BF* biofilm, *CS* shellfish of the control, *TS* shellfish of the treatment, *CM* macroalgae of the control, *TM* macroalgae of the treatment

shown in Fig. 2, the number of sequencing-matching genes varied. Adding PSB improved the nitrification process. All the genes in the sedimentation area were significantly higher than those in all the other areas of the system ($p < 0.05$). The most prevalent genes for denitrification were nor (*norB/C*), *nap (napA/B*), and *nir (nirK/S*). There was a significant increase in the *gdhA* gene in the shellfish-treated area, which can convert org-N into NH_4^+-N ($p < 0.05$). Because of this, it could not evaluate how they affected nitrification and denitrification differently. Nonetheless, the system contained the functional gene *nifH,* responsible for converting N_2 to NH_3 through nitrogen fixation. There was a significant increase in *ureC* concentration in the macroalgae treatment area ($p < 0.05$). In the phosphorus group, there was an increase in the quantity of *ppx* in the shellfish area and a significant increase in *phoD* in the macroalgae treated area ($p < 0.05$).

Abundance and network analysis of bacterial community and functional genes

Shotgun functional gene analysis showed the relative abundance of nitrogen genes in different areas (Fig. 3a). It indicates that the most abundant nitrogen gene *ureC* was present in the CS. Still, its concentration increased in the shellfish area treatment. After this, the urea concentration was slightly decreased in the other two areas (the control and treated area of the macroalgae). The *nosZ2* gene was found in both control and treated areas of shellfish and macroalgae. In addition, *nifH* was also present in these areas. *nosZ1* was also present

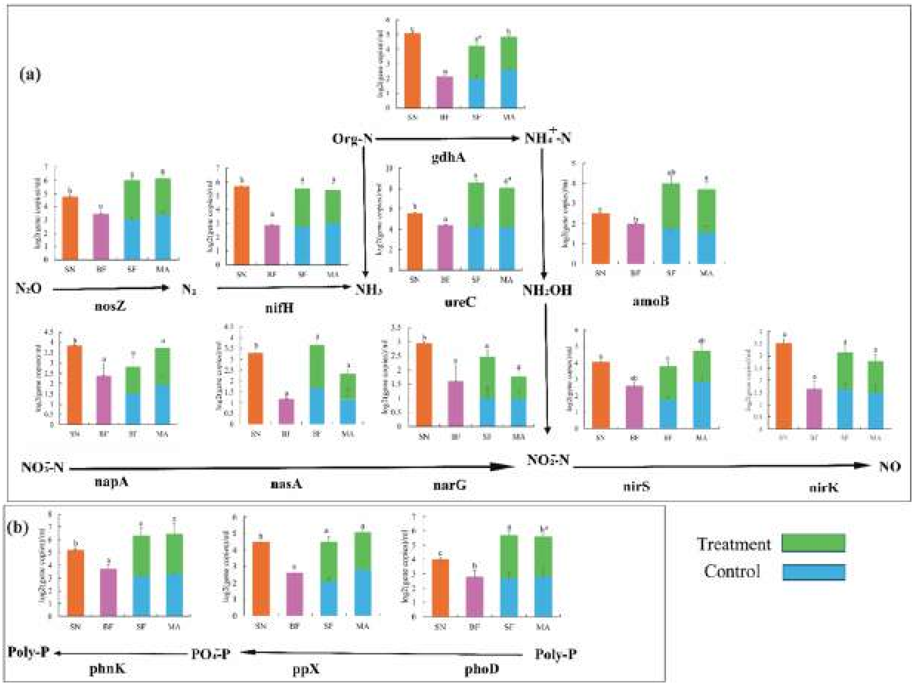

Fig. 2 Absolute abundance functional genes in the cycles of nitrogen (**a**) and phosphorus (**b**). Lowercase letters present the significant difference between all groups, and * presents the significant difference between control and treatment group; *SN* sedimentation area, *BF* biofilm area, *SF* shellfish area, *MA* macroalgae area

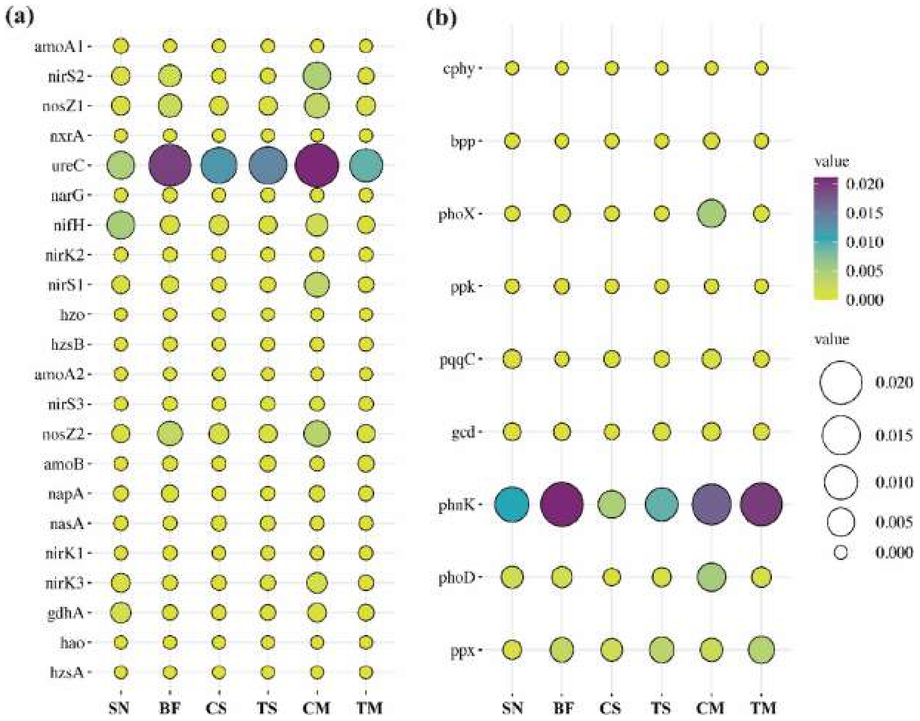

Fig. 3 Relative abundance of the nitrogen functional gene (**a**) and phosphorus functional gene (**b**). *SN* sedimentation area, *BF* biofilm, *CS* shellfish area of the control, *TS* shellfish area of the treatment, *CM* macroalgae area of the control, *TM* macroalgae area of the treatment

in shellfish and macroalgae areas. The relative abundances of the phosphorus genes are shown (Fig. 3b). The *phnK* gene is the most abundant in all areas, particularly in the treated shellfish area, where it is present at high concentrations. The *phoD* gene was slightly present in the TS unit but absent in other areas. *ppx* were found in the shellfish and macroalgal areas. The shellfish area *ppx* was the most abundant compared with the other areas.

A network analysis investigated the functional genes in all areas' control and treatment groups and their relationship with the bacterioplankton community (Fig. 4). This analysis indicated that the Spearman coefficients were greater than 0.8, and the *q* value was less than 0.05. In the control group, all functional genes were present but were less abundant, and a smaller number of bacterial phyla were attached to them than in the treatment group. The bacterial communities were more abundant in the treatment groups. This is evident from Fig. 3a which shows that genes such as *ureC* were associated with many bacterial phyla in the shellfish area of the treatment. Genes in the control group were less abundant than those in the treatment group. Similarly, many bacterial phyla associated with genes, such as *nirS2, nirZ2*, *nifK*, and *nosZ1*, were observed in the control group, but their abundance increased in the treatment group. More bacterial phyla were attached to the functional genes, indicating that the adding of PSB altered the bacterial community in the treatment groups, demonstrating the impact of PSB on the system.

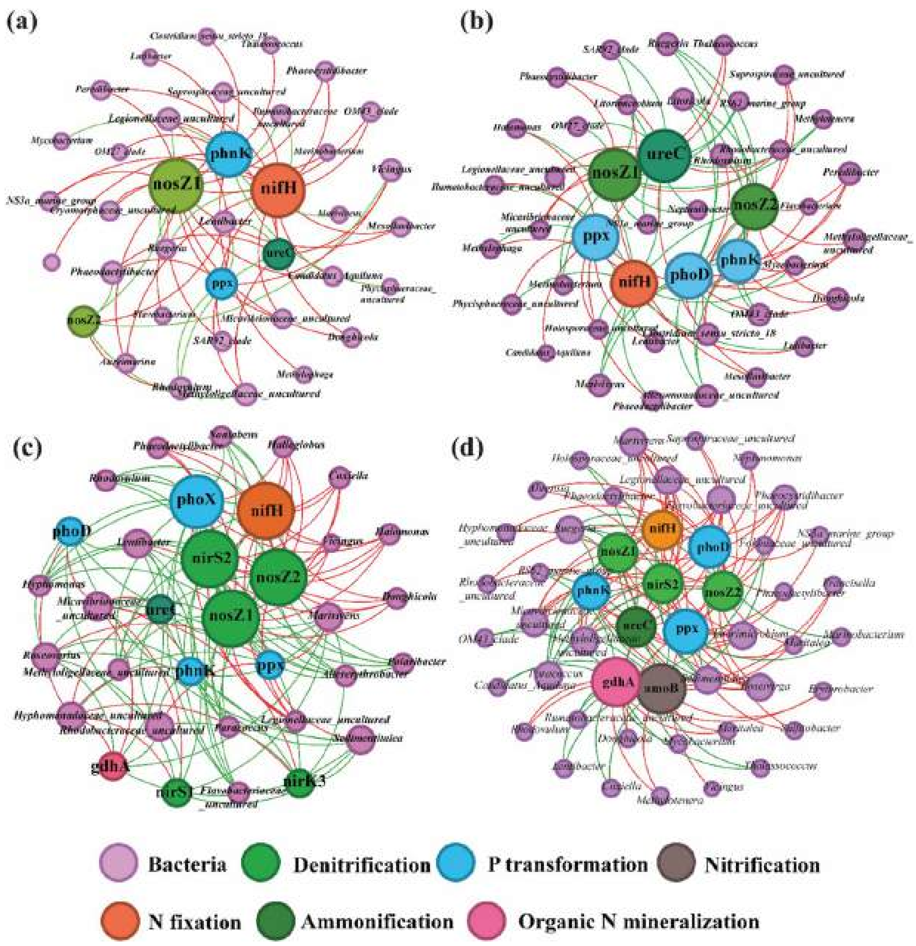

Fig. 4 Network analysis between the functional genes of nitrogen and phosphorus and bacterial genera in the system. **a** Shellfish area of the control; **b** shellfish area of the treatment; **c** macroalgae area of the control; **d** macroalgae area of the treatment. The red and green lines show the negative and positive relationships, respectively

Mycobacterium was positively correlated with *phnK*. *Rhodoyulum* positively linked to *ppx* and *ureC* levels. *nosZ2* group and *Vicingus* were positively correlated (Fig. 4a). *Methylotenera* was positively linked to *ureC* and *nosZ1* in the shellfish treatment area (Fig. 4b). Rugeria and *marivivens* were positively correlated with *phoD phnK* abundance. *Halomonas* was positively correlated with *nosZ1*. In the macroalgal area of the control, *Nonlabens*, *Rhodovulum*, *Hyphomonas*, and *Polaribacter* were positively correlated with *phoX, nifH, nirS2, nosZ2*, and *nosZ* (Fig. 4c). *GdhA* abundance was positively correlated with *nirS1* abundance. Sedimentation was positively interacted with *nirK3, nirS, phnK, ureC*, and *phoD*. In the macroalgal area of the treatment (Fig. 4d), *mycobacterium*, *thalassococcus*, *Coxiella*, om43-clade were positively connected with *amoB* and *gdhA*. *Marinobacterium* was positively linked with *nosZ2* and *ppx* levels. *Rugeria and Paracoccus* were positively connected with *ureC*, *nirS2, phnK, nosZ1*, and *nifH* levels.

Changes IAWBS effluents under PSB

The removal rate of NO_2^--N, NO_3^--N, PO_4^{3-}-P, NH_4^+-N, and COD_{Mn} were 79%, 74%, 83%, 90%, and 71% (Fig. 5). This data indicates that the removal efficiencies of these nutrients varied significantly. For NO_2^--N, the removal rate in the control group was less efficient, whereas the removal efficiency was higher in the treatment area with PSB. The treatment area has a 74% removal rate of NO_3^--N, which is significantly higher as compared to the control group ($p < 0.05$). NH_4^+-N removal rate was 90% in the PSB treatment area, whereas the control group, which did not exhibit PSB, showed a lower removal rate ($p < 0.05$). There was a significant difference in the PO_4^{3-}-P removal rate between the control and treatment areas; the removal rate of PO_4^{3-}-P was 83%, which was higher in the treatment group than in the control group ($p < 0.05$). COD_{mn} removal rate was significantly higher in the treatment area (71%) than in the control area ($p < 0.5$).

Discussion

Changes in bacterioplankton community and nutrient removal induced by PSB

Bioremediation is a natural self-cleaning mechanism where microorganisms undergo aerobic or anaerobic transformations, converting organic matter into biomass. Recent research has shown that holistic bioremediation strategies offer more efficient solutions for removing the nutrients from aquaculture wastewater (Lukwambe et al. 2019; Wang et al. 2018a, b). According to our studies on the bacterioplankton community in various groups, the most prevalent bacterial phyla in the system were Proteobacteria, Actinobacteria, and Bacteroidetes (Fig. 1a). In the shellfish area, Proteobacteria was the most abundant phyla and

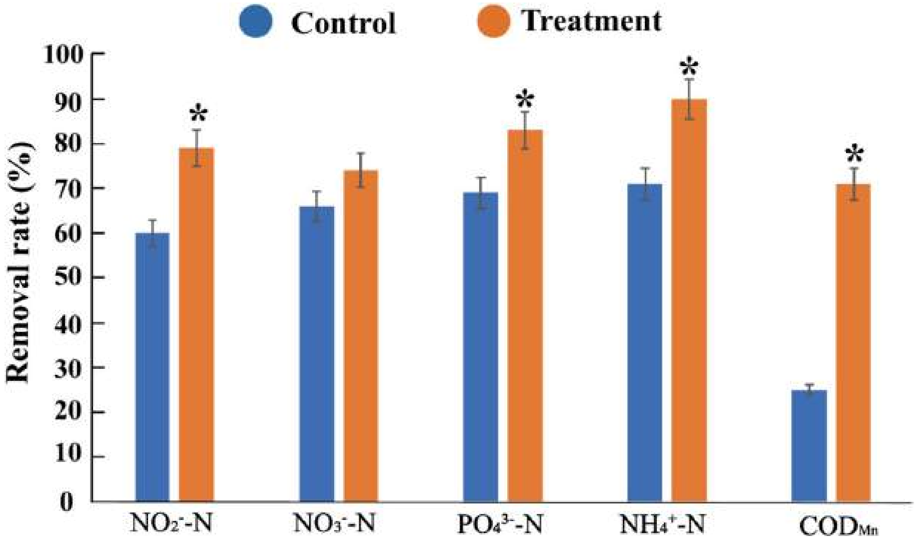

Fig. 5 This indicates the removal efficiency of nutrients in the control and treatment areas. *Significant difference between control and treatment areas

less prevalent in the sedimentation area (Fig. 1a). The combined relative abundance of these three phyla was more than 70% and occupied the critical position dominantly. Similar results were found in previous studies in which the most prevalent phylum was Proteobacteria and Actinobacteria (Xie et al. 2020; Li et al. 2017, 2020). The remarkable capacity of Proteobacteria to absorb phosphorus and nitrogen may be related to their complex survival mechanisms in high nutrient environments and to utilize a diverse range of energy sources (Lukwambe et al. 2019; Xie et al. 2020). Proteobacteria contain the most denitrifying bacteria (Li et al. 2015; Wang et al. 2018a, b). They are critical for removing nitrogen, phosphorus, and organic contaminants from aquatic habitats (Zhang et al. 2017). According to Gao et al. (2021) and Wang et al. (2022), Bacteroides are linked to the breakdown of macromolecules, carbohydrates, and pollution control, that remove nutrients and organic debris. Similar investigations carried out in other anammox systems revealed the existence of micro anaerobic zones (Huang et al. 2020; Wang et al. 2021).

Furthermore, previous research found that Acidobacteria favored environments with low nutrient levels (Zhang et al. 2014). Furthermore, it has been observed that under anaerobic conditions, both Bacteroidetes and Chloroflexi may convert high nitrogen and phosphorus into low nitrogenous and phosphorus compounds (Qiu et al. 2014; Fu et al. 2018). Further the denitrification process was carried out in the presence of Bacteroidetes in anoxic environments in addition to metabolizing protein and saccharides (Li et al. 2018a).

Alphaproteobacteria, Bacteriodia, Gammaproteobacteria, and Actinobacteria were the most abundant classes in our experiment (Fig. 3b). The macroalgae area was enriched in many algal-lysing bacteria, such as class Actinobacteria. In the IAWBS system, Actinobacteria can eradicate harmful bacteria and contribute to nutrient removal (Lukwambe et al. 2015), Furthermore, Alphaproteobacteria and Actinobacteria are also involved in removing nitrogen, improving aquaculture water quality (Wei et al. 2023) and suppressing harmful algal bacteria (Ghai et al. 2014). According to Lukwambe et al. (2015), members of AOB and NOB, mostly related to the Alphaproteobacteria, are typically involved in nutrient cycling. In wastewater treatment systems, these bacteria are widely recognized as the primary source of nitrogen removal (Zhang et al. 2019; Chang et al. 2022; Lukwambe et al. 2015).

The PSB displayed excellent removal capability, specifically for NH_4^+-N, with an average efficiency of 88–90% (Fig. 5). The nutrient removal performance of nitrogen, phosphorus, and COD_{Mn} under the impact of PSB is illustrated in (Fig. 5). The shellfish and macroalgae areas were analyzed concerning the control group. Adding PSB increased the clearance rates of NO_2^--N and NO_3^--N in our experiment. The bacteria present in the systems were strongly involved in the nitrogen and phosphorus cycle. Additionally, most of these bacteria are beneficial for eliminating nitrogen-containing chemicals found in wastewater (Deng et al. 2021; Raza et el. 2024b). These bacteria can convert nitrogen (N) through various processes such as nitrification, denitrification, uptake, anaerobic oxidation of ammonia by nitrate, and nitrogen fixation (Baneras et al. 2012). Macrophytes release oxygen through their stems, rhizomes, and roots and then can enhance the nitrification of NH_4^+-N, as demonstrated by (Van Hulle et al. 2010). Underneath these circumstances, autotrophic nitrifying bacteria carry out the aerobic oxidation of ammonium to nitrate. Subsequently, under oxygen-deprived conditions, nitrate is transformed by heterotrophic denitrifying bacteria into nitrogen gas or nitrous oxide (Rehman et al. 2017). In our earlier work, we discovered that razor clams caused an increase in the population of nitrifying bacteria in the sediment (Lukwambe et al. 2018). These bacteria are responsible for the oxidation of nitrites to nitrates. The inclusion of PSB expanded the food chain of the system by accelerating main productivity through the responses of microorganisms, thereby enhancing the population of heterotrophic bacteria (Actinobacteria). PSB has been recognized as a proficient aerobic denitrifier that breaks down NO_3^--N via assimilation and aerobic denitrification pathways (lv et al. 2022). Moreover, Alphaproteobacteria exhibit remarkable efficacy in the process of aerobic inorganic nitrogen transformation, successfully depleting NH_4^+-N, NO_3^--N, and NO_2^--N (Nan zhang et al. 2022; Raza et al. 2024a).

Shifts of nitrogen and phosphorus-related genes by network analysis

The effects of PSB exposure on functional genes of microbial metabolism related to N and P removal in all areas were measured (Fig. 3). The most abundant gene related to the nitrogen removal process was the *ureC* gene in macroalgae area (Fig. 3a). This gene encodes reductase enzymes which are involved in the reducing phase of the ammonification process (Wei et al 2015; Wei et al. 2024). In our studies, the most abundant gene in all the areas was *ureC*, which belongs to the class Alphaproteobacteria (Fig. 1b, 3a). Alphaproteobacteria, which contain almost 31–35% nitrifying bacteria, possess the *ureC* gene (Wang et al. 2024). According to previous reports (Casella et al. 2006; Ghosh et al. 2007; Jackson et al. 1991), when there is sufficient NO, the Cu-containing nitrite reductase encoded by *nirK* could replace *norS* to decrease NO to N_2O. Levy-Booth et al. (2014) discovered that N_2O emissions were associated with nitrifying and denitrifying populations by quantifying genes implicated in N cycling. According to Kandeler et al. (2009), the percentage of N_2O converted to N_2 increased with increasing *nosZ/nirK* ratios. The addition of PSB dramatically increased the appearance of functional genes required in denitrification (*gdhA*), ammonification (*ureC*), and nitrification (*amoB*) (Fig. 2). During wastewater treatment, the abundance and activity of genes associated with nitrogen metabolism can be influenced by the introduction of various bacterial species (Zhu et al. 2022). The metabolic pathways of nitrogen cycling genes experienced substantial alterations, mostly in shellfish and macroalgal areas. Simultaneously, there was a significant ($p<0.05$) increase in the demonstration of genes related to nitrification, such as amoA/B/C, and genes involved in denitrification, including *nosZ, ureC, nosZ2*, and *nirK*. The genes associated with nitrate reduction, namely *NosZ*, *NorB*, and *NirK*, showed a favorable correlation with *Marinobacterium* and *Marivivens* (Fig. 4). An intricate network can portray a pivotal role in enhancing the variety and resilience of microbial communities (Dai et al. 2017; Jiang et al. 2019; Lukwambe et al. 2015). This indicated that the anammox process was limited. The nitrogen content analysis revealed that the nitrification and denitrification efficiencies in the control group were lower compared to the treatment group. The concentrations of NO_3^--N and NH_4^+-N were significantly reduced in the treatment group relative to the control group (Fig. 5). These findings indicate that nitrogen metabolism in the IAWBS system was enhanced through nitrification and denitrification processes; however, nitrogen fixation and the anammox process were inhibited.

The understanding of phosphorus metabolism mechanisms is less advanced than that of nitrogen metabolism. Microorganisms involved in phosphorus cycling, such as polyphosphate-accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs), play a key role by releasing phosphorus and accumulating polyphosphate (poly-P). Furthermore, the functional genes related to phosphorus removal were *phnK* and *PhoD,* primarily affected by Photosynthetic bacteria (PSB) and highly common in macroalgae areas. The carbon phosphorus lyase complex is encoded by one of the crucial *Phnk* genes of bacteria. Regarding phosphorus functional genes (Fig. 2b) indicates that the most useful gene is *phnK,* which is involved in ATP binding and is essential for the growth of bacteria, such as *E. coli.* Furthermore, the increased phosphorus release during the anoxic phase were explained by the slightly higher *ppx* gene abundance in the SF and MA areas than in the others. These findings demonstrated that poly-P synthesis and hydrolysis were improved to maximize phosphorus elimination. This complex is essential for converting inactivated phosphonates into phosphate, which is necessary for metabolism (Yang et al. 2016). Transmembrane transport increased the uptake of *phnK* use of phosphorus-comprising substances (Tian et al. 2021). The *phnK* mediated in the P cycling (Wang et al. 2022, 2017), which conformed to the changes of the *phnK* gene. Furthermore, the *ppx* and *ppk* genes facilitate the accumulation and release of phosphorus in anaerobic and aerobic processes, respectively (Yang et al. 2019). In our studies, several phosphorus metabolic functional genes as the foundation for efficient P removal were identified. The abundance of the *phoD* gene was significantly higher in MA, suggesting that the primary mechanism of phosphorus metabolism in the MA area was the accumulation of poly-P to remove phosphorus. *phoD* often participates in transporting organic phosphorus (Willis et al. 2019). The treatment group exhibited more phosphorus absorption genes than the control group (Fig. 5), suggesting an enhanced removal impact of PO_4^{3-}-P in the system. The (*ppk*) gene was identified as poly-P, whereas the(*ppx*) gene was accountable for the breakdown of poly-P (Jha et al. 2021). Furthermore, it was shown that poly-P formation is associated with *ppk, ppk2*, and *ppa.* The ATP-binding protein encoded by *phnK* facilitates cellular metabolism and nutrient uptake by binding and recruiting ATP. Simultaneously, the genes *phoD* and *phoX*, which encode alkaline phosphatases, are upregulated in response to the elevated phosphate demand associated with active cellular metabolism (Lin et al. 2018). Certain phases of wastewater treatment systems exhibit an increased prevalence of these organisms. The closely interconnected bacterial community, characterized by shared ecological roles, likely promotes the growth of suitable bacteria for bioremediation (Zeng et al. 2019).

Conclusion

The current study evaluated the potential influence of PSB on the N and P-related functional genes in IAWBS. The findings revealed that PSB effectively eliminated nutrients NO_2^--N, NO_3^--N, NH_4^+-N, PO_4^{3-}-P, and COD_{Mn} with removal rates of 79%, 74%, 83%, 90%, and 71%, respectively. Adding PSB dramatically increased the representation of functional genes related to the P and N cycles, such as *nirK*, *nirS*, *ureC, nifH*, and *amoB.* In addition, the abundance of genes concerned with phosphorus transformation, such as *phnK, phoD,* and *phoX*, was enhanced following the addition of photosynthetic bacteria. This discovery will improve our understanding of the connections between functional genes and other bacterial phyla, the influence of PSB on IAWBS, and how the PSB controls the removal of nitrogen and phosphorus compounds from wastewater.

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Data availability Data availability will be made on request.

Declarations

Competing interests The authors declare no competing interests.

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