



Article Effects of Snail Bellamya purificata Farming at Different Stocking Densities on the Algal and Fungal Communities in Sediment

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Abstract: The snail Bellamya purificata is recognized as a potential bio-remediation species, and is commonly employed in polyculture to enhance resource utilization efficiency and realize culture environment regulation. In order to enrich the microbiome studies on elucidating the ecological effects of snail B. purificata farming, we assessed the effect of B. purificata farming activities, at varying stocking densities, on the algal and fungal communities in sediment. Four experimental groups were established in our study, each corresponding to a different stocking density: 0, 234.38, 468.75, and 937.5 g/m², represented as CON, LD, MD, and HD, respectively. High-throughput sequencing based on ITS and 23S ribosomal RNA (rRNA) genes was employed to analyze the variations in algal and fungal communities under B. purificata farming activities at different stocking densities. B. purificata farming activities had no significant effect on the alpha diversities of fungal and algal communities, but significantly altered the compositions of fungal and algal communities in sediments, especially B. purificata farming activity at low stocking density. B. purificata farming activities at low stocking density could significantly increase the relative abundances of fungal genera Paraconiothyrium and Penicillium compared with the CON group. The promoting effect diminished with increasing density. B. purificata farming activities at low or medium stocking density also could enhance the relative abundances of algal genera Microchloropsis, Scenedesmus, and Auxenochlorella. Hence, B. purificata farming activity at low stocking density might be the optimum density to enhance resource utilization efficiency and minimize environmental pollution.

Keywords: Bellamya purificata cultivation; aquaculture; algal community; fungal community; sediment

Key Contribution: The findings of this study provide valuable insights into the impacts of *B. purificata* farming activities at different stocking densities on the algal and fungal communities in sediment and contribute a broader understanding for the snail *B. purificata*'s ecological effects and serve as a valuable theoretical reference for the rational application and development of *B. purificata* farming.

1. Introduction

Global aquaculture production approached a record high of 122.6 million tons valued at USD281.5 billion in 2020, with China contributing nearly 70% to the total world aquaculture output [1]. The development of aquaculture has contributed to ensuring global food security and meeting the increasing demand of the growing world population for high-quality proteins [1,2]. Along with rapid development, the aquaculture industry is facing



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). several pressing challenges. Aquaculture ecosystems, including ponds, lakes, reservoirs, and rivers, play a vital role in maintaining the quality of aquatic products and ensuring food security supply. However, traditional aquaculture always pursues maximizing yield and benefits through blindly increasing stocking densities and feeding amounts, which in turn lead to a low utilization of resource, large amounts of residual organic waste, and various environment issues in its own and surrounding aquatic ecosystems [3–8].

To address this issue for sustainable aquaculture, integrated multi-trophic aquaculture (IMTA) has been studied and developed in recent years, which represents an innovative approach to aquaculture that aims to optimize resource utilization and minimize negative impacts on the environment [9–13]. IMTA refers to the polyculture of multiple species from different trophic levels in a mutually beneficial relationship, where waste products from one species serve as nutrients for others [9]. The snail Bellamya purificata is recognized as a potential bio-remediation species, and commonly employed in polyculture to enhance the resource utilization efficiency and realize the culture environment regulation [14–16]. The snail B. purificata is a highly representative freshwater snail and is widely distributed across ponds, lakes, reservoirs, rivers, and other aquatic ecosystems in China, which has a natural preference for inhabiting silt and consuming organic debris and algae in its surrounding environment [17,18]. There have been some studies on the ecological effects of *B. purificata* in aquaculture or water purification processes [14,19,20]. The snail *B. purificata* can enhance organic matter degradation within sediment and promote material circulation at the sediment–water interface, in addition to purifying the culture water [14,19,20]. We have attempted to explore the mechanisms or pathways of *B. purificata*'s ecological effects from a microbiological perspective by determining the bacterial communities in sediments [21]. However, fungi and algae are also important components of microorganisms, although the biomass of bacteria may be ten times that of fungi in the sediment [22].

Fungi and algae play a vital role in material circulation and biogeochemical processes in the aquatic ecosystem. Algae serve as crucial primary producer and food chain driver in aquatic ecosystems [23]. Fungi and algae can provide insight into dynamic variations in ponds, lakes, reservoirs, rivers, and other aquatic ecosystems through the algae's and fungi's structural, functional, and physiological features [23–26]. A previous study has revealed that different farming practices and farming species markedly affect the fungal communities in sediments [27]. Xu [25] first reported the dynamic variations of fungal community and diversity in the integrated rice–crab farming system to better understand and optimize the farming ecosystem. Even so, there is still a lack of studies focusing on the fungal and algal communities In aquaculture ecosystems, particularly the influences of *B. purificata* farming activities on the fungal and algal communities. We believe that enriching the content of microbiome studies to demonstrate the snail *B. purificata*'s ecological effects is crucial for the rational application and development of *B. purificata* farming.

Hence, we performed high-throughput sequencing based on ITS and 23S ribosomal RNA (rRNA) genes in sediment to assess the effects the *B. purificata* farming activities with different stocking densities on the fungal and algal communities. The findings of this study would lead to a broader understanding of the snail *B. purificata*'s ecological effects and serve as a valuable theoretical reference for the rational application and development of *B. purificata* farming.

2. Materials and Methods

2.1. Experiment Design

The experiments were conducted at the Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences (120.250479° E, 31.51581° N; Wuxi, China). The experimental snail and sediment were collected from aquaculture ponds located at the Dapu aquaculture facility (119.939129° E, 31.316981° N; Wuxi, China). Before commencing the experiment, a period of 14 days was allotted for the *B. purificata* snails to acclimatize to the controlled laboratory environment by placing them in a glass tank. To ensure homogeneity and consistency, the sediment used in the experiment underwent drying,

grinding, sieving, and mixing according to the pre-processing steps implemented in the previous studies [28–30]. Twelve glass tanks ($80 \times 40 \times 45$ cm) were employed in the experiment. All the glass tanks were covered with sediment to a depth of 7 cm on the bottom, filled with aerated and filtered tap water, and then left to be precipitated and stabilized for 14 d before the experiment. For the experiment, four separate groups, including one control group and three treatment groups, were established according to four different stocking densities and each group with three replicates. The four different stocking densities were 0, 234.38, 468.75, and 937.50 g/m², respectively. The corresponding groups were abbreviated as CON, LD, MD, and HD, respectively. After acclimation, healthy snails with an average wet weight of 2.53 ± 0.01 g were collected and randomly distributed between glass tanks. During the experiment period, the commercial feed (Zhejiang Haida Feed Co., Ltd., Shaoxing, China) was utilized as the experimental diet. The snails were fed every day at 4:00 pm, with the amount approximating 2% of their individual body weights. The experimental conditions were maintained at a constant water temperature of 26.5 ± 0.5 °C and a dissolved oxygen (DO) level of about 6.5 mg/L. One-third of the water in each glass tank was changed every two days. The experimental lighting condition was a natural light/dark cycle. The experimental period lasted for 80 days, during which all snails were observed to be in good health with no mortalities recorded.

2.2. Sample Collection

At the end of the experiments, sediment samples were collected using plastic tubes with a diameter of 2 cm from ten randomly chosen sampling points in each glass tank. The sediment samples were taken from the surface sediment layer, which was between 0–1 cm deep. To maintain consistency, all the sediment samples from each identical glass tank were mixed thoroughly. Sediment samples designated for the analysis of fungal and algal communities were promptly stored at -80 °C to preserve their integrity for further analysis.

2.3. PCR Amplification and Sequencing

The DNA of fungi and algae in the sediment samples was extracted using the E.Z.N.A.[®] soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocol. The quality and concentration of DNA were evaluated using 1.0% agarose gel electrophoresis and a NanoDrop[®] ND-2000 spectrophotometer (Thermo Scientific Inc., Waltham, MA, USA), and subsequently, the DNA was stored at -80 °C until further use. Specific primers were designed and synthesized to amplify the ITS1-ITS2 and 23S rDNA regions using an ABI GeneAmp[®] 9700 PCR thermocycler (ABI, CA, USA). The ITS1-ITS2 region was amplified with primer pairs ITS1 (5'–CTTGGTCATTTAGAGTAAGTAA–3') and ITS2 (5'–GCTGTGTTCATCGATGC–3'). The 23S rDNA region was amplified with two forward primers (A23SrVF1: 5'–AGACARAAAAGACCTATG–3' and A23SrVF2: 5'–CARAAAGACCTATTGMAGCT–3') and two reverse primers (A23SrVR1: 5'–AGATCAGC CTTTATCC–3' and A23SrVR2: 5'–TCAGCCTGTTATCCTAG–3') [31].

PCR amplification for ITS1-ITS2 region was carried out three times in 20 μ L reaction mixtures consisting of 2 μ L of 10× Buffer, 2 μ L of 2.5 mM dNTPs, 0.8 μ L of forward primer (5 μ M), 0.8 μ L of reverse primer (5 μ M), 0.2 μ L of rTaq Polymerase, 0.2 μ L of BAS, 10 ng of template DNA, and double-distilled H₂O to the final volume. PCR amplification for the 23S rDNA region was carried out three times in 20 μ L reaction mixtures including 4 μ L of 5× FastPfu Buffer, 2 μ L of 2.5 mM dNTPs, 0.8 μ L of forward primer (5 μ M), 0.8 μ L of 5× FastPfu Buffer, 2 μ L of 2.5 mM dNTPs, 0.8 μ L of forward primer (5 μ M), 0.8 μ L of reverse primer (5 μ M), 0.4 μ L of FastPfu Polymerase, 0.2 μ L of BAS, 10 ng of template DNA, and double-distilled H₂O to the final volume. The PCR amplification was performed using the following cycling conditions: initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s, and single extension at 72 °C for 10 min, and end at 10 °C. The PCR product was extracted with a 2% agarose gel. The extracted PCR product was then purified and quantified by the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City,

CA, USA) and Quantus[™] Fluorometer (Promega, WI, USA) following the manufacturer's protocol, respectively.

Purified amplicons were pooled in equimolar amounts and subjected to paired-end sequencing on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw sequencing reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA993837).

2.4. Data Processing

The raw FASTQ files underwent de-multiplexing through an in-house perl script. Subsequently, raw FASTQ files were subjected to quality filtering using fastp version 0.19.6 and merged using FLASH version 1.2.7 [32,33]. The optimized sequences were clustered into operational taxonomic units (OTUs) using UPARSE 7.1 with a 97% sequence similarity level [34]. The most abundant sequence for each OTU was chosen as a representative sequence. To ensure accuracy, the OTU table was manually filtered, and chloroplast sequences were eliminated from all samples. In order to mitigate the effects of sequencing depth on the alpha and beta diversity measure, the number of ITS DNA and 23S rDNA sequences from each sample were rarefied to 44,639 and 13,331, respectively. The taxonomy of each OTU representative sequence was analyzed using RDP Classifier version 2.2 against the ITS DNA and 23S rDNA database (Unite ITS 8.0 and NT v20210917) with a confidence threshold of 0.7, respectively [31,35].

2.5. Statistical Analysis

Bioinformatic analysis for the sediment samples was conducted by the Majorbio Cloud platform (https://cloud.majorbio.com (accessed on 1 December 2022)). Alpha diversity indices including the observed richness (Sobs), Shannon, Simpson, Chao1, and ACE were calculated using Mothur v1.30.1 [36]. The Sobs, Chao1, and ACE were used for accessing the richness of the fungal and algal communities, while Shannon and Simpson were employed to evaluate the diversity. Higher values of these indices indicate higher richness or diversity. One-way ANOVA followed by the Tukey-Kramer post hoc test was used to confirm differences in the alpha diversity indices of fungal and algal communities within sediment between CON, LD, MD, and HD groups. Subsequently, for the beta diversity, the Bray–Curtis distances among different samples were calculated and the principal coordinate analysis (PCoA) based on Bray-Curtis distances was conducted to reveal the differences in the fungal and algal communities between different groups. The Adonis test accompanying the PCoA analysis was performed to further determine the differences in the fungal and algal communities between different groups. The community bar plot was conducted to demonstrate the relative abundances of the dominate phyla and genera in the fungal and algal communities. The phylum or genus in the fungal and algal communities with a relative abundance greater than 1% would be defined as a dominant phylum or genus. One-way ANOVA followed by the Tukey-Kramer post hoc test was used to confirm differences in the relative abundances of all the phyla and genera within fungal and algal communities. All analyses and related figures were completed using the vegan and ggplot2 packages in R v. 4.0.3 (R Core Team, Vienna, Austria).

3. Results

3.1. Overview of Fungal and Algal Communities in Sediment

In the present study, 2295 distinct OTUs were obtained from the sediment samples through Illumina sequencing technology based on the fungal ITS gene, and subsequentially assigned into 12 phyla and 259 genera. As shown in Figure 1, there were 124 OTUs and 11 genera unique to the CON group, 515 OTUs and 108 genera unique to the LD group, 262 OTUs and 20 genera unique to the MD group, and 336 OTUs and 17 genera unique to the HD group. Moreover, the LD group exhibited 834 OTUs and 143 genera not included in the CON group, which were far more than those exhibited by MD and HD groups.



Figure 1. Venn diagrams of the fungal communities in CON, LD, MD, and HD groups at operational taxonomic unit (**a**) and genus (**b**) levels.

We also obtained 1391 algae-related OTUs based on the 23S rRNA gene, which were assigned into 44 phyla and 333 genera. As shown in Figure 2, there were 205 OTUs and 36 genera unique to the CON group, 156 OTUs and 21 genera unique to the LD group, 140 OTUs and 19 genera unique to the MD group, and 119 OTUs and 22 genera unique to the HD group. Compared with the CON group, the LD group owned 286 unique OTUs and 53 unique genera, the MD group owned 268 unique OTUs and 48 unique genera, and the HD group owned 248 unique OTUs and 53 unique genera.



Figure 2. Venn diagrams of the algal communities in CON, LD, MD, and HD groups at operational taxonomic unit (**a**) and genus (**b**) levels.

3.2. Alpha and Beta Diversities of Fungal Community in Sediment

Alpha diversity indices including Sobs, Shannon, Simpson, ACE, and Chao1 were calculated to evaluate the diversity and richness of the fungal community in sediment. As shown in Figure 3, no significant differences in the Sobs, Shannon, Simpson, ACE, and Chao1 between the CON, LD, MD, and HD groups were observed according to the results of one-way ANOVA followed by the Tukey–Kramer post hoc test (p > 0.05). For the beta diversity, PCoA analysis was conducted to investigate the differences in fungal community based on Bray–Curtis distances. As shown in Figure 4a, PC1 and PC2 explained 25.39% and 18.94% of the total variation in the fungal community within sediment, respectively. The CON, LD, MD, and HD groups were not obviously separated, as they all have overlapping areas. The result of the Adonis test also indicated no significant differences between the CON, LD, MD, and HD groups (p > 0.05).









Figure 4. Principal coordinate analysis (PCoA) of fungal (**a**) and algal (**b**) communities in sediment based on Bray–Curtis distances.

3.3. Alpha and Beta Diversities of Algal Community in Sediment

According to the results of one-way ANOVA followed by the Tukey–Kramer post hoc test, there were no significant differences in Sobs, Shannon, Simpson, ACE, and Chao1 between the CON, LD, MD, and HD groups as shown in Figure 5 (p > 0.05). For the beta diversity, as shown in Figure 4b, the first two PCs in the PCoA analysis explained 34.17% and 16.41% of the total variation in the algal community within sediment, respectively. The LD group was obviously separated from the other three groups as revealed by the PCoA results. The result of the Adonis test also confirmed the PCoA results and indicated a significant difference between the CON, LD, MD, and HD groups (p < 0.05).



Figure 5. Differences in the alpha diversity indices of algal communities including Sobs (**a**), Shannon (**b**), Simpson (**c**), ACE (**d**), and Chao1 (**e**) between CON, LD, MD, and HD groups. Different letters indicated significant differences between different groups.

3.4. Composition of Fungal Community in Sediment

There were in total 12 phyla and 259 genera assigned from 2295 distinct OTUs in the sedimentary fungal community in the present study. The dominate phyla and genera (relative abundance > 1%) in the fungal community are shown in Figure 6. Similar with previous studies, most fungi could not be effectively annotated at either the phylum or genus level and has been represented as unclassified_k_Fungi in Figure 6 [27,37]. Excluding the unclassified fungal taxa, the dominate phyla were Ascomycota, Rozellomycota,

(a)

HD

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Basidiomycota, and Chytridiomycota, and the dominate genera were *Scutellinia*, *unclassi-fied_p_Rozellomycota*, *unclassified_p_Chytridiomycota*, and *Apiotrichum* in the present study.

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📕 unclassified_k_Fungi 📕 Scutellinia 📕 unclassified_p_Rozellomycota 📕 unclassified_p_Chytridiomycota 📕 Apiotrichum 📗 others

O.A.

Figure 6. Fungal community composition at phylum (**a**) and genus (**b**) levels within sediment in CON, LD, MD, and HD groups.

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As shown in Figure 7, the relative abundances of genera *Paraconiothyrium* and *Penicillium* were significantly different among the CON, LD, MD, and HD groups (p < 0.05). The relative abundance of *Paraconiothyrium* in sediment of LD groups was significantly higher than that of CON group (p < 0.05). However, no significant differences in the relative abundance of *Paraconiothyrium* between the LD, MD, and HD groups were observed (p > 0.05). The relative abundance of *Penicillium* in the LD groups was significantly higher than in the CON, MD, and HD groups (p < 0.05).

Community barplot analysis at phylum level



Figure 7. The significantly different genera of fungal communities in the sediment between CON, LD, MD, and HD groups, including *Paraconiothyrium* (**a**) and *Penicillium* (**b**). Different letters indicated significant differences between different groups.

3.5. Composition of Algal Community in Sediment

There were a total of 44 phyla and 333 genera in the algal community in the present study. The dominate phyla and genera (relative abundance > 1%) in the algal community were shown in Figure 8. The dominate phyla ranked in descending order of relative abundance were Verrucomicrobia, unclassified_d_unclassified, Chlorophyta, Cyanobacteria, Ignavibacteriae, Firmicutes, unclassified_d_Bacteria, unclassified_d_Eukaryota, Bacillariophyta, Candidatus_Woesebacteria, unclassified, Proteobacteria, and Oomycota. There were 23 dominate genera. Excluding the unclassified algal genus, the top 5 dominate genera were *Pedosphaera, Prosthecobacter, Desmodesmus, Synechococcus*, and *Opitutus*.



Figure 8. Algal community composition at phylum (**a**) and genus (**b**) levels within sediment in CON, LD, MD, and HD groups.

Through One-way ANOVA, there were 1 phylum and 6 genera that were significantly different among the CON, LD, MD, and HD groups. As shown in Figure 9, the relative abundance of phylum Streptophyta in the sediment of the LD group was significantly higher than that of MD group (p < 0.05). However, the CON, LD, and HD groups showed similar relative abundances of Streptophyta (p > 0.05). The relative abundance of genus *Nibricoccus* in the HD group was significantly higher than in the MD group (p < 0.05). The LD and MD groups exhibited significantly higher relative abundances of *Scenedesmus* compared with the CON group (p < 0.05), but the relative abundance of *Scenedesmus* in the HD group was similar with that in the CON group (p > 0.05). The relative abundance of Microchloropsis in the LD group was significantly higher than that in the CON, MD, and HD groups (p < 0.05). The relative abundance of Auxenochlorella in the CON group was significantly lower than that in the MD group (p < 0.05), and no significant differences among the LD, MD, and HD groups were observed (p > 0.05). The relative abundance of *Choricystis* in the LD group showed a significant decreasing trend with increasing stocking density, while the relative abundance of *Chthoniobacter* showed a significant increasing trend with increasing stocking density and reached a maximum in the MD group (p < 0.05).



Figure 9. The significantly different phylum and genera of algal communities in the sediment between CON, LD, MD, and HD groups, including Streptophyta (**a**), *Nibricoccus* (**b**), *Scenedesmus* (**c**), *Microchloropsis* (**d**), *Auxenochlorella* (**e**), *Choricystis* (**f**), and *Chthoniobacter* (**g**). Different letters indicated significant differences between different groups.

4. Discussion

4.1. Fungal Community in Sediment Affect by B. purificata Farming Activities

Fungal community, as well as algal and bacterial communities, play a significant role in the biogeochemical cycle of aquatic ecosystems. Fungi have diverse morphological structures, complex community structures, and strong metabolic capabilities, the physiological and biochemical characteristics of which are affected by the surrounding environment [38]. In the present study, most fungi could not be well annotated, which could be attributed to the relatively limited DNA sequences in the existing fungal databases compared to the total amount of fungal DNA sequences [39,40]. The phyla Ascomycota, Rozellomycota, Basidiomycota, and Chytridiomycota were the dominate phyla in the present study, ranking among the top 5 in relative abundance, respectively. Fan [39] analyzed the fungal community in sediment from tilapia (Oreochromis niloticus) cultural ponds and reported the Basidiomycota, Ascomycetes, and Chytridiomycota as the dominate phyla. Zhang et al. [27] investigated dozens of fish, crab, and crayfish ponds and the dominant fungal phyla observed in these ponds were predominantly Ascomycota, Chytridiomycota, Rozellomycota, and Basidiomycota. Wang et al. [41] and Zhao et al. [42] also revealed the Ascomycetes and Basidiomycota as the dominant phyla in Poyang Lake and Hongze Lake. These similar results indicate that the present indoor simulation experiment could well simulate the real state of the fungal community in the cultural ecosystem.

B. purificata farming activities at different stocking densities had no obvious impacts on the fungal community diversity in the sediment, which was different with the variations in fungal community diversity under tilapia farming activities reported in a previous study [39]. Fan [39] pointed out that the fungal community in the cultural pond was not only sensitive to temperature and climate, but also to the external nutrient inputs. The accumulation of organic waste, including feed residues and faces, in sediment significantly affect the fungal community [39]. However, the snail *B. purificata* is a typical species which has been proven to play an important role in organic matter degradation in sediment [19]. Snails promote the degradation of organic matter, reduce the accumulation of organic matter in sediment, and enhance material cycling at the sediment-water interface [19]. Hence, the snail *B. purificata* might have the potential to maintain the consistency in sedimentary organic matter content between different groups by ingestion and promoting the degradation and recycling of organic matter, thereby avoiding the obvious impact derived from organic matter accumulation on the fungal community. Meanwhile, the unaffected fungal community also revealed that bioturbation by the snail B. purificata could not directly alter the fungal community in sediment, although *B. purificata* bioturbation could significantly change the physico-chemical properties of the sediment [19]. As the relevant physicochemical properties within the sediment were not measured in our study, this inference needs to be explored in further studies.

Although the overall impact of *B. purificata* farming activities on the fungal community in sediment is not significant according to the results of alpha and beta diversity, *B. purificata* cultivation affected several specific genera. *B. purificata* cultivation in the low stocking density (LD) group significantly increased the genera *Paraconiothyrium* and *Penicillium*. The genus *Paraconiothyrium* is widely distributed worldwide with diverse host habitats and has potential applications as a producer of antibiotics [43]. *Paraconiothyrium* can suppress the activity of harmful fungi such as *Sclerotinia sclerotiorum* in the soil, thus reducing the infection caused by pathogenic microorganisms [43]. The genus *Penicillium*, as a saprophytic fungus, is widely distributed in soil and sediment [44,45]. The secondary metabolites of the *Penicillium* are various compounds with antibacterial and antioxidant activities, which can inhibit the growth of pathogenic bacteria as well as plant-pathogenic fungi [44–50]. Hence, the significantly increased *Paraconiothyrium* and *Penicillium* in the LD group indicated that *B. purificata* farming activity at a low stocking density may effectively promote the enrichment of *Paraconiothyrium* and *Penicillium* in sediment, thereby inhibiting pathogenic microbe activity, improving the performance of cultured aquatic animals, and establishing

more sustainable aquatic food production [51]. However, this promoting effect diminished with the increasing density.

4.2. Algal Community in Sediment Affect by B. purificata Farming Activities

Algae play a significant role in autochthonous primary production, providing the basis for littoral secondary production, and act as crucial regulators of nutrient dynamics within aquatic ecosystems [52,53]. Despite their importance, variations in the algal community within the aquaculture ecosystem has received relatively little attention [52]. In the present study, *B. purificata* farming activities imposed a more pronounced effect on the algal community in sediment relative to the fungal community. In particular, *B. purificata* farming activity at a low stocking density had a stronger overall effect on the algal community than that at medium and high stocking densities according to the results of the PCoA. As reported in a previous study, phyla such as Chlorophyta and Cyanobacteria, were dominated in the algal community of the East Sea [54]. Similarly, the relative abundance of phyla Chlorophyta and Cyanobacteria were also dominated in the present study, both of which ranked among the top 5 in relative abundance without unclassified taxa.

Similar to the effects of *B. purificata* farming activities at different stocking densities on the fungal community's diversity, *B. purificata* farming activities also had no obvious impacts on the algal community's diversity in the sediment. However, there has been limited research on the interactions and effects of culture species with algal communities in sediments. The dynamics of algal communities are associated with various physical and chemical factors in the aquaculture environment, among which inorganic nutrients such as nitrogen and phosphorus are fundamental substances required for the growth and reproduction of algae [55,56]. *B. purificata* can enhance the organic matter degradation within sediment and promote material circulation at the sediment–water interface, which may facilitate the growth of algae [14,19,20]. On the other hand, *B. purificata* prefers to ingest organic debris and algae in its surroundings [17,18]. Hence, we hypothesized that the non-significant impacts were likely to be attributed to a combined effect of promoting algal growth by bioturbation and inhibiting the algal community by ingestion.

Moreover, in the present study, the algal genera Microchloropsis, Scenedesmus, and Auxenochlorella were significantly enhanced in the LD or MD group relative to those in the CON group. The algal genus Microchloropsis adapts to different nutritional conditions and can effectively utilize nitrate and organic nitrogen [57,58]. The genus *Scenedesmus* is capable of removing nitrate and phosphate from the surrounding environment [59]. In addition, the genus Auxenochlorella is an early-appearing single-celled eukaryotic green algae, which is an efficient primary producer in ecosystems and capable of removing ammonium [60,61]. The significantly increased relative abundances of *Microchloropsis*, Scenedesmus, and Auxenochlorella in the LD or MD groups might result from the enhanced degradation of organic matter in sediment and the improved cycling of nutrients at the sediment–water interface caused by *B. purificata* snail farming. Talib et al. [62] have investigated mitigating eutrophication in lakes through nutritional control and biological manipulation and discovered that the significant increase in the abundance of the harmless genus Scenedesmus is an important phenomenon during the process of reducing eutrophication levels. Meanwhile, the importance of *Scenedesmus* and *Auxenochlorella* has been confirmed and they are widely employed in wastewater treatment and environmental regulation [59–61]. This revealed that the enhanced degradation of organic matter in sediment and the improved migration of nutrients from sediment to the overlying water caused by *B. purificata* bioturbation would not induce an increase in the relative abundance of harmful algae. Instead, it increased the relative abundance of harmless algae that have an environmental regulatory significance.

5. Conclusions

B. purificata farming activities had no significant effect on the alpha diversities of fungal and algal communities, but significantly altered the compositions of fungal and algal communities in sediments, especially *B. purificata* farming activity at a low stocking density. *B. purificata* farming activities at a low stocking density could significantly increase the relative abundances of fungal genera *Paraconiothyrium* and *Penicillium*, thereby inhibiting pathogenic microbe activity and improving the performance of cultured aquatic animals. *B. purificata* farming activities at low or medium stocking densities could also enhance the relative abundances of harmless algal genera *Scenedesmus* and *Auxenochlorella*, which are widely employed in wastewater treatment and environmental regulation. Therefore, the low stocking density (234.38 g/m²) in the present study might be the most optimum density from the perspective of fungal and algal communities. Implementing *B. purificata* cultivation at a low stocking density might lead to more sustainable aquatic food production. The findings of this study might contribute to better understanding for the ecological effects of snail *B. purificata* farming and serve as a valuable theoretical reference for the rational application and development of *B. purificata* farming.

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