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# Nitrogen occurrence forms and bacterial community in sediment influenced by *Bellamyia purificata* bioturbation

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Endogenous water pollution and excessive nitrogen in ponds have always been prominent problems threatening aquaculture. To solve this problem, a 70-day indoor simulation experiment was conducted. The snail *Bellamyia purificata* was stocked at four different densities: 0, 15, 30, and 60 individuals/tank, represented as CON (control), LD (low density), MD (medium density), and HD (high density), respectively, to investigate the effects of *B. purificata* on the nitrogen occurrence forms and bacterial communities in the sediment. At the end of the experiment, the nitrate concentration was significantly higher, while the total nitrogen content was significantly lower in the MD group than in the other three groups. Ammonia monooxygenase activity was significantly lower in the CON group than in the other three groups, and hydroxylamine oxidase activity was significantly higher in the HD group than in the other three groups. The CON and MD groups showed the highest and lowest values in nitrate reductase activity, respectively. The hydroxylamine reductase activity decreased significantly with increasing density. Through 16S ribosomal RNA (rRNA) high-throughput sequencing, significantly affected bacterial communities by *B. purificata* were found. Alpha diversity results showed that, a significantly lower Shannon index was observed in the MD group than in the other three groups. The LD and MD groups showed the highest and lowest Chao1 index values, respectively. Phyla Nitrospinae and family Nitrosomonadaceae were significantly enriched in the HD and MD groups, respectively. Redundancy analysis (RDA) indicated a significant correlation between differential bacterial taxa and TN content. Predicted functional analysis based on FAPROTAX (Functional annotation of prokaryotic taxa) database showed that functional groups aerobic ammonia oxidation and aerobic nitrite oxidation were significantly enriched in the MD group. Overall, *B. purificata* significantly altered the bacterial community composition, increased hydroxylamine oxidase and ammonia monooxygenase activities, enhanced the bacterial nitrification process, and promoted the transformation of total nitrogen to nitrate. Moreover, *B. purificata* stocked at

30 and 60 individuals/tank appeared to have a significant promotion effect on the bacterial community and nitrogen occurrence forms in the sediment. Hence, co-culturing *B. purificata* may be a feasible and effective ecological restoration method to alleviate excess nitrogen and reduce water eutrophication.

#### KEYWORDS

*Bellamya purificata*, bioturbation, nitrogen occurrence forms, extracellular enzyme activity, bacterial community, sediment

## 1 Introduction

Pond aquaculture is the most important method of freshwater aquaculture in China (Zhang et al., 2020; Liu et al., 2021). In 2020, the total production and area of pond aquaculture accounted for 59.12% and 37.31% of total freshwater aquaculture, respectively (MARA, 2021). Aquaculture pond ecosystems are important freshwater ecosystems that provide valuable ecological services and economic benefits to humans (Li et al., 2009; Guo et al., 2021; Herbeck et al., 2021). However, traditional pond aquaculture is a labour-intensive industry, and the improvement of production and economic benefits relies on excessive feed input and high stocking density. Excessive nitrogen input severely affects pond nitrogen cycling and causes serious environmental problems (Zhu et al., 2019; Cao et al., 2020; Li Y et al., 2022). With the continuous accumulation of organic nitrogen in aquaculture ponds, the sediment, as a carrier for the migration and accumulation of biogenic elements, can release nutrients and toxic substances into the upper water, thus causing endogenous water pollution and eutrophication of the pond ecosystem (Decaestecker et al., 2007; Gold et al., 2017; Woodman et al., 2021).

To address this dilemma and reduce the risk of endogenous water pollution and eutrophication, researchers have attempted to alleviate organic waste accumulation and reduce nitrogen pollution in pond sediments by co-culturing economical macrobenthos according to their feeding habits and abilities to utilise organic debris (Yuan et al., 2008; Ge, 2014). Yuan et al. (2008) used the sea cucumber *Apostichopus japonicus* to remove organic particulate waste and promote the regeneration of nutrients in sediments of shellfish culture systems. Ge (2014) attempted to improve resource utilisation efficiency and purify the culture environment by co-culturing freshwater prawns, *Macrobrachium nipponense*, in aquaculture ponds.

Benthic fauna plays an important role in aquatic ecosystems, especially in the biogeochemical cycle at the sediment-water interface, as they can lead to variations in the physical structure and chemical properties of the sediment and affect nitrogen migration and transformation (Dong et al., 2009; Löfgren et al., 2014; Li et al., 2015; Zou et al., 2022). In addition to their impacts

on biogeochemistry, benthic fauna also contributes to the construction of most ecological communities through the redistribution of available resources (Modenutti and Pérez, 2001; Parro et al., 2019).

Microorganisms are the main driving factors involved in nitrogen migration and transformation (Kuypers et al., 2018; Zhang et al., 2022). Bacterial nitrogen cycling processes related to nitrogen elimination play an important role in controlling nitrogen balance in pond ecosystems (Feng et al., 2017; Wang et al., 2018; Wei et al., 2019). Bacteria produce various enzymes through metabolic processes to control nitrogen transformation. For example, ammonia monooxygenase and hydroxylamine oxidoreductase are involved in the first stage of nitrification, which converts ammonia to nitrite (Xu et al., 2016; Xiang et al., 2020). Previous studies report that the microbial community composition and function can also be regulated using benthic animals by altering the nutrient transport and physicochemical properties of sediments (Tuikka et al., 2016; Wu et al., 2017; Saxena et al., 2018). It may be an effective way to understand the pathways and mechanisms of benthic fauna affecting nitrogen transformation and transportation in sediments through a comprehensive study of nitrogen occurrence forms, extracellular enzyme activity, and bacterial community.

Studies on the effects of macrobenthos on nitrogen and its driving mechanisms from the perspective of bacteria and extracellular enzyme activity, however, are still limited.

The snail, *Bellamya purificata*, is an important freshwater macrobenthic organism with wide distribution in China and high nutritional value (Meng et al., 2013; Huang et al., 2021). The *B. purificata* is not only a popular aquatic product with high economic value but also an important ecological species (Liang et al., 2013). *B. purificata* can effectively improve water quality and provide important ecosystem services (Chen et al., 2012; Zhao, 2014). Hou et al. (2021) demonstrated that *B. purificata* can significantly promote the degradation of organic waste in sediments. However, there are few reports on how *B. purificata* affects the occurrence forms, migration, and transformation of nitrogen and the bacterial community. Determining the effects of *B. purificata* bioturbation on the nitrogen occurrence forms, extracellular enzyme activity, and

bacterial communities in sediment can provide new ideas and theories for mitigating nitrogen pollution from the perspectives of polyculture and bioremediation.

Hence, the aims of our study were to investigate the effects of *B. puzosii* on the nitrogen occurrence forms, related extracellular enzyme activity, and benthic bacterial communities, and to explore the pathways and mechanisms of *B. puzosii* affecting nitrogen transformation. In addition, we hope to provide theoretical support for mitigating nitrogen pollution and preventing endogenous water pollution in aquaculture ponds and surrounding aquatic ecosystems.

## 2 Materials and methods

### 2.1 Culture experiment

The experiment in this study was conducted at the Key Laboratory of Integrated Rice-Fish Farming Ecology, Ministry of Agriculture and Rural Affairs, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences (Wuxi, China). *Bellamyia purificata* and experimental sediments were collected from the Dapu and Qiting aquaculture facilities, respectively (119°55'56.06"E, 31°18'41.69"N; 119°49'17.47"E, 31°23'55.32"N). The *B. purificata* were healthy and energetic with an average wet weight of  $2.05 \pm 0.28$  g, shell length of  $2.03 \pm 0.15$  cm, and shell height of  $1.43 \pm 0.18$  cm. Before the experiment started, all snails were kept in glass tanks for 14 days to acclimatise them to laboratory conditions. In order to maintain the consistency and homogeneity of the experimental sediments, we dried all the sediments in the sun, grinded and passed all the sediments through a 100  $\mu$ m mesh sieve, and mixed all the sediments evenly before use. A 10 cm thick layer of sediment was spread on the bottom of each experimental tank (63  $\times$  30  $\times$  39 cm), after which they were filled with filtered freshwater. The sediment in each tank were allowed to settle and stabilise for 14 days before the start of the experiment. Three treatments and one control were set up, with three replicates for each treatment. Snails were cultured in the experimental tanks at four different densities: 0, 15, 30, and 60 individuals/tank, represented as CON (control), LD (low density), MD (medium density), and HD (high density), respectively. Apart from the differences in stocking density, other experimental conditions were kept strictly consistent. During the experiment, the snails were fed at 16:00 every day. The feeding amount (2% of the total weight of snails in MD group) was identical for all tanks. Commercial feed (Zhejiang Haida Feed Co., Ltd, China) was used as experimental diets after being ground and sieved through a 100- $\mu$ m mesh, which contained total organic matter ( $119.57 \pm 2.31$  mg/g), total nitrogen ( $41.02 \pm 0.98$  mg/g), and total phosphorus ( $7.07 \pm 0.87$  mg/g). One-third of the water was changed every two days. The water temperature was maintained at  $25.0 \pm 0.5^\circ\text{C}$ . The experiment was conducted under a natural light: dark cycle for 70 days.

### 2.2 Sample collection

Since the experimental sediments have been pre-processed for keeping consistency and homogeneity before the experiment started. We only collected the sediment samples at the end of the experiment to avoid the disturbances caused by sampling operation. Surface sediment samples (0-1 cm depth) were collected at nine random sampling points in each tank using a plastic centrifuge tube with a diameter of 2 cm. Then, all the surface sediment samples collected from the same tank were mixed well and stored at  $-80^\circ\text{C}$  for further determination of bacterial community, nitrogen occurrence forms, and extracellular enzyme activities.

### 2.3 Laboratory analyses

#### 2.3.1 Nitrogen form

Before determination, the sediment samples were dried in a lyophiliser (CHRIST LYO Alpha 1-4 LD plus, German) and ground to a powder in a mortar. Ammonia, nitrate, nitrite, total nitrogen (TN), fixed ammonium (FA) and exchangeable nitrogen (EN) concentrations were determined. Ammonia nitrogen content was determined using Nessler's reagent (Kolaćinska and Koncki, 2014). Nitrate nitrogen was determined using phenol disulfonic acid spectrophotometry (Kendüzler and Türker, 2005). Nitrite nitrogen was determined using naphthalenediamine spectrophotometry (Pozzobon et al., 2021). TN was determined using alkaline potassium persulfate (McCarthy et al., 1980). FA and EN were determined as described by Silva and Bremner (1966) and Brodrick et al. (1987), respectively.

#### 2.3.2 Enzyme activity

Five enzymes related to nitrification and denitrification were identified: nitrate reductase, nitrite reductase, hydroxylamine reductase, ammonia monooxygenase, and hydroxylamine oxidase. Nitrate reductase, nitrite reductase, and hydroxylamine reductase activities were determined using an enzyme activity kit from Grace Biotech International Group Co. Ltd. (Suzhou, China). Ammonia monooxygenase and hydroxylamine oxidase levels were determined using an ELISA kit from Shanghai Enzyme-linked Biotechnology Co. Ltd. (Shanghai, China).

#### 2.3.3 DNA extraction and PCR amplification

The selected sequencing region was 16S-V<sub>3</sub>-V<sub>4</sub> in this experiment. All samples were tested as required. According to the manufacturer's protocols, E.Z.N.A. Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) was used to extract the bacterial DNA from the collected sediment samples. We evaluated the DNA quality and quantity using a NanoDrop 2000 Spectrophotometer (Bio-Rad Laboratories Inc., USA). The V<sub>3</sub>-V<sub>4</sub> region of the bacterial 16S ribosomal RNA gene was amplified according to the polymerase chain reaction (PCR) method with the primers 341F 5'- CCTAYGGGRBGCASCAG-3' and 806R 5'-

GGACTACNNGGGTATCTAAT-3' as reported in Hou et al. (2021). After purification and quantification using AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and QuantiFluor™-ST (Promega, USA), the PCR products extracted from all the sediment samples were pooled averagely, and then used to Illumina Pair-End library based on Illumina's genomic DNA library preparation procedure. The amplicon library was paired-end sequenced ( $2 \times 250$ ) on an Illumina MiSeq platform (Shanghai BIOZERON Co., Ltd.) according to standard protocols. Raw reads were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (PRJNA863036).

### 2.3.4 Bioinformatics analysis

QIIME software was used to process sequencing data, and UCLOST was used to classify operational taxonomic units (OTUs) at the similarity level of 97%. The sequence with the highest abundance of each OTU was selected as the representative sequence of OTU. Then, based on the number of OTU sequences in each sample, a matrix file of OTU abundance in each sample was constructed. To ensure the reliability and accuracy of the analysis results, the barcode sequences information for each sample with the following criteria: (i) The 250bp reads were truncated at any site receiving an average quality score  $<20$  over a 10 bp sliding window, discarding the truncated reads that were shorter than 50bp. (ii) Exact barcode matching, 2 nucleotide mismatch in primer matching, 0.2 in overlap, reads containing ambiguous characters were removed. (iii) The reference and denovo were combined to remove singletons, the database was Silval 138, 27466 reads were left for each sample after normalization. The Ribosomal Database Project (RDP) Classifier (version 2.2 <http://rdp.cme.msu.edu/>) was used to determine the taxonomic identities of the phylotypes.

## 2.4 Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA), followed by Duncan's test for multiple comparisons to determine the differences between groups using SPSS Statistics software (version 22.0, International Business Machines Corporation, USA). The level of significance ( $\alpha$ ) was 0.05 (where  $P < 0.05$  was considered significant). Functional annotation of prokaryotic taxa (FAPROTAX) database was used to predict ecological and biological functions of bacteria based on the python. Principal component analysis (PCA) was using SIMCAP (version 11.5, Umetrics, MKS Instruments AB, USA) to analyze, and the distance was based on Bray-Curtis. Use Mothur (version 1.30.2, <https://www.mothur.org/wiki/Downl>, USA) to analyze Chao1, Shannon and Simpson index. Redundancy analysis (RDA, CANOCO 4.5 software) was applied to reveal the correlations between environmental factors and the differential bacterial taxa.

## 3 Results

### 3.1 Nitrogen occurrence forms in sediment

The different nitrogen occurrence forms in the sediment of the CON, LD, MD, and HD groups are shown in Figure 1. The nitrate nitrogen content in the MD group was significantly higher than that in the other three groups ( $P < 0.05$ ). Ammonia nitrogen content was significantly higher in the CON and LD groups than in the MD and HD groups ( $P < 0.05$ ). The total nitrogen content in the MD group was significantly lower than that in the other three groups ( $P < 0.05$ ). There was no significant difference in the nitrite nitrogen concentration in the sediments among the four groups ( $P > 0.05$ ). No significant differences in fixed ammonium and exchangeable nitrogen concentrations were observed among the four groups ( $P > 0.05$ ).

### 3.2 Extracellular enzyme activity in sediment

Extracellular enzyme activities, including nitrate reductase, nitrite reductase, hydroxylamine reductase, ammonia monooxygenase, and hydroxylamine oxidase in sediment of CON, LD, MD, and HD groups are shown in Figure 2. The CON and MD groups showed the highest and lowest nitrate reductase activities, respectively ( $P < 0.05$ ). No significant differences in the nitrate reductase activity were observed between the LD and HD groups ( $P > 0.05$ ). The CON and LD groups showed the lowest and highest nitrite reductase activity, respectively ( $P < 0.05$ ). No significant differences in nitrite reductase activity were observed between the MD and HD groups ( $P > 0.05$ ). The CON and HD groups showed the highest and lowest hydroxylamine reductase activity, respectively ( $P < 0.05$ ). No significant differences in hydroxylamine reductase activity were observed between the MD and LD groups ( $P > 0.05$ ). The HD and LD groups showed the highest and lowest hydroxylamine oxidase activity, respectively ( $P < 0.05$ ). No significant differences in hydroxylamine oxidase activity were observed between the CON and MD groups ( $P > 0.05$ ). Ammonia monooxygenase activity was significantly higher in the HD and MD groups than in the CON and LD groups ( $P < 0.05$ ).

### 3.3 Bacterial community composition in sediment

The present study obtained 9,582 OTUs from sediment samples using Illumina sequencing technology based on the bacterial 16S rRNA gene. The OTUs were assigned to 58 phyla, 606 families, and 1,066 genera. There were 456, 505, 325, and 462 OTUs unique to the CON, LD, MD, and HD groups, respectively (Figure 3). The CON, LD, MD, and HD groups contained 6,888,

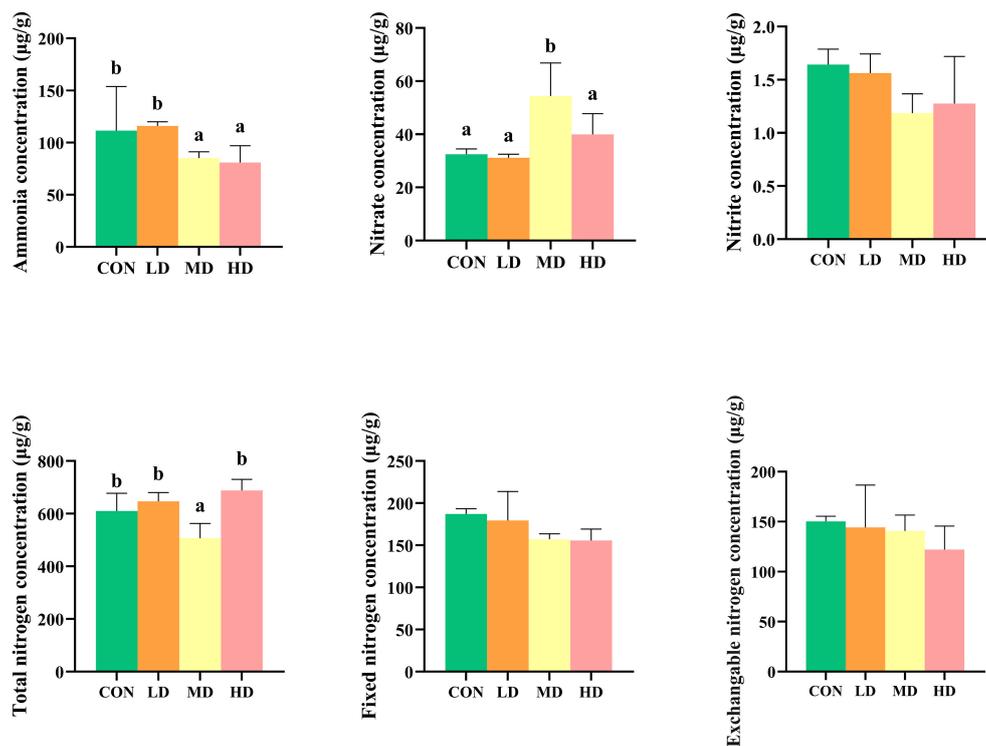


FIGURE 1

Differences in sedimentary nitrogen forms between the CON, LD, MD and HD groups during the experimental period, including ammonia, nitrate, and nitrite concentrations, total nitrogen, fixed nitrogen, and exchangeable nitrogen. Different lowercase letters represent significant differences between the CON, LD, MD, and HD groups ( $P < 0.05$ ).

7,165, 6,638, and 6,945 OTUs, respectively. The four groups shared 4,182 OTUs. Among these, there were 277 OTUs shared by the LD and MD groups, 393 OTUs shared between the LD and HD groups, and 252 OTUs shared between the MD and HD groups.

As shown in Figure 4, the ten most abundant phyla in the sediment at the end of the experiment were Proteobacteria, Acidobacteria, Bacteroidetes, Chloroflexi, Nitrospirae, Verrucomicrobia, Actinobacteria, Nitrospinae, Cyanobacteria, and unclassified. The ten most abundant families in the sediment were Burkholderiaceae, Rhodocyclaceae, *Thermodesulfovibrionia\_norank*, Syntrophaceae, Geobacteraceae, Nitrosomonadaceae, Steroidobacteraceae, Methylomonaceae, Pedosphaeraceae, and unclassified. The ten most abundant genera in the sediment were *Burkholderiaceae\_uncultured*, *Thermodesulfovibrionia\_norank*, *Desulfatiglans*, *Steroidobacteraceae\_uncultured*, Subgroup 6, *Anaerolineaceae\_uncultured*, *Pedosphaeraceae\_norank*, *P9X2b3D02\_norank*, *Sva0485\_norank*, and unclassified.

Significant differences in the ten most abundant phyla, families, and genera among the CON, LD, MD, and HD groups were observed (Figure 5). At the phylum level, the relative abundance of Nitrospinae was significantly higher in the HD group than that in the other three groups ( $P < 0.05$ ). The abundance of Proteobacteria was significantly lower in the HD

group than in the other three groups ( $P < 0.05$ ). At the family level, the relative abundance of Nitrosomonadaceae in the MD and CON groups was significantly higher than that in the LD and HD groups ( $P < 0.05$ ). At the genus level, the HD and CON groups exhibited the significantly lowest and highest relative abundances in *Thermodesulfovibrionia\_norank*, respectively ( $P < 0.05$ ), while *P9X2b3D02\_norank* was significantly more abundant in the HD group ( $P < 0.05$ ).

### 3.4 Bacterial community diversity in sediment

Bacterial community alpha diversity, including Chao1, Shannon, and Simpson indices, in the sediment of the CON, LD, MD, and HD groups are shown in Figure 6. A significantly lower Shannon index was observed in the MD group than in the other three groups ( $P < 0.05$ ). The LD and MD groups had the highest and lowest Chao1 index, respectively ( $P < 0.05$ ). However, no significant differences in the Simpson index were found among the four groups ( $P > 0.05$ ).

Principal component analysis (PCA) was used to investigate the differences in benthic bacterial communities among the

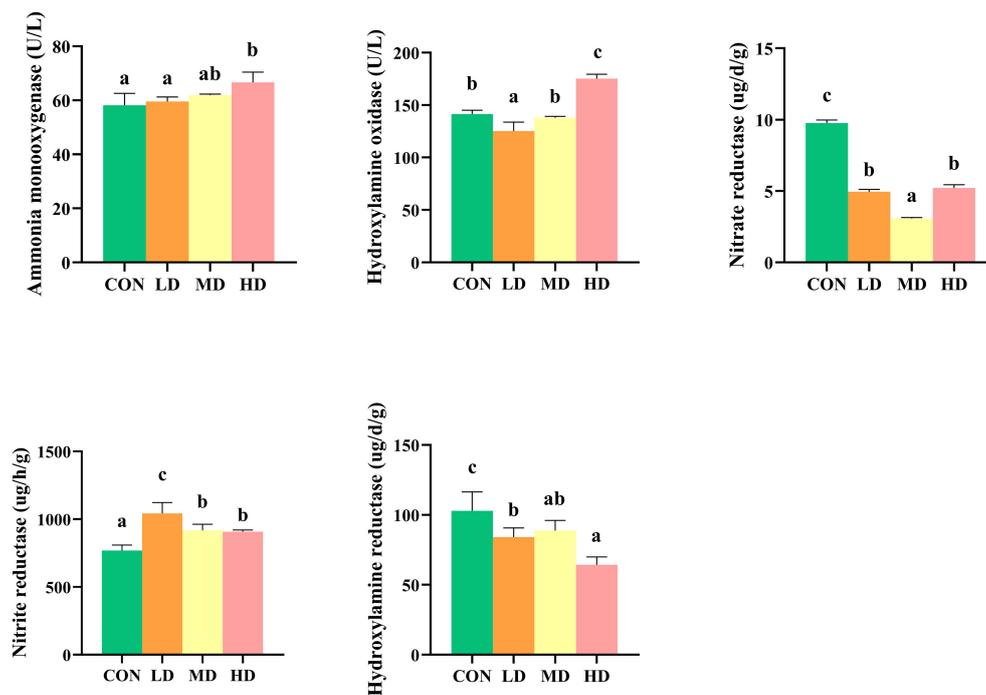


FIGURE 2

Differences in sedimentary extracellular enzyme activity between the CON, LD, MD and HD groups during the experimental period, including nitrate reductase, nitrite reductase, hydroxylamine oxidase, hydroxylamine reductase, and ammonia monoxygenase. Different lowercase letters represent significant differences between the CON, LD, MD, and HD groups ( $P < 0.05$ ).

CON, LD, MD, and HD groups, based on the Bray-Curtis distance. As illustrated in Figure 7, the PC1 axis explained 39.154% of the difference in the bacterial community composition, and the PC2 axis explained 15.118%. The bacterial communities of all sediment samples were categorised into four groups, as revealed by the PCA results. Visible differences in bacterial communities among the CON, LD, MD, and HD groups were observed at the end of the experiment.

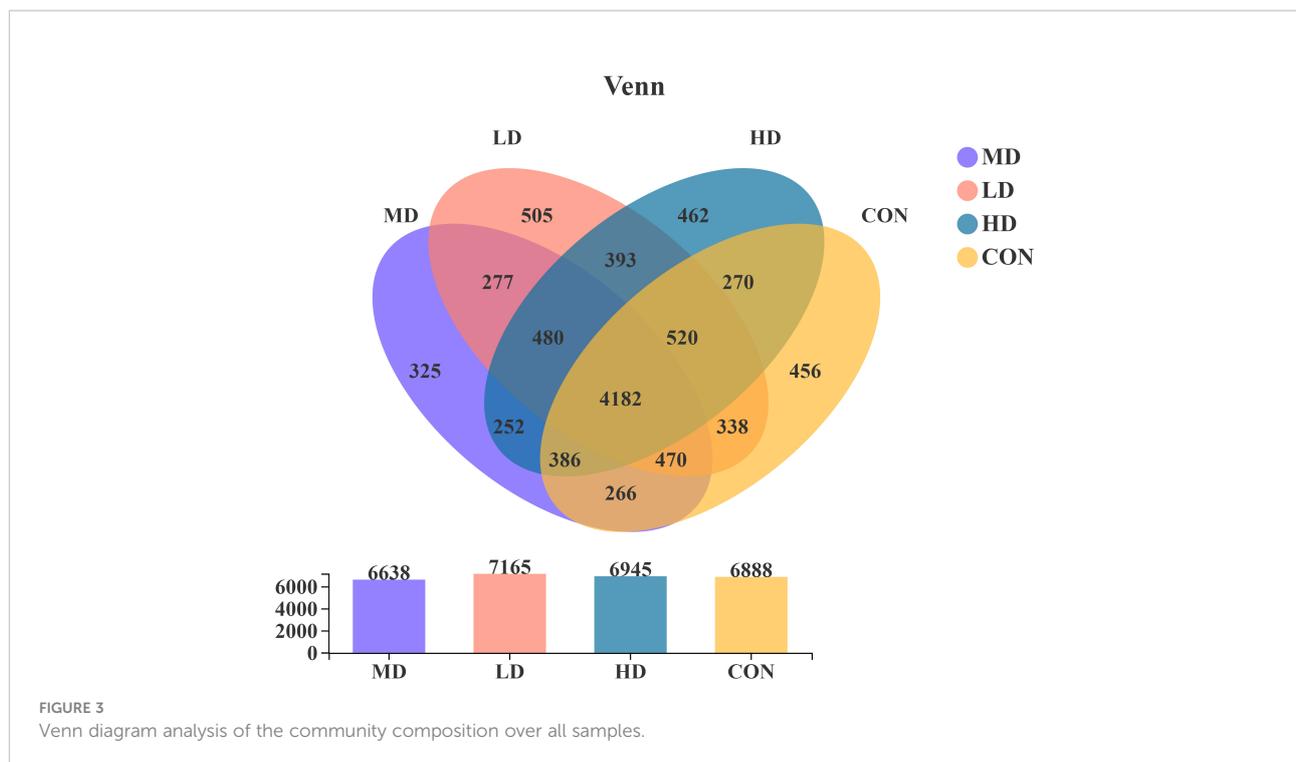
### 3.5 Correlation analysis between bacterial community and environmental factors

RDA analysis in the sediment of the CON, LD, MD and HD groups are shown in Figure 8. RDA<sub>1</sub> and RDA<sub>2</sub> accounted for 97.44% and 2.41% of the community changes, respectively. Based on the RDA, relationships between bacteria and each environmental factor were as follows: ammonia ( $r^2=0.258$ ,  $P=0.251$ ), nitrate ( $r^2=0.080$ ,  $P=0.702$ ), nitrite ( $r^2=0.166$ ,  $P=0.457$ ), TN ( $r^2=0.568$ ,  $P=0.02$ ), EN ( $r^2=0.339$ ,  $P=0.149$ ), FA ( $r^2=0.217$ ,  $P=0.329$ ). The above results showed that, TN had significant correlation with the bacterial community structure ( $P < 0.05$ ), while other environmental factors

had no significant correlation with the bacterial community structure ( $P > 0.05$ ). Specifically, at the phylum level, TN was negatively correlated with Proteobacteria and Nitrospinae. At the family level, TN was negatively correlated with Nitrosomonadaceae. At the genus level, TN was negatively correlated with *Thermodesulfovibronia\_norank* and *P9X2b3D02\_norank*.

### 3.6 Bacterial community function prediction in sediment

Based on the functional annotation of prokaryotic taxa (FAPROTAX) database, we found 61 functional groups in total, twelve of which were related to the nitrogen cycle: aerobic ammonia oxidation, aerobic nitrite oxidation, nitrification, nitrate denitrification, nitrite denitrification, nitrous oxide denitrification, denitrification, nitrogen fixation, nitrite respiration, nitrate respiration, nitrate reduction, and nitrogen respiration (Figure 9). Among the nitrogen cycling pathways mentioned above, two significantly different functional groups were observed in the CON, LD, MD, and HD groups (Figure 10). The MD group showed the highest aerobic ammonia oxidation ( $P < 0.05$ ). Aerobic nitrite oxidation was



also significantly higher in the MD group than in the other three groups ( $P < 0.05$ ).

## 4 Discussion

### 4.1 Nitrogen form and enzyme activity in sediments

Extracellular enzyme activities, such as ammonia monooxygenase, nitrate reductase, and hydroxylamine reductase, play an important role in the migration and transformation of nitrogen, by participating in nitrification and denitrification (Hong et al., 2019; Song et al., 2020; Sun et al., 2020; Zhao et al., 2021). Ammonia monooxygenase, which oxidises ammonia to hydroxylamine, is involved in the first step of nitrification (Aakra et al., 2001; Shinozaki & Fukui, 2002; Lin et al., 2015). Nitrate reductase and hydroxylamine reductase contribute to denitrification, nitrate reductase reduces nitrate ions to nitrite ions, and hydroxylamine reductase reduces hydroxylamine to ammonia (Jansson et al., 2008; Boutrin et al., 2012). In this study, the ammonia monooxygenase activity in the sediment was significantly increased with *B. purificata* bioturbation, whereas the nitrate reductase and hydroxylamine reductase activities were significantly decreased. This may indicate that *B. purificata* bioturbation could enhance the nitrification process and weaken denitrification in the sediment by affecting the related extracellular enzyme activities.

In addition, the results of nitrogen occurrence forms in this experiment were consistent with the results of extracellular enzyme activities discussed above. Nitrification refers to a reaction process in which ammonia nitrogen is oxidised to nitrite, which is further oxidised to form nitrate. The significantly higher nitrate content in the sediment of the MD group indicates the promotion of nitrification by *B. purificata*. Moreover, previous studies have reported that macrobenthos, including snails, can effectively promote degradation of organic matter in sediments (Blackburne et al., 2007; He et al., 2019; Hou et al., 2021). Organic nitrogen is degraded by ammonification to produce ammonia, which is the initial substrate for nitrification (Cui et al., 2019; Hu et al., 2021; Hou et al., 2021). Hence, the significantly decreased TN content in the sediment of the MD group may indicate that *B. purificata* promotes the transformation of total nitrogen to nitrate by enhancing nitrification and decomposition.

### 4.2 Bacterial community structure and functional prediction

Microorganisms are highly sensitive to changes in the ecological environment, and bacterial diversity is closely related to environmental changes (Escalas et al., 2017; Crowther et al., 2019; Sokol et al., 2022). Nitrogen transformation is closely associated with soil microorganisms (Yang et al., 2015; Pu et al., 2020; Shi et al., 2020). In this study, differences in PCA, phylum, family, and genus among the four groups revealed large impacts of *B. purificata* on the

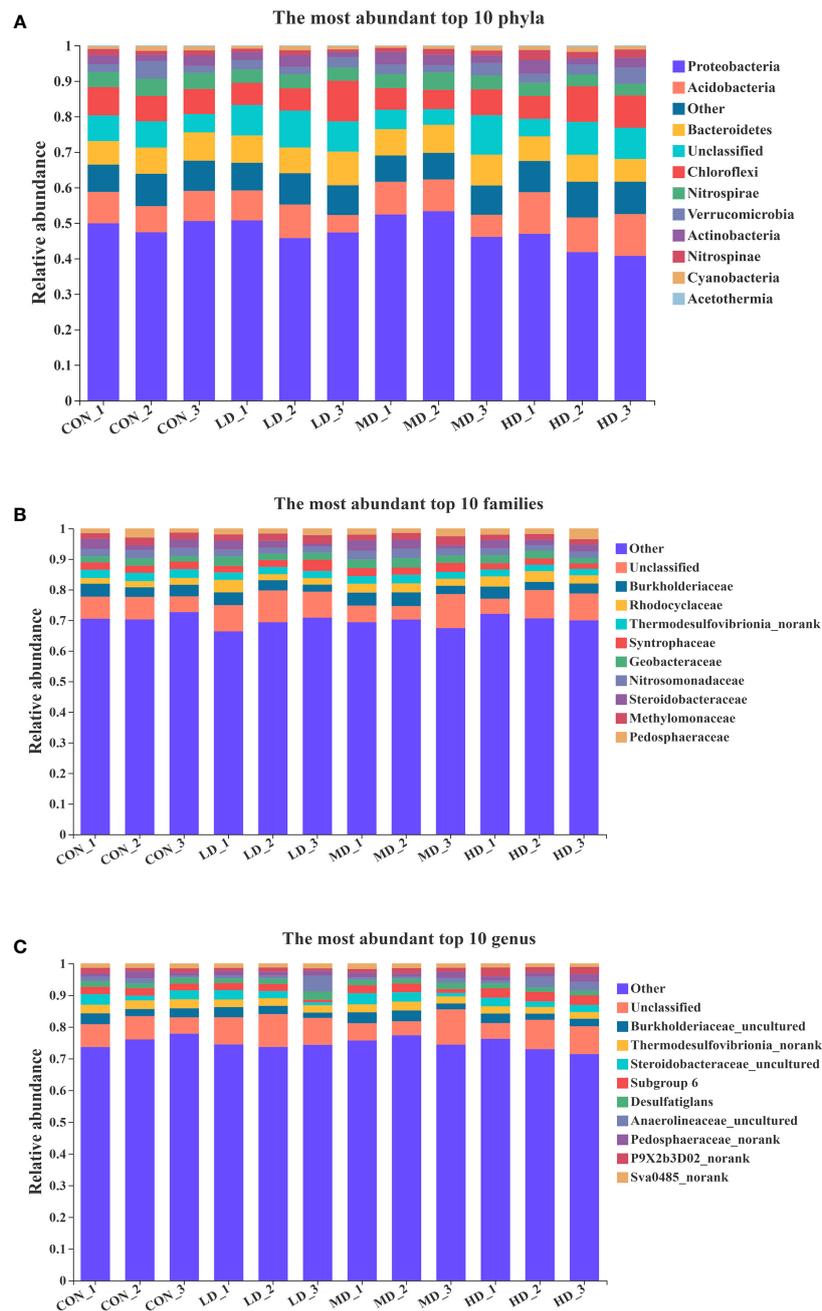
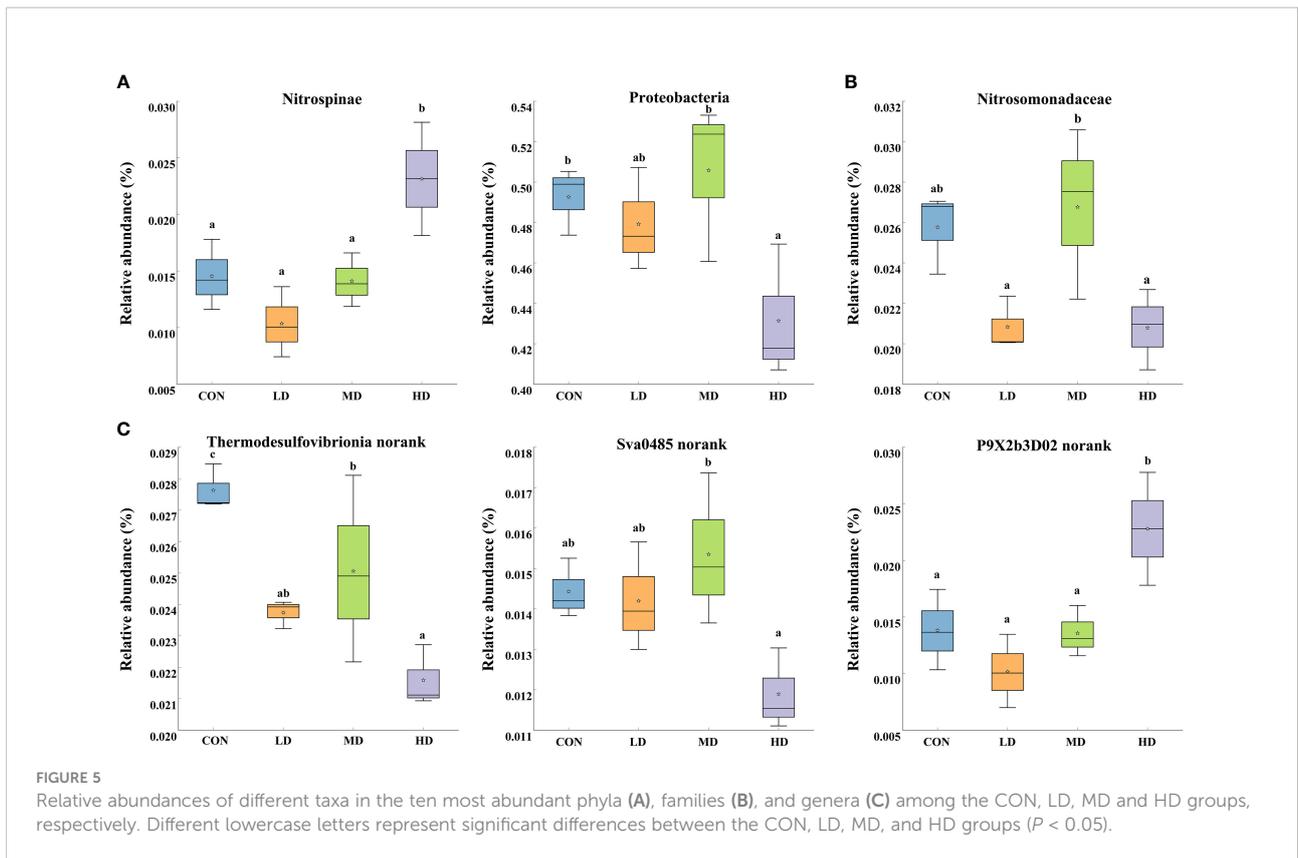


FIGURE 4 Ten most abundant phyla (A), families (B), and genera (C) in sediment of the CON, LD, MD, and HD groups at the end of the experiment.

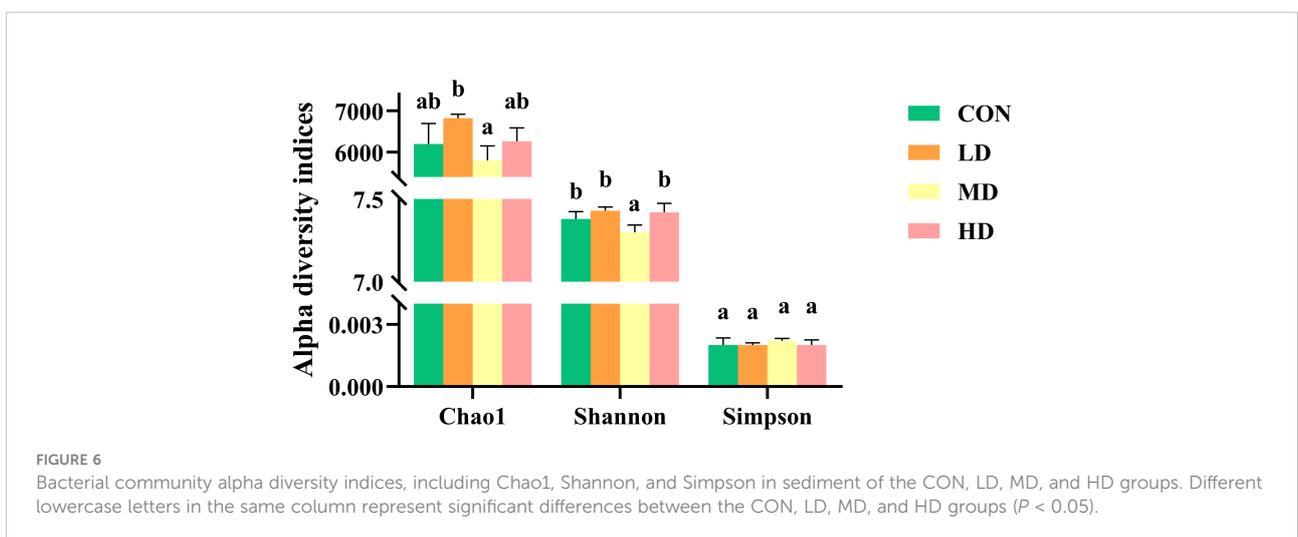
bacterial communities. Nitrospinae and Nitrosomonadaceae were significantly enriched in the HD and MD groups, respectively ( $P < 0.05$ ). Previous studies have shown that members of the phylum Nitrospinae are the most abundant and widely known nitrite oxidizing bacteria (NOB), and are distributed in oceans as well as

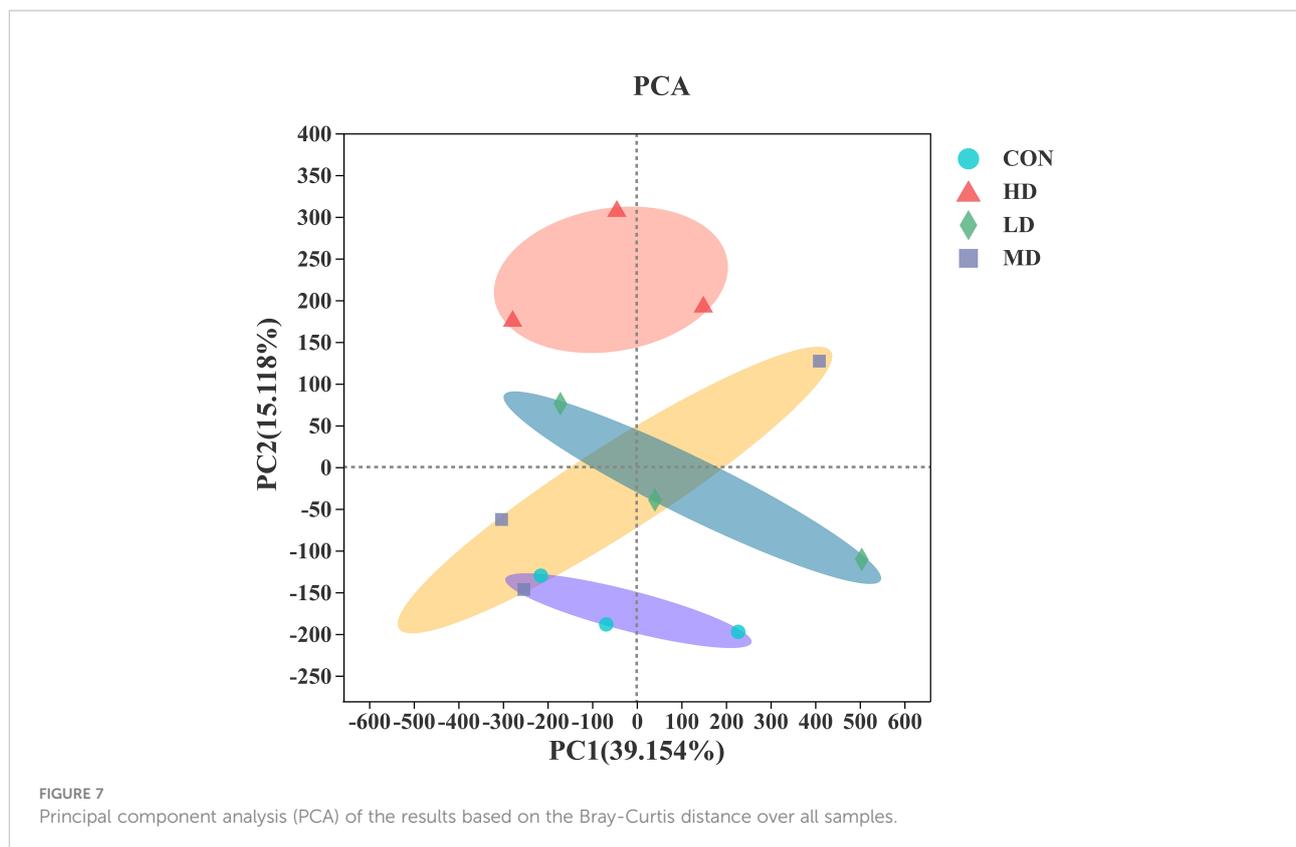
in freshwater bodies (Black and Just, 2018; Duan et al., 2019; Liu et al., 2021; Mueller et al., 2021). NOB are key players in the global nitrogen and carbon cycle, and can cooperate with microorganisms to improve the nitrification capacity of the system and enhance the degradation of ammonia nitrogen (Spieck et al., 2014; Pachiadaki



et al., 2017). Nitrosomonadaceae belong to the most abundant ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA) at the family level (Louca et al., 2016; Straka et al., 2019). Functionally, Nitrosomonadaceae results in nitrogen removal after aeration and contributes to ammonia removal (Clark et al., 2021; Wong et al., 2021; Li J. et al., 2022; Zhang et al., 2022). Hence, significantly higher

Nitrosomonadaceae in the MD group indicates a strong ability to oxidise ammonia to nitrite (Morse et al., 2018; Wang et al., 2021), and significantly higher Nitrospinae in the HD group indicates an enhanced nitrite oxidation ability (Sorokin et al., 2014). The bacterial communities in the sediment influenced by *B. purificata* demonstrated a stronger ability to remove ammonia and nitrogen. Significantly negative correlations





between TN and Nitrospinae and Nitrosomonadaceae also supported this discussion.

In addition, the results of the FAPROTAX function prediction in the present study were consistent with the discussion above. Aerobic ammonia oxidation and aerobic nitrite oxidation were significantly enriched in the MD group. Aerobic ammonia oxidation and aerobic nitrite oxidation are key functions of nitrification (Koch et al., 2014; Beman et al., 2021; Wu et al., 2022). Studies have shown that the potential application of these two microbial processes in wastewater treatment is of great significance (Chen et al., 2005; Rabaey et al., 2008). Specifically, aerobic ammonia oxidation converts  $\text{NH}_4^+$  to  $\text{NO}_2^-$  under aerobic conditions (Xia et al., 2013; Trener et al., 2021). Aerobic nitrite oxidation converts  $\text{NO}_2^-$  to  $\text{NO}_3^-$  under aerobic conditions (Xu et al., 2011; Ning et al., 2016). Their activities directly or indirectly affect sediment nutrient cycling, water eutrophication, greenhouse gases, and ecosystem functions (Oguz et al., 2007; Schlicht et al., 2019). This explains the higher nitrification capacity in the MD and HD groups than that in the CON group. The analysis of bacterial community composition and the FAPROTAX function prediction showed that the bioturbation caused by *B. purificata* was beneficial for the enrichment of aerobic nitrification bacteria and promoted the nitrification process.

Besides, oxygen is crucial for nitrification (Fan et al., 2021; Rodríguez-Gómez et al., 2021; Zhu et al., 2021), as it is carried

out under aerobic conditions, with oxygen as the electron acceptor and nitrogen as the electron donor (Daims et al., 2015; van Kessel et al., 2015). Previous studies have shown that macrobenthos bioturbation can affect the distribution of oxygen in sediments through bioturbation (Satoh and Okabe, 2013). During macrobenthos bioturbation processes, upper water with high dissolved oxygen is pumped into the sediments, which can increase the dissolved oxygen content and improve the redox potential in the sediment and then affect the bacterial activities and promote the nitrogen, phosphorus, and sulfur cycles at the sediment-water interface (Shull et al., 2009; Nguyen et al., 2022; Zhang et al., 2022). Meanwhile, extracellular enzyme activities in sediments are closely related to bacterial communities in sediments (Rietl et al., 2016). Therefore, in this study, significantly enhanced nitrification was reflected by the changes of extracellular enzyme activity and bacterial community structure. This may be a bioturbation from *B. purificata*.

## 5 Conclusion

In conclusion, *B. purificata* could significantly alter the bacterial community composition and functional groups related to nitrification and increase the hydroxylamine oxidase and ammonia monooxygenase activities, thus enhancing the

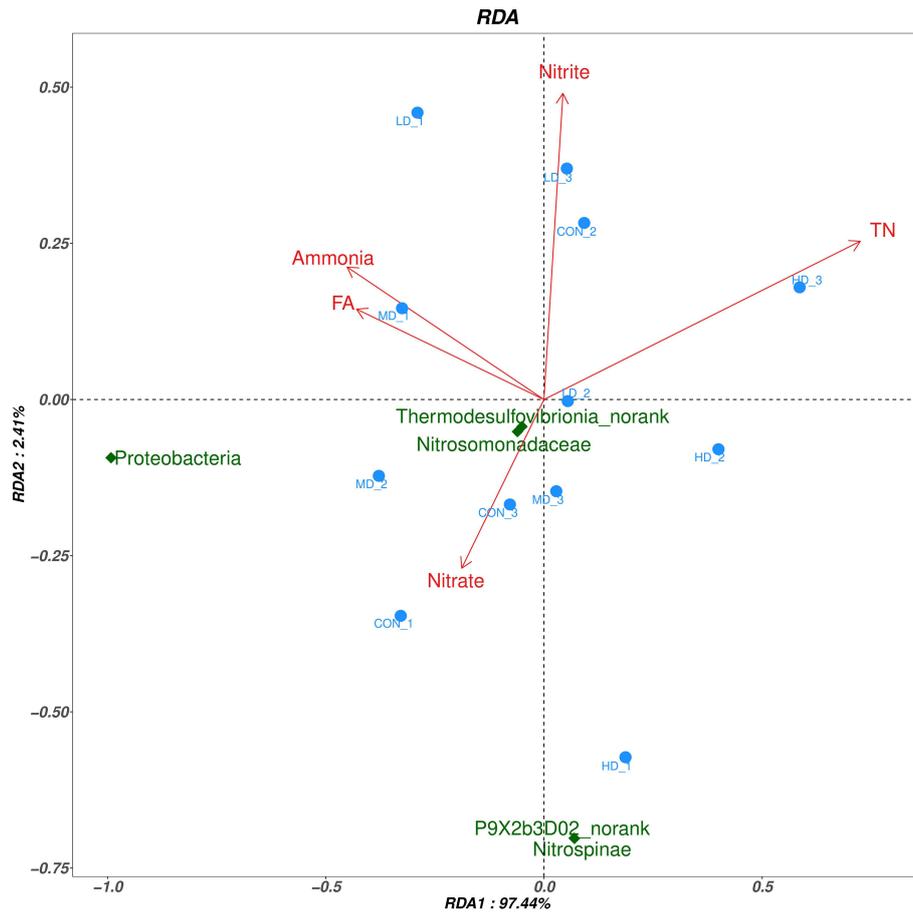


FIGURE 8 Redundancy analysis (RDA) to reveal the correlations between environmental factors and the differential bacterial taxa.

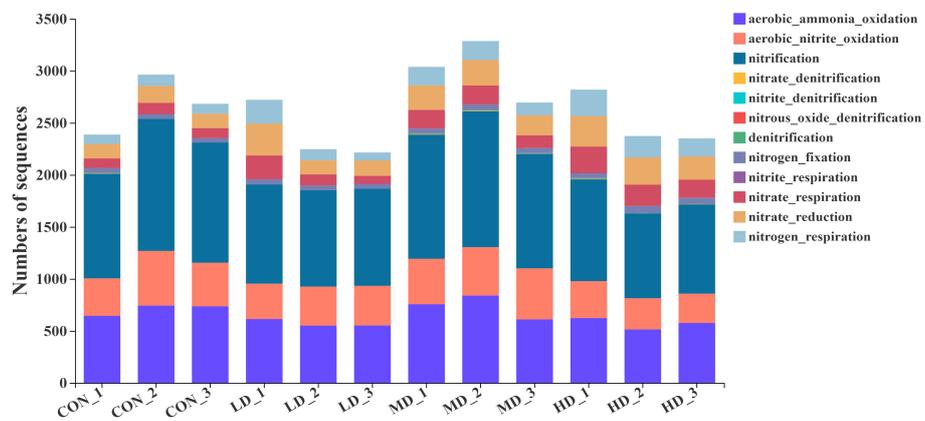


FIGURE 9 Functional groups related to nitrogen cycling in sediments of the CON, LD, MD and HD groups, respectively.

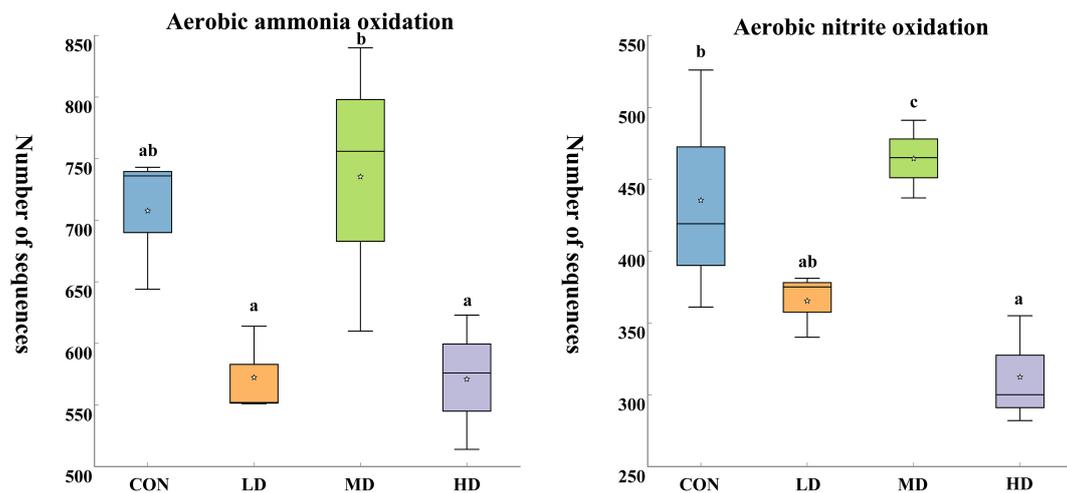


FIGURE 10

Significantly different functional groups related to nitrogen cycling in sediments of the CON, LD, MD, and HD groups at the end of the experiment. Different lowercase letters represent significant differences between the CON, LD, MD, and HD groups ( $P < 0.05$ ).

nitrification process in sediment and promoting the transformation of total nitrogen to nitrate. Moreover, *B. purificata* stocked at 30 and 60 individuals/tank appears to have a better promotion effect on the bacterial community and forms of nitrogen occurrence. Hence, co-culturing *B. purificata* at suitable stocking densities may be a feasible and effective ecological restoration method to alleviate excess nitrogen and reduce water eutrophication.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repository and accession number(s) can be found in the article/supplementary material.

## Author contributions

YZ: Methodology, formal analysis, data curation, and original draft preparation. YH: Conceptualisation, methodology, formal analysis, and original draft preparation. RJ and BL: Visualisation and investigation. JZ and XG: Resources, writing, reviewing, editing, and supervision. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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