Ecological Processes

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Dynamics of protist and bacterial communities during the nitrogen removal by ecological floating beds of *Sesuvium portulacastrum*

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Abstract

Background Ecological floating beds can restore eutrophic water, but few studies have focused on changes in microbial communities during the remediation process. To gain a deeper understanding of the restoration process, we used 16S/18S rRNA gene metabarcoding and metagenomic sequencing to investigate the changes in the structure and function of protist and bacterial communities.

Results By comparing seawater with or without floating beds, we found that *Sesuvium portulacastrum* can effectively remove nutrients and dissolved solids from water, with nitrate removal above 52% and phosphate removal above 34% within 33 days. *S. portulacastrum* increased the alpha diversity of both protists and bacteria, changed their community composition, and improved the community stability. The stochastic processes were critical in shaping the community assembly, and the contribution of stochastic processes in floating beds was lower in the treatment group than in the control group. In addition, changes in aquatic community structure further led to changes in community function, particularly nitrogen cycle processes. Among all nitrogen cycle-related functional genes, dissimilatory nitrate reduction genes (44.50%) and denitrification genes (62.44%) were the most common on day 1 and day 33, respectively. The enhanced denitrification process promoted the nitrogen removal in eutrophic water, contributing to ecological restoration and water quality improvement.

Conclusions Our results suggested that *S. portulacastrum* and associated microbial communities exhibited a synergistic role in the restoration process. The well-developed root system of *S. portulacastrum* acted as a carrier for microorganisms to play a crucial role in the removal of nutrients and other dissolved solids. This study can provide a reference for the optimization of ecological management of eutrophic seawater. Restoration efforts should integrate considerations of water physicochemical properties with the structure and function of aquatic community.

Keywords Ecological floating bed, Metagenome, Microbial diversity, Microbial community, Nitrogen cycling gene

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Background

One of the most significant biogeochemical processes on Earth is the cycling of nitrogen (Galloway et al. 2004). Nitrogen may exist in multiple redox states (−3 to+5), and microorganisms play a significant role in transformations between these states (Stein and Klotz 2016; Kuypers et al. 2018). Human activities have drastically changed the global nitrogen cycle with the rise of civilization which has resulted in a substantial increase of reactive nitrogen in aquatic ecosystems (Galloway et al. 2008; Wang et al. 2021). Nitrogen enrichment has recently led to several significant marine-related environmental problems, including eutrophication and algal blooms (Wang et al. 2018; Dai et al. 2023). Therefore, it is necessary to remove excess nitrogen from the water into the atmosphere through denitrification to relieve the environmental pressure.

In order to protect the aquatic environment and ecosystem, it is necessary to adopt appropriate methods for water purification. At present, a myriad of physical, chemical and biological techniques have been designed over the years for wastewater treatments. For instance, regulating water flow and mixing can be employed, along with chemical methods (such as the use of adsorbents and oxidants) to reduce the concentration of suspended particulate matter, excess nitrogen and phosphorus. Additionally, microorganisms, algae and other organisms can be used to degrade pollutants (Corbella and Puigagut 2018; Mohanty et al. 2018; Lucke et al. 2019; Lukwambe et al. 2024). The ecological floating bed technology is one of these techniques, which has been applied extensively to remove nitrogen from eutrophic water owing to its affordability, low energy requirements, lack of contamination, and appropriateness (Afzal et al. 2019; Garcia Chance et al. 2019; Huang et al. 2020). Nitrogen pollutants in seawater come from a wide range of sources and have complex compositions. Compared with fresh water, seawater is relatively short of nitrogen removing microorganisms, and its pollutant structure, salinity effect and ion strength effect will increase the difficulty and complexity of treatment. The high concentration of Cl^- and low carbon nitrogen ratio (C/N) of seawater determine the unique treatment environment and biological nitrogen removal mechanism (Tan et al. 2019; Ni et al. 2022). This increases the difficulty of wastewater treatment and poses a great challenge to traditional nitrogen removal techniques. According to the characteristics of seawater wastewater, selecting suitable salt-tolerant plants is the key to the treatment of seawater wastewater. Several large plants have been identified for the treatment of contaminated seawater and their efficacy has been demonstrated (Samal et al. 2018, 2019; Di Luca et al. 2019). *Sesuvium portulacastrum* is a perennial succulent herb that grows on the beach and mudflats of the estuary (Lonard and Judd 1997; Lokhande et al. 2009). As one kind of ideal plant for ecological floating beds, *S. portulacastrum* can grow normally under seawater for a long time, with a high tolerance to high salt, drought, and heavy metal (Slama et al. 2008; Boxman et al. 2017). To meet the requirements of plant growth, ecological floating beds may actively obtain nutrients from the water, such as nitrogen and phosphorus, through the roots of the plants (Vymazal 2007; Kong et al. 2019). Meanwhile, a symbiotic network of microorganisms (protists and bacteria), rhizomes, and roots develops in the surface water, offering a biologically active surface area for physical and biochemical activities including trapping and filtration (Bi et al. 2019; Di Luca et al. 2019). Aquatic plant roots secrete oxygen, which encourages the growth of plant roots and creates a microenvironment for microbial symbionts (Samal et al. 2019). Microorganisms can promote the absorption of nutrients by plants, protect plants from bacteria and viruses, and increase the mechanical stability of the floating beds (Samal et al. 2019). This is an ideal environment for the rhizosphere nitrogen cycle and the nitrogen transformation of aquatic plants, which speeds up the removal and transformation of macromolecular pollutants and achieves the function of purifying water (Abed et al. 2017; Samal et al. 2018; Yang et al. 2021).

The planktonic microbial community (including protist and bacterial communities) is crucial to the composition and function of global aquatic ecosystems and plays key roles in global nitrogen cycles (Liu et al. 2017, 2019; Machado et al. 2019). Previous studies have also shown that the process of nitrogen removal in eutrophic water by ecological floating beds can affect the planktonic microbial community structure and function (Liu et al. 2021; Chao et al. 2021; Zhang et al. 2024). For example, phytoplankton (algae) and bacteria can directly utilize nutrients, and planktonic protozoa may be affected by nutrients through their grazing on bacteria (Burson et al. 2018; Feitosa et al. 2019). Exploring the effects of ecological floating bed restoration on the composition and diversity of protist and bacterial communities is highly significant for elucidating the role of ecological floating beds in the overall restoration of aquatic ecosystems. A study used the aeration system to remove ammonium nitrogen and found that bacterial community composition and diversity changed significantly with the decrease of ammonium nitrogen (Sun et al. 2018b). Simultaneously, as an important feature of ecosystems, stable communities are crucial for providing healthy function (Griffiths and Philippot 2013). However, the effects of nutrient removal processes on the stability of microbial communities are largely unknown, especially in seawater (Amalfitano et al. 2017; Logares et al. 2018). Another

study investigated protist and bacterial communities during the nutrient removal in freshwater by using floating beds of *Canna indica* and found that both community compositions became more stable with the removal of nutrients (Liu et al. 2022). In addition, bacteria have a wider metabolic diversity and growth rate than protists, as a result, bacterial communities may adapt more readily than protists to particular environments, such as algal blooms, temperature changes, and lake thermal stratification (Berry et al. 2017; Frindte et al. 2019; Zhang et al. 2021). However, the community ecology study of protists lags far behind that of bacteria (Logares et al. 2020). Most previous studies have focused on changes in the composition and diversity of microbiomes during the removal of nutrients from water and the role of microbial communities in the removal of nutrients, for example, exploring the function of bacterial communities in the nitrogen cycle, especially denitrification (Li et al. 2018; Chao et al. 2021). We paid additional attention to whether the response patterns of protist and bacterial communities to nutrient removal are the same or different, which is currently lacking in field experimental data. Few studies have integrated the structure and function of protist communities to explore the dynamic changes of microorganisms in the process of nutrient removal.

In this study, 16S rRNA and 18S rRNA gene metabarcoding and metagenomic sequencing were used to investigate the protist and bacterial community compositions and nitrogen functional genes during the nitrogen removal by ecological floating beds of *S. portulacastrum*. We proposed the following hypotheses: (a) the diversity and community compositions of the protists and bacteria fluctuated with the removal of nitrogen by the use of ecological floating beds in eutrophic water; (b) the stability of the protist and bacterial community composition improved with the removal of nitrogen; and (c) the abundance of denitrification function genes increased during the removal of nitrogen by the use of ecological floating beds. The objective of this study was to enhance the understanding of the effects and mechanisms by which *S. portulacastrum* contributes to the remediation of eutrophic seawater, ultimately aiming to optimize the bioremediation process for seawater.

Methods

Mesocosm, sampling, and environmental variables

The culture experiments were conducted over 33 days from November 25 to December 27, 2022 (Table S1). The indoor culture device was built, which was composed of 4×3 glass culture tanks and alumina structural frame. The material was 12 mm ultra-white glass, and the size of a single culture tank was 80×65×40 cm. Above each culture tank, light (0.5W LED lamp, 42 white light + 6 blue light) and control system were set, and the illumination intensity was 1,200 lx (Fig. S1). *S. portulacastrum* plants were collected at Houhai in Putian, Fujian, Southeast China. The seawater was collected from the nearshore zone in Xiamen, Fujian, China on November 22, 2022. Before the experiment, the sediment was filtered step by step and finally filtered through a 200 μm sieve to remove the impurities and large particles. The same volume (70 L) of seawater was added to each experimental tank, and the initial nitrate, nitrite, and phosphate concentrations of seawater were measured. Referring to the recipe of F/2 Medium (Guillard and Ryther 1962; Guillard 1975), the natural seawater was adjusted to be the experimental seawater by adding $NaNO_3$ and NaH_2PO_4. We set up three concentrations of (A) N100P10, (B) N100P20, and (C) N200P20 (μM), and three parallel treatment groups and one blank control group (only seawater) were set up for each experimental concentration. The same size (0.5 m×0.5 m) floating beds were put in the experimental tank, and the floating beds were processed by a 10 mm expandable polyethylene foam board, and the culture density was 200 plants/m^2. Before planting on the floating beds, the roots and leaves at the stem joints were removed to prevent rot and facilitate rooting, and 1 to 2 stem segments were ensured to enter the water when planting. The floating aquaculture system is shown in Fig. S1.

The photoperiod in the incubation experiments was 12 L:12 D. Due to the evaporation of water and the transpiration of plants, part of the water would be lost every day, and sterilized ultra-pure water was supplemented to maintain the salinity of the water at 30.13‰. Sampling was performed every four days, on days 1, 5, 9, 13, 17, 21, 25, 29, and 33 (Table S1), to collect water quality parameters and nutrient salt concentrations. We used multiparameter water quality analyzer HORIBA-U-50 to measure the temperature, salinity, electric conductivity (EC), turbidity (NTU), total dissolved solids (TDS), dissolved oxygen (DO), pH using the pH meter, phosphate phosphorus (PO_4-P) using the phosphorus molybdenum blue spectrophotometry (Murphy and Riley 1962), nitrate nitrogen (NO_3-N) and nitrite nitrogen (NO_2-N) using the naphthalene ethylenediamine hydrochloride spectrophotometry (Ensafi et al. 2004), then the ratio of nitrate and nitrite nitrogen to phosphate phosphorus (N/P) was calculated. The growth of plants was assessed by weighing and measuring the longest root length.

DNA extraction and Illumina sequencing

Approximately 600 mL water was filtered using a 0.22 μm pore-size polycarbonate membrane (Millipore, Billerica, MA, USA) for microbial (protist and bacterial) DNA extraction. A total of 162 samples were obtained. The

total DNA of microbial communities was extracted from the membranes using the FastDNA® spin kit for soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions.

The 18S rRNA gene primer pair TAReuk454FWD1 (5′CCAGCASCYGCGGTAATTCC3′) and TAReukREV3 (5′ACTTTCGTTCTTGATYRA3′) (Stoeck et al. 2006) and the 16S rRNA gene primer pair 338F (5′ACTCCTACGGGAGGCAGCA3′) and 806R (5′GGACTACHVGGGTWTCTAAT3′) (Huse et al. 2008; Caporaso et al. 2011; Salas-González et al. 2021) were used to amplify the V4 region of eukaryotic 18S rRNA gene and the V3-V4 region of bacterial 16S rRNA gene, respectively. The DNA concentration and purity were determined by NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The DNA was sequenced using the PE250 strategy on the Illumina NovaSeq 6000 platform.

Six samples were selected on days 1 and 33 of N200/P20 treatment group for metagenomic sequencing. After the genomic DNA passed the quality test, interrupted the DNA, and then end-repaired the fragmented DNA, connected to the sequencing joint, and screened for the purification of the connected product. Amplified library and product was purified to form a sequencing library, which was sequenced using the PE150 strategy by Illumina NovaSeq 6000 platform after passing the quality inspection. The raw reads obtained from sequencing were quality-controlled and filtered to obtain clean reads for subsequent bioinformatics analysis. The fastp software was used to filter raw reads to obtain high-quality sequencing data (clean reads). The percentage of bases with Phred values greater than 20 in the total number of bases was more than 99%, and the percentage of bases with Phred values greater than 30 was more than 95%. Finally, each sample had more than 10 G of clean data.

Amplicon analysis

Amplicon sequence variants (ASVs) were obtained using the DADA2 plug-in in QIIME2 2020.6 software (Bokulich et al. 2018; Bolyen et al. 2019). For the 18S rRNA genes, the sequences were annotated with the Protist Ribosomal Reference (PR2) database (version 4.11.1) (Guillou et al. 2013), and the sequences that belong to Rhodophyta, Streptophyta, Fungi, Metazoa, Opisthokont, and unclassified eukaryotes were eliminated to obtain the protist communities (Zhao et al. 2019). For the 16S rRNA genes, the sequences were annotated with the SILVA database (version 138) (Quast et al. 2013), and the sequences that belong to unclassified phylum were eliminated to obtain the bacterial communities. For our data analyses, we used a randomly selected subset of the same number of reads from each sample to standardize the sequencing effort (13,794 protist sequences and 36,588 bacterial sequences for each sample, respectively).

Diversity analyses and correlation analysis

Alpha diversity indices, including abundance-based coverage estimators (ACE) index and Shannon-Wiener diversity, were calculated using the "vegan" package in R. Principal coordinate analysis (PCoA) was performed based on the Bray-Curtis distance using the "vegan" package in R (Brian et al. 2001). Mantel tests were used to determine correlations between environmental variables and selected characteristics of protist and bacterial communities in the "vegan" package in R.

Co-occurrence network construction

Spearman correlation coefficients (r) between ASVs were computed using the "psych" package and maintained strong correlation and statistical significance ($|r| > 0.6$, and $P < 0.01$). Network visualization, topological characterization, and modular analysis were then implemented using Gephi v. 0.10.1.

Temporal stability of alpha diversity and taxonomic members at class level

We quantified the temporal stability of alpha diversity indices as the ratio of the mean relative abundance of a diversity index to its standard error of each sample day during the experiment. We quantified the temporal stability of microbial taxonomic members at the class level as the ratio of the mean relative abundance of a microbial member to its standard error over 33 days of the experiment, as in many other studies (Ma et al. 2017).

Null model for stochastic and deterministic processes

The phylogenetic turnover analysis can predict underlying ecological processes by examining phylogenetic signals. We used the null model to quantify and compare the community assembly process in protist and bacterial communities of the treatment and control groups. We obtained the beta nearest taxon index (βNTI) to classify deterministic and stochastic processes. When $|\beta NTI| > 2$, community turnover would be impacted by a deterministic process; contrarily, a stochastic process would dominate community turnover when $|\beta NTI| < 2$. Heterogeneous selection would predominate in community turnover when $\beta NTI > +2$ and homogeneous selection would predominate when $\beta NTI < -2$. Then we obtained the Bray-Curtis based Raup-Crick (RC_{bray}) index for pairwise comparisons when $|\beta NTI| < 2$. Dispersal limitation and homogenizing dispersal would dominate community turnover when $RC_{bray} > +0.95$ or < -0.95, respectively. The undominated percentage

was dominated when $|RC_{bray}|$ was less than 0.95 (Stegen et al. 2013).

Metagenome assembly and nitrogen-cycling genes annotation

The MEGAHIT software was used for metagomic assembly and contig sequences shorter than 300 bp were filtered, and the QUAST software was used to evaluate the assembly results. Then the MetaGeneMark software (version 3.26) was used to identify coding region of the genome, and predict coding genes. Then the MMseqs2 software (version 12-113e3) was used to remove redundancy and set the similarity threshold to 95% and coverage threshold to 90% to construct non-redundant gene sets. Non-redundant gene sets were subjected to NR (Amino acid sequence of non-redundant protein) species annotation and KEGG (Kyoto Encyclopedia of Genes and Genomes) functional annotation, and screened for gene clusters and their relative abundance in the nitrogen cycle.

Results

Temporal dynamics of environmental variables

Nutrient concentrations exhibited a general downward trend, with occasional increases from day 1 to day 33 (Fig. 1). In the control group, the NO_3-N, PO_4-P,

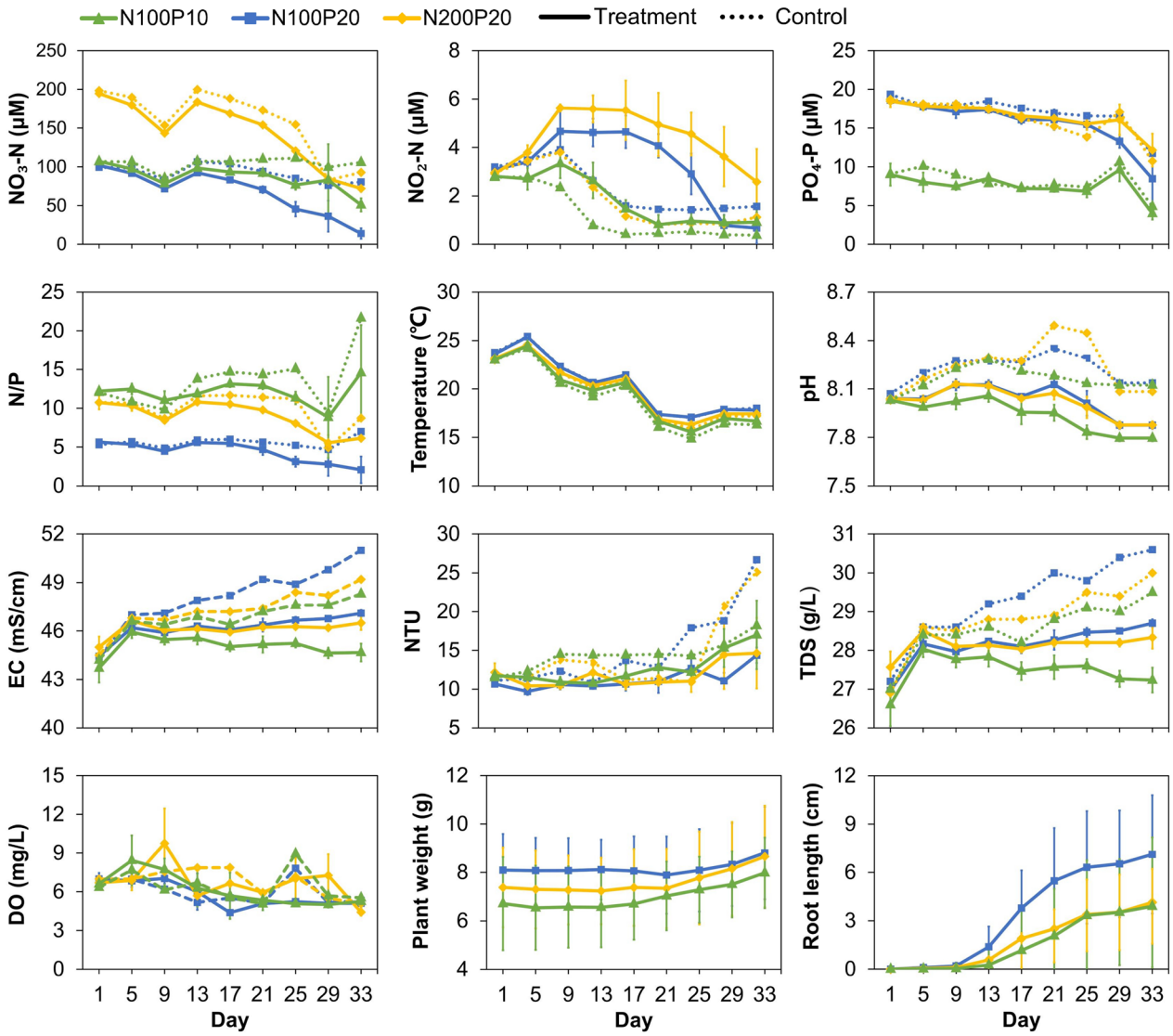

Fig. 1 Variations of environmental variables, plant weight, and root length from day 1 to day 33. Error bars represent the standard errors of the mean. Different colors represent different cultural concentrations. The solid line is the treatment group, and the dotted line is the control group. *EC* electric conductivity, *NTU* turbidity, *TDS* total dissolved solids, *DO* dissolved oxygen

and N/P were persistently higher than in the treatment group, but NO_2-N, conversely, was persistently lower during the experiment. The nitrate concentration of the N100P20 group decreased most obviously, the nitrate removal rate was 86.30%, and the nitrite removal rate was 78.21%. The nitrate removal rate and nitrite removal rate of the N200P20 group were 62.98% and 11.40%, and those of the N100P10 group were 52.37% and 67.51%, respectively. The phosphate concentration of the N100P10 group decreased most obviously, and the phosphate removal rate was 55.77%. The phosphate removal rate of the N200P20 group was 34.22%, and that of the N100P20 group was 54.58% (Fig. 1).

For the other factors, temperature showed a fluctuating downward trend during the experiment. In the control group, pH, EC, NTU, and TDS showed a wavelike increase from day 1 to day 33; whereas in the treatment group, EC and TDS increased from day 1 to day 5 and fluctuated decreased change from day 5 to day 33, pH fluctuated decreased from day 1 to day 33, and NTU fluctuated increased from day 1 to day 33. pH, EC, NTU, and TDS were persistently higher in the control group than in the treatment group (Fig. 1).

For plant growth, the root length of the plant continued to increase from day 1 to day 33. At the beginning of the experiment, the plants had their roots removed, the plants began to take root on day 9, and nearly half of the plants took root by day 13, and all the plants had roots by day 25. The weight of the plants remained relatively stable from days 1 to 17 and gradually increased from days 17 to 33 (Fig. 1).

Bray-Curtis similarities of protist and bacterial community

The samples in both control and treatment groups showed different dissimilarities at different periods according to the Bray-Curtis distance between the protist and bacterial communities. At the beginning of the experiment, the microbial communities were more similar between the control and treatment groups, and the dissimilarity between communities increased over time. Both protist and bacterial communities in the treatment group gradually separated from those in the control group over time within 33 days (Fig. 2).

Temporal dynamics of alpha diversity

Although the nutrient concentrations in the treatment group kept a downward trend during the experimental stage (from day 1 to day 33), both ACE and Shannon-Wiener index of protist community did not always maintain an upward or downward trend (Fig. 3). Concurrently, the ACE and Shannon-Wiener index of protist community were lower in the treatment group than in the control group on day 5, and higher on day 29 and 33. However, the ACE index of bacterial community was higher in the treatment group than in the control group from day 21 to day 33 and the Shannon-Wiener index was higher in the treatment group on days 21 and 25 (Fig. 3).

Temporal dynamics of microbial taxonomic members

Dinophyceae (26.94%) was the most abundant protist class in the treatment group, followed by Spirotrichea (17.03%), Coscinodiscophyceae (12.06%), and Oomycetes (6.01%). Coscinodiscophyceae (29.83%) was the most abundant protist class in the control group, followed by Dinophyceae (21.89%), Oomycetes (7.97%), and Spirotrichea (6.76%). Alphaproteobacteria (42.66% of the total

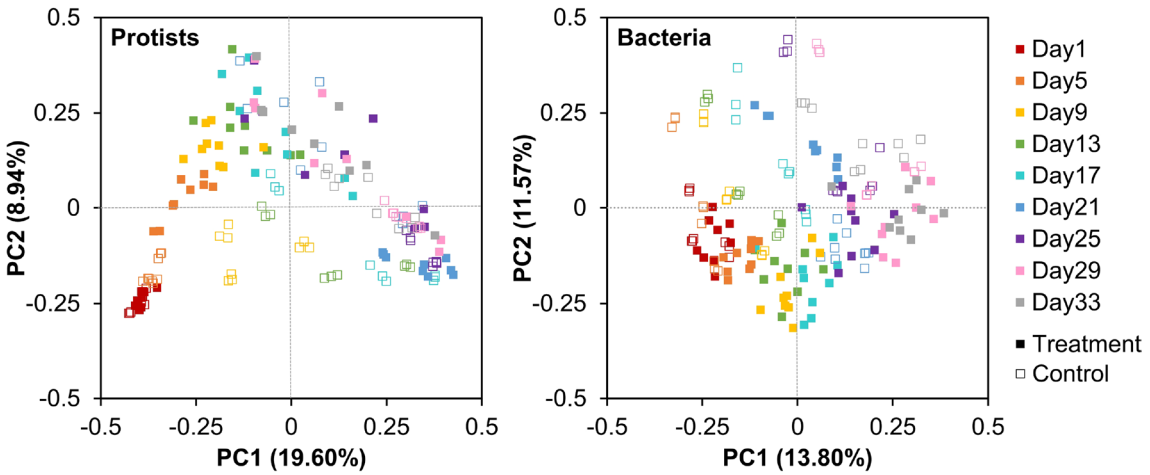

Fig. 2 Principal coordinate analysis (PCoA) of protist and bacterial communities from day 1 to day 33. Different colors represent different sampling times. The solid square is the treatment group and the hollow square is the control group

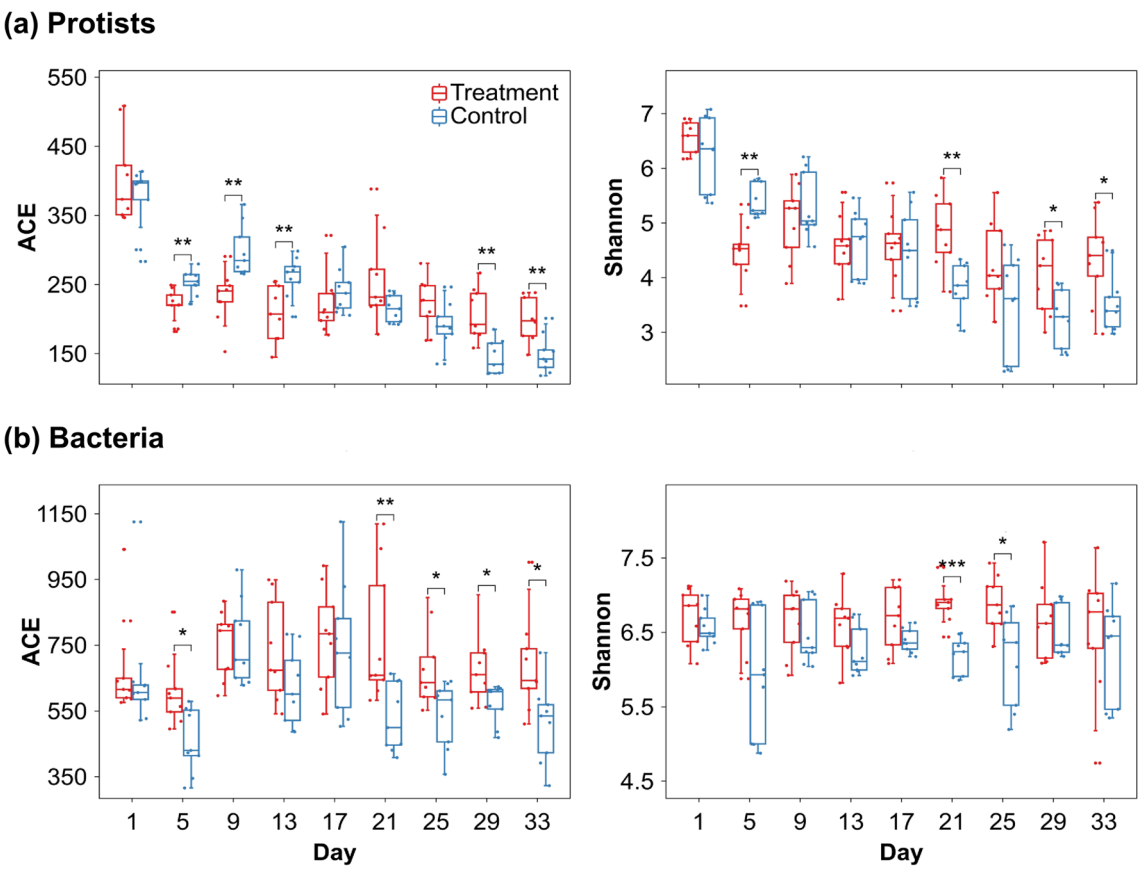

Fig. 3 Alpha diversity index (ACE and Shannon index) of protist (**a**) and bacterial (**b**) communities from day 1 to day 33. Error bars represent the standard error of the mean. The statistic is Wilcoxon test (* $P<0.05$, ** $P<0.01$, *** $P<0.001$)

bacterial abundance) was the most abundant bacterial class in both control and treatment groups, followed by Gammaproteobacteria (20.02%), Bacteroidia (8.72%), and Acidimicrobiia (8.10%). Clearly, the succession pattern of protists was different between the treatment and control groups (Fig. 4).

Environmental variables that affect protist and bacterial community

The Mantel test showed that all environmental variables were significantly correlated with the variations of community compositions of protists and bacteria in both the control and treatment groups. In most cases, however, these correlations were stronger in the control group than in the treatment group, and stronger in the protist community than in the bacterial community. Among these environmental variables, temperature was most closely related to the community compositions of protists and bacteria, followed by EC, TDS, NTU, and NO_2-N (Table 1).

In addition, all environmental variables showed significant correlations with the alpha diversity of the protist community in the control group, except for N/P. Among these environmental variables, PO_4-P showed the strongest correlation with the ACE index of protists in the treatment group, while temperature showed the strongest correlation with the Shannon-Wiener index. However, there was no significant correlation between environmental variables and the ACE index of bacteria in the treatment group, while TDS and EC showed a significant correlation with the Shannon-Wiener index. Both EC and DO showed significant correlations with the ACE index of bacteria in the control group, while only PO_4-P and NTU showed significant correlations with the bacterial Shannon-Wiener index (Table 1).

Co-occurrence networks

The protist network had more nodes and edges in the treatment group than in the control group, and the network average degree, modularity, and connectivity were larger. The network of protists was more complex in the treatment group than in the control group (Fig. S2; Table S2). The bacterial network in the treatment group had more nodes and connectivity, and the network

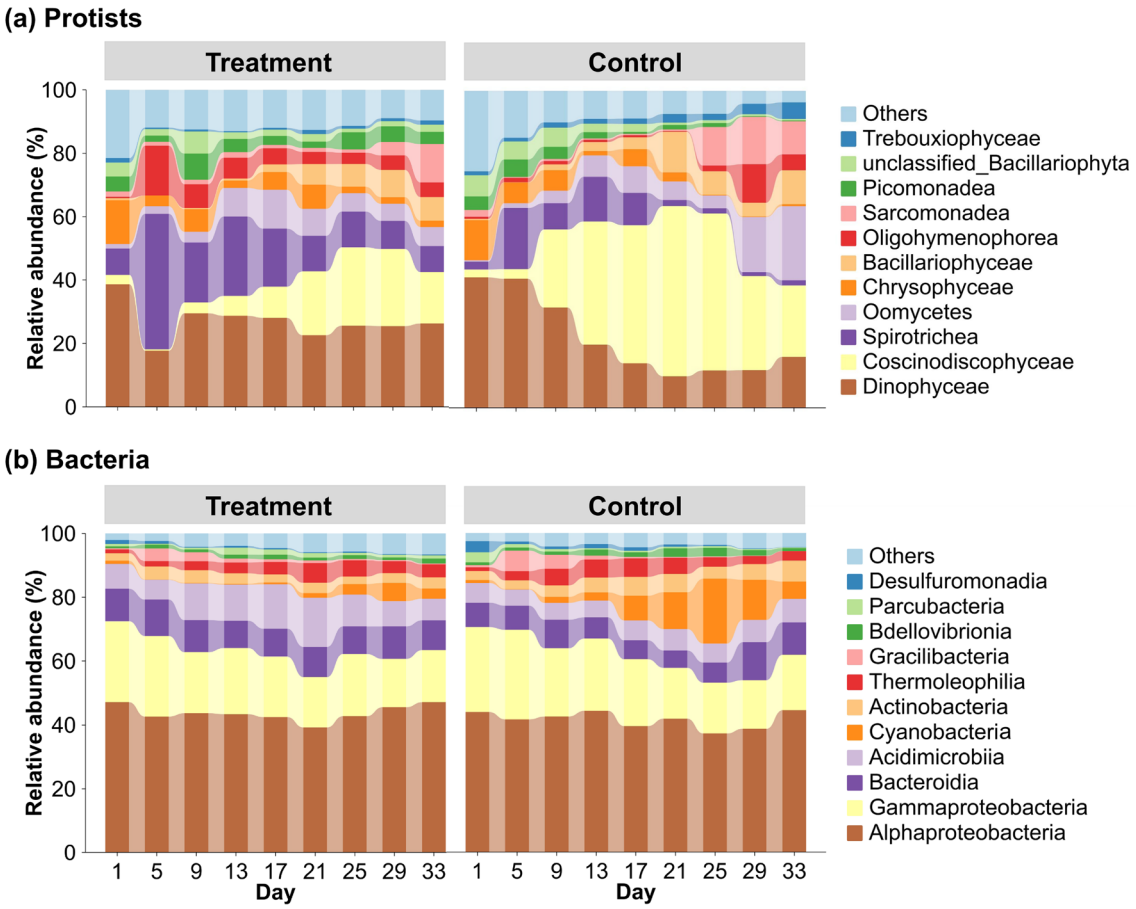

Fig. 4 Relative abundance of the main class of protists (**a**) and bacteria (**b**) in the treatment and control groups from day 1 to day 33

diameter and mean path length were larger; the bacterial network in the control group had more edges, and the network modularity index and average degree were higher.

Stability of community diversity

Overall, the temporal stability of protist and bacterial community diversity in the treatment group was higher than that in the control group (Fig. 5). The temporal stability of the control group fluctuated wildly, sometimes high and sometimes low, and the temporal stability of the treatment group fluctuated gently.

Stability of microbial taxonomic members

More than half of the taxonomic members at the class level of protist community had higher temporal stability in the treatment group than those in the control group; and more than half of the taxonomic members in bacterial class had lower temporal stability in the treatment group than those in the control group (Fig. 6). For protist communities, the temporal stability of Spirotrichea, Oomycetes, Oligohymenophorea, and Sarcomonadea increased, but the temporal stability of Dinophyceae, Coscinodiscophyceae, Chrysophyceae, Bacillariophyceae, and Picomonadea decreased in the treatment group (Fig. 6). For bacterial communities, the temporal stability of Gammaproteobacteria, Bacteroidia, Actinobacteria, and Gracilibacteria increased, but the temporal stability of Alphaproteobacteria, Acidimicrobiia, and Cyanobacteria decreased in the treatment group.

Ecological processes in the community assembly

The protist and bacterial communities were mainly driven by stochastic processes, and stochastic processes accounted for a higher percentage in the control group than in the treatment group. In the stochastic processes, the community of the treatment group was mainly driven by dispersal limitation, while the community of the control group was mainly driven by undominated processes (Fig. 7).

Microbial nitrogen cycling processes

The nitrogen cycling process involved in seawater microorganisms mainly includes five pathways, i.e.,

Table 1 Spearman correlations between community composition, alpha diversity, and environmental variables

	Treatment group			Control group		
	Community composition	Alpha diversity		Community composition	Alpha diversity	
		ACE	Shannon		ACE	Shannon
(a) Protists						
NO_3-N	**0.080**	**0.286**	0.132	**0.085**	**0.259**	0.138
NO_2-N	**0.112**	0.102	0.116	**0.540**	**0.228**	0.112
PO_4-P	**0.230**	**0.347**	**0.274**	**0.226**	**0.332**	**0.232**
N/P	**0.142**	−0.073	−0.173	**0.106**	−0.016	0.001
Temp	**0.586**	**0.276**	**0.430**	**0.621**	**0.706**	**0.744**
pH	**0.227**	**0.300**	**0.347**	**0.215**	−0.042	**−0.271**
TDS	**0.176**	−0.113	−0.015	**0.540**	**−0.807**	**−0.815**
NTU	**0.182**	**−0.225**	−0.199	**0.278**	**−0.514**	**−0.338**
EC	**0.187**	−0.107	−0.018	**0.521**	**−0.808**	**−0.821**
DO	**0.171**	0.201	0.164	**0.089**	**0.298**	0.247
(b) Bacteria						
NO_3-N	**0.174**	−0.066	0.033	**0.201**	−0.200	−0.008
NO_2-N	**0.159**	0.000	0.039	**0.365**	−0.167	−0.008
PO_4-P	**0.257**	−0.058	−0.094	**0.465**	0.035	**0.285**
N/P	**0.291**	−0.037	0.028	**0.294**	−0.137	−0.207
Temp	**0.384**	−0.140	−0.159	**0.385**	0.179	0.181
pH	**0.380**	0.156	−0.015	**0.163**	0.095	−0.198
TDS	**0.190**	−0.147	**−0.272**	**0.602**	−0.215	−0.009
NTU	**0.243**	−0.039	0.072	**0.518**	0.206	**0.242**
EC	**0.207**	−0.146	**−0.269**	**0.612**	**−0.222**	−0.027
DO	**0.126**	−0.137	−0.041	**0.143**	**−0.043**	0.000

Note: *Temp* temperature, *TDS* total dissolved solids, *NTU* turbidity, *EC* electric conductivity, *DO* dissolved oxygen. Bold numbers indicate significant correlations ($P < 0.05$)

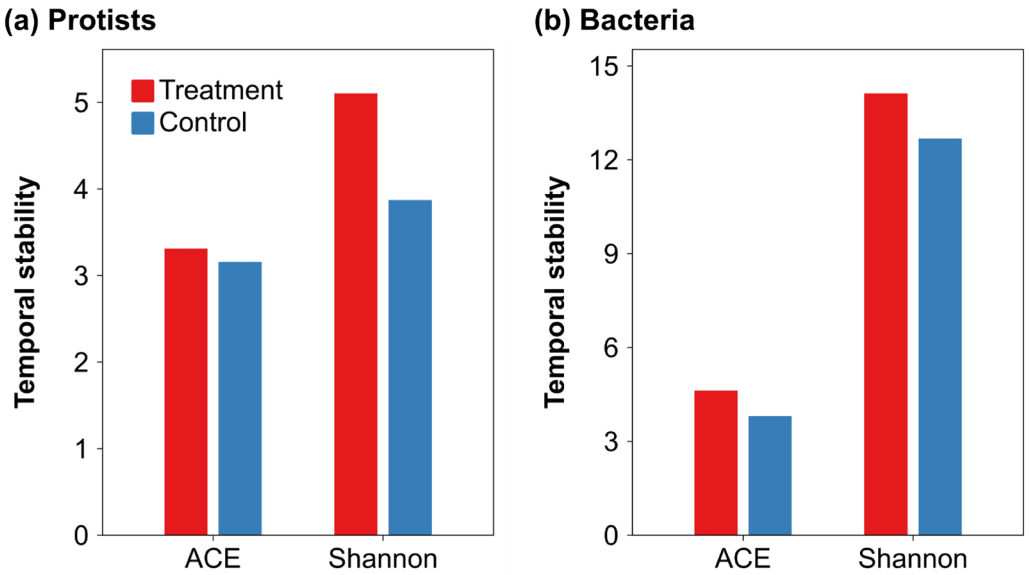

Fig. 5 Stability of alpha diversity (ACE and Shannon index) of protist (**a**) and bacterial (**b**) communities throughout the experiment period in the treatment and control groups

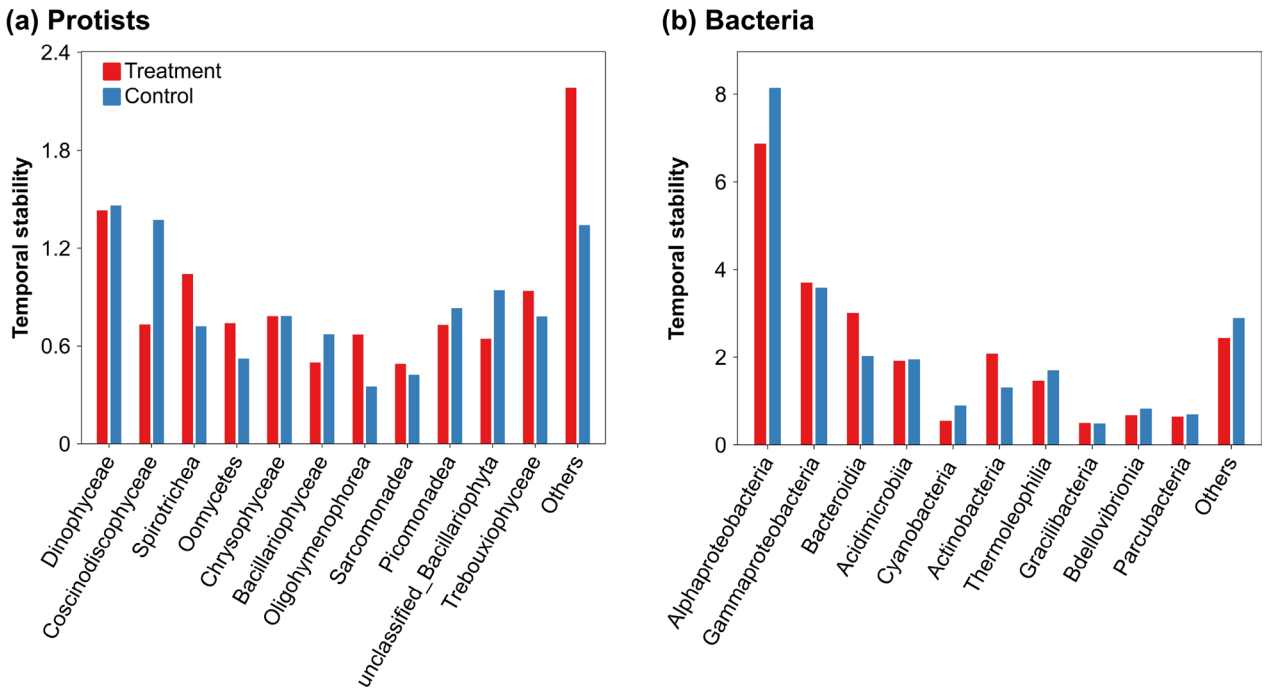

Fig. 6 Temporal stability of protist (**a**) and bacterial (**b**) taxonomic members at the class level throughout the experiment period in the treatment and control groups

assimilatory and dissimilatory nitrate reduction, denitrification, nitrogen fixation, and nitrification. Among all the nitrogen-cycling functional genes, dissimilatory nitrate reduction (44.50%) was the most abundant on day 1, followed by assimilatory nitrate reduction (24.00%) and denitrification (17.75%); however, on day 33, denitrification (62.44%) was the most abundant, followed by dissimilatory nitrate reduction (23.23%) and assimilatory nitrate reduction (11.91%) (Fig. S3). In the ecological floating bed water, the abundance of nitrogen-cycling functional genes pathways of nitrification, denitrification, dissimilatory, and assimilatory nitrate reduction, except for nitrogen fixation, were significantly changed ($P < 0.05$) between the beginning and the end of the experiment. Among all the functional genes involved in the nitrogen cycle, a total of 11 nitrogen cycle functional genes were detected to be significantly different ($P < 0.05$) in this experiment (Fig. 8). Among them, the relative abundance of 4 functional genes was significantly higher ($P < 0.05$) at the end of the experiment, including the dissimilatory nitrate reduction functional genes (*nrfA*), and denitrification functional genes (*napA*, *napB*, and *nosZ*). Besides, the relative abundance of 7 functional genes was significantly lower ($P < 0.05$) at the end of the experiment, including the dissimilatory nitrate reduction functional genes (*nirB* and *nirD*), the assimilatory nitrate reduction functional genes (*nasA* and *nasB*), and the nitrification functional genes (*amoA*, *amoB*, and *amoC*) (Fig. 8).

The relationship between the abundance of nitrogen-cycling functional genes and environmental variables was showed (Fig. 9). In the dissimilatory nitrate reduction, the relative abundance of *nirB* and *nirD* was positively correlated with pH, while the abundance of *nrfA* and *nrfH* was negatively correlated with DO. For the denitrification genes, the relative abundance of *narG*, *narH* and *narI* was negatively correlated with pH, while the relative abundance of *napA*, *norB* and *norC* was negatively correlated with NO_3-N and temperature, and the relative abundance of *norB* and *norC* was positively correlated with EC and TDS. Among these environmental variables, pH showed the strongest correlations with the abundance of functional genes, followed by NO_3-N and temperature (Fig. 9).

Discussion

The diversity and composition of the protist and bacterial communities fluctuated with the removal of nitrogen by the use of ecological floating beds in eutrophic water

Floating beds create favorable conditions for water remediation and create a large surface area for attached microorganisms to grow (Zhang et al. 2013; Xu et al. 2018). A growing number of studies have documented the phytoremediation of waste waters. For example, by

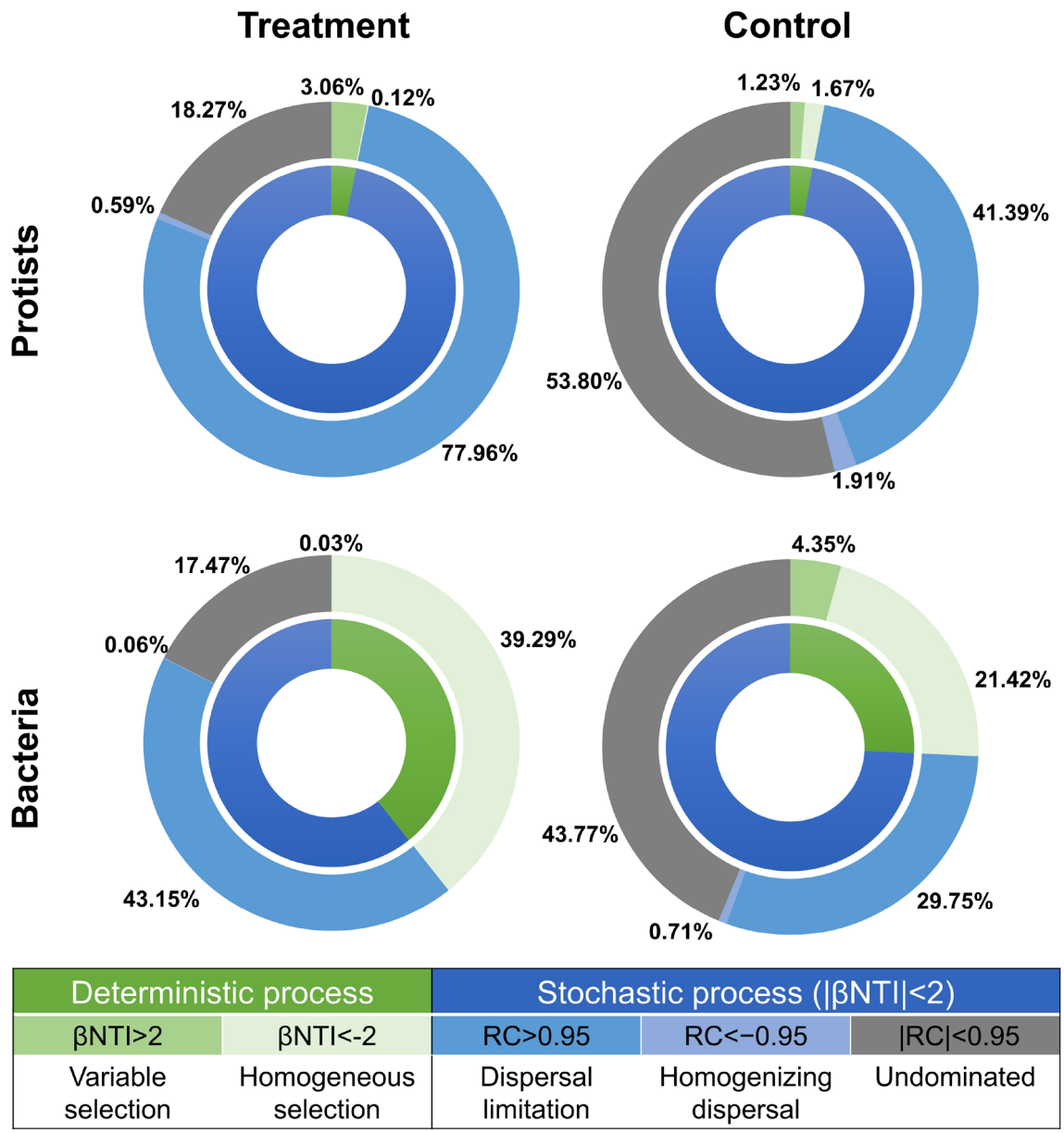

Fig. 7 Influence of deterministic and stochastic processes on protist and bacterial communities throughout the experiment period in the treatment and control groups

floating beds of *Acorus calamus*, 36% of TN and 35% of TP were removed from eutrophic lakes and reservoirs (Hu et al. 2010). *Oenanthe javanica* reduced the total nitrogen in the lake water by 31%–64% (Yang et al. 2008); while 23% TP was removed from polluted river water with a floating bed consisting of *Phragmites australis* and *Canna indica* (Saeed et al. 2016). In this study, the removal rate of nitrate and phosphate by *S. portulacastrum* was above 52% and above 34%, respectively (Fig. 1), indicating that *S. portulacastrum* is one of effective halophytes for removing nutrients. In addition to nutrients, there are other organic or inorganic ions and particles present in the water that cannot be directly absorbed by plants or microorganisms (Lynch et al. 2015), and the water flow promotes the removal of these dissolved solids by the roots through filtration. These solids are typically removed by sedimentation processes and deposited in the bottom region, from which the suspended solids need to be strategically removed (Kerr-Upal et al. 2000). When floating beds were set in the lake composed of *Typha orientalis*, *Eleocharis dulcis* and *Juncus effuses*, the suspended matter decreased by 80% and the EC decreased

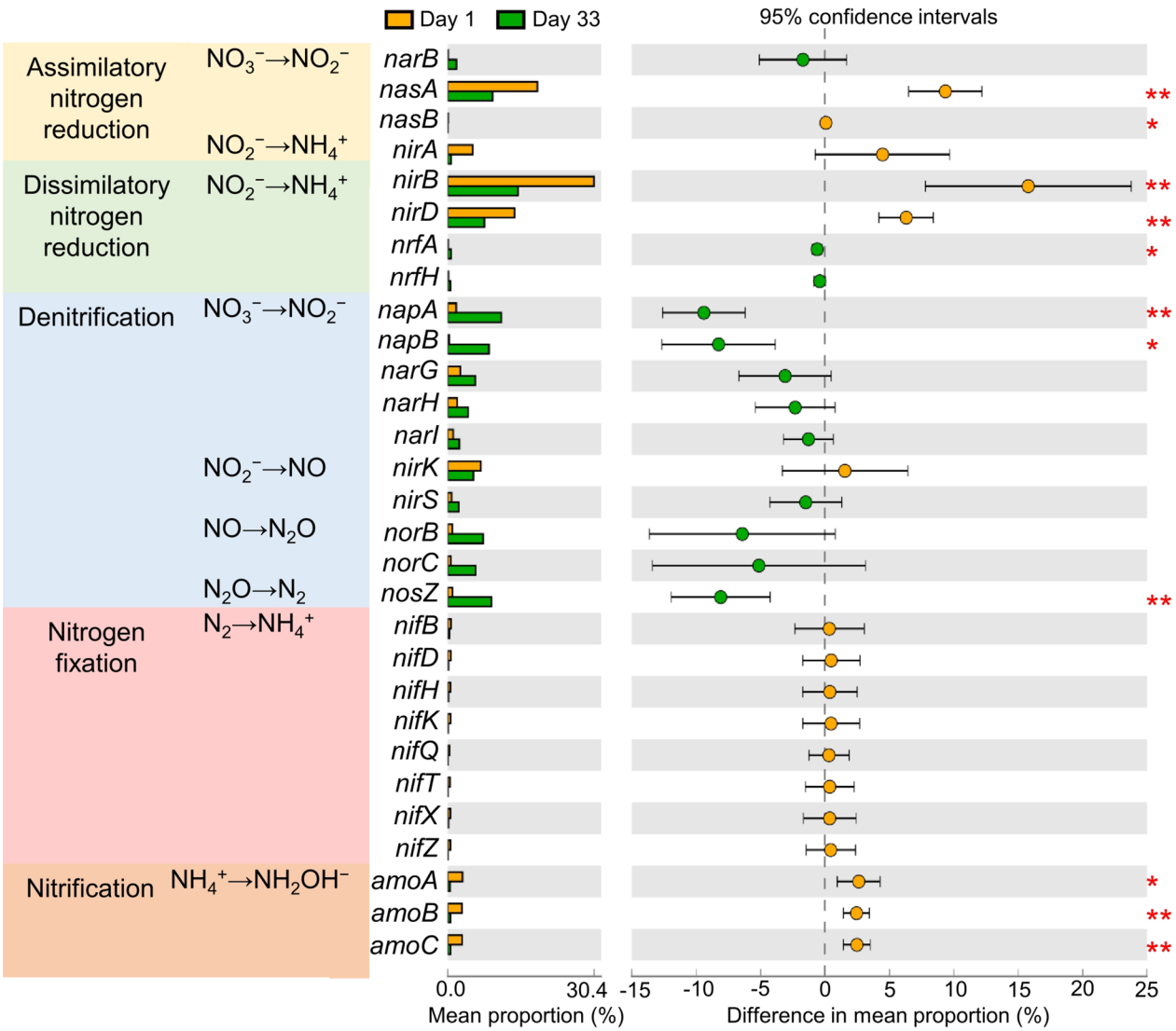

Fig. 8 Differential characteristics of microbial nitrogen cycle functional genes on day 1 and day 33. The statistic is Wilcoxon test (* $P<0.05$, ** $P<0.01$)

by 33% after four months (Lu et al. 2015). In this study, after 33 days of restoration experiments, the EC and TDS in water were significantly lower than those of the control group (Fig. 1), indicating that *S. portulacastrum* can effectively remove dissolved solids. It is worth noting that with the increase of salinity, the nutrient removal ability of plants decreases (Lymbery et al. 2013). Considering that the salinity of seawater in this study was maintained at 30.13‰, it can be concluded that *S. portulacastrum* has a stronger ability to repair waste water than the above-mentioned aquatic plants.

The microorganisms utilized nitrogen, phosphorus, and some organic matter in the water for their own growth. Synergistically with the plant, they facilitate the conversion of organic nitrogen to plant-available ammonium and nitrate nitrogen (Herman et al. 2006). Consequently, the microorganisms play a major role in removing of nutrients from waste water. Analyzing the community diversity and composition of microorganisms can effectively reflect the relationship between microorganisms and nutrients in wastewater. This study indicated that, over 33 days, the bacterial community of the treatment group had a continuously higher diversity than that of the control group (Fig. 3). Additionally, the application of *S. portulacastrum* changed the species composition in the water (Fig. 4). The abundant roots of *S. portulacastrum* provided a good growth environment for microorganisms, which is consistent

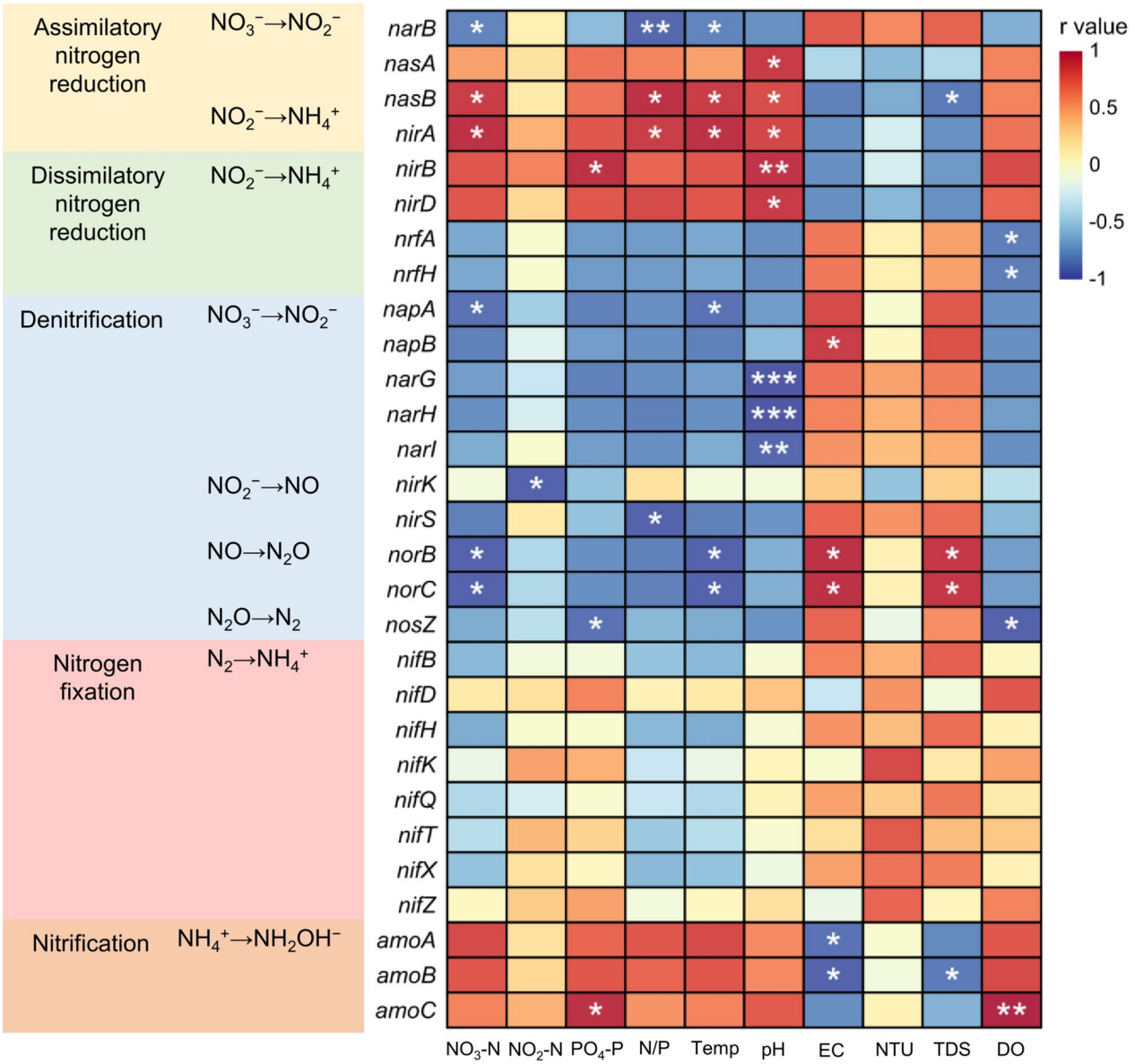

Fig. 9 Spearman correlations between abundance of nitrogen cycling function genes and environmental variables (* $P<0.05$, ** $P<0.01$, *** $P<0.001$). *Temp* temperature, *EC* electric conductivity, *NTU* turbidity, *TDS* total dissolved solids, *DO* dissolved oxygen

with the result that the species diversity of floating bed plant roots is higher than that of non-floating bed area in the waste water (Yuan et al. 2025). Furthermore, established roots can serve as attachment substrate for the growth of microbial communities, such as those of protists and bacteria, and can enhance the community diversity (Wang et al. 2020). In addition, increased diversity can enhance community stability and improve stress tolerance of microbiome (Sun et al. 2018a). Consequently, a well-developed root system can not only improve plant nutrient absorption, but also improve the diversity of the microbial community and change the community composition as a substrate.

It is noteworthy that the diversity index of the protist community showed a downward trend from day 1 to day 5, and began to rise after day 5, while the bacterial community did not show the same downward trend (Fig. 3), and the species composition of the protist community changed more significantly than that of the bacterial community from day 1 to 33 (Fig. 4). Dinoflagellata (mainly including *Gyrodinium*) was the most dominant protist community in the treatment group, followed by

Intramacronucleata and Bacillariophyta, while Bacillariophyta (mainly including *Cyclotella*) was the most dominant in the control group, followed by Dinoflagellata and Intramacronucleata (Fig. 4). This is different from the results of floating bed removal of nutrients in rivers, where Intramacronucleata was the most abundant protist phylum in both control and treatment groups, followed by Chlorophyta and Cryptophyta (Liu et al. 2022). In an experiment to add nutrients to phytoplankton blooms in seawater, it was found that Dinoflagellata was the most abundant in the protist community, and Bacillariophyta dominated when the concentration of silicates was high (Pearman et al. 2016). The protist community composition was different in saltwater or fresh water under different conditions, especially in Dinoflagellata and Bacillariophyta, which varied greatly under different temperature and nutrient salt conditions (Xiao et al. 2018). Our results showed that Alphaproteobacteria (mainly including *Rhizobiales* and *Marivita*) was the most abundant bacteria in the floating beds water, followed by Gammaproteobacteria, Bacteroidia, and Acidimicrobiia (Fig. 4). The absolute preponderance of Proteobacteria was consistent with the findings of floating bed water in many lakes, wetlands, rivers and oceans (Gao et al. 2018; Ni et al. 2018; Sun et al. 2018b; Yuan et al. 2025), suggesting Proteobacteria are the most common bacteria in wastewater treatment systems and have key functions involved in nitrogen metabolism (Chen et al. 2018; Hou et al. 2018). In addition, Mantel test showed that various environmental factors were significantly correlated with the community compositions of protists and bacteria in the control group and the experimental group, and the correlation between various environmental factors and community composition and diversity of protists was stronger than that of bacteria (Table 1). Bacteria had more vast metabolic versatility and growth rates than protists (Massana and Logares 2013). The bacterial communities could adapt to the new environment more easily than protist communities, and protist communities were more affected by environmental variables after eutrophication. Combined with the current research, protists are important hubs in the ecosystem which can form complex symbiotic relationships with a large number of bacteria in the environment, closely linking bacteria with organisms of higher trophic levels (Xiong et al. 2018). Protists can enhance the cooperation between beneficial bacteria, enhance the absorption of nutrients by plants, and promote plant growth (Guo et al. 2024); and protists are also major predators of bacteria, able to inhibit the spread of harmful bacteria and pathogens and improve plant performance (Guo et al. 2022). We focused on the dynamics of protist communities as environmental indicators and signals to play an earlier warning role.

The stability of community composition improved by the use of ecological floating beds in eutrophic water

Understanding how nitrogen removal in eutrophic water impacts microbial community stability is crucial, considering that stable microbial communities are assumed to sustain ecosystem services and functioning (Pennekamp et al. 2018; Puentes-Téllez and Salles 2020). In this study, we discovered that the use of ecological floating beds improved the composition stability of the microbial community during the nitrogen removal processes. There are two reasonable explanations for this result.

First, the removal of nutrients in this experiment from day 1 to day 33 was more obvious in the treatment group than in the control group, and nutrient concentrations in the control group appeared to be more stable than those in the treatment group (Fig. 1). The community diversity of the treatment group was higher than that of the control group during the late period (Fig. 3). Therefore, the stable nutrient conditions might decrease the species turnover rate of microbial communities and thus resulted in a stable microbial community. This finding permits us to suppose that stable microbial communities in a high nutrition environment might not always be correlated with stable nutrient concentrations over time.

Previous research revealed that the stochastic process produced random variation in colonization or extinction, which in turn produced an unstable ecological community composition (i.e., high spatiotemporal variability) (Conradi et al. 2017). Because nutrient input promotes community growth and decreases niche selection in communities, it may increase the relative importance of stochastic processes (Chase and Myers 2011). In this study, we discovered that although microbial communities in both control and treatment groups were governed by the stochastic process, the contribution of the stochastic process was higher in the control group than that in the treatment group (Fig. 7). Simultaneously, we examined the temporal stability of the diversity and composition of protist and bacterial communities, and found that the treatment group was higher than the control group (Figs. 5 and 6). Thus, we assume that the high nutrient concentrations in the control group might increase the random rates of random colonization or extinction, leading to high temporal instability in the microbial communities. Furthermore, the environmental variables in the control group showed stronger connections with the microbial communities than those in the treatment group (Table 1). The protist and bacterial communities in the control group were strongly affected by the great changes in the environment. However, in the treatment group, the continued decrease in nutrient concentrations did not become a limitation for the variations of protist and bacterial communities.

Generally, the recovery of an aquatic ecosystem following eutrophication might take several years or even decades. As a result, it is difficult to understand how the bioremediation initiatives affected the stability of the entire aquatic ecosystem in a short period (Liu et al. 2020). Within 33 days, the protist and bacterial communities in the treatment group in our research likewise gradually separated from those in the control group (Fig. 2). We may thus confidently infer that the ecological floating beds increased the stability of microbial community composition in eutrophic water, given the quick responses of microbial communities to environmental changes.

The abundance of denitrification functional genes increased during the removal of nitrogen by the use of ecological floating beds in eutrophic water

Normally, plants depend on reactive forms of nitrogen, including ammonium nitrogen and nitrate nitrogen, to sustain growth because they need nitrogen for several physiological activities, including growth and development and the production of essential cellular components like proteins and nucleic acids (Kuypers et al. 2018). To investigate the variations in the nitrogen cycle metabolism process of microorganisms in water under the situation of nitrogen excess, the concentration of nitrate nitrogen was regulated during the experiment's setup in this study. The differences in the main metabolic pathways were examined at KEGG level 3. In the ecological floating bed water, there were great changes in the abundance of several functional genes of the nitrogen cycling pathways between the beginning and the end of the experiment, mainly including denitrification, dissimilatory nitrate reduction, and assimilatory nitrate reduction (Fig. S3).

The denitrification process is a key way of biological nitrogen removal during water restoration (Pavlineri et al. 2017; Di Luca et al. 2019). According to previous research, 80% of nitrogen was removed by denitrification, and the remaining 20% was removed by bio-accumulation and sedimentation processes in three New Zealand experimental wetlands (Saunders and Kalff 2001). At the beginning of our experiment, the gene abundance of nitrate dissimilation to ammonium was higher than that at the end of the experiment, the concentration of nitrate nitrogen in water was higher, and the nitrate reduction reaction was stronger (Fig. 8 and S3). At the end of the experiment, the concentration of denitrification genes was higher, more nitrate was consumed, and the assimilation nitrate reaction and nitrification reaction in the water were less strong than they had been at the beginning (Fig. 8 and S3). In the dissimilated nitrate reaction, the dissimilated reduction to ammonium was weakened, while the denitrification process was strengthened. By the end of the experiment, denitrification was the primary method of NO_3^- consumption in water. Both the denitrification process and dissimilatory nitrate reduction to ammonium are competing methods of reducing nitrate nitrogen, and they will be competing with NO_3^- in the environment at the same time (Kraft et al. 2014; Wei et al. 2015). More accessible ammonium nitrogen can be produced by dissimilatory nitrate reduction to ammonium, enabling the transformation of endogenous nitrogen, therefore, dissimilatory nitrate reduction to ammonium is conducive to the maintenance of nitrogen in water while also aggravating the eutrophication of water (Minick et al. 2016; Rezvani et al. 2019). In the process of turning nitrate into ammonium and contributing to nitrogen fixation, bacteria of dissimilatory nitrate reduction to ammonium compete with denitrification bacteria for electron acceptors; and denitrification can reduce nitrate to nitrogen, so that nitrogen is removed from the water, thereby reducing the content of nitrogen, resulting in nitrogen loss (Kuypers et al. 2018; Rezvani et al. 2019).

Denitrification is the most common method to reduce nitrogen in waste water and nitrogen removal in the eutrophic water environment, however, denitrifying bacteria proliferate slowly, so how to enrich them effectively is the key of engineering technology (Lawson et al. 2017). In this study, at the end of the experiment, the abundance of denitrification functional genes (i.e., *nap*A, *nap*B, and *nos*Z) was significantly increased (Fig. 8), and the nitrate nitrogen in the cultured water was reduced to N_2, thus completing the biological nitrogen removal process. The change of community structure caused the change of community function (Fig. 10), laying ecological floating beds can promote nitrogen cycling in water, significantly improve the denitrification capacity of water, and achieve the nitrogen removal through the denitrification pathway of functional microorganisms.

Conclusions

Our experiment demonstrated that nitrogen and dissolved solids could be effectively removed in a few days by the ecological floating beds of *S. portulacastrum* in eutrophic water. The well-developed root system of *S. portulacastrum* can improve the diversity of the microbial community and change the community composition as a substrate. Furthermore, the protist and bacterial communities had a stronger symbiotic relationship and exhibited greater stability. Following the installation of the floating beds, the denitrification process was encouraged by the core microbiome, which allowed for the migration and transformation of nitrogen as well as the removal of pollutants containing nitrogen from the

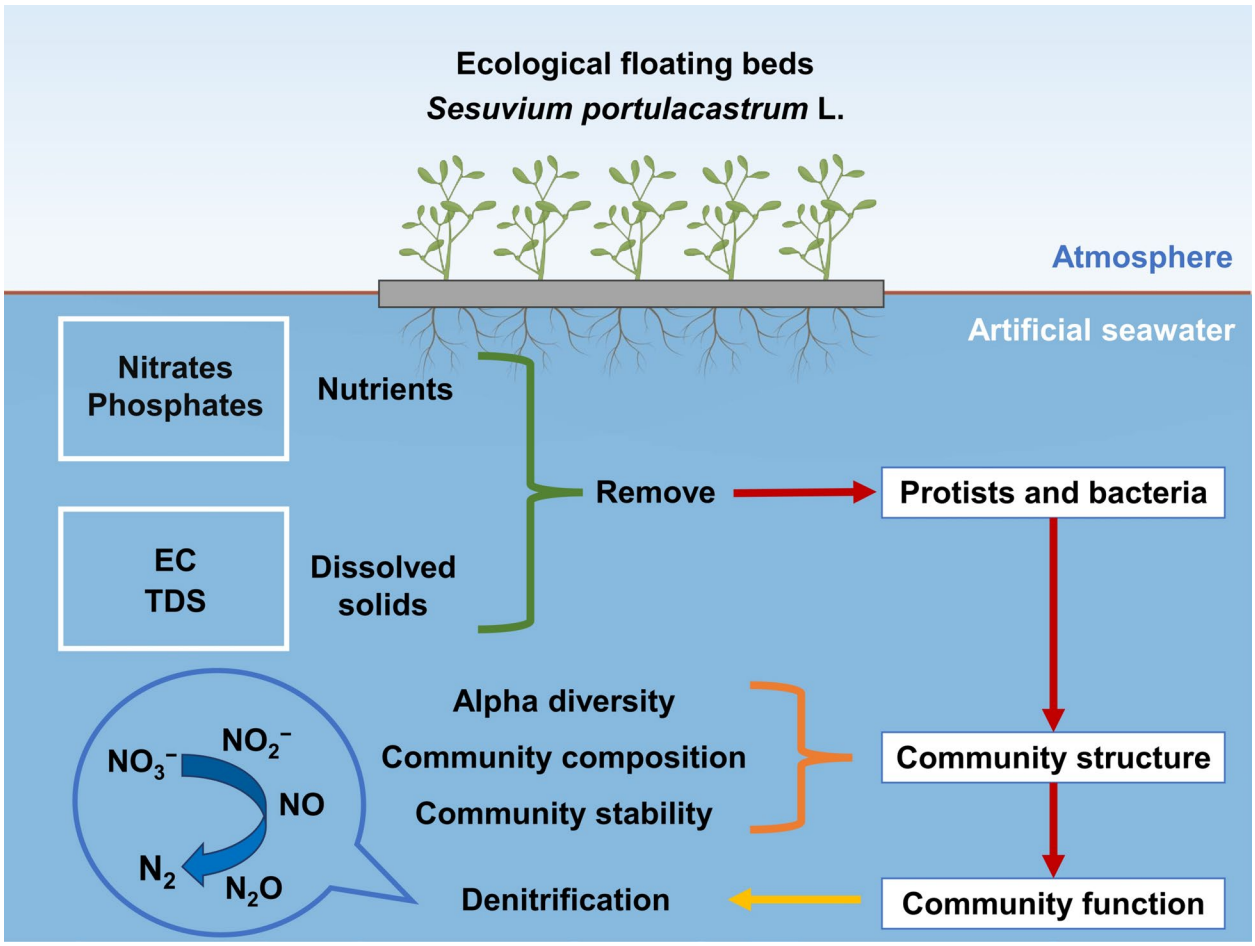

Fig. 10 The mechanism of *S. portulacastrum* floating beds in the process of water restoration

eutrophic water, ultimately improving the water quality. Our research offers a new perspective into the dynamics of microbial community structure and function during nitrogen removal by floating beds, which may contribute to the restoration of eutrophic aquatic ecosystems and further understanding of aquatic ecosystem stability.

Abbreviations

S. portulacastrum	*Sesuvium portulacastrum*
EC	Electric conductivity
NTU	Turbidity
TDS	Total dissolved solids
DO	Dissolved oxygen
PO_4-P	Phosphate phosphorus
NO_3-N	Nitrate nitrogen
NO_2-N	Nitrite nitrogen
N/P	Ratio of nitrate and nitrite nitrogen to phosphate phosphorus
ASVs	Amplicon sequence variants
PR2	Protist Ribosomal Reference
ACE	Abundance-based coverage estimators
PCoA	Principal coordinate analysis
βNTI	Beta nearest taxon index
RC_{bray}	Bray-Curtis based Raup-Crick metric
NR	Amino acid sequence of non-redundant protein
KEGG	Kyoto Encyclopedia of Genes and Genomes

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13717-025-00602-3.

Supplementary Material 1

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Author contributions
Rui Zhao has made a significant contribution to the experiments, sample collection, data analysis, and wrote the manuscript. Yi Shi has made a significant contribution to sample collection and reviewed the manuscript. Lingfeng Huang has constructed and sustained ecological floating beds, and provided the floating bed plant. Jun Yang has made a significant contribution to the experiment design, and reviewed the manuscript. Wenjing Zhang has made a significant contribution to the experiment design, sample collection, data analysis and writing. All authors read and approved the final manuscript for publication.

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Availability of data and materials
The raw sequences have been deposited in the NCBI project under accession numbers PRJNA1228346 (16S rRNA gene), PRJNA1228557 (18S rRNA gene) and PRJNA1228997 (metagenome). The raw sequences were also stored in the National Omics Data Encyclopedia (NODE) database under the Project ID: OEP00006075 (16S rRNA gene), OEP00006076 (18S rRNA gene) and OEP00006077 (metagenome).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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