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The effect of *Sesuvium portulacastrum* for reducing inorganic nitrogen pollution in coastal mariculture wetland

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Mariculture ponds are essential components of the coastal wetland, which are often criticized by eutrophication risk for the dissolved inorganic nitrogen (DIN) input to the coastal zone by the culture tailwater. However, the reduce of this DIN pollution was difficult because the tailwater is hard to collect and the treatment is inefficient and expensive. *Sesuvium Portulacastrum* is a coastal vegetation which has high efficiency in DIN absorption from the seawater and sediment. In this study, we use *Sesuvium Portulacastrum* as a tool species to study the control behavior of the DIN in mariculture ponds wetland. The change trend of DIN in pond water and benthic species in pond sediment was investigated. The results showed that *Sesuvium Portulacastrum* reduced NH₄₊, NO₃₋, and NO₂₋ in the pond water by 83.21%, 95.22%, and 91.32%, respectively. The species number of benthic organisms was enhanced from 2 to 5 and the species structure was more optimized in *Sesuvium Portulacastrum* pond than control pond. At the end of the experiment, eutrophication indicator species (*Capitella capitata*) was disappeared in the *Sesuvium Portulacastrum* pond. Those suggest that the coastal vegetation (*Sesuvium Portulacastrum*) have great potential to eliminate DIN pollutants in mariculture pond wetland.

KEYWORDS

Sesuvium portulacastrum, reduction, inorganic nitrogen, mariculture, pond water

1 Introduction

Mariculture ponds is an essential component of the coastal wetland because the large water body and proximity the coastal area. It supplies a large amount of quality protein for human about 30% of global mariculture production ([The State of World Fisheries and Aquaculture, 2022](#)). Meanwhile, it is often criticized by eutrophication risk for the input the effluents rich in N and P to the adjacent coastal zone. According to the World Food and Agriculture Organization (FAO), the growth in global marine and coastal aquaculture production will mainly come from mariculture in the next 30 years. With this rapid

development trend, adjacent coastal zones would receive a large amount of mariculture tailwater, which enhance the eutrophication risk in this area (Levy et al., 2017). However, human activities such as overfishing, especially the overharvesting of shellfish, have excessive destructed the coastal sediments. It resulted in the disappearance of indigenous habitats such as seagrass beds, macroalgae bed, and oyster reefs which express the key buffering and degradation capacity of coastal zones for the N and P pollutants (Planque et al., 2010). Furthermore, the restoration of indigenous habitats in coastal zone is often slow and difficult. Therefore, it is important to control the N and P level in mariculture pond wetlands to reduce the input of this contaminant to coastal zone.

Currently, the treatment of mariculture tailwater is difficult because it is rich in variety of pollutants including N, P, and a larger number of organic pollutants such as bait and animal limbs (Chen et al., 2016). These pollutants are prone to clogging treatment equipment, which increases the equipment consumed and cost. Furthermore, the high salinity of the tailwater will corrode treatment equipment made of steel. Taking the normal industrial treatment model, the cost of tailwater treatment is 1.47 RMB m⁻³ which could increase the cost of mariculture about 17.6 RMB kg⁻¹, about 50% of the original culture cost (Zeng et al., 2019). Hence, industrial treatment model is unsustainable which tends to reduce the expansion of mariculture by the high culture cost. Therefore, it is urgent to develop a low-costly and eco-friendly method to control the N and P pollution level of mariculture tailwater.

Artificial wetlands constructed by the salt marsh vegetations are often eco-friendly method applied for mariculture tailwater treatment (Hu et al., 2017; Cai et al., 2022). The removal rates of N and P pollution can be up to 92% and 72% or more in this kind of system (Wang et al., 2014). High removal capacity of N and P are from two pathways. First are the abundant microorganisms such as *Phytophthora nitrite*, *Vibrio vulnificus*, and *Platyhelminthes* supply by the root system of the salt marsh vegetation. Another was the uptake of N and P by the growth of salt marsh vegetation in wetland. The combination and cooperative interaction of microorganisms and the growth of salt marsh vegetation would make the efficiently removal capacity of artificial wetlands. In addition, the macro-vegetation surviving in salt marshes, such as *Salicornia europaea* L., *Suaeda salsa* (L.) Pall., and *Sesuvium portulacastrum*, has evolved the ability to absorb multiple forms of elemental N. Those vegetations could directly utilize peptide organic nitrogen in seawater, which is important for scavenging N in mariculture tailwater (Quintã et al., 2015; Zheng et al., 2016). However, there are still intractable limits for the application of artificial wetland in treatment mariculture ponds tailwater which constructed based by salt marsh vegetations. For example, the high temperatures and salinity of mariculture water make the salt marsh vegetations difficult survival in this harsh environment. It is necessary to select and breed of vegetations tolerant to high temperatures, high salinity, and high stress tolerance for application in the control of pollutants in mariculture wetland.

Sesuvium portulacastrum is a perennial herbaceous saline vegetations that grows in tropical and subtropical coastal areas (Fan et al., 2010). It often grows in high temperature, high salinity, and long-term flooding adverse environments and have potential to select as the

contaminant control species in mariculture wetlands. Some applications of the *Sesuvium portulacastrum* in mariculture tailwater treatment have been conducted. For example, wetlands constructed based by *Sesuvium portulacastrum* could increase the removal efficiency of DIN in mariculture effluents (Ma et al., 2021). *Sesuvium portulacastrum* could remove 98.5% of NH₄⁺ and 55.9% of total nitrogen in mariculture tailwater (Ying et al., 2018). In addition, the use of *Sesuvium portulacastrum* in a recirculating aquaculture system integrating *Chanos chanos* and *Holothuria scabra* allowed ammonia levels to be controlled and reliably decreased to < 1 mg L⁻¹ (Senff et al., 2015). Hence, *Sesuvium portulacastrum* has great potential applications for controlling N pollution in mariculture water environment.

In this work, we select open-air *Holothuria scabra* mariculture ponds in the Yellow River Delta as the study area. The role of *Sesuvium portulacastrum* in controlling inorganic nitrogen (NH₄⁺, NO₃⁻, and NO₂⁻) in pond water during the summer and early autumn growth season of *Holothuria scabra* was observed. Biodiversity parameters were investigated for the pond substrate. Meanwhile, the water temperature in this system was monitored because it is the key factor in ensuring that the *Holothuria scabra* safely survives in growing season in summer and early autumn.

2 Materials and methods

2.1 Study location

The Yellow River Delta is the important coastal *Holothuria scabra* culture zone in China. The culture scale has exceeded 333 km² and accounts for more than half of the pond culture area of sea cucumbers in Shandong Province. This region has become the largest sea cucumber pond culture area in China (Wang and Liu, 2023). However, the temperatures and rainfall are variable in the Yellow River Delta, which pose a potential threat to sea cucumber mariculture. In the summers of 2013, 2016, and 2017, the production of *Holothuria scabra* mariculture was significantly decreased due to the high temperatures and lack of oxygen by the short-time strong rainfall. It had seriously affected the development of *Holothuria scabra* mariculture and the living of mariculturist in this area. Meanwhile, the discharge of tailwater would be urgent problem for the culture of *Holothuria scabra* with the gradual tightening of environmental protection policies for mariculture. The experiment was conducted in the *Holothuria scabra* culture ponds in Dongying Kenli District Huilu Aquaculture Co. in the coastal area of Laizhou Bay (E 118°57'50.39", N 37°37'12.00") (Figure 1).

2.2 Experimental setup

2.2.1 *Sesuvium portulacastrum*

In summer, *Holothuria scabra* ponds are characterized by high temperature and salinity. Hence, we collected *Sesuvium portulacastrum* near the subtidal zone in Wenchang City, Hainan Province, which also has higher temperature and salinity. It is unable to overwinter and will not impact the local ecosystem in the Yellow River Delta. To maintain the activity of the *Sesuvium*

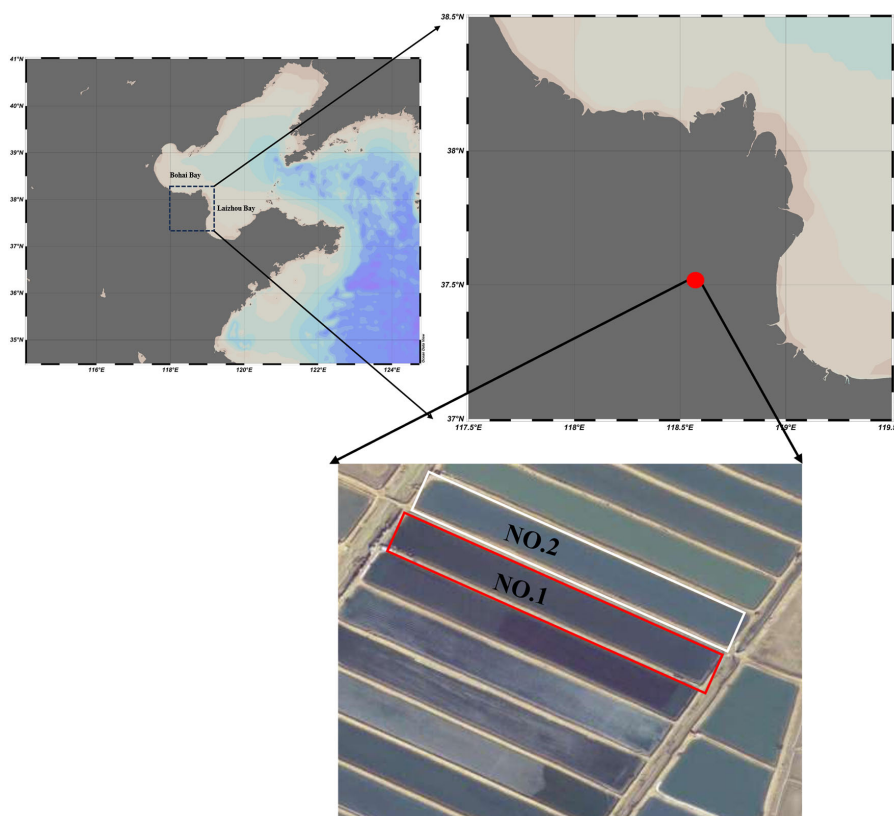


FIGURE 1

The location of the experiment in the sea cucumber ponds in Dongying Kenli District Huilu Aquaculture Co. in the coastal area of Laizhou Bay (E 118°57'50.39", N 37°37'12.00"). The red boxes in the figure represent the experimental area (NO. 1). The white boxes in the figure represent the control area (NO. 2).

portulacastrum seedlings, we conducted acclimatization process before the begin of the experiment in sea cucumber ponds for 15 d. The brief process was below: *Sesuvium portulacastrum* were staged in 30 L high-density polyethylene square boxes (60cm×50cm×10cm) contain about 20 L seawater. We used air to supply the dissolved oxygen for the *Sesuvium portulacastrum* at a rate of 1.5 m³ h⁻¹ and a light period of 16h:8h. Thick and stout *Sesuvium portulacastrum* with fat leaves was selected for the experiment.

2.2.2 Experiment in the ponds

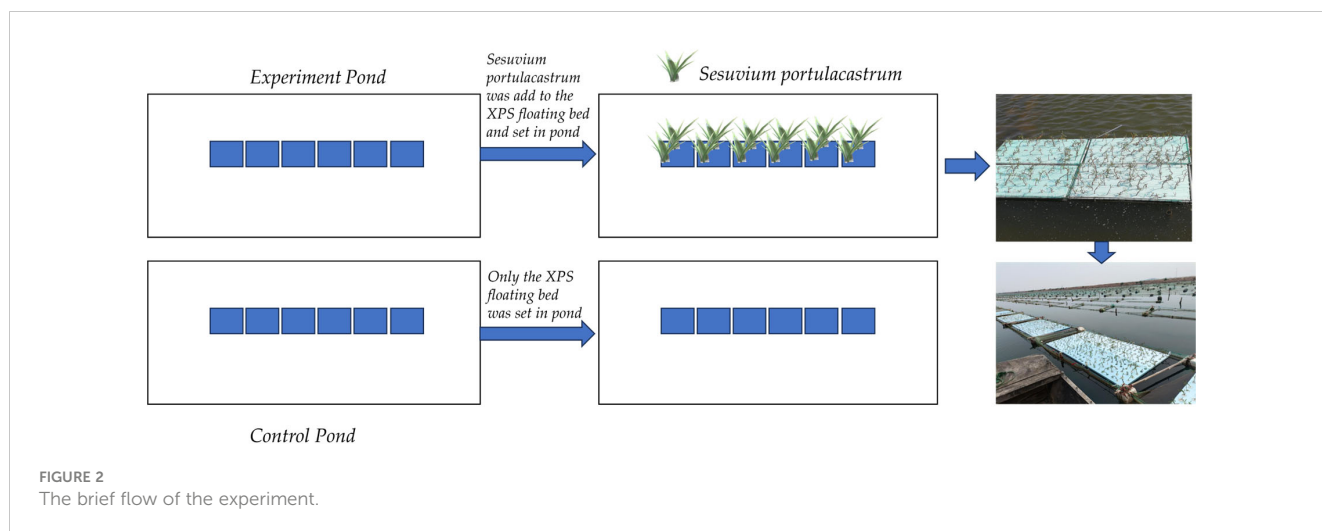
The experiment was carried out over a period of 120 d from June 5 to October 5 in 2023. The steps were as follows: Two ponds with the same area (1 hm²), specifications, and facilities were selected to carry out the experiment. Pond No. 1 served as the experiment pond and Pond No. 2 was as the control pond (Figure 1). The experimental ponds were rectangular in shape, east-west oriented, water depth up to 2.5 m, and water quality in accordance with The Water Quality Standards for Fishery (GB 11607-89). Six fish cages were set up in the experimental ponds, with specifications of 3m×1.5m×1m and a 30-purpose polyethylene mesh as the cage coat. Cages were fixed using bamboo poles and ropes, with a foam float tied to each of the four corners of the nets. The distance between every fish cage was 6m. The control pond also comprised six fish cages. At the beginning of June (June 5), we used

a bench scale (AHC-WL, T-scale[®]) to weigh about 0.25kg of *Holothuria scabra* seeding (0.08g/ins-0.10g/ins) and put into every cage. *Sesuvium portulacastrum* with robust plants and fat leaves were selected and transplanted onto XPS (extruded polystyrene foam board) floating beds in June 10. The floating beds were secured together with ties and then fixed above the *Holothuria scabra* seeding cages. About 30% of the surface water of the sea cucumber culture cages was covered by floating beds. The same process was followed for the control pond. The initial concentration of DIN in control pond and experiment pond was 0.927 mg L⁻¹ and 0.944 mg L⁻¹, respectively. Figure 2 shows a brief flow of the experiment.

2.3 Sampling and analysis

2.3.1 Sampling

The sampling process is according our previous study and briefly described below (Wang and Liu, 2023). The sample system consists of a collection unit, a filtration unit, and a sampling unit. The collection unit was 125 ml HDPE bottles (Nalgene[™], Thermo Fisher Scientific Inc., USA). The filtration unit was a pressure filtration unit (YY3014236, Filter Holder, Millipore[®]). Since the pond waters contained high concentrations of suspended particulate matter and organic matter, the diameter of the filter



membrane (0.22 μm , GPWP14250, Millipore[®]) was 142 mm. The units were fixed using a C-Flex tube. When sampling, the C-Flex-tube-fixed water intake was directly inserted into the pond water to collect the samples. Two parallel samples were collected. The fixed water intake sampling tubes were attached to a disc filter unit, and washed with ultrapure water before and after each sample. The collected water samples were stored in self-sealing PE bag at -20°C . Sampling devices and bottles were soaked in 3% HNO_3 for 48 h and then rinsed five times with ultra-pure water ($>18.2 \Omega$) and blown dry on an ultra-clean table (Class-100). Water samples were collected every 10 days from June 5 to September 5. The last sample was collected every 30d from September 5 to October 5 because the culture in the pond was over.

Benthic organisms were sampled according to the quantitative sampling method in the Zhang et al., 2007. The collection gear was a QNC7-1 box-type mud collector, the sampling area was 0.25m^2 , and each station was sampled 4 times. Samples were eluted with a 0.5 mm mesh set sieve and macrobenthic samples were collected and stored in 500 ml HDPE bottles containing formaldehyde solution. The samples were preserved, categorized, and weighed in accordance with the Specifications for oceanographic survey-Part 6: Marine biological survey (GB/T 12763.3-2007).

2.3.2 Analysis

Regular parameters such as temperature (T), salinity (S), and dissolved oxygen (DO) were collected using a multiparameter water quality analyzer (ProQuatro, YSI[®]). The concentrations of DIN ($\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$), active phosphate (PO_4^{3-}), and DSi (SiO_2) of the samples were detected by a continuous-flow nutrient analysis (AutoAnalyzer III, SEAL AnalyticalTM). The quantification limit of the nutrient analysis method for DIN, active phosphate (PO_4^{3-}), and DSi was 0.012 mg L^{-1} , 0.008 mg L^{-1} , 0.011 mg L^{-1} and the recoveries ranged from 95.5% to 101.2%. The samples were analyzed three times and the error must be less than 5%.

Indicator of coastal eutrophication potential (ICEP) was used to measure the nutrients risk of the N and P in the *Sesuvium portulacastrum* ponds to the coastal zone. This index was

proposed by Billen and Garnier (2007) and modified it to suit the study because the pond water was not exchanged with the coastal area in the culture process. The modified equation is as follows:

$$\text{ICEP(N)} = \left(\frac{C_N}{14 \times 16} - \frac{C_{Si}}{28 \times 20} \right) \times 106 \times 12 \quad (1)$$

$$\text{ICEP(P)} = \left(\frac{C_p}{31 \times 16} - \frac{C_{Si}}{28 \times 20} \right) \times 106 \times 12 \quad (2)$$

In this study, benthic community compositional diversity was expressed using the Shannon–Wiener species diversity index (H'), the Margalef species abundance index (D), and the Pielou species evenness index (J) (Lobon-Cervia et al., 2012). The following formula was used:

$$H' = -\sum_{i=1}^a N_i \ln N_i \quad (3)$$

$$J = \frac{H'}{\ln S} \quad (4)$$

$$D = \frac{S-1}{\ln N} \quad (5)$$

N_i is the ratio of the number of individuals of species i to the total number of individuals of all captured organisms (n_i/N); N is the total number of individuals of all captured organisms; and S is the total number of species of all captured organisms.

The Mcnaughton dominance index was used to determine the dominant species in the pond sediment (Lobon-Cervia et al., 2012). Community dominance was calculated using the following formula:

$$W = \frac{S_1 + S_2}{ST} \quad (6)$$

S_1 and S_2 are the biotic densities of the two most abundant species in the community; ST is the total benthic density.

3 Results

3.1 Basic geochemical parameters

The temperature (T), salinity (S), pH, and dissolved oxygen (DO) are key geochemical parameters related to the stability of the environment in *Holothuria scabra* pond. Especially for the temperature, it is related to the survival rate of the *Holothuria scabra* seedlings cultured in the pond. Figure 3 shows the change trend of basic geochemical parameters throughout the culture process. In all the ponds, the water temperature showed a trend of increasing and then decreasing, which was similar to the weather temperature changes during the experiment. However, changing amplitude of temperature was different from the experimental and control ponds (Figure 3A). In the control pond, the water temperature increased from 25°C to 30.9°C, whereas that of the experimental pond increased to 29.2°C. This indicates that the *Sesuvium portulacastrum* floating bed can effectively reduce the pond water temperature. It is important for the *Holothuria scabra* in the pond to survive the high temperature period in summer and early autumn.

Changes in DO are strongly influenced by the external environment such as wind around the pond because the water depth of the pond was only about 2m. In the *Sesuvium portulacastrum* pond, the average concentration of DO fluctuate around 4.53 mg L⁻¹, with a concentration fluctuation range of 0.32 mg L⁻¹ (Figure 3B). However, in the control pond, the average concentration of DO was 4.35 mg L⁻¹, with a fluctuation range of 0.65 mg L⁻¹. This fluctuation mainly occurred around 60d and 70d, and the lowest concentration of DO was 3.04 mg L⁻¹ (Figure 3B). pH showed similar changes trend to DO (Figure 3C). In the *Sesuvium portulacastrum* pond, pH varied from 8.01 to 8.23 with a fluctuation of 0.06. However, in the control ponds,

pH varied from 7.89 to 8.24 with a fluctuation of 0.09, and the severe changes were observed around 60 d and 70 d. For salinity, the change trend was almost identical between different ponds (Figure 3D). Salinity ranged from 30–34 and the highest salinity occurring during the maximum temperature in almost 32°C. Evaporation from the pond water body was high during high-temperature periods, resulting in higher salinity in the pond water (Figure 4D). During this period, *Sesuvium portulacastrum* still grew vigorously which indicating the salt and heat tolerance of this plant.

3.2 Dissolved inorganic nitrogen and reactive phosphate

Nitrate nitrogen (NO₃⁻N) is the most essential forms for vegetation uptake in the pond. It is also one of the more stable states of elemental N. Figure 4A. shows the changes in NO₃⁻N concentration in ponds with or without *Sesuvium portulacastrum*. The concentration of NO₃⁻N varied with different ponds. In the *Sesuvium portulacastrum* pond, NO₃⁻N was continued declining but the decrease rate gradually slowed with the decrease in temperature. The maximum concentration of NO₃⁻N was found in June, about 0.625 ± 0.005 mg L⁻¹. During the culture process, the concentration of NO₃⁻N showed three change stages (Figure 4A). There was rapid decline phase from 0 to 40d, a slow decline phase from 40 to 60d, and an equilibrium rise phase from 60 to 90d. About 88.12% of the NO₃⁻N was eliminated in the rapid decline phase, while there was only 5% in the slow decline stage. In the control pond, the minimum concentration of NO₃⁻N was 0.195 ± 0.002 mg L⁻¹ in August and was essentially unchanged by September. Notably, a strenuous fluctuation in NO₃⁻N

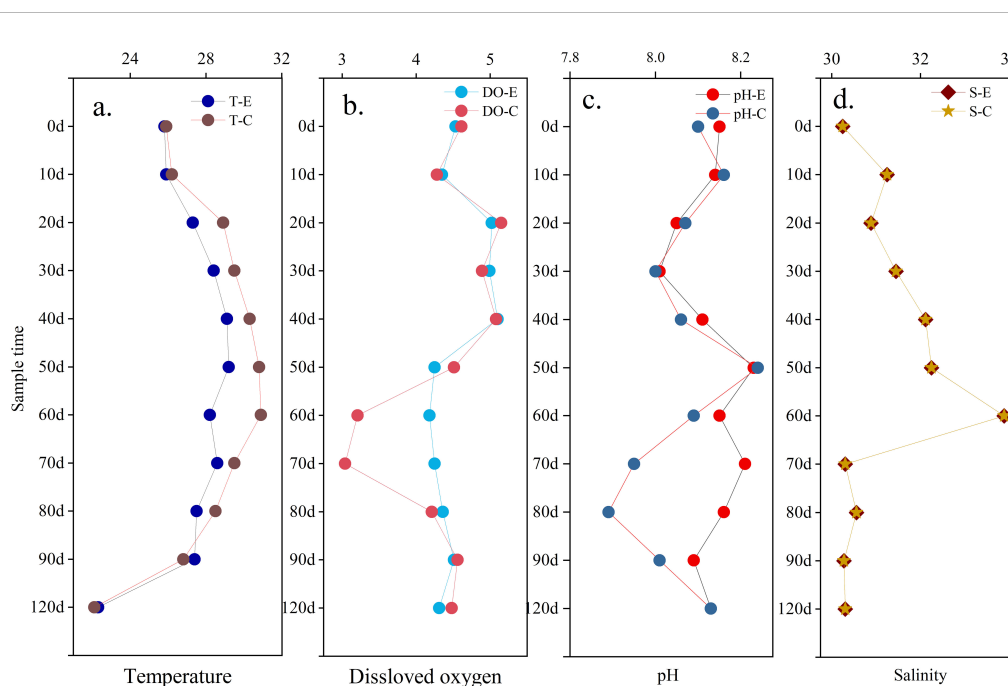


FIGURE 3
Change trend of basic biogeochemical parameters in ponds with or without *Sesuvium portulacastrum*.

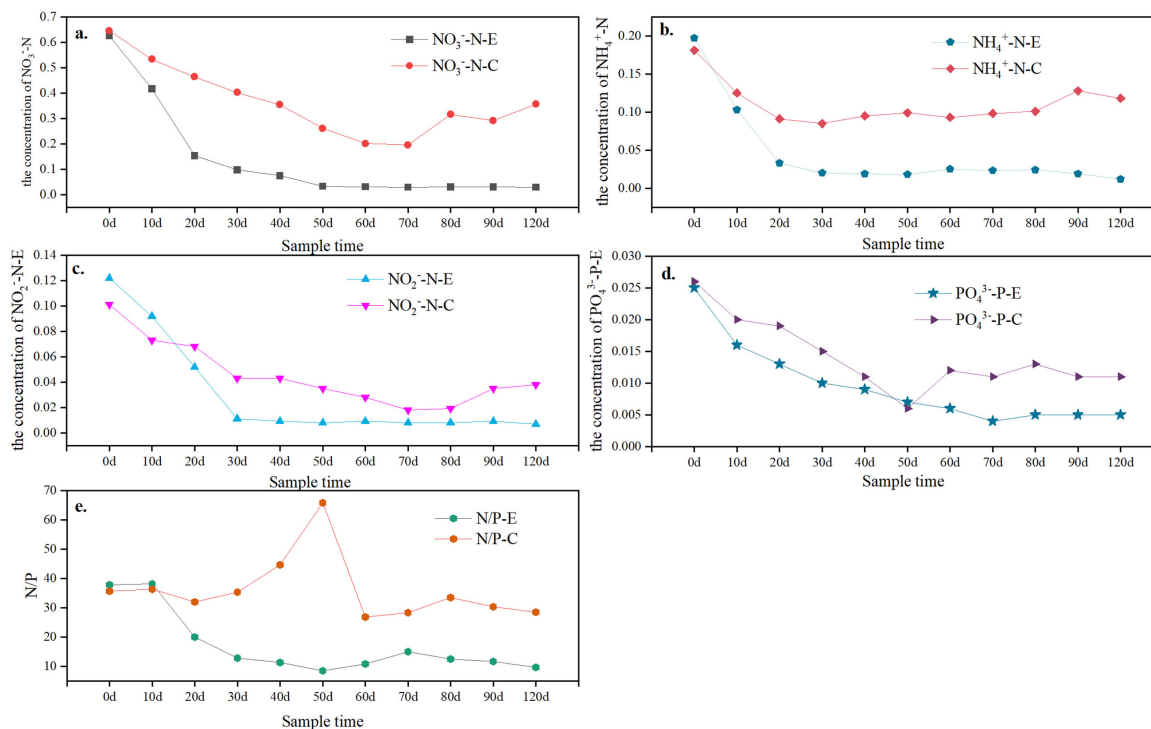


FIGURE 4

Concentration trend of dissolved inorganic nitrogen in the culture system with or without *Sesuvium portulacastrum*: (A) NO_3^- -N variation; (B) NO_2^- -N variation; (C) NH_4^+ -N variation; (D) PO_4^{3-} -P variation; (E) N/P ration variation. X-E represent the pond with *Sesuvium portulacastrum*; X-C represent the pond without *Sesuvium portulacastrum*.

concentration was observed (about $0.356 \pm 0.011 \text{ mg L}^{-1}$) in October. However, the NO_3^- -N in the *Sesuvium portulacastrum* pond was lower and more stable in the same period than the control pond. The elimination rate of NO_3^- -N was 95.22% in the *Sesuvium portulacastrum* system but it was 42.56% in the control pond.

The variation of NO_2^- -N was similar to NO_3^- -N but more drastic in the culture system. Figure 4B shows the changes in NO_2^- -N in the culture system with or without *Sesuvium portulacastrum*. A rapid decline stage was found from 0 to 30d in the *Sesuvium portulacastrum* system about $0.029 \pm 0.003 \text{ mg L}^{-1}$ (Figure 4B). However, in the control pond, this decline continued to 60d at a concentration amplitude of $0.019 \pm 0.006 \text{ mg L}^{-1}$. There was no increase in NO_2^- -N in the *Sesuvium portulacastrum* pond during September and October. However, for the control ponds, NO_2^- -N showed a significant increase and fluctuation. About 91.32% of the NO_2^- -N was consumed in the *Sesuvium portulacastrum* system in the rapid decline stage (0-30d). However, the removal rate was 81.67% in the control pond.

The change trend of NH_4^+ -N in the culture system was different to that of DIN in other forms. Figure 4C shows the trend of NH_4^+ -N in the culture system with or without *Sesuvium portulacastrum*. In the *Sesuvium portulacastrum* system, NH_4^+ -N showed a very rapid decrease from 0 to 20d, and about 83.21% was removed. After this stage, NH_4^+ -N showed a slow removal rate of only 8-10% from 20d to 40d. After 40d, the NH_4^+ -N stably fluctuated around $0.19 \pm 0.07 \text{ mg L}^{-1}$ in the experimental pond. In the control pond, NH_4^+ -N also

showed a relatively intense decline, about 50% in 20d. However, the concentration of NH_4^+ -N stably fluctuated around $0.10 \pm 0.05 \text{ mg L}^{-1}$ with the culture process.

Reactive phosphate levels in pond water were consistently low (Figure 4D). At the beginning of the experiment, the phosphate concentrations in the two ponds were 0.025 mg L^{-1} and 0.026 mg L^{-1} , respectively. In the *Sesuvium portulacastrum* ponds, reactive phosphate had a rapid declining phase from 0 to 30d. It then gradually decreased to minimum of 0.005 mg L^{-1} in 30-70d stage. After 70d, the phosphate concentration in the ponds fluctuated around 0.005 mg L^{-1} . In the control ponds, different trends of changes in phosphate concentration were observed. A sharp decline in phosphate occurred during the 0-50d period, although most of the decline was lower than in the experimental ponds. At 50d, the phosphate concentration reached minimum concentration (about 0.006 mg L^{-1}). However, after 50 days, there was a significant increase in phosphate concentration in the control pond. It eventually rose to 0.18 mg L^{-1} . This trend was similar to the changes in DIN in the control system. The N/P ratio was also expressed extremely varied in the ponds with the culture (Figure 4E). In the control ponds, the N/P ratio was changed from 26.8 to 65.8 and maximum value was observed in 40d to 50d. It was not found a clear change trend in the N/P ratio. However, in the experimental ponds, the N/P ratio decreased with the culture time. Furthermore, after 30d, the N/P was maintained in 11.5 ± 1.9 . Moreover, the N/P ratio in the experimental pond was lower than in the control pond.

3.3 Eutrophication risk

The ICEP index indicated the eutrophication risk of the water in *Holothuria scabra* ponds and could be used to measure the impact of *Holothuria scabra* culture tailwater on the offshore environment. The maximum eutrophication index of DIN (about 4.63) in the *Sesuvium portulacastrum* pond appeared at the beginning of the experiment (Figure 5). With the culture process, the ICEP gradually decreased until the index became negative after up to 50 d in the experimental pond. It is notably indicated that the eutrophication risk of the experimental ponds disappeared. In the control ponds, the change trend of ICEP was in another way. During the 0–60d, the ICEP changed similarly to the experimental ponds, which gradually decreased but the decreased amplitude was smaller. However, a significant increase of the ICEP about 6.43 was observed in the control ponds at 70d (Figure 5). And the ICEP then decreased and finally stabilized at about 2.4. In the whole experiment process, the ICEP of the control ponds was higher than that in the experimental ponds. These results suggest that *Sesuvium portulacastrum* plays an important role in controlling eutrophication risk in *Holothuria scabra* ponds. The ICEP index for phosphate was consistently negative in both the experimental and control ponds. This result clarified that the *Holothuria scabra* ponds are phosphate-limited in the Yellow River Delta. It is noteworthy that ICEP index for phosphate fluctuated less in the experimental ponds than in the control ponds. It also indicates that *Sesuvium portulacastrum* plays an important role in phosphate stabilization in *Holothuria scabra* pond like the control behavior in DIN.

3.4 Benthos community

The acquisition of benthic species in different ponds was compared at 0d, 30d, 60d, and 90d (Table 1 and Figure 6). In the control pond, only two benthic species were observed, which were mainly dominated by *Capitella capitata* and with a small number

of *Minicoraphium insidiosum*. The composition of benthic species was not change in this pond and the biodiversity parameters at low level. The Shannon–Wiener diversity index (H') was about 0.13–0.16, the Pielou evenness index (J) was about 0.24–0.26, and the Margalef species abundance index (D) was about 0.19–0.22. It indicated that the control pond was undergoing persistently eutrophication. In the *Sesuvium portulacastrum* pond, the benthic species was changed with the experiment process. The benthic species was consistent to the control pond in the preliminary stage of the experiment, dominated by *Capitella capitata* and small number of *Minicoraphium insidiosum*. However, as the experiment continued, the number of species increased to 3 and *Capitella capitata* was about 45.23% lower than in the control pond in 30d. The Shannon–Wiener diversity index (H') increased from 0.17 to 1.06, the Pielou evenness index (J) from 0.24 to 0.96, and the Margalef species abundance index (D) from 0.21 to 0.46. The change of benthic organisms in the *Sesuvium portulacastrum* pond was shown in Figure 6. Significant variations in species structure occurred during the 60 d. Although the number of species was decreased from 3 to 2, the *Capitella capitata*, a species indicative of eutrophication, decreased from 38.79% to 0% (Silva et al., 2017). *Capitella capitata* was replaced by the *Perinereis aibuhitensis* and *Pseudopolydora paucibranchiata*. Over the 90d, the number of species increased to 5 and some shellfish such as *Theora lata* appeared.

3.5 *Holothuria scabra* seedling

At 80 d, five *Holothuria scabra* fishfly cages were randomly selected for weighing in the experiment and control ponds, respectively. This result would best reflect the role of the *Sesuvium portulacastrum* system in protection of *Holothuria scabra* seedlings because the high temperature period has just

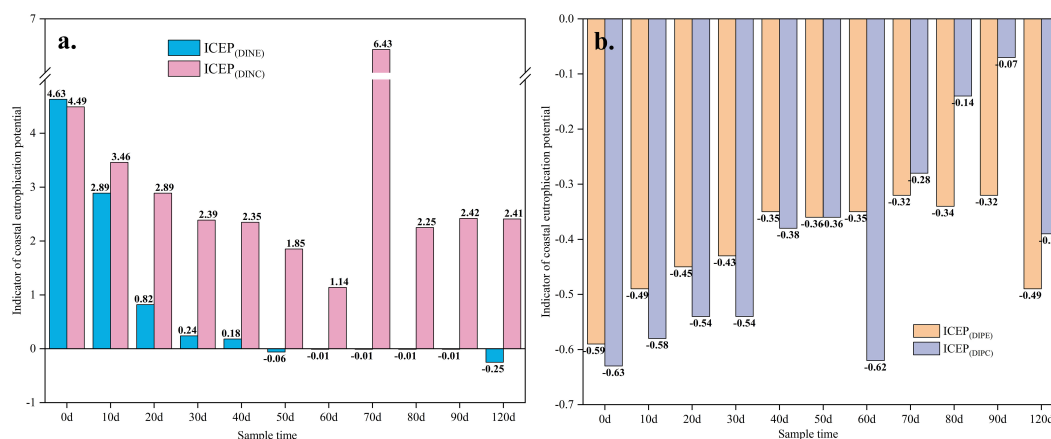


FIGURE 5

Indicator of coastal eutrophication potential in the culture system with or without *Sesuvium portulacastrum*. (A) ICEP_(DINE) and ICEP_(DINC) are the indicators of coastal eutrophication potential of dissolved inorganic nitrogen in the *Sesuvium portulacastrum* and control systems, respectively; (B) ICEP_(DIPE) and ICEP_(DIPC) are the indicators of coastal eutrophication potential of active phosphate in the *Sesuvium portulacastrum* and control systems, respectively.

TABLE 1 Structural parameters of benthic organisms in the *Sesuvium portulacastrum* pond and control pond at different times.

Sample time (d)	Species		Shannon–Wiener diversity index (H')		Pielou evenness index (J)		Margaief species abundance index (D)		Simpson dominance index (W)	
0	2 ^c	2 ^e	0.13 ^c	0.17 ^e	0.24 ^c	0.24 ^e	0.22 ^c	0.21 ^e	1.00 ^c	1.00 ^e
30	2 ^c	3 ^e	0.16 ^c	1.06 ^e	0.24 ^c	0.96 ^e	0.19 ^c	0.46 ^e	1.00 ^c	0.98 ^e
60	2 ^c	2 ^e	0.15 ^c	0.28 ^e	0.25 ^c	0.26 ^e	0.21 ^c	0.20 ^e	1.00 ^c	1.00 ^e
90	2 ^c	5 ^e	0.16 ^c	2.04 ^e	0.26 ^c	1.35 ^e	0.20 ^c	0.92 ^e	1.00 ^c	0.35 ^e

c is control pond; e is *Sesuvium portulacastrum* pond.

passed. The survival rate was calculated according to the seedling casting of 0.25 kg and the specification of 11,000 heads/kg. The survival rate results are shown in Table 2. In the experiment pond, the average harvest was 2.313 kg, with a single weight of 1.732 g ins⁻¹ and a survival rate of 61.45%. In the control pond, the average harvest was 1.762 kg, with an average weight of 1.598 g ins⁻¹ and a survival rate of 40.29%. The average harvest of the experimental pond was increased by 0.551 kg, the average weight of every seedling was increased by 0.078 g, and the survival rate was increased by 21.16% compared with the control pond.

4 Discussion

From the experiment results, the *Sesuvium portulacastrum* system in the *Holothuria scabra* ponds expressed positive influences in reducing pond eutrophication, increasing pond ecological stability, and conserving *Holothuria scabra* seedlings under high temperatures in summer and early autumn. The *Sesuvium portulacastrum* might be the key factor to induce those positive influences in the mariculture pond system. Figure 7 shows the potential ecological cycle built by *Sesuvium portulacastrum*.

4.1 For the dissolved inorganic nitrogen

In the begin of the experiment, the initially high concentration of NO₃⁻N reflected the transformation of NH₄⁺-N and NO₂⁻N by

microorganisms in the pond water (Diab et al., 1993). NO₃⁻N is the most stable state of elemental N and was more conservative removal compared to other forms (NH₄⁺-N and NO₂⁻N) in the seawater. It was mainly accomplished through the uptake behavior of algae and vegetations in the pond water (Pereira et al., 2020). Generally, the higher concentration of NO₃⁻N the faster uptake of *Sesuvium portulacastrum* in the pond water (Hao et al., 2024). When the NO₃⁻N in the system declines to certain level, the uptake of this form will gradually become slower (Pereira et al., 2020; Hao et al., 2024). However, when the NO₃⁻N is gradually depleted, the concentration balance between the sediment-water was broken induced the release of NO₃⁻N from sediment to water (Khoi et al., 2006). This release process is mainly dominated by the microbial activities in the sediment (Zhang et al., 2015). Under the strong adsorption and uptake of the *Sesuvium portulacastrum* system, the released nitrate will be rapidly consumed (Peng et al., 2022). NO₃⁻N in the pond will eventually reach the equilibrium with a relatively low concentration. In addition, epiphytic algae appeared on the roots of *Sesuvium portulacastrum* in the late stage of the experiment. This indicated that not only the *Sesuvium portulacastrum* but also the epiphytic environment created by the root system have an important influence on the NO₃⁻N removal in the system (Dou et al., 2011; Liu et al., 2019). Hence, *Sesuvium portulacastrum* play powerful absorber for NO₃⁻N in the mariculture pond because this form of nitrogen was easily and rapidly uptake by vegetations (Cai et al., 2022).

As an important intermediate state of nitrification in the *Sesuvium portulacastrum* pond system, the concentration of NO₂⁻N reflects the influence of the whole system on the cycling of

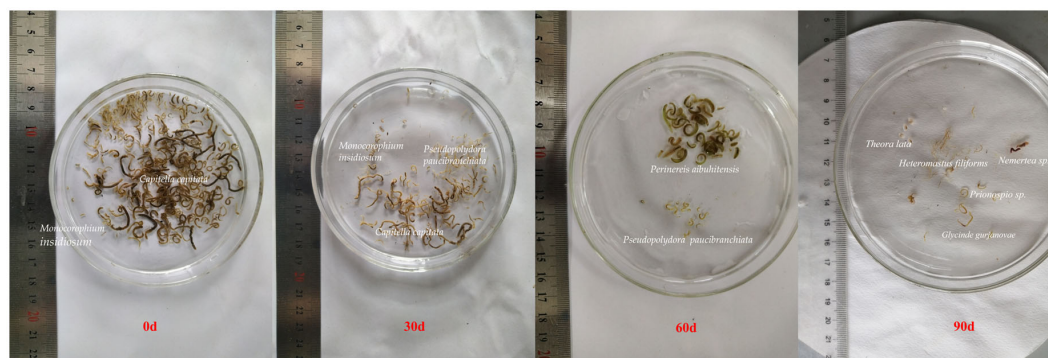


FIGURE 6 Change trend of benthic organisms in the *Sesuvium portulacastrum* pond.

TABLE 2 The total biomass, individual weight, and survival rate in the different ponds.

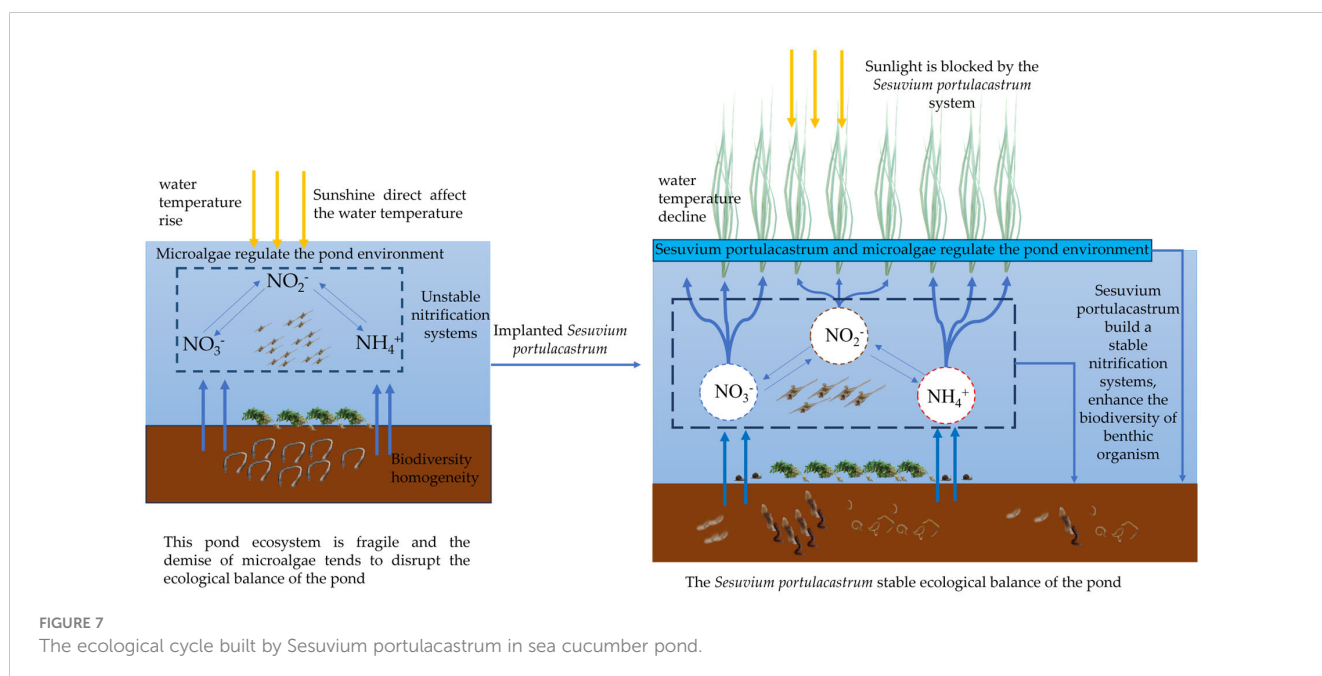
Pond	total biomass (kg)	individual weight (g/ins)	survival rate (%)
Experiment pond	2.313 ± 0.056	1.676 ± 0.155	61.45 ± 1.36
Control pond	1.762 ± 0.085	1.598 ± 0.187	40.17 ± 1.50

elemental N (Beman et al., 2013). The concentration of $\text{NO}_2^- \text{N}$ was in the rapid decline stage (0-20d) when the dissolved oxygen increased in the pond water. High dissolved oxygen would promote more active microbial activity, which lead to the conversion of $\text{NO}_2^- \text{N}$ to stable $\text{NO}_3^- \text{N}$ and N_2 (Rosamond et al., 2012). However, in this study, the decline in $\text{NO}_2^- \text{N}$ was smaller in the experimental pond than that in the control pond. This might be the *Sesuvium portulacastrum* system consumed a certain amount of dissolved oxygen during the early growth stages of this plants. It results in partial hypoxia in the pond where the *Sesuvium portulacastrum* are deployed which could reduce the level of nitrification and denitrification (Ni et al., 2021). After 20d of the experiment, the level of $\text{NO}_2^- \text{N}$ in the *Sesuvium portulacastrum* system decreased sharply. The nitrification system in the *Sesuvium portulacastrum* system was being constructed, resulting in a low level of intermediate nitrogen conversion in the system (Dou et al., 2011; Liu et al., 2019). Meanwhile, the damage of plant root by transplant during the construction of the *Sesuvium portulacastrum* system also caused the release and formation of nitrite in the pond water. When the construction of the microbial nitrification system was completed, the $\text{NO}_2^- \text{N}$ was rapidly removed. With the culture process, the free $\text{NO}_2^- \text{N}$ was transformed into a complete form in the pond water, and only a small amount was left to decompose continuously. This stable nitrification system would also

continuously eliminate $\text{NH}_4^+ \text{-N}$ in the water, which was rapidly converted to $\text{NO}_3^- \text{N}$ and $\text{NO}_2^- \text{N}$ under sufficient oxygen (Dou et al., 2011). In addition, the *Sesuvium portulacastrum* would directly utilize the ammonium nitrogen in the water as a nutrient source for plant growth. The *Sesuvium portulacastrum* system not only an absorber of the $\text{NH}_4^+ \text{-N}$ but also comprises a healthy nitrification system to maintain the cycling between different forms of DIN in the pond. This stable cycle would make the changes in nitrate nitrogen, nitrite nitrogen, and ammonia nitrogen leveled off.

4.2 For the microalgae

The *Sesuvium portulacastrum* system also maintained the microalgae balance in the pond. During the 60d-70d period, abnormal changes in dissolved oxygen, pH, and DIN occurred in the control pond. We believe that this phenomenon is mainly related to the massive death of microalgae in the *Holothuria scabra* ponds, i.e., algal inversion. Microalgae blooms easily induced a sharp decrease of nutrients in the pond by rapid multiplication uptake amount of nutrients (Bell et al., 2015). However, disordered proliferation of microalgae was not stable and often exceeded the survival capacity of the pond which could induce the mass and rapid death of this organism (Silva et al., 2017). In addition, sudden rainstorm following persistent high temperature also induced the algal inversion in the pond (Tayaban et al., 2018). The decomposition of microalgae led to sharply decrease in dissolved oxygen and the release of nutrient. Meanwhile, the microalgae gradually entered extinction also caused decrease in DO and pH in September (Sanchis-Perucho et al., 2018). However, this phenomenon did not occur in the *Sesuvium portulacastrum* system. This might be because the nutrient uptake behavior of *Sesuvium portulacastrum* suppressed the microalgae in the pond and make them could not disorderly growth (Smith and Horne, 1988). *Sesuvium portulacastrum* could balance the



changes in pH, dissolved oxygen, and nutrients timely when partial algal collapse or algal die-off in the pond. The ecosystem became more stable and the ecological buffering capacity for the DIN pollution were stronger in the *Sesuvium portulacastrum* pond.

4.3 For the organism

The *Sesuvium portulacastrum* system played a beneficial role and made positive influence on both cultured and benthic organisms. The continuous high temperature was often fatal to *Holothuria scabra*. In general, the pond water temperature once exceeded 33°C for more than 2d, the *Holothuria scabra* cultured in the pond would extinction due to intolerance to high temperatures ($\geq 33^\circ\text{C}$) (Dong and Dong, 2009; Zhang et al., 2022). Meanwhile, the high temperature would also induce hypoxia of the pond water further leading to the death of *Holothuria scabra* (Unmuth et al., 2000; Zhang et al., 2022; Hoblyn and Iversen, 2024). *Sesuvium portulacastrum* community could suppression of high temperatures of pond water by covering water surface to reduce water temperature in the pond. The *Sesuvium portulacastrum* system diminished the water temperature by 1–2°C to enhance the *Holothuria scabra* survival rate about 21.6%. Notably, the *Sesuvium portulacastrum* system changed the organism species and structure in the pond. Aquatic vegetation cover was a significant environmental variable to influence the variance in aquatic organisms in pond sediment (Natsumeda et al., 2015). The vegetation could primarily affect the chemistry characters of pond sediment such as uptake more N and P, and then manage the evolution of ponds benthic species for the direction more species and prefer the vegetation (Sinclair et al., 2021). Hence, the *Sesuvium portulacastrum* system will induce the ponds environment more biodiversity.

In general, the strong uptake behavior of *Sesuvium portulacastrum* diminished the DIN in the mariculture pond, which created nutrient deficit between surface waters. This deficit induced the release of DIN, DIP, and other eutrophication factors from sediment (Wu et al., 2021). Meanwhile, it also increased the decomposition of organic debris in sediments. Changes in sediment composition would cause variations in benthos species structure (Posey et al., 1993; Ni et al., 2021). In mariculture pond, the benthic organisms in pond sediment were often single and mostly represented by eutrophication indicator species such as *Capitella capitata*. The establishment of the *Sesuvium portulacastrum* system led to a succession of benthic organisms in the mariculture pond (Kaenel et al., 1998). For example, the replacement of the *Capitella capitata* by the *Perinereis aibuhitensis* in the pond sediment indicated more stable and health pond depositional environment with the *Sesuvium portulacastrum*.

5 Conclusions

The *Sesuvium portulacastrum* system established in the *Holothuria scabra* ponds demonstrated a very positive impact on the pond and around environment. It reduced the NH_4^+ , NO_3^- , and

NO_2^- in the pond water by 83.21%, 95.22%, and 91.32%, respectively. Active phosphate was suppressed to low levels ($<0.005 \text{ mg L}^{-1}$) in the pond water. The number of pond benthic organism species was enhanced from 2 to 5. *Capitella capitata*, the eutrophication indicator organism, was disappeared and *Perinereis aibuhitensis* appeared. These results suggest that the *Sesuvium portulacastrum* could both reduce DIN pollutants in *Holothuria scabra* culture pond and regulate the pond environment. However, studies on the effects of *Sesuvium portulacastrum* and other aquatic vegetations on the stability of mariculture pond ecosystems are still few and focused on nutrient depletion. Its effects on the microbial, microalgal, and elemental geochemical cycles and biodiversity in mariculture ponds still need to be further investigated. In addition, the wetland function of the mariculture pond around the coastal area should be taken seriously in future.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

KL: Writing – original draft, Writing – review & editing, Funding acquisition, Methodology, Software. WG: Formal analysis, Investigation, Visualization, Writing – review & editing. ZY: Investigation, Validation, Writing – review & editing. YH: Formal analysis, Investigation, Writing – review & editing. MZ: Formal analysis, Investigation, Writing – review & editing. CS: Investigation, Writing – review & editing. XZ: Investigation, Writing – review & editing. LW: Writing – original draft, Writing – review & editing, Conceptualization.

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Conflict of interest

Author XZ was employed by the company Dongying Haimu Agricultural Technology Co., Ltd.

The remaining 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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