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Original Research

The Elimination of Nitrogen by Oysters in *Litopenaeus Vannamei* Mariculture Ponds

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Abstract

Mariculture of white leg shrimp, *Litopenaeus vannamei*, provides a crucial protein source for humans. Aquaculture of this species also generates significant nitrogen (N) pollution, which can trigger harmful algal blooms in coastal environments. Although mariculture typically has low profitability, it is essential to establish ecologically and economically sound ways to minimize the release of N into the environment. Oyster polyculture within *L. vannamei* ponds presents a promising approach for reducing the release of N because oysters have a strong capacity to filter N from the environment while producing a marketable commodity. This study investigated spatiotemporal variations in dissolved N forms (NH_4^+, NO_2^-, NO_3^-, and total N) in shrimp pond water with and without oyster co-cultivation. The results demonstrated that the oyster-integrated pond exhibited significantly lower N pollutant concentrations when compared with the control pond. Specifically, NH_4^+, NO_2^-, NO_3^-, and total nitrogen content decreased by 39.8%, 52.6%, 27.3%, and 51.4%, respectively. Notably, these reductions lowered N concentrations below local regulatory thresholds for the discharge of mariculture wastewater. These findings highlight the potential for the use of oyster polyculture as an effective and dual-purpose method that can be used to mitigate N pollution while maintaining shrimp production yields for *L. vannamei* mariculture.

Keywords: mariculture, dissolved inorganic nitrogen, pollution, oyster, N elimination

Introduction

Mariculture serves as an important production method that allows people to obtain protein-rich marine food. With the increasing demand for protein from the sea, the output of marine aquaculture products in China has increased annually [1]. In 2024, marine production operations in China harvested 37.09 million tons of marine products, the largest marine aquaculture operation of any country worldwide [2]. About 2.24 million tons of mariculture products were produced from white leg shrimp (*Litopenaeus vannamei*) in China, representing about 21% of total world shrimp production [3]. The rapid development of mariculture has supplied large amounts of quality protein, which has improved the livelihood of the people in China. However, nitrogen (N) pollution from the process of mariculture is responsible for inducing harmful algal

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blooms in the surrounding coastal area, a fact that should not be ignored [4, 5].

In China, *L. vannamei* is often grown in intensive mariculture systems. Because cultivation space is limited, large amounts of feed are used to maximize the production of *L. vannamei*. This results in unconsumed feed being converted into contaminants [6]. Because N in feed is the main source of these contaminants, it has been shown that only about 20% of the added feed is consumed by shrimp. Most of the N (feed) that is present in the water comes in organic or inorganic forms or accumulates in pond sediments [7]. In addition, this accumulated N in the bottom sediments of aquaculture ponds returns to the water via continuous water agitation that is used to supply sufficient oxygen to shrimp [8]. Total nitrogen (TN) discharges from *L. vannamei* aquaculture ponds are much higher than tailwater discharge standards allow [9-11]. The direct discharge of untreated aquaculture water into the ocean may cause pollution and eutrophication of surrounding water bodies [12]. Therefore, a cost-effective water treatment method is urgently needed to address N pollution in aquaculture waters. When compared with physical and chemical approaches, biological tailwater treatment methods offer advantages such as low cost, long-term effectiveness, and the production of no secondary pollution [13-15]. This provides more effective approaches for treating eutrophic water during *L. vannamei* mariculture.

The use of specific organisms for treating mariculture wastewater has rarely been reported. It has been demonstrated that mussels at an appropriate density can effectively control the concentrations of nitrite, ammonia, and other harmful substances in pond water [16, 17]. In addition, shellfish possess a very strong ability to filter-feed on microalgae and particles (5-100 μm) commonly found in mariculture water [18, 19]. In summary, using shellfish to remediate N pollution in areas with *L. vannamei* mariculture may be a workable approach due to their strong filter-feeding capacity. However, it is first necessary to clarify how oysters filter these pollutants. The use of bivalve shellfish to remediate mariculture systems, especially in *L. vannamei* culture, has rarely been reported. This may be the result of the strict culture and tailwater conditions needed for *L. vannamei* mariculture, which are unfavorable for the survival of some shellfish [20]. Intensive *L. vannamei* mariculture conditions have resulted in large amounts of feed being deposited at the bottom of culture or tailwater ponds, leading to a highly hypoxic environment [21]. This limits the potential for demersal bivalve shellfish such as *Ruditapes philippinarum*, *Mactra veneriformis*, and *Scapharca broughtonii* to remediate the tailwaters of areas with *L. vannamei* mariculture. In contrast to demersal bivalve shellfish, oysters exhibit an adherent growth habit, which can eliminate the harmful effects of sedimentation during mariculture on their growth. They also have a strong N purification capacity for seawater, along with high environmental adaptability and economic value.

This study employed oysters (*Ostrea rivularis*) as the bioremediation species to eliminate N pollution in *L. vannamei* mariculture ponds. Juvenile oysters (attached to scallop shells, about 0.2-0.7 cm/ind.) were maintained, suspended in pond water, and cultivated for 4 months. The concentrations of TN and dissolved inorganic nitrogen (DIN, including NH_4^+, NO_2^-, and NO_3^-) in pond water were monitored. Furthermore, the relationship between oyster growth and the changing trends of the concentrations of different N forms in pond water was also monitored.

Materials and Methods

Oyster Polyculture in an *L. Vannamei* Production System

An oyster (*O. rivularis*) and *L. vannamei* polyculture system was established in a 6,000 m^2 long pond (water depth: 2 m) located at 37°52’17.031”N, 119°06’09.042”E in the Yellow River Delta. Concrete-anchored columns hung from buoys served as the structural anchors for the polyculture system (Fig. 1). Each column comprised two functional components: Part A (a stainless-steel bracket used to secure oyster seedling ropes) and Part B (the primary fiberglass-reinforced polymer column body) (Fig. 1). The experimental process proceeded as follows. Concrete columns for oyster deployment were installed along one long side of the pond, with an identical set symmetrically installed along the opposite side. Primary support ropes (about 33 m long) of the oyster culture structure were then stretched horizontally between opposing columns. Oyster seedling ropes, each tied to a scallop shell, were hung from these primary ropes and secured using stainless-steel cross buckles. A total of 100 spat ropes (60,000 spat in total, at a density of 10 ind./m^2) were evenly spaced along the primary support ropes. To counteract the gradual sinking caused by oyster growth and accumulated biomass, high-density polyethylene floats were later fastened to the main support ropes. The oyster spats (0.1-0.5 cm in size) were sourced from a commercial hatchery (Rongcheng Chenglong Oyster Farming Co., Ltd.) in Rongcheng City, Weihai, China (37°10′31.259″N, 122°34′35.567″E) and deployed on May 24, 2024 (Fig. 1). The control pond, identical in size and adjacent to the experimental unit, shared the same hydrological conditions. The experiment was conducted from May 24 to September 7, 2024, with both ponds managed under consistent protocols, including synchronized feeding schedules and equivalent feed-to-biomass ratios calibrated to maintain uniform *L. vannamei* densities.

Sampling

To resolve potential contamination during pond sampling, a previously published clean sampling system was adopted [22]. A pickup-truck-mounted bucket was

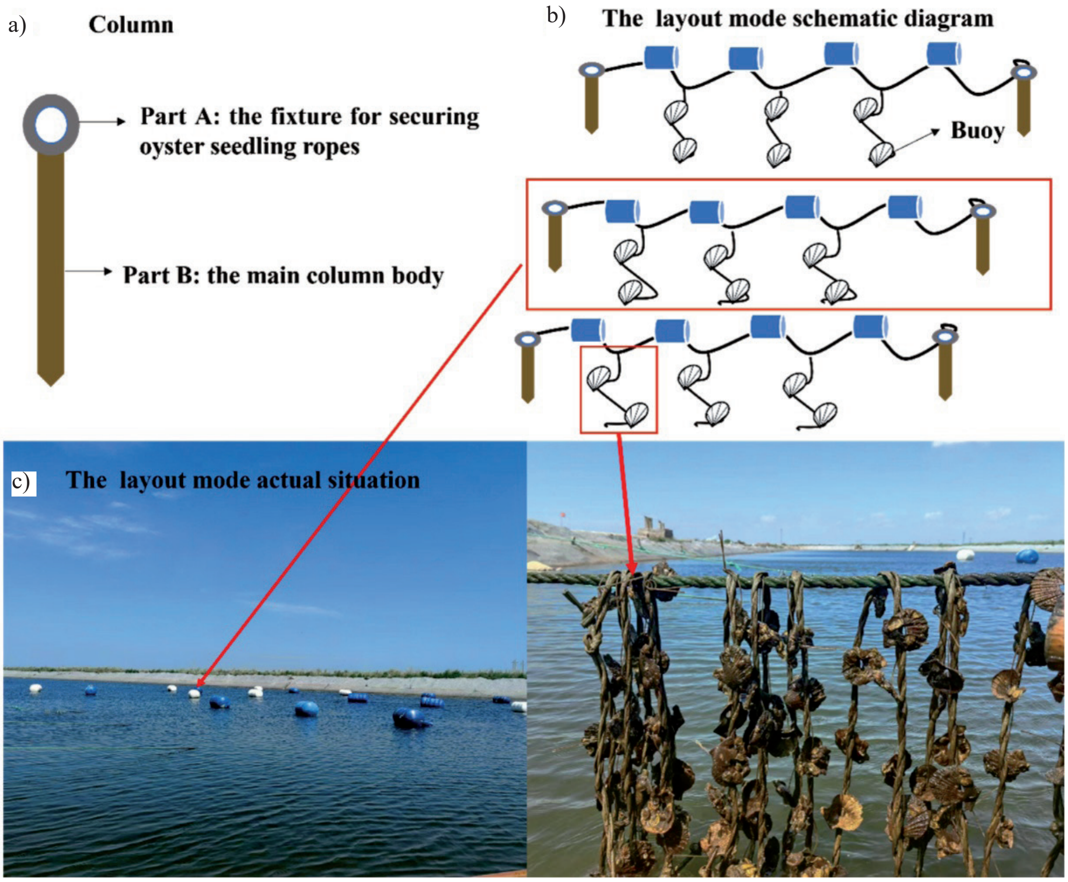

Fig. 1. Layout model of the polyculture system used for oysters in a *Litopenaeus vannamei* mariculture pond: a) hanging concrete column used to fix oyster culture ropes; b) schematic diagram of the grid layout of column bodies; c) photos of the grid layout pattern and oyster seedling ropes.

designed as the cleaning device; the sampling process is described in brief below (Fig. 2). For sampling, a sampling tube with a water outlet directly into the pond surface water was inserted at a depth of about 20 cm. A filtration pump facilitated sample collection, with pre-cleaned polyethylene bottles serving as sample containers. All bottles and connecting tubes underwent a rigorous cleaning workflow prior to use: triple rinsing with 10% Decon 90® detergent, followed by five rinses with deionized water. Containers were then sequentially soaked in 1% HCl (v/v) and 1% HNO_3 (v/v) for 48 h, rinsed more than 5 times with ultrapure water (>18.2 MΩ·cm), and dried in a Class-100 ultra-clean bench. After drying, the bottles and tubes were double-bagged in self-sealing high-density polyethylene bags to prevent contamination. Temperature, salinity, and dissolved oxygen content were measured using a multi-parameter water quality analyzer (YSI, ProQuatro, Yellow Springs, Ohio, USA); the accuracies of these parameters were ±0.05ºC (temperature), ±0.01 (salinity), and ±0.5 μmol L^{-1} (dissolved oxygen). Surface pond water samples were collected at three key culture stages: the early culture stage (May 24), the middle culture stage (June 12, June 25, July 11, and July 23), and the end culture stage (from August 7 to September 7).

Determination of N Content

The concentration of N, including TN, NO_3^-, NO_2^-, and NH_4^+, was measured using a continuous flow analyzer (QuAAtro, SEAL Analytical GmbH, Hamburg, Germany), with a detection limit of 0.02 μmol. During the sample testing, a standard solution of artificial seawater (provided by the Second Institute of Oceanography of the Ministry of Natural Resources of the People's Republic of China, Hangzhou, China) was used for the quality control. The relative standard deviations between the parallel samples were <5%.

Results and Discussion

Change Trend of TN in Different Ponds

In the oyster-free *L. vannamei* mariculture control pond, the TN concentration ranged from 1.78±0.51 to 4.73±0.56 mg L^{-1} (mean = 3.08±0.31 mg L^{-1}). Throughout the shrimp culture season, the TN concentration in the control pond showed an initial increase followed by a subsequent decrease (Fig. 3). From May 24 to July 23, the TN remained stable initially

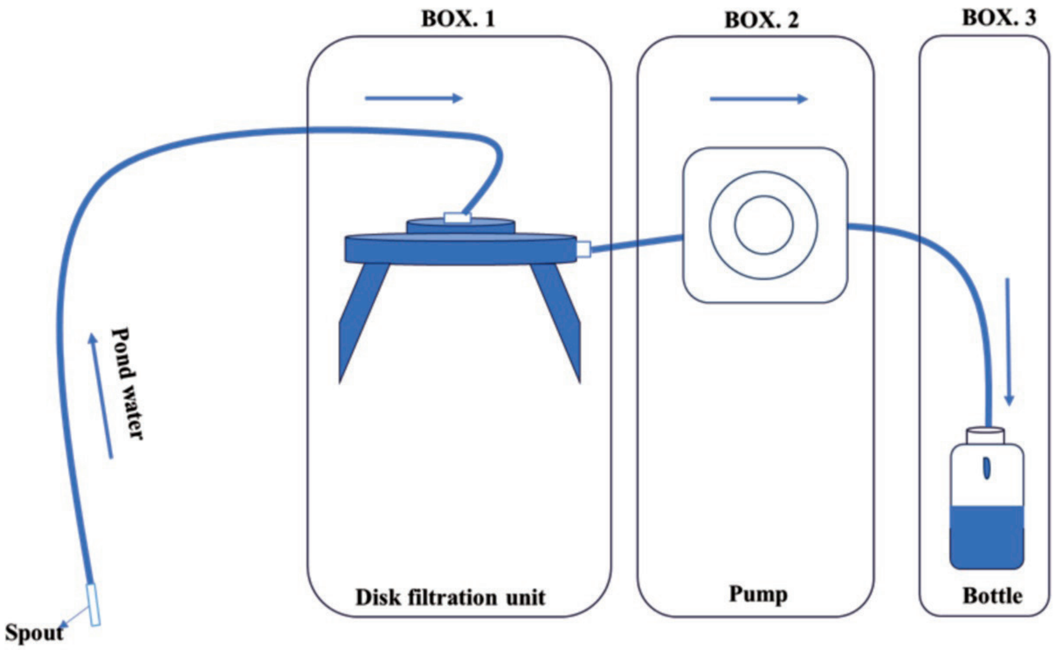

Fig. 2. Schematic diagram of the filtration unit: Box 1 shows the sampling unit consisting of a disk filtration unit (with a 40-cm-diameter filtration membrane) for treating river water containing high concentrations of a variety of suspended particulate matter; units are all connected and held in place by C-Flex tubing bottles. Box 2 shows the filtration equipment unit consisting of a vacuum filtration pump. Box 3 shows the sample collection unit consisting of the polyethylene sample collecting bottle.

before rising sharply; by July 23, it had increased by 147.6% compared to early culture levels, which was the highest concentration observed during the study period. Furthermore, this parameter decreased to about 68% of the peak concentration observed on August 7 (Fig. 3). Subsequently, TN in the control pond stabilized, with minor fluctuations. This phenomenon might be related to the growth and feed conversion ratio (FCR) of *L. vannamei*. During June–July (the peak growth period for *L. vannamei*), aquaculturists typically increase feed input to promote shrimp growth. However, data from the Food and Agriculture Organization of the United Nations show that global *L. vannamei* aquaculture has an average FCR of 1.2-1.5, meaning 16.7-33.3% of feed remains unconsumed in production systems [23]. Concurrently, the expansion of shrimp biomass increases the release of metabolic byproducts into the environment, leading to the accumulation of N pollutants in pond water [24]. By September, when the weight of *L. vannamei* exceeded 11 g/ind., the FCR fell below 1.0, with improved feed assimilation in mature shrimp [25]. This metabolic shift is driven by the growth of individuals, and it minimizes residual feed accumulation in aquaculture systems, leading to measurable declines in TN in seawater. To clarify the consistent concentration of TN observed in the control pond, the DIN concentration in pond water was also investigated. Dissolved inorganic nitrogen concentrations ranged from 0.25 mg L^{-1} to 0.61 mg L^{-1}, which accounted for only 9.6–13.9% of TN. This finding indicates that DIN provides a limited contribution to TN pollution (<11% of TN) in *L. vannamei* culture ponds. Thus, the authors believe that TN contamination might be principally driven by the dissolved organic nitrogen (DON) and particulate nitrogen (PN) fractions, which accounted for over 89% of total N.

Baseline TN concentrations (Fig. 3) showed no significant differences between the oyster-integrated polyculture system and the control pond. This similarity confirms that negligible operational discrepancies may occur with different culture practices. The concentration of TN in the experimental pond was consistently lower than that in the control pond (Fig. 3). Moreover, the concentration of TN exhibited temporal dynamics similar to those of the control pond. From May 24 to June 25, TN levels showed a decrease, but a marked increase was observed from July 11 to July 23, representing a 49.1% surge (Fig. 3). This pronounced elevation may also have coincided with intensive feed inputs and the accumulation of unconsumed feed in the ponds. Subsequently, TN declined to 1.63±0.03 mg L^{-1} by September 7, representing a 37.5% reduction (Fig. 3). Based on the dynamics of TN concentration, we inferred that shrimp in both the experimental pond and the control pond underwent similar growth and feeding processes. Excluding the initial convergence, the experimental pond showed substantially lower (34.36-58.78%) TN loading relative to the control. The introduction of oysters provided an excellent TN elimination effect in the *L. vannamei* culture ponds in this study. This reduction likely stems from the filtration activity of oysters [26]. It has previously been found that oysters can filter approximately 12-120 L of seawater per

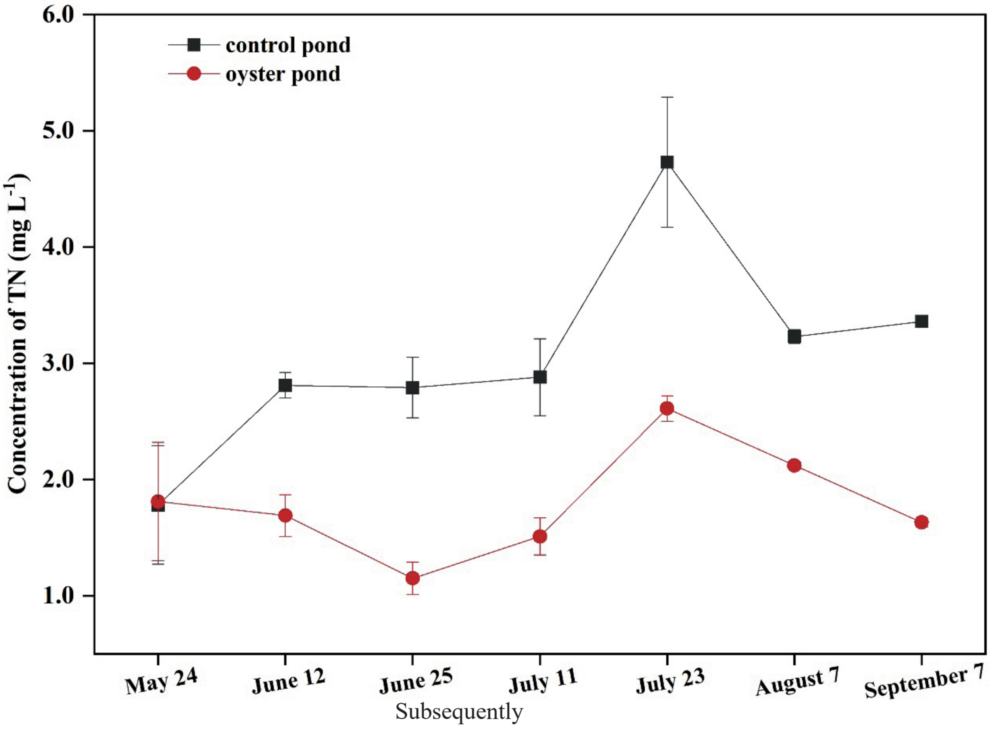

Fig. 3. Changes and trends in the concentration of TN in the control pond (black squares) and oyster pond (red dots) from May 24 to September 7, 2024.

day while removing up to 80% of suspended particulate matter (SPM) [27]. The sequestered SPM, including the unconsumed feed, phytoplankton, and other organic or inorganic matter, may have been partially assimilated by oysters through digestion or deposition as pseudofeces on pond sediments [28, 29]. Together, these mechanisms reduced the concentration of SPM and ultimately diminished the TN load in the water column.

Change Trend of DIN in Ponds

Dissolved inorganic nitrogen serves as an important and frequently monitored pollutant in marine ecosystems, where its concentration dynamics and interconversion between species govern the overall health of many aquatic ecosystems. This study tracked the three main DIN species: NH_4^+, NO_2^-, and NO_3^-. In the control pond, NH_4^+ and NO_3^- dominated DIN (combined 71.2-89.4%), while NO_2^- was a minor fraction (0.9-3.1%). Thus, NH_4^+ and NO_3^- served as the primary metrics for studying inorganic N and TN dynamics in this study. For NH_4^+, dynamics exhibited a distinct triphasic pattern: (1) Primary attenuation: Concentrations decreased from May 24 to June 12 (Fig. 4a)). (2) Subsequent enrichment: Concentrations increased from June 25 to July 11 (Fig. 4a)). (3) Eventual stabilization: Concentrations plateaued between 0.15 and 0.14 mg L^{-1} during the final monitoring period (Fig. 4a)). For NO_3^-, a gradual accumulation process was observed in the control pond. The NO_3^- concentration increased from 0.18±0.02 (May 24) to 0.76±0.1 mg L^{-1} (September 7) (Fig. 4b)). The NO_3^- dynamics showed two phases: a quasi-stable phase from May 24 to June 12, followed by a rapid enrichment phase spanning June 12-August 7. For NO_2^-, similar changes in concentration were observed. Nitrite (NO_2^-) experienced a progressive attenuation, declining from May 24 to June 11, with a subsequent rise to 0.031 mg L^{-1} (September 7) (Fig. 4c)). The NO_2^- concentration in the control pond consistently remained at diminished levels (0.002-0.031 mg L^{-1}), reflecting the stability of N cycling under standardized husbandry protocols. The level of NO_3^- enrichment suggested either a metabolic release of NO_3^- during the shrimp growth period or a nitrification-mediated conversion of $NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$. Meanwhile, NH_4^+ and NO_3^- from the shrimp mariculture were documented as major pollutants driving coastal N loading, which is an important contributor to the degradation of water quality (e.g., eutrophication) [30]. Effective strategies will be needed to sustain the development of mariculture [31]. Despite this, limited methods exist that can be used to degrade inorganic N pollutants in mariculture, although research on this type of mitigation strategy remains sparse.

In oyster-integrated systems, scientists typically monitor the constituents of DIN, specifically NH_4^+, NO_2^-, and NO_3^-. In this study, the NO_3^- concentration was significantly lower than that in the control pond. Except for the initial concentration, which was largely comparable or even slightly higher (only 0.01 mg L^{-1}), NO_3^- concentrations were consistently lower in the treatment area than in the control pond at all sampling

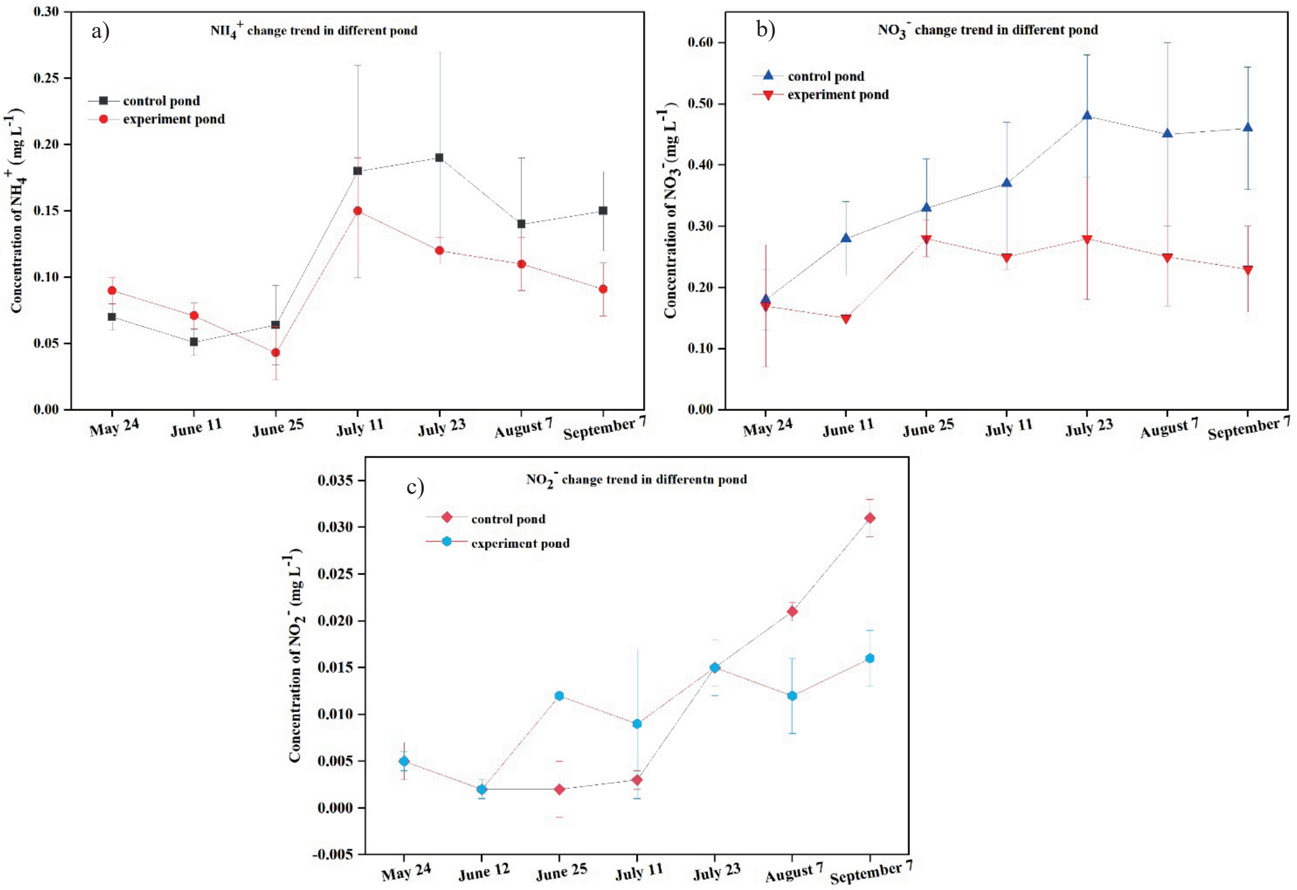

Fig. 4. Trends in the concentration change of dissolved inorganic nitrogen in the control and experimental ponds for: a) NH_4^+, b) NO_3^-, and c) NO_2^- from May 24 to September 7, 2024.

times. The reduction ranged from 5.53% to 57.89%, demonstrating the efficacy of oysters in removing nitrate from the pond water column.

During the growth phase, oysters exhibited a strong demand for nitrate N. This process might be primarily mediated through filter-feeding activities and denitrification occurring on the shell surface, which promotes the conversion of nitrate N in pond systems into N_2O [32]. The removal of NH_4^+ by oysters remained at a significant level throughout the study. It is worth noting that a slight increase in ammonia N levels was observed during the initial culture phase. In the early cultivation period (from May 24 to June 11), the concentration of ammonia N in the experimental pond increased, which was about 32% higher than that in the control pond during the same period (Fig. 4). This spike was probably the result of oyster growth and metabolism; at this early stage, low biomass and filtration capacity limited NH_4^+ removal rates below the pace of metabolic generation [33]. Consequently, oysters had a modest impact on the overall levels of pollutants in the pond, constrained by their limited filtration activity.

The use of oysters in the experimental system might alter the concentration dynamics for NO_2^-. No significant increase in nitrite N was observed in the initial phase of aquaculture. Throughout the experiment, NO_2^- in the experimental pond remained consistently lower than that in the control pond, with reductions of 42.89-60.81%. This decrease may be attributed to enhanced nitrification and denitrification processes mediated by oysters and their shell structures as follows [34]: the ridged morphology of oyster shells promotes microbial cycling in the water column, thereby intensifying both nitrification and denitrification activities [35]. This helped to maintain the intermediate inorganic N species (NO_2^-) at a higher consumption rate.

Implications for Controlling Total N Pollution in *L. Vannamei* Mariculture Ponds

Nitrogen contamination in shrimp aquaculture ponds primarily originates from three potential sources: DIN, PN capable of transforming into both organic or inorganic forms, and DON. In *L. vannamei* ponds, monitoring TN is critical because the three forms of N comprising TN ultimately contribute to the bioavailability of N in pools through processes such as sedimentation, suspension, nitrification, and decomposition. These bioavailable species often drive eutrophication and cause biological toxicity in aquatic systems. Furthermore, their decomposition processes often induce hypoxia, ultimately leading to mortality

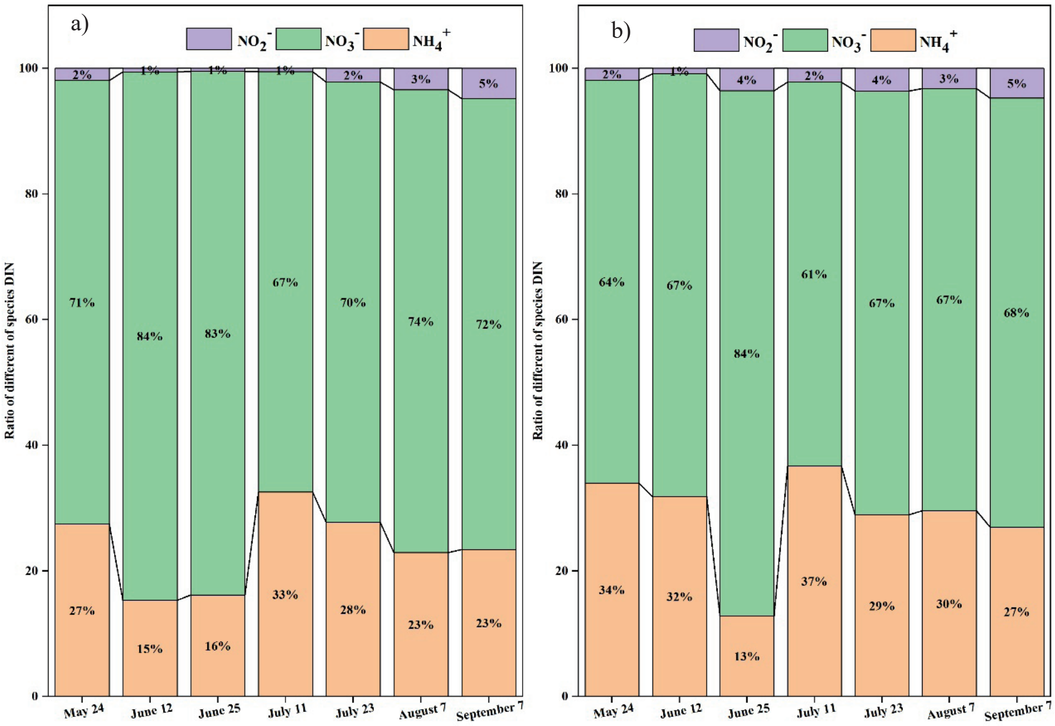

Fig. 5. Ratios of different species of dissolved inorganic nitrogen (DIN) in the a) control and b) experimental pond from May 24 to September 7, 2024.

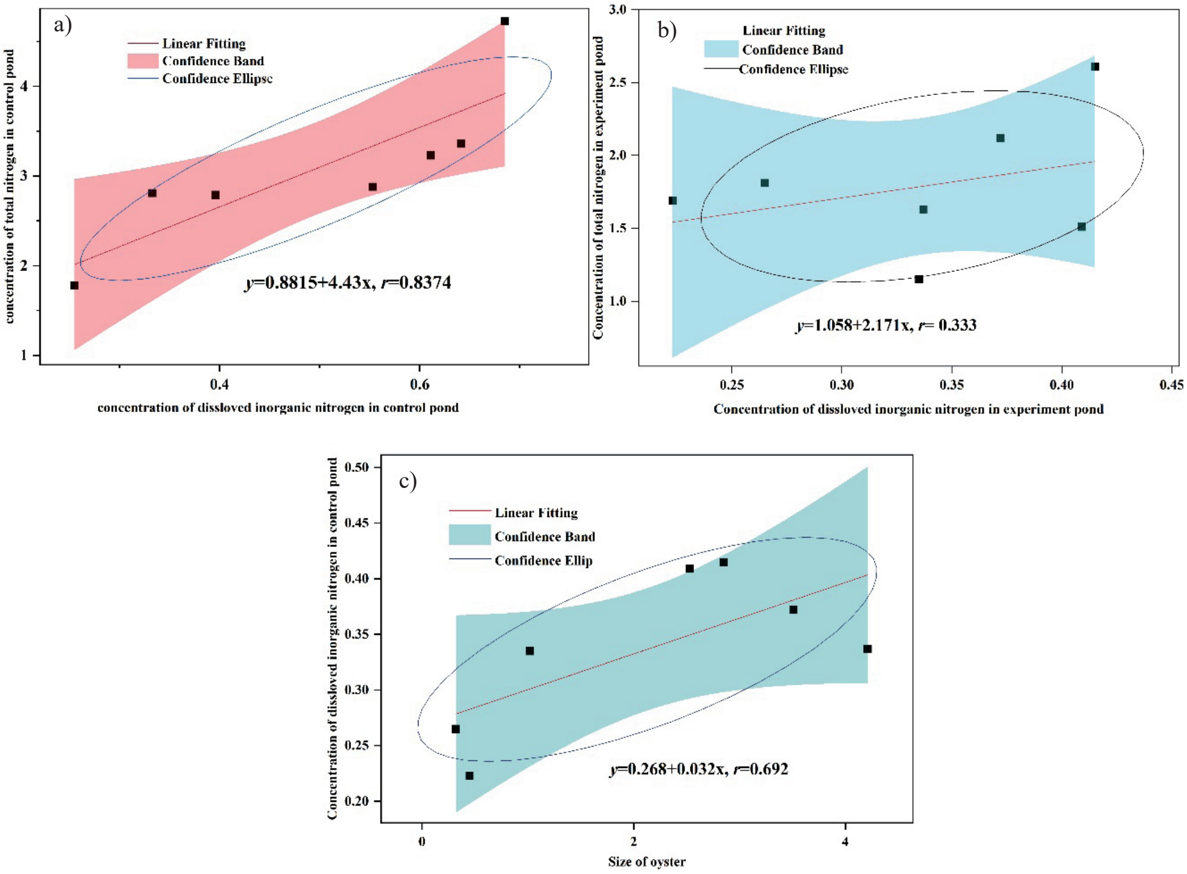

Fig. 6. Linear relationships among total nitrogen, inorganic nitrogen, and oyster size: linear relationships between a) total nitrogen and inorganic nitrogen in the control pond; b) total nitrogen and inorganic nitrogen in the experimental pond; c) oyster growth and inorganic nitrogen.

in other aquatic organisms [36]. Additionally, as an important anthropogenic stressor in marine waters, the presence of DIN necessitates rigorous monitoring due to its dual role in eutrophication and biogeochemical cycling. Spatiotemporal variability in DIN speciation (NH_4^+/NO_2^-/NO_3^-) directly modulates aquatic trophic status, with concentration thresholds dictating ecosystem resilience [37]. Therefore, the effective management of these N pollutants during aquacultural operations is essential to prevent their discharge into coastal environments and mitigate the associated environmental risks.

In this study, the ratio of inorganic nitrogen (DIN) to TN and the correlations among parameters were further evaluated to assess the relationship between TN and DIN. In the control pond with *L. vannamei* but no oysters, NH_4^+ and NO_3^- dominated the DIN pool with a combined 95.31-99.30% of TN, while NO_2^- represented a minor component (0.70-4.69%) (Fig. 5a)). Their proportional contributions to TN pools were as follows: NH_4^+ at 1.81-4.46%, NO_3^- at 9.96-13.63%, and NO_2^- at 0.07-0.92%. The NH_4^+ and NO_3^- portions represent the primary sources of the inorganic fraction within TN. The negligible NO_2^- fraction (<1%) indicates that this fraction has insignificant impacts on TN pollution. The DIN:TN ratio decreased from 14.32% (May 24) to 11.85% (June 12) and then rebounded to 26.11% (July 11), showing that active N speciation transitions were occurring, including DIN-DON interconversion and particulate sequestration (DIN/DON→PTN). These data confirm the marginal role of DIN in TN pools, where DON and particulate total nitrogen (PTN) dominated contamination. In the treatment pond, inorganic N remained predominantly composed of NH_4^+ and NO_3^-. The proportion of inorganic N relative to TN remained low; nitrate and ammonia accounted for 13.20-29.13%, and NO_2^- contributed about 1%. However, when compared with the control pond, the proportion of inorganic N within TN exhibited an initial increase followed by a decrease, yet remained low overall (Fig. 5b)). The DIN:TN ratio decreased between May 24 and June 12 and then rebounded to 29.13% (Fig. 5b)). This pattern suggests that oysters assimilated significant amounts of N pollutants, with a potentially greater removal efficiency for inorganic N than for TN. Notably, the initial DIN levels were significantly elevated in the treatment pond, which was likely attributable to excretory fluxes from bivalves. However, post-initialization monitoring revealed accelerated declines in NH_4^+ and NO_3^- (excluding NO_2^-) relative to the control. During May 24-September 7, the treatment pond showed 39.8%, 52.6%, and 27.3% reductions in NH_4^+, NO_2^-, and NO_3^-, respectively. Conversely, the control pond demonstrated 214.29% and 255.56% increases in NH_4^+ and NO_3^-, respectively. These dynamics demonstrate bivalve efficacy in DIN removal within shrimp aquaculture systems.

A significant Pearson correlation was observed between DIN and TN in the control pond ($r = 0.837$, $p<0.05$) (Fig. 6a)), indicating that TN (including PN and DON constituents) serves as a major source of DIN in the mariculture environment. This underscores the critical need for aquacultural management strategies that simultaneously reduce both TN and DIN to mitigate risks associated with pond aquaculture effluent. In contrast, the correlation weakened ($r = 0.333$, $p<0.05$) in the experimental pond (Fig. 6b)), indicating that the presence of oysters altered the N cycle processes within the entire pond ecosystem. Concurrently, the relationship between oyster growth (measured as average shell size) and DIN concentrations was examined; a positive correlation emerged ($r = 0.692$, $p<0.05$), indicating that oyster growth generates a demand for inorganic N. These findings indicate that oysters act as inorganic N sinks within the aquatic environment (Fig. 6c)).

Together, these results confirm the feasibility of using oysters to mitigate N pollution in *L. vannamei* mariculture systems; oysters participate in nutrient cycling, regulate N transformation pathways, and directly influence N concentrations. Our findings reveal how oysters impact the N cycle via two important mechanisms. First, they do this via robust filter-feeding and the creation of microbial habitats. Specifically, oysters consume diverse nutrient sources (phytoplankton, zooplankton, organic detritus, bacteria, and dissolved organic matter) [38]. Through feeding and bio-deposition, they reduce nutrient loads in aquaculture areas. Second, oysters perform N sequestration via their own physiology and aspects of their shell structure: their high amino acid and protein content requires substantial N for biosynthesis [39], while their multi-layered shells provide microenvironments for microbial processes (e.g., nitrification, denitrification) that further reduce N pollutants [40]. Active filter-feeding also promotes water circulation, enhancing these microbial functions.

Conclusions

Nitrogen is a critical pollutant requiring stringent control in *L. vannamei* mariculture because it serves as a primary driver of eutrophication and hypoxia. This study demonstrated that inorganic N accounted for a relatively small proportion (approximately 10%) of TN pollutants, while particulate and organic nitrogen constituted the dominant fraction (~90%), acting as the principal source of DIN in the pond ecosystem. The introduction of oysters significantly enhanced the mitigation of N pollution, leading to targeted reductions of DIN: NH_4^+ (39.8%), NO_2^- (52.6%), and NO_3^- (27.3%) alongside a 51.4% drop in TN. A strong positive correlation between oyster soft tissue growth and DIN reduction provided compelling evidence that oysters preferentially assimilate inorganic N, highlighting their role as efficient sinks for this bioavailable fraction. In essence, integrated oyster-shrimp aquaculture delivers profound ecological purification capacity alongside sustainable economic benefits, establishing this aquaculture method as a promising model that advances environmental health and socioeconomic progress in tandem.

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Conflict of Interest

The authors declare no conflict of interest.

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