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Dissimilatory nitrate reduction processes in surface sediments of shrimp ponds during the culture period

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Intensive aquaculture in estuaries and coasts has resulted in several ecological and environmental problems. Among various nitrogen transformation pathway, dissimilatory nitrate (NO_3^-) reduction is considered to be highly important in regulating reactive nitrogen. However, there are relatively few studies on the processes and contribution of NO_x^- reduction in sediment during the shrimp pond culture period. Three sediment NO_3^- reduction processes, denitrification (DNF), anaerobic ammonium oxidation (ANA), and dissimilatory NO_3^- reduction to ammonium (DNRA), were surveyed in eight shrimp ponds across three subtropical estuaries using ^{15}N isotope tracing experiments. The rates of DNF, ANA and DNRA ranged from 2.87–18.11, 0.10–1.92, and 0.21–1.25 nmol N g $^{-1}$ h $^{-1}$, respectively. DNF was responsible for 64.2–91.6% of the total NO_3^- reduction. Regarding environmental factors, C and N substrates, as well as salinity, significantly affected NO_3^- reduction. In general, the N losses were approximately 32.43–131.64 g N m^{-2} yr^{-1} for DNF and 2.38–15.85 g N m^{-2} yr^{-1} for ANA in this study, indicating that coastal reclamation is a nonnegligible way to remove nitrogen. Our results provide a scientific foundation for understanding the mechanism of nitrogen cycling in the artificial aquatic environment of shrimp ponds.

KEYWORDS

denitrification, anammox, DNRA, shrimp ponds, sediment

Introduction

With rapid economic development and the influence of human activities, large amounts of reactive nitrogen from upstream have been carried to estuarine and coastal systems by atmospheric transport and river runoff in recent years (Galloway et al., 2008; Canfield et al., 2010; Chen et al., 2016b). Reactive nitrogen mainly exists in the form of nitrate (NO_3^-), which has a significant influence on the ecology and functions of estuaries and coastal environments (Kennison and Fong, 2014; Macdonald et al., 2018). Such as eutrophication and algal blooms caused by increased NO_3^- concentrations, and even pose a potential threat to human heath (Birch and McCaskie, 1999; Wang et al., 2020). Thus, further understanding of the transformation processes in estuarine and coastal systems is required.

Dissimilatory NO_3^- reduction is an important pathway for removing reactive nitrogen and mainly includes three processes: denitrification (DNF), anaerobic ammonium oxidation (ANA), and dissimilatory NO_3^- reduction to ammonium (DNRA) (Thamdrup and Dalsgaard, 2002; Deng et al., 2015; Huang et al., 2021). Among these processes, DNF, which converts NO_3^-/NO_2^- to N_2 or N_2O, has long been considered the main pathway for NO_3^- removal progress (Seitzinger et al., 2006; Burgin and Hamilton, 2007). ANA oxidises ammonia (NH_4^+) into dinitrogen gas by reducing NO_3^-/NO_2^-, which has recently been thought to play an important role in the regulation of the sediment nitrogen cycle (Trimmer et al., 2003; Dale et al., 2009; Hou et al., 2015). While both DNF and ANA would remove NO_3^-/NO_2^- by conversion to gaseous nitrogen, DNRA converts NO_3^- to bioavailable NH_4^+, inducing the net retention of reactive N in the environment (Silver et al., 2005; Huygens et al., 2007; Dong et al., 2011). The contributions of the three NO_3^- reduction processes to nitrogen cycling differ depending on the type of ecosystem and sediment (Thamdrup and Dalsgaard, 2002; Laverman et al., 2007; Minick et al., 2016). Previous studies have shown that DNF was the main pathway of NO_3^- reduction processes in aquatic ecosystems (Deegan et al., 2012; Shan et al., 2016). However, studies have shown that DNRA plays an important role in mangrove systems (Cao et al., 2016). Studying the mechanism of NO_3^- reduction would help us to further understand the nitrogen transformation process in aquatic ecosystems.

As a key area of land-marine interaction, estuarine tidal flat wetlands are hotspots for the nitrogen cycle (Osburn et al., 2016; Hou et al., 2018). In recent years, because of the increasing demand for seafood products, large areas of tidal flat wetlands in China's coastal estuaries have been reclaimed as artificial aquaculture ponds (Chen et al., 2016a; Yang et al., 2017b). Owing to the addition of feed, aquaculture significantly increases carbon and nitrogen-based substances, changing carbon and nitrogen cycle processes, such as CH_4 and N_2O emissions (Yang et al., 2017a; Gao et al., 2018). According to previous research, the NO_3^- reduction route and proportion changed after the reclamation of estuarine tidal flat wetlands into aquaculture ponds (Gao et al., 2019). The process of NO_3^- reduction were closely related to environmental factors such as NO_3^-, TOC, and NH_4^+ (Deng et al., 2015; Cheng et al., 2016; Damashek and Francis, 2018). However, the detailed process of dissimilatory NO_3^- reduction in aquaculture ponds during the culture period remains unclear. With the continuous expansion of farming scale, it is necessary to develop the understanding of this special ecosystem.

This study selected shrimp ponds in three different regions of the subtropical estuarine and analysed the dissimilatory NO_3^- reduction process during the culture period using nitrogen isotopic techniques. In addition, the primary environmental factors influence DNF, ANA, and DNRA processes were studied. Furthermore, we compared the relative contributions of the three processes following the culture period in shrimp ponds. Our study provides a deeper understanding of nitrogen dynamics and the progress of aquaculture ponds in estuaries and coasts.

Materials and methods

Study area and samples collection

Three main estuaries in Fujian Province were selected as the study area: From north to south, the Min River, Mulan River, and Jiulong River. The three estuaries all have a subtropical monsoon climate, with an average annual rainfall of more than 1300 mm and an average annual temperature of 19.6 to 21.0°C (Zhang et al., 2011; Tong et al., 2012; Luo et al., 2019). In August 2017, eight sampling points were selected for shrimp culture ponds near the three estuaries, and surface sediments were collected underwater using a Plexiglas tube (Figure 1). The shrimp ponds at the sampling points were all reclaimed from estuary swamp wetlands, with similar land-use transformation years (7–9 years), and all were muddy pond slopes and pond bottoms. The shrimp ponds contained white shrimp (*Litopenaeus vannamei*). Shrimp seedlings are put in at the end of May every year, and farming ends after all the shrimps are harvested in mid- to late October. Shrimp farming ponds use local river water as a source of farming water. Each shrimp farming pond is equipped with two impeller aerators with a power of 1.5 kW, and the startup time is about 20:00 to 2:00 the next morning. The switch of the aerator during the day depends on the weather, the growth of fish and shrimp, and the water quality. The feed was provided twice a day at 7:00 and 17:00, and the feeding amount was in accordance with the conventional requirements. The basic features of shrimp ponds are listed in Table 1.

Analysis of sediment properties

Sediment density was measured using the syringe method (Percival and Lindsay, 1997), and the moisture content was dried

FIGURE 1
Study area and sampling sites.

using the weight loss method at 80°C to a constant weight. After removing sedimentary carbonate using 0.1 M HCl, the concentration of total organic carbon (TOC) and total nitrogen (TN) in the sediments were determined using a carbon-hydrogen-nitrogen elementary analyser (VVarioELIII, Elementary, Germany) and C N elemental analyser (Elementar Vario MAX CN, Germany), respectively (Lin et al., 2017; Hu et al., 2022). The sediment concentrations of NH_4^+ and NO_3^- were extracted with 2 M KCl solution and then determined using a continuous flow analyser (SAN Plus, Skalar Analytical B.V., The Netherlands) (Sun et al., 2022).

Determination of potential DNF, ANA, and DNRA rates

The potential DNF, ANA, and DNRA rates were measured using the nitrogen isotope tracing method (Yin et al., 2014; Deng et al., 2015; Gao et al., 2017). In brief, the slurry was prepared by mixing sediment into helium-purged water with a sediment/water volume ratio of 1:7 and then transferring to a helium-purged 12-mL vial (Labco Exetainers) (Hou et al., 2015). The vials were then pre-incubated for 36 h to remove surplus NO_3^-, NO_2^-, and O_2 at *in situ* temperatures. After pre-incubation,

TABLE 1 Environmental parameters in surface sediments of the shrimp ponds.

	MA	MB	MC	PG	PH	JD	JE	JF
Bulk density ($g\ cm^{-3}$)	1.29 ± 0.06	1.45 ± 0.02	1.59 ± 0.15	1.60 ± 0.06	1.63 ± 0.04	1.4 ± 0.02	1.5 ± 0.03	1.65 ± 0.76
Water content(%)	52.17 ± 3.03	48.68 ± 1.25	35.68 ± 0.98	39.57 ± 0.62	34.04 ± 2.89	55.62 ± 1.51	51.59 ± 2.65	36.62 ± 3.05
Salinity(ppt)	0.46 ± 0.01	0.91 ± 0.01	1.16 ± 0.05	0.07 ± 0.01	1.28 ± 0.05	1.60 ± 0.01	1.76 ± 0.03	1.93 ± 0.01
TN(%)	0.14 ± 0.01	0.15 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.08 ± 0.01
TOC(%)	1.76 ± 0.11	1.78 ± 0.11	1.24 ± 0.08	1.67 ± 0.07	0.92 ± 0.03	0.76 ± 0.04	0.62 ± 0.05	0.78 ± 0.08
TOC/TN	11.97 ± 0.78	11.66 ± 0.47	10.14 ± 0.09	11.53 ± 0.06	7.62 ± 0.13	6.86 ± 0.04	4.72 ± 0.18	10.47 ± 2.87
NH_4^+(mg N kg^{-1})	5.73 ± 0.11	7.06 ± 0.11	3.47 ± 0.13	7.86 ± 0.01	5.21 ± 0.04	3.72 ± 0.33	5.29 ± 0.11	2.88 ± 0.42
NO_3^-(mg N kg^{-1})	0.09 ± 0.02	0.13 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.02 ± 0.01	0.09 ± 0.01

sterile anoxic solutions of $^{15}NO_3^-$ (^{15}N at 99%) was added to all vials *via* the septa, with the final content of ^{15}N being approximately 100 μM (Hou et al., 2013). Then, 200 μL of $ZnCl_2$ solution (50%) was added to half of the replicates (as initial samples). Then, half of the slurries were incubated for 8 h, and 200 μL of $ZnCl_2$ solution (50%) was added at the end of incubation to terminate the reaction (Lin et al., 2017). Both of the contents of $^{29}N_2$ and $^{30}N_2$ were measured by membrane inlet mass spectrometry (MIMS) during incubation, to calculate the DNF and ANA rates based on the difference in $^{29}N_2$ and $^{30}N_2$ produced among the final and initial results (Gao et al., 2019).

The DNF and ANA rates were approximated based on the accumulations of $^{29}N_2$ and $^{30}N_2$, respectively (Deng et al., 2015). The contributions of DNF and ANA to $^{29}N_2$ production were calculated using Equation (1).

$$P_{29} = A_{29} + D_{29} \tag{1}$$

Here, P_{29}, D_{29}, and A_{29} (nmol N g^{-1} h^{-1}) denote the total $^{29}N_2$ production rate and the production rate of $^{29}N_2$ from DNF and ANA during the slurry experiments, respectively. The ratio of ^{14}N and ^{15}N produced by $^{14}NO_3^-$ or $^{15}NO_3^-$ with random isotope pairing and D_{29} was calculated using equation (2) (Risgaard-Petersen et al., 2003).

$$D_{29} = P_{30} \times 2 \times (1 - F_N) \times F_N^{-1} \tag{2}$$

Here, P_{30} (nmol N g^{-1} h^{-1}) represents the production rate of total $^{30}N_2$, and F_N (%) denotes the proportion of ^{15}N in NO_3^-, which was estimated from the measured content of added $^{15}NO_3^-$ and surplus NO_3^- (Shan et al., 2016). Finally, the DNF potential rates were calculated using Equation (3), and the ANA was calculated using Equation (4).

$$D_{\text{total}} = D_{29} + 2 \times D_{30} \tag{3}$$

$$A_{29} = P_{29} - D_{29} \tag{4}$$

Here, D_{total} and A_{29} (nmol N g^{-1} h^{-1}) denote DNF and ANA rates, respectively.

The DNRA rate was calculated using the $^{15}NH_4^+$ oxidation and MIMS analysis (OX/MIMS) method (Yin et al., 2014). First, the sediment slurry was pre-incubated. After the preincubation, 100 μL of $^{15}NO_3$ (^{15}N at 99.6% and a final content of approximately 100 μM ^{15}N) was added to all the vials. Immediately, half of the slurry was saved (as initial sample) with 200 μL of $ZnCl_2$ solution (50%). The rest of the vials (as final samples) were further incubated 8 h before adding 200 μL of $ZnCl_2$ solution (50%). The potential rates of DNRA were calculated using Equation (5).

$$R_{\text{DNRA}} = \left[\left({}^{15}\text{N H}_4^+\right)_{\text{Final}} - \left({}^{15}\text{N H}_4^+\right)_{\text{Initial}}\right] \times V \times W^{-1} \times T^{-1} \tag{5}$$

Here, R_{DNRA} (nmol N g^{-1} h^{-1}) is the total potential rate of DNRA; $(^{15}NH_4^+)_{Final}$ and $(^{15}NH_4^+)_{Initial}$ (nmol N L^{-1}) are the content of $^{15}NH_4^+$ in the final and initial sample, respectively; and V (L), W (g), and T (h) denote the volume of the vial, dry weight of the sediment, and time, respectively (Gao et al., 2019).

Statistical analyses

The differences between the NO_3^- reduction rates among all points were analysed by one-way analysis of variance (ANOVA) (homogeneity of variance was tested by the LSD test, and Dunnett's T3 was used to test for heterogeneity of variance). The relationships between the environmental variables and DNF, ANA, and DNRA rates were revealed by Pearson correlation analyses. SPSS 22.0 was used for one-way ANOVA and pearson analysis. While Redundancy analysis (RDA) was used to evaluate variations in NO_3^- reduction rates with respect to environmental variables using software Canoco 4.5 (Sun et al., 2020). The significance level was set at 0.05.

Results

Physiochemical characteristics of the site

The physicochemical characteristics of each site are presented in Table 2. Sediment bulk density varied from 1.27 to 1.78 g·cm^{-3} in

TABLE 2 Basic characteristics of all shrimp ponds in the estuaries of Fujian Province.

Sampling Site	Name	Area (ha)	Average depth (m)	Unit product output(kg ha^{-1})	Food coefficient
MA	Shanyutan wetland of Min River estuary	0.753	1.3	9.77	1.38
MB	Bianfuzhou wetland of Min River estuary	0.982	1.9	12.3	1.90
MC	Culu Island of Min River estuary	0.521	1.4	10.2	1.77
PG	Dongxiang of Mulan River	1.113	2.1	12.1	1.76
PH	Zhe lang of Mulan River	1.255	2.8	13.0	1.86
JD	Changzhou of Jiu long River	0.226	1.6	10.3	1.82
JE	Junkennongchang of Jiu long River	0.749	1.7	11.2	1.64
JF	Xumaozhou of Jiu long River	0.739	1.6	10.6	1.56

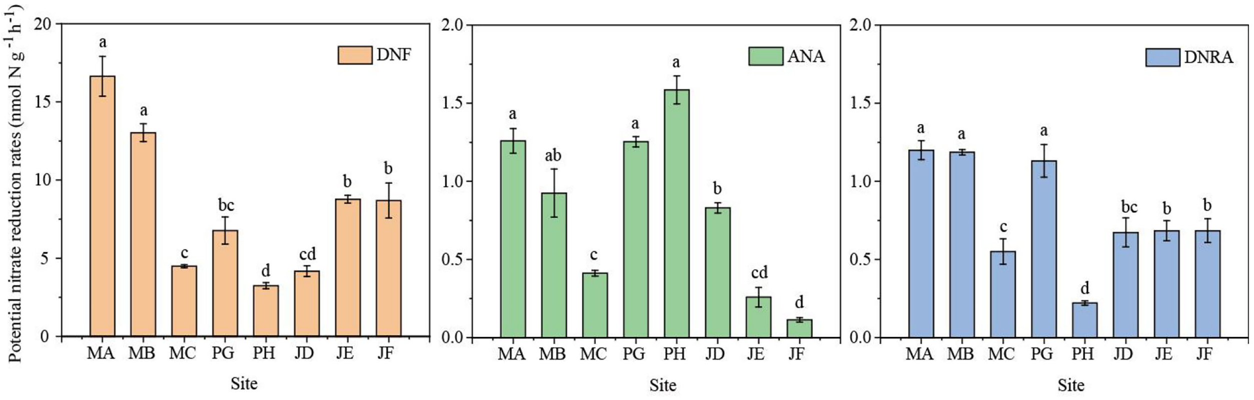

FIGURE 2
Sediment potential DNF, ANA and DNRA rates in the shrimp ponds. Different letters indicate significant differences ($p < 0.05$) among different sites. Error bars represent the standard deviation(n = 3).

the study area, and the water content in the sediment varied from 31 to 59%. The salinities of the shrimp ponds ranged from 0.06 to 1.93 ppt. The TOC, TN, and TOC/TN ratios in the sediment varied between 0.58 to 1.97%, 0.07 to 0.16%, and 4.51 to 13.75, respectively. The concentration of NH_4^+ and NO_3^- in sediments varied from 2.42 to 8.41 and 0.02 to 0.14 μmol·g^{-1}, respectively. In addition to the physicochemical characteristics of the sediments, the area and average depth of each shrimp pond were also different, and the largest area (1.113–1.255 ha) and average depth (2.1–2.8 m) of shrimp ponds were found in the Mulan estuary. The unit water output varied between 9.77–10.6 kg ha$^{−1}$, and the food coefficient ranged from 1.38–1.86 in the shrimp pond (Table 1).

Dissimilatory NO_3^- reduction processes

The sediment potential rates of DNF ranged from 2.87 to 18.11 nmol N g^{-1} h^{-1}, and there were significant differences between the all sites ($n = 24$, $p < 0.05$). The highest DNF rate was found at site MA, while the lowest DNF rate was at site PH (Figure 2). DNF contributed 64.2–91.5% of the total NO_3^- reduction rate (Figure 3). The rates of ANA varied from 0.10 to 1.92 nmol N g^{-1} h^{-1} in the study area, and a distinct spatial difference in the ANA rates was observed among sites ($n = 24$, $p < 0.05$). The highest ANA rate occurred at site PH, whereas the lowest ANA rate occurred at site JF. Compared to DNF, ANA

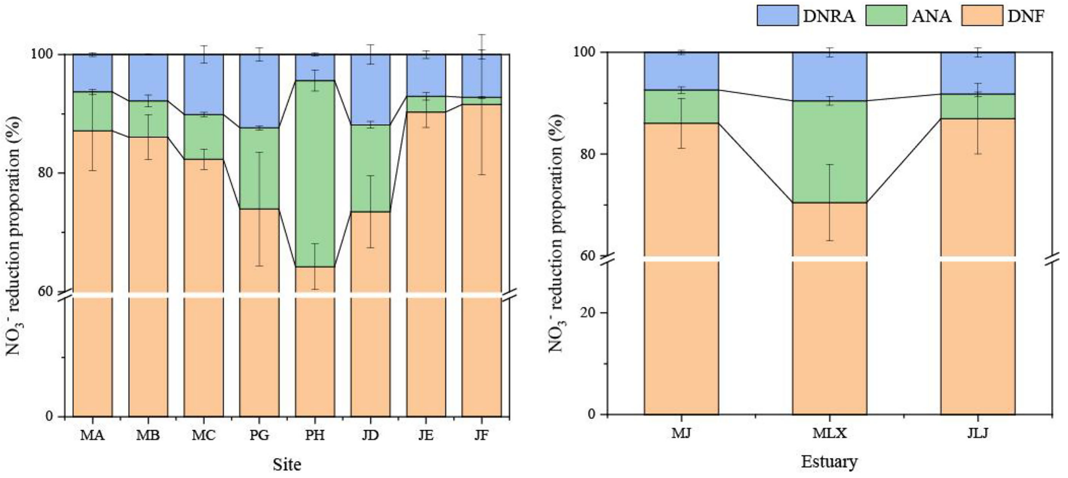

FIGURE 3
Relative contributions of DNF, ANA and DNRA to total NO_3^- reduction in the shrimp ponds. Error bars represent the standard deviation.

had less effect on NO_3^- reduction and contributed 1.2–31.4% to total nitrogen loss. Potential DNRA rates varied from 0.21 to 1.25 nmol N g^{-1} h^{-1}. Significant differences were observed among the sites in the study area (n = 24, $p < 0.05$). DNRA and DNF showed the same distribution, with the highest value appearing at the MA site and the lowest value appearing at the PH site. The contribution of DNRA accounted for 4.38–12.35% of the total NO_3^- reduction.

Influences of physiochemical characteristics on NO_3^- reduction rates

The RDA was implemented to evaluate variations in the NO_3^- reduction rate with respect to environmental variables. The first two RDA dimensions were found to account for 70.56% of the cumulative variance, and the first and second axes contributed 55.12 and 15.44%, respectively (Figure 4). The RDA results showed that salinity and TOC were significantly associated with various NO_3^- reduction process. The DNF rates were significantly and negatively correlated with bulk density (r = - 0.559, $p < 0.05$, n = 24) and positively correlated with water content (r = 0.464, $p < 0.05$, n =24), TOC (r = 0.590, $p < 0.01$, n = 24), TOC/TN (r = 0.495, $p < 0.05$, n = 24), and NO_3^- (r = 0.525, $p < 0.01$, n = 24) (Table 3). Of the detected environmental factors, salinity and the ANA rate showed a significant negative correlation (r = - 0.656, $p < 0.01$, n = 24), and there was a positive correlation with TN (r = 0.498, $p < 0.05$, n =24), TOC (r =0.506, $p < 0.05$, n = 24) and NH_4^+ (r = 0.577, $p < 0.05$, n = 24). The sediment potential rate of DNRA was negatively associated with bulk density (r = 0.441, $p < 0.05$, n = 24) and salinity (r = - 0.637, $p < 0.01$, n = 24) and positively related to water content (r = 0.472, $p < 0.05$, n = 24), TOC (r = 768, $p < 0.01$, n = 24), TN (r = 0.546, $p < 0.01$, n = 24), TOC/TN (r = 0.608, $p < 0.01$, n = 24), NH_4^+ (r = 0.604, $p < 0.05$, n = 24), and NO_3^- (r = 0.508, $p < 0.05$, n = 24) (Table 3). There are positive correlations of most of the dissimilatory NO_3^- reduction processes with TOC, TOC/TN, NH_4^+, and NO_3^- were found (Table 3).

Discussion

In coastal wetlands, reclaiming natural wetlands for aquaculture activities is a major anthropogenic disturbance that threatens intrinsic N balance. The addition of feed and the growth and excretion of shrimp during the breeding process increased C and N substrates in the sediment of shrimp ponds; at the same time, the rates of the dissimilatory NO_3^- reduction process were promoted (Wu et al., 2014; Chen et al., 2016b; Gao et al., 2019). The addition of bait to coastal wetlands can change

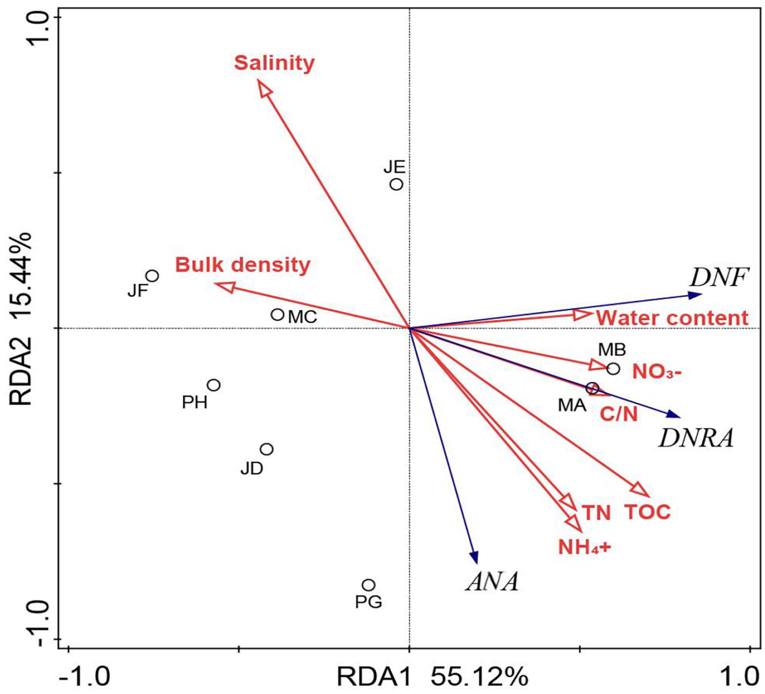

FIGURE 4
Ordination diagram showing the results of RDA of NO_3^- reduction processes and soil physicochemical characteristics. The hollow circles represent individual sediment samples from the eight shrimp ponds in the subtropics Estuary. The red arrows represent soil physicochemical characteristics, and blue arrows represent NO_3^- reduction processes.

TABLE 3 Person's correlations of sediment NO_3^- reduction rates with physico-chemical properties.

	DNF	ANA	DNRA
Bulk density	**-0.559** *	n.s.	**-0.441***
Water content	**0.464***	n.s.	**0.472***
Salinity	-0.339	**-0.656****	**-0.637****
TN	0.402	**0.498***	**0.546****
TOC	**0.590****	**0.506***	**0.768****
TOC/TN	**0.495***	0.355	**0.608****
NH_4^+	0.358	**0.577***	**0.604***
NO_3^-	**0.525****	0.304	**0.508***

* Significant at $p < 0.05$, ** Significant at $p < 0.01$, $n = 24$. The meaning of the bold values represent significant difference. The n.s. indicates a no statistical significance.

the microbial community structure in sediments and contribute to greenhouse gas (GHGs) emissions (Lin and Lin, 2022). DNF, ANA, and DNRA are the most crucial processes in dissimilar NO_3^- reduction processes in aquatic environments (Rysgaard et al., 2004; Song et al., 2013; Yang et al., 2022). We researched the distribution of dissimilatory NO_3^- reduction processes in shrimp ponds and analysed the main influencing factors controlling the process. In the present study, most of the dissimilatory NO_3^- reduction processes with TOC, TOC/TN, NH_4^+, and NO_3^- were showed a positive correlations. Therefore, the contribution of these environmental factors to NO_3^- reduction in shrimp ponds requires further elucidation.

NO_3^- reduction process and the influence of environmental factors

DNF rate were measured by ^{15}N tracer techniques according to the assumption of N_2, because of the ratio of N_2O to N_2 from DNF in aquatic ecosystems is very low (Dong et al., 2002). Salinity affected the DNF rate to some extent, although no statistically significant correlation was observed, and a decrease in denitrification activity associated with higher salinity was observed. RDA showed a negative correlation between salinity and DNF rate. Salinity may result in physiological stress on DNF, which in turn affects the DNF rate, which generally decreases with an increase in salinity (Rysgaard et al., 1999; Seo et al., 2008). In general, the DNF rate was positively related to the TOC content in previous studies, such as in lakes, rice fields, estuaries, and coastal environments (Vymazal, 2007; Deng et al., 2015; Yang et al., 2022). Our study showed that the DNF rate showed a positive relationship with the TOC content in the shrimp ponds, which is similar to previous research (Gao et al., 2019). Because shrimp ponds have been in a high organic carbon environment for a long time, they are beneficial for the growth of denitrifying-associated bacteria and promote the DNF rate (Canion et al., 2014; Plummer et al., 2015; Yang et al., 2017b). A remarkable relationship between the DNF rate and NO_3^- was observed in our study (Table 3), and it was confirmed that NO_3^- is a major driver of the rate of NO_3^- reduction in shrimp ponds. DNF microorganisms use NO_3^- as an electron acceptor and substrate, which is strongly dependent on the NO_3^- concentration (Giles et al., 2012; Shan et al., 2016; Palacin-Lizarbe et al., 2020).

Spatial variations in ANA rates have also been found, and numerous studies have reported that salinity is a crucial environmental factor affecting ANA (Wang et al., 2012; Hou et al., 2015). Salinity was significantly correlated with the ANA rate in this study, suggesting that high salinity might inhibit the rate of ANA activity. It has been reported that ANA activity is not directly caused by an energy source, but is influenced by C and N substrates in sediments (Hou et al., 2015). A strong correlation was found between ANA and the C and N substrates in our study; this C and N matrix mainly comes from the decomposition of a large amount of feed and manure residues in the culture period (Wu et al., 2014). Studies have shown that TOC and NH_4^+ concentrations at suitable concentrations can promote the ANA rate (Cheng et al., 2016; Damashek and Francis, 2018), as well as a significant positive correlation between TOC, NH_4^+, and ANA. However, it should be noted that high TOC concentrations may inhibit ANA activity (Bettazzi et al., 2010).

DNRA is often considered to be closely related to salinity, and previous studies have shown that increasing salinity accelerates the DNRA rate (Gardner et al., 2006; Giblin et al., 2010). However, some studies have found that the abundance of the *nrfA* gene associated with DNRA rates is not significantly affected by salinity (Yin et al., 2017). Interestingly, our results showed that DNRA and ANA rates declined significantly with increasing salinity, as both showed similar results (Table 3). RDA also showed a negative correlation between salinity and ANA, DNRA rates. TOC and NH_4^+ could be important factors influencing DNRA in sediments (Bu et al., 2017), which was also proven by the significant positive correlation between the rate of DNRA and TOC in our study. High TOC concentrations may provide adequate organic substrates for the growth of DNRA-associated microorganisms (Deng et al., 2015). DNRA can more efficiently utilise NO_3^- as an electron acceptor in a carbon-rich environment, thereby increasing the DNRA rate (Kraft et al., 2014; Cheng et al., 2016).

Relationship between different NO_3^- reduction processes

The DNF rate was compared with the ANA and DNRA rates to identify potential connections among them (Figure 5). These correlation analyses show that DNF is closely related to ANA and DNRA in the study area. During the interaction between ANA and DNF, DNF is a major source of NO_2^- for ANA in coastal wetland sediments (Meyer et al., 2005), and there was a significant relationship found between DNF and ANA rates in the shrimp

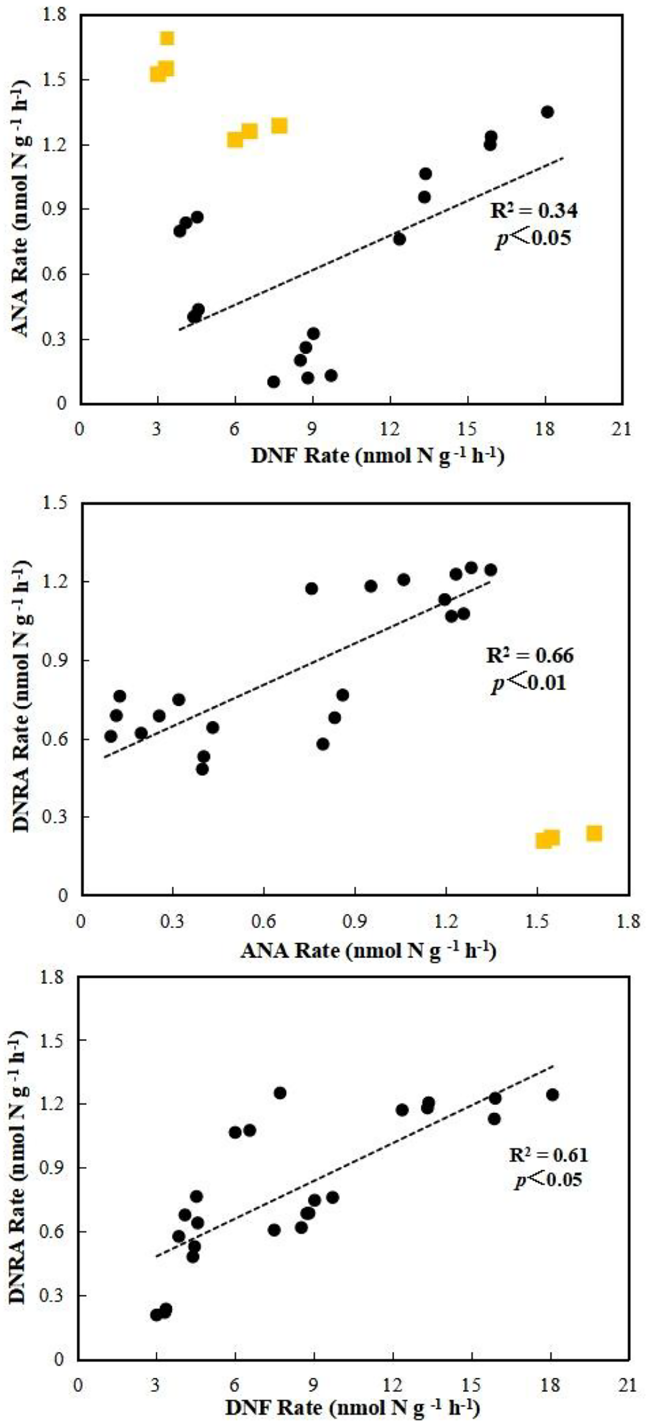

FIGURE 5
Correlations between DNF, ANA and DNRA rate. The linear regression is shown for the relation between DNF, ANA and DNRA rate. R^2 represents regression coefficient, and Yellow points represents not included in the statistics.The significance level was set at 0.05 and 0.01.

pond (R^2 = 0.34, $p < 0.05$). Studies have also found a coupling related process between ANA and DNF (Hou et al., 2015). The ANA rates were significantly associated with DNRA rates (R^2 = 0.66, $p < 0.01$), indicating that DNRA can produce NO_2^- as an intermediate product, which is an alternative substrate for ANA (Lam et al., 2009). DNF and DNRA compete for NO_3^- under hypoxic or anaerobic conditions. Our results showed that the DNF rates were significantly related with the DNRA rates in the sediment (R^2 = 0.61, $p < 0.05$) (Figure 5), indicating that the growth of NO_3^- substrate promoted both of them.

Competition among DNF, ANA, and DNRA can determine the fate of NO_3^- owing to their diverse roles in dissimilatory NO_3^- reduction. DNF was the dominant route contributing 64.2–91.6% (81.14%) of the total the dissimilatory NO_3^- reduction processes in the shrimp pond, while the contributions of ANA and DNRA were 1.2–31.4% (10.48%) and 4.34–12.4% (8.38%), respectively (Figure 6). According to several studies, DNF plays a major role in removing nitrogen from various aquatic systems, while the proportions of ANA and DNRA differ between ecosystems (Deng et al., 2015; Shan et al., 2016; Yang et al., 2022). Different aquatic environments contribute different amounts of dissimilatory NO_3^- reduction, as shown in Table 4. Studies have shown that the DNF, ANA, and DNRA rates in urban rivers and seas are higher than those in shrimp ponds (Song et al., 2013; Cheng et al., 2016). However, the rate of DNF in a paddy field was between 2.37 and 8.30 nmol N g^{-1} h^{-1}, and ANA and DNRA were also relatively low (Shan et al., 2016). The DNF rate in estuarine and lake regions ranges from 0.09 to 11.47 nmol N g^{-1} h^{-1}, which is lower than that in shrimp ponds (Deng et al., 2015; Yang et al., 2022). Compared with the estuary wetland, the dissimilation NO_3^- reduction rate increased after reclamation for shrimp ponds, showing an increasing trend with increasing reclamation years (Gao et al., 2019). It was found that the biggest difference between the shrimp ponds system and other systems may be more dependent on the difference caused by the high concentration of C and N matrix input. We found that the rate of DNF ranged from 3.24 to 16.64 nmol N g^{-1} h^{-1} in shrimp ponds, while the rate of ANA was slightly higher than that of DNRA (Figure 2). Further analysis showed that DNRA was higher than ANA in aquaculture ponds in the Min and Jiulong River estuaries, indicating that the contribution of DNRA to the total NO_3^- reduction was still higher than that of natural wetlands (Gao et al., 2019). ANA was found to be much higher than DNRA in the Mulan River estuary, especially the PH site at 31.4% (Figure 3). This may be related to the large size and depth of shrimp ponds in the estuary of the Mulan River, however, specific causes need to be identified.

If the average DNF and ANA rates were extrapolated to the study area, the N losses were approximately 32.43–131.64 g N m^{-2} yr^{-1} for DNF and 2.38–15.85 g N m^{-2} yr^{-1} for ANA. According to the area of aquaculture ponds in China's coastal wetland (2.6 $\times 10^6$ ha) (Chen et al., 2016a; Chen et al., 2016b), approximately 2.18 × 10^6 t N can be removed from shrimp ponds by this process annually, indicating that coastal reclamation is a considerable way to remove nitrogen. Recent results suggest that if natural wetlands are gradually converted into shrimp ponds, reactive nitrogen may be retained (Murphy et al., 2016). Shrimp ponds may be more important than nitrogen loss (Gao et al., 2019). Consequently, sediment N loads increase, resulting in decreased water quality and shrimp disease outbreaks (Castillo Soriano et al., 2013).

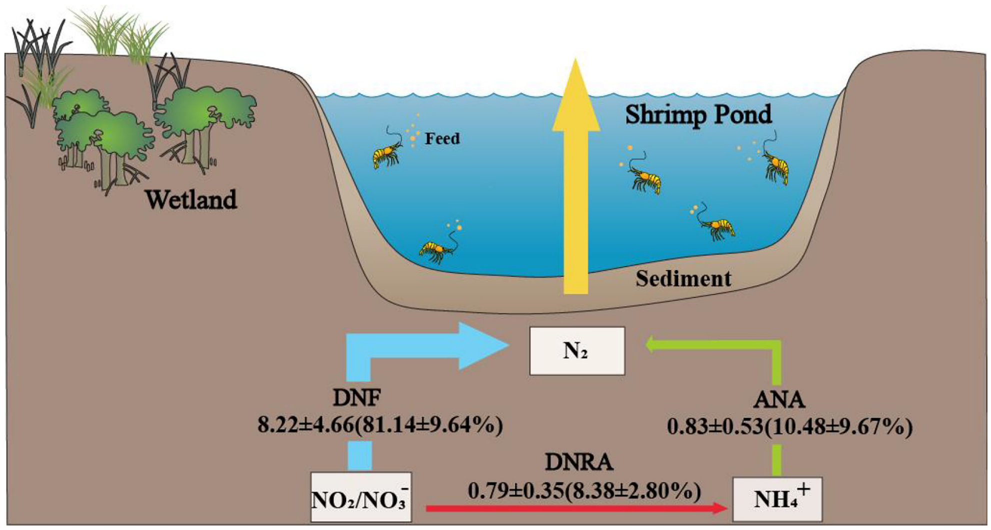

FIGURE 6
The conceptual map on NO_3^- reduction processes in the shrimp pond sediment. The unit of DNF, ANA, and DNRA rates were nmol N g^{-1} h^{-1}.

TABLE 4 Contributions of Denitrification, ANAMMOX, and DNRA to total nitrate reduction in our study and other aquatic ecosystems.

Aquatic system	DNF		ANA(%)		DNRA(%)		References
	Ratenmol N g^{-1} h^{-1}	Contribution (%)	Ratenmol N g^{-1} h^{-1}	Contribution (%)	Ratenmol N g^{-1} h^{-1}	Contribution (%)	
Paddy soil	2.37-8.30	76.8-92.5	0.15-0.77	4.5-9.2	0.03-0.54	0.5-17.6	(Shan et al., 2016)
Urban river	0.193-98.7	11.5-99.5	0.0387-23.7	0.343-81.6	0-10.3	0-52.3	(Cheng et al., 2016)
Urban lakes	3.54-11.47	58.87-63.89	0.07-1.60	3.72-8.86	1.10-4.18	29.38-35.28	(Yang et al., 2022)
East China Sea	0.6-20^{a}	17-85 (65)	0.4-4^{a}	8-66 (20)	2.6-9.7^{a}	8-22 (15)	(Song et al., 2013)
Yangtze Estuary	0.09-4.52	66.2	0.01-0.52	8	0.03-0.89	25.8	(Deng et al., 2015)
Coastal wetlands	1.52-17.58	70-92.41	0.31-1.27	2.49-15.27	0.14-2.01	5.10-20.75	(Gao et al., 2017)
shrimp pond	2.5-14.3	57.2-82.2	0.6-2.1	10.3-17.3	1.6-3.8	7.5-27.4	(Gao et al., 2019)
Shrimp pond	2.87-18.11	64.2-91.6	0.10-1.92	1.2-31.4	0.21-1.25	4.4-12.4	This study

^{a}means that the unit for nitrate reduction rates is nmol N cm^{-3} h^{-1}

Conclusion

This study investigated the process of NO_3^- dissimilation and reduction in shrimp pond sediments in a subtropical estuary area and identified the main factors influencing the process. Studies have shown that DNF is the main pathway, and that ANA and DNRA also play important roles. The feed added during the culture promoted the C and N matrices in the sediment, which in turn promoted the reduction of various NO_3^-. This process of nitrogen removal under intensive aquaculture in estuarine and coastal areas should receive more attention in future studies.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

DS: Investigation, Formal analysis, Writing and editing. JH: Conceptualization, Methodology, Funding acquisition. ML: Methodology. XL, CC, WH: Formal analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

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