Study on the mechanism of the increase/inhibition of nitrate ammonium by the ways of ecological restoration in the Pearl River Estuary

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Abstract. This paper studies the bottom sludge and water of fishing ports near Yamen Waterway in Taishan City, and Xi-Nanyong Riverway in Foshan City, which are one of the most polluted areas in the Pearl River Estuary. By changing different ecological repair methods, we have studied the changing trend of nitrate nitrogen ammonium ~ anaerobic ammonia oxidation ~ denitrification pathway, and clarified the fate of nitrogen from the macroscopic view; we have established a continuous culture flow simulation device, which can Clarify the weight of ammonia nitrogen, which is generated by ammonium nitr ate nitrogen. We changed the water quality parameters and concentration during the operation of the continuous flow device, including salinity, temperature and dissolved oxygen, aeratation, flow rate, ammonia nitrogen, nitrate nitrogen, etc. We analyzed the relationship between the conversion rate of ammonia nitrogen and environmental factors. We obtained the DNRA rate through a continuous flow device. We analyzed the effects of different removal methods on nitrogen removal. The research results show that under the environment of salinity (0.48ppt), higher dissolved oxygen, lower temperature, higher ORP (greater than 300mV), it is conducive to the discharge of reactive nitrogen. On the contrary, the rate of ammonia nitrogen generated by DNRA increases, the max is19nmol/(g h), making Nitrogen stays in the water. This conclusion can provide a theoretical basis for the accurate evaluation of the denitrification effects and the effectiveness of the methods for ecological restoration of the Pearl River Estuary and other similar estuaries.

1. Introduction
The interaction of brackish and fresh water is significant in the estuary area. During their tidal cycle, salinity is one of the most drastically changing environmental factors in the estuary coastal environment [1]. Salinity has an inhibitory effect on microbial denitrification [2-4], and this research result has been recognized by many scholars. At present, it is believed that as the salinity rises in the water body, the sulfate concentration gradually decreases due to reduction. Sulfate is reduced to obtain low-valent sulfide, which is an electron donor for ammonium nitrate nitrogen. This process accelerates the accumulation of ammonium nitrogen in the estuary water body caused by the ammonium nitrate nitrogen [5-8]. Laverman et al. [9] studied the hydrodynamic response mechanism of the nitrate ammonium ammonium microbial community. They observed that as the salinity increases, the nitrate ammonium ammonium microbial community...
ammonium reaction rate is 35 nmol/cm$^3$/h higher than in a freshwater environment. In the simulation experiment of nitrogen cycle in sediment water and soil system in the saltwater-freshwater confluence area. Gidlin et al. [10] monitored the respiration of estuary sediments, the nutrient flux at the sediment-water interface, denitrification and nitrification rates in the sediments of the Parker estuary in the United States. The results showed that: The nutrient flux at the water-soil interface of the sediment is mainly in the form of ammonia. Ammonia flux has a high correlation with salinity. The seasonal change pattern of ammonium nitrate nitrogen is consistent with the time-scale change of salinity. Other environmental factors in the estuary environment accompanied by tidal cycle changes include oxidation-reduction potential (ORP), water temperature, pH, phosphate, sulfate, and soluble nitrogen in the water. We have reported research abroad[11], and concluded that nitrate nitrogen ammonium is easy to occur in an environment with low redox potential and high water temperature, and the impact of other environmental factors has not been involved[11,12]. In China, there is no report on the research work on the influence of environmental factors in the estuary on the ammonium nitrate nitrogen. In this study, combined with the environmental characteristics of the Pearl River Estuary, the rate of nitrate ammonium ammonium / anammox / denitrification was studied in the anaerobic environment of the sediment system. The results of this study will explore the ultimate fate of nitrogen in the water ecological environment in the estuary area, and provide technical guidance for the effectiveness evaluation of water ecological restoration technologies in tidal areas and the macro-range setting of technical parameters.

2. Study area
Huangmao Sea is located in the west of the Pearl River Estuary, between 112º56’~113º15’ east longitude and 21º52’30”~22º52’30” north latitude. Huangmao Sea is connected to Yamen and Hutiaomen, and is connected to Tanjiang and Xijiang. Huangmao Sea is shaped like a horn, and it is the main channel for flood discharge and tide absorption. Therefore, the safety of water quality in Yamen is very important, and it determines the sustainable development of the water area as hydrodynamic environment, water ecology and biodiversity.
XiNan Yong is located at the lower reaches of the Beijiang River, passing through Foshan and Guangzhou. The starting point of XiNan Yong is in the Sanshui District of the Southwest Gate. XiNan Yong has a total length of 41.46km. XiNan Yong is an important waterway from Foshan to Guangzhou, and it is also an important tidal reach for aquatic ecological habitat. The water quality of XiNan Yong has been in Class V water quality (GB3838-2002) for a long time. The existence of the harsh environment reduces the standard of living for the surrounding people. Therefore, we are very deliberate and worthwhile to do this work. It can provide a basis for solving nitrogen and phosphorus pollution in the tidal reach. (Figure 1)

3. Materials and instrument reagents

3.1. Sample collection
Sample collection location: fishing port near Yamen Waterway in Taishan City, XiNanyong Riverway in Foshan City; collection samples: sediment and water. When collecting samples, we did the following methods: 1) Our equipment for collecting samples meets the requirements of Chinese standards and has been inspected to be qualified. 2) The sampling points and collected samples are in line with our plan. 3) The sample collection process is in compliance with Chinese standards. 4) After the sample is collected, it is smoothly transported to the testing room within the Chinese water quality preservation standard period.

3.2. Instrument reagents and Test conditions
The test adopts the continuous flow recording experiment method, and the laboratory windows are shaded by shutters. During the test, the water is not changed and no nitrogen-related chemicals are added. All sampling and parameter detection methods refer to the national general detection and analysis methods, pH meter method (detection limit is 0.01), salinity adopts salinometer method (detection limit is 0.01NTU), water temperature adopts thermometer method, and ammonia nitrogen adopts water quality Determination of ammonia nitrogen Nessler's reagent spectrophotometric method (HJ 535-2009), nitrate nitrogen using water quality determination of nitrate nitrogen UV spectrophotometry (HJ/T 346-2007), nitrite nitrogen using water quality nitrite nitrogen Determination of spectrophotometry (GBT 7493-1987); Determination of total nitrogen by water quality Determination of total nitrogen by alkaline potassium persulfate digestion UV spectrophotometry (HJ 636-2012), the key test methods are described below: Columnar plexiglass cylinder with a diameter of 15 cm, corresponding size sealing plug, water inlet pipe, peristaltic pump, water inlet storage tank, outlet pipe, water outlet storage tank, designed filling sediment and water gap ratio (5:1), filling peristaltic pump Collect the bottom water body circularly. After running for 24 hours, the entire system is stable. Add 1mg/L $^{15}$NO$_3$ ($^{15}$N content greater than 99%), continue to incubate for 12 hours to stabilize the flow state, and then observe for 72 hours. Take samples of the inlet and outlet every 8 hours, and take 3 samples for each sampling Parallel samples, continuous flow culture is completed, (each sediment column sample needs to take 120 samples, of which 60 water samples are stored in 15ml glass bottles with stoppers and 310ul 50% ZnCl$_2$. All water samples should be tested within 6h after sampling N$_2$/Ar, O$_2$/Ar, $^{28}$N$_2$, $^{29}$N$_2$, $^{30}$N$_2$; Membrane sampling mass spectrometer completed.

4. Results and discussion

4.1. DNRA results trend
Establish a laboratory simulated culture flow experimental device. We will connect the bottom seawater sample container and sediment sample, and use a peristaltic pump (Rainin RP-1) to circulate the water sample and control the flow rate. We put water samples and sediments in different environments as planned. We realized the continuous flow of water at the sediment-water interface in the column sample driven by the peristaltic pump. Our goal is the biogeochemical characteristics of the sediment-water interface in situ. The schematic diagram of the device is shown in Figure 2. The
simulated tidal channel is used as the research water body, and the dissolved oxygen of the experimental device (aeration rate 3.5L/min and no aeration), salinity (0.48~11.3 PPT), temperature (high temperature, normal temperature and low temperature), oxidation-reduction potential (50mV and 350mV), detection of nitrate nitrogen, nitrite nitrogen, ammonia nitrogen, reactive nitrogen and isotope ammonia nitrogen ($^{15}$NH$_4^+$) Concentration changes. The results is Table 1-Table 3.

**Figure 2.** Experimental setup.

**Table 1.** Changes in ammonia N in different environmental conditions.

| Condition     | 0h  | 24h  | 48h  | 72h  | 96h  | 120h | 144h | 192h | 264h |
|---------------|-----|------|------|------|------|------|------|------|------|
| Salinity0.48  | 10.3| 1.01 | 5.31 | 0.835| 0.740| 0.772| 0.21 | 0.12 | 0.19 |
| Salinity0.96  | 10.5| 1.51 | 1.18 | 0.642| 0.652| 0.627| 0.23 | 0.24 | 0.10 |
| Salinity3.68  | 11.1| 5.57 | 6.17 | 5.73 | 3.71 | 0.882| 0.11 | 0.13 | 0.22 |
| Salinity4.8   | 11.4| 4.73 | 6.28 | 6.61 | 6.12 | 2.02 | 0.94 | 0.07 | 0.24 |
| Salinity11.3  | 10.5| 5.25 | 6.41 | 7.48 | 6.99 | 6.83 | 4.09 | 1.65 | 0.26 |
| High ORP      | 8.78| 3.59 | 2.23 | 1.57 | 1.73 | 0.632| 0.17 | 0.11 | <0.025|
| Low ORP       | 10.1| 6.68 | 6.53 | 5.15 | 6.30 | 6.61 | 3.64 | 1.42 | 0.53 |
| High temperature | 10.7| 1.15 | 1.12 | 0.875| 0.678| 0.703| 0.430| 0.250| 0.110|
| Low temperature | 8.50| 3.75 | 4.81 | 4.89 | 5.82 | 5.69 | 2.27 | 5.53 | 3.06 |
| High DO1      | 7.47| 0.718| 0.708| 0.707| 0.750| 0.683| 0.30 | 0.17 | 0.04 |
| High DO2      | 5.25| 0.745| 1.04 | 0.632| 0.668| 0.598| 0.26 | <0.025| 0.09 |
| Low DO1       | 8.59| 6.23 | 4.89 | 5.15 | 2.99 | 0.582| <0.025| 0.16 | 0.06 |
| Low DO2       | 6.79| 5.49 | 6.61 | 5.82 | 3.14 | 0.540| 0.09 | <0.025| <0.025|

**Table 2.** Changes in Nitrite N in different environmental conditions.

| Condition     | 0h  | 24h  | 48h  | 72h  | 96h  | 120h | 144h | 192h | 264h |
|---------------|-----|------|------|------|------|------|------|------|------|
| Salinity0.48  | 0.51| 0.28 | 0.83 | 0.16 | 0.03 | 0.09 | 0.010| 0.016| 0.023|
| Salinity0.96  | 0.51| 1.02 | 0.78 | 0.37 | 0.01 | 0.01 | 0.036| 0.020| 0.0027|
| Salinity3.68  | 0.39| 0.90 | 0.77 | 1.66 | 3.19 | 0.82 | 0.058| 0.053| 0.051|
| Salinity4.8   | 0.39| 0.67 | 0.11 | 0.88 | 1.55 | 3.99 | 1.789| 0.038| 0.069|
| Salinity11.3  | 0.36| 0.39 | 0.37 | 0.43 | 0.50 | 0.92 | 0.766| 1.870| 1.588|
| High ORP      | 1.03| 1.26 | 1.85 | 2.93 | 0.39 | 0.02 | 0.060| 0.033| 0.032|
| Low ORP       | 0.61| 0.57 | 0.99 | 0.62 | 0.51 | 2.36 | 1.284| 1.277| 0.062|
| High temperature | 0.54| 1.70 | 3.32 | 1.42 | 0.07 | 0.08 | 0.06 | 0.10 | 0.07 |
| Low temperature | 0.39| 0.44 | 0.35 | 0.36 | 0.35 | 0.36 | 0.175| 0.171| 0.276|
| High DO1      | 0.58| 0.08 | 0.10 | 1.40 | 0.31 | 0.02 | 0.041| 0.026| 0.021|
| High DO2      | 0.49| 0.20 | 2.11 | 2.37 | 1.26 | 0.05 | 0.667| 0.032| 0.028|
| Low DO1       | 0.41| 0.68 | 0.68 | 1.22 | 2.48 | 2.82 | 0.083| 0.024| 0.024|
| Low DO2       | 0.41| 0.82 | 0.82 | 1.66 | 3.24 | 1.21 | 0.008| 0.105| 0.016|
Table 3. Changes in Nitrate N in different environmental conditions.

|          | 0h  | 24h | 48h | 72h | 96h | 120h | 144h | 192h | 264h |
|----------|-----|-----|-----|-----|-----|------|------|------|------|
| Salinity0.48 | 5.65 | 9.10 | 8.70 | 9.15 | 9.15 | 9.20 | 7.65 | 7.62 | 7.77 |
| Salinity0.96 | 4.90 | 9.20 | 9.05 | 9.45 | 8.90 | 9.45 | 7.83 | 8.03 | 8.03 |
| Salinity3.68 | 3.90 | 4.95 | 4.20 | 5.35 | 6.45 | 9.45 | 6.90 | 6.73 | 6.64 |
| Salinity4.8 | 3.60 | 3.95 | 3.65 | 3.75 | 3.60 | 6.60 | 6.43 | 7.86 | 7.72 |
| Salinity11.3 | 3.45 | 3.45 | 3.60 | 3.80 | 3.20 | 3.30 | 2.84 | 3.68 | 4.44 |
| High ORP | 7.80 | 7.75 | 8.75 | 8.35 | 3.80 | 9.80 | 7.98 | 8.09 | 8.15 |
| Low ORP | 4.60 | 4.10 | 4.50 | 4.40 | 5.05 | 3.00 | 1.03 | 4.32 | 6.27 |
| High temperature | 4.85 | 9.00 | 9.00 | 8.65 | 8.90 | 9.50 | 8.70 | 9.10 | 9.90 |
| Low temperature | 4.30 | 5.55 | 6.55 | 6.10 | 5.00 | 5.05 | 3.74 | 3.60 | 4.25 |
| High DO1 | 6.75 | 9.45 | 9.40 | 9.10 | 9.00 | 9.55 | 7.48 | 7.68 | 7.70 |
| High DO2 | 5.10 | 8.95 | 8.10 | 7.80 | 8.20 | 8.80 | 7.56 | 7.22 | 7.44 |
| Low DO1 | 4.10 | 4.40 | 3.75 | 3.90 | 4.50 | 7.35 | 7.53 | 6.80 | 7.52 |
| Low DO2 | 4.05 | 4.75 | 4.55 | 6.15 | 7.00 | 10.7 | 8.49 | 8.46 | 8.64 |

4.2. Analysis and evaluation

Figure 3. Salinity effects on ammonia nitrogen (A), nitrite nitrogen (B), nitrate nitrogen (C) and DNRA rate (D).

4.2.1. Changes of ammonia N, nitrate N and nitrite N under different salinity

We dynamically simulated the water environment in the study area, and the experimental conditions were salinity 0~11.3 PPT (initial concentrations were 0, 0.48, 0.96, 3.39, 4.8, 11.3 PPT), and the initial dissolved oxygen was 4.3 mg/L in a continuous flow device. The concentration of ammonia nitrogen is 10.3~11.4 mg/L, the concentration of nitrite nitrogen is 0.36~0.51 mg/L, the concentration of nitrate nitrogen is 3.45~5.65 mg/L, and the concentration of CODMn is 3.84~4.78 mg/L. The sampling time is 0h, 24h, 48h, 72h, 96h, 120, 144h, and the water flow rate is 200ml/min. We have the following conclusions. Under different salinity conditions, both ammonia nitrogen and reactive nitrogen have a downward trend (Figure 3). In the study of different salinities, nitrate nitrogen has an upward trend. We suspect that it may be related to the higher activity of nitrifying bacteria than denitrifying bacteria. As the salinity becomes higher, the rate of increase in the concentration of nitrate nitrogen is lower. Salinity has a great influence on the isotope of ammonia nitrogen generated by DNRA. The salinity is
0 and 0.48 PPT. The rate of ammonia nitrogen generation is the highest at 96 hours, which is 5.33 nmol/(g h), but it drops significantly after 144 hours.

4.2.2. Changes of ammonia N, nitrate N and nitrite N under dissolved oxygen As the incubation time increases (Figure 4), under the conditions of high dissolved oxygen and low dissolved oxygen, ammonia nitrogen, nitrate nitrogen and nitrite nitrogen will eventually decrease. In high dissolved oxygen, nitrate nitrogen increases significantly after 24 hours. The reason is that the nitrifying bacteria in the environment are related to aerobic bacteria. In the non-aerated state, it is more beneficial to generate ammonia nitrogen through DNRA.

![Figure 4](image1.png)

**Figure 4.** Dissolved oxygen effects on ammonia nitrogen (A), nitrite nitrogen (B), nitrate nitrogen (C) and DNRA rate (D).

![Figure 5](image2.png)

**Figure 5.** Temperature effects on ammonia nitrogen (A), nitrite nitrogen (B), nitrate nitrogen (C) and DNRA rate (D).
4.2.3. Changes of ammonia N, nitrate N and nitrite N under Temperature

In this experiment, the temperature is set as low temperature between 4-10°C, normal temperature during the test period is 22-27°C, and high temperature is 32-34°C. From the analysis of the concentration change trend of ammonia nitrogen, nitrate nitrogen and nitrite nitrogen, at low temperature in the state, the microbial activity of nitrogen conversion is low, and the change trend of nitrogen is small. The ammonia nitrogen accumulated through DNRA also fluctuates greatly at high temperature. (Figure 5)

4.2.4. Changes of ammonia N, nitrate N and nitrite N under Oxidation-reduction potential

In different ORP experiments, we tested the high ORP of 400mV, the medium ORP of 148-216mV, and the low ORP of 40-80mV. To increase ORP we use potassium perman ganate solution, and to reduce ORP we use sodium sulfite. Solution proceed. The results of the study showed that ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen showed a downward trend, increasing with the cultivation time (Figure 6).

Figure 6. ORP effects on ammonia nitrogen (A), nitrite nitrogen (B), nitrate nitrogen (C) and DNRA rate (D).

We adopted the following steps in our design. First, we selected Yamen as one of our research areas. As Yamen serves as the entrance to the sea of Zhuhai and Jiangmen, it plays an important role in the economic development of the two places. The sustainable development of Maohai can also promote the sound and healthy development of the Greater Bay Area. Since XiNnang Yong is an important transportation link between Guangzhou and Foshan, and Guangzhou and Foshan are the main core areas for the construction of the Greater Bay Area, the improvement and promotion of XiNnang Yong's water quality is very important. So we chose XiNnang Yong as another research area. Secondly, we assessed the current state of the water environment in the two places before sampling, and sampled and saved the samples in accordance with the water environment sampling specifications. Finally, we continuously input saturated oxygen to distinguish different dissolved oxygen in the sample water through an oxygenator and without any means. We sampled 0h, 24h, 48h, 72h, 96h, 120h, 144h, 192h, 264h according to the design. Time sampling, we determined that salinity, temperature, ORP did not change in the experiment. We distinguished 6 different salinity environments by adding different concentrations of sodium chloride to our experimental samples. According to our design time sampling, we determined that the dissolved oxygen, temperature and ORP did not change in the experiment. The result of the experiment is that the DNRA rate is the
fastest in the low concentration environment, especially in the 0-0.48ppt environment. We controlled three different temperature environments of 4-10 °C, normal temperature 24-27 °C and high temperature 32-34 °C through the constant temperature control room. According to our design time sampling, we determined that the dissolved oxygen, salinity and ORP did not change in the experiment of. We have obtained that at high temperature, the fluctuation of nitrogen conversion is very large, and the activity is very low at low temperature. We used 1M potassium permanganate to increase the ORP in the experimental water, and controlled it above 400mV as the high ORP experimental object, while the medium ORP object was the water without any treatment. Our family's 1M sodium sulfite reduced the ORP in the water body and made the ORP lower than 80mV as a low ORP test object. Sampling according to our design time, we determined that the dissolved oxygen, salinity and temperature did not change in the experiment. Experimental results show that in the case of high ORP, the rate of DNRA production is much lower than that of low ORP.

The water environment in the estuary area is complex and changing. After the above series of experiments, we have preliminarily judged that 0-0.48ppt at an appropriate salinity, increased dissolved oxygen, appropriate temperature, and low-high ORP environment are beneficial to the estuary Active nitrogen output from the tidal reach.

5. Conclusions
We have done tests for different salinities, different dissolved oxygen, different ambient temperatures, different ORPs, etc. We have conducted continuous flow device experiments, and we have made an evaluation of the changing trend. Regarding ammonia nitrogen, nitrate nitrogen and nitrite nitrogen, the change trend of nitrogen is small, and the change of DNRA generation rate is relatively large at high temperature. Ammonia nitrogen, nitrate nitrogen, and reactive nitrogen show a downward trend and increase with time. DNRA is easier to generate, and low ORP is compared with high ORP. Based on these conclusions, we can combine the information of the existing water ecological restoration technology to provide a theoretical basis for the accurate evaluation of the denitrification effect and the effectiveness of the water ecological restoration technology in the Pearl River Estuary and other similar estuaries.

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