

Article

Coupling Imports of Dissolved Inorganic Nitrogen and Particulate Organic Matter by Aquaculture Sewage to Zhangjiang Estuary, Southeastern China

Shuang He ¹, Ta-Jen Chu ^{1,2} , Zhiqiang Lu ^{1,2,*} and Danyang Li ^{1,2,*}

¹ Fisheries College, Jimei University, Jimei District, Xiamen 361021, China; 202121063039@jmu.edu.cn (S.H.); chutajen@gmail.com (T.-J.C.)

² State Key Laboratory of Mariculture Breeding, Jimei University, Xiamen 361021, China

* Correspondence: zqlu@jmu.edu.cn (Z.L.); lidanyang@jmu.edu.cn (D.L.)

Abstract: Estuary ecosystems serve as crucial connectors between terrestrial and marine environments, thus playing vital roles in maintaining the ecological balance of coastal marine ecosystems. In recent years, the eutrophication in estuaries caused by aquaculture sewage has been revealed, highlighting the necessity to understand its influence on the nutrient conditions and carbon storage of estuaries. In this study, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ were used to indicate the contribution of aquaculture-derived sewage to dissolved inorganic nitrogen in Zhangjiang Estuary, and $\delta^{13}\text{C}$ and C:N ratio were used to reveal its effects on the particulate organic matter. The major results are as follows: (1) Aquaculture water contributed 62~86% and 60~100% of the total nitrate and ammonium in Zhangjiang Estuary, respectively, and the drainage periods of the cultured species has a great influence on the content and composition of dissolved inorganic nitrogen. (2) Aquaculture water was also the major source of particulate organic matter (24~33% of the total content) here, most of which may be derived from crab ponds. (3) The imports of nutrients by aquaculture water may potentially regulate particulate organic matter in Zhangjiang Estuary by promoting the growth of phytoplankton and zooplankton. Our study revealed the coupling effects of aquaculture activities on the nitrogen and carbon storage in an estuarine ecosystem. It also indicates that isotopes may be efficient in the monitoring of a coastal environment, which may further aid the management of inshore cultivation.

Keywords: dissolved inorganic nitrogen; particulate organic matter; stable isotopes; aquaculture; Zhangjiang Estuary



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1. Introduction

Due to the increasing world population, the demand for seafood has continued to increase in recent decades, thus stimulating the expansion of aquaculture. Up until 2022, global fisheries and aquaculture production surged to 223.2 million tonnes, with 62% harvested in marine areas and 31% from inland waters [1]. China has the world's largest mariculture industry, accounting about 36% of the global total aquaculture production [1]. In southeastern China, aquaculture ponds are usually set around estuaries, causing a wide variety of environmental problems, among which the contaminants of water were the primary focus [2–4]. As an estuary ecosystem serves as the link between terrestrial and marine environments, it plays a vital role in maintaining the ecological balance of coastal marine ecosystems [5,6]. Thus, it is necessary to reveal the long-time impacts of aquaculture pollutants on the environment of estuaries.

Aquaculture pollutants mainly consist of a large amount of dissolved inorganic nitrogen (DIN) and organic matters derived from excessive feeds, residual baits, and animal wastes [7]. The exportation of DIN and organic matter from aquaculture ponds may affect regional environment by the following paths: (1) The large amounts of nutrients may stimulate the production of algae [8–10] and hence increase the amount of biogenic organic

matter, which is usually characterized by higher bio-availability [11,12]. (2) The residual baits and the animal wastes in aquaculture waters and sediments may directly increase the amount of particulate organic matter (POM) in the surface of the estuary [8,13]. (3) The simultaneous increases of the contents and the bio-availability of organic matter, as well as the contents of DIN, may accelerate the coupling cycles of carbon (C) and nitrogen (N) in such areas [8,9,11]. The decomposition of the imported organic matter and algae residues may deplete water oxygen, thus posing a threat to fish, shellfish, and other marine organisms [14,15]. Additionally, the coupling imports of DIN and organic matter may further promote heterotrophic microbial N removal here, thus increasing the release of N_2O , the by-product of microbial N removal [9,16–18]. It has been reported that N_2O is approximately 300 times more potent than CO_2 in holding heat in a 100 year timescale [19]. Therefore, it is an urgent need to reveal the coupling effects of aquaculture water on DIN and organic matter in the estuary environment. However, until now, quantifying the sources of DIN and organic matter to the river and linking these sources to specific land uses and point-sources of pollution has been less reported. As most of the residual feeds and animal baits are in particulate forms, the source analysis of DIN and POM based on isotopic characteristics ($\delta^{15}\text{N}$ for NO_3^- , NH_4^+ and PN, $\delta^{18}\text{O}$ for NO_3^- , and $\delta^{13}\text{C}$ for POC) may provide help in this region [20–24].

Zhangjiang Estuary (117.40–117.50° E, 23.88–23.93° N) is a semi-enclosed estuary in southeastern China [25]. The climate in this area is subtropical with an annual precipitation of 1680 mm and seasonal mean temperatures ranging from 15.9 to 28.8 °C. The eutrophication caused by the imports of human-derived sewage has been reported here for several years [13,16,25,26], and thus it may provide an ideal site to reveal the effects of aquaculture water on DIN and POM in estuary. During our previous study in December 2021, the IsoSource mixing model was used to reveal the sources of POM here, indicating that sewage was the major source of POM [27]. However, it has been suggested that the Mixsir mixing model may be more suitable for the source analysis of organic matter or nutrients based on isotopic values [28,29]. Therefore, in this study, the Mixsir mixing model was employed for the DIN and POM source analysis, and the major source of POM in December 2021 was also re-analyzed. The main aims of this study are as follows: (1) to observe the distribution characteristics of nitrate (NO_3^-), ammonium (NH_4^+), and POM in Zhangjiang Estuary; (2) to observe the specific contribution of aquaculture water to the total DIN and POM here, as well as reveal its possible regulators. Our results will provide new insights in the monitoring and management of the coastal environment and offer more information regarding the sustainable development of aquaculture.

2. Materials and Methods

2.1. Sample Collection

Field sampling was conducted in May 2023. Water samples were collected at 8 stations, among which S1 was set at the shrimp ponds, and S2~S8 were set from upstream to downstream of Zhangjiang Estuary (Figure 1). In this study, S1 and S2 were set to observe the isotopic values of DIN and POM derived from aquaculture water and freshwater. Another station was set at the up in the neighboring Dongshan Bay to observe the isotopic values of DIN and POM derived from seawater. Surface water (≈ 0.5 m) at all stations was collected by Niskin bottles in one day to observe the contents of NO_3^- , NH_4^+ , POC, PON, and their isotopic values ($\delta^{15}\text{N}$ for NO_3^- , NH_4^+ , and PN; $\delta^{18}\text{O}$ for NO_3^- ; and $\delta^{13}\text{C}$ for POC). All samples were treated with ZnCl_2 after collection and kept at 4 °C until analysis [20,22]. Basic parameters, including temperature (T), salinity (S), the contents of dissolved oxygen (DO), phycoerythrin, and chlorophyll *a* (Chl *a*) were measured in situ using YSI 6600 multi-probe sensors (Yellow Springs Instrument Co., Yellow Springs, Ohio, OH, USA). Apart from samples in May 2023, the source of POM in December 2021 was re-analyzed by the Mixsir mixing model. The details of stations in December were shown in our previous study [27].

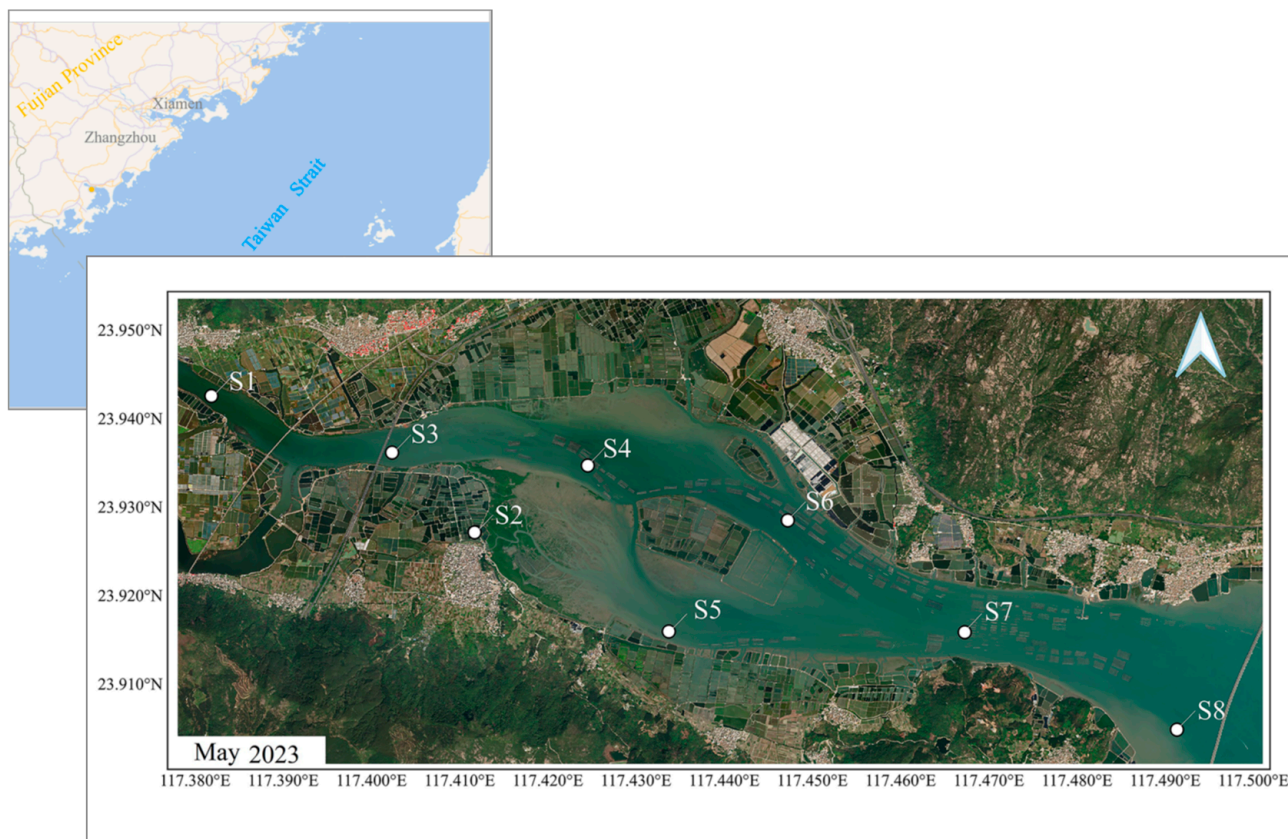


Figure 1. The sampling stations in Zhangjiang Estuary in May 2023.

2.2. Contents and Isotopic Characteristics of Nitrate and Ammonium

Water samples were filtered by precombusted (450 °C, 4 h) GF-75 filters (47 mm, 0.3 µm pore size). Concentrations and $\delta^{15}\text{N}$ of NO_3^- and NH_4^+ were determined using a MAT 253 gas stable isotope ratio mass spectrometer interfaced with Gas-Bench II (Thermo Fisher, Waltham, MA, USA). The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- were measured using the “denitrifier method” [30,31], with the analytical precisions of the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ less than 0.5‰ and 1‰, respectively. The $\delta^{15}\text{N}$ of NH_4^+ were determined following the methods described by Koba et al. [32]. In brief, NH_4^+ was concentrated on the glass fiber filter using the diffusion method and digested to NO_3^- using persulfate [33–35]. After that, the $\delta^{15}\text{N}$ of the converted NO_3^- from NH_4^+ was measured using the denitrifier method [30,31]. The analytical precisions for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ were less than 0.2‰ and 1‰, respectively.

2.3. Particulate Organic Matter

The large detritus in the water samples was removed with a 200 µm mesh, and then the particulate organic matter (POM) in water samples was collected by filtering 4 L of water through GF-75 filters (47mm, 0.3 µm pore size). The filters were previously combusted for 4 h at 450 °C to remove organic matter. All water samples were then filtered through a 200 µm mesh sieve to remove large detritus. Then, water samples (without large detritus) were filtered by the precombusted GF-75 filters to collect particulate organic carbon (POC) and nitrogen (PN). The GF-75 filters were quickly washed using Milli-Q water following filtration and frozen at −20 °C until analysis. In the laboratory, the filters were treated with HCl vapor (48 h) to remove inorganic carbon and then dried at 60 °C. The content of POC, PN, and the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of POM ($\delta^{13}\text{C}_{\text{POM}}$, $\delta^{15}\text{N}_{\text{POM}}$) were measured using a Finnigan Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) interfaced with a Carlo Erba NC 2500 elemental analyzer (CE Instruments Ltd., England, United Kingdom). The analytical precision was <0.2‰ in this study.

2.4. Statistical Analysis

Pearson's correlations analysis was conducted using the Statistical Package for Social Sciences program (version 19.0). The source analysis of nitrate and POM based on the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- and the $\delta^{13}\text{C}$ and C:N ratio of POM were conducted by the Mixsir mixing model, using R (4.3.3 version).

3. Results

3.1. Physicochemical Parameters

The basic physicochemical parameters of the surface water in Zhangjiang Estuary (S2 to S8) are shown in Table 1. Surface temperatures (T) and surface salinity (S) in Zhangjiang Estuary ranged from 23.7 to 25.5 °C (24.4 ± 0.7 °C, $n = 7$) and 12.7 to 30.1‰ (21.4 ± 6.4 ‰, $n = 7$). Generally, surface T decreased from upstream to downstream, and surface S had the opposite distribution. The content of dissolved oxygen (DO) ranged from 6.6 to 7.4 mg L⁻¹ (6.9 ± 0.3 mg L⁻¹, $n = 7$), with the maximum observed at S2 and the minimum at S4. The contents of phycoerythrin and Chl *a* ranged from 0.6 to 10.1 µg L⁻¹ (2.9 ± 3.4 µg L⁻¹, $n = 7$) and 2.0 to 6.4 µg L⁻¹ (3.5 ± 1.5 µg L⁻¹, $n = 7$), respectively, with the maximum observed at S2 and S4, and the minimum at S5 and S7, respectively. When compared with the values observed in December 2021 [27], the contents of DO, phycoerythrin, and Chl *a* were obviously higher in May 2023 (Figure 2).

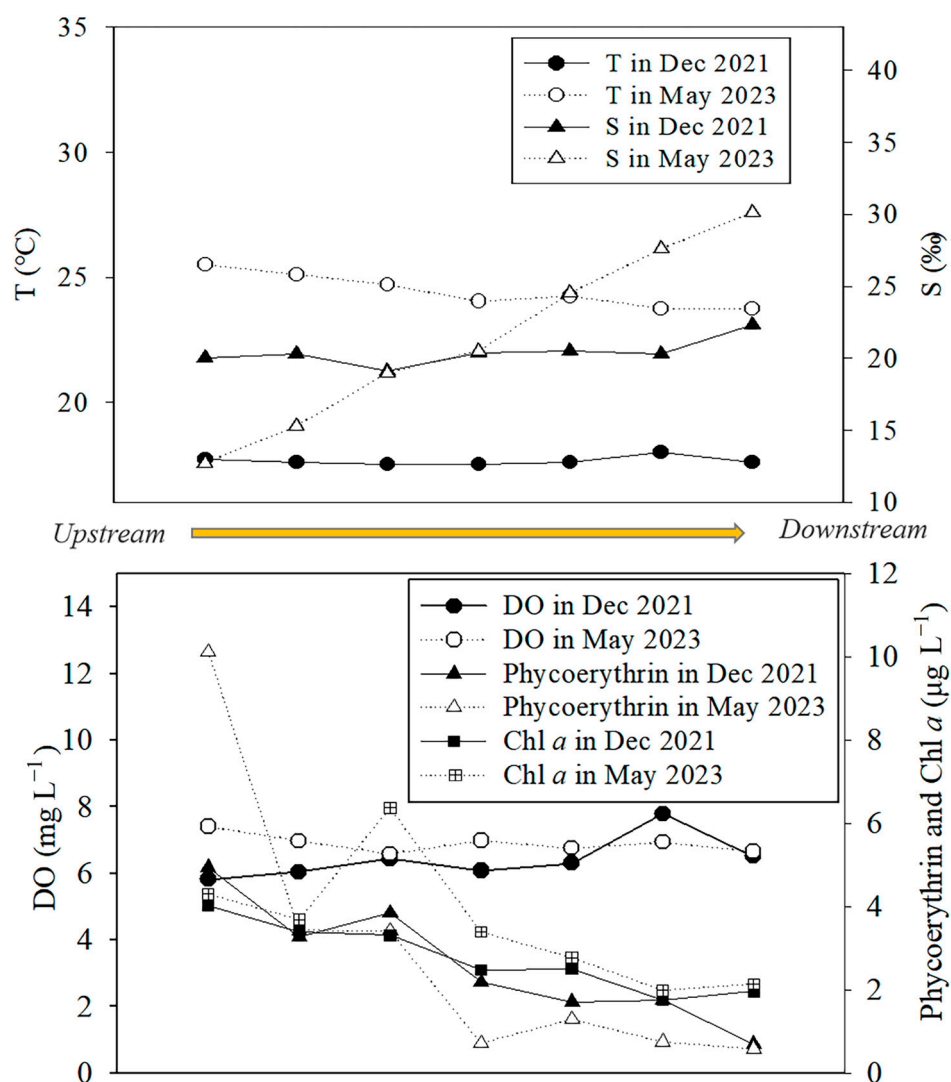


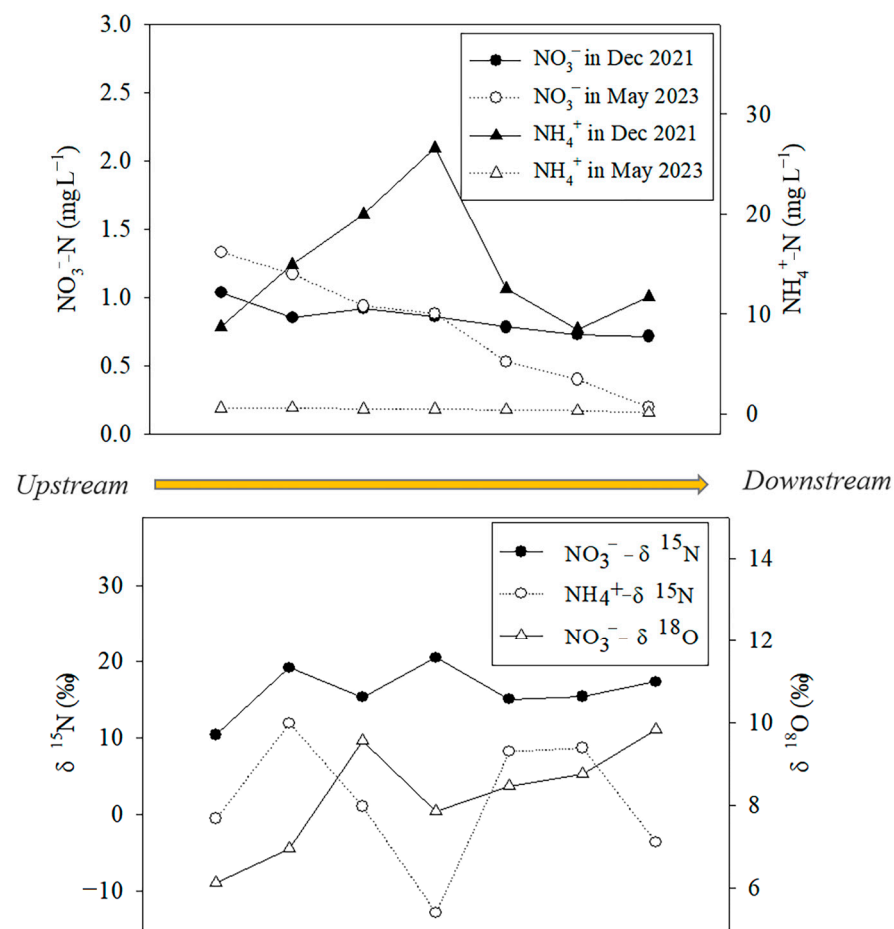
Figure 2. Comparison of physicochemical parameters observed in May 2023 and December 2021.

Table 1. The physicochemical parameters in Zhangjiang Estuary in May 2023.

Station	T (°C)	S (‰)	DO (mg L ⁻¹)	Phycoerythrin (µg L ⁻¹)	Chl <i>a</i> (µg L ⁻¹)
S2	25.5	12.7	7.40	10.1	4.30
S3	25.1	15.3	6.97	3.43	3.69
S4	24.7	19.0	6.58	3.42	6.37
S5	24.0	20.5	6.98	0.72	3.39
S6	24.2	24.5	6.75	1.29	2.77
S7	23.7	27.6	6.93	0.75	1.99
S8	23.7	30.1	6.65	0.58	2.14

3.2. Concentration and Isotopic Values of Nitrate and Ammonium

The concentrations of NO_3^- -N and NH_4^+ -N in May 2023 ranged from 0.20 to 1.33 mg L^{-1} ($0.78 \pm 0.42 \text{ mg L}^{-1}$, $n = 7$) and 0.13 to 0.65 mg L^{-1} ($0.44 \pm 0.17 \text{ mg L}^{-1}$, $n = 7$), respectively. Our previous study indicates that the content of NO_2^- -N in the study area was nearly under the detection limits [27], and thus it was not considered in this study. The content of total dissolved inorganic nitrogen (DIN) was calculated by summing the contents of NO_3^- -N and NH_4^+ -N, which ranged from 0.32 to 1.90 mg L^{-1} ($1.22 \pm 0.58 \text{ mg L}^{-1}$, $n = 7$). NO_3^- -N was the major type of DIN, accounting for 55~72% of the total DIN (Figure 3). Generally, the concentrations of NO_3^- -N and NH_4^+ -N both decreased from S2 to S8 (Figure 3). Compared to the results in December 2021, the contents of NO_3^- -N and NH_4^+ -N observed in May 2023 was lower, with relatively less spatial variation (Figure 3). Another difference was that the content of NH_4^+ -N in November 2021 was obviously high, which accounted for more than 90% of the total DIN at most stations [27].

**Figure 3.** The contents and isotopic values of nitrate and ammonium.

The $\delta^{15}\text{N}$ of NO_3^- and NH_4^+ and the $\delta^{18}\text{O}$ of NO_3^- were only determined in May 2023. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of NO_3^- ranged from 10.40 to 20.47‰ (16.16 ± 3.28 ‰, $n = 7$) and 6.13 to 9.83‰ (8.22 ± 1.34 ‰, $n = 7$), with the maximums observed at S3 and S4, respectively, and the minimums both observed at S2. The $\delta^{15}\text{N}$ of NH_4^+ ranged from 0.13 to 0.65‰ (0.44 ± 0.17 ‰, $n = 7$), with the maximum observed at S3 and the minimum at S8 (Table S1). Generally, there were no obvious characteristics for the distribution of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$.

3.3. The Contents and the Isotopic Characters of Particulate Organic Matter

The concentrations of POC and PN in May 2023 ranged from 7.8 to 57.6 $\mu\text{mol L}^{-1}$ (24.9 ± 16.3 $\mu\text{mol L}^{-1}$, $n = 7$) and 2.3 to 9.7 $\mu\text{mol L}^{-1}$ (5.5 ± 2.6 $\mu\text{mol L}^{-1}$, $n = 7$), respectively, with the maximum both observed at S2, and the minimum at S4 and S8, respectively (Figure 4a). The C:N ratio of POM ranged from 3.0 to 6.0 (4.3 ± 1.0 , $n = 7$), with the maximum observed at S2 and the minimum at S4 (Figure 4a). The $\delta^{13}\text{C}$ of POM in May 2023 ranged from -27.4 to -24.5 ‰ (-26.1 ± 1.0 ‰, $n = 7$), with the maximum observed at S6, and the minimum at S3 (Figure 4a). Compared to December 2021, the content of PN was higher in May 2023, while the content of POC and the C:N ratio were lower (Figure 4). The $\delta^{13}\text{C}$ of POM in May 2023 was similar to that observed in December 2021.

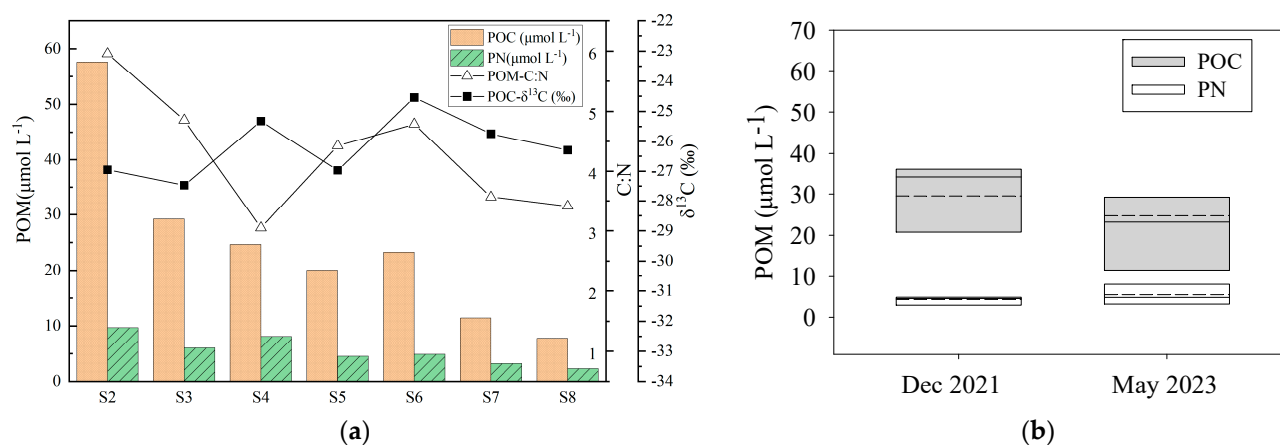


Figure 4. The contents and isotopic values of particulate organic matter: (a) the results in May 2023; (b) the comparison of POM content in December 2021 and May 2023.

4. Discussion

4.1. Aquaculture Sewage as the Major Source of Dissolved Inorganic Nitrogen

During our investigation, the content of DIN ($0.32\text{--}1.90$ mg L^{-1} , Table S1) was about five times higher than the reported value in the neighboring Dongshan Bay, which ranged from 0.12 to 0.35 mg L^{-1} [36]. The densely distributed aquaculture ponds around the study area may be the major reason for the high content of DIN here. The content and the composition of DIN exhibited obvious seasonal variations, with a notably higher content and contribution of NH_4^+ observed in December 2021. This discrepancy may be attributed to varying drainage periods of the aquaculture species cultivated in this region. The major cultured species in the study area include crab, clam, and shrimp [13]. The cultivation of crab was carried out entire the year, while that for shrimp and clam were carried out from May to November and October to July, respectively. Another notable distinction between the two sampling periods is that the sampling in November 2021 was conducted after the removal of the sediments in clam ponds, which typically are carried out in August–September [13]. The residues and the metabolic N of the cultured species primarily exist as $\text{NH}_4^+\text{-N}$ and dissolved organic nitrogen (DON) [37,38], and thus the exports of aquaculture water or sediments may significantly increase the content of $\text{NH}_4^+\text{-N}$ in the study area. Apart from NH_4^+ , aquaculture ponds may also be an important source of NO_3^- , as higher levels of NH_4^+ in aquaculture ponds may promote the microbial nitrification here, thus

producing more NO_3^- [37–41]. In this study, positive correlations between NO_3^- -N and T, DO, and NH_4^+ -N were observed in May 2023 (Figure 5a), indicating the possible active nitrification in Zhangjiang Estuary. Nitrifying bacteria prefer to utilize ^{14}N from the NH_4^+ pool, leading to a gradual enrichment from ^{14}N to ^{15}N in NO_3^- with the reduction of the NH_4^+ pool [42]. Thus, the areas with active nitrification are typically characterized by opposite variations in NO_3^- - $\delta^{15}\text{N}$ and the content of NH_4^+ -N, as well as the $\delta^{15}\text{N}$ of NH_4^+ [42,43]. However, such variation was not observed in the study area. This suggests that though microbial nitrification may be facilitated by relatively higher T and DO, along with the high availability of NH_4^+ in the study area, it seems not to be the major source of NO_3^- -N here. Thus, we propose that the positive correlation between NO_3^- -N and NH_4^+ -N (Figure 5b) may be mainly caused by their common major source, rather than active microbial nitrification. Due to the higher density of aquaculture ponds in the upstream of Zhangjiang Estuary, the decreasing S from downstream to upstream may reflect the increasing effects of aquaculture sewage on NO_3^- and NH_4^+ , and the negative correlation between NO_3^- -N and S can further support this (Figure 5a).

Previous investigation has indicated that the flux of the exported N derived from the aquaculture ponds in Zhangjiang Estuary was about $97\sim 714 \text{ Kg N ha}^{-1} \text{ yr}^{-1}$, with the maximum observed from the shrimp ponds [13]. Our previous study in December 2021, based on the statistical correlation between POM and DIN, indicated that human-derived sewage contributed 12.2% to NO_3^- and 100% to NH_4^+ . Much of the N-fertilized water enters into the surface streams and then is transported downriver to the estuary [39], thus causing many problems such as the bloom of algae, as well as oxygen deficit [9,13,40]. In addition, it may also promote the microbial removal of N and thus increase the release of N_2O [9]. Apart from N_2O , the microbial transformation of NO_3^- and NH_4^+ were also reported to be closely related with the emission of CH_4 [19]. As N_2O and CH_4 have been reported to be about 300 and 200 times more potent in holding heat than CO_2 [19], it is an urgent need to evaluate the specific effects of aquaculture sewage to the content and composition of DIN in the study area. The Mixsir mixing model based on the isotopic values of NO_3^- may provide help. In this study, the human-derived sewage was categorized into aquaculture, agriculture, and domestic sewage, and $\delta^{15}\text{N}$ - NO_3^- and $\delta^{18}\text{O}$ - NO_3^- were used to directly observe their contribution to NO_3^- . Though the $\delta^{15}\text{N}$ value of NH_4^+ was also observed, it is hard to trace its source by a single isotope, and thus the content of NH_4^+ derived from aquaculture was observed based on the statistical correlation between NO_3^- and NH_4^+ contents (Figure 5b). The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- derived from the above sources are presented in Table 2. As the largest exporting flux of N was observed from shrimp ponds [13], S1 was set at the shrimp pond to observe the isotopic values of NO_3^- derived from aquaculture ponds. Generally, the observed $\delta^{15}\text{N}$ - NO_3^- and $\delta^{18}\text{O}$ - NO_3^- at S3~S8 were closer to the cover range of aquaculture water (Figure 5c). Based on the Mixsir mixing model, the specific contribution of aquaculture water to the total NO_3^- was observed.

Table 2. $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate derived from different sources.

Source	$\delta^{15}\text{N}$ (‰)	$\delta^{18}\text{O}$ (‰)	References
Seawater	7.15	8.97	This study
Freshwater	10.40	6.13	This study
Aquaculture sewage	20.75	5.24	This study
Agriculture sewage	0.30	0.88	[24,44]
Domestic sewage	8.01	8.74	[45,46]

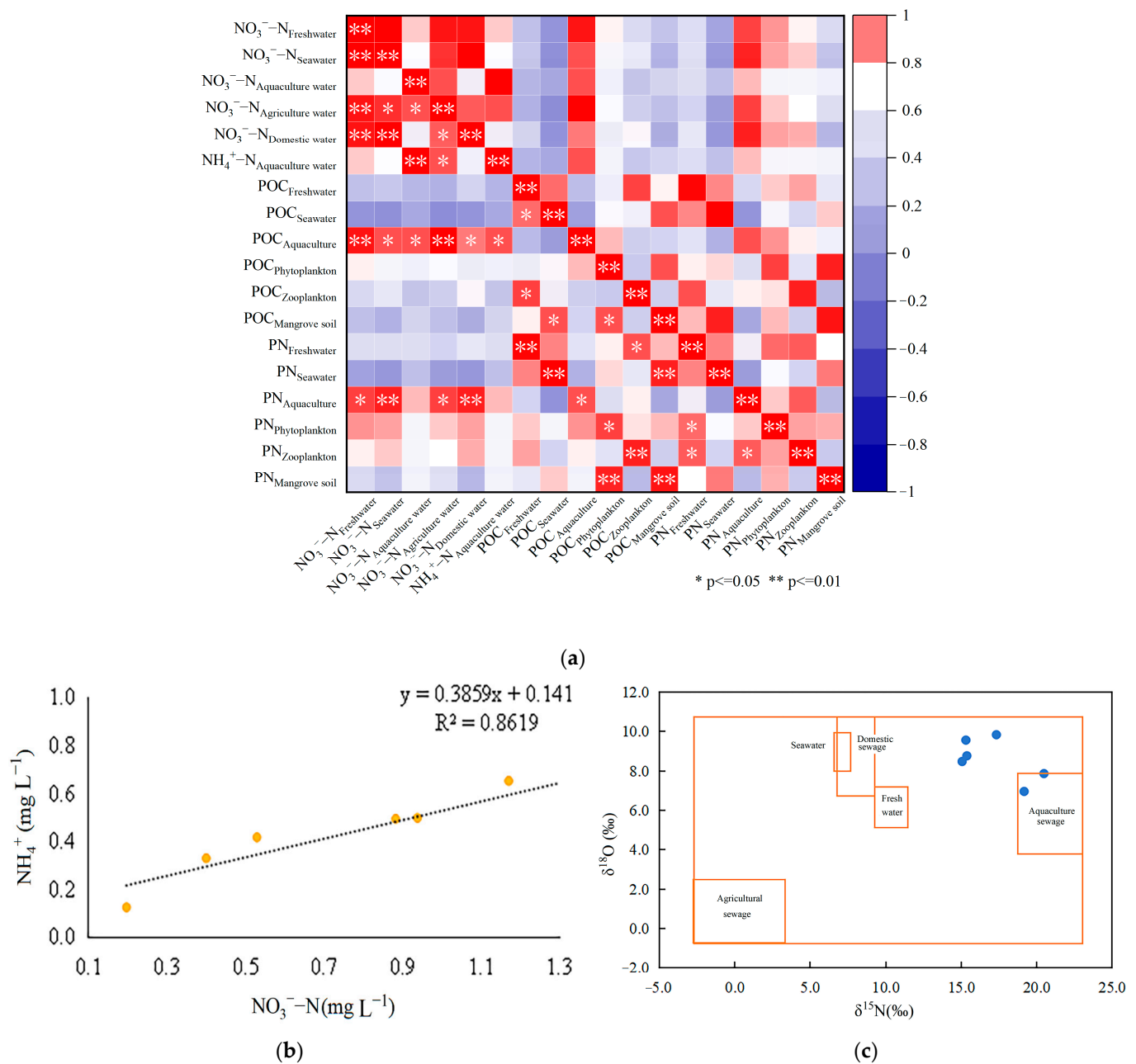


Figure 5. The content correlation and isotopic values of nitrate and ammonium in May 2023: (a) the correlation matrix of the observed parameters in May 2023; (b) the correlation between nitrate and ammonium contents; (c) the isotopic characteristics of nitrate and ammonium observed in May 2023, which was shown by the blue dot.

The major results are as follows (Figure 6a): (1) In May 2023, most of the NO_3^- in the study area was derived from aquaculture water, accounting for 62~86% ($71 \pm 9\%$, $n = 6$) of the total NO_3^- . (2) Domestic sewage had a similar contribution to seawater and freshwater, which accounted for 4~13% ($10 \pm 4\%$, $n = 6$), 4~12% ($9 \pm 3\%$, $n = 6$), and 4~10% ($7 \pm 2\%$, $n = 6$) of the total NO_3^- , respectively. (3) Agricultural water accounted for only 2~3% ($3 \pm 1\%$, $n = 6$) of the total NO_3^- . Compared to the results observed in December 2021 (8~31%), the contribution of aquaculture water to the total NO_3^- was higher. The most possible reason was that the nitrification in aquaculture ponds may be promoted by the relatively higher T in May 2023, thus exporting more NO_3^- [41–43]. Based on the correlation between NO_3^- and NH_4^+ (Figure 5b), the contents of NH_4^+ derived

from aquaculture sewage can also be observed, which ranged from 0.20 to 0.48 mg L⁻¹ (0.33 ± 0.11 mg L⁻¹, n = 6), accounting for 60% ~ 100% of the local NH₄⁺ (Figure 6b). Compared to the results in November 2021, the contribution of aquaculture to NH₄⁺ in May 2023 was lower. There are three possible reasons: (1) during the sampling in May 2023, the cultivation of shrimp was at the initial period, and thus the producing of NH₄⁺ was obviously less than that in November 2021 [13]; (2) the relatively higher T in May 2023 may also promote the conversion of N from NH₄⁺ to NO₃⁻, mainly by microbial nitrification [41–43], thereby increasing the flux of NO₃⁻ and decreasing the flux of NH₄⁺ from aquaculture ponds; (3) the sediment of clam ponds was typically washed out in August or September [13], which could increase the content of NH₄⁺. Such influence may last for months and thus cause significantly higher NH₄⁺ levels in December 2021. Conversely, the different methods for the source analysis of DIN may also be one of the reasons for the different results observed in December 2021 and May 2023, highlighting the needs of long-time monitoring of N-signal derived from aquaculture sewage.

Overall, the above results reveal the significant effects of aquaculture water on DIN in Zhangjiang Estuary. The drainage period of the cultured species here, as well as water temperature, may be the major regulator for the content and composition of DIN. The larger flux of drainage and the lower T in winter may cause the increasing content of NH₄⁺ in Zhangjiang Estuary, while the relatively higher T in summer may promote microbial removal of NH₄⁺. Furthermore, our results also indicate that stable isotopes may provide help in observing the specific effects of aquaculture water on the regional environments, which may be considered in the long-time monitoring and management of coastal aquaculture.

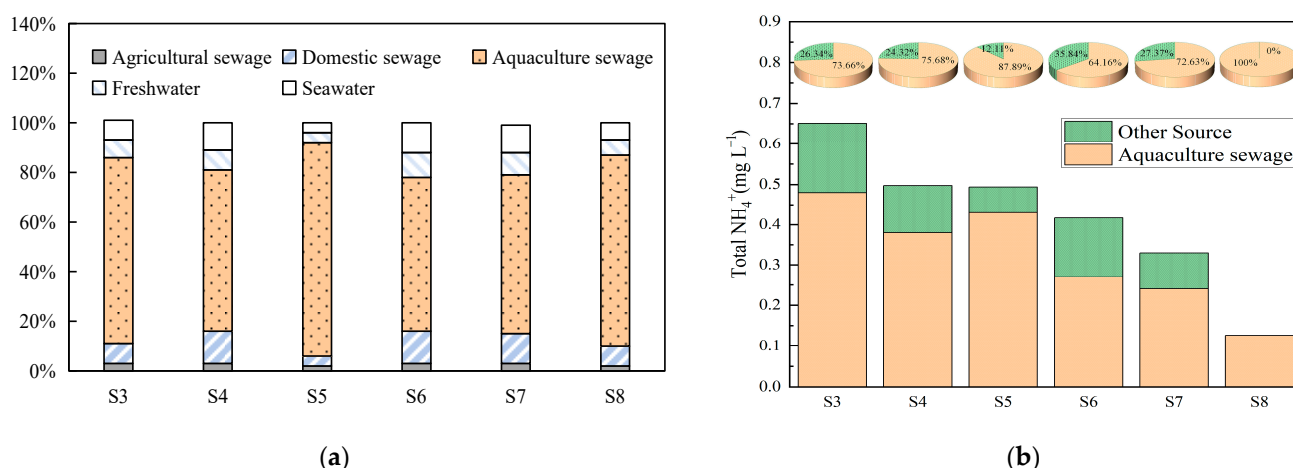


Figure 6. The significant contribution of aquaculture sewage to the total (a) nitrate and (b) ammonium in May 2023.

4.2. Aquaculture Increased the Contents of Particulate Organic Matter

The $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and the C:N ratio of POM have been widely used to indicate the possible source of organic matter and the incorporation of nutrients into bacteria and phytoplankton in estuary ecosystems [28,29]. In Zhangjiang Estuary, aquaculture water may also be the major source of POM, as large amounts of bio-debris, such as the fecal and shell of the cultured species, as well as the residual algae, were exported in company with the exports of DIN [5,9,47,48]. Besides the direct exports of POM by aquaculture water, the potential increasing of POM by the nutrients derived from aquaculture ponds may also be considered. The results in Section 4.1 have revealed the significant contribution of aquaculture water on DIN in Zhangjiang Estuary, indicating its possible important contribution to POM by promoting the growth and production of algae [49,50]. Our previous study has indicated that phytoplankton contributed about 21.8% of the total POM in Zhangjiang Estuary [27]. To observe more ideal results, the Mixsir mixing model was used instead of the IsoSource mixing model [28,29] to observe the major source of POM

in May 2023, and POM in December 2021 was re-analyzed to observe comparable results. Generally, the major source of POM in the study area includes the imports of freshwater, seawater, human-derived sewage, and the production of algae [51–58]. The contribution of zooplankton was also considered, as the promoted production of algae may further facilitate the growth and the metabolism of zooplankton. In addition, as Zhangjiang Estuary is fringed by a large area of natural mangroves, the contribution of mangrove sediments was also considered. The $\delta^{13}\text{C}$ and C:N ratios of POM were used as tracers during the source analysis of POM, and the characteristic values of the above sources are shown in Table 3. As the $\delta^{13}\text{C}$ and C:N ratio of aquaculture, agriculture, and domestic sewage have similar range [59–62], it is hard to distinguish their contribution of POM. Considering that aquaculture water has been identified as the primary source of DIN here, we suggest that its contribution to POM may be more significant than that of the agriculture and domestic sewage. Thus, the reported $\delta^{13}\text{C}$ and C:N ratio of human-derived sewage was defined as the characteristic values of aquaculture water in this study. Generally, the observed $\delta^{13}\text{C}$ and C:N ratio of POM during the two sampling periods fell into the cover range of aquaculture sewage, freshwater, and seawater (Figure 7).

Table 3. $\delta^{13}\text{C}$ and C:N ratio of particulate organic matter derived from different sources.

Source	$\delta^{13}\text{C}$ (‰)	C:N Ratio	References
Mangrove soil	−25.24	12.34	This study
Aquaculture sewage	−27.68	4.05	This study, [59–62]
Phytoplankton	−21.10	7.77	[63–72]
Freshwater	−25.38	4.49	This study, [73]
Seawater	−23.17	4.05	This study, [73]
Zooplankton	−28.90	3.45	[64,67,68,74–79]

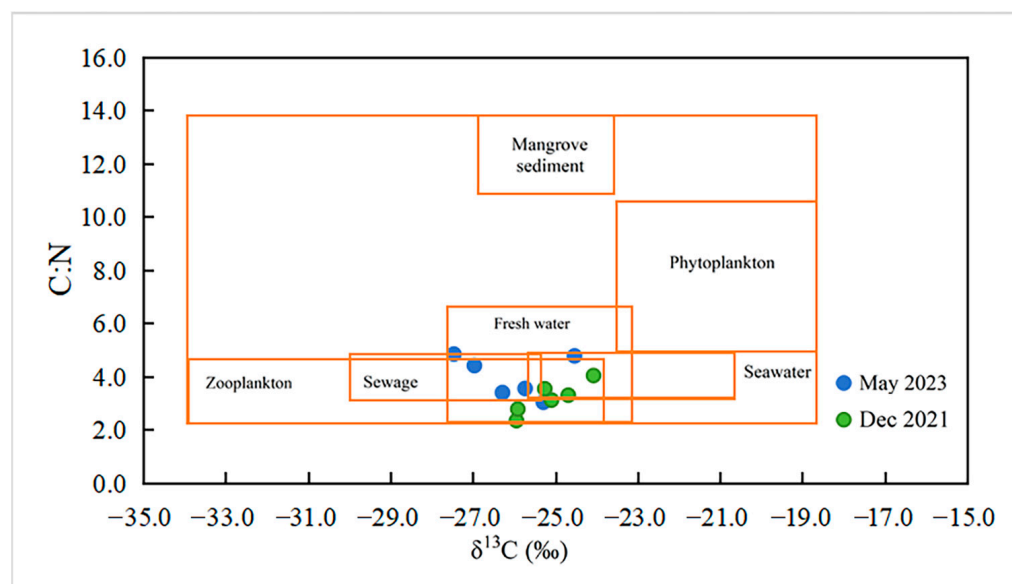


Figure 7. The $\delta^{13}\text{C}$ and C:N ratio of particulate organic matter in May 2023.

The major results are as follows (Figure 8): (1) Aquaculture sewage was the major source of POM, accounting for 24~33% ($29 \pm 3\%$, $n = 6$) of the total POM in November 2021 and 24~32% ($29 \pm 3\%$, $n = 6$) of that in May 2023. (2) Freshwater, seawater, and zooplankton had similar contribution ratios with each other, accounting for 20~23% ($22 \pm 1\%$, $n = 6$), 16~25% ($20 \pm 3\%$, $n = 6$), and 16~23% ($19 \pm 2\%$, $n = 6$) of the total POM in November 2021, and 22~24% ($23 \pm 1\%$, $n = 6$), 17 ~ 25% ($20 \pm 3\%$, $n = 6$), and 15~23% ($19 \pm 3\%$, $n = 6$) of that in May 2023. (3) Phytoplankton contributed only 5~9% ($7 \pm 1\%$ in November 2021 and $6 \pm 1\%$ in May 2023) of the total POM in the study area. (4) In comparison, mangrove soil

had less contribution to the total POM in Zhangjiang Estuary. Compared the results of the two models, the contribution ratio of aquaculture sewage and phytoplankton observed by the Mixsir mixing model were obviously lower, even during the same sampling period. As the Mixsir mixing model cited multiple reported values (POM- $\delta^{13}\text{C}$ and POM-C: N) and considered their variance [28,29], we propose the results in this new study may be more ideal. Comparing the results in this study, a similar contribution of aquaculture sewage to POM in November 2021 and May 2023 was observed (Figure 8).

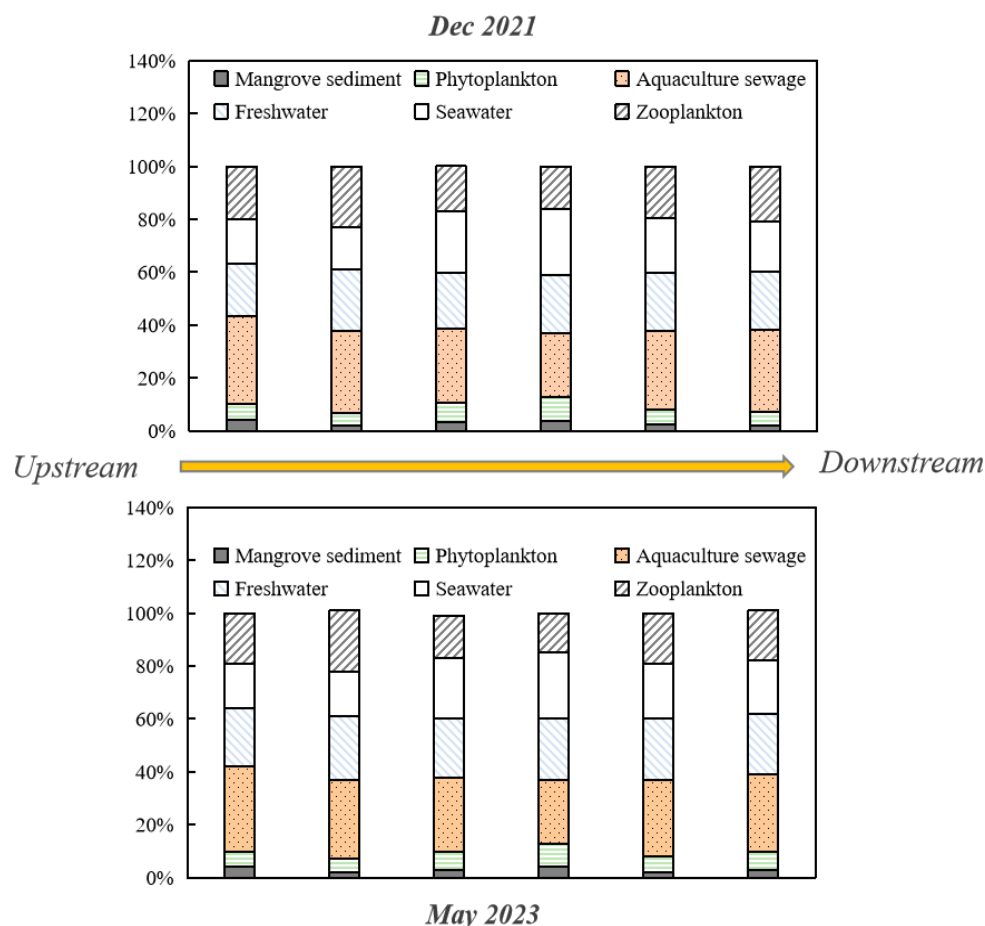


Figure 8. The contribution ratio of the possible sources to particulate organic matter in Zhangjiang Estuary.

The source analysis of DIN has revealed the influence of the cultured species and their drainage period on the content and composition of DIN (Section 4.1). In August–September, the sediments in clam ponds were washed out, thus producing higher contents of NH_4^+ in December 2021 (Figure 3). However, such a difference may have a limited influence on the contribution of aquaculture water to POM (Figure 8). Thus, we propose that the drainage of shrimp ponds and clam ponds may have relatively less effects on the contents of POM in Zhangjiang Estuary, and the direct contribution of POM by aquaculture in Zhangjiang Estuary may be mainly derived from crab ponds. Apart from the direct contribution, the potential contribution of aquaculture water, mainly by promoting the growth of phytoplankton and zooplankton, may also be important. The positive correlation between POC, PN, and DIN derived from aquaculture was observed (Figure 9). On one hand, the correlations between POC, PN, and DIN derived from aquaculture waters indicate the coupling imports of POM and DIN by aquaculture water. On the other hand, the correlations between POC and PN derived from phytoplankton with DIN derived from aquaculture revealed the enhanced production of phytoplankton by the imported DIN. However, the contribution of phytoplankton to the total POM was not as high as expected.

The most possible reason for this is the ingesting of zooplankton, which can be further supported by the negative correlation between the contribution ratio of phytoplankton and zooplankton ($r = -0.88$, $p < 0.05$, $n = 6$). The contribution of phytoplankton and zooplankton may vary with seasons and nutrient conditions; thus, the long-time monitor is needed to reveal their temporal variation and response to N derived from aquaculture.

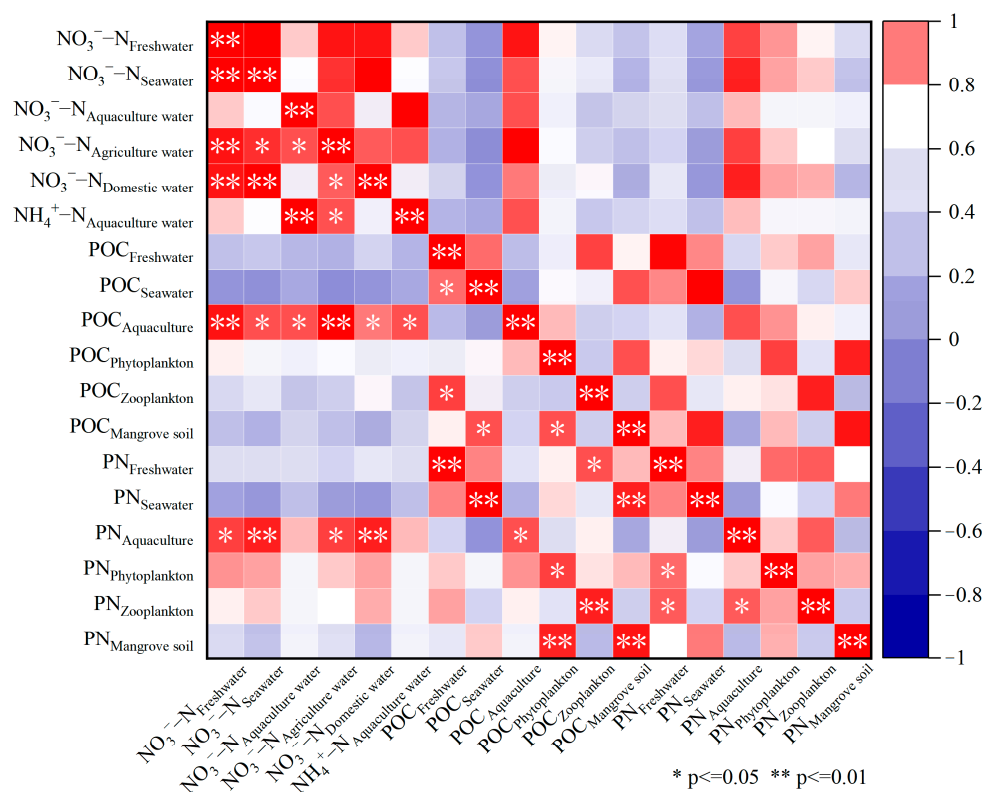


Figure 9. The correlation matrix of particulate organic matter and dissolved inorganic nitrogen derived from aquaculture water and phytoplankton in May 2023.

Generally, the above results indicate that aquaculture water was the primary source of POM in Zhangjiang Estuary, and most of the POM contributed by aquaculture water could be derived from crab ponds. In addition, this study also revealed the potential contribution of aquaculture water to POM, mainly by promoting the growth and metabolism of phytoplankton and zooplankton successively. As such, bio-derived POM play important roles in the biogeochemical cycle of carbon, highlighting the need of long-time investigation of aquaculture water effects on the coastal carbon cycle.

5. Conclusions

This study revealed the coupling imports of DIN and POM by aquaculture ponds in Zhangjiang Estuary. The major conclusions are as follows: (1) aquaculture sewage was the major contributor of DIN (62~86% of the total NO_3^- and 60~100% of the total NH_4^+), and the cultured species and their drainage periods have great effects on the content and component of DIN here; (2) aquaculture water also has great contribution to POM (24~33% of the total POM) in the Zhangjiang Estuary, most of which may derived from crab ponds; (3) DIN derived from aquaculture may promote the growth of phytoplankton and zooplankton in succession, thus increasing the content and bioavailability of POM in Zhangjiang Estuary. This study also indicates that the stable isotopes may be efficient in the monitoring of the coastal environment and the management of coastal aquaculture.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/w16142054/s1>. Table S1: The major data in this study. Table S2: DIN, POM from different sources.

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