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Composition, Dynamic Changes, and Carbon Sequestration Effects of Organic Carbon in the Water of a *Penaeus vannamei* Culture Pond

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Abstract: To investigate the composition, dynamic changes, and carbon sequestration effects of organic carbon in the water of *Penaeus vannamei* aquaculture ponds, changes in organic carbon were assessed in the water of the *P. vannamei* pond monoculture and integrated *P. vannamei–Mercenaria mercenaria* pond aquaculture systems during an aquaculture period. RDOC (refractory dissolved organic carbon) was determined using the DOC (dissolved organic carbon) degradation method, and the organic carbon composition in the water and its relationship with environmental factors were analyzed. The results showed the following: (1) The aquaculture activities significantly increased the DOC, POC (particulate organic carbon), and RDOC contents in the water and decreased the proportions of RDOC in the water. The DOC, POC, RDOC contents, and RDOC proportions in the monoculture systems were higher than those of the integrated aquaculture systems. (2) DOC, POC, RDOC contents, and RDOC proportions in the water were significantly positively correlated with chlorophyll-*a* contents in both aquaculture systems and significantly negatively correlated with water temperature and salinity. (3) Based on the average content and proportion of RDOC in the water of the two aquaculture systems, it was calculated that approximately an average of 108.64 kg of RDOC was present per hectare of *P. vannamei* aquaculture water during the aquaculture period.

Keywords: *Penaeus vannamei*; *Mercenaria mercenaria*; pond aquaculture; dissolved organic carbon; refractory dissolved organic carbon; water environmental factors

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1. Introduction

As a result of the irrational use of natural resources and explosive population growth, atmospheric CO_2 and CH_4 concentrations have risen since the Industrial Revolution to 143% and 254% of their pre-industrial (ca. 1750) levels, respectively (WMO, 2015). Ongoing research has been examining the global carbon cycle and the effects of carbon sequestration in different types of ecosystems to reduce emissions and sequester carbon to mitigate worsening global warming trends. Aquatic ecosystems, as ecosystems that have always played an important role in providing an abundance of resources to humankind, also have an important influence on the greenhouse gases in the atmosphere [1]. For example, the oceans have absorbed approximately 41% of the carbon emitted by human industrial activity since the Industrial Revolution [2]. As such, carbon-related research in various types of aquatic ecosystems has become a hotspot for scientists.

Aquaculture systems, as a special class of aquatic ecosystems, have also begun to receive more attention from carbon researchers. Research on the dynamics of greenhouse gases (GHGs) such as CO_2 or CH_4 in aquaculture systems has been very popular in recent years, which is crucial for assessing carbon footprints and climate impacts [3]. Although aquaculture systems have been recognized as a source of carbon dioxide emissions by a significant portion of studies [4–7], opinions are divided. Some studies demonstrate that aquaculture systems emit relatively low levels of CO_2 and are a relatively low source of CO_2 [8]. Some studies have even suggested that aquaculture systems for specific species such as shellfish or shrimp and crabs are CO_2 sinks [9–11]. It can be seen that the use of CO_2 to assess carbon sources/sinks in aquaculture systems is still unstable and controversial.

In addition to the dissolved gas form, elemental carbon is present in aquaculture systems in the form of organic carbon. In 1968, Barber et al. first noted that dissolved organic carbon (DOC) contents were relatively constant over a wide range of deeper depths in many sea areas of the world and proposed that DOC in deep seawater is biochemically refractory. This refractory dissolved organic carbon (RDOC) is DOC that is resistant to biodegradation and photodegradation [12–14] and is difficult to chemically oxidize [15,16]. This may be because the remaining DOC itself is resistant to further reactions, or it may be because the concentration of the DOC substrate is below the threshold concentration for further reactions [17]. However, one thing is sure, RDOC itself is very stable and difficult to break down and utilize, and although RDOC may contain chelating agents and may represent important transport pathways or sinks for some heavy metals, RDOC is not a pollutant in itself, but does contribute to carbon sequestration [18]. Thus, once carbon is preserved in water in the form of RDOC, it can be said to be fixed as a carbon sink [19,20], thus reducing potential emissions of carbon-containing greenhouse gases.

Servais et al. took note of the fact that biodegradable dissolved organic carbon (BDOC) can be metabolized by microorganisms, such as bacteria, within a few days to a few months, then published and improved a method for the determination of BDOC contents that uses the differences between the mean values of the initial and the final DOC contents during the incubation process [21,22]. In these studies, DOC is incubated in an environment where microorganisms are present, and during the incubation process, the amount of DOC gradually decreases until a relatively small amount remains unchanged, and this remaining amount is the DOC that is difficult for microorganisms to degrade and utilize, and can be regarded as the amount of RDOC. This DOC degradation method has been widely used in assessing the bioavailability of DOC in rainwater [23], drinking water [21,24], as well as some natural water such as oceans [25], rivers [26], streams [27,28], groundwater [29,30], and salt marshes [31]. In aquaculture systems, studies on RDOC have mainly focused on coastal shellfish and algal aquaculture [32–34], while research on aquaculture ponds has been rare. There are approximately 110,830 km^2 of aquaculture ponds worldwide [35], and many coastal wetlands have been converted to aquaculture ponds, driven by economic interests and population growth [36]. According to statistics in 2022, the total area of aquaculture ponds in China was 71,075 km^2, with a year-on-year growth of 1.40%, of which the area of mariculture ponds was 20,744 km^2. With the concept of carbon sink fisheries put forward alongside other concepts [10,37], low-carbon aquaculture has become prioritized in the development of the aquaculture industry. Therefore, it is very important to actively carry out research on the effects of carbon sequestration in various aquaculture systems to reduce emissions, increase the sequestration of carbon, and realize the "double carbon" goal.

In this study, *Penaeus vannamei* pond monoculture (P) and integrated *P. vannamei*–hardshell clam (*Mercenaria mercenaria*) pond (PM) aquaculture systems were used as research objects. The dynamic changes in various environmental factors and indicators of organic carbon in pond water during the aquaculture period (June~September) were measured, and changes in RDOC contents of the pond water during the aquaculture period were determined using the DOC degradation method. The composition of organic carbon in the aquaculture ponds was also analyzed, as well as its relationship with the water environment factors. This research can provide a foundation for studying the formation of RDOC in marine aquaculture ponds, offer insights and methodologies for studying RDOC in the water bodies of other aquaculture systems, and contribute to the understanding of carbon sequestration effects. Furthermore, it can elucidate the role of seawater pond aquaculture in carbon sequestration, suggest potential methods for contributing to greenhouse gas emission reduction, and serve as a reference for developing low-carbon aquaculture strategies.

2. Materials and Methods

2.1. Pond Conditions and Aquaculture Management

The experiments were carried out from June to September 2022 at the Dongying Delta Farming Co., Ltd. (Dongying, China) facilities in Shandong Province, with a pond area of approximately 0.20 hm^2 and a water depth of approximately 1.20 m. *P. vannamei* fingerlings were released on 12 June 2022 and hardshell clam juveniles on 13 July, with a culture cycle of 85 days in this study. No water was exchanged during the aquaculture period, each pond was equipped with two oxygenators, and prawns were regularly fed with artificial compound feed twice a day (6:00 and 18:00) at a daily rate of 3~5% of the body mass of the prawns. The sampling area, locations of ponds and sampling points are shown in Figure 1.

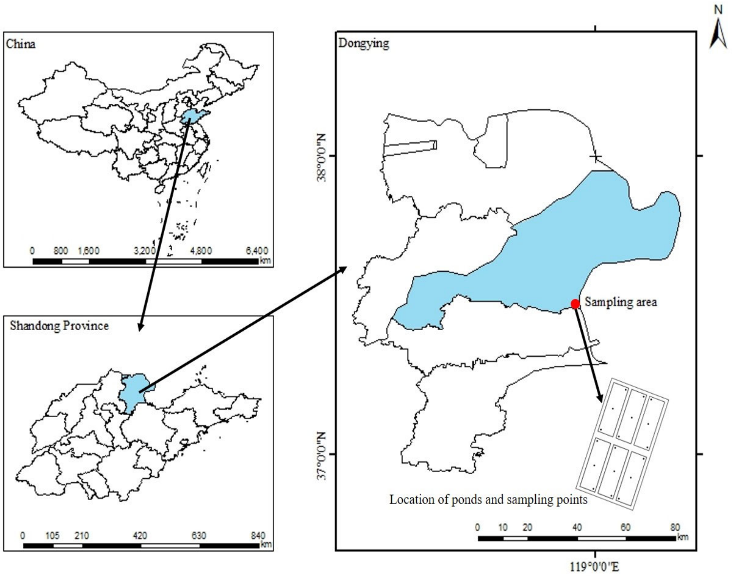

Figure 1. Sampling area, locations of ponds and sampling points.

2.2. Experimental Design

The experiments were set up with two aquaculture systems, a *P. vannamei* pond monoculture system (P) and an integrated *P. vannamei*–hard shell clam pond aquaculture system (PM), and three replicates were set up for each culture system. The stocking density of *P. vannamei* was 30×10^4 ind/hm^2 for both aquaculture systems (mean body length of *P. vannamei* fingerlings was 1.60 ± 0.21 cm and mean body mass was 0.02 ± 0.01 g) and the stocking density of hardshell clams in the PM systems was 450 kg/hm^2 (mean shell length of hardshell clam juveniles was 1.79 ± 0.18 cm, the mean shell height was 0.83 ± 0.07 cm, and the mean body mass was 1.44 ± 0.35 g).

2.3. Sample Collection and Measurements

Initial water samples were collected from the ponds of the two aquaculture systems at the start of the aquaculture period on 15 June 2022 and every 10 days from 17 July onwards, resulting in a total of seven collection times during the experimental period. Three sampling points were set up along the central axis of the aquaculture ponds, and water temperature (WT), dissolved oxygen content (DO), salinity (S), pH, and transparency (SD) were measured at each point at around 8:00 a.m. on each sampling date. Water temperature was determined using a seawater thermometer. Dissolved oxygen contents, salinity, and pH were determined using a YSI portable water quality analyzer (Incorporated, Yellow Springs, OH, USA). Additionally, transparency was determined using a transparency disc. At each collection time, 2 L of surface water samples were collected at 50 cm below the surface at each sampling point, mixed and brought back to the laboratory for the determination of alkalinity (Alk), chlorophyll-*a* (Chl-*a*), nitrate nitrogen (NO_3–N), nitrite nitrogen (NO_2–N), ammonia nitrogen (NH_4–N), reactive phosphorus (PO_4–P), total nitrogen (TN), and total phosphorus (TP). Alkalinity was determined via acid–base titration, Chl-*a* by acetone extraction, nitrate nitrogen via zinc–cadmium reduction, nitrite nitrogen via diazo–azodyne photometry, ammonia nitrogen via indophenol blue, reactive phosphate via ammonium molybdate–antimony potassium tartrate-ascorbic acid method, and the total nitrogen and total phosphorus contents were determined via potassium persulphate–boric acid–sodium hydroxide combined digestion under the pressure method [38,39].

Surface water samples from 50 cm deep were also collected using 500 mL polythene bottles, which were rinsed three times with sample water before collection, and the samples were kept in the dark and returned to the laboratory for processing. The water samples were immediately filtered through 0.45 μm glass fiber filter membranes, which had been pre-combusted in a muffle furnace at 450 °C for 3 h. After filtration, the water samples were immediately transferred into 500 mL stoppered conical flasks, which had been acid-washed with dilute hydrochloric acid, washed with purified water, and dried, and the flasks were wrapped with tinfoil. Since Tranvik and Höfle et al. showed that DOC bioavailability is independent of the microbial inoculum [40,41], to ensure that heterotrophic microorganisms capable of utilizing DOC were actually present in the water samples, 5 mL (100:1) aliquots of in situ water sample were filtered through 2 μm glass fiber membranes, which removed large particulate matter and protozoa while leaving bacteria, and added to each bottle [21,22]. The samples were incubated in complete darkness at 25 °C for 20 days (480 h), during which time the conical flasks were shaken with the stopper open at regular intervals to ensure that the flasks were adequately oxygenated and small water samples were taken to determine the DOC content using a Total Organic Carbon analyzer (TOC, Multi N/C 2100, Analytik Jena AG, Jena, Germany). Any remaining DOC contents after 480 h of microbial degradation were determined to be the RDOC contents in the water samples.

The filtered 0.45 μm glass fiber membrane was dried in an oven at 60 °C. An appropriate amount of 1 $mol \cdot L^{-1}$ dilute hydrochloric acid was poured into a drying dish, and the water sample membranes and blank membranes were placed 5 cm above the hydrochloric acid to fumigate with hydrochloric acid for 6 h. After fumigation, the membranes were washed three times with purified water to remove the residual acid, dried again in an oven at 60 °C, and wrapped in tin. Using these processed samples, the particulate organic carbon (POC) contents of the water samples were determined using an elemental analyzer (Vario EL cube, Elementar, Langenselbold, Germany) and estimated by repeated analyses using an in-laboratory standard sample of acetanilide; the standard deviation of the repeated analytical measurements was determined to be ±0.3%.

2.4. Data Processing and Analysis

Data are expressed as mean ± standard deviation (mean ± SD). Data were tested for normality (Shapiro–Wilk) and variance chi-squared (Levene) prior to analyses, and if the variance chi-squared requirement was not met, the data were further transformed for analysis. Significance analyses were performed using Tukey's test (*t*-test) and one-way analysis of variance (ANOVA) for samples that met normality and chi-square, and Brown–Forsythe non-parametric tests for samples that did not meet normality or chi-square. The DOC degradation was fitted using the ExpDecay function to obtain the degradation function. The Tangent plug-in in the Origin 2019 software was used to find the tangent slope (k) of each point on the degradation function, defined as when $k \leq -0.005$, which was regarded as the point when DOC degradation had reached completion. The incubation time that had elapsed at this point was taken as the time required for DOC degradation to complete. Pearson's bivariate correlation was used to analyze the relationship between organic carbon in water and environmental factors, and stepwise multiple regression was used to establish the optimal regression equations between organic carbon in water and environmental factors. $p < 0.1$ was regarded as a significant difference, and $p < 0.05$ was regarded as a highly significant difference. All data analyses were performed using SPSS 26.0 software, and figures were produced using Origin 2019 software.

3. Results

3.1. Physico-Chemical Indicators in Pond Water during the Aquaculture Period

Changes in water temperature, dissolved oxygen contents, salinity, alkalinity, pH, transparency, and Chl-*a* contents in pond water of both aquaculture systems during the aquaculture period are shown in Table 1. The range of water temperature was 22.2 ± 0.1~31.2 ± 0.1 °C, with an average of 26.1 ± 3.1 °C, and there was a tendency to increase and then decrease as the culture period progressed ($p < 0.05$). The highest water temperature (31.1 ± 0.1 °C) occurred in the first half of August. Dissolved oxygen contents ranged from 6.03 ± 0.07 to 8.98 ± 0.06 $mg \cdot L^{-1}$, with an average of 7.56 ± 0.91 $mg \cdot L^{-1}$. The oxygenators were not switched on during the pre-farming period, and the highest dissolved oxygen content (8.82 ± 0.16 $mg \cdot L^{-1}$) was observed in the first half of August after the oxygenators were turned on, after which the dissolved oxygen gradually reduced as the culture progressed ($p < 0.05$). Salinity ranged from 24.51 ± 0.07 to 28.91 ± 0.02, with a mean salinity of 26.40 ± 1.49. Alkalinity ranged from 1.01 ± 0.02 to 2.67 ± 0.05 $mmol \cdot L^{-1}$, with a mean alkalinity of 1.83 ± 0.53 $mmol \cdot L^{-1}$. Alkalinity showed a gradual increasing trend as the culture progressed ($p < 0.05$), and at the end of the aquaculture period, the alkalinity was higher in the PM system than in the P system ($p < 0.05$). The pH values ranged from 8.26 ± 0.03 to 8.96 ± 0.02, with a mean pH of 8.55 ± 0.19, with a tendency to increase gradually as the culture progressed ($p < 0.05$). The transparency ranged from 20.9 ± 0.2 to 43.6 ± 0.1 cm, with an average transparency of 30.0 ± 7.9 cm. Transparency exhibited a gradually decreasing trend as the culture progressed ($p < 0.05$). The range of Chl-*a* contents was 24.51 ± 0.72~218.03 ± 6.09 $\mu g \cdot L^{-1}$, with an average of 92.39 ± 65.17 $\mu g \cdot L^{-1}$, showing a gradual increasing trend as the culture progressed ($p < 0.05$), and the P system had higher Chl-*a* contents than the PM system ($p < 0.1$).

Table 1. Physico-chemical indicators in pond water of both aquaculture systems during the aquaculture period.

Treatments	Physico-Chemical Indicators in Pond Water during the Aquaculture Period						
	WT/°C	**DO/$mg \cdot L^{-1}$**	**S**	**Alk/$mmol \cdot L^{-1}$**	**pH**	**SD/cm**	**Chl-*a*/$\mu g \cdot L^{-1}$**
P	26.1 ± 3.1 (22.3~31.2)	7.53 ± 0.80 (6.52~8.66)	27.09 ± 1.43 (24.53~28.91)	1.74 ± 0.48 (1.01~2.53)	8.55 ± 0.23 (8.26~8.96)	28.6 ± 7.8 (21.1~42.9)	110.59 ± 78.21 ^{a} (24.51~218.03)
PM	26.1 ± 3.1 (22.2~31.0)	7.60 ± 1.02 (6.03~8.98)	25.71 ± 1.19 (24.51~27.79)	1.92 ± 0.55 (1.03~2.67)	8.54 ± 0.15 (8.36~8.77)	31.4 ± 7.8 (20.9~43.6)	74.20 ± 41.42 ^{b} (25.14~124.85)

Note(s): Different letters in the same column represent significant differences ($p < 0.05$), the same as below.

Changes in nitrate nitrogen, nitrite nitrogen, ammonia nitrogen, reactive phosphorus, total nitrogen, and total phosphorus contents in the ponds of the two aquaculture systems during the aquaculture period are shown in Table 2. The nitrate nitrogen contents ranged from 0 to 0.117 ± 0.028 $mg \cdot L^{-1}$, with an average content of 0.036 ± 0.045 $mg \cdot L^{-1}$, showing a decreasing trend as the culture progressed ($p < 0.05$). The nitrite nitrogen contents ranged from 0.002 ± 0.001 to 0.009 ± 0.001 $mg \cdot L^{-1}$, with an average of 0.006 ± 0.003 $mg \cdot L^{-1}$, showing an increasing trend as the culture progressed ($p < 0.05$). The ammonia nitrogen contents ranged from 0.015 ± 0.005 to 0.226 ± 0.008 $mg \cdot L^{-1}$, with an average of 0.079 ± 0.077 $mg \cdot L^{-1}$, showing an increasing and then decreasing trend as the culture progressed, and at the end of the aquaculture period, ammonia nitrogen content was higher in the P system than in the PM system ($p < 0.05$). The reactive phosphorus contents ranged from 0.002 ± 0.002 to 0.062 ± 0.001 $mg \cdot L^{-1}$, with an average of 0.022 ± 0.017 $mg \cdot L^{-1}$, showing an increasing trend as the culture progressed ($p < 0.05$), and at the end of the aquaculture period, the reactive phosphorus content of the PM system was higher than that of the P system ($p < 0.05$). The total nitrogen contents ranged from 2.83 ± 0.05 to 7.97 ± 0.17 mg·L^{-1}, with an average of 5.30 ± 1.61 mg·L^{-1}, showing an increasing trend as the culture progressed ($p < 0.05$), and at the end of the aquaculture period, the total nitrogen content of the P system was higher than that of the PM system ($p < 0.05$). The total phosphorus contents ranged from 0.12 ± 0.06 to 0.69 ± 0.03 mg·L^{-1}, with an average content of 0.35 ± 0.16 mg·L^{-1}, showing an increasing trend as the culture progressed, and the total phosphorus content in the PM system was higher than that in the P system at the end of the aquaculture period ($p < 0.05$).

Table 2. Nutrient contents in pond water of both aquaculture systems during the aquaculture period.

Treatments	Nutrient Contents in Pond Water during the Aquaculture Period					
	NO_3–N/mg·L^{-1}	NO_2–N/mg·L^{-1}	NH_4–N/mg·L^{-1}	PO_4–P/mg·L^{-1}	TN/mg·L^{-1}	TP/mg·L^{-1}
P	0.032 ± 0.040 (0~0.092)	0.006 ± 0.003 (0.002~0.008)	0.090 ± 0.089 (0.024~0.226)	0.020 ± 0.019 (0.002~0.062)	5.52 ± 1.89 (2.83~7.97)	0.28 ± 0.11 (0.12~0.44)
PM	0.040 ± 0.050 (0~0.117)	0.006 ± 0.002 (0.002~0.009)	0.068 ± 0.061 (0.015~0.199)	0.025 ± 0.015 (0.006~0.054)	5.07 ± 1.22 (2.86~6.62)	0.42 ± 0.17 (0.23~0.69)

3.2. Dynamic Changes in Organic Carbon Contents in Pond Water during the Aquaculture Period

3.2.1. Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC), and Particulate Organic Carbon (POC)

The variation in TOC, DOC, and POC contents in pond water of both aquaculture systems during the aquaculture period is illustrated in Figure 2. The TOC contents of the P and PM systems ranged from 6.11 ± 0.12 to 28.47 ± 0.20 mg·L^{-1} (mean: 19.72 ± 7.55 mg·L^{-1}) and 6.09 ± 0.07 to 25.31 ± 0.35 mg·L^{-1} (mean: 18.00 ± 5.68 mg·L^{-1}), respectively. Both aquaculture systems showed trends of gradually increasing as the culture progressed ($p < 0.05$). The TOC contents of the water at the end of aquaculture were 4.66 times (22.36 mg·L^{-1} increase) and 4.16 times (19.22 mg·L^{-1} increase) those at the beginning, respectively, and the TOC content of the P system was higher than that of the PM system ($p < 0.05$). The DOC contents of the P and PM systems ranged from 3.71 ± 0.07 to 17.81 ± 0.13 mg·L^{-1} (mean: 12.08 ± 4.57 mg·L^{-1}) and 3.70 ± 0.03 to 16.46 ± 0.26 mg·L^{-1} (mean: 11.13 ± 3.62 mg·L^{-1}), respectively. Both aquaculture systems showed trends of gradually increasing as the culture progressed ($p < 0.05$). The DOC contents at the end of the aquaculture period were 4.80 times (14.10 mg·L^{-1} increase) and 4.45 times (12.77 mg·L^{-1} increase) those at the beginning, respectively, and the DOC content of the P system was higher than that of the PM system ($p < 0.05$). The POC contents of the P and PM systems ranged from 2.39 ± 0.05 to 12.15 ± 0.09 mg·L^{-1} (mean: 7.63 ± 3.17 mg·L^{-1}) and 2.39 ± 0.04 to 8.84 ± 0.10 mg·L^{-1} (mean: 6.88 ± 2.20 mg·L^{-1}), respectively. Both aquaculture systems showed trends of gradually increasing as the culture progressed ($p < 0.05$). The POC contents at the end of the aquaculture period were 4.45 times (8.26 mg·L^{-1} increase) and 3.70 times (6.45 mg·L^{-1} increase) higher than those at the beginning, respectively, and the POC content of the P system was higher than that of the PM system ($p < 0.05$).

3.2.2. Refractory Dissolved Organic Carbon (RDOC)

The variation in DOC contents in the pond water during the 20 days (480 h) of incubation and preservation period at 25 °C in complete darkness and the fitted curves using the ExpDeacy function are shown in Figure 3. The equations and correlation coefficients of the ExpDecay function are shown in Table 3. The function equations represent the degradation of DOC contents in the water with time, and based on the fitted correlation coefficients (Table 3), the functional model could be considered to fit the data well. It could be seen that DOC, as it was decomposed and utilized by microorganisms, presents a trend of rapidly decreasing at first and then basically tending to stabilize. There was a significant difference in DOC contents before and after degradation ($p < 0.05$). There was no significant difference in DOC degradation between the two aquaculture systems before the release of hard-shelled clam juveniles (Figure 3A), but after the release of the hard-shelled clam juveniles, there were significant differences in the time to completion of DOC degradation, the rate of degradation, and the contents of degraded DOC between the two systems (Figure 3B–G).

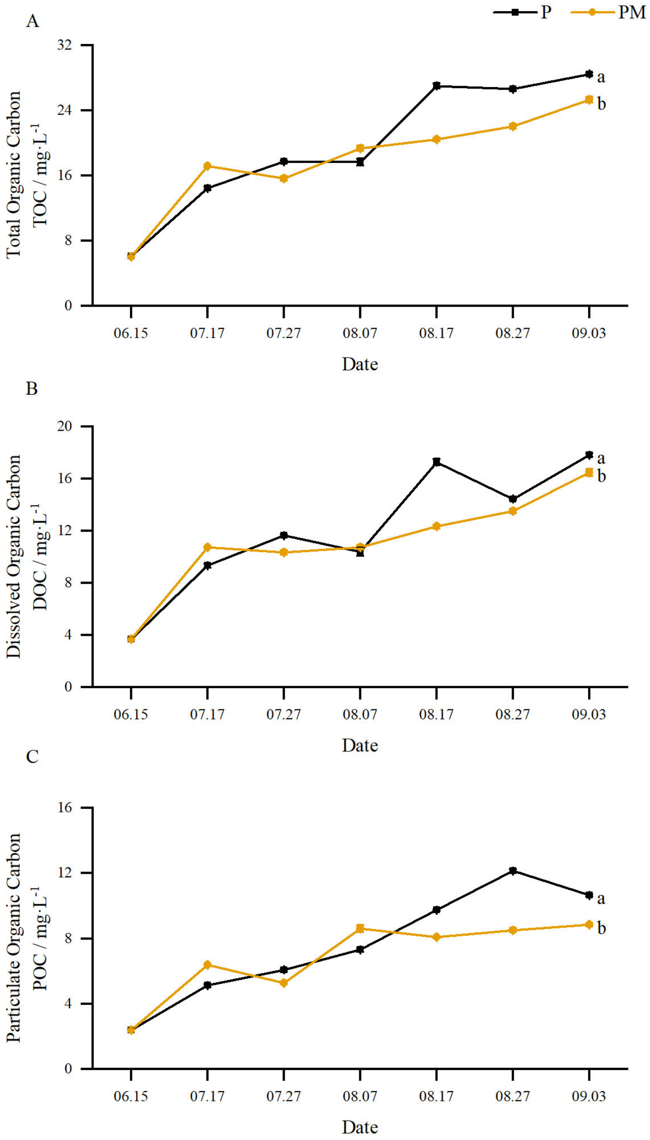

Figure 2. TOC (**A**), DOC (**B**), and POC (**C**) contents in pond water of both aquaculture systems during the aquaculture period. Note(s): Different letters in the same figure represent significant differences ($p < 0.05$), the same as below.

The times for the completion of DOC degradation and the rates of degradation are shown in Table 4. The time for the completion of DOC degradation in the P and PM systems ranged from 67.11 ± 0.91 to 388.07 ± 29.18 h (mean time 200.98 ± 104.52 h) and 68.39 ± 2.94 to 245.05 ± 1.04 h (mean time 189.29 ± 56.36 h), respectively, and both showed gradual decreasing trends as the culture progressed ($p < 0.05$). The average time was longer in the P system than in the PM system ($p < 0.05$). The DOC degradation rates of the P and PM systems ranged from 0.011 ± 0.000 to 0.030 ± 0.003 $\mathrm{mg \cdot L^{-1} \cdot h^{-1}}$ (mean rate of 0.018 ± 0.007 $\mathrm{mg \cdot L^{-1} \cdot h^{-1}}$) and 0.012 ± 0.001 to 0.022 ± 0.000 $\mathrm{mg \cdot L^{-1} \cdot h^{-1}}$ (mean rate of 0.017 ± 0.004 $\mathrm{mg \cdot L^{-1} \cdot h^{-1}}$), respectively, and both showed trends of gradually increasing as the culture progressed ($p < 0.05$). At the end of the aquaculture period, the rate of DOC degradation was higher in the PM systems than in the P system ($p < 0.05$).

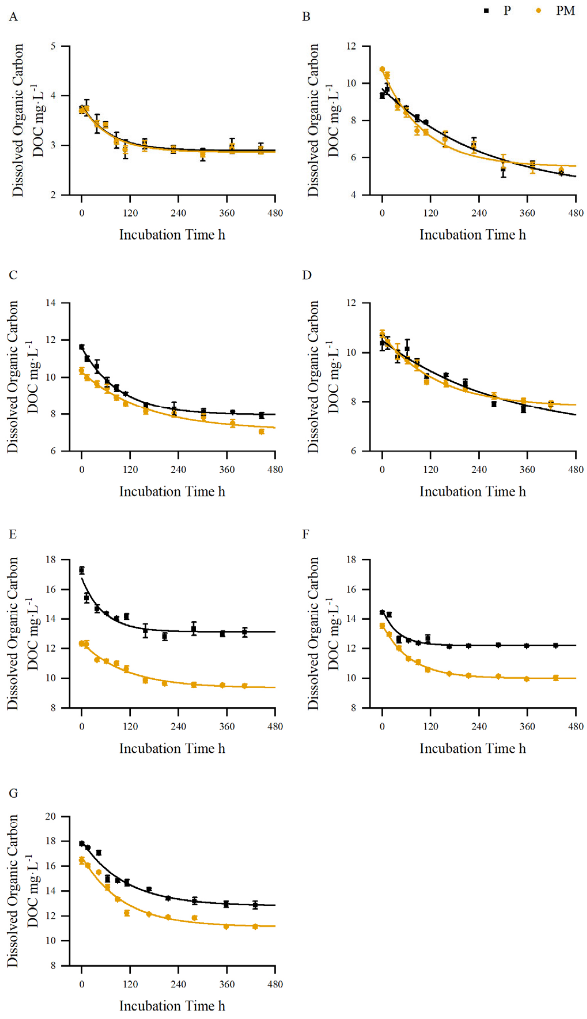

Figure 3. Degradation of DOC in the pond water of both aquaculture systems. Notes: The sampling date in (**A**) is 15 June; the sampling date in (**B**) is 17 July; the sampling date in (**C**) is 27 July; the sampling date in (**D**) is 7 August; the sampling date in (**E**) is 17 August; the sampling date in (**F**) is 27 August; and the sampling date in (**G**) is 3 September.

Table 3. Fitting formulas and correlation coefficients of ExpDecay function for DOC degradation of both aquaculture systems.

Dates	Treatments	Functional Equation	R^2
06.15	P	$y = 0.915 \times \exp(-x/68.545) + 2.895$	0.903
	PM	$y = 0.930 \times \exp(-x/70.947) + 2.864$	0.917
07.17	P	$y = 5.609 \times \exp(-x/262.458) + 4.094$	0.970
	PM	$y = 5.201 \times \exp(-x/104.356) + 5.505$	0.973
07.27	P	$y = 3.660 \times \exp(-x/92.638) + 7.950$	0.993
	PM	$y = 3.180 \times \exp(-x/161.279) + 7.106$	0.975
08.07	P	$y = 3.794 \times \exp(-x/302.667) + 6.691$	0.935
	PM	$y = 2.926 \times \exp(-x/136.747) + 7.791$	0.983
08.17	P	$y = 3.664 \times \exp(-x/54.385) + 13.110$	0.908
	PM	$y = 3.064 \times \exp(-x/109.447) + 9.357$	0.967
08.27	P	$y = 2.436 \times \exp(-x/37.783) + 12.205$	0.885
	PM	$y = 3.615 \times \exp(-x/68.840) + 10.003$	0.994
09.03	P	$y = 5.237 \times \exp(-x/106.457) + 12.801$	0.956
	PM	$y = 5.673 \times \exp(-x/101.434) + 11.109$	0.958

Table 4. Completion time of DOC degradation and DOC degradation rate in pond water of both aquaculture systems.

Treatments	**Items**	**Date**							
		06.15	**07.17**	**07.27**	**08.07**	**08.17**	**08.27**	**09.03**	**Mean ± SD**
P	Time for completion of DOC degradation/h	67.11 ± 0.91	388.07 ± 29.18	191.55 ± 6.38	278.36 ± 4.32	140.94 ± 14.09	97.22 ± 7.02	243.60 ± 11.17	200.98 ± 104.52 ^{a}
	DOC degradation rates/mg·L^{-1}·h^{-1}	0.011 ± 0.000	0.011 ± 0.001	0.019 ± 0.001	0.010 ± 0.000	0.030 ± 0.003	0.023 ± 0.002	0.020 ± 0.001 ^{a}	0.018 ± 0.007
PM	Time for completion of DOC degradation/h	68.39 ± 2.94	240.40 ± 2.94	221.34 ± 0.91	199.08 ± 2.16	188.67 ± 2.89	162.08 ± 0.99	245.05 ± 1.04	189.29 ± 56.36 ^{b}
	DOC degradation rates/mg·L^{-1}·h^{-1}	0.012 ± 0.001	0.022 ± 0.000	0.014 ± 0.000	0.015 ± 0.000	0.016 ± 0.000	0.022 ± 0.000	0.022 ± 0.000 ^{b}	0.017 ± 0.004

Note(s): Different letters in the same column represent significant differences ($p < 0.05$), the same as below.

The variation in the RDOC contents in the pond water of both aquaculture systems during the aquaculture period is shown in Figure 4. The RDOC contents in the P and PM systems ranged from 2.99 ± 0.23 to 13.11 ± 0.34 mg·L^{-1} (mean 8.80 ± 3.74 mg·L^{-1}) and 2.86 ± 0.07 to 11.12 ± 0.11 mg·L^{-1} (mean 7.70 ± 2.63 mg·L^{-1}), respectively, and both showed gradual increasing trends as the culture progressed ($p < 0.05$). The RDOC contents at the end of the culture were 4.31 times (9.87 mg·L^{-1} increase) and 3.88 times (8.26 mg·L^{-1} increase) higher than that at the beginning in the P and PM systems, respectively, and the RDOC content of the P system was higher than that of the PM system ($p < 0.05$).

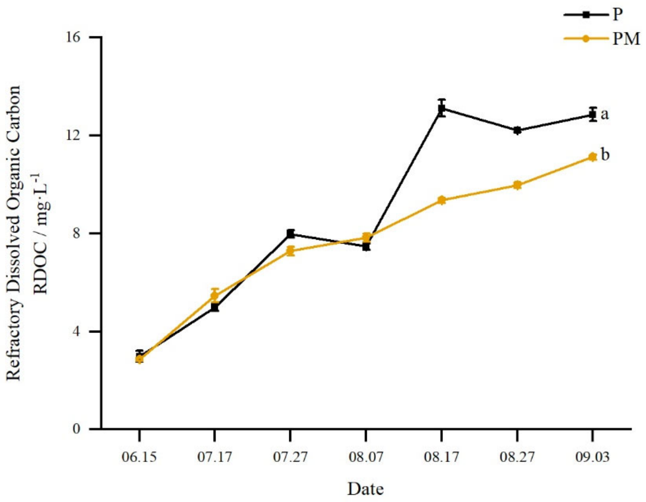

Figure 4. RDOC contents in pond water of both aquaculture systems during the aquaculture period.

3.2.3. Proportions Made up by Different Organic Carbon Components

The contents and proportions of each organic carbon component in the pond water of both aquaculture systems during the aquaculture period are shown in Table 5. The DOC:POC:TOC of the P and PM systems were 0.615:0.385:1 and 0.617:0.383:1, respectively, and the DOC contents were 1.58 and 1.62 times the POC contents; there were no significant differences between the two aquaculture systems ($p > 0.05$). At the end of the aquaculture period, the proportion of the DOC content was lower ($p < 0.05$), and the proportion of the POC content was higher ($p < 0.05$) in the P system than in the PM system.

Table 5. Contents and proportion of each organic carbon component of both aquaculture systems during the aquaculture period.

Treatments	**Components**	**Contents/mg·L^{-1}**								**Proportions to TOC/%**
		15 June	**17 July**	**27 July**	**7 August**	**17 August**	**27 August**	**3 September**	**Mean ± SD**	
P	DOC	3.71 ± 0.07	9.34 ± 0.14	11.63 ± 0.09	10.38 ± 0.31	17.27 ± 0.23	14.45 ± 0.08	17.81 ± 0.13	12.08 ± 4.57	61.51 ± 3.67
	POC	2.39 ± 0.05	5.12 ± 0.08	6.07 ± 0.10	7.31 ± 0.14	9.74 ± 0.06	12.15 ± 0.09	10.66 ± 0.07	7.63 ± 3.17	38.49 ± 3.67
	TOC	6.11 ± 0.12	14.46 ± 0.22	17.71 ± 0.19	17.68 ± 0.43	27.01 ± 0.29	26.60 ± 0.18	28.47 ± 0.20	19.72 ± 7.55	100.00
PM	DOC	3.70 ± 0.03	10.75 ± 0.07	10.35 ± 0.18	10.76 ± 0.15	12.34 ± 0.15	13.53 ± 0.16	16.46 ± 0.26	11.13 ± 3.62	61.71 ± 3.12
	POC	2.39 ± 0.04	6.40 ± 0.05	5.29 ± 0.08	8.61 ± 0.20	8.08 ± 0.07	8.52 ± 0.12	8.84 ± 0.10	6.88 ± 2.20	38.29 ± 3.12
	TOC	6.09 ± 0.07	17.15 ± 0.11	15.64 ± 0.26	19.36 ± 0.34	20.42 ± 0.21	22.05 ± 0.28	25.31 ± 0.35	18.00 ± 0.35	100.00

The variation in RDOC as a proportion of DOC in the pond water of both aquaculture systems during the aquaculture period is shown in Figure 5. The proportion made up by RDOC contents before the start of aquaculture was 78.91 ± 3.68%. The proportions of RDOC contents after the start of culture ranged from 53.35 ± 0.92 to 84.44 ± 0.13% (mean proportion 71.06 ± 9.40%) and 51.78 ± 2.03 to 75.50 ± 0.39% (mean proportion 68.75 ± 8.04%) in the P and PM systems, respectively, and both showed gradual increasing trends as the culture progressed ($p < 0.05$). The proportions of the RDOC contents were higher before the start of the culture than after the start of the culture ($p < 0.05$), and at the end of the aquaculture period, the proportion of the RDOC contents was higher in the P system than in the PM system ($p < 0.05$).

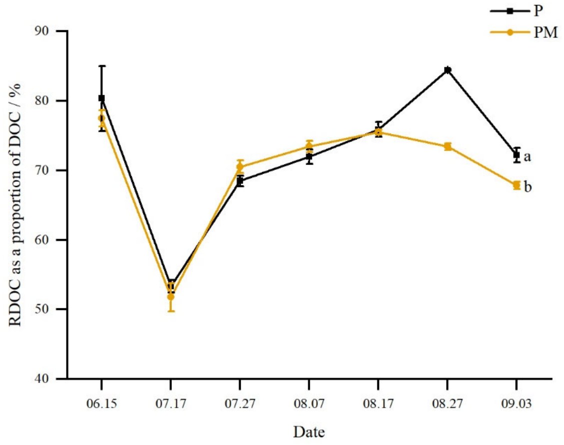

Figure 5. RDOC as a proportion of DOC in pond water of both aquaculture systems during the aquaculture period.

3.3. Correlation Analysis between Organic Carbon and Environmental Factors in Water

The results of the correlation analysis between organic carbon and environmental factors in pond water of the two aquaculture systems are shown in Table 6. During the aquaculture process, the DOC contents in pond water of both aquaculture systems showed significant negative correlations with water temperature, dissolved oxygen, salinity, and transparency ($p < 0.05$) and significant positive correlations with alkalinity and Chl-*a* contents ($p < 0.05$). The POC contents showed significant negative correlations with dissolved oxygen, salinity, and transparency ($p < 0.05$) and significant positive correlations with alkalinity and Chl-*a* contents ($p < 0.05$). RDOC contents showed significant negative correlations with water temperature, dissolved oxygen, salinity, and transparency ($p < 0.05$), and significant positive correlations with alkalinity and Chl-*a* contents ($p < 0.05$). The proportions of RDOC showed significant negative correlations with both salinity and transparency ($p < 0.05$) and a significant positive correlation with Chl-*a* contents ($p < 0.05$). In addition, there was no significant correlation between organic carbon and nutrient indicators in this study.

Table 6. Correlation analysis results of organic carbon and environmental factors in pond water of both aquaculture systems.

Environment Factors	DOC Contents			POC Contents			RDOC Contents			RDOC Proportion		
	P	PM	Mean	P	PM	Mean	P	PM	Mean	P	PM	Mean
WT	−0.696 **	−0.840 **	−0.723 **	−0.721 **	NS	−0.523 **	−0.681 **	−0.685 **	−0.644 **	NS	NS	NS
DO	−0.690 **	−0.886 **	−0.731 **	−0.753 **	−0.506 *	−0.590 **	−0.664 **	−0.687 **	−0.621 **	NS	NS	NS
S	−0.863 **	−0.575 *	−0.547 **	−0.848 **	−0.711 **	−0.563 **	−0.855 **	−0.877 **	−0.594 **	−0.605 **	−0.864 **	−0.530 **
Alkalinity	0.751 **	0.755 **	0.650 **	0.717 **	0.511 *	0.523 **	0.761 **	0.753 **	0.631 **	0.631 **	NS	0.437 **
pH	NS	0.855 **	NS	NS	NS	0.367 *	NS	0.878 **	0.341 *	NS	NS	NS
SD	−0.604 **	−0.859 **	−0.700 **	−0.798 **	−0.823 **	−0.761 **	−0.757 **	−0.977 **	−0.815 **	−0.886 **	−0.596 **	−0.733 **
Chl-*a*	0.685 **	0.821 **	0.727 **	0.903 **	0.804 **	0.884 **	0.821 **	0.949 **	0.858 **	0.848 **	0.593 **	0.744 **

Note(s): NS indicates insignificant correlation, ** indicates significant correlation at 0.01 level, * indicates significant correlation at 0.05 level.

The results of the multiple linear regression analysis between organic carbon and environmental factors in the water of the two aquaculture systems are shown in Table 7. It can be seen that DOC contents in the pond water during the aquaculture period were mainly affected by Chl-*a* contents and water temperature, POC contents were mainly affected by Chl-*a* contents and salinity, RDOC contents were mainly affected by Chl-*a* contents, water temperature, and salinity, and the proportions of RDOC were mainly affected by Chl-*a* contents and salinity. Therefore, the main factors affecting organic carbon contents in pond water were Chl-*a* contents, water temperature, and salinity.

Table 7. Multiple linear regression analysis between organic carbon and environmental factors in pond water.

Dependent Variable	Multiple Linear Regression Equation	R^2	*p*
DOC contents/mg·L^{-1}	DOC = 23.828 + 0.023 × Chl-*a* − 0.496 × WT	0.733	<0.05
POC contents/mg·L^{-1}	POC = 15.524 + 0.025 × Chl-*a* − 0.384 × S	0.848	<0.05
RDOC contents/mg·L^{-1}	RDOC = 21.780 + 0.028 × Chl-*a* − 0.220 × WT − 0.369 × S	0.861	<0.05
RDOC proportions/%	RDOC proportion = 106.425 + 0.088 × Chl-*a* − 1.376 × S	0.616	<0.05

4. Discussion

4.1. Comparison of Organic Carbon Composition and Contents in Different Aquaculture Ecosystems

In the present study, the mean water DOC content in the *P. vannamei* pond monoculture system (P) was 12.08 ± 4.57 mg·L^{-1}, and that in the integrated *P. vannamei*–hardshell clam pond culture system (PM) was 11.13 ± 3.62 mg·L^{-1}, both of which were slightly higher than those observed by Liu (9.95 ± 2.04 mg·L^{-1}) and You (8.53 ± 8.25 mg·L^{-1}) in Chinese prawn enclosure culture experiments [42,43], and much higher than those observed in natural water bodies, including the open ocean (0.69 ± 0.28 mg·L^{-1}, [44], karstic rivers (1.49 ± 0.14 mg·L^{-1}, [26], and streams (2.34 ± 0.78 mg·L^{-1}, [27]. This was consistent with a gradual decrease in carbon inputs moving from headwater streams to the open ocean due to a gradual decrease in artificial interventions [45]. The mean POC contents in pond water were 7.63 ± 3.17 mg·L^{-1} and 6.88 ± 2.20 mg·L^{-1} for the P and PM systems, respectively. At the end of the aquaculture, the DOC and POC contents were higher in the P system than in the PM system ($p < 0.05$), which may have been because the factors affecting DOC contents in the water were mainly Chl-*a* contents and water temperature and the factors affecting POC contents in the water were mainly Chl-*a* contents and salinity (Table 7). This is important because the filter-feeding hard-shelled clams would reduce Chl-*a* contents in water by consuming phytoplankton, thus indirectly resulting in lower DOC and POC contents in the PM system.

The mean water RDOC contents were 8.80 ± 3.74 mg·L^{-1} and 7.70 ± 2.63 mg·L^{-1} in the P and PM systems, respectively. The mean proportions of RDOC to DOC in the water of the P and PM systems were 72.39 ± 9.45% and 70.00 ± 8.05%, respectively. At the end of the aquaculture, the contents and proportions of RDOC were higher in the P system than in the PM system ($p < 0.05$), and the degradation completion time and degradation rate corroborated this experimental result (Table 4). On the one hand, this may have been because the hardshell clams directly altered the composition of the DOC, which can be degraded or affect the community composition and number of heterotrophic microorganisms, either of which would have altered the DOC degradation processes [46]. However, further experimental validation regarding the specific composition of DOC and the community structure, composition, dynamics of heterotrophic microorganisms and digestive enzyme activities in water of both aquaculture systems would be required to determine if this were the case. On the other hand, this may have been because the filter feeding of phytoplankton by hardshell clams reduced the Chl-*a* contents in the water. In this study, the mean proportion of RDOC contents in the pond water of the two aquaculture systems was 71.19 ± 8.86%, but the final proportions of the RDOC contents in pond water may be even lower. Even though in the degradation incubation experiment, which was only carried out for 20 days (480 h), the rate of degradation of DOC tended to be very slow and close to zero after about 200 h, very small amounts of DOC were still degraded every day near the end of the aquaculture period. Therefore, the proportion of RDOC contents in these systems will likely be lower than in natural waters such as oceans (about 94%, [44], karstic rivers (67~93%, [26], streams (77~97%, [27], and groundwater (88~96%, [47]. These relatively low levels may result from the high degree of artificial intervention in aquaculture ponds compared to natural water bodies, where there is almost no intervention at all. On the one hand, daily feeding and the use of oxygenators can disturb the stability of the pond water, which may affect the composition and contents of organic carbon in pond water; on the other hand, feeds, fertilizers, etc., are exotic organic carbon inputs that are designed to be more readily utilized by various organisms, hence resulting in more abundant and readily degraded DOC in pond waters compared to natural waters.

4.2. Factors Affecting Changes in Refractory Organic Carbon Contents

In the oceans, carbon sinks can act as a “solubility pump” that moves CO_2 deeper into the ocean through chemical equilibriums and physical transport [48]. A “carbonate pump” is based on the balance of the seawater CO_2 system and the precipitation and deposition of carbonate [49]. A“biological pump (BP)” transfers POC from the surface to the deeper layers of the ocean or even to the seafloor through a series of biological processes [50,51], and a “microbial carbon pump (MCP)” refers to microbial processes that utilize DOC and converts it to RDOC [19,52]. All of these pumps contribute to transporting CO_2 deeper into the ocean. Since this study was conducted in a pond with a water depth of about 1 m, the roles of the “solubility pump” and “biological pump” were not examined, and since RDOC is the more recalcitrant form of elemental C, the microbiological carbon pump may be the most important carbon sink in this system.

In this study, Chl-*a* contents showed significant positive correlations with DOC and RDOC contents and RDOC proportions (Tables 6 and 7, $p < 0.05$). The influences of Chl-*a* contents and the phytoplankton it represents on elemental C are multi-faceted. Phytoplankton absorb CO_2 in the water and convert it into organic carbon through photosynthesis. Phytoplankton also indirectly influence CO_2 in water by influencing the pH of the water, thus affecting the chemical balance of a series of C elements [53]. Indeed, studies have shown that the degradation of certain algae, such as seaweed (*Ulva prolifera*), will initially drive rapid increases in DOC content and various DOC species and promote rapid microbial growth, but over the long term, 1.6% of the carbon biomass of the algal debris will become RDOC [34]. Therefore, the Chl-*a* content is considered a key driver of RDOC contents in the water.

In this study, water temperature showed a significant negative correlation with both DOC and RDOC contents (Tables 6 and 7, $p < 0.05$), which was consistent with the findings of McDonough et al. [54]. The effects of water temperature on elemental C are also multi-faceted and comprehensive. Elevated water temperatures may lead to increased CO_2 emission from the water, resulting in lower CO_2 utilization by phytoplankton and reduced synthesis of organic carbon. Additionally, water temperature also affects the chemical balance of a series of C elements. Through these influences, high or low water temperatures can affect the utilization of organic carbon by phytoplankton, microorganisms, and even aquaculture organisms [55–57] and ultimately affect the DOC contents in the water of aquaculture ponds.

In this study, salinity showed a significant negative correlation with both RDOC contents and proportions (Tables 6 and 7, $p < 0.05$), which was consistent with the findings of Kubo et al. [25]. This may be because the community composition of heterotrophic microorganisms in water changes in response to salinity gradients [58], with bacterial communities in water with higher salinities being able to preferentially utilize unstable and recently produced DOC [59]. However, some studies have suggested that the bioavailability of DOC is related only to DOC contents and not to heterotrophic microbial community species composition [17,60]. In this study, the degradation experiments directly used microorganisms in in situ water, but the microbial community in water was not cultured and analyzed. Therefore, it could not be determined whether the composition of the heterotrophic microbial community affected the contents and proportions of RDOC. Further experimental validation would be necessary to elicit the mechanisms underlying the relationship between salinity and the contents and proportions of RDOC.

4.3. Analysis of Carbon Sequestration Effects of Integrated Prawn Aquaculture Systems

Integrated *P. vannamei*–hardshell clam pond culture systems have been shown, scientifically, to be a reasonable aquaculture system in terms of utilization of waste and water quality maintenance. By mixing the benthic and filter-feeding shellfish in the ponds used to primarily cultivate *P. vannamei*, the nutrient salt cycle in the aquaculture system is accelerated, the accumulation of nutrients in water is reduced, the utilization rate of nutrients by aquaculture organisms is improved, and water purity is enhanced, all of which contribute to improving the stability of the aquaculture system along with other aquaculture benefits [61]. However, in this study, the Chl-*a* content was the key driver affecting the contents and proportions of organic carbon in the water. Therefore, based on our results, the higher the mixing density of hardshell clams, the higher the proportion of RDOC in water, which may not be preferential in terms of utilizing RDOC for carbon sequestration, all other factors being equal. However, the growth of hardshell clams utilizes elemental C in the water and contributes directly and indirectly to the function of carbon sinks through pathways such as shell calcification [10,11], and from this point of view, as the density of hardshell clams increases, the carbon sink capacity of the culture pond will also increase [9]. This is despite the fact that RDOC in aquaculture ponds can be more difficult to utilize than shellfish shells, making the elemental C element in RDOC less likely to eventually be emitted to the atmosphere as a greenhouse gas, which makes this a relatively paradoxical issue. However, aquaculture ponds primarily function as farms for human food production and are not solely carbon sinks, so their carbon sequestration capacities should be considered by-products of food acquisition [62]. Therefore, further research is still necessary to determine the densities of *P. vannamei* and hardshell clams to be stocked in ponds to optimize the balance of food production, economic efficiency, and carbon sequestration capacity. In addition, sediments are an important component of pond ecosystems, and the presence and proportion of RDOC in sediments and its contribution to carbon sequestration needs to be investigated to more comprehensively assess the carbon sequestration effect of integrated aquaculture models in prawn ponds.

5. Conclusions

The presence of RDOC in *P. vannamei* pond culture system water has been discovered. Aquaculture activities significantly increased the contents of DOC, POC, and RDOC while reducing the proportion of RDOC in this water. The contents of DOC, POC, and RDOC, as well as the proportions of RDOC, were found to be significantly higher in the monoculture systems compared to the integrated aquaculture system. Additionally, these indicators were significantly positively correlated with chlorophyll-a contents in both aquaculture systems while exhibiting significant negative correlations with water temperature and salinity. By calculating the average content and proportion of RDOC across both aquaculture systems, it was determined that approximately 108.64 kg of RDOC is present per hectare of *P. vannamei* aquaculture water during the aquaculture period. Future research will focus on analyzing the specific composition of DOC in the water column, investigating the outcomes, composition, dynamics, and digestive enzyme activities of heterotrophic microorganisms to further determine the suitable density of *P. vannamei* and hard-shelled clams placed in the ponds, as well as the presence of RDOC in the sediment. This analysis aims to provide a more comprehensive assessment of the carbon sequestration capabilities of integrated shrimp aquaculture systems in ponds, thereby offering valuable insights for the development of low-carbon aquaculture strategies.

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