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A Study on Nitrogen and Phosphorus Budgets in a Polyculture System of *Oreochromis niloticus*, *Aristichthys nobilis*, and *Cherax quadricarinatus*

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Abstract: Polyculture is an effective way to achieve efficient utilization of nutrient resources in high-density intensive aquaculture systems. In order to study the optimal culture mode of *Oreochromis niloticu*, *Aristichthys nobilis*, and *Cherax quadricarinatus*, the budget of nitrogen and phosphorus in various polyculture systems (CH, CHC1, CHC2, CHC3) was studied with land-based enclosures. The results showed that all the three polyculture systems had higher total yields of cultured animals than the control group (two polyculture systems) ($p < 0.05$). The co-cultured organisms absorbed artificial feed or organic matter (such as plankton and sediment) from the polyculture system to different degrees. Feed was the main input of nitrogen (98.22–98.33%) and phosphorus (99.43–99.56%) in all systems. Considering the N and P outputs, 46.64–64.58% and 81.60–84.79%, respectively, accumulated in the sediment, and 34.43–52.55% and 14.89–17.30% of the N and P outputs, respectively, were harvested by aquaculture organisms. The pollution production coefficients of TN and TP in the *O. niloticus* polyculture ponds were 5.35–6.26 g/m^2 and 1.17–1.61 g/m^2, respectively. The TN production coefficients of *O. niloticus* and the ternary polyculture groups (CHC1, CHC2, and CHC3) were lower than that of the control group (CH). The TP production coefficients showed the opposite pattern. The N and P utilization efficiencies in the group with the optimal ratio of *O. niloticus*, *A. nobilis*, and *C. quadricarinatus* (4, 0.15, and 3 ind/m^2, respectively) were 2.56–12.82% and 6.62–11.03% higher, respectively, compared with those of the other groups. The N utilization efficiency was effectively improved in this group with the optimum stocking density for the polyculture systems, resulting in improved ecological efficiency and economic benefits.

Keywords: *Oreochromis niloticus*; *Aristichthys nobilis*; *Cherax quadricarinatus*; polyculture system; N and P budget

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1. Introduction

Oreochromis niloticus is the most common commercially farmed fish species worldwide; it is characterized by fast growth, easy domestication, and tolerance to transportation [1]. It is also a major farmed species in China, particularly in south China. *O. niloticus* aquaculture is expanding yearly: the stocking density is increasing, and greater amounts of feeds and fertilizers are being applied in response to the ever-increasing market demand. This increases the N and P contents in the water body, exacerbates eutrophication, and causes frequent diseases, with decreasing yields. These issues affect the sustainable development of intensive *O. niloticus* aquaculture [2].

Polyculture is a production method wherein different aquaculture organisms are proportionally cultured within the same system based on the principles of ecological balance,

mutually beneficial species relationships, and multi-nutrient utilization [3]. Polyculture improves ecological stability and nutrient recycling, which may result in synergistic benefits to the participating species. In aquaculture, this synergism can result in increased profitability through improved growth rates and/or reduced feed inputs [4]. Integrated aquaculture may improve the resource utilization efficiency in aquaculture systems, reduce environmental pollution, increase the biodiversity and yields of aquaculture organisms, and decrease the incidence of diseases [5,6]. In traditional Chinese pond culture, pond nesting is the main mode of *A. nobilis* culture in Guangdong and even the whole of China. The first purpose of pond nesting is to improve the added value of the main fish culture. The second is to share a certain proportion of pond rent, electricity and other breeding costs, or to make more profit from nesting *A. nobilis* [7]. Third, bighead carp exhibit filter-feeding behavior, consuming particulate matter and plankton in the aquatic environment. This behavior promotes relative stability and balance among plankton in the water column, thereby effectively controlling water blooms caused by eutrophication [8]. In the Guangdong area, grass carp, tilapia, white shrimp and some other intensive culture ponds all contain *A. nobilis*, and the stocking density is mostly 750~3000 ind/hm^2 [7]. At the same time, our previous experiments showed that the co-culture of *O. niloticus* + *A. nobilis* (biomass ratio = 15~25:1) + shrimps (*Macrobrachium rosenbergii*, *Penaeus vannamei* and *Metapenaeus ensis*) could significantly improve the economic benefits of *O. niloticus* [9]. Hence, this paper established the biomass ratio in reference to the above studies. Since their introduction to China in 1992, *C. quadricarinatus* have been utilized in polyculture models due to their diverse feeding habits, including consuming bottom-dwelling organisms, algae, and detritus [10–12]. Polycultures of tilapia and *C. quadricarinatus* can efficiently utilize resources, improving culture efficiency, regulating water quality, and reducing pollution through their diurnal cross-activities, ecological niches, and feeding composition [13–15]. The polyculture of these three species is a new farming model for adding value and decreasing pollutant emissions in China, as they have relatively good complementary feeding habits and growth space [9,16]. However, the stocking density and ratio of the three species lack relevant norms and theoretical guidance and are mostly determined based on experience during actual production. Therefore, studying N and P budgets in *O. niloticus*, *A. nobilis*, and *C. quadricarinatus* polyculture systems and comparing differences in environmental parameters between different polyculture models can provide data to support the optimization of polyculture models and improve the related aquaculture management.

Nitrogen and P are generally the limiting nutrient elements in aquaculture, and their levels can directly reflect changes in the aquaculture environment [17]. Nitrogen and P budgets (and their utilization efficiencies) can be used to quantify the pollution level in an aquaculture system to evaluate its strengths and weaknesses [18]. The N and P budget in aquaculture systems is widely studied, including in channel catfish (*Ictalurus punctatus*) ponds in America [19], intensive fishponds of gilthead seabream (*Sparus aurata*) in Israel [20], shrimp (*Penaeus monodon*) culture ponds [18], *Litopenaeus vannamei* and *Portunus trituberculatus* culture ponds [21], scampi (*Macrobrachium rosenbergii*) culture ponds [22] in India, crab culture ponds [23], and polyculture culture ponds of snakehead [24]. Relevant domestic and foreign research about *O. niloticus* has mainly focused on the N and P budgets of *O. niloticus*—fish polyculture, prawn—*O. niloticus* polyculture, and *O. niloticus* monoculture ponds [25–29], and there are few reports on N and P budgets of *O. niloticus*—*A. nobilis* polyculture systems. Therefore, this study established a series of farming models with different polyculture ratios based on the different growth spaces and feeding habits of the three freshwater aquaculture organisms. Land-based enclosure experiments were used to study the N and P budgets of four different polyculture systems of *O. niloticus*, *A. nobilis*, and *C. quadricarinatus*. We compared N and P accumulation in the sediments and water under different culture models and the utilization rates of N and P in each system. Together, we hope to provide a theoretical basis to optimize polyculture systems based on *O. niloticus*.

2. Materials and Methods

2.1. Experimental Ponds and Materials

This study was conducted at the aquaculture base of Zhuhai Deyang Aquaculture Co., Ltd., located in Zhuhai, Guangdong, China, from 12 May 2020 to 12 November 2020, and completed within 184 days.

The experiments were conducted using enclosure ecosystems in a freshwater pond with an area of approximately 1 ha that had a sediment bottom and a water depth of 1.8 to 2.0 m during the experiment. Twelve small ponds which were divided into four groups with three replicates per group were created with a 2 m distance from the edge of the freshwater pond as land-based experimental enclosures. The enclosures were constructed using impervious polyvinyl plastics and supported with timber piles. The area of each enclosure was 64 m^2 (8 m × 8 m). Separation of the pond area by enclosures avoided water exchanges among different experimental systems. The specific structure of the enclosure was previously reported in Deshang [30].

The *O. niloticus*, *A. nobilis*, and *C. quadricarinatus* individuals used in the experiment were sourced from Zhuhai Deyang Aquaculture Co., Ltd. (Zhuhai, China), and their mean stocking sizes were 6.2 ± 0.44, 20 ± 1.56, and 1.25 ± 0.09 g, respectively (Table 1). Four treatments were established, with three replicates of each treatment based on the species and densities of the experimental animals in the small ponds. There were three test treatments (CHC1, CHC2, and CHC3) and one control treatment (CH). The composition and stocking density of each treatment are shown in Table 1.

Table 1. Stocking information for different systems (CH: monoculture of *Oreochromis niloticus*; CHC1: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 1.5 ind/m^2; CHC2: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 3 ind/m^2; CHC3: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 4.5 ind/m^2).

	Oreochromis niloticus		***Aristichthys nobilis***		***Cherax quadricarinatus***	
	Weight (g)	**Density (ind/m^2)**	**Weight (g)**	**Density (ind/m^2)**	**Weight (g)**	**Density (ind/m^2)**
CH	6.22 ± 0.48	4	21.04 ± 3.56	0.15		
CHC1	6.21 ± 0.52	4	20.27 ± 6.28	0.15	1.26 ± 0.09	1.5
CHC2	6.18 ± 0.53	4	22.85 ± 4.37	0.15	1.26 ± 0.23	3
CHC3	6.19 ± 0.48	4	21.14 ± 5.15	0.15	1.25 ± 0.21	4.5

2.2. Aquaculture Management

O. niloticus was fed with a commercial pellet feed manufactured by Guangdong Haid Group Co., Ltd. (Guangzhou, Guangdong, China), and the feed amount was equivalent to 5% of body mass. The feed intake was observed daily, and the feed amount was adjusted based on the weather conditions, water temperature, and fish growth. An aeration tray was placed in each small enclosure pond to ensure that the dissolved oxygen was greater than 5 mg/L in the water. The water was not replaced during the experiment; however, the water lost owing to evaporation, filtration, and sampling was replenished as needed. Harvesting conditions were determined using a previously reported method [24].

2.3. Response Variables and Analytical Methods

2.3.1. Determination of Dissolved Oxygen, pH Value, and Temperature of Pond Water

The dissolved oxygen content was measured once every 15 days with a HACH LDO™ portable dissolved oxygen tester, and the pH value and temperature (°C) were measured with an HI9813-6 pH-EC-TDS-°C.

2.3.2. Measurement of Nutrients of Pond Water Samples and Interstitial Water

One liter of water (including suspended solids and phytoplankton) was collected from the upper, middle, and lower water layers at the four corners and center of each small

pond before, during, and after the experiment using a water sampler (Rong Sheng, China) every 15 days. The depths of the sites where water samples were collected was 0.5 m. Ten-centimeter-thick sediment was collected using a mud collector at the bottom of the pond and centrifuged for 10 min at 8000 r/min. Then, the interstitial water was collected, and the seepage of N and P was calculated according to the concentration of interstitial water. The water samples of each group were mixed prior to determining the total ammonia nitrogen (TAN), nitrite (NO_2^--N), nitrate (NO_3^--N), active phosphate (PO_4^{3-}-P), total nitrogen (TN), and total phosphorus (TP) contents according to previously reported methods [31,32].

2.3.3. Collection and Measurement of Breeding Organisms, Sediment, and Feed

Biological samples were collected before and after stocking the organisms. TN and TP were also collected and their contents determined using the above methods. At the same time, prior to stocking with aquaculture species, three sediment collection tubes were placed in each small pond, with the openings 1.0 m below the water surface. A cylindrical polyvinyl chloride (PVC) tube (diameter and height: 90 and 550 mm, respectively) was used to collect sediment, and the tubes' openings were covered with a nylon net (mesh 2a = 0.8 cm) to prevent aquaculture organisms from entering. At the end of the experiment, the sediment collection tubes were removed, the upper layer of water was siphoned out, and the sediment height (containing settled phytoplankton) was measured. Then, the sediment was poured into a large culture dish (15 cm), oven-dried at 60 °C for 48 h, crushed, and sieved using a 100-mesh screen. The TN content was determined using an elemental analyzer (Elementar III, Varian, Germany), and the TP content was determined using the acid-soluble molybdenum–antimony anti-colorimetric method [33]. The stocked animals, feed, and the harvested animals were dried at 60 °C to a constant weight, smashed, and sieved with a sample sifter (0.15 mm pore size). The TN of these samples was determined using a Vario ELIII Elemental Analyzer (Elementar, Dortmund, Germany). The TP levels of the stocked animals, feed, and harvested animals were determined using the molybdenum yellow spectrophotometer method [34].

2.3.4. Determination of the Growth Parameters of Breeding Organisms

The feeding situation of breeding organisms was observed every day. The amount of bait cast and death rates were recorded in detail, and the weight of raised organisms was measured at the end of breeding. Three samples were obtained from each experimental enclosure every 5 days, and their growth was recorded.

2.3.5. Determination of Other Indicators

In the period before and after the breeding cycle, the enclosures were analyzed to assess nutrient absorption, and the nutrient content (also known as adsorption amount) was calculated. Rainwater was collected outdoors, and the inputs of N and P from rainwater were determined by estimating their amounts in rainfall.

2.3.6. Calculation Formula

Nitrogen and P budgets and the system N and P utilization rate were calculated as previously described [24] for the harvest of breeding organisms. The N and P pollution production coefficients of the aquaculture ponds were estimated [35] according to the material scale algorithm, and the pollution production coefficient of N and P in the sediment was calculated according to the area method [36].

2.4. Statistical Analysis of Data

The data are presented as the mean and standard deviation (mean ± SD). The data were analyzed using one-way analysis of variance (ANOVA) by LSD multiple comparison tests using the computer software of SPSS 21.0, to determine whether there were significant differences between all the four systems. Cases with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Environmental Parameters

The initial environmental parameters did not differ between the treatment groups ($p > 0.05$) (Table 2). The environmental levels of TN and TP in the sediment were 0.74–0.793 g/L and 0.617–0.683 g/L respectively. The variation in water environmental parameters is shown in Table 2. The temperature fluctuation was 23.5–33.4 °C, the dissolved oxygen contents and pH were in the range of 4.83–13.1 mg/L and 7.07–8.39, respectively, and the TAN, NO_3^--N, NO_2^--N, TN, PO_4^{3-}-P, and TP contents were 0.024–1.49, 0.019–1.46, 0.002–0.171, 0.504–2.23, 0.005–0.219, and 0.009–0.254 mg/L, respectively.

Table 2. Variation in environmental parameters in different treatment groups (CH: monoculture of *Oreochromis niloticus*; CHC1: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 1.5 ind/m^2; CHC2: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 3 ind/m^2; CHC3: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 4.5 ind/m^2). DO: dissolved oxygen; TAN: total ammonia nitrogen; NO_3^--N: nitrate nitrogen; NO_2^--N: nitrite nitrogen; TN: total nitrogen; PO_4^{3-}-P: soluble reactive phosphorus; TP: total phosphorus; (): Initial environmental parameters.

Environmental Compartment	Parameter	CH	CHC1	CHC2	CHC3
Water	T (°C)	23.5–33.4 (27.2 ± 0.4)	23.5–33.4 (27.2 ± 0.4)	23.5–33.4 (27.2 ± 0.4)	23.5–33.4 (27.2 ± 0.4)
	DO (mg/L)	4.83–8.54 (4.98 ± 0.21)	4.97–10.2 (5.17 ± 0.2)	5.35–12.4 (5.72 ± 0.75)	4.98–13.1 (5.58 ± 0.54)
	pH	7.07–8.04 (7.27 ± 0.02)	7.14–8.31 (7.36 ± 0.01)	7.54–8.39 (7.58 ± 0.06)	7.32–8.15 (7.41 ± 0.03)
	TAN (mg/L)	0.043–0.586 (0.331 ± 0.036)	0.024–1.285 (0.348 ± 0.031)	0.036–1.24 (0.369 ± 0.037)	0.028–1.49 (0.368 ± 0.029)
	NO_3^--N (mg/L)	0.019–0.087 (0.081 ± 0.038)	0.067–1.12 (0.083 ± 0.052)	0.033–1.08 (0.084 ± 0.041)	0.124–1.46 (0.083 ± 0.043)
	NO_2^--N (mg/L)	0.018–0.059 (0.003 ± 0.001)	0.002–0.044 (0.003 ± 0.001)	0.004–0.171 (0.004 ± 0.001)	0.003–0.129 (0.003 ± 0.001)
	PO_4^{3-}-P (mg/L)	0.005–0.131 (0.091 ± 0.04)	0.035–0.179 (0.093 ± 0.007)	0.037–0.202 (0.097 ± 0.010)	0.027–0.219 (0.107 ± 0.012)
	TN (mg/L)	0.69–1.731 (1.29 ± 0.041)	0.504–1.98 (1.304 ± 0.068)	0.815–2.23 (1.32 ± 0.053)	0.607–2.16 (1.21 ± 0.073)
	TP (mg/L)	0.009–0.217 (0.091 ± 0.007)	0.042–0.201 (0.122 ± 0.021)	0.064–0.254 (0.112 ± 0.013)	0.043–0.217 (0.143 ± 0.017)
Sediment	TN (g/L)	(0.740 ± 0.114)	(0.792 ± 0.107)	(0.793 ± 0.078)	(0.762 ± 0.087)
	TP (g/L)	(0.683 ± 0.067)	(0.629 ± 0.054)	(0.617 ± 0.063)	(0.621 ± 0.058)

3.2. Harvesting Conditions

Table 3 shows that feeds and feed coefficients of *O. niloticus* were similar between the treatment groups at the time of harvesting ($p > 0.05$). In CHC2, the average body weight was 0.684 ± 0.009 kg/ind, the survival rate was 93.2 ± 2.16%, and the yield was 25.5 ± 0.803 Ton/hm^2. These data were noticeably higher than those in CH ($p < 0.05$), but not significantly different from those in CHC1 and CHC3 ($p > 0.05$). The survival rate of *A. nobilis* in all groups was 100%, and the size and yield did not differ ($p > 0.05$). The size of *C. quadricarinatus* did not differ between the treatment groups at the time of harvesting ($p > 0.05$). The survival rates in CHC1 and CHC2 were greater than that in CHC3 ($p < 0.05$), and the yield in CHC2 was higher than those in CHC1 and CHC3 ($p < 0.05$). There was no significant difference in total aquaculture output between the CHC2 and CHC3 groups ($p > 0.05$), but it was significantly higher than that of CH and CHC1 groups ($p < 0.05$).

Table 3. Harvesting conditions of cultured animals in different treatment groups (CH: monoculture of *Oreochromis niloticus*; CHC1: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 1.5 ind/m^2; CHC2: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 3 ind/m^2; CHC3: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 4.5 ind/m^2). Data are presented as the mean ± standard deviation (SD), and different superscripts in the same column indicate significant differences ($p < 0.05$). FCR: feed conversion ratio.

Group	*Oreochromis niloticus*					*Aristichthys nobilis*			*Cherax quadricarinatus*			Total Yield (Ton/hm^2)
	Size (kg)	Survival Rate (%)	Yield (Ton/hm^2)	Feed (Ton/hm^2)	FCR	Size (kg)	Survival Rate (%)	Yield (Ton/hm^2)	Size (kg)	Survival Rate (%)	Yield (Ton/hm^2)	
CH	0.654 ± 0.017 ^{a}	89.4 ± 2.23 ^{a}	23.3 ± 0.734 ^{a}	30.4 ± 1.15	1.30 ± 0.11	0.515 ± 0.011	100	0.772 ± 0.043	-	-	-	24.1 ± 0.654 ^{a}
CHC1	0.670 ± 0.011 ^{ab}	90.5 ± 1.72 ^{ab}	24.2 ± 0.759 ^{ab}	30.3 ± 1.04	1.25 ± 0.70	0.516 ± 0.011	100	0.774 ± 0.058	0.059 ± 0.007	52.1 ± 6.76 ^{a}	0.458 ± 0.009 ^{a}	25.4 ± 0.749 ^{a}
CHC2	0.684 ± 0.009 ^{b}	93.2 ± 2.16 ^{b}	25.5 ± 0.803 ^{b}	30.6 ± 1.10	1.20 ± 0.05	0.517 ± 0.012	100	0.776 ± 0.103	0.057 ± 0.007	50.3 ± 8.13 ^{a}	0.865 ± 0.017 ^{b}	27.1 ± 0.804 ^{b}
CHC3	0.665 ± 0.014 ^{ab}	92.5 ± 1.45 ^{ab}	24.6± 0.784 ^{ab}	30.3 ± 0.989	1.23 ± 0.06	0.511 ± 0.014	100	0.766 ± 0.089	0.050 ± 0.006	32.1 ± 7.85 ^{b}	0.721 ± 0.012 ^{c}	26.1 ± 0.814 ^{b}

3.3. Dry Matter and Nitrogen and Phosphorus Contents of Feed and Breeding Organisms

The feed and *O. niloticus*, *A. nobilis*, and *C. quadricarinatus* stocking dry matter and nitrogen and phosphorus contents in Table 4 show that harvest experimental biological dry matter and N and P contents have different degrees of increase ($p < 0.05$) compared with those of stocked organisms. This shows that grazing organisms absorbed artificial feed or organic matter (such as plankton and sediment) from the breeding system to different degrees.

Table 4. Contents of dry matter, N, and P in feed, in *Oreochromis niloticus*, and in stocked and harvested species in the experiment. Data are presented as the mean ± standard deviation (SD), and different superscripts in the same column indicate significant differences ($p < 0.05$).

Item	Sampling Time	Dry Matter (%)	N (Dry Matter)	P (Dry Matter)
Oreochromis niloticus	Stocked	21.8 ± 1.34 ^{a}	14.4 ± 0.69 ^{a}	1.46 ± 0.05 ^{a}
	Harvested	22.6 ± 1.64 ^{a}	15.6 ± 0.77 ^{b}	1.78 ± 0.02 ^{b}
Aristichthys nobilis	Stocked	20.7 ± 1.94 ^{a}	11.3 ± 0.48 ^{a}	1.06 ± 0.009 ^{a}
	Harvested	21.3 ± 1.63 ^{b}	12.3 ± 0.55 ^{b}	1.17 ± 0.024 ^{b}
Cherax quadricarinatus	Stocked	21.4 ± 1.82 ^{a}	10.8 ± 0.32 ^{a}	0.95 ± 0.08 ^{a}
	Harvested	22.8 ± 2.07 ^{b}	11.7 ± 1.05 ^{b}	1.15 ± 0.06 ^{b}
Feed		93.7	6.94	1.31

3.4. Contents of Dry Matter, N, and P, and Densities in the Sediment of Different Enclosures before and after the Experiment

The moisture content of the sediment significantly increased ($p < 0.05$), and the density decreased ($p < 0.05$) after the experiment (Table 5). Furthermore, the N and P contents in the sediment accumulated to a large extent ($p > 0.05$).

Table 5. Contents of mud dry matter, N, and P and densities in the sediment of different enclosures before and after the experiment. Data are presented as the mean ± standard deviation (SD), and different superscripts in the same column indicate significant differences ($p < 0.05$).

Item	Sampling Time	Treatments			
		CH	CH1	CH2	CH3
Dry matter %	Before	63.8 ± 3.05 ^{a}	63.8 ± 3.05 ^{a}	63.8 ± 3.05 ^{a}	63.8 ± 3.05 ^{a}
	After	40.3 ± 2.68 ^{b}	38.3 ± 2.61 ^{b}	37.5 ± 3.01 ^{b}	40.5 ± 2.46 ^{b}
Density/g.cm^{-3}	Before	1.83 ± 0.06 ^{a}	1.83 ± 0.06 ^{a}	1.83 ± 0.06 ^{a}	1.83 ± 0.06 ^{a}
	After	1.31 ± 0.03 ^{b}	1.38 ± 0.04 ^{b}	1.47 ± 0.03 ^{b}	1.56 ± 0.05 ^{b}
N (Dry matter)	Before	0.27 ± 0.02 ^{a}	0.27 ± 0.02 ^{a}	0.27 ± 0.02 ^{a}	0.27 ± 0.02 ^{a}
	After	1.56 ± 0.08 ^{b}	1.44 ± 0.06 ^{b}	1.31 ± 0.02 ^{b}	1.12 ± 0.03 ^{b}
P Dry matter	Before	0.019 ± 0.001 ^{a}	0.019 ± 0.001 ^{a}	0.019 ± 0.001 ^{a}	0.019 ± 0.001 ^{a}
	After	0.094 ± 0.006 ^{b}	0.103 ± 0.003 ^{b}	0.097 ± 0.002 ^{b}	0.104 ± 0.002 ^{b}

3.5. N and P Inputs

The N and P were primarily derived from the feed in all treatment groups. The inputs were 203.6–211.7 g/m^2 and 46.7–47.02 g/m^2, respectively, constituting 98.2–98.3% and 99.4–99.6% of the N and P inputs into the system, respectively (Table 6). The inputs of N and P from the initial water body were 1.98–2.12 g/m^2 and 0.13–0.18 g/m^2, respectively, accounting for 0.93–1.02% and 0.28–0.39% of the N and P inputs of the system, respectively. The stocked organisms and rainfall marginally contributed to the N and P inputs in all treatment groups.

Table 6. Inputs of nitrogen and phosphorus (g/m^2) in different treatment groups (CH: monoculture of *Oreochromis niloticus*; CHC1: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 1.5 ind/m^2; CHC2: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 3 ind/m^2; CHC3: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 4.5 ind/m^2). Data are presented as the mean ± SD, and different superscripts in the same column indicate significant differences ($p < 0.05$).

Systems		Treatments			
		CH	CH1	CH2	CH3
N (g/m^2)	Feed	203.6 ± 8.43	211.6 ± 13.03	204.9 ± 11.65	211.7 ± 14.37
	Animals	0.490	0.520	0.545	0.558
	Water	1.98 ± 0.09	2.03 ± 0.11	2.12 ± 0.15	2.00 ± 0.09
	Rain	1.04	1.04	1.04	1.04
	Total	207.1 ± 8.78	215.2 ± 13.04	208.6 ± 11.38	215.3 ± 14.28
P (g/m^2)	Feed	47.0 ± 3.22	46.5 ± 2.89	47.0 ± 2.98	46.5 ± 2.16
	Animals	0.057	0.06	0.065	0.068
	Water	0.13 ± 0.01	0.14 ± 0.04	0.16 ± 0.05	0.18 ± 0.03
	Rain	0.02	0.02	0.02	0.02
	Total	47.2 ± 0.11 ^{a}	46.7 ± 0.07 ^{b}	47.2 ± 0.02 ^{c}	46.8 ± 0.03 ^{c}

3.6. N and P Outputs

The primary N output was sediment deposition. The output of N to sediment was 65.6–121.4 g/m^2, which constituted 55.2–65.6% of the N output of the system in all treatment groups (Table 7). The secondary N output was the harvested aquaculture organisms. It reached 65.8–74.0 g/m^2, which accounted for 33.8–52.2% of the N output in the system. The seepage was 0.34–2.51 g/m^2, which accounted for 0.20–1.29% of the output of the system N and P. The output of N by ammonia volatilization and absorption and the amount of N accumulated in the water body accounted for non-significant proportions of the TN output.

Table 7. Outputs of nitrogen and phosphorus (g/m^2) in different treatment groups (CH: monoculture of *Oreochromis niloticus*; CHC1: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 1.5 ind/m^2; CHC2: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 3 ind/m^2; CHC3: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 4.5 ind/m^2). Data are presented as the mean ± standard deviation (SD), and different superscripts in the same column indicate significant differences ($p < 0.05$).

Systems		Treatments			
		CH	CH1	CH2	CH3
N (g/m^2)	Sediment accumulatio	123.4 ± 8.43 ^{a}	94.8 ± 7.54 ^{b}	65.6 ± 6.36 ^{c}	90.5 ± 6.19 ^{b}
	Harvested animals	65.8 ± 2.28 ^{a}	69.4 ± 2.67 ^{ab}	74.0 ± 2.70 ^{b}	71.1 ± 2.36 ^{b}
	Water accumulation	0.620 ± 0.09 ^{a}	0.410 ± 0.116 ^{b}	0.080 ± 0.05 ^{c}	0.250 ± 0.04 d
	Absorption	1.01 ± 0.17 ^{a}	0.96 ± 0.09 ^{a}	0.71 ± 0.04 ^{b}	0.76 ± 0.11 ^{b}
	Seepage	2.52 ± 0.21 ^{a}	1.65 ± 025 ^{b}	0.37 ± 0.12 ^{c}	0.34 ± 0.16 ^{c}
	Volatilization	1.28 ± 0.06 ^{a}	1.18 ± 0.28 ^{a}	1.06 ± 0.19 ^{ab}	1.04 ± 0.25 ^{b}
	Total	194.6 ± 9.61 ^{a}	168.4 ± 7.27 ^{b}	141.8 ± 6.68 ^{c}	164 ± 7.12 ^{b}

Table 7. *Cont.*

Systems		Treatments			
		CH	CH1	CH2	CH3
P (g/m^2)	Sediment accumulation	37.0 ± 3.22 [a]	36.6 ± 3.35 [a]	34.9 ± 3.90 [a]	35.1 ± 2.47 [a]
	Harvested animals	6.50 ± 0.022 [a]	6.85 ± 0.024 [b]	7.31 ± 0.026 [c]	7.02 ± 0.026 d
	Water accumulation	0.14 ± 0.01 [a]	0.10 ± 0.01 [a]	0.08 ± 0.01 [b]	0.90 ± 0.02 [b]
	Absorption	0.27 ± 0.05 [a]	0.18 ± 0.03 [a]	0.11 ± 0.02 [b]	0.15 ± 0.04 [b]
	Seepage	0.48 ± 0.11 [a]	0.35 ± 0.07 [b]	0.14 ± 0.02 [c]	0.17 ± 0.03 [c]
	Volatilization	-	-	-	-
	Total	44.4 ± 3.23 [a]	44.1 ± 3.31 [a]	42.5 ± 2.73 [a]	43.34 ± 2.26 [a]

The output of P to the sediment in all treatment groups was 34.9–37.0 g/m^2, which constituted 81.0–83.4% of the P output of the system. The P output through the harvesting of aquaculture organisms was 6.50–7.31 g/m^2, which accounted for 14.6–17.2% of the P output of the system. The seepage was 0.17–0.48 g/m^2, which accounted for 0.33–1.08% of the N and P output of the system. The amount of P accumulation and absorption in the water body accounted for a non-significant proportion of the TP output.

The amount of N accumulated in the water body and sediment in CHC2 was notably lower than in other groups ($p < 0.05$) (Table 7). The amount of P accumulated in the water body in CHC3 was the highest among the groups ($p < 0.05$), yet the amounts of P accumulated in the sediment were similar for all groups ($p > 0.05$).

3.7. N and P Utilization Efficiencies

The N utilization efficiency of the treatment groups increased in the following order: CH (31.20%) < CHC1 (32%) < CHC3 (32.8%) < CHC2 (35.2%). The P utilization efficiency of the treatment groups increased in the following order: CH (13.6%) < CHC3 (13.6%) < CHC1 (14.5%) < CHC2 (15.1%) (Figure 1). The P utilization efficiency in CHC2 was the highest and was substantially greater than the P utilization efficiencies in CH and CHC3 ($p < 0.05$), and similar to that in CHC1 ($p > 0.05$).

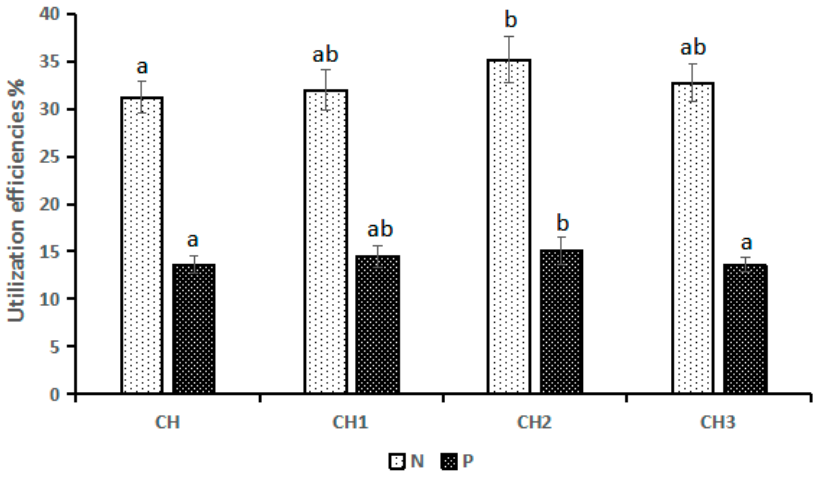

Figure 1. N and P utilization efficiencies by cultured animals in different treatment groups (CH: monoculture of *Oreochromis niloticus*; CHC1: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 1.5 ind/m^2; CHC2: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 3 ind/m^2; CHC3: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 4.5 ind/m^2). Data are presented as the mean ± standard deviation (SD), and different superscripts indicate significant differences ($p < 0.05$).

3.8. Pollution Production Coefficients of the Ponds (PPCP) and Sediment Deposition (PPCSD)

The calculated TN and TP pollution production coefficients of *O. niloticus* farming with different treatment groups are presented in Table 8. The TN pollution coefficients of the ponds were 6.01 g/m^2, 5.35 g/m^2, and 5.41 g/m^2 in the CHC1, CHC2, and CHC3 groups, respectively. The TN pollution coefficient in CHC1 was not significantly different from that of the CH group ($p > 0.05$), whereas the values in all other groups were significantly lower than the value of 6.26 g/m^2 in the CH group ($p < 0.05$). The coefficients of TP pollution were 1.61 g/m^2, 1.52 g/m^2, and 1.56 g/m^2 in the CHC1, CHC2, and CHC3 groups, respectively. Therefore, they were significantly higher than the 1.17 g/m^2 observed in the CH group ($p < 0.05$). The coefficients of TN contamination in the CHC1, CHC2 and CHC3 groups were 13.5 g/m^2, 12.5 g/m^2 and 11.9 g/m^2, respectively, and these values were significantly lower than the value of 14.75 g/m^2 in the CH group ($p < 0.05$). There were significant differences among other groups ($p < 0.05$), except between CHC2 and CHC3 groups ($p > 0.05$). However, there was no significant difference in the TP production coefficients between the treatment groups ($p > 0.05$).

Table 8. Pollution production coefficients of the ponds (PPCP) and sediment deposition (PPCSD) of tilapia farming with different culture models (CH: monoculture of *Oreochromis niloticus*; CHC1: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 1.5 ind/m^2; CHC2: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 3 ind/m^2; CHC3: *O. niloticus*, 4 ind/m^2, *A. nobilis*, 0.15 ind/m^2, *C. quadricarinatus*, 4.5 ind/m^2). Data are presented as the mean ± standard deviation (SD), and different superscripts indicate significant differences ($p < 0.05$).

Group	PPCP (g/m^2)		PPCSD (g/m^2)	
	K_{TN}	K_{TP}	K_{TN}	K_{TP}
CH	6.26 ± 0.45 ^{a}	1.17 ± 0.02 ^{a}	14.8 ± 0.41 ^{a}	4.64 ± 0.29 ^{a}
CH1	6.01 ± 0.36 ^{a}	1.61 ± 0.03 ^{b}	13.5 ± 0.22 ^{b}	4.57 ± 0.32 ^{a}
CH2	5.35 ± 0.26 ^{b}	1.52 ± 0.01 ^{b}	12.5 ± 0.14 ^{c}	4.49 ± 0.09 ^{a}
CH3	5.41 ± 0.32 ^{b}	1.56 ± 0.04 ^{b}	11.9 ± 1.02 ^{c}	4.36 ± 0.11 ^{a}

Note(s): K_{TN}, total nitrogen pollution coefficient; K_{TP}, total phosphorous pollution coefficient.

4. Discussion

4.1. N and P Inputs and Outputs

The common sources of N and P inputs in pond aquaculture systems are the feeds and fertilizers applied, the farmed animals, the initial water body, and rainfall [37]. Feeds and fertilizers are the major sources of N and P inputs, constituting 70–95% of the TN and P inputs in aquaculture systems [24,38]. This result was confirmed in studies of the intensive *O. niloticus* farming model [26] and polyculture ponds of tilapia [26,27], in which the inputs of N and P from feed constituted 84.3–92.6% and 81.9–98.7% of the N and P inputs to the system, respectively. In this study, the inputs of N and P from feed constituted 98.2–98.3% and 99.4–99.6% of the N and P inputs to the system, respectively. This indicates that the feed was the main source of N and P in the aquaculture ponds. The slightly higher levels recorded in this study compared with those recorded in previous studies may be related to differences in the initial amount of ammonia nitrogen in the water body, the lack of water exchange throughout the whole experiment, and the administration of only one type of artificial compound feed.

Previous reports indicate that N and P output by percolation can constitute 0.10–1.28% and 0.11–1.3% of the TN and P input, respectively [21,39]. In this study, the seepage outputs in relation to total output of N and P of the system were 0.2–1.29% and 0.33–1.08%, respectively. This is consistent with the results reported above. The absorption was less and had little influence on the experiment. Furthermore, studies suggest that sediment accumulation plays an integral role in the N and P outputs in aquaculture systems, with the outputs of N and P via sediment accumulation accounting for 19.4–73.4% and 39.5–93.5% of the TN and TP outputs of the system, respectively [40,41]. In this study, the outputs of N

and P through sediment accumulation constituted 55.2–65.6% and 81.00–83.4% of the N and P outputs, respectively. This result is consistent with those from previous research of intensive tilapia (*Oreochromis niloticus*) culture ponds [26,28]. The proportions of N and P accumulated in sediment in the TN and TP outputs were considerably different. This may be related to differences in the N and P cycles. In the N cycle, some N is released into the air in gaseous forms (N_2, N_2O, and NO), whereas the P cycle is sedimentary: P does not exist in gaseous forms and is primarily adsorbed to the surfaces of suspended particulate matter and sediments in water [24].

Tables 6 and 7 show that 7.74–32.5% of the N input was "missing." Numerous studies on N and P budgets reported similar results [42]. This may be related to denitrification and percolation [43]. Previous reports indicate that N output by denitrification and percolation can constitute 7–30.0% and 0.1–1.28% of the TN input, respectively [21,39]. Denitrification involves microorganisms using organic compounds as carbon sources and electron donors to reduce nitrites and nitrates into gaseous N [44], which can be categorized as anaerobic or aerobic [6]. In addition, aerobic denitrification processes are more obvious when the dissolved oxygen (DO) concentration is above 2.0 mg/L, and a high DO concentration can improve the aerobic denitrification process [45]. The aquaculture water body has a high DO content (DO: 4.85–5.35 mg/L) (Table 2). Therefore, a completely aerobic denitrification process took place in this study, and its main product was N_2. Denitrification is affected by multiple environmental parameters, such as temperature, pH, DO, microbial abundance, and community composition [46], resulting in significant differences in the proportions of "missing" N between various treatment groups. The difference between P input and output is marginal because no gaseous phase is present in the P cycle, and such differences are primarily associated with percolation or calculation errors [47].

4.2. N and P Accumulation

The water in the aquaculture system is periodically replaced to maintain a good aquaculture environment, and the amounts of N and P accumulated in the water are generally low. Nevertheless, the replacement of water in aquaculture systems requires high consumption of water resources. An estimation by Yang [48] shows that approximately 4.77×10^4 and 3.75×10^3 tons of N and P, respectively, are discharged every year when the water in mariculture systems in China is replaced. This adversely affects the surrounding environment. Although the water was not replaced during the aquaculture process in this study, the amounts of N and P accumulated in the final water body were 0.08–0.62 g/m^2 and 0.08–0.90 g/m^2 (Table 7), respectively, which were significantly lower than those in *O. niloticus* monoculture ponds [26] and tilapia-Chinese Carps polyculture ponds [28]. This indicates that the polyculture of *O. niloticus*, *A. nobilis*, and *C. quadricarinatus* could effectively reduce the water pollution caused by pond aquaculture. The amounts of N and P accumulated in the water body in treatment CHC2 were notably lower than those in the other treatment groups ($p < 0.05$), and the amounts of N and P accumulated in the water body in treatment CHC2 were reduced by 68.0–87.1% and 20.0–91.1%, respectively. This suggests that the CHC2 model effectively decreased the amounts of N and P accumulated in the water body (Table 7).

Sediment accumulation plays an essential role in N and P outputs. In this study, more N accumulated in the sediment for CH than in those of the other treatments ($p < 0.05$). This was 23.2–46.8% higher than that in the polyculture model of three species. This can be explained by the residual feed and feces of *O. niloticus* settling directly at the bottom of the pond and promoting plankton growth. The inorganic nutrients released by the residual feed and feces of *O. niloticus* promote the growth of plankton, which is an important source of sediment and detritus [6]. In the polyculture model of three species, *C. quadricarinatus* could feed on and utilize detritus (such as residual feed and feces) to reduce the amount of N accumulated in the sediment. However, the amounts of P accumulated in sediment were similar in the different treatments, ($p > 0.05$), which could be attributed to the sedimentary cycle caused by the strong affinity of sediment for phosphorus.

4.3. Suitable Breeding Ratio and N and P Utilization Efficiencies

Compound pond breeding of tilapia culture is a common aquaculture practice in China. However, people still rely on experience in the selection of different mixed breeding varieties and stocking proportions. This study showed that there were some differences in the growth, survival rate, and yield of cultured fishes under different mixed breeding modes. In each treatment group, the size, survival rate, and yield of *O. niloticus* and *C. quadricarinatus* and the total output of breeding organisms showed first an increasing and then a decreasing trend with the increases in the *C. quadricarinatus* mixed breeding ratio (Table 3). Among them, the CHC2 group had the highest yield, which was significantly higher than that of the other treatment groups, while the CHC3 group had the lowest yield. Therefore, in the compound breeding system of *O. niloticus*, *A. nobilis*, and *C. quadricarinatus*, the excessive stocking density of *C. quadricarinatus* has a negative effect on the system output value. The comprehensive benefit and yield of the CHC2 group were significantly higher than those of other the other compound breeding models. They were especially higher than those measured for tilapia single culture [26,28], tilapia co-culture with *Hypophthalmichthys molitrix* and *A. nobilis* [13], and tilapia co-culture with *A. nobilis* + shrimps (*Macrobrachium rosenbergii*, *Penaeus vannamei* and *Metapenaeus ensis*) [9]. Our findings are consistent with the results of a previous study on N and P budgets in the polyculture systems of snakehead with bighead carp [24].

Nitrogen and P are important nutrient elements affecting the aquaculture system. Their effective utilization rates and conversion accumulation are often used as important indicators to evaluate the aquaculture level, aquaculture mode, and the pollution degree of aquaculture. They have important theoretical and practical significance for in-depth study of the inputs and outputs, income and expenditure conversion, and utilization rate of aquaculture ponds [49]. The contents of dry matter, N, and P of the experimental organisms increased to different degrees ($p < 0.05$) at harvest (Table 4), and the content of dry matter, N, and P in the sediment decreased to different degrees ($p < 0.05$) (Table 5). This indicates that breeding organisms absorb organic matter from artificial feed or breeding systems (such as plankton and sediment). This is consistent with the results of a tilapia single culture pond study [50]. In addition, the N and P utilization efficiencies in CHC2 were greater than those in the control group (CH) ($p < 0.05$). The N and P utilization efficiencies in the polyculture model of the three species (CHC2) were 2.56–12.8% and 6.62–11.0% greater than those of the control, respectively. Various forms of N can be utilized by aquaculture organisms in a polyculture system. *Oreochromis niloticus* consumes pellet-based feed, and its residual feed and feces settle to the bottom of the water body to provide food for *C. quadricarinatus*. The nutrients released by residual feed for *O. niloticus* can promote plankton growth to provide food for *A. nobilis*. *Cherax quadricarinatus* dwells in the benthic zone, causing the bioturbation of sediment to promote the re-release of N and P [51], which promotes plankton growth once again so that the N and P can be repeatedly recycled at various nutrient levels.

In this study, the N and P utilization efficiencies of farmed animals were 31.20–35.20% and 13.6–15.1%, respectively. These results are consistent with those obtained by Tian [52] and Sahu [18], and greater than those obtained in intensive *O. niloticus* aquaculture systems [26] and other fish fishing systems [24,27,41]. The ratio of different organisms is a critical factor in polyculture systems. This study revealed that the N and P utilization efficiencies of *C. quadricarinatus* varied at different stocking densities; CHC1 could not fully utilize N and P at a low stocking density, while an excessive *C. quadricarinatus* stocking density (CHC3) led to the inhibition of the growth and survival of the farmed fish, and decreased the N and P utilization efficiencies. CHC2 (*O. niloticus*, 4.0 ind/m^2; *A. nobilis*, 0.15 ind/m^2; and *C. quadricarinatus*, 3.0 ind/m^2) exhibited high ecological efficiency and possible economic benefits could be inferred. This suggests that the polyculture of the three species may be a highly effective aquaculture model.

4.4. Pollution Production Coefficient and Environmental Impact of Different O. niloticus and Breeding Modes

An aquaculture pond is an artificial or semi-artificial ecosystem, and the productivity of the system, the material cycling characteristics and output, the degree of pollution, and its impact on the surrounding environment are closely related to its aquaculture mode. Different aquaculture varieties and aquaculture modes have a great impact on the N and P loads in the aquaculture environment [44]. In this study, the pollution production coefficients of TN and TP in *O. niloticus* and the polyculture pond were 5.35–6.26 g/m^2 and 1.17–1.61 g/m^2, respectively, according to the material balance method. Among the treatments, the TN production coefficients of *O. niloticus* and the ternary polyculture groups (CHC1, CHC2, and CHC3) were lower than that of the control group (CH), and this result was lower than the values for *O. niloticus* in a single raised pond [28], in a prawn + tilapia mixed pond [53], and in a shrimp and crab mixed pond [54]. The TP production coefficient was slightly higher in the control group (CH); this is consistent with the results of a study by Zhong [28]. Pond sediment is an important reservoir for the main biogenic factors such as N and P in ponds, and it is also a secondary pollution source of aquaculture water eutrophication. Disturbances or changes in the environmental conditions have an important influence on the aquaculture water environment [36]. Therefore, the pond sediment production coefficient can be used to evaluate the pond breeding model. This study showed that the N and P pollution coefficients (KTN and KTP) of the CHC1, CHC2, and CHC3 groups were lower than those of the control group (CH), according to the area method. The values were lower than those of a tilapia monoculture pond reported by Meng [36] and prawn intensive ponds reported by Yang [55]. Meanwhile, they were consistent with the values of the complex pond reported by Zhong [28]. The latter study concluded that the pollution coefficients of N and P pollution in breeding ponds and sediments are related to factors such as breeding varieties, breeding modes, breeding pond age, and feeding strategy. The pond compound breeding mode is significantly better than the single breeding mode [28,36]. Our findings confirm this view, with the CHC2 group being the optimal breeding mode in terms of the breeding benefit, N and P utilization rate, and pollution coefficient.

5. Conclusions

This study conducted land-based enclosure experiments to investigate the N and P budgets of *O. niloticus*, *Aristichthys nobilis*, and *C. quadricarinatus* at different stocking densities in a polyculture system. The statistical analyses indicated that the inputs of N and P from feed were 98.2–98.3% and 99.4–99.6%, respectively, and 55.2–65.6% of the N and 81.0–83.4% of the P outputs accumulated in the sediment. Hence, this study indicates that the feed was the main source of N and P inputs in the pond system. The CHC2 group (stocking density: *O. niloticus*, 4 ind/m^2; *A. nobilis*, 0.15 ind/m^2; and *C. quadricarinatus*, 3 ind/m^2) was the optimal mode for breeding benefits, N and P utilization rates, and pollution coefficient, and had greater ecological efficiency and economic benefits. Meanwhile, further research is required to determine the underlying mechanisms of the nutrient salt utilization efficiency of *A. nobilis* and *C. quadricarinatus* by performing C and N stable isotopic analysis. This should include the utilization of suspended particulate waste and sedimented particulate matter generated in the Nile tilapia composite culture pond system.

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Data Availability Statement: The data that support the findings of this study are openly available.

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