


Article

Crayfish–Fish Aquaculture Ponds Exert Reduced Climatic Impacts and Higher Economic Benefits than Traditional Wheat–Rice Paddy Cultivation

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Abstract: In pursuit of higher economic profits, an increasing number of conventional rice paddies are being converted into aquaculture ponds in Southeast China. Due to the lack of field observations, the greenhouse gas (GHG) emissions caused by this change are not clear. A parallel field experiment in Southeast China was performed to compare CH₄ and N₂O emissions from rice paddies and rice-paddy-converted freshwater crayfish–fish aquaculture ponds that had previously been rice paddies. The annual fluxes of CH₄ and N₂O fluxes from inland crayfish–fish aquaculture averaged 0.36 mg m⁻² h⁻¹ and 45.55 µg m⁻² h⁻¹, which amounted to 31.50 kg CH₄ ha⁻¹ and 3.99 kg N₂O ha⁻¹, respectively. Compared with traditional rice paddies, such conversions significantly reduced the emissions of CH₄ and N₂O emissions by 46.4% and 67.5%, respectively, but greatly increased the net ecosystem economic budget (NEEB) by 485%. The fluxes of both CH₄ and N₂O fluxes from aquaculture ponds were positively correlated with water/sediment temperature and dissolved organic carbon in the sediment, but were negatively correlated with the concentration of oxygen that is dissolved in the water. In addition, the emissions of CH₄ and N₂O were closely associated with the chemical oxygen demand of water and the content of N in the sediment, respectively. The results of this study suggest that converting rice paddies to freshwater crayfish–fish aquaculture ponds could cause a reduction in the impacts on the climate and result in greater economic benefits. There is an urgent need worldwide for more field studies on the emissions of CH₄ and N₂O emissions from aquaculture ponds, including more types of fish species and management practices. These results will help researchers to comprehensively evaluate whether such conversions of agricultural land use are ecologically and economically feasible.

Keywords: rice paddy; aquaculture; CH₄; N₂O; NEEB



Citation: Hu, Z.; Gu, C.; Maucieri, C.; Shi, F.; Zhao, Y.; Feng, C.; Cao, Y.; Zhang, Y. Crayfish–Fish Aquaculture Ponds Exert Reduced Climatic Impacts and Higher Economic Benefits than Traditional Wheat–Rice Paddy Cultivation. *Agriculture* **2022**, *12*, 515. <https://doi.org/10.3390/agriculture12040515>

Academic Editor: Nour Ahmadi

Received: 5 March 2022

Accepted: 31 March 2022

Published: 5 April 2022

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1. Introduction

Global warming that is caused by greenhouse gas (GHG) emissions is a serious environmental problem worldwide. Two of the key GHGs are methane (CH₄) and nitrous oxide (N₂O). Their global warming potential (GWP) over a 100-year period on a mass basis is 28- and 265-fold greater than that of carbon dioxide (CO₂), respectively. Paddy fields, as typical agricultural wetlands, have been proven to be major sources of CH₄ and N₂O [1–3]. China produces the largest amount of rice in the world, accounting for approximately one fifth of the area of the world's rice production, and it contains 23% of

all the cultivated land [4,5]. In recent decades, many field experiments and model studies have studied the emissions of greenhouse gases from Chinese paddy fields, and their estimates suggest that the emissions of CH₄ and N₂O emissions were 6–10 Tg yr⁻¹ and 32–51 Gg yr⁻¹, respectively [5–10].

For agricultural wetlands, GHG emissions from aquaculture ponds have attracted increasing attention because of the increased culture area and more intensive feed input [1,2,11–13]. Unfortunately, current knowledge of the GHGs produced from aquaculture ponds remains limited. The updated report from the Intergovernmental Panel on Climate Change [11] provides methodological guidance on CH₄ emissions due to a lack of direct field measurement data and robust emission factors, as well as a methodology to estimate N₂O emissions from aquaculture ponds [1,13]. These modeling estimates have major uncertainties, which require validation and calibration using direct field measurements [1,13].

China is one of the major producers (35%) and exporters (19%) of aquatic products in the world [14]. To meet the current large market demands and rich economic returns, Chinese inland aquaculture ponds have developed rapidly in recent years [1,2]. Limitations in resources and space have resulted in the increasing conversion of some paddy fields in Southeast China to inland aquaculture ponds. A total of 1.32 million ha of paddy fields have been converted to more than half of the total area of inland pond aquaculture (2.57 million ha) in China over the past few decades [15]. Among them, the scale of freshwater crayfish aquaculture has increased rapidly over the years; it has become the sixth most common freshwater aquaculture species in China (the top five are all major freshwater fish species) [16]. China's freshwater crayfish export markets are mainly concentrated in the United States and Europe, accounting for more than 90% of the export share [16]. In contrast, the fluxes of CH₄ and N₂O have rarely been studied in inland aquaculture. However, these limited field measurements indicated that emissions of CH₄ and N₂O were greatly affected by the aquaculture product variety, culture methods, water management, and feeding material [1]. Therefore, there is a need for more field measures to determine the extent to which these changes occur when agricultural land use is converted to rice paddies and inland aquaculture. It is imperative to understand whether these agricultural changes can decrease the levels of CH₄ and N₂O and/or enrich the pools of soil organic carbon (SOC) to mitigate the level of net GHG emissions to generate a net ecosystem economic budget (NEEB) [1,17–19].

Here, fluxes of CH₄ and N₂O were measured simultaneously in conventional rice paddies and neighboring 5-year-old aquaculture ponds for freshwater crayfish–fish farming that had been converted from rice paddies. Overall, the primary goals of this study were the following: (1) to discern the annual production of fluxes of CH₄ and N₂O from inland aquaculture ponds; (2) to determine how dependent seasonal CH₄ and N₂O fluxes were on the parameters of water and soil/sediment in inland aquaculture ponds; and (3) to compare the global warming potential (GWP) and net ecosystem economic budget (NEEB) among rice paddies and aquaculture ponds that had been converted from rice paddies. These results are helpful for us to comprehensively evaluate whether such a conversion of agricultural land use is ecologically and economically feasible.

2. Materials and Methods

2.1. Site Description

In 2019, this study performed a parallel field experiment simultaneously in conventional winter wheat–rice paddy and 5-year-old ponds that had been converted from rice paddies to freshwater crayfish–fish farming aquaculture ponds. These bodies of water were located on the experimental farm of Nanjing Agricultural University (Xinghua, Jiangsu Province, China) (32°52' N, 119°50' E). These two agricultural farming systems were situated approximately 300 m apart.

The experimental region has a subtropical monsoon climate. Its annual mean precipitation is 1130 mm, and the air temperature averaged 17.7 °C during the annual observation period. A cropping system of a rice–winter wheat rotation overwhelmingly dominated the

field site, but some conventional rice paddies had been converted to freshwater crayfish–fish farming aquaculture ponds.

The freshwater crayfish–fish farming aquaculture ponds monitored in this study had been converted from conventional rice paddy fields in 2014. Since then, they had served to continuously cultivate integrated crayfish–fish for the 5-year period before these field experiment measurements. The initial physicochemical properties of the soil/sediment at the experimental site are summarized in Table 1. The soil was classified as gleisil or hydromorphic. For the freshwater crayfish farming pond, the concentration values of the dissolved oxygen (DO) and chemical oxygen demand (COD) on the surface water at a depth of 0–30 cm averaged between 7.4 mg L⁻¹ and 15.1 mg L⁻¹ and varied from 6.2 to 10.0 mg L⁻¹ and 9.30 to 28.7 mg L⁻¹ over the whole waterlogging stage, respectively, during the study periods. In the freshwater crayfish–fish aquaculture pond sediments, the mean dissolved organic carbon (DOC) concentration during the annual observation period was 130 mg kg⁻¹ (with a range from 54.7 to 307 mg kg⁻¹).

Table 1. Physicochemical properties of the initial sediment and soil at a depth of 0–20 cm.

Agroecosystem	pH	Bulk Density g cm ⁻³	Dissolved Organic Carbon mg kg ⁻¹	Total Nitrogen g kg ⁻¹	Total Organic Carbon g kg ⁻¹
Winter wheat–rice paddy	6.8 ± 0.05	1.10 ± 0.16	218.5 ± 13.1	1.71 ± 0.15	16.1 ± 1.09
Crayfish farming wetland	7.0 ± 0.02	1.25 ± 0.26	190.6 ± 8.8	1.42 ± 0.14	14.0 ± 0.68

2.2. Field Experiment Management

2.2.1. Winter Wheat–Rice Rotation Cropping System

This study utilized a local conventional winter wheat–rice paddy field with three individual replicated plots that were 60 × 80 m each and close to the observed freshwater crayfish–fish farming aquaculture system as a reference. We followed the typical local regimes for fertilizer and water over the course of a year (Table 2). Winter wheat (*Triticum aestivum* L. cv. Yangmei 20) was sown on 13 November 2019 and was harvested on 2 June 2020. In accordance with the conventional local fertilization management, the seasonal application of chemical N fertilizer was 200 kg N ha⁻¹, and this fertilizer was applied in split applications as basal fertilizer (45%), at the stage of turning green (30%), and the booting stage (25%). After the winter wheat had been harvested, there was a shortened fallow period from 3 to 23 June 2020. At the end of this period, the plots were plowed, and the surface soil was mixed. Finally, the ground was leveled before the transplantation of rice. The rice (*Oryza sativa* L. cv. Wuyunjing 24) seedlings were transplanted to the rice paddies on 25 June 2020, and harvested on 23 October 2020.

Farming customs involved flooding in midseason and drainage-reflooding-moisture irrigation without waterlogging (F–D–F–M) and were implemented during the season in which the rice was grown (Figure 1). Synthetic N fertilizer (urea) was applied at 250 kg N ha⁻¹ cumulative input to all the field plots during the season in which the rice was grown. The percentages applied included basal fertilizer (40%), tillering stage (40%), and the heading stage (20%). In addition, calcium superphosphate (P₂O₅) and potassium chloride (KCl) (100 kg ha⁻¹ and 150 kg ha⁻¹, respectively) were applied as basal fertilizers during both the wheat and rice growing seasons [2]. Crop residues were pulverized by machines and returned to the fields after crops were harvested. Crop management techniques, i.e., seed rate, plant spacing, irrigation, and weed management, were consistent with those of local farmers.

Table 2. Agricultural practices in the two compared treatments.

Winter Wheat–Rice Rotation		Freshwater Crayfish–Fish Aquaculture	
Date	Key Practice Involved	Date	Key Practice Involved
12 November 2019	Basal fertilizing (45%)	13 November 2019	Drying pond
13 November 2019	Wheat sowing	13 December 2019	Pond dredging and sunning
17 December 2019	Fertilizing at turning-green stage (30%)	5 March 2020	Pond flooding
27 March 2020	Fertilizing at booting stage (25%)	7 March 2020	Pond disinfection
2 June 2020	Harvest	22 March 2020	Planting <i>Elodea canadensis</i>
3 June 2020–23 June 2020	wheat–rice fallow	2 April 2020	Freshwater crayfish stocking
24 June 2020	Basal fertilizing (40%)	18 April 2020	Planting <i>Hydrilla verticillata</i>
25 June 2020	Rice transplanting	20 April 2020	Snail stocking
7 July 2020	Fertilizing at tillering stage (40%)	25 April 2020	Planting <i>Vallisneria natans</i>
2 August 2020	Drainage initiated	28 April 2020	Bighead carp stocking
12 August 2020	Fertilizing at heading stage (20%)	1 May 2020	Mandarin fish stocking
23 October 2020	Harvest	5 June 2020	Freshwater crayfish and fish harvest
24 October 2020–11 November 2020	Rice–wheat fallow	12 November 2020 5 November 2020–10 November 2020	Drying ponds again Fish harvest

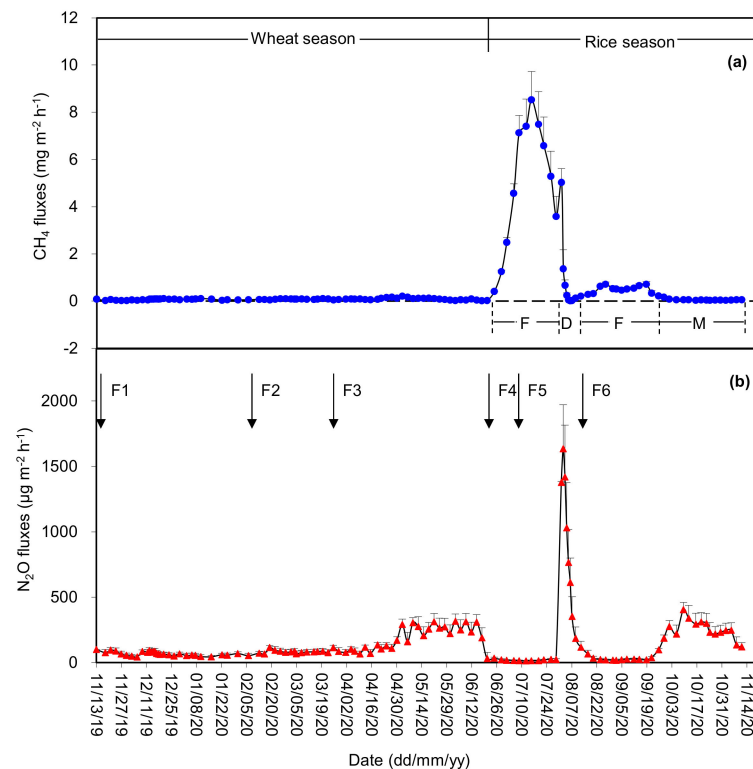


Figure 1. Annual dynamics of CH₄ (a) and N₂O (b) fluxes from rice–wheat rotation. F–initial flooding; D–midseason drainage; M–moisture irrigation without waterlogging. F1–F6 solid arrows indicate N fertilizer application events. The bars indicate the standard error of the means (\pm SE).

2.2.2. Freshwater Crayfish Farming Aquaculture System

The parallel field experiment on freshwater crayfish–fish farming aquaculture ponds was simultaneously performed from November 2019 to November 2020. This study utilized

three freshwater crayfish–fish farming ponds as experimental replicates. The ponds were 60×650 m. In accordance with typical local aquaculture methods, the common sequential polyculture model dominated by freshwater crayfish (*Procambarus clarkii*) was integrated with bighead carp (*Aristichthys nobilis*) and mandarin fish (*Siniperca chuatsi*) as supplements, as well as mixed snails (*Bellamya quadrata*). The stocking density of larval freshwater crayfish (body length 2–4 cm) was 2.1×10^5 – 2.25×10^5 tail ha^{-1} on 2 April 2020. To improve the water environment utilization and economic profits, bighead carp (body length 2.5–4.0 cm) and mandarin fish (body length > 5 cm) were successively introduced to the aquaculture pond at densities of 800–1300 and 900–1400 tail ha^{-1} on 28 April and 1 May 2020, respectively. In addition, snails were introduced into the ponds, so that the water quality improved, and there was extra food for the freshwater crayfish. The ponds were stocked with approximately 5000 kg ha^{-1} of snails on 20 April 2020, to provide extra food for the freshwater crayfish and improve the quality of the water.

To provide food and molting shelters, each freshwater crayfish–fish farming pond was split into two portions based on the presence (WAV) or absence of aquatic vegetation (OAV). Three kinds of aquatic vegetation (*Elodea canadensis*, *Hydrilla verticillate*, and *Vallisneria spiralis*) were transplanted in sequence on 22 March, 18 April, and 25 April 2020, respectively. Most of them were submerged plants that covered nearly 60% across the bottom of the aquaculture pond. In general, the WAV covered about 60% of the entire freshwater crayfish–fish farming pond, whereas the OAV covered 40%. To optimize the survival environment for freshwater crayfish and fish, the DO concentration was kept >5 mg L^{-1} during the flooding period through the use of micropore aerators that were 10 cm above the sediment surface on the bottom of aquaculture ponds. Freshwater crayfish are omnivorous. To be consistent with local aquacultural practices, the freshwater crayfish and fish were fed twice daily at 9:00 and 16:00 with a formulated feed (made in Linda Feed Technology Company, Ltd., China), corn, and trash fish. The amount of feed daily was adjusted based on their stage of growth stage and how they responded to the previous feeding. Overall, the total input of N (420 kg N ha^{-1}) provided to the freshwater crayfish–fish as feed was similar to that applied to the winter wheat–rice rotation treatment over the entire course of a year. The depth of water in the freshwater crayfish–fish aquaculture ponds constantly varied from 0.6 to 1.8 m during the whole flooding period. More details of the management techniques for the annual aquaculture cycle are shown in Table 2.

2.3. CH_4 and N_2O Flux Measurements

The annual in situ field measurements of the N_2O and CH_4 fluxes from both the winter wheat–rice paddy fields and the freshwater crayfish–fish farming aquaculture ponds were performed simultaneously using a static closed opaque chamber from 13 November 2019 to 13 November 2020. Special boardwalks were constructed to guarantee the ability of smoothly reaching and minimally disturbing selected representative gas sampling points at both sites for the whole observation period. They were installed above the potential surface of water and soil before the initial flooding. For each plot, three collars that were 50 cm long \times 50 cm wide \times 20 cm high were constructed from PVC. They were permanently sunk into the soil/sediment near the boardwalks, so that the gas-collecting chambers could be reproducibly placed during successive measurements of GHG emissions throughout the entire period of observation. Each collar had a 5 cm deep groove on the top edge, which could be sealed with a level surface by filling with water. Consistent with the collar, the cross-sectional area of the chamber was 50 cm \times 50 cm (0.25 m²). A circulating fan was located in each chamber to completely mix the gas. Aluminum foil and sponges were wrapped as layers, so that potential changes in the air temperature inside the chamber were largely minimized when the gas was sampled. The temperature inside the chamber was recorded each time and showed almost no difference among different chambers. When the gas was sampled, the chamber rim of fit into the collar's groove, and the chamber was placed over the surface area of water or the crop vegetation. This study preestablished a height-adjustable stainless-steel stand before the initial flooding at each gas sampling

point, which was firmly fixed into the sediment, so that the flux collar was sufficiently supported. The potential water surface was then adjusted, so it was level and could adapt to the fluctuations in the level of water. Thus, the disturbance of water was minimized during gas sampling in the freshwater crayfish–fish aquaculture ponds. The height of each stand was adjusted on the day before each freshwater crayfish–fish farming pond was sampled for gas to guarantee that all the flux collars were situated just below the water surface. This ensured that the chamber rim was sealed with a leveled surface.

The fluxes of N_2O and CH_4 were usually measured twice per week throughout the whole observation period. In particular, they were evaluated more frequently (once per day) during the midseason drainage and after precipitation events or fertilization in paddy fields and initial drainage in the freshwater crayfish–fish farming aquaculture ponds, respectively. The gas samples were always collected from 08:00 to 11:00 in the morning for the rice growing season and from 14:00 to 17:00 in the evening for the wheat growing season because the temperatures of the water/sediment and soil during this period were close to those of the mean daily soil and water/sediment; this minimized the effect of variations in the fluxes of N_2O and CH_4 [1,12,20,21]. Special 1.5 L-volume air-sampling bags were used to collect the gas from each chamber. Inert aluminum-coated plastic was used to create the bags, which were operated by a battery-operated pump made by Delin Ventures (London, UK) with a flow of 3 L min^{-1} at 0, 5, 10, 15, and 20 minutes after the chamber was closed. The samples in the air–sampling bags were sent immediately to the laboratory for analysis by gas chromatography (GC) that was conducted within a few hours.

The chamber GC method was used to simultaneously measure the fluxes of N_2O and CH_4 using a modified GC (Agilent 7890A; Agilent Technologies, Santa Clara, CA, USA) [20,21]. The GC was equipped with two detectors, including an electron capture detector (ECD) and a flame ionization detector (FID), as described by Zou et al. (2005) [21] and Liu et al. (2014) [22]. The standard deviations and average fluxes of the GHGs were calculated from three replicates of each treatment. The cumulative emissions of N_2O and CH_4 accumulated sequentially from the fluxes that occurred between every two adjacent intervals of measurements [20,21,23].

2.4. GWP and NEEB Calculation

Lashof and Ahuja introduced the concept of global warming potential (GWP) in 1990 [24]. This metric is a simplified index that is based on radiative properties and is used to estimate the potential future impacts of the emissions of different gases on the climate system in a relative sense. When the GWP is estimated, CO_2 is usually considered to be the reference gas, and increases or reductions in the emissions of N_2O and CH_4 are transformed into CO_2 equivalents based on their GWPs [22]. Researchers recently used estimates of the net GWP to completely recognize the impacts of agricultural practices on radiative forcing [17,25–27]. In this study, combined GWPs from emissions of N_2O and CH_4 were calculated for both winter wheat–rice paddy and freshwater crayfish–fish farming aquaculture treatments with IPCC factors over a 100-year time scale (GWP: $\text{CH}_4 \times 45 + \text{N}_2\text{O} \times 270$) [11,28].

Li et al. (2015) [29] and Sun et al. (2019) [30] proposed the net ecosystem economic budget (NEEB) to evaluate the economic feasibility of eco–agriculture. This study calculated it using the following equation:

$$\text{NEEB} = \text{yield gain} - \text{agricultural activity cost} - \text{carbon cost}$$

The yield gain (paddy fields, $4.35 \times 10^4 \text{ CNY ha}^{-1}$; freshwater crayfish–fish farming aquaculture ponds, $3 \times 10^5 \text{ CNY ha}^{-1}$) was calculated based on the current prices for rice, wheat, freshwater crayfish, fish, and their respective yields. The agricultural activity costs were $2.76 \times 10^4 \text{ CNY ha}^{-1}$ and $2.1 \times 10^5 \text{ CNY ha}^{-1}$ for the winter wheat–rice paddy and freshwater crayfish–fish farming aquaculture treatments, respectively. The costs consisted of mechanical tillage and harvesting, fertilizers, pesticides and herbicides, crayfish frying,

fish frying, forage, site rent, and labor costs. The carbon cost was the product of the carbon trade price ($103.7 \text{ CNY t}^{-1} \text{ CO}_2\text{-eq}$) and their net GWP.

2.5. Other Data Measurements

The 0–20 cm layers of the sediment or surface soil were collected prior to wheat sowing and the flooding of aquaculture ponds to measure the pH, soil texture, bulk density (BD), total nitrogen (TN), DOC, and total organic carbon (TOC) [1]. The Chinese Soil Society Guidelines were used to determine the physicochemical properties of the soil [31]. The temperatures of the water and soil were measured during gas sampling using electronic temperature recorders. A JENCO0-9010 M digital DO meter (Jenco Instruments, San Diego, CA, USA) was used to determine the concentration of DO in situ, whereas the COD concentration was measured using HACH reaction kits (HACH, Loveland, CO, USA). The contents of soil/sediment $\text{NO}_3^- \text{-N}$ were monitored by UV spectroscopy at 220 and 275 nm, and the contents of $\text{NH}_4^+ \text{-N}$ were determined using the indophenol blue method (U-2900; Hitachi, Tokyo, Japan). This study measured the dynamics of DOC concentration in the soil/sediment using infrared detection (Phoenix 8000; Teledyne Tekmar, Mason, OH, USA) and ultraviolet-enhanced persulfate digestion. The sampling frequencies of soil/water temperatures and water DO were consistent with those of gas sampling, and all the other environmental parameters were monitored 2–3 times a month over the whole observation period.

2.6. Statistical Analyses

Differences in the annual emissions of N_2O and CH_4 between these two agricultural systems were examined using a one-way analysis of variance (ANOVA). Tukey's multiple range tests were used to examine the differences between treatments in more detail. Regression curves were computed to examine the dependence of N_2O and CH_4 on the parameters of soil/sediment and water fluxes. All the statistical analyses were performed using SPSS v. 22.0 (IBM, Inc., Armonk, NY, USA), and statistical significance was determined at $p < 0.05$.

3. Results

3.1. CH_4 Emissions

In the winter wheat–rice rotation cropping system, CH_4 emissions only occurred during the rice-growing season, while no significant emissions and/or uptake of methane were detected during the wheat-growing season (Figure 1a). The emissions of CH_4 gradually increased over the rice-growing season until the peak fluxes were observed under waterlogged field conditions approximately 2–3 weeks after the rice had been transplanted. This was attributed to organic residues that remained from previous seasons in concert with the application of crop residues. After this, the fluxes of CH_4 decreased dramatically by midseason drainage, and they remained at a lower rate of approximately $0.03\text{--}0.34 \text{ mg m}^{-2} \text{ h}^{-1}$ until the rice was harvested (Figure 1a).

The aquatic vegetation in the freshwater crayfish–fish farming ponds did not modify the seasonal patterns of fluxes of CH_4 , which were greatly dependent on the temperature of water temperature and the parameters of the water/sediment over the entire observation period (Figures 2a, 3a and 4). The initial peak CH_4 flux was captured in the early stage of drainage, which was mainly due to the explosive emissions of methane generated during the flooding period and stored in the sediment. Thereafter, the fluxes of CH_4 from the WAV and OAV treatment plots rapidly declined and remained at a low rate of emission until the freshwater crayfish were stocked in the waterlogged ponds. After flooding, the CH_4 fluxes of the two treatment plots typically increased as the level and water temperature of the pond water increased over the yearly cultivation cycle, and the level of CH_4 emissions peaked during the highest water temperature period (from mid-July to early August). Subsequently, the CH_4 fluxes gradually decreased in parallel with the water temperature and level over the whole cycle of observation until the freshwater crayfish and fish were harvested. Although the trend in methane emissions was very similar between seasons,

there was a substantial difference in the total seasonal flux of CH₄ between the WAV and OAV treatment plots (Figure 2a and Table 3).

Table 3. Seasonal and annual total CH₄ and N₂O emissions and their net GWP and NEEB.

Wetlands ^a	CH ₄ (kg ha ⁻¹)			N ₂ O (kg ha ⁻¹)			GWP ^b (kg CO ₂ -eq ha ⁻¹ yr ⁻¹)	NEEB ^c (CNY ha ⁻¹)
	Wheat Season/ Drainage Period	Rice Season/ Flooding Period	Annual	Wheat Season/ Drainage Period	Rice Season/ Flooding Period	Annual		
Winter wheat–rice rotation	4.56 ± 0.69 ^a	54.42 ± 8.79 ^a	58.80 ± 9.45 ^a	7.50 ± 1.82 ^a	4.78 ± 1.16 ^a	12.28 ± 2.98 ^a	5962 ± 1221 ^a	15.28 ± 1.25 ^a
Freshwater crayfish–fish aquaculture ^d	1.36 ± 0.36 ^b	30.14 ± 8.35 ^{bc}	31.50 ± 8.70 ^{bc}	1.95 ± 0.54 ^b	2.04 ± 0.58 ^b	3.99 ± 1.11 ^b	2494 ± 691 ^b	89.38 ± 12.7 ^b
WAV	1.46 ± 0.44 ^b	38.13 ± 11.60 ^{ab}	39.59 ± 12.04 ^b	1.74 ± 0.52 ^b	2.07 ± 0.62 ^b	3.81 ± 1.13 ^b	2810 ± 844 ^b	
OAV	1.21 ± 0.23 ^b	18.15 ± 3.50 ^c	19.36 ± 3.73 ^c	2.26 ± 0.58 ^b	1.99 ± 0.51 ^b	4.25 ± 1.08 ^b	2019 ± 461 ^b	

Different letters in a single column represent significant differences between treatments at $p < 0.05$. ^a The annual wheat–rice rotation cycle consisted of the wheat season (wheat growing plus short fallow) and rice season (rice growing plus short fallow), and annual freshwater crayfish–fish aquaculture cycle was divided into drainage and flooding periods. ^b IPCC GWP factors (mass basis, kg CO₂-eq ha⁻¹) for CH₄ and N₂O are 45 and 270 over the time period of 100 years, respectively (IPCC, 2013). ^c NEEB refers to the net ecosystem economic budget (CNY ha⁻¹). ^d The whole pond mean during each aquaculture stage was obtained by the equation of WAV × 60% (percentage pond area with aquatic vegetation cover) + OAV × 40% (percentage pond area without aquatic vegetation cover) based on the individual treatment values. WAV—with aquatic vegetation; OAV—without aquatic vegetation.

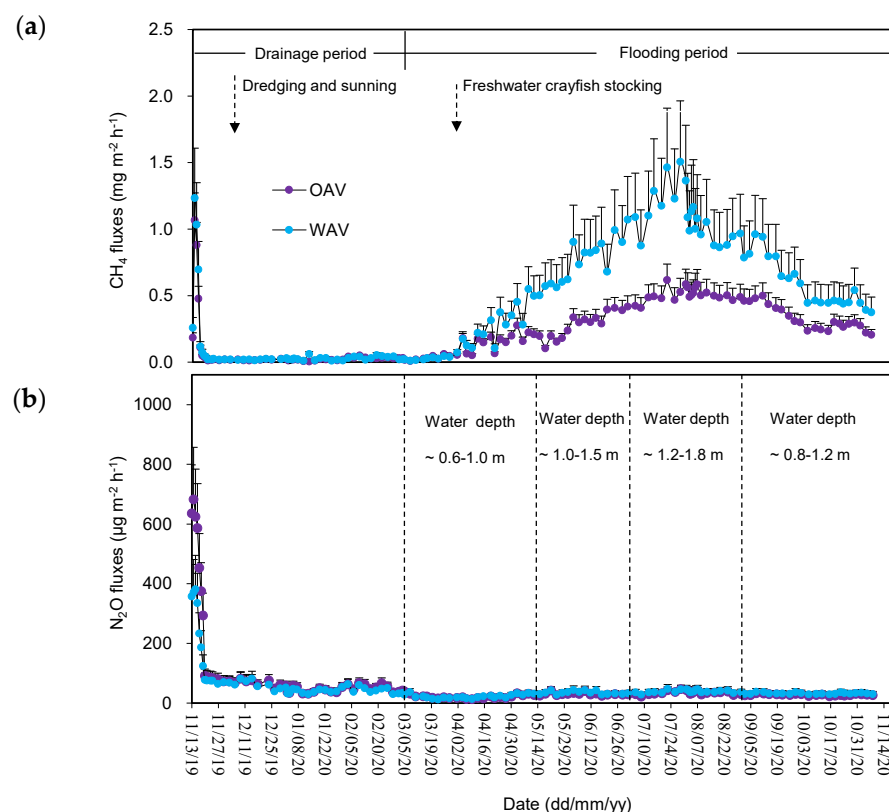


Figure 2. Annual dynamics of CH₄ (a) and N₂O (b) fluxes from freshwater crayfish–fish aquaculture ponds. WAV and OAV—treatments with and without aquatic vegetation in crayfish–fish aquaculture ponds, respectively. Dotted arrows indicate the major management throughout the year.

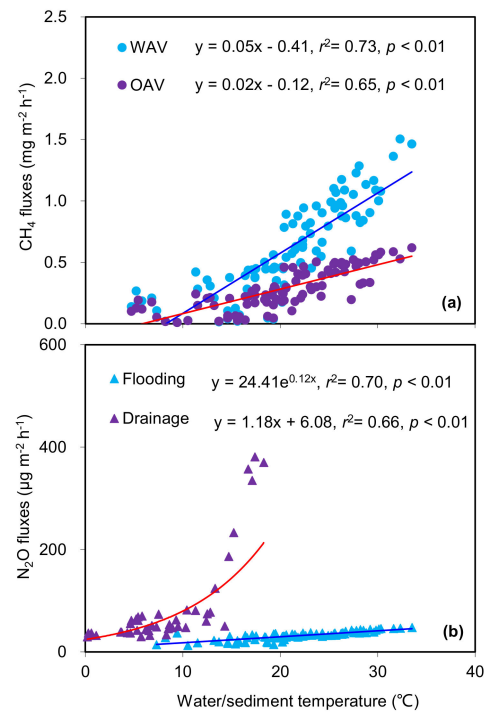


Figure 3. Dependence of CH₄ (a) and N₂O (b) fluxes on water/sediment temperature in the freshwater crayfish–fish aquaculture ponds.

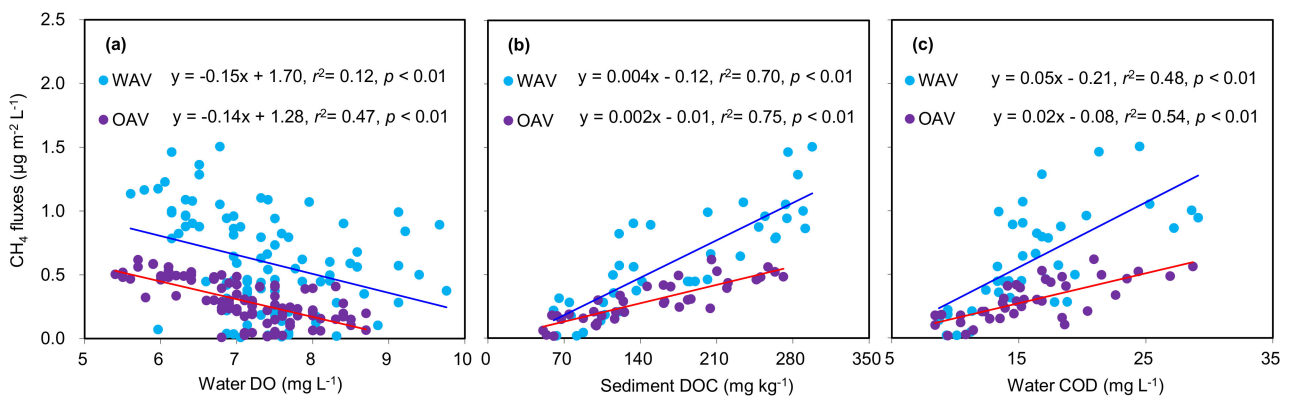


Figure 4. Dependence of CH₄ fluxes on water and sediment parameters in the waterlogging freshwater crayfish–fish aquaculture ponds. (a) Dissolved oxygen (DO) in water; (b) dissolved organic carbon (DOC) in sediment; (c) water chemical oxygen demand (COD).

3.2. N₂O Emissions

In the winter wheat–rice rotation cropping system, changes in the N₂O fluxes during the seasons were mainly regulated by the water regime and application of synthetic fertilizer during the rice–growing season (Figure 1b). A high level of N₂O emissions was apparent during the nonwaterlogged periods of the rice growing season, such as the midseason drainage (D) and moist (M) periods. In particular, there was an obvious tradeoff between the emissions of N₂O and CH₄ during the midseason drainage that facilitated pronounced peak fluxes of N₂O emissions. In contrast, only a small level of N₂O emissions was observed under the waterlogged conditions throughout the rice growing season. However, the peaks of N₂O fluxes were commonly detected after topdressing synthetic fertilizer was applied during the winter wheat season.

In the freshwater crayfish–fish aquaculture ponds, a similar seasonal trend in N₂O emissions was observed between the two treatments that had aquatic vegetation or lacked it;

this trend was more closely affected by the water parameters and temperature of the pond (Figures 2b, 3b and 5). Similar to the CH₄ emissions, substantial N₂O fluxes occurred during pond drainage, which were triggered by the fierce nitrification/denitrification process of N in the sediment under suitable moisture and temperature. In addition, intensive emissions of N₂O were produced during waterlogging and stored in the sediment at that moment. Thereafter, as the practice of drainage was extended, the N₂O fluxes gradually decreased and leveled off. Clearly different from the drastic variation in N₂O emissions at the initial stage of drainage, the N₂O fluxes of the freshwater crayfish–fish aquaculture ponds across treatments tended to be relatively stable throughout the flooding period.

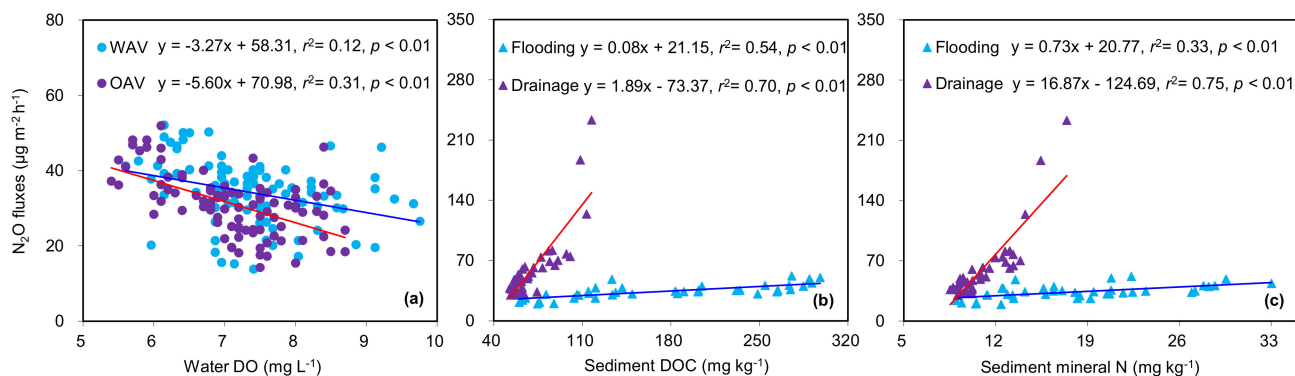


Figure 5. Dependence of N₂O fluxes on water and sediment parameters in the freshwater crayfish–fish aquaculture ponds. (a) Dissolved oxygen (DO) in water; (b) dissolved organic carbon (DOC); in sediment; (c) sediment mineral N (NH₄⁺ + NO₃⁻).

3.3. GWP and NEEB

There was a significant effect on the GWP and NEEB following the conversion of paddy fields into crayfish–fish aquaculture (Tables 3 and S1). Compared with the conventional winter wheat–rice rotation, crayfish–fish aquaculture significantly decreased the GWP by 58.2%. However, the presence or absence of aquatic vegetation had no effect on the GWP of crayfish–fish aquaculture ponds. Relative to the low NEEB (15.28 CNY ha⁻¹) in the winter wheat–rice rotation, crayfish–fish aquaculture significantly increased the NEEB by 484.9%.

4. Discussion

4.1. Impact of Aquatic Vegetation on CH₄ and N₂O Emissions

Aquatic vegetation plays an important role in crayfish culture during the flooding period of freshwater crayfish–fish farming ponds [32]. It is worth noting that aquatic hydrophytes have a significant impact on the production and emissions of GHGs [1,33]. Indeed, vegetation might enhance anaerobic environments and methanogenesis conditions by both direct root system oxygen consumption and root exudate release in the sediment, which increase microbial activity with subsequent increases in DO consumption and methanogenesis [34,35]. Finally, aquatic hydrophytes also served as a major pathway for the transportation of CH₄ to the atmosphere from the soil/sediment through their complex conduits, thus avoiding oxidation in the water [36].

In contrast, the presence of aquatic vegetation did not notably change the N₂O emissions from freshwater crayfish–fish aquaculture ponds; significant N₂O emissions appeared in the drainage period instead of the waterlogging stage, as already reviewed by Maucieri et al. (2017) [37] for constructed wetlands. Considerably higher N₂O emissions occurred in the OAV treatment than WAV during the drainage period, which was probably because N₂O can be transported through the tracheal tissue of aquatic vegetation, thus generating higher N₂O emissions in the OAV treatment during the drainage period.

4.2. Effect of Aquaculture Parameters on CH₄ and N₂O Fluxes

In general, there was a close relationship between the fluxes of N₂O and CH₄ from freshwater crayfish–fish aquaculture ponds and the parameters of water and sediment, including DO and COD concentrations, mineral N concentrations, water temperature, and sediment DOC. In this study, both the fluxes of N₂O and CH₄ strongly positively correlated with the sediment and water temperature. As previously documented, water temperature acts as a sensitive factor, which directly influences the rate of CH₄ flux and efficiency of transport through the water column [38–40]. Moreover, the fluctuating water temperature could also be accompanied by variations in the DO concentrations in water, which could affect the production of CH₄ [41]. The temperature and N₂O flux correlations in the freshwater crayfish–fish farming ponds over the whole annual cycle are consistent with previous studies on other agricultural wetlands [2,12,42]. Of particular interest is that the ANOVA determined that the regression slopes differed significantly, thus suggesting that the N₂O flux was more dependent on temperature under drainage conditions than waterlogging ones in the freshwater crayfish–fish farming ponds. Different results were observed by Paudel et al. (2015) [43]. They found that the N₂O flux and temperature negatively correlated in an intensive aquaculture system at the bench scale. Presumably, there was no sediment at the bottom in their system, and the temperature range was much lower and was between 15 °C and 24 °C. Thus, this might not reflect the conditions in normal-scaled inland aquaculture ponds.

Over the course of the yearly cycle of waterlogged aquaculture, the N₂O and CH₄ fluxes were both negatively associated with the concentration of water DO, and areas with more aquatic vegetation responded more robustly to the DO in water (Figures 4 and 5). As shown by Huttunen et al. (2006) [44], water DO has a critical role in influencing the fluxes of N₂O and CH₄ from aquatic environments. Such a strong negative relationship between the concentrations of DO in the water and CH₄ was also proposed in other closely related aquatic wetlands [1,2,12,13,44,45]. High DO contents in the water could promote the oxidation of CH₄ during the passageway in transportation or at the sediment–water interface but could also suppress methanogenesis that was in the same microcosm [46]. Most likely, the concentration of DO was higher in the WAV than in the OAV treatment because of the photosynthetic activities of aquatic hydrophytes. Thus, CH₄ fluxes tended to be more DO–dependent when aquatic vegetation was growing. High DO concentrations could weaken denitrification, which is the primary process for the production of N₂O in anoxic water environments [1,2,13]. As in a previous study [47], low oxygen and high conditions of NO₃[−] generally favored the most intensive production of N₂O in water systems, where denitrification is always stimulated.

CH₄ and N₂O fluxes increased in parallel with the concentrations of DOC in the sediment in the freshwater crayfish–fish farming ponds over the annual observation cycle. As emphasized by Yagi and Minami (1990) [48] and Singh et al. (2000) [49], a relatively higher labile C fraction drives CH₄ emissions. In this study, sediment DOC positively affected the CH₄ fluxes during the whole observation period, which was a measurement of highly labile C in the sediment [13]. To date, limited research has correlated the fluxes of N₂O with the DOC in sediment in wetlands, and they significantly positively linearly correlated over the yearly cycle in this study. Possible reasons could be explained as follows: (1) sediment DOC could provide an energy source for heterotrophic bacteria [13]; and (2) the decomposition of DOC in the sediment in aquatic environments could provide more of a substrate to produce N₂O and deplete the available DO.

The contents of DO in the water positively affected the CH₄ emissions in the inland freshwater crayfish–fish aquaculture ponds, and this dependent relationship became greater in the vegetated areas. Presumably, the COD status in water can function as an index of overall activities of anaerobic microbes and sufficiently indicate the rate of accumulation of soluble microbial products (SMPs), such as methanogenesis [50]. Alternatively, the rate of production of SMPs correlated with the bacterial abundance and their age or retention time in a water column [51]. Previous studies in paddy soil and river sediment found similar

results [42], and we also found that fluxes of N_2O positively correlated with the mineral N in the sediment of freshwater crayfish–fish farming ponds because of the activities of denitrifiers that increase the production of N_2O and a decrease in the metabolism of N_2O by bacteria [1,52]. For example, a higher ratio of $N_2O:N_2$ from denitrification has been documented in estuaries that are rich in nitrates [1,52]. Moreover, Barnes and Upstill-Goddard (2011) [53] summarized that denitrification by nitrifiers can be strongly enhanced by abundant NO_3^- and NO_2^- and that the processes of the decomposition of hydroxylamine mainly contribute to N_2O production in sediments. In contrast, the production of N_2O can be promoted by sufficient NH_4^+ in either sediment or the water column. However, we failed to find a significant correlation between N_2O fluxes and water mineral N, which could be associated with the differences in aquatic water regulation, quality, and depth.

4.3. Comparison of GWP and NEEB from Agricultural Land Use Conversion

Our study indicated that the amounts of N_2O and CH_4 emitted annually from the paddy fields totaled 58.80 kg ha^{-1} and $7.82 \text{ kg } N_2O\text{-N ha}^{-1}$, respectively. Overall, such observations were consistent with previous research from our group [1,2,9,12,21,22,26]. To our knowledge, this is the first study to be carried out in freshwater crayfish–fish farming aquaculture ponds with static opaque chamber gas chromatography methods to simultaneously measure fluxes of N_2O and CH_4 in situ. As confirmed in previous studies, the use of appropriately designed chambers might serve as an operative approach to determine the greenhouse gases from water bodies, including both ebullition and diffusive fluxes [54]. Compared with early modeling approaches that simulated fluxes of N_2O and CH_4 from dissolved concentrations of N_2O and CH_4 , this direct measurement could be more realistic, which nearly overlooked large uncertainties in ebullitive fluxes and the calculations of diffusive parameters. To date, the research methods of this study have been successfully applied to measure greenhouse gases in similar aquaculture ponds [2,12,13,29,30].

In general, the conversion of conventional paddy fields to inland crayfish–fish farming ponds that is taking place has resulted in a significant decrease in the emissions of N_2O and CH_4 by 67.5% and 86.7%, respectively. The following reasons could be attributed to the lower fluxes of N_2O and CH_4 due to this conversion in agricultural land use. First, the soil/sediment substrates for microbes (TN and DOC/TOC) that include methanogens, denitrifiers, and nitrifiers are reduced to relatively lower amounts in the freshwater crayfish–fish aquaculture ponds than in paddy fields. In contrast, larger amounts of crop residues from long-term rice–winter wheat rotation systems are regularly retained from the current and former cropping seasons [21]. However, annual dredging must be conducted during the early drainage period of freshwater crayfish–fish farming ponds to reduce the load of pollution and avoid water eutrophication, which can guarantee the healthy growth of freshwater crayfish and fish [55]. Compared with rice paddies, such annual aquaculture operations could undoubtedly substantially reduce the N and C substrates in the sediment that are available for the production of N_2O and CH_4 . Second, the eventual fate or rates of the emissions of N_2O and CH_4 from aquatic wetlands mainly depend on how efficiently the sediment–water interface is transported to the atmosphere, which is negatively related to the depth of water [56–58]. Over the course of the study, the depth of water in the freshwater crayfish–fish farming ponds was continuously higher relative to that in paddy fields and was dominated by the F–D–F–M water regime. Third, in our study, the fluxes of N_2O and CH_4 and the concentrations of DO in the water strongly negatively correlated. Therefore, a higher water DO concentration ($6.2\text{--}10.0 \text{ mg L}^{-1}$) in freshwater crayfish–fish aquaculture ponds would certainly reduce the fluxes of N_2O and CH_4 . Finally, as a kind of zoobenthos, it is necessary for freshwater crayfish to aggressively move around and consume much biodebris on the sediment surface, which would reduce the substrates available for the production of N_2O and CH_4 [39,59]. In particular, freshwater crayfish prefer to burrow physiologically, intensively destroying the structure of the sediment in freshwater crayfish–fish farming ponds, which substantially affects the production of

CH₄ and N₂O production and emissions. We adopted the net GWP to comprehensively compare the climate impacts of the emissions of N₂O and CH₄ from both agricultural systems under local typical farming management practices, so that we could estimate the future potential impacts of the different GHGs emissions on the climate system based on radiative forcing [24,28]. As shown in Table 3, the trend of traditional paddy fields being converted to inland intensive crayfish–fish aquaculture ponds significantly decreased the GWP by 58.2%. In addition, the net ecosystem economic budget (NEEB) is an important index for completely estimating the ecological and agricultural benefits and has great guiding significance for the formulation of policies and the development of agricultural production [29,30,60]. This study showed that the NEEB of inland crayfish–fish aquaculture ponds was nearly 5.85-fold higher than that of rice–winter wheat cropping systems. In conclusion, higher ecosystem economic profits and lower climatic impacts could be attained concurrently by the contemporary agricultural land use shift of converting paddy fields into inland crayfish–fish farming aquaculture ponds in Southeast China.

4.4. Limitations of This Study and Future Research Perspectives

China currently supplies most of the global freshwater crayfish and fish, which mainly depend on aquaculture [14]. These findings indicate that when traditional paddy fields are converted to intensive inland aquaculture ponds, this not only substantially decreases the CH₄ and N₂O fluxes but also dramatically improves the ecosystem and economic benefits. Compared with rice paddies, the lower emissions of N₂O and CH₄ from the freshwater crayfish–fish farming aquaculture ponds were mainly due to consecutive waterlogging, high water levels, and fallow season dredging, among others, based on the results of this experimental research. Nevertheless, the field measurements in this study were limited by the local climate, water exchange, stocking density, and fish species. Due to these limitations, it would not be appropriate to simply scale up the average flux from the observation of this study to a global scope and calculate the global CH₄ and N₂O emissions. In contrast to natural wetlands, inland aquaculture ponds are more complex agricultural wetland systems where GHG production and emissions are substantially affected by aquaculture management. To date, relatively few studies have focused on the emissions of N₂O and CH₄ from inland aquaculture ponds. To more accurately understand the emissions of N₂O and CH₄ from aquaculture ponds, more types of aquaculture ponds, considering regional climates, feeding rates, methods, and typical management practices, should be assessed with direct field measurements prioritized.

5. Conclusions

These findings provide the first knowledge obtained by comparing the emissions of N₂O and CH₄ after the conversion of conventional paddy fields to freshwater crayfish–fish farming aquaculture ponds in Southeast China. During the whole annual cycle, this study found that the current changes in land use from paddy fields to freshwater crayfish–fish farming aquaculture ponds resulted in a significant decrease in the emissions of N₂O and CH₄ but simultaneously greatly increased the NEEB. Both fluxes of N₂O and CH₄ strongly positively correlated with the water/sediment temperature and DOC in the sediment but were negatively correlated with the water DO. Fluxes of N₂O and CH₄ positively associated with mineral N and water COD in the sediment, respectively. In addition, aquatic vegetation was found to significantly affect both the production and emissions of CH₄ and N₂O during the period of flooding in aquaculture ponds. Future direct field research in aquaculture ponds, including more fish varieties and management practices, is highly needed worldwide.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture12040515/s1>, Table S1: Wheat, rice and fish yield during the study period.

Author Contributions: Conceptualization, Z.H. and Y.Z. (Yaojun Zhang); methodology, Z.H.; formal analysis, Z.H., Y.Z. (Yaojun Zhang) and C.M.; investigation, C.G., F.S., Y.Z. (Yufei Zhao), C.F. and Y.C.; writing—original draft preparation, Z.H., C.G., F.S., Y.Z. (Yufei Zhao), C.F. and Y.C.; writing—review and editing, Y.Z. (Yaojun Zhang) and C.M.; visualization, Z.H., Y.Z. (Yaojun Zhang) and C.M.; supervision, Y.Z. (Yaojun Zhang); project administration, Z.H.; funding acquisition, Z.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Science Foundation of the Jiangsu Higher Education Institutions of China, grant number 19KJB610025; the Science and Technology Support Program (Agriculture) of Taizhou, grant number TN202010; the Research Foundation for Talented Scholars of Taizhou University, grant number QD2016043.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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