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Optimal fertilization strategy promotes the sustainability of rice–crayfish farming systems by improving productivity and decreasing carbon footprint

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Abstract

Rice-crayfish farming systems (RCs), a novel rice cropping system, have gained rapid popularity in many countries due to their economic advantages. Fertilizers tend to be applied in large quantities for higher profits, but has high burden on resources and environment, especially in terms of the carbon emissions. It is crucial to explore an optimal fertilization strategy with high productivity and low carbon emissions for the sustainable development of RCs. However, information about C emissions is incomplete, regarding the indirect C emissions during the rice growing season as well as C emissions during the crayfish culture period. We conducted field experiments to investigate the effects of five fertilization strategies including no fertilization (CK), farmer's practices (FP), optimized fertilization (OPT), organic fertilization only (OF), and organic fertilizer substitution (OPTOF) on the productivity, economic benefits, greenhouse gas (GHG) emissions, carbon footprint, and sustainability index of RCs. Results showed that OPT reduced direct (by 6.7%) and indirect (by 37.0%) GHG emissions during the rice growing season while maintaining rice (95%) and crayfish (104%) yields compared with that of FP. Additionally, the soil organic carbon storage and annual economic benefit of the OPT increased by 20.1% and 4.7%, respectively, whereas the carbon footprints of unit area, unit grain yield, unit energy yield, and unit of economic output decreased by 29.5%, 27.2%, 24.5%, and 32.7%, compared to the FP, respectively. The sustainability index (0.78) of the OPT treatment was significantly higher than that of other treatments due to its higher productivity and lower the carbon footprint. In conclusion, optimal fertilization strategy in RCs could achieve to increase productivity while reducing carbon footprint. This is conducive to the sustainability of RCs. Future attention in RCs should be focused on the development and promotion of such strategies.

Keywords Rice-crayfish farming systems \cdot Fertilization strategies \cdot Carbon footprint \cdot Greenhouse gas emissions \cdot Sustainability index

1 Introduction

Ensuring food security and developing low-carbon (C) agriculture by 2050 are critical global challenges for both researchers and policymakers (Cassman and Grassini

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Jianwei Lu lunm@mail.hzau.edu.cn 2020). Rice (*Oryza sativa* L.) is among the most important food crops worldwide. Conventional rice systems produce high yields through high agrochemical inputs and energy consumption (Bennett et al. 2012); this approach sustains more than half of the global population (Yuan et al. 2021).

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However, increasing rice production through adding more agrochemical inputs is costly and poses a high burden on resources and the environment, particularly in terms of increasing greenhouse gas (GHG) emissions (Sun et al. 2021b). About 30% and 11% of global agricultural nitrous oxide (N₂O) and 30% methane (CH₄), respectively, are emitted from rice fields (Linquist et al. 2012). Therefore, a pressing need exists to obtain a win-win rice production strategy for maintaining grain stabilization and reducing carbon footprint (CF). Currently, the adoption of appropriate rice cropping systems and fertilization strategies are two commonly adopted ways to sustain or increase rice yield while minimizing C emission (Monjardino et al. 2022; Li et al. 2023a, b).

The rice–crayfish (*Procambarus clarkii*) farming system (RC) is a practice where rice and crayfish are cultivated in a single field, offering a potential alternative to conventional single or double cropping of rice (Fig. 1. Jiang and Cao 2021). RCs offers multiple benefits, including the production of both rich carbohydrates (rice) and high-quality animal proteins (crayfish), improved soil structure to enhance soil organic carbon (SOC) sequestration (Zhang et al. 2022), increased stability of the soil microbial communities to maintain soil biological fertility (Li et al. 2022), and enhanced agroecosystem services (Xu et al. 2021). RCs become prosperous in China with the increasing demand for rich carbohydrates and high-quality animal proteins, with

the RCs production area exceeding 1.40 million hectares (Yu et al. 2023). More importantly, RCs have great potential for reducing C and nitrogen (N) losses, while ensuring stable grain production compared to conventional rice systems (Sun et al. 2021a). For example, RCs could reduce C emissions on the premise of stabilizing food production (rice and crayfish) and increasing the farmer's income (Fang et al. 2021a, b). Liu et al. (2022) reported that N fertilizer is deposited in the peripheral trench surrounding the RC, decreasing soil and paddy water ammonium-N content in the rice paddies, which together contribute to decreased ammonia (NH₃) volatilization and N₂O emissions in the paddy fields. Additionally, bioturbation from crayfish activities such as foraging and digging burrows, increases the rate of gas exchange between soil, floodwater, and the atmosphere, thereby reducing CH_4 emissions (Sheng et al. 2018). Nonetheless, previous studies on RCs have focused mostly on monitoring GHG emissions during the rice growth season only, and less on reporting GHG emissions during the crayfish culture period (Xu et al. 2023; Li et al. 2023a, b; Zhang et al. 2023). The crayfish culture period produces GHG emissions due to higher organic inputs (crayfish feeds) and long-term anaerobic environments. For example, feeding crayfish bait reduced CH4 emissions by 13.9-18.7% and increased N₂O emission by 24.4-32.2%, resulting in a higher global warming potential (GWP), compared to no feeding (Sun et al. 2019). Additionally, GHG emissions in



Fig. 1 Location map of experiment site (**a**), experimental field (**b**), schematic of rice-crayfish farming system of production field structure (**c**), rice-crayfish farming system of production management process (**d**), and static chamber for greenhouse gas collecting (**e**).

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agroecosystem come not only directly from cropland soils, but also indirectly from fertilizer, pesticide, as well as seed production and from machinery operation and irrigation (Wright et al. 2011; Jiang et al. 2019). However, previous studies have mainly focused on monitoring direct GHG emissions from rice fields (Fang et al. 2023), while ignoring indirect GHG emissions from rice production activities. These studies could lead to an underestimate of GHG emissions associated with rice and crayfish production, thereby limiting a comprehensive sustainability evaluation of RCs. Therefore, it is essential to comprehensively and quantitatively assess annual direct and indirect C emissions during the rice growing season and the crayfish culture period in RCs to inform decisions on sustainable rice production and agricultural development.

Fertilization is highly effective measures for supplementing nutrients and increasing rice yield (Bargaz et al. 2018). The GHG emissions in rice production are substantially affected by fertilization, which should be considered when quantifying the environmental effects of fertilization (Chen et al. 2020). A recent study reported that the optimal N fertilizer rate for rice production was 20-39% lower than that required to obtain the maximum yield, resulting in an N loss of 21-45%, while maintaining 95-99% of the maximum yield (Zhang et al. 2018a, b). Similarly studies suggested that replacing partial synthetic nitrogen (N) fertilizer with organic fertilizer would not only meet crop nutrient requirements but also reduce N loss in farmland (Qaswar et al. 2020). For example, chemical fertilizer combined application and organic fertilizer can stimulate microbial growth and trigger the immobilization of soil nitrate-N by soil microbes, resulting in strong competition for available C and nitrate-N with denitrification, and subsequently reduced N₂O emissions (Chen et al. 2021). Importantly, chemical fertilizers could indirectly affect the C input to the soil by influencing the additions of plant residues and root and rhizodeposition; besides, organic fertilizers directly influence C input to the soil and over long-term assist the SOC sequestration (Ren et al. 2021). These studies indicated that rational fertilization strategy would not only meet crop nutrient requirements to increase crop yield, but also reduce GHG emissions by increasing SOC sequestration. RCs are a representative sustainable agricultural system, and the optimal fertilization strategy for ensuring a stable rice yield while reducing C emissions and increasing soil C sequestration remains unknown. Theoretically, the nutrients supplement from internal circulation of excreta and residual feeds, crayfish necromass, and crayfish shells within RCs could reduce reliance on external fertilizers (Xu et al. 2022). However, in RCs actual production, due to a lack of theoretical knowledge and scientific fertilization guidance, farmers remain faithful to conventional water and fertilizer management approaches in RCs, which is not conducive to resource effective use and sustainable development of RCs (Guo et al. 2022). Therefore, it's necessary to explore an optimal fertilization strategy to ensure rice and crayfish yields stabilization while reducing GHG emissions.

To address these knowledge gaps, we conducted a 2-year field experiment to evaluate the effects of different fertilization strategies on productivity, GHG emissions, the CF, and sustainability index (SI). Our objectives were to: (1) determine the response of annual productivity and annual soil GHG emissions to different fertilization strategies; (2) comprehensively assess CF of RCs from unit hectare, unit kg rice yield, unit energy yield (EY), and economic output; and (3) determine the optimal fertilization strategy for maintaining grain stabilization and reducing CF to enhance RCs sustainability.

2 Materials and methods

2.1 Study sites

The field trial was conducted in Jianli Country, Hubei Province, central China ($30^{\circ}05'42''$ N, $114^{\circ}52'04''$ E) and initiated in 2019 (Fig. 1a). The field was under a conventional rice cropping pattern (rice-fallow rotation) for a long time before this trial. The field experiment was performed in period 2021-2022. The experiment site was located in a subtropical monsoon zone, where the average temperature and total rainfall during rice season were 18.3°C and 1235 mm in 2021 and 18.6°C and 1063 mm in 2022 (Fig. S1); the rainy season concentrated from June to August. The soil parent material was modern ricer alluvium and lacustrine alluvium, which is a sandy clay loam, with 23.6% sand, 60.7% silt and 15.7% clay. The soil properties in the 0–20 cm soil layer was pH 7.8, 24.6 g kg⁻¹ organic matter, 1.4 g kg⁻¹ total N, 14.2 mg kg⁻¹ Olsen-P, and 244.0 mg kg⁻¹ readily available K.

2.2 Experimental design and management

The field experiment consisted of five treatments with three replications that were arranged in a randomized complete block design. The treatments including: (1) no fertilization (control, CK); (2) local farmer's practices (FP), fertilizer application rates were determined according to local farmer's practices of 150 kg N ha⁻¹, 112.5 kg P₂O₅ ha⁻¹, and 112.5 kg K₂O ha⁻¹. The 75% of the N fertilizer, and all P and K fertilizer were applied as base fertilizer (compound fertilizer, N-P₂O5-K₂O=15%-15%-15%) 1 day before transplanting and the 25% of N fertilizer as tillering fertilizer (Urea, 46% N) 10-days after transplanting; (3) optimized fertilization (OPT), the fertilizers were applied according to the quantity of nutrients required to obtain the local target rice yield (6.00-6.75 t ha⁻¹) and the quantity of soils



nutrients supplied, where 100 kg N ha⁻¹, 27.3 kg P₂O₅ ha⁻¹, and 54.5 kg K_2O ha⁻¹; all fertilizers were applied as a base fertilizer (rice-crayfish special compound fertilizer, N-P₂O5-K₂O=22%-6%-12%, incorporating humic acid, urease and nitrification inhibitors and bacillus subtilis in raw material); (4) organic fertilization only (OF), organic fertilizer with an equal N level with the OPT treatment; the organic fertilizer application rate was 4.55 t ha⁻¹ (100 kg N ha⁻¹, 88 kg P₂O₅ ha^{-1} , and 84 kg K₂O ha^{-1}); the test organic fertilizer type was a commercial organic fertilizer (products of mixed plant and animal origin) with nutrients content of 2.22% N, 0.86% P, 1.54% K, and 45.8% organic matter; all fertilizer were applied as base fertilizer; and (5) organic fertilizer substitution base on OPT (OPTOF), substitution of 30% chemical N fertilizer with organic fertilizer based on the OPT treatment. The fertilizer rates were 100 kg N ha⁻¹, 45.5 kg P_2O_5 ha⁻¹, and 63.5 kg K₂O ha⁻¹; all fertilizer were applied as base fertilizers.

The rice variety used in this study was Huagui No.7, which was mechanically transplanted at a density of 210,000 hills ha⁻¹. Seedlings were planted on May 29 and June 1, and plants were transplanted on June 17 and 21 in 2021 and 2022, respectively. Other field agronomic practices, including tillage, herbicide application, pest and disease control followed local methods to prevent limitations in crop growth due to management practices. Rice straw was directly returned to the field.

The area of plot was 600-700 m². Approximately 20% of the plot area comprised a ring-shaped ditch around the paddy field as a crayfish habitat, enclosed by a 0.4-m-high crayfish escape net (Fig. 1b-c). Young crayfish (4.5-5 g each) were released into the filed plots at a standard abundance of 45,000 juveniles ha⁻¹ at the end of February (during the crayfish culturing season) every year. Special crayfish feed (containing 3.79% N, 1.76% P, 7.49% K, and 37.8% C) was supplied at a daily rate of approximately 4%-5% of crayfish body weight or a total of 1500 kg ha⁻¹ year⁻¹.

2.3 Measurements and calculations

2.3.1 Product yield and EY

At maturity, a 10-m² area at the center of each plot was handharvested and machine-threshed to determine rice grain and straw yields (fresh-yield). The grain and straw were oven-dried and weighed (dried-yield) for EY determination of grain and straw. The actual rice yield was adjusted to 14% moisture content. the grain moisture content was determined by a digital moisture tester (Li et al. 2021a, b). Rice yield determined according the rice planted area (83% in total area). Crayfish production was determined according to the accumulated crayfish caught daily during the crayfish culturing season. Crayfish



yield determined according total field area (rice planted area and the crayfish ditched culture area).

The EY of each product was determined by multiplying the product yield (kg ha⁻¹) by its corresponding calorific value (MJ kg⁻¹). The EY provides a measure of the productivity of the various components of the RC system, including rice grain, straw, and crayfish. The equation follows:

$$EY_{Rice} = Y_R \times UEV_R + Y_S \times UEV_S$$

 $EY_{Crayfish} = Y_C \times UEV_C$

Where Y_R , Y_S , and Y_C are rice grain and straw yields, and crayfish yields, respectively. The UEV_R , UEV_S , and UEV_C are the calorific values for rice grain (15.1 MJ kg⁻¹), straw (14.5 MJ kg⁻¹), and crayfish (6.45 MJ kg⁻¹), respectively (Hou et al. 2021).

2.3.2 Economic analysis

The net economic benefit of RC system was calculated as the total economic output minus the total economic input. In this study, the economic output consisted of the economic value of harvested rice grain and crayfish, and the total economic input included seeds, juvenile crayfish, fertilizers, crayfish fees, herbicides, pesticides, crayfish medicine, fuel, electricity, and labor. The rice grain price was 0.35 USD kg⁻¹, and the crayfish price was 2.80-5.59 USD kg⁻¹ based on market prices at the time of sold.

2.3.3 Direct GHG emissions

CH₄ and N₂O flux rates were measured using a static chamber (PHDMATE CO., LTD, SUZHOU, CHINA) and gas chromatography. During the rice season, a static chamber (60 cm \times 60 cm \times 120 cm) was placed on a stainless-steel frame (56 $cm \times 56 cm \times 25 cm$; the height of the frame was adjusted with changes in water depth (Fig. 1e). During the crayfish culture period, a static chamber (30 cm and 40 cm in diameter and height) was placed on a base fitted with a flotation device (inner diameter, 30 cm) immersed 2-3 cm into the water surface. Gas samples were collected once every 10-15 day (increasing in frequency after fertilization) at 8:00-11:30 A.M. Gas samples were collected in syringes and then injected into vacuum bottles after 0,10,20, and 30 min of static chamber closure. The gas samples were analyzed by chromatography. The soil CH₄ and N₂O emission fluxes were determined as follows (Zhong et al. 2021):

$$F = \rho \times \frac{V}{S} \times \frac{\Delta c}{\Delta t} \times \frac{273}{273 + t}$$

where F is the CH₄ or N₂O flux; ρ is the CH₄ or N₂O density at standard atmospheric pressure (mg m⁻³); V is the static chamber volume (m³); *S* is the area of the bottom static chamber (m²); $\Delta c/\Delta t$ is the rate of change in the CH₄ or N₂O concentration in the chamber (mg m⁻³ h⁻¹ or µg m⁻² h⁻¹); and *t* is the mean temperature in static chamber.

 CH_4 and N_2O emission were estimated by linear interpolation between successive sampling days using following formula (Musafiri et al. 2020);

GHG emission =
$$\sum \left[\frac{F_{i+1} + F_i}{2} \times t_{i+1} + t_i\right] \times 24 \times \frac{1}{100}$$

where F_i and F_{i+1} are the gas fluxes for two consecutive samplings; t_i and t_{i+1} are two consecutive sampling dates. The value 24 (h day⁻¹) and 1/100 value were used to convert mg m² to kg ha⁻¹.

2.3.4 Indirect GHG emissions

Indirect GHG emissions are mainly derived from management practices and agrochemical inputs such as production and transportation during agricultural production. We recorded various agrochemical inputs as well as the electricity, pesticide, and labor used for field-management practices during the experimental period (Table S1). The indirect GHG emissions were estimated using a life-cycle analysis methods, as follows:

$$GHG_i = \sum I_i \times C_i$$

where GHG_i represents indirect GHG emissions (kg CO₂-eq ha⁻¹), I_i is the amount of agrochemical inputs, and C_i is the CO₂-equivalent emissions of each index. The inputs and CO₂-equivalent emissions index values for this study are listed in Table S1.

2.3.5 CFs estimation

We estimated the CFs of different fertilization strategies including the CF per unit area (CF_A; kg CO₂-eq ha⁻¹ yr⁻¹), CF per kg grain yield (CF_{GY}; kg CO₂-eq kg⁻¹ yr⁻¹), CF per unit of EY (CF_{EY}; kg CO₂-eq GJ⁻¹ yr⁻¹), and CF per unit of economic output (CF_{EC}; kg CO₂-eq USD⁻¹ yr⁻¹), as follows:

$$CF_A = CE_d + CE_i - \Delta SOC$$

$$CF_{GY} = \frac{CF_A}{Grain yield}$$

 $CF_{EY} = \frac{CF_A}{Energy yield}$

$$CF_{EC} = \frac{CF_A}{Economic output}$$

2.3.6 Variation in SOC storage

Composite soil sample (consisting of 10 soil cores mixed) each plot was collected from topsoil (0-20 cm) after rice harvest in 2020 and 2022. The samples were air-dried, crushed and passed through a sieve (inner1-mm) for SOC content measurement using element analyzer. The soil bulk density (0–20 cm) was measured using the ring knife method. The SOC storage and the its variation were calculated as (Sun et al. 2021b):

SOC storage = $C \times BD \times Z$

$$\Delta \text{SOC} = \frac{\text{SOC}_{\text{a}} - \text{SOC}_{\text{b}}}{2} \times \frac{44}{12}$$

where C, BD, and Z are the SOC content (g kg⁻¹), soil bulk density (1.10-1.24 g cm⁻³ across different treatments in this study), and soil depth (cm), respectively, and SOC_a and SOC_b are the SOC storage values following rice harvest in 2022 and 2020, respectively.

2.3.7 Net soil GHG balance

The net soil GHG balance (NGHGB; kg CO_2 -eq ha⁻¹ yr⁻¹) was determined according to the direct soil GHG emissions and variation in SOC storage (ΔSOC ; kg CO_2 -eq ha⁻¹ yr⁻¹), as follows (Wang et al. 2021):

$$NGHGB = \overline{GHG_d} - \Delta SOC$$

where the $\overline{GHG_d}$ (kg CO₂-eq ha⁻¹ yr⁻¹) is the average of direct GHG emissions from each treatment during the 2-year study period.

2.3.8 SI evaluation

The SI is used to evaluate the effectiveness and sustainability of an agricultural production system, where a high SI value indicates maximum productivity and economic benefit with the lowest C emissions. In this study, the SI was determined based on the productivity, economic benefit, CF, and soil SOC sequestration. To allow for quantitative comparisons, we normalized the relevant variables to obtain dimensionless SI values. The SI index was calculated as follows (Gou et al. 2022):

$$\alpha_{xi} = \frac{x_{ij}}{x_{max}} \begin{pmatrix} i = 1, 2, 3, 4, 5\\ j = 1, 2, 3, 4, 5 \end{pmatrix} \text{or } \alpha_{xi} = \frac{x_{min}}{x_{ij}} \begin{pmatrix} i = 1, 2, 3, 4, 5\\ j = 6, 7, 8, 9, 10 \end{pmatrix}$$

where α_{xi} is a standardized value ($0 < \alpha_{xi} < 1$) at row i × column j. EC, GY, EY, and Δ SOC are assigned to j=1, 2, 3, 4 and CF_A, CF_{GY}, CF_{EY}, CF_{EC}, and NGHGB are assigned to j=5, 6, 7, 8, 9, respectively. x_{ij} is the corresponding actual value; and x_{max} and x_{min} are the maximum and minimum values.



$$\beta X_{ij} = \frac{1}{\alpha_1 X_{ij}} \times \sqrt{\frac{1}{m}} \sum_{i=1}^{m} (\alpha_1 X_{ij} - \alpha_2 X_{ij})^2 {i = 1, 2, 3, 4, 5 \ j = 1, 2, 3 \dots 9, 10}$$

where βX_{ij} is the coefficient of variation for each index and m is the maximum value for i or j. The SI is calculated as follows:

$$SI = \sum_{J=1}^{M} \left(\alpha_1 X_{ij} \times \frac{\beta X_{ij}}{\sum_{j=1}^{m} \beta X_{ij}} \right) \begin{pmatrix} i = 1, 2, 3, 4, 5\\ j = 1, 2, 3 \dots 9, 10 \end{pmatrix}$$

2.4 Statistical analysis

All the data were statistical analyzed using SPSS (IBM 26) software. To compare the significant difference of different treatment, we used one-way ANOVA followed by LSD test (p < 0.05 was considered statistically significant). All figures were drawn by Origin 8.0 software (OriginLab Corporation, Northampton, MA, USA) in this manuscript.

3 Results

3.1 Rice and crayfish yields and economic benefit

The fertilization treatments (FP, OPT, OF, and OPTOF) resulted in significant increase in average rice and crayfish yields by 21.3–49.3% and 24.1–60.6% compared to CK, respectively (Table 1), indicating that fertilization is still an important way to increase RC yield. Neither OPT nor

Table 1 The actual yield and annual energy yield of rice and crayfish among different fertilization strategies during the 2021 and 2022. The CK, FP, OPT, OF, and OPTOF are no fertilization, local farmer practices, optimized fertilization, organic fertilization only, and organic

OPTOF had a significant effect on the rice or crayfish yields compared to the FP treatment, while OF reduced yield significantly by 15.8% and 6.9%. Similarly, the FP, OPT, and OPTOF treatments demonstrated significant increases in the annual average EY over the 2 years compared to CK (58.0%, 47.2%, and 60.6%, respectively), whereas no significant differences were observed among FP, OPT, and OPTOF. Compared to FP, the OPT and OPTOF had no significant effect on the annual average EY, whereas there was significantly reduced in OF. The economic benefit of the FP, OPT, and OPTOF treatments were significantly higher than that of CK (32.0%, 38.2.2%, and 39.8%, respectively) and OF (45.7%, 52.5%, and 54.2%, respectively), and there were no significant differences were observed among the FP, OPT, and OPTOF treatments (Table S2).

3.2 Soil direct GHG emissions

Seasonal CH₄ and N₂O flux patterns were dependent on the fertilizer application and feed input in each treatment (Fig. 2). CH₄ and N₂O fluxes typically peaked after fertilization and high-frequency feeding during the rice growing season and crayfish culture period, respectively, with higher CH₄ flux in the OF and OPTOF treatments than in CK, FP, and OPT treatments; whereas N₂O flux followed the order FP > OPT > OPTOF > OF > CK. During crayfish culture period, CH₄ and N₂O fluxes were lower than those during rice growing season, and no significant differences were observed in the CH₄ and N₂O fluxes among the FP, OPT, and OPTOF treatments.

fertilizer substitution base on OPT, respectively. The data are presented as means (n=3) with standard errors. Different letters following the values in the same column indicated there is a significant difference in the different fertilization strategies (P < 0.05).

Year	Treatment	Products actual yield (t ha ⁻¹)		Energy yield (GJ ha ⁻¹)		
		Rice grain	Crayfish	Rice (grain + straw)	Crayfish	Total
2021	СК	5.10±0.28c	1.25±0.05a	153.17±11.75c	8.13 <u>+</u> 0.33a	161.29±12.1c
	FP	7.58 <u>+</u> 0.67a	1.35±0.09a	256.87±13.38a	8.78±0.58a	265.66±12.9a
	OPT	6.59 <u>+</u> 0.39b	1.40 <u>+</u> 0.11a	219.55±12.53b	9.12 <u>±</u> 0.71a	228.67±11.9b
	OF	5.68±0.12c	1.33 <u>+</u> 0.08a	177.02±17.03c	8.61±0.49a	185.63±17.1c
	OPTOF	7.28 <u>+</u> 0.65ab	1.42 <u>+</u> 0.10a	237.00±18.10ab	9.21±0.68a	246.21±18.2ab
2022	СК	4.72±0.57c	1.38±0.09bc	144.39±12.30c	8.94±0.59bc	153.33±12.82c
	FP	6.58 <u>+</u> 0.53ab	1.53 <u>+</u> 0.11ab	221.51±21.63ab	9.91±0.71ab	231.42 <u>+</u> 21.73ab
	OPT	6.86 <u>+</u> 0.66ab	1.60 <u>±</u> 0.05a	224.19±5.33ab	10.40 <u>+</u> 0.33a	234.59±5.50a
	OF	6.24 <u>+</u> 0.26b	$1.35 \pm 0.10c$	196.10±15.25b	8.78±0.65c	204.88±15.72b
	OPTOF	7.39 <u>±</u> 0.08a	1.60 <u>±</u> 0.05a	248.76±15.06a	10.40 <u>+</u> 0.33a	259.16±14.77a
AVE	СК	4.91±0.37c	1.31±0.03c	148.78±7.81c	8.53±0.21c	157.31±7.97c
	FP	7.08 <u>+</u> 0.43a	1.44 <u>+</u> 0.04ab	239.19±11.45a	9.35±0.28ab	248.53±11.72a
	OPT	6.72 <u>+</u> 0.52a	1.50 <u>±</u> 0.07a	221.87±4.32a	9.76 <u>+</u> 0.44a	231.63 <u>+</u> 4.14a
	OF	5.96 <u>+</u> 0.17b	1.34 <u>+</u> 0.09bc	186.56±15.12b	8.70±0.57bc	195.25±15.35b
	OPTOF	7.33 <u>+</u> 0.32a	1.51±0.05a	242.89±16.24a	9.81±0.34a	252.69±16.09a

Fig. 2 Soil CH₄ and N₂O emission flux rates among different fertilization strategies during the 2021 and 2022. The error bars indicate the standard errors of the means (n=3). CH₄ and N₂O emission amount from April to June 2022 was not available due to the COVID-19.



CH₄ and N₂O emissions during the rice growing season were significantly lower in CK than in the fertilization treatments (Fig. 3). N₂O emissions during the rice growing season were significantly lower by 21.3–48.1% in the OPT, OF, and OPTOF treatments than in the FP. Conversely, CH₄ emissions during the rice growing season were 24.2% and 13.5% higher in the OF and OPTOF treatments, respectively. The OPT treatment decreased GHG emissions by

6.2%. However, no significant differences in GHG emissions were detected among the treatments during crayfish culture period.

Overall, the global warming potential (GWP) varied across treatments during the rice growing season and crayfish culture period, with significantly higher values (75.8–130.1%) in the fertilization treatments during the rice season. However, there was no significant difference in GWP among treatments



Fig. 3 Soil CH₄ and N₂O cumulative emissions, and global warming potential (GWP) among different fertilization strategies during the 2021(**a-c**), and 2022 (**d-f**). The error bars indicate the standard errors of the means (n=3). Different letters indicated there is a significant

difference in the different fertilization strategies (P < 0.05). CH₄ and N₂O emission amount from April to June 2022 was not available due to the COVID-19.



during crayfish culture period. Annual GWP was significantly higher in the OF and OPTOF treatments than in FP (16.0% and 11.0%, respectively) and OPT (22.5% and 11.7%, respectively). By contrast, annual GWP was 5.3% lower in the OPT treatment than in the FP treatment.

3.3 Indirect GHG emissions

We estimated the annual indirect GHG emissions resulting from the inputs of all agricultural materials and processes (Fig. 4a). All fertilization treatments (FP, OPT, OF, and OPTOF) increased the average indirect GHG emissions by 16.0–34.7% compared with CK from 2021 to 2022. Compared to FP, the average annual indirect GHG emissions of OPT, OF, and OPTOF were significantly reduced by 16.2%, 15.1%, and 15.8%, respectively. These differences were mainly due to different fertilizer rates during the rice season. Additionally, the feed and electricity used in agricultural management lead to higher indirect GHG emissions, accounting for 49.3–66.5% and 16.6–22.3%, respectively, of total indirect emissions among the treatments (Fig. 4b).

3.4 SOC and soil net GHG balances

The fertilization treatments (FP, OPT, OF, and OPTOF) increased SOC storage in the RCs by 48.9–87.7% compared to CK; with greater effects in the OPT and OPTOF treatments than in the FP treatment (Fig. 5a). The NGHGB was lower in the OPT and OPTOF treatments than in the FP (54.7% and 18.4%, respectively) and OF (65.0% and 37.0%, respectively) treatments; the NGHGB was the lowest in CK (Fig. 5b).

3.5 CFs

We comprehensively evaluated the CFs of the RCs under different fertilization strategies from multiple perspectives (Fig. 6). The fertilization treatments (FP, OPT, OF, and OPTOF) significantly increased CF_A by 6.8–51.5% compared to CK. Overall, the CFs of OPT and OPTOF were significantly lower than that of FP. Specifically, the OPT and OPTOF treatments had lower CF_A (29.5% and 19.0%, respectively), CF_{GY} (27.2% and 22.3%, respectively), CF_{EY} (24.5% and 20.5%, respectively), and CF_{EC} (32.7% and 23.5%, respectively)



Fig. 4 The indirect greenhouse gas (GHG) emissions of different inputs (a) and its contributions (b) among different fertilization strategies.

Fig. 5 Variation in soil organic carbon, and soil net greenhouse gas balance (NGHGB) among different fertilization strategies. The error bars indicate the standard errors of the means (n=3). Different letters indicated there is a significant difference in the different fertilization strategies (P < 0.05).





b

b

d

d

EY

EC

∆SOC

values than the FP treatment. Among them, the OPT had the lowest CFs. RCs also have showed low CF_A in other integrated rice-production systems, such as the double-rice system, the ratoon rice system, and the rice-maize rotation system, particularly under the OPT treatment, which had a lower CF.

3.6 SI

We integrated 10 key productivity, economic, and CFrelated factors to determine the SI values for each treatment. As shown in Fig. 7a, the SI values of OPT and OPTOF treatments were significantly higher than that of CK (by 25.8% and 8.1%, respectively), FP (by 34.5% and 15.5%, respectively), and OF (by 62.5% and 39.6% respectively), and no significant differences were observed among CK, FP, and OF. Overall, the SI values of all treatments

Fig. 6 Annual average carbon footprint among different fertilization strategies. The CF_A , CF_{GY} , CF_{EY} , CF_{EC} are the carbon footprint per unit area (a), per kg grain yield (b), per unit energy yield (c), and per unit economic output (d), respectively. The error bars indicate the standard errors of the means (n=3). Different letters indicated there is a significant difference in the different fertilization strategies (P < 0.05).

Fig. 7 The sustainability index (SI, a), and the different evaluated parameter contributions to SI (b) among different fertilization strategies. The error bars indicate the standard errors of the means (n=3). Different letters indicated there is a significant difference in the different fertilization strategies (P < 0.05).

OF

0.0

0.2

0.4

0.6

Sustainability index (SI)

0.8

1.0

 $|CF_A|$ (kg CO₂-eq ha⁻¹ yr⁻¹) 1.8 CF_{GY} (kg CO₂-eq kg⁻¹ yr⁻¹) 12000 а а 1.6 ล а 10000 a b 1.4 b с 1.2 8000 b с 1.0 6000 0.8 0.6 4000 0.4 2000 0.2 0.0 70 5.0 CF_{EY} (kg CO₂-eq GJ⁻¹ yr⁻¹) CF_{EC} (kg CO₂-eq US\$⁻¹ yr⁻¹) С 45 60 а а 4.0 50 3.5 b b 3.0 40 b С С 2.5 с 30 d 2.0 1.5 20 1.0 10 0.5 0 0.0 сĸ FΡ OPT OF OPTOF ĊК FΡ OPT OF OPTOF GYR а b 0.2 GYc NGHGB OPT a 0.15 0.10 OPTOF ab CFEC CK bc 0.0 CFEY FP bc

CK

-FP

-OF

OPT

← OPTOF

CF_{GY}



CFA



4 Discussion

4.1 Optimal fertilization strategy improves productivity and economic benefits of RCs

The fertilization treatments (FP, OPT, OF, and OPTOF) showed significant increase in rice yield and annual EYs

of 21.3-49.3% and 24.1-60.6%, respectively, compared to the CK treatment (Table 1). These results indicate that fertilization is essential for achieving stable high rice yields in RCs. The primary reason for this result may be the inability of nutrients from excreta and residual bait feed, crayfish necromass, and crayfish shells in the RCs to meet the nutrient demands of targeted rice yield (Yuan et al. 2022). Additionally, the slow fertilizer efficiency release rate of crayfish residual organic material may fail to satisfy the nutrient demands of rice during vegetative growth phase, hindering the reproductive growth and yield increases of rice (Moe et al. 2019). However, OPT and OPTOF had lower fertilizer application rates without impairing the rice or crayfish yields, compared to the FP treatment (Table 1). This implies that overapplication of fertilizer under the FP treatment. Fertilizer over-application leads to the production of excess tillers, which causes competition for light and nutrients, resulted in a limitation of rice growth (Sui et al. 2013; Argento et al. 2022). Similar, fertilizer over-application can also cause waterbody eutrophication and GHG emissions (Fig. 3), which adversely affect crayfish growth and soil health (Yuan et al. 2022). Furthermore, the fertilizers in the FP treatment were not reasonably formulated (15%-15%-15%) and were not adapted to the nutrient demand pattern of rice, resulting in partial nutrient losses and thus limiting further increases in rice yield (Wang et al. 2022). In contrast, despite the lower fertilizer application in the OPT and OPTOF treatments, their rational fertilizer formulation modulated the nutrient supply by adjusting fertilizer retention and release processes, reducing nutrient losses (Fig. 3, N₂O emissions), improving nutrient availability and promoting plant nutrient uptake during the cropping season (Mi et al. 2018; Zhang et al. 2018a, b), thereby stabilizing or increasing rice yields. Similar studies have shown that rice yield can be maximized by proper fertilization while reducing nutrient surpluses and losses (Fang et al. 2021a, b; Wang et al. 2022). More importantly, optimizing the nutrient formulation (OPT) or adding organic fertilizers (OPTOF) improved the nutrient stoichiometry and increased soil microbial metabolism efficiency, thereby reducing nutrient losses and increasing the rice yields (Qaswar et al. 2020; Zhang et al. 2020). Notably, fertilization increased the crayfish yields (Table 1), possibly because it encouraged rice straw and plankton growth and provided feed and a favorable aquatic environment for the crayfish, thereby increasing the yields (Yuan et al. 2022). This supposition was further supported by the significant positive correlation between rice-straw biomass and crayfish yield in this study (Fig. S3). Moreover, all fertilization treatments produced significantly greater economic benefits than the CK treatment (Table S2), as

fertilization improved the economic output by increasing the rice and crayfish yields. The economic benefit was higher in the OPT and OPTOF treatments than in the FP treatment, due to the cost reductions associated with fertilization and labor.

4.2 Effects of different fertilization strategies on soil direct GHG emissions and C sequestration in RCs

In this study, CH_4 and N_2O emissions during the rice growing season were significantly higher by 75-132% and 36-162%, in the fertilization treatments than in CK, respectively (Fig. 3). These increases may have been attributed to promoting rice and crayfish growth, increasing root exudates and organic residues, including rice straw, crayfish feed residues, excreta, and necromass, which provided a rich substrate and an energy source for soil methanogens and ammonia-oxidizing archaea (Fan et al. 2021), leading to increased CH₄ and N₂O emissions. A significant positive correlation was observed between SOC and TN contents and CH₄ and N₂O emissions (Fig. S2), further supporting this hypothesis. Nevertheless, the OPT and OPTOF treatments significantly reduced N2O emissions compared to FP (Fig. 3). This because the soil N substrates (e.g., NH_4^+ –N and $NO_3^{-}-N$) for N₂O production decreased due to the 30% reduction in the N supplement (Wang et al. 2022), which was consistent with previous findings that N2O emissions are significantly positively correlated with the N application rate and soil NO₃⁻-N content (Fig. S2) (Jiang et al. 2019). Both organic fertilizer treatments (OPTOF and OF) promoted microbial immobilization of labile N by increasing the supply of organic C (Wang et al. 2015). This immobilized N was released during the crop growing season, improving N uptake and reducing N losses as N₂O emissions (Xia et al. 2017). Similar studies reported that combined application chemical and organic fertilizer could stimulate microbial growth and trigger the immobilization of nitrate-N by soil microbes, resulting in strong competition for available C and nitrate-N with denitrifies, and subsequently, reduced N₂O emissions (Chen et al. 2021). However, the OF and OPTOF treatments exhibited higher CH₄ emissions than the FP and OPT treatments (Fig. 3), potentially due to the stimulation by methanogens, which increased the C substrates due to the added organic fertilizer in the OF and OPTOF treatments, leading to an increase in CH_4 emissions (Shang et al. 2011). This result is consistent with previous studies showing that both bioorganic fertilizer and straw return high CH4 emissions during the rice growing season (Shen et al. 2014). Additionally, CH₄ and N₂O emissions were higher during the rice growing season than during the crayfish culture period in this study (Fig. 3), perhaps due to the water depth

in the field. Compared to the phreatic layer during the rice growing season, the deep water (100-120 cm) maintained during the crayfish culture period weakened the efficiency of CH₄ and N₂O emissions outward through bubble and liquid diffusion channels (Xu et al. 2023; Li et al. 2023a, b), reducing CH₄ and N₂O emissions during crayfish culture period. The anaerobic environment caused by the deep-water conditions during crayfish aquaculture is not conducing to the production of N₂O, and even if the N₂O produced is reduced to N₂ due to impeded diffusion, thus reducing N₂O emissions (Chen et al. 2018). Similar study has reported that the CH₄ and N₂Oemissions increased with an increase in the depth of the water layer; however, when the depth of the water layer was larger than 14.3 cm, CH₄ and N₂Oemissions decreased with an increase in the depth of the water layer (Xu et al. 2023).

Notably, fertilization significantly increased SOC storage (Fig. 5), possibly by adding exogenous C. For example, fertilization increased SOC storage by promoting the growth of rice and crayfish and increasing the return of rice straw (2.8-3.8 t C kg ha⁻¹ yr⁻¹) and crayfish feed residues (about 480 C kg ha⁻¹ yr⁻¹) (Pan et al. 2004). Moreover, adding organic fertilizer improved soil aggregate stability, which physically protects SOC and promotes C storage (Pei et al. 2023). More importantly, combined chemical and organic fertilizer could increase soil microbial activities and metabolism efficiency by improving labile C fractions accessibility, thereby promoting soil microbial necromass accumulation and stability, ultimately lead to higher stable C storage (Chen et al. 2023; Spohn et al. 2016). These processes may explain why the OPTOF treatment had better effect on SOC storage than the other treatments (Fig. 5a). The NGHGB reflects the capacity of farmland for C sequestration. Higher soil NGHGB was detected in the fertilization treatments (FP, OPT, OF, and OPTOF) than in CK (Fig. 5b), which was attributed to the lower GHG emissions in CK (Fig. 3). Moreover, soil NGHGB was lower in the OPT and OPTOF treatments than in the FP treatments, likely due to the increased GWP in paddies through increased CH_4 emissions (Fig. 3a, d), which was partially offset by the decrease in N₂O emissions (Fig. 3b, e) and the increase in SOC sequestration (Fig. 5a) induced in the OPT and OPTOF treatments than the FP treatment (Maillard and Angers 2014; Zhang et al. 2020). These findings suggested that optimized fertilization contributes to mitigating the NGHGB and improving soil C sequestration.

4.3 Effects of different fertilization strategies on indirect GHG emissions and CFs in RCs

In rice production, indirect GHG emissions stemming from the agricultural material inputs and various agricultural operations, in addition to direct GHG emissions from soil, contribute to overall emissions (Li et al. 2021a, b). Our findings showed that the fertilization treatments increased indirect GHG emissions compared to CK, whereas the OPT and OPTOF treatments resulted in lower indirect GHG emissions compared to the FP and OF treatments (Fig. 4a). Fertilizer inputs and artificial-use fertilizers were the primary contributors to such differences in emissions (Fig. 4a). Moreover, the feed inputs and electricity used for crayfishculture practices contributed to the higher proportion of indirect GHG emissions, accounting for 47.9-65.1% and 17.0–22.3%, respectively, among the treatments (Fig. 4a). These sources differed from the main source of indirect GHG emissions in conventional rice-rotation systems, such as the rice-rapeseed rotation system, in which fertilizers contribute approximately 67.3% of GHG from farm inputs (Huang et al. 2019). Therefore, optimizing electricity management during the crayfish farming season is a key measure to further reduce CFs.

We assessed the CFs of the different fertilization strategies from multiple perspectives, thereby reducing the limitations of basing conclusions on a single index (Chai et al. 2014). Our results revealed significantly higher CFs (CF_A , CF_{GY} , CF_{EY} , and CF_{EC}) in the FP and OF treatments than in CK (Fig. 6), which was attributed to differences in GHG emissions and productivity among the different fertilization treatments. Specifically, the average GWP value was 70.5% and 98.1%, higher in the FP and OF treatments than in CK, respectively (Fig. 3). In contrast, the CFs (CF_A , CF_{GY} , CF_{FY} , and CF_{FC}) were significantly lower in the CK and OPT treatments than in the OPTOF, FP, and OF treatments, indicating that optimized fertilization strategy would decrease CFs compared to FP in RCs. The reasons for this are that the OPT and OPTOF treatments decreased GHG emissions (Fig. 3), increased SOC sequestration (Fig. 5), and improved rice yield, EY (Table 1) and economic benefits (Table S2). Similar studies have reported that applying controlled-release urea or substituting N fertilizer with organic fertilizer can maintain crop yields, enhance soil C sequestration, and reduce CFs (Pei et al. 2023; Xu et al. 2023). Thus, the CF_{Δ} of the RCs was further reduced by adopting an optimized fertilization strategy (e.g., OPT), which had a lower CF in this study than most current rice-rotation systems, including double-rice, ratoon rice, rice-maize, rice-rapeseed, and rice-wheat (Fig. S4).

4.4 Optimal fertilization strategy in RCs

In the context of increasingly stringent agro-environmental constraints, the rice-production systems and fertilization strategies with high productivity and low C emissions have attracted increasing attention (Chai et al. 2021). Ricecrayfish farming systems are ecologically sound agricultural systems that achieve the dual goals of stabilizing food



production and reducing the environmental footprint (Hu et al. 2021). However, an optimal fertilization strategy for RCs has remained elusive. In this study, we integrated 10 key factors related to productivity and economic and environmental benefits to assess different fertilization strategies based on the SI values to determine an optimal fertilization strategy for RCs. We found that the OPT treatment had the highest SI (0.78), which was significantly higher than those of all other treatments (Fig. 7a). In terms of the individual components of the SI, the OPT treatment had a lower NGHGB (Fig. 5) and CF (Fig. 6), and higher productivity (Table 1), indicating enhanced productivity and SOC sequestration with lower CFs. In contrast, the FP treatment achieved a comparable or better crop yield than OPT, but ultimately led to a declining in the SI (Fig. 7), indicating that it would not be profitable, generating economic cost (Table S2) and would have a higher CF (Fig. 6) in RCs. In summary, OPT was the optimal fertilization strategy for maintaining productivity and reducing CF in RCs. Nonetheless, as is often the case, there is much talk and little action in the actual production of RCs, particularly for the optimal fertilization strategy. It is essential to quantify the actual benefits of OPT related to productivity, environmental and economic factors, as such data would aid policymakers in formulating policies that would lead to real-world applications. Converting conventional fertilization to optimal fertilization for all RCs in China would lead to an 8.4-9.8millionton increase in crayfish yields and a USD 262-329 million increase in economic benefits, accompanied by a 2.40-4.23 Tg CO_2 -eq yr⁻¹ decrease in C emissions (Table S3). We conclude that further optimizing the fertilization strategy could realize the dual goals of maintaining productivity and reducing the CFs of RCs in China.

5 Conclusion

This study analyzed the effects of five fertilization strategies on the productivity, economic benefits, annual GHG emissions (rice growing season and crayfish farming period), carbon footprint, and sustainability index of RCs. The results confirmed that optimized fertilization allowed for equal or higher crayfish yields (104%) and maintained a stable high rice yield (95%) compared to the current farmer's practices, while reducing fertilizer application (30%). Additionally, optimized fertilization produced synergistic effects, such as increasing economic benefits (4.7%) and soil C sink (20.1%) and reducing annual GHG emissions (5.3%) and carbon footprints (24.5–32.7%), resulting in a higher sustainability index (0.78). Therefore, optimized fertilization strategy was deemed the most effective fertilization practice for increasing productivity while reducing carbon footprint. Importantly, the crucial ecological benefit differed between



different fertilization strategies in RCs. For instance, optimized fertilization strategy mainly reduced annual GHG emissions, while organic fertilizer substitution strategy mainly increased food productivity and soil carbon sink. Although the implementation of organic fertilization only strategy significantly contributed to soil carbon sinks, it simultaneously leads to elevated carbon emissions. Therefore, our findings highlight the crucial roles of implementation of categorized management strategies for reducing environmental emissions and promoting the sustainability of RCs. In conclusion, optimal fertilization strategy in RCs could achieve to increase productivity while reducing carbon footprint. This is conducive to the sustainability of RCs. Future attention in RCs should be focused on the development and promotion of such strategies.

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Data availability The authors confirm that the data supporting the fndings of this study are available within the article. Further raw data is available from the corresponding author, upon reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The author declare to have no competing interests.

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