

Effects of Rice-Crayfish Co-culture on Greenhouse Gas Emissions from Paddy Fields Following Straw Incorporation: Postprint

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Date: 2017-11-08T00:00:00+00:00

Abstract

The rice-crayfish co-culture model constitutes an important component of integrated rice-cropping and aquaculture systems, characterized by complete straw return to the field, continuous flooding during the non-rice season, and year-round cultivation of red swamp crayfish (*Procambarus clarkii*). Currently, the impacts of this rice-crayfish model on greenhouse gas emissions from paddy fields remain unclear. This study investigated the effects of straw return and rice-crayfish co-culture on CH₄, N₂O, and CO₂ emissions from paddy field systems, using winter flooding without straw return (W) as the control in the Jiangnan Plain, with additional treatments of winter flooding with straw return (WS) and winter flooding with straw return plus crayfish farming (WSC), thereby providing data support and theoretical basis for accurate assessment of greenhouse gas emissions from paddy fields. The results demonstrated that during the field monitoring period, the cumulative CH₄ emissions from the winter flooding with straw return treatment increased significantly compared with the winter flooding without straw return treatment ($P < 0.05$), with increases of 27.23% and 60.08% in 2015 and 2016, respectively. The cumulative CH₄ emissions from the winter flooding with straw return plus crayfish farming treatment decreased significantly compared with the winter flooding with straw return treatment ($P < 0.05$), with reductions of 29.02% and 41.19% in 2015 and 2016, respectively. The cumulative CO₂ emissions from the winter flooding with straw return treatment increased significantly compared with the winter flooding without straw return treatment. Compared with the winter flooding without straw return treatment, both the winter flooding with straw return treatment and the winter flooding with straw return plus crayfish farming treatment had no effect on cumulative N₂O emissions. From the perspective of greenhouse effect, the winter flooding with straw return treatment substantially increased the

greenhouse effect compared with the winter flooding without straw return treatment, whereas conducting crayfish farming on the basis of winter flooding with straw return could substantially reduce CH₄ emissions, thereby mitigating the increased greenhouse effect resulting from straw return. Rice yields showed no significant differences among all treatments. Compared with the winter flooding with straw return treatment, winter flooding with straw return plus crayfish farming could significantly reduce greenhouse gas emission intensity. Compared with the winter flooding without straw return treatment, both winter flooding with straw return and winter flooding with straw return plus crayfish farming had no significant effects on soil dissolved organic carbon (DOC), acetic acid, and NH₄⁺-N. Winter flooding with straw return plus crayfish farming could significantly increase economic returns per unit area.

Full Text

Effect of Rice-Crayfish Co-Culture on Greenhouse Gas Emission in Straw-Puddled Paddy Fields

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Abstract

Rice-crayfish co-culture represents an important integrated rice-aquaculture system characterized by complete straw return, continuous flooding during the non-rice season, and year-round crayfish (*Procambarus clarkii*) cultivation. However, its impact on greenhouse gas emissions from paddy fields remains unclear. This study investigated CH₄, N₂O, and CO₂ emissions under three treatments: winter waterlogging without straw return (W), winter waterlogging with straw return (WS), and winter waterlogging with straw return plus crayfish (WSC), using winter waterlogging without straw return as the control. The results demonstrated that cumulative CH₄ emissions under WS increased significantly compared to W ($P < 0.05$), with increases of 27.23% in 2015 and 60.08% in 2016. In contrast, WSC significantly reduced CH₄ emissions compared to WS ($P < 0.05$), with decreases of 29.02% in 2015 and 41.19% in 2016. Straw return significantly enhanced CO₂ emissions, while both WS and WSC had no significant effect on N₂O emissions compared to W. From a global warming potential perspective, WS substantially increased greenhouse effect, whereas integrating crayfish cultivation with straw return effectively mitigated this enhancement by

reducing CH₄ emissions. No significant differences in rice yield were observed among treatments, but WSC significantly decreased greenhouse gas emission intensity compared to WS. Compared to W, both WS and WSC had no significant impact on soil dissolved organic carbon (DOC), acetic acid, and NH₄⁺-N content. However, WSC dramatically improved economic returns per unit area.

Keywords: Rice-crayfish co-culture; Straw return; Methane; Nitrous oxide; Greenhouse gas

Introduction

Methane (CH₄) and carbon dioxide (CO₂) are the most important greenhouse gases. Atmospheric CH₄ concentration has increased from 0.72 μmol · mol⁻¹ before the Industrial Revolution to 1.82 μmol · mol⁻¹ in 2012, a 1.53-fold increase, while CO₂ increased by 40% during the same period [1]. Currently, CH₄ contributes 20%-39% to global warming [2]. Paddy fields represent a significant source of atmospheric CH₄, with annual emissions of 31-112 Tg, accounting for 5%-19% of global total emissions [2]. Chinese paddy fields emit 7.2-9.5 Tg of CH₄ annually [3]. Strict anaerobic conditions resulting from long-term flooding promote CH₄ production and emission, while intermittent irrigation and mid-season drainage during rice growth can substantially suppress CH₄ emissions [4]. Non-rice season management practices, such as water and organic matter management, significantly influence CH₄ emissions during the subsequent rice season [5]. Straw return, a widely promoted practice in China for improving soil organic matter and structure while utilizing crop residues effectively, simultaneously stimulates CH₄ emissions [6-7].

Integrated rice-aquaculture systems combining rice cultivation with fish, shrimp, or ducks offer mutualistic benefits, saving space and generating considerable economic returns [8]. Traditional rice-fish systems exist worldwide [9-10] and have evolved into diversified integrated rice cultivation models [11-14]. Rice-crayfish co-culture, one such integrated system, yields exceptionally high economic benefits of 44,700 yuan per hectare (internal data from National Integrated Rice Cultivation Conference), significantly surpassing rice-fish and rice-duck systems [12,15]. By 2016, its adoption exceeded 200,000 hectares in the Jiangnan Plain alone. This system features: (1) year-round crayfish presence in ditches or paddies; (2) complete straw return; (3) non-rice season flooding depth exceeding 50 cm; (4) application of oxygen enhancers and disinfectants during flooding to increase dissolved oxygen; and (5) crayfish burrowing during the rice season that increases soil-water interface area. Previous research indicates that non-rice season flooding combined with straw return substantially increases CH₄ emissions during the rice season [16], yet the greenhouse gas emissions after introducing crayfish remain unknown. We hypothesized that crayfish burrowing increases the soil-water interface, allowing soil pores to accommodate more oxygen during wetting-drying cycles and prolonging dissolved oxygen consumption

after reflooding, potentially reducing CH_4 emissions while increasing CO_2 emissions, ultimately decreasing net greenhouse effect. To test this hypothesis, we monitored greenhouse gas emissions for two consecutive years under three treatments—winter waterlogging (W), winter waterlogging plus straw return (WS), and winter waterlogging plus straw return plus crayfish (WSC)—to characterize emission patterns and greenhouse effects, providing data support for accurate assessment of greenhouse gas emissions from Chinese paddy fields.

1.1 Study Site

The experiment was conducted at Houhu Farm in Qianjiang City, Hubei Province, located in the low-lying lake region of the Jiangnan Plain with a static winter groundwater table of 40–60 cm. The area experiences a north subtropical monsoon humid climate with an average annual temperature of 16.1°C , frost-free period of 246 days, and annual precipitation of 1,100 mm. The soil, developed from lacustrine deposits, is a fluvio-aquic paddy soil. The previous cropping system was middle rice followed by winter fallow. Baseline soil fertility characteristics were: total nitrogen $2.4 \text{ g} \cdot \text{kg}^{-1}$, total phosphorus $0.45 \text{ g} \cdot \text{kg}^{-1}$, total potassium $19.5 \text{ g} \cdot \text{kg}^{-1}$, organic matter $26.43 \text{ g} \cdot \text{kg}^{-1}$, available nitrogen $129.50 \text{ mg} \cdot \text{kg}^{-1}$, available phosphorus $9.13 \text{ mg} \cdot \text{kg}^{-1}$, available potassium $178.67 \text{ mg} \cdot \text{kg}^{-1}$, and pH 7.12.

1.2 Experimental Design and Field Management

The experiment was initiated in 2014, with greenhouse gas emissions monitored during the 2015 rice season (late June to late September) and throughout 2016 (late March to late November). The total experimental area was 900 m^2 , comprising three treatments: winter waterlogging without straw return (W), winter waterlogging with straw return (WS), and winter waterlogging with straw return plus crayfish (WSC). Each treatment had three replicates, totaling nine plots of 100 m^2 each. To prevent water and fertilizer leakage, 60 cm wide and 40 cm high ridges wrapped with plastic film were constructed around each plot. For WSC plots, a 3.0–4.0 m wide and 0.8–1.0 m deep crayfish ditch was excavated along one side, surrounded by nylon netting (buried 1.0 m underground with 0.3 m aboveground, supported by bamboo stakes) to prevent escape.

After rice harvest, straw was returned at $3,750 \text{ kg} \cdot \text{hm}^{-2}$ using a high-stubble plus surface mulching method. During the rice season, nitrogen ($180 \text{ kg} \cdot \text{hm}^{-2}$), phosphorus (P_2O_5 $90 \text{ kg} \cdot \text{hm}^{-2}$), and potassium (K_2O $144 \text{ kg} \cdot \text{hm}^{-2}$) were applied. Nitrogen was split as basal:tillering:panicle fertilizer at 5.8:1.8:2.4, phosphorus was applied entirely as basal fertilizer, and potassium was split 5:5 between basal and panicle stages. Basal fertilizer consisted of compound fertilizer (N: P_2O_5 : K_2O = 25:10:16) plus superphosphate ($\text{P}_2\text{O}_5 \geq 12\%$), while topdressing used urea (N $\geq 46\%$) and potassium chloride ($\text{K}_2\text{O} \geq 60\%$). Basal fertilizer was applied before transplanting, tillering fertilizer 15 days after transplanting, and panicle fertilizer 50 days after transplanting. Additionally, $6 \text{ kg} \cdot \text{hm}^{-2}$ of

granular zinc ($\text{Zn} \geq 25\%$) and $60 \text{ kg} \cdot \text{hm}^{-2}$ of granular silicon ($\text{SiO}_2 \geq 20\%$) were applied basally.

Field management details are provided in Table 1. The rice variety was 'Jianzhen 2' (middle rice), and crayfish (*Procambarus clarkii*) were stocked. In October 2014, broodstock crayfish ($\sim 40 \text{ individuals} \cdot \text{kg}^{-1}$) were introduced; 2015 production was not recorded due to natural mortality. Broodstock were supplemented in October 2015. From March to May 2016, crayfish feed was applied at $1,800 \text{ kg} \cdot \text{hm}^{-2}$ (average), with feed composition containing $46.6 \text{ g} \cdot \text{kg}^{-1}$ total nitrogen, $11.0 \text{ g} \cdot \text{kg}^{-1}$ total phosphorus, and $10.5 \text{ g} \cdot \text{kg}^{-1}$ total potassium. Harvesting began in mid-April and concluded in early June. Immature juveniles migrated to the ditches and re-entered the paddies after field preparation, transplanting, tillering drainage, and reflooding, with a second harvest conducted before rice harvest.

1.3 Gas Sampling and Analysis

Gas samples were collected using static chambers and analyzed with an Agilent 7890A gas chromatograph. The static chamber system consisted of a base ($42 \text{ cm} \times 42 \text{ cm} \times 20 \text{ cm}$), middle section, and top section, all stainless steel. The base featured a 3 cm deep \times 2 cm wide water seal channel and two rows of 2 cm diameter holes 10 cm from the top to facilitate water and nutrient movement. Bases were installed before transplanting, leaving only the water channel above-ground. The middle and top sections ($42 \text{ cm} \times 42 \text{ cm} \times 50 \text{ cm}$) also had water seal channels. During flooding periods, the middle section was placed on the base, and after 2 hours of equilibrium, the top section was added. A thermal insulation layer covered the chamber exterior. Four rice hills were maintained inside each base at the same density as outside.

Gas sampling occurred from June 29 to September 10, 2015, and March 14 to November 22, 2016, with one chamber per plot (three chambers per treatment). Samples were collected every 7–10 days between 8:00–10:00 AM. During collection, water was injected into the base channel, the middle and top sections were sealed, and gas samples were drawn at 0, 5, 10, 15, and 20 minutes using 30 mL syringes, then analyzed within 24 hours. In one plot per treatment, redox potential reference electrodes were installed 20 cm and 40 cm deep, 20 cm from the chamber base, with redox conditions recorded periodically.

The gas flux calculation formula was:

$$F = \rho \times \frac{V}{S} \times \frac{dC}{dt} \times \frac{273}{273 + T}$$

where F represents gas flux (FCH_4 in $\text{mg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, FCO_2 in $\text{mg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, FN_2O in $\text{mg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$), ρ is gas density at standard state ($\text{kg} \cdot \text{m}^{-3}$), V is chamber effective volume (m^3), S is base area (m^2), dC/dt is concentration

change rate, and T is mean chamber temperature. N_2O was not measured in 2015 due to instrument limitations.

Cumulative emissions were calculated by interpolation: average flux between adjacent monitoring days multiplied by the interval days, then summed across the entire monitoring period. Global warming potential (GWP) was calculated as [16]:

$$\text{GWP} = 25 \times \text{CH}_4 \text{ cumulative emission} + 298 \times \text{N}_2\text{O cumulative emission} + \text{CO}_2 \text{ cumulative emission} \quad (2)$$

1.4 Soil Sampling and Analysis

Concurrent with gas sampling, soil samples (0–20 cm) were collected using a stainless steel corer, with three points per plot composited into one sample. Samples were transported to the laboratory, visible roots and stones removed, and fresh samples analyzed for dissolved organic carbon (DOC), NO_3^- -N, NH_4^+ -N, and acetic acid content.

For DOC and acetic acid determination: soil moisture was measured gravimetrically, then 10 g fresh soil (oven-dry equivalent) was extracted with deionized water at a 1:5 soil:water ratio (accounting for water in fresh soil). Samples were shaken at 25°C for 30 minutes, centrifuged at $8,000 \text{ r} \cdot \text{min}^{-1}$ for 10 minutes at 4°C, and supernatant filtered through 0.45 μm membrane and stored at -18°C. DOC was measured using an Elementar total organic carbon analyzer, and acetic acid was determined by Waters HPLC following Sun et al. [17]: C18 reverse-phase column (4.6 mm \times 250 mm, 5 μm), mobile phase 0.1% phosphoric acid:acetonitrile (98:2), detection wavelength 210 nm, flow rate $1.0 \text{ mL} \cdot \text{L}^{-1}$, injection volume 20 μL , column temperature 35°C. All reagents were chromatographic grade.

NO_3^- -N and NH_4^+ -N were extracted with $1 \text{ mol} \cdot \text{L}^{-1}$ KCl (soil:water = 1:10) and analyzed by flow injection analyzer. Soil temperature was measured with a probe digital thermometer, and Eh values were determined using platinum electrodes.

1.5 Yield Calculation and Data Analysis

Rice yield was determined by actual harvest per plot and valued at $2.8 \text{ yuan} \cdot \text{kg}^{-1}$. Crayfish were harvested, counted, and sold per plot, with revenue calculated from actual sales at an average price of $24 \text{ yuan} \cdot \text{kg}^{-1}$. Inputs including feed, labor, anti-escape nets, fishing gear, and water/soil detoxifiers were recorded. Data processing and calculations were performed in Excel, with significance testing conducted using SPSS software via one-way ANOVA, two-way analysis, LSD test, and repeated measures ANOVA with Greenhouse-Geisser correction.

2 Results

2.1 CH₄ Emissions

CH₄ emission results (Figure 1 [Figure 1: see original paper], Table 2) showed cumulative emissions of $(9.95 \pm 1.24) \text{ g} \cdot \text{m}^{-2}$, $(9.22 \pm 2.77) \text{ g} \cdot \text{m}^{-2}$, and $(12.66 \pm 1.20) \text{ g} \cdot \text{m}^{-2}$ for W, WSC, and WS treatments, respectively, during the 2015 monitoring period. Midseason drainage caused sharp CH₄ emission reductions that did not recover after reflooding, with pre-drainage emissions accounting for 75.3%, 81.1%, and 86.1% of total observed emissions in W, WS, and WSC treatments. In 2016, cumulative emissions were $(28.48 \pm 5.21) \text{ g} \cdot \text{m}^{-2}$, $(26.81 \pm 2.05) \text{ g} \cdot \text{m}^{-2}$, and $(45.59 \pm 2.30) \text{ g} \cdot \text{m}^{-2}$, respectively. Rice transplanting in early June, drainage in late July, and drainage in early October all caused sharp emissions compared to W ($P < 0.05$) by 27.23% in 2015 and 60.08% in 2016. WSC significantly reduced CH₄ emissions compared to WS ($P < 0.05$) by 29.02% in 2015 and 41.19% in 2016, primarily by decreasing pre-transplantation emissions.

2.2 CO₂ and N₂O Emissions

CO₂ emission results (Figure 1, Table 2) showed cumulative emissions of $(2.14 \pm 0.15) \text{ kg} \cdot \text{m}^{-2}$, $(2.27 \pm 0.22) \text{ kg} \cdot \text{m}^{-2}$, and $(4.34 \pm 0.20) \text{ kg} \cdot \text{m}^{-2}$ for W, WSC, and WS in 2015. Drainage and reflooding caused a temporary CO₂ emission decrease followed by a sustained increase. In 2016, cumulative emissions were $(3.61 \pm 0.23) \text{ kg} \cdot \text{m}^{-2}$, $(4.20 \pm 0.29) \text{ kg} \cdot \text{m}^{-2}$, and $(4.34 \pm 0.20) \text{ kg} \cdot \text{m}^{-2}$. Straw returns significantly increased CO₂ emissions ($P < 0.05$), while crayfish effects on this enhancement varied between years.

N₂O emission results (Figure 1, Table 2) showed cumulative emissions of $(0.273 \pm 0.024) \text{ g} \cdot \text{m}^{-2}$, $(0.308 \pm 0.050) \text{ g} \cdot \text{m}^{-2}$, and $(0.285 \pm 0.022) \text{ g} \cdot \text{m}^{-2}$ for W, WS, and WSC in 2016. Emissions remained low and stable before the first drainage (July 20), then increased after drainage, with subsequent flooding and drainage activities amplifying flux variability until harvest. WS increased N₂O emissions by 12.8% compared to W, while WSC decreased emissions by 7.5% compared to WS, though these effects were not statistically significant.

2.3 Soil NO₃⁻-N and NH₄⁺-N Dynamics

Soil NO₃⁻-N and NH₄⁺-N were monitored across different periods (Figure 2 [Figure 2: see original paper]). Both nutrients generally decreased during the rice season. In 2016, drainage before transplanting increased NO₃⁻-N, basal fertilizer application increased total inorganic nitrogen, and post-harvest levels remained low. In 2015, mean NO₃⁻-N concentrations ranked W > WSC > WS, while NH₄⁺-N ranked WS > WSC > W. In 2016, NO₃⁻-N ranked WSC > WS > W, and NH₄⁺-N ranked WSC > W > WS. Inconsistent trends between years may relate to differing monitoring durations, interannual climate variability, and drainage timing/severity. Table 3 shows NO₃⁻-N was significantly positively correlated with CH₄ flux in W treatment, while NH₄⁺-N was

significantly positively correlated with N_2O flux in WSC treatment.

2.4 Soil DOC and Acetic Acid Dynamics

Two-year results (Figure 3 [Figure 3: see original paper]) showed DOC concentrations first decreased, then increased, then decreased again during the rice season, with a sharp post-harvest increase in 2016 due to straw return coupled with flooding. Table 3 indicates DOC content was only significantly correlated with N_2O flux in WSC treatment. Acetic acid concentrations showed inconsistent interannual patterns and no significant correlation with CH_4 flux.

2.5 Soil Temperature Variations

Soil temperature at 5 cm depth showed no clear differences among treatments but varied between years (Figure 4 [Figure 4: see original paper]), primarily influenced by air temperature. For example, late June 2015 had average air temperature of 27°C versus 25°C in 2016. Soil temperature on July 17, 2015 was $\sim 24^\circ\text{C}$ following maximum air temperatures of 25°C and 27°C on July 16-17, while on July 18, 2016 it was $\sim 28^\circ\text{C}$ following maximum temperatures of 33°C and 30°C on July 17-18. Table 3 shows no significant relationship between 5 cm soil temperature and CH_4 or CO_2 fluxes, but a significant negative correlation with N_2O flux in WSC treatment. Excluding data from drainage to 25 days post-reflooding progressively strengthened the correlation between soil temperature and CH_4 emissions, indicating a prolonged lag effect of drainage on CH_4 flux. Appropriate drainage timing could significantly reduce CH_4 emissions.

2.6 Soil Redox Potential (Eh) Dynamics

Soil Eh at 20 cm and 40 cm depths was monitored during rice seasons (Figure 5 [Figure 5: see original paper]). In 2015, Eh values remained relatively stable between -30 and -50 mV. In 2016, greater fluctuations occurred, with two distinct peaks in the 20 cm layer; WS peaks appeared ~ 20 days earlier than W and WSC. The 40 cm layer also showed two peaks with consistent timing across treatments, though WSC maintained higher Eh values. Mean Eh values in 2015-2016 were -40.98 mV, -38.10 mV, -39.62 mV (20 cm) and -36.20 mV, -33.48 mV, -28.20 mV (40 cm) for WSC, W, and WS, respectively. After two years of crayfish cultivation, WSC showed higher Eh in the 40 cm than 20 cm layer, suggesting that burrowing activities substantially improved oxidation status in deeper soil layers.

2.7 Soil Environmental Factors

Treatment effects on soil parameters are shown in Table 4. In 2016, WSC significantly increased NO_3^- -N content compared to W and WS, while other parameters showed no significant differences. Repeated measures ANOVA with Greenhouse-Geisser correction (required due to significant sphericity test results,

$P < 0.01$) revealed that all soil parameters varied significantly over time. In 2015, both DOC and acetic acid were significantly affected by treatment.

2.8 Rice Yield and Greenhouse Effect

Table 5 shows WSC had the lowest greenhouse effect from CO_2 and CH_4 emissions in 2015, significantly lower than WS. In 2016, W had significantly lower CO_2 greenhouse effect than WSC and WS, while WSC had the lowest CH_4 greenhouse effect, significantly lower than WS. Overall, WSC reduced total greenhouse effect by 7.8% compared to WS in 2015, while W reduced it by 21.3% and WSC by 11.1% in 2016. WS substantially increased greenhouse effect, but WSC effectively suppressed this enhancement primarily through CH_4 emission reduction. Two-way ANOVA showed significant treatment \times year interactions for CO_2 and CH_4 GWP ($F = 5.380$, $P = 0.021$ and $F = 14.088$, $P = 0.001$, respectively), likely due to interannual climate differences and varying monitoring durations.

Table 6 presents two-year combined yields. No significant differences in rice yield or value were observed among treatments. Crayfish production increased net output value by 32,200 yuan $\cdot \text{hm}^{-2}$ in WSC, making its per-unit-area revenue 1.78-1.82 times that of W and WS. WS increased greenhouse gas emission intensity, while WSC significantly reduced it (Table 8) due to decreased greenhouse effect.

3 Discussion

3.1 Effects on CH_4 Emissions

While straw return enhances soil carbon sequestration, it strongly stimulates CH_4 emissions [18-19]. Continuous flooding combined with straw return has a powerful priming effect on CH_4 emissions [20]. This study confirmed that WS significantly increased CH_4 emissions compared to W. Previous studies on integrated rice-aquaculture systems report varying effects: Yuan et al. [15] found rice-fish reduced CH_4 by 12%-18% and rice-duck by 23%-26%; Zhan et al. [12] reported rice-duck reduced CH_4 GWP by 18%; while Frei et al. [21] found rice-fish increased CH_4 emissions. Deep non-rice season flooding in rice-crayfish systems substantially reduces CH_4 emission via bubbling and diffusion [22]. Although deep flooding lowers soil Eh, crayfish burrowing greatly increases soil-water interface area and oxygen content, which can elevate Eh. This dual effect may prevent major Eh changes while reducing methanogen abundance and increasing methanotroph abundance [23], thereby altering CH_4 production and emission. Compared to rice-rape rotation, rice-crayfish reduced CH_4 emissions by 39.5%-64.7% [13-14]. This study's WSC reduced CH_4 by 29.02% (2015) and 41.19% (2016) compared to WS, smaller than Cheng's [14] findings, possibly because that study compared rice-rape rotation (a dry-wet rotation) without straw return, whereas this study involved continuous flooding plus straw return, which significantly enhances CH_4 emissions compared to dry-wet rotation [20].

3.2 Effects on CO₂ and N₂O Emissions

Straw return effects on CO₂ emissions are inconsistent across studies, with some reporting increases [24-25] and others decreases [26-27], primarily due to different return methods [24]. Surface mulching, with loose soil-straw contact, promotes aerobic decomposition and CO₂ emission. Different integrated systems have varying effects: rice-duck significantly increases CO₂ emissions due to duck activity enhancing soil-oxygen contact and microbial aerobic respiration [12], while Cheng [14] found rice-crayfish reduced CO₂ compared to rice-rape rotation, likely because the dry-wet rotation's aerobic fallow period provides a long buffering period preventing sharp CO₂ drops, whereas continuous flooding in rice-crayfish strongly suppresses aerobic respiration. This study found WS had no significant CO₂ effect in 2015 but increased emissions significantly in 2016 compared to W, while WSC had no significant effect on WS, suggesting that under long-term flooding, straw return affects annual but not seasonal CO₂ emissions, with crayfish having no additional effect.

Qin et al. [28] reported straw return had no significant effect on N₂O emissions, while Zhang et al. [19] found wheat straw return significantly reduced N₂O emissions. These inconsistent results are strongly influenced by return methods: Xiao et al. [29] showed no-tillage straw return significantly reduced N₂O compared to rotary and plow tillage. Different aquaculture species also affect N₂O differently: rice-duck increased N₂O emissions [12,15], while Datta et al. [30] found rice-fish decreased N₂O, possibly due to species-specific environmental impacts. This study showed WS increased N₂O emissions while WSC reduced them to levels comparable to W, likely because crayfish culture commonly uses lime disinfection, which raises water pH and negatively correlates with N₂O emissions [30].

3.3 Effects on Soil Environment

Integrated rice-aquaculture systems substantially disturb paddy ecosystems, increasing dissolved oxygen and reducing reductive substances [12,14,31]. This study found WSC had higher Eh at 40 cm than 20 cm depth, indicating crayfish burrowing affects deeper layers more than ducks or fish, substantially improving deep soil oxidation status and likely reducing CH₄ production while increasing oxidation. Except for NO₃⁻-N in 2016, soil acetic acid, NH₄⁺-N, and DOC showed no significant differences among treatments (Table 5), though they varied significantly over time (Table 4), suggesting that under long-term flooding, straw return and crayfish have limited effects on these parameters, which are more strongly influenced by temperature, water management, nutrient uptake, and root exudates. Peng et al. [32] reported continuous flooding plus straw return significantly increased DOC and organic acids, promoting CH₄ emissions. In this study, the high-stubble plus surface mulching method limited soil-straw contact, restricting microbial decomposition. Crayfish likely reduced DOC by feeding on straw and altered microbial community structure [33], increasing organic acid-utilizing microbes and reducing acetic acid accumulation.

4 Conclusions

Compared to winter waterlogging without straw return, straw return significantly increased CH_4 emissions, while integrating crayfish cultivation substantially mitigated this enhancement. CH_4 emissions were primarily regulated by soil temperature during flooding and by multiple factors during wetting-drying cycles. Straw return increased annual CO_2 emissions. No significant differences in rice yield were observed among treatments, but rice-crayfish co-culture significantly reduced greenhouse gas emission intensity compared to straw return alone.

Rice-crayfish co-culture in winter-flooded paddies with straw return significantly increased soil NO_3^- -N content but had no significant effect on DOC, acetic acid, or NH_4^+ -N, though these parameters varied significantly over time. The system substantially improved economic returns per unit area to 1.78-1.82 times that of non-crayfish systems.

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