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Integrated Effects of Straw Incorporation and N Application on Rice Yield and Greenhouse Gas Emissions in Three Rice-Based Cropping Systems

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Abstract: Crop straw and N fertilizer applications impact paddy rice yield and greenhouse gas (GHG) emissions. However, their interactive effects have not been well documented. This study investigated the effects of straw (S), no straw incorporation (NS), and three levels of N fertilization rates (N0, N1, and N2) on single rice (SR), double rice (DR), and rice-wheat (RW) cropping systems. Straw incorporation significantly increased total CH_4 emissions by 118.6%, 8.0%, and 79.0% in the SR, DR, and RW, respectively, compared to the NS. The total GHG emissions in DR are significantly 72.6% and 83.5% higher than those in RW and SR, respectively. Compared to NS, straw incorporation significantly increased yield-scaled emissions by 27.8%, 15.0%, and 89.0% in SR, DR, and RW, respectively. Straw with N application significantly increased average rice yield over N1 and N2 by 39.4%, 50.0%, and 6.7% in SR, DR, and RW, respectively. There was a significant correlation between methyl coenzyme M reductase (mcrA) and CH_4 emissions in $r_{SR} = 0.87$ ($p < 0.05$) and $r_{RW} = 0.85$ ($p < 0.05$), except in $r_{DR} = 0.06$ ($p > 0.05$). This study scientifically supports straw incorporation combined with a moderate N application rate in rice-based cropping systems to maintain high rice yields and mitigate GHG emissions.

Keywords: straw; N fertilizer; rice yield; CH_4 emissions; yield-scaled emissions; mcrA; cropping systems

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1. Introduction

China is a major rice-producing country, accounting for approximately 26% of global production [1]. Given the vital role rice plays in feeding the world's increasing population, it is crucial to double the rice production intensity [2]. As the world's leading rice producer, China makes an unwavering effort towards continuous rice production by adopting improved rice varieties and efficient agro-management practices. This has contributed immensely to the rice farm yield increase from 5.2 to 8.8 t ha^{-1} from 2016 to 2021 [3]. However, producing more rice to ensure food security is still a challenge worth considering [4]. Therefore, a further increase in rice production would lead to the generation of more crop straws in the future.

Asia accounts for 600 to 800 million metric tons of crop straw annually [5]. Moreover, increased rice production has led to increased straw production by 59.3% in recent years in rice-based cropping systems [6]. However, the huge amount of leftover crop straw has not been effectively put to good use across the rice-based cropping systems, which creates problems for rice farmers before the next planting season. Crop straw, a by-product of rice production, is a nutrient-rich resource that can improve soil fertility and may serve as an alternative to synthetic fertilizer usage. For example, Wang et al. [7] showed that the incorporation of crop straw with reduced nitrogen (N) fertilizer enhanced rice yield. In most cases, crop straw incorporation and fertilizer application rates are typically cropping system-specific in China. Moreover, the recalcitrant nature of rice straw causes slow nutrient release from the straw and hinders crop growth. For instance, Marschner et al. [8] reported that the recalcitrant nature of rice straw due to hemicellulose, cellulose, lignin, and water-soluble polysaccharides makes straw decomposition difficult and impedes crop growth [9]. In addition, straw incorporation increases greenhouse gas (GHG) emissions. For example, decomposable carbon from straw-incorporated paddy fields is the most important driver of greenhouse gas emissions [7], specifically the cumulative CH_4 emissions [10]. It was mostly soil methanogenic bacteria growth that caused the positive effects of straw incorporation on greenhouse gas emissions [11]. These bacteria caused a large amount of CH_4 to be released from paddy fields. Nevertheless, balancing straw incorporation and GHG emissions effectively remains a challenge.

Several studies have highlighted the importance of the C/N ratio in organic amendments to manipulate their decomposition. Straw incorporation enhanced the immobilization of N fertilizer, contributing to a better synchronization of crop N demand [12]. A possible reason could be variations in the microbial community and the response of cells to environmental changes due to N fertilization [13]. It has been previously shown that N fertilization increases fungal biomass, which is beneficial to straw degradation [14]. In addition, significant shifts in microbial composition and community abundance in response to long-term N addition have been reported [7]. For instance, adequate N addition may alleviate N limitation for some microbes that use inorganic N as energy sources or may decline N_2-fixing microbes due to excess N [15]. Therefore, the adequate threshold for N application to regulate microbial demand and aid straw incorporation to maintain rice yield at a low environmental cost should be considered. Moreover, more information is needed to avoid excessive N application and reduce the cost of rice production with adequate straw management in rice-based cropping systems.

The rice paddy field has been identified as a significant source of GHG, accounting for 15% to 20% of total global anthropogenic CH_4 emissions [16]. Therefore, GHG emission reduction is the key to achieving a 1.5 °C climate stabilization target. To date, there are many inconsistencies with some previous results on GHG mitigation and rice yield maintenance under straw incorporation and N application across the rice-based cropping systems of China. This might be because of several mechanisms that may operate at different time scales, making long-term predictions of CH_4 emissions with straw incorporation challenging [17]. However, there is a paucity of information on fertilizer application optimization to complement the N demand for microbial straw decomposition, plant growth, and rice yield in paddy fields. Moreover, the interactive effects of straw incorporation and N application rate on rice yield and CH_4 emission have not been thoroughly evaluated and well documented. Therefore, the objectives of this study were to (i) determine the effects of N application levels and straw incorporation on rice yield in the rice-based cropping systems of China and (ii) find out how different levels of N application and straw incorporation affect greenhouse gas emissions in rice-based cropping systems.

2. Materials and Methods

2.1. Site Description

The field study was conducted in 2019 in three rice-based cropping systems in China, namely; single rice (SR), double rice (DR), and rice-wheat (RW) cropping systems.

In the single rice cropping system, the experiment was carried out at the National Modern Agricultural Demonstration Park in Minzhu Town, Harbin, Heilongjiang Province, China (45°49′ N, 126°48′ E). This region is one of the coolest climate zones in China, and only one rice season is often practiced in rice planting areas between May and October throughout the year. The soil is classified as Chernozem soil (Mollisols in USA-ST). The basic characteristics of the soil were: soil organic matter (27.3 g kg^{-1}), available N (78.9 mg kg^{-1}), available P (24.2 mg kg^{-1}), available K (184.7 mg kg^{-1}), and pH 8.6. The climate is northern temperate, with an average yearly precipitation of 508 mm to 583 mm and an effective cumulative temperature of 2600 °C to 2700 °C (Figure 1), a frost-free period of 131–146 days, and 2668 h of sunshine annually.

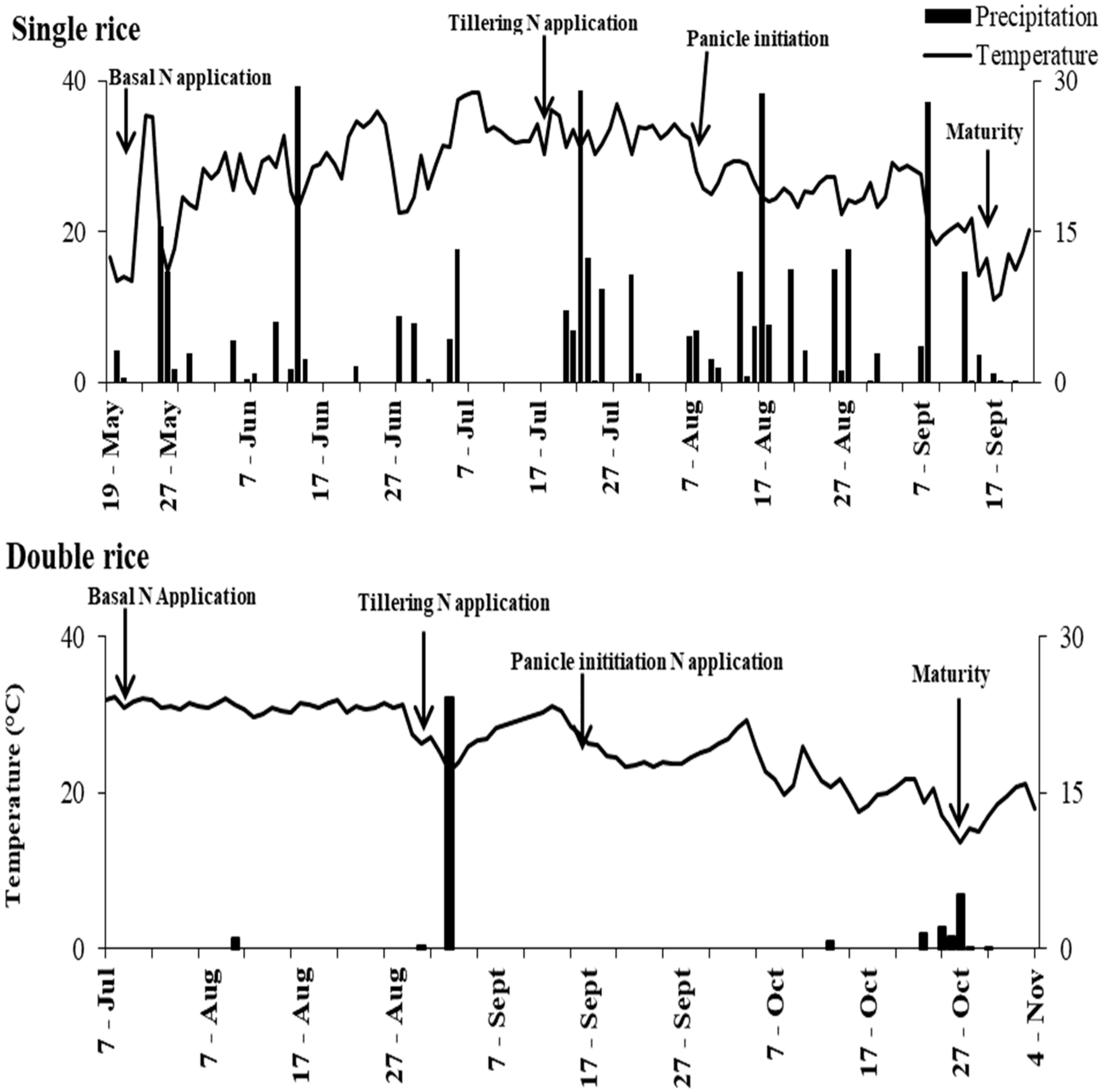


Figure 1. *Cont.*

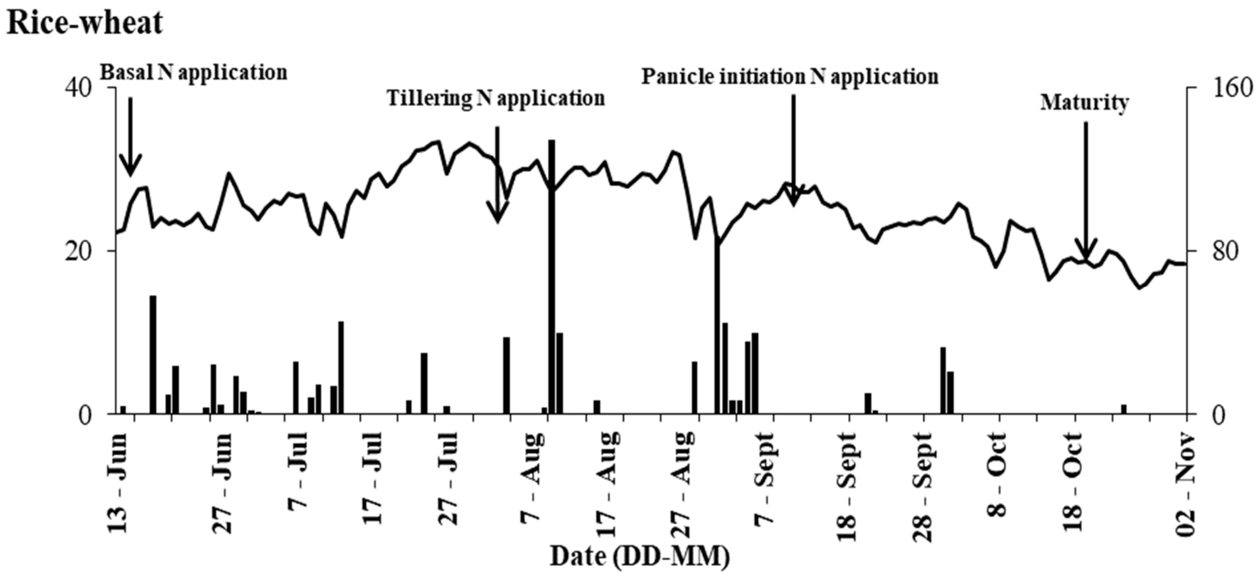


Figure 1. Variations in daily average atmospheric precipitation, temperature, and management practices during the rice-growing season in the single rice, double rice, and rice-wheat cropping systems.

In the double rice cropping system, the early rice season spans from April to July, and the late rice season spans from July to November. This study focused only on the late rice cropping season because early rice straw is the major issue with this system. The experiment was conducted at the Jiangxi Institute of Red Soil in Jinxian, Jiangxi Province of China (28°37′ N, 116°26′ E) on Stagnic Anthrosols soil. This region is characterized by a typical subtropical climate with an average of 262 frost-free days a year, 1537 mm of rainfall, and a temperature of 18.1 °C (Figure 1). The soil characteristics were silty loam texture, soil organic matter 22.6 g kg^{-1}, available N 128.5 mg kg^{-1}, available P 57.2 mg kg^{-1}, available K 100.3 mg kg^{-1}, and a pH of 5.9.

In the rice-wheat cropping system, the experiment was conducted at Taican town, south of the Yangtze River, Suzhou, Jiangsu Province, China (31°33′50 N, 121°10′26 E) on Fluvisols soil. In this region, wheat and rice are planted one after the other, with a minimum gap of 2 weeks between the two seasons. The wheat season ranged from mid-November to May, while the rice season was cultivated from late June to early November. The soil properties were: soil organic matter 26.1 g kg^{-1}, available N 135.3 mg kg^{-1}, available P 28.6 mg kg^{-1}, available K 92.2 mg kg^{-1}, and pH 6.8. The climatic conditions are warm and temperate, with a mean annual precipitation of 1070 mm. The average annual temperature is 15.6 °C (Figure 1), with a frostless period of 224 days. The weather data for the three cropping systems is shown in Figure 1.

2.2. Experimental Design

The experiments in the three cropping systems were randomized in a split-plot design with three replications. The main plot was straw incorporation (S) and no straw incorporation (NS), while three levels of N fertilization (N0, N1, and N2) were adopted as the subplot. The treatments were (SN0, SN1, and SN2) and (NSN0, NSN1, and NSN2) in the three different rice-based cropping systems. These cropping systems adopted in this study varied primarily in climate, cultivar, and soil properties. We selected three representatives of rice-based cropping systems for the evaluation of straw and nitrogen (N) application levels. The corresponding N application rates per hectare for the single rice, double rice, and rice-wheat cropping systems are shown in Table 1. The nitrogen application standard for N2 followed normal application rates in each cropping system. In this study, we adopted a 30 kg N ha^{-1} reduction from N2 as the N1 treatment, with the target of reducing N fertilizer application and supplementing with crop straw in rice-based cropping systems in China. Plot sizes of 30 m^2 in SR, 30 m^2 in DR, and 44 m^2 per plot in RW cropping systems were adopted. All plots in the three cropping systems were irrigated in line with the local management practices for high rice yield in single rice, double rice, and rice-wheat cropping systems, following standard procedures in both straw and no straw fields. The straw return rates in single rice, double rice, and rice-wheat cropping systems were 7.5 t ha^{-1}, 7.0 t ha^{-1}, and 6.0 t ha^{-1} per treatment, respectively, using conventional tillage. In the single rice cropping systems, rice straw from the previous season was adopted. Moreover, the rice straw from the early rice season was incorporated into the late rice season of the double rice cropping system. Meanwhile, in the rice-wheat cropping system, the wheat straw was harvested and incorporated into the rice cropping season. The crop straw in each cropping system was cut into approximately 5–7 cm before being incorporated into the soil. Before transplanting, the rice fields were puddled and submerged with water for 3–4 days to meet the soil moisture conditions required for transplanting under continuous flooding.

Table 1. Straw and fertilizer management in the three rice-based cropping systems.

Cropping Systems	Crop Straw Incorporation		Total NPK Fertilizer Application Rate (kg ha^{-1})			Fertilizer Application Ratio in Different Stages (kg^{-1} plot^{-1})						
						N				P_2O_5	K_2O	
	Straw	No Straw	N	P_2O_5	K_2O	Basal	Tillering	Panicle	Flowering	Basal	Basal	Panicle
Single rice	SN0	NSN0	0	70	60							
	SN1	NSN1	150	70	60	0.21	0.25	0.25	0.10	0.45	0.18	0.18
	SN2	NSN2	180	70	60	0.29	0.29	0.29	0.12			
Double rice	SN0	NSN0	0	75	75							
	SN1	NSN1	165	75	75	0.54	0.21	0.32		1.87	0.19	0.19
	SN2	NSN2	195	75	75	0.64	0.25	0.38				
Rice-Wheat	SN0	NSN0	0	67.5	67.5							
	SN1	NSN1	240	67.5	67.5	0.69	0.37	0.55	0.69	2.47	0.25	0.25
	SN2	NSN2	270	67.5	67.5	0.77	0.41	0.62	0.77			

NS: No straw; S: Straw; Single rice: (N0: 0 kg ha^{-1}, N1: 150 kg ha^{-1}, and N2: 180 kg ha^{-1}): Double rice: (N0: 0 kg ha^{-1}, N1: 165 kg ha^{-1}, and N2: 195 kg ha^{-1}) and Rice-wheat: (N0: 0 kg ha^{-1}, N1: 240 kg ha^{-1}, and N2: 270 kg ha^{-1}).

Varieties of rice planted in the three cropping systems were Japonica rice of Longjing 21 in single rice, hybrid Indica rice of Taiyou 871 in double rice, and Nanjing 44 in the rice-wheat cropping system. Raised rice seedlings were manually transplanted in the single rice cropping system on 12 May 2019, the double rice cropping system on 12 August 2019, and the rice-wheat cropping system on 20 June 2019. The transplanting was performed at a density of two seedlings per hill, and the fertilizer application in the cropping systems followed local agronomic management recommendations with moderate modifications to the N levels. In the single rice cropping system, nitrogen in the form of urea was applied in four splits as follows: 40% basal, 25% at tillering, 25% at panicle initiation, and 10% at the flowering stage, while P_2O_5 was applied 100% at once, and K_2O was applied at 50% as basal and 50% at the panicle initiation stage of rice growth. In the double rice cropping system, nitrogen (N) was applied in three splits: 50% basal, 20% at the tillering stage, and 30% at the panicle initiation stage. Meanwhile, P_2O_5 was applied 100% at once, and K_2O was applied at 50% as basal and 50% at the panicle initiation stage. In the rice-wheat cropping system, the urea was applied in four splits: 30% as basal, 16% at early tillering, 24% at tillering, and 30% at the panicle initiation stage, while the P_2O_5 was applied 100% at once, and K_2O was applied at 50% as basal and 50% at the panicle initiation stage. The rate of NPK fertilizer applied in each cropping system is shown in Table 1.

2.3. Gas Sampling and Measurement

Gas samplings for CH_4 and N_2O were carried out using the static closed chamber technique. At the tillering stage, the chamber with dimensions of 50 cm × 50 cm × 50 cm and the dimensions of 50 cm × 50 cm × 100 cm at the panicle initiation stages were equipped with an internally battery-powered fan and utilized for the gas collection, respectively.

Gases were extracted weekly between 9 a.m. and 11 a.m. from the chamber headspace using a 50-mL airtight syringe at 0, 5, 10, and 15 min for four different sampling times after closure and pumped into pre-evacuated butyl rubber stopper-mounted vials (40 mL) as previously described [18], but with little modifications with sampling minute intervals.

The air temperature of the chamber was measured during gas collection using a mercury-in-glass thermometer connected to the chamber. A gas chromatograph (Agilent 7890A, Agilent Technologies, Wilmington, DE, USA), fitted with a flame ionization detector and an electron capture detector, was used to simultaneously analyze the concentration of the two gases in a gas sample. The weekly fluxes of CH_4 and N_2O were computed using the linear regression of CH_4 or N_2O concentrations.

Cumulative N_2O and CH_4 emissions were calculated using the formula described by Cai et al. [19].

$$\text{Cumulative } (CH_4 \text{ or } N_2O) \text{ emission} = \sum_{i=1}^{n} (F_i + F_{i+1})/2 \times (t_{i+1} - t_1) \times 24$$

where F_i is the CH_4 or N_2O flux (mg N_2O/CH_4 m^{-2} h^{-1}), i is the ith measurement, the term $(t_{i+1} - t_i)$ is the time in days between two adjacent measurements, and n is the total number of measurements. The total greenhouse gases (TGHG) were generated using the radioactive forcing of 27.9 for CH_4 and 273 for N_2O. The total GHG was calculated as CO_2 equivalent (CO_2-eq) based on a 100-year time horizon [20] using the formula:

$$\text{TGHG (kg } CO_2\text{-eq ha}^{-1}\text{ yr}^{-1}) = 27.9 \times CH_4 \text{ (kg } CH_4 \text{ ha}^{-1}) + 273 \times N_2O \text{ (kg } N_2O \text{ ha}^{-1}),$$

where the CH_4 and N_2O reflect the combined seasonal cumulative emissions of the two gases.

Yield-scaled emission (kg CO_2-eq kg^{-1}) was calculated as the ratio between TGHG and rice yield.

2.4. Yield Determination

Rice from the single rice cropping system was harvested on 24 September 2019. Late rice was harvested on 29 October 2019 in the double rice cropping system; the rice harvest in the rice-wheat cropping system was completed on 31 October 2019. The grain yields were determined in each plot from all plants in a 1 m^2 area, and the grain moisture content was adjusted to 13.5% and 14.5% fresh weight for japonica and indica rice, respectively.

2.5. Soil Sample Determination in Rice Paddy Soils

Due to the different maturation periods of the planted rice varieties in the cropping systems, initial soil samples in three replicates, at a depth of 0 cm to 15 cm per plot, were collected using three randomized auger points at the rice tillering stage (5th week in single rice, 6th week in double rice cropping system, 3rd week in rice-wheat). The composite soil samples were air-dried and ground to pass through a 2 mm sieve. The air-dried soil samples were placed in an ice-pack container for onward transportation and storage in a freezer. The portions of the composite soil samples were stored at −80 °C for molecular microbial assay. The microbial abundances of methanotrophs and methanogens were quantified to estimate the dynamics of methyl coenzyme M reductase (*mcrA*) and particulate methane monooxygenase (*pmoA*) during the rice-growing season. The Line-Gene 9600 Plus Real-time PCR was used to determine the copy numbers of the *mcrA* and *pmoA* genes (Bioer, Hangzhou, China). This was conducted using the primer pairs of MLf (5′-GGTGGTGTMGGATTC ACACARTAYGC WACAGC-3′) and MLr (5′-TTCATTGCRTAGTTWGGRTAG TT-3′) [21], and A189F (5′-GGNGACTGGGACTTCTGG-3′) and Mb661R (5′-CCGGMGCAACGTCYTTACC-3′) [22], respectively. The qPCR amplifications were carried out at various cycles of 95 °C for 30 s in a total volume of 20 µL with a SYBR@Premix Ex TaqTM (Takara, Dalian, China) with the reaction mixture of 10 µL ChamQ SYBR Color qPCR, 0.4 µL each for the forward and reverse primers.

2.6. Statistical Analysis

Analyses of variance were performed for the cropping systems, nitrogen (N) application, and straw incorporation patterns. Data were analyzed among the three cropping systems, different N rates, and straw incorporation patterns in each cropping system. The mean significance differences at $p < 0.05$ were tested with Duncan's Multiple-Range Test (DMRT) using SAS 9.0. Regression models, weekly gas analyses, and graphs were performed using Microsoft Excel 2019.

3. Results

3.1. CH_4 Emissions

In this study, cropping systems and straw incorporation significantly impacted cumulative CH_4 emissions ($p < 0.01$; Table 2). Moreover, there were significant interactions between straw incorporation and N application on cumulative CH_4 emissions ($p < 0.01$; Table 2). In the single rice cropping system, SN0 produced significantly higher CH_4 flux than SN1, SN2, NSN0, NSN1, and NSN2 on the 3rd week after rice transplanting ($p < 0.05$; Figure 2). Compared to SN0, SN2 significantly reduced the cumulative CH_4 emissions by 129.0% ($p < 0.05$; Table 2). In the double rice cropping systems, there was no significant difference between nitrogen and straw incorporation on cumulative CH_4 emissions. In the rice-wheat cropping system, compared to SN1, SN2 significantly decreased cumulative CH_4 emissions by 31.4% in the rice-wheat cropping system ($p < 0.05$; Table 2).

Table 2. Impacts of straw incorporation and fertilizer application rates on rice yield and greenhouse gas emissions in the three rice-based cropping systems.

Cropping Systems	Treatments	Cumulative CH_4 (kg ha^{-1})	Cumulative N_2O kg ha^{-1}	Total Greenhouse Gas kg CO_2-eq ha^{-1}	Yield (t ha^{-1})	Yield-Scaled Emission kg CO_2-eq kg^{-1}
Single rice	NSN0	173.4 ± 80.6 ^{c}	0.2 ± 0.1 ^{a}	4904.5 ± 2219.0 ^{c}	7.6 ± 0.2 ^{b}	0.7 ± 0.3 ^{b}
	NSN1	230.6 ± 75.0 ^{c}	0.4 ± 0.1 ^{a}	6541.5 ± 2099.8 ^{c}	10.9 ± 0.2 ^{a}	0.6 ± 0.1 ^{b}
	NSN2	351.0 ± 85.4 ^{abc}	0.4 ± 0.3 ^{a}	9904.3 ± 1544.2 ^{abc}	10.7 ± 0.1 ^{a}	0.9 ± 0.1 ^{b}
	SN0	700.5 ± 170.4 ^{a}	0.1 ± 0.2 ^{a}	19,571.5 ± 4698.5 ^{a}	7.1 ± 0.4 ^{b}	2.8 ± 0.6 ^{a}
	SN1	661.2 ± 204.0 ^{ab}	0.2 ± 0.4 ^{a}	18,513.0 ± 5713.8 ^{ab}	9.6 ± 0.1 ^{a}	2.0 ± 0.6 ^{ab}
	SN2	288.7 ± 60.3 ^{bc}	0.3 ± 0.1 ^{a}	8139.6 ± 1660.0 ^{bc}	10.2 ± 0.8 ^{a}	0.8 ± 0.1 ^{b}
	Average	400.9 ± 65.1 B	0.3 ± 0.1 C	11,262.1 ± 1810.4 B	9.3 ± 0.4 A	1.3 ± 0.2 B
Double rice	NSN0	687.4 ± 155.3 ^{a}	1.2 ± 0.8 ^{a}	19,502.5 ± 4572.59 ^{ab}	6.8 ± 0.2 ^{b}	2.9 ± 0.8 ^{a}
	NSN1	576.0 ± 93.4 ^{a}	0.9 ± 0.6 ^{a}	16,331.1 ± 2582.40 ^{b}	10.1 ± 0.8 ^{a}	1.6 ± 0.2 ^{a}
	NSN2	839.7 ± 44.3 ^{a}	1.0 ± 0.7 ^{a}	23,702.4 ± 1376.56 ^{ab}	10.7 ± 0.6 ^{a}	2.3 ± 0.2 ^{a}
	SN0	634.7 ± 72.9 ^{a}	1.0 ± 0.4 ^{a}	17,964.7 ± 1376.56 ^{a}	6.3 ± 0.5 ^{b}	2.9 ± 0.5 ^{a}
	SN1	891.0 ± 121.2 ^{a}	1.1 ± 0.7 ^{a}	25,165.9 ± 3174.29 ^{ab}	9.0 ± 0.5 ^{a}	2.8 ± 0.2 ^{a}
	SN2	746.3 ± 136.1 ^{a}	1.8 ± 0.3 ^{a}	21,313.8 ± 3725.70 ^{ab}	9.9 ± 0.9 ^{a}	2.1 ± 0.2 ^{a}
	Average	729.2 ± 46.4 A	1.2 ± 0.2 B	20,663.4 ± 1302.1 A	8.8 ± 0.5 A	2.5 ± 0.2 A
Rice-Wheat	NSN0	226.2 ± 9.2 ^{e}	2.5 ± 0.6 ^{a}	6988.0 ± 237.0 ^{d}	7.5 ± 0.2 ^{a}	0.9 ± 0.1 ^{e}
	NSN1	285.6 ± 34.6 ^{de}	2.4 ± 0.6 ^{a}	8619.9 ± 787.7 ^{cd}	7.8 ± 0.1 ^{a}	1.1 ± 0.1 ^{de}
	NSN2	367.1 ± 72.1 ^{cd}	2.3 ± 1.0 ^{a}	10,865.5 ± 2273.5 ^{bc}	7.7 ± 0.3 ^{a}	1.4 ± 0.3 ^{cd}
	SN0	504.6 ± 27.6 ^{ab}	1.6 ± 1.4 ^{a}	14,513.0 ± 753.0 ^{b}	6.7 ± 0.2 ^{b}	2.2 ± 0.1 ^{b}
	SN1	633.8 ± 76.2 ^{a}	1.9 ± 0.4 ^{a}	18,190.7 ± 2066.1 ^{a}	6.8 ± 0.2 ^{b}	2.7 ± 0.2 ^{a}
	SN2	434.9 ± 12.5 ^{bc}	1.8 ± 0.6 ^{a}	12,635.7 ± 527.6 ^{b}	7.5 ± 0.1 ^{a}	1.7 ± 0.1 ^{c}
	Average	408.6 ± 36.0 B	2.1 ± 0.3 A	11,968.8 ± 989.5 B	7.3 ± 0.1 B	1.7 ± 0.2 B
Source of variations						
Cropping systems (Cs)		21.76 **	10.94 **	21.85 **	30.99 **	96.7 0 **
Straw incorporation (S)		17.76 **	0.29 ^{NS}	17.46 **	11.48 **	13.83 **
Nitrogen (N)		0.56 ^{NS}	0.12 ^{NS}	0.57 ^{NS}	50.31 **	1.66
Cs × S		2.42 ^{NS}	0.63 ^{NS}	2.32 ^{NS}	0.03 ^{NS}	4.31 *
Cs × N		0.92 ^{NS}	0.06 ^{NS}	0.93 ^{NS}	9.06 **	2.04
S × N		6.37 **	0.22 ^{NS}	6.27 **	0.76 ^{NS}	3.55 *
Cs × S × N		1.25 ^{NS}	0.08 ^{NS}	1.29 ^{NS}	0.20 ^{NS}	1.14 ^{NS}

NS: No straw; S: Straw; Single rice: (N0: 0 kg ha^{-1}, N1: 150 kg ha^{-1}, and N2: 180 kg ha^{-1}): Double rice: (N0: 0 kg ha^{-1}, N1: 165 kg ha^{-1}, and N2: 195 kg ha^{-1}) and Rice-wheat: (N0: 0 kg ha^{-1}, N1: 240 kg ha^{-1}, and N2: 270 kg ha^{-1}). Different small alphabets in the same column at each cropping system mean a significant difference at $p < 0.05$ and different capital alphabets mean a significant difference at $p < 0.05$ among the three cropping systems. *: significant at 0.05; **: significant at 0.01.

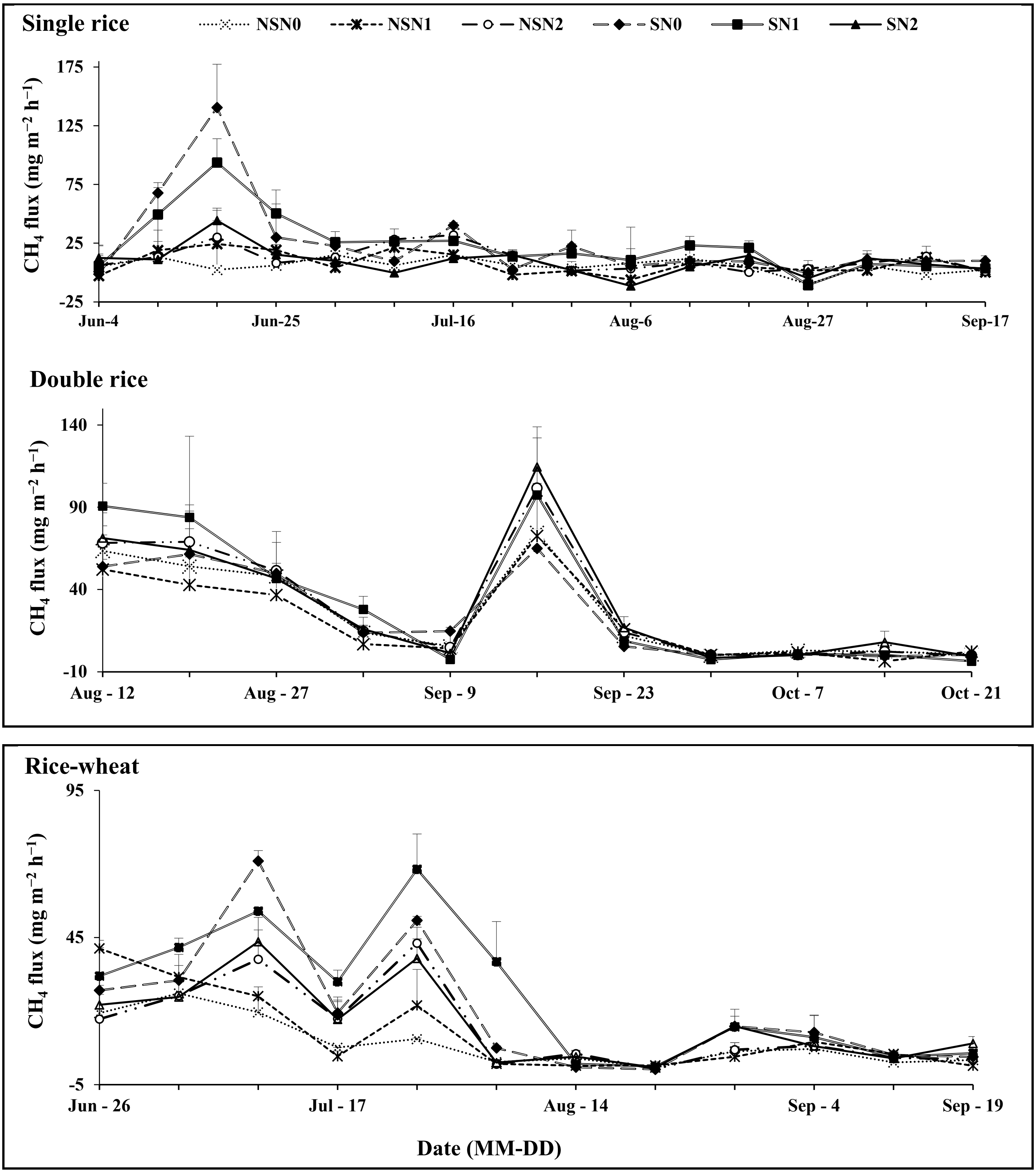


Figure 2. Seasonal variations of CH_4 fluxes for the single rice, double rice, and rice-wheat cropping systems under N application and straw incorporation. NS: No straw; S: Straw; Single rice: (N0: 0 kg ha^{-1}, N1: 150 kg ha^{-1}, and N2: 180 kg ha^{-1}): Double rice: (N0: 0 kg ha^{-1}, N1: 165 kg ha^{-1}, and N2: 195 kg ha^{-1}) and Rice-wheat: (N0: 0 kg ha^{-1}, N1: 240 kg ha^{-1}, and N2: 270 kg ha^{-1}). Bars indicate the standard error of three replicates.

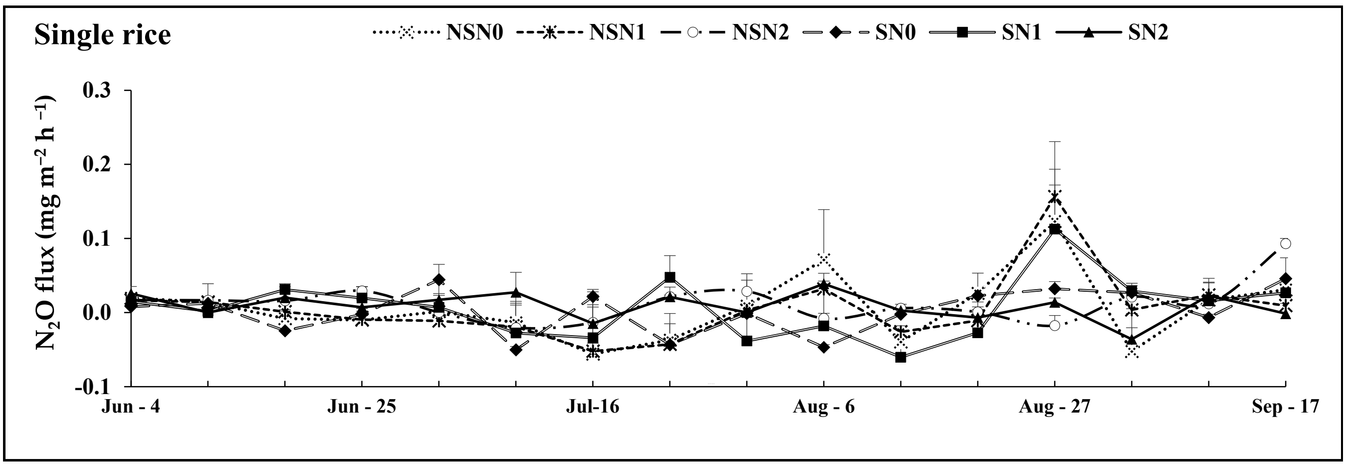
The cumulative CH_4 emissions across the cropping systems were in the order of double rice > rice-wheat > single rice cropping systems. Compared to rice-wheat and single rice cropping systems, straw incorporation in the double rice cropping system significantly increased CH_4 emissions ($p < 0.01$) by 78.4% and 81.8%, respectively. It is also noteworthy to show that, relative to no straw incorporation, straw incorporation significantly increased the cumulative CH_4 emission by 118.6%, 8.0%, and 79.0% in SR, DR, and RW cropping systems, respectively.

3.2. N_2O Emissions

A significant effect of the cropping systems was noted in cumulative N_2O emissions ($p < 0.01$; Table 2). The cumulative N_2O emissions across the cropping systems were in the order of rice-wheat > double rice > single rice cropping systems. There was a significant 317.9% increase in N_2O emissions in the DR cropping system compared to the SR cropping system under the management of straw and N applications. Meanwhile, compared to DR and SR cropping systems, the RW cropping system significantly increased N_2O emissions ($p < 0.01$) by 76.9% and 639%, respectively. In the SR cropping system, NSN1 had the highest N_2O flux during the 13th week of gas sampling ($p < 0.05$; Figure 3). In the DR cropping system, the highest flux of N_2O gas sampling was noted in the 9th week of late rice planting ($p > 0.05$; Figure 3). Meanwhile, SN2 in the RW cropping system recorded the highest N_2O flux in the 8th week of rice growth ($p < 0.05$; Figure 3).

3.3. Rice Yield

Significant effects of cropping systems, straw incorporation, and N applications were observed on rice yield ($p < 0.01$; Table 2). There was a significant interaction between the cropping systems and N application on rice yield in this study ($p < 0.01$; Table 2). Straw incorporation significantly decreased the rice yield by 7.9~8.6% in three rice cropping systems. In the single rice and double rice cropping systems, there were no significant differences in rice yield between SN1 and SN2, except for the rice-wheat cropping system. Under straw incorporation in the single rice cropping system, compared to SN0, SN1 and SN2 significantly increased rice yield by 35.2% and 43.7% ($p < 0.05$; Table 2), respectively. In addition, SN1 and SN2 at the double rice cropping system significantly increased rice yield by 42.9% and 57.1% ($p < 0.05$; Table 2), respectively. Whereas, SN2 at the rice-wheat cropping system significantly increased rice yield by 11.9% ($p < 0.05$; Table 2) when related to SN0.



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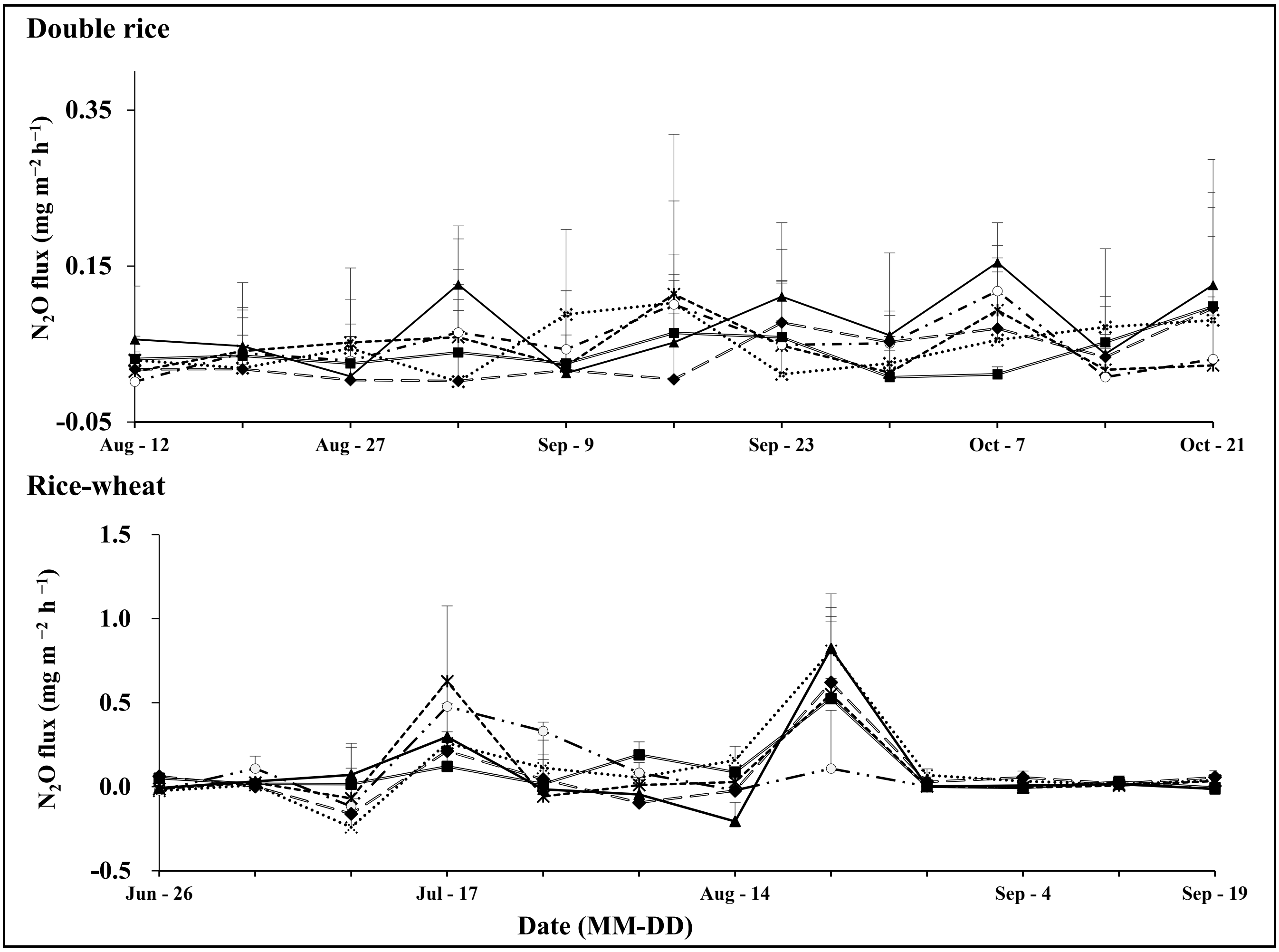


Figure 3. Seasonal variations of N_2O fluxes for the single rice, double rice, and rice-wheat cropping systems under N application and straw incorporation. NS: No straw; S: Straw; Single rice: (N0: 0 kg ha^{-1}, N1: 150 kg ha^{-1}, and N2: 180 kg ha^{-1}): Double rice: (N0: 0 kg ha^{-1}, N1: 165 kg ha^{-1}, and N2: 195 kg ha^{-1}) and Rice-wheat: (N0: 0 kg ha^{-1}, N1: 240 kg ha^{-1}, and N2: 270 kg ha^{-1}). Bars indicate the standard error of three replicates.

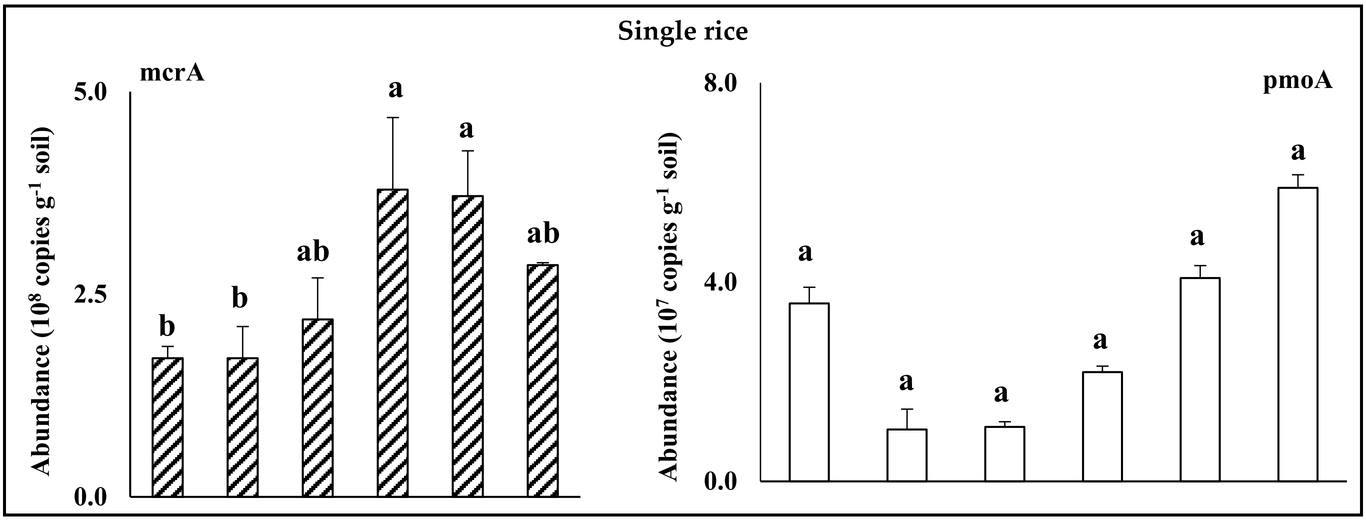
3.4. Total Greenhouse Gas and Yield-Scaled Emissions

Straw incorporation and cropping systems had a significant effect on total GHG emissions ($p < 0.01$; Table 2). The straw incorporation significantly increased the total GHG emissions by 72.6% and 83.5% ($p < 0.05$) in the double rice cropping systems when compared to rice-wheat and single rice cropping systems. Under straw incorporation, compared to SN0, SN2 decreased significantly by 58.4% ($p < 0.05$) in the single rice cropping system. Moreover, SN1 in the rice-wheat cropping system significantly increased the total GHG by 25.3% and 43.5% ($p < 0.05$), compared to SN0 and SN2, respectively. Straw incorporation and cropping systems significantly affect yield-scaled emissions ($p < 0.01$; Table 2). Crop straw incorporation across the cropping systems impacted the yield-scaled emissions in the order of DR > RW > SR (Table 2). The yield-scaled emissions under straw incorporation in the double rice cropping system are significantly higher (47.1% and 92.3%) than in the rice-wheat and single rice cropping systems, respectively. However, there were no significant differences between the rice-wheat and single rice cropping systems.

Relative to no straw incorporation, straw incorporation significantly increased yield-scaled emissions by 27.8%, 15.0%, and 89.0% in SR, DR, and RW, respectively. The SN2 significantly reduced the yield-scaled emission ($p < 0.05$) by 71.4% in the single rice cropping system relative to the SN0, respectively (Table 2). In the double rice cropping system, no significant yield-scaled emissions were produced. Whereas, in the rice-wheat cropping system, SN2 contributed to a significant reduction in yield-scaled emissions of 22.7% and 37.0% ($p < 0.05$) compared to SN0 and SN1, respectively.

3.5. Methanogens and Methanotroph Abundance

We checked gene abundance at 15 cm soil layer depth and found that there were significant changes in methanogen abundance in the single rice and double rice cropping systems ($p < 0.05$; Figure 4). There is no significant effect on the rice-wheat cropping system. Meanwhile, there were no significant differences in the methanotrophs in SR and RW, except for DR ($p < 0.05$; Figure 4). At the same level of N application, methanogen abundance was higher with straw incorporation than without straw incorporation in the single rice cropping system ($p < 0.05$; Figure 4). Compared to SN0, SN2 in the double rice cropping system significantly reduced the methanogen abundance by 58.7% ($p < 0.05$; Figure 4). The regression model showed a significant positive relationship between methanogens and CH_4 emissions in the single rice cropping system ($r_{SR} = 0.87$; $p < 0.05$) and in the rice-wheat cropping system ($r_{RW} = 0.85$; $p < 0.05$).



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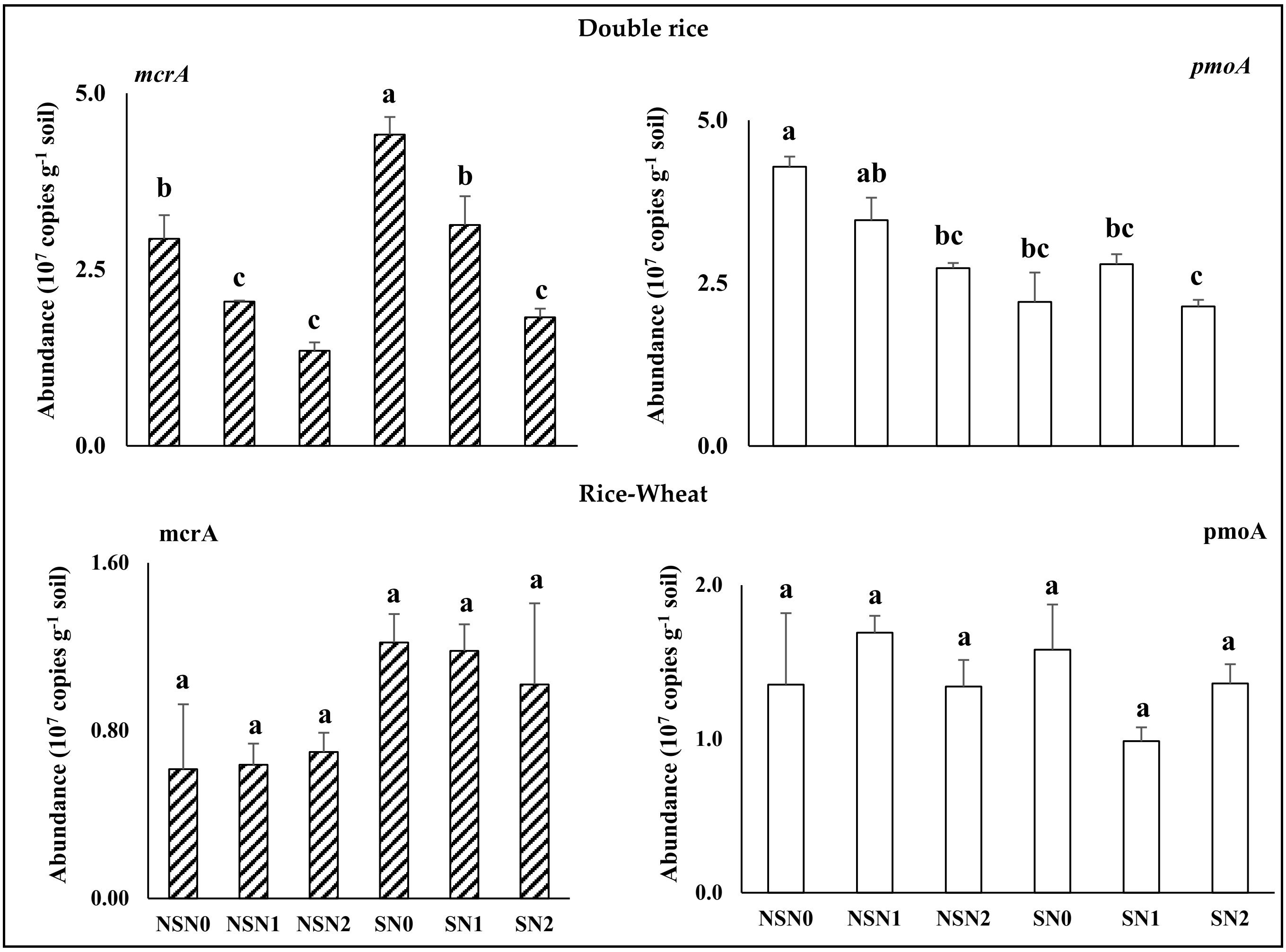


Figure 4. Impacts of straw incorporation and N application on *mcrA* and *pmoA* gene abundances. *mcrA* gene abundance; *pmoA* gene abundance; NS: No straw; S: Straw; Single rice: (N0: 0 kg ha^{-1}, N1: 150 kg ha^{-1}, and N2: 180 kg ha^{-1}); Double rice: (N0: 0 kg ha^{-1}, N1: 165 kg ha^{-1}, and N2: 195 kg ha^{-1}); Rice-wheat: (N0: 0 kg ha^{-1}, N1: 240 kg ha^{-1}, and N2: 270 kg ha^{-1}). Bars indicate the standard error of three replicates. Different alphabets on the columns of *mcrA* and *pmoA* at each cropping system indicate significant differences among treatments at a 0.05 probability level.

4. Discussion

Straw's return to the field is a sustainable approach to mitigating environmental challenges. However, the impact of straw incorporation, N application rates, and their interactive effects stimulated cumulative CH_4 emissions, GHG emissions, and rice yield in the single rice, double rice, and rice-wheat cropping systems. The emissions differences among the three rice-based cropping systems could be attributed to regional differences, which could regulate the biogenic processes of methane production [23]. The cumulative CH_4 emissions in the double rice cropping system are almost double those of the rice-wheat and single rice cropping systems. The treatments within the rice-based cropping systems showed that straw incorporation triggered cumulative CH_4 emission faster than applying nitrogen application alone in the double rice, rice-wheat, and single rice cropping systems. This might be explained by the varied organic carbon substrate generated from microbial straw decomposition and environmental factors like precipitation and temperature in paddy fields [24–26].

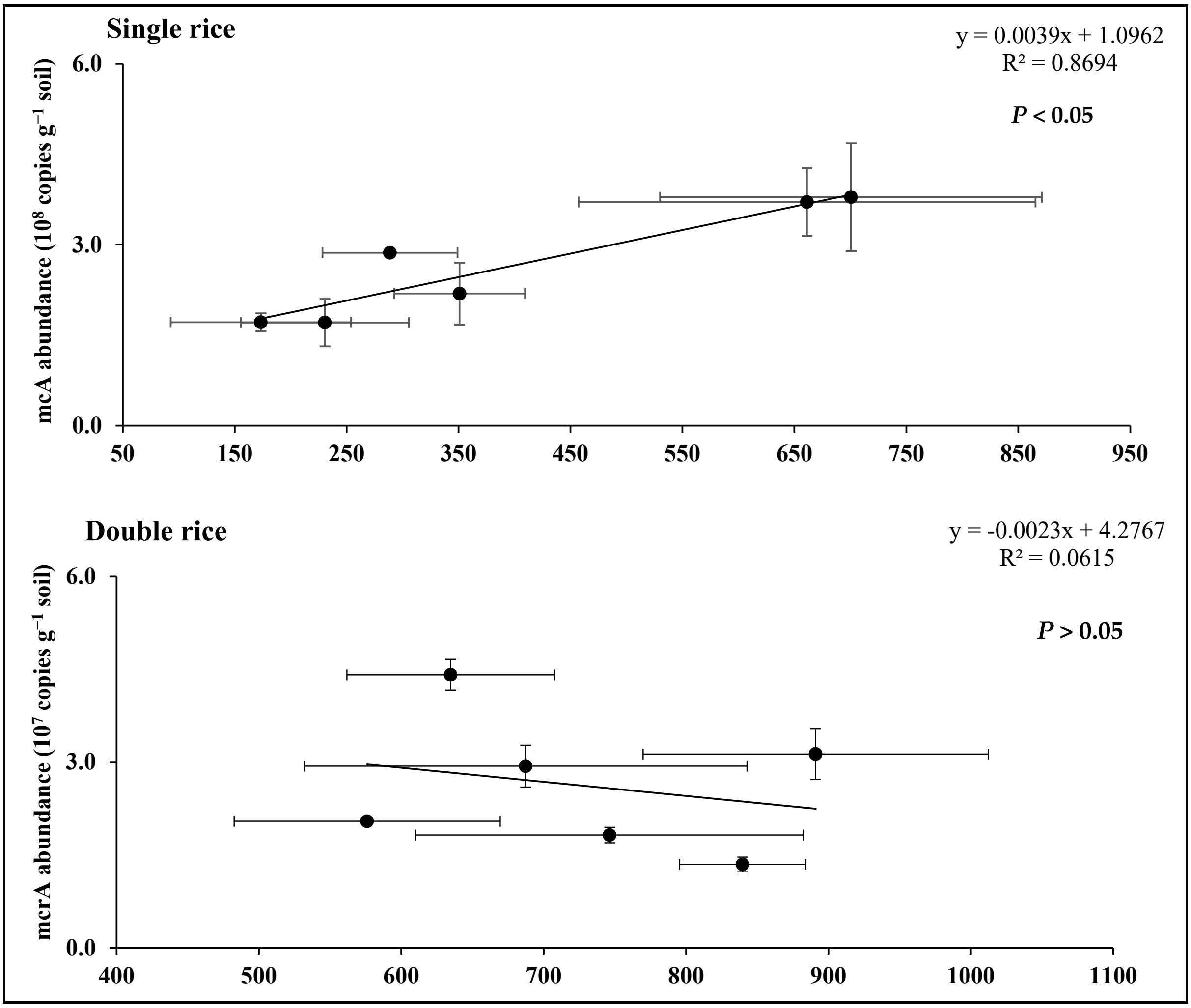
In this study, SN1 did not significantly impact cumulative CH_4 emissions in these three rice-based cropping systems. On the other hand, SN2 considerably reduced CH_4 emissions at single rice and rice-wheat cropping systems, indicating that the applied N may also play a role in regulating microbial activities, leading to a significant reduction in greenhouse gas emissions [15,27]. Although increasing the N application rate from SN1 to SN2 resulted in lower cumulative CH_4 emissions across the three cropping systems, it was consistent with the report from Linquist et al. [28]. However, Sun et al. [29] stated that nitrogen application significantly enhanced CH_4 emissions between 50–100 kg ha^{-1}, while N application had an inhibitory effect on CH_4 emissions between 200 and 250 kg ha^{-1}. Earlier reports from Tang et al. [30] also attributed this to higher competition between rice plants' N uptake and microbial N consumption, thus reducing straw decomposition rate and CH_4 emission.

The effect of straw and nitrogen application on cumulative N_2O emissions was negligible in this study due to the continuous flooding adopted across the three cropping systems. Although there have been conflicting reports regarding the effects of straw application on N_2O emissions [31], similar findings in this study have been reported by Li et al. [32], who showed that straw application under the premise of optimal fertilizer application tended to reduce N_2O emissions. The average cumulative N_2O emissions in the three rice-based cropping systems differ significantly. This was due to N application rates at each cropping system, which plays a dominant role in the nitrification (loss of N as N_2O) and denitrification processes (reduction of NO_3 to N_2), culminating in cumulative N_2O emission generation [33]. While quite a few studies have checked the N_2O emission differences amongst the three rice-based cropping systems of China, many studies have linked an increase in N_2O emissions to fertilizer application rates [34], tillage management [35], and variations in precipitation [36]. In this study, N_2O emissions from the rice-wheat cropping system are higher than those from the double rice and single rice cropping systems. This could be attributed to the N application rate, as earlier stated in Table 1, corroborating the study from Millar et al. [37], who showed an exponential increase in N_2O emissions due to fertilizer application.

Biogenic CH_4 emissions contribute largely to the total GHG emissions in rice paddy fields. Significant differences between the cropping systems have been reported. The CH_4 emissions in the double rice cropping system were higher than those in the single rice cropping system [38]. This is possibly due to increased temperature, enhanced photosynthetic production, and crop residue, which act as substrates for methanogens [39]. In this study, the double rice cropping system dominates the total GHG emissions among the three cropping systems. In addition, the synergistic effect of straw and N applications significantly stimulated total GHG gas emissions. This has been largely attributed to the regulatory effect of N on crop straw, which contributes to a substantial increase in CH_4, a prominent contributor to total GHG gas emissions in paddy fields [7]. The cumulative CH_4 emissions from the straw-incorporated N treatments contributed more to the increased total GHG emissions in the rice-based cropping systems. However, the higher rice yield between SN1 and SN2 compensated for this, leading to lower yield-scaled emissions. Therefore, obtaining straw incorporated with an N fertilization threshold of N2 is crucial for minimizing environmental impacts and maintaining rice yield.

This study's significant decrease in yield-scaled emissions at the single rice cropping system could be attributed to a significant increase in rice yield under straw incorporation with N application, corroborating the reports from Jiang et al. [40]. This indicates that the single rice cropping system has the exploitable potential to increase rice yield and the capacity to ensure straw incorporation at a reduced environmental cost. Moreover, this will reduce the field cost of straw management by farmers within the cropping cycle's short time frame and increase farmers' crop yield and income. In addition, straw incorporation has also been recommended as a cost-effective option for farmers when incorporated about 30 days before crop establishment [41]. Meanwhile, the higher yield-scaled emission observed in the double rice cropping system relative to the single rice and rice-wheat cropping systems underscores the imperative for meticulous consideration of the factors that significantly influenced the CH_4 emission in the double rice cropping system. This emphasis is essential for adopting a forward-thinking approach to climate change mitigation while concurrently improving rice yield in the context of a double rice cropping system and a rice-wheat cropping system. Moreover, since the promotion of straw incorporation and nitrogen has a wide range of potentials, Li et al. [32] have suggested that there is a need to pay more attention to the intrinsic mechanistic links between management practices and greenhouse gas emissions, particularly in double rice cropping systems.

A positive correlation between cumulative CH_4 emissions and methanogenic abundance has been documented [42–44]. These are suggestive of the different rates of N and straw incorporation rates, which could allow methanogens to build up for the generation of CH_4 (Figure 5). According to Jiang et al. [17], the non-significant straw effect on methanotrophs in SR and RW could be linked to an increased response of CH_4 oxidation rates over time in soils that previously received straw. Meanwhile, a significant effect of straw and nitrogen on the methanotrophs in DR is suggestive of improved O_2 transport into the rice rhizosphere. Other factors like differences in irrigation regimes, temperature and rainfall patterns, N application rates, increased microbial consumption, and root exudates might have contributed to CH_4 emissions aside from the methanogens across the three rice-based cropping systems [38,45,46]. There are some limitations in this study that could be addressed in future research. For instance, the expenses incurred by farmers for managing straw incorporation, and the effects on farmers' income. Furthermore, the impact of straw incorporation and nitrogen management on carbon sequestration and net carbon emissions also needs to be explored in rice-based cropping systems in the future.



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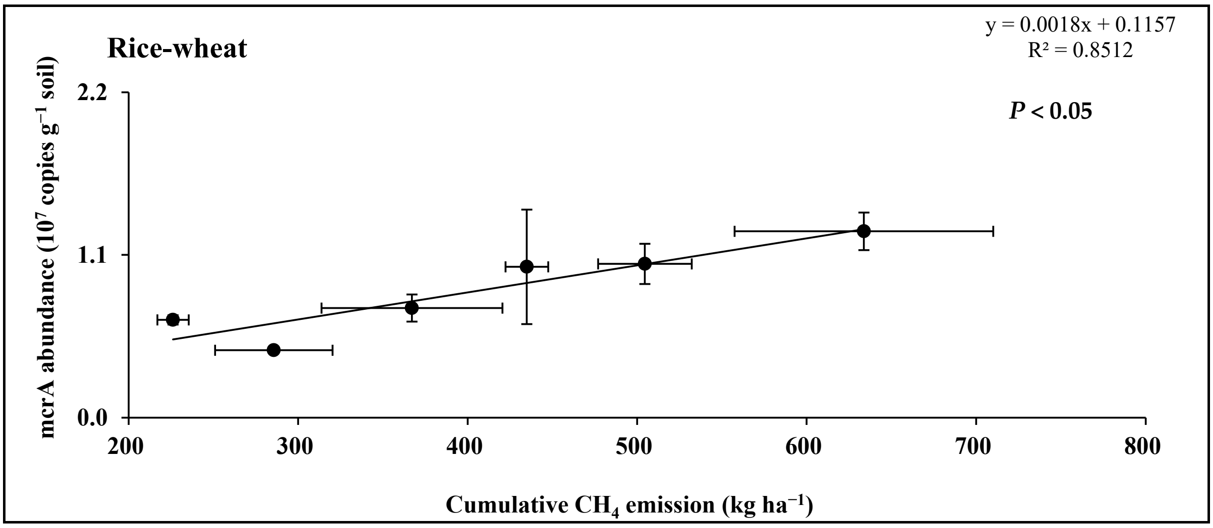

Figure 5. The correlation analysis of methanogens and CH_4 emissions in the single rice, double rice, and rice-wheat cropping systems. *mcrA*: methyl coenzyme M reductase. Bars indicate the standard error of each value from *mcrA* and Cumulative CH_4 emissions.

5. Conclusions

Straw incorporation significantly increased the cumulative CH_4 emissions and yield-scaled emissions in rice-based cropping systems. However, the effects of straw incorporation on cumulative CH_4 and total GHG emissions in the double rice cropping system were significantly higher than those of the single rice and rice-wheat cropping systems. Under straw incorporation, the N application rate significantly increased the average rice yield but reduced the cumulative CH_4 in single rice and rice-wheat cropping systems, resulting from lower methanogen abundance, but did not have a significant effect in the double rice cropping system. This study could provide scientific evidence that straw incorporation into rice-based cropping systems with moderate nitrogen management would benefit rice yields and lower greenhouse gas emissions. Further research on the long-term effects of nitrogen application and straw incorporation should be addressed on net carbon emissions in rice-based cropping systems in the future.

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Data Availability Statement: Data are contained within the article.

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

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