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Original Research

Partial Substitution of Synthetic Nitrogen Fertilizer with Pig Manure in Karst Paddy Fields of Guizhou, China: Trade-offs among Yield, Nitrogen Recovery Efficiency, and Greenhouse Gas Intensity

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Abstract

Soil degradation and greenhouse gas emissions caused by excessive application of synthetic nitrogen fertilizers severely constrain the sustainable development of rice agriculture in karst mountainous regions. To identify an appropriate organic fertilizer substitution ratio that balances food security and environmental benefits, a randomized block experiment was conducted in karst paddy fields of Guizhou, China. Five treatments were established in total: a zero-N control (CK), 100% synthetic nitrogen fertilizer (CF), and three substitution treatments in which 20%, 40%, and 60% of synthetic nitrogen fertilizer was replaced by pig manure (designated OM20, OM40, and OM60, respectively). Soil health indicators, rice yield, nitrogen recovery efficiency (NRE), and CH_4 and N_2O emission fluxes were systematically measured. The results showed that the OM20 treatment achieved the numerically highest grain yield and the significantly highest aboveground NRE (45.2%), although this yield difference was not statistically significant. Cumulative CH_4 emissions increased significantly with increasing substitution ratios, while cumulative N_2O emissions decreased significantly. Greenhouse gas intensity (GHGI) assessment showed that the GHGI of OM20 did not differ significantly from that of CF, whereas the GHGI values of OM40 and OM60 were significantly higher by 21.8% and 37.2%, respectively. In summary, under the conditions of this single-season experiment, among the 0%-60% pig manure substitution gradients tested, 20% substitution of synthetic nitrogen fertilizer with pig manure exhibited the optimal overall performance. It could maintain a relatively low greenhouse gas intensity while achieving a high rice yield and nitrogen recovery efficiency. It should be noted that these findings are based on a single growing season of field observation, and the long-term effects of continuous pig manure application require further multi-year verification.

Keywords: pig manure substitution, karst paddy fields, rice yield, nitrogen recovery efficiency, greenhouse gas intensity, comprehensive evaluation

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Introduction

The long-term excessive application of synthetic nitrogen fertilizers has led to a series of ecological and environmental problems, including accelerated soil acidification in global agricultural lands, decreased soil organic matter content, nutrient imbalances, and increased greenhouse gas emissions, severely constraining the sustainable development of agriculture [1, 2]. As one of the world's most important food crops, rice (*Oryza sativa L.*) is both the foundation of food security and a major source of agricultural greenhouse gas emissions [3-5]. It is estimated that, using IPCC AR5 century-scale global warming potential (GWP) factors [5], methane (CH_4) accounts for 50%-80% of total greenhouse gas emissions from rice cropping systems. Accurate quantification of paddy field greenhouse gas emissions under different fertilization regimes is a critical prerequisite for formulating agricultural climate change mitigation strategies in China [6]. In single-cropping rice systems, N_2O emissions are primarily concentrated in short windows following dry-wet alternation and nitrogen fertilizer application [3, 7, 8]. In China, the nitrogen recovery efficiency (NRE) of paddy fields is only 30%-42% [9], far below the 52%-68% levels in developed countries [10]. Excessive nitrogen application not only wastes resources but also causes a cascade of environmental problems such as aquatic eutrophication and air pollution [11]. The karst mountainous regions of Southwest China have infertile soils and fragile ecosystems [12-14]. In these areas, soil degradation and non-point source pollution caused by excessive synthetic fertilizer use are particularly severe. The karst paddy ecosystems in Southwest China differ notably from those in plain rice regions in soil properties and biogeochemical processes. Developed from limestone parent material, karst paddy soils in this region have undergone long-term paddy cultivation and excessive synthetic fertilization, resulting in severe acidification and significant leaching of carbonate minerals. They are characterized by inherently low organic carbon storage, weak nutrient retention capacity and high vulnerability to further acidification [12, 14]. Compared with plain paddy soils dominated by iron-aluminum oxides, these acidified karst paddy soils have distinct clay mineral compositions and organic carbon turnover patterns, leading to different responses to organic fertilizer inputs.

Moreover, the relatively low annual temperature in mountainous karst areas slows organic matter mineralization and alters nitrogen transformation pathways, shaping unique microbial community structures and activities and leading to distinct greenhouse gas emission patterns compared with plain paddy soils [15]. Nevertheless, most existing studies on organic substitution have been conducted in plain rice regions, and systematic data on yield-environment trade-offs in karst paddy fields remain scarce [16]. In ecologically fragile karst areas, land quality is a core factor restricting agricultural production efficiency [17], and optimized fertilization strategies are crucial for improving land productivity while reducing environmental risks. In these areas, soil degradation and non-point source pollution caused by excessive synthetic fertilizer use are particularly severe, highlighting an urgent need for region-specific environmentally friendly fertilization technologies [18, 19].

In recent years, partial substitution of synthetic nitrogen fertilizers with organic fertilizers has been widely recognized as a key strategy for coordinating the trade-off between crop yield improvement and environmental protection [7, 20]. Numerous studies have demonstrated that combined organic-inorganic fertilization can significantly increase soil organic carbon content, improve soil physicochemical properties, and enhance microbial diversity and enzyme activities, while maintaining or even increasing crop yields [21]. Meta-analyses indicate that replacing synthetic nitrogen fertilizers with animal manure can increase grain yields of wheat, maize, and rice in China by 3.3%-3.9% and improve nitrogen recovery efficiency by 6.3%-10.0% [10]. Appropriate organic substitution ratios can reduce environmental risks such as greenhouse gas emissions and nutrient leaching while ensuring crop yields [22]. Long-term field experiments have shown that continuous multi-year organic substitution can increase yields of crops such as rice and maize by 3%-10%, while increasing soil organic carbon by 10%-30% and total soil nitrogen content by 5%-20% [23].

Although organic substitution strategies are widely advocated, significant controversy persists regarding the determination of optimal substitution ratios, and their effects vary significantly across different crop types and soil conditions. In paddy field systems, the effects of high-proportion organic substitution are particularly complex. On the one hand, organic fertilizer application provides an abundant carbon source for methanogens, increasing the risk of CH_4 emissions; on the other hand, organic fertilizers may suppress CH_4 production by improving soil redox potential and regulating methanotrophic bacterial communities [24]. Studies have shown that moderate organic substitution can increase cumulative CH_4 emissions from paddy fields by 44.8%; conversely, other studies have indicated that high-proportion substitution does not result in additional CH_4 emissions, but rather enhances soil CH_4 oxidation capacity by improving soil aeration [25]. In double-cropping rice systems, 50% substitution of synthetic nitrogen fertilizer with pig manure leads to significant increases in CH_4 emissions and GWP; however, lime application can reduce CH_4 emissions by more than 36% without significantly affecting crop yield [26].

The impact of organic substitution on N_2O emissions is equally complex. Combined organic-synthetic fertilization can significantly reduce N_2O emissions by promoting soil organic nitrogen sequestration, while simultaneously increasing rice yields and grain protein content [27]. However, the effects of different substitution ratios show a nonlinear response [28].

Studies on karst paddy fields in Southwest China indicate that reducing synthetic nitrogen input by 20%-40% combined with appropriate organic substitution can significantly enhance soil microbial activity and nutrient supply capacity, thereby maintaining the sustainability of rice production in ecologically sensitive areas [15, 16].

Notably, existing studies have largely focused on single crop species or individual environmental indicators, and comprehensive trade-off assessments integrating yield, nitrogen recovery efficiency, and greenhouse gas emissions remain limited [29, 30]. In particular, the variation patterns of "carbon footprint per unit yield" (greenhouse gas intensity, GHGI) under different substitution ratios warrant further elucidation [2]. Furthermore, systematic empirical studies on the multidimensional regulatory mechanisms underlying the effects of organic substitution on soil–crop–microbial–climate interactions are still lacking [16].

Based on the current state of research, we conducted a field experiment at the Jiuzhou Rice Science and Technology Demonstration Village, Taijiang County. The experiment included one zero-N control (CK) and four iso-nitrogen treatments with 0%, 20%, 40%, and 60% of synthetic nitrogen fertilizer substituted by pig manure to systematically investigate the effects of different fertilization regimes on rice yield, nitrogen recovery efficiency, soil health indicators, and greenhouse gas (CH_4 and N_2O) emissions. We propose the following three hypotheses: (1) A moderate organic substitution ratio (20%-40%) can improve nitrogen recovery efficiency (NRE) while maintaining or increasing rice yield. This hypothesis is proposed based on previous meta-analyses showing that the optimal substitution ratio for paddy fields across China is generally around 30% at the national scale [10]. Considering the lower annual temperature in karst mountainous areas, which slows organic nitrogen mineralization, we infer that the locally optimal ratio may be slightly lower than the national average, and thus the 20%-40% range is expected to achieve the best balance between yield benefit and environmental performance; (2) CH_4 emissions will increase with increasing organic substitution ratio, whereas N_2O emissions will decrease; (3) A comprehensive evaluation integrating grain yield, nitrogen recovery efficiency and greenhouse gas intensity can identify an optimal substitution ratio that achieves superior overall benefits, balancing food security and environmental performance. The results of this study will provide a scientific basis for optimizing low-carbon and high-yield fertilization technologies in karst mountainous paddy fields.

Materials and Methods

Experimental Site

This experiment was conducted in 2025 at the Jiuzhou Rice Science and Technology Demonstration Village in Shidong Town, Taijiang County, Qiandongnan Miao and Dong Autonomous Prefecture, Guizhou Province. The experimental site is located in a typical karst mountainous area in Southwestern China (26°32′N, 108°18′E) at an elevation of 556 m. The region has a subtropical monsoon climate, with an average annual temperature of approximately 16ºC and an average annual precipitation of approximately 1200 mm, primarily concentrated during the growing season from April to September. Prior to the experiment, the basic physicochemical properties of the plow layer soil (0-20 cm) were as follows: pH (H_2O) 5.2, organic carbon 14.8 g kg^{-1}, total nitrogen 1.5 g kg^{-1}, available phosphorus (Olsen-P) 10.2 mg kg^{-1}, and available potassium (NH_4OAc-K) 58.3 mg kg^{-1}. The soil was classified as a paddy soil derived from limestone parent material, with a clay loam texture.

Experimental Design

A randomized block design was adopted for this experiment, consisting of five treatments with three replicates per treatment. Each plot had an area of 25 m² (5 m × 5 m). Plastic film was laid along the inner sides of the plot bunds, buried 20 cm below the soil surface and extending 30 cm above the field surface, to prevent cross-contamination of water and nutrients between adjacent plots. The three blocks were arranged along the fertility gradient of the experimental field, and the five treatments were randomly assigned within each block.

This randomized block design was adopted specifically to control the inherent spatial heterogeneity of karst paddy soils. Pre-experiment soil testing confirmed that there were significant gradients in basic soil fertility (organic carbon and total nitrogen) among the three blocks, verifying that the block design effectively captured the background spatial variation and reduced its interference with treatment effects.

Three biological replicates per treatment are the standard and widely accepted setup for paddy field plot experiments, and this setup has been commonly used in previous field studies in similar karst rice regions [15, 16]. Combined with the block effect included in our statistical model, this setup provides sufficient statistical power for the analysis of variance.

Nevertheless, given the higher soil heterogeneity in karst areas compared with plain paddy fields, increasing the number of replicates in future long-term studies could further improve the stability and robustness of the results.

The rice cultivar used was the locally dominant cultivar "Zhongzheyou 8" (*Oryza sativa* L. cv. Zhongzheyou 8).

The total nitrogen application rate for all nitrogen-amended treatments (excluding CK) was 150 kg N ha^{-1}. This rate was selected to represent the conventional fertilization level for single-cropping rice in karst paddy regions of Guizhou Province. It falls within the recommended nitrogen application range for

medium-fertility paddy soils according to local soil testing and fertilizer recommendation guidelines, and is consistent with the application rates used in previous field studies in similar karst rice-growing areas [15, 16]. The specific treatments were as follows:

CK: No nitrogen fertilizer applied; phosphorus and potassium fertilizer rates were identical to those in all other treatments.

CF: 100% of nitrogen supplied as synthetic nitrogen fertilizer (urea).

OM20: 20% of nitrogen supplied as pig manure, 80% as synthetic nitrogen fertilizer (urea).

OM40: 40% of nitrogen supplied as pig manure, 60% as synthetic nitrogen fertilizer (urea).

OM60: 60% of nitrogen supplied as pig manure, 40% as synthetic nitrogen fertilizer (urea).

The organic fertilizer consisted of pig manure collected from a local large-scale pig farm, which was thoroughly composted via windrow aerobic composting for approximately 90 days. After maturation, the material was dark brown and odorless, with a C/N ratio of 17.5 and a seed germination index (GI) of 87.3%. It complied with the Chinese National Standard for Organic Fertilizers (NY 525-2021). The measured concentrations of major heavy metals in the composted pig manure were as follows: arsenic (As) 2.3 mg kg^{-1}, cadmium (Cd) 0.21 mg kg^{-1}, chromium (Cr) 12.5 mg kg^{-1}, lead (Pb) 11.2 mg kg^{-1}, and mercury (Hg) 0.08 mg kg^{-1}. All values were well below the maximum permissible limits specified in the standard. Its basic nutrient content was as follows: N 20 g kg^{-1}, P_2O_5 15 g kg^{-1}, K_2O 12 g kg^{-1}, and organic carbon 350 g kg^{-1}. Nitrogen in the composted pig manure was dominated by organic nitrogen, accounting for approximately 76% of total nitrogen, with the remaining fraction mainly present as ammonium nitrogen. This morphological composition determines the slow-release characteristics of manure nitrogen, and its mineralization rate is highly sensitive to ambient temperature.

The total application rates of phosphorus and potassium were kept consistent across all treatments at 75 kg ha^{-1} P_2O_5 and 90 kg ha^{-1} K_2O, respectively. Phosphorus and potassium contributed by the organic fertilizer were counted toward the total amounts, and deficits were supplemented using calcium magnesium phosphate (12% P_2O_5) and potassium chloride (60% K_2O), respectively. The synthetic nitrogen fertilizer used was urea (46% N).

All organic fertilizer, calcium magnesium phosphate, and potassium chloride were uniformly broadcast as basal fertilizers one day before transplanting and immediately incorporated into the 0-20 cm soil layer via rotary tillage. Synthetic nitrogen fertilizer (urea) was applied in three splits: 40% as basal fertilizer, applied simultaneously with organic fertilizer one day before transplanting, 30% as tillering fertilizer (7 days after transplanting), and 30% as panicle fertilizer (35 days after transplanting, at the second-to-last leaf stage). Immediately after each application, irrigation was applied. The overall experimental procedure of this study is shown in Fig. 1.

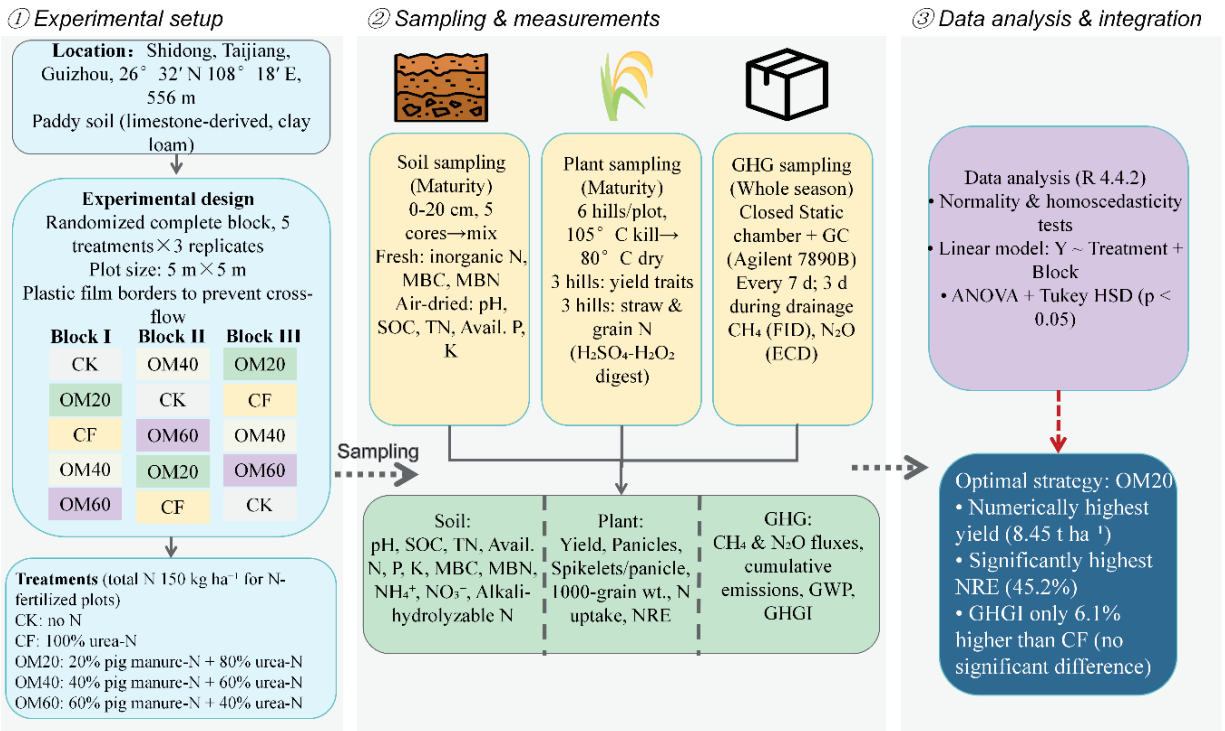


Fig. 1. Experimental design and technical flowchart.

Field Management and Sample Collection

Water management for all treatments followed the typical intermittent irrigation regime in the local area: a water depth of 2-4 cm was maintained from transplanting until the end of the tillering stage, followed by natural drainage and field drying, and then by alternating wet and dry irrigation until water was withheld one week before harvest. Rice was transplanted on 26 May 2025 and harvested on 28 September 2025, with a total growth period of 125 days. The row and hill spacing was 30 cm × 15 cm, with 2-3 seedlings per hill. Drainage and field drying were initiated when the tiller number in the field reached approximately 2.4 million tillers ha^{-1} (roughly 80% of the expected effective panicle number). Irrigation was resumed when 1-2 cm cracks appeared on the field surface and no footprint was left upon walking. Subsequently, alternating wet and dry irrigation was implemented. Other field management practices were conducted according to local standard practices.

At rice maturity, soil samples from the 0-20 cm plow layer were collected at five points arranged in an "S" shape across each plot and homogenized into one composite sample per plot. Each sample was split into two subsamples. One fresh subsample was sieved through a 2 mm mesh and stored at 4ºC, and analyzed for inorganic nitrogen, microbial biomass carbon and microbial biomass nitrogen within 7 days. The other subsample was air-dried, ground and sieved through a 0.15 mm mesh for determination of organic carbon, total nitrogen and other soil properties. Concurrently, six representative hills were randomly selected from each plot. All samples were oven-dried at 105ºC for 30 min to inactivate enzymes, then dried at 80ºC to a constant weight. Three of these hills were used for crop yield component analysis, while the remaining three were separated into straw and grain; after weighing, they were ground for nitrogen content analysis. One border row was removed from each side of every plot as a buffer zone, and the six sampled hills were also excluded. A 2.5 m × 2.0 m (5 m^2) harvest area was delineated in the center of the remaining plot area; all plants in this area were harvested, threshed, air-dried, and weighed. Grain moisture content was determined using the 105ºC oven-drying method, and grain yields were adjusted to a standard moisture content of 13.5%.

Sample Analysis and Indicator Determination

Soil physicochemical properties: Soil pH was measured with a pH meter (Sartorius PB-10, Germany) at a soil-to-water ratio of 1:2.5. Soil organic carbon was determined using the potassium dichromate oxidation-external heating digestion method. Total soil nitrogen was measured using a Kjeldahl nitrogen analyzer (FOSS Kjeltec 8400, Denmark). Soil alkaline-hydrolyzable nitrogen was determined using the 1.0 M NaOH alkaline hydrolysis diffusion method (incubated at 40ºC for 24 h). Soil available phosphorus was extracted with 0.5 M $NaHCO_3$ (pH 8.5) (shaken at 25ºC and 180 rpm for 30 min) and determined using the molybdenum-antimony colorimetric method. Soil available potassium was extracted with 1 M NH_4OAc (pH 7.0) (shaken at 25ºC and 180 rpm for 30 min) and determined by flame photometry [31, 32].

Soil inorganic nitrogen and microbial biomass carbon and nitrogen: Fresh soil samples were extracted with 2 M KCl (soil-to-liquid ratio 1:5, shaken at 25ºC and 180 rpm for 1 h), and NH_4^+-N and NO_3^--N concentrations were determined using a continuous flow analyzer (AA3, Seal Analytical, Germany). Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined using the chloroform fumigation–K_2SO_4 extraction method [33, 34]: Fresh soil was subjected to chloroform fumigation in the dark at 25ºC for 24 h, followed by extraction with 0.5 M K_2SO_4 (soil-to-liquid ratio 1:4, shaken at 25ºC and 180 rpm for 30 min). Organic carbon in the extract was determined using a TOC analyzer (Elementar vario TOC, Germany), and total nitrogen was determined using a Kjeldahl nitrogen analyzer. The calculation equations were as follows: MBC = EC/0.45, MBN = EN/0.54, where EC and EN are the differences between fumigated and unfumigated samples.

Yield and yield components: After recording the number of effective panicles in the sampled plants, grains were manually threshed to separate filled and unfilled grains. The total number of grains per panicle and the number of filled grains were counted separately, and the seed-setting rate (number of filled grains / total number of grains) was calculated [35]. Filled grains were dried at 80ºC to a constant weight for the determination of thousand-grain weight. Based on the planting density (30 cm row spacing × 15 cm hill spacing, 222,200 hills ha^{-1}), the number of effective panicles per hill was converted to the number of effective panicles per unit area ($\times 10^4$ panicles ha^{-1}). Theoretical yield (kg ha^{-1}) = Number of effective panicles ($\times 10^4$ panicles ha^{-1}) × Total number of grains per panicle × Seed-setting rate × Thousand-grain weight (g) $\times 10^{-2}$.

Plant nitrogen content and nitrogen recovery efficiency: Straw and grain samples at maturity were dried and ground, then digested with H_2SO_4-H_2O_2 (heated to 350ºC and maintained until the solution became clear and transparent, approximately 2 h). Total nitrogen content was determined using a Kjeldahl nitrogen analyzer. Straw nitrogen accumulation (kg N ha^{-1}) and grain nitrogen accumulation (kg N ha^{-1}) were calculated as the dry matter yield of the respective organ multiplied by its nitrogen content, then converted to per hectare values based on planting density. Total plant nitrogen accumulation (kg N ha^{-1}) = Straw nitrogen accumulation + Grain nitrogen accumulation. In this study, total plant nitrogen accumulation included only aboveground parts (straw + grain) and excluded roots. Therefore, the calculated nitrogen recovery efficiency (NRE) represented the aboveground nitrogen recovery efficiency.

This calculation approach is widely used in paddy field experiments. Complete root sampling under flooded field conditions is technically difficult and introduces large measurement uncertainty, making aboveground-based NRE the standard and comparable indicator in most regional agronomic studies.

In terms of absolute values, aboveground-only NRE is generally 10%-15% lower than whole-plant NRE, as root nitrogen accumulation accounts for approximately 10%-15% of total plant nitrogen uptake at rice maturity.

Nevertheless, since the same calculation method was consistently applied across all treatments, the relative differences in NRE among treatments and the overall variation trend are fully reliable. This methodological consistency ensures that the comparison of nitrogen utilization efficiency among different substitution ratios is valid.

The equation for nitrogen recovery efficiency (NRE, %) was [27, 35]: NRE (%) = (Total plant nitrogen accumulation in N-applied treatment − Total plant nitrogen accumulation in zero-N control) / Nitrogen application rate × 100%.

Greenhouse gas sampling was performed at 7-day intervals starting one day after rice transplanting. The sampling frequency was increased to once every three days during field drying periods, and sampling continued until crop harvest. A total of 19 sampling campaigns were conducted across the entire rice growing season.

The sealed static chamber-gas chromatography method was adopted [8]. The static chamber system consisted of a stainless steel base (L × W × H = 50 cm × 50 cm × 20 cm) and an opaque PVC chamber (L × W × H = 50 cm × 50 cm × 120 cm). The base was buried 10 cm into the soil before rice transplanting and remained fixed throughout the entire rice growing season. A small 12 V DC fan (rotation speed 1200 rpm) was mounted on the top of the chamber to homogenize the internal air. The side walls were fitted with gas sampling ports sealed with silicone stoppers and connectors for PT100 temperature sensors. During sampling, the PVC chamber was placed over the base, and the joint was sealed with water.

Sampling was performed between 9:00 and 11:00 a.m. This time window was selected following the standard protocol for static chamber measurements in paddy field studies [8, 27]. Between 9:00 and 11:00 a.m., soil temperature and air temperature rise steadily and are close to the daily average value, and soil microbial activities as well as crop physiological processes remain relatively stable. Fluxes measured in this time window have been widely verified to be representative of the average daily emission rate, which ensures the scientific validity of cumulative greenhouse gas emissions calculated throughout the entire growing season. After the chamber was placed over the plants, gas samples were withdrawn from the chamber using an airtight syringe at 0, 15, 30 and 45 min, and then transferred into pre-evacuated 12 mL headspace vials. All samples were analyzed within 48 h. Concentrations of CH_4 and N_2O were determined using a gas chromatograph (Agilent 7890B, USA) [27]. CH_4 was quantified with a flame ionization detector (FID), and N_2O with an electron capture detector (ECD). A Porapak Q packed column (80/100 mesh, 2 m × 3 mm) was used. The column temperature was set to 60ºC, while the detector temperatures were 200ºC for FID and 300ºC for ECD. High-purity nitrogen was used as the carrier gas at a flow rate of 30 mL min^{-1}. During each sampling event, air temperature inside the static chamber, soil temperature at 5 cm and 10 cm depths, and volumetric soil water content in the 0-10 cm layer were monitored simultaneously with a portable TDR device (FieldScout TDR 350, USA). The ponding water depth near the chamber was measured at three random points using a steel ruler, and the average value was calculated.

Gas emission fluxes were calculated from the slope of the linear regression of concentration against time. Only flux data with a coefficient of determination (R^2) > 0.90 were retained for further analysis. A total of 285 flux data points were obtained in this study, among which 98.6% satisfied the criterion of R^2>0.90. Only four data points were excluded due to instrumental fluctuations, and missing values were replaced by the average of measurements at adjacent time points. Cumulative emissions of CH_4 and N_2O were calculated using the trapezoidal integration method. Specifically, the average flux between two consecutive sampling points was multiplied by the time interval (in days), and all results were summed to obtain the total cumulative emissions over the entire rice growing season [36].

Global Warming Potential (GWP) was calculated using the 100-year scale factors from IPCC AR5 [37]: GWP (kg CO_2-eq ha^{-1}) = cumulative CH_4 emissions × 28 + cumulative N_2O emissions × 265. Greenhouse Gas Intensity (GHGI, kg CO_2-eq kg^{-1} grain) = GWP / grain yield.

Data Analysis

All data were analyzed using R 4.4.2. First, data normality and homogeneity of variances were verified using the Shapiro–Wilk test and Levene's test, and natural logarithmic transformation was applied when necessary. Significant outliers were removed using the Grubbs test ($p<0.05$). Linear models accounting for block effects (Y ~ Treatment + Block) were fitted using the base stats package, and analysis of variance (ANOVA) was performed using the aov() function. Differences among treatments were analyzed by two-sided multiple comparisons using the Tukey HSD test in the agricolae package (v1.3-7) ($p<0.05$). All figures were plotted using the ggplot2 package (v3.5.1).

Results and Discussion

Soil Health Indicators

Pre-experiment soil testing was conducted before transplanting to verify the homogeneity of the experimental baseline. The initial basic physicochemical properties of the 0-20 cm plow layer soil were as follows: pH (H_2O) 5.2, soil organic carbon 14.8 g kg^{-1}, total nitrogen 1.5 g kg^{-1}, available phosphorus 10.2 mg kg^{-1}, and available potassium 58.3 mg kg^{-1}. Statistical analysis confirmed that there were no significant differences in any of the initial soil indicators among the five treatment groups, ensuring that the experimental baseline was consistent.

After one rice growing season, different fertilization treatments significantly altered soil chemical properties at rice maturity (Table 1). Compared with CK, sole synthetic fertilizer application (CF) exerted no significant effects on soil pH, total nitrogen or soil C/N ratio. Soil pH values of organic substitution treatments (OM20, OM40, OM60) were 0.03-0.10 units greater relative to CF; OM40 and OM60 exhibited significantly higher pH than CF ($p<0.05$), whereas OM20 did not differ significantly from CF. Total nitrogen increased gradually with rising organic substitution ratios. OM60 had the highest total nitrogen concentration (1.68 g kg^{-1}), which was significantly greater than CF (1.52 g kg^{-1}) and CK (1.48 g kg^{-1}). The soil C/N ratio increased with rising organic substitution ratios; OM60 showed a markedly higher C/N ratio than CF ($p<0.05$), whereas no significant differences were detected among the remaining treatments.

The response of soil organic carbon (SOC) to organic substitution ratios was more evident (Fig. 2). Compared with CF, SOC concentrations in OM20, OM40 and OM60 increased by 4.2%, 8.1% and 12.3%, respectively. SOC contents of OM40 and OM60 were significantly higher than those of CF and CK ($p<0.05$), suggesting that pig manure incorporation effectively improved the soil organic carbon pool.

Table 1. Initial and mature soil physicochemical properties under different fertilization treatments.

Treatment	pH (H_2O)	Total nitrogen (g kg^{-1})	C/N Ratio
Initial baseline	5.2	1.5	9.9
CK	5.18±0.05 bc	1.48±0.06 c	9.59±0.32 ab
CF	5.12±0.06 c	1.52±0.07 bc	9.54±0.35 b
OM20	5.15±0.05 abc	1.55±0.06 abc	9.74±0.38 ab
OM40	5.20±0.06 ab	1.62±0.07 ab	9.63±0.40 ab
OM60	5.22±0.07 a	1.68±0.08 a	9.64±0.42 a

Note: Different lowercase letters within the same column denote significant differences among treatments according to Tukey's HSD test at $p<0.05$. The initial baseline value represents the average soil properties before transplanting, and pre-experiment tests confirmed no significant differences among the plots (n = 15).

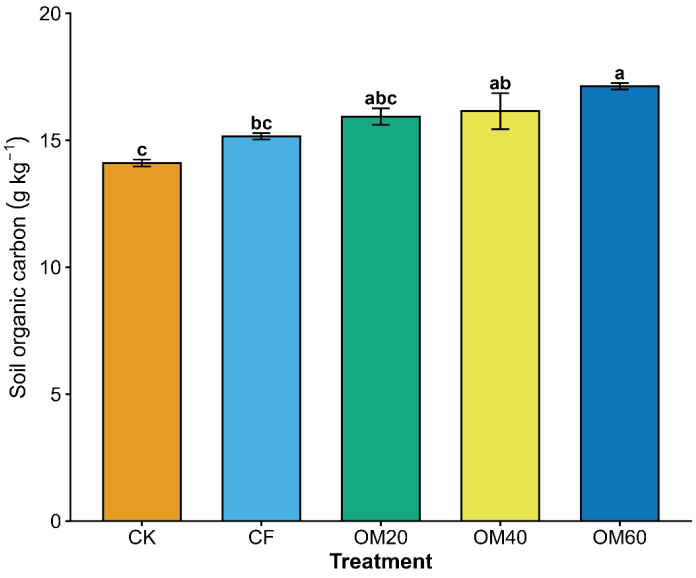


Fig. 2. Soil organic carbon content under different fertilization treatments.

The measured levels of soil inorganic nitrogen, microbial biomass carbon and nitrogen further corroborated the above variations (Fig. 3). Compared with CF, ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) in OM20 rose by 12.3% and 8.7%, respectively ($p<0.05$), whereas NO_3^--N in OM60 was markedly lower than that in OM20. Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) increased progressively with rising organic substitution ratios. OM60 exhibited significantly greater MBC and MBN than CF, with increments of 32.5% and 31.2%, respectively ($p<0.05$). The variation trend of alkaline-hydrolyzable nitrogen matched that of total nitrogen; OM60 had a significant 15.2% increase relative to CF ($p<0.05$). No significant inter-treatment differences were observed for soil available phosphorus and available potassium.

Yield and Yield Components

Grain yields of all N-amended treatments were significantly greater than that of the zero-N control (CK), with yield improvements ranging from 45.4% to 62.5% (Table 2, Fig. 4). Among the fertilized treatments, grain yield first increased and then declined with rising organic substitution ratios. OM20 produced the numerically maximum grain yield (8.45 t ha^{-1}), which was 4.1% higher than that of CF (8.12 t ha^{-1}), yet the difference was non-significant ($p>0.05$). OM40 (7.88 t ha^{-1}) had a comparable grain yield to CF, whereas OM60 (7.56 t ha^{-1}) had a significantly lower grain yield than CF and OM20 ($p<0.05$) and a higher grain yield only than CK. These results suggested that 20% organic substitution optimized grain yield, while 60% substitution caused significant yield loss owing to inadequate nitrogen availability.

Analysis of yield components explained the variation in grain yield (Table 2). Compared with CF, effective panicle numbers declined across all organic substitution treatments, and OM60 had significantly fewer effective panicles (228.7×10^4 ha^{-1}), a value 14.8% lower than that of CF. In contrast, grains per panicle increased markedly by 7.3% (OM20) and 4.4% (OM40) relative to CF ($p<0.05$). This improvement offset the moderate reduction in effective panicles and sustained grain yields at or above the CF level. No significant difference in grains per panicle was detected between OM60 and CF, but OM60 showed a 14.8% reduction in effective panicles ($p<0.05$) alongside a decreased seed-setting rate (84.5% vs. 87.6%). Collectively, these declines resulted in a significantly lower grain yield for OM60. Thousand-grain weight ranged narrowly from 25.8 g to 26.2 g with no inter-treatment differences, implying that thousand-grain weight was barely influenced by fertilization regimes. Theoretical yield followed a similar changing pattern to grain yield; OM20 achieved the numerically maximum theoretical yield (8397 kg ha^{-1}), consistent with its measured grain yield.

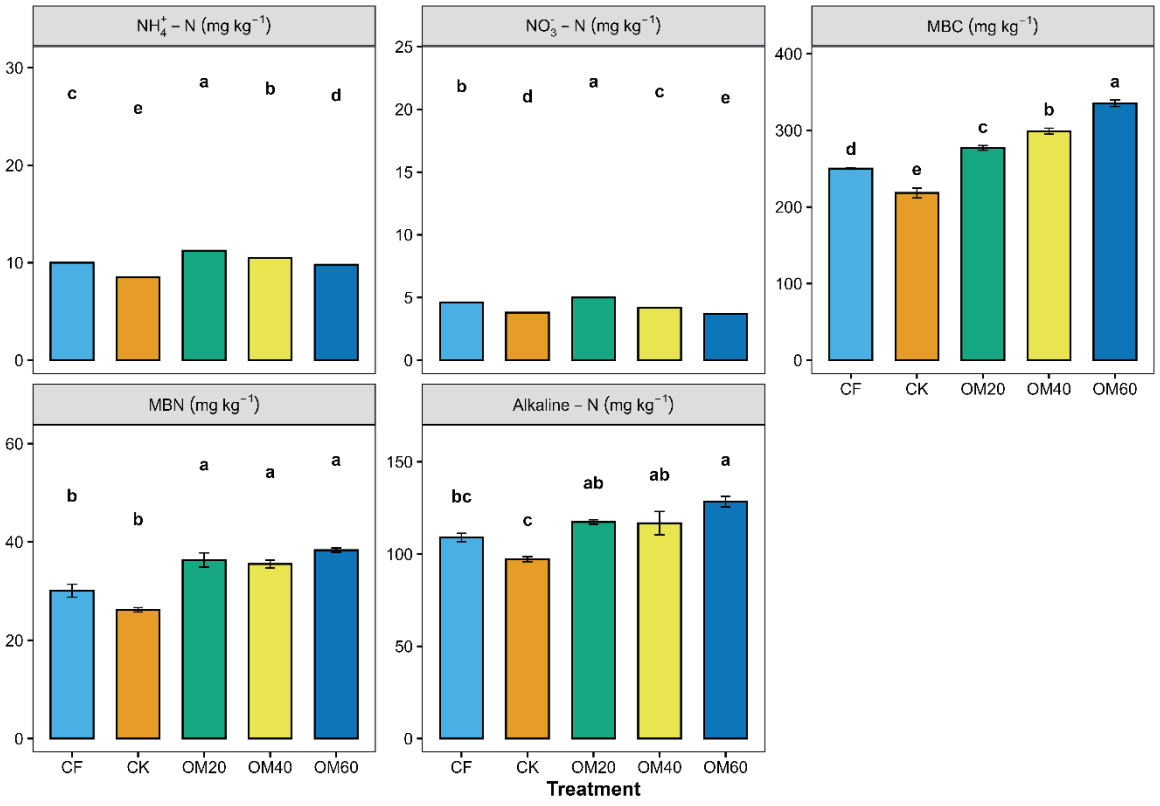


Fig. 3. Contents of soil inorganic nitrogen, alkaline-hydrolyzable nitrogen, MBC and MBN across fertilization treatments.

Table 2. Grain yield and yield components of rice under different fertilization treatments.

Treatment	Effective panicles ($\times 10^4$ ha^{-1})	Grains per panicle	Filled grains per panicle	Seed-setting rate (%)	1000-grain weight (g)	Theoretical yield (kg ha^{-1})
CK	185.2±8.4 d	108.5±5.2 c	89.3±4.7 c	82.3±1.8 a	25.9±0.4 a	4283
CF	268.4±10.1 a	132.6±6.1 b	116.2±6.0 b	87.6±2.1 ab	26.1±0.3 a	8138
OM20	255.8±9.7 ab	142.3±5.8 a	125.5±5.8 a	88.2±1.9 a	26.2±0.4 a	8411
OM40	242.3±8.9 bc	138.5±6.4 ab	120.6±6.4 ab	87.1±2.3 ab	25.9±0.3 a	7568
OM60	228.7±9.5 c	134.2±5.9 b	113.4±5.9 b	84.5±2.4 bc	25.8±0.5 a	6691

Note: Different lowercase letters within the same column denote significant differences among treatments according to Tukey's HSD test at $p<0.05$. Filled grains per panicle = total grains per panicle × seed-setting rate; standard deviation was calculated by error propagation. Theoretical yield = effective panicles × filled grains per panicle × 1000-grain weight × 0.01, calculated using the mean values of each treatment. All data are expressed as mean±standard deviation (n = 3).

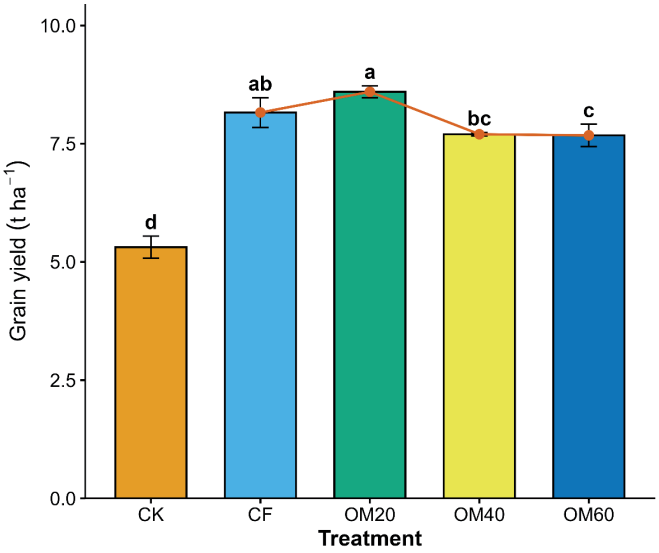


Fig. 4. Changes in grain yield along different organic substitution ratios.

The variations in theoretical and grain yields were generally consistent across treatments, further verifying the reliability of yield data. The discrepancy between theoretical and grain yields for OM60 was approximately 13%, which exceeded the discrepancies of other N-amended treatments (<5%). Such divergence was possibly induced by uneven organic manure incorporation, causing pronounced spatial variability within OM60 plots. In this study, pig manure was uniformly broadcast on the soil surface and then thoroughly incorporated into the 0-20 cm plow layer via two passes of rotary tillage, which follows the standard operational procedure for organic fertilizer application in paddy field experiments. However, complete homogenization of high-rate solid organic manure is technically difficult under field conditions, which is a common limitation in plot-scale fertilization trials. This spatial heterogeneity may also have increased the within-treatment variation of greenhouse gas flux data to some extent. Nevertheless, the static chamber bases were placed at representative positions in each plot, and three replicates were set for each treatment. The overall variation trends and significant differences in greenhouse gas emissions among treatments remain stable and reliable, and the core conclusions of this study are not affected. Consequently, the three sampled hills for yield component measurement presented fewer effective panicles and a lower seed-setting rate relative to the plot average. Since grain yield was determined via full-plot harvesting and weighing and was less prone to sampling bias, this study adopted grain yield as the core indicator for inter-treatment comparison. In addition, OM60 exhibited larger replicate-to-replicate variation in grain yield than the remaining treatments, further confirming obvious within-plot spatial heterogeneity.

Nitrogen Recovery Efficiency

Analysis of nitrogen recovery efficiency (NRE) further clarified the intrinsic mechanisms governing yield responses to organic substitution ratios (Fig. 5). OM20 attained the maximum NRE (45.2%),

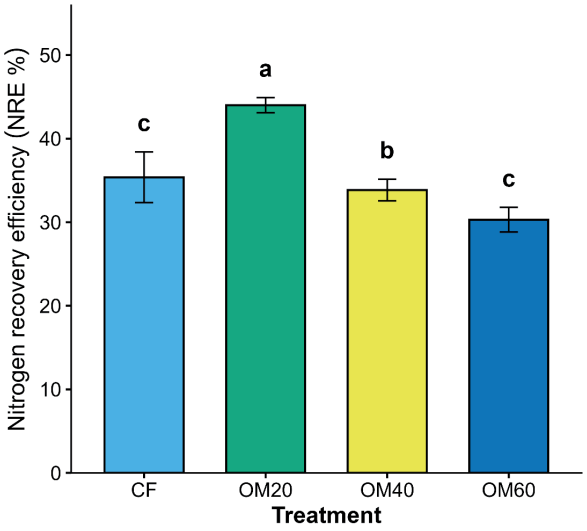


Fig. 5. Nitrogen recovery efficiency under different organic substitution ratios.

with a significant 34.5% improvement compared with CF (33.6%) ($p<0.05$). OM40 also had a significantly higher NRE (35.7%) than CF, while OM60 exhibited significantly lower NRE (29.3%) relative to OM20 and was statistically similar to CF. These results suggested that organic substitution at 20%-40% substantially promoted translocation of fertilizer N to aboveground rice tissues, whereas excessive substitution lowered seasonal available N due to the slow mineralization of organic-bound nitrogen.

Greenhouse Gas Emissions and GWP

Seasonal dynamics of CH_4 and N_2O fluxes differed markedly across the rice growing season (Fig. 6). CH_4 fluxes peaked at 20-30 days after transplanting (active

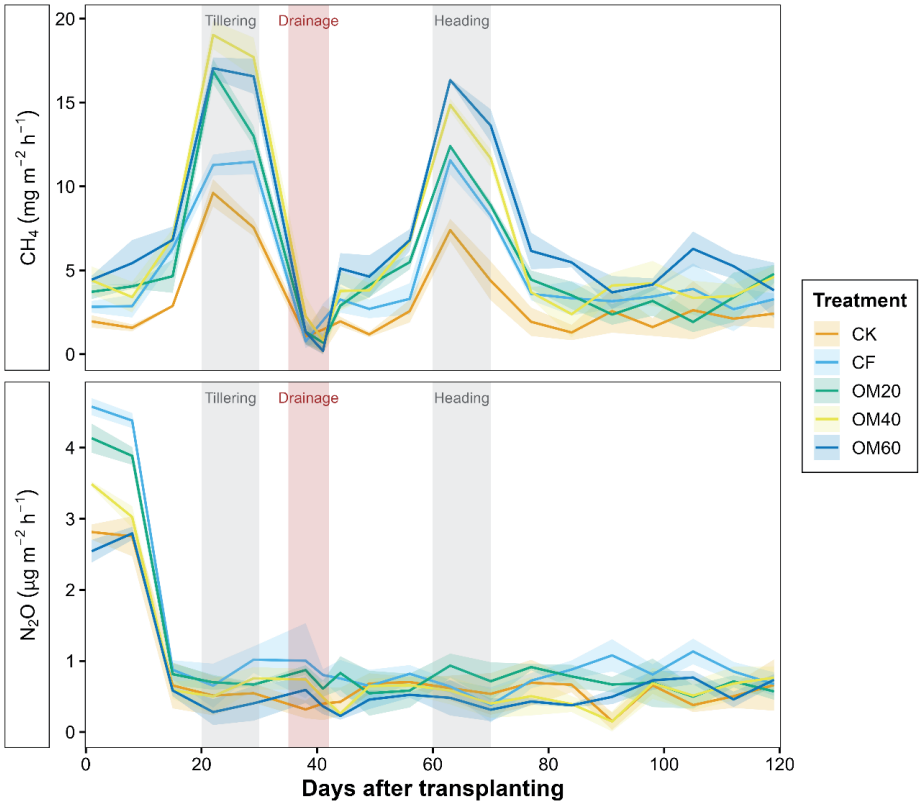


Fig. 6. Seasonal dynamics of CH_4 and N_2O fluxes under different organic substitution ratios.

tillering stage) and 60-70 days after transplanting (heading stage). Field drainage commenced at approximately 35 days after transplanting; CH_4 emissions declined sharply in all treatments during drainage and recovered rapidly following subsequent re-flooding. The maximum CH_4 flux occurred at the active tillering stage. The peak flux of the OM60 treatment was significantly higher than that of the CF treatment, while the peak flux of OM20 showed no significant difference from that of CF. The timing of the two CH_4 emission peaks was consistent across all treatments, indicating that the substitution ratio mainly affected the emission intensity rather than the temporal pattern. During the field drainage period, CH_4 fluxes in all treatments decreased sharply, confirming the strong regulatory effect of water management on methane emissions. The magnitude of the flux reduction was slightly larger in high-substitution treatments, suggesting that water management could partly offset the CH_4 increase caused by organic manure application. N_2O fluxes occurred as episodic pulses after N fertilization, especially after basal and tiller-stage fertilization. CF produced the maximum N_2O flux peaks, while organic substitution treatments had significantly lower and smoother peaks owing to the slow-release characteristic of organic-derived nitrogen.

Cumulative greenhouse gas emissions are summarized in Fig. 7 and Table 3. CK exhibited the lowest cumulative CH_4 emission (85.3 kg ha^{-1}), and N fertilization significantly stimulated CH_4 production. Among N-amended treatments, cumulative CH_4 emissions rose with increasing organic substitution ratios; OM60 emitted 168.5 kg ha^{-1} CH_4, which was 41.2% greater than CF ($p<0.05$). By contrast, cumulative N_2O emissions followed the reverse trend. CF had the maximum N_2O emission (2.85 kg ha^{-1}), whereas OM20, OM40 and OM60 decreased N_2O by 14.4%, 22.5% and 30.5% relative to CF, respectively ($p<0.05$).

Global warming potential (GWP) was collectively governed by cumulative CH_4 and N_2O emissions. Although organic substitution markedly lowered N_2O fluxes, elevated CH_4 emissions dominated variations in overall GWP. GWP rose significantly with increasing organic substitution ratios. OM60 yielded a GWP of 5243 kg CO_2-eq ha^{-1}, 27.9% greater than CF (4098 kg CO_2-eq ha^{-1}) ($p<0.05$). Similarly, OM20 and OM40

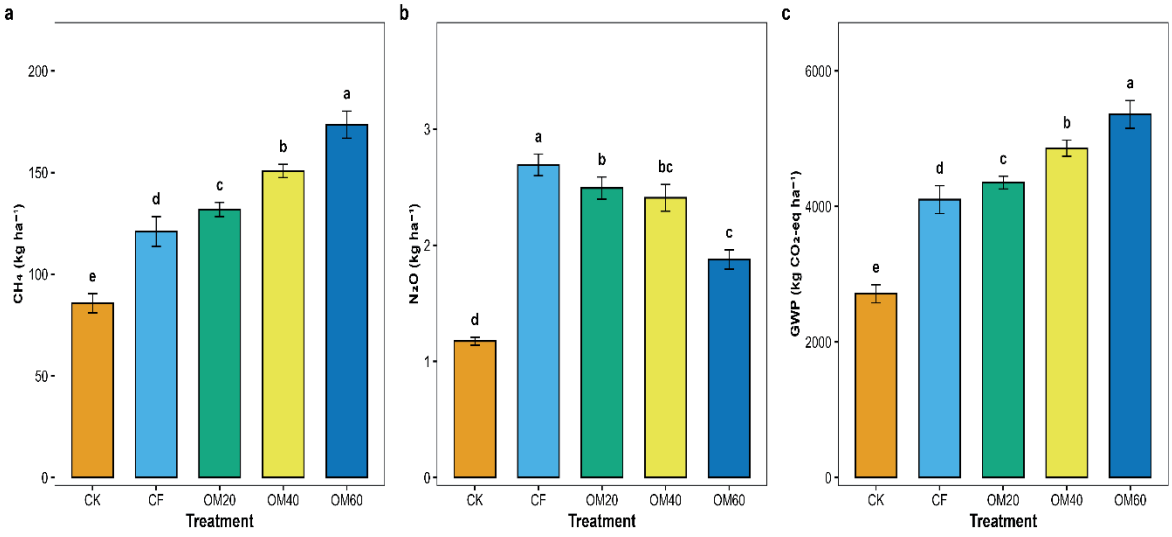


Fig. 7. Cumulative CH_4 and N_2O emissions and global warming potential across fertilization treatments.

Table 3. Cumulative greenhouse gas emissions and GWP under different fertilization treatments.

Treatment	Cumulative CH_4 (kg ha^{-1})	Cumulative N_2O (kg ha^{-1})	GWP (kg CO_2-eq ha^{-1})
CK	85.3±6.5 e	1.12±0.10 d	2686±202 e
CF	119.4±8.2 d	2.85±0.22 a	4098±298 d
OM20	138.6±9.5 c	2.44±0.18 b	4527±325 c
OM40	152.3±10.1 b	2.21±0.16 bc	4850±362 b
OM60	168.5±11.3 a	1.98±0.15 c	5243±385 a

Note: Different lowercase letters within the same column denote significant differences among treatments according to Tukey's HSD test at $p<0.05$.

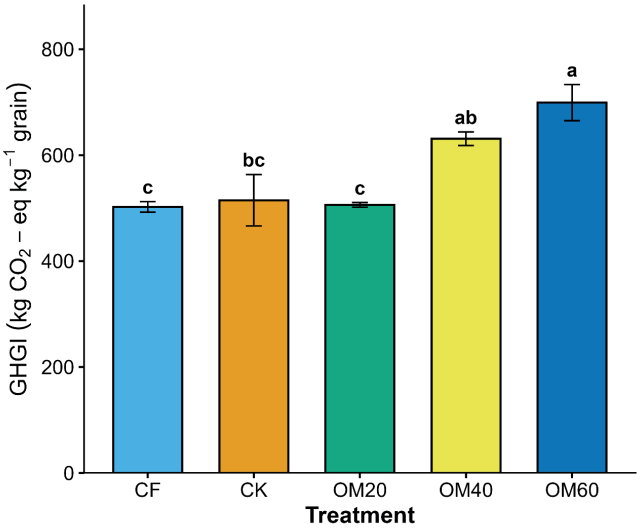


Fig. 8. Greenhouse gas intensity under different organic substitution ratios.

had 10.5% and 18.3% higher GWP relative to CF, respectively, and both differences were statistically significant. These findings suggested that despite improved soil carbon sequestration under organic substitution, the practice incurred higher aggregate greenhouse gas emissions.

Comprehensive Evaluation: GHGI and Optimal Substitution Ratio

After incorporating grain yield and environmental impacts into the calculation of greenhouse gas intensity (GHGI), obvious disparities were observed across treatments (Fig. 8). CF had a GHGI of 0.505 kg CO_2-eq kg^{-1} grain. OM20 produced the numerically maximum grain yield, leading to a non-significant difference in GHGI (0.536 kg CO_2-eq kg^{-1}) relative to CF. By contrast, OM40 and OM60 exhibited significantly higher GHGI values (0.615 and 0.693 kg CO_2-eq kg^{-1}, respectively) than CF and OM20. These results suggested that despite elevated total greenhouse gas emissions under OM20, yield improvement diluted its yield-scaled carbon footprint.

Although CF had the numerically minimum GHGI, its nitrogen recovery efficiency was markedly inferior to OM20, and grain yield was statistically comparable between the two treatments. OM20 had a GHGI of 0.536 kg CO_2-eq kg^{-1} grain, merely 6.1% above CF, while delivering the numerically maximum grain yield (8.45 t ha^{-1}) and the highest nitrogen recovery efficiency (45.2%). According to multiple comparison results, 20% organic substitution represented the optimal fertilization practice with comprehensive benefits within the tested substitution gradients in this single-season field experiment.

Overall, the above results demonstrated clear gradient responses of soil properties, rice yield, nitrogen recovery efficiency, and greenhouse gas emissions to increasing pig manure substitution ratios. In the following sections, we interpret these findings in combination with published studies, explore the underlying regulatory mechanisms, and further evaluate the comprehensive agronomic and environmental benefits of different fertilization regimes.

Effects of Organic Substitution on Soil Properties

The improvements in soil pH, SOC, and microbial biomass with rising manure substitution ratios are consistent with previous findings [36] and can be explained by the following mechanisms. Relative to sole synthetic nitrogen fertilization (CF), OM40 and OM60 significantly elevated soil pH by 0.08-0.10 units, attributable to abundant alkaline constituents (carbonates, organic acid salts) in manure counteracting soil acidification [21]. Paddy soils in karst mountain regions are intrinsically prone to acidification; long-term exclusive urea application accelerated soil acidification [27], whereas pig manure amendment alleviated such acidification trends to a certain degree [1]. Meanwhile, soil total nitrogen and soil organic carbon (SOC) gradually rose with increasing organic substitution ratios. SOC under OM60 was significantly higher (by 12.3%) than that under CF, owing to exogenous organic carbon inputs from pig manure [36]. The tested pig manure contained 350 g kg^{-1} carbon; even at the 20% substitution level, around 525 kg organic carbon was imported per hectare, laying the material basis for soil carbon pool accumulation.

Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) are sensitive indicators responding to organic substitution [21]. Relative to CF, OM60 increased MBC by 32.5% and MBN by 31.2%, with the rising amplitude obviously larger than that of SOC. These findings suggested that manure amendment enlarged soil carbon pools and, more crucially, boosted

soil microbial proliferation via readily degradable carbon substrates [38]. At maturity, OM60 exhibited significantly lower soil nitrate-N than OM20 and a markedly higher soil C/N ratio than CF. This discrepancy implied distinct soil N transformation patterns under high manure input. The pig manure used in this study had a C/N ratio of 17.5. High rates of manure application stimulated soil microbes to immobilize native mineral nitrogen to support organic carbon decomposition and microbial growth, thereby promoting net nitrogen retention in soil [34]. This microbial immobilization is pronounced during rice tillering: low ambient temperatures slow organic N mineralization, triggering fierce competition for mineral N between microbes and rice plants [29]. Such competition restricts available soil N at tillering and further limits the development of effective panicles.

Compared with results from paddy field studies in the middle and lower reaches of the Yangtze River Plain, the magnitude of SOC increase under pig manure substitution was relatively smaller in the present study. This difference is primarily associated with the mineral compositional characteristics of karst paddy soils derived from limestone. After long-term paddy cultivation and acidification, the clay mineral composition and aggregate structure of these soils are significantly different from plain paddy soils dominated by iron-aluminum oxides, leading to distinct organic carbon stabilization and turnover patterns, which further result in different SOC response amplitudes to exogenous organic manure input [39]. Furthermore, the increase in MBC was substantially higher than that in SOC in this study. This finding is consistent with the general pattern of short-term single-season organic fertilizer application: the labile carbon fractions in pig manure preferentially stimulate microbial growth and reproduction, whereas the accumulation of stable organic carbon requires longer periods of continuous application [40].

Nonlinear Response of Grain Yield to Organic Substitution Ratios

The parabolic yield response to increasing substitution ratios observed in this study aligns with the general pattern reported in national-scale meta-analyses [10, 22]. OM20 produced the numerically maximum grain yield (8.45 t ha^{-1}), which was statistically similar to CF; by contrast, OM60 significantly reduced grain yield relative to CF and OM20 ($p<0.05$). This finding partly verified the first hypothesis: moderate organic substitution (20%-40%) maintained grain yield comparable to synthetic nitrogen fertilization, and the 20% substitution level achieved numerically peak yield. Superior yield under OM20 mainly stemmed from a marked rise in grains per panicle (7.3% higher than CF, $p<0.05$), which offset the mild decline in effective panicle number. Synergistic N supply from organic and inorganic sources accounted for this beneficial effect [2, 27]. Urea supplied readily available mineral N to satisfy rice demands during tillering and panicle initiation, whereas sustained mineralization of pig manure guaranteed late-season N for grain filling, facilitating spikelet development and grain plumpness [35, 38].

However, grain yield decreased significantly when the substitution ratio increased to 60%. Yield-component analysis revealed that the yield loss of OM60 originated from a pronounced drop in effective panicles (14.8% lower than CF) alongside a reduced seed-setting rate. Organic N mineralization is highly susceptible to ambient temperature and soil moisture. In the cool karst mountain climate (mean annual temperature = 16ºC), the in-season mineralization efficiency of pig manure N is typically only 40%-60% [34]. For the OM60 treatment, 60% of total N was derived from pig manure, meaning approximately 36-54 kg N ha^{-1} remained immobilized in organic forms and cannot be used by rice within a single growing season. Such delayed N release strongly restricted rice tillering (7-35 days after transplanting), a critical period governing effective panicle formation and characterized by an intensive N requirement. It should be noted that this explanation is mainly inferred from yield component characteristics and published mineralization patterns, as soil inorganic nitrogen dynamics during key growth stages were not continuously monitored in this study. The significant reduction in effective panicles in OM60, which is highly sensitive to nitrogen supply during the tillering stage, provides indirect supporting evidence for this inference. The spatio-temporal mismatch between manure N mineralization and crop N demand frequently occurs under high organic replacement [10], suppressing tiller development and reducing effective panicle count by 14.8% relative to CF. This observation agrees with published findings: organic substitution exceeding 50% limits seasonal available N owing to slow organic N mineralization, diminishing yield benefits or triggering yield decline [22]. Accordingly, optimal organic substitution for cool karst paddy soils should fully account for the mineralization dynamics of pig manure-derived nitrogen. It should be noted that all pig manure was applied as a single basal dressing in this study, while synthetic nitrogen fertilizer was split into three applications. This fertilization regime follows the common farming practice in local karst rice regions, where organic manure is generally incorporated once before transplanting. This application pattern may have coupled with the substitution ratio effect and exacerbated the early-season nitrogen deficiency in high-substitution treatments to some extent.

At the national scale, the optimal ratio of pig manure substitution for synthetic nitrogen fertilizer in paddy fields is generally around 30% [10], whereas the 20% substitution ratio achieved the best overall performance in this study. This discrepancy is mainly driven by regional climatic conditions. The mean annual temperature in karst mountainous regions is only 16ºC, which is significantly lower than that in eastern

rice-growing areas. Low temperature markedly reduces the mineralization rate of nitrogen from organic fertilizers, resulting in insufficient nitrogen supply in the early growth stage under high-proportion substitution [41, 42]. This finding further indicates that there is no universally optimal ratio for organic fertilizer substitution. The substitution scheme must be locally adapted to regional temperature and soil types. The results of this study can provide preliminary empirical reference for karst rice-growing regions in Southwest China.

Mechanisms Underlying Improved Nitrogen Recovery Efficiency

OM20 achieved the maximum nitrogen recovery efficiency (NRE) of 45.2%, which was 34.5% higher than that of CF. OM40 also exhibited a markedly higher NRE (35.7%) than CF (33.6%). These findings fully validated the first hypothesis of this study. The improvement in NRE driven by organic substitution can be explained by three core mechanisms. First, pig manure amendment optimized soil physical conditions [1], facilitated rice root growth and development, and strengthened root nitrogen uptake capacity [35]. Second, organic substitution enhanced soil microbial activity, as evidenced by the significantly elevated MBC and MBN observed in this study [38]. Microbial metabolites (e.g., organic acids and extracellular polysaccharides) further promoted rhizosphere nitrogen activation and crop absorption [31]. Third, the spatio-temporal complementary N supply of organic and inorganic nitrogen reduced nitrogen loss pathways [7], allowing a greater proportion of fertilizer nitrogen to be absorbed and utilized by rice plants.

Notably, OM60 had a nitrogen recovery efficiency (NRE) of 29.3%, which was statistically comparable to CF but significantly lower than OM20. These results suggested that high-rate organic substitution caused an asynchronous N supply relative to rice nutrient demand. Early-season N shortage at tillering not only restricted effective panicle formation [34] but also suppressed total aboveground N uptake by rice [22]. In the present study, NRE was calculated from aboveground N accumulation. Although this approach tends to produce higher absolute values than conventional whole-plant calculations including root biomass, the relative variation trends across different treatments remained reliable.

Trade-offs in Greenhouse Gas Emissions

The observed trade-off between cumulative CH_4 and N_2O emissions across substitution ratios verified our second hypothesis and is broadly consistent with recent syntheses of paddy field fertilization studies [30]. Cumulative CH_4 emissions increased significantly with elevated organic substitution ratios, with OM60 exhibiting a 41.2% higher CH_4 emission level than CF. Readily decomposable organic carbon in pig manure provides abundant substrates for methanogenic bacteria [4], and the anaerobic environment of flooded paddy fields further promotes methanogenic processes [43]. In this study, CH_4 fluxes peaked at the active tillering and heading stages, which closely matched the seasonal patterns of field water management and root exudate release in paddy soils. The sharp drop in CH_4 emissions during field drainage periods further confirmed the critical regulatory effect of water management on CH_4 production and emission [3].

In contrast, cumulative N_2O emissions declined gradually as organic substitution ratios rose; OM20, OM40 and OM60 cut N_2O emissions by 14.4%, 22.5% and 30.5% relative to CF, respectively. Three potential mechanisms may explain the N_2O mitigation induced by pig manure substitution. First, the slow-release characteristic of manure-derived nitrogen avoids rapid accumulation of inorganic nitrogen in topsoil, which limits substrate availability for nitrification and denitrification [7]. Second, enhanced microbial nitrogen immobilization may serve as an important pathway: high rates of pig manure input may promote soil microbes to transform mineral nitrogen into microbial biomass nitrogen, thereby reducing the substrate pool for N_2O production [2]. This inference is indirectly supported by the significantly elevated soil MBC and MBN observed in this study, but direct confirmation via measurements of net nitrogen mineralization rates or gross microbial nitrogen immobilization rates is still required. Third, improved soil aggregation after manure application, which has been widely reported in previous organic fertilization studies, may optimize soil aeration conditions and further regulate denitrification processes. This pathway remains to be verified by direct aggregate composition measurements in karst paddy soils.

Integrated GWP results demonstrated that elevated CH_4 emissions dominated the overall greenhouse gas balance under organic substitution. OM20, OM40 and OM60 had significantly higher GWP values than CF, with increments of 10.5%, 18.3% and 27.9%, respectively. These findings suggested that for karst paddy ecosystems, the environmental advantages of manure substitution (lower N_2O emission and enhanced soil carbon sequestration) were partially counterbalanced by rising CH_4 fluxes.

The increase in CH_4 emissions with rising substitution ratios in this study was larger than that reported for most sandy paddy fields. This discrepancy is closely associated with the clay loam texture of karst paddy soils: heavy-textured soils exhibit poor aeration and maintain more stable anaerobic conditions, which favor the activity of methanogens [44]. The continuous decline in N_2O emissions can be attributed not only to the slow-release effect of organic nitrogen, but also to enhanced microbial nitrogen immobilization. High rates of pig manure input drive soil microbes to transform mineral nitrogen into microbial biomass nitrogen, thereby reducing substrate availability for nitrification and denitrification [45]. This mechanism is indirectly

supported by the elevated soil MBC and MBN observed in this study, consistent with the inference of enhanced microbial nitrogen immobilization.

Integrated GHGI Assessment and Identification of the Optimal Substitution Ratio

The GHGI-based integrated assessment provides a more holistic perspective for evaluating fertilization strategies than single environmental indicators. The results demonstrated that despite the higher total GWP under OM20, its GHGI (0.536 kg CO_2-eq kg^{-1} grain) was statistically comparable to CF (0.505 kg CO_2-eq kg^{-1} grain). These findings suggested that improved grain yield effectively diluted yield-scaled emission intensity for OM20 [30]. By contrast, OM40 and OM60 significantly elevated GHGI by 21.8% and 37.2%, respectively. Such variation revealed that coupled yield decline and rising CH_4 fluxes drove remarkable growth in yield-based environmental cost as organic substitution ratios increased.

When only direct in-field greenhouse gas emissions are considered, sole synthetic nitrogen fertilization (CF) resulted in the lowest GHGI. Nevertheless, crop production pursues dual targets of food security and ecological sustainability. The current GHGI calculation excluded upstream emissions originating from fertilizer manufacturing and transportation, indirect emissions during pig manure composting, as well as the carbon sink benefit derived from soil organic carbon sequestration. Full life-cycle carbon accounting would further enlarge the comprehensive carbon-footprint superiority of organic substitution: less synthetic fertilizer input cuts industrial carbon expenditure accompanied by promoted soil C sequestration [46]. Even restricted to direct field emission data, CF produced numerically lower grain yield than OM20 (non-significant difference) and significantly inferior nitrogen recovery efficiency (NRE). Selecting fertilization options merely based on minimum GHGI would ignore losses in grain productivity and nitrogen recovery efficiency [47]. Accordingly, three core indicators (GHGI, grain yield and NRE) were comprehensively combined in this study, identifying 20% organic substitution as the optimal fertilization regime. OM20 delivered the numerically maximum grain yield and remarkably improved NRE, with only a minor 6.1% rise in GHGI. This outcome partly verified the third hypothesis that 20% manure substitution sustains low GHGI alongside stable grain output, confirming GHGI's practicability as a multi-objective integrated evaluation index.

Limitations and Future Perspectives

This study bears several limitations. First, all observations originated from a single rice growing season. Pig manure application has cumulative effects on soil organic carbon accumulation, microbial community succession and greenhouse gas emission patterns. Single-season data can only reflect the agronomic and environmental effects in the first year of application, and cannot directly represent the long-term effects of continuous annual application. Long-term multi-year positioning experiments are required to verify the stability of the optimal substitution ratio and the cumulative environmental risks. Second, the mechanistic interpretation of this study has certain constraints. On the one hand, only soil microbial biomass indicators were determined, and the functional microbial pathways driving CH_4 and N_2O variations were not elaborated; on the other hand, soil inorganic nitrogen dynamics at key growth stages were not continuously monitored, so the nitrogen supply mechanism of manure substitution and the microbial nitrogen immobilization pathway for N_2O mitigation are mainly inferred from indirect evidence. Direct quantification of gross nitrogen transformation rates and functional gene abundance is needed in future studies to fully elucidate the underlying mechanisms. Third, the GHG inventory of this study only covers direct in-field CH_4 and N_2O emissions, ignoring upstream carbon costs from fertilizer manufacture and transport as well as the soil organic carbon sink benefit. A full life-cycle assessment is required to comprehensively evaluate the net climate effect of different fertilization strategies. Fourth, there are still uncertainties in the experimental design and promotion of findings. The tested organic substitution gradients are spaced broadly, and more precise rates within the 10%-30% interval need to be further screened. In addition, all pig manure was applied as a single basal dressing in this study, which follows local conventional farming practices but may exacerbate early-season nitrogen deficiency under high substitution ratios. The coupling effect of fertilization timing and substitution ratio deserves further exploration. Fifth, the potential long-term risk of heavy metal accumulation was not evaluated. This study only determined the heavy metal content of the applied pig manure and confirmed that it met the national standard, but did not monitor changes in soil heavy metal concentrations after planting. The cumulative risk of heavy metals under long-term continuous manure application requires further long-term positioning monitoring. In addition, the uneven spatial distribution of high-rate solid manure is an objective limitation of field plot experiments, which may increase within-treatment variation to some extent.

Future research can be advanced along three orientations. First, establish long-term field monitoring to track multi-year dynamics of soil carbon cycling, microbial community succession and GHG fluxes for clarifying long-term fertilization effects of manure substitution. Second, integrate life-cycle assessment (LCA) to encompass upstream fertilizer-related emissions and soil C sequestration, enabling holistic evaluation of net GHG balances across diverse fertilization strategies. Third, combine pig manure with mitigation practices including biochar, nitrification inhibitors and lime, and explore split application

strategies for organic manure, to reduce GHG emissions without compromising rice productivity.

Conclusions

It should be emphasized that all conclusions in this study are drawn from a single-growing-season field experiment. Given that continuous manure application has cumulative effects on soil carbon pools, microbial communities and greenhouse gas emissions, the patterns observed in this study need to be validated by long-term positioning monitoring.

This study targeted prevalent constraints in karst mountainous areas of Southwest China, including degraded soil quality, fragile ecosystems and over-reliance on synthetic fertilizers. Via field plot experiments, we systematically quantified the yield, nitrogen recovery and greenhouse gas effects of gradient pig manure substitution in karst paddy fields. The core findings can be summarized as follows: A 20% substitution ratio achieved the best comprehensive performance, maintaining grain yield and low greenhouse gas intensity while significantly improving nitrogen recovery efficiency; excessive substitution (60%) caused significant yield loss due to insufficient early nitrogen supply, and substantially increased yield-scaled greenhouse gas intensity.

These findings have important scientific and practical implications for the green transformation of rice production in karst mountainous regions.

First, in terms of scientific value, this study supplements systematic empirical data on yield-environment trade-offs of organic fertilizer substitution in karst paddy ecosystems derived from limestone parent material, filling the gap of region-specific research. It verifies that the response of karst paddy soils to organic substitution is significantly different from that of plain paddy soils, and conclusions from plain rice regions cannot be directly applied to karst ecologically fragile areas. Second, in terms of methodological value, the GHGI-based multi-dimensional comprehensive evaluation framework adopted in this study provides a reliable tool for balancing agronomic benefits and environmental impacts, which can be extended to the optimization of fertilization strategies in other ecologically fragile regions. Third, in terms of practical application, the optimal substitution ratio of 20% proposed in this study can provide preliminary empirical reference and data support for fertilizer management optimization in karst rice-growing areas of Southwest China. It supports the implementation of synthetic fertilizer reduction and agricultural low-carbon transition, and helps to improve land production efficiency while reducing environmental risks in ecologically fragile areas.

Future long-term field monitoring is recommended to elucidate the cumulative effects of continuous manure substitution on soil carbon pools and greenhouse gas budgets, and to develop synergistic technologies combining organic amendment with greenhouse gas mitigation practices.

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AI Usage Disclosure Statement

We declare that artificial intelligence tools were only used for English language polishing during the manuscript preparation process, and did not participate in any core research work such as study design, data analysis, result interpretation and conclusion generation. AI tools do not qualify for authorship, and all academic responsibilities of this manuscript are borne by the authors.

Conflict of Interest

The authors declare no conflict of interest.

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