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THE EFFECTS OF ORGANIC FERTILIZERS AS A FRACTION SUBSTITUTE TO CHEMICAL FERTILIZERS ON RICE YIELD AND QUALITY, GREENHOUSE GAS EMISSIONS, AND MICROBIAL DIVERSITY IN SOIL

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Abstract

With the implementation of China's zero-growth policy for chemical fertilizer application, increasing attention has been directed toward exploring organic fertilizers as partial substitutes for chemical fertilizers. This study investigated rice cultivation under four fertilization regimes: conventional chemical fertilization (CK) and organic fertilizer substitution at 10% (T1), 20% (T2), and 30% (T3) of total chemical fertilizer equivalents. Key findings include: (1) Comparised with CK, the T1, T2, and T3 treatments increased rice yields by 6.22%, 9.06%, and 4.68%, respectively. Organic fertilizer substitution significantly improved grain quality through reduced chalkiness (8.78%-19.49%), decreased amylose content (9.56%-12.95%), and increased protein levels (10.06%-17.29%), collectively enhancing palatability. (2) Seasonal greenhouse gas (GHG) emission patterns showed treatment-independent consistency, but cumulative emissions varied with substitution ratios. Notably, T2 and T3 achieved 10.84% and 25.92% reductions in GHG intensity (GHGI) respectively compared to CK, indicating effective emission mitigation. (3) Organic amendments significantly altered soil microbial community structure. Dominant phyla across treatments included Proteobacteria (8.85%-10.51%), Acidobacteria (10.16%-33.82%), Bacteroidetes (10.82%-71.38%), Chloroflexi (17.05%-22.70%), and Patescibacteria (212.15%-308.41%), The precise mechanisms underlying these microbial-mediated emission processes require further investigation. These findings demonstrate that 20% organic substitution (T2) optimally balances yield enhancement (9.06%) with environmental sustainability (11.00%), providing an effective strategy for green agricultural development in rice production systems.

Key words: Rice; Greenhouse gas emissions; Microorganisms; Rice quality.

Introduction

Rice (*Oryza sativa* L.) serves as a staple cereal crop critical for ensuring national food security in China. Rational fertilizer management constitutes a fundamental agricultural practice for maintaining sustainable rice productivity. However, excessive chemical fertilizer application induces multiple adverse effects, including soil acidification, organic matter depletion, and reductions in both grain yield and quality (Liu *et al*., 2021; Anas *et al*., 2020). Conversely, inadequate fertilization compromises crop growth parameters and final productivity. Of particular concern, nitrogen surplus from chemical overapplication generates substantial environmental burdens through atmospheric release of N_2O and hydrological dispersion of reactive nitrogen species, with less than 40% typically assimilated by crops (Cao *et al*., 2013). These unsustainable practices not only threaten agroecosystem stability but also constrain the development of environmentally conscious rice cultivation systems. Organic fertilizers, derived from processed plant/animal residues, offer distinct advantages through their rich organic matrix containing essential macronutrients (N, P, K) and micronutrients. Unlike rapidly available chemical fertilizers, organic amendments demonstrate gradual nutrient release kinetics that sustain long-term soil fertility. Emerging evidence suggests that integrated nutrient management-strategically combining chemical and organic fertilizers - can optimize nutrient synchronization with crop demand while mitigating environmental impacts. This synergistic approach capitalizes on the immediate nutrient availability of inorganic sources and the sustained-release characteristics of organic amendments, potentially addressing both agronomic and ecological requirements throughout critical growth stages (Iqbal *et al*., 2021; Case *et al*., 2017).

Growing recognition of organic fertilizer substitution has prompted extensive investigation into its agronomic and environmental implications. Empirical studies demonstrate that 20% chemical fertilizer replacement with organic amendments maintains soil fertility while enhancing photosynthetic efficiency, ultimately increasing grain yield by 8.3-12.7% compared to conventional practices (Shi *et al*., 2021). Hou *et al*., (2020) established that integrated organic-chemical fertilization enhances nitrogen use efficiency (NUE) by 18-24% while boosting productivity, with optimal substitution ratios showing soil fertility dependence: 30% for medium-low fertility soils versus 50-70% in high-fertility paddies (Hou *et al*., 2020). Divergent emission patterns emerged among major GHGs (i.e. methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O)) under organic substitution regimes A three-year longitudinal field trials revealed organic amendments increased methane emissions differentially based on source material, with pig manure (+83.46 kg ha^{-1} yr^{-1}), chicken manure

(+293.06 kg ha^{-1} yr^{-1}), and rice straw (+635.66 kg ha^{-1} yr^{-1}) showing progressive enhancement compared to chemical fertilization (Wu *et al.*, 2018). While organic fertilization enhances soil organic carbon sequestration, it may concurrently stimulate CO_2 efflux through accelerated microbial mineralization (Zhai *et al.*, 2011). Conversely, N_2O emissions decrease by 22-38% under combined fertilization due to reduced nitrification substrates and suppressed denitrification enzyme activity (Wei *et al.*, 2014; Gao *et al.*, 2017). Furthermore, the application of organic fertilizers provides carbon and energy for the life activities of soil microorganisms, enhancing their metabolic activity and enhancing biodiversity. Soil microorganisms are a vital component of the soil ecosystem, playing a pivotal role in the processes of soil formation, material circulation and fertility evolution (Dobrovol'skaya *et al.*, 2015). When the soil environment is disturbed externally, the functional stability of their communities is of great significance in maintaining soil health and regulating plant growth, and is recognized as a key biological signal of soil health (Ike *et al.*, 2019). The use of bio-organic fertilizers can alter the fungi-to-bacteria proportion in the soil, contributing to the health and stability of the soil ecosystem (Black *et al.*, 2003). It has been demonstrated that the application of organic fertilizer also enhances the relative abundance of favorable bacteria (Streptomyces and Burkholderia) in the soil, thereby promoting the growth of plant-associated microorganisms in the rhizosphere (Lin *et al.*, 2019). It is well established that the integration of organic and inorganic fertilizers can enhance both soil fertility and the ecological environment, increase soil microbial diversity and activity, coordinate the supply relationship of soil nutrients, and improve nutrient utilization and crop yield and quality (Wei *et al.*, 2016). The reduction of fertilizer utilized is also imperative when considering environmental protection and sustainable development.

Within chemical fertilizer reduction frameworks, precise optimization of organic substitution ratios emerges as critical for maximizing yield potential while maintaining environmental stewardship. Current research on organic-inorganic fertilization synergies predominantly emphasizes grain productivity and quality improvements, yet systematic quantification of substitution impacts on three critical parameters - greenhouse gas flux dynamics, soil microbiome restructuring, and their integrated relationships - remains limited. This knowledge gap impedes the development of substitution protocols balancing agronomic productivity with ecological sustainability. Therefore, exploring the combined response of varying ratios of organic and inorganic fertilizers to the rice yield and quality, soil microbial structure, and greenhouse gas effluences from rice fields would be beneficial to sustainable agriculture. Our objective is to investigate the outcomes of replacing chemical fertilizers with organic fertilizers at different ratios in terms of greenhouse gas emissions, soil microbial community structure, and diversity, including rice yield and quality in rice fields. We aim to find the optimal ratio of organic fertilizers to replace chemical fertilizers. The objective is to furnish scientific substantiation for the establishment of sustainable fertilization techniques and the promotion of environmentally renewable and sustainable agriculture.

Materials and Methods

Test materials-rice variety: The test site was situated in Chuzhou City, Anhui Province, China. in the paddy field of Anhui Science and Technology University (E 117°33'39", W 32°52'49"). The rice varieties selected were "Quan9you 063", a one-season indica rice variety adapted to planting in Anhui Province, which was sown on May 21, transplanted on June 30, and harvested on October 7, 2021. The row spacing of transplanted plants was 25 cm × 25 cm.

Experimental organic manure was composed of crushed cow manure and wheat straw in a ratio of 2:1, and the manure and straw were mixed fully and evenly by repeatedly turning the pile artificially. The ingredients are mixed and piled into a cone mound. The water content of the heap was meticulously regulated to a range of 55-65%, while the C/N ratio was maintained at 25-30. The oxygen content was kept within the range of 8-18%, and the pH was adjusted to a precise level between 6.5 and 8.0. After 50 to 60 days of high-temperature compost fermentation, black-brown granular compost with good maturation was obtained. The content of nitrogen, phosphorus, potassium, organic matter, and pH were 1.03%, 0.87%, 1.35%, 47.8%, and 6.67, respectively.

Experimental design: The experiment consisted of four treatment groups: CK treatment: Compound fertilizer (18-18-18) at a rate of 750 kg/hm^2 and urea at a rate of 450 kg/hm^2. T1 treatment: In the first instance, the chemical fertilizer was substituted with compost. This treatment included compound fertilizer (18-18-18) at a rate of 675 kg/hm^2, urea at a rate of 405 kg/hm^2, and compost at a rate of 5 t/hm^2. T2 treatment: 20% of the chemical fertilizer was substituted with compost. This treatment included compound fertilizer (18-18-18) at a rate of 600 kg/hm^2, urea at a rate of 360 kg/hm^2, and compost at a rate of 10 t/hm^2. T3 treatment: 30% of the chemical fertilizer was substituted with compost. This treatment included compound fertilizer (18-18-18) at a rate of 525 kg/hm^2, urea at a rate of 315 kg/hm^2, and compost at a rate of 15 t/hm^2. The experimental design incorporated a replication of each treatment on three occasions, meticulously arranged within a randomised complete block configuration. The plot area was 14 m^2, and plastic film was used to separate the field ridges between each plot. The fertilizer for each plot was added to the soil once, seven days before transplanting rice. Except for the varying fertilizer application rates, it is evident that all other operations were managed in a consistent manner in accordance with the local high-yield cultivation model.

Greenhouse gas collection and measurement: The emission fluxes of CH_4, CO_2, and N_2O from paddy fields were measured using the closed static box method. The sampling device consisted of a top box, middle section box, and base. After transplanting rice, the base (50 cm × 25 cm × 25 cm) was buried between crop rows; the groove was the only element exposed, and this did not move during the length of the rice growing season. In the box, sized 50 cm × 25 cm × 75 cm, a tiny fan was positioned inside to ensure uniform distribution of gas in the box, and yellow sponge plastic paper covered the outside of the box for reflective heat insulation. Then, a thermometer attached to the outside of the box is used to measure the temperature inside the box. The middle box (50 cm × 25 cm × 25 cm) was used to increase the box height at the later stage of rice growth. Gas samples were collected at the rice tillering stage (July 25, 2021), rice heading stage (August 09, 2021), rice full heading stage (August 24, 2021), rice filling stage (September 09, 2021), and rice maturity stage (September 24, 2021). The sampling time was fixed at 8:00-11:00 a.m. During gas collection, water was injected into the water tank at the base, and samples were collected every 6 minutes with a 60 ml syringe after being put into the sampling box. Three gas samples were collected in total. Simultaneously, the air temperature and the temperature in the box were observed and recorded (Fig. 1).

After sample collection, Agilent 7890B gas chromatograph is used for analysis in the laboratory. The following formula is employed to calculate greenhouse gas emission flux:

$$F = \rho \times h \times dc/dt \times 273/(273 + T) \quad (1)$$

The location is as follows: The mean temperature recorded in the box during the process of sampling is indicated by the preceding statement. F is the measured emission flux of CO_2. ($mg \cdot m^{-2}h^{-1}$), CH_4 ($mg \cdot m^{-2} \cdot h^{-1}$) or N_2O ($ug \cdot m^{-2} \cdot h^{-1}$); h is the height of the sampling box (m); and dc/dt is the slope of the regression curve of gas concentration with time while sample collection;

The following is the formula used to calculate total greenhouse gas emissions:

$$f = \sum[(Fi+1 + Fi)/2] \times (Di+1 - Di) \times 24/1000 \quad (2)$$

where: f is the greenhouse gas emission ($kg \cdot hm^{-2}$); Fi and Fi+1 are the emission fluxes $mg \cdot m^{-2} \cdot h^{-1}$ at the time of i and i+1 sampling, respectively; Di and Di+1 are the sampling times of the i and i+1 times respectively. The emission fluxes are Fi and Fi+1($mg \cdot m^{-2} \cdot h^{-1}$), which are at the time of i and i+1 sampling, respectively; Di and Di+1 are the sampling times of the i and i+1 periods; and f is the greenhouse gas emission ($kg \cdot hm^{-2}$)

The global warming potential (GWP) calculation formula is:

$$GWP = CO_2 + 34 \times CH_4 + 298 \times N_2O \quad (3)$$

The Global Warming Potential (GWP) is a measure of the magnitude of the warming effect caused by a particular quantity of a greenhouse gas compared to the warming effect caused by the same quantity of another greenhouse gas. ($CO_2/kg \cdot hm^{-2}$), and the total emissions of CH_4, CO_2 and N_2O ($kg \cdot hm^{-2}$).

Calculation formula of greenhouse gas emission intensity (GHGI):

$$GHGI = GWP/ Y \quad (4)$$

where: GHGI is the greenhouse gas emission intensity ($CO_2/kg \cdot kg^{-1}$), and Y is the crop yield (kg/hm^2).

Fig. 1. Greenhouse gas pictures were collected in the field.

Soil sampling and analysis: The collection of soil samples was made during the rice maturity period, and three 0 ~ 20 cm soil samples were randomly collected from each plot in accordance with the “z-shaped” sampling route. After removing impurities such as surface floating soil, gravel, and plant residues, the collected soil samples were mixed and packed into sterile self-sealing bags and stored at -80°C for microbiological analysis. Total soil DNA was extracted using a kit (MO BIO Laboratories, Carlsbad, USA). Primers 515F (5'-GTGCCAGCMGCCGCCGTAA-3’) and 806R (5’- GGACTACHVGGGTWTCTAAT-3’) were used to amplify the 16S rRNA of the soil bacteria. The amplified products were purified with 2% agarose gel electrophoresis, and the purified products were used to construct an amplicon library and sequenced using Illumina Nova 6000 PE250. The obtained raw sequences were quality-controlled and optimized using Fast (version 0.14.1) software based on the overlap between PE reads to obtain valid data. Afterward, all the sequences were divided OTU at a 97% similarity level using the Usearch software platform to generate OTU tables. A taxonomic analysis of the OTU representative sequences at a 97% similarity level was performed using the RDA classifier Bayesian algorithm.

Rice yield and quality indicators: At the rice maturity stage, nine sample plants were selected from each plot and placed into a net bag for seed testing. We followed these specific operations: after drying in the shade, we manually threshed and counted the total grains, emptied the grains and full grains of each rice plant, calculated the grains per spike, seeded the setting rate, determined the 1000 grain weight, and calculated the theoretical yield.

The harvested rice was then subjected to a drying process involving the application of air, after which it was stored at ambient temperature for a period of three months. The objective of this storage period was to ascertain the quality of the grain. Processing quality: 3 repeats were randomly selected from each treatment. Firstly, brown rice was obtained by a ridging machine, and polished rice was obtained by an intelligent milled rice machine. The ratio of brown rice and milled rice was calculated, respectively. Appearance-quality: approximately 200 hulled rice grains were selected from each treatment at random and placed in an imager for chalkiness (chalkiness ratio times chalkiness size). Nutrition and taste quality: gum consistency, protein content, and amylose content were determined using a PertonDA7200 near-infrared analyzer.

Statistical analysis: The data was organised using Excel software, statistically analyzed using DPS software, and plotted using Origin software. Shannon and Chao1 indices of the samples were completed using the Megabio Cloud platform, the microbial species composition of the samples was analyzed using the box plot method, and the differences in the community composition between samples at the phylum level were analyzed using principal coordinates and principal components, The present study sought to analyze the impact of environmental elements on the microbial community structure at the phylum level. To this end, the RDA method was employed.

Results

The present study investigates the impact of diverse fertilization treatments on rice yield and its constituent components: The yield and composition factors of rice under different fertilizer treatments are shown in Table 1. The results indicate that the rice yield follows the order T2 > T1 > T3 > CK. Organic amendments demonstrated superior yield performance relative to exclusive chemical fertilization regimes. Specifically, compared to CK, the yield of T1, T2, and T3 increased by 6.22%, 9.06%, and 4.68%, respectively, with T2 showing the highest and most significant yield increase. Regarding the yield constitutive elements, there was no significant difference in rice thousand-grain weight and the set rate between treatments with organic fertilizer and chemical fertilizer alone. However, when compared to CK with chemical fertilizer alone, T1, T2, and T3 showed respective increases of 2.68%, 1.13%, 1.13% in effective spike count, and 5.41%, 6.19%, and 3.09% in grains per spike count. Therefore, using a reasonable proportion of organic fertilizers instead of chemical fertilizers is beneficial for boosting the number of effective spikes and spike grains, ultimately leading to higher rice yields.

Effects of different fertilizer treatments on rice quality: The quality of rice is a comprehensive set of attributes, primarily including processing quality, such as the percentage of brown rice and the percentage of milled rice; appearance quality, such as the percentage of bran; flavor quality, such as consistency of stickiness; amylose content; and protein content. Figure 2 shows the results of differing fertilizer treatments on the final quality of the rice. When comparing the appearance quality, it was found that the highest chalkiness was found in the CK treatment with conventional fertilizer (Fig. 2B). As demonstrated in Figure 2, the implementation of organic fertilizer resulted in a large reduction in the rice's chalkiness. Additionally, the application of the treatment ranging from T1 to T3 led to a marked improvement in the acceptability levels, with a reduction in the unpleasantness rating by 16.19%, 8.76%, and 19.50%, respectively. Improving the appearance quality of the rice. For the eating quality of rice under various treatment types, there was no measurable, significant difference in the glue consistency (Fig. 2C) content between a conventional fertilization treatment and other organic fertilizer treatment ($p<0.05$). The protein (Fig. 2A) content was the highest in the T3 treatment and the lowest in the CK treatment, and the difference between the two was significant ($p<0.05$). The protein content in T1 and T2 treatments was greater than those in the CK treatment, but there was not a significantly great difference ($p<0.05$). The organic fertilizer application significantly reduced the rice straight-chain starch content (Fig. 2F), with T1–T3 treatments reducing the rice straight-chain starch content by 9.56%, 12.98%, and 11.00%, respectively, compared to CK, with the T2 treatment having the most significant reduction effect. Comparing the processing quality of different treatments, it was found that whether organic fertilizer was applied or not and different proportions had no significant effects on the brown rice percentage (Fig. 2D) and milled rice percentage (Fig. 2E) ($p<0.05$). The overall results indicated that the use of organic fertilizer could improve the appearance-quality and taste quality of rice to different degrees, and the effect on the processing quality was insignificant. The T2 and T3 fertilisation treatments were relatively optimal.

Effect of fertilizer treatments on greenhouse gas emission fluxes from rice fields: Figure 3 shows the dynamics of the flux of greenhouse gas emissions from the rice fields during rice fertility. Figure 3a shows the dynamics of CH_4 emissions from the rice fields from various fertilizer treatments. Regardless of the treatment approach, the pattern of CH_4 emissions flux remains consistent, exhibiting a gradual decline until reaching a plateau as the rice plants mature and develop. Under different fertilizer treatments, the peak CH_4 emissions flux appeared 25 d after rice transplanting, and the emissions of each treatment in the order of T3 > T1 > T2 > CK ranged from 10.94 to 100.95 $mg\cdot m^2\cdot h^{-1}$. The addition of organic fertilizers may increase CH_4 emissions compared to CK. Figure 3b shows the CO_2 emissions flux dynamics of rice fields under different fertilizer treatments. The CO_2 emission features of the rice show a bimodal trend, with the first small peak occurring 40 d after rice transplantation and the other large peak occurring 70 d after transplantation. Figure 3c shows the dynamic pattern of N_2O emissions in the paddy field under different fertilization treatments, which is different from the emissions pattern of CO_2 and CH_4. N_2O emissions were lower in the early stage and increased significantly in the late stage. Under varying fertilization treatments, the peak value of N_2O emissions was CK > T3 > T2 > T1; the results demonstrated that the inclusion of organic fertilizer decreased the emissions of N_2O in the paddy field. Different fertilizer treatments do not change the pattern of GHG emissions from rice paddies, but they do affect the cumulative GHG emissions from rice paddies.

Table 1. The following study will examine the relationship between rice yield and its constituent factors in the context of different fertilization treatments.

Treatment	RPN (10^4)	GNPP	RSSP (%)	TSW (g)	Yield ($kg\cdot hm^{-2}$)
T1	13.44 ± 0.10a	198.80 ± 4.55a	87.63 ± 1.37a	27.17 ± 0.63a	9535.50 ± 104.27b
T2	13.23 ± 0.14ab	200.43 ± 1.70a	90.36 ± 0.47a	27.37 ± 0.47a	9833.40 ± 69.67a
T3	13.23 ± 0.08ab	194.03 ± 5.49ab	89.77 ± 2.05a	27.17 ± 0.34a	9382.05 ± 162.05b
CK	13.08 ± 0.05b	188.03 ± 1.50b	89.13 ± 1.83a	27.20 ± 0.08a	8942.70 ± 122.36c

Note: RPN: Rice panicle number per mu. GNPP: Grain number per panicle. RSSP: Rice seed setting percentage. TSW: Thousand-seed weight. The presence of lowercase letters within a given column indicates a significant difference (LSD test, $p<0.05$). The error values are representative of the standard deviation of three biological replicates

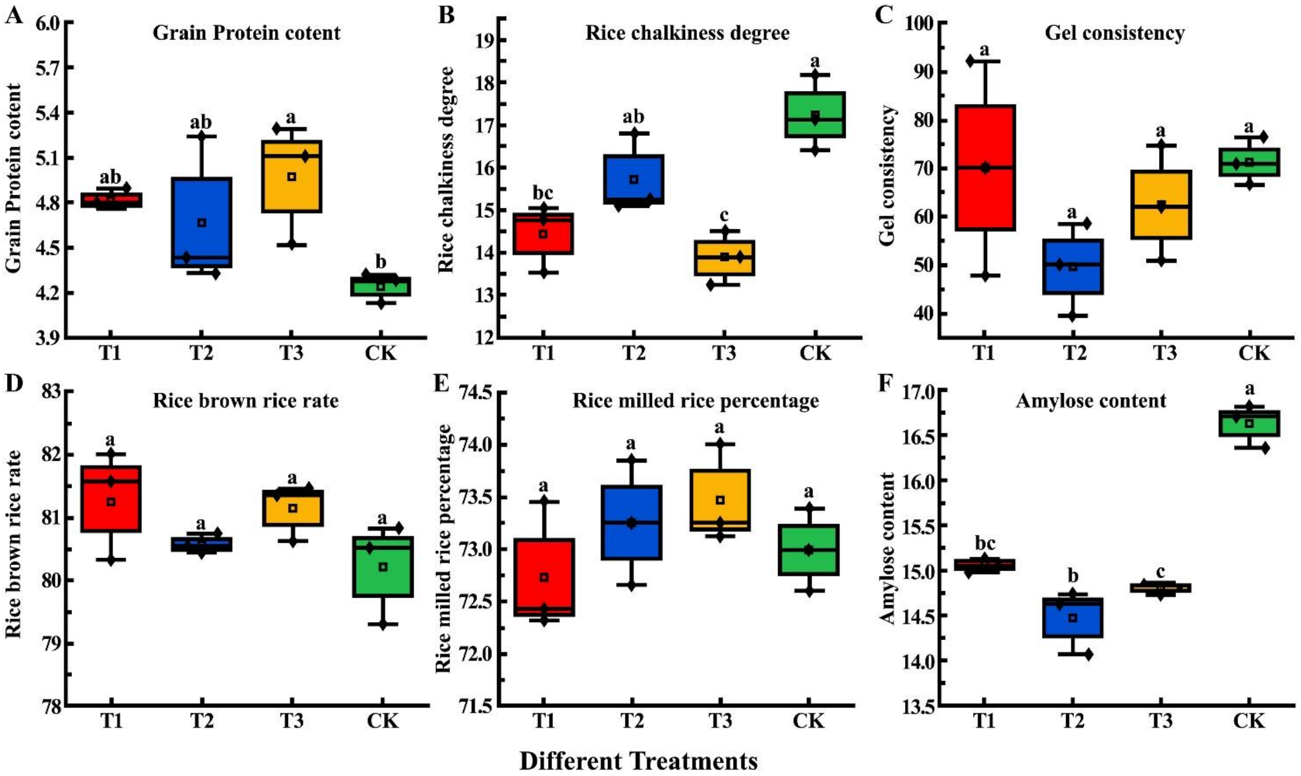

Fig. 2. Picture of rice quality analysis under different treatments. (A) rice protein content. (B) rice chalkiness. (C) rice gel consistency. (D) rice brown rice percentage. (E) rice milled rice percentage. (F) amylose content of rice.

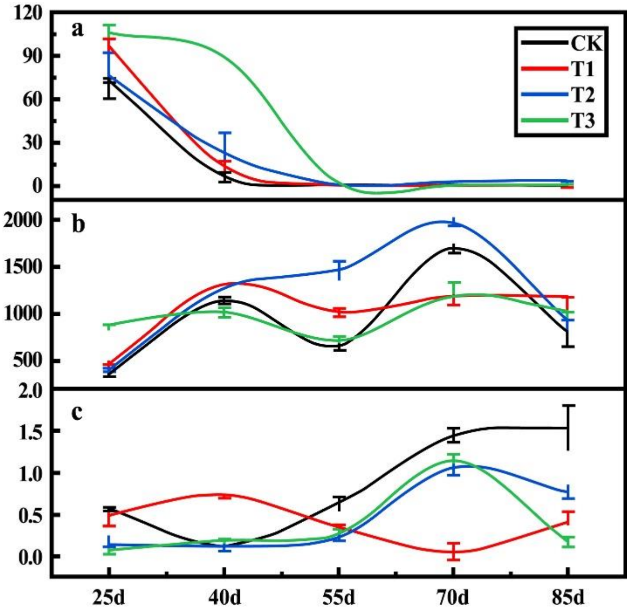

Fig. 3. Dynamic picture of greenhouse gas emission fluxes in rice fields under different treatments. (a) Dynamic picture of CH_4 emission flux in paddy field. (b) Dynamic picture of CO_2 emission flux in paddy field. (c) Dynamic picture of N_2O emission flux in paddy field.

An examination of the repercussions of disparate fertilization strategies on the overall greenhouse gas (GHG) emissions, global warming potential (GWP), and greenhouse gas inventory (GHGI) from rice fields: The present study investigates the effects of the utilization of organic fertilizer on cumulative GHG emissions, GWP, and GHGI in rice fields. As can be seen from Table 2, the cumulative CH_4 emissions of each treatment ranged from high to low during the rice fertility period: T3 > T2 > T1 > CK. The use of organic fertilizers to replace some fertilizers increased the cumulative CH_4 emissions from rice fields, and they progressively increased as the percentage of chemical fertilizers that are replaced by organic fertilizers grew, and the cumulative CH_4 emissions of every treatment ranging between 15.98 to 52.07 $kg·hm^{-2}$. The cumulative CO_2 emissions of the treatments ranged from 1400.46 to 1935.18 $kg·hm^{-2}$, with a significant ($p<0.05$) increase of 23.91% in the cumulative CO_2 emissions of the T2 treatment in comparison to the non-organic fertilizer CK treatment. The disparities between the T1 and T3 treatments, in contrast with the CK (non-organic fertilizer) treatment, were deemed not to be statistically significant. The cumulative N_2O emissions from the treatments ranged from 0.57 to 1.18 $kg·hm^{-2}$. The use of organic fertilizer significantly reduced the cumulative N_2O emissions from the rice fields, with the cumulative emissions of treatments T1, T2, and T3 significantly ($p<0.05$) reduced by 43.22%, 47.46%, and 51.69% compared to CK without organic fertilizer application. Therefore, utilizing organic fertilizers can raise the cumulative emissions of CH_4 and CO_2 and reduce the cumulative emissions of N_2O from rice fields, but differences will occur depending on the percentage of organic fertilizer substitution. Among all treatments, CK with conventional fertilizer had the lowest GWP, and applying organic fertilizer could further raise GWP, and the order of treatment was T3 > T2 > T1. The GWP value increased by 5.21%, 19.64%, and 29.56% compared with CK treatment, respectively. It is evident that rises in GWP values of paddy fields correspond to an increase in the proportion of organic fertilizer that is utilized in place of chemical fertilizer. The application of organic fertilizer brought a significant yield increase to the paddy fields. The integrated yield calculation of GHGI values revealed that the GHGI values of paddy fields in T2 and T3 treatments were significantly ($p<0.05$) reduced by 10.85% and 25.92% compared to the CK treatment, showing that the use of organic fertilizer could reduce the GHGI of paddy fields, but the difference between T1 treatment and conventional fertilizer CK treatment was significant. The T2 and T3 treatments increased the yield while increasing the GWP of the rice field but significantly reduced the GHGI. The judicious utilization of organic fertilizers as an alternative to chemical fertilizers has been demonstrated to be an efficacious strategy for achieving a harmonious equilibrium between economic and ecological benefits.

Effect of different fertilizer treatments on the alpha diversity of soil bacteria in rice fields: As can be seen in Table 3, the coverage index for the various treatments of bacteria exceeded 0.98. It can be deduced from the results of the sequencing process that the data are accurate and reflect the true microbial population found in the soil samples. The abundance of bacterial communities in the soil was represented by the Chao1 and Ace indices, where larger values denoted a greater number of communities. For the various fertilizer treatments T1–T3, the Ace (6682.11-6850.82) and Chao1 indices (5873.1-6153.93) were much higher than those of the CK treatment group (5988.57 and 5145.30). Specifically, the soil indices of Ace or Chao1 were considerably ($p<0.05$) higher in the T1 and T2 treatment groups than in the CK treatment group. The diversity of the bacterial community in the soil was also evaluated using the Simpson and Shannon indices; the higher the Simpson index, the weaker the microbial diversity, and the higher the Shannon index, the greater the microbial diversity. The findings of this study demonstrated a lower bacterial Shannon Index in the T1 and T3 treatments (10.4–10.63) when compared with the CK treatment group (10.67). Whilst the T2 treatment group demonstrated a greater mean value of 10.73, this did not reach statistical significance when compared to the CK treatment group, which exhibited a mean value of 10.67 ($p<0.05$). The Simpson index was found to be lower in the T1–T3 treatment groups (0.0018-0.0022) when compared to the CK treatment group (0.0030). The T2 treatment group exhibited a significant decrease in comparison to the CK treatment group ($p<0.05$).

The following study investigates the relationship between fertilizer treatments and the relative abundance of soil bacterial communities: A subsequent examination of the soil bacterial community composition under various fertilisation treatments was conducted. The bacterial communities under different treatments were analyzed at the phylum level, as illustrated in Figure 4. The top eight dominant bacterial phyla were Proteobacteria (34.3%-38.4%), Acidobacteria (11.7%-17.7%), Patescibacteria (4.2%-17.4%), Bacteroidetes (6.0%-10.3%), Chloroflexi (7.3%-9.5%), Actinobacteria (5.1%-8.5%), Gemmatimonadetes (3.3%-5.8%), and Verrucomicrobia (2.6%-3.6%). These eight bacterial clades accounted for more than 93% of the total reads sequence. The relative abundance of Patescibacteria in the sampled soil was significantly higher ($p<0.05$) in treatments T1, T2, and T3 as opposed to the control group (CK) without organic fertilizer. However, the relative abundance of Chloroflexi was noticeably lower ($p<0.05$) in these treatments compared to the CK. In treatment T3, the relative abundance of Acidobacteria and Gemmatimonadetes was significantly lower ($p<0.05$) compared to treatments T1 and T2. A comparative analysis revealed that no statistically significant differences among the four treatment groups were present in terms of the relative abundance of Proteobacteria, Bacteroidetes, and Verrucomicrobia ($p<0.05$). These three dominant bacterial phyla were selected for investigation due to their established significance in this field.

Table 2. Analysis table of total greenhouse gas emissions, GWP, and GHGI in paddy fields under different fertilisation treatments.

Treatment	Cumulative CH$_4$ emission (kg·hm^{-2})	Cumulative CO$_2$ emission (kg·hm^{-2})	Cumulative N$_2$O emission (kg·hm^{-2})	GWP	GHGI
ck	15.98 ± 0.49b	1472.40 ± 26.29bc	1.18 ± 0.12a	2362.96 ± 20.17c	3.78 ± 0.03a
T1	22.33 ± 1.89b	1564.2 ± 49.069b	0.67 ± 0.06b	2492.83 ± 16.90c	3.83 ± 0.06a
T2	23.72 ± 8.51b	1935.18 ± 46.58a	0.62 ± 0.05b	2940.37 ± 240.46b	3.37 ± 0.29b
T3	52.07 ± 1.36a	1400.46 ± 35.75c	0.57 ± 0.03b	3354.58 ± 12.52a	2.80 ± 0.05c

Note: As indicated by a lowercase letter difference in a given column, a significance level of $p<0.05$ is indicated, in accordance with a LSD test. The error values are representative of the standard deviation of three biological replicates

Table 3. The following analysis table provides an overview of the alpha diversity index of soil bacteria in response to different fertilization treatments.

Treatment	Coverage	Ace	Simpson	Chao1	Shannon
CK	0.98 ± 0.0008a	5988.57 ± 112.37b	0.0030 ± 0.0005a	5145.30 ± 315b	10.67 ± 0.33a
T1	0.98 ± 0.0017a	6850.82 ± 277.02a	0.0020 ± 0.0004ab	6153.93 ± 319a	10.40 ± 0.16a
T2	0.98 ± 0.0007a	6835.33 ± 242.25a	0.0018 ± 0.0002b	6013.47 ± 3461a	10.73 ± 0.17a
T3	0.98 ± 0.0016a	6682.11 ± 216.52a	0.0022 ± 0.0005ab	5873.1 ± 386ab	10.63 ± 0.19a

Note: As indicated by a lowercase letter difference in a given column, a significance level of $p<0.05$ is indicated, in accordance with a LSD test. The error values are representative of the standard deviation of three biological replicates

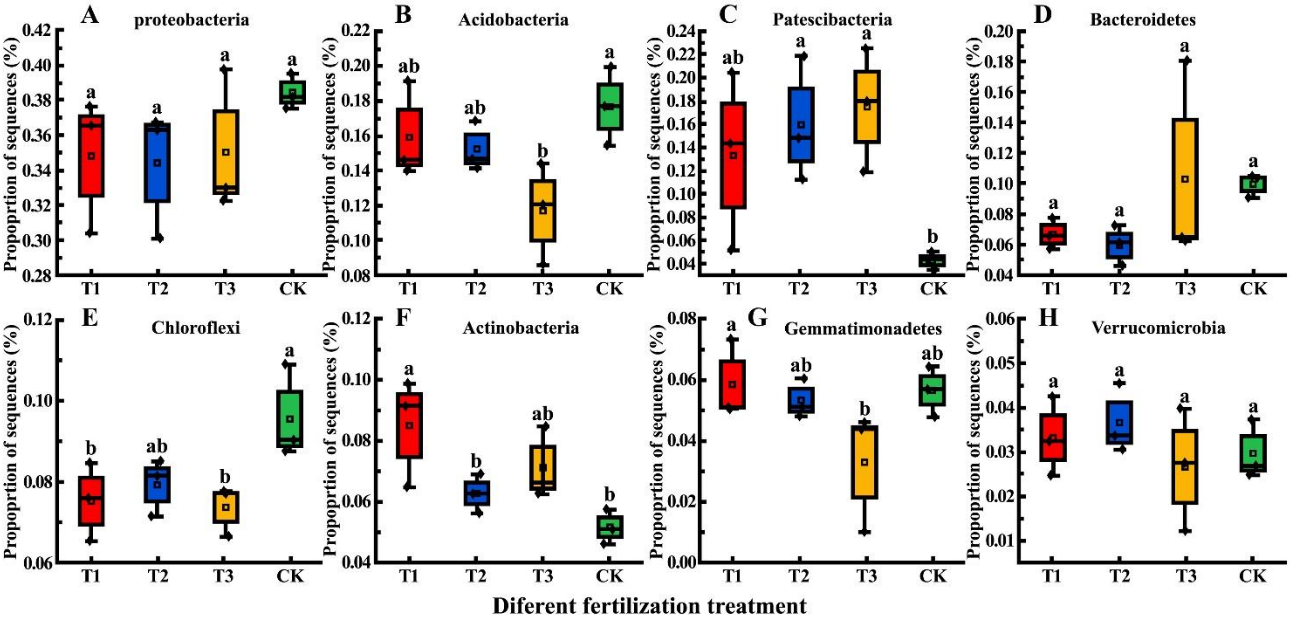

Fig. 4. The following figure presents the relative bacterial community abundance at the phylum level in soil treated with different fertilizers. The proportion of Proteobacteria (A), Acidobacteria (B), Patescibacteria (C), Bacteroidetes (D), Chloroflexi (E), Actinobacteria (F), Gemmatimonadetes (G) and Verrucomicrobia (H) in different treatments.

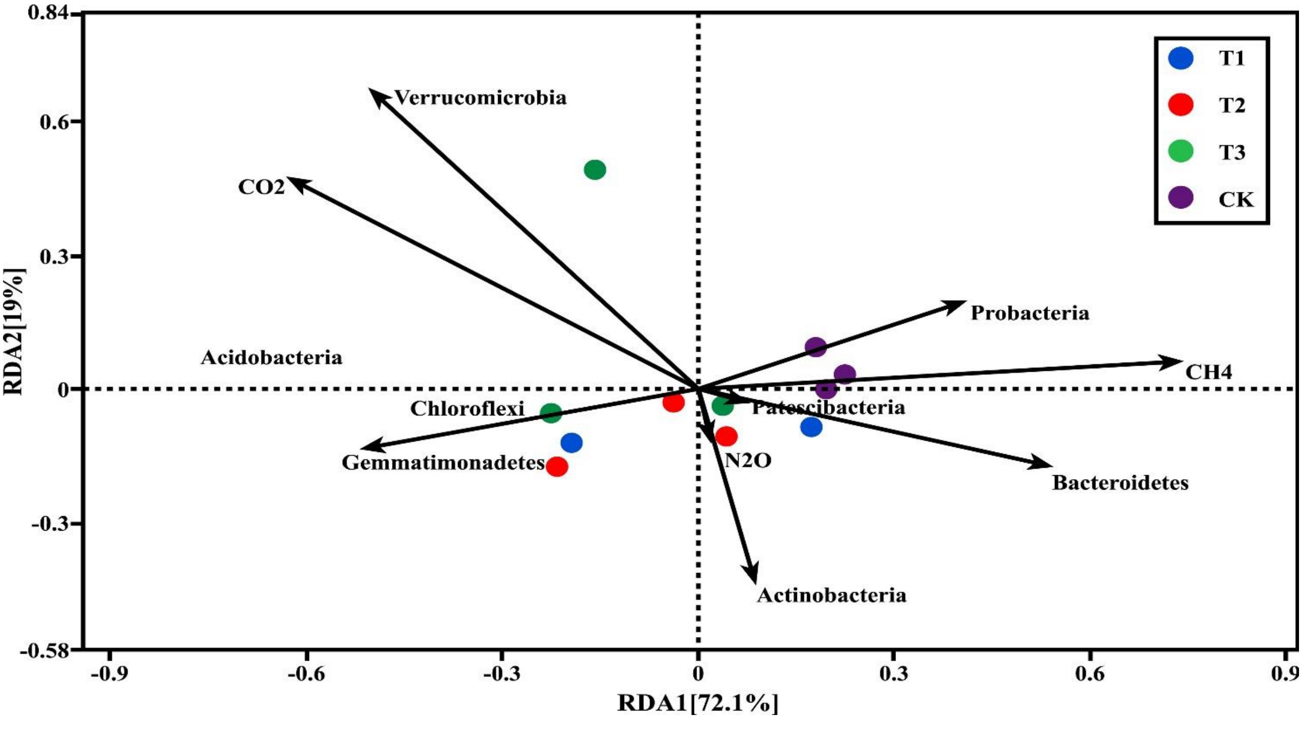

Fig. 5. Redundancy analysis of soil microorganisms and greenhouse gases in rice fields under different fertilizer treatments.

Study on the correlation between soil greenhouse gas emissions and microbial communities under different fertilisation conditions: To achieve a more complete and nuanced comprehension of the relationship between soil microbes and their role in greenhouse gas emanations, this study employed RDA redundancy analysis. The RDA ranking (Fig. 5) showed that the conventional fertilizer CK was primarily situated in the first quadrant, whereas the treatments involving organic fertilizer were predominantly located in the third and fourth quadrants. It can be seen from the figure that the arrow lengths of CH_4 and CO_2 are the longest, indicating that CH_4 and CO_2 are more closely correlated with the soil bacterial community. The main dominant phylum in each treatment was Proteobacteria. The distribution of the subject was observed to occur in the first quadrant, and an identifiable positive correlation was seen between the subject and CH_4. Furthermore, a positive correlation was found between Actinobacteria and Bacteroidetes on the one hand and N_2O on the other. Finally, a positive correlation was identified between Verrucomicrobia and CO_2, and a negative correlation between Verrucomicrobia and both CH_4 and N_2O.

Discussion

The substitution of chemical fertilizers with organic fertilizers can, in part, enhance rice yield and quality while concomitantly reducing the intensity of greenhouse gas emissions from rice fields. China is a large producer of agriculture with sufficient organic fertilizer resources. The judicious utilization of organic fertilizers can curtail the reliance on nitrogen fertilizers, thereby ensuring the balance of the physical and chemical qualities of soil and concomitantly effectuating a reduction in environmental pollution, a phenomenon of considerable significance. Throughout the maturation and development of rice, the amount and type of fertilizer used seriously affects the dynamics of stem tillering and the rate of spike production. Excessive fertilizer application not only increases the number of ineffective tillers but also leads to nutrient losses, causing damage to the ecological environment. Too little fertilizer will not meet the normal growth requirements of the crop, resulting in lower yields. Therefore, compared with chemical fertilizers alone, the inclusion of organic fertilizers is a benefit to overall plant root growth, increasing the effective number of rice spikes and promoting rice growth and development (Lin *et al*., 2023). In this study, organic fertilizers were found to be capable of increasing the yield of rice by replacing some of the chemical fertilizers, with the most significant result of organic fertilizers replacing 20% of chemical fertilizers ($p<0.05$). A comparison of the yield components revealed that increases in effective spike number and grains per spike were the main factors in the yield improvement (Table 1), which is in line with outcomes from other studies (Moe *et al*., 2019; Elkholy *et al*., 2010). Rice quality is a comprehensive indicator used to evaluate the appearance, taste, and nutrition of rice. Aside from the genetic characteristics inherent to variety, the quality of the rice is also influenced by various cultivation practices. Among the most significant of these is fertilizer application. (Lanning *et al*., 2011). The findings from a comprehensive two-year trial conducted by Huang *et al*. It was demonstrated that the implementation of organic cultivation practices resulted in a substantial increase in the protein and gum consistency content while concurrently reducing the straight-chain starch content of rice when compared to methods of conventional cultivation (Huang *et al*., 2016). Hong *et al*. found that organic fertilization as a base fertilizer reduced the straight-chain starch content of rice compared to conventional fertilization, resulting in an increase in palatability(Hong *et al*., 2013). The incorporation of organic fertilizer in the experiment yielded multiple benefits. Not only did it effectively decrease the chalkiness of rice and enhance its overall appearance and quality, but it also resulted in a notable increase in the protein content of the rice. Additionally, the organic fertilizer reduced the straight-chain starch content and improved the taste quality, although it did not have a significant impact on the brown rice percentage and gum consistency (Fig. 2). The findings of the experiment demonstrate that substituting chemical fertilizer with organic fertilizer can enhance seed yield and rice quality. The most substantial impact is observed when organic fertilizer is substituted for 20% of the chemical fertilizer. Nevertheless, the underlying mechanisms through which the substitution of organic fertilizers affect rice yield and quality continue to be elucidated and require continued investigation.

Rice paddies represent a significant source of greenhouse gas emissions, serving as a primary location for the production, oxidation, and diffusion of soil greenhouse gases. This study found that the greenhouse gas emissions law was basically the same in all treatment rice fields, and the cumulative emissions showed different differences due to the proportion of organic fertilizer replacement. From Figure 3a, it is evident that the peak of CH_4 emissions flux occurred during the tillering stage (25 days after transplantation) and showed a significant decrease during the middle and late stages of rice growth. It is easy to form an anaerobic environment under the early flooding condition of the paddy field, which can meet the growth demand of CH_4 bacteria, promote the proliferation of CH_4 bacteria, and produce a large amount of CH_4 (Zu-Cong *et al*., 2005). The decreased CH_4 emissions flux during the middle and late stages of rice growth is primarily due to the thinner or absence of a water layer in the later stages of the rice field. As the water dries up, there is sufficient oxygen supply, which promotes CH_4 oxidation and subsequently reduces emissions (Sander *et al*., 2014). The utilization of organic fertilizers resulted in a significant increase in the cumulative methane emissions from paddy fields in comparison with the control group (CK). Furthermore, substituting a proportion of chemical fertilizers with organic fertilizers resulted in an increase in methane emissions. (Table 2). The primary methods through which methane is produced in rice fields can be categorized as follows: firstly, methanogens employ organic acids derived from the decomposition of organic matter to produce CH_4. Secondary to this process is the production of CH_4 via a different route: the action of methanogens on organic matter results in H_2 and CO_2, which, in turn, can be converted back into CH_4 (Kimura, 1997).The experiment revealed a clear correlation between the addition of organic fertilizers and an increase in methane emissions within rice fields. The introduction of organic matter into the paddy soil resulted in a substantial enrichment of the soil's organic composition, thereby stimulating methane production in the paddy field. The CO_2 emission data collected from the rice field trials under varying conditions exhibited a bimodal distribution across the rice development phase (Fig. 3b). The application of organic fertilizers led to a significant ($p<0.05$) rise in the cumulative CO_2 emissions within the T1 and T2 treatment groups (Table 2). The underlying reasons for this phenomenon may be associated with the dynamics of soil temperature and the specific characteristics of the rice growth process.(Iqbal *et al*. 2009). Relevant studies have proved that CO_2 emissions from rice fields are strongly influenced by soil respiration and microbial activity. In the early stage of rice transplanting, the fragile vitality of the root system was small, and the CO_2 emissions from rice fields were usually low; in the middle and late stages, the root system of rice grew and developed maturely, and the application of organic fertilizer effected an increase in the carbon source, which enhanced the plant growth vitality as well as microbial activity and raised CO_2 emissions from rice fields (Yang *et al*., 2018; Zhai *et al*., 2011). N_2O production in rice fields is the result of a combination of soil nitrification and denitrification, which then diffuses into the atmosphere through plant aeration tissues, voids, etc., (Kim *et al*. 2002). The application of nitrogen fertilizer provides the substrate for nitrification and denitrification reactions, and dramatic changes in field moisture can also accelerate nitrification and denitrification rates. Therefore, the application of nitrogen fertilizer and field moisture are key factors affecting N_2O emissions (Xiong *et al*., 2007). In this experiment, N_2O emissions were relatively low in the early stages of rice growth (Fig. 3c), probably because the flooded state of the field in the early stages blocked the emissions of N_2O gas to the atmosphere, while at the same time, the activity of nitrifying and denitrifying bacteria in the soil was also limited by water, reducing N_2O emissions. N_2O emissions rose significantly in the middle and late stages of rice growth (Fig. 3c). When there is good aeration and moderate water content in alternating wet and dry fields, it facilitates the reproduction of nitrifying and denitrifying bacteria, which greatly stimulated N_2O emissions from rice fields. The application of organic fertilizer significantly ($p<0.05$) reduced cumulative N_2O emissions compared to the conventional fertilizer application of CK, but the differences between treatments were not significant (Table 2). The analysis may be due to the fact that the further decomposition of organic fertilizers in the soil requires the consumption of oxygen, thus reducing the emissions of N_2O (Tao *et al*., 2018), and secondly being able to supply an abundant source of carbon, the soil consumes NO_3^- to replenish the nitrogen source in order to maintain the balance of the carbon-to-nitrogen ratio, which in turn reduces the amount of NO_3^- and thus the amount of N_2O produced during denitrification (Baruah & Baruah, 2015).

In summary, the replacement of some chemical fertilizers with organic fertilizers in this experiment increased the emissions of CH_4 and CO_2 in the rice field, but it also significantly reduced the cumulative emissions of N_2O. To measure the combined greenhouse effect of the different treatments in the rice field, the overall effect of these three major greenhouse gasses needs to be taken into account. GWP and GHGI are important indicators for the comprehensive evaluation of greenhouse gas warming capacity and emissions intensity (Allen *et al*., 2016). This study concluded that the addition of organic fertilizer contributed to higher total soil CO_2 and CH_4 emissions, which ultimately resulted in an increase in global warming potential (GWP). This is largely consistent with the discoveries of Yang *et al* (Dan *et al*., 2018). Although organic fertilizer resulted in an increase in GWP, it also had a significant yield increase and, therefore, did not necessarily result in an increase in GHGI values. The GHGI varied between 2.8 and 3.83 $kg \cdot hm^{-2}$ in the different treatments, with GHGI being significantly less than CK under T2 and T3 treatments, mainly due to the increased rice yield (Table 2). Overall, the use of organic fertilizers can increase rice yields and reduce the intensity of greenhouse gas emissions, contributing to safe food production and environmental sustainability.

Application of organic fertilizer increased soil bacterial diversity and changed microbial community structure: Microorganisms significantly affect soil, not only by affecting healthy crop growth but also by participating in the organic matter and nutrient cycling decomposition processes (Dobrovol'skaya *et al*., 2015). Studies have demonstrated that prolonged use of chemical fertilizers can have detrimental effects on the soil environment, impacting the community structure and diversity of soil bacteria; however, this can be mitigated by the application of organic fertilizers (Cui *et al*., 2018). This study was based on high-throughput sequencing technology to study the microbial bacterial community under different fertilizer treatments. The alpha index is an important indicator of soil microbial diversity. In this study, we observed significant increases in the Chao1 and Ace indices of bacteria in the treatment with organic fertilizer, as compared to the control group (CK), as indicated in Table 3; this indicates organic fertilizer has the capacity to increase the diversity of soil bacteria. The utilization of organic fertilizer brings about the rich organic matter and nutrient content of nitrogen, phosphorus, and potassium to the soil, promotes microbial multiplication, and increases the diversity of soil microorganisms to a certain extent (Sadet-Bourgeteau *et al*., 2018), which is consistent with the outcomes of this experimental study. At the phylum classification level, Proteobacteria (34.3%-38.4%), Acidobacteria (11.7%-17.7%), Patescibacteria (4.2%-17.4%), Bacteroidetes (6.0%-10.3%), Chloroflexi (7.3%-9.5%), Actinobacteria (5.1%-8.5%), Gemmatimonadetes (3.3%-5.8%), and Verrucomicrobia (2.6%-3.6%) were the dominant bacterial phylum in the rice fields of the different treatments, but the percentage of the same dominant phylum varied among the treatments (Fig. 4). Patescibacteria have a small genome and may have a lifestyle categorised as symbiotic or parasitic. A high count of Patescibacteria in the soil after the application of organic fertilizer indicates a sizable number of other microorganisms for attachment (Sánchez-Osuna *et al*., 2017), whereas Actinobacteria can promote the decay of plants, animals, and residues in soil, which holds a key role in the further decomposition of organic fertilizers while also having great importance in the soil nitrogen cycle (Lauber *et al*. 2008). The results of soil analysis showed that organic fertilizer replaced part of chemical fertilizer and changed the structure and variety of the microbiome, which was similar to the results of past studies (Ding *et al*., 2018; Dang *et al*., 2022).

It has become an indisputable fact that greenhouse gases in rice fields are affected by soil microbial activities. Previous studies have provided confirmation that N_2O emissions stem from the processes of nitrification and denitrification. These processes are characterised by the involvement of ammonia-oxidising archaea (AOA) and ammonia-oxidising bacteria (AOB). Further studies have shown that amoA was able to catalyze NH_3/NH_4^+ in the soil to NO_3^- and NO_2^- and then reduce it to N_2O or N through the denitrification of narG, nirK, nirS, and nosZ (Limpiyakorn *et al*., 2006; Henry *et al*., 2006). Therefore, only a coupled soil microbiome can more accurately estimate greenhouse gas emissions and their global warming effects and provide a theoretical basis for developing better emissions reduction measures. To further explore the relationship between dominant species in microbial communities and GHG emissions under different treatments, the dominant phylum of soil bacteria was analyzed redundantly with GHG (Fig. 5). The RDA analysis indicated that the RDA1 and RDA2 axes explained 72.1% and 19% of the soil bacterial communities in the different treatments, respectively, with a total explanation rate of 91.1%. Schutz *et al*. concluded that soil microbial abundance and activity significantly influenced CH_4 emissions from rice fields(Schütz *et al*., 1989) and that soil methanogenic bacteria promoted CH_4 emissions through the consumption of organic matter in an anaerobic environment (Le Mer & Roger, 2001). The application of organic fertilizers into the soil increases the carbon-to-nitrogen ratio content, resulting in microorganisms being forced to fix the active state of nitrogen in the soil, reducing the nitrogen substrate from which N_2O is produced and thus reducing N_2O emissions (Shan & Yan, 2013). The top five dominant bacterial phyla in paddy soil observed in this study were Proteobacteria, Acidobacteria, Patescibacteria, Bacteroidetes, and Chloroflexi, where Proteobacteria and Acidobacteria are beneficial to soil bacteria that can participate in the carbon and nitrogen cycle of soil organic matter (López-Mondéjar *et al*., 2015), and Chloroflexi anaerobic cordate bacteria are capable of decomposing humic acids, reducing sulphate to produce methane and changing the environmental conditions in the soil (Noguchi *et al*., 2014). The dominant bacterial phylum Proteobacteria was positively correlated with CH_4, Actinobacteria, and Bacteroidetes with N_2O, and Verrucomicrobia was positively correlated with CO_2 and negatively correlated with CH_4 and N_2O. This implies that the impact of various organic fertilizer treatments on greenhouse gas emissions is not solely determined by changes in the soil bacterial community. Instead, it has a multifaceted influence on microorganisms present in the soil, leading to various degrees of positive and negative feedback on soil GHG emissions. The utilization of organic fertilizer has altered the diversity and activity of the soil microbial community structure. However, continued research is necessary to determine the extent and driving factors of the changes in soil microbial community structure and function. as well as their impact on greenhouse gas emissions when partially replacing chemical fertilizers with organic fertilizer, are still unclear. An in-depth study of soil physical and chemical properties and microbial community changes may be the key to uncovering the significance of organic fertilizer replacement on soil carbon and nitrogen emissions. It is imperative that subsequent studies focus on the examination of organic fertilizer as a substitute for soil microbial community diversity and functional verification. Moreover, the interaction mechanism between soil physical and chemical properties and greenhouse gases must be investigated, thus providing a scientific foundation for the appraisal of soil greenhouse gas emissions and carbon sequestration and emissions reduction.

Conclusions

The discoveries and outcomes of this study demonstrated that (1) the utilization of organic fertilizer in lieu of chemical fertilizers resulted in an augmentation of the effective number of spikes and grains of rice, thereby enhancing the rice yield by 4.68% to 9.06%. It was observed that treatment T2 exhibited the most substantial yield enhancement. The implementation of organic fertilizers had a significant impact on the appearance, quality, and palatability of the rice. The straight-chain starch content was lowered, and the protein content was raised, resulting in higher palatability. (2) The pattern of greenhouse gas emissions from paddy fields in regard to different organic fertilizer treatments was found to be similar, but the cumulative emissions of N_2O were greatly reduced, and the cumulative emissions of CH_4 and CO_2 were increased. The utilization of organic fertilizers led to an augmentation in the GWP value of the paddy fields, accompanied by a substantial enhancement in yields. Furthermore, a notable decrease in the intensity of GHG emissions was observed from the paddy fields under the T2 and T3 treatments. (3) the top five dominant bacterial phyla in each treatment were Proteobacteria, Acidobacteria, Patescibacteria, Bacteroidetes, and Chloroflexi, but the proportion of the same dominant bacterial phyla varied among the different fertilizer treatments, which in turn influenced the greenhouse gas emissions, though the exact mechanism of action requires further investigation. Combining the changes in the rice yield, quality, soil greenhouse gas emissions, and microbial communities in each treatment, the replacement of 20% chemical fertilizers with organic fertilizers can better balance the ecological and economic benefits of rice. It is an established fact that this method is suitable not only for the purpose of producing rice of a consistently high calibre, but also for the reduction of greenhouse gas emissions from rice fields, with the additional benefit to the agricultural development of increased environmental sustainability and the adoption of a greener approach.

Glossary:

T1	Incorporating organic fertilizer to substitute for 30% of chemical fertilizer
T2	Incorporating organic fertilizer to substitute for 30% of chemical fertilizer
T3	Incorporating organic fertilizer to substitute for 30% of chemical fertilizer
CK	Only applying fertilizers
GHGI	Greenhouse Gas Inventory
GWP	Global Warming Potential
GHG	Greenhouse Gas
NEP	Rice panicle number per mu
GNPP	Grain number per panicle
SSR	Rice seed setting percentage
TSW	The number of seeds from a given quantity
SPAD	Single Point Active Determination

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