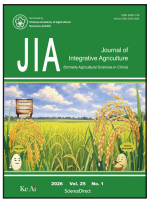
Journal of Integrative Agriculture 2026, 25(1): 273–289

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

Partial organic fertilizer substitution and water-saving irrigation can reduce greenhouse gas emissions in aromatic rice paddy by regulating soil microorganisms while increasing yield and aroma

Ligong Peng[1, 2, 3*], Sicheng Deng[1, 2, 3*], Wentao Yi[1, 2, 3], Yizhu Wu[1, 2, 3], Yingying Zhang[1, 2, 3], Xiangbin Yao[1, 2, 3], Pipeng Xing[1, 2, 3], Baoling Cui[1, 2, 3], Xiangru Tang[1, 2, 3#]

[1] *State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, College of Agriculture, South China Agricultural University, Guangzhou 510642, China*

[2] *Scientific Observing and Experimental Station of Crop Cultivation in South China, Ministry of Agriculture and Rural Affairs, Guangzhou 510642, China*

[3] *Guangzhou Key Laboratory for Science and Technology of Fragrant Rice, Guangzhou 510642, China*

Highlights

- Partial organic fertilizer substitution and water-saving irrigation significantly reduced CH_4 emissions and global warming potential, but increased N_2O emissions.
- Partial organic fertilizer substitution and water-saving irrigation affected CH_4 and N_2O emissions by altering the structure of microbes and the activities of key enzymes in metabolic pathways of CH_4 and nitrogen.
- Partial organic fertilizer substitution and water-saving irrigation significantly increased nitrogen use efficiency, yield, and 2-AP content.

Abstract

As the global leader in rice production, China's paddy fields contribute substantially to greenhouse gas emissions through methane (CH_4) and nitrous oxide (N_2O) releases. Aromatic rice cultivation practices have been optimized to enhance the aroma, so the relationship between its cultivation and greenhouse gas emissions from paddy fields is unclear. To investigate how aroma-enhancing cultivation practices drive microbial community dynamics in aromatic rice paddies and their implications for greenhouse gas emissions, a two-year experiment in five ecological locations (Xingning, Nanxiong, Conghua, Luoding, and Zengcheng) compared two farming practices: partial organic substitution for inorganic fertilizers combined with water-saving irrigation (IOF+W) and traditional cultivation (CK). The CH_4 and N_2O emissions, soil microbial composition and function, global warming potential (GWP), nitrogen use efficiency, yield, and the content of 2-acetyl-1-pyrroline (2-AP) were measured and analyzed. The main purpose was to investigate the impact of IOF+W on CH_4 and N_2O emissions and their relationship with soil microorganisms. The results showed that IOF+W significantly reduced CH_4 emission fluxes and totals (36.95%) and GWP (31.29%), while significantly increasing N_2O emission fluxes and totals (14.82%). The soil microbial community structure was reshaped by the IOF+W treatment, which suppressed methanogens but enhanced the abundances of nitrifying and denitrifying bacteria. Key enzymatic activities involved in CH_4 production, such as methyl-coenzyme M reductase, formylmethanofuran dehydrogenase, and methyltransferase, decreased. In contrast, the activity of the key CH_4-oxidizing enzyme methanol dehydrogenase increased. This shift led to an overall attenuation of the CH_4 production metabolism while enhancing the CH_4 oxidation metabolism. In addition, the activities of pivotal enzymes involved in denitrification and nitrification were improved, thus enhancing nitrogen nitrification and denitrification metabolism. Moreover, the IOF+W treatment significantly increased nitrogen use efficiency (47.83%), yield (14.77%), and 2-AP content (13.78%). Therefore, the IOF+W treatment demonstrated good efficacy as a sustainable strategy for achieving productive, green, resource-efficient, and premium-quality aromatic rice cultivation in South China.

Keywords: greenhouse gas, soil microbial composition, 2-AP, water-saving irrigation, nitrogen use efficiency

Received 13 December 2024; Received in revised form 23 February 2025; Accepted 19 May 2025; Available online 9 June 2025
Ligong Peng, E-mail: plg@stu.scau.edu.cn; #Correspondence Xiangru Tang, Tel/Fax: +86-20-85280021, E-mail: tangxr@scau.edu.cn
* These authors contributed equally to this study.

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2025.06.012

1. Introduction

Agricultural activities contribute nearly 24% of total global greenhouse gas emissions (IPCC 2019). Rice paddies serve as the primary emission sources for three major greenhouse gases: methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2). Although CO_2 emissions from paddy fields are far higher than those of CH_4 and N_2O, the global warming potentials (GWP) of CH_4 and N_2O are 34 and 298 times greater than CO_2, respectively, and they contribute to over 16 and 7% of global warming (Han *et al.* 2023). The CH_4 and N_2O in the atmosphere primarily originate from farmland systems, with paddy fields being the primary emission sites for both (Jiang *et al.* 2018). The CH_4 emissions from rice paddies constitute about 33% of global CH_4 releases, while their N_2O contributions are approximately 69% of worldwide totals (Schreiber *et al.* 2012). Therefore, in addition to focusing on CO_2 emissions, it is equally crucial to address CH_4 and N_2O emissions for achieving greenhouse gas reductions in paddy fields. With the rapid growth of the global economy and population, the gradual reductions in available arable land and water resources, coupled with further environmental degradation (Yang 2015), realizing green and efficient rice production is of paramount importance for China's food security and sustainable agricultural development.

As the world's largest rice producer, China accounts for 20% of the total rice cultivation area globally and approximately 30% of the world's rice output (FAO 2023). China's paddy fields contribute approximately 30% of the global CH_4 emissions from paddy fields annually (Yan *et al.* 2009), while the N_2O emissions from paddy fields account for roughly 11% of the world's agricultural N_2O emissions (Tang *et al.* 2022). Rice paddies have become a major contributor to China's greenhouse gas production, so emission reductions are necessary to address climate change and restore the C-nitrogen (N) cycle equilibrium in agricultural ecosystems.

As living standards continue to improve, the demand for high-quality rice is growing. Known for its distinctive and intense aroma, aromatic rice is regarded as a delicacy among rice varieties (Shan *et al.* 2015). Aromatic rice currently represents an estimated 15–18% of global rice trade volumes, with both market prices and consumer demand demonstrating consistent annual growth (Hashemi *et al.* 2013). Given the higher profits associated with cultivating aromatic rice (Qi J Y *et al.* 2023), many regions in Guangdong Province have embraced the large-scale cultivation of this variety. The aroma intensity is the most crucial evaluation metric for aromatic rice, with a stronger aroma translating into higher value. 2-Acetyl-1-pyrroline (2-AP) is the characteristic aroma compound responsible for varying degrees of aroma intensity (Luo *et al.* 2020; Luo *et al.* 2024). The concentration of 2-AP demonstrates significant responsiveness to cultivation techniques, particularly fertilization regimes and water management strategies. According to previous studies, appropriate fertilization rates, fertilizer application ratios, and mild drought stress can all contribute to augmenting 2-AP levels while improving yield performance in aromatic rice cultivars (Imran *et al.* 2023).

Research has shown that the production of CH_4 and N_2O in paddy fields is primarily determined by soil microbial activity, and the microbial communities are particularly responsive to agricultural management, including both inorganic and organic fertilization as well as the water regime (Qi J Y *et al.* 2023). The cultivation methods for enhancing the aroma of aromatic rice are different from conventional cultivation methods. Studies have shown that optimized NPK fertilization (including application rates and nutrient ratios) combined with water-efficient irrigation practices can substantially enhance grain productivity and 2-AP accumulation in aromatic rice varieties (Imran *et al.* 2023). Moreover, these agricultural practices exert substantial impacts on CH_4 and N_2O emissions in rice cultivation systems (Guo *et al.* 2019; Bhatia *et al.* 2023). Zhang *et al.* (2023) and Wu Q G *et al.* (2022) pointed out that water-saving irrigation in rice cultivation significantly reduces CH_4 emissions but increases N_2O emissions. Both the ratio (the NPK ratio) and amount of fertilizer significantly regulate N_2O and CH_4 emissions from rice paddies (Yang *et al.* 2024). A higher proportion of nitrogen fertilizer can enhance nitrification and denitrification processes, thereby increasing N_2O emissions. An appropriate nitrogen fertilizer ratio can indirectly influence rhizosphere organic carbon and oxygen levels by affecting rice root growth, which can also regulate the activities of methanogens and methanotrophs, ultimately impacting CH_4 emissions (Li *et al.* 2022). However, excessive nitrogen application may cause soil acidification, indirectly promoting CH_4 emissions (Dai *et al.* 2023). While the proportions of phosphorus and potassium fertilizers have a relatively minor direct impact on N_2O and CH_4 emissions, they can influence these emissions indirectly by affecting rice plant vitality, soil structure, and aeration (Yang *et al.* 2024).

The amount of fertilizers applied determines soil nutrient levels, thereby influencing microbial activity and greenhouse gas emissions (Yan *et al.* 2005). For varying N application amounts, a single drying event is more suitable for N application rates below 115 kg ha^{-1}, while multiple drying events are advantageous in reducing greenhouse gas emissions at higher N application rates (≥115 kg ha^{-1}), with the mitigation effect diminishing as the N application rate increases (Wu Q G *et al.* 2022). Excessive application of N fertilizer can lead to greater N_2O emissions from paddy fields (Xu *et al.* 2023; Guo *et al.* 2024). A meta-analysis by Zhang *et al.* (2024) (n=172) found that both inorganic and organic fertilizers significantly improved rice yield, while N fertilizer, organic fertilizer, and water-saving irrigation all increased N_2O emissions from rice paddies. Applying organic fertilizer alone significantly increases CH_4 emissions, while water-saving irrigation significantly reduces CH_4 emissions. Combining inorganic and organic fertilizers often has a better effect in regulating soil microbial structure and abundance (Zhao *et al.* 2016). Furthermore, water-saving irrigation can help coordinate the microbial changes induced by chemical and organic fertilization in rice paddies. Regarding the impact on CH_4 and N_2O related bacteria in rice paddies, inorganic fertilizers, organic fertilizers, and irrigation conditions primarily influence nitrifying bacteria, denitrifying bacteria, methanogens, and methanotrophs by affecting rice plant

growth, the availability of metabolic substrates (such as nitrogen sources and organic carbon), and the soil oxygen level (Wu Q G *et al.* 2022; Dai *et al.* 2023; Yang *et al.* 2024).

To enhance both the productivity and aroma of aromatic rice, an adequate nutrient supply must be ensured during cultivation. Consequently, reducing chemical fertilizer usage while incorporating organic amendments and water-saving irrigation methods constitutes a viable strategy for boosting aromatic rice production and quality while reducing greenhouse gas emissions. Nevertheless, few studies have systematically investigated the distinctive cultivation techniques that enhance the aroma in aromatic rice varieties, particularly their impacts on the abundance, composition, and function of soil microorganisms in rice fields, as well as their correlations with greenhouse gas emissions. In addition, the vast geographical area of South China encompasses significant ecological diversity. To ensure the representativeness and adaptability of the treatment and facilitate the promotion of green cultivation practices for aromatic rice that enhance both yield and aroma, this study selected five ecologically distinct sites for a field experiment. At those sites, this study implemented a treatment of partial organic substitution for inorganic fertilizers combined with water-saving irrigation and analyzed the mechanisms of CH_4 and N_2O emissions, with the aim of providing a theoretical and practical basis for reducing greenhouse gas emissions, saving fertilizer, increasing yield, and enhancing the aroma of aromatic rice. Specifically, this study systematically investigated how the IOF+W treatment influenced bacterial community structure, abundance, and functional metabolism in the aromatic rice cultivation systems, while clarifying its association with greenhouse gas emissions. A complete assessment of the influence of IOF+W on aromatic rice soil ecosystems demands accurate microbial community data, including the structure, abundance, and metabolic characteristics. Metagenomic analysis offers significantly enhanced characterization of microbial diversity compared to traditional 16S/18S rRNA sequencing methods (Li *et al.* 2023).

Our expectation was that the partial substitution of inorganic fertilizers with organic fertilizers and water-saving irrigation would increase yield, aroma, and resource use efficiency, while also reducing greenhouse gas emissions. The mitigation of greenhouse gas emissions stems not merely from modifications in the cultivation environment, but also from transformations in the soil microbial community structure and functionality.

2. Materials and methods

2.1. Experimental details

The tested variety was the locally dominant high-quality aromatic rice cultivar “19 Xiang” (19X), with a total growth period of 114–117 days. The experimental trials were conducted in 2023 and 2024 at multiple research locations of South China Agricultural University located in Xingning (XN) City (115°13′E, 24°16′N), Nanxiong (NX) City (114°38′E, 25°16′N), Conghua (CH) District (113°45′E, 23°33′N), Luoding (LD) City (111°58′E, 22°38′N), and Zengcheng (ZC) District (113°34′E, 23°24′N) in Guangdong Province, China. These selected sites collectively encompass the predominant soil and climatic conditions characteristic of South China’s primary aromatic rice growing regions. The research areas share a subtropical monsoon climate regime with predominantly clay soils in the plow layer (0–20 cm). The essential physical and chemical properties of the soil are provided in Appendix A.

Each ecological site employed two management methods: partial organic substitution for inorganic fertilizers combined with water-saving irrigation (IOF+W) and traditional cultivation (CK). The IOF+W treatment included the application of 157.5 kg N, 42 kg P_2O_5, and 63 kg K_2O per hectare, along with an additional 105 kg of organic fertilizer (dry matter of the organic fertilizer), maintaining the ratio of N:P_2O_5:K_2O:organic fertilizer at 15:4:6:10. Water-saving irrigation was applied, involving shallow water during seedling establishment and controlled soil drying during the tillering, panicle initiation, and grain filling stages. Once the soil water potential at the 5 cm depth dropped to −20±5 kPa, a 1–2 cm shallow irrigation was applied, followed by natural drying to maintain the water-saving conditions. During other growth stages, a water layer of 2–3 cm was maintained, and excess precipitation was drained to maintain controlled water levels. In the CK treatment, fertilizers were applied at rates of 170 kg N, 42.5 kg P_2O_5, and 127.5 kg K_2O per hectare, maintaining an N:P_2O_5:K_2O ratio of 20:5:15, with deep water irrigation maintained at a water depth of ≥5 cm. The N, phosphorus (P), and potassium (K) fertilizers used were urea (containing 46% N), calcium superphosphate (containing 12% P_2O_5), and potassium chloride (containing 60% K_2O), respectively. The organic fertilizer used in this study was processed pig manure, which was air-dried, mechanically crushed, passed through an 8-mesh sieve, and co-applied with the inorganic fertilizers. Both cultivation systems employed split fertilizer applications with a 5:2 ratio between the basal and tillering fertilizers. The basal fertilizer was mechanically side-deep placed (10 cm depth) during transplanting, while the tillering dose was surface-broadcast at the tillering stage. The IOF+W treatment represented a 19.7% reduction in inorganic fertilizer usage compared to CK, comprising reductions of 7.4% for N, 1.2% for P_2O_5, and 50.6% for K_2O.

A completely randomized block design was employed, with each block covering 100 m^2 (5 m×20 m) and replicated three times at each ecological site (totaling six blocks). Adjacent plots were separated by small ridges (30 cm in both height and width) to maintain independent irrigation and drainage systems. Other field management measures were consistent across the two cultivation management systems. Prior to sowing, seeds underwent a 24-h soaking period followed by a 12-h incubation at a constant 34°C. The germinated seeds were then placed in PVC seedling trays for raising the seedlings. Fifteen days later, mechanical transplanters were used to transfer the seedlings to paddy fields. In 2023, seedlings were raised on March 13th, transplanted on April 1st, and harvested on July 13th. In 2024, seedlings were raised on March 15th, transplanted on April 2nd, and harvested on July 15th.

2.2. Determination of greenhouse gas emission parameters

A rectangular static chamber (0.34 m×0.34 m×1.1 m) with a base (0.36 m×0.36 m×0.20 m) was employed to enclose a group of rice plants (Appendix B). The system incorporated an automated controller for periodic collection of CH_4 and N_2O emissions from the paddy fields. A 12V miniature fan was installed within the chamber to ensure complete gas homogenization. During sampling events, two temperature sensors simultaneously monitored the chamber air and soil temperatures. The internal chamber pressure was recorded by pressure sensors. Chamber bases were pre-installed at the soil surface in each experimental block prior to rice transplantation. Samples were obtained at 20-day intervals during mid-morning hours (09:00–12:00) under clear-sky conditions. During chamber base assembly, the base grooves were carefully filled with water to ensure proper sealing. The chamber was subsequently connected to a gas sampling bag, and 200 mL samples were collected at 10-min intervals (four samples total over 30 min). After collection, the gas valve bag was immediately sealed to prevent leakage, while concurrent temperature and air pressure measurements were recorded. Gas sample analysis was performed using an Agilent 7890A GC System (Agilent Technologies, Wellington, DE, USA). CH_4 was quantified by an FID maintained at 250°C, with gas flow rates set to 50 mL min^{-1} (H_2), 500 mL min^{-1} (air), and 2 mL min^{-1} (make-up gas). An ECD detector operating at 300°C was used for N_2O detection, with a make-up gas flow rate of 2 mL min^{-1}. The column was maintained at 50°C, and data were collected at 5 Hz. The reference standard gas was obtained from the National Center for Standard Reference Materials of China.

The greenhouse gas flux was calculated using the formula (Han *et al.* 2023):

$$F=\rho\times[273/(273+T)]\times H\times dc/dt\times p/p_0 \quad (1)$$

The flux calculation incorporated the parameters: F, greenhouse gas flux (mg m^{-2} h^{-1}); ρ, gas density (CH_4= 0.714 kg m^{-3}; N_2O=1.964 kg m^{-3} at STP); H, net chamber height (m); dc/dt, concentration change rate (μL L^{-1} h^{-1}); T, in-chamber temperature (°C); and p/p_0, pressure ratio (in-chamber/standard atmosphere).

The formula for calculating total greenhouse gas emissions was:

$$c=\sum_{i=1}^{n}\left(\frac{F_i+F_{i+1}}{2}\right)\times(t_{i+1}-t_i)\times 24 \quad (2)$$

where c represents the total greenhouse gas emissions (kg ha^{-1}), F_i and F_{i+1} are two adjacent gas emission fluxes (kg ha^{-1} h^{-1}), t_i and t_{i+1} are two adjacent sampling times, and 24 is the conversion coefficient between hours and days.

Global warming potential (GWP) represents the cumulative radiative forcing of a unit mass of a greenhouse gas over a specified time horizon. Greenhouse gas intensity (GHGI) refers to the GWP generated by the production of rice per unit mass. Using CO_2 as the reference gas, emissions of CH_4 and N_2O can be converted into CO_2 equivalents (CO_2-eq) through their GWP values. On a 100-year time horizon, the GWPs of CH_4 and N_2O per unit mass are 34 and 298 times that of CO_2, respectively (Han *et al.* 2023). The formulas for calculating GWP and GHGI were:

$$GWP=34\times FCH_4+298\times FN_2O \quad (3)$$

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

where GWP is global warming potential (kg ha^{-1}), FCH_4 is the total emission of CH_4 (kg ha^{-1}), FN_2O is the total emission of N_2O (kg ha^{-1}), GHGI is greenhouse gas intensity (kg kg^{-1}), GWPs is the sum of GWP (kg ha^{-1}), and Y is the total yield (kg ha^{-1}).

2.3. Properties and metagenomic sequencing of paddy soils

During the rice flowering period in June 2024, composite soil samples (1 kg/block) were obtained from the 0–20 cm rhizosphere zone (2–10 cm from the root center) using a sterile soil sampler with a diameter of 3 cm. Following the five-point sampling, samples were screened to remove roots and debris and either stored at −80°C for metagenomic sequencing analysis or air-dried for physicochemical analysis.

The soil organic carbon (SOC) content was determined using the potassium dichromate oxidation method. The concentration of dissolved organic carbon (DOC) in the extract after distilled water extraction was determined by colorimetry (Qi J Y *et al.* 2023). The concentrations of NO_2^-, NO_3^- and NH_4^+ in the extraction solution after 2 mol L^{-1} KCl extraction were determined by spectrophotometry. The total nitrogen (TN) concentration was quantified using a TOC analyzer (Multi N/C 3100 TOC, Analytik, Jena, Germany). Total phosphorus (TP) content was quantified through $HClO_4$ digestion, whereas total potassium (TK) content was determined by NaOH fusion followed by flame photometric detection. SM content was determined by measuring the mass loss after oven-drying fresh soil samples at 105°C to a constant weight, and pH was measured at a soil-to-water ratio of 1:2.5 (w/v) using a calibrated FE20 pH meter (FE20, Mettler-Toledo Instruments, China).

The Mag-Bind® Soil DNA Kit (Omega Bio-Tek, USA) was employed for DNA extraction. Post-extraction quality control included concentration assessment and purity evaluation through 1% agarose gel electrophoresis. DNA was fragmented to approximately 400 bp using a Covaris M220 System (Gene Company Limited, USA), after which paired-end (PE) libraries were prepared employing the NEXTFLEX Rapid DNA-Seq (Bioo Scientific, USA). Following library preparation, the samples underwent metagenomic sequencing using the Novaseq 6000 platform (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China) as previously described (Dai *et al.* 2017). The metagenomic sequencing data were submitted to the NCBI Short Read Archive database (accession no. SRP523021).

2.4. Determination of nitrogen use efficiency in aromatic rice

For each treatment, a nitrogen-free control (without any fertilizer application) was established adjacent to the main plots. At maturity, three uniformly growing plants were randomly selected from each treatment. The roots were removed, and the aboveground parts were placed in an oven at 105°C for 30 min to deactivate enzymes, followed by drying

at 80°C until a constant weight was achieved to determine the aboveground dry matter weight. A portion of the mature plants was uniformly collected, ground, and dried at 75°C to a constant weight. Approximately 0.2 g of the ground sample was digested using concentrated H_2SO_4-H_2O, and the nitrogen content in the plant dry matter was determined using the Kjeldahl method. Nitrogen use efficiency was calculated using the following formulas (Hou *et al.* 2019):

Nitrogen accumulation (kg ha^{-1})=Dry matter accumulation of rice plants×Nitrogen content in dry matter (5)

Nitrogen recovery efficiency (%)=(Nitrogen accumulation in the fertilized treatment−Nitrogen accumulation in the nitrogen-free treatment)/Total nitrogen application rate×100 (6)

Nitrogen agronomic efficiency (kg kg^{-1})=(Yield of the fertilized treatment−Yield of the nitrogen-free treatment)/Total nitrogen application rate (7)

Nitrogen partial factor productivity (kg kg^{-1})=Yield/Total nitrogen application rate (8)

2.5. Determination of yield and 2-AP content in aromatic rice

During maturity, five rice plants per block were randomly selected for the determination of effective panicles, and then representative samples were transported to the laboratory for measurements of spikelet number per panicle, seed setting rate, and grain weight. Yield assessment involved harvesting per unit area, with three replicates per treatment. Grain moisture content was determined with a moisture meter (PM-8188 A, Japan), and final yields were standardized to 14% moisture.

During aromatic rice harvesting, ten panicles exhibiting uniform growth were collected and immediately stored in an ultra-low temperature freezer. The panicle samples were mechanically ground, with 2 g portions accurately weighed into 20 mL glass vials equipped with sealing rings. Chromatography-grade dichloromethane (10 mL) was introduced into each vial, which was sealed prior to 4 h of ultrasonication at 40 Hz and 40°C. Following extraction, the sample was cooled to ambient temperature before adding anhydrous Na_2SO_4 for residual water removal. A 1 mL portion of the solution was filtered through an organic syringe filter membrane (pore size 0.22 μm) into headspace vials using sterile syringes. The internal standard 2,4,6-trimethylpyridine (TMP, 0.2 mg kg^{-1}) was added, and samples were immediately analyzed by gas chromatography-mass spectrometry (GC-MS) analysis. The 2-AP concentration was determined using a GC-MS QP 2010 plus (Shimadzu Corporation, Japan) with TMP as an internal reference standard, following the calibration methodology described by Luo *et al.* (2023).

2.6. Data analysis

A three-way analysis of variance (ANOVA) was performed to analyze the effects of years, ecological locations, and treatments. The least significant difference (LSD) test was applied to assess significant variations (P<0.05) between treatments within each site. Data are presented as means of triplicate measurements, and figures were generated using Origin 2023b (OriginLab, Northampton, MA, USA). Following metagenomic data acquisition, species and functional variations were examined by generating bacterial gene sets. The Kruskal-Wallis H test and Wilcoxon rank-sum test were employed to evaluate significant differences in microbial composition, enzymes, and metabolic pathways among the treatments. For multiple testing, P-values were adjusted using the false discovery rate (FDR) correction method. Special attention was given to parameters showing statistical significance after FDR correction. The R package 2.4.3 was used to perform various statistical analyses such as principal component analysis (PCA), principal coordinate analysis (PCoA), random forest modeling, and non-metric multidimensional scaling (NMDS). Data analysis was performed *via* the web-based Majorbio Cloud Platform (accessible at www.majorbio.com).

3. Results

3.1. Yield and 2-AP content

The yield and yield components all exhibited significant responses (P<0.05) to the main effects of year, ecological sites, and treatments, as well as their interactions. The IOF+W treatment at the five ecological sites increased yield by enhancing effective panicles, spikelets per panicle, and seed setting rate, with yields significantly higher (P<0.05) than those of the CK. In 2023, the yield increase ranged from 10.79 to 19.52%, with an average increase of 15.32%; and in 2024, the yield increase ranged from 10.54 to 21.04%, with an average increase of 14.77%. Among all ecological sites, XN achieved the highest yield (8.92 t), whereas ZC had the lowest yield (6.25 t). Statistical analysis revealed that the 2-AP content was significantly influenced (P<0.05) by year, ecological sites, and treatments. The effects of different years, ecological sites and treatments on 2-AP content reached significant levels (P<0.05) (Table 1). Compared with CK, the IOF+W treatment significantly (P<0.05) increased the 2-AP content in rice. In 2023 (Fig. 1-A), the 2-AP contents increased by 12.29 to 18.22%, with an average increase of 15.67%; and in 2024 (Fig. 1-B), the 2-AP contents increased by 9.05 to 17.69%, with an average increase of 13.78%.

3.2. N use efficiency

The statistical analysis revealed significant impacts (P<0.05) of year, ecological sites, treatments, and their interactive effects on multiple measured parameters related to N use efficiency (Table 2). The dry matter accumulation, N accumulation, N recovery efficiency (NRE), N agronomic efficiency (NAE), and N partial factor productivity (NPFP) in the IOF+W treatment were significantly (P<0.05) higher than those in CK. Compared with CK, the NAE and NPFP in the IOF+W treatment increased by 31.27–57.66% and 17.12–32.36%, respectively. The XN, CH, and NX ecological sites exhibited higher NAE and NPFP. These results indicated that IOF+W can significantly enhance NAE and NPFP, with average increases of 47.83 and 23.70%, respectively.

3.3. CH_4 and N_2O emissions from rice paddies

As the growth cycle progressed, CH_4 emission fluxes

Table 1 Yield and yield components of aromatic rice under different ecological sites and treatments

Year (Y)	Ecological site (E)[1)]	Treatment (T)[2)]	Effective panicle number	Spikelets per panicle	Seed setting rate (%)	1,000-grain weight (g)	Grain yield (t ha^{-1})
2023	XN	IOF+W	18.20 a	186.05 a	87.12 a	19.88 b	8.90 a
		CK	12.20 b	151.68 b	75.12 b	23.46 a	7.90 b
	NX	IOF+W	19.80 a	200.92 a	80.62 a	19.47 a	8.73 a
		CK	16.60 a	155.71 b	68.26 b	20.51 a	7.88 b
	CH	IOF+W	14.60 a	220.68 a	66.40 a	20.57 a	7.94 a
		CK	11.40 b	165.01 b	62.81 a	19.34 a	6.65 b
	LD	IOF+W	19.60 a	158.32 a	74.47 a	19.83 a	8.04 a
		CK	14.80 b	130.89 b	70.84 a	17.56 b	6.73 b
	ZC	IOF+W	13.20 a	202.34 a	62.25 a	19.66 a	7.13 a
		CK	9.40 b	178.05 b	59.84 a	18.18 b	6.25 b
2024	XN	IOF+W	21.00 a	197.13 a	69.02 a	18.89 a	8.92 a
		CK	15.33 b	182.69 b	57.90 b	18.03 a	7.89 b
	NX	IOF+W	19.67 a	198.21 a	62.68 a	22.72 a	8.08 a
		CK	13.00 b	170.23 a	56.68 b	21.45 a	6.84 b
	CH	IOF+W	17.67 a	187.37 a	66.28 a	21.45 b	8.70 a
		CK	11.33 b	160.06 b	54.64 b	23.87 a	7.84 b
	LD	IOF+W	15.33 a	181.33 a	61.44 a	21.50 b	8.26 a
		CK	13.00 a	176.26 a	55.91 a	20.14 a	7.47 b
	ZC	IOF+W	16.33 a	181.49 a	57.80 a	20.98 a	8.02 a
		CK	9.33 b	172.75 a	51.09 b	19.66 a	6.63 b
ANOVA							
Y			ns	*	**	ns	**
E			**	*	**	**	**
T			**	**	**	ns	**
Y×E			*	**	*	**	**
Y×T			ns	*	**	ns	**
E×T			ns	*	**	**	ns
Y×E×T			ns	ns	*	**	ns

[1)] XN, NX, CH, LD, and ZC represent the ecological sites of Xingning, Nanxiong, Conghua, Luoding, and Zengcheng, respectively.
[2)] IOF+W and CK represent the inorganic fertilizer reduction combined with organic fertilizer and water-saving irrigation, and conventional cultivation, respectively.
The lowercase letters indicate significant differences (P<0.05) in cultivation methods within the same ecological site. * and ** indicate significant differences at P<0.05 and P<0.01, respectively, and ns indicates no significant difference.

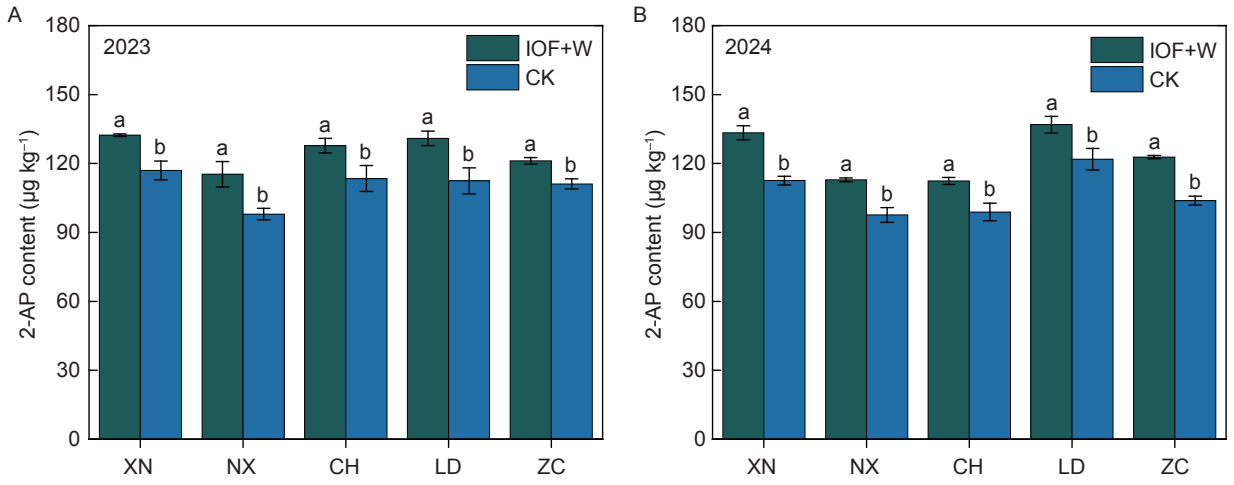


Fig. 1 2-AP content in aromatic rice under different ecological sites and treatments. XN, NX, CH, LD, and ZC represent the ecological sites of Xingning, Nanxiong, Conghua, Luoding, and Zengcheng, respectively. IOF+W and CK represent the inorganic fertilizer reduction combined with organic fertilizer and water-saving irrigation, and conventional cultivation, respectively. The lowercase letters above the bars indicate significant differences (P<0.05) between different treatments within the same ecological site. Error bars represent standard errors (n=3).

exhibited a trend of first increasing and then decreasing, peaking during the booting stage and reaching the lowest point during the maturity stage. All five ecological sites demonstrated reduced CH_4 emission fluxes under the IOF+W treatment when compared to the CK (Fig. 2-A–E). The IOF+W treatment achieved significant (P<0.05) reductions in

Table 2 Nitrogen use efficiency of aromatic rice under different ecological sites and treatments

Year (Y)	Ecological site (E)[1)]	Treatment (T)[2)]	Dry matter accumulation (g)	Nitrogen accumulation (kg ha^{-1})	Nitrogen recovery efficiency (%)	Nitrogen agronomic efficiency (kg kg^{-1})	Nitrogen partial factor productivity (kg kg^{-1})
2023	XN	IOF+W	78.38 a	66.40 a	19.83 a	17.50 a	57.78 a
		CK	67.50 b	47.11 b	7.07 b	11.17 b	46.94 b
	NX	IOF+W	74.83 a	90.11 a	22.68 a	18.06 a	54.83 a
		CK	64.17 b	78.83 b	14.48 b	12.29 b	46.81 b
	CH	IOF+W	74.16 a	115.75 a	35.02 a	15.95 a	49.01 a
		CK	60.74 b	99.37 a	22.98 a	11.41 b	37.91 b
	LD	IOF+W	71.40 a	61.93 a	17.73 a	16.01 a	50.44 a
		CK	63.77 b	47.26 b	7.85 b	10.15 b	38.11 b
	ZC	IOF+W	59.66 a	59.77 a	20.93 a	13.30 a	42.95 a
		CK	45.25 b	41.61 b	8.78 c	10.13 b	36.03 b
2024	XN	IOF+W	62.22 a	175.14 a	61.26 a	18.51 a	57.33 a
		CK	45.97 b	125.54 b	27.78 b	13.33 b	45.84 b
	NX	IOF+W	64.09 a	124.16 a	36.12 a	16.52 a	51.64 a
		CK	46.15 b	82.55 b	9.06 c	10.64 b	41.14 c
	CH	IOF+W	63.83 a	136.78 a	34.53 a	24.33 a	57.31 a
		CK	57.86 b	128.44 a	27.29 b	16.65 b	48.22 b
	LD	IOF+W	51.89 a	178.92 a	61.40 a	19.05 a	52.10 a
		CK	43.97 b	126.90 b	26.48 b	12.38 b	43.81 b
	ZC	IOF+W	52.05 a	79.37 a	25.49 a	23.47 a	51.46 a
		CK	34.01 b	48.76 b	5.56 b	15.46 b	40.28 b
ANOVA							
Y			**	*	*	*	ns
E			**	**	**	ns	**
T			**	**	**	**	**
Y×E			**	**	**	**	**
Y×T			ns	**	**	ns	ns
E×T			**	ns	ns	ns	ns
Y×E×T			**	ns	ns	**	**

[1)] XN, NX, CH, LD, and ZC represent the ecological sites of Xingning, Nanxiong, Conghua, Luoding, and Zengcheng, respectively.
[2)] IOF+W and CK represent the inorganic fertilizer reduction combined with organic fertilizer and water-saving irrigation, and conventional cultivation, respectively.
The lowercase letters indicate significant differences (P<0.05) in cultivation methods within the same ecological site. * and ** indicate significant differences at P<0.05 and P<0.01, respectively, and ns indicates no significant difference.

total CH_4 emissions across all five ecological sites compared to the CK (Fig. 2-F), with reductions ranging from 26.55 to 47.69% (average: 36.95%) in 2023 and from 16.92 to 28.08% (average: 23.21%) in 2024. Among the sites, ZC had the highest total CH_4 emissions, while LD and CH showed the most and least significant reductions under the IOF+W treatment, respectively.

The N_2O emissions followed an opposite trend of decreasing initially and then increasing throughout the growth cycle, peaking during the maturity stage and reaching their lowest levels during the booting and flowering stages. At all five ecological sites, the IOF+W treatment resulted in higher N_2O emission fluxes compared with CK (Fig. 3-A–E). In addition, the total N_2O emissions under the IOF+W treatment significantly (P<0.05) increased at all five sites compared with CK (Fig. 3-F), with increases ranging from 7.92 to 17.34% (average: 13.91%) in 2023 and from 7.79 to 21.63% (average: 14.82%) in 2024. Among all ecological sites, total N_2O emissions were the highest at XN and the lowest at ZC. Notably, the IOF+W treatment induced the strongest enhancement of N_2O emissions at NX, with LD showing the least response.

GWP was significantly affected (P<0.05) by the different ecological sites and treatments (Table 3). The GWP of rice paddies was primarily contributed by CH_4 emissions, accounting for 98.01–99.37% of the total, while N_2O contributed a relatively small portion (0.63–1.99%). Both GWP and GHGI reached their highest levels at ZC and their lowest at CH among the ecological sites. Compared with CK, the IOF+W treatment across all five ecological sites significantly (P<0.05) reduced GWP (26.85–43.76%) and GHGI (34.64–51.84%), with average reductions of 31.29 and 40.30%, respectively.

3.4. Soil microbial diversity

Based on the metagenomic NR annotation results, significant differences (P<0.05) were observed in the α-diversity indices of soil microbial communities, including Chao, Shannon, and Simpson, between the two different treatments. Specifically, the IOF+W treatment effectively reduced the species abundance (Chao index) of soil microorganisms (Fig. 4-A). The dominant bacteria at the phylum level included Proteobacteria, Chloroflexi, Actinobacteria, Acidobacteria, Planctomycetota, Nitrospirae, and Euryarchaeota (Fig. 4-B). Venn analysis revealed 5,938 shared species among all treatments and ecological sites. Notably, the species composition differed between the IOF+W treatment and the

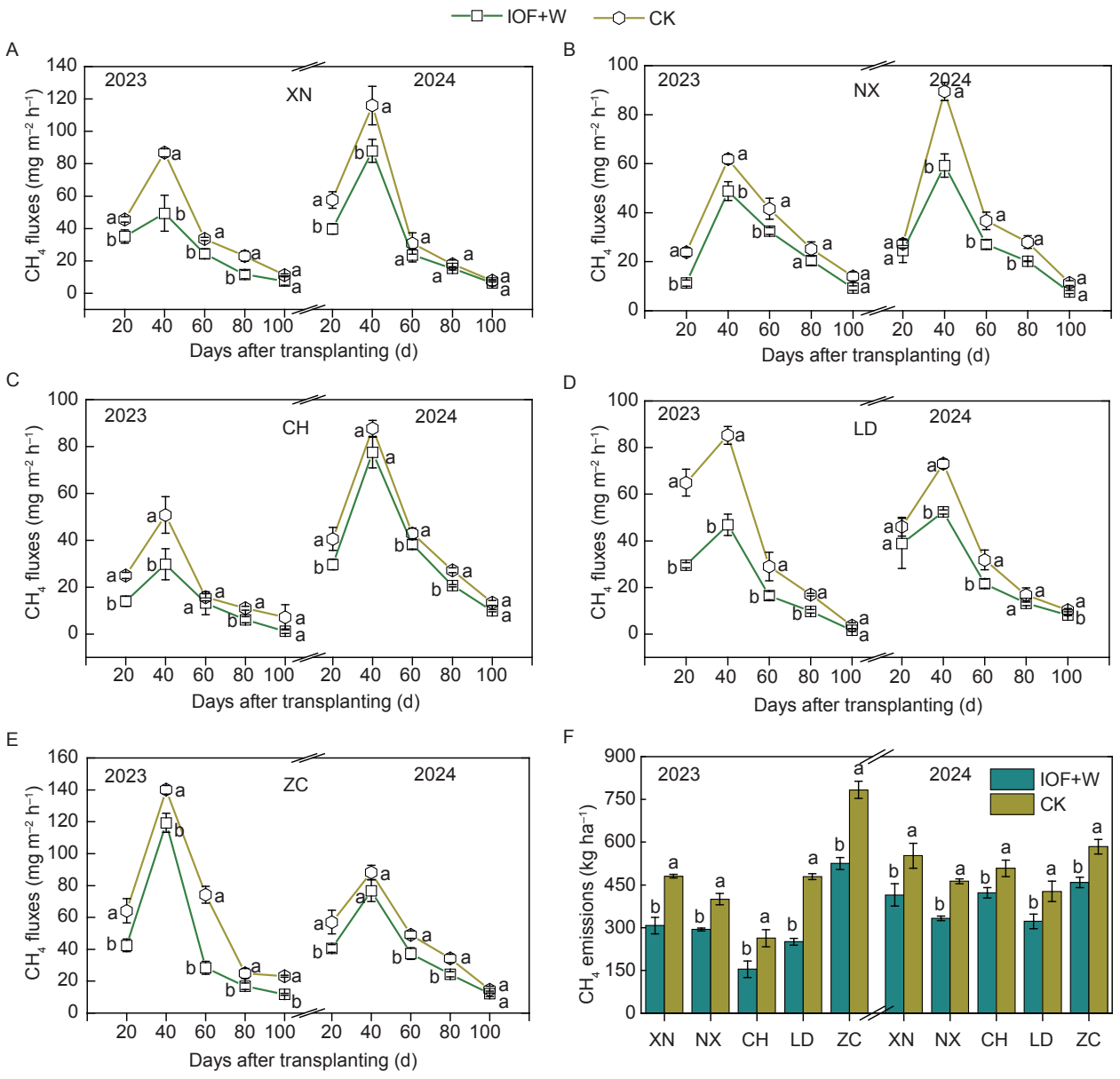


Fig. 2 CH_4 emission flux and total emissions in paddy fields under different ecological sites and treatments. XN, NX, CH, LD, and ZC represent the ecological sites of Xingning, Nanxiong, Conghua, Luoding, and Zengcheng, respectively. IOF+W and CK represent the inorganic fertilizer reduction combined with organic fertilizer and water-saving irrigation, and conventional cultivation, respectively. The lowercase letters above the lines and bars indicate significant differences (P<0.05) between different treatments within the same ecological site. Error bars represent standard errors (n=3).

CK, with 353 unique species in the IOF+W treatment and 288 unique species in CK (Fig. 4-C). Although no significant differences in β-diversity were detected between the treatments, the IOF+W treatment increased β-diversity in all ecological sites except XN (Fig. 4-D).

3.5. CH_4 and N_2O emissions-related microorganisms

An analysis of the species composition and differences in methanogens and methanotrophs revealed that the IOF+W treatment reduced the abundance of methanogens (Chao index) at all ecological sites except ZC (Fig. 5-A). Similarly, the IOF+W treatment reduced the abundance of methanotrophs (Chao index) at all ecological sites except XN (Fig. 5-B). PCA analysis showed that the compositions of methanogens and methanotrophs could be separated based on the different ecological sites and treatments in the PCA plot, with reasonable grouping (R≥0.599, P=0.001). The Anosim test indicated significant differences between groups, with higher credibility (P<0.05). An analysis of compositions of microbiomes (ANCOM) between the different treatments within the same ecological site showed that the abundances of Methanomicrobiales, Methanocellales, Methanosarcinales, Methanobacteriales, and Methanotrichales were significantly lower in the IOF+W treatment compared with CK (Fig. 5-C). The abundances of *Methyloceanibacter*, *Methylocystis*, and *Methylobacter* were lower in the IOF+W treatment than in CK, with *Methyloceanibacter* and *Methylobacter* showing significantly lower abundances. In contrast, the abundances

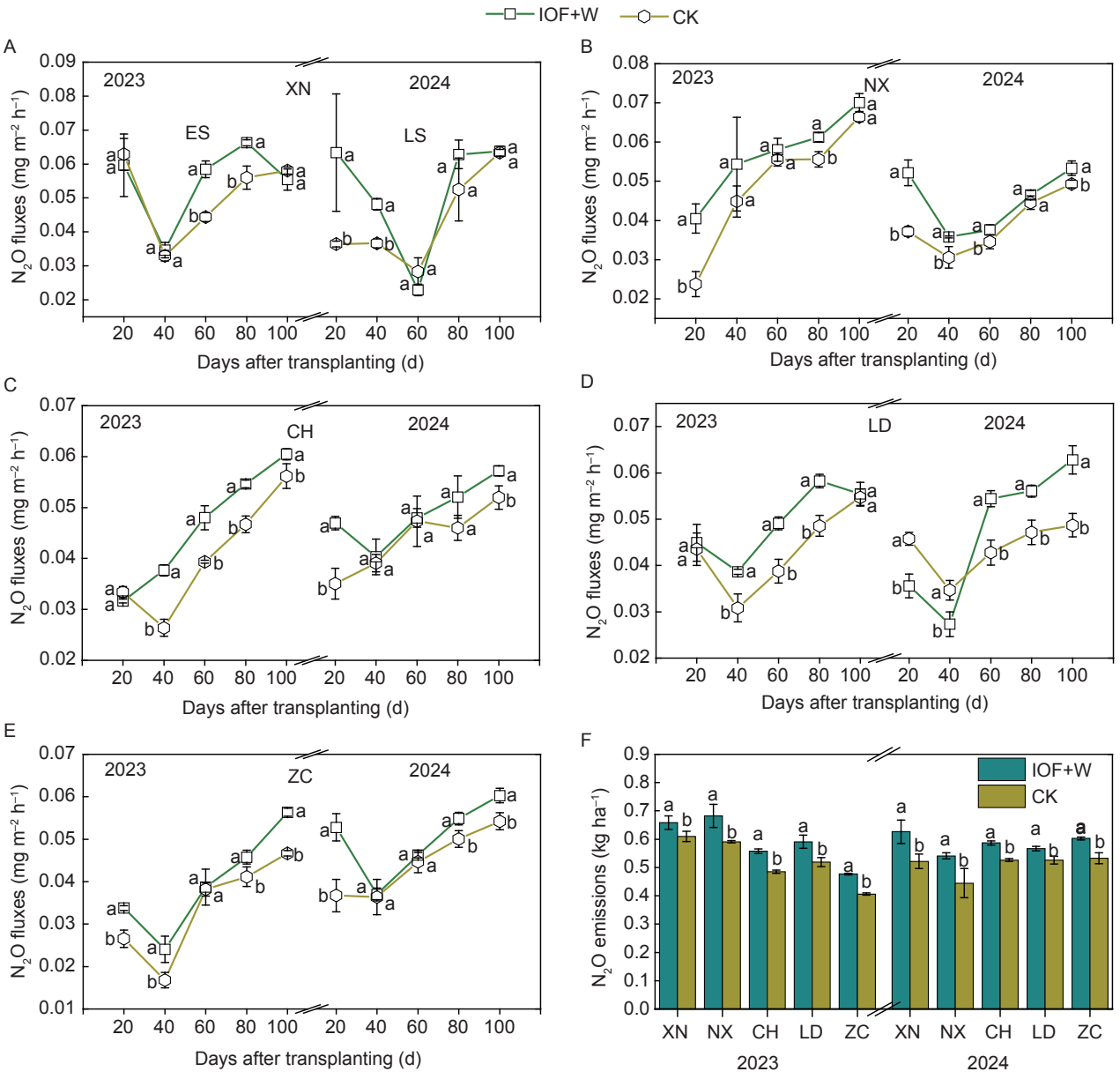


Fig. 3 N_2O emission flux and total emissions in paddy fields under different ecological sites and treatments. XN, NX, CH, LD, and ZC represent the ecological sites of Xingning, Nanxiong, Conghua, Luoding, and Zengcheng, respectively. IOF+W and CK represent the inorganic fertilizer reduction combined with organic fertilizer and water-saving irrigation, and conventional cultivation, respectively. The lowercase letters above the lines and bars indicate significant differences (P<0.05) between different treatments within the same ecological site. Error bars represent standard errors (n=3).

of *Methylosinus*, *Methylococcus*, and *Methylocella* were higher in the IOF+W treatment (Fig. 5-D). The correlation heatmap indicated significant positive correlations between Methanomicrobiales, Methanotrichales, and CH_4 emissions and DOC content. Methanocellales was significantly positively correlated with CH_4 emissions, while *Methylocystis* was significantly positively correlated with both CH_4 emissions and DOC content (Fig. 5-E). Random forest analysis showed that Methanocellales, Methanococcales, Methanomicrobiales, and Methanobacteriales were the main microorganisms contributing to CH_4 production, while *Methylocystis*, *Methyloceanibacter*, and *Methylobacter* were the main microorganisms involved in CH_4 oxidation (Fig. 5-F). In summary, the IOF+W treatment reduced the total abundance of methanogens primarily by reducing the abundances of Methanomicrobiales, Methanocellales, Methanosarcinales, Methanobacteriales, and Methanotrichales. The effect of the IOF+W treatment on methanotrophs was less pronounced, but the total abundance of methanotrophs decreased.

An analysis of the species composition and differences in nitrifying and denitrifying bacteria revealed that the IOF+W treatment significantly increased the abundances of nitrifying bacteria (Chao index) at the XN and CH ecological sites (Fig. 6-A). Similarly, the IOF+W treatment significantly increased the abundances of denitrifying bacteria (Chao index) at the XN, CH, and ZC ecological sites (Fig. 6-B).

Table 3 Warming potential of paddy fields under different ecological sites and treatments

Ecological site (E)[1]	Treatment (T)[2]	GWP (CH_4) (kg ha^{-1})[3]		GWP (N_2O) (kg ha^{-1})		GWPs (kg ha^{-1})[3]	Total yield (t ha^{-1})	Contribution to GWPs (%)		GHGI (kg kg^{-1})[3]
		2023	2024	2023	2024			CH_4	N_2O	
XN	IOF+W	8,600.79 b	11,613.02 b	179.80 a	171.00 a	20,564.61 b	18.13 a	98.29	1.71	1.13 b
	CK	13,446.37 a	15,462.03 a	166.60 a	142.38 b	29,217.38 a	15.66 b	98.94	1.06	1.87 a
NX	IOF+W	8,223.21 b	9,314.11 b	186.23 a	147.69 a	17,871.24 b	16.77 a	98.13	1.87	1.07 b
	CK	11,196.49 a	12,950.50 a	161.32 b	121.43 b	24,429.74 a	14.84 b	98.84	1.16	1.65 a
CH	IOF+W	4,308.88 b	11,820.72 b	152.27 a	160.18 a	16,442.05 b	16.75 a	98.10	1.90	0.98 b
	CK	7,362.75 a	14,227.88 a	132.35 b	143.73 b	21,866.71 a	14.53 b	98.74	1.26	1.51 a
LD	IOF+W	6,543.86 b	9,017.33 b	161.37 a	154.77 a	15,877.33 b	16.15 a	98.01	1.99	0.98 b
	CK	15,986.40 a	11,962.05 a	141.8 b	143.5 9b	28,233.83 a	13.82 b	98.99	1.01	2.04 a
ZC	IOF+W	14,704.14 b	12,832.90 b	130.17 a	164.57 a	27,831.78 b	14.90 a	98.94	1.06	1.87 b
	CK	23,957.02 a	16,356.87 a	110.93 b	145.51 b	40,570.33 a	12.91 b	99.37	0.63	3.14 a
ANOVA										
Y		**		ns						
E		**		**						
T		**		**						
Y×E		**		**						
Y×T		**		ns						
E×T		**		ns						
Y×E×T		**		ns						

[1] XN, NX, CH, LD, and ZC represent the ecological sites of Xingning, Nanxiong, Conghua, Luoding, and Zengcheng, respectively.
[2] IOF+W and CK represent the inorganic fertilizer reduction combined with organic fertilizer and water-saving irrigation, and conventional cultivation, respectively.
[3] GWP is global warming potential, GWPs is the sum of GWP and GHGI is greenhouse gas intensity.
The lowercase letters indicate significant differences (P<0.05) in cultivation methods within the same ecological site. * and ** indicate significant differences at P<0.05 and P<0.01, respectively, and ns indicates no significant difference.

PCA analysis showed that the composition of nitrifying and denitrifying bacteria could be separated based on the different ecological sites and treatments in the PCA plot, with reasonable grouping (R≥0.565, P=0.001). The Anosim test indicated significant differences between groups, with higher credibility (P<0.05). The ANCOM analysis showed that the IOF+W treatment increased the abundances of *Nitrospira*, *Nitrobacter*, *Nitrososphaera*, *Nitrosococcus* and *Nitrospirillum*, thereby enhancing the total abundance of nitrifying bacteria (Fig. 6-C). In addition, the IOF+W treatment increased the abundances of *Pseudomonas*, *Bacillus*, *Agrobacterium*, *Acinetobacter* and *Achromobacter*, contributing to an increase in the total abundance of denitrifying bacteria (Fig. 6-D). *Nitrospira*, *Nitrobacter*, *Nitrosococcus* and *Nitrospirillum* were significantly positively correlated with the NO_3^--N content, while *Acinetobacter* and *Agrobacterium* were significantly positively correlated with the TN and NO_2^--N contents (Fig. 6-E). Random forest analysis indicated that *Nitrososphaera*, *Nitrosococcus*, *Nitrospira*, *Thiobacillus*, *Paracoccus* and *Agrobacterium* were the main microorganisms involved in nitrification and denitrification (Fig. 6-F). In summary, the IOF+W treatment increased the abundances of both nitrifying and denitrifying bacteria.

3.6. Comparison of microorganism functions

The NMDS analysis of KEGG pathway level3 for methanogens and methanotrophs (Fig. 7-A) showed that the distributions of metabolic functions were separated under the different treatments, with distinct functional differences within the grouped treatments, indicating good representativeness of the grouping (stress<0.05). The PCoA analysis of KEGG pathway level3 for nitrifying and denitrifying bacteria (Fig. 7-B) indicated that the metabolic functions under different treatments were separated in the PCA plot based on ecological sites and treatments, suggesting a reasonable grouping (R=0.897, P=0.001). The Anosim test showed that the differences between groups were greater than within groups, with high reliability (P<0.05). The analysis of metabolic pathway differences revealed that compared with CK, the IOF+W treatment exhibited lower C metabolism and CH_4 metabolism but significantly enhanced N metabolism (Fig. 7-C). The differential analysis of KEGG enzymes showed that compared with CK, the IOF+W treatment significantly reduced the abundances of key enzymes involved in CH_4 production: formylmethanofuran dehydrogenase [EC:1.2.7.12], methyltransferase [EC:2.1.1.86], and methyl-coenzyme M reductase [EC:2.8.4.1], while significantly increasing the abundance of the key enzyme involved in CH_4 oxidation: methanol dehydrogenase [EC:1.1.2.10] (Fig. 7-D). This indicated that the IOF+W treatment effectively reduced CH_4 production and promoted CH_4 oxidation in paddy fields. These enzymes were ubiquitous in the CH_4 metabolic pathway, and there were significant differences in the CH_4 metabolic pathway between the different treatments. Compared with CK, the IOF+W treatment increased the abundances of key enzymes involved in denitrification: nitrite reductase [EC:1.7.5.1] [EC:1.7.2.1] [EC:1.7.1.15], and nitrification: ammonia monooxygenase [EC:1.14.99.39] and hydroxylamine oxidoreductase [EC:1.7.99.1] (Fig. 7-E). These enzymes were ubiquitous in the N metabolic pathway. The phenomenon

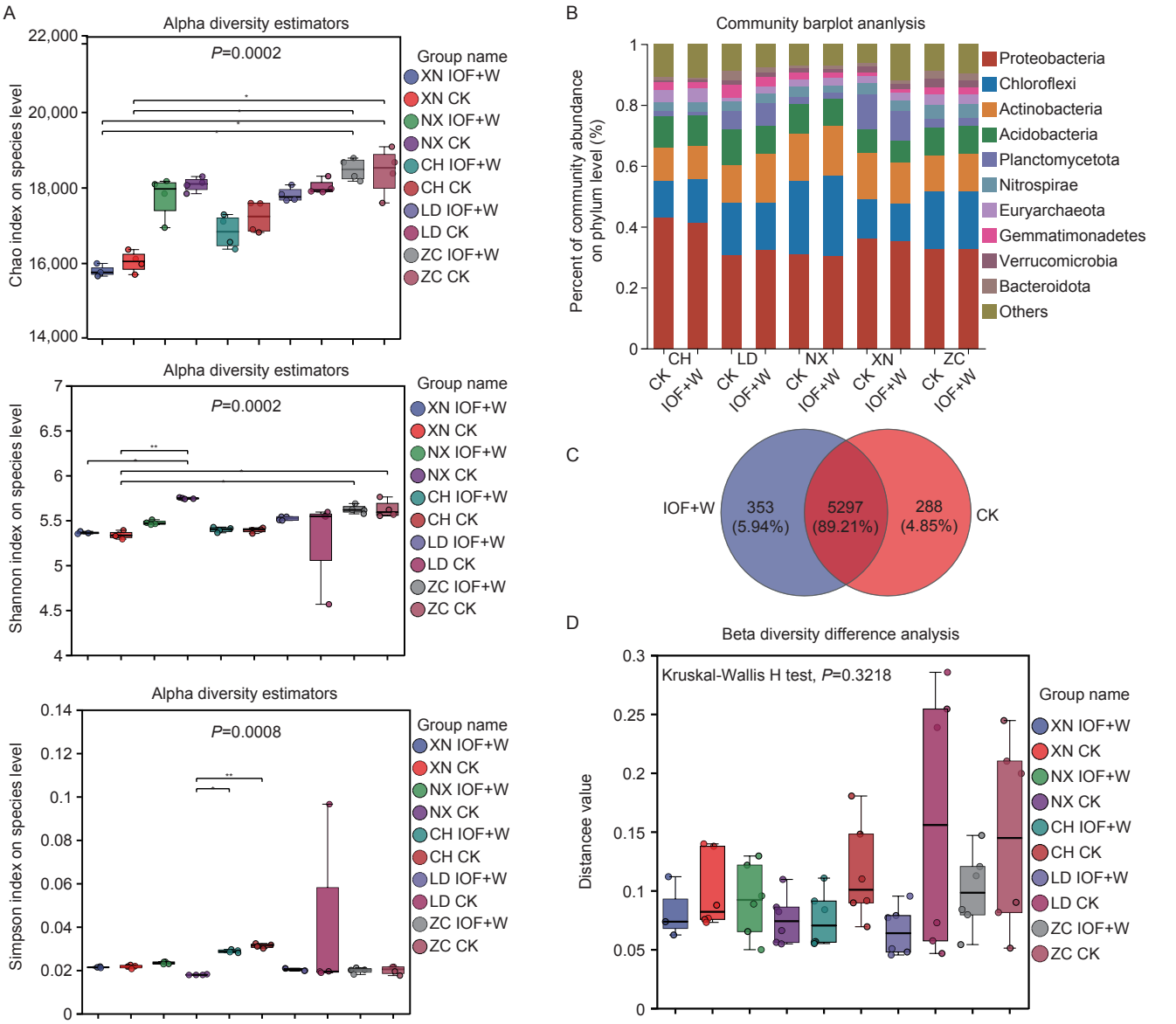


Fig. 4 Bacterial α-diversity and relative abundance in paddy soil under different ecological sites and treatments. A and B, the bacterial α-diversity (A) and relative abundance (B) at the phylum level, respectively. C, Venn diagram of the shared and unique species between the different treatments. D, β-diversity. XN, NX, CH, LD, and ZC represent the ecological sites of Xingning, Nanxiong, Conghua, Luoding, and Zengcheng, respectively. IOF+W and CK represent the inorganic fertilizer reduction combined with organic fertilizer and water-saving irrigation, and conventional cultivation, respectively.

of a higher NO_2^--N content despite the reduced N fertilizer application in the IOF+W treatment (Appendix C), was consistent with the upregulation of N metabolic enzymes and pathways. In summary, the IOF+W treatment altered the metabolic pathways of CH_4 and N by changing the abundances of bacterial metabolism-related enzymes, resulting in lower CH_4 metabolism and higher N metabolism, which in turn affected the production of CH_4 and N_2O.

4. Discussion

4.1. Effect of partial organic substitution for inorganic fertilizers combined with water-saving irrigation on CH_4 emissions

The production and oxidation of CH_4 are primarily microbially-driven processes (Ding *et al.* 2022; Qi J Q *et al.* 2023). The emissions of CH_4 are significantly correlated with the composition of related microorganisms and the expression of their metabolic functions (Ding *et al.* 2022; Ma *et al.* 2022; Qi J Y *et al.* 2023). To explain why the IOF+W treatment significantly reduced CH_4 emissions, this study used metagenomic sequencing to determine the structure and function of soil microorganisms. Our findings revealed that the IOF+W treatment limited the α-diversity (Chao and Shannon indices) of paddy soil (Fig. 4), which indicated that both the abundance and diversity of paddy soil microorganism were altered. Xie *et al.* (2022) showed that inorganic fertilizers, combined with organic fertilizers, and irrigation levels significantly impact soil bacterial abundance (Chao index) and diversity (Shannon index), and

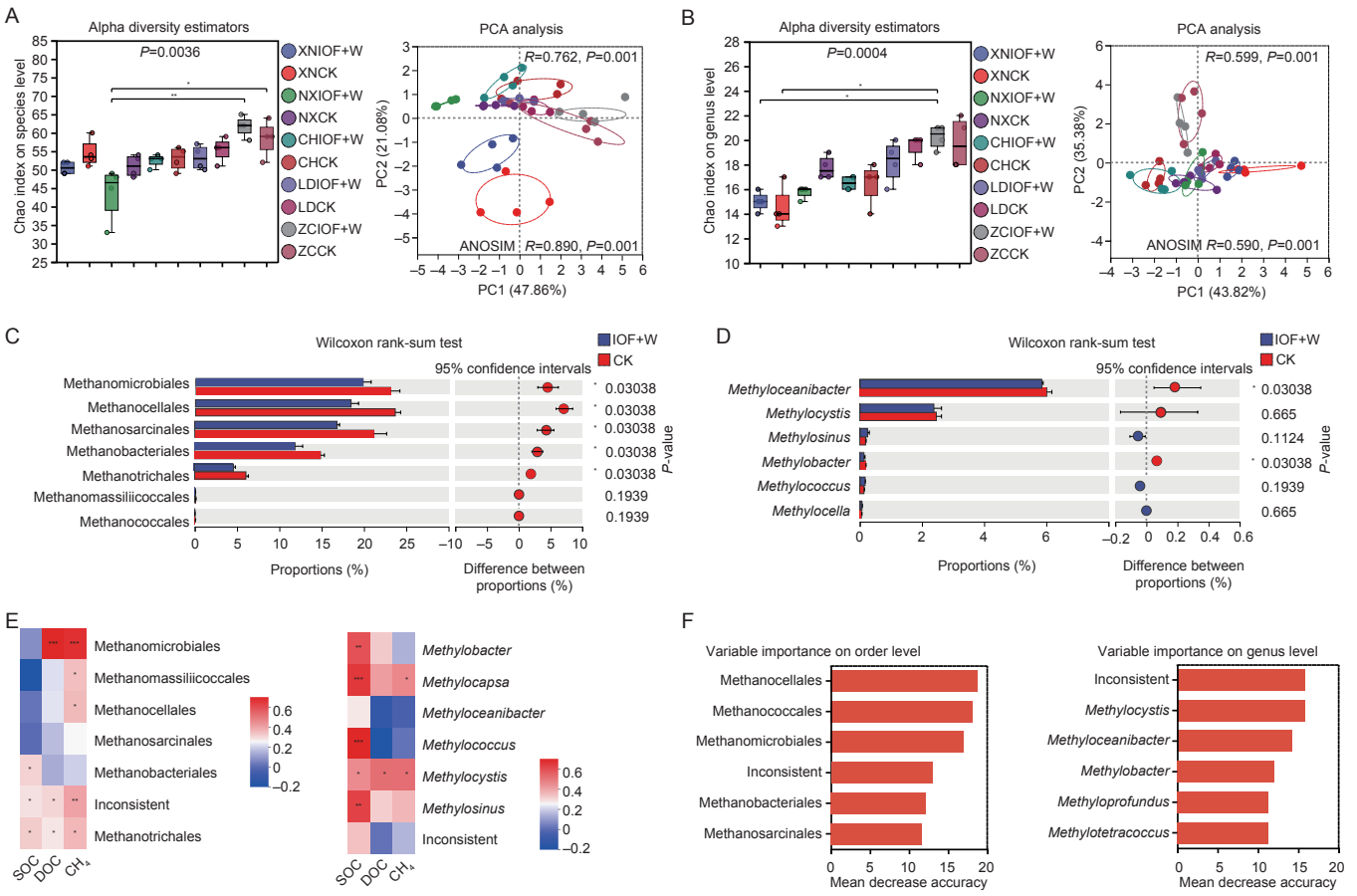


Fig. 5 Abundances of methanogens and methanotrophs in paddy soil under different ecological sites and treatments. A and B, the PCA analysis of Chao index and order-level abundances of methanogens and methanotrophs, respectively. C, Wilcoxon rank-sum test based on Bootstrap at the order level. D, Wilcoxon rank-sum test based on Bootstrap at the genus level. E, correlation heatmap. F, random forest analysis based on ten-fold cross-validation. XN, NX, CH, LD, and ZC represent the ecological sites of Xingning, Nanxiong, Conghua, Luoding, and Zengcheng, respectively. IOF+W and CK represent the inorganic fertilizer reduction combined with organic fertilizer and water-saving irrigation, and conventional cultivation, respectively.

the combined application of inorganic and organic fertilizers reduced α-diversity (Chao and Shannon indices). This may account for the reduction in α-diversity observed in the IOF+W treatment in this study. The changes in soil microbial composition and abundance may explain the reduced CH_4 emissions and increased N_2O emissions in the paddy field, so we further investigated the structural and functional differences of the microbial communities associated with CH_4 production and oxidation in the paddy ecosystem.

CH_4 production in paddy fields mainly occurs through the acetate pathway, H_2/CO_2 pathway, and methyl-containing pathway (Yu *et al.* 2022), which utilize acetic acid, H_2/CO_2, and methyl-containing compounds as substrates, respectively. Therefore, CH_4 production is primarily associated with the metabolism of methanogens that utilize these substrates, and the emitted CH_4 represents the portion that is not oxidized by methanotrophs. Previous studies have mostly focused on the relationship between the composition of soil microorganisms in conventional rice paddies and greenhouse gas emissions. In this study, we further analyzed the composition and functions of soil bacteria in aromatic rice paddies under specific cultivation conditions for aroma enhancement in conjunction with greenhouse gas emissions. Our findings revealed that compared with CK, IOF+W reduced the activities of key enzymes involved in CH_4 production, including formylmethanofuran dehydrogenase, methyltransferase and methyl-coenzyme M reductase, while increasing the activity of the key CH_4-oxidizing enzyme lanthanide-dependent methanol dehydrogenase (Fig. 7). Consequently, under the IOF+W treatment, the soil produced less CH_4 and exhibited higher CH_4 oxidation activity. Although CK exhibited a more extensive CH_4 metabolic pathway, the total amount of unoxidized CH_4 was greater than that under IOF+W. As a result, CH_4 emissions from the IOF+W treatment were significantly lower than those from CK, with a reduction of 36.95% (Fig. 2). Thus, the primary reasons for CH_4 emission reduction under IOF+W treatment were the limitation of CH_4 production and the promotion of CH_4 oxidation. Like CH_4 metabolism, the IOF+W treatment in this study led to reduced methanogen abundance, while its impact on methanotrophs was less pronounced (Fig. 5). Fertilization and irrigation practices significantly influence the abundances of methanotrophs and methanogens in paddy fields (Wu Q G *et al.* 2022; Xu *et al.* 2022). Generally, the application of organic fertilizers, as well as the combined application of organic and inorganic fertilizers, will increase the abundance

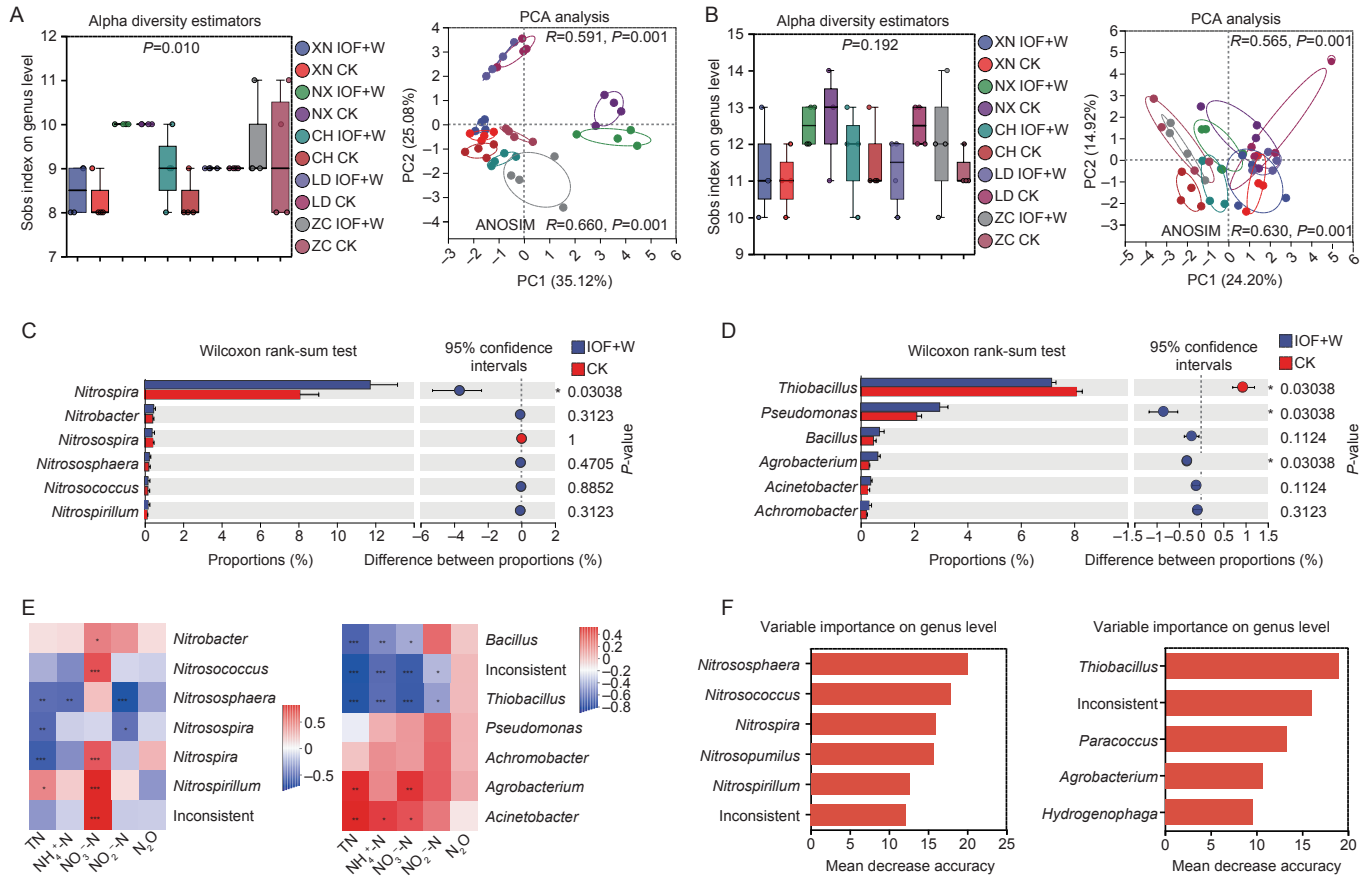


Fig. 6 Abundances of nitrifying bacteria and denitrifying bacteria in paddy soil under different ecological sites and treatments. A and B, principal component analysis (PCA) of Chao index and genus-level abundances of nitrifying and denitrifying bacteria, respectively. C and D, Wilcoxon rank-sum test based on Bootstrap at the genus level for nitrifying and denitrifying bacteria, respectively. E, correlation heatmap. F, random forest analysis based on ten-fold cross-validation. XN, NX, CH, LD, and ZC represent the ecological sites of Xingning, Nanxiong, Conghua, Luoding, and Zengcheng, respectively. IOF+W and CK represent the inorganic fertilizer reduction combined with organic fertilizer and water-saving irrigation, and conventional cultivation, respectively.

of methanogens. However, the impact on the abundance of methanotrophs has been inconsistent across different studies (Kong *et al.* 2019). The impact of inorganic fertilizers (N, P, and K) alone on the abundances of methanotrophs and methanogens in paddy fields has also been inconsistent (Xu *et al.* 2022). Organic fertilizers have the most pronounced effect on regulating soil microbial composition and abundance. Previous research showed that organic fertilizer application significantly alters the composition and abundance of soil bacteria in paddy fields (Dong *et al.* 2014; Song *et al.* 2022), increasing the abundances of Proteobacteria, Bacteroidetes, and Acidobacteria, among others (Wang *et al.* 2017). In addition, organic fertilizer application can increase the soil organic carbon content, thus providing more substrates for methanogens and thereby enhancing their abundance (You *et al.* 2022). Considering the goal of increasing rice yield, the combined application of inorganic and organic fertilizers has a more favorable effect on regulating the soil microbial structure and abundance (Zhao *et al.* 2016). In this study, the IOF+W treatment significantly reduced the abundance of methanogens, which contrasts with previous findings (Kong *et al.* 2019). This discrepancy may be attributed to the fact that while the application of reduced inorganic fertilizer in combination with organic fertilizer in the IOF+W treatment would typically increase the abundance of methanotrophs, the implementation of multiple drainage events under water-saving irrigation severely inhibited their growth, ultimately leading to a reduction in their abundance. Similar conclusions were drawn by Wu J F *et al.* (2022), who found that alternate wetting and drying irrigation reduced the abundances of Euryarchaeota, methanogens, and methanogen-related genes in paddy fields. Since water management primarily affects soil gas exchange, the improved aeration significantly enhances the abundance and activity of aerobic microorganisms while inhibiting strict anaerobes, particularly methanogens. The influence of irrigation on the abundances of methanogens and methanotrophs in paddy soils is even more pronounced than that of organic fertilizer (Ma *et al.* 2022). In conclusion, water-saving irrigation can not only reduce the abundance of methanogens but also increase the abundance of methanotrophs, leading to a significant reduction in CH_4 emissions (Chen *et al.* 2021; Leon and Izumi 2022). In this study, the IOF+W treatment significantly reduced the abundance of methanogens but did not show a clear trend in the abundance of methanotrophs, which even decreased slightly (Fig. 5). This may be due to the higher

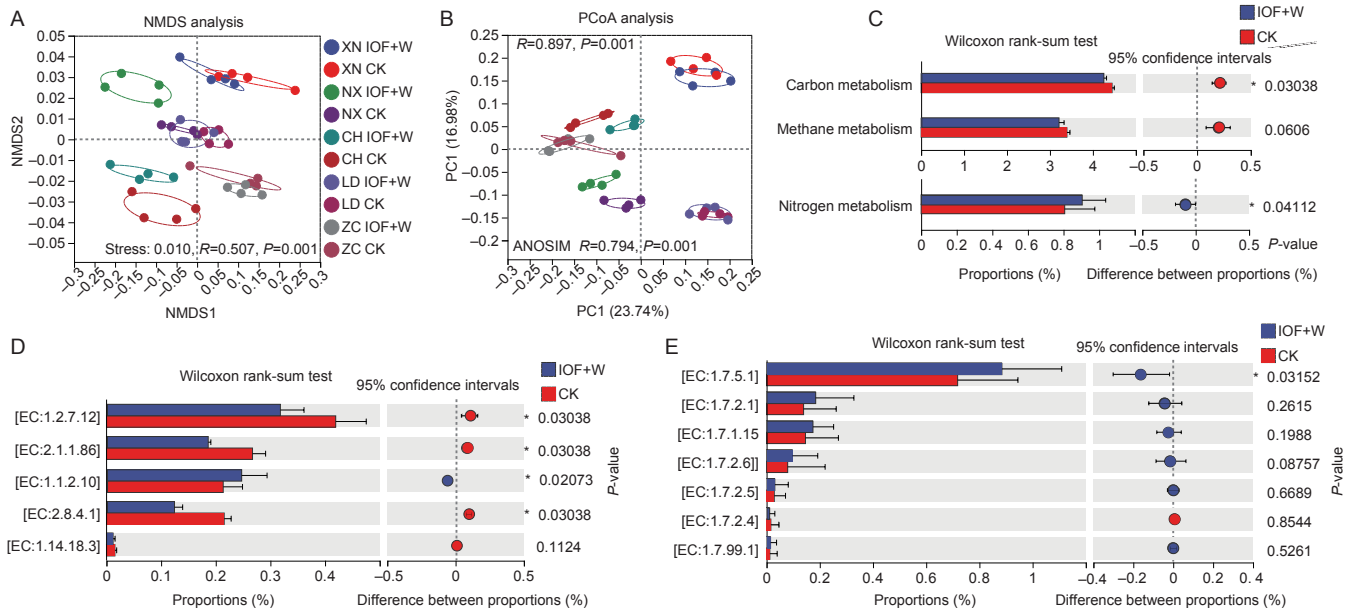


Fig. 7 Differences in bacterial metabolic pathways in paddy soil under different ecological sites and treatments. A, NMDS analysis based on the Bray-Curtis distance matrix at KEGG pathway level3. B, PCoA analysis based on Bray-Curtis at KEGG pathway level3. C, Wilcoxon rank-sum test based on Bootstrap at KEGG pathway level3. D and E, Wilcoxon rank-sum test based on Bootstrap at the KEGG enzyme level. XN, NX, CH, LD, and ZC represent the ecological sites of Xingning, Nanxiong, Conghua, Luoding, and Zengcheng, respectively. IOF+W and CK represent the inorganic fertilizer reduction combined with organic fertilizer and water-saving irrigation, and conventional cultivation, respectively.

CH_4 concentrations produced under CK, which favored the growth of methanotrophs. This hypothesis is supported by Okubo *et al.* (2014), who found that low CH_4 concentrations limited the growth of methanotrophs, thereby hindering CH_4 oxidation, while high CH_4 concentrations had the opposite effect.

In summary, the IOF+W treatment reduced CH_4 emissions by reducing methanogen abundance and the activities of key enzymes involved in methanogenesis, while simultaneously increasing the activities of key enzymes related to methane oxidation. This not only led to lower CH_4 production but also enhanced CH_4 oxidation in the rice paddy, significantly reducing the amount of unoxidized CH_4 and overall CH_4 emissions. Furthermore, the CH_4 emissions in this experiment were significantly positively correlated with soil DOC content (Appendix D), consistent with the findings of Qi J Q *et al.* (2023). This correlation may also explain the variation in CH_4 emissions across the five ecological sites, with the ZC site exhibiting the highest CH_4 emissions, corresponding to its highest DOC content.

4.2. Effect of partial organic substitution for inorganic fertilizers combined with water-saving irrigation on N_2O emissions

Like CH_4 emissions, N_2O emissions are primarily generated through microbially-driven processes and N_2O emission fluxes show significant correlations with the composition of relevant microbial communities and the expression of their metabolic functions (Ding *et al.* 2022). The observed alterations in soil microbial composition and abundance may explain the increased N_2O emissions from paddy fields, prompting further investigation into the structural and functional characteristics of the N_2O emission-associated microbial communities in the paddy ecosystem. In paddy fields, N_2O is produced during nitrification and denitrification processes (Butterbach-Bahl *et al.* 2013). Denitrification is an anaerobic microbial process that reduces nitrate (NO_3^-) to nitrogen gas (N_2) or nitrous oxide (N_2O) (Qin *et al.* 2019), while nitrification is an aerobic process whereby microorganisms sequentially oxidize ammonia (NH_3/NH_3^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-). Under certain conditions, these two processes can occur simultaneously in soil ecosystems (Liu *et al.* 2012). Compared with CK, the IOF+W treatment promoted the activity of the key denitrification enzyme (nitrite reductase) and moderately increased the activities of the key nitrification enzymes (ammonia monooxygenase and hydroxylamine oxidoreductase). These shifts stimulated both the nitrification and denitrification metabolic pathways (Fig. 7), ultimately leading to a 14.82% increase in N_2O emissions (Fig. 3). These results may be attributed to the combined effect of inorganic fertilizer reduction along with organic fertilizer and water-saving irrigation under the IOF+W treatment. Wu J F *et al.* (2022) found that alternate wetting and drying irrigation not only reduced the abundances of methanogens and CH_4-producing genes but also increased the abundances of genes related to the nitrification and denitrification pathways, thereby enhancing the N metabolism-related genes, which aligns with the observed enhancement of the nitrification and denitrification processes under the IOF+W treatment in this study. In addition, the combined application of inorganic and organic fertilizers has a better effect on regulating the soil microbial structure and abundance, albeit with increased

nitrification and denitrification functions (Zhao *et al.* 2016), which aligns with our findings that the IOF+W treatment enhanced the nitrification and denitrification processes. The IOF+W treatment also significantly increased the abundances of nitrifying and denitrifying bacteria compared to CK, consistent with the enhanced nitrification and denitrification processes (Fig. 6). Other studies have also reported that fertilization and irrigation practices significantly affect the abundances of nitrifying and denitrifying bacteria in paddy fields (Wu Q G *et al.* 2022; Yang *et al.* 2024). Greater nitrogen application can enhance the abundances of nitrifying and denitrifying bacteria, as nitrogen fertilizers provide ample ammonium (NH_4^+) and nitrate (NO_3^-), which serve as metabolic substrates for these bacteria (Chen *et al.* 2021). Organic fertilizer application increases the abundances of nitrifying and denitrifying bacteria by providing nitrogen sources and influencing soil oxygen levels, which affect their growth (Wang *et al.* 2017). The combined application of inorganic and organic fertilizers also increases the abundances of nitrifying and denitrifying bacteria (Zhao *et al.* 2016). Continuous flooding inhibits nitrifying bacteria while promoting denitrifying bacteria due to the anaerobic conditions, which are unsuitable for the nitrifying bacteria. In contrast, water-saving irrigation favors nitrifying bacteria (Wang *et al.* 2017). In this study, the IOF+W treatment increased the abundances of both nitrifying and denitrifying bacteria, consistent with previous findings (Zhao *et al.* 2016). Although the IOF+W treatment applied slightly less nitrogen from chemical fertilizer than the CK treatment, the nitrogen sources released from organic fertilizer decomposition also provided substrates for these bacteria, and the alternating wet and dry conditions in the IOF+W treatment favored the growth of both nitrifying and denitrifying bacteria.

In summary, the IOF+W treatment increased the abundances of both nitrifying and denitrifying bacteria, enhanced the activities of key enzymes involved in nitrification and denitrification, and ultimately promoted nitrogen metabolism (both nitrification and denitrification), leading to higher N_2O emissions. In addition, the N_2O emissions were positively correlated with nitrite levels in this study (Appendix D). Nitrite-N is an intermediate product of nitrification and denitrification. The IOF+W treatment exhibited higher nitrite-N concentrations (Appendix C), which suggested that the enhanced nitrification and denitrification under IOF+W resulted in elevated soil nitrite-N levels and increased N_2O emissions. Moreover, differences in nitrogen content may explain the variation in N_2O emissions across the five ecological sites, with the XN site showing the highest N_2O emissions, corresponding to its highest soil nitrogen content. Huang *et al.* (2020) demonstrated that changes in soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) levels influence N_2O emissions, as higher NH_4^+ concentrations in paddy soils enhance nitrification, while elevated NO_3^- concentrations promote denitrification (Alam and Jia 2012). Moreover, the soil DOC content also affects nitrification and denitrification, with increased DOC levels strengthening denitrification and accelerating NO_3^- reduction (Qin *et al.* 2017). Therefore, variations in soil nitrogen content and its different forms across the five ecological sites contributed to the observed differences in N_2O emissions.

4.3. Integrated benefits of partial organic substitution for inorganic fertilizers combined with water-saving irrigation

The IOF+W treatment reduced the GWP and GHGI by 26.29 and 35.99%, respectively (Table 3). The combination of fertilization and irrigation demonstrated significant advantages in balancing yield improvement and greenhouse gas mitigation. Wu Q G *et al.* (2022) noted that adding organic amendments during drainage can achieve greater reductions in greenhouse gas emissions (N_2O and CH_4). The organic fertilizer applied in the IOF+W treatment may have acted as a soil amendment, contributing to the overall reduction in greenhouse gas emissions. Notably, although the IOF+W treatment significantly increased N_2O emissions, its contribution to the overall GWP was minimal (1.99%), whereas CH_4 contributed significantly (98.01%). These differences resulted in substantial reductions in overall GWP and GHGI, thereby allowing the IOF+W treatment to achieve greenhouse gas mitigation in paddy fields.

Compared with CK, the IOF+W treatment significantly increased the rice yield (Table 1). This could be attributed to the appropriate fertilization rate and ratio, and the application of organic fertilizer (slow-release) in the IOF+W treatment, which promoted rice growth. Moderate drought stress not only enhances tillering but also facilitates the transition from vegetative to reproductive growth and improves grain filling (Wu Q G *et al.* 2022; Yang *et al.* 2024), ultimately increasing the yield through higher effective panicles, spikelets per panicle, and seed setting rate. Previous studies have shown that the fertilization rate, ratio, and drought conditions influence the content of 2-AP precursors (e.g., proline). Appropriate fertilization and drought conditions can increase 2-AP precursor levels and enhance 2-AP synthase activity, ultimately boosting the 2-AP content in rice (Imran *et al.* 2023). In this study, the IOF+W treatment significantly increased the 2-AP content in rice (Fig. 1), as it employed the appropriate fertilization rate and ratio for aromatic rice growth and aroma enhancement in South China. In addition, mild drought stress promoted the accumulation of 2-AP precursors and increased 2-AP synthase activity, leading to the higher 2-AP content in aromatic rice (Imran *et al.* 2023). The differences in the yield and 2-AP content of the aromatic rice across the five ecological sites may be attributed to the variations in soil fertility, temperature and light conditions. Furthermore, the IOF+W treatment significantly improved nitrogen agronomic efficiency and partial factor productivity, which could be attributed to the rational fertilization rate and ratio (Chen *et al.* 2022; Qiao *et al.* 2022), and moderate water stress (Wang *et al.* 2022; Hamoud *et al.* 2023), all of which enhanced nitrogen use efficiency.

5. Conclusion

This study focused on high-quality aromatic rice in South China and investigated the effects of partial organic substitution for inorganic fertilizers combined with water-saving irrigation (IOF+W) on CH_4 and N_2O emissions, as

well as soil bacterial diversity, abundance, and functionality, across five ecological sites. This study elucidated the underlying mechanisms of CH_4 and N_2O emissions under IOF+W conditions. The results demonstrated that the IOF+W treatment reduced CH_4 emissions by 36.95% by reducing the abundance of methanogens, increasing the abundance of methanotrophs, inhibiting key enzymes involved in CH_4 production, and promoting key enzymes involved in CH_4 oxidation, thereby altering CH_4 metabolism. Meanwhile, the IOF+W treatment increased the abundances of both nitrifying and denitrifying bacteria, enhanced key enzymes involved in nitrification and denitrification, and strengthened nitrogen metabolism, leading to a 14.82% increase in N_2O emissions. Although the IOF+W treatment increased N_2O emissions, its contribution to the GWP was significantly lower than that of CH_4. Compared to CK, the IOF+W treatment reduced the GWP by 31.29% and the GHGI by 40.30%, thereby achieving effective greenhouse gas mitigation. In addition, the IOF+W treatment increased the aromatic rice yield by 14.77%, raised the 2-AP content by 13.78%, reduced the application of inorganic fertilizers, and improved nitrogen use efficiency (Appendix E). In conclusion, the IOF+W approach is an effective strategy for enhancing yield, aroma, efficiency, and greenhouse gas mitigation in aromatic rice cultivation in South China.

Acknowledgements

Funding was provided by the Guangdong Province Low-Carbon Fragrant Rice Cultivation Demonstration Project, China (F23032).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2025.06.012

References

Alam M S, Jia Z J. 2012. Inhibition of methane oxidation by nitrogenous fertilizers in a paddy soil. *Frontiers in Microbiology*, **3**, 246.

Bhatia A, Cowan N J, Drewer J, Tomer R, Kumar V, Sharma S, Paul A, Jain N, Kumar S, Jha G, Singh R, Prasanna R, Ramakrishnan B, Bandyopadhyay S K, Kumar D, Sutton M A, Pathak H. 2023. The impact of different fertiliser management options and cultivars on nitrogen use efficiency and yield for rice cropping in the Indo-Gangetic Plain: Two seasons of methane, nitrous oxide and ammonia emissions. *Agriculture*, *Ecosystems and Environment*, **355**, 108593.

Butterbach-Bahl K, Baggs E M, Dannenmann M, Kiese R, Zechmeister-Boltenstern S. 2013. Nitrous oxide emissions from soils: How well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society of London* (Series B: Biological Sciences), **368**, 20130122.

Chen H H, Li X C, Hu F, Shi W. 2021. Soil nitrous oxide emissions following crop residue addition: A meta-analysis. *Global Change Biology*, **27**, 3640–3656.

Chen J, Li C, Kong D L, Geng Y J, Wang H, Fang X T, Li S Q, Hu Z Q, Liu S W, Zou J W. 2021. Incorporating DNA-level microbial constraints helps decipher methane emissions from Chinese water-saving ground cover rice production systems. *Field Crops Research*, **260**, 107992.

Chen Z M, Wang Q, Ma J C, Zhao J, Huai Y, Ma J W, Ye J, Yu Q G, Zou P, Sun W C, Zhang G M, Zhao Y J. 2022. Combing mechanical side-deep fertilization and controlled-release nitrogen fertilizer to increase nitrogen use efficiency by reducing ammonia volatilization in a double rice cropping system. *Frontiers in Environmental Science*, **10**, 1–15.

Dai H L, Dai Z Q, Peng L H, Wu Y F, Zou H M, Lu X W. 2017. Metagenomic and metabolomic analysis reveals the effects of chemical phosphorus recovery on biological nutrient removal system. *Chemical Engineering Journal*, **328**, 1087–1097.

Dai X L, Sun J P, Zhao Z J, Ma R P, Zheng Z Y, Liu Y H, Wang X B, Zhou W. 2023. Linking the potential activities of methanogens and methanotrophs to their communities under different fertilization regimes in coastal saline paddy soils. *Applied Soil Ecology*, **192**, 105102.

Ding H N, Hu Q Y, Cai M L, Cao C G, Yang J. 2022. Effect of dissolved organic matter (DOM) on greenhouse gas emissions in rice varieties. *Agriculture*, *Ecosystems and Environment*, **330**, 107870.

Dong W Y, Zhang X Y, Dai X Q, Fu X L, Yang F T, Liu X Y, Sun X M, Wen X F, Schaeffer S. 2014. Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China. *Applied Soil Ecology*, **84**, 140–147.

FAO (Food and Agriculture Organization). 2023. Online statistical database: Crops and livestock products. FAOSTAT. [2023-10-1]. https://www.fao.org/faostat/en/#data/QCL

Guo C, Ren T, Li P F, Wang B, Zou J L, Hussain S, Cong R H, Wu L S, Lu J W, Li X K. 2019. Producing more grain yield of rice with less ammonia volatilization and greenhouse gases emission using slow/controlled-release urea. *Environmental Science and Pollution Research International*, **26**, 2569–2579.

Guo S, Guo H T, Zhang Y L, Qian Z H, Wang Z J, Lu J M, Wang Y, Zhao C, Wang W L, Zhang H C, Yang F P, Huo Z Y. 2024. Effects of side deep placement of controlled release nitrogen management on rice yield, NH_3, and greenhouse gas emissions. *Acta Agronmica Sinica*, **50**, 1525–1539. (in Chinese)

Hamoud Y A, Shaghaleh H, Guo X P, Zhang K. 2023. pH-responsive/sustained release nitrogen fertilizer hydrogel improves yield, nitrogen metabolism, and nitrogen use efficiency of rice under alternative wetting and moderate drying irrigation. *Environmental and Experimental Botany*, **211**, 105376.

Han Y, Zhang Z X, Li T C, Chen P, Nie T Z, Zhang Z H, Du S C. 2023. Straw return alleviates the greenhouse effect of paddy fields by increasing soil organic carbon sequestration under water-saving irrigation. *Agricultural Water Management*, **287**, 108434.

Hashemi F S G, Rafii M Y, Ismail M R, Mahmud T M M, Rahim H A, Asfaliza R, Malek M A, Latif M A. 2013. Biochemical, genetic and molecular advances of fragrance characteristics in rice. *Critical Reviews in Plant Sciences*, **32**, 445–457.

Hou W F, Khan M R, Zhang J L, Lu J W, Ren T, Cong R H, Li X K. 2019. Nitrogen rate and plant density interaction enhances radiation interception, yield and nitrogen use efficiency of mechanically transplanted rice. *Agriculture*, *Ecosystems and Environment*, **269**, 183–192.

Huang R, Wang Y Y, Gao X S, Liu J, Wang Z F, Gao M. 2020. Nitrous oxide emission and the related denitrifier community: A short-term response to organic manure substituting chemical fertilizer. *Ecotoxicology and Environmental Safety*, **192**, 110291.

Imran M, Shafiq S, Ashraf U, Qi J Y, Mo Z W, Tang X R. 2023. Biosynthesis of 2-acetyl-1-pyrroline in fragrant rice: Recent insights into agro-management, environmental factors, and functional genomics. *Journal of Agricultural and Food Chemistry*, **71**, 4201–4215.

IPCC (Intergovernmental Panel on Climate Change). 2019. Online statistical database: Climate change and land (An intergovernmental panel on climate change special report on climate change). [2025-3-10]. https://www.ipcc.ch/srccl/

Jiang Y, Guan D H, Zhang W J. 2018. The effect of rice plant traits on methane emissions from paddy fields: A review. *Chinese Journal of Eco-Agriculture*, **26**, 175–181. (in Chinese)

Kong D L, Li S Q, Jin Y G, Wu S, Chen J, Hu T, Wang H, Liu S W,

Zou J W. 2019. Linking methane emissions to methanogenic and methanotrophic communities under different fertilization strategies in rice paddies. *Geoderma*, **347**, 233–243.
Leon A, Izumi T. 2022. Impacts of alternate wetting and drying on rice farmers' profits and life cycle greenhouse gas emissions in An Giang Province in Vietnam. *Journal of Cleaner Production*, **354**, 131621.
Li P, Ye S F, Chen J, Wang L Y, Li Y J, Ge L, Wu G G, Song L L, Wang C, Sun Y, Wang J B, Pan A H, Quan Z X, Wu Y F. 2023. Combined metagenomic and metabolomic analyses reveal that *Bt* rice planting alters soil C-N metabolism. *ISME Communications*, **3**, 4.
Li S Y, Chen Y, Yu F, Zhang Y J, Liu K, Zhuo X X, Qiu Y Y, Zhang H, Gu J F, Wang W L, Yang J C, Liu L J. 2022. Reducing methane emission by promoting its oxidation in rhizosphere through nitrogen-induced root growth in paddy fields. *Plant and Soil*, **474**, 1–20.
Liu J B, Hou H J, Sheng R, Chen Z, Zhu Y J, Qin H L, Wei W X. 2012. Denitrifying communities differentially respond to flooding drying cycles in paddy soils. *Applied Soil Ecology*, **62**, 155–162.
Luo H W, He L X, Du B, Pan S G, Mo Z W, Duan M Y, Tian H, Tang X R. 2020. Biofortification with chelating selenium in fragrant rice: Effects on photosynthetic rates, aroma, grain quality and yield formation. *Field Crops Research*, **255**, 107909.
Luo H W, Imran M, Yao X B, Zhang S M, Yi W T, Xing P P, Tang X R. 2023. Foliar application of glutamate and phenylalanine induced regulation in yield, protein components, aroma, and metabolites in fragrant rice. *European Journal of Agronomy*, **149**, 126899.
Luo H W, Zhang Q Q, Lai R F, Zhang S M, Yi,W T, Tang X R. 2024. Regulation of 2-acetyl-1-pyrroline content in fragrant rice under different temperatures at the grain-filling stage. *Journal of Agricultural and Food Chemistry*, **72**, 10521–10530.
Ma C L, Wu J F, Li F S. 2022. Impacts of combined water-saving irrigation and controlled-release urea on CH_4 emission and its associated microbial communities and function. *Science of the Total Environment*, **830**, 154724.
Okubo T, Tokida T, Ikeda S, Bao Z, Tago K, Hayatsu M, Nakamura H, Sakai H, Usui Y, Hayashi K, Hasegawa T, Minamisawa K. 2014. Effects of elevated carbon dioxide, elevated temperature, and rice growth stage on the community structure of rice root-associated bacteria. *Microbes and Environments*, **29**, 184–190.
Qi J Q, Yuan H Y, Zhuang Q, Zama E F, Tian X F, Tao B X, Zhang B H. 2023. Effect of different types of biochar on soil properties and functional microbial communities in rhizosphere and bulk soils and their relationship with CH_4 and N_2O emissions. *Frontiers in Microbiology*, **14**, 1292959.
Qi J Y, Yao X B, Jian L, He L X, Cao J L, Kan Z R, Wang X, Pan S G, Tang X R. 2023. A 40 % paddy surface soil organic carbon increase after 5-year no-tillage is linked with shifts in soil bacterial composition and functions. *Science of the Total Environment*, **859**, 160206.
Qiao J, Wang J, Zhao D, Zhou W, Schwenke G, Yan T M, Liu D L. 2022. Optimizing N fertilizer rates sustained rice yields, improved N use efficiency, and decreased N losses via runoff from rice–wheat cropping systems. *Agriculture*, *Ecosystems and Environment*, **324**, 107724.
Qin H L, Xing X Y, Tang Y F, Hou H J, Yang J, Shen R, Zhang W Z, Liu Y, Wei W X. 2019. Linking soil N_2O emissions with soil microbial community abundance and structure related to nitrogen cycle in two acid forest soils. *Plant and Soil*, **435**, 95–109.
Qin S P, Hu C S, Clough T J, Luo J F, Oenema O, Zhou S G. 2017. Irrigation of DOC-rich liquid promotes potential denitrification rate and decreases $N_2O/(N_2O+N_2)$ product ratio in a 0–2 m soil profile. *Soil Biology & Biochemistry*, **106**, 1–8.
Schreiber F, Wunderlin P, Udert K M, Wells G F. 2012. Nitric oxide and nitrous oxide turnover in natural and engineered microbial communities: Biological pathways, chemical reactions and novel technologies. *Frontiers in Microbiology*, **3**, 372.
Shan Q W, Zhang Y, Chen K L, Zhang K, Gao C X. 2015. Creation of fragrant rice by targeted knockout of the *OsBADH2* gene using TALEN technology. *Plant Biotechnology Journal*, **13**, 791–800.
Song W F, Shu A P, Liu J A, Shi W C, Li M C, Zhang W X, Li Z Z, Liu G R, Yuan F S, Zhang S X, Liu Z B, Gao Z. 2022. Effects of long-term fertilization with different substitution ratios of organic fertilizer on paddy soil. *Pedosphere*, **32**, 637–648.
Tang Z W, Zhang J, Deng A X, Zhang W J. 2022. Spatiotemporal characteristics and reduction approaches of methane emissions from rice fields in China. *Chinese Journal of Eco-Agriculture*, **30**, 582–591. (in Chinese)
Wang J C, Song Y, Ma T F, Raza W, Li J, Howland J G, Huang Q W, Shen Q R. 2017. Impacts of inorganic and organic fertilization treatments on bacterial and fungal communities in a paddy soil. *Applied Soil Ecology*, **112**, 42–50.
Wang Z C, Liu J J, Hamoud Y A, Wang Y S, Qiu R J, Agathokleous E, Hong C, Shaghaleh H. 2022. Natural ^{15}N abundance as an indicator of nitrogen utilization efficiency in rice under alternate wetting and drying irrigation in soils with high clay contents. *Science of the Total Environment*, **838**, 156528.
Wu J F, Ma C L, Li F S. 2022. Microbial community structure and function in paddy soil as affected by water-saving irrigation mode. *European Journal of Soil Biology*, **113**, 103450.
Wu Q G, He Y, Qi Z M, Jiang Q J. 2022. Drainage in paddy systems maintains rice yield and reduces total greenhouse gas emissions on the global scale. *Journal of Cleaner Production*, **370**, 133515.
Xie Y, Wang Z C, Cheng X X, Qiu R J, Hamoud Y A, Hong C, Zong X Y, Wang Y S, Agathokleous E, Guo X P. 2022. Dissecting the combined effects of cultivar, fertilization, and irrigation on rhizosphere bacterial communities and nitrogen productivity in rice. *Science of the Total Environment*, **835**, 155534.
Xu C, Shen S S, Zhou B B, Feng Y Y, He Z, Shi L L, Wang Y, Wang H H, Mishra T, Xue L H. 2022. Long-term non-phosphorus application increased paddy methane emission by promoting organic acid and methanogen abundance in Tai Lake region, China. *Science of the Total Environment*, **864**, 161146.
Xu Q, Dai L X, Zhou Y, Dou Z, Gao W Y, Yuan X C, Gao H, Zhang H C. 2023. Effect of nitrogen application on greenhouse gas emissions and nitrogen uptake by plants in integrated rice–crayfish farming. *Science of the Total Environment*, **905**, 167629.
Yan X Y, Akiyama H, Yagi K, Akimoto H. 2009. Global estimations of the inventory and mitigation potential of methane emissions from rice cultivation conducted using the 2006 Intergovernmental Panel on Climate Change Guidelines. *Global Biogeochemical Cycles*, **23**, 1–15.
Yan X Y, Yagi K, Akiyama H, Akimoto H. 2005. Statistical analysis of the major variables controlling methane emission from rice fields. *Global Change Biology*, **11**, 1131–1141.
Yang J C. 2015. Approaches to achieve high grain yield and high resource use efficiency in rice. *Frontiers of Agricultural Science and Engineering*, **2**, 115–123.
Yang Y C, Yao F F, Sun Y B, Yang Z P, Li R, Bai G, Lin W X, Chen H F. 2024. Appropriately reduced nitrogen and increased phosphorus in ratooning rice increased the yield and reduced the greenhouse gas emissions in Southeast China. *Plants* (Basel, Switzerland), **13**, 438.
You X X, Wang S, Du L N, Wu H, Wei Y. 2022. Effects of organic fertilization on functional microbial communities associated with greenhouse gas emissions in paddy soils. *Environmental Research*, **213**, 113706.
Yu F, Li S Y, Qiu Y Y, Zhuo X, Huang J, Wang H, Zhu A, Liu K, Liu L J. 2022. Microbiological mechanism of methane emission in paddy field and influence of water-saving cultivation on methane emission. *Chinese Journal of Rice Science*, **36**, 1–12. (in Chinese)
Zhang G B, Yang Y T, Wei Z J, Zhu X L, Shen W Y, Ma J, Lv S H, Xu H. 2023. The low greenhouse gas emission intensity in water-saving and drought-resistance rice in a rainfed paddy field in Southwest China. *Field Crops Research*, **302**, 109045.
Zhang L, Zhang F, Zhang K P, Liao P, Xu Q. 2024. Effect of agricultural management practices on rice yield and greenhouse gas emissions in the rice-wheat rotation system in China. *Science of the Total Environment*, **916**, 170307.
Zhao J, Ni T, Li J, Lu Q, Fang Z Y, Huang Q W, Zhang R F, Li R, Shen B, Shen Q R. 2016. Effects of organic-inorganic compound fertilizer with reduced chemical fertilizer application on crop yields, soil biological activity and bacterial community structure in a rice-wheat cropping system. *Applied Soil Ecology*, **99**, 1–12.

Executive Editor-in-Chief Jinshui Wu
Managing Editor Lujuan Sun