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USE OF ORGANIC COMPOST WITH *PARACOCCUS DENITRIFICANS* SUSPENSIONS IMPROVE RICE AND WHEAT YIELDS WITH LOWER EMISSIONS OF GREENHOUSE GASES

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Abstract

In this experiment, we evaluated the effects of different compost proportions combined with *Paracoccus denitrificans* nitrate-reducing suspensions on greenhouse gas emissions and soil microbiota associated with rice and wheat plants, focusing on yield and quality outcomes. The treatments were as follows: T0 (single application of organic fertilizer), T1 (10% compost instead of fertilizer), T2 (20% compost replacement), T3 (30% compost replacement), W0 (clear water), W1 (*P. denitrificans* suspension MHZ006), W2 (*P. denitrificans* suspension MHZ007). Compost application improved crop yield ,quality of rice and wheat and the physical and chemical properties of the soil. Compared to T1W0, T2W0 and T3W0 increased soil proteobacteria by 7.34% and 12.03%. Firmicutes increased by 3.35% and 0.15%, respectively Nitrous oxide (N_2O) emissions decreased by 16.98% and 48.27%, while methane (CH_4) emissions rose by 1.36% and 23.62%, respectively. The application of *P. denitrificans* suspensions significantly reduced CH_4 emissions. The combination of 30% compost and microbial suspension MHZ007, was the most effective fertilization measure, reducing CH_4 and N_2O by 80.75% and 96.12%, respectively while increasing yield by 15.77%, compared with that of a single fertilizer. Thus, 30% compos with microbial suspension MHZ007 effectively improves and enhances the yield and quality of rice and wheat, reduces N_2O and CH_4 emissions, and improves soil vitality.

Key words: Compost; Greenhouse gas; Rice-wheat rotation; Microbial suspension.

Introduction

Rice and wheat are the world's most important food crops, feeding 90% of the global population (Blümmel *et al*., 2020). While crop production is greatly dependent on chemical fertilizers, their use has significant environmental drawbacks (Li *et al*., 2023b). As a result there is a dire need to reduce chemical fertilization while enhancing agricultural efficiency to maintain crop yields and promote sustainable agricultural growth.

China began large-scale fertilizer application in the 1990s, and by 2015, the country was using 54.16 million tons of agricultural fertilizer. This is 3.4 times more than the United States and 27 times more than Africa (Kuang & Xie, 2022). Although nitrogen fertilizer can increase rice and wheat yields, declining nitrogen use efficiency leads to poor fertilizer utilization, soil compaction and decreased soil fertility (Zhang *et al*., 2008). This decline in turn leads to reduce crop quality and soil productivity but also disrupts the broader ecological balance.

To address these challenges, researchers are exploring to employ organic fertilizer or crude organic compost as alternatives to chemical fertilizers. The application of organic fertilizers improves soil's physical and chemical properties, enriches soil microorganisms, and enhances nitrogen cycle, leading to healthier soils and increased crop yields. (Deblina *et al*., 2022). However, the impact of organic fertilizers on greenhouse gases emissions, requires further study and remains unclear. Greenhouse gas emissions of N_2O and CH_4 are critical as they account for 10% and 50% of the total global emissions, respectively (Liu *et al*., 2016).

In compost, nitrogen fertilizer should be released slowly to lower the soil's available nitrogen content and thereby reduce N_2O emissions (Sun, 2020). However, compost also contains abundant methanogenic bacteria, which is more conducive to the production and emission of CH_4 (Sun, 2020). For instance, the total greenhouse gas emissions from 10% straw compost, increased by 12.3% compared to conventional fertilizer (Wu, 2020). Liu *et al*., (2016) found that N_2O emission from 100% organic fertilizer increased by 56.3% compared to single fertilizer. Conversely, Sun, (2020) reported that annual cumulative emissions of N_2O from 40% organic fertilizer were significantly reduced by 25.32% but the yearly cumulative emissions of CH_4 increased by 7.67% compared to conventional fertilizer.

A metanalysis by Li *et al*., (2019) suggested that CH_4 emissions increased by 79.4% during the early rice season and 81.2% in the late rice season. Yang *et al*., (2018) found that reducing chemical fertilizer and incorporating compost decreased N_2O emission by 4.89%, butCH_4 emission increased by 13.08% compared to chemical fertilizer alone. Interestingly, Fang *et al*., (2021) showed that applying liquid bactericides reduced the emissions of N_2O and CH_4 by 36.9% and 39.2%, respectively. Therefore, microbial bacterial suspensions may help mitigate the greenhouse gas emission associated with straw incorporation. Chen *et al*., (2022) demonstrated that the application of microbial suspensions can replicate the benefits of compost while reducing greenhouse gas emissions. According to Wu *et al*., (2019), straw compost partially substituting chemical fertilizer combined with digestive inhibitors had no significant adverse impact on yield and quality, while effectively reducing greenhouse gas emissions.

Previous studies on the impact of composting on greenhouse gas emissions have been inconsistent in the context of factors such as dry and wet farming, seasonal cropping, temperature, crop varieties and regional precipitation. Suppose a full-season study covering rice and wheat would allow for a better assessment of the effects of different compost ratios combined with microbial suspensions. In order to analyze the correlation between greenhouse gas emissions and crop yield and quality, and to provide theoretical and practical guidance for reducing emissions by replacing chemical fertilizers with compost and bacterial suspensions.

Materials and methods

Test material: The experiments were conducted at the experimental station of Anhui Institute of Science and Technology, located in Fengyang County of Chuzhou City, Anhui Province (E 117°33'39", W 32°52'49"), in a transition zone between the northern subtropical and southern temperate climates. The average annual temperature of the region is 16℃ and an average yearly precipitation of 885 mm (Table 1).

Rice was cultivated from May to November 2021, and wheat from December 2021 to June 2022 in the Western Planting Park of Anhui University of Science and Technology. The previous harvested crop in the experimental field was wheat. The soil within the top 20 cm contained 20.7g/kg of organic matter, 110.8mg/kg of alkali-hydrolyzed nitrogen, 25.7mg/kg of rapidly available phosphorus, and 115.1mg/kg available potassium.

The wheat variety TW9you063 = and rice variety Huaimai-44 were selected for the experiment. TW9you063 is characterized by a moderate growth period, strong tolerance to fertilizer, lodging resistance, flourishing growth , stout stalks, large spikes with numerous grains, excellent late-stage color transformation, high quality grain and stable yield. The seedling stage of the selected variety, Huaimai-44, exhibits semi-creeping with short leaves and green color. The plant type is more compact after growing, the blade is straight as were the leaves. It has a stronger tillering capacity, higher heading rate, earlier yellowing, cold tolerance, and lodging resistance.

Experimental design: The experiment was repeated three times, with the base fertilizer application differing according to crop requirements. The primary focus was to study the dynamic change of greenhouse gas emission flux in rice and wheat and its effects on the microbial community, yield, and crop quality.

The compost used in this experiment was a crude organic fertilizer, composed of cow manure and straw, which were provided by local farmers and Xiaogang Village in Fengyang County, respectively, and the compost raw materials were straw and cow dung, with a ratio of 2:1. The contents of nitrogen, phosphorus and potassium were 1.03%, 0.87% and 1.35%, respectively. The organic matter content was 47.8%; PH of 6.67. Each plot utilized seedling trays for one month of seedling raising. 60% of the base fertilizer which included compost and organic fertilizer, was applied at the time of transplanting with the remaining 40% applied two months later during the critical growth stage., Standard field irrigation and weed control were carried out according to different growth stages. The bacterial strains MHZ006 and MHZ007 were isolated in our laboratory as soil microorganisms that can inhibit greenhouse gas emissions. MHZ006 is a *Paracoccus denitrificans* strain capable of performing denitrification under aerobic conditions. MHZ007 is a strain of *Pseudomonas monteilii* strain, which belongs to the root growth-promoting bacteria. It can fix nitrogen, increase soil fertility, and reduce chemical fertilizers' usage, which contributes to reducing environmental pollution, improving disease resistance, and enhancing overall crop (Table 2).

Sample collection and determination methods: The static box method was used to measure the emission fluxes of N_2O and CH_4 in rice fields. The sampling device consisted of a top box, a middlebox and a base, all made of opaque organic plastic. The box dimensions (50cm × 50cm × 50cm) with a small fan placed inside to ensure uniform distribution of gas. The box was covered with sponge aluminum foil for reflective heat insulation, and a thermometer was inserted through the top to monitor internal temperature. Grooves surround the base (50cm × 50cm × 25cm), where the base was buried in the crop row, leaving only the grooves exposed, Water was injected into the grooves during gas sampling to prevent air leakage and the box was placed into the grooves. Gas samples were extracted through the sampling port.

Sampling began 20 days after crop planting and was conducted between 8:00 and 11:00 a.m. Samples were collected at 5-minute intervals, using 60 mL syringes to extract gas at 0, 5, and 10 minutes. A total of three gas samples were collected per session. Both the ambient air temperature and the box's internal temperature were recorded during each sampling session.

Data processing: After collecting the samples and analyzing them in the laboratory using gas chromatograph (Agilent, USA), the greenhouse gas emission flux is calculated by the following formula:

$$F=\frac{dc}{dt}\times\frac{M}{V_0}\times\frac{P}{P_0}\times\frac{T_0}{T}\times H$$

where F = gas emission flux (mg/(m^2 · h));
dc/dt is the slope of the regression curve of gas volume fraction with time.

V_0 is the molar volume of gas under standard gas (22.41L/mol);
P and P_0 are the air pressure (Pa) at the sampling point and the air pressure (101.325kPa) at the standard state, respectively.
T and T_0 are the absolute temperature (K) at the sampling time and the absolute temperature (273.15K) at the standard state, respectively.
H is the height of the sampling box (m).
M is the molar mass of the gas (g/mol);

Table 1. Weather conditions during the experimentation (2021-2022).

Date	Maximum temperature (℃)	Minimum temperature (℃)	Average Temperature (℃)	Total rainfall (mm)	Total sunshine (h)
2021.05	33	12	22.5	79.9	106
2021.06	38	17	27.5	24.8	168
2021.07	36	22	29	76.2	194
2021.08	35	20	27.5	98.1	158
2021.09	34	17	25.5	113.6	256
2021.10	34	6	20	69.5	155
2021.11	22	-2	10	17.5	145
2021.12	18	-6	6	13.4	133
2022.01	14	-3	5.5	60.2	138
2022.02	22	-5	8.5	29.2	134.3
2022.03	28	1	14.5	187.8	165.9
2022.04	32	4	18	2.8	194.1
2022.05	31	10	20.5	0	203.5
2022.06	36	18	27	3.4	173.3

Table 2. Experimental treatment.

No.	Handle	Fertilizer application (kg/hm^2)	Amount of compost (t/hm^2)	Apply a bacteriostatic spray
1.	T0W0	Compound fertilizer (18-18-18)750，Urea 450	0	not sprayed
2.	T0W1	Compound fertilizer (18-18-18)750，Urea 450	0	MHZ006
3.	T0W2	Compound fertilizer (18-18-18)750，Urea 450	0	MHZ007
4.	T1W0	Compound fertilizer (18-18-18)675，Urea 405	5	not sprayed
5.	T1W1	Compound fertilizer (18-18-18)675，Urea 405	5	MHZ006
6.	T1W2	Compound fertilizer (18-18-18)675，Urea 405	5	MHZ007
7.	T2W0	Compound fertilizer (18-18-18)600，Urea 360	10	not sprayed
8.	T2W1	Compound fertilizer (18-18-18)600，Urea 360	10	MHZ006
9.	T2W2	Compound fertilizer (18-18-18)600，Urea 360	10	MHZ007
10.	T3W0	Compound fertilizer (18-18-18)525，Urea 315	15	not sprayed
11.	T3W1	Compound fertilizer (18-18-18)525，Urea 315	15	MHZ006
12.	T3W2	Compound fertilizer (18-18-18)525，Urea 315	15	MHZ007

Note: The experimental treatment were as follows: T0☹conventional fertilizer control), T1(compost to replace 10% fertilizer), T2 (compost to replace 20% fertilizer), T3 (compost to replace 30% fertilizer) W0(Treatment without microbial suspension,) W1 (with microbial suspension MHZ006), W2 (with microbial suspension MHZ007)

Table 3. Cumulative greenhouse gas emissions, combined warming potential and emission intensity under different compost substitution ratios with microbial suspensions.

Treatment	Cumulative emission (tN·hm-2)	CH_4 cumulative emission (tC·hm-2)	GWP (tCO_2-eq.hm-1)	GHGI (gCO_2-eq·kg-1)	Output (t/hm^2)
T0W0	21.97 ± 0.08a	0.43 ± 0.04b	8327.04a	670.16a	19.42 ± 0.05f
T0W1	10.86 ± 0.24e	0.25 ± 0.05cd	3804.26f	366.83d	20.93 ± 0.03d
T0W2	6.43 ± 0.35g	0.23 ± 0.05cd	2122.46h	205.96f	21.05 ± 0.05d
T1W0	18.99 ± 0.43b	0.44 ± 0.07b	6431.85b	655.22b	18.52 ± 0.1h
T1W1	12.44 ± 0.12d	0.28 ± 0.02c	5803.07d	340.86e	19.01 ± 0.17g
T1W2	4.41 ± 0.15i	0.22 ± 0.03cd	1604.97j	99h	20.02 ± 0.04e
T2W0	18.09 ± 0.21c	0.48 ± 0.03b	6023.94c	596.95c	19.89 ± 0.09e
T2W1	6.83 ± 0.24f	0.24 ± 0.01cd	3715.44g	150.42g	21.46 ± 0.13c
T2W2	2.35 ± 0.29j	0.21 ± 0.02cd	800.38k	66.13i	21 ± 0.12d
T3W0	12.15 ± 0.26d	0.88 ± 0.07a	5484.96e	370.22d	21.49 ± 0.45c
T3W1	5.06 ± 0.28h	0.29 ± 0.04c	2056.04i	146.31g	24.23 ± 0.06b
T3W2	0.47 ± 0.04k	0.19 ± 0.02d	223.05l	9.89j	24.67 ± 0.13a

During the rice growth period, the cumulative greenhouse gas emissions are calculated by multiplying the average of the emission fluxes between the adjacent two sampling periods by the interval between them and summing the results. Calculation of soil emission gas warming potential and greenhouse gas emission intensity.

To account for the gas warming potential (GWP), greenhouse gas emissions are converted into equivalent CO_2 to compare their climate. GWP is calculated from the cumulative emissions of each gas and their corresponding twarming coefficient.

$$GWP=CO_2+25\times CH_4+298\times N_2O$$

Greenhouse gas emission intensity (GHGI) is used to evaluate the combined greenhouse effect of each treatment. GHGI's algorithm is:

$$GHGI=GWP/Y$$

where GHGI is the treatment's greenhouse gas emission intensity, and Y is the crop yield of each treatment ($kg\cdot hm^{-2}$).

Determination and correlation calculation of soil microorganisms: Soil samples were collected in November 2021 after the rice harvest. After removing the topsoil, samples were taken from each plot following a five-point pattern, using a soil sampler to take 5 pieces of 20cm soil, mixing evenly and impurities were removed. The fresh soil sample was then divided into two portions: one was air-dried to determine the physical and chemical properties; One was stored at -80°C to extract soil genomic DNA. The soil sample of 0.5g was weighed, and the total soil DNA was extracted with a DNA extraction kit according to the instructions. It was sent to Guangdong Mege Gene Technology Co., Ltd. for sequencing.

The Spearman's rank correlation coefficient was used to calculate the correlation between two variables. The formula is:

$$\rho=1-\frac{6\sum d_i^2}{n(n^2-1)}$$

where,
ρ denotes Spearman grade correlation coefficient;
d is the sum of the squares of the difference between the rankings of the two variables, and
n is the number of samples

The above experiment was repeated three times to confirm the accuracy of results. The test data was processed and analyzed using Excel2010 and DPS7.05 software, and the significance was tested using the LSD method. The mapping software is Excel and R, and R uses the packages "pheatmap" and "ggplot2". The correlation between soil physicochemical properties and greenhouse gases and their functional bacteria was analyzed using the "psych" package in R language. Principal coordinates (PCoA) and redundancy analysis (RDA) of differences in community structure and effects of environmental factors on community structure were completed by the "vegan" package in R language.

Results

Experiment overview: Field tests were conducted, as shown in (Fig. 1), to compare and analyze the degree of greenhouse gas emissions from rice and wheat under different compost substitution ratio combined with microbial suspension treatment and its impact on yield and quality. Additionally, the application of compost treatment enhanced the activity of the soil environment and its microbial communities.

Effects of different compost substitution ratios combined with bacterial suspension on soil N_2O emission: The flux value of N_2O fluctuated, exhibiting both positive and negative values. During the rice-wheat growing period, emissions peaked at 20 days postemergence, with the reductions reaching their maximum after 40 days of emergence and then subsequently stabilizing. In the main zone, emissions followed the order T0>T1>T2>T3, while in the split zone were W0>W1>W2. Notably, N_2O emissions from wheat were higher than those from rice. N_2O emissions were low at 40 days of emergence, and the emission flux of N_2O treated by T0W0 was 104.75μgCO_2/($m^2\cdot h$). Compared with T0W0 treatment, T0W1, T0W2, T1W0, T2W0 and T3W0 treatment, the emission flux of N_2O decreased by 9.37%, 51.77%, 10.75%, 13.40% and 22.96%, respectively. The N_2O emission flux of T0W1 treatment was 94.94μgCO_2/($m^2\cdot h$), and the emission flux of T1W1, T2W1 and T3W1 treatment was reduced by 7.27%, 15.20% and 17.11%, respectively, compared with T0W1 treatment. The N_2O emission flux of T0W2 treatment was 50.52μg CO_2/($m^2\cdot h$), and the flux for treatments T1W2, T2W2 and T3W2 were 56.08%, 71.83% and 590.97% lower than that of T0W2 treatment, respectively.

Effects of different compost substitution ratios combined with bacterial suspension on soil CH_4 emission: Under various compost substitution ratios combined with bacterial suspensions, the CH_4 emission patterns exhibited varying seasonal variations (Table 3). Cumulative CH_4 emissions from rice and wheat gradually increased 40 days before emergence and then gradually declined. The highest emission from T0, followed by T1, the lowest emission from T3 was followed by T2, and the emission from the split zone is W0>W1>W2 from large to small. The cumulative emissions of rice and wheat CH_4 ranged from highest to lowest: T3W0>T2W0>T1W0> T0W0> T3W1> T1W1> T0W1>T2W1> T0W2> T1W2> T2W2>T3W2; This trend can be attributed to the fact that the cumulative CH_4 emission of rice is higher than that of wheat.

The maximum CH_4 emission flux was 19.85μg CO_2/($m^2\cdot h$) under the T0W0 treatment, and the CH_4 emission flux under T0W1 and T0W2 treatment was 47.20% and 58.54% lower than that under the T0W0 treatment, respectively. Compared with the T0W0 treatment, T1W0, T2W0, and T3W0 treatment increased the CH_4 emission fluxes by 3.38%, 19.50%, and 37.33%, respectively. The CH_4 emission flux of T0W1 treatment was 10.48μgCO_2/($m^2\cdot h$), and the CH_4 emission flux of T1W1 and T3W1 treatment was increased by 9.92% and 1.91%, respectively, compared with T0W1 treatment and the CH_4 emission flux of T2W1 treatment was reduced by

20.99% compared with T0W1 treatment. The CH_4 emission flux of T0W2 treatment was 8.23μgCO_2/(m^2·h). Compared to T0W2 treatment, the CH_4 emission flux of T2W2 and T3W2 treatment was reduced by 18.96% and 29.53%, respectively, and that of T1W2 treatment saw an increase of 27.34%.

After 100 days of emergence, CH_4 emissions were relatively low, with cumulative emissions in the main area decreasing from T2>T0> T1>T3, which are 4.64μg CO_2/(m^2·h), 3.84μg CO_2/(m^2·h), 3.67 μgCO_2/(m^2·h), 3.04μg CO_2/(m^2·h), respectively. In the fissure area cumulative emissions ranked as, W0>W1>W2, with values of 11.72μg CO_2/(m^2·h), 5.65μg CO_2/(m^2·h), -2.18μg CO_2/(m^2·h).

Effects of different compost substitution ratios combined with bacterial suspension on the comprehensive warming potential of greenhouse gases and greenhouse gas emission intensity: The results of estimating the comprehensive warming potential of greenhouse gases and greenhouse gas emission intensity under various compost substitution ratios combined with microbial suspension during the rice and wheat growth period are shown in (Table 3). Among all treatments, T3W2 treatment contributed the most significantly to the comprehensive warming potential of the paddy field, and the N_2O emission of T0W2 treatment was 48.80%, 173.62% and 1268.09% higher than those of T1W2, T2W2 and T3W2, respectively. Additionally, the CH_4 emissions treated by T0W2 were 4.55%, 9.52% and 21.05% higher than those treated by T1W2, T2W2 and T3W2, respectively. The results indicate that N_2O and CH_4 emissions from the MHZ007 bacterial suspension decreased gradually with increasing compost substitution ratio. Compared to T0W2, TIW2, T2W2, and T3W2 treatments decreased by 24.38%, 42.34%, and 89.49%, respectively; Compared with T0W0, T1W0, T2W0 and T3W0 treatments were 22.76%, 27.66% and 34.13% lower, respectively. The greenhouse gas emission intensity and GWP have the same changing trend when the yield of each treatment has apparent differences. The GHGI value of T0W0 treatment with different compost substitutions combined with microbial suspension was the highest (670.16gCO_2-eq·kg^{-1}), higher than all other treatments.

Compared with the T1W0 treatment, the yield increased by 2.65% and 5.31%, respectively. Compared with T2W0 treatment, the yield of T2W1 and T2W2 treatment was increased by 7.89% and 5.58%, respectively. Compared with the T3W0 treatment, the yields of the T3W1 and T3W2 treatments increased by 12.75% and 14.80%, respectively. The results showed that the combined application of bacterial suspension inhibits greenhouse gas emissions and does not reduce the yield.

Based on the experimental data, it can be concluded that composting can partially replace chemical fertilizer and that bacterial suspension can inhibit greenhouse gas emissions without adversely affecting yield. Compared with W2, the treatment of MHZ007 combined with microbial suspension is the most effective, but it must be attained under the condition of replacing 30% fertilizer with compost treatment of T3.

Effects of different compost substitution ratios combined with bacterial suspension on soil microbial community composition: Figure 2 (a) shows the relative abundance of microbial community structures with different compost substitution ratios and bacterial suspension levels. The microbial community composition of different treatments was mainly *Proteobacteria, Acidobacteria, Patescibacteria, Bacteroidetes, Chloroflexi, Actinobacteria, Gemmatimonadetes, Verrucomicrobia, Firmicutes* and *Nitrospirae*.

As the compost substitution ratio increased, the relative abundance of *Proteus* at T0W0, T1W0, T2W0 and T3W0 treatment levels gradually decreased at first but those of *Proteus* increased with compost substitutions of 20% and 30% combined with microbial suspension. The relative abundance of *Proteus* was notably lower with non-compost alternatives and 10% compost alternatives. The highest relvatie abundance of *Proteus* occurred when composting replaced 30% with MHZ007 microbial suspension and composting replaced 20% with MHZ007 microbial suspension.

With the increase of compost substitution ratio, the relative abundance of *Acidbacterium* gradually increased. Levels of *Acidibacterium* with a 30% compost substitution combined with MHZ007 microbial suspension were higher than other treatments. The relative abundance of *Bacteroides* increased gradually with the increase of compost substitution ratio. The relative abundance of Bacteroides in compost substitution was 20%. The highest levels of *Bacteroides* were again seen with the 30% and compost substitution was combined with microbial suspension.

Additionally, the relative abundance of *Chloroflexi* in the mixture of MHZ007 and MHZ006 was higher than that in the mixture of MHZ007 and MHZ006. The relative abundance of *Chloroflexi* in the mixture of 30% compost substitute was the highest than that of other treatments. The relative abundances of other genera were not significantly changed by different compost substitution ratios combined with microbial suspensions.

The relative abundance of *Sporomonas* increased gradually with the increase of compost proportion, and the relative abundance of *Sporomonas* in compost instead of unapplied microbial suspensions was the smallest.

Figure 2(b) shows the relative abundance of different compost substitution ratios and bacterial suspension microbial community structures at the genus level. They are mainly *Sphingomonas, Pseudolabrys, Candidatus solibacter, Bryobacter, Anaerolinea, Gemmatimonas*, RB41 and *Haliangium*.

The relative abundance of *Sphingomonas* showed an increase with the increase in compost substitution ratio. The relative abundance of *Pseudocheilus* in mixed MHZ007 suspension was higher than that of the unmixed and mixed MHZ006 suspension. The relative abundance of *Candidatus solibacter* decreased first and then gradually increased, the relative abundance of *Candidatus solibacter* increased with different compost substitution ratios. The relative abundance of *Candidatus solibacter* in 30% compost was the highest, greater than with MHZ007 microbial suspensions.

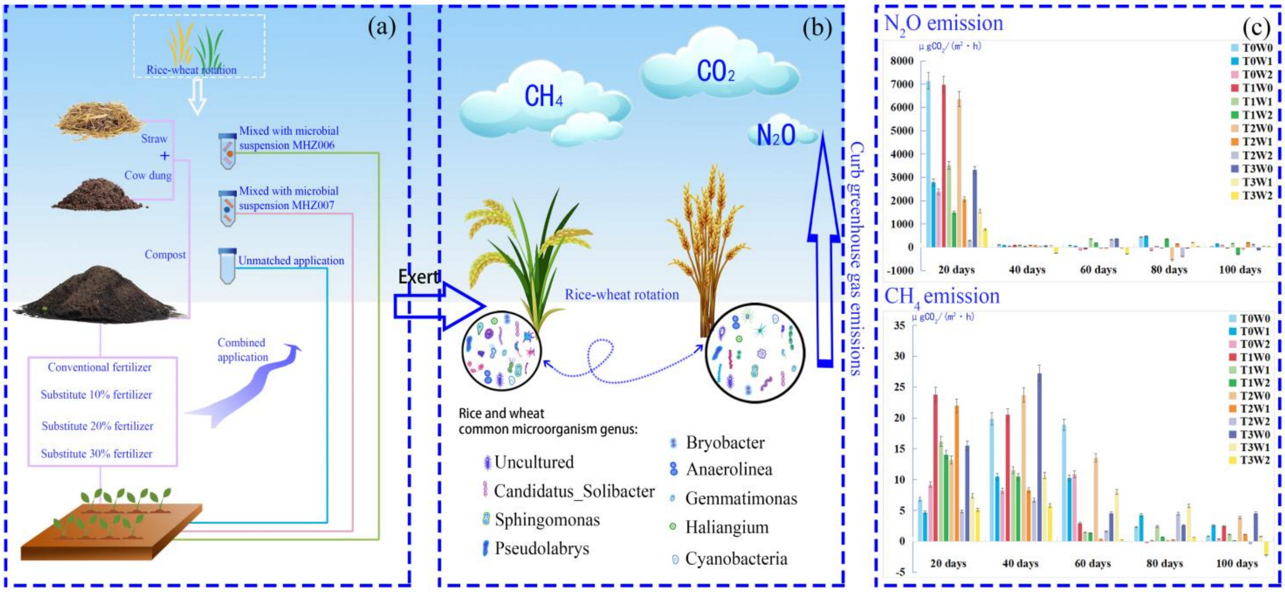

Fig. 1. Overall overview of the experimental design Note: Treatment flow chart (a); microbial genera common to rice-wheat rotation (b); emission of N_2O and CH_4 greenhouse gases during rice-wheat rotation (c).

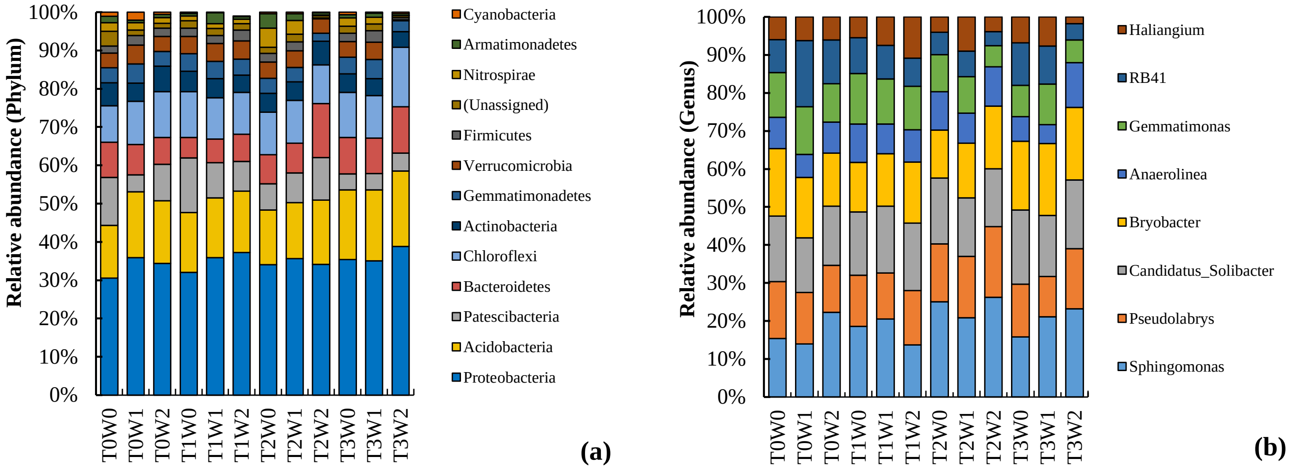

Fig. 2. The relative abundance (a), the relative abundance of dominant functional bacteria (>1%) (b).

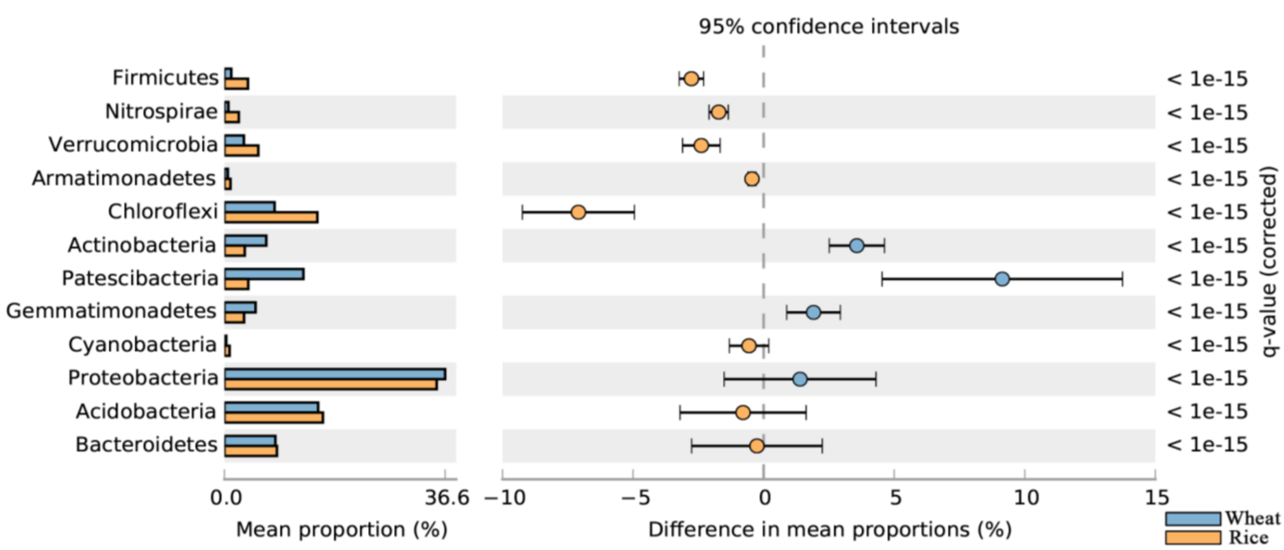

Fig. 3. Comparative analysis of different compost treatments with relative abundance of common bacterial phylum in rice and wheat.

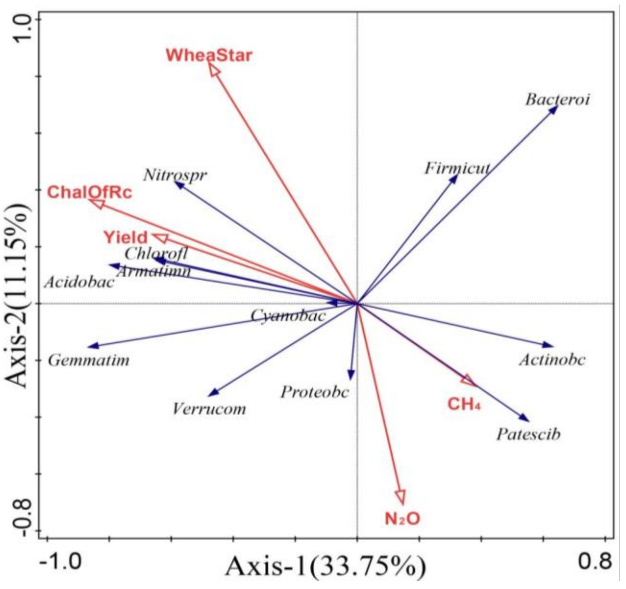

Fig. 4. Redundancy analysis of environmental factors, rice yield quality and soil microbial community structures.

The relative abundance of *Anthoxylus* was found to be highest when 30% of the fertilizer was replaced with compost in conjunction with the MHZ007 microbial suspension.

With the increase in the compost substitution ratio, the relative abundance of *Bryobacter* also exhibited an upward trend. The relative abundance of *Bryobacter* was the smallest in 20% of unapplied microbial suspensions replaced by compost, and the relative abundance of *Bryobacter* was increased by 30% of compost and combined microbial suspensions compared with unapplied microbial suspensions. Notably, the highest relative abundance of *Bryobacterium* was recorded when 30% compost was applied alongside the MHZ007 microbial suspension.

Both rice and wheat microbiomes were examined to identify common bacteria taxa associated with various compost substitution ratios. The relative abundances of both treatment bacterial taxa at phylum and genus levels are illustrated in Figures 2(a) and 2(b). Significant differences were observed in the abundance of 12 bacterial phyla in rice and wheat composting treatment. Comparative analysis using STAMP (Fig. 3) was performed on all these gates, and revealed strong correlations among the different composting treatments for rice and wheat. There were high *Firmicutes* in rice, *Nitrospirae, Verrucomicrobia, Armatimonadetes, Chloroflexi, Cyanobacteria, Acidobacteri*a and *Bacteroidetes. While, Actinobacteria, Patescibacteria, Gemmatimonadetes* and *Proteobacteria* were abundant in wheat.

RDA analysis suggested five dominant bacterial phyla (*Nitrospirae, Armatimonadetes, Acidobacteria, Chloroflexi* and *Gemmatimonadetes*) were positively correlated with crop yield and quality while exhibiting negative correlations with greenhouse gas emissions. *Nitrospirae* demonstrated a significant positive correlation with total soil carbon. Despite its low energy utilization rate and slow growth, *Nitrospirae* can continuously provide available nutrients for rice and wheat, thereby enhancing the energy cycle of crops and mitigating greenhouse gas emissions from waste. *Acidobacteria* and *Chloroflexi* were positively correlated with soil ammonium nitrogen, enhancing the metabolic activity of the soil community. This strong metabolism is the key to vigorous growth and formation of high-yield rice. Furthermore, with the increase of nitrogen fertilizer, the relative abundance of *Gemmatimonadetes* increases, which has growth promoting effect, that can significantly promote the growth of wheat seedlings, improve the induction resistance of wheat, and increase the yield by approximately10%. In summary, this experiment highlights the importance of understanding the relationship between the compost environment and these five key bacterial taxa. The dominant bacteria play a crucial role in improving soil performance, suggesting that these key bacteria significantly influence the soil environment and greenhouse gas emissions.

Correlation analysis between microbial community structure and environmental factors and rice and wheat yield and quality: The correlation analysis results concerning the microbial community structure, environmental factors and rice yield and quality are presented in (Fig. 4) Collectively, the two axes account for 50% of the relationship between microbial community structure and environmental factors and yield and quality of rice and wheat. Further analysis revealed that the explanatory contributions of RDA1 and RDA2 were 35.69% and 14.33%, respectively. Notably the responses of soil microbial communities to environmental factors, as well as to rice yield and quality varied under different compost substitution ratios in conjuction with microbial suspensions.

Discussion

Compost produced through high-temperature fermentation of crop straw and animal manure represents an effective alternative to chemical fertilizer (Zheng *et al*., 2014; Majid & Elina, 2014; Bian, 2020). In the present experiment, a combination of straw and chemical fertilizer was utilized as raw materials for compost production, enabling a partial replacement of chemical fertilizers. This approach enhances the diversity and activity of soil microorganisms while improving the nitrogen supply in the soil. Consequently, it contributes to the increased yield and quality of rice and wheat (Zhang *et al*., 2012; Wang *et al*., 2017; Cao *et al*., 2019; Dong, 2022). In addition, while composting will increase the greenhouse gas emission trend of rice-wheat rotation (Zou *et al*., 2006; Hou *et al*., 2012; Xu *et al*., 2020a), the application of microbial suspension (Lu *et al*., 2019) has been applied tomitigate greenhouse gas emission.

In this study, increasing the proportion of compost as a substitute for fertilizer was associated with improved physico-chemical properties of the soil, enhanced yield and quality of rice and wheat, and a reduction in cumulative N_2O emissions. However, the overall trend in greenhouse gas emissions was found to be increasing. The treatment involving 30% compost substitute with MHZ007 microbial suspension was the best, =maintaining the advantages of compost substitute fertilizer and inhibiting greenhouse gas emissions simultaneously. Specifically, the N_2O emission was 91.71% lower than that of a single fertilizer, and the yield was 14.80% higher.

Research by Li *et al*., (2023a) indicated that organic fertilizer can achieve the effect of increasing production up to 30%, and will reduce production if it exceeds 30% or even 50%. Zheng *et al*., (2023) demonstrated that when the percentage of organic fertilizer replacing chemical fertilizer was controlled within 20% to 50%, soil nitrogen leaching could be effectively controlled and ensured crop yield. Ji *et al*., (2019) found that the higher application of organic fertilizer when substituting 20% of chemical fertilizer, resulted in improved yield and quality, with increases ranging from 5.6% to 20.8% compared to sole chemical fertilizer application. Sun *et al*., (2020) reported that the cumulative N_2O emission of organic fertilizer replaced by fertilizer was reduced by 17.72% ~ 38.74% compared with single fertilizer These findings align with the results of this study.

Compost substitution for fertilizer can effectively reduce the leaching loss of soil nitrogen, as the release of fertilizer effect on the compost was slow, providing a sustained availability of fertilizers for crops and minimizing losses. Additionally, compost not only carries a large number of microorganisms to provide nutrients to the crops and increase microbial metabolic activities in soil but can also reduce N_2O emission, decreasing nitrogen loss, and improving yield and quality (Tang *et al*., 2019; Liu., 2020; Wang, 2020). Du *et al*., (2019) reported that the nitrogen use efficiency of organic fertilizer substitution increased to 32.4% ~ 37.8% compared to single fertilizer application, effectively reducing the apparent leaching loss ratio of nitrogen fertilizer. Li *et al*., (2010) demonstrated that the organic carbon content of rice-wheat rotation soil compost increased by 1.7 to 2.4 times, thereby improving soil nutrients and the yield and quality of rice and wheat.

In this study, the use of compost as a substitute for chemical fertilizer, combined with microbial suspension, was found to reduce the comprehensive warming potential of greenhouse gases in rice and wheat cultivation. Compared with other treatments, the comprehensive warming potential under 30% composting alternative treatment had the lowest N_2O emission because nitrification and denitrification led by microorganisms in soil were the main ways to produce N_2O, and the application of chemical fertilizer provided a prerequisite for nitrification and denitrification. Consequently, it promotes the emissions of N_2O.

When compost is utilized to partially replace chemical fertilizers, its slow nutrient release rate reduces the total available nitrogen content in the soil. This decrease in nitrogen availability hinders the processes of nitrification and denitrification, thereby mitigating the production and emission of N_2O (Miao *et al*., 2020). Additionally, the high ratio of 30% compost to replace fertilizer, the concentration of nitrate and total soluble nitrogen in the leaching solution of farmland soil was reduced. The leaching loss of soil nitrogen was effectively prevented and the emission of N_2O was diminished. Overall, the combined warming potential of 30% composting fertilizer was 53.20% lower than that of conventional fertilizer, indicating that the proportion of compost applied significantly influences the soil's organic nitrogen dynamics.

Replacing chemical fertilizer with compost significantly impacted the soil microbial community, resulting in an increase in the abundance of dominant bacterial phyla such as Proteobacteria, Bacteroides, Firmicutes, and Actinobacteria. Proteobacteria grew in a highly nutritious environment, indicating that the application of bio-compost subsequently, increased the nutrient content of soil and then affected its physical and chemical properties. Both *Bacteroides* and *Firmicutes* are negatively correlated with N_2O emission. Therefore, substituting composting can increase the activity and species of soil microorganisms and improve the utilization of nitrogen fertilizer while reducing N_2O emissions (Lu & Wu, 2018; Nie *et al*., 2018; Kong, 2020; Xu, 2020b).

Liu, (2022) demonstrated that increasing dominant bacteria in soil can improve soil biological activity and fertility. Aligning with the findings of this study. Moreover, Ma. (2019) reported that a relative abundance of *Bacillus* with small relative abundance increased significantly with fertilizer application, indicating that compost promoted crop growth and development. Studies had shown that nitrogen-fixing microorganisms mainly include α-type, β-type and γ-type Proteus types (Beatty & Good 2011). Among these populations, *Firmicutes* are crucial for decomposing crop straw and are amongst the most abundant bacteria in the soil. They play a pivotal role in nitrogen utilization and compete with nitrifying bacteria for nitrogen sources. Consequently, the fixation of available nitrogen in crops is promoted, the substrate for N_2O production was reduced (Das & Adhya, 2014; Yao *et al*., 2013), and N_2O emissions were suppressed.

Furthermore, the substitution of compost for chemical fertilizers significantly enhanced the yield and quality of rice and wheat. As the proportion of compost substitution increased, the nitrogen concentration in crop straw decreased; however, the nitrogen content in the grain remained unchanged, resulting in improved yield and quality, even when combined with microbial suspension applications. Under the alternative condition of compost, the treatment with the highest yield increase was 30%, which enriched soil microorganisms, promoted soil nitrogen fixation, improved soil fertility, and reduced greenhouse gas emissions, consistent with the research findings of Tao *et al*., (2017), which also indicated that compost substitution is an effective fertilization strategy that enhances the soil environment, mitigates environmental pollution, and increases both yield and quality.

Conclusion

Through out the whole rice-wheat rotation, composting substituted with microbial suspension significantly reduced the emission of CH_4 and N_2O. Specifically, CH_4 emissions of conventional fertilizer in main zone were the smallest, that of 30% compost was the largest, that of MHZ007 microbial suspension in split zone was the smallest, and that of unapplied microbial suspension was the largest. For N_2O the smallest accumulation occurred with 30% compost substitution, and the largest accumulation was associated with conventional fertilizer. The emission of MHZ007 microbial suspension in split zone was the smallest, and the emission of N_2O from unmixed microbial suspension was the largest.

Regarding GWP and GHGI, composting replaced 30% with MHZ007 microbial suspension can reduce GHGI and increase 17.87% without affecting the yield. The greenhouse gas emission reduction effect of crops was mainly achieved under the condition that the compost replaced 30% and MHZ007 microbial suspension treatment was applied. This approach demonstrates that composting, when paired with microbial suspensions, is advantageous for both economic and environmental sustainability. Future fertilization strategies should emphasize the cultivation of microorganisms and the utilization of natural fertilizers in place of chemical fertilizers and pesticides, aiming to reduce greenhouse gas emissions, enhance soil health, and improve agricultural productivity and quality.

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