

BIOMASS COMPOSTING COMBINED WITH A BIOLOGICAL NITRIFICATION INHIBITOR INFLUENCES GREENHOUSE GAS EMISSION AND MICROBIAL COMMUNITY IN RICE PADDY

CLEVIE THERTULLY BALONGANA MAYASSI¹, CECE QIAO¹, YACHUN WANG¹, XINGGHEN HUANG¹, HONGZHI MIN¹, YUNING ZOU¹, BING HAO², LANTIAN REN^{1*} AND WENGE WU^{1,3*}

¹Xiaogang Village Institute of modern ecological agriculture, Anhui Institute of science and Technology Serving Local Development Research Institute. University of Science and Technology of Anhui, Fengyang 233100, China

²Key Laboratory of Bio-organic Fertilizer Creation Ministry of Agriculture, Bengbu 233400, China

³Rice Research Institute, Anhui Academy of Agricultural Sciences, Hefei 230031, China

*Corresponding author's email sky1981007@163.com

Abstract

Characterizing the composition of the microbial community in the rhizosphere associated with higher crop yields and (or) greenhouse gases is an important first step in understanding the role of microbiota in paddy fields. The aim of these studies was to decipher the effects of straw tea composting in combination with the addition of biological nitrification inhibitors on greenhouse gas emissions and the microbial community in paddy fields. Quan 9 you 063 varieties of rice in Fengyang, Anhui Province were used as the study object, the amount of conventional fertilizer as the control CK. The results showed that the addition of biological nitrification inhibitors could reduce gas emissions and significantly increase rice yield by 41.70%, as well as improve root system growth and development. In addition, compost products can reduce greenhouse gas emissions. However, the biological nitrification inhibitor affected communities of nitrogen-fixing bacteria (Proteobacteria), nitrogen-eating bacteria (Nitrospirae) and Verrucomicrobia, which is known to be one of the most common and diverse species in soil habitats. As with bacteria, different community structures and distribution patterns of archaea have been observed in soil; The Thaumarchaeota type was the most important of all treatments.

Key words: Biomass composting; Greenhouse Gas Emission; Microbial community; Rice paddy.

Introduction

Rice (*Oryza sativa* L.) is one of the most important crops in the world and is a staple food crop for at least 65% of the Chinese population. Rice is typically cultivated in China with an average annual rice planting area of 35 million ha and contributes about 30% of total world rice production (Bandumula, 2018). Generally, rice is grown in soils deficient in N. As the world's largest consumer of N fertilizer; China currently covers 30% of the world's N consumption while a low nitrogen use efficiency (NUE) of about 30-35% in rice paddies (Peng *et al.*, 2006). However, humanity is facing the dual challenges of both ensuring food productivity while concomitantly minimizing the significant environmental impacts such as greenhouse gas (GHG) emissions of intensive agriculture (Devendra *et al.*, 2013) due to excessive use of N fertilizer (Ren *et al.*, 2020).

Composting is an intensive treatment process for organic waste that implements, by optimizing them, aerobic biological processes of degradation and stabilization of complex organic matter (Gobat *et al.*, 2003). The raw materials for composting are composed mainly of plant remains and relatively few animal remains or mineral substances (Fuchs *et al.*, 2001). The resulting composts have a dual nature: amendment, because they contain organic compounds that are precursors of humus, and fertilizer, because of their content of fertilizing elements (Gobat *et al.*, 2003). They therefore make it possible to make up for the deficit of overexploited soils and to improve their long-term fertility. The goal of composting is the production of stable products which can be stored without additional treatment and which can be applied to the soil without

causing damage to the crops and which, on the contrary, improve the fertility of the soil and the health of the plants. The decrease in soil fertility following over-intensive or inappropriate agriculture is observed in both industrialized and developing countries. This results in a loss of stable organic matter in the soil and an increased susceptibility of plants to diseases, due to the microbiological imbalance of the soil.

The increasing use of chemical fertilizers has deteriorated soil fertility and the quality of agricultural products, which has not only affected the overall productive capacity of soils, but also affected the ecological environment (Rawluk *et al.*, 2001). Since composting is a rich source of nutrients with a high organic matter content, it has become an effective method to replace chemical fertilizers (Turmel *et al.*, 2015). Composting can be beneficial for improving the condition of organic matter and microbial characteristics, it can also reduce CH₄ emissions (Farmaha, 2014). The use of compost is therefore a need of the time. However, as global warming worsens and the greenhouse effect intensifies, emissions of N₂O and CH₄ in rice paddy harbored more concerns Awasthi *et al.*, (2017). Relevant studies have shown that CH₄ and N₂O PRG on scale of 100 is 25 times and 298 times that of CO₂ (Anon., 2007). Dicyandiamide (DCD) is known to limit speed of the nitrification-denitrification process, thus decreasing the losses of nitrogen (N) and the ubiquitous production of N₂O in soil and other environments.

In agro-ecosystems, soil microorganisms represent the largest reservoir of biodiversity. The soil microbiome performs various ecological services such as mineralization of organic matter, nutrient cycling, and the promotion of plant growth (Saravanakumar *et*

al., 2017). Remarkably, the microorganisms involved in the nitrification reaction are autotrophic Bacteria which participate in the oxidation of an inorganic substrate as the sole source of energy and mineral carbon (Zhou *et al.*, 2015). Nitrosation and nitrification constitute the fundamental processes of nitrification in farmland soils, during which the intermediate product NH_4^+ was converted into NO_2^- and NH_2OH that mainly completed by Ammonia-Oxidizing Archaea (AOA) and Ammonia-Oxidizing Bacteria (AOB). Accordingly, AOB plays an important role in the transformation of NO_2^- into NO_3^- (Wu *et al.*, 2020). During the process, the first step is ammonia which is oxidized to nitrite by *Nitrosomonas*, *Nitrococcus*, and *Nitrospira*. When nitrite in turn is oxidized to nitrate by *Nitrobacter*, *Nitrococcus*, and *Nitrospira*, which form the second step. It should be noted that this process only happens with relatively low organic matter. The compositions of AOA and AOB in paddy soils can be influenced by multiple physicochemical properties, such as organic carbon and nitrogen contents, soil type, pH, and flooding-drying cycle. For example, AOB are well adapted to eutrophic environments with high loads of organic carbon and total ammonium (NH_4^+ and NH_3), Such as agricultural soils and wastewaters (Erguder *et al.*, 2009). However, in some paddy soils, AOA is one to three orders of magnitude more abundant than AOB, although paddy soils generally have high nitrogen and organic carbon contents, especially after intense fertilization over long periods of the term. The net nitrification rate was significantly related to the abundance of AOB (Chen *et al.*, 2015), and DCD exerted more significant inhibition on AOB growth than AOA (Shen *et al.*, 2013). Besides, DCD was mainly studied in croplands and grasslands, where amendment of DCD ($10\text{-}30 \text{ kg ha}^{-1}$) was indicated to be effective in mitigating N_2O emissions (Belastegui *et al.*, 2003). Despite the fact that nitrification inhibitors are very effective in reducing N_2O emissions, they are not widely used in production due to their high cost and toxicity; As a result, some researchers have turned their attention to biological nitrification inhibitors, the application of which is seen as an important new strategy for mitigating nitrogen losses from agricultural soils. MHPP (methyl3-(4-hydroxyphenyl) propionate) has been identified as a BNI of sorghum root exudate to inhibit nitrification in soil (Zakir *et al.*, 2008). The studies conducted by (Ren *et al.*, 2019) regarding the analysis of the effect of the biological nitrification inhibitor MHPP on nitrogen fixation and emission reduction for composting of straw and slag manure, The results showed that the addition

of 0.6% MHPP extract solution gave better results for nitrogen fixation and emission reduction and should therefore be used widely in production activities. Nardi *et al.*, 2013 in their study showed that sorghum MHPP was able to suppress nitrification in soil while reducing the abundance and activity of ammonia oxidizing microorganisms, suggesting that AOB and AOA are the targets of the biological nitrification inhibitor. However, few studies focused on the short-term shifts in the rhizosphere microbial community under the combinatory of straw compost and biological nitrification inhibitor to better understand the impact on soil quality, improve agro-ecosystem productivity, and mitigate greenhouse gas emissions.

Therefore, this study aimed to investigate effect of straw composting on the growth and development of rice by substituting organic fertilizer and amendment of biological nitrification inhibitors. The objectives were to: (i) To minimize the N_2O emissions in rice paddies; (ii) To identify the rhizosphere microbial structure; (iii) To decipher the impact of the application of composts as means of restoring soil fertility.

Material and Methods

Site description and experiment layout: The field experiment was located in the plantation of Anhui Science and Technology University (E117 - 33-39 ", W32 - 52-49 ") from July 2019 to November 2019. Sowing was conducted on June 12th, Transportation in the field was done on July 2nd, rice was harvested on November 1st. The frost-free period of this region is 230 days, with an average annual temperature and precipitation of 15°C and 1200 mm, respectively. The soil was characterized as cinnamon soil, with pH 6.02, and contains 20.8 g kg^{-1} organic matter, 2.14 g kg^{-1} total N, 3.21 g kg^{-1} total P, 7.53 g kg^{-1} total K, and 25.8 mg kg^{-1} available P, 115 mg kg^{-1} available K of arable soil layer. The variety of rice was Quan 9 You 063. The rice straw used was obtained after harvesting in our field.

The field experiment was performed in a completely randomized block design with three replicates for each of the following treatments (Table 1), and the plots 2 m*2 m whose control CK, treatment T1, T2, T3 and T4 were isolated between any two adjacent plots for preventing water and fertilizer exchange. P-hydroxybenzoic acid (MHPP) sorghum root exudate was used as biological nitrification inhibitors, it was prepared by hydroponic cultivation of sweet sorghum, The variety of sweet sorghum was Hercules. The nutrient composition of straw compost was, N: 1.03%, P_2O_5 : 0.87%, K_2O : 1.35%.

Table 1. Treatment setup.

Treatment	The concentration
CK	50 kg 15-15-15 compound fertilizer + 30 kg urea per 666.67 m ²
T1	10 t/ha compost + nitrification inhibitor DCD (3.5% of 50 kg)
T2	10 t/ha compost + biological nitrification inhibitor spray 6 times (4l/time)
T4	10 t/ha compost + straw compost tea 15 times + biological nitrification inhibitor spray 6 times

Note: Other treatments reduced compound fertilizer and urea by 20% and increased compost by 10t/ha compared with CK

Gas sampling method: According to the temperature, the gas collection was eight times from July to September. The sampling in the static chamber and back box method was adopted. The gas prefer every five minutes during sampling with a syringe, three times in total for each treatment. The temperature in the static chamber and the soil surface was recorded each time the gas was withdrawn. The main trial greenhouse gas emission flow note (CO_2 , NH_4 , N_2O) were measured using a gaseous phase chromatograph Agilent 798A (Agilent, USA); the interpolation method was used to calculate unlocked daily emission flows according to (Chen *et al.*, 2017). Hence, the relative CO_2 , NH_4 , and N_2O emissions were obtained by calculating daily values following the given equation below:

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

Note: F, greenhouse gas emission flux ($\text{mg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$), dc/dt , slope of the regression curve of gas volume fraction with time during sampling, M, molar mass of the gas ($\text{g} \cdot \text{mol}^{-1}$), V_0 , molar volume of the gas under the standard gas ($22.41 \text{ l} \cdot \text{mol}^{-1}$), P and P_0 , pressure of sampling point (Pa) and standard state, respectively, T and T_0 are the absolute temperature (K) at the time of sampling and the absolute temperature under standard conditions (273.15 K), H is the height (m) of the sample box.

DNA extraction and quantitative PCR analysis: Three rhizosphere sub-samples were collected from each replicate plot and mixed as a soil sample at the harvest time. All soil samples were stored at $-70 \text{ }^\circ\text{C}$ for subsequent DNA extraction. Soil DNA was extracted from 0.25 g soil subsamples using the Power Soil DNA Isolation Kits (MoBio Laboratories Inc., Carlsbad, USA). Genomic DNA was extracted according to the instructions of DNA extraction kits corresponding to various samples. DNA integrity and purity were detected by 1% agarose gel electrophoresis, and DNA concentration and purity were detected by Nano-Drop-One. PCR amplification and electrophoresis detection of the products were conducted using genomic DNA as the template, and based on the selection of sequencing regions, barcode primers and PremixTaq (TaKaRa) were used for PCR amplification. Using Gene Tools Analysis Software (Version 4.03.05.0, SynGene) concentration of PCR products after contrast, calculated in accordance with the principle of quality such as the sample size required, each PCR product. The PCR mixture was recovered using the E.Z.N.A.[®]GelExtractionKit gel recovery kit, and the target DNA fragment was eluted with TE buffer. After carrying on library according to the NEBNext[®] Ultra[™] DNA Library Prep Kit for Illumina[®] standard process for building operation, is completed by high-throughput sequencing platform Miseq sequence on the computer. The Raw image data files obtained by sequencing are analyzed by (Base Calling) and converted into (Raw Reads), The results are stored in FASTQ (FQ for short) file format, which contains sequence information of sequencing sequences (Reads) and their corresponding sequencing quality information.

Statistical analysis: As statistical test were considered statistically significant at $p < 0.05$. The sequences retained were analyzed using the UPARSE pipeline to generate an OTU table with representative sequences (Edgar, 2013).

Subsequent analyses (Alpha and Beta diversities) were based on OTUs by Unifrac distance and map analysis evaluating the similarity of bacterial communities in different samples. Principal coordinates analysis (PCoA) based on Bray-Curtis distances were conducted to evaluate beta-diversity patterns using the vegan package of R (version 3.5.1, R Core Team, 2015). The classification of the representative sequences for each OTU was performed using the RDP classifier against the RDP Bacterial 16S rRNA database (Cole *et al.*, 2009). The realization of the ternary figure was proceeded using the ggtern package. Co-occurring networks were constructed using ggplot2 packages of R (3.3.1; Duncan Murdoch, 2016) and was derived by defining a boundary between pairs of coexisting OTUs, using as an input matrix with Spearman correlations ($p < 0.4$).

Results

N_2O , CO_2 , CH_4 emission characteristics: The N_2O emission in rice paddies sprayed with biological nitrification inhibitors and straw compost. It had two peaks, one occurred on August 11th, and a smaller peak occurred on August 30th. The rank of N_2O emissions was $\text{CK} > \text{T3} > \text{T4} > \text{T1} > \text{T2}$ on August 11th. The N_2O emissions from CK processing were $517.75 \text{ t C} \cdot \text{hm}^{-2}$, the N_2O emission of T3 is $481.10 \text{ t C} \cdot \text{hm}^{-2}$, which is 7.08% lower than the N_2O emission of CK. Another smaller peak was on August 21st. The lowest emissions were T1 ($55.88 \text{ t C} \cdot \text{hm}^{-2}$), followed by T4 ($61.36 \text{ t C} \cdot \text{hm}^{-2}$). As a result, T1 with the application of DCD and T2 with the application of biological nitrification inhibitors effectively mitigate the gas emission of rice paddies, T4 with the application of biological nitrification inhibitors and compost tea harbored slight fewer gas emissions (Fig. 1A).

The CO_2 emissions were characterized by two peaks (Fig. 1B). One occurred on July 21st, which the highest CO_2 emissions were T3 and CK, followed by T4, T2, and T1, the CO_2 emissions of T3 were $1608 \text{ t C} \cdot \text{hm}^{-2}$, which is 129% of T1. Another peak occurred on August 21st, and the lowest emissions were treatment T1 ($522.00 \text{ t C} \cdot \text{hm}^{-2}$).

The lowest value of CH_4 emission occurred on August 11th, rank in gas followed order $\text{T2} (-0.10 \text{ t C} \cdot \text{hm}^{-2}) > \text{T4} (-0.18 \text{ t C} \cdot \text{hm}^{-2}) > \text{T3} (-0.66 \text{ t C} \cdot \text{hm}^{-2}) > \text{CK} > \text{T1} (-1.16 \text{ t C} \cdot \text{hm}^{-2})$. The application of DCD (T1) and biological nitrification inhibitor (T2) effectively inhibited gas emissions in rice paddies compared to compost tea and biological nitrification inhibitor combined with compost tea. Overall, T1 with the application of DCD and T2 with the application of biological nitrification inhibitors indicated high efficiency from the perspective of gas emission of rice paddies (Fig. 1A).

Total greenhouse gas emissions and analysis of rice yield and yield composition: Straw compost combined with nitrification inhibitors throughout the rice growth period significantly migrated CH_4 emissions in rice paddies. T1 significantly reduced CH_4 emissions, followed by T2 with biological nitrification inhibitors compared with CK. The reduction of N_2O emissions in the rice paddies was made by using the combined application of straw compost instead of chemical fertilizers and nitrification inhibitors. N_2O emissions from T1, T2, T3, and T4 decreased by 51.56%, 46.71%, 13.84%, and 30.10%, respectively (Table 2).

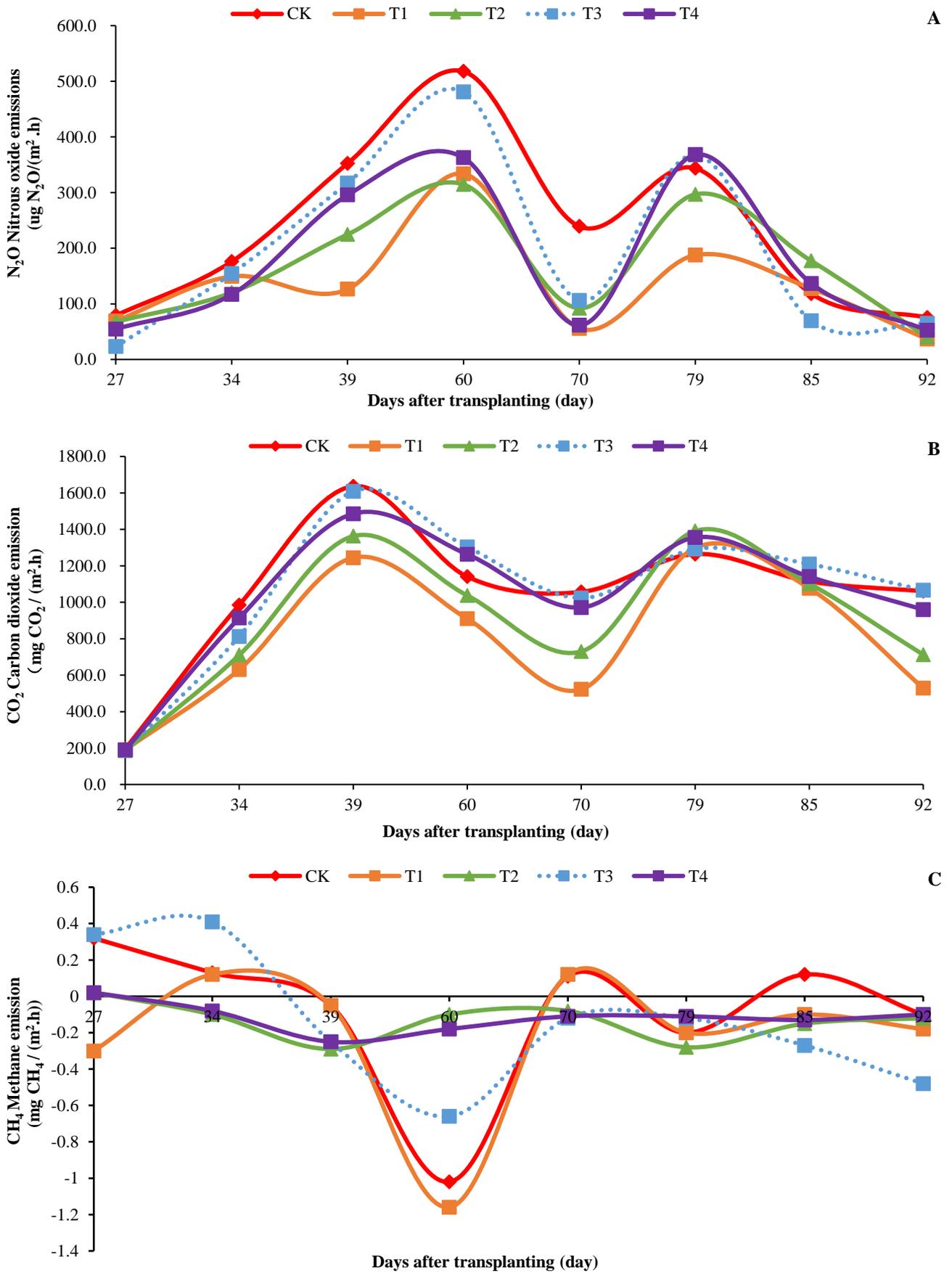


Fig. 1. The effects of straw compost combined with nitrification inhibitor treatments on the N₂O (A); CO₂ (B); CH₄ (C) emission rate of rice field soil

Note: CK, purified fertilizer; T1, nitrification inhibitor DCD; T2, biological nitrification inhibitor; T3, straw compost tea; T4, straw compost tea + biological nitrification inhibitor. The same as below.

Table 2. Combination of straw compost and nitrification inhibitor treatment on rice field gas emissions and effects of straw compost combined with nitrification inhibitor on rice yield components.

Treatment	CO ₂ emission (t C·hm ⁻²)	CH ₄ emission (kgc/hm ²)	N ₂ O emission (kgc/hm ²)	Total emission (t C·hm ⁻²)	Spike number / (ten thousand /hm ²)	Kernels per spike / (per spike)	1000-grain weight (g)	Yield (kg/hm ²)
CK	40.57 ± 1.01a	-5.56 ± 0.14b	2.89 ± 0.07a	40.57 ± 1.01ab	8.20 ± 0.21b	208.20 ± 5.21e	22.09 ± 0.55b	5897.49 ± 10.34e
T1	30.72 ± 0.77c	-9.56 ± 0.24c	1.40 ± 0.03b	30.72 ± 0.77e	9.70 ± 0.24a	240.50 ± 6.01ab	22.36 ± 0.56ab	7288.50 ± 12.51c
T2	34.71 ± 0.87b	-5.36 ± 0.13b	1.54 ± 0.04b	34.71 ± 0.87d	9.00 ± 0.23ab	246.38 ± 6.16a	22.36 ± 0.56ab	7710.25 ± 13.35b
T3	40.81 ± 1.02a	-5.62 ± 0.14b	2.49 ± 0.06a	40.81 ± 1.02a	8.50 ± 0.21b	232.13 ± 5.80c	22.37 ± 0.56ab	6774.72 ± 11.73d
T4	39.74 ± 0.99a	-4.50 ± 0.11a	2.02 ± 0.05b	39.74 ± 0.99b	8.67 ± 0.22b	239.51 ± 5.99ab	22.86 ± 0.57a	8356.78 ± 38.23a

Note: Different lowercase letters indicate significant differences at $p < 0.05$

Table 3. Composting straw compost combined with nitrification inhibitors and their potential for comprehensive warming in rice fields.

Treatment	CO ₂ GWP (kg/hm ²)	CH ₄ GWP (kg/hm ²)	N ₂ O GWP (kg /hm ²)	Total GWP (kg /hm ²)
CK	40572.15	-139.03	861.37	41294.49
T1	30726.33	-238.88	416.20	30903.65
T2	34710.88	-134.01	460.37	35037.24
T3	40814.00	-140.38	741.64	41415.26
T4	39739.99	-112.43	601.21	40228.78

Table 4. Effects of straw compost combined with nitrification inhibitor on rice quality.

Treatment	Protein (%)	Chalkiness degree (%)	Chalky rice rate (%)	Glue consistency (%)	Indica rice amylose (%)	Brown rice rate (%)	Head rice yield (%)	Luminousness (%)
CK	7.00 ± 0.17a	14.74 ± 0.37c	7.93 ± 0.20b	119.41 ± 2.99b	10.88 ± 0.27b	80.17 ± 2.00ab	72.58 ± 1.81b	0.82 ± 0.02b
T1	6.00 ± 0.15bc	19.77 ± 0.49a	19.53 ± 0.649a	120.30 ± 3.01b	10.44 ± 0.26b	80.73 ± 2.02ab	73.64 ± 1.84a	0.85 ± 0.02ab
T2	5.37 ± 0.13c	20.08 ± 0.50a	15.34 ± 0.38ab	130.15 ± 3.25a	11.73 ± 0.29ab	81.48 ± 2.04a	73.64 ± 1.84a	0.88 ± 0.02a
T3	5.90 ± 0.15bc	16.83 ± 0.42ab	9.63 ± 0.24b	117.98 ± 2.95c	10.95 ± 0.27ab	80.50 ± 2.01bc	72.55 ± 1.81b	0.84 ± 0.02ab
T4	6.40 ± 0.16ab	18.38 ± 0.46ab	18.75 ± 0.547a	120.25 ± 3.01b	12.26 ± 0.31a	81.27 ± 2.03ab	73.43 ± 1.84a	0.83 ± 0.02b

Note: Different lowercase letters indicate significant differences at $p < 0.05$

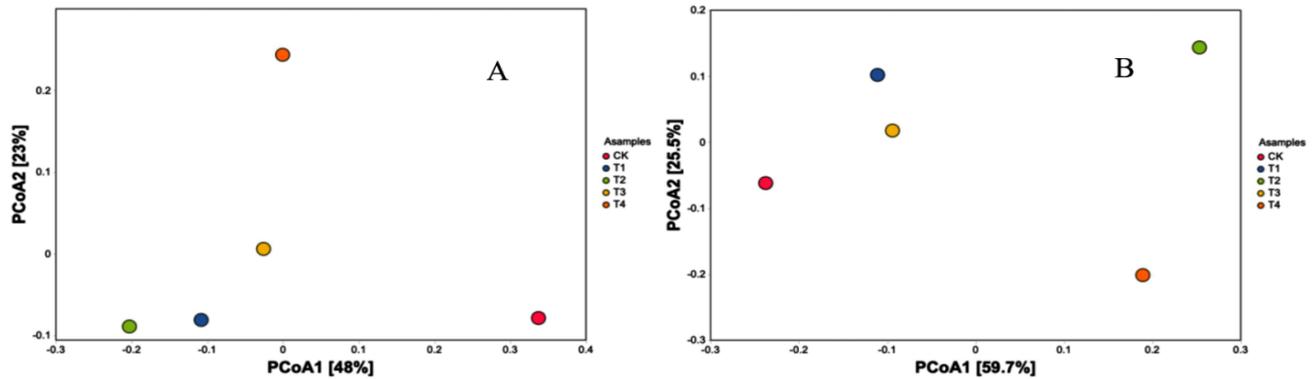


Fig. 2. Analysis of the principal coordinates (PCoA) of Bacteria (A) and Archaea (B) over the weighted Unifrac distance.

Comprehensive warming potential: Analysis in Table 3 shows that the warming potential varies significantly between treatments. The replacement of part of the fertilizers with straw compost has made it possible to significantly reduce the warming potential in rice fields. The lowest warming potential was observed in T1 treatment, which was 33.62%, 13.38%, 34.01% and 30.17% lower than CK, T2 and T4 treatments, respectively. The low total emissions of greenhouse gases from T1 treatment are mainly due to the fact that CH₄, CO₂ and N₂O have a lower warming potential. Among them, the warming potential of CO₂ T1 is 32.04% lower than CK, the temperature increase in temperature T1 CH₄ is 41.80% lower than CK and the temperature increase potential T1 N₂O is 106.96% lower than CK.

Rice quality analysis: The protein content of treatments T1, T2, T3, and T4 was significantly lower than that of CK (Table 4). Spraying biological nitrification inhibitors and straw compost tea decreased significantly, but spraying biological nitrification inhibitors and straw compost tea can effectively improve the degree of protein reduction. The chalkiness rate of treatments T1, T2, T3, and T4 increased significantly. Only treatment CK and T3 met the requirements (High-quality premium rice chalky kernel ratio < 10%), and all other treatments had reached second-class high-quality rice requirements. The amylose content of indica rice in each treatment was significantly higher than that of the control group. Except for T1, the amylose content of high-quality indica rice reached the national standard (13.0%-22.0%) and was sprayed with biological nitrification inhibitor and straw. The treatment of composted tea was significantly higher than that of DCD, indicating that biological nitrification inhibitors can effectively replace DCD. Overall, T1, T2, T3, and T4 conformed to the high-quality national rice (brown rice rate > 80%, whole rice rate > 66%). It comprehensively shows that the quality of rice obtained by using biological nitrification inhibitor instead of DCD and combined with straw compost tea has reached the national high-quality rice standard.

Microbial diversity analysis

β -diversity: In order to visualize the differences in the composition of bacterial communities and archaea, the analysis of the principal coordinates (PCoA) was carried out, respectively. The ordination of PCoA revealed a distinct sample grouping based on different treatments (PERMANOVA, pseudo-F = 12.09, $p < 0.001$; ANOSIM, $p < 0.001$). Regarding the analysis of the main coordinates,

the bacterial communities were separated by the first component (PCoA1) with a total of 48% and the second component (PCoA2) with a total of 23% (Fig. 2A). In archaea, the total of the first component was 59%, and that of the second was 25.5% (Fig. 2B).

Microbial community taxonomic composition of rhizosphere soil bacteria and Archaea communities: Bacteria are one of the most abundant microorganisms in soil, play a very important role in soil nutrient cycling, and are also able to fix nitrogen. Similarly, these have a role in soil fertility for agriculture. The taxonomic sequences of all species were classified into 30 bacterial phyla. The dominant bacteria were: Proteobacteria, Gemmatimonadetes, Acidobacteria, Chloroflexi, Nitrospirae, Verrucomicrobia, accounting for more than 75.0% of the total bacterial 16 rRNA gene sequences. Considering the CK and T3 treatments, most OTUs belonged to Acidobacteria and Proteobacteria (Fig. 3A). It may indicate that Acidobacteria played a key role in the denitrifying activity and used nitrites as a source of nitrogen, suggesting their involvement in the nitrogen cycle. T4 was the highest in OTU-39, followed by T2. OUT-11, belonging to the phylum Chloroflexi, can increase organic carbon and participate in nitrification. After all these analyses, T4 treatment of straw composting tea and biological nitrification inhibitor was the highest, followed by T1 treatment of DCD.

Figure 3B shows an abundance of two phyla Thaumarchaeota and Nanoarchaeota, of CK and T3. The relative abundances of OUT-4, OUT-45, OUT-72, OUT-161, and OTU-1 were high; Nitrososphaeracea usually participates in the soil nitrogen cycle. OUT-105 presents a class of Bathyarchaeia, a class of archaea exhibiting various metabolic modes in the phylum of Crenarchaeota; this can degrade proteins, carbohydrates, fatty acids, and aromatic compounds. Acting as a heterophilic metabolism of organic matter like methyl compounds, this class has the power to perform autotrophic metabolism while using H₂ and CO₂ to produce acetic acid. It also has the ability to conceal the reduction of nitrates and sulfate while participating in the metabolic cycle of methane. OTU-105 was also used in the T4. OTU-161, OTU-188, and OTU-14 were all Archean microorganisms of the phylum Woesearchaeia and Nanoarchaeota; they were affected by pore size and were most abundant in T4 treatment. Besides, the nitrogen and organic carbon content have not influenced the composition of these two communities and have contributed to supporting our rice plant's growth.

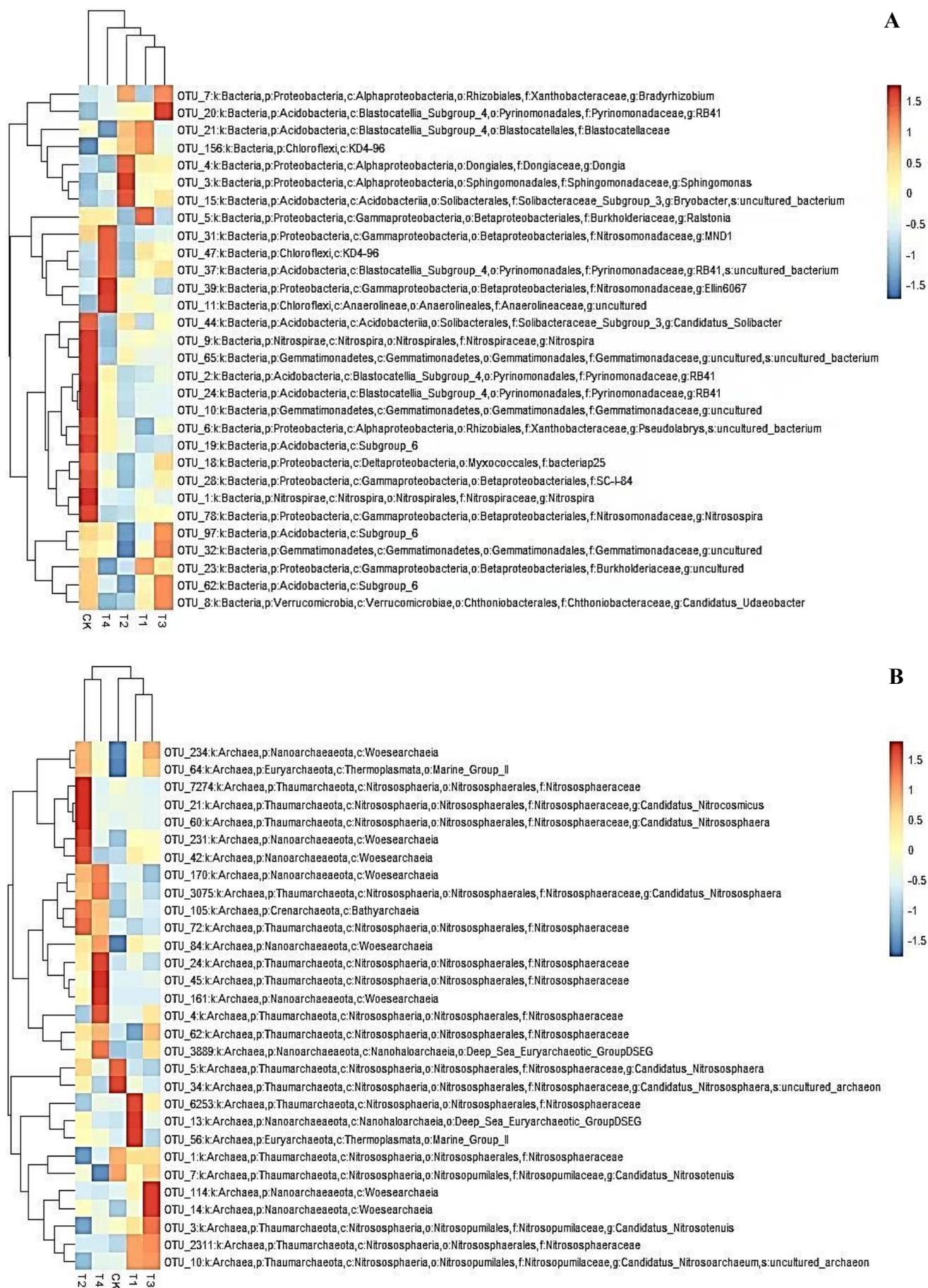


Fig. 3. Cluster analysis of species abundance of bacterial (A) and archaeal (B).

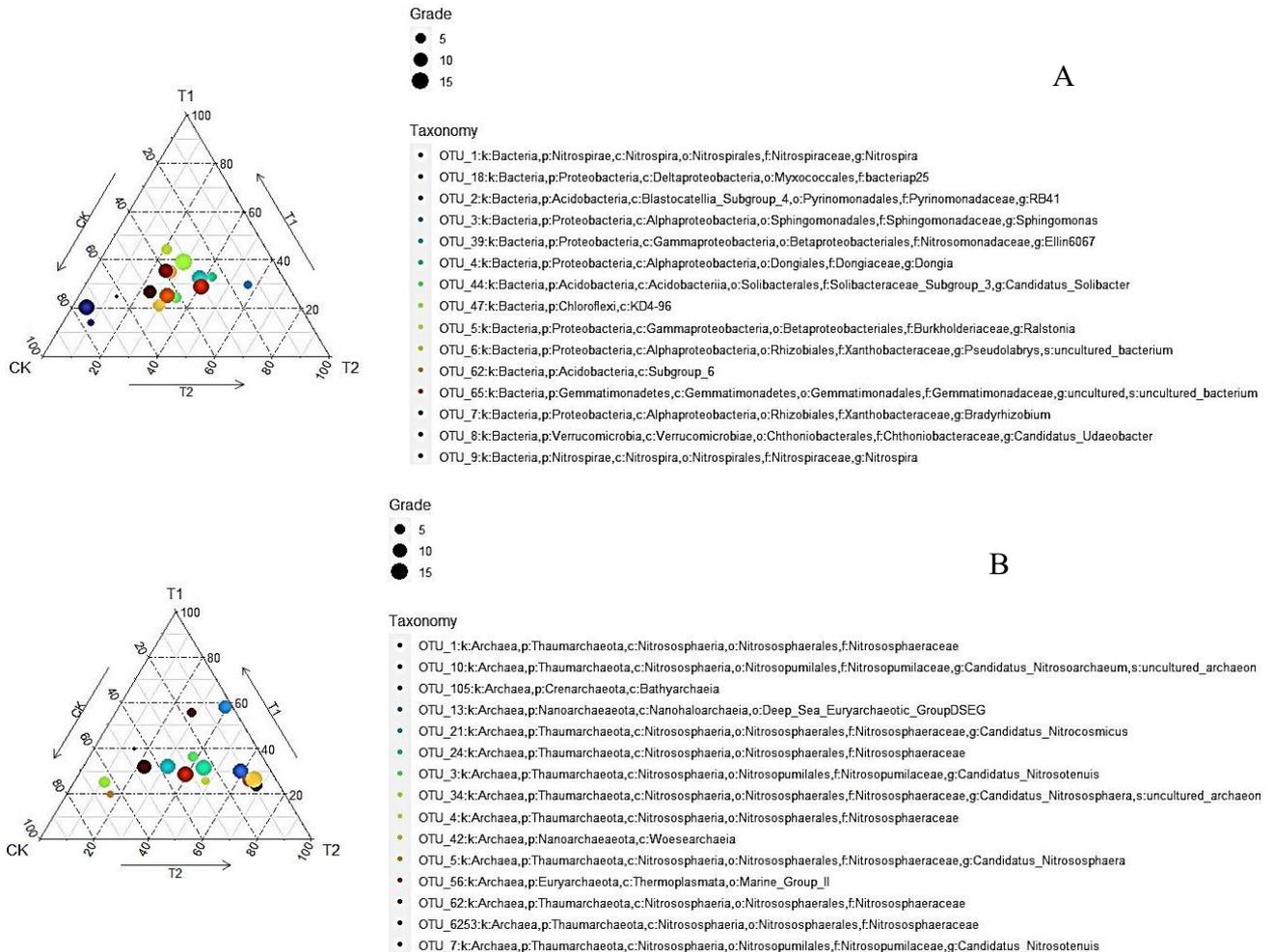


Fig. 4. Ternary phase diagram of Bacteria (A) and Archaea (B).

Ternary plot of OTUs showing the distribution of OTUs in different sample categories: The portions of taxa of Bacteria and Archaea participated due to the high intraspecies variability (Fig. 4). The ternary plot showed off that it participated advanced portions of bacterial communities and the advanced portions of Archaea. Proteobacteria have usually been set up as essential corridor of the rhizosphere community. Besides the cube type, they were also present-day at the position of the phylum. The skimp phylum Chloroflexi was significantly swelled in the soil of the T2 treatment, whereas in control CK and T1, the bacterial community of the slices sheltered more Acidobacteria, Gemmatimonadetes, Nitrospirae, and Verrucomicrobia (Fig. 4A). Ternary plots for Archaeal OTUs indicated for the top and topsoil (Fig. 4B) that the rhizosphere made not house specialized OTUs, which would be located at the separate tip area of the ternary path. For all the treatments, specialized archaea were set up when the face and the topsoil were assimilated, which were codified as the Euryarchaeota. still, the maturity of arched OTUs were located in the middle ground of the ternary plot, substantially home to Thaumarchaeota involving 1, 10, 21, 24, 3, 34, 4, 5, 62, ubiquitous OTUs (all Nitrososphaera), which were present-day in all slices and contributed to at least 85 of readings dissection.

Discussion

Rice is unique among cereals in that it withstands a wide variety of climatic, soil and hydrological conditions. Nowadays, with the improvement in living standards and the trend of population growth and reduction of arable land, increasing rice production in china has become an important issue. Over time, the excessive use of chemical fertilizers has led not only to soil degradation, but also to large-scale ecosystem degradation and long-term productivity losses. To address these concerns, replacing chemical fertilizers with organic fertilizers becomes a useful way to increase the efficiency of plant resource use as well as improve the quality of agricultural products. More and more attention is paid to the excessive accumulation of straw resources, efficient use of straw and sustainable development.

Dicyandiamide (DCD) is known to suppress the limiting first step of the nitrification-denitrification process, thereby decreasing nitrogen (N) losses and ubiquitous nitrous oxide (N₂O) production in soils and other environments. The nitrification inhibitor dicyandiamide (DCD) has been shown to be effective in decreasing N₂O emissions when applied in the field with mineral fertilizers (Zaman *et al.*, 2009). In this study, spraying with organic nitrification inhibitors and tea was used in an experiment

instead of DCD and chemical fertilizers to study the effects on rice yield and greenhouse gas emissions. They were linked to the rhizome microbiota. The effect of dicyandiamide on greenhouse gases from paddy fields showed that nitrification inhibitors significantly reduced N_2O emissions compared to DCD. This finding agrees with Wang *et al.*, (2017), who examined that DCD change had no significant impact on CO_2 emissions from paddy fields, but a significant reduction in the amount CH_4 emissions down to 20.7% and N_2O emissions of 31.9%, and a significant increase in rice yield of 10.0%. In general, the biological nitrification inhibitor is a liquid secreted by sorghum roots, suggesting it is more suitable use than DCD. In this study, the results showed that CH_4 emission from rice field according to DCD at late growth stage of rice has the best effect on reducing CO_2 and N_2O . These results are confirmed by Wu *et al.*, (2020), who studied the use of straw manure to partially replace chemical fertilizers with DCD and found that straw manure can reduce greenhouse gas emissions glass while stabilizing wheat yield and improving wheat quality.

Regarding the effect of returning rice straw to the field as an alternative to some chemical fertilizers on rice field greenhouse gases, in this experiment the rice straw returned to the field was mainly used to compost tea straw, which can reduce greenhouse gas emissions. The results showed that the application of compost tea in combination with nitrification inhibitors could significantly increase rice plant height, chlorophyll content and number of branches and nodes. Similar results were found by Yu *et al.*, (2020) when combined application of inhibitors and rice straw (UIS) reduced urea-N retention in soil and decreased urea-N recovery rate by 41.29% compared to the United States at tillering stage. As such, the straw reverts to the immobilization stimulus and thus, alters the way fertilizer N is retained and distributed (Yu *et al.*, 2020; Cui *et al.*, 2021; Isabela *et al.*, 2021). Similarly, with the addition of urease inhibitors and nitrification inhibitors, inorganic N forms were structured in the soil and under feed conditions, the continuity of the NH_4^+ fertilizer affecting the retention pattern and N distribution in the soil, especially in combination with the application of straw. Therefore, it is possible to use biological nitrification inhibitors instead of DCD which can significantly increase rice yield, thousand grain weight, growth and root development at seedling stage and plant height.

Proteobacteria, Germmatimonadetes, Acidobacteria, Chloroflexi Nitrospirae and Verrucomicrobia were the major phyla present in all samples (Fig. 3A), for archaea, Thaumarchaeota and Nanoarchaeota were the most abundant (Fig. 3B), consistent with studies Previously, modified compost increased microbial diversity compared with common chemical diseases Chaudhry *et al.*, (2012), which may be due to the increased input of organic carbon substrates. However, the diversity and richness of did not differ much when it came to payouts. Thus, the addition of diseases and natural obstacles to nitrification did not completely affect the rhizosphere microbial communities in terms of diversity, abundance and abundance. The results show that there is a lot of acid bacteria in our soil with further refinement in T3 treatment and CK control. These

results are consistent with Eichorst *et al.*, (2018), who confirmed that acid bacteria are ubiquitous in degraded farmland and argued that acid bacteria are indeed fully equipped with genes mediates the metabolism of inorganic and organic sources. Regarding the dicyndiamide-related compost biomass, our results did not affect the acidobacteria community; However, we have confirmed that this combination plays an important role in Nitrogen Obsession. According to Salam *et al.*, (2020), acidic bacteria can effectively reduce nitrates and can also be nitric oxide; this is proven by genomic data which supports their active participation in nitrogenous nutrient circuits.

Many other bacteria have been cultivated on the soil of our wet rice civilization, indeed if this bone is rare due to the influence of the diseases used, it is clearly the phylum Proteobacteria, the class Alphaproteobacteria of the known Bradyrhizobium group. to other rhizobia bacteria take atmospheric nitrogen and fix it in ammonia (NH_3) or ammonium (NH_4), Gustavo (1997); Betaproteobacteria are known to be a more abundant class, essentially excluding organics and nutrients. While Acidobacteria and Proteobacteria are abundant, we grow Nitrospirae, which grow in most treatments but in small numbers, which function to recover nitrogen (Rodríguez *et al.*, 2015). Furthermore, at the site of the sector, we observed a significant reduction in the Germmatimonadetes abundance in the soil from the T3 treatments. It seems to say that these tend to be favored by moisture, Germmatimonadetes accumulates polyphosphates and reduces the amount of phosphate available to the plant. Verrucombia is another phylum present in our arable soil and present even deeper; it is the only phylum, among many others, to have significantly increased soil volume from the T3 treatment, although little is known about the physiology of these bacteria. Their abundance decreases with excess nitrogen. These bacteria play an essential role in the depletion of organic carbon (Nixon *et al.*, 2019). Chloroflexi is another phylum found in our rice soil; this was also not found to be an abundant group but was more expressed in treatments T1 and T4 (Fig. 3B). These results are consistent with other studies that have shown that these bacteria are synthetic oxidizing acetates and belong to methanazation bacteria (Sánchez *et al.*, 2017).

The soil tested in this study showed another microorganism called Archaea, including Euryarchaeota and Crenarchaeota, between which is the root of the Archean flora. In addition, the results showed the presence of four branches; therefore, the most abundant is Thaumarchaeota. Spang *et al.*, (2010) the plant that phylum Thaumarchaeota revealed by its molecular network of 16S rRNA genes is an archaea capable of oxidizing airborne ammonia. The secondary phylum is Nanoarchaeota. Therefore, Nanogearchaeum isolates were obtained only from underwater hydrothermal reflections and terrestrial hot springs. Euryarchaeota present in our soil from all treatments are significantly less abundant; again, the phylum contains all the methane production. This can be based on David *et al.*, (2001) who argue that archaea produce methane CH_4 through methanogenesis. Some Euryarchaeota archaea are aerobic and anaerobic hyperthermophiles. The last phylum was the Crenarchaeota, a less numerous thermoplasma than the others, which differs from the other archaea in ribosomal

RNA sequence. Cubonova *et al.*, (2005) showed that some histones are missing in the genome. This crop contains numerous compulsively anaerobic species that provide sulfur for its development.

Conclusion

Straw combined with biological nitrification inhibitors has played an important role in reducing greenhouse gas emissions but has also favored the yield and quality of rice. At the same time, T2 and T3 treatments containing biological nitrification inhibitors, whether for bacteria or archaea, increases the number of proteobacteria, gematimonade, acidobacteria, nitrospirae, thaumarchaeota and nanoarchaeota. Therefore the addition of biological nitrification inhibitors is the best method. During this experiment, we saw the biological nitrification inhibitors improved the development of rice and greenhouse gas emissions and the microbial community. In further studies, the impact of adding microbial agents and biological nitrification inhibitors to greenhouse gas emissions and the rice microbial community could be explored.

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