



Research article



Mechanisms of N₂O emission reduction during agricultural straw composting: functional gene regulation and bacterial community dynamics mediated by exogenous microbial inoculants

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ABSTRACT

This study investigated the effects of exogenous microbial inoculation on nitrous oxide (N₂O) emissions, N₂O-related gene expression, and bacterial community structure during straw composting. The study found that the inoculation of thermophilic bacteria and the combined inoculation of thermophilic bacteria and decomposition agent reduced N₂O emissions by 33.3 % and 50.8 %, respectively. The inoculation of exogenous microbial agents altered the physicochemical properties, functional genes, and the contribution of bacterial communities to N₂O emissions. The *amoA* gene was a key gene significantly affecting N₂O emissions ($P < 0.001$). Proteobacteria was the main potential hosts of N₂O-related functional genes and significantly influenced the gene abundances of *nxrA* and *nirS* ($P < 0.01$). Through the Partial least squares path modelling model, it was found that physicochemical properties were the most direct and crucial factors in regulating N₂O emissions. The research findings offer novel ideas and theoretical support for applying microbial agents to the resource utilization of straw and the regulation of N₂O emissions during composting.

1. Introduction

Over the past few years, the cultivation area and production of vegetables and fruits in China have been steadily increasing, accompanied by a large amount of forestry and agricultural waste, which affects the surrounding ecology and environment. Composting, a prevalent method for processing organic residues, not only achieves the minimization, detoxification, and recycling of such residues but also enriches soil fertility via microbial processes, thereby facilitating the sustainable progression of agriculture (Wang et al., 2025; Zhou et al., 2024a). Nevertheless, the composting process releases N₂O, which has a global warming potential (GWP-100) of 265–298 times that of CO₂, while depleting the ozone layer (Xiong et al., 2021). The release of large

amounts of N₂O not only reduces the quality of compost but also contributes to global climate change and poses a threat to ecosystems.

N₂O is formed as an intermediate during nitrification and denitrification, with its production and release being affected by numerous factors (microbial activity, functional gene expression, environmental conditions, etc.) (Yang et al., 2019). In the nitrification process, some NH₄⁺ is lost as N₂ and N₂O, while another part is transformed into NO₃⁻ and NO₂⁻ through nitrification. Ammonia oxidation is a crucial step in the nitrification reaction. This reaction is mainly propelled by the ammonia monooxygenase-encoding gene (*amoA*), and the *amoA* gene abundance shows a strong correlation with N₂O flux (Lin et al., 2017). NO₃⁻ is converted to N₂ and N₂O through denitrification (Su et al., 2023). The denitrification process starts with NO₃⁻ being reduced to NO₂⁻, and

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then NO_2^- is further reduced to NO. Genes for *narG*, *nirS*, and *nirK* facilitate this reduction process (Caceres et al., 2018). Immediately thereafter, the *norB* gene produces a NO reductase that converts NO to N_2O , and the *nosZ* gene produces a N_2O reductase that transforms N_2O into N_2 (Yu et al., 2020). Therefore, a detailed examination of the variations in key functional genes and microorganisms throughout the composting process is vital to uncover the mechanisms of nitrogen loss and N_2O emission.

Many studies have shown that exogenous functional bacteria, along with mineral additives, are capable of efficiently curbing N loss during composting (Wang et al., 2022a). The introduction of phosphorus slag into the sludge digestion process prolongs the thermophilic phase and reduces N_2O emissions (Xu et al., 2024). Furthermore, research revealed that introducing biochar at the onset of composting decreased N_2O emissions by 55 %. Meanwhile, microbial inoculation enhanced the total nitrogen content (TN) in the compost product by 26.8 % (Liu et al., 2024a; Lin et al., 2023). Functional microbial agents are frequently employed in composting for nitrogen-loss reduction because they are cost-effective, highly efficient, and free from secondary contamination (Liang et al., 2024). It has been shown that microbial inoculation both enhances nitrogen holding capacity and optimizes the composition of microbial communities, while also regulating the expression of nitrogen-functional genes (Tian et al., 2023). The inoculation of lignocellulose-degrading microorganisms reduced the concentration of NO_3^- -N during the composting maturity period and decreased the substrate supply for the N_2O emission reaction (Yu et al., 2020). A study by Wang et al. (2024) showed that inoculation with exogenous thermophilic bacteria increased diversity during composting, competed with denitrifying bacteria, inhibited denitrification, and consequently decreased N_2O emissions. Inoculation with actinomycetes could increase the organic and effective nitrogen content of compost products, indicating that microorganisms are the key factors influencing nitrogen metabolism during composting, as shown by Zhang et al. (2022). Thus, choosing suitable microbial agents and examining the impact on compost quality, N loss, and the structure of microbial communities is crucial.

In the present study, composite microbial agents and a commercial decomposition agent, which were screened from the thermophilic stage of compost piles, were inoculated into a mixture composed of melon fruit, vegetable straw, and mushroom waste at the start of the compost process. The dynamic characteristics of N_2O emission, the regulatory mechanism of nitrogen cycling functional gene expression, and the evolution of bacterial community composition with different microbial inoculation strategies were investigated during composting. This research will supply a potential scientific basis and technological guidance for optimizing the straw composting process and N_2O emission reduction.

2. Materials and methods

2.1. Sample collection and experimental

The raw materials, including melon fruits, vegetables straw and mushroom residue, were sourced from a farm in Liuyang, China (Main physicochemical properties of raw materials see supplementary material). Thermophilus bacterial (THB) is a *Geobacillus kaustophilus* complex microorganism isolated from samples from thermophilic stage of composting reported by previous study (He et al., 2024). The decomposition agent (DPA) is primarily composed of *Bacillus subtilis* and *Aspergillus oryzae*, with its function being to accelerate lignocellulose degradation. The straw was cut into pieces 2–3 cm, combined with mushroom residue in a 1:1 fresh weight ratio, and water was added to bring the moisture level to about 65 %. Piles were turned daily during the mesophilic and thermophilic stages (0–14 days) of composting and every 2–3 days during the cooling and maturing stages (15–45 days). The composting was conducted for 45 days at the experimental base of Hunan Academy

of Agricultural Sciences.

Four treatments were carried out as follows: control group (CK), Straw + mushroom residue (25 Kg each); treatment group 1 (T1), Straw + mushroom residue + 5 g DPA (the bacterial activity of 500 million· g^{-1}); treatment group 2 (T2), Straw + mushroom residue + 25 mL THB (each milliliter of about 100 million bacteria); treatment group 3 (T3), Straw + mushroom residue + 5 g DPA + 25 mL THB. Compost samples were taken at 1, 3, 7, 15 and 45 days, and preserved at 4 °C and –20 °C for subsequent determination.

2.2. Physicochemical analysis

The pile temperature was recorded using a stainless-steel probe thermometer (Sanyun, China). TN in samples was detected using an Elemental Analyzer (vario macro cube, GER) (Liu et al., 2024a). Samples were leached out using a 2 M/L KCl solution. Subsequently, the contents of NO_3^- -N and NH_4^+ -N were determined by a spectrophotometer (Lin et al., 2023). N_2O gas samples were collected in aluminum foil sampling bags on days 1, 3, 5, 7, 10, 15, 20, 25, 35, and 45, with three replicates. The gas from the bag was transferred to a gas bottle (30 mL) and analyzed with a gas chromatograph (Shimadzu, GC-2014, Japan). For this study's analysis, other physicochemical properties were drawn from those acquired in previous research (He et al., 2024).

2.3. Microbial community analysis

Genomic DNA from samples was extracted with a DNA SPIN Kit (Germany) according to the instructions, and the extracted DNA was preserved at –80 °C for subsequent analysis (Yin et al., 2022). In the V4-V5 region, primers 515F-907R were selected for amplification of bacteria. Polymerase chain reaction products were sequenced on the Novaseq-PE150 platform provided by Shenzhen Micro Science Alliance Technology Co., Ltd. Biotechnology Co (He et al., 2023). All original sequences have been submitted to the National Center for Biotechnology Information (NCBI) under registration number PRJNA1071281.

2.4. Quantification of target genes

Quantification of nitrogen functional genes using Gene Chip QMEC technology. The amplification process in detail was elucidated in prior research (Wang et al., 2022b). An external standard curve was constructed, and the Ct value of the sample was substituted into the standard curve equation to solve for the copy number of the target gene in the sample (*amoA*, *hao*, *nxrA*, *nirS*, *nirK*, *narG*, *nosZ I*, and *nosZ II*). Meanwhile, the 16S rRNA gene served as an internal reference for converting the target genes' absolute copy number.

2.5. Statistical analysis

The experimental data were statistically analyzed with SPSS (Version 22.0) (He et al., 2023). The data is tested for normality and variance homogeneity before performing ANOVA, with significance established at $P < 0.05$. Graphs were plotted using the software Origin (Version, 2021). Partial least squares path modeling (PLS-PM), redundancy analysis (RDA), and network analysis were applied to investigate the intrinsic connections between N_2O emissions, bacterial communities, and functional genes (Liu et al., 2024a; Wang et al., 2025).

3. Results and discussion

3.1. Pile temperature and nitrogen transformation

The pile temperature dynamics during composting in this experiment are shown in Fig. 1a. Compost from all treatments reached the thermophilic stage (above 50 °C) on day 6. Compared to CK, the high temperature of compost from T1, T2 and T3 treatments lasted longer,

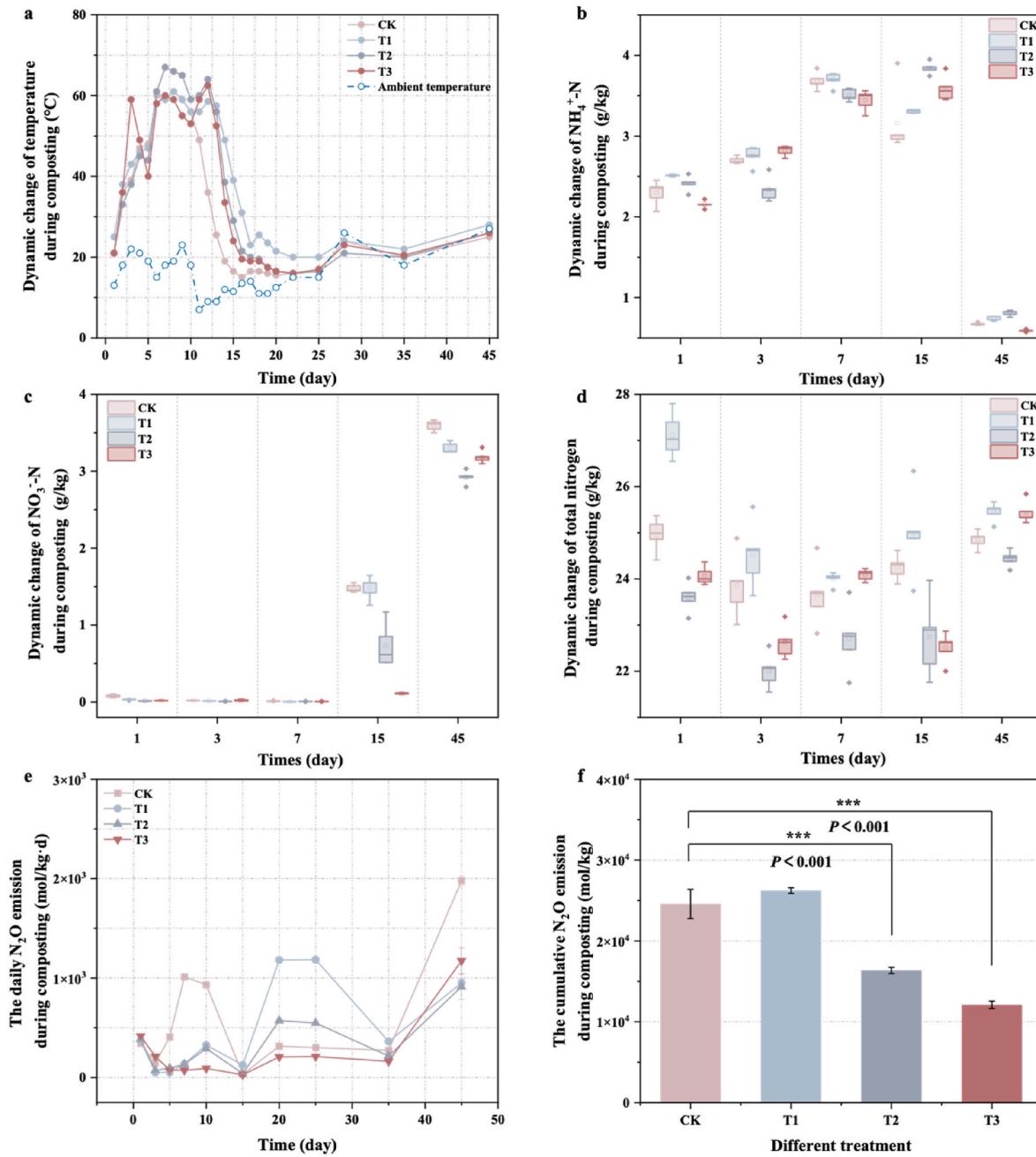


Fig. 1. Dynamic changes in physicochemical properties during composting: temperature (a), ammonium nitrogen (b), nitrate nitrogen (c), total nitrogen (d). Daily N_2O emissions (e) and cumulative N_2O emissions (f) under different treatments (CK: Raw materials; T1: Addition with 5g DPA; T2: Addition with 25 mL THB; T3: Addition with 5g DPA and 25 mL THB; *** $P < 0.001$).

with the highest temperature of compost from T3 treatment reaching 67 °C. The overall trend of NH_4^+ -N during composting showed a raising and then lowering trend, and the NH_4^+ -N content of all treatments fell below 1 g·Kg⁻¹ at the completion of composting (Fig. 1b). It is worth noting that the NH_4^+ -N levels under microbial inoculation (T1, T2 and T3) were all higher than those of CK on the 15th day of composting. From the start of composting to the thermophilic stage, the level of NO_3^- -N remained at a low level, but it increased rapidly during the cooling phase. However, it increased rapidly during the cooling phase, with the NO_3^- -N level being higher in the CK treatment than in the other three treatments (Fig. 1c). Throughout the composting process, the TN level (Fig. 1d) showed a rapid decrease in the mesophilic and thermophilic periods and a gradual rebound in the cooling and maturing stages, which is like the results of Liu et al. (2023).

Similar to most aerobic composting studies (Luo et al., 2014; Zhou et al., 2024b), all treatments went through four typical phases: mesophilic, thermophilic, cooling and maturing. The duration of the thermophilic stage of composting is strongly linked to the use and safety of compost products (Xu et al., 2022). In this research, the main reason for the prolongation of the thermophilic period in the compost under T1, T2 and T3 treatments was that the microbial inoculation could promote microbial activity and metabolism, accelerate the degradation of organic substance in the compost and generate large amounts of heat in the process (Chen et al., 2020). Meanwhile, the increase in the number of thermophilic microorganisms (such as Firmicutes and Proteobacteria) also helped to maintain the thermophilic phase. The variations in TN, NH_4^+ -N and NO_3^- -N reflect the morphological distribution and conversion process of nitrogen in the compost (Yan et al., 2023). NH_4^+ -N is an

inorganic nitrogen source that is available for use by microorganisms. In the early phases of composting, the NH_4^+ -N level increases due to the biodegradation of large amounts of nitrogenous organic matter and the nitrification process. Then, NH_4^+ -N levels gradually decreased in all treatments. The main reason for this may be that the inoculation of microbial agents promoted the conversion of NH_4^+ -N to nitrite nitrogen and NO_3^- -N through the activity of the relevant nitrosation organisms (Chen et al., 2017). Unlike NH_4^+ -N, NO_3^- -N content remained low in the early phase of composting and gradually increased during composting. The initial phase of composting features high temperatures and low dissolved oxygen levels, which are unfavorable for the development and metabolism of nitrifying microbial, thus impeding the nitrification rate. The TN level is very significant in assessing the quality and fertilizer efficiency of compost. In this study, the significant reduction in TN content during the thermophilic stage was attributed to the rapid decomposition of nitrogen in organic matter (OM), which makes it easy for NH_4^+ in the pile to escape in the form of ammonia gas, causing N loss (Huang et al., 2024).

3.2. N_2O emission from composting

The N_2O emission trends and cumulative emissions for each treatment are shown in Fig. 1e and f. N_2O emission under microbial inoculation (T1, T2, T3) was low in both the mesophilic and thermophilic stages of composting. Particularly in the case of the T3 treatment, the daily N_2O emission remained below 500 $\mu\text{mol}/\text{kg}$ for 35 days prior to the commencement of composting. It is noteworthy that in the maturation phases of composting (after 35 days), daily N_2O emissions rose markedly in all treatments. The cumulative N_2O emissions during composting were 24.6 mmol/kg, 26.2 mmol/kg, 16.4 mmol/kg and 12.1 mmol/kg for CK, T1, T2 and T3, respectively. The amount of N_2O emissions to the environment was significantly reduced in both T2 and T3 treatments, by 33.3 % and 50.8 %, respectively. In other words, THB and the combined addition of THB and DPA are beneficial in reducing N_2O emissions from compost.

During composting, N_2O emissions are the primary way nitrogen is lost and have a strong greenhouse effect (Guo et al., 2020). Microbial activity determines N_2O emissions during composting, with incomplete nitrification and denitrification being the key biological processes for N_2O production (Chen et al., 2023). The low level of N_2O emissions in the initial period of composting is probably caused by the high concentration of NH_4^+ during this period, which inhibits the activity of nitrifying organisms and slows down the rate of the nitrification reaction. Meanwhile, *Bacillus subtilis* in DPA and THB rapidly assimilates NH_4^+ into microbial proteins (biological nitrogen fixation), reducing the amount of free NH_4^+ converted to NO_2^- and thereby decreasing N_2O emission (Sun et al., 2020). In addition, the high temperature is also one of the reasons why N_2O emissions are low in the beginning period of composting (Liu et al., 2024b). A significant quantity of N_2O was emitted during the maturation period. This phenomenon could be explained by the alteration of microbial activity towards the conversion of N-containing organic matter and the intensification of denitrification, giving rise to an elevation in N_2O emission (Wang et al., 2013). This is like the findings of Guo et al. (2020) and Liu et al. (2023) that N_2O is predominantly emitted during the cooling phase. The microbial inoculation treatments (T1, T2 and T3) significantly reduced the emission rate of N_2O during the compost maturation period in contrast to the CK. The reason could potentially be that the inoculation of THB and DPA altered the bacterial community structure of the compost at the maturation stage, which enhanced complete denitrification, converted NO_3^- to N_2 , and diminished the generation of N_2O as an intermediate product. On the other hand, the lower NO_3^- content compared to the CK also weakens the substrate supply for denitrification, thereby reducing N_2O emissions.

3.3. Functional genes

The changes in the abundance of N_2O -related functional genes (*amoA*, *hao*, *nxrA*, *nirS*, *nirK*, *narG*, *nosZ I*, and *nosZ II*) throughout the composting process are depicted in Fig. 2. It was found that N_2O related functional genes were most active in the thermophilic phase of compost. The bacterial *amoA* gene abundance peaked on the 7th day of composting and was markedly greater in the CK compared to other groups (Fig. 2a). The number of *hao* genes exhibited a start upward trend followed by a downward trend during the whole composting process (Fig. 2b). The abundance of the *nxrA*, *nirS*, and *nirK* genes reached a maximum on day 3. Specifically, the abundance of *nxrA*, *nirS*, and *nirK* genes was highest in the T1 treatment on day 3 of the composting, while the abundance of these genes was markedly reduced in the T3 treatment contrast to the other three treatments (Fig. 2c–e). In the T3 treatment, the abundance of *nxrA*, *nirS* and *nirK* genes decreased by 68.8 %, 63.2 % and 75.0 %, respectively, compared to the CK. The levels of *narG*, *nosZ I*, and *nosZ II* genes exhibited a pattern of increasing and then decreasing during composting (Fig. 2f–h). The difference was that these three genes reached their peak abundance in the T2 treatment.

As far as nitrification genes are concerned, the bacterial *amoA* gene is a key gene in the nitrification process, and its abundance during composting is strongly correlated to NH_4^+ -N and NO_3^- -N (Yu et al., 2020). The start low levels of the *amoA* gene during composting, especially with DPA and THB treatments, may be attributed to the increased microbial activity induced by external microbial inoculation. The compost heated up quickly due to this activity, which suppressed the function of ammonia-oxidizing bacteria. On day 7, the rapid increase in NH_4^+ -N provided substrate for the ammonia oxidation process, resulting in a transient rise in *amoA* gene abundance (Wang et al., 2018). The decomposition of easily decomposable OM in the initial stage of composting generates many nitrogen-containing intermediate products. Among them, hydroxylamine (NH_2OH), which is generated from the breakdown of nitrogenous organic compounds, serves as the substrate for the hydroxylamine oxidoreductase encoded by the *hao* gene. Microorganisms respond to the rise in substrate by inducing the expression of the *hao* gene to metabolize it, resulting in a higher abundance of the *hao* gene (Domeignoz-Horta et al., 2018). The significant increase in *nxrA* gene abundance in the early phases of composting was also attributable to the breakdown of nitrogen-rich organic substances, which led to an increase in the production of nitrite in the substrate, resulting in an increase in *nxrA* gene abundance. With substrate consumption during composting, the expression of *hao* and *nxrA* genes is inhibited and their abundance decreases. During composting, denitrification is the primary source of N_2O production, with the key genes in this process being *nirS*, *nirK*, *narG*, *nosZ I*, and *nosZ II* (Maeda et al., 2017). In this research, by the 3rd day of composting, the *nirS* and *nirK* gene abundances were notably greater under T1 and T2 treatments than in the CK. The possible reason is that, at this stage, the microorganisms capable of efficiently utilizing nitrite for denitrification and carrying the *nirK* and *nirS* genes gradually became the dominant flora (Cui et al., 2019). The *nosZ I* and *nosZ II* genes act in the final and critical step of denitrification, encoding nitrous oxide reductases that reduce N_2O to N_2 . Among them, *nosZ II* gene abundance may be more closely related to N_2O production during composting (Hiis et al., 2024). The addition of DPA and THB enhanced the proliferation of the *nosZ II* gene, which accounts for the higher N_2O emissions from CK compared to the other three treatments on day 7 of composting.

3.4. Variation of bacterial community

To evaluate bacterial diversity and composition during composting, this study employed bacterial phylum and genus abundance mapping, alpha diversity analysis (Shannon, Simpson and Chao1 indices), and beta diversity analysis (principal coordinate analysis, PCoA) (Fig. 3). Both the Shannon and Chao1 indices exhibit a slowly decreasing trend

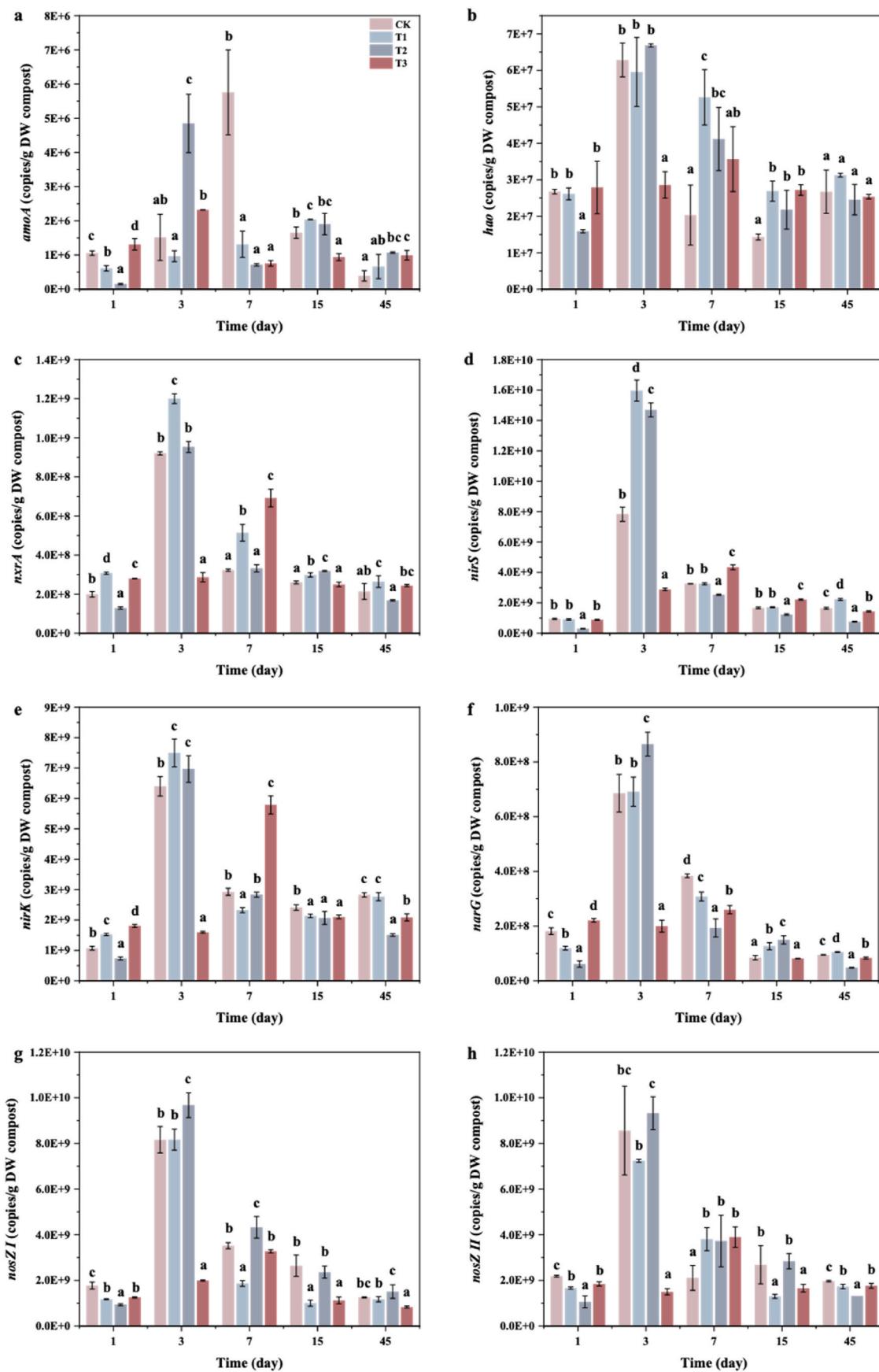


Fig. 2. Changes of N₂O-related genes abundance during composting. (a) *amoA*, (b) *hao*, (c) *nxrA*, (d) *nirS*, (e) *nirK*, (f) *narG*, (g) *nosZI*, (h) *nosZII* (CK: Raw materials; T1: Addition with 5g DPA; T2: Addition with 25 mL THB; T3: Addition with 5g DPA and 25 mL THB).

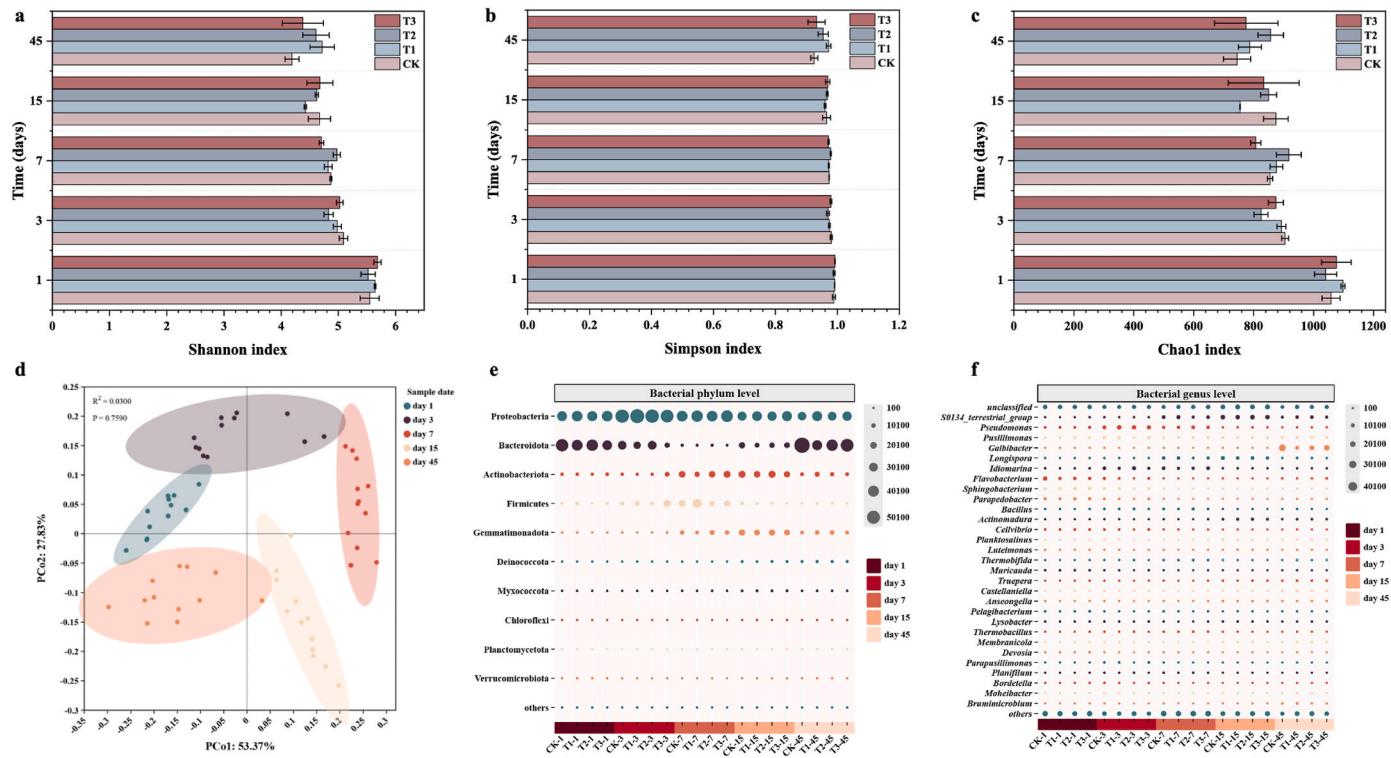


Fig. 3. Shannon, Simpson and Chao1 analysis of bacterial community (a, b, c); PCoA analysis of bacterial community (d); Bacterial community composition at the level of (e) phylum and (f) genus at different stages of composting (CK: Raw materials; T1: Addition with 5g DPA; T2: Addition with 25 mL THB; T3: Addition with 5g DPA and 25 mL THB).

during the whole composting process, and the treatments of T1, T2, and T3 all increase the Shannon and Chao1 index during the mature stage of composting (Fig. 3a–c). For all treatments, the Simpson index remained unchanged significantly from the compost warming period to the maturation stage, and it stayed above 0.9 throughout (Fig. 3b). PCo1 and PCo2 explained 53.7 % and 27.83 % of the variation in the bacterial communities, respectively (Fig. 3d). The findings showed notable alterations in the bacterial community composition at 3, 7, and 15 days of composting. As depicted in Fig. 3e and f, the dynamics of the absolute abundance at both the bacterial phylum and genus levels are presented. The results showed Proteobacteria, Bacteroidota, Actinobacteriota and Firmicutes as the dominant phyla, whose share remained above 75 % throughout the composting process, reaching a maximum of 97.1 %. Firmicutes showed the highest activity during the thermophilic period, especially under THB inoculation. During composting, the abundance of Bacteroidota initially decreased and then increased, eventually surpassing Proteobacteria as the most abundant phylum during the compost maturation stage. The study showed that *Pseudomonas*, *Pusillimonas*, *Galbibacter*, and *Longispora* were the most abundant bacteria genera in terms of the taxonomic genus classification. Similar to the dynamics at the bacterial phylum level, *Galbibacter* (belong to Bacteroidota) was the most abundant genus at the maturation stage, with a peak of 34.1 %.

The Shannon, Simpson, and Chao1 index are important metrics in alpha diversity and are frequently used to evaluate biodiversity and species richness (Li et al., 2024). Shannon and Simpson indices revealed that composting reduced bacterial community diversity, and a decrease in the Chao1 index also indicated a decrease in community richness, whereas exogenous microbial inoculation (DPA and THB) enhanced bacterial diversity and richness. PCoA analysis is often used to investigate the similarity of bacterial community structure (Liu et al., 2024c). Fifteen days into the composting process, the temperature fluctuated sharply during the cooling period, resulting in the differential distribution of bacterial communities, which means that temperature is a key

factor for the differences in bacterial communities. Proteobacteria and Bacteroidota are the two most abundant phyla of bacteria. Some members of Proteobacteria can participate in nitrogen cycle processes (nitrification and denitrification) and carbon cycle processes, playing an integral role in nitrogen conversion and organic carbon decomposition (Lv et al., 2024). During the maturation phases of composting, complex organic macromolecules are broken down into simple compounds, which can be used by Bacteroidota to proliferate. *Galbibacter*, as a member of the phylum Bacteroidota, can utilize a wide range of carbohydrates for growth and metabolism, making it the dominant genus during the compost maturation stage (Yin et al., 2024). Additionally, *Galbibacter* may indirectly regulate N₂O emissions during compost maturation by influencing nitrogen metabolic pathways (e.g., nitrification or denitrification). Specifically, metabolic skewing toward incomplete nitrification could enhance N₂O emissions (Guo et al., 2025; Liu et al., 2024b). During the compost maturation period in this study, the highest *Galbibacter* abundance was found in the CK treatment, which corresponded to the high N₂O emission, whereas the *Galbibacter* abundance was reduced in all the exogenous microbial inoculation (T1, T2, T3) treatments, and thus the N₂O emission was also reduced. Firmicutes is a key phylum in the degradation and transformation of OM, and the inoculation of THB effectively elevated the abundance of Firmicutes during the thermophilic phase of composting, which in turn enhanced the decomposition efficiency of OM during composting and promoted the composting progress (Zhou et al., 2024c).

3.5. Analysis of factors related to N₂O emissions

In this study, RDA, network analysis, and Mantel analysis were used to examine the association between N₂O emissions and physicochemical parameter, bacterial communities, and N functional genes. The findings indicated that N₂O emissions were significantly correlated with pH, OM, NH₄⁺-N, NO₃⁻-N, and *amoA* gene ($P < 0.001$) (Fig. 4a). Previous studies indicated that lower pH value could reduce N₂O emission from compost,

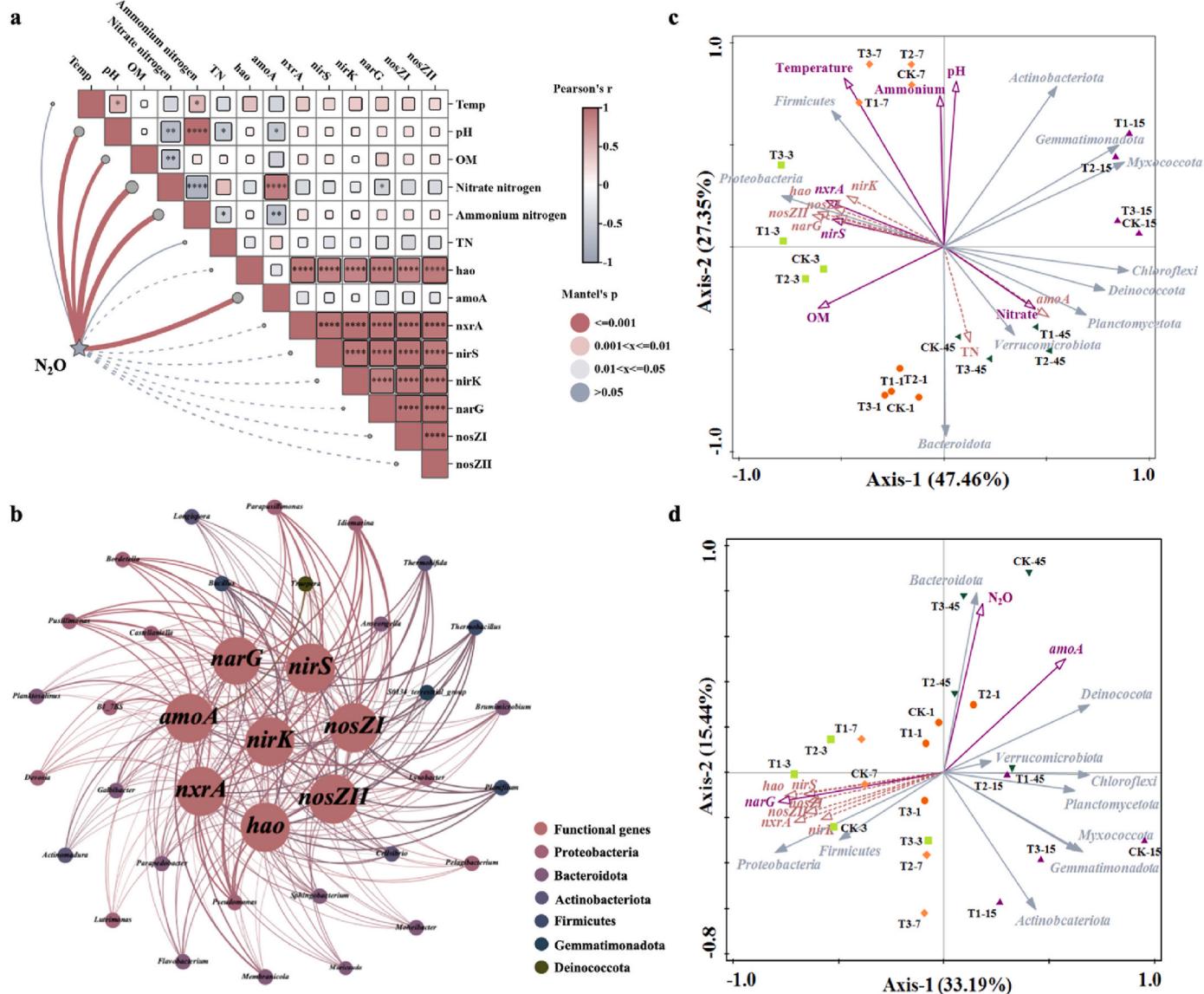


Fig. 4. Mantle analysis of N₂O emissions, physicochemical parameters and N₂O-related functional genes (a); Network analysis of N₂O functional genes and bacterial communities (b); RDA of physicochemical parameters (c) and N₂O emissions (d) with bacterial communities and N₂O-related functional genes, respectively (CK: Raw materials; T1: Addition with 5g DPA; T2: Addition with 25 mL THB; T3: Addition with 5g DPA and 25 mL THB).

which is like the results of this study (Liu et al., 2024a; Cao et al., 2020). Unlike the study of Hou et al. (2024), the positive correlation between both NH₄⁺-N, NO₃⁻-N, and N₂O emission was found in the present study, which implies that lower NH₄⁺-N and NO₃⁻-N contents also represent lower N₂O emission. The *amoA* gene plays a role in converting NH₄⁺-N to NO₂ by coding for the α -subunit of ammonia monooxygenase, marking the initial and crucial step in nitrification (Yang et al., 2024). Through the present investigation, the *amoA* gene was found to be the only one of the eight nitrification and denitrification genes that was significantly associated with N₂O emissions ($P < 0.001$), leading to the hypothesis that nitrification is the key factor driving N₂O emission. Proteobacteria (29 %) and Bacteroidota (26.3 %) were the two major potential hosts of N₂O-related functional genes. Among them, Proteobacteria was strongly positively associated with *nirS* ($P < 0.01$) and *nrxA* ($P < 0.05$). Bacteroidota has an extremely close relationship with the emission of N₂O ($P < 0.01$). *amoA* ($P < 0.05$) and *narG* ($P < 0.01$) are two N₂O-related functional genes significantly influenced by the bacterial community. DRA analysis revealed that Proteobacteria and Firmicutes impacted multiple N₂O-related functional genes (Fig. 4b-d). It was also observed

that temperature, pH, NH₄⁺-N, NO₃⁻-N, and OM were significant factors influencing the bacterial community. Especially, NO₃⁻-N exerted an evident influence on the abundance and composition of multiple bacterial communities (such as Bacteroidota, Verrucomicrobiota, and Planctomycetota), which further elucidated the reason why NO₃⁻-N significantly affected N₂O emissions during composting.

A PLS-PM model was built to thoroughly analyze the direct and indirect impacts of various factors on N₂O emissions (Fig. 5a and b). The model found that physicochemical properties had a direct impact on both N₂O emissions and the bacterial community's abundance ($P < 0.001$; direct = -0.9691, indirect = -0.8063). Integrating the results of the Mantle analysis above, it could be inferred that the extent of OM degradation directly influences N₂O release. In addition, the abundance of bacterial communities also had a direct impact on the N₂O functional gene abundance ($P < 0.01$; direct = -0.8832), and indirectly affected N₂O emissions. Based on the results of previous RDA and network analyses, Proteobacteria and Bacteroidota are key bacterial groups potentially responsible for influencing N₂O emissions. Most previous studies have identified N-related functional genes as the key variables in

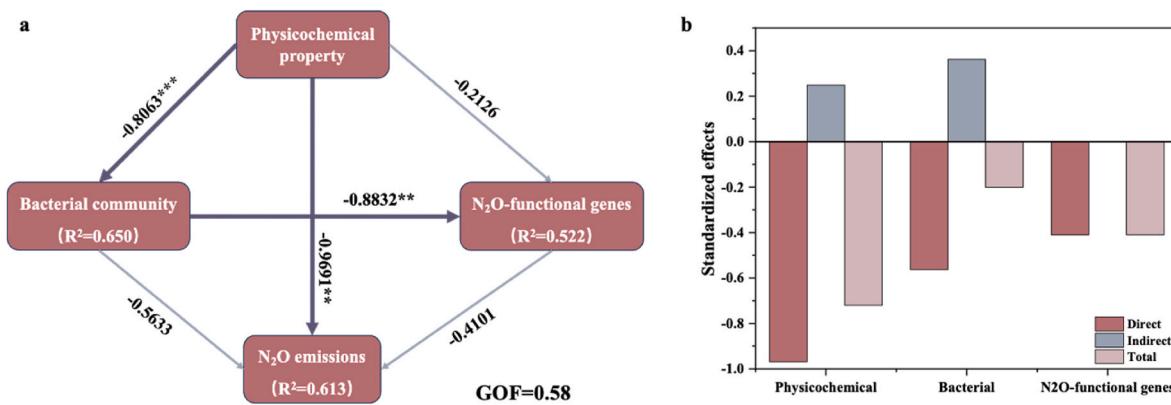


Fig. 5. Partial least squares path modeling (PLS-PM) showing the effects of different factors on N₂O emissions (a, **P < 0.01, ***P < 0.001); Standardized direct and indirect mean effects derived from the PLS-PM (b).

regulating N₂O emissions (You et al., 2022). However, the findings of this research show that physicochemical properties are the most important factors influencing N₂O emissions. This outcome is due to the direct impact of physicochemical properties on the makeup and distribution of N₂O-related bacterial communities and the expression of functional genes, which subsequently exert a significant influence on N₂O emissions during composting (Xu et al., 2017). Consequently, modulating the physicochemical characteristics and the microbial community structure of compost via microbial inoculation represents an efficacious approach for controlling N₂O emissions during composting.

4. Conclusions

In this research, the inoculation of exogenous microorganisms effectively prolonged the thermophilic periods of compost and reduced nitrogen loss. It directly or indirectly affects N₂O emissions and can do so by changing the physicochemical characteristics, bacterial community, and functional genes of the compost. The results showed that the combined inoculation of THB and DPA can cut N₂O emissions by 50.8 %. Proteobacteria and Bacteroidota were the key hosts of N₂O-related functional genes, and *amoA* was a key gene affecting N₂O emissions (P < 0.001). PLS-PM analysis indicated that the physicochemical characteristics of the compost pile were the most direct and crucial factors in regulating N₂O emissions. This study promotes the further application of microbial agents, providing scientific and economic solutions for reducing greenhouse gas emissions during the utilization of straw resources.

CRediT authorship contribution statement

Yuewei He: Writing – original draft, Methodology, Investigation, Formal analysis. **Fenglong Hu:** Methodology, Investigation. **Zan Du:** Methodology, Investigation. **Ping Liu:** Methodology, Investigation. **Xinwei Cui:** Writing – review & editing, Supervision, Resources. **Yixiang Cai:** Writing – review & editing, Validation, Resources, Formal analysis. **Xichen Zhao:** Writing – review & editing, Validation, Supervision, Project administration, Methodology. **Renli Qi:** Writing – review & editing, Resources. **Hongli Huang:** Writing – review & editing, Resources, Methodology. **Binghua Yan:** Writing – review & editing, Software, Resources. **Anwei Chen:** Writing – review & editing, Validation, Resources. **Jiachao Zhang:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they do not have any commercial or associative interest that represents a conflict of interest in connection with

the work submitted.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2025.126663>.

Data availability

Data will be made available on request.

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