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Biochar

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Effects of biochar carried microbial agent on compost quality, greenhouse gas emission and bacterial community during sheep manure composting

Zhe Wang[1†], Yilin Xu[1†], Tong Yang[2], Yongqi Liu[2], Tingting Zheng[2] and Chunli Zheng[1,2*]

Abstract

Although composting is a very effective way to dispose agricultural wastes, its development is greatly limited by the low compost quality and greenhouse gas emissions. At present, there is a lack of effective means to solve these two problems simultaneously. Here, the effects of three additives of compound microbial agent, biochar and biochar carried microbial agent on the composting performance, nitrogen transformation, greenhouse gas and ammonia emissions, and bacterial communities were investigated in sheep manure composting during 28 days. Results showed that biochar carried microbial agent prolonged the thermophilic stage and promoted compost maturity. At the same time, it was confirmed by the increase of the decomposition of organic nitrogen and the transformation of NH_4^+-N to NO_3^--N. Besides, adding biochar carried microbial agent decreased CH_4, NH_3 and N_2O emissions by 65.23%, 42.05% and 68.64%, respectively. The gas emissions were mainly correlated to *Chloroflexi*, *Myxococcota*, *Acidobacteriota*, *Firmicutes*, and *Gemmatimonadota*. Redundancy analysis showed that EC and TKN were closely related to bacterial community. Therefore, biochar carried microbial agent is recommended as an effective additive to enhance compost quality and reduce gas emissions during sheep manure composting.

Highlights

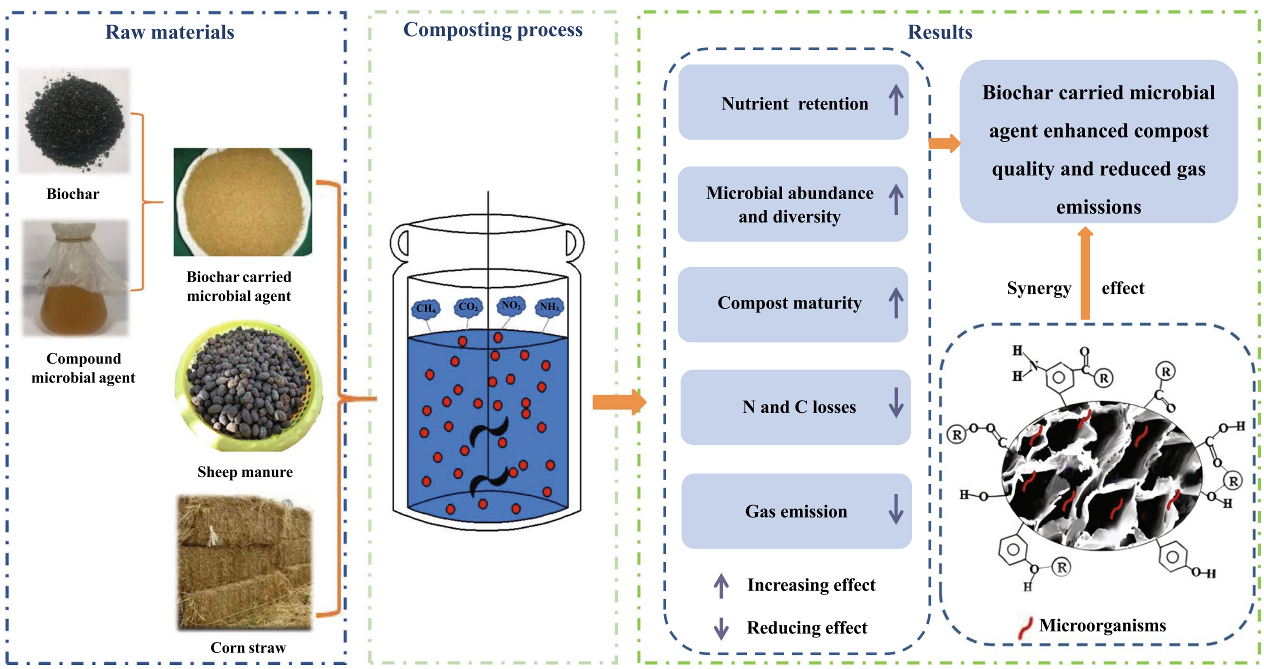
- Biochar carried microbial agent (BCMA) was applied into sheep manure composting.
- BCMA prolonged thermophilic phase, reduced N losses and promoted compost maturity.
- Physical properties were improved by preventing the formation of large clumps.
- Adding BCMA reduced CH_4 by 65.23%, NH_3 by 42.05% and N_2O by 68.64%.
- BCMA affected the microbial structure.

Keywords Biochar carried microbial agent, Sheep manure, Composting, Nitrogen transformation, Bacterial community, Greenhouse gases

†Zhe Wang and Yilin Xu are co-first authors

*Correspondence:
Chunli Zheng
zhengchunli1979@163.com
Full list of author information is available at the end of the article

Graphical Abstract



1 Introduction

The development of specialization, large-scale and intensification of livestock industry has generated a large volume of animal manure. Currently, the amount of livestock manure produced is approximately 3.8×10^9 t every year, but the comprehensive utilization rate is less than 60% in China (Yao et al. 2021). A large number of livestock manure is stacked, which causes a series of harms to the soil, water and atmospheric environment, and constantly threatens the sustainable development of the ecological environment (Wang et al. 2018). So, we must find a feasible method to solve the problem of livestock manure. Aerobic composting can convert livestock manure into a safe and mature fertilizer due to its high content of organic matter, nitrogen, phosphorus, potassium and trace elements, achieving the reduction, detoxification and recycling of livestock and poultry manure (Ravindran et al. 2018). However, aerobic composting of livestock manure is usually accompanied by the production of greenhouse gases and other gases, including methane (CH_4), carbon dioxide (CO_2), nitrous oxide (N_2O), ammonia (NH_3), etc. (Chan et al. 2016). A large amount of gas emissions can lead to the loss of carbon and nitrogen during compost (Tong et al. 2019). Among them, NH_3 and N_2O emissions account for 73.68–92.91% and 1.23–4.16% of the total nitrogen loss, respectively (Yuan et al. 2019). During composting, 14–51% of the organic carbon in the compost material can be released into the atmosphere as CH_4 and CO_2 (Barrington et al. 2002). Generally, the emission of gases will lead to the reduction of compost quality (Wang et al. 2022). Therefore, it is crucial to develop a method to reduce gas emissions and improve the quality of compost at the same time.

A number of studies have indicated that the addition of additives is a very available way for reducing carbon and nitrogen losses and gas emissions during the composting process (Ren et al. 2020; Pan et al. 2019; Soudejani et al. 2019). Because of its particular porous structure and abundant surface functional groups, biochar can reduce gas emissions (Yin et al. 2021a). Besides, biochar can also improve the environmental conditions of the heap, which is beneficial to the composting quality improvement (Zainudin et al. 2020). Awasthi et al. (2017) found that biochar significantly reduced CH_4, N_2O and NH_3 emissions by 92.85–95.34%, 95.14–97.30% and 58.03–65.17%. Hagemann et al. (2018) reported that addition of three different biochars increased nutrient retention, moisture content and total organic carbon content in the mature compost. Wang et al. (2021) showed that 10% biochar addition reduced nitrogen losses to 25.69% and increased organic matter degradation, thereby shortening composting period and promoting microbial succession in the composting process.

As we know, composting is a series of complex biological, physical and chemical processes (Guo et al. 2020), and microorganisms play a crucial role in composting systems. Many researchers have pointed out that the inoculation of native or exogenous microorganisms improves the composting process, especially the introduction of exogenous compound microbial agents (Wang et al. 2022; Xu et al. 2022a, 2022b; Zhao et al. 2022). In addition, biochar is a biocompatible material, which is conducive to the reproduction and habitat of microorganisms, and can increase the number and activity of microorganisms (Wang et al. 2022). Therefore, it is supposed that in the perspective of engineering practice and microbial inoculation storage, biochar carried microbial agent (microbial agent was sprayed on the biochar) is beneficial for the storage, transportation and utilization of microbial inoculation in practice, and thus benefits its utilization in high value compost production (Tu et al. 2019). Tu et al. (2019) used the bamboo biochar loaded commercial bacteria agent for enhancing the pig manure composting. However, the composing process was strongly related to the feedstock, composing system and amendment (including biochar) types (Wang et al. 2022). So far, the application of corn straw biochar carried compound microbial agent in sheep manure composting is still limited, and its effects on composting quality, greenhouse gas emissions and bacterial community changes are not clear.

Therefore, the regulatory effect of biochar carried microbial agent on the biological stabilization process of sheep manure compost was investigated. The aims of this study were (1) to evaluate the effect of biochar carried microbial agent on physicochemical parameters, nitrogen transformation, gas emissions and bacterial community succession during composting; (2) to reveal the relationship between bacterial community, environmental factors and gas emission. The results of this study are expected to provide valuable information for the promotion of livestock manure composting technology.

2 Materials and methods

2.1 Collection and preparation of raw materials

Fresh sheep manure and corn straw came from Guanjiang village (Baotou, Inner Mongolia, China). Corn straw was cut into 2 cm lengths as a conditioning agent, which can regulate the C/N ratio and moisture content of compost. The biochar was prepared from corn straw by slow pyrolysis at 550 ℃ in a muffle furnace (SK16BYL, Nanjing Boyun Tong Instrument Technology Company, Nanjing, China). The basic physical and chemical properties of composting materials are shown in Table 1.

The sheep manure extract medium and corresponding selective solid medium for inorganic phosphorus dissolving bacteria, organic phosphorus dissolving bacteria, potassium dissolving bacteria and nitrogen fixing bacteria were prepared. The single bacteria isolation and purification experiments were carried out. Six strains of bacteria were obtained by dilution and culture of solid separation medium. Then, the six strains were crossed in pairs for antagonistic experiment. The strains could grow well, and there was no antagonistic reaction between them. They could coexist for mixed culture so as to prepare compound microbial agents. The detailed preparation methods are provided in the Additional file 1. After mixed fermentation, the numbers of nitrogen-fixing, inorganic phosphorus dissolving, organic phosphorus dissolving and potassium dissolving bacteria were 10^5, 10^5, 10^8 and 10^7 mL^{-1}, respectively.

The biochar carried microbial agent was prepared by spraying and mixing the compound microbial agents and biochar according to the mass ratio of 1:10, inoculating and multiplying for 2 weeks. The scanning electron microscope images of biochar and biochar carried microbial agent are shown in Fig. 1. The pore structure of corn straw biochar was clear and rich because it contained cellulose and hemicellulose (Fig. 1a). It can be obviously observed that microorganisms were well loaded on the surface of biochar (Fig. 1b).

2.2 Composting experiment

2.2.1 Composting device

The aerobic composting was conducted in 25 L self-designed cylindrical devices made of stainless steel (with

Table 1 The physico-chemical properties of the raw materials

	TN (g kg^{-1})	TC (g kg^{-1})	pH	EC (mS cm^{-1})	OM (%)	Moisture (%)	C/N
Sheep manure	18.01 ± 0.55	392.82 ± 4.12	8.52 ± 0.03	3.23 ± 0.03	69.84 ± 0.11	71.69 ± 0.55	21.82 ± 0.12
Corn straw	7.61 ± 0.05	508.03 ± 5.34	6.41 ± 0.02	0.28 ± 0.01	68.52 ± 0.25	6.21 ± 0.02	66.84 ± 1.06
Biochar	5.27 ± 0.02	693.22 ± 5.56	7.62 ± 0.03	0.21 ± 0.01	90.62 ± 1.15	3.52 ± 0.01	131.54 ± 2.13

Values indicate mean ± standard deviation based on determination with three replications. TN (total nitrogen), TC (total carbon), EC (electricity conductivity), OM (organic matter)

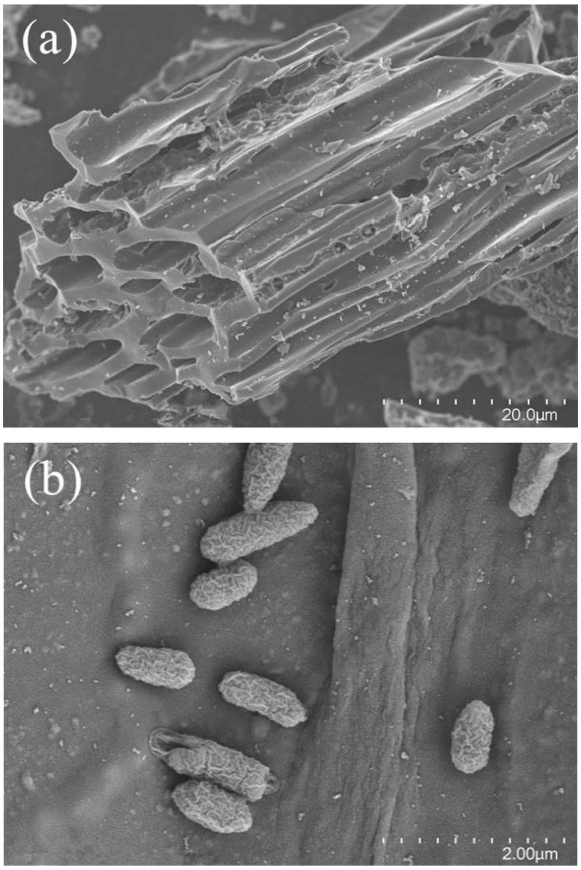


Fig. 1 SEM images of biochar (**a**) and biochar carried microbial agent (**b**)

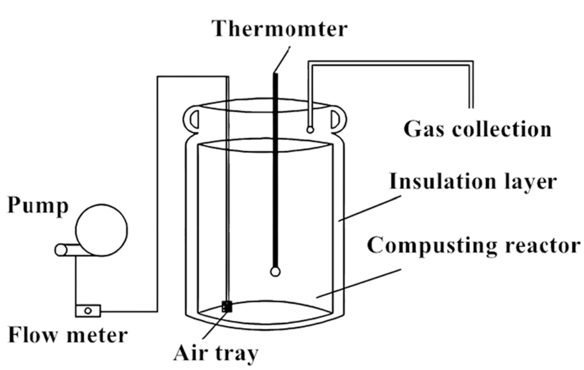


Fig. 2 The diagram of the composting reactor

the height of 46 cm and the inner diameter of 30 cm). There were 4 cm thick insulation layers on the outer wall of the reactors. The devices had sealing covers at the top. Fresh air was supplied at a gas flow rate of 0.5 L h^{-1} kg^{-1} (dry weight basis) at the bottom of the composting reactors (Fig. 2).

2.2.2 Experimental design

The sheep manure and corn straw were mixed evenly in a ratio of 8:1 (w/w, dry weight basis). The mixture was further adjusted to a moisture content of 60% and a C/N ratio of 25 prior to the addition of biochar, compound microbial agent and biochar carried microbial agent. Four aerobic composting treatments were (i) sheep manure + corn straw (CK), (ii) CK + 0.1% biochar (B), (iii) CK + 0.01% compound microbial agent (M), and (iv) CK + biochar carried microbial agent (BM, adding 0.01% compound microbial agent to 0.1% biochar before natural air drying). All of the treatments were replicated three times. About 4.5 kg of compost material for each treatment was loaded into reactor and composted for 28 days. Temperature was monitored every day between 14:00 and 15:00. Samples weighing about 200 g were collected from each treatment on 0, 4, 8, 12, 16, 20, 24 and 28 days after mixing them well, and then homogenized to obtain a representative sample. The samples were stored in a refrigerator at 4 ℃ for the determination of physicochemical and maturity indexes. 0.5 g of sample from each treatment group on 6, 14, and 28 days was frozen at − 20 ℃ for microbial analyses. Gas samples were collected every 5 days using a 1 L gas sampling bag.

2.2.3 Samples analysis

Fresh compost samples and deionized water were mixed at a ratio of 1:10 (w/v), and the mixture was shaken for 30 min to analyze pH, electric conductivity (EC), E_4/E_6, and seed germination (GI). The pH and EC were measured using a pH meter (pHS-3C, China) and conductivity meter (DDS-307A, China). The absorbance of E_4/E_6 was determined at 465 and 665 nm using an ultraviolet–visible spectrophotometer (UH4150, Japan). GI test was performed with *Glycine max* (Linn.) Merr. seeds as described by Li et al. (2020). The GI was calculated according to Eq. (1). Organic matter (OM), moisture content, total Kjeldahl nitrogen (TKN), NH_4^+-N, and NO_3^--N were determined according to a standard test, the Test Method for the Examination of Composting and Compost (TMECC 2002). The contents of total carbon (TC) and total nitrogen (TN) were determined using a Vario MACRO elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). To assess the formation of clumps within the pile, composts smaller than 70 mm were screened and weighed to quantify the different compositions (70–25, 25–4 and < 4 mm). The gas produced in the composting process was collected by the gas bag. The concentrations of CH_4, CO_2 and N_2O were measured by gas chromatography (Agilent 6890N, USA). The NH_3 in compost was adsorbed in boric acid solution and detected by titration with 1 mol L^{-1} HCl according to Mao et al. (2019). The compositions and succession of the bacterial communities were assessed on samples taken at three major stages, including thermophilic (day 6), cooling (day 14) and mature composts (day 28) by 16S rRNA gene high-throughput sequencing using the Illumina MiSeq platform at Majorbio (Shanghai, China). According to the manufacturer's instructions, DNA of compost samples was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). The hypervariable regions V3-V4 of the bacterial 16S rDNA gene were amplified with primer pairs 338F (5′-ACT CCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTA CHVGGGTWTCTAAT-3′) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA).

$$\mathrm{GI} = \frac{\text{Seed germination rate of treatment (\%)} \times \text{Root length of treatment (mm)}}{\text{Seed germination rate of control (\%)} \times \text{Root length of control (mm)}} \times 100\% \quad (1)$$

2.3 Statistical analysis

All analyses were conducted in triplicate to obtain the average value. Figures were drawn by Origin 2022. All statistical data were tested for normality and homogeneity of variance. Significant differences between the treatments were determined by a one-way analysis of variance (ANOVA) using the SPSS 26.0 software package (SPSS for Windows, Version 26.0, USA), and multiple comparisons using the least significance difference (LSD) post hoc test were done whenever the ANOVA indicated significant differences ($p \le 0.05$). The relationships between gaseous emissions and microbial community were analyzed by Pearson's correlation analysis. Redundancy analyses (RDA) were performed by the CANOCO 5.0 to recognize environmental factors and microbial communities during thermophilic stage, cooling stage and maturity stage.

3 Results and discussions

3.1 Effect of additive on the basic parameters during composting

3.1.1 Changes in temperature

Temperature is an important index reflecting the maturity of composting process, as it may affect microbial metabolism and activity (Yang et al. 2020). The ambient temperature was maintained between 26.8 ℃ and 38.5 ℃. Figure 3a shows the changes of temperature during the composting process. All treatment groups showed the same trends of change, which were heating, thermophilia, cooling and maturity stages. The temperature of all treatments rose sharply within the first 5 days. It could be because of the rapid degradation of available organic matter in sheep manure (Awasthi et al. 2016). The thermophilic stage (> 50 ℃) of CK, M, B and BM treatments was reached on day 6, 5, 4 and 2, which lasted 5, 8, 6 and 9 days, and the peak temperatures were 60.1, 60.3, 60.9 and 63.9 ℃, respectively. All four treatments met the criteria for harmless composting (Jain et al. 2018). After that, the temperature decreased gradually and finally reached ambient temperature. It was discovered that the BM treatments reached faster peak temperature, higher peak temperature and longer thermophilic period. Adding biochar carried microbial agent to the compost could rapidly heat up and prolong the duration of high temperature, indicating that it was beneficial to composting. This was probably because the biochar carried microbial agent promoted the decomposition of organic matter, thus generating more heat (Zhao et al. 2020). Also, biochar carried microbial agent filled the pores between composting materials and reduced heat loss (Awasthi et al. 2017).

3.1.2 Changes in pH

Figure 3b shows the change trend of pH value during the composting. The initial pH values of B, M, BM and CK were 8.67, 6.88, 8.61 and 8.48, respectively. The pH increased slightly, reaching a maximum value for all treatments on day 4. This might be related to the rapid decomposition of organic matter during this period, with organic nitrogen being ammoniated and mineralized (Gao et al. 2010). The maximum pH values of the four treatment groups were B > BM > CK > M. The pH value of M was significantly lower than that of B and BM treatments ($p < 0.05$). Adding biochar increased the pH in the composting while adding microbial agents showed the opposite trend, which was closely related to the pH of raw materials. The pH values of microbial agent, biochar and biochar carried microbial agent were 6.89, 10.01 and 8.16, respectively. The pH values of treatment groups were affected by alkaline biochar and acidic microbial agents. From day 4 to day 12, the ammoniation was weakened, the nitrification was gradually enhanced, and the pH of compost gradually decreased with low-molecular-weight organic acids and CO_2 accumulation (Chen et al. 2019). On days 12–24, the continuous degradation of organic acids further increased pH values (Wang et al. 2022; Xue et al. 2021). As the compost slowly reached maturity, the pH decreased in 24–28 days. The final pH values of BM, B, M and CK treatment groups were 7.58, 7.8, 7.38 and 7.58, respectively, which satisfied the pH value range of the Chinese industry standard (5.5–8.5).

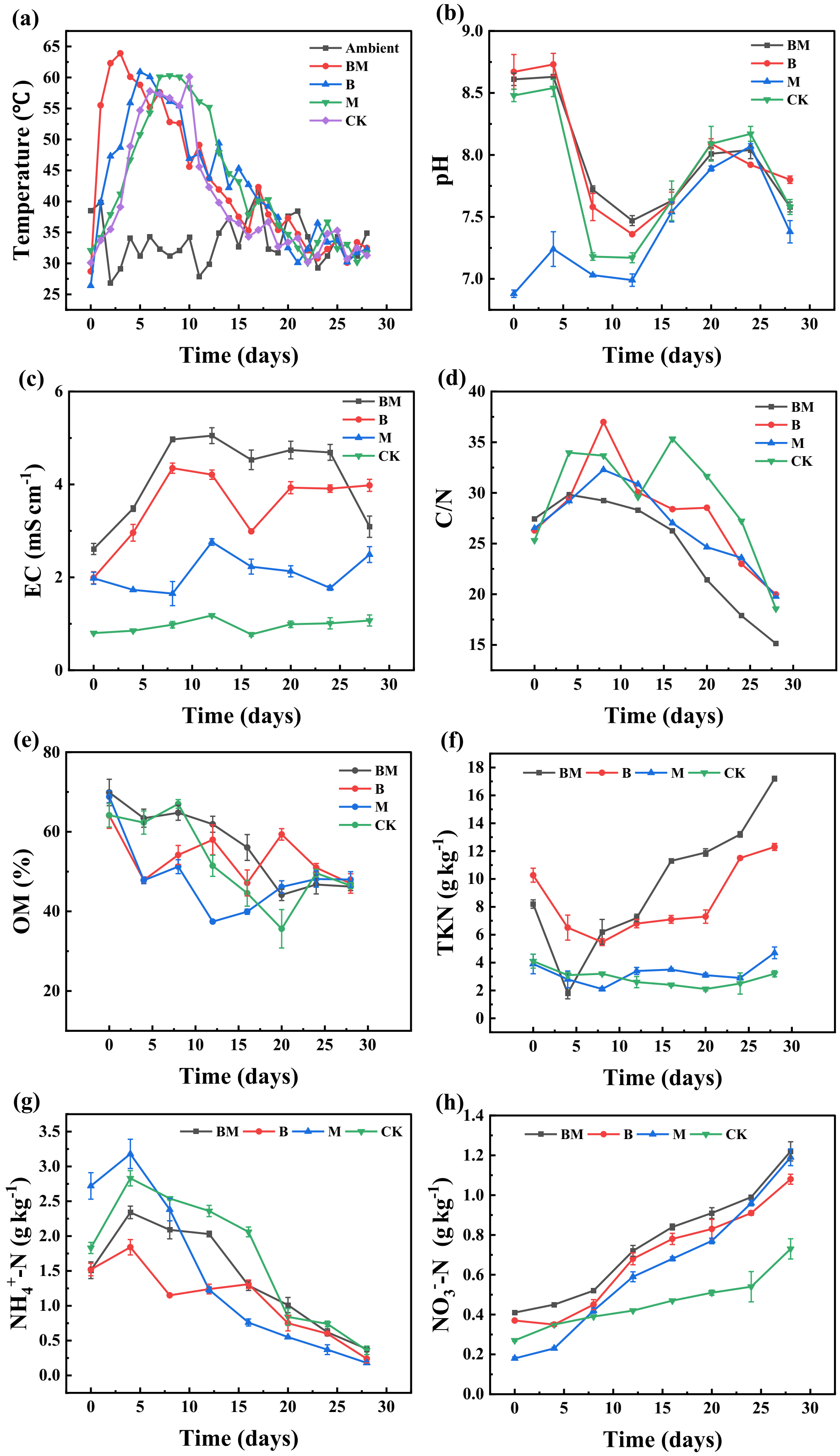


Fig. 3 Changes in temperature (**a**), pH (**b**), EC (**c**), C/N (**d**), OM (**e**), TKN (**f**), NH_4^+-N (**g**) and NO_3^--N (**h**) in different treatments during composting. CK: sheep manure + corn straw; M: CK + 0.01% compound microbial agents; B: CK + 0.1% biochar; and BM: CK + biochar carried microbial agent (0.01% compound microbial agent to 0.1% biochar before being naturally dried). Results are the mean of three replicates and error bars indicate standard deviation

3.1.3 Changes in EC

The EC value represents the soluble salt content in compost, which is one of the criteria for whether the compost would cause phytotoxicity on plant growth (Gao et al. 2010). Figure 3c shows the water-soluble products in the piles mostly came from free water in the early stage of composting, and the EC contents remained at a low level. Owing to the fast decomposition of organic matter and the loss of water, the EC values began to rise (Singh and Kalamdhad 2013). Then, the four composting groups displayed a downward trend owing to the precipitation of mineral salts and the volatilization of large amounts of NH_3 (Waqas et al. 2017). Although there were moderate fluctuations, the final EC values of BM, B, M and CK groups reached 3.09, 3.98, 2.49 and 1.07 $mS{\cdot}cm^{-1}$, respectively, which were under 4 mS cm^{-1}, indicating that the compost could be applied to the soil safely (Wang et al. 2020). It is obvious that compared with the CK treatments, addition of biochar carried microbial agent significantly increased the EC values ($p<0.05$). Qu et al. (2020) found that the dilution effects and/or salt adsorption potential of biochar could reduce EC values. However, the addition of the biochar carried microbial agent might be conducive to microbial reproduction, accelerate the degradation rate of organic matter, and accumulate a large amount of inorganic substances, thus resulting in a higher EC values in this study.

3.1.4 Changes in C/N

The variation of C/N ratio in the four composting treatment groups is shown in Fig. 3d. In the early stage, C/N values increased slightly due to nitrogen losses from NH_3 emissions (Qiu et al. 2020). After that, the C/N ratios of BM, B and M treatment groups decreased until the end of composting with the degradation of carbon-containing materials and accumulation of organic nitrogen (Chang et al. 2019). The C/N ratios in the CK treatment group increased within 10 to 16 days. The final C/N values for the BM, B, M and CK treatment groups were 15.13, 19.98, 19.78, and 18.56, respectively. The C/N values in all four treatments were less than 20, indicating the compost reached maturity (Chung et al. 2021). Generally, the BM treatment group was the most effective, probably because of the higher nitrogen conservation and OM degradation in the pile, resulting in lower C/N values (Jiang et al. 2015).

3.1.5 Changes in OM

As shown in Fig. 3e, the initial OM contents of the BM, B, M and CK were 69.87%, 64.08%, 68.79% and 64.15%, respectively. The OM contents showed a downward trend, although there were fluctuations during the composting process. This might be due to the decomposition of OM into small molecules such as CO_2 and water, increasing the reactor temperature and the synthesis of humic acid through microbial action (Ren et al. 2019). Therefore, the OM content of each treatment decreased gradually in the composting process. The final OM values for the BM, B, M and CK groups were 46.23%, 47.01%, 47.99% and 46.47%, respectively. Moreover, the OM values for the BM, B, M and CK groups decreased by 33.83%, 26.63%, 30.24% and 27.56%, respectively. OM degradation rate was faster in the BM group than in the CK group, which may be due to the promotion of microbial activity by biochar carried microbial agent addition (Yin et al. 2021b). In addition, materials with a high initial C/N ratio have been reported to result in higher OM degradation rates (Zhang et al. 2020).

3.1.6 Changes in the formation of clumps

In this study, it was observed that the four treatments formed clumps of variable sizes from the initial stage. In order to evaluate the formation of clumps in the piles, particle size distributions of the four treatments were measured after composting and the results are shown in Additional file 1: Table S1. Most of the particle size distribution was less than 4 mm. In the BM treatment group, the proportion of particles smaller than 4 mm in size was 81.93%. was 81.93%, which was much larger than in the other groups. This showed that the addition of biochar carried microbial agent increased the proportion of small clump fraction, avoided the generation of anaerobic environment, and promoted the diffusion of O_2 (Sánchez-García et al. 2015).

3.2 Effect of additives on nitrogen transformation during composting

TKN content is a significant parameter in assessing the quality of compost products. The change of TKN of compost in each treatment is shown in Fig. 3f. In all four treatments, TKN values decreased initially and then increased gradually. During the thermophilic phase, TKN levels were reduced due to the decomposition of organic nitrogen and the release of NH_3 (Santos et al. 2018). In the later stages of composting, the increase of TKN content was related to the enrichment of nitrogen-containing organic substances and rapid decomposition of OM (Tong et al. 2019). The final TKN contents of the BM, B, M and CK treatment groups were 17.2, 12.3, 4.7 and 3.2 g kg^{-1}, respectively. Compared with the initial stage of composting, the the content of TKN in BM, B and M groups increased by 109.76%, 19.77% and 20.51%, while that in CK group decreased by 21.95%. Compared with the CK group, the addition of biochar carried microbial agent significantly increased the TKN content ($p<0.05$).

Nitrogen transformation in compost mainly includes ammonification, nitrification and denitrification reaction (Liu et al. 2020). The NH_4^+-N content showed an increase trend during the first five days in all treatments and then decreased until composting was completed (Fig. 3g). In the initial stage, the increase of NH_4^+-N content was mainly owing to the change of organic nitrogen to NH_4^+-N by ammonification reactions at higher temperatures and pH (Guo et al. 2012). The highest values of NH_4^+-N were 2.34, 1.84, 3.18 and 2.83 g kg^{-1} for the BM, B, M and CK groups, respectively. The M treatment group had the highest NH_4^+-N content compared to CK. This suggested that compound microbial agent promoted rapid degradation of organic nitrogen (Li et al. 2019a). After that, with the volatilization of ammonia and the nitrification and denitrification of bacteria in the composting process, the NH_4^+-N contents began to decrease until the end (Wang et al. 2016a). Finally, the NH_4^+-N contents in BM, B, M and CK groups were 0.38, 0.24, 0.18 and 0.36 g kg^{-1}, respectively, and the final compost products of each treatment satisfied the maturity standard of organic fertilizer (less than 0.4 g kg^{-1}).

The NO_3^--N content of all treatments showed an increase trend from day 0 to the end of composting (Fig. 3h), which was consistent with the results of Jiang et al. (2015). In the beginning of composting, the values of temperature, pH and NH_4^+-N were higher, which inhibited the activity and growth of nitrifying bacteria, and NO_3^--N content was lower (Ren et al. 2019). As composting temperature and pH decreased, NH_4^+-N was gradually transformed into NO_3^--N, and the content of NO_3^--N increased (Chung et al. 2021; Wang et al. 2017a). After composting, the NO_3^--N contents in the BM, B, M and CK treatment groups were 1.22, 1.08, 1.19 and 0.73 g kg^{-1}, respectively. The NO_3^--N content in BM group was significantly higher than that in CK group ($p < 0.05$).

After composting, the difference in NH_4^+-N and NO_3^--N content was little, while TKN showed great difference among treatments, indicating that the difference came from organic nitrogen. The TKN in the BM treatment was higher, which meant that the organic nitrogen of this treatment was higher than that of other treatments. The reason for the decrease of nitrogen loss may be related to the decrease of organic nitrogen loss in BM group. In short, biochar carried microbial agent had the synergistic effect of biochar and microbial agent in nitrogen retention and quality enhancement of compost (Kolton et al. 2016).

3.3 Effect of additives on greenhouse gas emissions

Composting is a complex biochemical process in which microorganisms degrade organic matter and eventually obtain stable humus (Cáceres et al. 2018). However, the composting process produces many adverse by-products, including greenhouse gases from microbial metabolic activities (Fig. 4), which have received much attention in recent years (Yin et al. 2021a).

3.3.1 CH_4 emission

CH_4 is an important greenhouse gas and produced by deoxidizing CO_2/H_2 or acetic acid of methanogenic bacteria under hypoxic/anaerobic conditions (Yang et al. 2013). As can be seen in Fig. 5a, CH_4 emissions rapidly increased during the first five days and then gradually decreased until the end of composting. At the initial stage of composting, the degradation of OM consumed a lot of oxygen and formed a certain anaerobic area, increasing the activity of methanogenic bacteria (Yun et al. 2018). The peak values of CH_4 emission on day 5 were 0.46, 4.02, 1.56 and 2.11 g day^{-1} for BM, B, M and CK groups, respectively. As composting proceeded, CH_4 emissions decreased gradually until the end of composting because of the decrease of temperature and degradable carbon substances.

Cumulative CH_4 emissions in BM, B, M and CK groups were 1.08, 6.68, 2.80 and 3.16 g, respectively. The findings showed that the addition of biochar increased CH_4 emissions which was inconsistent with most research conclusions. In general, the addition of biochar might suppress methanogenic activity and CH_4 emissions by improving compost structure and changing the redox potential (Liu et al. 2017). Besides, the adsorption of NH_4^+ by biochar can also reduce the availability of methanogenic bacteria and reduce CH_4 emissions (Karhu et al. 2011). However, in previous studies, the biochar addition was relatively high, and in this study, the biochar addition was only 0.1%, which may not be sufficient to improve the ventilation conditions of the piles. This was consistent with the distributions of compost particle size. The proportion of particles less than 4 mm in size in BM treatment group was the largest, while the proportion of B group was close to that of the CK group. This showed that BM group could better promote the diffusion of O_2, avoid anaerobic environment and reduce the release of CH_4.

3.3.2 CO_2 emission

CO_2 emissions reflect the activity of microbial and the efficiency of the composting process (Wang et al. 2017b; Li et al. 2020). Figure 5b shows the changes in CO_2 emission rates. The CO_2 emissions of all four treatments sharply increased in the first 10 days, which was same with the change trend of composting temperature. At the beginning of composting, an increase in temperature promoted microbial activity, and then metabolically

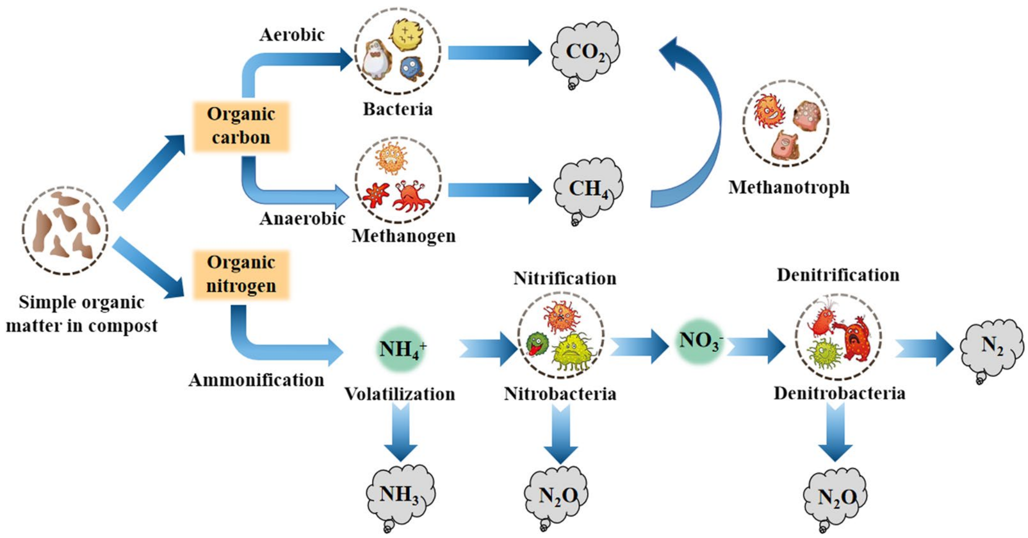


Fig. 4 Flow chart of greenhouse gas emissions during composting

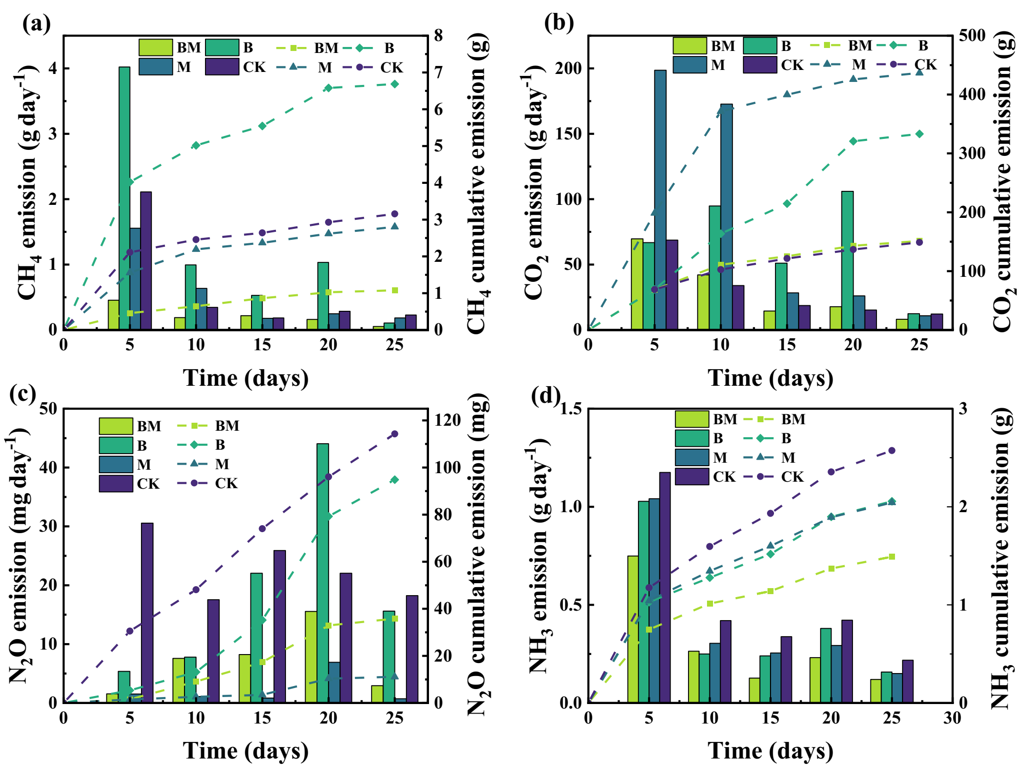


Fig. 5 Emissions of CH_4 (**a**), CO_2 (**b**), N_2O (**c**), and NH_3 (**d**) in different treatments during composting. CK: sheep manure + corn straw; M: CK + 0.01% compound microbial agents; B: CK + 0.1% biochar; and BM: CK + biochar carried microbial agent (0.01% compound microbial agent to 0.1% biochar before being naturally dried)

active microorganisms converted OM to CO_2 (Ren et al. 2019). Thereafter, the CO_2 emissions of the BM, M and CK treatment groups gradually decreased until the end of the composting process. This was attributed to the rapid consumption of soluble organic matter, leading to a reduction in carbon sources (Ma et al. 2020). However, a smaller emission peak occurred on day 20 in the B and BM groups. This might be due to the fact that OM was not fully degraded at an early stage. As OM continued to degrade, CO_2 emissions increased again.

The final cumulative emissions from the BM, B, M and CK treatment groups were 151.32, 333.21, 436.73 and 148.91 g, respectively. The CO_2 emissions of the other three treatment groups increased compared with CK treatment group, and the emission of BM treatment group increased the least, indicating the synergistic effect of biochar and compound microbial agent on CO_2 emissions. On the hand, the combination of biochar and compound bacterial agent can improve the adsorption capacity, resulting in limited CO_2 emissions. On the other hand, the compound microbial inoculum could adjust pH and water content around the biochar (Mishra et al. 2013), and accelerate the release of unstable aliphatic compounds (which can be used as carbon source of microorganisms) in the biochar (Steiner et al 2016), thus improving the effectiveness of biochar carried microbial agent in the loss of CO_2.

3.3.3 N_2O emission

N_2O is an important greenhouse gas in composting and can be produced during incomplete nitrification and denitrification under aerobic and anaerobic conditions (Yang et al. 2019). Figure 5c shows the peak of N_2O emission in the CK group occurred in the early composting stage, while the peaks in the other treatment groups occurred during the cooling stage. This was different from the results of Xue et al. (2021), who found that the peak of N_2O emission occurred at the early stage of composting in all treatment groups. In this study, it might be because the CK group was more likely to form local anaerobic environment at the beginning, resulting in a large amount of N_2O emissions through denitrification (Yang et al. 2015). There was a large release of N_2O from day 15 to day 20 in the BM, B and M treatment groups, which may be related to nitrification of nitrogenous compounds with the increase of NO_3^--N concentration (Wang et al. 2016b).

The cumulative emissions of N_2O in the BM, B, M and CK treatment groups were 35.85, 94.86, 11.09 and 114.30 mg, respectively. Compared with CK, the cumulative N_2O emissions of BM, B and M were reduced by 68.64%, 17.01% and 90.29%, respectively. Microbial additives greatly reduced the emissions of N_2O in the composting process. This might be attributed to the addition of exogenous compound microbial agent prevented the growth of nitrifying microorganisms and the activity of related enzymes, thus inhibiting the nitrification of N_2O production (Cui et al. 2019).

3.3.4 NH_3 emission

NH_3 is an indirect greenhouse gas that can cause nitrogen loss (Chen et al. 2019). The trends of NH_3 emissions in the four treatment groups are shown in Fig. 5d. In the first 5 days, NH_3 emissions increased sharply with the rapid increase of temperature and pH, which can be explained by the conversion of large amounts of NH_4^+-N to NH_3 (Yang et al. 2015). On the fifth day, NH_3 emission rates reached peak, attaining maximum values of 0.75, 1.03, 1.04 and 1.18 g day^{-1} for BM, B, M and CK, respectively. After five days, the NH_3 release rates from the four treatments gradually slowed down with the OM stabilized. NH_3 release pattern was similar with the results of Maulini-Duran et al. (2014).

The final cumulative emissions for the BM, B, M and CK treatment groups were 1.49, 2.06, 2.04 and 2.57 g, respectively. Compared with CK, the cumulative NH_3 emissions of BM, B and M groups decreased by 42.05%, 20.09%, and 20.59%, respectively. BM treatment group had the greatest inhibition effect on NH_3 emission. Adding biochar carried microbial agent can effectively reduce NH_3 volatilization of compost. First, the porous structure and large specific surface area of biochar were highly favorable for NH_3 adsorption (Yin et al. 2021b). Secondly, the abundant reactive functional groups on the surface of biochar could availably adsorb NH_4^+ and reduce the emission of NH_3 (He et al. 2019). In addition, adding exogenous compound microbial agent could inhibit the release of NH_3 by altering the metabolism of carbon and nitrogen (Chen et al. 2019).

It is estimated that the loss of nitrogen during composting accounts for 21–77% of the initial total nitrogen (Chan et al. 2016). The loss of nitrogen is mainly mediated by microorganisms, so it is inevitably accompanied by the loss of carbon due to microbial growth and maintenance respiration (Tong et al. 2019). The carbon loss during composting is 34–77% of the initial total carbon (Guo et al. 2012). In this composting process, the cumulative emissions of gases were $CO_2 > CH_4 > NH_3 > N_2O$. CO_2 and NH_3 emissions were the main causes of total carbon and nitrogen losses, which was consistent with most research conclusions (Li et al. 2018; Yuan et al. 2019; Ba et al. 2020; Sun et al. 2020). The cumulative emissions of gases compared with the CK group are listed in Table 2. The three treatment groups had different emission reduction effects on different gases. Compared with CK group, CH_4 and NH_3 emissions in BM group were reduced by 65.23% and 42.05%, respectively, which was the best among the three treatment groups. In terms of CO_2 emissions, all three treatment groups increased, while BM treatment group only increased by 1.61%, which was the lowest increase. Generally speaking, biochar carried

Table 2 The cumulative emissions of gases compared with the CK group

	CH_4	CO_2	NH_3	N_2O
BM	− 65.23% ± 5.83 c	1.61% ± 1.43 b	− 42.05% ± 5.57 b	− 68.64% ± 9.68 b
B	111.81% ± 5.52 a	123.77% ± 29.02 a	− 20.09% ± 1.52 a	− 17.01% ± 2.58 a
M	− 11.18% ± 2.15 b	193.28% ± 32.41 a	− 20.60% ± 2.59 a	− 90.30% ± 14.14 b

Results are the mean of three replicates ± standard deviation. M: CK + 0.01% compound microbial agents; B: CK + 0.1% biochar; and BM: CK + biochar carried microbial agent (0.01% compound microbial agent to 0.1% biochar before being naturally dried)

microbial agent could effectively reduce gas emissions and had good environmental benefits (Jindo et al. 2012; Tu et al. 2019).

3.4 Effect of additives on bacterial communities

The samples of four treatments on days 6 (thermophilic stage), 14 (cooling stage) and 28 (maturity stage) were collected to analyze the changes of bacterial diversity and richness by 16S rDNA gene sequences. All sequences of each sample are clustered as Operational Taxonomic Units (OTU), and the recognition rate exceeds 97% (Xue et al. 2021). Chao 1 index can reflect the bacterial community richness (Mao et al. 2019). Shannon and Simpson indexes are used to assess the species diversity and evenness. The better diversitycorresponds to higher Shannon and lower Simpson index (Liu et al. 2018). As shown in Additional file 1: Table S2, the coverage index was above 0.99, ensuring the accuracy of the results in the real state. According to the OTU number, Shannon, Simpson, and Chao 1 index, biochar carried microbial agent could increase the abundance and diversity of bacterial communities in compost. It also showed that the addition of biochar carried microbial agent in compost had a positive impact on the activity of microbial community, which might be because the porous structure of biochar was suitable for the growth and reproduction of microorganisms (Sun et al. 2016). At the same time, biochar could also provide inorganic nutrients for the microorganisms, which was more conducive to the survival and reproduction of endogenous and exogenous microorganisms in the compost heap (Steiner et al. 2016).

Relative abundance of the dominant bacterial taxonomic groups in phyla level is shown in Fig. 6a. The microbial communities in each stage of composting showed similar change patters, but there were also some differences. The main bacterial phyla in the thermophilic phase were *Proteobacteria*, *Actinobacteria* and *Firmicutes*, and their relative abundance exceeded 70%. Zhang et al. (2014) reported that *Firmicutes* could grow in the heat by producing thick spores and mainly exist in the thermophilic stage in the composting process. Pandey et al. (2013) showed that *Firmicutes* were able to degrade cellulose, hemicellulose and lignin, and hydrolyze sugars and proteins. As the same time, it could produce all kinds of organic acids, alcohols and lipids. Wang et al. (2016b) showed that thermophilic *Actinobactenia* were important in the biodegradation of lignocellulose in cow manure composting. The relative abundance of *Firmicutes* and *Actinobactenia* in the BM treatments was the highest at the thermophilic stages, reaching 39.24% and 24.79%, respectively, which also explained that BM treatment group had higher peak temperature, longer thermophilic period and faster degradation rate of OM compared with the CK. With the decrease of pile temperature, the relative abundance of *Firmicutes* in the maturity stage dropped to 14.78%, 14.42%, 9.82% and 12.32% for BM, B, M and CK, respectively. In the cooling and maturity stages , the abundance of *Proteobacteria* in BM and B treatment groups was much higher than that in CK. Some *Proteobacteria* had the functions of nitrogen fixation and reducing nitrogen loss, which are related to the conservation of nitrogen in the compost (Xi et al. 2016). This was consistent with the findings that the addition of biochar carried microbial agent and biochar reduced N_2O and NH_3 emissions and increased the TKN contents in the compost. *Chloroflexi* played a major role in the metabolism of amino acids and carbohydrates (Li et al. 2019b). In this study, the relative abundance of *Chloroflexi* in all treatments increased as composting progressed, probably because of the ability of *Chloroflexi* to utilize metabolites and cellular compounds extracted from the dead biomass (Xu et al. 2019).

The dominant bacterial abundances in genus level are shown in Fig. 6b. In this study, legends with relative abundance less than 1% were not shown and were classified into other groups. During the thermophilic period, *Rhizobiaceae*, *norank_f_JG30_KF_CM45*, *Pseudomonas*, *Cellvibrionaceae*, *Bacillus* and *Cellulomonas* were the dominant bacteria, accounting for more than 20%. In the thermophilic stage, *Bacillus* can produce spores to resist high temperature and high osmotic pressure. However, the survival of *Bacillus* in the four treatments was reduced due to a decrease in OM at the end of composting (Grata et al. 2008). Both *Pseudomonas* and *Rhizobiaceae* have nitrogen fixation. The relative amounts of these two bacteria were higher in the BM and B treatments than in the CK group throughout the composting process, which further

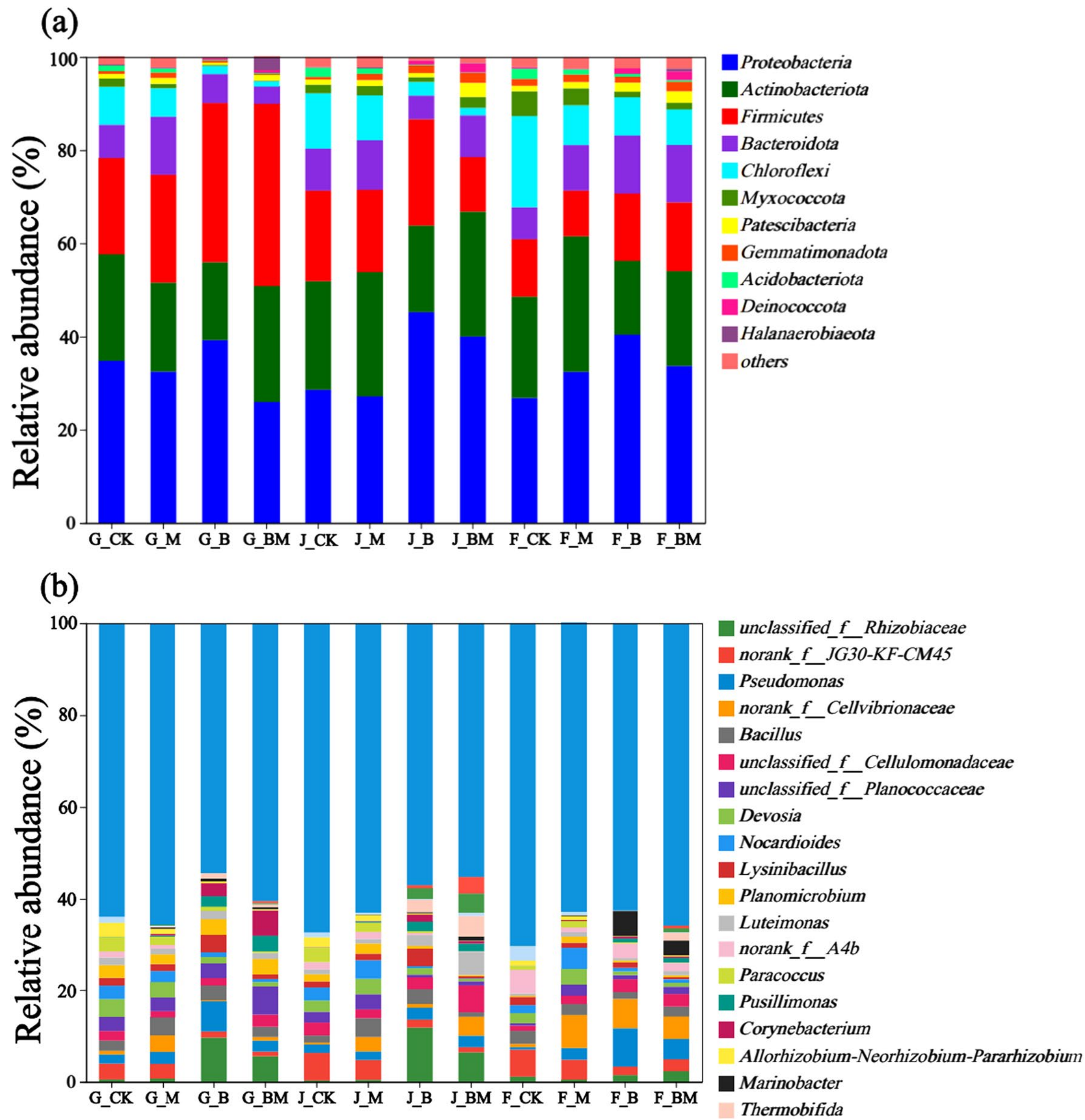


Fig. 6 Bacterial abundance at phyla level (**a**), and bacterial abundance at genus level (**b**) in different treatments during composting. CK: sheep manure + corn straw; M: CK + 0.01% compound microbial agents; B: CK + 0.1% biochar; and BM: CK + biochar carried microbial agent (0.01% compound microbial agent to 0.1% biochar before being naturally dried)

explained that the addition of biochar carried microbial agent and biochar had a promoting effect on nitrogen conservation.

3.5 Effect of additives on maturity parameters

The ratio of E_4/E_6 usually reflects the condensation and aromatization of humus in compost (Ren et al. 2020). E_4/E_6 value is negatively correlated with the degree of maturation in the compost. As shown in Additional file 1: Fig. S4(a), E_4/E_6 ratios decreased rapidly in the initial stage due to the mineralization of carbohydrates and oxidation of phenolic compounds (Vieyra et al. 2009). After that, E_4/E_6 showed a trend of first rising and then declining. In the end, the E_4/E_6 ratios of the BM, B, M and CK treatment groups were 2.84, 3.47, 3.68 and 3.32, respectively. Compared to CK, the BM treatment group showed a lower ratio, indicating that biochar carried microbial agent promoted the condensation and aromatization of humus very well.

Seed germination index (GI) is a significant indicator to assess phytotoxicity and maturity of compost (Qiu et al. 2020). Additional file 1: Fig. S4(b) shows that the GI curves for the four treatments increased steadily in the compost. All treatment groups had low initial GI values. This is likely because of the rapid decomposition of OM in the initial stages of composting, producing large amounts of volatile fatty acids (VFAs) and ammonium, which were toxic to seed germination (Jiang et al. 2018). Finally, the GI values in BM, B, M and CK were 97.92%, 88.25%, 94.32%, and 84.66%, respectively. All the GI values were above 80%, indicating that the composts reached maturity and elimination of phytotoxicity (Yang et al. 2015). The final GI values of the other three treatments were all increased compared with CK, especially BM treatment. This indicated that the addition of biochar carried microbial agent accelerated the stabilization and reduced the phytotoxicity of the compost. The result could be attributed to the higher peak temperature and longer thermophilic period of BM treatment, which was beneficial for the killing of pathogenic bacteria and improvement of compost quality.

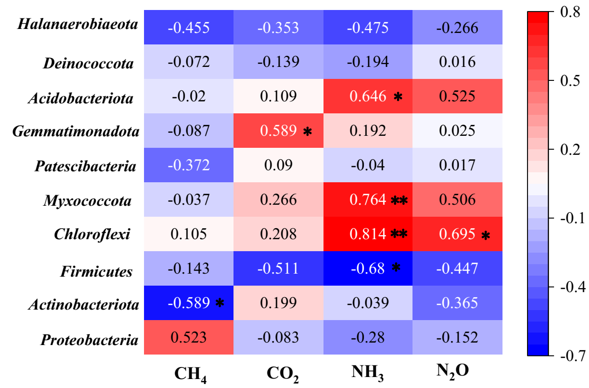


Fig. 7 Correlation analysis between gaseous emissions and microbial relative abundance in phyla level based on the spearman correlation. The right side of the legend is the color range of different *r* values. Red is positive and blue is negative correlation. The value of $p < 0.05$ is marked with *, $p < 0.01$ is marked with **

3.6 The relationship between microbial community and selected factors

The Pearson correlation coefficient of gas emissions and microbial relative abundance in phyla level was calculated to explore the relationship between them. The results are shown in Fig. 7. Correlation analysis revealed that N_2O emissions were positive related with *Chloroflexi*. Yang et al. (2019) and Xue et al. (2021) believed that the anaerobic environment in composting was positively correlated with the relative abundance of *Chloroflexi*, which would increase the emission of N_2O, which was consistent with the results of this study. NH_3 emissions were positively related with the relative abundance of *Myxococcota*, *Chloroflexi* and *Acidobacteriota*, but negatively with that of *Firmicutes*. The results indicated that these microorganisms likely played important roles in the NH_3 release processes. Lei et al. (2021) also showed a strong and significant relationship between the main phyla (*Firmicutes*, *Acidobacteriota*, *Proteobacteria*, and *Chloroflexi*) and variations in the nitrogen loss (NH_3 and N_2O) in the composting process. CO_2 emissions were positively correlated with *Gemmatimonadota*. *Actinobacteria* was negatively related with the CH_4 emissions, which suggested that *Actinobacteria* could inhibit the proliferation of methanogens to reduce CH_4 emissions from compost (Xue et al. 2021).

Environmental factors will affect bacterial communities and their functions, and redundancy analysis (RDA) can express the correlation among environmental factors and microbial communities during composting. Figure 8 shows the effects of composting factors (temperature , pH, OM, C/N, EC, TKN, NH_4^+-N, NO_3^--N, E_4/E_6 and GI) on the abundant phyla. The total contribution rates of the two axes on the distribution of bacterial community structure in thermophilic, cooling and maturity stages were 96.20%, 96.46% and 97.56%, respectively, and the analysis results were relatively reliable. A certain distance between the points of different treatments was observed at different stages of composting, indicating that additives can change the microbial structure in compost. The correlation order of environmental factors affecting bacterial community structure in thermophilic, cooling and maturitystages were EC > E_4/E_6 > NO_3^--N > NH_4^+-N > OM > TKN > C/N > Temperature > pH > GI, EC > TKN > NO_3^--N > pH > OM > NH_4^+-N > C/N > Temperature > E_4/E_6 > GI, TKN > Temperature > OM > NH_4^+-N > EC > NO_3^--N > E_4/E_6 > C/N > pH > GI, respectively. During thermophilic and cooling stages, EC was the critical factor influencing bacterial community structure. The relative abundance of most bacteria has a negative correlation with the EC value, indicating that the excessive salt content in compost has a significant inhibitory effect on the growth and reproduction of microorganisms, thus affecting the microbial community structure. The main reason is that salt affects the effectiveness of water or the physiological and metabolic processes of microbial cells. The amount of TKN mainly controlled the change of bacterial community structure in the maturity stage. Duan et al. (2019) reported that temperature was the main factor influencing microbial community structure in thermophilic and maturity stages of composting. In this study, although temperature was not the most relevant environmental factor, it also played an important part in changing microbial community structure throughout affecting the biological activity of microorganisms.

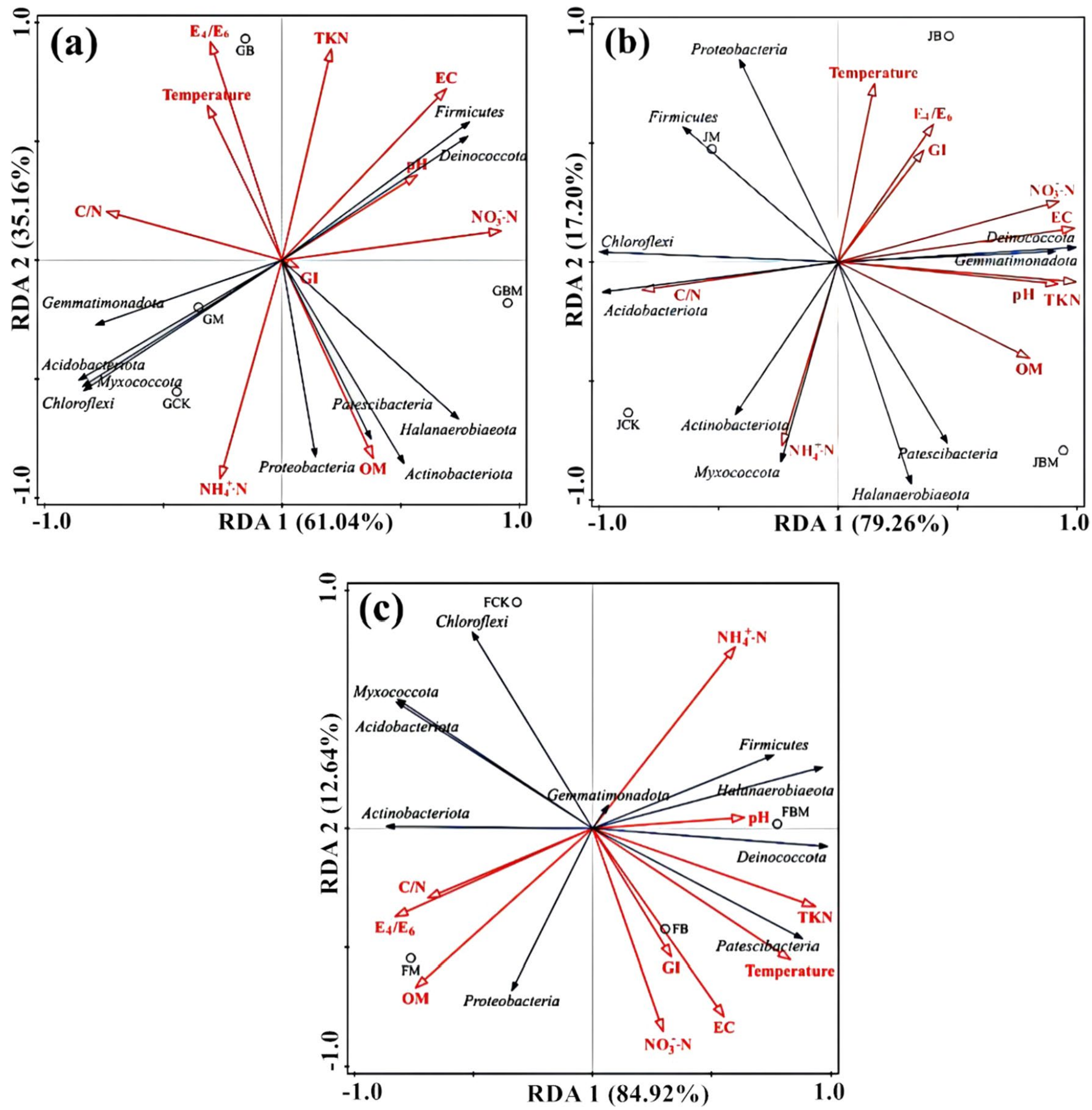


Fig. 8 Redundancy analysis (RDA) between environmental factors and microbial communities during thermophilic stage (**a**), cooling stage (**b**), maturity stage (**c**)

Significant relationships were determined between the main phyla (*Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Deinococcota*, and *Acidobacteriota*) and variations in the nitrogen contents (NH_4^+-N and NO_3^--N) in the composting process. In thermophilic stage, *Proteobacteria* had significant and positive correlations with NH_4^+-N ($p < 0.05$), *Firmicutes* and *Deinococcota* were positively correlated with NO_3^--N, indicating that these three phyla played an important role in nitrogen transformation and release in this stage. Xu et al. (2022a) also showed that *Firmicutes* and *Proteobacteria* were the main host bacteria for *nifH* gene of nitrogen-fixing microorganisms. In the cooling and maturation periods, *Chloroflexi* and *Acidobacteriota* revealed highly negative correlation with NO_3^--N, but positive relationship with NH_4^+-N, which might have played important roles in the transformation of nitrogen. In subsequent studies, functional microorganisms related to nitrogen transformation in composting process can be further explored through the level of microbial genera and related functional genes.

4 Conclusions

This study showed that all four treatments satisfied the sanitation standards and requirements of compost maturity during the 28-day aerobic composting. BM treatment significantly extended the high temperature stage of compost, improved the degradation capacity of OM and minimized the formation of large clumps. It also reduced nitrogen losses and increased nutrient retention during composting. Compared with CK, BM group reduced CH_4 by 65.23%, NH_3 by 42.05% and N_2O by 68.64%, respectively. The gas emission was mainly correlated to *Chloroflexi*, *Myxococcota*, *Acidobacteriota*, *Firmicutes*, and *Gemmatimonadota*. The RDA result showed that EC was associated with samples in the thermophilic and cooling stages, and TKNwas associated with samples in the maturing stage. *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Deinococcota*, and *Acidobacteriota* played important roles in the transformation of nitrogen. Therefore, biochar carried microbial agent is recommended as an effective additive to enhance compost quality and reduce gas emissions during sheep manure composting.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-022-00202-w.

Additional file 1. Supplementary material.

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Author contributions

ZW: Conceptualization, supervision, writing—review and editing, Funding acquisition, project administration. YX: Formal analysis, data curation, writing—original draft. CZ: Supervision, conceptualization, funding acquisition. QJ: Supervision, conceptualization. TY: Formal analysis, data curation. YL: Formal analysis. TZ: Formal analysis. All authors read and approved the final manuscript.

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Availability of data and materials

The authors declare that the data supporting the findings of this study are available within the article and its additional information files.

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author details

[1]School of Energy and Environment, Inner Mongolia University of Science and Technology, Baotou 014010, China. [2]Inner Mongolia Engineering Research Center of Evaluation and Restoration in the Mining Ecological Environment, Inner Mongolia University of Science and Technology, 014010 Baotou, China.

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