The impact of ammonifying microorganisms on the stabilization and carbon conversion of cow dung and wheat husk co-composting

Zhiming Xu a, Ronghua Li b, Xiu Zhang c, Xuerui Xu a, Shaowen Wang a, Feng gao a, Guoping Yang c, Yiqing Yao d, Zengqiang Zhang b, Yong Zhang a, Fusheng Quan a*

a College of Veterinary Medicine, Northwest A&F University, Key Laboratory of Animal Biotechnology of the Ministry of Agriculture and Rural Affairs, Yangling, Shaanxi 712100, China

b College of Natural Resources and Environment, Northwest A&F University, Yangling, Shaanxi 712100, China

c Key Laboratory for the Development and Application of Microbial Resources in Extreme Environments, North Minzu University, Yinchuan, Ningxia 750021, China

d Northwest Research Center of Rural Renewable Energy Exploitation, College of Mechanical & Electronic Engineering, Northwest A&F University, Yangling, Shaanxi 712100, China.

*Correspondence author:
Fusheng Quan

Tel: (225) 578-1360; E-mail: quanfusheng@nwafu.edu.cn

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Abstract:

Ammonifying microorganisms (Amm) play a crucial role in the global utilization of agricultural solid residue, but the mechanism of their action in the stabilization and carbon transformation of agricultural solid residue composting remains unclear. In this work, different treatments, including Control, Amm-1, Amm-2, Amm-3, and Amm-4, were established for co-composting of cow dung and wheat husk to investigate the action mechanism of Amm in composting stabilization and carbon conversion. The findings indicated that the Amm inoculation facilitated the decrease of total organic carbon, dissolved organic carbon, CO\(_2\) productions, and CH\(_4\) emissions by 35.13~37.28%, 15.61~16.78%, 1.75%~32.38%, and 10.30%~18.01%, respectively. Among the treatments, the inoculation of Amm-4 immensely showed the highest improvement effect on the organic solid residue humification and carbon transformation process, with the final compost product showed high stability and favorable performance on improving crop growth. Further mechanism analysis suggested that the Amm inoculation optimized humification-related enzyme activity and fungal community structure, enhanced the humus formation, increased the humification index, and promoted the stabilization of final product. In conclusion, this investigation verified the Amm inoculation can improve compost quality and promote the green application of agricultural solid residue as resource.

Keywords: Ammonifying microorganisms; Agricultural solid residue; Compost; Stabilization; Carbon transformation
1. Introduction

Large amounts of agricultural solid residue will inevitably be produced as a result of global agricultural production. If improperly disposed of, it will become harmful substances in the environmental ecosystem. The efficient utilization of livestock manure and straw is one of the pressing issues in agricultural solid residue today [1]. Several studies have discovered that composting can convert these organic solid residues into humic substances (C_{EX}), realizing resource and humification [2]. Composting involves the biological degradation of organic solid residues through microbial succession and enzyme action, transforming organic matter (OM) into stable C_{EX} [3]. However, composting materials contain many complex and hard-to-degrade macromolecular substances (MS), including lignin and cellulose. Cow dung has the highest cellulose and lignin contents, ranging from 40 – 50% and 10 – 20%, respectively. Wheat husk contains 35-50% cellulose and 20-30% lignin [4, 5]. These higher levels of MS can hinder the microbial biodegradation process in composting, reducing the quality of compost products and the humification process [1, 2]. Therefore, improving the humification of cow dung and wheat husk co-composting is essential to achieve the resource utilization and harmless application of agricultural solid residue, and improve the quality of compost products.

The microbial-mediated transformation process in composting includes MS decomposition and transformation, enzymatic reactions, CO_{2} and CH_{4} mineralization, and humification [6]. Bacteria and fungi accelerate organic matter degradation by secreting metabolites and extracellular enzymes, forming C_{EX} precursors, promoting
C\textsubscript{EX} polymerization, and providing the driving force for carbon conversion in composting [7]. Microorganism inoculation in composting can promote the conversion of MS into C\textsubscript{EX} in agricultural solid residue [2]. For example, \textit{G. trabeum} can enhance the activity of laccase (LA) and polyphenol oxidase (POA) in pig manure and straw co-composting, while \textit{filamentous fungi} can produce cellulase (CA), and \textit{Bacillus} can produce various amylases (AA) [1]. Inoculating white-rot fungi can promote the conversion of carbon-containing compounds such as cellulose [8]. Firmicutes and Bacteroidota can degrade long-chain cellulose into small polysaccharides to increase humification [9]. However, most native microbial activity is inhibited during the thermophilic phase of composting (CTP), and high temperatures can hinder the mesophilic microbial transformation of agricultural solid residue [2], resulting in incomplete degradation of MS, reduced C\textsubscript{EX} content, increased CO\textsubscript{2} and CH\textsubscript{4} emissions (greenhouse gases), and carbon loss [10].

Researchers have developed highly efficient microbial inoculants to address this issue. For example, inoculating thermophilic fungal consortiums (\textit{Trichoderma} and \textit{Aspergillus}) can accelerate the mineralization of carbon-containing substances and promote the humification process [11]. Moreover, several studies have discovered that thermophilic \textit{actinomycetes} play a crucial role in lignin degradation; while thermophilic \textit{Bacillus} plays an important role in cellulose degradation in vegetable compost [11, 12]. [13] Researcher also discovered that thermophilic bacteria (\textit{Ureibacillus spp.}, \textit{Bacillus spp.}, \textit{Geobacillus spp.}, \textit{Brevibacillus spp.}) can effectively utilize carbon-containing aromatic hydrocarbons and protein substances in cow dung.
In conclusion, extracellular enzyme production by microorganisms is closely related to MS degradation, carbon conversion, and humification. Consortium inoculation (thermophilic bacteria and fungi) can effectively mitigate the detrimental effects of temperature on biological transformation [1, 3, 14, 15].

Ammonifying microorganisms (Amm) play an important role in composting by degrading complex macromolecules such as OM and MS into simple compounds, producing metabolic products such as amino acids, amines, and soluble sugars, which serve as $C_{EX}$ precursors to enhance compost humification [16]. Furthermore, the metabolic activity of Amm can promote the temperature rise of composting materials, microbial metabolic rate, and $C_{EX}$ formation. Meanwhile, Amm metabolism proliferation can release growth factors, enzymes, and other substances that promote $C_{EX}$ formation [16]. Therefore, Amm is an essential microorganism in humification process. However, no definitive conclusion exists about the effects of Amm consortium (thermophilic bacteria and fungi) on the key enzymes (POA, LA, AA, and CA) in humification process, necessitating additional research and discussion.

Furthermore, more research is required to determine how Amm influences the structure of indigenous microbial communities to provide the driving force for carbon conversion in composting. In summary, although Amm plays an important role in humification process, its mechanism in humification and carbon conversion in composting remains unknown and requires further research.

Therefore, this study aims to investigate the impact of different Amm consortium on the stabilization and carbon conversion of cow dung and wheat husk co-
composting. The specific objectives are: (i) to evaluate the carbon conversion efficiency and humification process; (ii) to investigate the impact on carbon loss control and microbial community succession, and the relationship between these communities and humification; (iii) to understand the dynamic changes of specific enzymes and SFS; and (iv) to investigate the interaction between inoculated Amm, enzymes, microorganisms, and humification, and to understand the ecological mechanisms of compost products' stabilization, harmless application and its contribution to carbon conversion.

2. Materials and Methods

2.1. Experimental Materials and Design

Collect cow dung and wheat husk (<1 cm) from the farm in Shaanxi province, the physicochemical properties were measured (Table S1). After thoroughly mixing cow dung and wheat husk, the C: N ratio was adjusted to approximately 30 and the moisture content to approximately 65%. After the composting material was in the stable balance for 2 h, five experimental groups including Control, Amm-1, Amm-2, Amm-3, and Amm-4 were set up and treated with different additives. Amm-1, Amm-2, Amm-3, and Amm-4 were prepared as NAMC according to strain enrichment and isolation (S1). 16S rRNA gene sequence was used to identify the three microorganisms (Table S3) and the ammoniation performance between bacteria and fungi were determined by Nessler reagents and AUTO Analyzer 3 (Table S2). The composting materials was thoroughly mixed and placed in a 100 L composting container for 42 days. The container was regularly ventilated at a rate of 0.4 L/min. Specific materials and operational parameters
for the composting device can be found in [17]. Samples were thoroughly mixed and collected from the compost mixture at 1, 3, 7, 14, 21, 28, 35, and 42 days during the composting period. The samples were divided into three parts: one was air-dried for measuring humic substances, another was studied fresh for dissolved organic carbon (DOC) measurement, and the third was stored at -80 °C for measuring enzymes and microorganisms.

2.2. Physicochemical property analysis

Samples for CO2 and CH4 measurements were collected daily during the first 14 days and every two days thereafter. The samples were analyzed using a gas chromatograph (Agilent Technologies 7890B Network GC system, America). Fresh compost samples were thoroughly mixed with sterile distilled water (1:10, w/v), and DOC and total organic carbon (TOC) concentrations were measured according to the method [2]. For CEX measurement, dried sample : solution = 2 g: 20 mL of solution (8 g NaOH + 89.212 g sodium pyrophosphate + 2 L distilled water). The mixture was oscillated at 12000 r/min for 16 h and then centrifuged at 4000 r/min for 15 minutes. The supernatant was collected, and 20 mL solution was added. This process was repeated three times to collect the centrifugal liquids, which were then filtered and extracted to remove C_HA and C_FA. The filtered solution pH was adjusted to 7 using 6 mol/L HCl and stored for 12 h to measure C_HA. The solution was washed with deionized water until the silver nitrate test yielded no precipitate. The C_FA was calculated as the difference between CEX and C_HA [18].

2.3. Synchronous fluorescence spectra and enzyme analysis
V1: the total reaction volume, V2: the total volume of the sample solution, V3: the sample volume, A: the absorbance value, W: the sample weight, T: the total reaction time, ε: the molar absorptivity, D: the dilution factor, and d: the optical path length. The AA, POA, LA, and CA were measured using a microplate reader at 540, 474, 420, and 540 nm, respectively [1, 3, 14, 19, 20]. The calculation was performed as follows:

\[
\text{AA (mg min}^{-1} \text{g}^{-1}) = \text{Amylase activity} = 5 \left( \frac{A + 0.18}{3.72 * V1} \right) / T
\]

\[
\text{POA (nmol h}^{-1} \text{g}^{-1}) = \text{Polyphenol oxidase activity} = \frac{A / (\varepsilon \times d) * V1 * 10^9}{W \times T} * D
\]

\[
\text{CA (mg min}^{-1} \text{g}^{-1}) = \text{Cellulase activity} = \left( \frac{A + 0.07}{15.16 * V1 / V3} \right) / W \times T
\]

\[
\text{LA (mg min}^{-1} \text{g}^{-1}) = \text{Laccase activity} = 5 \left( \frac{A + 0.18}{1.67 * V1} \right) / W \times V3 / V2
\]

The DOC solutions for different treatments were adjusted to 7 mg/L to avoid errors in the measurement of Synchronous Fluorescence Spectra (SFS). The SFS was measured at 250 – 600 nm using an F-4600 spectrophotometer (scanning speed: 1200 nm/min; wavelength interval: 10 nm; response time: 0.1 s).

2.4. Microbial sequencing analysis

Analysis of microbial succession in composting processes by sequencing. The bacterial primers to amplify the V3 - V4 region (341F, 5'-CCTACGGGNGGCWGCAG-3'; 805R, 5'-GACTACHVGGGTATCTAATCC-3') of the 16S rRNA gene, and the fungi primers to amplify the ITS1 region (F, 5'-GAACCGCGGARGGATCA -3'; R, 5'-GCTGCGTCCCTTCATCGATGC-3').
Sequencing analysis with QIIME2 (NovaSeq PE250 platform).

2.5. Plant growth experiment

In the pot experiment, the soil was selected as poor soil of 0-20 cm in the topsoil (Yangling, Shaanxi Province), which was air-dried and fully mixed with 2.5% well-rotted compost. Each treatment was replicated six times, with 10 pakchoi seeds sown in each pot. After cultivation for 50 days in a greenhouse, ten pakchoi growing cabbage plants were randomly selected from each treatment for evaluation. The heavy metal content in pakchoi was determined by digestion with HNO$_3$-H$_2$O$_2$, and the concentrations of Cu and Zn were measured using atomic absorption spectroscopy (Z-2000). Chlorophyll content was analyzed using a chlorophyll meter (SPAD 502).

Seed Germination index (GI): fresh compost samples were weighed and mixed with deionized water at a ratio of 1:10 (W/V), and filtrate was taken and cultured in a petri dish for 48 hours at 25°C to determine the germination rate and root length of the seeds.

2.6. Statistical analysis

Calculation of all humification indices-HI (RH, RI, RP, and RD) according to [2].

$$R_H = \text{Humification rate} = \frac{C_{EX}}{TOC} \times 100\%$$

$$R_I = \text{Humification index} = \frac{C_{HA}}{TOC} \times 100\%$$

$$R_P = \text{The percentage of HA} = \frac{C_{HA}}{C_{EX}} \times 100\%$$

$$R_D = \text{The degree of polymerization} = \frac{C_{HA}}{C_{FA}} \times 100\%$$
All data were analyzed (three replications) and plotted using SPSS (v26.0) and OriginPro 2023, respectively. The significance of the results was determined using one-way ANOVA. SEM was performed using IBM SPSS AMOS 25.0. The circular plots and Sankey diagrams were drawn using R software (version 2.15.3).

3. Results and discussion

3.1. Effect of the carbon conversion

The mineralization of organic carbon by microorganisms resulted in a rapid decrease in TOC content in compost during the composting process (Fig. 1a). The TOC in all treatments ranged from 46.00 – 46.98% at the CFP stage. The TOC gradually decreased as the composting progressed until it reached a stable level at the end of co-composting. This decrease was caused by the rapid degradation of carbon-containing substances, such as lipids and carbohydrates, by microorganisms and OM mineralization (Yan et al., 2023). The TOC in the Control, Amm-1, Amm-2, Amm-3, and Amm-4 at the end of experiment was 38.08, 37.29, 37.02, 36.03, and 35.12%, respectively. These results indicate that Amm inoculation promoted TOC degradation. This could be attributed to the metabolic proliferation caused by Amm inoculation and endogenous microorganisms during composting, which requires a large amount of energy (Wu et al., 2024). Therefore, OM and MS must be completely degraded to provide energy for microbial activity. Moreover, [9] Researcher demonstrated that the metabolic proliferation of microorganisms requires a large amount of energy in pig manure composting.
Microorganisms in compost preferentially utilize DOC as a carbon source [21]. The DOC content increased during the first three days of composting, most likely due to the rapid increase in temperature that caused carbon-containing organic compounds to become soluble (Fig. 1b). Subsequently, the DOC content decreased rapidly at day 14, likely due to the microbial degradation of numerous solid polymers into $\text{CO}_2$ and other substances, releasing a large amount of energy to sustain the growth and activities of microorganisms during composting [9]. The final DOC content at the end of trail was 19.36 (Con), 16.79 (Amm-1), 16.79 (Amm-2), 16.01 (Amm-3), and 15.60% (Amm-4). The indicated that Amm inoculation promoted DOC degradation, with Amm-4 showing the most effective results. This could be because carbon is essential for microbial growth, and Amm inoculation improved the proliferation and metabolism of local microbial communities (bacteria and fungi), promoting the degradation of carbon-containing substances in compost. [22] Researcher have demonstrated that carbon can increase the number of bacteria and fungi involved in carbon metabolism.

$\text{CO}_2$ and $\text{CH}_4$ emissions are related to the dynamics of carbon metabolism, including mineralization and humification [9]. The peak $\text{CO}_2$ emission values for the Control, Amm-1, Amm-2, Amm-3, and Amm-4 are 343.17 (8d), 265.45 (7d), 298.19 (7d), 269.65 (7d), and 210.34 g d$^{-1}$ (8d), respectively (Fig. 1c). It is possible that Amm inoculation during the composting had an effect and suppressed $\text{CO}_2$ emissions. The $\text{CO}_2$ emissions peaked at 21d and 35d, which may be attributed to increased porosity caused by compost turning, providing sufficient oxygen for the microbes to utilize.
OM and produce CO₂ [23]. The gradual decrease in CO₂ emissions (Fig. 1e) is related to soluble OM degradation and changes in the dominant microbial community [9].

Furthermore, the cumulative CO₂ emissions for each treatment were: Control (2321.90 g), Amm-1 (1663.40 g), Amm-2 (2281.34 g), Amm-3 (1921.89 g), and Amm-4 (1570.09 g). The Amm inoculation resulted in varying degrees of decreased CO₂ emissions in all treatments. Amm-1 and Amm-4 treatments showed significantly lower CO₂ emissions than the other three treatments ($p < 0.05$), which may be due to the presence of fungi. Numerous studies have shown that the fungal community mainly contributes to CO₂ emissions [9, 24]. The results indicated that Amm could achieve carbon sequestration and efficiently utilize CO₂, thereby reducing CO₂ emissions.

The majority of CH₄ emissions occurred during the first 21 days of the composting (Fig. 1d). The temperature, moisture content, and material pores were comparatively higher during this period, resulting in an insufficient oxygen supply. Furthermore, the rapid degradation of OM, such as organic nitrogen, fat, and protein, requires a large amount of oxygen [25]. Subsequently, the composting temperature dropped below 50°C, and the carbon content of degradable materials decreased, resulting in a rapid decline in CH₄ emissions. This trend is consistent with pig manure composting [21]. The cumulative CH₄ emissions for each treatment during experiment were: Control (9.16 g), Amm-1 (8.11 g), Amm-2 (8.03 g), Amm-3 (8.21 g), and Amm-4 (7.51 g) (Fig. 1f). The CH₄ emissions of Amm-1, Amm-2, Amm-3, and Amm-4 were reduced by 11.46, 12.34, 10.30, and 18.01%, respectively,
compared to the Control. This may be because the Amm inoculation primarily
improves ammoniation during composting, which requires oxygen in the subsequent
stages of nitrification and denitrification, leading to insufficient oxygen supply for
anaerobic methanogens and a decrease in CH$_4$ production. In summary, Amm
inoculation can promote MS degradation during composting. Few carbohydrates may
undergo acid-catalyzed hydrolysis or dehydration reactions during MS degradation,
leading to a large amount of C$_{EX}$ production. This could also be the intermediate
product of CO$_2$ and CH$_4$ conversion produced during the degradation of OM and MS
into aromatic C, which is facilitated by the interaction between Amm and indigenous
microorganisms and promotes the conversion of carbon-containing substances to
humic polymers [18].

3.2. The effect of the humification process during composting

HI (R$_{HI}$, R$_I$, R$_P$, and R$_D$) is an indicator for measuring the OM humification level. The humification of MS and OM, such as cellulose, can be understood by studying the changes in HI [2]. Furthermore, HI can accurately assess the degree of humification and reflect the quality of compost products (Fig. 2) [26]. All treatments showed a significant increase in HI in comparison to Control, indicating a high degree of organic carbon humification during composting [26, 27]. The increase in R$_I$
indicates that the C$_{HA}$ structure complexity is enhanced (Fig. 2b). This is attributed to the participation of humification precursors in C$_{EX}$ formation, whereby the active component of simple OM is transformed into a spheroidal structure containing stable C$_{EX}$, which eventually condenses to form a complex 3D structure [18]. R$_H$ (Fig. 2a),
R_D (Fig. 2c), and R_P (Fig. 2d) continued to increase during composting, indicating the sequestration of biomass, like woody cellulose, with organic carbon during the maturation process. The values R_H, R_I, R_D, and R_P of the Amm treatment during composting were 21.85 – 24.37% (p < 0.05), 19.62 – 22.65% (p < 0.05), 8.79 – 13.10 (p < 0.05), and 89.79 – 92.91%, respectively, with Amm-4 showing the highest HI values. Moreover, the R_H, R_I, R_D, and R_P of the Amm treatment increased by 6.38 – 18.65% (p < 0.05), 7.45 – 24.04% (p < 0.05), 9.66 – 63.38% (p < 0.05), and 0.95 – 4.42%, respectively, with Amm-4 showing significantly higher HI values than the control. The results indicate that NAMC inoculation, especially Amm-4, can increase the HI of compost, which may be attributed to the positive effects of Amm on the degradation of OM and the synthesis of humic substances (C_HA). Fig. 2 demonstrates that the humification degree of Amm-1 and Amm-4 treatments is higher than that of other treatments, which may be related to the efficient degradation of cellulose by fungi during the humification process. Amm inoculation may have a synergistic effect with indigenous microorganisms during the composting process, decomposing C_EX precursors into structurally simple organic compounds, which are then metabolized by microorganisms, forming complex and stable macromolecular organic products. This explains the lower DOC and TOC values shown in Fig. 1. Therefore, the dynamic changes in enzyme production by microorganisms during the humification process requires further research.

Based on the previous studies on the mineralization and humification reactions, it can be concluded that the addition of ammonifying microorganisms in composting...
induced the degradation of organic matter due to the increased microbial activity and 
availability of nitrogen. This can lead to a reduction in CO\textsubscript{2} and CH\textsubscript{4} emissions, as the 
organic matter is more efficiently converted into stable humus instead of being 
released as greenhouse gases. Ammonification is a key process in the nitrogen cycle, 
where organic nitrogen is converted to ammonium by a group of microorganisms 
called ammonifying bacteria. Balancing the mineralization and humification reactions 
during composting can be achieved by the ammonifying microorganisms providing 
the necessary nitrogen for microorganisms' growth and ammonification.

3.3. Dynamic changes in enzyme activities related to humification and carbon 
conversion during composting

The carbon transformation kinetics and humification process in compost can be 
revealed by the dynamic changes in typical enzymes (Wu et al., 2024). The 
polyphenol oxidase and laccase systems of microorganisms are the main pathways for 
lignin and polycyclic aromatic hydrocarbons degradation, and the activity of these 
two enzymes can reflect the degree of compost humification [1, 19]. POA and LA 
significantly affect microbial community changes in compost using SW as a carbon 
source [14]. The changing trend of POA (Fig. 3a) and LA (Fig. 3c) activity is similar, 
which increases and peaks at 21d and then decreases during composting. This may be 
due to the availability of sufficient material during composting, which provides 
nutrients for microbial proliferation and metabolism, resulting in higher enzyme 
activity. After the composting enters the stage at 21d, POA and LA cause many C- 
containing materials to condense and polymerize into C\textsubscript{EX}. The POA values at 21d of
composting for different treatments were: 2013.36 (Control), 2245.43 (Amm-1), 2145.56 (Amm-2), 2200.12 (Amm-3), and 2321.34 (Amm-4) nmol h\(^{-1}\) g\(^{-1}\). During the cooling stage, the POA values of Amm-4 (1940.39 nmol h\(^{-1}\) g\(^{-1}\)) and Amm-1 (1497.18 nmol h\(^{-1}\) g\(^{-1}\)) were significantly higher than those in other treatments (1284.45 – 1340.46 nmol h\(^{-1}\) g\(^{-1}\)) (\(P < 0.05\)). This may be due to Amm inoculation, especially Amm-4, which can enhance the ammoniation process in composting, resulting in the condensation of simple compounds and microbial metabolites produced by the oxidation of nitrogen-containing proteins, amino acids and other substances with polyphenols to form \(C_{EX}\). As an enzyme can promote lignin degradation, LA values for different treatments at 21d of composting were: 25.34 (Control), 27.98 (Amm-1), 26.75 (Amm-2), 26.88 (Amm-3), and 28.32 (Amm-4) mg min\(^{-1}\) g\(^{-1}\). The LA increased by 7.02 – 11.61% in the Amm treatments during the cooling stage of composting in comparison to the Control. Several studies have demonstrated that extracellular laccase can oxidize and polymerize humus precursors like amino acids and phenols [28]. Furthermore, inoculation with bacteria, fungi, and actinomycetes can change the microbial community structure and increase the LA, which can polymerize MS into phenols, amino acids, and other small molecules such as proteins and lignin [28, 29]. [30] Researcher have demonstrated that the reduction in gas emissions, such as CO\(_2\) and NH\(_3\), is related to the enhanced LA through microorganism inoculation. This is consistent with reduced gas emissions shown in Fig. 1. Amm inoculation, especially Amm-4, resulted in a relatively high LA in all stages of composting because Amm provided a nitrogen source for microbial growth by degrading nitrogen-containing...
organic substances into small molecules. Amm inoculation enhanced LA through synergistic effects with indigenous microorganisms to degrade MS and provide carbon sources for microbial growth. Furthermore, Amm inoculation can produce amino acids through enhanced nitrogen transformation during composting, an important link to promote the degree of LA-induced humification and polymerization and generate nitrogen-containing humic products [26]. Several studies have also demonstrated that POA and LA can synergistically degrade lignin and promote humification [30].

AA can indicate the degree of mineralization during composting and has a mineralizing effect on diverse starch substrates. CA can convert cellulose into amino compounds and small sugar molecules (humus precursors) [3]. AA and CA peak at 7d and then decrease gradually, indicating that starch substrate and cellulose undergo rapid degradation during heating stage [15]. The AA values in the Amm-treated groups increased by 14.34 – 29.91% at 7d, compared to the Control (Fig. 3b), and the CA values increased by 17.22 – 30.43% (Fig. 3d). During the cooling stage, the AA values of Amm-1 and Amm-4 were 10.56% and 10.25% higher than those in the Control, respectively ($P < 0.05$). However, there was no significant difference in CA values among different treatment groups during cooling stage ($P > 0.05$). The results indicate that the AA and CA values of Amm treatments were significantly higher than those in the Control during cooling stage. This can be attributed to the complementary effects with indigenous microorganisms after Amm inoculation, which can degrade MS such as cellulose and starch into simple small molecules during heating stage,
provide materials for microbial growth and metabolism, and promote the composting
process and humification further in composting.

We observed higher enzyme activities for Amm-4 and Amm-1 in all treatments
throughout the composting process. This may be due to the presence of white-rot
fungi in both Amm-1 and Amm-4. [31] Researcher discovered that fungi can
depolymerize substances by producing intracellular and extracellular enzymes.
Moreover, [32] Researcher demonstrated that white-rot fungi can degrade lignin and
cellulose and promote humification process. [30] Researcher discovered that white-rot
fungi could improve the activities of phenol oxidase and laccase. The results indicate
that Amm-4 has the best impact, as the inoculated bacteria and fungi adapt to the
changing, harsh environment in composting through complementary effects and
produce synergistic effects to promote organic matter degradation and humification
process [33].

3.4. Simultaneous fluorescence spectroscopy and analysis of material evolution
Simultaneous fluorescence spectrum can accurately reflect the evolution of
various substances during composting, especially changes in humic-like substances
[10]. A peak appears near 280 nm throughout the composting process, while another
peak appears near 370 nm during cooling stage (D42) (Fig. 4). A main peak was
observed at 250-308 nm wavelength in all stages of composting (Fig. 4a, b), with
Control-D1 (2036 au) > Control-D7 (1525 au) > Amm-1-D7 (1336 au) > Amm-2-D7
(1515 au) > Amm-3-D7 (1353 au) > Amm-4-D7 (1161 au) > Control-D42 (761 au) >
Amm-2-D42 (755 au) > Amm-4-D42 (660 au) > Amm-1-D42 (642 au) > Amm-3-
The peak values at 250-308 nm decreased in all treatments as the composting progressed, which may be due to the presence of a large amount of OM and MS, such as Protein-like substances, during the cooling stage, followed by their degradation. Furthermore, the lower peak values at 250 – 308 nm in the Amm treatment group during heating stage indicate that Amm inoculation promotes MS degradation, such as proteins. Studies have also demonstrated that substances such as carbon aromatic hydrocarbons and proteins have peak values at wavelengths of 250 – 308 nm [10]. Humic substances predominate in the wavelength range of 308 – 363 nm [34]. Each treatment in cooling stage showed a clear peak in the 308 – 363 nm range, with the fluorescence intensity reaching its peak (Fig. 4c). The fluorescence intensity of the Amm treatment increased by 18.04 – 65.80% at 363 nm compared to the control group. This study demonstrates that the Amm inoculation promotes the formation of humic substances and improves the degree of humification. The results of this study are consistent with those of HI (Fig. 2).

The Synchronous Fluorescence Spectroscopy Area (SFSA) can be used to study the fluorescence signals of organic substances in a specific wavelength range. Within the 250 – 600 nm range, SFSA can be divided primarily into three parts, representing protein-like substances (\(A_{250-308}\)), \(C_{FA}\) (\(A_{308-363}\)), and \(C_{HA}\) (\(A_{363-600}\)) [10]. Specific data for each part is shown in Table 1. The change in \(A_{250-308}\) during the composting process showed a continuous decrease. The \(A_{250-308}\) of heating stage and cooling stage decreased by 18.08 – 27.94% and 54.99 – 64.17%, respectively, compared to the composting starting stage (74708). This is because protein-like substances
gradually degrade as the composting progresses. Moreover, the \( A_{308-363}(21995) \) and \( A_{363-600}(19350) \) in composting mature stage increased by 53.06 – 153.78% and 156 – 309.59%, respectively, compared to those in composting initiating stage. This is because humic substances (\( C_{FA} \) and \( C_{HA} \)) extensively polymerize during composting. Moreover, we observed that the \( A_{363-600} \) in mature stage was significantly higher than the \( A_{308-363} \), consistent with [27], indicating that the composting starting stage provides energy for microbial growth and metabolism in composting and converts into more stable \( C_{HA} \) via condensation and polymerization. The \( A_{308-363} \) was observed in composting mature stage in the following order: Control-D42 (33665) < Amm-2-D42 (39738) < Amm-3-D42 (45000) < Amm-1-D42 (50037) < Amm-4-D42 (55818); whereas, the \( A_{363-600} \) was detected in the following order: Control-D42 (49535) < Amm-2-D42 (51572) < Amm-3-D42 (68956) < Amm-1-D42 (72164) < Amm-4-D42 (79255). This indicates that AmmC inoculation promotes the formation of \( C_{FA} \) and \( C_{HA} \) in composting. Both Amm-4 and Amm-1 exhibited high SFSA, indicating that the white-rot fungi in Amm-1 and Amm-4 positively affect the formation of humic-like substances. In summary, Amm inoculation (especially Amm-4) promotes the degradation of protein-like substances and the formation of stable humic-like substances through the complementary action of bacteria and fungi, which is consistent with the conclusion in Fig. 3.

3.5. Microbial community succession

Composting is a controlled biological transformation process, which refers to the organic waste biodegradation by microbial communities under solid aerobic
conditions. In this study, the top five bacterial and fungal communities at the phylum level accounted for 70.73 – 94.84% and over 99% of the total abundance, respectively. The top five bacterial phyla in order of abundance are Chloroflexi > Firmicutes > Proteobacteria > Bacteroidota > Actinobacteriota (Fig. 5a), which is consistent with the results of pig manure composting [9]. The highest abundance was observed in Firmicutes (7.45% – 63.57%) and Bacteroidota (0.94% – 34.37%) during the high-temperature stage of composting. This may be because many bacteria in Firmicutes and Bacteroidota (such as Bacillus and Clostridia) have a rigid cell wall and can produce spores to tolerate the high temperature (Wu et al., 2024). The results show that Amm inoculation, especially Amm-4, can increase the number of these bacterial communities. This may be due to the complementary effect of inoculated Amm and native bacterial communities in the high-temperature stage of composting, which can promote MS degradation and provide sufficient nutrients for bacterial growth and reproduction. Moreover, the researchers demonstrated that Firmicutes and Bacteroidota could degrade long-chain cellulose into small polysaccharides, improving the degree of humification [35]. The dominant bacterial phyla during composting mature stage were Chloroflexi (27.60% – 34.49%) and Proteobacteria (18.14% – 20.57%), mainly because most bacteria in these phyla (such as Nitrospirae and Nitrosomonas nitrosa) are mesophilic and can participate in the simple oxidation of organics, as well as the generation of ammonium ions, oxidation of nitrite, reduction of nitrogen oxides, and synthesis of extracellular polysaccharides [36]. The results indicate that the differences in bacterial community abundance mainly occur in
the high-temperature stage, and inoculating Amm promotes the abundance of specific microbiota in this period, which is consistent with the results shown in Fig. 3. These finding indicated that inoculating of Amm increases the number of specific bacterial communities at different stages, improves their metabolic activity, and generates a large amount of heat, increasing composting temperature. Moreover, some heat-resistant enzymes secreted by microorganisms that died due to high temperatures may remain active, promoting the degradation of organic substances. Moreover, thermophilic microorganisms can decompose residual refractory organic matter (such as lipids and cellulose), gradually forming humic-like substances.

The top 10 bacterial genera included **Candidatus Chloroploca**, **Limnochordaceae**, **Chryseolinea**, **Ruminofilibacter**, **Ureibacillus**, **Actinomarinales**, and **Rhodothermaceae** (Fig. 5b). The abundance of **Ruminofilibacter** in Amm-4 during the thermophilic phase significantly increased ($P < 0.01$) compared to the control group (0.11%), indicating that inoculating Amm-4 can promote the growth and metabolism of **Ruminofilibacter** in the composting high-temperature stage, making it the dominant genus of this period. Moreover, the abundance of **Ruminofilibacter** in each treatment during composting mature stage was: Control (0), Amm-1 (0.16%), Amm-2 (1.13%), Amm-3 (0.39%), and Amm-4 (0.20%). Furthermore, the abundance of **Ruminofilibacter** in all treatments during composting mature stage was: Control (0), Amm-1 (0.16%), Amm-2 (1.13%), Amm-3 (0.39%), and Amm-4 (0.20%). **Ruminofilibacter** was not detected in the control group during mature stage but detected in all treatment groups inoculated with Amm. This may be
because \textit{Ruminofilibacter} degrades organics such as lignocellulose and starch during composting and forms complex and stable C\textsubscript{EX}. Since \textit{Ruminofilibacter} belongs to Bacteroidota, this confirms, in conjunction with Fig. 3a, that inoculating Amm can promote humification during the composting process.

The main fungal phyla included Ascomycota (15.40 – 97.54%), Basidiomycota (1.02 – 40.96%), Zygomycota (0 – 0.22%), and Neocallimastigomycota (0 – 0.43%) (Fig. 5c). Ascomycota exhibited the highest abundance and an increasing trend during the composting process, which was also observed in chicken manure composting [7]. The number of Zygomycota and Neocallimastigomycota decreased and eventually disappeared as the composting progressed. The anaerobic fungi Neocallimastigomycota was discovered more abundant during the composting starting stage [37], primarily due to the high density of the initial material in the pile and the high moisture content, providing suitable growth conditions for anaerobic fungi. However, no Neocallimastigomycota were detected during composting mature stage in all treatments, mainly because of a high degree of composting materials humification, the pore sizes of the materials increased, and sufficient oxygen was provided. Most Zygomycota are saprophytic fungi that cannot adapt to high temperatures; therefore, most of them die or enter dormancy as the compost heap temperature increases. Basidiomycota plays crucial role in the degradation of MS, such as cellulose, hemicellulose, and lignocellulose [38]. After Amm inoculation, the abundance of Basidiomycota throughout the composting process was higher than Control. The highest abundance of Basidiomycota was observed in the Amm-4
(9.42%) and Amm-1 (2.70%) treatments during the high-temperature and mature stages of composting, which may be due to the presence of White rot fungi in both Amm-1 and Amm-4, as White rot fungi can generate \( \text{H}_2\text{O}_2 \) to degrade MS like lignin via the presence of peroxidase genes and peroxidase [39]. Furthermore, White rot fungi can produce lignocellulolytic enzymes through a Fenton-based oxidation mechanism to degrade cellulose [40]. White rot fungi belong to Basidiomycota. Therefore, inoculation of treatments containing White rot fungi (especially White rot fungi -4) can induce the production of endogenous Basidiomycota spores to resist harsh composting conditions [37].

The genera with the highest abundance of fungi included Chaetomium, Chaetomiaceae, Wallemia, Puccinia, Candida, Alternaria, Cryptococcus, Mycothermus, and Microascaceae (Fig. 5d). The abundances of Wallemia and Cryptococcus in Amm-4 (3.74% and 1.04%) significantly increased during the high-temperature stage of composting (\( P < 0.01 \)) when compared to Control (0.13% and 0.43%) (\( P < 0.05 \)), indicating that Amm-4 inoculation can promote the proliferation and metabolism of Wallemia and Cryptococcus, making them the dominant genera at this stage. The abundance of Mycothermus differed significantly among treatments and increased during the composting mature stage. [38] Researcher also demonstrated that Mycothermus is the dominant fungal genus during maturation. Compared to the control group, the abundance of Mycothermus in each treatment increased as follows: Amm-1 (30.13%), Amm-2 (33.93%), Amm-3 (85.35%), and Amm-4 (105.38%). The results indicate that Amm inoculation can increase the abundance of specific fungal
genera during mature stage of composting. This may be because *Wallemia* and *Cryptococcus* belong to Basidiomycota and exhibit complementary effects during composting, increasing their abundance [37]. This is consistent with the results at the phylum level shown in Fig. 5c. The abundance of unclassified fungi in each treatment during the thermophilic stage was 2.76 (Amm-1), 1.01 (Amm-2), 6.60 (Amm-3), and 9.56 (Amm-4) times higher than that of Control. The results indicate that Amm inoculation can increase the abundance of specific fungal groups in composting, with the most significant effects in Amm-4. Sankey diagrams have many advantages, including strong visualization ability and clear hierarchical relationships (Fig. 5e and Fig. 5f). They allow for overall correlation analysis of samples at microbial phylum and genus levels, providing a more intuitive display of the bacterial and fungal community succession process. This result is consistent with that shown in Fig. 5a-d.

### 3.6 Analysis of the correlation and mechanisms of humification and biotransformation processes.

Structural equation modeling (SEM) was used to evaluate the relationship between various factors and HI conversion (Fig. 6a). The formation of humic-like substance (HI) was significantly correlated with CS ($\lambda = -0.82, P < 0.001$), enzymes ($\lambda = 0.1, P < 0.001$) and bacterial ($\lambda = -0.27, P < 0.05$). Fungi were significantly correlated with enzymes ($\lambda = 0.33, P < 0.05$) and CS ($\lambda = -0.82, P < 0.05$). Furthermore, the overall effect showed that CS had a greater impact on HI (Fig. 6a-1), which may be because the CS degradation products can form humus precursor substances, which are then metabolized by microorganisms to form stable $C_{EX}$. 

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promoting humification process (Fig. 2). Furthermore, bacteria can degrade MS such as cellulose and starch and form humic-like substance under the action of complex and stable cyclic organic compounds [35]. [7] Researcher also demonstrated that fungi and bacteria could secrete various metabolites and extracellular enzymes to accelerate organic residue degradation, form $C_{EX}$ precursors, and promote humification process (Fig. 5). CG formation was significantly correlated with CG ($\lambda = -0.82, P < 0.05$) and enzymes ($\lambda = 0.1, P < 0.01$). [18] Researcher showed that CG emissions are related to carbon metabolism kinetics, including mineralization and humification. This is because when MS is degraded, the CG produced may be sequestered into $C_{EX}$ intermediates - aromatic C, promoting the conversion of carbon-containing substances to humification process (Fig. 1). [9] Researcher also reported that enzymes produced by microbial metabolism play a key role in CG mineralization and can indirectly influence humification process. Enzymes and fungi were key factors in improving HI in this study, possibly because Amm inoculation can increase fungal diversity in compost and use enzymes produced by complementary effects with endogenous microorganisms to degrade complex MS such as cellulose and starch into simple small molecular substances, providing raw materials for microbial proliferation and metabolism in the composting process, promoting the composting process and humification process further. This is consistent with the results shown in Fig. 1-5.

The fungal genus *Mycothermus* was significantly correlated with DOC, CO$_2$, CH$_4$, AA, CA, HI, *Candidatus_Chloroploca*, *Chryseolinea*, *Ureibacillus*, *
Actinomarinales, and Chaetomiaceae (Fig. 6b). The study demonstrated that 
Mycothermus is the dominant fungal genus in mature stage of composting,
significantly affecting carbon transformation and humification process in compost and
has complementary effects with other fungal communities [38]. This is consistent
with the results shown in Fig. 5d. The bacterial genus Ruminofilibacter was
significantly correlated with DOC, CO₂, CH₄, AA, CA, HI, Candidatus Chloroploca,
Ureibacillus, Actinomarinales, Chaetomiaceae, and Mycothermus. This suggests that
Ruminofilibacter significantly affects carbon transformation and humification process
during composting. This may be because Ruminofilibacter belongs to Bacteroidota.
[35] demonstrated that Bacteroidota could degrade MS, such as long-chain cellulose,
into small polysaccharides to improve humification process, which is consistent with
Fig. 5b. DOC, CO₂, and CH₄ were significantly correlated with HI. This is because
the degradation of DOC into small molecules provides a skeleton for microbial
proliferation and raw materials for humification [15, 21]. CO₂ and CH₄ can be
converted into humus C⁻EX intermediates - aromatic C, promoting the conversion of
carbon-containing substances to humification process [9]. Moreover, microorganisms
have synergistic effects that can stimulate the breakdown and synthesis of enzymes
[28, 29].

Network analysis was conducted on the treatment with Amm inoculation to
reveal the impact mechanism of Amm on the composting process (Fig. 6c). The
correlation between each factor and humification was as follows: DOC (0.88 –
0.96) > bacterial (0.78 – 0.89) > fungi (0.65 – 0.88) > CO₂ (0.76) > CH₄ (0.74 –
Following the inoculation of Amm, the strongest association among humification, DOC, and bacterial and fungal communities was observed. This may be because DOC provides materials for the proliferation and metabolism of exogenous Amm and enhances the proliferation and metabolism activity of local microbial communities (bacteria and fungi), promoting humification [21]. The study discovered that fungi are involved in the degradation and transformation of OM and MS in composting by producing various enzymes (LA, CA, and POA). Bacteria in composting directly degrade OM into CO$_2$ and minerals by producing various enzymes (e.g., AA) and metabolites [14]. Furthermore, *Mycothermus* and CA, with 17 linked nodes each, significantly influence various factors in composting. Moreover, the correlation between *Mycothermus* and CA and humification are 0.78 – 0.80 and 0.62 – 0.67, respectively. The results showed that *Mycothermus* and CA are not only important influencing factors in the composting process but also affect compost humification process. [41] reported that *Mycothermus* degrades organic compounds and releases heat by producing cellulase and other enzymes, promoting composting and humification. Moreover, the ammonification of Amm can improve the MS degradation rate by *Mycothermus* in composting and promote humification process [38]. In summary, a comprehensive analysis of previous research shows that Amm inoculation (especially Amm-4) can adapt to the changes in the composting environment by enhancing the complementary effects of endogenous bacteria and fungi in the compost and collaborating with endogenous microorganisms to promote carbon transformation and humification.
3.7 Stability of composting products and application in soil

We have proven in the laboratory that the germination index of seeds treated with compost products 80%, meeting the standards for application in soil. The direct impact of carbon conversion significantly influences the quality of compost products. Pot experiments can be conducted for the harmless evaluation and quality assessment of compost products (Fig. 7g). Compared to the control (Con), pakchoi chlorophyll, plant height, root length, and dry biomass increased significantly by 8.18%-36.76%, 46.78%-98.18%, 40.49%-52.62%, and 60.88%-84.04%, respectively (Fig. 7a-d).

Indicating that compost products inoculated with Amm are more conducive to the development of plant roots and promote nutrient enrichment. The higher chlorophyll content also demonstrates that compost products inoculated with Amm are conducive to the synthesis of plant chlorophyll, directly impacting photosynthesis and thereby promoting plant growth.

The content of Cu and Zn in lettuce serves as one of the indicators reflecting the harmless standards of compost products. Compared to Con, the compost products inoculated with Amm showed a decrease of 2.52%-20.19% and 2.46%-22.81% in Cu and Zn content in lettuce, respectively (Fig. 7e-f). This indicates that Amm inoculation reduces heavy metal concentrations in compost, leading to a decrease in the accumulation of Cu and Zn in pakchoi. In summary, inoculation with Amm, especially Amm-4, promotes carbon conversion, influences the formation of humus, reduces harmful substances in compost, and improves crop growth. This provides a reference for the harmless application of high-value compost products after Amm.
4. Conclusion

Amm inoculation accelerated TOC and DOC degradation, reduced CO$_2$ and CH$_4$ emissions, increased HI and enzyme activity, improved microbial community structure, and improved crop growth. Mechanism study showed that enzymes and fungi play pivotal roles in the humification reaction, and the associated between DOC and microbial communities was strongest with HI after inoculating Amm. In summary, inoculating Amm, especially Amm-4 can promote carbon conversion and humification by improving microbial structure in compost. This research offers theoretical backing for the harmless application of Amm in global agricultural organic residue resources.

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Author contributions

Zhiming Xu: Investigation, Data curation, Methodology, Writing- original draft.
Ronghua Li: Conceptualization, Writing-review & editing original draft. Xiu Zhang: Analysis, Writing - review & editing. Xuerui Xu, Shaowen Wang, and Feng Gao: Data curation. Guoping Yang, Yiqing Yao, Yong Zhang, and Zengqiang Zhang: Methodology, Review & editing. Fusheng Quan: Supervision, Funding acquisition, Project administration, Writing - review & editing.

Conflicts of interest
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.

Data availability
Data will be made available on request.

Appendix A. Supplementary data
E-supplementary data for this work can be found in e-version of this paper online.
Reference


