

The Dynamic Changes of Nutrient and Microbial Succession
in Nanomembrane Aerobic Composting of Tomato Straw

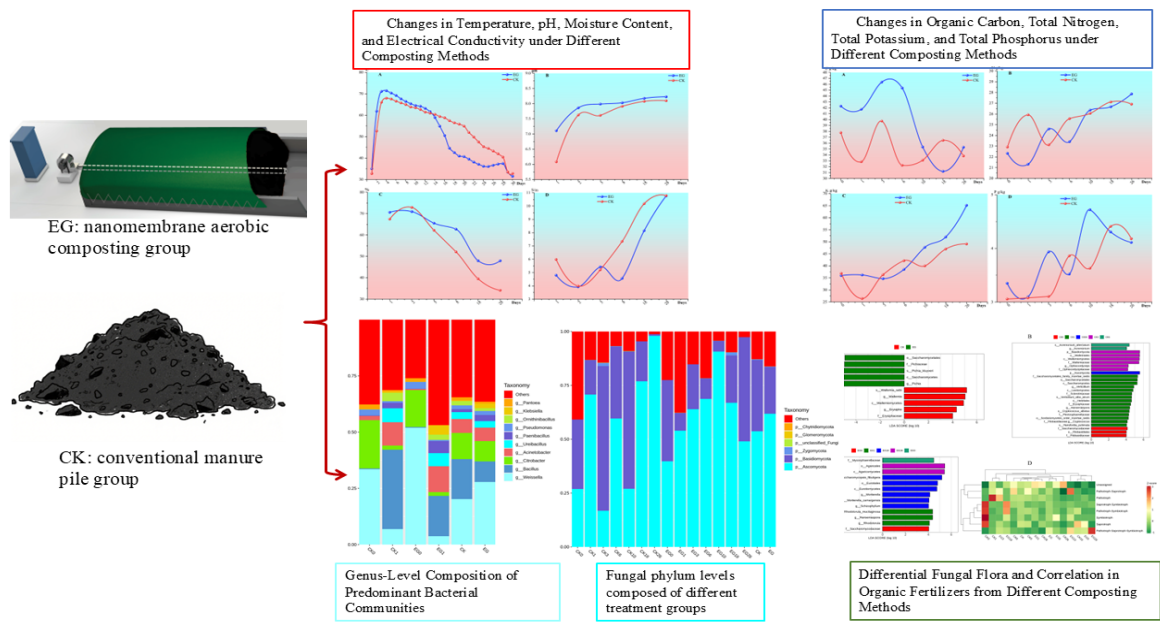
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Abstract

As the demand for sustainable agriculture grows, the use of agricultural by-products for organic fertilizer production has garnered substantial attention. This study investigates the nutrient dynamics and microbial diversity during the composting of tomato straw using nanomembrane aerobic composting and conventional composting methods. Over 28 days, we measured temperature, pH, moisture, electrical conductivity, and nutrient levels. The results indicate that nanomembrane aerobic composting achieved a higher initial temperature, thereby accelerating the decomposition of organic matter and promoting nutrient conversion. The nanomembrane treatment maintained higher organic carbon content and increased total nitrogen, phosphorus, and potassium relative to conventional composting. Microbial profiling revealed marked differences in fungal diversity between treatments. Key fungal phyla were more abundant in nanomembrane compost, suggesting enhanced microbial activity. In contrast, bacteria were undetectable on the third day of fermentation, raising questions about their role in the middle and late stages of composting. This study demonstrates that nanomembrane aerobic composting can enhance composting efficiency and organic fertilizer quality by optimizing environmental conditions and promoting microbial activity. Nanomembrane aerobic composting promotes a more controlled succession of fungal communities, which may play a crucial role in the fermentation process. It is noteworthy that during the composting process, bacteria gradually became undetectable as fermentation progressed, whereas fungi were consistently present throughout the experiment. It might suggest to the researchers that fungi, rather than bacteria, may play a more significant role in the fermentation process of organic fertilizers.



Key words: organic fertilizer, tomato straw, nutritional components, bacterial diversity, fungal diversity

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Introduction

The urgency of sustainable agricultural production is increasing, due to the growing global population and intensified agricultural practices. Agricultural waste management is an important aspect of this challenge. Improper disposal of agricultural waste (such as crop straw) not only leads to waste of resources but also causes environmental pollution problems. Crop straw contains abundant nutrients that can be utilized by plants, making it a valuable resource for sustainable agriculture once properly treated (Janczak et al. 2017; Yin et al. 2021). China, as one of the world's agricultural powerhouses, generates a large amount of agricultural waste annually. For instance, the production of tomato straw alone is substantial.

Composting, a process that converts organic waste into nutrient-rich organic fertilizer, offers a promising solution to agricultural waste management (Sathiyapriya et al. 2024). Meanwhile, composting technology is also a practical and environmentally friendly method for handling biodegradable organic waste on a global scale. Compared with other disposal methods such as landfilling and incineration, it is more friendly to the air, water and soil. (Arslan Topal et al. 2016; Argun et al. 2017). It can effectively inactivate insect eggs, and pathogenic bacteria during the composting process (Argun et al. 2017; Manea and Bumbac 2024). Organic fertilizers, as essential soil conditioners, provide essential nutrients for plant growth, enhance soil structure and biological activity, and support ecosystem cycling processes (Azarbad et al. 2014), and maintain a favorable environment for the survival of soil microorganisms.

Tomato straw, an agricultural by-product, is a high-quality raw material for organic fertilizer production due to its rich organic matter and nutrient content (Janczak et al. 2017). However, traditional composting methods have several drawbacks, such as lengthy fermentation cycles, significant nutrient losses, and potential secondary pollution. To address these issues, nanomembrane aerobic composting technology has emerged as a practical approach. This technology regulates the micro-environment during composting by controlling humidity, temperature, and oxygen supply, thereby enhancing the efficiency of organic matter decomposition and nutrient conversion.

The composting process is fundamentally a biological transformation driven by microorganisms utilizing organic matter as a substrate. During this process, microorganisms play a crucial role in breaking down

complex organic compounds (Nakasaka et al. 2019). The efficiency and outcome of composting are significantly influenced by the metabolic capabilities and community structure of these microorganisms, which are crucial determinants of the composting process (Yin et al. 2021; Peng et al. 2022). There are significant differences in the main microbial communities, depending on the conditions of composting and the different stages of composting (Luo et al. 2023).

Nanomembrane aerobic composting has multiple advantages over traditional composting. It creates a controlled microenvironment, enhancing organic matter decomposition and nutrient retention. Research shows it retains nutrients better and reduces losses (Cao et al. 2022). Its high molecular selectivity allows precise temperature and oxygen control, promoting beneficial microbes and speeding up composting (Xiong et al. 2021). Studies confirm it improves composting efficiency and final product quality. Additionally, it is more environmentally friendly, reducing odor emissions and pathogen spread risks, with nanomembrane systems effectively controlling odors and harmful gas releases (Xiong et al. 2023, Cao et al. 2022).

In this study, we aimed to explore the effects of two composting methods, nanomembrane aerobic composting and conventional composting, on the nutrient composition and microbial diversity of organic fertilizer derived from tomato straw. By systematically comparing key parameters such as temperature, pH, moisture content, electrical conductivity, and nutrient composition during fermentation, we assessed the impact of these methods on organic fertilizer quality. Additionally, we analyzed the changes in microbial community structure to uncover differences in microbial diversity under varying composting conditions. This study provides a scientific foundation for understanding the microbial mechanisms involved in composting offering theoretical insights and practical guidance for optimizing the composting process. Our findings may improve the utilization efficiency of organic fertilizers and promote sustainable agricultural development.

Experimental

Materials and Methods

Experimental design. Tomato straw served as the substrate to compare nanomembrane-aerobic and conventional composting. Upon completion of the final tomato harvest, the tomato stalks were uniformly

collected. The collected stalks were air-dried outdoors and subsequently crushed into particles smaller than 1 cm using a crusher. The moisture content of the crushed stalks was adjusted to approximately 70% for composting purposes. The experiment comprised two composting treatments, each with three replicates.

Composting treatment 1 (EG). Covered with a nanomembrane and supplied air for 10 minutes every 1 hour (at a supply air volume of 1,200 m³/h). The compost pile dimensions were 1.4 m in height, 2 m in width, and 4 m in length, with a moisture content of approximately 70%.

Composting treatment 2 (CK). Conventional composting without aeration or membrane coverage. The compost pile dimensions were identical to Treatment 1, with a moisture content of approximately 70%. Sampling was conducted at 10:00 a.m. on Days 1, 2, 5, 8, 18, and 28. A five-point composite sampling method was employed, involving four points along the diagonal and the center point of the compost core. The depth was kept consistent for all sample collections. Take samples from the restored composting body each time. Immediately after collection, samples were reduced to approximately 200 g using the quartering method. The samples were divided into two portions: the first for analysis of pH, electrical conductivity (EC), organic matter, total nitrogen, total phosphorus, total potassium, and moisture content, and the second was immediately preserved in liquid nitrogen for microbial diversity analysis.

Test equipment. The nanomembrane composting system has four key components: nanomembrane, controller, oxygen supply unit, and fermentation pad. We used a commercial nanomembrane (Biomintec Environment, Shanghai). It features an ePTFE core sandwiched between two polyester films (pore size 50–200 nm). Key specifications: tensile strength 3800 N (warp)/2900 N (weft), breaking force 194 N/215 N, shrinkage ≤ 3 %, hydrostatic head > 20,000 mm H₂O, air permeability 3.2 mm s⁻¹, MVTR 6950 g m⁻² day⁻¹, splash rating 5, PTFE thickness ≤ 0.07 mm, pore size 0.2–0.3 μm (all per cited standards).

Analytical methods. Physical analyses. Temperature: The temperature of the compost pile was measured using a thermometer at approximately 30 cm below the surface at 10:00 daily. The pH, EC, organic matter, total nitrogen, total phosphorus, and total potassium were analyzed according to the standards for organic fertilizers (NY525-2021). Specifically, pH was measured using a pH meter. Organic matter was determined by the potassium dichromate titration method.

Total nitrogen, total phosphorus, and total potassium were extracted using H₂SO₄-H₂O₂ (SSH) digestion.

Chemical analyses. Total nitrogen was measured using a Kjeldahl nitrogen analyzer, total phosphorus was determined by the vanadomolybdenum yellow colorimetric method, and total potassium was measured using a flame photometer. Electrical conductivity (EC) was assessed using a conductivity meter. In this study, a Kjeldahl nitrogen analyzer (Model K1100; Shandong HaiNeng Scientific Instrument Co., Ltd., China) was used for total nitrogen analysis. A flame photometer (Model FP640; Shanghai Yuefeng Instrument Co., Ltd., China) was employed for the determination of total potassium. Total phosphorus was measured using a spectrophotometer (Model I3; Shandong HaiNeng Scientific Instrument Co., Ltd., China). Electrical conductivity was assessed with a conductivity meter (Model DS-307A; Shanghai Precision & Scientific Instrument Co., Ltd., China).

Amplified sequencing. DNA amplification and sequencing: DNA extraction was performed by lysing sample cells using beads in a mixture of 4% (w/v) SDS, 500 mM NaCl, and 50 mM EDTA. This buffer protects the released DNA from degradation by DNase, which is highly active in the sample. Impurities and SDS were removed by ammonium acetate precipitation, and nucleic acids were isolated via isopropanol precipitation. Genomic DNA (gDNA) was purified using the Zymo Research BIOMICS DNA Microprep Kit (Zymo Research, USA). Illumina sequencing technology was employed for additional sequencing (Caporaso et al. 2010). Prokaryotes were identified using the 16S rRNA gene, fungi using the ITS gene, and specific functional genes (Kuczynski et al. 2011).

For PCR amplification of the V4 region of the 16S rRNA gene, universal primers 515F (5'-GTGYCAG-CMGCCGCGGTAA3') and 806R (5'-GGACTACH-VGGGTWTCTAAT3') were used. For ITS gene amplification, primers ITS3 (5'-GATGAAGAACG-YAGYRAA3') and ITS4 (5'-TCCTCCGCTTATTGATATGC3') were employed. TOYOBO KOD-Plus-Neo DNA Polymerase (KOD-401B; TOYOBO Co., Ltd., Japan) was used, and PCR was conducted using the GeneAmp® PCR System 9700 (Applied Biosystems, Thermo Fisher Scientific Inc., USA). The PCR conditions were: 94°C for 1 min (initial denaturation), followed by 30 cycles of 94°C for 20 s, 54°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 10 min. Three replicates per sample were pooled for processing.

Libraries were constructed using the NEB-Next® Ultra™ II DNA Library Prep Kit for Illumi-

na[®] (NEB#E7645L; New England Biolabs, USA). High-throughput sequencing was performed using PE250 with the Illumina Hiseq Rapid SBS Kit v2 (FC-402-4023 500 Cycle).

Data analysis. The offline sequencing data were processed to generate high-quality sequences for subsequent analysis. Bioinformatics tools, including USEARCH (Edgar 2010) and QIIME (Caporaso et al. 2010) were employed for data processing, while R, Python, and Java used for statistical analysis and visualization (Brown et al. 2015).

Data quality control involved splicing sequences using FLASH (Magoč and Salzberg 2011), isolating sequences based on barcodes, and filtering out low-quality sequences with an average mass < 25 or length < 200 bp. Chimeras were removed using the UCHIME algorithm and the gold database to obtain effective data tags. Community composition was analyzed using R and the ggplot2 package (Wickham 2016) for data transformation and visualization.

Species differences were analyzed using LefSe tools (Khleborodova et al. 2024) and the random Forest package (Liaw and Wiener 2002) in R for random forest analysis, with analyses conducted using R scripts. Community function prediction was performed by clustering and annotating 16S rRNA and ITS gene sequences based on the SILVA database (Quast et al. 2013), and transforming the results using the KEGG database (Kanehisa et al. 2025) . Corrections were made based on the copy numbers of 16S rRNA and ITS genes in different bacterial and fungal genomes from NCBI, and classification information was predicted based on microbial functional gene profiles in the KEGG database.

MS Excel was used to organize the data, and Origin 2021 (OriginLab Corporation, USA) was employed for variance analysis, linear regression, correlation analysis, curve fitting, and Duncan’s multiple comparison tests. Software and database versions used include QIIME v1.9.0, USEARCH v10.0.240, R v3.6.0, Python

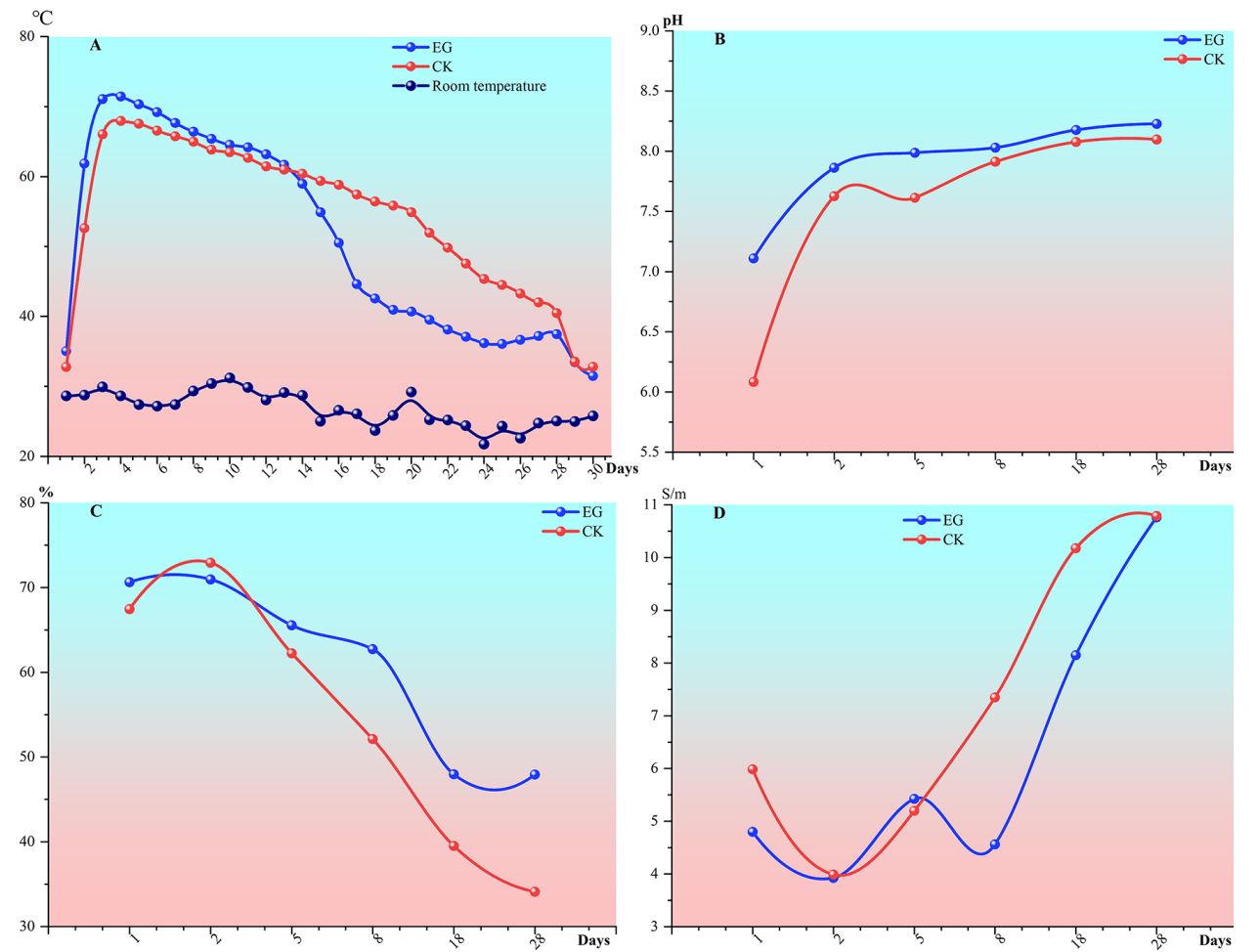


Fig. 1. Changes in temperature, pH, moisture content, and electrical conductivity under different composting methods. A) Temperature changes of tomato straw in different composting methods; B) pH changes of tomato straw in different composting methods; C) moisture content changes of tomato straw in different composting methods; D) electrical conductivity changes of tomato straw in different composting methods. CK – conventional manure pile group; EG – nanomembrane aerobic composting group.

v3.7.4, and SILVA database v132. Data records are accessible via the following links after the release date: PRJNA1256098, PRJNA1256112.

Results

Changes in temperature, pH, moisture content, and electrical conductivity under different composting methods. The temperature changes of tomato straw under different composting methods are shown in Fig. 1A. The temperature of the EG group increased sharply in the first 3 days and reached a peak of 71.44°C on the fourth day. The temperature of the EG group began to drop sharply after the 14th day and remained above 50°C for 15 days. Similarly, the temperature of group CK rose sharply in the first three days, reached a peak of 67.95°C on the fourth day, and then slowly dropped,

maintaining above 50°C for 20 days. The temperature of the EG group was higher than that of the CK group during the first 14 days. After 14 days, both groups experienced a rapid decrease in temperature.

The pH variations of tomato straw across various composting methods are depicted in Fig. 1B. Throughout the composting process, the pH of both groups initially increased and then stabilized. The final pH of both groups shifted towards alkalinity by the end of composting. The moisture content is illustrated in Fig. 1C. The initial moisture content of the two treatments in this experiment was approximately 70%. As composting progressed, the moisture content of the two treatment groups gradually decreased. The electrical conductivity (EC) changes are depicted in Fig. 1D. The EC of both groups initially decreased, then increased, and finally reached approximately 10 mS/cm.

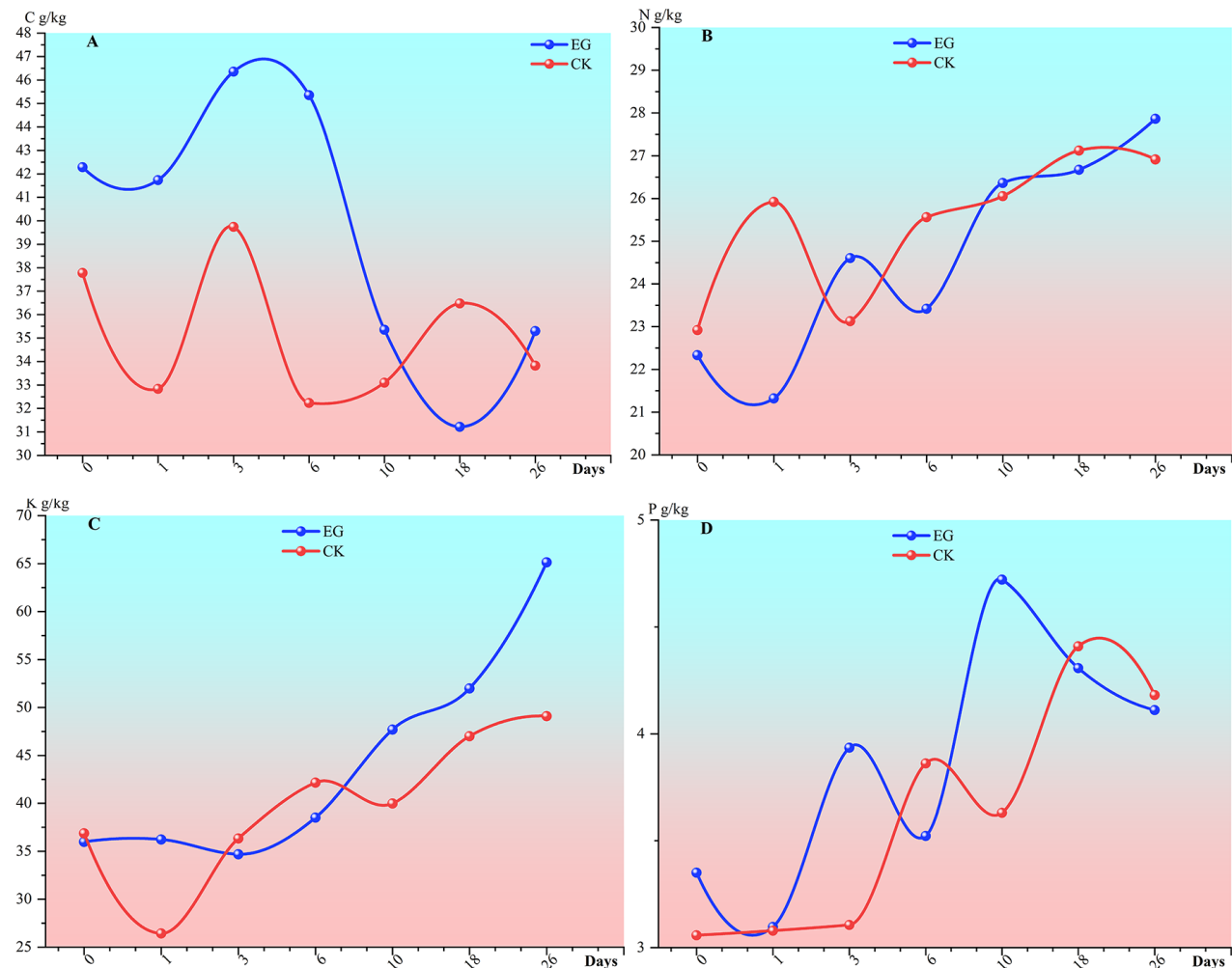


Fig. 2. Changes in organic carbon, total nitrogen, total potassium, and total phosphorus under different composting methods. A) Changes in organic carbon of tomato straw under different composting methods; B) changes in total nitrogen of tomato straw under different composting methods; C) changes in total potassium of tomato straw under different composting methods; D) changes in total phosphorus of tomato straw under different composting methods. CK – conventional manure pile group; EG – nanomembrane aerobic composting group.

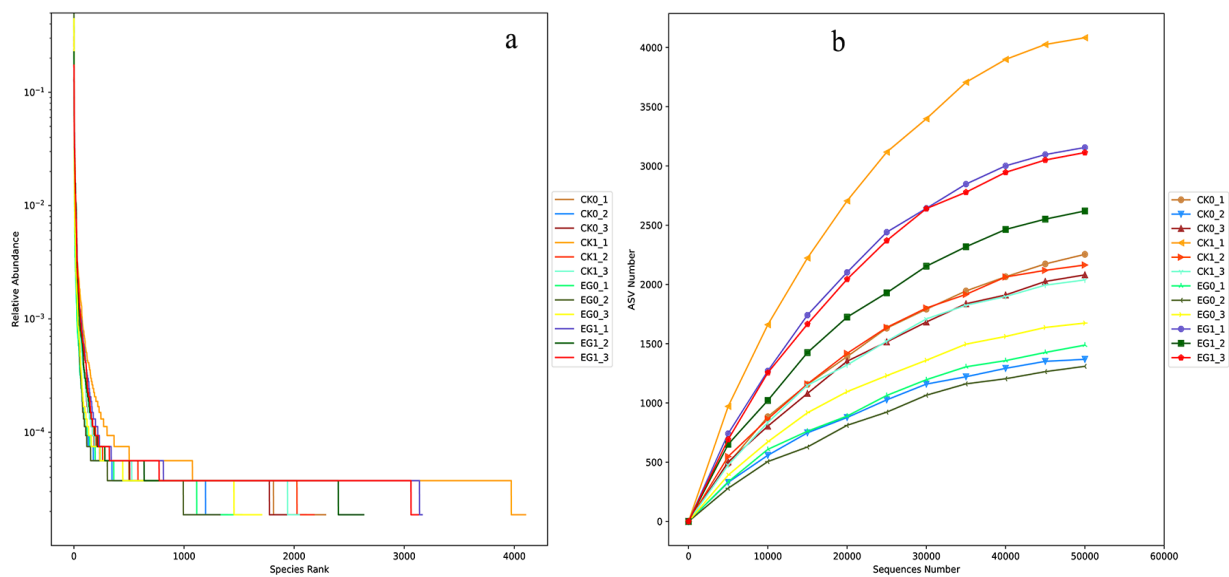


Fig. 3. Rank-abundance rarefaction curve and dilution curve. a) Rank Abundance curve; b) dilution curve.

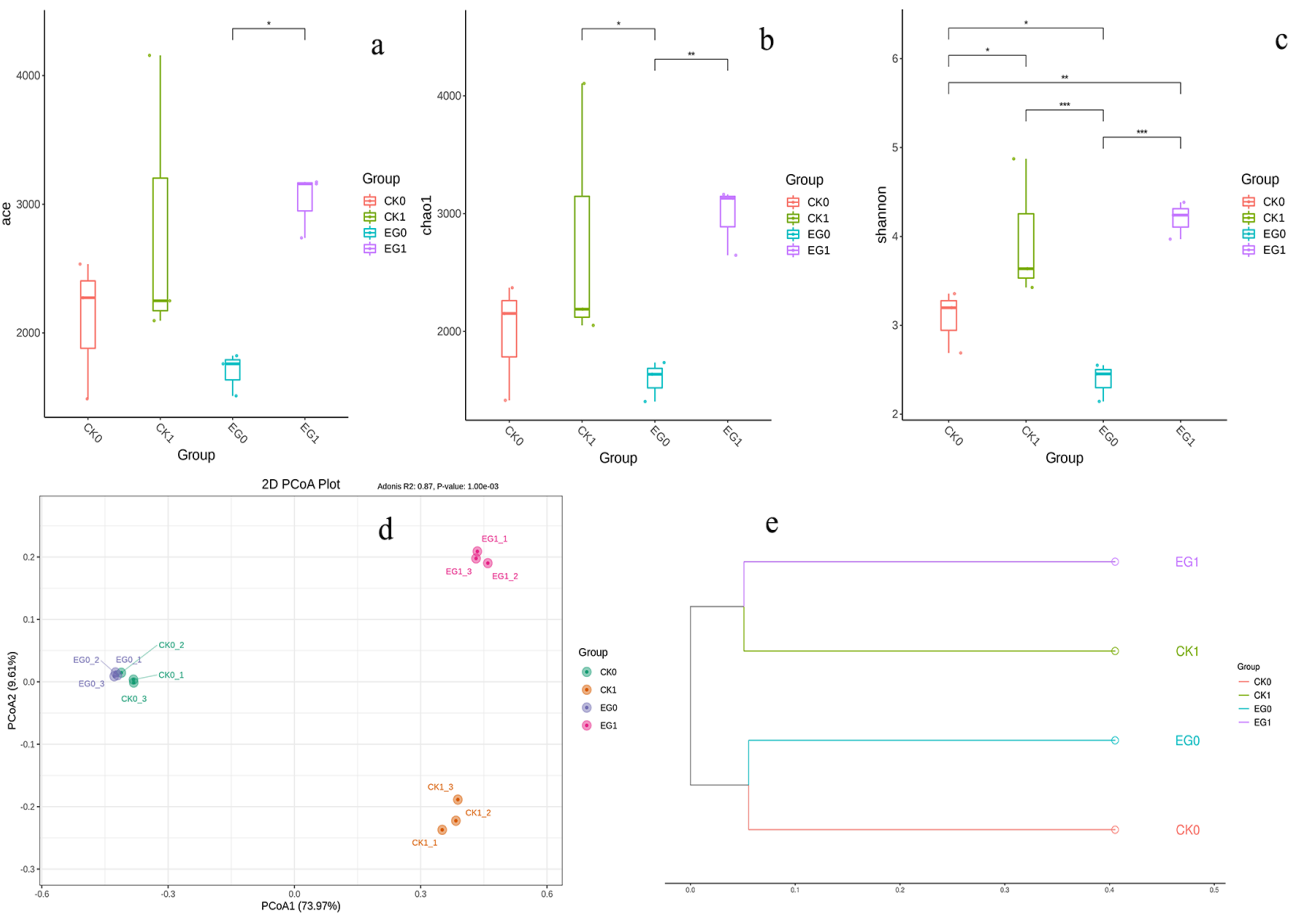


Fig. 4. Alpha diversity index, principal coordinate analysis, and UPGMA clustering tree of two treated composts. a) ACE index of bacterial alpha diversity across composting methods; b) Chao1 index of bacterial alpha diversity across composting methods; c) Shannon index of bacterial alpha diversity across composting methods; d) PCoA plot of bacterial alpha diversity across composting methods; e) UPGMA dendrogram of bacterial alpha diversity across composting methods. The experimental groups represented by CK and EG are the same as those in Fig. 1.

Changes in organic carbon, total nitrogen, total potassium, and total phosphorus under different composting methods. The changes in organic carbon during the composting process can be seen in Fig. 2A. There was a general trend of fluctuating decline, with the CK group dropping to a minimum of 31.2% on day 18. The decrease was greater in the EG group than in the CK group, with values of 7% and 4%, respectively.

The changes in total nitrogen are shown in Fig. 2B, which exhibit a general trend of fluctuation and increase. At the end of composting, the total nitrogen content of the EG group and CK group was 27.86g/kg and 26.91g/kg, respectively.

The changes in total phosphorus are presented in Fig. 2C, generally displaying a trend of initial increase followed by a decrease. The EG group reached a maximum of 4.71 g/kg on day 12 and then declined. The CK group reached a maximum of 4.41g/kg on day 18 and then decreased.

The changes in total potassium are illustrated in Fig. 2D, indicating an overall upward trend. The total potassium content in the EG group was higher than

that in the CK group, with values of 65.14 g/kg and 49.09 g/kg, respectively.

Bacterial diversity in different composting methods. Rank-abundance rarefaction curve and dilution curve. As shown in Fig. 3a, the rank-abundance curve exhibited a flat and declining trend across all samples. The OTU values detected in each sample ranged from 1,000 to 4,000. The dilution curve tends to flatten, with additional data yielding only a small number of new species (Fig. 3b). This confirms the rationality of the sequencing data volume to some extent.

Alpha diversity of bacteria in different composting methods. As shown in Fig. 4a, 4b, and 4c, the abundance-based coverage estimator (ACE), Chao1, and Shannon indices were higher on the first day than on day 0 in all experimental groups. This suggests that as fermentation progresses, both the number of individuals within the bacterial community and the species richness, as well as species evenness, increase.

Fig. 4d displays the principal coordinate analysis (PCoA), revealing distinct clustering among the various experimental treatments. The first principal com-

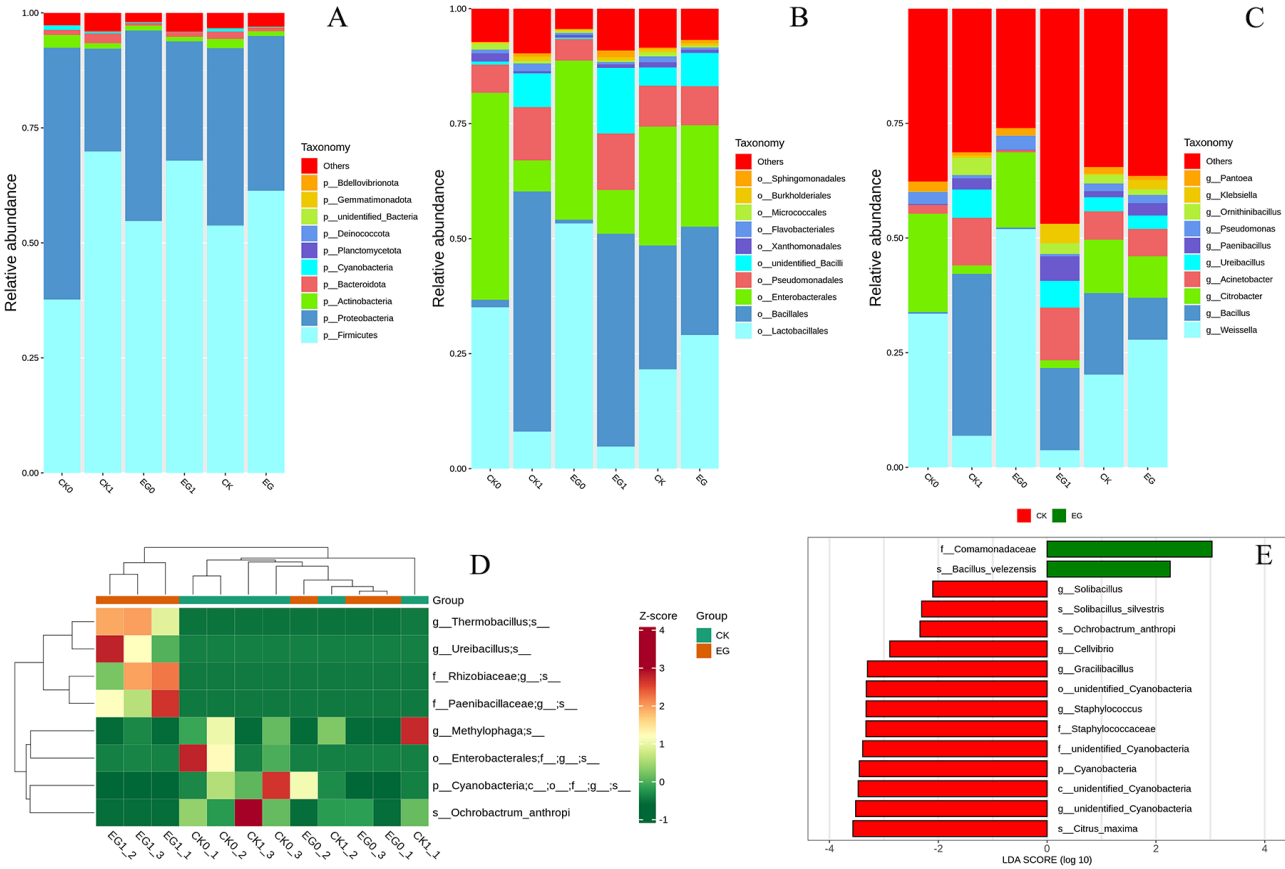


Fig. 5. Relative abundance and differential flora of bacteria across composting methods. A) Phylum-level composition of predominant bacterial communities; B) order-level composition of predominant bacterial communities; C) genus-level composition of predominant bacterial communities; D) correlation heatmaps between bacteria and different samples; E) LDA scores for microbial groups (determined by linear discriminant analysis). The experimental groups represented by CK and EG are the same as those in Fig. 1.

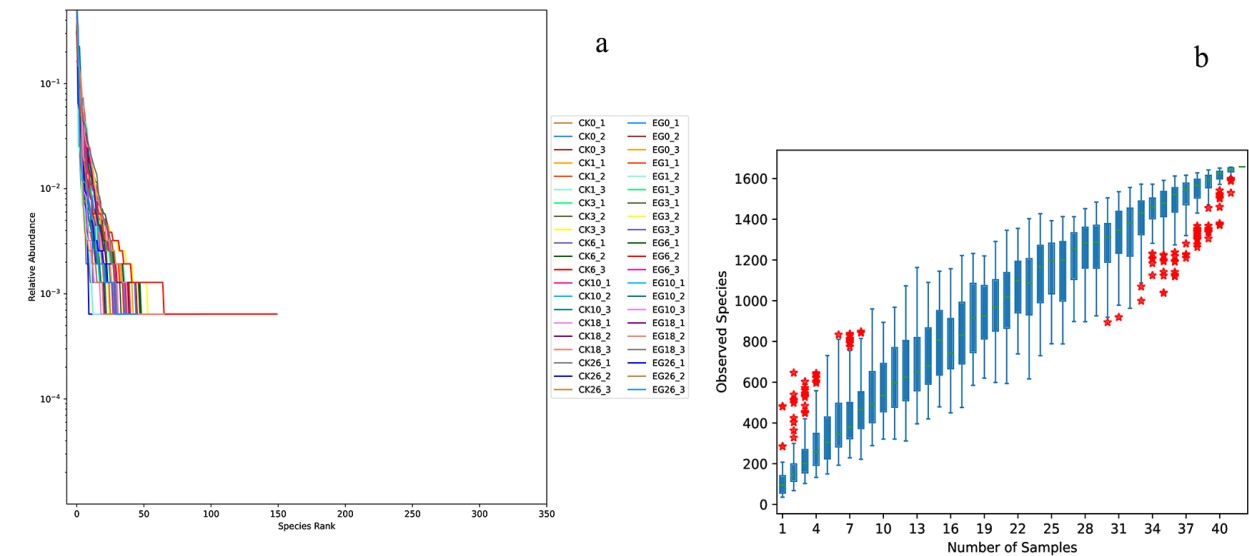


Fig. 6. Relative abundance curve and observed species curve. a) Relative Abundance curve; b) observed species curve.

ponent explains 73.97% of the variation, indicating a well-designed experimental setup. Samples clustered together on day 0, with the CK and EG groups forming distinct clusters as fermentation progressed. Fig. 4d also illustrates the unweighted pair-group method with arithmetic mean (UPGMA). On day 0, all samples showed similar clustering, and the CK and EG groups continued to exhibit similar clustering patterns throughout the fermentation process. Interestingly, no relevant data were detected on the second day following the initiation of fermentation.

Phylum-level bacteria diversity during organic fertilizer fermentation. Fig. 5A shows that the predominant bacterial phyla are Firmicutes, Proteobacteria, Actinobacteria, Bacteroidota, Cyanobacteria, Planctomycetota, Deinococcota, Gemmatimonadota, and Bdellovibrionota, with Firmicutes and Proteobacteria as the core microbiota.

As shown in Fig. 5B, the predominant bacterial orders are Sphingomonadales, Burkholderiales, Micrococcales, Flavobacteriales, Xanthomonadales, Bacilli, Pseudomonadales, Enterobacterales, Bacillales, and Lactobacillales.

Fig. 5C illustrates that the predominant bacterial genera are *Pantoea*, *Klebsiella*, *Ornithinibacillus*, *Pseudomonas*, *Paenibacillus*, *Ureibacillus*, *Acinetobacter*, *Citrobacter*, *Bacillus*, and *Weissella*.

Fig. 5D shows that the genera *Thermobacillus* and *Ureibacillus*, as well as the families *Rhizobiaceae* and *Paenibacillaceae*, exhibit higher correlations with experimental groups EG1_1, EG1_2, and EG1_3.

Fig. 5E highlights the differential microbial populations: the EG group features the family *Comamonad-*

aceae and the species *Bacillus velezensis*, the CK group includes *Solibacillus*, *Solibacillus silvestris*, *Ochrobactrum anthropi*, *Cellvibrio*, *Gracilibacillus*, *Cyanobacteria* (across order, family, phylum, and genus levels), *Staphylococcus*, the family *Staphylococcaceae*, and the species *Citrus maxima*.

Overall, the abundance of Firmicutes increased while that of Proteobacteria decreased with extended fermentation time in both the EG and CK groups. Notably, no bacteria were detected after the third day of fermentation.

Fungal diversity in different composting methods. Relative abundance curve and observed species curve. As shown in Fig. 6a, the horizontal coordinate widths of the Relative Abundance of each sample are similar. This suggests that the abundance of fungal species in each sample is similar. This may indicate the presence of a relatively abundant core fungal community in each group.

The observed species index indicates the number of species contained in the sample. As evident from Fig. 6b, the observed species curve flattens out, suggesting that the growth rate of species in the entire sample diminishes with an increase in sample size.

According to Fig. 7a, the first principal component explained 34.1% of the data variation, while the second principal component explained 22.97%. The samples of EG0, EG1, CK0, and CK1 are more dispersed, indicating that there are significant differences among the samples at the initial stage of fermentation. In contrast, Samples EG18, EG26, CK18, and CK26 are relatively concentrated in the Fig., indicating that the differences among the samples at the end of fermentation have decreased.

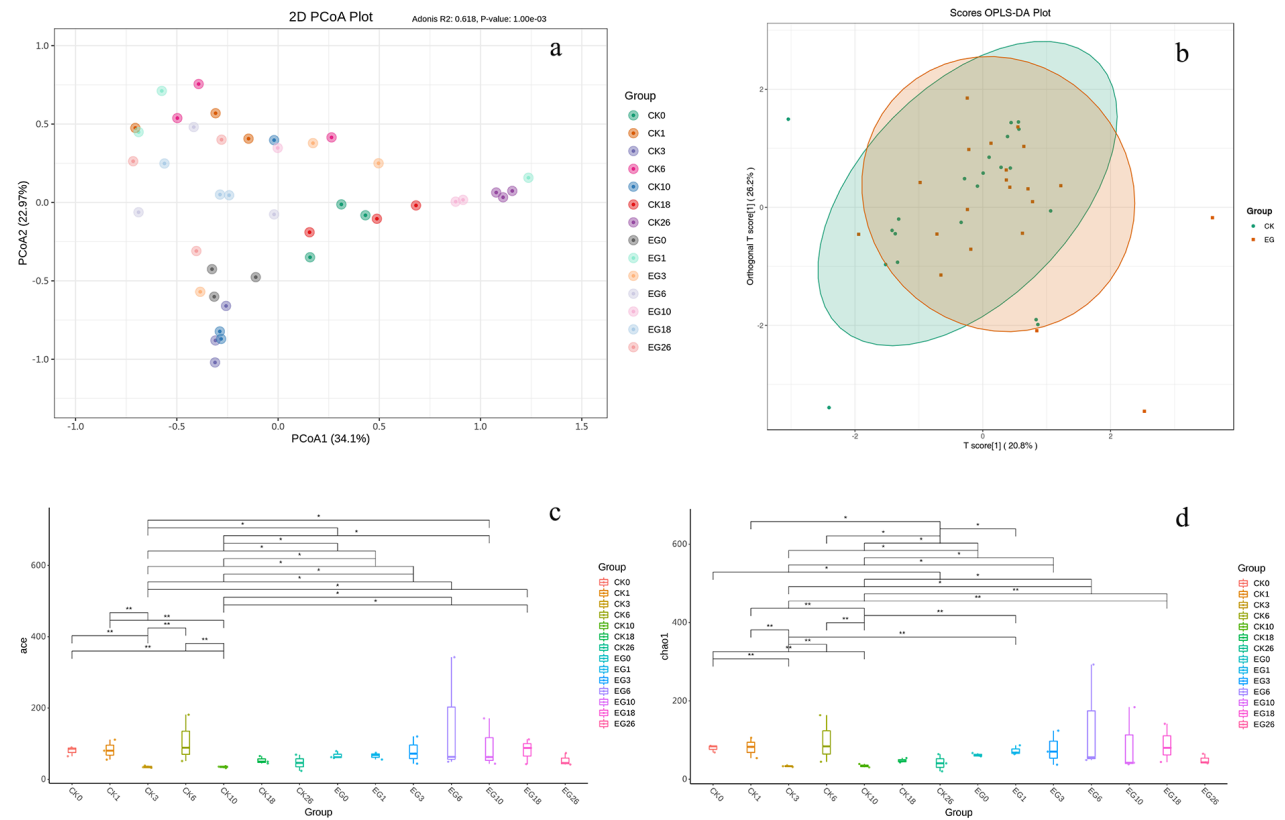


Fig. 7. Multivariate statistical analysis and alpha diversity index plots. a) Principal component analysis (PCA) plot; b) OPLS-DA score plot; c) ACE index box plot; d) Chao1 index box plot. The experimental groups represented by CK and EG are the same as those in Fig. 1.

Fig. 7b shows the score chart of OPLS-DA, where the first principal component accounts for 20.8% and the second principal component accounts for 26.2%. The clustering between different treatment groups was not obvious. Similar to Fig. 7a, the samples were relatively dispersed and overlapped between the two groups, which may explain the low principal component values.

The ACE bar chart in Fig. 7c shows that the estimated number of ASVs in the community changed significantly between groups as the fermentation process progressed.

The Chao1 bar chart in Fig. 7d shows that the trend of Chao1 data is similar to that of ACE. According to Fig. 7c and 7d, the differences between EG0 and CK0, EG1 and CK1, and EG26 and CK26 were not significant. This is consistent with the results of principal component analysis to some extent. However, the difference between EG0 and EG26 was significant, indicating that the fermentation process was normal and efficient. The difference between CK10 and EG10 was significant, indicating a significant difference between the experimental groups.

The bar charts in Fig. 8A illustrate the relative abundance of microbial communities at the phylum level across various treatment groups. In all treatment groups, the phylum Ascomycota is predominantly abundant, consistently occupying the largest proportion of the microbial community. This is followed by Basidiomycota and Zygomycota, which also show significant representation across the samples. The relative abundance of Chytridiomycota, Glomeromycota, and unclassified Fungi is considerably lower compared to the top three phyla.

In general, the core fungal groups in both the CK and EG groups were Ascomycota and Basidiomycota. However, there were significant differences in the core fungal composition between CK26 and EG26. The relative abundance of Ascomycota in CK26 was significantly higher than that in EG26, while the relative abundance of Basidiomycota in CK26 was significantly lower than that in EG26.

Fig. 8B illustrates the genus-level composition of microbial communities across different treatment groups. The relative abundance of various genera was quantified, revealing *Wallemia* as the most prevalent

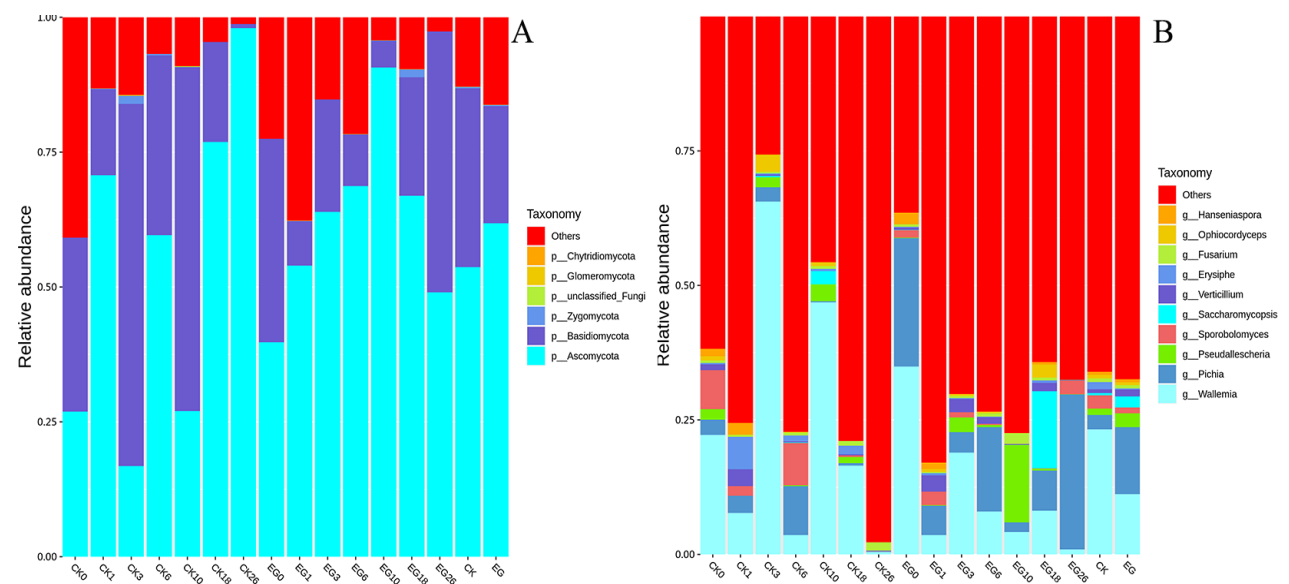


Fig. 8. Relative abundance and composition of fungi in organic fertilizers from different composting methods. A) Fungal phylum levels composed of different treatment groups; B) fungal genus levels composed of different treatment groups. The experimental groups represented by CK and EG are the same as those in Fig. 1.

genus, followed by *Pichia*, *Pseudallescheria*, *Sporobolomyces*, *Saccharomycopsis*, *Verticillium*, *Erysiphe*, *Fusarium*, and *Ophiocordyceps*.

A comparative analysis of the treatment groups, designated as CK (control) and EG (experimental), revealed significant differences in the relative abundance of certain genera. Specifically, *Walleimia* and *Sporobolomyces* exhibited higher relative abundances in the CK group than in the EG group. This suggests that the control conditions may be more conducive to the proliferation of these genera.

Conversely, the genera *Pichia* and *Saccharomycopsis* were found to have lower relative abundances in the CK group than in the EG group. This indicates that the experimental treatment may favor the growth and/or detection of these genera.

Fig. 9A Linear Discriminant Analysis (LDA) of Microbial Genera in CK and EG Groups. The genera *_Walleimia_* (*s_Walleimia_sebi* and *g_Walleimia*) and *_Erysiphaceae_* (*f_Erysiphaceae*) exhibit higher LDA scores in the CK group, indicating significantly higher relative abundance than in the EG group. Conversely, the genera *_Saccharomycetales_* (*o_Saccharomycetales*), *_Pichia_* (*f_Pichia*), and *_Ascomycota_* (*p_Ascomycota*) are more prevalent in the EG group.

As shown in Fig. 9B, there are differences in fungal flora in the CK group at different times. Distinct fungal communities were observed in CK0, CK1, CK3, CK6, and CK26. *Acremonium_* (*s_Acremonium_alteratum* and *g_Acremonium*) is prominent in CK0, while *_Basidiomycota_* (*p_Basidiomycota*) and *_Walleimia_*

(*o_Walleimia*) are more abundant in CK1. The EG group shows a distinct pattern, with *_Ophiocordyceps_* (*g_Ophiocordyceps*) and *_Verticillium_* (*g_Verticillium*) being particularly enriched.

Similar to Fig. 9B, Fig. 9C shows the difference in fungal flora in the EG group at different times. Distinct fungal communities were observed in EG0, EG1, EG3, EG18, and EG26. *Myxogaster_* (*f_Myxogaster*) and *_Aganicetes_* (*o_Aganicetes*) are significantly more abundant in EG0, while *_Eurotiales_* (*o_Eurotiales*) and *_Eurotiomycetes_* (*c_Eurotiomycetes*) are enriched in EG1. The EG18 and EG26 subgroups exhibit unique compositions, with *_Mortierellales_* (*g_Mortierellales*) and *_Schizophyllum_* (*g_Schizophyllum*) standing out, respectively.

As shown in Fig. 9D, a clear distinction is observed between the CK and EG groups, with the EG group showing a higher relative abundance of saprotrophs and pathotrophs, while the CK group is enriched with symbiotrophs. Pathotroph-Symbiotroph is positively correlated with EG10 and negatively correlated with CK10. Saprotroph-Symbiotroph (Pathotroph-Symbiotroph) and Pathotroph-Symbiotroph (Pathotroph) are positively correlated with EG18 and negatively correlated with CK18. Autotroph-Saprotroph-Symbiotroph (Saprotroph-Symbiotroph) was positively correlated with EG26 and negatively correlated with CK26. Saprotroph-Symbiotroph, Pathotroph-Symbiotroph, Symbiotroph, and Saprotroph are positively correlated with CK3 and negatively correlated with EG3. Pathotroph is negatively associated with CK3 and positively associated with EG3.

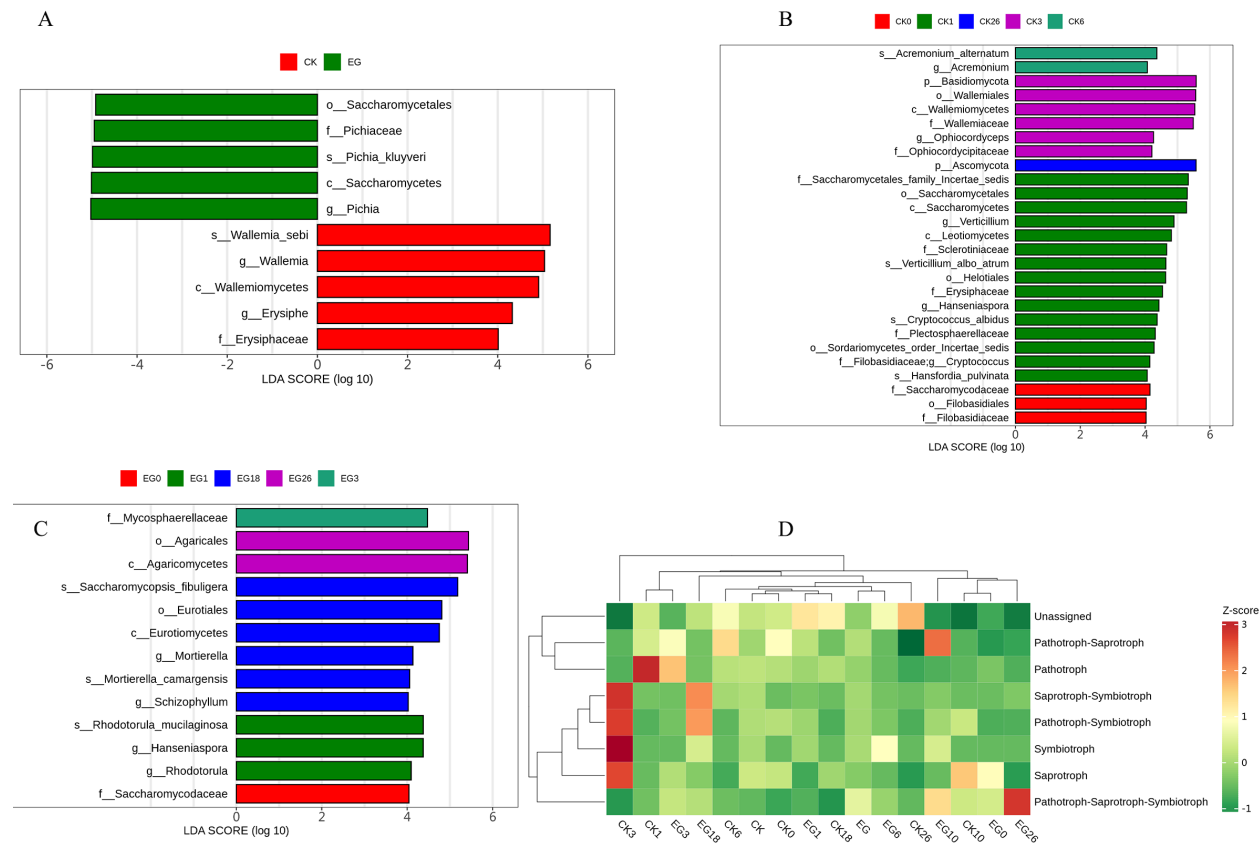


Fig. 9. Differently fungal flora and correlation in organic fertilizers from different composting methods. A) Linear discriminant analysis (LDA) of fungi in different test groups; B) LDA of fungi in CK group; C) LDA of fungi in EG group; D) Clustering heatmaps of fungal flora and different treatments. The experimental groups represented by CK and EG are the same as those in Fig. 1.

Discussion

In this study, we compared nanomembrane aerobic composting and conventional composting to analyze the nutrient composition and microbial diversity of tomato straw during fermentation. The results are consistent with previous studies in some aspects, and also reveal several new findings.

Firstly, the results showed that on the fifth day of fermentation, the temperature of the EG group exceeded 71°C, while that of the CK group was close to 68°C. The final moisture content of the EG group was significantly higher than that of the CK group. This may be attributed to the coating reducing heat loss. Water will continue to volatilize throughout the entire fermentation process (Castro-Alba et al. 2019). However, the moisture loss in the EG group is more minor, which may also be due to the film coating reducing water volatilization.

Secondly, during this fermentation process, the carbon (C) content decreases, while the nitrogen (N), potassium (K), and phosphorus (P) contents increase.

These changes are consistent with the elemental changes observed in organic fertilizer fermentation. The fluctuations in organic carbon and total nitrogen content may be related to the decomposition of organic matter and the mineralization process of nitrogen during the composting process. The increase in total phosphorus and total potassium content likely results from the release of nutrients from the straw during composting, along with other factors (Yaser et al. 2024).

When microorganisms decompose organic matter, they consume a large amount of carbon sources to obtain energy, resulting in reduced organic carbon content in organic fertilizer (Leconte et al. 2011). This phenomenon is commonly observed in the fermentation process of organic fertilizer. The test results show that the carbon (C) content of organic fertilizer is lower than that of raw materials, which conforms to the law of microbial decomposition of organic matter. Concurrently, the changes in carbon (C) and nitrogen (N) content observed by Raphael et al. in their organic fertilizer fermentation test were consistent with those in this experiment (Masunga et al. 2016). During fer-

mentation, microbial activity can release more potassium, making it available to plants (Rosolem et al. 2005). The potassium in fermented organic fertilizer is more easily absorbed (Li et al. 2020).

Notably, the decrease in moisture content throughout the composting process also significantly impacts the concentration of these elements. As composting progresses and moisture is lost, a concentrating effect occurs, elevating the relative concentrations of non-volatile elements. This effect is crucial for understanding the changes in nutrient content during composting.

Temperature is an important indicator for measuring the composting process, and the rapid temperature rise is a direct reflection of enhanced microbial metabolic activities (Yang et al. 2018; Wei et al. 2019). In this experiment, the nanomembrane treatment achieved higher temperatures at the initial stage of fermentation. This is consistent with the higher temperatures and longer duration in the high-temperature phase of aerobic composting reported in previous studies. The high-temperature phase facilitates the accelerated decomposition of organic matter and the inactivation of pathogenic microorganisms (Zucconi et al. 1981; Wang et al. 2022). Second, the two treatments we observed tended to converge in temperature towards the end, possibly related to the microbial community succession reported in the literature (Hartl et al. 2017). Although the microbial abundance varied throughout the fermentation process and even in the final fermentation of the two treatments, the species composition remained consistent. Regardless of the type of fermented organic fertilizer, its temperature will continue to approach ambient temperature once fermentation is terminated.

The composting process relies on microbes, which secrete enzymes that biochemically convert organic matter into stable humus (Liu et al. 2017; Zhang et al. 2024). However, it is noteworthy that bacterial data could not be detected on the second day after the start of fermentation in this study. This is inconsistent with many previous studies. Some researchers have detected bacteria throughout the fermentation process of organic fertilizers and reported changes in their diversity (Schloss et al. 2005; Fracchia et al. 2006; Partanen et al. 2010; Cao et al. 2019).

In the current study, the fermentation process exhibited rapid temperature increases, with temperatures reaching 61.88°C and 52.65°C on the second day, 71.09°C and 66.06°C on the third day, and peaking at 71.44°C and 67.95°C on the fourth day. Although the

temperatures subsequently declined, they remained elevated, with values of 61.67°C and 60.98°C recorded on the 13th day. Notably, the thermophilic stage (temperature > 50°C) persisted for over 15 days throughout the entire fermentation process. This prolonged thermophilic phase is indicative of the intense microbial activity and efficient degradation of organic matter, which are hallmarks of successful composting processes.

During the fermentation process, the thermophilic stage (temperature exceeding 50°C) lasted for more than 15 days. Such high temperatures pose significant challenges to the survival of most bacteria. Although previous studies have reported that certain thermophilic bacteria can maintain the stability of their cell membranes, nucleic acids, and other cellular structures under high-temperature conditions, and their proteins remain active at elevated temperatures (Pollo et al. 2015), these characteristics are generally absent in mesophilic bacteria. Temperature has a pronounced impact on microbial community succession and the changes in metabolic products during fermentation. In some fermentation processes, microbial diversity and abundance tend to decrease with increasing temperature (Pollo et al. 2015; Wang et al. 2022). These findings suggest that most bacteria are unable to survive under high-temperature conditions.

This might suggest that bacteria may not play a significant role in the fermentation process of organic fertilizers. Although this is not consistent with the results of past researchers. Considering the fermentation temperature, it is plausible that the prolonged high-temperature period may have caused a significant reduction in bacterial populations, resulting in DNA extraction levels below the detection threshold. The occurrence of sustained high temperatures is consistent with the patterns observed in the fermentation of organic fertilizers. The sharp decline in bacterial survival under prolonged high-temperature conditions aligns with the biological characteristics of bacteria. This is merely a speculation based on the results of this experiment. The absence of bacterial detection in the compost might also be due to other factors, such as the detection method.

Unlike bacteria, fungi were detected throughout the experiment. The composition of fungi changed significantly in different periods of the same treatment, as indicated by the Chao1 index. Additionally, the composition of fungi differed significantly between different treatments at the same time. This demonstrates that the fungal community is in a dynamic state

throughout the entire fermentation process, which is also supported by Fig. 8. Overall, the horizontal composition of mycophyla is the same between the CK and EG groups. However, the dynamic changes of fungi in the EG group were more controlled.

Pichia pastoris can rapidly degrade organic acids in organic fertilizers, thereby increasing the pH value (Nakasaki et al. 2013). The abundance of *Pichia* in the EG group was higher than that in the CK group. This was consistent with the higher pH in the EG group compared to the CK group. Meanwhile, changes in pH are closely related to soil microbial activity and organic acid consumption (Wu et al. 2014; Wu et al. 2020). Moreover, the degradation rate of organic fertilizer decreases when the pH is low in compost (Cheung et al. 2010).

The final alkaline pH of the organic fertilizer in this study is consistent with increasing soil pH and improving soil structure. This consistency suggests that tomato straw is a suitable material for organic fertilizer production.

Wallemia is a genus of fungi within the phylum Basidiomycota. This fungus belongs to a group of fungi that are highly tolerant (Zajc and Gunde-Cimerman 2018). *Wallemia* spp. is considered an important spoilage fungus (Pitt and Hocking 1987; Paterson and Lima 2017). *Wallemia* spp. is commonly found in high concentrations in air and dust in outdoor, indoor, and agricultural environments (Jančič et al. 2016; Díaz-Valderama et al. 2017). This indicates that *Wallemia* spp. can be sourced from a variety of environments (Zalar et al. 2005). Therefore, it is reasonable to find this fungus in the organic fertilizer raw materials used in this experiment. *Wallemia* spp. was twice as abundant in the CK group as in the EG group. *Wallemia* spp. is one of the most drought-tolerant fungal groups (Jančič et al. 2016; Zajc and Gunde-Cimerman 2018). This characteristic is consistent with the water content finally observed in this experiment.

The results indicate that the microbial community structure varies significantly across different treatment conditions. The dominance of Ascomycota and Basidiomycota at the phylum level, along with *Wallemia* and *Pichia* at the genus level, suggests that these taxa may be key players in the microbial ecology of the studied system. The fluctuation in the 'Others' category highlights the presence of a diverse and dynamic microbial community that may respond to changes in environmental conditions or treatment applications (Rastogi et al. 2020).

These findings provide valuable insights into the microbial community dynamics and could have implications for understanding the ecological roles of different microbial taxa in response to various treatments. Further studies are warranted to explore the functional significance of these communities and their potential applications in biotechnology or ecological management.

While this study provides valuable experimental results, it also has certain limitations. For example, there was no single composting treatment variable, and the experimental group was compared only to the traditional control group. At what point did the bacteria repopulate after the experiment?

Future studies should further explore the long-term effects of different compost parameters on organic fertilizer quality and microbial diversity (Wang et al. 2018; Qiao et al. 2021). And how to optimize the composting process to reduce gas emissions, improve soil quality, and enhance crop health (De Corato 2020; Xu et al. 2022). Additionally, considering the complexity of microbial communities in the composting process, it is essential to use metagenomics and other technologies to analyze the functional potential of microbial communities further (Hoang et al. 2022; Zhu et al. 2024).

Conclusions

Nanomembrane composting markedly improves both the efficiency and quality of organic-fertilizer production. Its rapid initial heating accelerates organic-matter decomposition and nutrient transformation. It retains more organic carbon and yields higher total N, P, and K than conventional composting. Microbial profiling showed that the composting method strongly shaped the abundance of key taxa. Nanomembrane aerobic composting promotes a more controlled succession of fungal communities, which may play a crucial role in the fermentation process.

It is noteworthy that during the composting process, bacteria gradually became undetectable as fermentation progressed, whereas fungi were consistently present throughout the experiment. This might suggest to the researchers that fungi, rather than bacteria, may play a more significant role in the fermentation process of organic fertilizers. This experiment provides a real-case reference for nanomembrane aerobic composting and the role of fungi in the fermentation process of organic fertilizers.



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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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