

Research article

Engineering the composting microbiome with a synthetic microbial community to accelerate lignocellulose degradation and humus synthesis



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ABSTRACT

Bioaugmentation with synthetic microbial communities (SynComs) presents a promising engineering strategy to overcome the bottleneck of lignocellulose recalcitrance in organic waste valorization. However, the mechanisms by which SynComs modulate indigenous microbial networks and steer metabolic fluxes remain elusive. Here, we deconstruct these mechanisms by investigating the impact of a rationally designed five-member bacterial-fungal SynCom on the co-composting of cattle manure and mulberry branches. Through an integrated multi-omics approach, we reveal that SynCom inoculation acts as a potent ecological engineer, accelerating the process by significantly elevating pile temperatures and shortening the maturation period by accelerating entry into the maturation phase by approximately 7 days. Compared with the control, the SynCom treatment enhanced the overall degradation rates of lignin, cellulose, and hemicellulose by 19.3 %, 7.9 %, and 12.0 %, respectively, and boosted humus content by 34.4 %. Metagenomics revealed that the SynCom profoundly restructured the native microbiome, enriching for key functional genera such as *Thermobifida* and *Actinomadura*. This engineered community possessed an enhanced genetic toolkit, with a significantly increased abundance of crucial carbohydrate-active enzymes (CAZymes), including cellulases (GH5, GH12), hemicellulases (CE1, CE3), and lignin-modifying auxiliary activity enzymes (AA1, AA6). Untargeted metabolomics further identified a distinct metabolic footprint in the SynCom treatment, characterized by the enrichment of key humification precursors like protocatechuic acid and sinapic acid. Integrated Procrustes and correlation analyses confirmed a tight coupling between the engineered microbiome, its functional gene repertoire and metabolic output. This study deciphers the multi-layered mechanism by which a designed SynCom enhances biowaste valorization and provides a mechanistic blueprint for engineering microbial consortia for advanced biotechnology applications in sustainable agriculture.

1. Introduction

The global imperative to transition from a linear to a circular bioeconomy has placed immense focus on developing sustainable technologies for waste valorization (Razza et al., 2018). The agricultural sector alone generates billions of tons of lignocellulosic residues annually, such as straws, stalks, and manures. These materials, if mismanaged, lead to environmental pollution, but they also represent a vast and largely untapped reservoir of renewable carbon (Sadh et al., 2018; Yu et al., 2022). Composting stands out as a robust, scalable, and cost-effective aerobic biotechnology to close this loop, transforming

organic waste into a stabilized, humus-rich soil amendment known as organic fertilizer (Huang et al., 2022; Liu et al., 2025). The application of organic fertilizer is critical for sustainable agriculture, as it improves soil structure, enhances water retention, provides essential nutrients, and contributes to long-term carbon sequestration, thereby mitigating climate change and improving food security (McNicol et al., 2020; Pergola et al., 2018; Wang et al., 2022).

Despite its widespread application, the engineering and optimization of composting face a formidable challenge, i.e., the inherent recalcitrance of the lignocellulose matrix (Chen et al., 2025). This complex biopolymer consists of crystalline cellulose microfibrils encased in a

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sheath of amorphous hemicellulose and a rigid, aromatic network of lignin, a structure that has evolved to resist enzymatic and microbial degradation (Hatfield and Fukushima, 2005; Terrett and Dupree, 2019). Consequently, conventional composting processes are often slow, requiring months to reach maturity, and result in incomplete degradation and a final product of variable quality (Wang et al., 2025). The efficiency of this biotransformation is entirely dependent on the metabolic capabilities of the indigenous microbial communities, which must work in synergy to secrete a vast arsenal of extracellular enzymes to deconstruct the complex substrate (Qin et al., 2025). However, this natural process is rarely optimized for the speed and efficiency required by industrial applications.

To overcome these limitations, bioaugmentation, i.e., the strategic introduction of functionally specialized microorganisms, has emerged as a promising engineering tool to steer the composting process (Li et al., 2024; Wang et al., 2025). Early approaches using single-strain inoculants showed promise, but often suffered from inconsistent performance due to poor survival and competition with robust native microbiomes (Feng et al., 2024). The field is now advancing towards the use of rationally designed synthetic microbial communities (SynComs), which leverage principles of microbial ecology such as metabolic complementarity and division of labor (Chen et al., 2025; Liu et al., 2025). By combining multiple strains with distinct, synergistic capabilities (e.g., cellulolytic, hemicellulolytic, and ligninolytic specialists), SynComs can achieve greater functional robustness and perform complex tasks more efficiently than individual strains (Qian et al., 2020). However, despite the clear potential, the application of SynComs in complex environmental systems like compost remains largely empirical. While previous studies have demonstrated the efficacy of SynComs in improving composting (Chen et al., 2025; Liu et al., 2025), they often treat the native microbiome as a black box. The specific ecological and molecular mechanisms by which an introduced SynCom integrates with, and subsequently re-engineers, the native microbial ecosystem to enhance overall process performance remain poorly understood (Ma et al., 2023). Unraveling this knowledge gap is essential for moving from trial-and-error to predictable, model-driven engineering of microbial processes.

While the individual use of metagenomics or metabolomics in composting research is growing (Liu et al., 2023; Ma et al., 2023; Zhang et al., 2025b), the integration of these approaches to build a comprehensive, multi-layered model explaining how a bioaugmentation strategy works is still nascent. Our study's novelty lies in using this integrated multi-omics framework to connect the engineered inoculum to the cascading changes in community structure (metagenomics), functional gene potential (CAZymes), and the resulting chemical phenotype (metabolomics), thereby providing a complete mechanistic pathway from microbe to metabolic output. The integration of multiple omics technologies, particularly metagenomics and metabolomics, offers an unprecedented opportunity to deconstruct these complex biological systems (Liu et al., 2023; Ma et al., 2023; Zhang et al., 2025b). Metagenomics provides a census of the community members and their collective genetic potential, including a comprehensive profile of carbohydrate-active enzymes (CAZymes) (Chen et al., 2025). Concurrently, untargeted metabolomics captures a snapshot of the system's chemical phenotype, identifying the small-molecule intermediates and products that constitute the metabolic currency of the ecosystem (Wang et al., 2024b). In this study, we deployed a rationally designed five-member bacterial-fungal SynCom in a pilot-scale composting system. We hypothesized that the SynCom would act as an ecological engineer, initiating a cascade of events that (1) reshapes the indigenous microbial network by creating strong selective pressures, (2) enhances the collective genetic toolkit for lignocellulose degradation, and (3) steers metabolic pathways towards accelerated humification. By integrating time-series multi-omics data, we aimed to construct a comprehensive, mechanism-based model that explains how a rationally designed SynCom can be used to engineer and optimize a complex

environmental biotechnology process.

2. Materials and methods

2.1. SynCom assembly and composting setup

The composting experiment was performed at Huanjiang Observation and Research Station for Karst Ecosystems, Chinese Academy of Sciences. The feedstock was prepared by mixing cattle manure and air-dried, pulverized mulberry branches at a dry weight ratio of 8:2. The SynCom consisted of five previously isolated and characterized strains: three bacteria (*Bacillus cereus*, *Achromobacter* spp., *Pseudomonas* sp.) and two fungi (*Cladosporium* sp. and *Trichoderma harzianum*) (Chen et al., 2025). While simpler consortia have shown promise, our five-member design was rationally constructed to ensure a high degree of functional complementarity and redundancy, which is critical for robustness in a complex, non-sterile environment. This SynCom combines potent cellulase/hemicellulase producers (*Bacillus*, *Trichoderma*), a known lignin-modifier (*Achromobacter*), and versatile degraders (*Cladosporium*, *Pseudomonas*) to create a synergistic consortium capable of attacking all components of the lignocellulose matrix simultaneously. These strains were selected based on their proven high individual performance in plate-based assays for cellulase, xylanase, and laccase activity, as well as their robust growth at varying temperatures and their compatibility in co-culture, indicating synergistic potential for lignocellulose degradation. The bacterial and fungal strains were cultured separately in Luria-Bertani (LB) and Potato Dextrose Broth (PDB) (HuanKai Microbial, Guangzhou, China), respectively, to the late logarithmic phase, harvested by centrifugation, and resuspended in sterile phosphate-buffered saline (PBS) to a concentration of 1×10^{10} cells mL⁻¹. The final SynCom inoculum was prepared by mixing equal proportions of each strain suspension (Chen et al., 2025).

Two treatments were established in trapezoidal piles (1.5 m length × 1.5 m base width × 1.2 m height), each with six replicates arranged in a randomized block design: (1) Control: composting with 400 kg of cattle manure and 100 kg of mulberry branches (dry weight basis); and (2) SynCom treatment (SynCom): composting with 400 kg of cattle manure and 100 kg of mulberry branches inoculated with the SynCom inoculum to achieve a final density of approximately 1×10^8 cells g⁻¹ of dry compost material. The initial moisture content was adjusted to 55–60 % using tap water, and the C/N ratio was 25–28. The detailed initial physicochemical properties of the feedstock are described in a companion paper (Chen et al., 2025) and in Table S1.

2.2. Sampling and physicochemical analysis

Temperature was recorded daily at five different points (center, top, bottom, and two sides at mid-depth) in each pile using a long-stem digital thermometer. Composite samples were collected at days 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60. The piles were turned manually every three days to ensure aeration. To prevent cross-contamination, the Control and SynCom treatment blocks were physically separated by a 2-m buffer zone. All tools used for turning and sampling were thoroughly cleaned between treatments. Each composite sample consisted of approximately 500 g of material obtained by mixing subsamples from the five temperature-monitoring locations. Samples were divided into two portions: one was air-dried for physicochemical analysis, and the other was immediately stored at –80 °C for molecular analyses.

The pH and electrical conductivity (EC) were determined in a 1:10 (w/v, dry weight basis) aqueous extract using a SevenExcellence S470 pH/Conductivity meter (Mettler Toledo, Switzerland). Total organic carbon (TOC) and total nitrogen (TN) were measured using a Various MAX cube (Elementar, Germany). The germination index (GI) was assessed to evaluate phytotoxicity using Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) seeds according to the method of Li et al. (2024).

2.3. Lignocellulose and humic substance quantification

The concentrations of hemicellulose, cellulose, and lignin were quantified using the sequential detergent fractionation method of Van Soest with a Fibretherm FT12 automated fiber analyzer (C. Gerhardt, Germany) (Chen et al., 2025). The samples were mixed with 0.1 M Na₄P₂O₇·10H₂O and 0.1 M NaOH in a ratio of 1:10 to extract humic substances (HS), and followed by acidification with 6 M HCl to separate humic acid (HA, precipitate) from fulvic acid (FA, supernatant) (Pan et al., 2021). The carbon content in the humic substances, humic acid, and fulvic acid fractions was quantified using a Shimadzu TOC-vwp analyzer (Kyoto, Japan). The degree of polymerization was calculated as the humic acid/fulvic acid ratio (Wu et al., 2023).

2.4. Metagenomic DNA extraction, sequencing, and bioinformatic analysis

Total genomic DNA was extracted from 0.1 g (wet weight) of frozen compost samples from the initial, mesophilic, thermophilic, and maturation phases (6 replicates per phase per treatment) using the Mag-Bind® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocol. DNA integrity and quantity were verified using 1 % agarose gel electrophoresis and a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). High-quality DNA was used to construct paired-end libraries, which were sequenced on an Illumina NovaSeq 6000 platform (PE150) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China), generating approximately 10 Gb of data per sample.

Raw sequencing reads were subjected to quality control using Fastp (v0.20.0) to remove adapters and low-quality reads (Phred score <20, length <50 bp). The high-quality reads were assembled into contigs using MEGAHIT (v1.1.2) with a length ≥300 bp. Open reading frames (ORFs) were predicted from contigs ≥500 bp using Prodigal (v2.6.3). The predicted protein sequences were clustered at 95 % identity to construct a non-redundant gene catalog using CD-HIT (v4.6.8). Gene abundance was calculated by mapping high-quality reads back to the catalog using Salmon (v0.14.1). Taxonomic annotation was assigned to genes by aligning them against the NCBI NR database (release 2022-01) using DIAMOND (v0.8.3.5). Functional annotation of CAZymes was performed by searching the protein sequences against the CAZy database (v11) using dbCAN2 (e-value < 1e⁻⁵) (Chen et al., 2025).

2.5. Metabolite profiling

Metabolites were extracted from 10.0 g of frozen samples from the maturation phase (6 replicates per treatment). Briefly, samples were homogenized in a pre-chilled 80 % methanol solution, sonicated at 40 kHz for 30 min at 5 °C, and centrifuged at 13,000 g for 15 min at 4 °C (Ma et al., 2023). The supernatant was collected and lyophilized for LC-MS/MS analysis.

The analysis was performed on a liquid chromatograph system (Agilent, 7890, California, USA) and a mass spectrometer (Thermo Fisher, Pegasus HT time-of-flight, Hudson, USA) according to Liu et al. (2023). Chromatographic separation was achieved on a Waters ACQUITY UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 µm). The mobile phase consisted of 0.1 % formic acid in water (A) and acetonitrile (B). The mass spectrometer was operated in both positive and negative ion modes. Raw data were processed using Progenesis QI (Waters Corporation, Milford, USA) for peak picking, alignment, and normalization. Metabolites were identified by matching the accurate m/z values and fragmentation patterns against public databases, including the KEGG (Kyoto Encyclopedia of Genes and Genomes) and Metlin databases (<https://metlin.scripps.edu/>).

2.6. Statistical analysis

All statistical analyses and visualizations were performed in R (v4.4.2). Significant differences between the two treatments for physicochemical variables, lignocellulose content, and humic fractions were determined using Student's t-tests. Microbial alpha diversity (Chao1, Shannon) and beta diversity (NMDS on Bray-Curtis dissimilarity) were calculated using the 'vegan' package. Bubble plots of species abundance were shown with 'reshape2' and 'ggplot2' packages. LEfSe (Linear discriminant analysis Effect Size) was used to identify statistically significant biomarker taxa with an LDA score threshold of 2.0 ($p < 0.05$). Random Forest analysis ('rfPermute' package) was used to identify the most important microbial genera predicting lignocellulose degradation and humus formation. For metabolomics data, Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) was performed to visualize group separation. Differential metabolites were identified based on a Variable Importance in Projection (VIP) score >1.0 and an FDR-corrected p-value (q -value) < 0.05. For all analyses involving multiple comparisons (e.g., differential metabolites and CAZyme families), p -values were adjusted using the Benjamini-Hochberg false discovery rate (FDR) correction, and a corrected p -value (q -value) < 0.05 was considered significant. Correlation heatmaps were performed to examine the correlations between humic content, physicochemical variables, microbial genera, and metabolites by using the 'psych' package and 'Complex Heatmap' package. Procrustes analysis, which served to evaluate the correlation between the microbial community composition (NMDS ordination) and the metabolite profiles (OPLS-DA ordination), as well as KEGG enrichment analysis, were both performed on the free online platform of Majorbio Cloud Platform (www.majorbio.com).

3. Results and discussion

3.1. SynCom inoculation intensifies thermal dynamics and accelerates compost maturation

The temperature profile of a compost pile is a direct proxy for the intensity of microbial metabolic activity and serves as a primary indicator of process efficiency (Bernal et al., 2009; Zhu et al., 2021b). In our study, the SynCom inoculation acted as a potent biostimulant, dramatically altering the thermal dynamics (Fig. S1a). Both treatments rapidly entered the thermophilic phase (>50 °C), a critical stage for pathogen inactivation and organic matter decomposition, by day 4 (Ma et al., 2023). However, the SynCom treatment demonstrated a significantly more vigorous process, reaching a higher temperature peak (71.7 °C vs. 70.9 °C in the control) one day earlier. More importantly, the SynCom treated pile maintained a significantly higher temperature ($p < 0.05$) for the majority of the thermophilic phase (days 6–25). This sustained period of intense heat generation is indicative of accelerated and more complete oxidation of organic matter, directly attributable to the enhanced metabolic output of the SynCom-engineered microbiome (Finore et al., 2023; Sun et al., 2021). This intensification of the thermophilic phase is a key engineering objective, as it directly correlates with more effective sanitization and faster degradation rates of recalcitrant materials (Bernal et al., 2009). The subsequent rapid cooling phase in the SynCom treatment further suggests a quicker depletion of readily available substrates, signaling a more rapid progression towards maturation and a shorter overall operational time, which has significant economic implications for industrial-scale composting facilities (Ansar et al., 2025; Peng et al., 2022). Based on the temperature profile, the high-heat thermophilic phase (>50 °C) was shortened by approximately 7 days in the SynCom treatment (Fig. S1a). Furthermore, the SynCom treatment achieved a germination index (GI) of 174.4 % by the end of the thermophilic phase, far exceeding the maturity benchmark of 80 % (Li et al., 2024), while the control group was only 131.1 % (Fig. S1e). This indicates a significant acceleration of the maturation process, shortening the effective composting time.

This accelerated transformation was mirrored in the key physicochemical indicators of compost maturity (Fig. S1b–e). The control group maintained significantly higher pH during both the initial and thermophilic phases (Fig. S1b), likely due to faster ammonification; conversely, during the maturation phase, the SynCom treatment exhibited a significantly higher pH, possibly reflecting a more advanced stage of humification and stabilization where organic acid consumption surpasses production (Ren et al., 2025). The electrical conductivity of both treatment groups gradually increased (Fig. S1c), likely due to the release of small organic molecules containing soluble ions from the macromolecular organic matter (Zhu et al., 2021a). The C/N ratio, a critical metric for compost stability, decreased more sharply in the SynCom treatment, reaching a final value of 12.9 (Fig. S1d), well below the typical threshold of 20 for mature compost and significantly lower than the control's 13.4 (Li et al., 2024; Zhu et al., 2021b). This reflects more efficient carbon mineralization relative to nitrogen immobilization, preventing nitrogen limitation for subsequent soil applications (Huang et al., 2022). Perhaps most compellingly, the germination index, a crucial measure of phytotoxicity, provides unequivocal evidence of superior product quality (Cui et al., 2024). The final germination index of the SynCom treated compost reached 181.5 %, significantly higher than the control (130.1 %) (Fig. S1e), and far exceeding the 80 % standard for safe agricultural application (Li et al., 2024). This demonstrates that the SynCom-driven process not only accelerated decomposition, but also more effectively eliminated phytotoxic intermediate compounds, such as certain organic acids and phenols, which are often generated during the initial stages of decomposition (Cui et al., 2024; Ren et al., 2025). Germination index was significantly positively correlated with humus components (Fig. S1f, $p < 0.05$), suggesting that the higher germination index reflects a completer and more balanced metabolic network within the engineered community, capable of efficiently turning over potentially inhibitory compounds.

3.2. SynCom drives superior lignocellulose valorization and humus synthesis

The primary engineering goal of bioaugmentation in this context is to overcome the recalcitrance of lignocellulose. The SynCom inoculation achieved this with remarkable efficiency (Fig. 1). Throughout the thermophilic and maturation phases, the residual contents of lignin, cellulose, and hemicellulose were significantly lower in the SynCom treatment (Fig. 1a–c). By the end of the process, the overall degradation rates for these components in the SynCom treatment were 38.9 %, 50.5 %, and 45.2 %, respectively, representing a 19.2 %, 7.9 %, and 12.0 % improvement over the control (Fig. 1d). This superior degradative capacity confirms that the introduced SynCom, selected for its lignocellulolytic potential, successfully established a highly effective functional niche, likely through both its own enzymatic contributions and the stimulation of synergistic native degraders (Chen et al., 2025; Xing et al., 2024). Such enhanced degradation is a critical step in unlocking the chemical energy and carbon stored within agricultural waste for bioconversion.

The accelerated breakdown of lignocellulose provides a larger pool of chemical precursors for humification, a process that improves soil physiochemical and biological properties (Yu et al., 2019). Consequently, the SynCom treatment showed a significantly greater accumulation of total humic substances, reaching 120.0 mg g^{-1} , a 34.4 % increase over the control (89.3 mg g^{-1}) (Fig. 1e). The true measure of humification quality, however, lies in the composition of these substances. Humic acid represents the most stable, high-molecular-weight, and agronomically valuable fraction of humus, while fulvic acid is a more transient, lower-molecular-weight fraction (Guo et al., 2019). The humic acid content in the SynCom treatment increased by a remarkable 169.6 %, far outpacing the 123.5 % increase in the control (Fig. 1f). This was reflected in the degree of polymerization, a key indicator of humus maturity (Zhang et al., 2018). The degree of polymerization in the

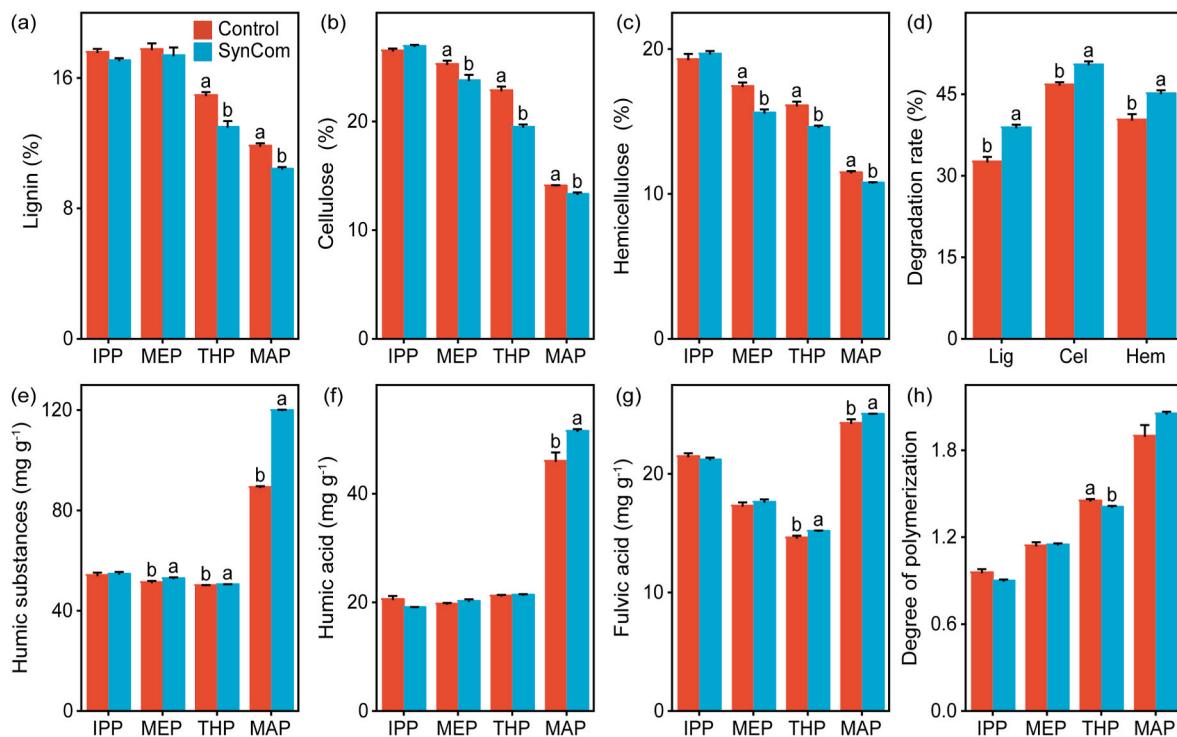


Fig. 1. SynCom inoculation accelerates lignocellulose degradation and enhances humus synthesis. Absolute contents (% of the whole composting substrate) of residual (a) lignin (Lig), (b) cellulose (Cel), and (c) hemicellulose (Hem). (d) Final degradation rates of lignocellulose components. (e) Humic substances (HS), (f) Humic acid (HA), and (g) Fulvic acid (FA) contents. (h) Degree of polymerization (DP), calculated as the HA/FA ratio. IPP, MEP, THP, and MAP represent the initial, mesophilic, thermophilic, and maturation phases, respectively. Different lowercase letters indicate significant differences between Control and SynCom treatments within the same phase ($p < 0.05$).

SynCom treatment rose to 2.1, higher than the control's 1.9 (Fig. 1h), indicating that the SynCom-engineered process not only produced more humus but actively promoted the polymerization of simpler fulvic acid into more complex, stable humic acid. This transformation is highly

desirable, as higher humic acid content is linked to improved soil water retention, nutrient chelation, and long-term carbon sequestration (Li et al., 2025; Zhou et al., 2022). In summary, the SynCom inoculation accelerated lignocellulose degradation, which in turn enhanced the

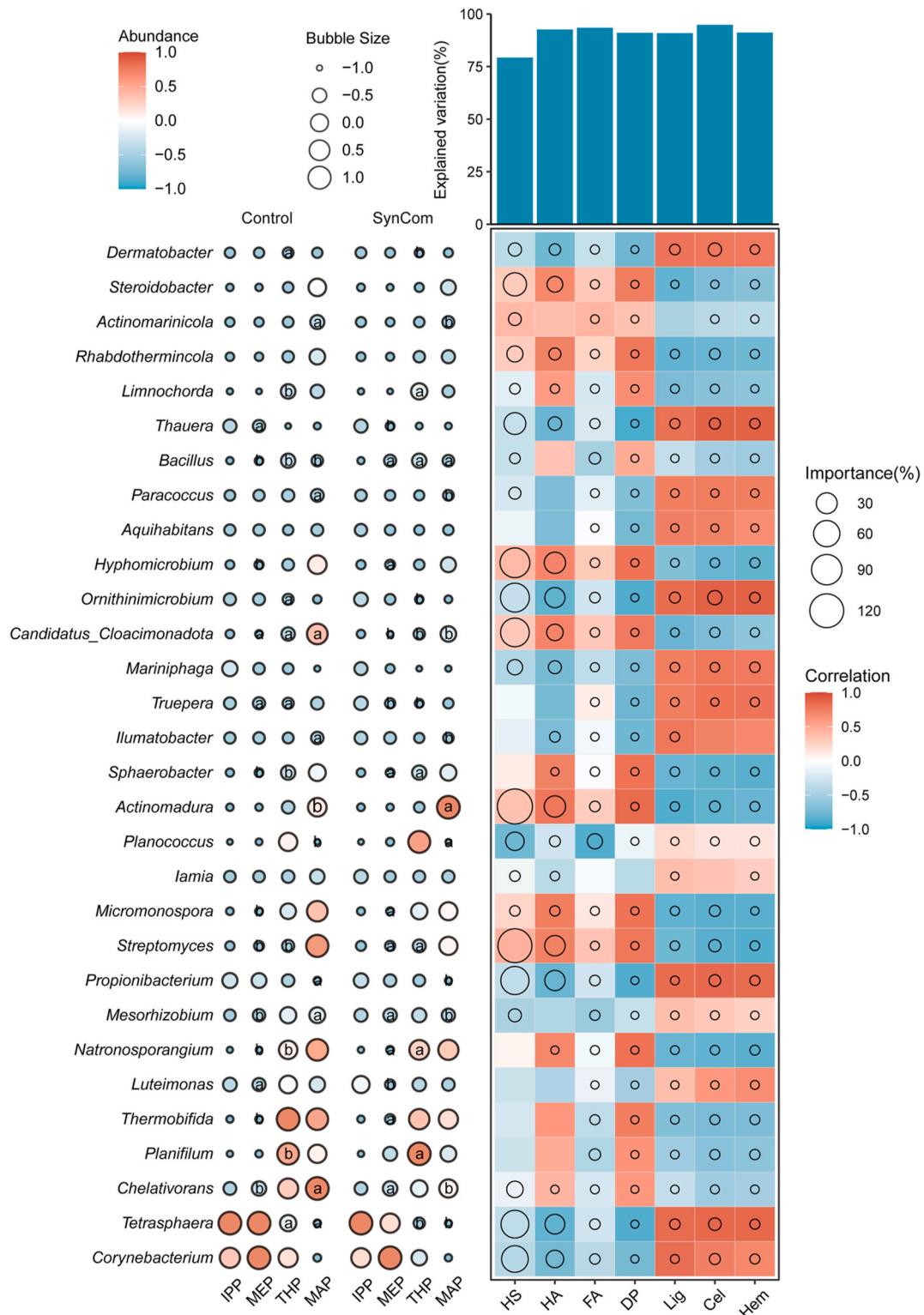


Fig. 2. Keystone microbial genera identified by Random Forest modeling are correlated with lignocellulose degradation and humification. The bubble chart (left) shows the relative abundance of the top 30 genera across the four composting phases; circle size corresponds to abundance, while color indicates the Spearman correlation with composting day. The heatmap (right) displays the importance of each genus in the Random Forest model (circle size) and its Spearman correlation with final compost properties (color). HS, humic substances; HA, humic acid; FA, fulvic acid; DP, degree of polymerization; Lig, lignin; Cel, cellulose; Hem, hemicellulose. Different lowercase letters indicate significant differences between Control and SynCom treatments within the same phase ($p < 0.05$).

accumulation and maturity of humic substances, thereby boosting the value of the final compost product.

3.3. SynCom as an ecological engineer reshaping the composting microbiome

To uncover the biological drivers of the enhanced performance, we tracked the microbial community dynamics. The SynCom inoculation acted as a powerful ecological engineering force, substantially altering the community's structure and diversity trajectory (Fig. S2). During the

intense thermophilic phase, both species richness (Chao1) and diversity (Shannon) were significantly suppressed in the SynCom treatment (Fig. S2a and b). This is not a negative outcome; rather, it reflects strong “cause-and-effect” loop. The SynCom inoculation itself, through its rapid metabolic activity, is the driver for the accelerated carbon decomposition and the resulting higher temperatures (Fig. S1a). This self-created thermal environment then acts as a strong selective filter, which filters for a highly specialized and efficient thermophilic consortium, eliminating less adapted competitors (Gu et al., 2017; Zhu et al., 2021a). The NMDS analysis confirmed this profound shift, showing a distinct and

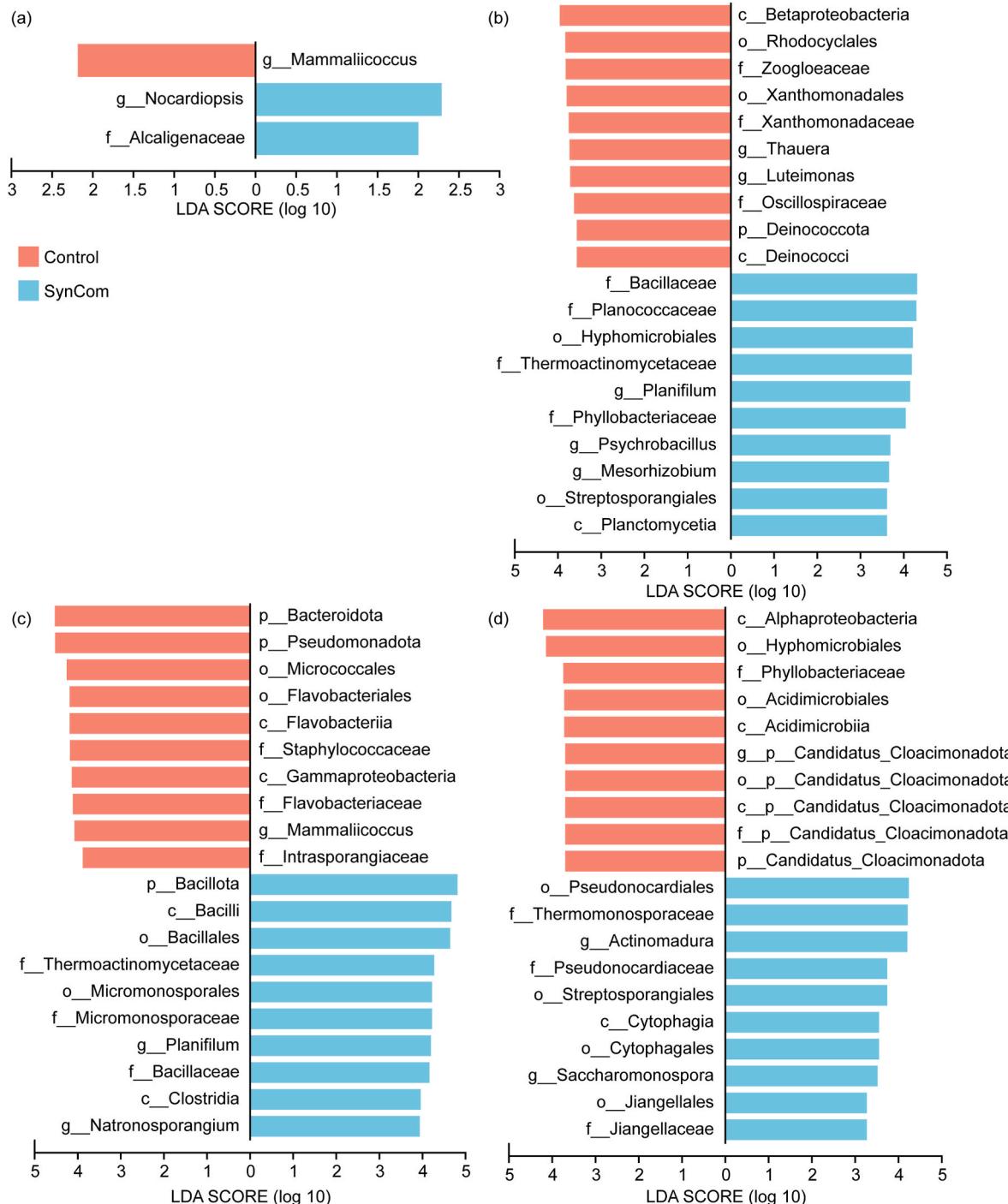


Fig. 3. LEfSe analysis identifies key bacterial biomarkers distinguishing the SynCom-engineered microbiome. The plots show differentially abundant taxa (biomarkers) between Control and SynCom treatments at the (a) Initial, (b) Mesophilic, (c) Thermophilic, and (d) Maturation phases. Only taxa with an LDA score ≥ 2.0 ($p < 0.05$) are shown. The length of the bar represents the effect size.

permanent divergence of the SynCom community trajectory from the control, particularly in the thermophilic and maturation phases (Fig. S2c). Taxonomic analysis revealed the specific nature of this engineered community. While the dominant phyla (Actinobacteriota, Pseudomonadota, Bacillota) were consistent with other composting studies (Fig. S2d) (Guo et al., 2021), the community composition at the genus level was dramatically different.

To further explore the succession of microbial communities, the abundances of the top 30 genera were shown in Fig. 2. During the thermophilic stage of the SynCom treatment, the relative abundance of genera such as *Planifilum*, *Streptomyces*, *Sphaerobacter*, and *Bacillus* was significantly higher compared to the control group. As composting advanced into the maturation stage, *Actinomadura*, *Bacillus*, and *Planococcus* exhibited significantly higher relative abundances than the control. To investigate the role of major genera in lignocellulose degradation and humus synthesis, we conducted random forest models and correlation analyses. These analyses revealed that *Actinomadura* and *Streptomyces* are significant contributors to humic substances and humic acid, though their impact on fulvic acid is relatively minor. Other studies have also noted the role of these genera in promoting humus synthesis (Xing et al., 2024). The key genera involved in fulvic acid formation were *Actinomadura* and *Actinomarinicola*. Notably, *Streptomyces*, *Actinomadura*, *Micromonospora*, and other genera exhibited significant negative correlations with residual lignocellulose and were highly predictive of its degradation. The Random Forest modeling, used to link taxonomy directly to function, identified *Streptomyces*, *Actinomadura*, *Sphaerobacter*, and *Actinomarinicola* as the most critical microbial genera driving the degradation of lignocellulose and the synthesis of humic acid and humic substances (Fig. 2). The significantly higher abundance of these keystone taxa in the SynCom treatment provides a direct biological explanation for its superior performance. This evidence strongly supports our hypothesis that the SynCom acted as an ecological engineer, creating environmental conditions and competitive interactions that selectively favored the proliferation of the most functionally important native microorganisms, thereby optimizing the entire community's metabolic output (Meng et al., 2021; Wang et al., 2025; Zhang et al., 2025b).

LEfSe analysis identified key bacterial biomarkers that distinguished the treatments (Fig. 3, Fig. S3). During the thermophilic phase, the SynCom treatment was overwhelmingly enriched with members of the phylum Bacillota, particularly the class *Bacilli* and order *Bacillales*. This is highly significant, as this group contains many well-known thermophilic species that are prolific producers of cellulases and other hydrolases (Wang et al., 2024a). As the compost matured, the SynCom treatment became significantly enriched in taxa renowned for their ability to degrade complex polymers, such as the family *Thermomonosporaceae* and the genus *Actinomadura* (Kroppenstedt and Goodfellow, 2006; Li et al., 2024). Both *Thermomonosporaceae* and *Actinomadura* are well-known Actinobacteriota, famous for their role as "late-stage" decomposers. They produce a wide array of extracellular enzymes, including peroxidases and laccases, that are crucial for attacking the complex aromatic structure of lignin, as well as cellulases and hemicellulases to degrade the shielded polysaccharides. Their enrichment in the SynCom treatment, particularly in the maturation phase (Figs. 2 and 3d), is a strong indicator of a more advanced and thorough degradation of the most recalcitrant lignocellulosic components. The enrichment of these specific taxa, which are often considered late-stage decomposers, suggests the SynCom accelerated the entire ecological succession, allowing the community to reach a more mature functional state more quickly (Meng et al., 2022). These results demonstrate that SynCom inoculation reshaped the bacterial community structure and enriched functional taxa, thereby facilitating the degradation and transformation of organic constituents during composting.

It should be pointed that this study employed a whole-community metagenomic approach, which captures the collective functional potential of the entire microbiome but does not easily distinguish between

the inoculants and native strains of the same genera (e.g., *Bacillus*). As suggested by our data (Figs. 2 and 3), the SynCom appears to function in two ways: by contributing its own enzymatic capabilities and, perhaps more significantly, by acting as an ecological engineer that creates selective pressures (e.g., higher temperature) to recruit and enrich highly efficient native specialists like *Actinomadura* and *Thermobifida*. Future studies using strain-specific tracking methods, such as qPCR or mapping to specific reference genomes, would be valuable to disentangle these two mechanisms further.

A limitation of the current metagenomic analysis is its focus on the bacterial community, while the fungal community dynamics were not explicitly detailed. This is a particularly relevant point as our SynCom included two fungal strains (*Cladosporium sp.* and *T. harzianum*), both selected for their potent lignocellulolytic capabilities. *T. harzianum* is a well-known and aggressive producer of cellulases and hemicellulases. It is highly probable that the *T. harzianum* inoculum played a critical role in the initial, rapid deconstruction of cellulose and hemicellulose, contributing to the fast temperature rise and "pre-processing" the substrate for the later-stage bacterial specialists. The *Cladosporium sp.* was likely included for its lignin-modifying abilities. While our analysis did not track their persistence, their functional contribution is a key component of the SynCom's success. Future studies incorporating fungal-specific analyses, such as ITS sequencing or metatranscriptomics, would be valuable to fully elucidate the synergistic bacterial-fungal interactions within the engineered community.

3.4. Functional metagenomics reveals an increased enzymatic toolkit

To translate the observed taxonomic shifts into functional mechanisms, we analyzed the community's genetic potential for carbohydrate degradation using the CAZyme database. The metagenomic data revealed that the SynCom-engineered community was equipped with a significantly more potent enzymatic arsenal for attacking lignocellulose (Fig. 4). At the class level, polysaccharide lyases (PLs: 1.2–1.7 %) and carbohydrate-binding modules (CBMs: 1.7–1.9 %) exhibited the lowest abundance. The higher abundance of glycoside hydrolases (GHs: 32.2–34.2 %), glycosyl transferases (GTs: 32.9–35.2 %), carbohydrate esterases (CEs: 18.5–20.4 %) and auxiliary activities (AAs: 9.4–11.2 %) may be attributed to their direct involvement in the degradation of lignocellulosic compounds or the re-polymerization of degradation products (Zhang et al., 2025a). The SynCom treatment showed a higher abundance of CEs during the thermophilic phase, enzymes essential for removing acetyl and other groups from hemicellulose, thereby increasing its accessibility to other hydrolases (Fig. 4a) (Armendáriz-Ruiz et al., 2018). This de-shielding step is often a rate-limiting step for the complete degradation of hemicellulase and, by extension, the overall deconstruction of the entire lignocellulose matrix. Its enhancement is a key indicator of improved degradation efficiency and a prerequisite for the action of downstream enzymes (Chen et al., 2025).

A detailed analysis of specific CAZyme families provided even clearer insights. The metagenome for the SynCom treatment was significantly enriched in genes encoding AA enzymes, including those from families AA1, AA4, AA6, AA7, and AA10, which are essential for lignocellulose degradation (Ma et al., 2022). As shown in Fig. 4b, these enzymes facilitated the hydrolysis and oxidation of cellulose and lignin. AA10 enzymes, classified as lytic polysaccharide monooxygenases (LPMOs), catalyze the oxidative cleavage of β-1,4-glycosidic bonds in cellulose, enhancing the efficiency of traditional hydrolytic enzymes (Lu et al., 2024). Increased LPMO activity was observed in the presence of lignin-derived electron donors, indicating a synergy between lignin and cellulose degradation. AA1 enzymes, including laccases and ferroxidases, are involved in lignin degradation, while AA4 enzymes catalyze the transformation of phenolic compounds in the aromatic ring. AA7 enzymes oxidize sugar residues at the reducing ends of oligosaccharides, contributing to the degradation of both cellulose and hemicellulose

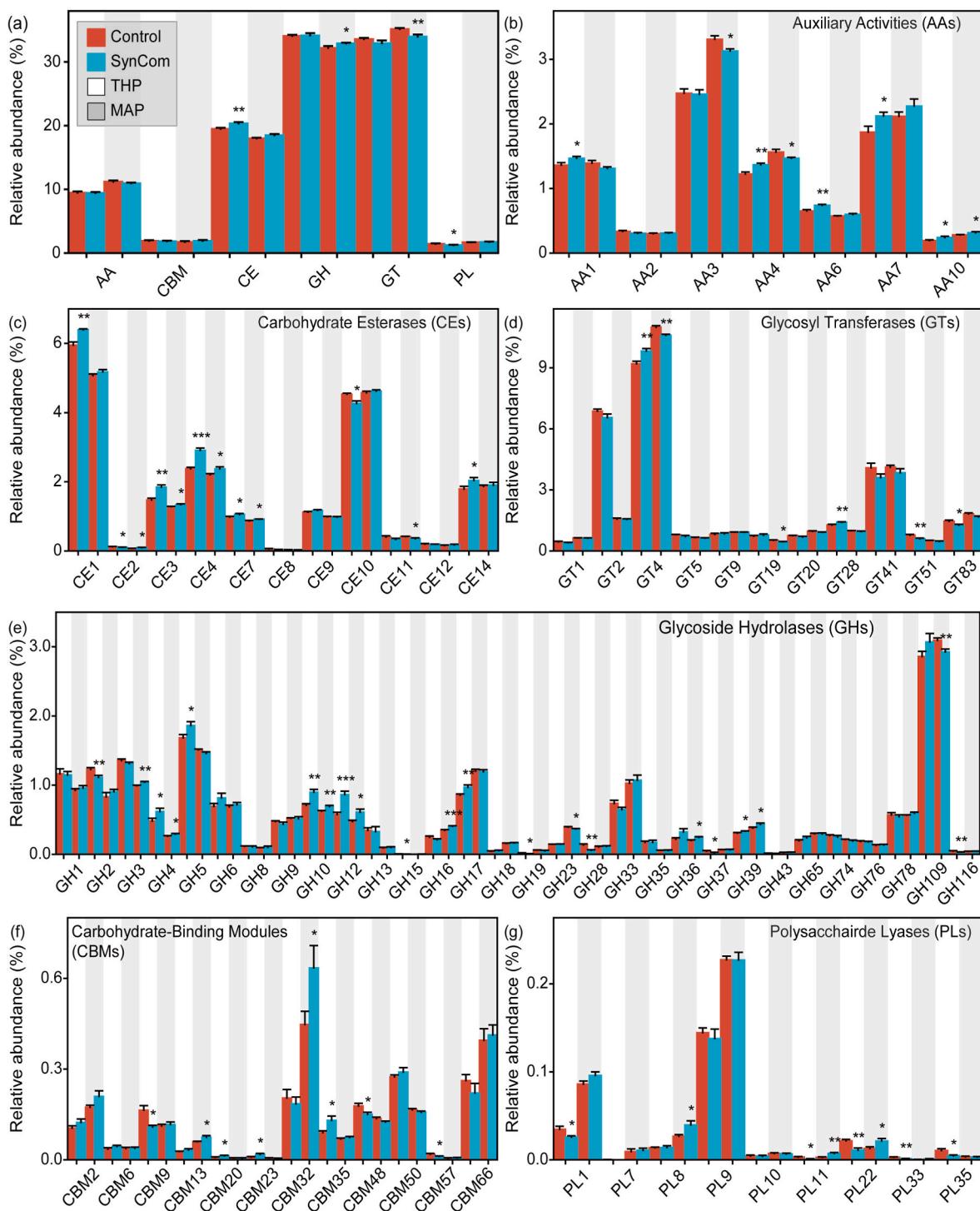


Fig. 4. Relative abundance of CAZyme genes identified in the metagenomes from the thermophilic (THP) and maturation (MAP) phases. (a) Abundance at the class level. (b-g) Abundance of specific families within (b) Auxiliary Activities (AAs), (c) Carbohydrate Esterases (CEs), (d) Glycosyl Transferases (GTs), (e) Glycoside Hydrolases (GHS), (f) Carbohydrate-Binding Modules (CBMs), and (g) Polysaccharide Lyases (PLs). Asterisks indicate significant differences between treatments within the same phase (* $q < 0.05$, ** $q < 0.01$, *** $q < 0.001$).

(Meng et al., 2021). The coordination of these oxidative AA enzymes with glycoside hydrolases (GHS) represents a key mechanism in efficient lignocellulose deconstruction. The SynCom treatment effectively upregulated this synergistic system, which was further supported by significant upregulation of key cellulases (e.g., GH5, GH12) and hemicellulases (e.g., CE1, CE4, CE7) (Fig. 4c-e), enhancing lignocellulose degradation. The coordinated upregulation of this entire suite of synergistic enzymes, both oxidative and hydrolytic, provides a clear

molecular mechanism for the enhanced lignocellulose degradation rates observed in the SynCom treatment. The SynCom effectively programmed the community to express a more diverse and efficient ‘enzymatic toolkit’, perfectly tailored to deconstruct the specific feedstock (Chen et al., 2025; Lu et al., 2024).

3.5. Metabolomics deciphers the chemical pathways to enhanced humification

While metagenomics reveals the functional potential, metabolomics unveils the real-time chemical reality of the system. Our analysis of the metabolite pool during the maturation phase, the peak period of humification, captured the distinct chemical signature of the SynCom-engineered process. OPLS-DA analysis showed a clear and statistically significant separation between the SynCom and control groups, confirming a fundamentally altered metabolic landscape (Fig. S4a). We identified 328 metabolites with differential abundance (Fold change >1 or fold change <-1 , VIP >1 , $q < 0.05$), categorized into 9 classes, including lipids and lipid-like molecules, organic acids and derivatives, organoheterocyclic compounds, organic oxygen compounds, benzeneoids, nucleosides, nucleotides, and analogues, phenylpropanoids and polyketides, organic nitrogen compounds, and alkaloids and derivatives. Among these, 188 metabolites were significantly upregulated in the SynCom treatment (Fig. S4b, Supplementary dataset). A total of 276 differential metabolites were annotated into seven primary metabolic pathways for cellular processes (CP), drug development (DD), environmental information processing (EP), genetic information processing (GP), human diseases (HD), metabolism (ME), and organismal systems (OS), with metabolite numbers of 3, 1, 22, 2, 21, 187, and 40, respectively (Fig. S4c). A total of 135 secondary pathways were identified, of which 90 were metabolic pathways (Fig. S4d).

A substantial portion of these upregulated metabolites are known precursors and intermediates in the humification process (Fig. 5). Of the 49 key metabolites analyzed, 28 were upregulated and 21 were downregulated (Supplementary dataset). The 28 upregulated metabolites include phenolic compounds, quinoline compounds, aromatic amino acid compounds, and lipid compounds, etc. The SynCom treatment showed a significant accumulation of lignin-derived phenolic compounds such as protocatechuic acid, sinapic acid, and coniferaldehyde. These aromatic molecules are the primary building blocks in the phenol-protein theory of humus formation, where they undergo microbial or enzymatic oxidation and polymerization to form the stable aromatic core of humic acid (Fuchs et al., 2011; Qi et al., 2022). A strong, positive correlation between these specific precursors and the final concentrations of humic substances and humic acid confirmed their direct role in the enhanced humification observed in the SynCom treatment (Fig. 5b, $p < 0.05$). This provides the missing chemical link, connecting accelerated lignocellulose degradation to increased humus synthesis (Liu et al., 2023).

Microorganisms depolymerize lignocellulose by initiating microbial metabolism to form humus precursors, which then polymerize to form humic substances (Yang et al., 2023). Procrustes analysis revealed a strong, significant synergistic relationship between the microbiome and metabolome profiles (Fig. S5, $M^2 = 0.68$, $p = 0.01$), indicating that the community structure was tightly coupled to its metabolic output. Most importantly, protocatechuic acid, sinapic acid, and coniferaldehyde were closely associated with the genera *Bacillus*, *Planococcus*, and *Actinomadura* (Fig. 5c, $p < 0.05$), suggesting that changes in metabolism made a significant contribution to humus accumulation. The preferential formation of high-molecular-weight humic acid over fulvic acid is likely not just a consequence of higher precursor availability, but also a result of the enhanced oxidative enzyme profile. The significant enrichment of laccase-encoding genes (AA1) in the SynCom-engineered microbiome likely created a more oxidative environment, catalyzing the polymerization of phenolic precursors into more complex and stable humic acid structures. In maize straw and canola residue composting, inoculation with *Phanerochaete chrysosporium* enriched fungal genera associated with lignocellulolytic enzyme production and elevated the levels of carbohydrates, amines, glycine, and aromatic metabolites (Wang et al., 2024b). Similarly, inoculating the SynCom in bagasse and cow manure co-composting up-regulated glycoside hydrolase genes, boosted carbohydrate and amino-acid metabolism, and promoted

humification (Lu et al., 2024).

KEGG pathway enrichment analysis provided a systems-level view of this metabolic reprogramming (Fig. S6, Supplementary data). Pathways related to the degradation of aromatic compounds (e.g., benzoate degradation, polycyclic aromatic hydrocarbon degradation, and bisphenol degradation), biosynthesis of phenylpropanoids, and the metabolism of aromatic amino acids (e.g., tyrosine, tryptophan), which can also serve as phenolic precursors, were significantly enriched in the SynCom treatment. This indicates that the engineered microbiome was not only more effective at liberating phenolic monomers from lignin, but was also actively funneling them, along with other key intermediates, into polymerization reactions (Liu et al., 2023; Wei et al., 2023). The enrichment of pathways for amino and nucleotide sugar metabolism further supports this, as these compounds are known to be incorporated into humic structures via Maillard reactions or enzymatic polymerization, increasing their nitrogen content and stability, which is crucial for the long-term agronomic value of the compost (Guo et al., 2019; Zhou et al., 2022). This metabolic reprogramming is the ultimate manifestation of the upstream alterations in microbial community and genetic function (Yang et al., 2023).

3.6. An integrated multi-omics model of engineered composting

By systematically integrating the multi-layered datasets, we can move beyond simple correlations to construct a cohesive, mechanistic model that explains how a rationally designed SynCom engineers a composting ecosystem for superior performance (Fig. 6). The model follows a clear causal chain. First, the SynCom acts as both a catalyst and a niche constructor. Its initial metabolic activity rapidly modifies the local environment, most notably by accelerating the entry into and intensification of the thermophilic phase. Second, the act of niche construction creates a strong selective filter, fundamentally altering the trajectory of microbial succession to favor the enrichment of highly adapted, indigenous thermophilic specialists, such as the potent lignocellulose-degrading genera *Bacillus*, *Thermobifida*, and *Actinomadura*. Third, this newly assembled, high-performance consortium is equipped with a significantly enhanced and functionally diverse genetic toolkit. The coordinated expression of powerful oxidative enzymes like LPMOs (e.g., AA10) and laccases (e.g., AA1), alongside a suite of complementary hydrolases, enables a synergistic and highly efficient attack on the recalcitrant lignocellulose matrix. Fourth, this enhanced degradation releases a large flux of monomeric sugars and, crucially, aromatic compounds, leading to a system-wide metabolic rewiring. The community's metabolic network is consequently rewired to funnel these molecules, particularly key phenolic acids like protocatechuic acid, into pathways that serve as direct precursors for humus synthesis. The intense microbial activity also modulates local abiotic conditions, such as the significantly higher temperature, creating an environment that favors the subsequent biotic (enzymatic) and abiotic polymerization of these precursors. Ultimately, the resulting high concentration of these building blocks drives their transformation into complex, stable humic substances, leading to a faster, more efficient composting process that yields a final product with a significantly higher content of agronomically valuable humic acid.

4. Conclusions

This study successfully demonstrates the power of a SynCom-based bioaugmentation strategy to engineer and optimize the complex process of lignocellulosic waste composting. By employing an integrated multi-omics approach, we constructed a detailed, multi-layered mechanistic model. Our findings reveal that the rationally designed SynCom acts as a potent ecological engineer, initiating a cascade of events that includes the targeted restructuring of the native microbiome, the functional enhancement of the community's enzymatic machinery, and the precise steering of metabolic pathways. This orchestrated process results

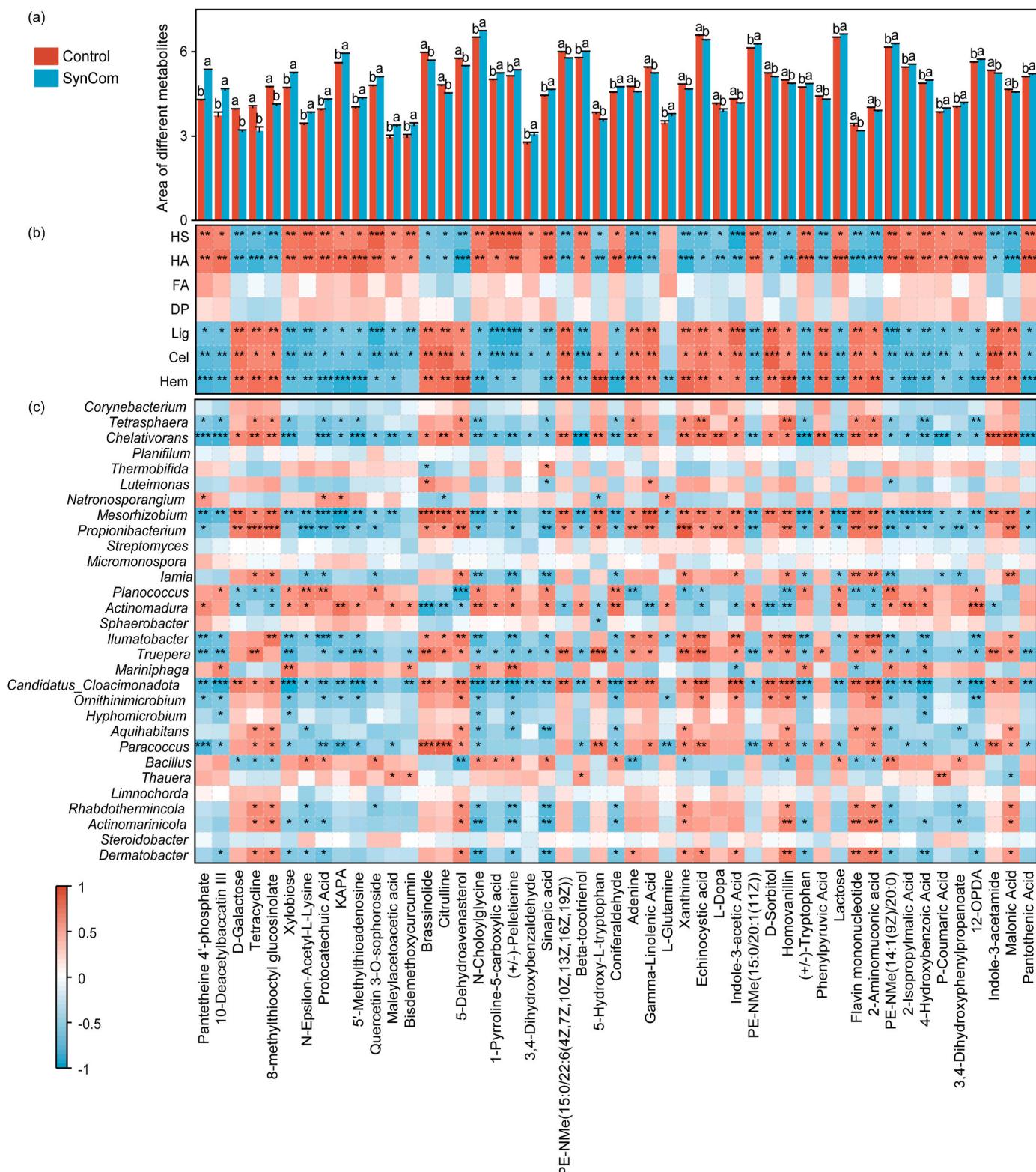


Fig. 5. The SynCom-engineered metabolome is enriched in humification precursors. (a) Relative abundance of key differential metabolites identified during the maturation phase. (b) Spearman correlation heatmap showing the relationship between these metabolites and the final content of humic and lignocellulose fractions. (c) Spearman correlation heatmap showing the relationship between key metabolites and the top 30 most abundant microbial genera. HS, humic substances; HA, humic acid; FA, fulvic acid; DP, degree of polymerization; Lig, lignin; Cel, cellulose; Hem, hemicellulose. Different lowercase letters in panel (a) indicate significant differences between treatments ($p < 0.05$). Asterisks on the heatmaps indicate the significance of the correlation (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

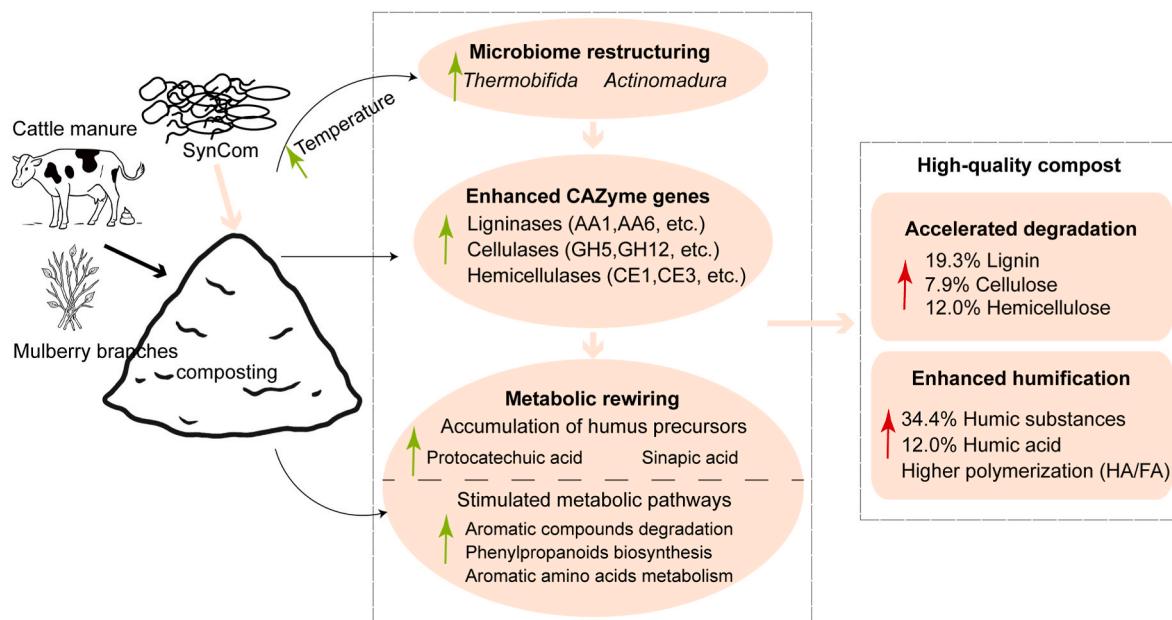


Fig. 6. An integrated, multi-level model of SynCom-engineered composting. The schematic illustrates how the initial SynCom inoculation acts as an ecological engineer, initiating a cascade that (1) modifies the physical environment (temperature), (2) restructures the native microbiome by enriching for keystone taxa, (3) upregulates a synergistic CAZyme genes, (4) rewires metabolic pathways to accumulate humus precursors, and ultimately (5) promotes the formation of stable, high-quality humus through coupled biotic and abiotic processes.

in accelerated lignocellulose degradation and the enhanced synthesis of stable, high-value humic substances. The mechanistic insights and the integrated model presented here provide a robust scientific foundation for the future design of next-generation microbial consortia for a wide range of applications in environmental engineering and the circular bioeconomy.

CRediT authorship contribution statement

Shuangshuang Chen: Writing – original draft, Methodology, Investigation. **Qiumei Liu:** Writing – review & editing, Methodology. **Dejun Li:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing 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.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2025.128088>.

Data availability

Data will be made available on request.

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