

ORIGINAL RESEARCH

Open Access



Manganese-modified biochar enhances white-rot fungi-mediated lignocellulose humification: insights from molecular transformation and multi-omics analyses

Feng Wang¹, Linjie Zhang¹, Feng Zhou¹, Wenyue Wang¹, Yinglong Su¹, Dongmei Zhou², Seungkwan Hong³, Min Zhan^{3*} and Bing Xie^{1*}

Abstract

Humic substance formation is essential for soil health, yet natural humification is slow, and synthetic production is often inefficient. White-rot fungi (WRF)-mediated lignocellulose humification shows potential but is limited by slow substrate degradation. Given the high biocatalytic potential of Mn(II) and its compatibility with the enzyme system of WRF, manganese-modified biochar (MBC) was utilized to enhance the degradation and conversion efficiency of lignocellulose. A 35-day solid-state fermentation experiment showed that 5% MBC addition increased lignin degradation by 93.4%, humic acid content by 47.7%, and polymerization degree by 112.4% compared with CK. Mechanistically, first, MBC enhanced extracellular lignocellulose-degrading enzymes and Mn-associated Fenton-like oxidation, thereby improving lignocellulose depolymerization. Second, interpretable machine learning and mass difference-based association analysis identified key molecular reactivity traits, suggesting that MBC facilitated humification by promoting the selective generation and conversion of reactive precursor molecules. In particular, enhanced DOM reactivity and increased precursor formation through deamination, dealkylation, and delignification were associated with the generation of more aromatic, oxygen-functionalized, and unsaturated humic-like substances, likely through polyphenol-dominated pathways. Finally, transcriptomic and metabolomic analyses indicated that MBC upregulated the expression of CAZyme-related genes and metabolic pathways associated with precursor generation, including phenolic/quinone biosynthesis, indicating that enhanced substrate depolymerization and subsequent precursor transformation jointly contributed to lignocellulose humification. Overall, this study provides mechanistic insights into how MBC enhances WRF-mediated lignocellulose humification and offers a theoretical basis for optimizing the bioconversion of lignocellulose-rich organic wastes.

Highlights

- MBC enhanced *P. chrysosporium*-mediated lignocellulose humification.
- FT-ICR MS and interpretable machine learning revealed key DOM traits linked to humification.

*Correspondence:

Min Zhan
pobabyzhan@korea.ac.kr
Bing Xie
bxie@des.ecnu.edu.cn

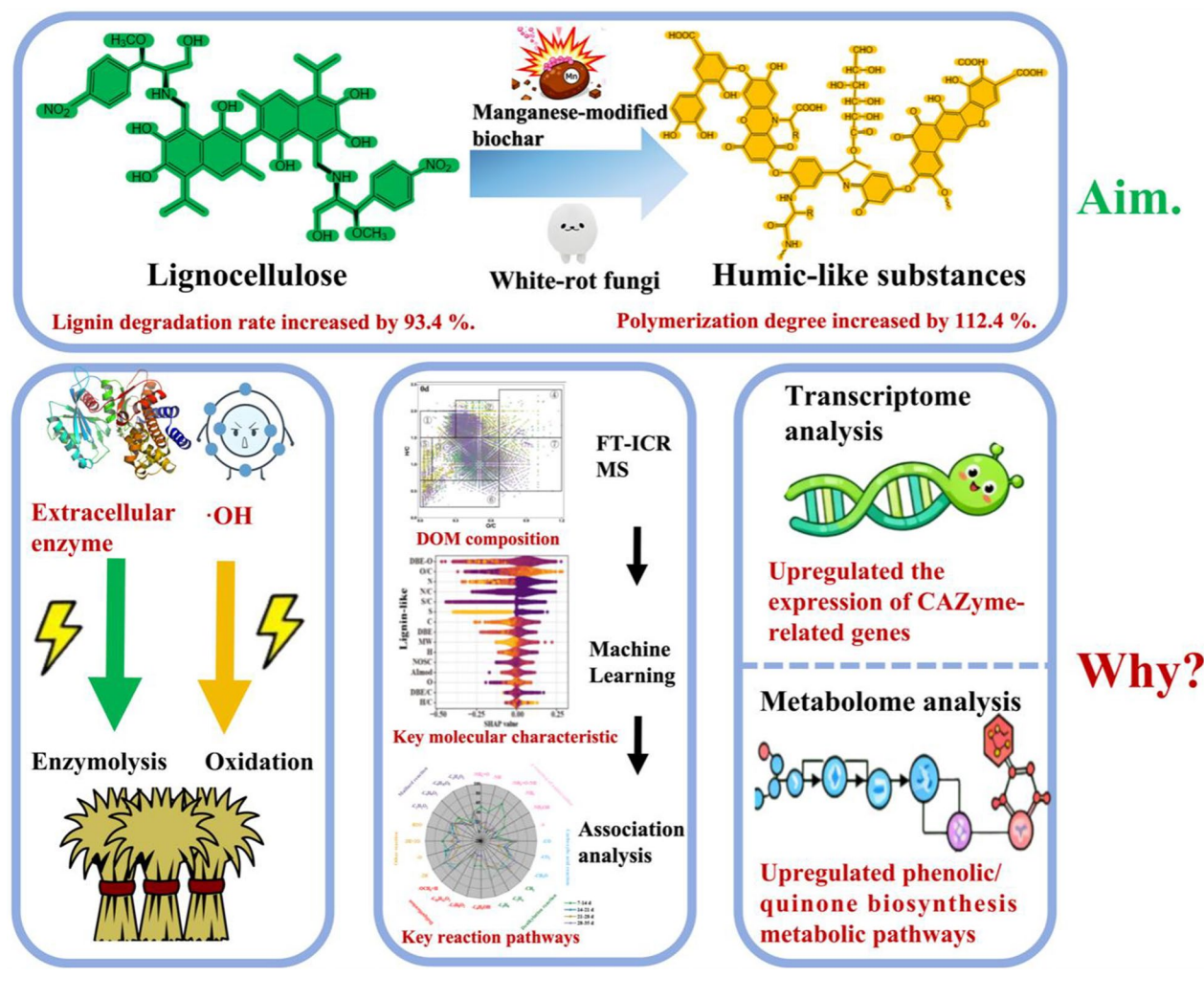
Full list of author information is available at the end of the article

© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

- This study provides new mechanistic insights into how MBC promotes white-rot fungi-mediated lignocellulose humification.

Keywords White-rot fungi, Lignocellulose, Manganese-modified biochar, Molecular transformation, Polyphenol humification pathway

Graphical Abstract



1 Introduction

Humic substances (HS) play critical roles in agricultural ecosystems by immobilizing pollutants, improving soil properties, and regulating carbon balance (Sun et al. 2024). However, prolonged overuse of fertilizers in intensive agriculture has reduced soil carbon stability, decreasing total carbon storage and disrupting HS composition, which leads to soil degradation and pollution accumulation (Svensson et al. 2022). Exogenous HS inputs are

urgently needed to address these issues. Unfortunately, the slow formation of natural HS and inefficient extraction make commercial HS products insufficient to meet market demand (Lee et al. 2019). Due to structural similarities between lignin and HS, along with abundant lignocellulose reserves, artificial humification of lignin offers a promising solution. This process involves oxidative degradation of lignin molecules and repolymerization of lignin-derived compounds. However, the

recalcitrance of lignocellulose hinders conversion efficiency and remains a significant economic challenge (Himmel et al. 2007).

Artificial humification of lignocellulose is achieved through two main pathways: microbial fermentation and physicochemical methods. Abiotic techniques (e.g., hydrothermal treatment, advanced oxidation) enable rapid humification but face challenges such as high energy use and release of inhibitory byproducts (Zhu et al. 2024). In contrast, biological processes operate under mild conditions with greater sustainability, despite being slower (Sun et al. 2024). White-rot fungi (WRF) are particularly promising due to their specialized enzymatic systems (e.g., manganese peroxidase, lignin peroxidase, laccase), which facilitate both lignocellulose breakdown and humic-like substance synthesis more reliably and economically than exogenous enzyme approaches (Benavides et al. 2025). However, their practical application remains limited due to: (1) significant fluctuations in ligninolytic activity and limited substrate decomposition efficiency during bioreactor operation, and (2) complexity in enzymatic regulation mechanisms, involving the dynamic balance of intracellular and extracellular enzyme systems sensitive to culture conditions, as well as redox-mediated metabolite production and transformation (Benavides et al. 2025; Mir-Tutusa et al. 2018). Therefore, the directional optimization of the culture system and metabolic regulation strategy will become a key breakthrough to release the biotransformation potential of WRF.

Significant research has been directed toward enhancing microbial activity and function through an exogenous additive strategy to accelerate humic-like substance formation. These additives can significantly improve the stability and rate of lignin degradation by regulating microbial transcription factors, inducing target enzyme synthesis, and enhancing electron transfer efficiency between extracellular enzymes and substrates (Liu et al. 2023c). However, cost-effective and environmentally benign additives must be selected for broad application. Biochar, characterized by its aromatic structures, oxygen-containing functional groups, and porous properties, is widely utilized as a composting amendment and has been demonstrated to promote humification by modulating microbial community structure and metabolic function (Liu et al. 2023c). However, the functionality of conventional pyrolyzed biochar remains limited, which has driven the development of modified biochars with enhanced reactivity (Qi et al. 2024). As a typical heavy metal inducer, Mn(II) can effectively enhance the activity of WRF and stimulate extracellular enzyme production (Zhao et al. 2022). Loading Mn species onto biochar produces an integrated amendment that combines the

physicochemical properties of biochar with the potential biochemical and redox functions of Mn (Tang et al. 2025). Compared with other modified biochars, such as iron-enhanced biochar, manganese-modified biochar (MBC) exhibits higher compatibility with the unique enzyme systems of WRF and demonstrates more efficient and specific catalytic performance in humification (Chen et al. 2023). However, the efficacy and mechanisms of MBC in the humification process within the biotransformation system mediated by WRF remain insufficiently studied and require further systematic investigation.

Dissolved organic matter (DOM) represents the most reactive fraction in solid-state fermentation systems (SSF), serving as both a key substrate and intermediate in microbial metabolism, thereby directly governing the pathways of organic matter transformation. Dynamic changes in its concentration, composition, and molecular characteristics are critical indicators for elucidating the humification process (Cai et al. 2022). In recent years, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) has provided molecular-level insights into the biochemical transformation of organic matter and the mechanisms of humification, owing to its exceptional ability to resolve molecular formulas and fingerprints of DOM (Liu et al. 2023b; Zhou et al. 2026). Given the complexity and diversity of humification pathways, distinguishing the preference between biotic and abiotic pathways in artificial humification systems is particularly important. The precise mass difference analysis enabled by FT-ICR MS allows inference of potential precursor-product pairs and reaction networks, thereby facilitating the identification of dominant reaction pathways (Liu et al. 2023a). The rapid development of machine learning facilitates the precise prediction of DOM molecular reactivity. Using unsupervised learning (e.g., clustering), molecular constituents are classified by their transformation characteristics from FT-ICR MS data, pinpointing their transformation propensity. By linking this propensity to molecular features, the importance of each feature is quantified via the SHapley Additive Explanations (SHAP) model. This offers a new method for revealing how microbial-derived carbon enhances lignocellulose humification (Dwinandha et al. 2023). Therefore, an in-depth investigation into DOM transformation is essential for elucidating the humification mechanisms in MBC-WRF systems, optimizing process parameters, and improving carbon sequestration potential.

Accordingly, this study investigated the effects of MBC on WRF-mediated lignocellulose degradation and humification-related transformation. First, the potential of MBC to enhance the humification process was evaluated by determining lignocellulose degradation rates and HS

contents, together with spectroscopic analyses. Subsequently, FT-ICR MS combined with mass difference analysis was employed to investigate the effects of MBC on the molecular properties and transformation pathways of DOM. Transcriptomic and metabolomic analyses were further integrated to elucidate the biological regulatory processes associated with lignocellulose humification in the WRF-mediated biotransformation system. This study further elucidates the underlying mechanisms by which MBC promotes WRF-mediated lignocellulose humification from the perspectives of molecular transformation and biological regulation.

2 Materials and methods

2.1 Material preparation and experiment set-up

Wheat stover was selected as the substrate for the SSF system using WRF due to its representative lignocellulosic composition and structure. The wheat stover, purchased from an agricultural product processing plant in Jiangsu, China, was cut to approximately 1.5 cm lengths, washed, and dried prior to use. The *Phanerochaete chrysosporium* (USA (NDM3-2)) was obtained from the Beijing Biological Depository Center (Beijing, China). Third-generation cultures were utilized for experiments. After cultivation on potato dextrose agar medium for 5 days, spores were harvested, mixed with sterile ultrapure water to create a spore suspension, and adjusted to a concentration of 2.0×10^6 spores mL^{-1} . MBC was prepared by loading Mn(II) onto bamboo-derived biochar (BC) via the impregnation method. Detailed preparation procedures and physicochemical characteristics are described in the Supplementary Information. Characterization results showed that Mn(II) was successfully loaded onto the surface of MBC, with a total Mn content of 11.61%, of which approximately 89.1% was present in the Mn(II) form (Fig. S1). Compared with BC, MBC exhibited a slight decrease in pH but a marked increase in EC; meanwhile, both the specific surface area and average pore size increased (Table S1).

Wheat straw (10 g) was weighed into 500 mL Erlenmeyer flasks, and the substrate C/N ratio was adjusted to 20 using $(\text{NH}_4)_2\text{SO}_4$. After sterilization at 121 °C, the moisture content was adjusted to 60% with a trace mineral solution, the composition of which is detailed in the Supplementary Information. Subsequently, 10 mL of spore suspension was inoculated, and the flasks were incubated at 30 °C for 35 days. The experiment included four treatments: CK (WRF only), SC (5% MBC without WRF inoculation), T1 (5% BC with WRF inoculation), and T2 (5% MBC with WRF inoculation). T1 was included to characterize the contribution of pristine biochar to WRF-mediated biotransformation, while comparison between T1 and T2 enabled assessment of

the additional effect associated with manganese modification. The SC was included to evaluate whether MBC alone caused observable lignocellulose transformation in the absence of fungal inoculation. The addition doses of biochar and manganese-modified biochar were determined based on the literature review and preliminary screening experiments. Sampling was performed on days 7, 14, 21, 28, and 35. All operations were conducted on a sterilized laminar flow bench to prevent microbial contamination.

2.2 Physicochemical properties, characterization of lignocellulose degradation, and humic substance determination

The methodologies for determining lignocellulose degradation efficiency, reactive oxygen species, and Mn speciation are provided in the Supplementary Information. Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and ^{13}C solid-state nuclear magnetic resonance (^{13}C NMR) spectroscopy were employed to comprehensively characterize the morphological features, surface functional group transformations, crystallinity changes, and molecular structural evolution of lignocellulosic components during wheat stover degradation. These analyses were integrated to elucidate the degradation mechanisms synergistically. HS were extracted according to the method of a previous study with subsequent separation into humic acid (HA) and fulvic acid (FA) fractions (Wang et al. 2025). The HA/FA ratio was calculated to evaluate the polymerization degree of the HS. Detailed extraction procedures are provided in the Supplementary Information.

2.3 Physiological parameters and extracellular enzyme secretion of *P. chrysosporium*

The wheat stover samples collected at different stages were immersed in PBS and ultrasonicated for 10 min to dislodge biofilms. Mycelia were filtered through a Buchner funnel equipped with a 0.22 μm membrane, followed by drying in a vacuum oven at 105 °C for 24 h. The growth of *P. chrysosporium* was assessed based on dry mycelial mass. Extracellular ligninolytic enzymes, including lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac), were determined following the manufacturer's protocols.

2.4 Extraction and characterization of dissolved organic matter

DOM solutions were obtained by mixing freeze-dried substrate samples with ultrapure water at a 1:10 ratio (w/v), followed by 24 h of continuous shaking and filtration through a 0.22 μm membrane. The dissolved organic

carbon concentration of DOM solutions was adjusted to $10 \text{ mg}\cdot\text{L}^{-1}$ prior to three-dimensional excitation-emission matrix (3D-EEM) spectroscopic analysis. Parallel factor analysis (PARAFAC) was performed using MATLAB 2021b to identify major fluorescent components. The pH of the diluted DOM solutions was adjusted to 2, followed by solid-phase extraction using a PPL cartridge (BondElute PPL, 6 mL, 1 g; Agilent Technologies, USA) (Yang et al. 2024). The molecular composition of DOM was determined via FT-ICR MS with negative ion mode detection (Solarix 15.0 T; Bruker, USA). Mass calibration and molecular formula assignment were performed according to established protocols (Liu et al. 2023a; Yang et al. 2024). Assigned formulas were categorized into seven major classes: lignin-like, lipid-like, unsaturated hydrocarbon-like, condensed aromatic structure-like, protein-like, carbohydrate-like, and tannin-like compounds (Table S2). Molecular characteristics, including H/C, O/C, N/C, S/C ratios, double bond equivalent (DBE), DBE minus oxygen (DBE-O), DBE/C, nominal oxidation state of carbon (NOSC), and modified aromaticity index (AI_{mod}), were calculated using R software. To elucidate DOM transformation during SSF, compounds were classified into three categories: precursors, resistant, and products. This categorization was based on comparative analysis of molecular presence/absence and peak intensity differences between pre-treatment and post-treatment samples. Molecular masses were recalibrated using atomic weights of C, H, O, N, and S as defined by the International Union of Pure and Applied Chemistry. Potential precursor-product pairs were identified through exact mass difference matching (Cai et al. 2022). All computations were conducted in Python, with mass difference network visualization implemented in the Gephi software. Detailed instrumental parameters and computational methodologies are provided in the Supplementary Information. Molecular properties determining the reactivity of DOM during SSF were investigated using machine learning. A binary classification task was performed in R using a random forest model to predict "reactive" ("precursors", labeled 0) and "non-reactive" ("resistant", labeled 1) compounds. Model interpretation was further conducted using SHAP (Dwinandha et al. 2023). Detailed information on machine learning model construction, parameter optimization, validation procedures, and the related data files is provided in the Supplementary Information.

2.5 Transcriptome analysis of CAZyme gene expression

Transcriptomic sampling was performed on day 21, representing the active lignocellulose degradation stage, to characterize transcriptional responses associated with lignocellulose biotransformation. After harvesting

P. chrysosporium mycelia from the surface of the wheat straw, total RNA extraction was performed using the RNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol. RNA purification, reverse transcription, library construction, and sequencing were performed at Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. (Shanghai, China) according to the manufacturer's instructions (Illumina, San Diego, CA). Clean reads were obtained through trimming and quality control of raw sequencing data, followed by alignment in directional mode to the *P. chrysosporium* reference genome (GCA000167175v1, https://fungi.ensembl.org/Phanerochaete_chrysosporium/Info/Index). Differential expression analysis was performed using DESeq2, with genes exhibiting $|\log_2(\text{fold change})| \geq 1$ and adjusted $p < 0.05$ classified as differentially expressed genes (DEGs). These DEGs were annotated against the CAZy database to identify differentially expressed CAZyme genes. Subsequently, GO and KEGG functional enrichment analyses were conducted for the annotated CAZyme genes. All bioinformatic analyses were executed on the Majorbio Cloud Platform (<https://www.majorbio.com>).

2.6 Identification of metabolic characteristics

Untargeted metabolomic profiling was performed on day 35, when HS content reached its peak, to characterize metabolic features associated with late-stage precursor transformation and humification. Metabolite identification was achieved by querying mass spectral databases, primarily the Human Metabolome Database (HMDB; <http://www.hmdb.ca/>), METLIN (<https://metlin.scripps.edu/>), and the proprietary MJDB database (Majorbio Biotechnology Co., Ltd., Shanghai, China). The resultant data matrix was uploaded to the Majorbio Cloud Platform for computational analysis. Significantly differential metabolites were defined as those exhibiting both a Variable Importance in Projection (VIP) score > 1 from the OPLS-DA model and a p -value < 0.05 from Student's t -test. Subsequently, metabolic enrichment and pathway topology analyses were conducted based on the KEGG database.

2.7 Statistical analysis

All experiments were conducted with three independent replicates unless otherwise stated. Data are presented as mean $\pm$ standard deviation. Statistical analyses were performed using SPSS. Differences among treatments were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

3 Results and discussion

3.1 Manganese-modified biochar enhanced

the degradation of lignocellulose by *P. chrysosporium*

After 35 days of cultivation, degradation by *P. chrysosporium* induced varying degrees of macroscopic fragmentation in treated straw groups (Fig. 1a). T2 exhibited the most pronounced structural disintegration, with its original rod-like morphology fragmenting extensively into fine debris and fibrous structures. SEM further revealed localized surface fragmentation in CK straw,

whereas T2 straw displayed a transformation from initially smooth and compact surfaces to highly roughened textures featuring irregular pores, deep cavities, and dissociated fiber bundles (Fig. 1b). In addition, no obvious macroscopic or microscopic fragmentation of the straw was observed in the SC group, indicating that MBC alone exerted only a limited direct depolymerization effect on straw. The lignocellulose degradation results showed that, compared with CK, the degradation rates of lignin, cellulose, and hemicellulose in T1 increased by 53.7%,

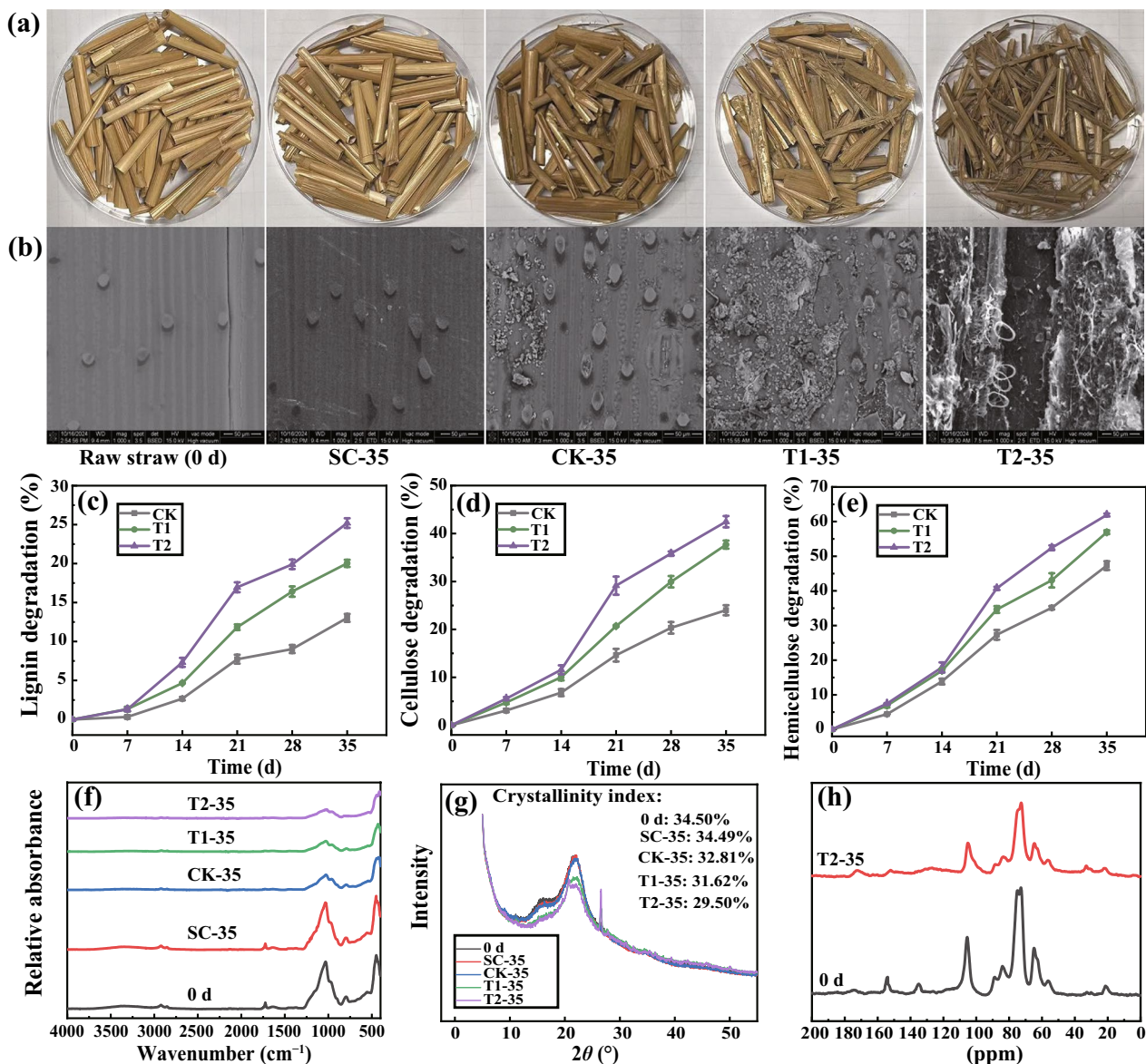


Fig. 1 Characterization of straw degradation. **a** Apparent and microscopic **b** morphology of straw. Degradation rate of **c** lignin, **d** cellulose, and **e** hemicellulose. **f** FTIR spectra, **g** XRD patterns, and **h** ^{13}C NMR spectra of straw. SC: Add manganese-modified biochar under sterile conditions; CK: Inoculated with white-rot fungi only; T1: Inoculated with white-rot fungi and added biochar; T2: Inoculated with white-rot fungi and added Mn-modified biochar

57.0%, and 20.48%, respectively, whereas the corresponding increases in T2 were 93.4, 77.0, and 30.9% (Fig. 1c–e). These results indicate that both BC and MBC promoted *P. chrysosporium*-mediated lignocellulose degradation, while the strengthening effect of MBC was more pronounced, further suggesting that Mn loading endowed biochar with greater enhancement potential.

FTIR spectroscopy indicated unaltered functional group types but significantly reduced characteristic peak intensities after *P. chrysosporium* degradation (Fig. 1f). Key peaks (2924, 1732, 1108, and 896 cm^{-1}) showed greater attenuation in T1/T2 versus CK. Specifically, diminished intensity at 2924 cm^{-1} reflected disruption of aliphatic chains in cellulose/hemicellulose; 896 cm^{-1} reduction indicated β -glycosidic bond cleavage; 1732 cm^{-1} decline suggested hemicellulose acetyl group dissociation (Zhang et al. 2024); and 1108 cm^{-1} attenuation implied lignin side-chain cleavage or aromatic condensation (Liang et al. 2018). Cellulose, as a heterogeneous polymer comprising crystalline and amorphous domains, exhibited higher susceptibility of amorphous regions to microbial attack (Lu et al. 2022). XRD analysis confirmed significant reductions in the crystallinity index (CrI), with CK, T1, and T2 showing decreases of 5.0%, 8.3%, and 14.5% compared with pristine straw (Fig. 1g), indicating depolymerization of the amorphous region and disruption of the crystalline hydrogen-bond network. Notably, MBC (T2) tripled the CrI reduction observed in CK, demonstrating enhanced deconstruction of cellulose crystalline structures. Solid-state ^{13}C NMR spectroscopy further validated the enhancement effect of MBC on lignocellulose degradation by *P. chrysosporium* (Fig. 1h). Characteristic polysaccharide resonances predominantly appeared within the 60–115 ppm region. Signals at 154, 135, and 106 ppm (the latter overlapping with carbohydrate peaks) were assigned to aromatic units in lignin (Khatami et al. 2019), while a weak resonance at about 20 ppm corresponded to hemicellulose components (Lu et al. 2022). Compared with CK, T2 exhibited substantially attenuated signal intensities across these diagnostic regions, indicating accelerated depolymerization of lignocellulosic constituents. Integrated visual-spectroscopic analyses confirmed that MBC enhanced *P. chrysosporium*'s lignocellulosic degradation efficacy by potentiating crystalline matrix disintegration and macromolecular bond scission.

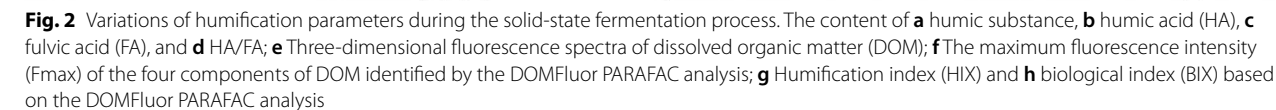
3.2 Manganese-modified biochar enhanced lignocellulose humification mediated by *P. chrysosporium*

Quantitative dynamics of HS and their major components (HA and FA) were analyzed (Fig. 2a–c). Throughout SSE, HS and HA contents were observed to increase progressively, while FA content declined

continuously. On day 35, the highest HS and HA contents (15.4% and 47.7% above the control, respectively) along with the lowest FA content were recorded in T2. Given that FA represents a low-molecular-weight, highly soluble transitional fraction, it is readily metabolized as a microbial carbon source and can be transformed into structurally complex HA via polycondensation with quinones, polyphenols, and amino acids (Wang et al. 2025). Consequently, *P. chrysosporium* may have facilitated the conversion of reactive FA into high-molecular-weight, aromatic, and stable HA, forming recalcitrant carbon. The HA/FA ratio, a key indicator of HS polymerization, was elevated by 42.8% and 112.4% in T1 and T2 versus CK (Fig. 2d), providing supportive evidence that MBC addition favored humification and the formation of more polymerized humic fractions.

3D-EEM was employed to characterize fluorescent components (Fig. 2e). Initial samples were dominated by peaks in Regions I and II, indicative of protein-like derivatives (tyrosine/tryptophan). By day 14, significant fluorescence intensification in T2 was detected across Region III (fulvic-like substances), IV (soluble microbial byproducts), and V (humic acid-like substances), signaling activated *P. chrysosporium* metabolism and humification initiation. Comparable signals were not observed in other treatments until day 28. Notably, the intense fluorescence peaks in the T2 were mainly concentrated in regions III and V by day 28, indicating progressive molecular transformation of microbial byproducts and protein-like derivatives toward more humic-like components.

PARAFAC analysis identified four fluorescent fractions as humic-like substances (C1, C4), protein-like substances (C2), and soluble microbial byproducts (C3) (Fig. S2). Spectral disparities between C1 and C4 were attributed to structural and formation-mechanism differences (Zhu et al. 2021). Quantification revealed protein-like dominance (C2) initially, followed by progressive enrichment of humic-like components (C1, C4) and microbial byproducts (C3) (Fig. 2f), supporting a transition from rapid decomposition toward a later stage of organic matter transformation associated with humification. On day 35, maximal humic-like substances and microbial byproducts were quantified in T2, suggesting that MBC significantly enhanced the humification of lignocellulose during SSF by improving lignin degradation efficiency and promoting the polymerization of small molecules (Gao et al. 2019). Consistent with the HA/FA ratio, the humification index (HIX) reached its highest value in T2 (Fig. 2g), providing supportive evidence for enhanced humification-related transformation in this treatment. Furthermore, elevated biological index values



Given the limitations of conventional analytical techniques, such as spectroscopy, in elucidating the transformation mechanisms of low-molecular-weight DOM,

this study employed FT-ICR MS to conduct detailed molecular-level characterization of DOM composition and properties during SSF, thereby improving the understanding of how MBC enhances the humification process. DOM was primarily composed of CHO compounds, followed by CHON, CHOS, and CHONS compounds (Fig. 3a, b). Significant changes in DOM composition during SSF were characterized by a decrease in the proportion of nitrogen- and sulfur-containing compounds (CHON, CHOS, CHONS) and

a concurrent increase in CHO compounds. On day 35, compared with CK, a higher proportion of CHON compounds was observed in T2, while lower proportions of CHO, CHOS, and CHONS compounds were detected. Van Krevelen diagram analysis indicated that the increase in CHO compounds was primarily attributed to lignin-like and tannin-like substances (Fig. 3a), suggesting the generation of substantial quantities of these recalcitrant macromolecular organic compounds during straw degradation by *P. chrysosporium*. Lignin-like

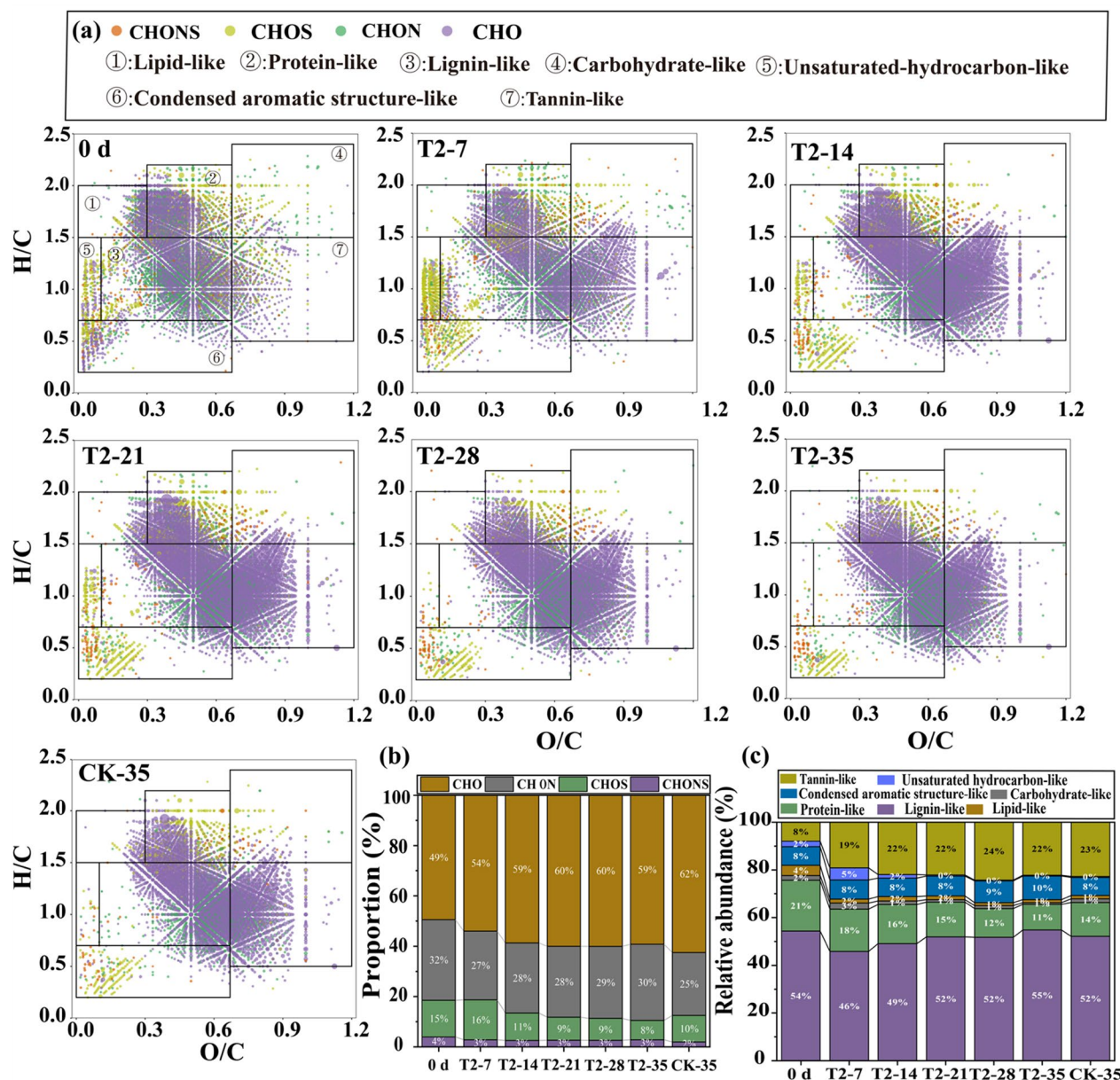


Fig. 3 Changes in the composition of DOM during solid-state fermentation. Van Krevelen diagrams of the **a** four major subcategories and seven biochemical classes. DOM composition of **b** subcategories and **c** biochemical classes based on molecular counts

and condensed aromatic structure-like compounds, typically containing reducible moieties coupled with alkoxy- or hydroxy-substituted aromatics, or primarily consisting of carbonyl compounds containing aliphatic ketones and carboxyl groups, constitute the fundamental structural units of HS (Cai et al. 2025). The relative enrichment of lignin-like and condensed aromatic structure-like compounds in T2 DOM samples reflected a molecular condensation trend (Fig. 3c). Furthermore, the relative abundance of tannin-like substances increased significantly from an initial 8% to 23% in CK and 22% in T2, thereby providing the necessary foundation for the progression of the phenolic oxidation-polymerization pathway (Cai et al. 2025). Notably, the relative abundance of CHON compounds in T2 DOM exhibited an initial decrease followed by an increase during fermentation, reaching 30% on day 35, higher than the CK value of 25% (Fig. 3b). Combined with the observation that protein degradation in T2 was greater than in CK (Fig. 3c), it is suggested that protein degradation products may be involved in the formation of humification-related precursors through carbonyl-amine condensation reactions, and that T2 generated more abundant nitrogen-containing precursor substances (Gao et al. 2019).

The overall trend of molecular transformation during SSF was assessed by calculating the weighted average compositional values of DOM molecules (Table S4). Spearman correlation analysis between DOM molecular characteristics and humification indicators (HA/FA, HIX) revealed significant positive correlations for O/C, DBE, DBE/C, NOSC, and AI_{mod} (Fig. S3). This demonstrates that DOM molecular characteristics serve as valuable indicators for evaluating the humification degree of samples. Throughout the SSF process, a continuous decrease in H/C_{wa} and S/C_{wa} was observed for T2 DOM molecules, while O/C_{wa} , DBE_{wa} , DBE/C_{wa} , $NOSC_{wa}$, and AI_{modwa} demonstrated continuous increases. These changes indicate elevated molecular unsaturation, enhanced aromaticity, and a higher oxidation state. Notably, a rapid decline in H/C_{wa} concurrent with a rapid increase in O/C_{wa} during the initial 14 days suggests dehydrogenation/cyclization reactions dominated this phase, consistent with the transformation pathway of lignin oxidative dehydrogenation forming quinone structures. After day 14, the sustained rise in DBE_{wa} and AI_{modwa} reflects increased aromatic ring condensation and aliphatic chain loss, providing supportive evidence for the progressive transformation of lignin-derived fragments toward humic-like structures (Cai et al. 2022). Furthermore, comparative analysis of DOM molecular characteristics on day 35 revealed higher O/C_{wa} , DBE_{wa} , and AI_{modwa} values in T2 than in CK. These

results provide supportive evidence that MBC promoted DOM transformation toward more oxidized, condensed, and aromatic humic-like structures.

3.3.2 Alterations in the reaction pathways of dissolved organic matter

To elucidate the transformation characteristics of DOM during humification, DOM molecules were classified into three categories by comparing initial and final samples: precursor molecules (disappeared), resistant molecules (retained), and product molecules (newly formed). The results indicated that the precursors were predominantly located in regions of low O/C and high H/C, whereas the products were enriched in regions of high O/C and low H/C (Fig. S4a, b). This distribution suggests that *P. chrysosporium* likely drives DOM evolution toward stable humic-like structures through dehydrogenation/condensation and side-chain oxidation (Cai et al. 2022). Furthermore, CHON and CHO compounds exhibited a higher proportion of reactive molecules (precursors) and resistant molecules compared with other subcategories. At the biochemical class level, lignin-like compounds contained the highest proportion of reactive and resistant molecules, followed by tannin-like and protein-like compounds (Fig. S4c–f). Consequently, elucidating the key factors controlling the reactivity of these molecules is crucial for regulating molecular transformations in lignocellulosic humification processes.

A random forest model was employed to identify 15 key molecular features for predicting DOM reactivity in CK and T2. The results showed that molecules with high N and S contents, high DBE-O, and low O/C consistently exhibited higher reactivity in both groups (Fig. S5a), indicating that lignocellulose humification is not a random process but is selectively governed by specific molecular structures. Molecules enriched in N and S may provide more reactive sites, such as amino, amide, and sulfur-containing functional groups, whereas high DBE-O and low O/C suggest relatively reduced and unsaturated aromatic structures with greater potential for oxidative activation. Accordingly, humification may preferentially consume heteroatom-rich, low-oxidation aromatic precursors and convert them into stable humic-like structures through oxidation, side-chain cleavage, and polymerization. Further subgroup analysis in T2 revealed that the key features controlling reactivity varied across molecular classes (Fig. 4a). At the elemental level, CHO molecules with high DBE-O and H but low O/C and DBE/C, CHON molecules with low O/C and NOSC but high N and DBE-O, and CHOS molecules with high DBE-O, C, DBE, and larger molecular weight were more prone to transformation. These patterns consistently point to the preferential transformation of molecules with low oxidation

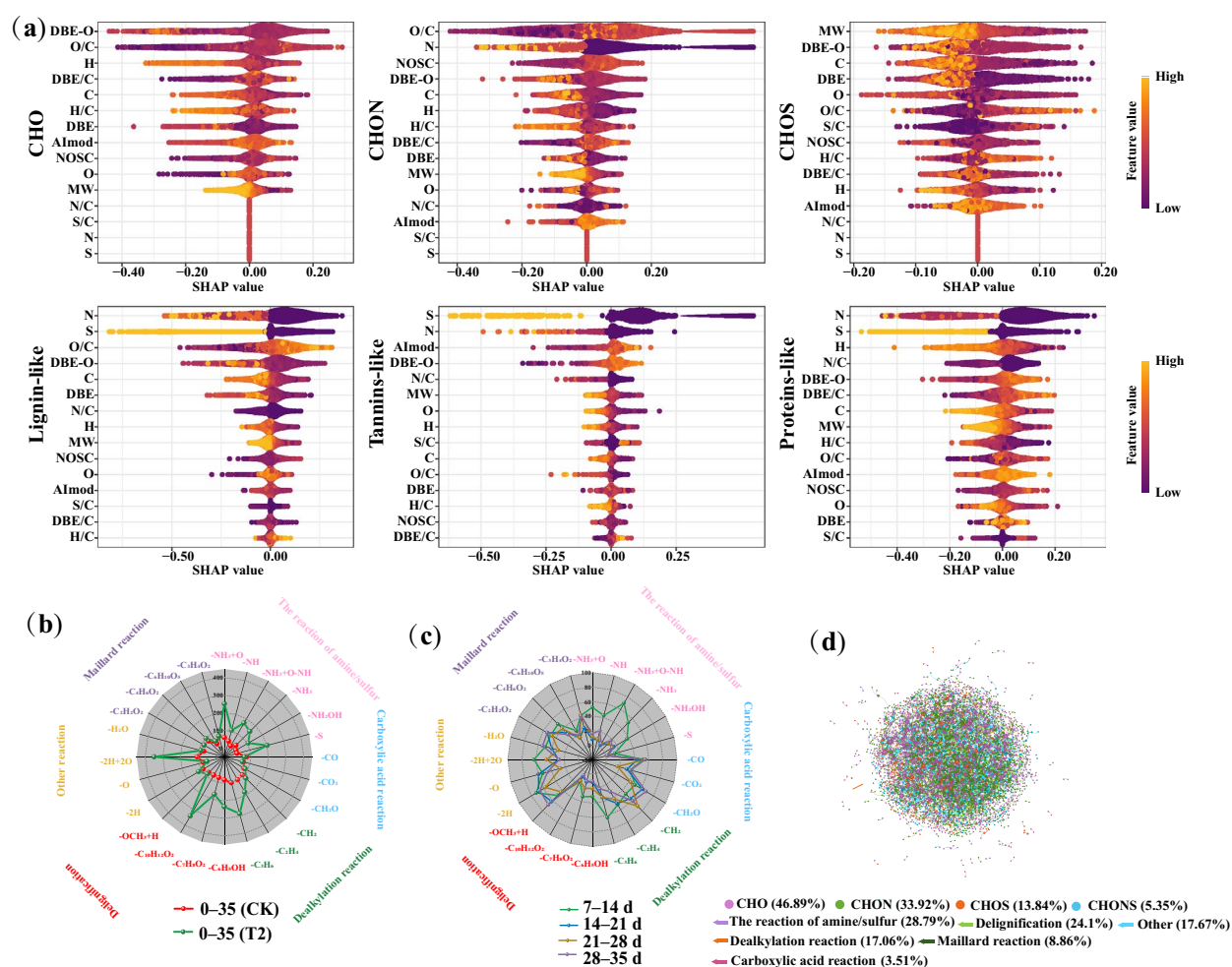


Fig. 4 **a** Machine learning explains the importance of each molecular property to the model output (formula reactivity) based on different molecular compositions and biochemical classes. **b** Radar plot illustrating the number of possible precursor-product pairs during solid-state fermentation in CK and T2. **c** The number of possible precursor-product pairs at different phases in T2. **d** Paired mass distance-based network indicating some of the possible reactions of CHO, CHON, CHNS, and CHONS molecular formulas in T2

degree, high unsaturation, and strong structural activation potential. At the biochemical level, N and S contents were common determinants of reactivity for lignin-like, tannin-like, and protein-like compounds, suggesting that heteroatom enrichment enhances DOM transformation potential. In addition, lignin-like molecules with low O/C and high DBE-O were more reactive, implying that relatively reduced and insufficiently oxidized aromatic lignin derivatives are more susceptible to oxidative activation and subsequent conversion into the aromatic backbone of humic-like substances (Hu et al. 2025). Tannin-like molecules with low AI_{mod} and low DBE-O exhibited higher reactivity, indicating that less condensed and more labile polyphenols are more readily oxidized into quinone-like intermediates and incorporated into the polyphenol-driven humification pathway (Cai et al. 2025). Protein-like molecules with high H content and high N/C were

also more reactive, suggesting that reduced nitrogenous compounds can serve both as readily utilizable substrates and as nitrogen donors for condensation with oxidized phenolic or quinone intermediates (Xie et al. 2023). Overall, DOM reactivity appears to be jointly regulated by elemental composition, oxidation state, degree of unsaturation, and biochemical origin. Lignin-like compounds primarily provide aromatic skeletons, tannin-like compounds contribute polyphenolic and quinone intermediates, and protein-like compounds supply nitrogen-containing substrates. The selective transformation of these molecular groups may collectively drive DOM evolution toward more aromatic, oxygen-functionalized, and structurally stable humic-like structures. These findings further suggest that MBC may promote humification by enhancing the selective activation and transformation of key reactive precursors.

To gain deeper insights into DOM transformation pathways during lignocellulosic humification, this study employed mass difference analysis to conduct chain analysis of 24 key reaction types. The T2 treatment demonstrated significantly higher overall DOM reactivity than CK (Fig. 4b). Within T2, deamination/desulfurization reactions exhibited the highest activity (28.79%), followed by delignification (24.1%) (Fig. 4d). Deamination activity was particularly pronounced during the initial 14 days (Fig. 4c).

Oxidative deamination may have contributed to the release of amino-containing intermediates and the restructuring of carbon skeletons, thereby favoring the formation of humification-related precursors (Schmidt et al. 2011). As SSF progressed, *P. chrysosporium* degraded lignocellulose to release methoxy groups ($-\text{OCH}_3$) and monophenolic derivatives (e.g., phenol, guaiacol, syringol) (Fig. 4c), which are generally regarded as important structural precursors in humification processes (Cai et al. 2022). Concurrently, dealkylation remained a substantial contributor (17.06% in T2), suggesting continued removal of alkyl side chains and activation of aromatic structures. While dicarbonyl compounds can undergo aromatization and polymerization with amino acid structures via the Maillard reaction to form humic-like substances (Cai et al. 2025), this pathway contributed significantly less in T2 (8.86%) than in CK (17.02%) (Fig. S6). This reduction is likely attributable to T2's enhanced lignocellulose degradation capacity, which generated more abundant polyphenolic precursors that strengthened polyphenol-mediated humification pathways, thereby diminishing the relative contribution of Maillard reactions. Decarboxylation was also inferred, which is consistent with oxidative cleavage of lignin side chains and the generation of phenolic lignin-derived fragments that may subsequently participate in humification-related transformations (Wei et al. 2022). Given that lignin-like substances primarily comprise CHO and CHON DOM molecules (Fig. 3a), Kendrick mass defect analysis was further applied to elucidate carboxyl group transformations in T2. Throughout SSF, increased formation of carboxyl-containing macromolecules was observed alongside decreased small carboxyl-containing molecules (Fig. S7). This shift may reflect microbial utilization of low-molecular-weight compounds by *P. chrysosporium*, together with the incorporation of carboxyl-containing intermediates into more complex functional structures during humification (Liu et al. 2022).

3.4 Manganese-modified biochar enhanced lignocellulose-degrading enzyme activity and $\cdot\text{OH}$ production

WRF biofilm formation directly impacts the degradation efficiency of straw. Higher biomass and mature biofilms typically indicate a more extensive active hyphal network,

enhancing extracellular enzyme secretion and substrate penetration (Wu et al. 2023). Macroscopic examination on day 35 of SSF revealed that the straw surface and internal pores were colonized entirely by mycelia in T2, whereas only sparse *P. chrysosporium* colonization was observed in the CK (Fig. S8a). The biofilm formation process was further characterized by measuring dry biomass. Biomass increased over time for all treatments. Biomass accumulation entered an exponential growth phase after day 14 in T1 and T2, while a low-level linear increase was maintained in CK. T2 exhibited the highest accumulation rate, achieving a final biomass that was significantly 2.82-fold higher than that of CK (Fig. S8b). The rapid biomass accumulation in T2 indicates that MBC addition effectively promoted biofilm maturation.

Lignocellulose degradation by WRF is highly dependent on the synergistic action of extracellular enzymes and $\cdot\text{OH}$. This synergy enables efficient degradation through specific biocatalysis combined with non-selective oxidative attack (Wang et al. 2025). Significant differences in extracellular enzyme activities and $\cdot\text{OH}$ levels were observed among treatments during SSF. Specifically, LiP, MnP, and Lac activities and $\cdot\text{OH}$ production followed the order $\text{T2} > \text{T1} > \text{CK}$ (Fig. S9a–e). The higher extracellular enzyme activities in T2 than in T1 indicate an additional effect associated with manganese modification. As Mn was successfully loaded onto MBC and was predominantly present as Mn(II), MBC-associated Mn species may have contributed to the enhanced extracellular enzyme activity in T2. In addition, the greater fungal biomass and biodegradation activity in T2 may have supported sustained extracellular enzyme production (Wu et al. 2023). The temporal variation in extracellular enzyme activities in the T2 treatment was generally consistent with the trend of lignocellulose degradation. LiP and MnP activities increased continuously throughout the SSF process, whereas Lac activity declined after day 21; nevertheless, all three enzymes exhibited their most pronounced increases during the 14–21-day period. During the same period, the lignocellulose degradation rate also increased markedly, suggesting that this stage may represent a critical period for oxidative depolymerization of lignocellulose in T2. This temporal consistency suggests that the coordinated enhancement of LiP, MnP, and Lac activities may have promoted the oxidative depolymerization of lignocellulose.

The higher $\cdot\text{OH}$ level observed in T2 was likely associated with the combined effects of active *P. chrysosporium* metabolism and MBC-associated Mn processes. On the one hand, the degradation of lignocellulose by WRF depends not only on their non-specific extracellular enzyme system, but also on non-enzymatic oxidative processes that can generate $\cdot\text{OH}$ and thereby promote

lignocellulose conversion (Guillén et al. 1992). On the other hand, Mn(II) can react with H_2O_2 to produce $\cdot OH$ through Fenton-like reactions (Watts et al. 2005). In T2, the enhanced fungal activity may have provided a more sufficient supply of H_2O_2 , while the addition of MBC created favorable conditions for Mn-involved redox cycling (Forrester et al. 1988). Their combined effects therefore likely strengthened Fenton-like oxidation, ultimately resulting in a higher $\cdot OH$ level. Meanwhile, the synergistic action of $\cdot OH$ and extracellular enzymes may have contributed not only to the initial oxidative depolymerization of lignocellulose, but also to the further oxidation of lignin-derived phenolic precursors into more reactive quinone-like or oxygen-enriched intermediates, thereby increasing their potential for subsequent condensation and polymerization and promoting the humification process (Wang et al. 2025). Quantitative analysis of Mn speciation in T2 further showed that exchangeable Mn was transformed into reducible Mn fractions during SSF (Fig. S9f), a change that may be related to the involvement of Mn(II) in both MnP-mediated oxidation and Fenton-like reactions.

3.5 Effect of manganese-modified biochar on CAZyme gene expression of *P. chrysosporium*

CAZymes primarily drive lignocellulose depolymerization and precursor release, thereby providing the substrate basis for the subsequent condensation and stabilization processes associated with humification. DEG expression analysis between CK and T2 groups identified 451 significantly upregulated genes and 354 significantly downregulated genes (Fig. 5a). Among these, 37 DEGs were annotated as CAZyme genes (based on the CAZy database), primarily encoding glycoside hydrolases (GHs; 20 upregulated) and auxiliary activities (AAs; 12 upregulated) (Fig. 5b). Microbial degradation and modification of lignin rely on redox-active enzymes predominantly classified within the auxiliary activities (AA) class. These enzymes act synergistically with glycoside hydrolases (GHs) to facilitate lignocellulose depolymerization (Chirania et al. 2022). In T2, genes encoding LiP and MnP from the AA2 family exhibited higher expression levels than in CK, consistent with greater enzyme production. Lytic polysaccharide monooxygenases from the AA9 and AA10 families primarily catalyze the oxidative cleavage of cellulose and, in some cases, hemicellulose, and thus mainly contribute to polysaccharide degradation during lignocellulose deconstruction. They may also indirectly facilitate lignin transformation by enhancing polysaccharide deconstruction and increasing cell-wall accessibility. In addition, some studies suggest that LPMO-mediated oxidation may be associated with Fenton chemistry and may assist in the oxidative cleavage of lignin-related structures (Li et al. 2021). Quinone oxidoreductases

(AA6 family) subsequently transform quinones to reactive hydroquinones, driving polymerization reactions. The AA3 and AA7 families encompass enzymes involved in Fenton reaction-based lignin degradation and modification (Xu et al. 2018). Within the GH class, families GH5, GH6, GH7, GH43, GH51, GH74, GH92, and GH116 were characterized as primary cellulolytic and hemicellulolytic enzymes (Auer et al. 2023). The upregulated expression of these AA and GH CAZyme genes in T2 provides molecular evidence for enhanced lignocellulose depolymerization and humification-related precursor generation.

Further functional analysis of the 37 differentially expressed CAZyme genes revealed 71 significantly enriched GO terms (Table S7), predominantly within biological processes (39 terms) and molecular functions (31 terms), with only one term related to cellular components. As shown in Fig. 5c, the top 20 most significant pathways (lowest *p*-values) were primarily associated with carbohydrate metabolism (e.g., cellulose metabolism), oxidoreductase activity, and catalytic functions. KEGG pathway enrichment analysis identified six significantly enriched metabolic pathways (Fig. 5d), mainly linked to carbohydrate metabolism, amino acid metabolism, and quinone compound biosynthesis. The activity of these pathways was significantly stronger in T2 than in CK, indicating that MBC addition may have helped enhance metabolic processes associated with humification-related precursor generation.

Notably, compared with T1, T2 exhibited significantly enhanced expression of AA and GH CAZyme genes related to lignocellulose degradation, along with strengthened carbohydrate metabolism, amino acid metabolism, and quinone biosynthesis pathways (Fig. S11). This enhanced effect may be attributed to the stimulation of metabolic activity in *P. chrysosporium* by Mn(II). As an essential cofactor for MnP, the presence of Mn(II) is generally observed to induce the transcriptional upregulation of MnP-encoding genes in *P. chrysosporium*, thereby promoting lignin degradation (Wang et al. 2025). The phenolic compounds generated in this process can subsequently act as inducers, further activating the transcription of genes encoding AA family enzymes. Additionally, Mn(II) participates in the regulation of intracellular pH and redox homeostasis, leading to the activation of specific transcription factors that promote the upregulation of CAZyme gene expression (Dong et al. 2025).

3.6 Manganese-modified biochar drives metabolic changes during lignocellulose humification mediated by *P. chrysosporium*

Metabolomic profiling provides additional stage-specific insights into the metabolic responses associated with lignocellulose humification. At the late fermentation stage

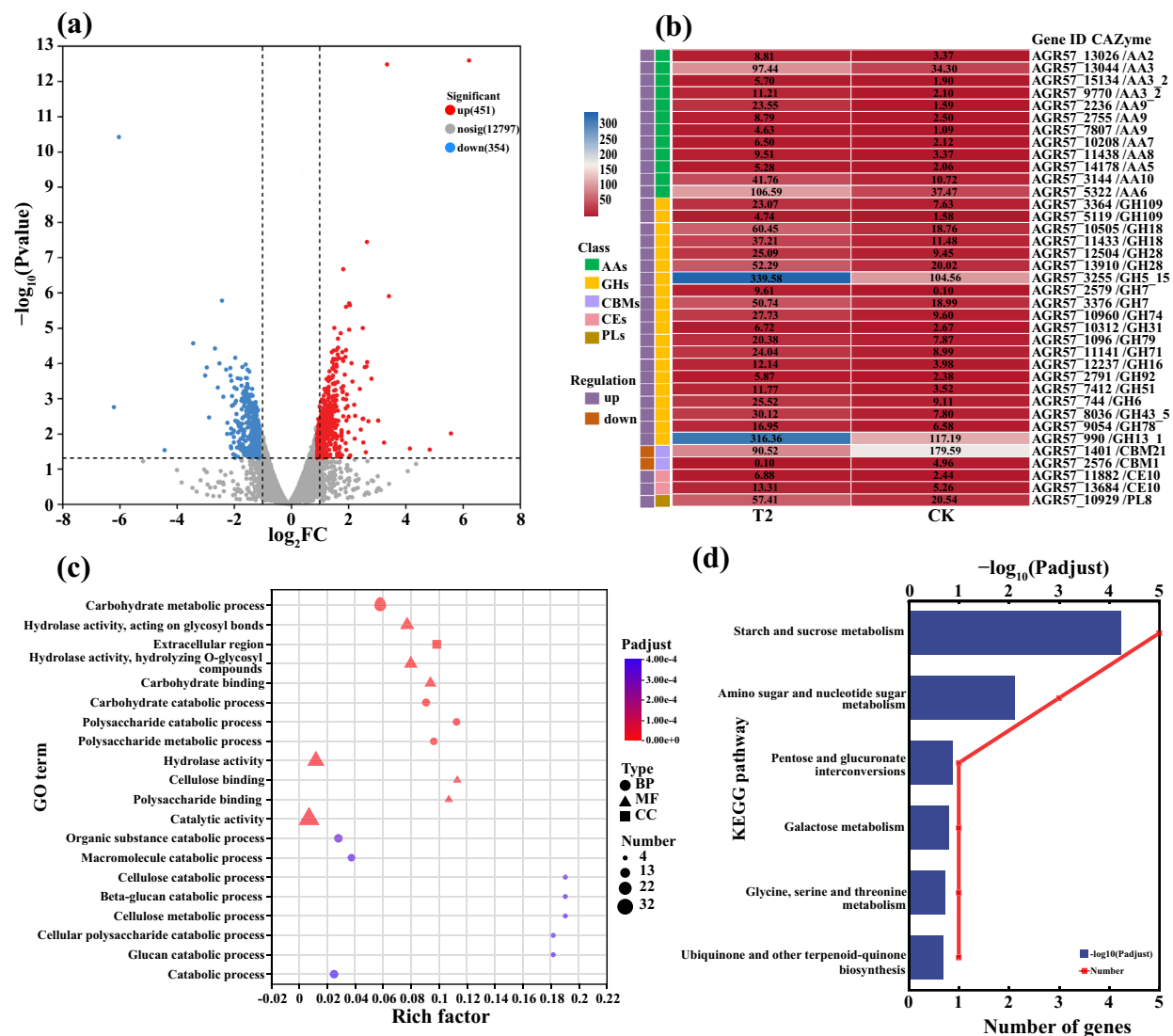


Fig. 5 Transcriptome analysis of *P. chrysosporium* of T2 vs. CK. **a** Volcano plot of differentially expressed genes (DEGs) up-regulation and down-regulation. **b** Expression of CAZyme DEGs. **c** Go enrichment analysis and **d** KEGG pathway enrichment analysis of up-regulation of CAZyme DEGs

(day 35), 314 differentially abundant metabolites were identified between CK and T2, with 183 significantly upregulated and 131 downregulated in T2 compared with CK (Fig. S12). Upregulated metabolites, including phenolic compounds, phenolic acids, glycosides, terpenoids, other organic acids, and amino acids (Table S8), primarily originated from degradation products and metabolic intermediates of lignocellulose, proteins, and lipids, thereby supplying key precursors for humification-related transformation.

KEGG enrichment analysis of upregulated metabolites revealed 39 significantly enhanced metabolic pathways, predominantly comprising amino acid

metabolism (13 pathways), carbohydrate metabolism (6 pathways), biosynthesis of other secondary metabolites (5 pathways), and lipid metabolism (4 pathways) (Table S9). During the lignocellulose humification process, amino acid metabolism supplies nitrogen sources and reactive substrates, carbohydrate metabolism provides energy and carbon skeletons, and the biosynthesis of other secondary metabolites may support the generation of humification-related precursors (Qi et al. 2022). These results provide supportive evidence that MBC addition enhanced microbial metabolic activity and promoted pathways associated with humification-related precursor generation.

The polyphenol humification pathway represents one of the major routes for lignocellulose humification. Phenolic compounds, as key precursors in this pathway, originate not only from extracellular pathways (e.g., enzymatic oxidation, free radical chain reactions) but also from crucial intracellular microbial synthesis (Wu et al. 2021). Polysaccharide hydrolysis products serve as essential substrates for intracellular polyphenol synthesis, being converted to polyphenols and phenolic derivatives (e.g., quinones) through metabolic pathways including glycolysis (map00010), phenylalanine biosynthesis (map00400), and ubiquinone/terpenoid-quinone biosynthesis (map00130). Enhanced activity of these pathways in T2 compared with CK may have contributed to a greater supply of precursors for polyphenol humification. Expression analysis of polyphenol synthesis-related enzyme genes revealed widespread upregulation in T2, particularly for genes encoding chorismate and phenylalanine synthesis (Fig. S14). Chorismate functions as a key precursor for quinone and phenylalanine biosynthesis, while phenylalanine serves as the direct substrate for polyphenol synthesis (Gao et al. 2022). The accumulation of these differential metabolites underpinned the increased polyphenol and quinone content in T2, ensuring a sustained endogenous polyphenol supply that directly influenced polyphenol humification. Quinones derived from polyphenols are considered highly reactive intermediates and may promote polymerization, thereby contributing to the formation of more complex and stable humic-like structures (Liu et al. 2023c).

Integrating the transcriptomic and metabolomic results provides a more comprehensive understanding of the potential mechanism by which MBC enhances lignocellulose humification. The upregulation of GH and AA family genes at the transcriptional level indicated enhanced hydrolytic and oxidative potential toward lignocellulose, while the enrichment of carbohydrate and amino acid metabolism and the accumulation of glycosides, organic acids, phenolic compounds, and quinone-related metabolites reflected enhanced precursor generation and transformation at the metabolic level. In particular, the increased abundance of phenolic and quinone-related metabolites was functionally consistent with the enhanced lignocellulose-degrading capacity indicated by CAZyme expression, whereas enhanced amino acid metabolism suggested an increased availability of nitrogen-containing substrates potentially involved in humification. Collectively, these stage-specific observations reveal functional concordance between lignocellulose deconstruction and subsequent precursor transformation, providing further insights into the biological processes underlying MBC-enhanced lignocellulose humification.

3.7 Mechanistic insights into manganese-modified biochar enhanced humification of lignocellulose in white-rot fungal biotransformation systems

Comprehensive analysis of the physicochemical parameters, DOM molecular transformation, enzyme activities, and reactive oxygen species dynamics, together with multi-omics results, indicated that the enhancement of lignocellulosic humification by MBC was associated with coordinated contributions more likely attributable to the synergistic coupling among the interfacial regulatory role of biochar, Mn-mediated abiotic oxidation, and WRF-mediated enzymatic conversion.

During the lignocellulose depolymerization stage, MBC addition markedly promoted the initial deconstruction of lignocellulose. On the one hand, MBC provided a more favorable microenvironment for WRF, promoted their colonization and biofilm maturation on the straw surface, and upregulated the expression of lignin-degradation-related enzyme genes, thereby enhancing the secretion of extracellular oxidases such as Lac, LiP, and MnP and promoting the depolymerization of lignocellulose. On the other hand, the Mn(II) loaded onto the biochar surface could participate in $\cdot\text{OH}$ -associated oxidation through redox cycling, further improving the oxidative depolymerization efficiency of lignocellulose. Notably, under sterile conditions, no obvious macroscopic or microscopic fragmentation was observed in the treatment amended with MBC alone, suggesting that Mn-mediated radical oxidation could not exert a pronounced effect independently in this system and was more likely to rely on synergistic participation by WRF.

During the precursor generation and condensation/polymerization stage, MBC mainly promoted humic-like substance formation by accelerating precursor generation and molecular condensation. At the metabolic level, in addition to enhancing extracellular oxidation, MBC upregulated pathways related to amino acid metabolism, carbohydrate metabolism, and the biosynthesis of other secondary metabolites. As a result, lignin-derived precursors were released through extracellular oxidation, while phenolics, quinones, aromatic structural units, and nitrogen-containing intermediates were continuously synthesized and transformed through intracellular metabolism, thereby providing the structural basis for humic-like substance formation. At the molecular level, this was reflected by enhanced DOM reactivity, particularly through increased deamination, dealkylation, and delignification reactions. The resulting precursors could undergo self-condensation, or use lignin-derived intermediates as core scaffolds to form humic-like substances via lignin-protein coupling pathways, or participate in humic-like substance formation through

Maillard reactions and polyphenol humification pathways (Cai et al. 2022; Qin et al. 2024). Meanwhile, Mn-related redox cycling, radical reactions, and extracellular enzymatic catalysis further promoted the conversion of reactive precursors into humic-like substances. Considering the relatively lower contribution of the Maillard pathway, the more active delignification reactions, and the greater abundance of phenolic and quinone precursors in T2, it can be inferred that the polyphenol humification pathway was more likely dominant in this system.

In summary, the potential mechanisms underlying the enhancement of lignocellulosic humification by MBC can be proposed as follows (Fig. 6): during the depolymerization stage, biochar-associated effects on fungal growth, together with the potential contribution of MBC-associated Mn species to extracellular enzyme responses and Mn-related redox/radical processes, were accompanied by enhanced lignocellulose deconstruction; during the precursor generation and condensation/polymerization stage, MBC promoted the continuous supply of precursor compounds by regulating the metabolic network of WRF, while Mn-associated enzymatic and redox processes may have further contributed to precursor activation and transformation, thereby favoring the polymerization of humic-like substances.

3.8 Environmental impact, limitations, and future perspectives

Previous studies have primarily focused on the lignin-degrading capacity of WRF and their roles in pollutant remediation, whereas comparatively less attention has been paid to their potential to promote lignocellulosic humification (Table S6). Compared with studies involving biochar alone or the co-application of Mn salts and biochar to enhance humification, the present study established a MBC-WRF synergistic system and investigated the potential mechanisms underlying its enhancement of lignocellulose humification across two sequential stages, namely lignocellulose depolymerization and subsequent precursor generation/condensation-polymerization. Earlier studies have mainly emphasized composting performance, humification indices, or microbial community shifts, while giving relatively limited attention to DOM molecular reactivity, the selective generation of precursor molecules, and their subsequent transformation patterns. In contrast, this study systematically analyzed, at the molecular, transcriptional, and metabolic levels, the process-level linkages among substrate deconstruction, precursor release, metabolic regulation, and late-stage condensation/polymerization. These findings deepen the understanding of lignocellulosic humification mechanisms and provide theoretical support for the optimization of artificial humification technologies.

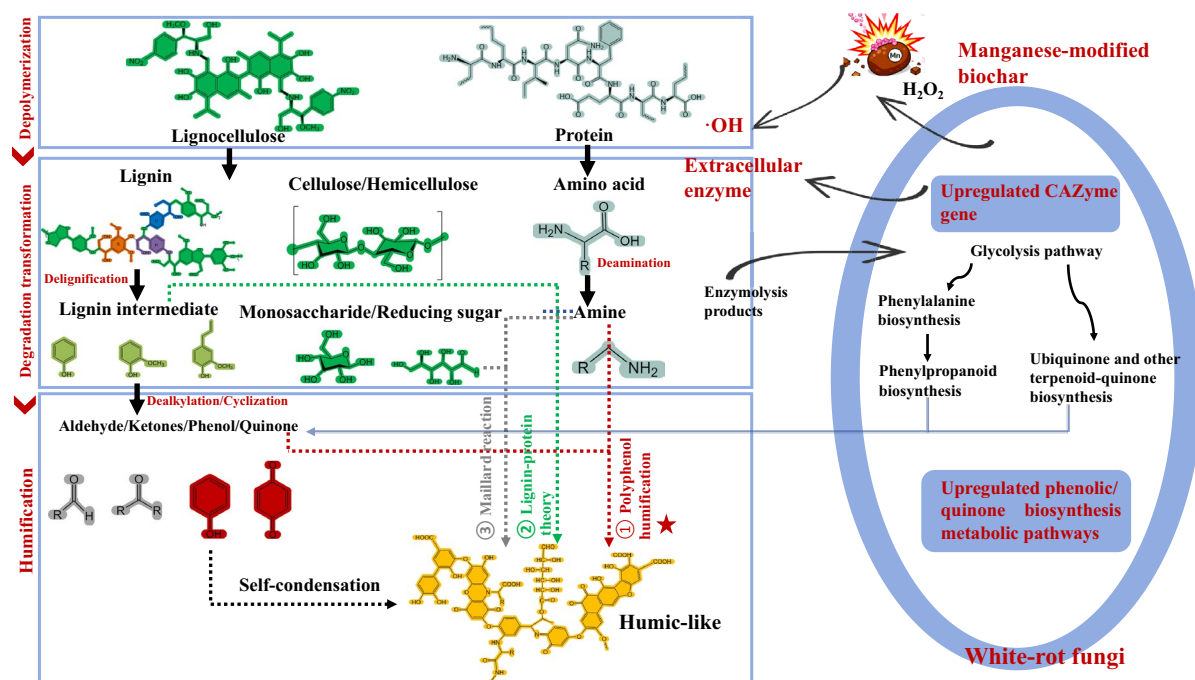


Fig. 6 Mechanism of manganese-modified biochar in enhancing humification during lignocellulose-based solid-state fermentation by *P. chrysosporium*

Furthermore, an exploratory data analysis method based on paired mass distance-associated analysis and interpretable machine learning was proposed to reveal the transformation mechanisms of DOM molecules during the MBC-regulated biotransformation of lignocellulose by *P. chrysosporium*, by linking the macro-phenomenon of "accelerated humification" to a molecular mechanism defined by "directional formation and efficient conversion of preferential precursor molecules". This not only clarifies the efficiency-enhancing role of MBC but also establishes a structure-based framework for predicting and optimizing SSF processes, laying a foundation for precision bioconversion of organic waste.

However, the analytical framework used in this study still has inherent inferential limitations. The machine learning results essentially reflect predictive associations among variables rather than strict causal proof. The proposed deamination, dealkylation, and delignification pathways, together with the associated precursor–product relationships, were mainly inferred from FT-ICR MS molecular formula information and exact mass-difference matching, rather than being directly verified as specific structural conversion processes. Likewise, the interpretation of humification pathways was based on a combined analysis of the roles of precursor substances in the polyphenol humification pathway and the Maillard reaction, together with the overall tendency of DOM to evolve toward higher oxidation, greater aromaticity, and stronger condensation. Given the possibility of false positives in mass-association analysis, future studies should further validate the reliability and chemical plausibility of these inferred relationships using approaches such as stable isotope labeling. Nevertheless, this analytical framework still offers clear advantages for identifying the major transformation directions of DOM and the possible mechanisms underlying humification. Furthermore, future studies could incorporate an equivalent free-Mn(II) treatment and a physical mixture of pristine biochar and Mn salts, together with Mn-release kinetics, to better distinguish the relative contributions of the biochar matrix, Mn species, and fungal metabolism, thereby providing deeper mechanistic insights into MBC-enhanced WRF-mediated humification.

From a practical perspective, the MBC-WRF synergistic system shows potential for enhancing lignocellulosic humification, although its environmental safety and applicability boundaries still require further evaluation. Although Mn loading helped improve oxidative activation in the present system, the risks of Mn release, migration, and leaching in soil environments cannot be neglected, and their long-term ecological effects remain to be clarified. In addition, while 5% MBC showed a favorable promoting effect in the present model system,

the optimal application rate in practical composting may still be influenced by amendment cost, Mn loading, and substrate heterogeneity. Therefore, future pilot-scale studies across different organic waste systems are still needed to optimize the minimum effective dosage and evaluate its economic feasibility, while its long-term stability and application potential under soil environmental conditions should also be further verified.

4 Conclusions

This study demonstrated that MBC effectively enhanced WRF-mediated lignocellulose humification. MBC promoted lignocellulose depolymerization by improving the fungal colonization environment and strengthening the synergistic interaction between extracellular enzymatic activity and Mn-mediated radical processes, while also accelerating precursor generation and transformation, thereby driving organic matter toward more oxidized, aromatic, and stable humic-like substances. Combined with FT-ICR MS, paired mass-difference association analysis, interpretable machine learning, and multi-omics analyses, this study provides new mechanistic insights into how MBC enhances WRF-mediated lignocellulose humification and offers a theoretical basis for optimizing artificial humification technologies and the valorization of lignocellulose-rich organic wastes.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s42773-026-00659-z>.

Supplementary Material 1.

Supplementary Material 2.

Acknowledgements

We acknowledge funding support from the National Natural Science Foundation of China, the International Cooperation Project of Shanghai Science and Technology Commission, and the National Research Foundation of Korea (NRF) by the Ministry of Education.

Author contributions

Feng Wang: Investigation, Validation, Data curation, Visualization, Writing—Original draft. Linjie Zhang: Data curation. Feng Zhou: Investigation. Wenyue Wang: Investigation. Yinglong Su: Writing—Reviewing and Editing. Dongmei Zhou: Writing—Reviewing and Editing. Seungkwan Hong: Writing—Reviewing and Editing. Min Zhan: Supervision, Writing—Reviewing, and Editing. Bing Xie: Conceptualization, Methodology, Writing—Reviewing, and Editing. All authors read and approved the final manuscript.

Funding

This research was supported by the National Natural Science Foundation of China (22276059 and 22676067), the International Cooperation Project of Shanghai Science and Technology Commission (24230712100), and the National Research Foundation of Korea (NRF) by the Ministry of Education (RS-2024-00463837).

Data availability

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare no competing financial interest.

Author details

¹Shanghai Engineering Research Center of Biotransformation on Organic Solid Waste, School of Ecological and Environmental Sciences, East China Normal University, Shanghai 200241, China. ²State Key Laboratory of Pollution Control and Resources Reuse, School of Environment, Nanjing University, Nanjing 210023, China. ³Department of Civil, Environmental and Architectural Engineering, Korea University, 145, Anam-Ro, Seongbuk-Gu, Seoul 02841, Republic of Korea.

Received: 20 November 2025 Revised: 19 August 2026 Accepted: 14 September 2026

Published online: 09 October 2026

References

- Auer E, Lazuka A, Huguenin-Bizot B, Jehmlich N, Déjean S, Lombard V, Henrisat B, O'Donohue M, Hernandez-Raquet G (2023) Horizontal metaproteomics and CAZymes analysis of lignocellulolytic microbial consortia selectively enriched from cow rumen and termite gut. *ISME Commun* 3:134
- Benavides V, Serrano A, Pinto-Ibieta F, Rubilar O, Ciudad G (2025) Biodegradation of olive mill solid waste by *Anthracoophyllum discolor* and *Stereum hirsutum*: effect of copper and manganese supplementation. *Bioresour Bioprocess* 12:18
- Cai SY, Liu M, Zhang Y, Hu AB, Zhang WJ, Wang DS (2022) Molecular transformation of dissolved organic matter and formation pathway of humic substances in dredged sludge under aerobic composting. *Bioresour Technol* 364:128141
- Cai DQ, Lu YZ, Zhu YP, Wang DF, Shi JP, Liu L, Li JY, Zhan XP, Zhang WJ, Xu H (2025) Inducing hour-level humification of *Enteromorpha prolifera* to fabricate fulvic-like acid fertilizer with Fenton's reagent. *Nat Commun* 16:5860
- Chen L, Chen YN, Li YP, Liu YH, Jiang HJ, Li H, Yuan Y, Chen YR, Zou B (2023) Improving the humification by additives during composting: a review. *Waste Manag* 158:93–106
- Chirania P, Holwerda EK, Giannone RJ, Liang XY, Poudel S, Ellis JC, Bomble YJ, Hettich RL, Lynd LR (2022) Metaproteomics reveals enzymatic strategies deployed by anaerobic microbiomes to maintain lignocellulose deconstruction at high solids. *Nat Commun* 13:3870
- Dong ZQ, Wu Q, Huang Y, Zhao YF, Yang E, Xu HN, Wei DQ, Yan JP (2025) Strategies for designing microbial platforms to produce white rot fungal lignin-modifying enzymes: Insights from protein engineering and transcriptional regulation. *Bioresour Technol* 437:133114
- Dwinandha D, Elsamadony M, Gao RJ, Fu QL, Liu JB, Fujii M (2023) Interpretable machine learning and reactivities assisted isotopically labeled FT-ICR-MS for exploring the reactivity and transformation of natural organic matter during ultraviolet photolysis. *Environ Sci Technol* 58:816–825
- Forrester IT, Grabski AC, Burgess RR, Leatham GF (1988) Manganese, Mn-dependent peroxidases, and the biodegradation of lignin. *Biochem Biophys Res Commun* 157:992–999
- Gao XT, Tan WB, Zhao Y, Wu JQ, Sun QH, Qi HS, Xie XY, Wei ZM (2019) Diversity in the mechanisms of humin formation during composting with different materials. *Environ Sci Technol* 53:3653–3662
- Gao J, Li L, Yuan SJ, Chen SS, Dong B (2022) The neglected effects of polysaccharide transformation on sludge humification during anaerobic digestion with thermal hydrolysis pretreatment. *Water Res* 226:119249
- Guillén F, Martínez AT, Martínez MJ (1992) Substrate specificity and properties of the aryl-alcohol oxidase from the ligninolytic fungus *Pleurotus eryngii*. *Eur J Biochem* 209:603–611
- Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD (2007) Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Science* 315:804–807
- Hu J, Liu CG, Zhang WK, Liu XW, Dong B, Wang ZD, Xie YG, Hua ZS, Liu XW (2025) Decomposing the molecular complexity and transformation of dissolved organic matter for innovative anaerobic bioprocessing. *Nat Commun* 16:4859
- Khatami S, Deng Y, Tien M, Hatcher PG (2019) Formation of water-soluble organic matter through fungal degradation of lignin. *Org Geochem* 135:64–70
- Lee JG, Yoon HY, Cha JY, Kim WY, Kim PJ, Jeon JR (2019) Artificial humification of lignin architecture: top-down and bottom-up approaches. *Biotechnol Adv* 37:107416
- Li F, Zhang JL, Ma FY, Chen Q, Xiao QY, Zhang XY, Xie SX, Yu HB (2021) Lytic polysaccharide monooxygenases promote oxidative cleavage of lignin and lignin-carbohydrate complexes during fungal degradation of lignocellulose. *Environ Microbiol* 23:4547–4560
- Liang JJ, Fang XX, Lin YQ, Wang DH (2018) A new screened microbial consortium OEM2 for lignocellulosic biomass deconstruction and chlorophenols detoxification. *J Hazard Mater* 347:341–348
- Liu YF, Wang ML, Yin SJ, Xie LK, Qu XL, Fu HY, Shi Q, Zhou F, Xu FL, Tao S, Zhu DQ (2022) Comparing photoactivities of dissolved organic matter released from rice straw-pyrolyzed biochar and composted rice straw. *Environ Sci Technol* 56:2803–2815
- Liu JB, Wang CL, Hao ZE, Kondo G, Fujii M, Fu QL, Wei YS (2023a) Comprehensive understanding of DOM reactivity in anaerobic fermentation of persulfate-pretreated sewage sludge via FT-ICR mass spectrometry and reactivities analysis. *Water Res* 229:119488
- Liu JB, Wang N, Fujii M, Bao Z, Wei JY, Hao ZE, Fu QL, Gao RJ, Liu JF, Wei YS (2023b) Insights into the roles of DOM in humification during sludge composting: comprehensive chemoinformatic analysis using FT-ICR mass spectrometry. *Chem Eng J* 475:146024
- Liu QM, He XY, Wang KL, Li DJ (2023c) Biochar drives humus formation during composting by regulating the specialized metabolic features of microbiome. *Chem Eng J* 458:141380
- Lu HY, Liu SY, Shi Y, Chen QH (2022) Efficient delignification of sugarcane bagasse by Fenton oxidation coupled with ultrasound-assisted NaOH for biotransformation from *Agaricus sinodeliciosus* var. Chaidam. *Chem Eng J* 448:137719
- Mir-Tutusaus JA, Baccar R, Caminal G, Sarra M (2018) Can white-rot fungi be a real wastewater treatment alternative for organic micropollutants removal? A review. *Water Res* 138:137–151
- Qi HS, Wang JL, Zhang LY, Chen LH, Zhao Y, Wei ZM (2022) Activation effect of catechol on biotic and abiotic factors of humus formation during chicken manure composting. *Waste Manag* 149:146–155
- Qi HS, Gao WF, Xie LN, Zhang GG, Song CH, Wei ZM, Hu N, Li T (2024) Effect of MnO₂-biochar composites on promoting humification during chicken manure composting. *Biochar* 6:32
- Qin XY, Huang WY, Li QL (2024) Lignocellulose biodegradation to humic substances in cow manure-straw composting: characterization of dissolved organic matter and microbial community succession. *Int J Biol Macromol* 283:137758
- Schmidt F, Koch BP, Elvert M, Schmidt G, Witt M, Hinrichs KU (2011) Diagenetic transformation of dissolved organic nitrogen compounds under contrasting sedimentary redox conditions in the Black Sea. *Environ Sci Technol* 45:5223–5229
- Sun K, Niu ZY, Xiao SH, Qi XM, Li SY, Chen MH, Dai LZ, Si YB (2024) Artificially regulated humification in creating humic-like biostimulators. *Npj Clean Water* 7:47
- Svensson T, Redon PO, Thiry Y, Montelius M, Bastviken D (2022) Chlorination of soil organic matter: the role of humus type and land use. *Sci Total Environ* 806:150478
- Tang RL, Yao S, Liu Y, Ren TY, Ma JY, Gong XY, Li GX, Ma RN, Yuan J (2025) Iron-modified biochar enhanced nitrogen retention during composting: bridging chemisorption and microbiome modulation. *Chem Eng J* 513:162761
- Wang F, Zhou F, Zhang LJ, Liu WJ, Su YL, Zhang YC, Hong SK, Zhan M, Xie B, Zhou YB (2025) Mechanisms of manganese-modified biochar and white-rot fungi in enhancing compost humification: boosting polyphenol pathway by lignocellulose degradation. *Chem Eng J* 507:160637
- Watts RJ, Sarasa J, Loge FJ, Teel AL (2005) Oxidative and reductive pathways in manganese-catalyzed Fenton's reactions. *J Environ Eng* 131:158–164
- Wei ZM, Mohamed TA, Zhao L, Zhu ZC, Zhao Y, Wu JQ (2022) Microhabitat drive microbial anabolism to promote carbon sequestration during composting. *Bioresour Technol* 346:126577

- Wu D, Xia TY, Zhang YX, Wei ZM, Qu FT, Zheng GR, Song CH, Zhao Y, Kang KJ, Yang HY (2021) Identifying driving factors of humic acid formation during rice straw composting based on Fenton pretreatment with bacterial inoculation. *Bioresource Technol* 337:125403
- Wu FY, Guo Z, Cui KP, Dong DZ, Yang X, Li J, Wu ZZ, Li LL, Dai YD, Pan T (2023) Insights into characteristics of white rot fungus during environmental plastics adhesion and degradation mechanism of plastics. *J Hazard Mater* 448:130878
- Xie T, Zhang ZH, Yu YL, Tian Y, Wang F, Li DY, Nan J, Feng YJ (2023) Aeration intensity drives dissolved organic matter transformation and humification during composting by regulating the organics metabolic functions of microbiome. *Chem Eng J* 476:146645
- Xu R, Zhang K, Liu P, Han HW, Zhao S, Kakade A, Khan A, Du DL, Li XK (2018) Lignin depolymerization and utilization by bacteria. *Bioresource Technol* 269:557–566
- Yang XS, Feng K, Wang S, Yuan MM, Peng X, He Q, Wang DR, Shen WL, Zhao B, Du XF, Wang YC, Wang LL, Cao D, Liu WZ, Wang JJ, Deng Y (2024) Unveiling the deterministic dynamics of microbial meta-metabolism: a multi-omics investigation of anaerobic biodegradation. *Microbiome* 12:166
- Zhang ZH, Rao YF, Ye MY, Zou DX, Liu RF, Liu YP (2024) Reduction of inhibitory effect of lignin via degradation and improvement of anaerobic digestion performance of maize straw by oxidative pretreatment with Fe²⁺-activated persulfate. *Renew Energ* 231:120795
- Zhao XY, Xu KL, Wang JW, Wang ZQ, Pan RK, Wang Q, Li SL, Kumar S, Zhang ZQ, Li RH (2022) Potential of biochar integrated manganese sulfate for promoting pig manure compost humification and its biological mechanism. *Bioresource Technol* 357:127350
- Zhou F, Wang F, Xing H, Li S, Kim Y, Li P, Su Y, Lee Y, Zhou D, Fujii M, Zhan M, Xie B, Jiang G (2026) Landfill leachate management toward zero liquid discharge: Molecular-level characterization, process selectivity, and engineering regulation of DOM. *Water Res* x 32:100591
- Zhu YC, Jin Y, Liu XS, Miao TL, Guan QK, Yang R, Qu JJ (2021) Insight into interactions of heavy metals with livestock manure compost-derived dissolved organic matter using EEM-PARAFAC and 2D-FTIR-COS analyses. *J Hazard Mater* 420:126532
- Zhu YP, Cao YX, Fu BB, Wang CJ, Shu SH, Zhu PJ, Wang DF, Xu H, Zhong NQ, Cai DQ (2024) Wastemilk humification product can be used as a slow release nano-fertilizer. *Nat Commun*. <https://doi.org/10.1038/s41467-023-44422-5>