



## Synthetic microbial community enhances lignocellulose degradation during composting by assembling fungal communities

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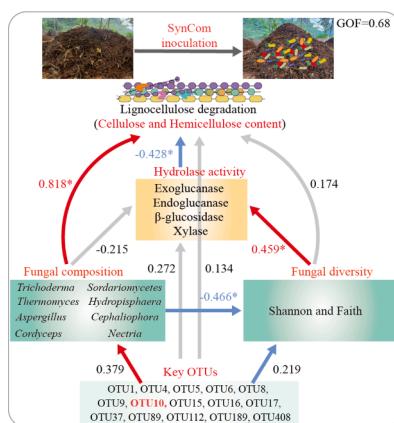
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### HIGHLIGHTS

- SynComs enhanced cellulose and hemicellulose degradation by 26.2% and 14.3%.
- OTU10 is critical in fungal diversity increase and lignocellulose degradation.
- The altered fungal community stimulated the fungal function of secreting hydrolase.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

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### ABSTRACT

Inoculating synthetic microbial community (SynCom) has been proposed as an eco-friendly approach for lignocellulose degradation in composting to enhance organic fertilizer quality. However, the mechanisms responsible for SynCom-regulated lignocellulose degradation during composting remain unclear. Here the SynCom inoculation decreased cellulose and hemicellulose contents by 26.2% and 14.3%, respectively, at the mature phase, while increasing endoglucanase, exoglucanase, and  $\beta$ -glucosidase activities significantly. SynCom inoculation increased the abundance of *Cephaliophorales* and *Thermomyces* at the mesophilic phase, *Sordariomycetes* at the thermophilic phase, and *Thermomyces*, *Acremonium*, *Aspergillus*, and *Sordariomycetes* at the mature phase, as well as increased the abundance of numerous Operational Taxonomic Units (OTUs), with OTU10 (*Hydropiophora*) being responsible for lignocellulose degradation. The altered fungal community stimulated functions of

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the wood saprotroph, undefined saprotroph, and litter saprotroph were responsible for lignocellulose degradation via changing microbial community. The results suggest that SynCom inoculation effectively stimulate lignocellulose degradation, so that benefits quality improvement of organic fertilizer.

## 1. Introduction

Approximately five billion tons of nutrient-rich agricultural waste are produced each year globally (Golovko et al., 2022). If inadequately treated, these wastes can serve as breeding grounds for pathogen growth, heavy metal accumulation, antibiotics aggregation, toxic gas production (such as SO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O), and carcinogen biosynthesis (e.g., polycyclic aromatic hydrocarbons, furans, and dioxins), resulting in resource waste, environmental pollution, soil quality reduction, and human health threat (Gaur et al., 2020). Composting has been found to be a viable, efficient, and environmentally friendly approach for degrading hazardous substances, thereby facilitating the safe conversion of organic waste into beneficial organic fertilizers (Li et al., 2022). Lignocellulose is the most abundant organic components of agricultural waste that play a pivotal role in organic matter transformation, N<sub>2</sub>O reduction, and humus formation during composting (Greff et al., 2022). As the major constituents of lignocellulose, cellulose and hemicellulose degradation presents a significant obstacle to the large-scale industrial application of biological composting. This is attributed to the highly resistant molecular hydrogen bonds among glucose units, resulting in the formation of compounds characterized by high crystallinity, mechanical strength, and chemical stability (Deng et al., 2023). Therefore, more efficient strategies are urgently required to enhance lignocellulose degradation during composting.

The degradation of lignocellulose necessitates the coordination of several hydrolase enzymes, including endoglucanase, exoglucanase, β-glucosidase, and xylanase (Gharechahi et al., 2023). The abundant microbiomes such as *Trichoderma reesei*, *Aspergillus fumigatus*, *Myceliophthora thermophila*, and *Penicillium verruculosum*, involved in the composting process play a pivotal role in independently or collaboratively facilitating the degradation of lignocellulose and the transformation of organic matter by secreting specific hydrolase enzymes (Hu et al., 2019; Qu et al., 2023). The inoculation of lignocellulose-degrading microbes could successfully survive in complex composting environment by resisting bio/abiotic stress, thereby coordinating various native microbiomes to encode cellulose-degrading genes for promoting lignocellulose degradation (Rastogi et al., 2020). However, only a small proportion of microbes can effectively drive lignocellulose degradation. The hydrolase enzymes secreted during composting are prone to denaturation and inactivation due to high temperature, alkaline pH, and other biotic and abiotic stresses encountered during composting (Baig, 2020). Therefore, exploring effective strategies is undoubtedly urgent for maintaining microbial activity and preventing hydrolase inactivation.

Inoculation of lignocellulose-degrading microbes present a promising solution to accelerate lignocellulose degradation. These microbes harbor genes of carbohydrate-active enzymes can produce stable hydrolases during composting (Nigussie et al., 2021). Various microbial taxa, predominantly fungi such as *Trichoderma reesei*, *Actinomyces cellulosae*, *Aspergillus fumigatus*, *Globodera rosroehiensis*, *Clostridium thermocellum*, *Leucotermess peratus*, *Myrothecium* spp., *Trichoderma koningii*, and *Bacillus subtilis*, have been reported to promote lignocellulose degradation during composting (Liu et al., 2023b). Nevertheless, the effectiveness of inoculation with single microbial strain in promoting lignocellulose degradation is unstable owing to the limited survival and colonization capacity in composting (Bolan et al., 2023). Evidence shows that the inoculation with synthetic communities composed of multiple lignocellulose-degrading fungal and/or bacterial taxa is more effective than that with single strain (Nigussie et al., 2021). Synthetic microbial community can enhance the activity of hydrolases and the

survival rate of inoculants during composting by regulating the structure of composting microbiota. However, the underlying mechanisms by which SynCom stimulate the degradation of lignocellulose remain unclear.

Mulberry branches and silkworm excrement are typical agricultural wastes with rich lignocellulose in South China, which have the potential to produce high-quality organic fertilizer due to their rich nutrients. In the present study, the lignocellulose-degrading microbes consisting of *Bacillus cereus*, *Bjerkandera adusta*, *Trichoderma harzianum*, *Cladosporium cladosporioides*, and *Cladosporium tenuissimum* were isolated and constructed SynCom from composting samples. Subsequently, the SynCom was inoculated in composting of mulberry branches and silkworm excrement to explore their efficiency and underlying mechanism on lignocellulose degradation using fluorescence spectrometry and high-throughput sequencing techniques. As mentioned earlier, fungal microbes are the key promoters of lignocellulose degradation. Hence, the current study hypothesized that SynCom inoculation would effectively accelerate lignocellulose degradation by secreting hydrolase and regulating fungal communities. The main objectives were to investigate: (1) the efficiency of SynCom inoculation in accelerating lignocellulose degradation; and (2) the biological mechanisms underlying the effects of SynCom inoculation on lignocellulose degradation during composting.

## 2. Materials and methods

### 2.1. Isolation of lignocellulose-degrading strains of composting

In our previous study on composting mulberry branches and silkworm excrement (Liu et al., 2022), culturable lignocellulose-degrading strains were successfully isolated from the four phases of composting using the gradient dilution coating method. A 1.0 g fresh sample of composting material was added to 10 mL of sterilized phosphate buffered saline (PBS) buffer, which was consisted of 0.15 M phosphate buffer and Tween 80, with a pH level of 7.0. The mixture was shaken at 180 rpm for 60 min as concentration of 10<sup>-1</sup> dilution, and then they were subsequently diluted into different concentration as follows: 100 μL of 10<sup>-1</sup> dilution was evenly mixed into 900 μL PBS as concentration of 10<sup>-2</sup> dilution, 100 μL of 10<sup>-2</sup> dilution was evenly mixed into 900 μL PBS as concentration of 10<sup>-3</sup> dilution, 100 μL of 10<sup>-3</sup> dilution was evenly mixed into 900 μL PBS as concentration of 10<sup>-4</sup> dilution, and 100 μL of 10<sup>-4</sup> dilution was evenly mixed into 900 μL PBS as concentration of 10<sup>-5</sup> dilution. Different concentrations of the mixture were prepared, and 200 μL of each diluent was subsequently plated on Potato Dextrose Agar (PDA) mediums supplemented with 1 % Carboxymethylcellulose sodium (CMC-Na) and 0.01 % congo red to determine the hydrolysis circle of different strains at 28 °C for 5 days. The single colony exhibiting a distinct hydrolysis circle was selected and transferred to a PDA plate for purification at 28 °C for 2 days. The selected strains were then inoculated in a sodium carboxymethyl cellulose medium and cultured at 28 °C for 5 days to determine their hydrolase production capacity, which allowed us to optimize the lignocellulose-degrading strains. Genomic DNA of the lignocellulose-degrading strain was extracted from 0.1 g of mycelium sample by the DNeasy Plant Mini Kit. The fungal primers ITS1F and ITS2 were used to amplify the fungal ITS1 regions for obtaining sequences (see Supplementary Materials). Data were retrieved from the BLAST database of the National Center for Biotechnology Information (NCBI) for DNA comparison, and MEGA (MEGA 11) was used to construct phylogenetic trees to identify species information and establish phylogenetic relationships.

## 2.2. Composting experiment

The composting experiment was conducted at Huanjiang Observation and Research Station for Karst Ecosystems, Chinese Academy of Sciences. Mulberry branches and silkworm excrement were used as raw materials for the composting experiment. The mulberry branches were crushed into fragments ranging from 0.2–1 cm. A composting pile of ~500 kg was prepared using a 1:9 ratio (w/w, dry weight basis) of mulberry branches to silkworm excrement, resulting in a C: N ratio of 25–28. Two treatments were included, i.e., composting with 450 kg silkworm excrement and 50 kg mulberry branches (Control), and composting with 450 kg silkworm excrement, 50 kg mulberry branches, and SynCom fermentation with a concentration of  $1 \times 10^8$  cells g<sup>-1</sup> compost (SynCom). Each treatment performed three replicates. The lignocellulose-degrading strains were isolated and inoculated on sterilized PDB medium (1000 mL) at 28 °C, 180 rpm min<sup>-1</sup> for 7 days. After shaking, these fermented liquid cultures were adjusted a density of  $1 \times 10^{10}$  cells mL<sup>-1</sup> based on dilution coating counting method, and then they were mixed in equal proportions to a SynCom. The composting pile was inoculated with the SynCom at a final density of  $1 \times 10^8$  cells g<sup>-1</sup>. Moisture content of the compost was initially adjusted to approximately 55–60 %, and there were no further adjustments throughout the composting process. Initial size of the compost was 500 kg (dry weight equivalent) for the two treatments. Each compost was equipped with five temperature probes at different locations to detect the temperature during composting process. The compost piles were turned up and down every 3 days. The composting phases in the current study were defined according to temperature variation (see *Supplementary Materials*), with the initial phase being 40–50 °C (day 1), the mesophilic phase being 50–55 °C (day 2 to 3), the thermophilic phase being 55–70 °C (day 4 to 16), and the mature phase being 35–50 °C (day 17 to 60). Sampling was performed at day 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60, respectively. Samples were obtained in different composting positions, and mixed as a composite sample for each compost. The composite samples were stored at –80 °C for the further determination of fungal community, lignocellulose content, and enzyme activities.

## 2.3. Detection of lignocellulose content

0.5 g of dried composting sample (W1) was extracted and defatted using acetone. The solid compounds were washed with boiling neutral detergent for 50 min to determine the dry weight (W2) of lignocellulose content, which included insoluble residues such as hemicellulose, cellulose, lignin, and silicate. The mixed precipitates were further washed with boiling acidic detergent for 50 min, followed by a rinse with distilled water to determine the dry weight (W3), which represented the reduced weight corresponding to the hemicellulose content. The remaining precipitates were hydrolyzed with 25 mL of 72 % H<sub>2</sub>SO<sub>4</sub> for 180 min to determine the reduced weight (W4) of the cellulose content. Finally, the residual precipitates were subjected to a muffle furnace for 180 min at 600 °C to detect the content of lignin (W5). Hemicellulose content was calculated as the difference between W2 and W3, and cellulose content was the difference between W3 and W4.

## 2.4. Assessment of lignocellulose structure changes

The changes in lignocellulose structure in composting samples were assessed using lectin Concanavalin A (200 mg L<sup>-1</sup>) as a fluorescent stain, employing different excitation and emission spectra. The method described in previous study was used to measure the change of lignocellulose structure (Liu et al., 2023b).

## 2.5. Determination of enzyme activities

0.1 mL of crude enzyme extract was added to 2.0 mL of 1 % sodium carboxymethyl cellulose solution and incubated in a 50 °C water bath for

60 min. Then 3 mL of DNS buffer was added to the reaction solution and boiled for 10 min before measuring the absorbance at 520 nm. The activities of exoglucanase and β-glucosidase were determined using 4-Nitrophenyl-β-D-glucopyranoside (pNPG) and P-Nitrobenzene cellobioside (pNPC) as substrates, respectively (Wang et al., 2019). The absorbance at 402 nm was measured after adding 100 μL of 1 M Na<sub>2</sub>CO<sub>3</sub> buffer. The activity of xylanase was determined by a previous study (Wu et al., 2020). 200 μL of crude enzyme extract was added to 100 μL of 1 % xylan solution and 80 μL of acetate buffer. The reaction solution was then added with 200 μL of 1 M dinitrosalicylic acid (DNS) buffer, with the total volume adjusted to 2.5 mL. Glucose was used to detect standard curve of endoglucanase, exoglucanase, β-glucosidase, and the xylan was used to detect standard curve of xylanase.

## 2.6. Analysis of Illumina sequencing

Genomic DNA was extracted from 0.25 g of composting samples collected from different phases. The fungal ITS1 region was amplified and sequenced on the Illumina MiSeq platform (Majorbio Co., Ltd., Shanghai, China) for high-throughput MiSeq sequencing (Illumina). All sequences were deposited in the NCBI Sequence Read Archive database, with the accession number PRJNA1184388. Raw fungal sequences were split by using QIIME (<https://view.qiime2.org/>). Fungal sequences with a length shorter than 200 bp or errors > 1.0 were removed to generate operational taxonomic units (OTUs) by using the UPARSE. To investigate potential differential taxa and explore the core microbes, taxonomic networks of the selected fungal OTUs were generated by removing sequences with an abundance < 150, a fold change < 4, and a  $p > 0.05$ .

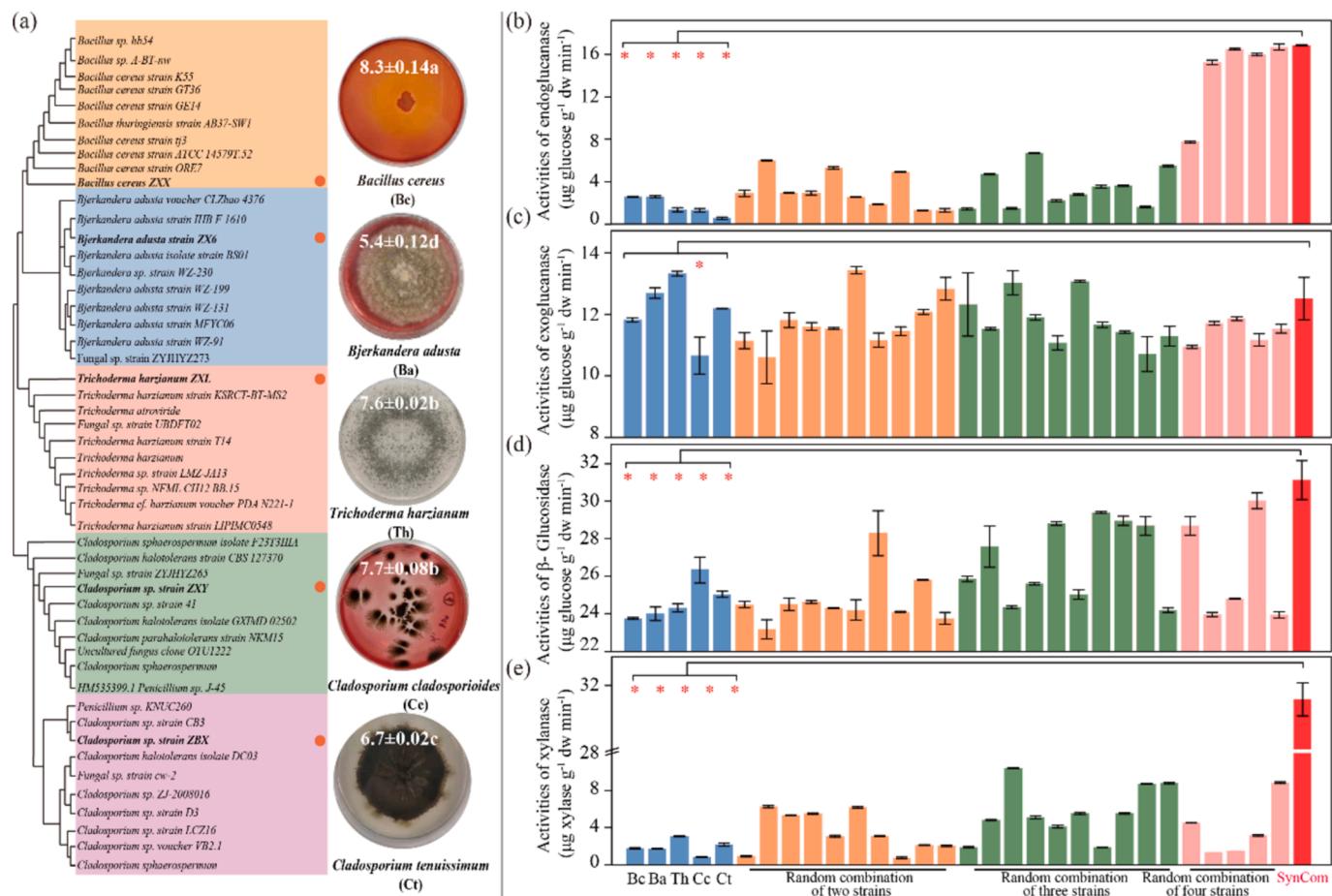
## 2.7. Statistical analyses

One way analysis of variance (ANOVA) with the least significant difference (LSD) test was used to examine the difference for each variable ( $p < 0.05$ ) among the lignocellulose-degrading microbes. The significance between the inoculation and un-inoculation of SymCom was examined by Student's *t*-test. The dates were visualized using R software (Version 3.5.0). Indicator OTUs were displayed on a taxonomic tree generated by iTOL (<https://itol.embl.de/>) at the phylum level (see *Supplementary Materials*). Spearman rank correlation values were calculated using the corrr function in R from the "psych" package. α-diversity indices of the fungal community, including Shannon, Simpson, Chao1, and richness, were calculated using the "vegan" package. Fungal community functions were annotated based on FUNGuild analysis using the "STAMP" package. Random Forest analysis was used to explore the main microbial predictors of lignocellulose degradation using the "rfPermute" package. The Partial Least Squares Path Modeling (PLS-PM) was constructed using the "plspm" package to illustrate the mechanism underlying effects of SynCom inoculation on lignocellulose (Ma et al., 2023).

## 3. Results and discussion

### 3.1. Effects of lignocellulose-degrading microbes on lignocellulose degradation

Based on the analysis of community composition and selective cultivation of composting samples in the present study, five culturable strains including *Bacillus cereus*, *Bjerkandera adusta*, *Trichoderma harzianum*, *Cladosporium cladosporioides* and *Cladosporium tenuissimum* were isolated during composting in the present study, with the hydrolysis circle diameters being  $8.3 \pm 0.14$  cm,  $5.4 \pm 0.12$  cm,  $7.6 \pm 0.02$  cm,  $7.7 \pm 0.08$  cm and  $6.7 \pm 0.02$  cm, respectively (Fig. 1a). The hydrolase activities of lignocellulose-degrading strains and their random combinations under solid fermentation were evaluated. Inoculating SynCom and random combinations of four strains significantly increased the



**Fig. 1.** Isolation of lignocellulose-degrading strains and their effects on hydrolase activities during solid fermentation. (a) Sequence identification and phylogenetic tree construction of lignocellulose-degrading strains. The red data (mean  $\pm$  standard error) indicate the hydrolysis circle values ( $n = 6$ ). Effects of lignocellulose-degrading strains and their random interaction combinations on the activities of exoglucanase (b), endoglucanase (c),  $\beta$ -glucosidase (d), xylanase (e) during solid fermentation. Bc, Ba, Th, Cc and Ct represent the lignocellulose-degrading strains of *Bacillus cereus*, *Bjerkandera adusta*, *Trichoderma harzianum*, *Cladosporium cladosporioides* and *Cladosporium tenuissimum*, respectively. Values from panels (b) to (e) are presented as means  $\pm$  standard errors ( $n = 6$ ). The asterisk \* denotes significant difference at  $p < 0.05$  among treatments.

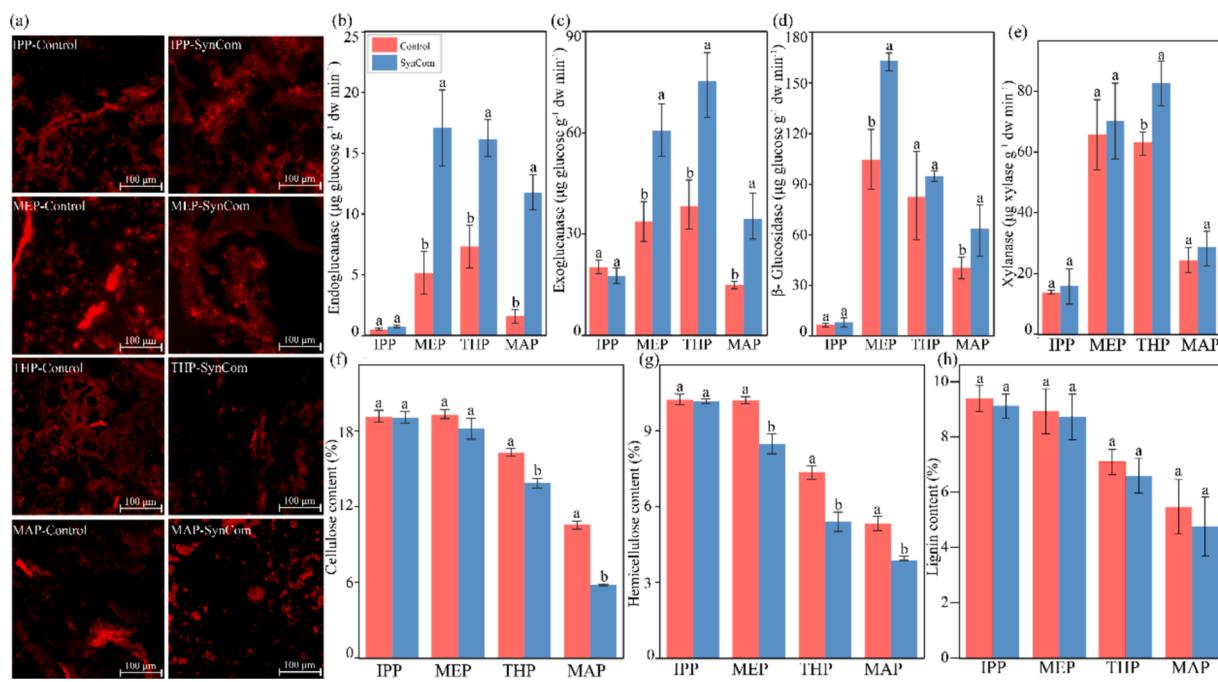
activities of endoglucanase compared to single strain inoculation and random combinations of two or three strains (Fig. 1b). However, exoglucanase activity under SynCom inoculation did not show a significant advantage compared to that under inoculation with one to four strains (Fig. 1c). The activities of  $\beta$ -glucosidase and xylanase were highest under SynCom inoculation compared to single strain inoculation or random combinations of two or three strains (Fig. 1d, e). Therefore, the combination of multiple strains significantly enhanced lignocellulose degradation compared to single strain inoculation by secreting hydrolases.

The construction of SynComs enables cooperation with native lignocellulosic microbes. This cooperation leads to the production of hydrolase enzymes by successfully colonizing ecological niches and assembling the compost microbiome that supports the survival of native microbes through metabolite acquisition and exchange (Negi et al., 2024). Efficient lignocellulose degradation relies on the expression of carbohydrate-active enzyme (CAZyme) genes and the activity of extracellular hydrolases (Shinde et al., 2022). A previous study reported that lignocellulose-degrading microbes such as *Bacillus cereus*, *Bjerkandera adusta*, *Trichoderma harzianum*, and *Cladosporium* spp. inoculation increased six families and 57 subfamilies of carbohydrate-active enzyme CAZyme by effectively occupying cellulose-rich niches, encoding 145 genes for lignocellulose degradation, and withstanding high-temperature stress (Dang et al., 2021). Considering the complexity and heterogeneity of the composting environment, lignocellulose

degradation under SynCom inoculation demonstrated superior colonization and growth compared to single strain inoculation. The interaction of synthetic microbial communities ensures spatiotemporal coordination, stability of enzyme systems, cell energy, metabolic exchange, reinforcing microbial community functions by combining various hydrolytic enzymes with high efficiency, strong functionality, and good controllability (Großkopf and Soyer, 2014). It has been suggested that SynCom inoculation can increase the capacity of stress resistance and organic matter transformation of composting microbial community. This process can lead to an increased expression of CAZyme genes and hydrolase activities, thereby promoting lignocellulose degradation.

### 3.2. Effects of SynCom inoculation on lignocellulose degradation during composting

In the current study, the degree of lignocellulose fragmentation was significantly increased by SynCom inoculation at the mature phases of composting, compared to the control, indicating a synergistic effect among SynCom strains on lignocellulose degradation (Fig. 2a). The activities of endoglucanase, exoglucanase, and  $\beta$ -glucosidase were significantly higher under SynCom inoculation at the mesophilic and mature phases, while the activities of endoglucanase, exoglucanase, and xylanase were significantly higher at the thermophilic phase (Fig. 2b–e). The cellulose and hemicellulose contents were significantly reduced by 26.2 % and 14.3 %, respectively, under SynCom inoculation compared to the



**Fig. 2.** Effects of synthetic microbial community inoculation on lignocellulose degradation and hydrolase enzyme activities at different composting phases. (a) Fluorescence intensity of lignocellulose structure treated with Calcofluor at different composting phases. Effects of synthetic microbial community inoculation on the activities of endoglucanase (b), exoglucanase (c),  $\beta$ -glucosidase (d), xylanase (e) at different composting phases. Effects of synthetic microbial community inoculation on cellulose content (f) and hemicellulose content (g) at different composting phases. IPP represent initial phase, MEP represent mesophilic phase, THP represent thermophilic phase and MAP represent mature phases of composting, respectively. Different letters represent significant differences at the level  $p < 0.05$  between synthetic microbial community inoculation and control.

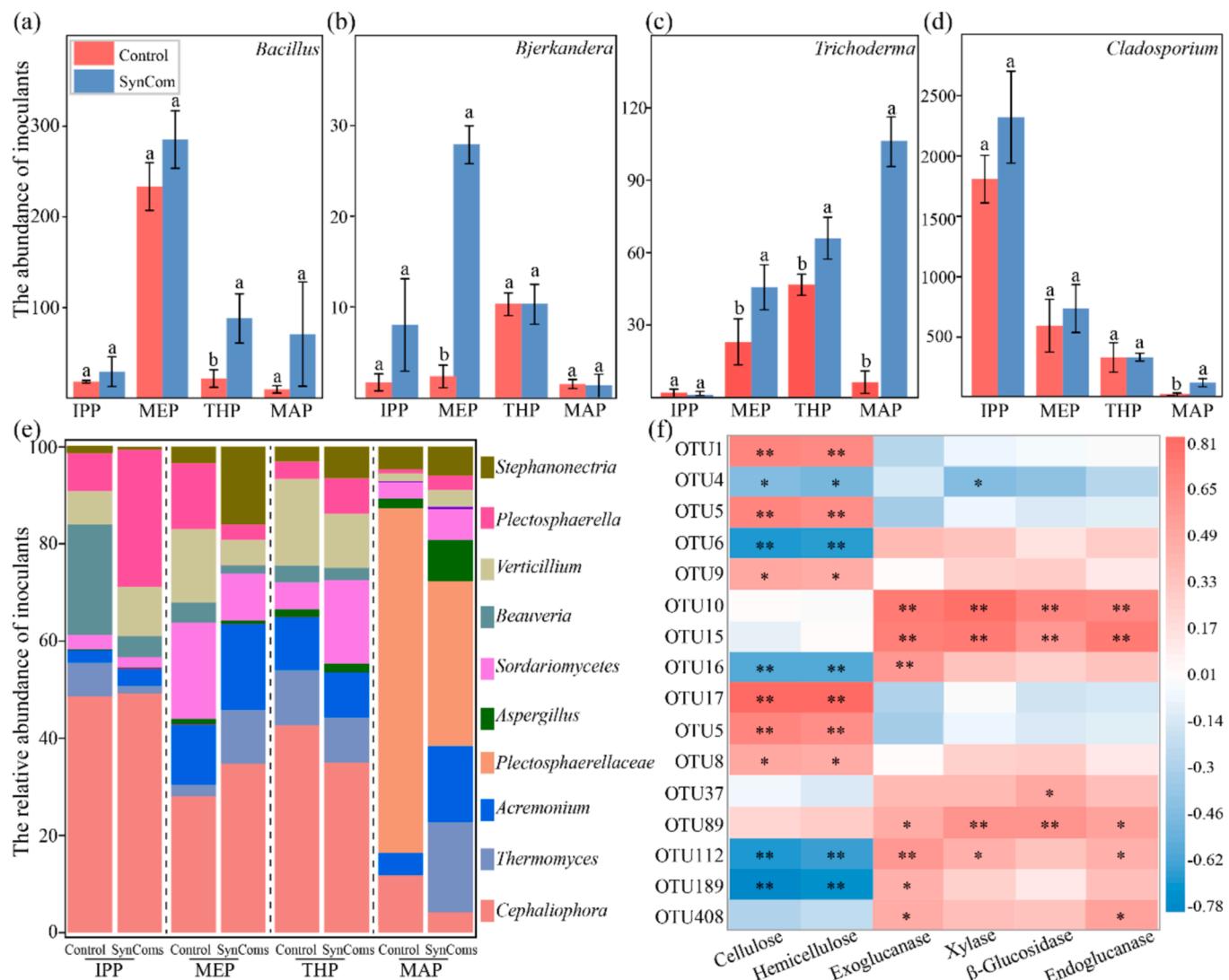
control (Fig. 2f, g). However, the lignin content did not significantly decrease under SynCom inoculation (Fig. 2h). Lignocellulose degradation primarily occurs at the thermophilic phase of composting, providing nutrients for microbial survival at the mesophilic phase and synthesizing precursor substances for humus formation at the mature phase (Liu et al., 2023a). At the thermophilic phase of composting, many lignocellulose degradation microbes poorly adapt to environmental changes and either die or become dormant. However, the strains resistant to high temperatures and complex environmental stresses remain active in lignocellulose degradation (Ezugworie et al., 2021). Previous study reported that the inoculation of SynCom promoted lignocellulose degradation by stimulating hydrolase enzyme production via successfully colonizing more ecological niches, and increasing the survival of native microbes through metabolite acquisition and assembling the compost microbiome (Qian et al., 2020). This enhancement mainly involved synergistic effects to increase hydrolase activity, ensure microbial colonization, and withstand environmental stress, ultimately altering the biochemistry metabolism of lignocellulose degradation and shaping specific microbial structures (Zhao et al., 2021). Therefore, the increased activities of endoglucanase,  $\beta$ -glucosidase, and xylanase, as well as the degradation of cellulose and hemicellulose following SynCom inoculation, suggested that SynCom could efficiently stimulate the production of lignocellulose degradation-related enzymes, thereby facilitating the degradation process.

Previous studies also have provided supporting evidence for the role of specific microorganisms in promoting lignocellulose degradation during composting. For instance, *Gloeophyllum* sp., *Actinomycetes* sp., *Trichoderma longibrachiatum*, *Bacillus* sp., *Aspergillus fumigatus*, *Penicillium* sp., and *Azotobacter* sp. have been identified as key contributors to the biosynthesis of hydrolases and the enhancement of lignocellulose degradation at different phases of composting (Zhu et al., 2021). Thermophilic *Aspergillus fumigatus* and *Geobacillus stearothermophilus* have been shown to increase temperature at the mesophilic phase, promote cellulose and hemicellulose degradation through the secretion of

lignocellulolytic enzymes at the thermophilic phase, and improve humus formation at the maturity phase (Liu et al., 2022). Similarly, *Bacillus thermoamylorans*, *Geobacillus thermodenitrificans*, *Trichoderma lanuginosus*, and *Aspergillus fumigatus* have been identified as potential candidates for producing thermozyomes and high enzyme activities (López et al., 2021). Moreover, the inoculation of *Clostridium thermocellum* and *Acetivibrio cellulolyticus* formed a large multienzyme complex, which increases their resistance to high temperatures and adverse stresses, thereby promoting lignocellulose degradation (Xu et al., 2016). However, the systematic biological mechanisms responsible for stimulating lignocellulose degradation through SynCom inoculation have not yet been fully investigated.

### 3.3. Effects of SynCom inoculation on fungal community during composting

The population of SynCom inoculants during composting indicated that the abundance of *Bacillus* genus significantly increased during composting, suggesting that SynCom of *Bacillus* genus inoculants effectively colonized the composting process (Fig. 3a). The abundance of *Trichoderma* genus at the mesophilic phases increased for both treatments. At the thermophilic and mature phases, the abundance of *Trichoderma* was significantly higher under SynCom inoculation compared to the control. Additionally, the abundance of *Bjerkandera* genus significantly increased at the mesophilic phase under SynCom inoculation while, there was no significant difference in the abundance of *Cladosporium* genus between the two treatments (Fig. 3b-d). Based on high-throughput sequencing, the most abundant genera of composting under SynCom inoculation were *Cephaliphoras* and *Acremonium*. The abundances of *Cephaliphoras* and *Thermomyces* were higher at the mesophilic phase, and the abundance of *Sordariomyces* increased at the thermophilic phase. The abundances of *Thermomyces*, *Acremonium*, *Aspergillus*, and *Sordariomyces* were higher at the mature phase. However, under SynCom inoculation, the abundances of *Sordariomyces*



**Fig. 3.** Effects of synthetic microbial community inoculation on fungal community composition and diversity at different composting phases. Abundance of synthetic microbial community, including *Bacillus* (a), *Trichoderma* (b), *Bjerkandera* (c) and *Cladosporium* (d) at the genus level at different composting phases. (e) Relative abundance at genus level. (f) Correlation between key operational taxonomic units (OTUs) and enzymatic activities, cellulose content and hemicellulose content. IPP represent initial phase, MEP represent mesophilic phase, THP represent thermophilic phase and MAP represent mature phases of composting, respectively. Different letters represent significant differences at the level  $p < 0.05$  between synthetic microbial community inoculation and control. \*\* and \* denote significant difference at  $p < 0.01$  and  $p < 0.05$  levels, respectively.

and *Verticillium* decreased at the mesophilic phase, and the abundance of *Cephaliophoras* decreased at the thermophilic phase. Additionally, the abundances of *Cephaliophoras* and *Plectosphaerellaceae* were decreased under SynCom inoculation (Fig. 3e). These findings suggested that SynComs inoculation played an important role in assembling the compost microbiome.

The successful colonization and survival of microbial inoculants in a complex environment are crucial processes for ensuring the secretion of hydrolases and the regulation of microbial community function (Papin et al., 2024). Previous research has reported that *Bacillus* possesses desirable properties such as high-temperature resistance, acid resistance, oxidation resistance, revival ability, and stability. These properties contribute to survival and activity during composting, thereby accelerating organic transformation, promoting the production of extracellular hydrolases, and facilitating lignocellulose degradation (Mitra et al., 2021). The high microbial metabolic activity observed at the mesophilic phases can be attributed to the favorable temperature and adequate nutrient availability, which allowed most microbes to thrive (Witfeld et al., 2021). During the thermophilic phase, *Trichoderma*

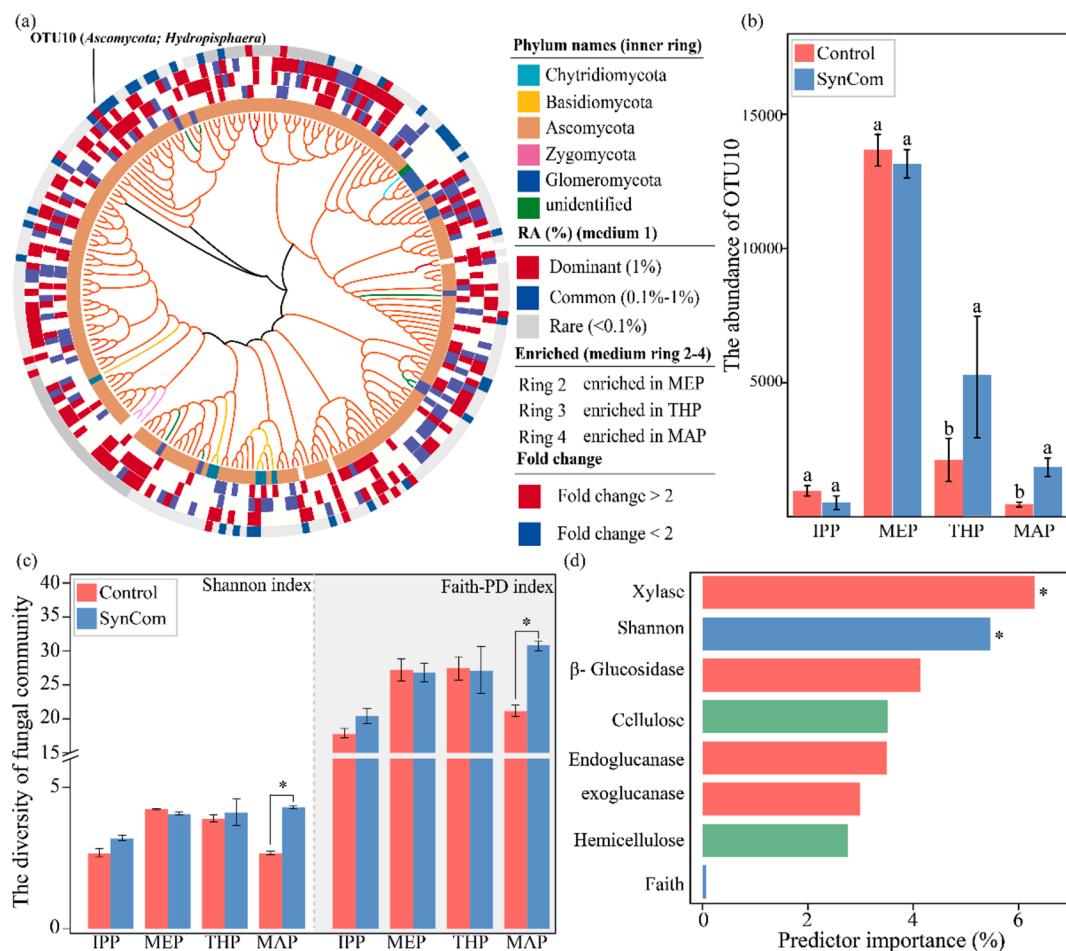
spp. could adapt to high temperatures and nutrient-poor conditions, leading to the secretion of large amounts of hydrolytic enzymes. In contrast, *Bjerkandera* has weaker resistance to high temperature, which may partially explain the current findings (Li et al., 2022; Moody et al., 2018). The changes in fungal community composition observed under SynCom inoculation could be attributed to the presence of abundant microbial inoculants during composting. These inoculants act as microbial regulators, selectively influencing microbial communities and altering microbial activities by stimulating interactions among microbial taxa (Zhao et al., 2021). Moreno et al. (2021) also reported that *Thermomyces*, *Cephaliophora*, *Sordariomycetes*, *Aspergillus*, and *Acremonium* were key players in hydrolase secretion and lignocellulose degradation during composting, with *Thermomyces* and *Cephaliophora* being frequent thermotolerant species, and *Sordariomycetes*, *Aspergillus*, and *Acremonium* displaying strong decomposing abilities. Therefore, SynCom inoculation and its induced alteration in fungal community composition could partly explain the increase of hydrolase activities and lignocellulose degradation during composting in the current study.

### 3.4. Effects of SynCom inoculation on key OTUs on lignocellulose degradation

A total of 2663 operational taxonomic units (OTUs) were selected for the two treatments (see [Supplementary Materials](#)). Among them, a few OTUs showed significant alterations at different phases of composting. Specifically, OTU9, OTU17, OTU15, OTU6, OTU16, OTU37, OTU8, OTU112, and OTU406 were significantly altered at the mesophilic phase, while OTU37 was significantly enriched at the thermophilic phase. OTU4, OTU9, OTU10, and OTU17 showed significant alterations at the mature phase. Some of these fungal taxa, including OTU1, OTU4, OTU5, OTU6, OTU8, OTU9, OTU16, OTU17, OTU112, and OTU89, were significantly correlated with cellulose and hemicellulose content. Furthermore, OTU10, OTU15, OTU89, and OTU112 were significantly correlated with the activities of exoglycanase, xylase, endoglucanase, and  $\beta$ -glucosidase ([Fig. 3f](#)). The fungal taxa *Sordariomycetes* (OTU8, OTU9, OTU15, and OTU17) and *Thermomyces* (OTU6) possess numerous genes encoding lignocellulose hydrolases for participating in carbohydrates degradation of composting ([Wang et al., 2020](#)). Based on phylogenetic relationships, fold change, relative abundance at the phylum level, and *p*-value, a total of 219 OTUs exhibited differences between SynCom inoculation and the control (see [Supplementary Materials](#)).

Among these fungal taxa, one dominant taxon, OTU10, which was assigned as *Hydropisphaera* sp., showed a particularly striking pattern during composting (SynCom inoculation vs control at the mesophilic, thermophilic, and mature phases: ANOVA; Fold change > 2 or < 2; relative abundance > 1%; [Fig. 4a](#)). OTU10, OTU89, and OTU112, on the other hand, demonstrated the ability to survive high temperatures and other stressful conditions. The abundance of OTU10 was significantly higher under SynCom inoculation than the control at the thermophilic and mature phases ([Fig. 4b](#)). As dominant thermotolerant genera, they decomposed lignocellulose by secreting lignocellulolytic enzymes during composting ([Gołębiewski et al., 2019](#); [Wilhelm et al., 2019](#)). Consequently, the changes in these OTUs are likely responsible for stimulating lignocellulose degradation by influencing microbial activity, reshaping the fungal community, and changing hydrolase enzyme activities in the current study.

SynCom inoculation significantly increased the Shannon or Faith-PD indices at the mature phase. However, the Chao1, evenness, and phylogenetic diversity were not significantly changed under SynCom inoculation during composting ([Fig. 4c](#)). As composting progressed, most microbes poorly survived in composting environment, but some microbes can resist high temperatures and biotic/abiotic stress and became active in cellulose degradation ([He et al., 2022](#); [Meng et al.,](#)



**Fig. 4.** Key operational taxonomic units (OTUs) of fungal community related to lignocellulose degradation. (a) Cladogram showing phylogenetic relationships among OTUs of fungal community composition during composting. Leaf labels indicate representative sequence IDs. Rings, from the inner to the outside circles, represent: 1) phylum-level taxonomy of OTUs. 2) fold change of OTUs between SynCom inoculation vs control at the thermophilic phase. 3) fold change of OTUs between SynCom inoculation vs control at the mature phase. 4) *p* value of OTUs between SynCom inoculation vs control both at thermophilic and mature phases. 5) variable pattern of OTU relative abundance (> 1% or 0.1%). (b) the abundance of OTU10. (c) Shannon index and Faith-PD index at different composting phases. (d) contributions of OTU10 abundance to lignocellulose degradation (enzymatic activities, cellulose, and hemicellulose content) and fungal community diversity according to Random Forest analysis. IPP represent initial phase, MEP represent mesophilic phase, THP represent thermophilic phase and MAP represent mature phases of composting, respectively. Different letters represent significant differences at the level of  $p < 0.05$  between synthetic microbial community inoculation and control.

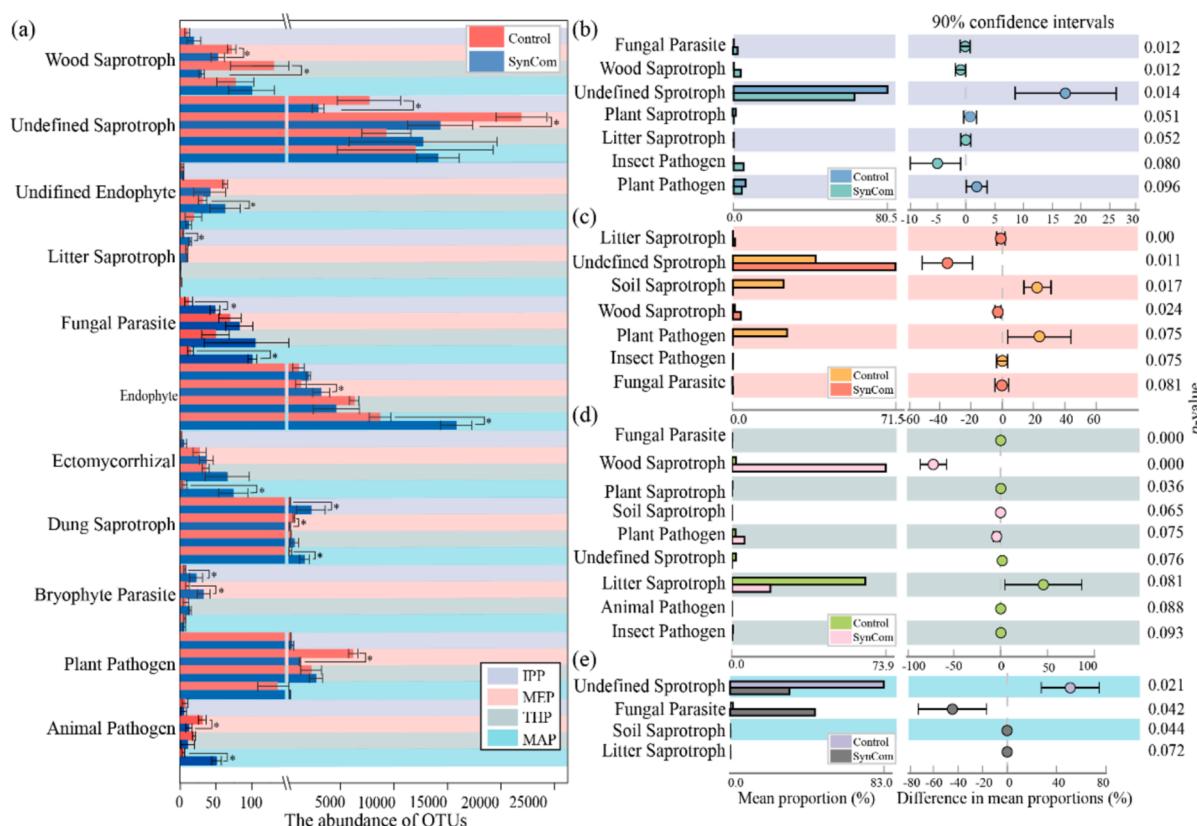
2019). The increased Faith-PD or Shannon indices under SynCom inoculation may be due to the assemble of microbial communities for lignocellulose degradation (Rizzo et al., 2022). Therefore, the mechanisms underlying changes in fungal community composition and diversity under SynCom inoculation were likely related to alterations in microbial composition, lignocellulose content, and enzyme activities.

### 3.5. Effects of core fungal species on lignocellulose degradation under SynCom inoculation

Random forest analysis revealed that activities of xylanase and  $\beta$ -glucosidase, and Shannon index were the strong predictors of the change in OTU10, with an increase in mean squared error (MSE) of 6.3 %, 5.5 %, and 4.1 %, respectively (Fig. 4d). This implied that OTU10 substantially stimulated the activities of xylanase and  $\beta$ -glucosidase, and Shannon index under SynCom inoculation. Previous study has provided evidence that *Hydropisphaera* genus could successfully hyperparasitize organic matter and encode numerous CAZyme genes, such as glycoside hydrolases (GH2, GH5, GH9, GH13, GH15, and GH79), carbohydrate esterases (CE4, CE7, CE43), auxiliary activities (AA1, AA2, and AA3), glycosyl transferases (GT1, GT2, GT4, GT9, GT20, GT22, GT26, and GT51), carbohydrate-binding modules (CBM6, CBM9, CBM13, CBM20, CBM35, CBM57, and CBM66), and polysaccharide lyases (PL9 and PL35), which are involved in lignocellulose degradation (Wardman et al., 2022). The present study highlighted significant response of OTU10 (*Hydropisphaera*) to SynCom inoculation, indicating its key role in lignocellulose degradation. Based on the Fungi Functional Guild

(FunGuild) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database, the fungal community was annotated and categorized into 11 functions (see Supplementary Materials). The four most dominant functions at the primary pathways for both treatments were Undefined Saprotoph, Endophyte, Dung Saprotoph, and Plant Pathogen (Fig. 5a). At the initial phase, the second layer pathways of fungal parasite and wood saprotoph significantly increased under SynCom inoculation compared to the control. At the mesophilic phase, the litter saprotoph, undefined saprotoph, and wood saprotoph showed significant increases. The fungal parasite, wood saprotoph, and plant saprotoph significantly increased at the thermophilic phase, while the undefined saprotoph and fungal parasite significantly increased at the mature phase (Fig. 5b–e). These findings suggest that SynCom inoculation acts as a highly active and hydrolase-rich microbial assistant, benefiting microbial growth and accelerating cellulose degradation in composting materials.

The function of the undefined saprotoph usually promotes organic matter breakdown and evolutionary strategies of microbes in agro-ecosystems (Kumar and Chandra, 2020). The higher presence of wood saprotrophs at the thermophilic and mature phases may be attributed to their higher lignocellulose degradation capacity, fighting ability, adaptability to complex and high-temperature stress, abundant hydrolase production, and successful colonization, as observed in this study (Marañón-Jiménez et al., 2021). Previous studies have also reported that undefined saprotrophs and wood saprotrophs were dominant fungal taxa responsible for organic matter degradation (Candotti Carniel et al., 2021; Qiao et al., 2021). Therefore, the altered fungal communities, the



**Fig. 5.** Effects of synthetic microbial community inoculation on the functions of fungal community at different composting phases. (a) Functions of fungal community for synthetic microbial community inoculation and control treatments based on FUNGuild. The background color of grey represents initial phase of composting; The background color of red represents mesophilic phase of composting; The background color of green represents thermophilic phase of composting; The background color of blue represents mature phases of composting. (b) Significant difference in functions in layer2 at initial phase for the two treatments. (c) Significant difference in functions in layer2 at mesophilic phase for the two treatments. (d) Significant difference in functions in layer2 at thermophilic phase for synthetic microbial community inoculation and control treatments. (e) Significant difference in function in layer2 at mature phase synthetic microbial community inoculation and control treatments. IPP represent initial phase, MEP represent mesophilic phase, THP represent thermophilic phase and MAP represent mature phases of composting, respectively. The asterisk (\*) represent significant differences at the level of  $p < 0.05$  between synthetic microbial community inoculation and control.

increased abundance of OUT10 and the accelerated function of wood saprotrophs further may contribute to stimulated lignocellulose degradation under SynCom inoculation.

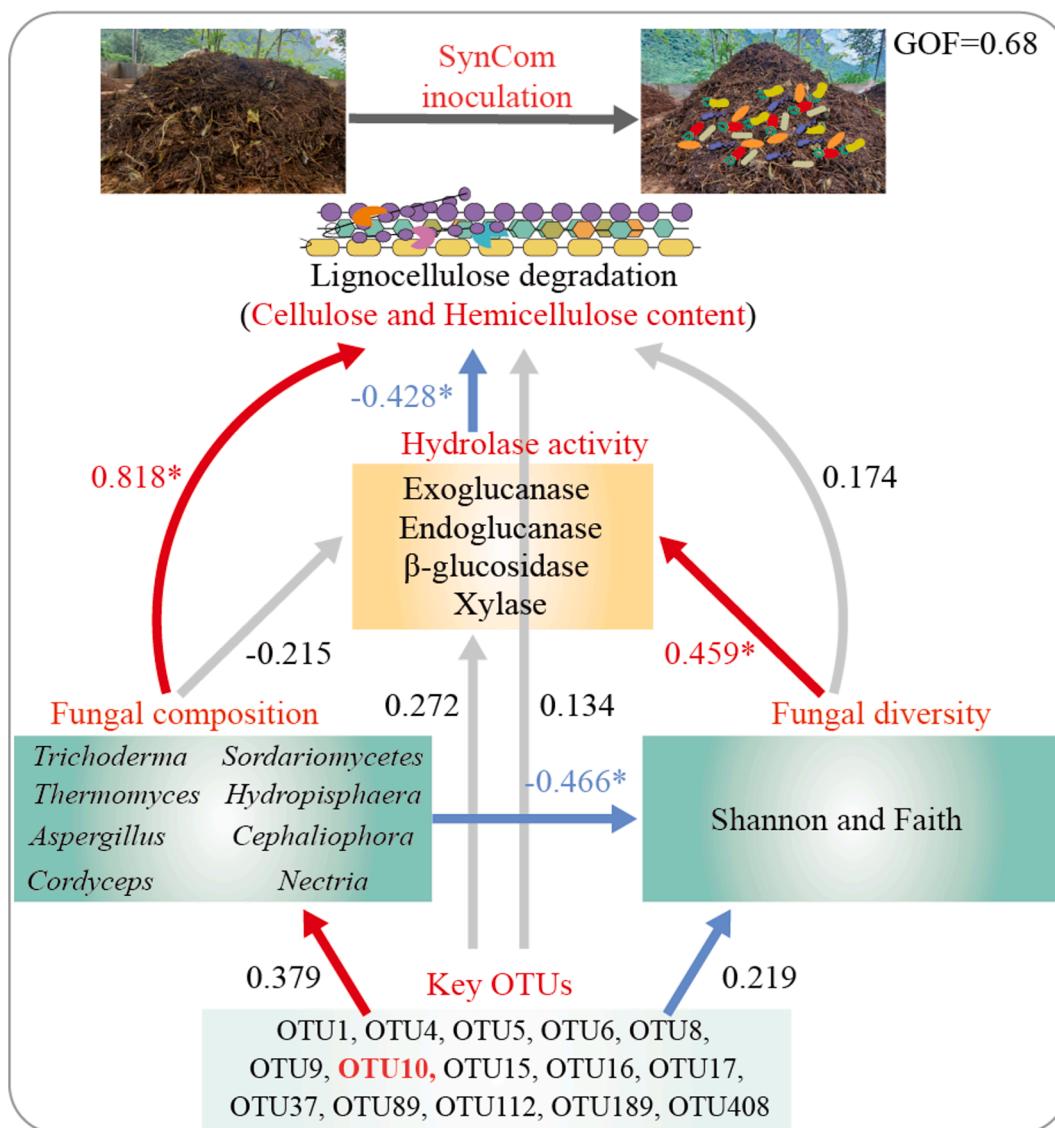
Partial Least Squares Path Modeling (PLS-PM) demonstrated that fungal community composition (*Sordariomycetes*, *Hydropisphaera*, *Cordyceps*, *Nectria*, *Thermomyces*, and *Cephaliophora*) was the strongest driver explaining lignocellulose degradation ( $r = 0.82$ ,  $p = 0.002$ ). Furthermore, fungal key OTUs (OTU1, OTU4, OTU5, OTU6, OTU8, OTU9, OTU10, OTU15, OTU16, OTU17, OTU37, OTU89, OTU112, OTU189, and OTU408) positively influenced fungal community composition ( $r = 0.379$ ,  $p < 0.01$ ) (Fig. 6). Hydrolase activity directly contributed to the decrease in cellulose and hemicellulose content ( $r = -0.428$ ,  $p < 0.020$ ), where the rhizosphere fungal community and endosphere fungal diversity (Shannon and Faith indices) played a significant role in determining hydrolase activity (exoglucanase, endoglucanase,  $\beta$ -glucosidase, xylanase) ( $r = 0.459$ ,  $p < 0.01$ ).

#### 4. Conclusions

Synthetic microbial community inoculation significantly enhanced lignocellulose degradation during composting by increasing activities of endoglucanase, exoglucanase and  $\beta$ -glucosidase to accelerate lignocellulose degradation through cooperating with native microbes. Synthetic microbial community inoculation selectively assembles fungal communities by changing microbial community composition and diversity and increasing the microbial functions of wood saprotroph, undefined saprotroph, and litter saprotroph. The stimulated abundance of OUT10 (*Hydropisphaera*) contributed to the microbial community and enzyme activities for lignocellulose degradation. Overall, the findings offer a cost-effective strategy for promoting lignocellulose degradation, thereby improving soil fertility and enhancing soil health through the application of organic fertilizers.

#### CRediT authorship contribution statement

Qiumei Liu: Writing – original draft, Project administration,



**Fig. 6.** Results from structural equation model illustrating the mechanism of synthetic microbial community inoculation on lignocellulose degradation via lignocellulose degradation, hydrolase enzymes, fungal community composition, fungal diversity, and key OTUs. The blue lines represent significant negative relationships, the pink lines represent significant positive relationships, and the grey lines represent no significant relationships. Width of arrows represents the strength of the relationships. Numbers beside the lines are standardized path coefficients with asterisks indicating their significance (\* $p < 0.05$ ).

**Methodology, Investigation.** **Zhouling Xie:** Investigation. **Siyu Tang:** Methodology, Investigation. **Qingquan Xie:** Methodology, Investigation. **Xunyang He:** Methodology, Conceptualization. **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 A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2025.132068>.

## Data availability

Data will be made available on request.

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