Characteristics of bacterial and fungal communities and their impact during cow manure and agroforestry biowaste co-composting

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Research article

Characteristics of bacterial and fungal communities and their impact during cow manure and agroforestry biowaste co-composting

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ABSTRACT

Microbial communities and environmental conditions are both of great importance for efficient utilization of agroforestry resources. Nevertheless, knowledge about the role of soluble nutrients and enzymatic properties, and their inner links with microbial communities remain limited. This is especially the case for the co-composting of agricultural and forestry biowaste. Here, we investigate the succession of key microbes during co-composting (sawdust + cow manure, SA; straw + cow manure, ST), employing amplicon sequencing, enzyme assays, and physicochemical analyses. N-fixing bacteria (*Pseudomonas*) and C-degrading fungi (*Acaulium*) have been identified as dominant taxa during such co-composting. Although eight antibiotic resistance genes were found to persist during composting, pathogenic microbes declined with composting time. NO_3^--N content was screened as a determinant structuring the bacterial and fungal communities, with importance also shown for C-degrading enzymes such as cellulose, laccase, and peroxidase activity.

These results identify the key microbial taxa and their main interactive environmental factors, which are potentially valuable for the development of a mixed microbial inoculant to accelerate the maturation of agroforestry biowastes composting.

1. Introduction

Abundant agricultural bioresources including crop straw, grain husk, sawdust, and animal wastes, can be used as renewable materials (Zeng et al., 2007; Parker and Philip, 2010). China produced 900 million tons of crop straw residues in 2020, but most were burned (Gadde et al., 2009; He et al., 2020a). In forest ecosystems, timber processing produces plentiful sawdust, with ~50% of the harvested wood ending up as waste (Škrbić et al., 2018). These agroforestry biowastes cause serious resource waste and environmental pollution (Škrbić et al., 2018; He et al., 2020a) and therefore need to be utilized efficiently.

Co-composting agroforestry biowastes with livestock manure is an optimized method to convert complex organics into harmless humus (Wei et al., 2020; Jiang et al., 2020; Yue et al., 2022; Chen et al., 2022). Traditionally, livestock manure was treated as a nutrient reservoir rich in organic matter, macronutrients (N, P, K), and trace elements (Chen et al., 2013) but it is also a source of antibiotic resistance genes and human pathogens (Guo et al., 2021; Zhang et al., 2022; Zhu et al., 2022). Compost maturity improvement and harmless utilization of livestock manure as organic fertilizers are therefore of great importance (Ro et al., 2016; Guo et al., 2020; Li et al., 2020a). Applying agroforestry byproducts such as straw, cornstalks, and sawdust is an efficient way to accelerate compost maturity through exogenous C-additives (Huang et al., 2004; Zhang et al., 2021a). Moreover, the main components (cellulose, hemicellulose and lignin) of C-additives could support microbial metabolism and accelerate compost maturation (Huang et al.,

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2004; Yu et al., 2019; Zhang et al., 2021a). During composting, bacterial and fungal communities substantially vary (Meng et al., 2019; Bello et al., 2020; Zhu et al., 2021a), and their community structure and C-component-related metabolic activity are key driving factors (Meng et al., 2019; Bello et al., 2020). Overall, the interaction between enzyme and microbe is close and simultaneously co-regulated by various factors including moisture, pH, composting substrates, as well as the sugar fractions (i.e., polysaccharides and reducing sugars) that are derived from organic matter decomposition (Chefetz et al., 1998; Pérez et al., 2002; Zhang and Lynd, 2010; Li et al., 2020b). However, compared to classical composting studies, our understanding on the associations among these factors are still limited. In particular, the following questions remain outstanding:

i. What are the dominant microbial differences induced by exogenous agroforestry C-additives with varied carbon to nitrogen (C/N) ratios (important factors influencing compost quality (Eiland et al., 2001; Huang et al., 2004; Zhu, 2007; Guo et al., 2012; Qiao et al., 2021));
ii. How are carbon components (hemicellulose, cellulose, and lignin) and C-degrading enzymes targeting those fractions simultaneously, especially for the co-composting of cow manure and agroforestry biowastes?
iii. Are there some remaining latent risks (antibiotic resistance genes and pathogenic microorganisms) after compost maturation?

Thus, in the present study, we explore variations in bacterial and fungal communities and their related C-compounds, C-degrading enzymes, and physicochemical properties during the co-composting of cow manure and different agroforestry biowastes (sawdust and straw).

The detailed aims of this study were (1) to investigate the changes of physicochemical, nutrients and C-degrading enzyme activities, and their inner links with microbial communities over co-composting; (2) to track the characteristics of the main bacterial/fungal genera and pathogenic microorganisms involved in cow manure co-composting processes; and (3) to assess the persistence of pathogenic organisms and antibiotic resistance genes, as well as potential risks in co-composting. This work is of great importance for improving compost quality, minimizing related environmental risks, and subsequent application of livestock manures.

2. Material and methods

2.1. Experimental setup

Two composts were prepared in December 2020 in Kunming Institute of Botany, Yunnan, China. Cow manure was blended with wood sawdust or straw (corn + rice) and composted in a greenhouse on a concrete surface. Cow manure and straw were collected from Kunming and Hongyan Village, Huize County, Yunnan, China (103°29′E; 26°41′N). There were three biological replicates (as individual piles of 100 kg dry weight, DW) for each compost type. Sawdust (SA) was made using 44.45 kg DW sawdust (oak, *Quercus variabilis*; and alder, *Alnus japonica*) in a ratio of 1:1 DW, 44.45 kg DW cow manure, 3.70 kg DW corn cob (Wuding Chunjiang Rare Fungus Co. Ltd., Yunnan, China), 3.70 kg gypsum (Yunnan Taishan Gypsum Co., LTD, Yunnan, China), and 3.70 kg urea (Tianyi Chemical Fertilizer Co. Ltd., Yunnan, China). Straw compost (ST) was made by 44.45 kg DW straw (corn: rice = 1:1 DW) with the same amounts of cow manure, corn cobs, gypsum and urea as in the SA. The characteristics of those materials used for composts are shown in Table S1. The composting pile (length × width × height = 1.0 × 1.0 × 0.8 m) was mixed by inverting at days 10, 20, and 30. Four successive stages during composting were determined by changes in temperatures: initial stage (0–1 day, 27.86 °C), thermophilic stage (1–12 days, peaking at 70 °C), cooling stage (12–25 days, 46.35 °C), and maturity stage (25–35 days, 27.11 °C). Compost samples were directly collected from the surface, center, and lower positions from the core of the composting piles and then manually mixed as a composite sample, at day 0 (D_0), day 10 (D_{10}), day 20 (D_{20}), and day 30 (D_{30}). The thoroughly mixed compost sample from each composting day was divided into two parts: one part was stored at −20 °C for DNA extraction and the other was stored at 4 °C for physicochemical analysis.

2.2. Physicochemical analysis

Temperature and humidity of the environment and the composting piles (with probe inserted at the heap center) were measured daily with HOBO bio-recorders (Onset, Arkansas, USA) (Fig. S1). pH was measured using a pH meter (PHS–3C, Shanghai, China) (Yang et al., 2012). Compost moisture content was determined by mass change after oven-drying at 105 °C for 48 h. Cellulase activity was determined by anthrone colorimetric method (Xu, 1986). Laccase and Mn-dependent peroxidase activity was determined using a UV/VIS spectrophotometer (JingHua 752, Shanghai, China) at 420 nm (Zhang et al., 2019). Neutral Xylanase was determined based on the method provided by Savoie and Libmond (1994). Total sugar and total reducing sugar contents were measured using the anthrone-based method (Wen et al., 2005). Compost sample hemicellulose was hydrolysed with 2 M hydrochloric acid and determined by a UV/VIS spectrophotometer (Xiong et al., 2005), and cellulose and lignin contents were measured by anthrone-sulfate colorimetry (Wang, 2006) and the concentrated sulfuric acid method (Xiong et al., 2005). Total nitrogen (TN) and carbon (TC) contents were quantified using an Elementar Vario MICRO cube (Elementar, Hanau, Germany) (Schumacher et al., 2009). Concentrations of ammonium (NH_4^+-N) and nitrate (NO_3^--N) were analyzed by a Dual channel flow analyzer (SEAL Analytical GmbH, Norderstedt, Germany) (Wang et al., 2018). All measurements were conducted in triplicate.

2.3. DNA extraction and Illumina MiSeq sequencing of 16S rRNA and ITS1 amplicons

Total DNA from 0.5 g fresh compost was extracted using a FastDNA Spin Kit for Soil (Qbiogene, Irvine, CA, USA) according to the instructions of the manufacturer. The quality and quantity of extracted DNA was determined using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) (Wang et al., 2018).

Illumina MiSeq sequencing was employed to investigate the shifts of bacterial and fungal communities in composts. The V4–V5 region of the 16S rRNA gene was amplified using the primer set 515F (5′-GTGCCAGCMGCCGCGGTAA-3′)/907R (5′-CCGTCAATTCMTTTRAGTTT-3′), which produces accurate taxonomic information with little bias for different bacterial classes. The ITS1 gene was amplified using the primer set ITS 5F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS 1R (5′-GCTGCGTTCTTCATCGATGC-3′) (He et al., 2020b), which assigns accurate taxonomic information for fungal classes. All PCR amplifications were conducted and visualized on 1.0% agarose gels with GoldView™ (0.005%, v/v; SBS Genetech, Beijing). Purified PCR amplicon concentrations were determined prior to MiSeq sequencing. The QIIME 2 software package was used for sequencing data analysis, and the pipelines were followed as previously described (Bokulich et al., 2017; Wang et al., 2018). Raw sequence data were deposited in the NCBI Sequence Read Archive under accession numbers SUB10865378 (Bacterial sequences) and SUB10863577 (Fungal sequences).

2.4. Statistical analysis

Excel and SPSS (SPSS Inc., Chicago, USA, V20) were employed for statistical analysis. Significant differences between data (means ± SD, $n = 9$) of treatments were compared with the Least Significant Difference at the $P < 0.05$. Bray–Curtis distance-based PCA (principal component analysis) and redundancy analysis (RDA) were performed in R4.1.0 using the packages of vega, devtools and an ade4. Origin 2021b (OriginLab, USA) software was used for graphic production.

3. Results

3.1. Physicochemical and nutrient changes during co-composting

Composting properties were tracked across initial stabilization (Day0/D_0), thermophilic (Day10/D_{10}), cooling (D_{20}), and maturation stages (D_{30}). At the thermophilic (D_{10}) stage, the two composts reached their significantly highest temperature ($P < 0.05$, Fig. S1D) and the compost pH was greater than 8 and changed similarly with temperature (Table 1). At the cooling stage (D_{20}), NH_4^+-N content was significantly highest ($P < 0.05$). At the maturation stage (D_{30}), the NO_3^--N concentration reached the highest value but the contents of total nitrogen and total carbon (TC) of the two co-composting gradually decreased by ~10%, averaging 23 mg g^{-1} and 330 mg g^{-1}, respectively (Table 1). Total and reducing sugar concentrations slowly decreased during composting (Fig. 1). A similar trend was also observed for cellulose and hemicellulose, ranging from 40 mg g^{-1} to 50 mg g^{-1} (Fig. 1). Chemical and physical properties of composting raw materials were generally found to vary similarly between the two composts (sawdust + cow manure, SA; straw + cow manure, ST) and can be seen in Supplementary File 1.

3.2. Enzyme activities and their correlations with other parameters

Carbon components such as hemicellulose (neutral xylanase), cellulose (cellulase), and lignin (laccase and Mn-dependent peroxidase) were measured with the aim of understanding C-dynamics during composting (Fig. 2). Specifically, neutral xylanase activity decreased (~5 U g^{-1}) in the thermophilic stage, but increased (averaged 12 U g^{-1}) in the cooling stages (Fig. 2B). The change of cellulase activity had a similar trend (sharply increased to ~30 U g^{-1} and then decreased to ~10 U g^{-1}) with composting temperature (Fig. 2A). Peroxidase activity showed an obviously opposite trend as compared to the change of cellulase, and ranged from 2 to 6 U g^{-1} (Fig. 2C). Laccase activity generally decreased from 10 U g^{-1} to 4 U g^{-1} over the composting process (Fig. 2D).

Correlation analyses showed, in both cow manure co-composts, that the key physicochemical parameters (pH and temperature) and the total amount of carbon and nitrogen nutrients (TC and TN) all had significant positive correlations with each other (Fig. 3). The two forms of inorganic N (NH_4^+-N and NO_3^--N), however, had no significant relationships with each other. The accumulation of NO_3^--N was closely associated with the decomposition of cellulose over the composting process (negative correlation, $P < 0.05$; Fig. 3), but NH_4^+-N concentration tended to increase along with the total sugar consumption during composting (negative correlation, $P < 0.01$; Fig. 3). The increases of lignin-degrading enzyme activities (laccase and peroxidase) were significantly correlated with consumption of total sugar. Cellulase, laccase, and peroxidase activities were significantly positively related with composting temperature ($n = 24$, $P < 0.01$; Fig. 3); but the xylanase (a thermo-tolerant enzyme) had a negative association with temperature (Fig. 3).

3.3. Characteristics of bacterial and fungal communities during composting

A total of 1,505,668 and 2,435,692 high-quality bacterial and fungal sequences were obtained from all samples, with an average of 62,736 (bacterial) and 8,545 (fungal) sequences per sample (Table S2). During each composting stage, the ratio of shared/unique microbial ASVs was stable at ~30% in the two composts (Figs. S2 and S5).

For bacterial ASVs, there were 39 bacterial phyla detected with the dominance of Proteobacteria (average ~50%), Bacteroidetes (20%), and Firmicutes (~15%), and a lower representation from other phyla (<10%) including Acidobacteria, Actinobacteria, Chloroflexi, Deinococcus-Thermus, and Planctomycetes (Fig. S3A). Compared with the initial stage (D_0), the average relative abundance of Bacteroidetes increased by ~45% at the end of composting (D_{30}) in both composts (Fig. S3A). Other phyla showed an inconsistent variation between the two composts (Supplementary file 1). Dominant bacterial genera were *Pseudomonas, Fermentimonas*, *Luteimonas*, *Myroides*, *Pusillimonas,* and *Ruminofilibacter* (Fig. S4A). Substantial differences were found at the initial stage (Fig. 4A). The relative abundances of *Pseudomonas, Moheibacter, Algoriphagus, Ruminofilibacter,* and *Sporosarcina* were 6-, 23-, 34-, 80-, and 143-fold higher in ST-compost than in SA-compost, respectively (Fig. 4A).

For fungal ASVs, a total of 10 fungal phyla were detected, and the dominant Ascomycota, Basidiomycota, Mortierellomycota, and Mucoromycota accounted for 72.95 ± 26.20% of the total sequences (Fig. S3B). The relative abundances of Ascomycota predominated and showed consistent increases in the two composts from ~50% to ~80%, whereas Basidiomycota decreased gradually from ~15% to ~5% (Fig. S3B). The fungal genera were significantly different between the two composts as revealed by PCoA analysis, and the prominent genera were *Acaulium*, *Aspergillus*, *Lophotrichus*, and *Kernia* (Fig. S4). The heatmap of the top 50 genera showed unique fungal composition variations during each cow manure co-composting stage (Fig. 4B). The dominant genera were *Acaulium* (12.50%), *Lophotrichus* (9.52%), and *Wickerhamomyces* (7.04%) in ST co-compost, while in SA, *Aspergillus*

Table 1
Variations in physicochemical properties at the center of the composting pile between SA and ST composts over a one-month composting period.

Composting days	Temperature (°C)	moisture (%)	pH (1:2.5 H_2O)	Total C (mg g^{-1})	Total N (mg g^{-1})	NO_3^--N (mg g^{-1})	NH_4^+-N (mg g^{-1})	C/N (%)
SA*								
D_0	23.5 ± 2.7c,x	59.48 ± 3.08 c,x	8.61 ± 0.04 b,x	362.7 ± 19.4a,x	25.47 ± 1.57 a,x	0.08 ± 0.00b,x	2.68 ± 0.29b,x	14.3 ± 1.2 a,x
D_{10}	53.2 ± 1.2a,y	67.17 ± 2.70 ab, y	8.97 ± 0.05 a,x	349.5 ± 32.8a,x	24.00 ± 0.80 ab, x	0.10 ± 0.00b,x	3.23 ± 0.33b,x	15.3 ± 0.3 a,x
D_{20}	35.7 ± 2.5b,y	63.03 ± 1.45 bc, y	8.70 ± 0.05 b,x	339.3 ± 8.1a,x	23.83 ± 1.17 ab, x	0.08 ± 0.00b,x	5.10 ± 0.03a,x	14.3 ± 0.4 a,y
D_{30}	24.9 ± 2.5c,x	68.98 ± 2.54 a,y	7.70 ± 0.19 c,x	325.9 ± 50.3a,x	22.73 ± 1.34 b,x	0.14 ± 0.03a,x	3.78 ± 0.07b,x	14.3 ± 1.5 a,x
ST								
D_0	24.9 ± 1.6c,x	63.57 ± 3.98 b,x	8.53 ± 0.22 ab, x	349.4 ± 17.5a,x	22.33 ± 2.15 a,x	0.07 ± 0.00a,y	2.27 ± 0.02d,y	15.7 ± 0.9 a,x
D_{10}	64.8 ± 3.7a,x	74.83 ± 2.66 a,x	8.90 ± 0.16 a,x	360.2 ± 6.5a,x	24.03 ± 0.67 a,x	0.09 ± 0.00a,y	3.10 ± 0.39b,y	15.0 ± 0.4 ab, x
D_{20}	46.2 ± 2.3b,x	73.92 ± 4.82 a,x	8.37 ± 0.31 b,y	350.5 ± 11.2a,x	23.70 ± 0.69 a,x	0.08 ± 0.00a,x	3.61 ± 0.22a,y	14.8 ± 0.3 ab, x
D_{30}	28.6 ± 3.4c,x	77.17 ± 1.11 a,x	7.61 ± 0.17 c,x	335.0 ± 19.6a,x	23.77 ± 1.50 a,x	0.31 ± 0.13a,x	2.71 ± 0.08c,y	14.1 ± 0.1 b,x

Data are means ± SD, $n = 9$.
a, b, c and d show significant differences ($P < 0.05$) between different composting turning days for the same compost.
x and y show significant differences ($P < 0.05$) between different composts for the same composting turning day.
*Abbreviations: SA, sawdust + cow manure; ST, straw + cow manure; D0, day0; D10, day10; D20, day20; D30, day30.

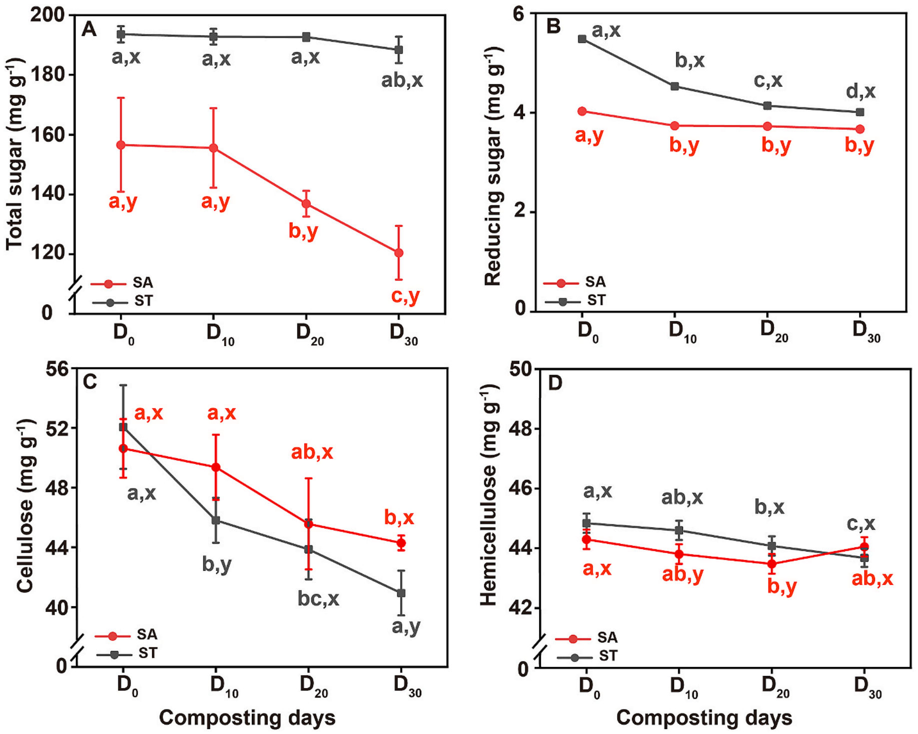

Fig. 1. Differences in total sugar (A), reducing sugar (B), cellulose (C), and hemicellulose (D) between SA and ST composts over the composting days. Data are means ± SD, $n = 9$. SA, sawdust + cow manure; ST, straw + cow manure.

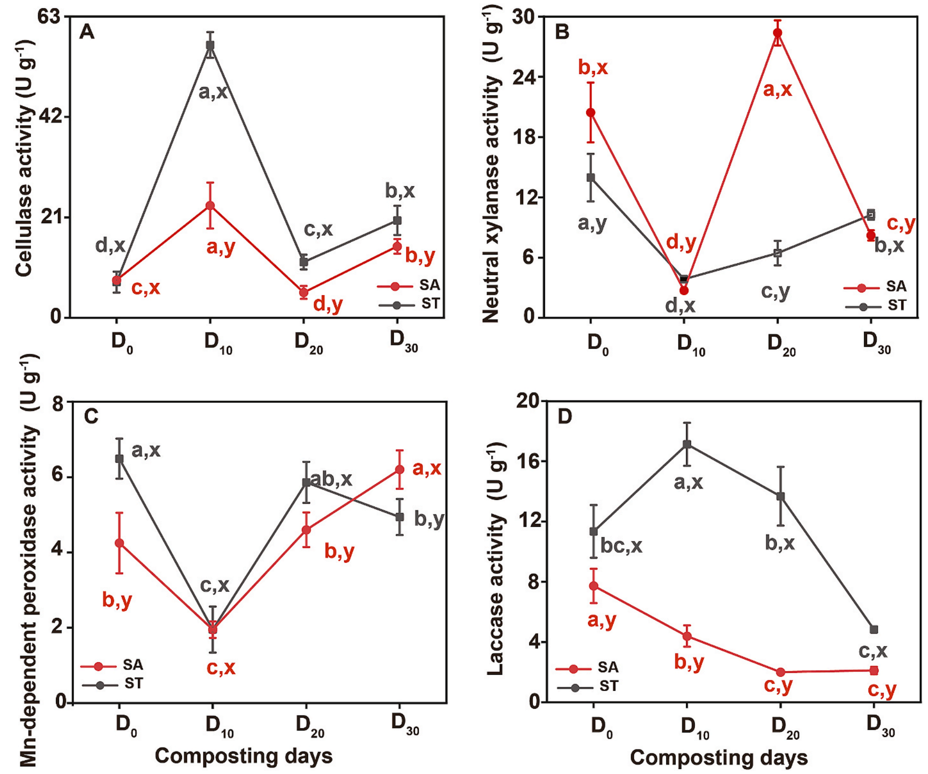

Fig. 2. Variations in activity of cellulase (A), neutral xylanase (B), Mn-dependent peroxidase (C), and laccase (D) and their differences between SA and ST composts over the composting days. Data are means ± SD, $n = 9$. SA, sawdust + cow manure; ST, straw + cow manure.

(15.38%), *Acaulium* (9.93%), and *Ophiostoma* (9.22%) dominated (Fig. 4B). In both composts, we found that the relative abundance of *Acaulium* increased by 5-fold by the end of composting (Fig. 4B).

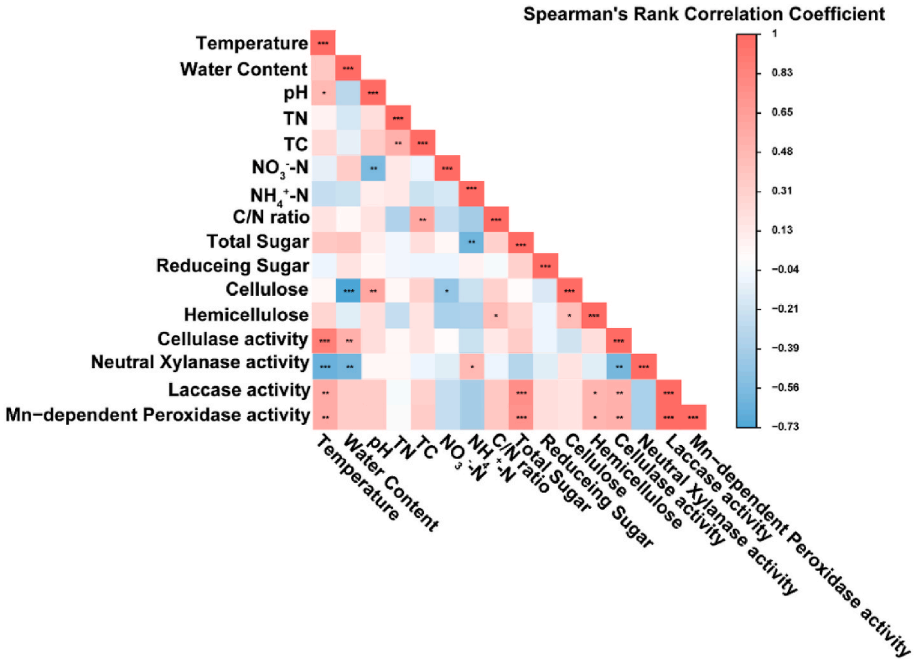

Fig. 3. Heatmap for correlation analyses (Pearson, $|r| > 0.6$) of physiochemical and biological factors in SA and ST composts. $*P < 0.05$, $**P < 0.01$ $***P < 0.001$. SA, sawdust + cow manure; ST, straw + cow manure.

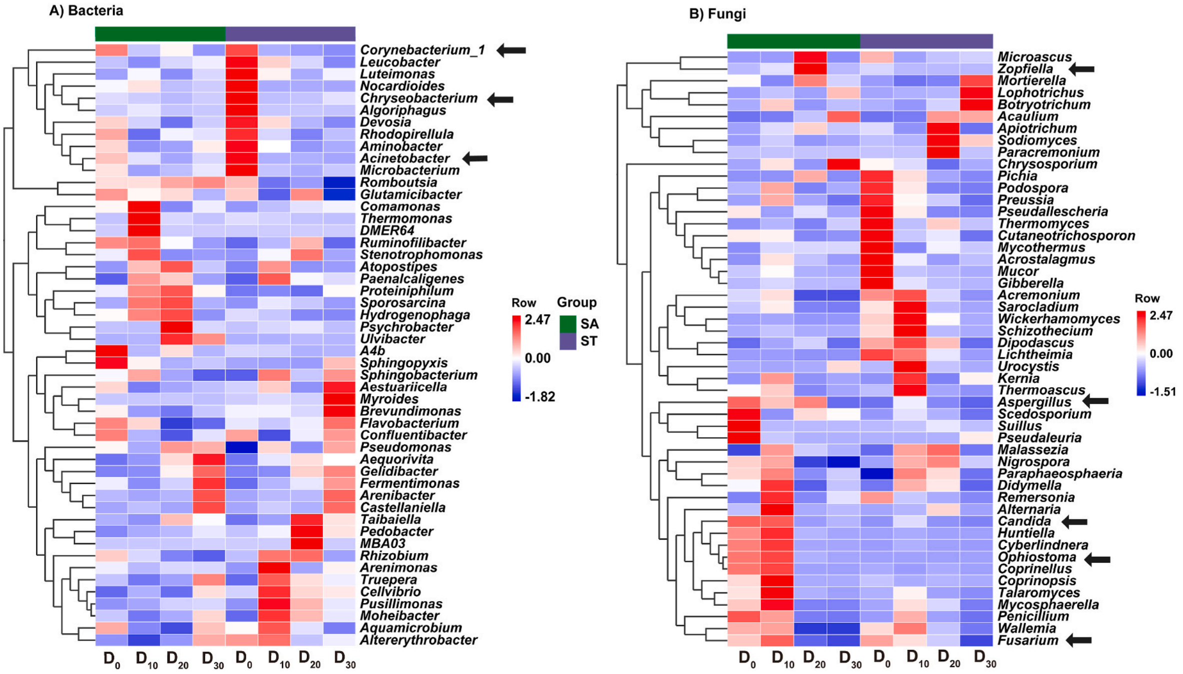

Fig. 4. Variations in hierarchically clustered heatmaps based on the top 50 genera within bacterial (A) and fungal (B) community compositions and their differences between SA and ST composts over the duration of the composting period. The relative abundances of the genera are the average of triplicated MiSeq sequencing data. Pathogenic taxa are indicated by arrows after their names. Data are means ± SD, $n = 3$. SA, sawdust + cow manure; ST, straw + cow manure.

3.4. *Correlations between microbial communities and environmental variables*

For the bacterial community in manure-sawdust (SA) compost, hemicellulose and total carbon and nitrogen contents (TC and TN) explained ~80% of the bacterial community variation (Fig. 5A, Fig. S6A). However, NO_3^--N was the most significant driver during manure-straw (ST) composting, contributing ~40% of the total variance (Fig. 5A, Fig. S6B). By comparison, ~80% of the variation in fungal community composition was explained by RDA (Figs. S5C and D). For both composts, NO_3^--N was the most significant factor (Fig. 5B) and contributed ~40% of the total variance in fungal community variation during the composting process (Figs. S6C and D).

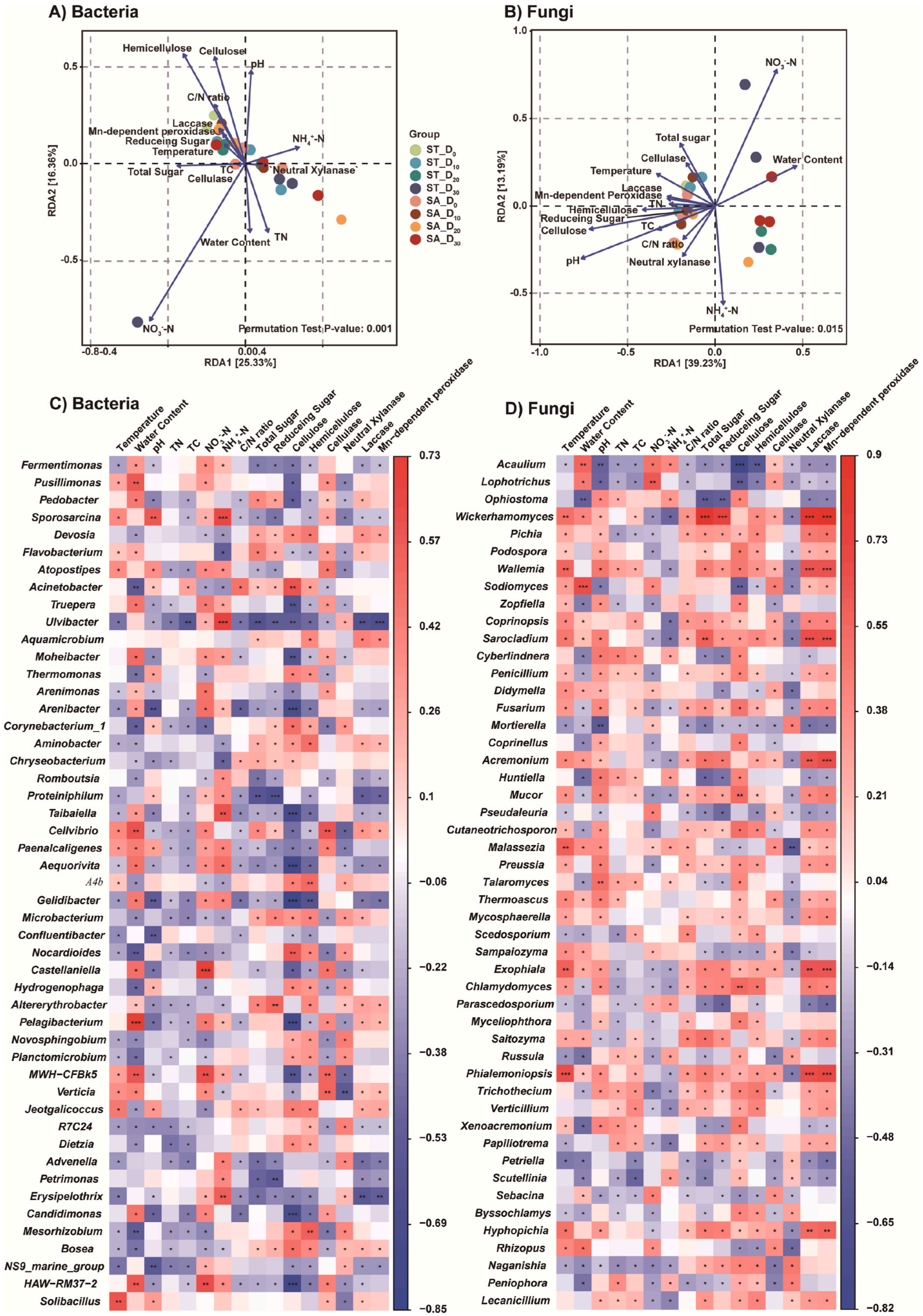

Fig. 5. Redundancy analyses based on the bacterial (A) and fungal (B) community composition and physicochemical variables for both SA and ST composts. Heatmap showing correlations between physicochemical properties and representative bacterial or fungal genera based on the Spearman correlation coefficient in the bacterial (C) and fungal (D) community composition. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. SA, sawdust + cow manure; ST, straw + cow manure.

4. Discussion

4.1. The changes of key environmental variables, enzyme activities, and their intrinsic links with microbial communities

Composting is a process of humification and mineralization (Zhu et al., 2020). Microbial behavior and organic matter transformation are affected by physicochemical parameters (Guo et al., 2012; Wu et al., 2017).

Compost initial C/N ratio affects the degree of maturity, and the succession of dominant microbes (Guo et al., 2012; Zhang et al., 2020). In our study, the cow-manure C:N ratio was relatively lower (15) as compared with other studies (~25). It may have some potential drawbacks such as slower heating rate, a lower maximum temperature and a shorter thermophilic period (Eiland et al., 2001; Zhu, 2007; Guo et al., 2012; Zhang et al., 2020). However, we observed that the average maximum temperature of manure-straw and manure-sawdust in the thermophilic phase reached 72 °C and 57 °C, respectively, and lasting 6–8 days (Fig. S1). This was in line with the requirements of harmless sanitation (>50 °C, 5–10 days) of aerobic manure composts (Zhang et al., 2021b; Vinnerås, 2013). In addition, Miyatake and Iwabuchi (2005) observed the highest level of thermophilic bacterial activity at 54 °C. Economic analyses showed that a lower initial-C/N, rather than a higher-C/N, reduces the weight of straw used per ton of fresh manure, and therefore, more manure can be treated (Zhu, 2007; Vinnerås, 2013; Zhang et al., 2021). Moreover, the removal efficiency of antibiotic resistance genes in low C/N compost was higher than that in high C/N compost (Zhu et al., 2021b). Therefore, in this case, our work provides a new co-composting pathway for the harmless treatment of lower C/N cow manure and agroforestry biowastes.

Changes in composting pile core temperature are commonly caused by the activities of microorganisms, human disturbance, and exothermic ammonium nitrogen conversion (Liang et al., 2003; Goyal et al., 2005; Zhu et al., 2020). Compared to the initial stage, the total N content in the mature stage of manure co-composting was reduced, indicating a potential change in N form, as the main organic N can be assimilated by N-recycling microorganisms, or converted to ammonia, nitrogen oxides, protein nitrogen, and NO_3^--N (Maleki et al., 2022). We further found that these N changes were closely related to specific microbial activities, as confirmed by a significant positive relationship between TN and N-fixing *Pseudomonas* (Fig. 5C). In both types of cow manure co-composting, the NH_4^+-N content was stable, but the NO_3^--N content accumulated. Based on the relationships among NO_3^--N, cellulase activity, and cellulose concentration, our results indicated that the accumulation of NO_3^--N was closely associated with cellulose breakdown over the duration of composting, which can be closely related to i) a continuous process of organic matter decomposition and utilization by C-degrading microorganisms (Hao et al., 2005); and ii) improved physicochemical properties, porosity, and oxygen concentration in co-composting piles which enhances nitrifying bacterial activity (Maleki et al., 2022). Apart from bacteria taxa, in our cow manure co-composting system, NO_3^--N was also an important factor affecting the change in fungal community structure. This was in accordance with Meng et al., (2019) who reported that fungal communities are strongly associated with N transformation and contributed to major variation in fungal community composition (Guo et al., 2012; Zhu et al., 2020).

In our study, there was a steady decrease of readily available C-fractions (total sugar and reducing sugar) and relatively recalcitrant cellulose over composting. Changes in sugar consumption during composting have also been reported by Wu et al., (2017), and decreases are accompanied by increases in lignin-degrading enzyme activities (laccase and peroxidase) in our case. Laccase is the most ubiquitous enzyme produced by white-rot fungi, and peroxidase is involved closely in lignin degradation, biobleaching, and oxidation of hazardous organic pollutants (Jarosz-Wilkolazka et al., 2007). As straw contains a higher C content, laccase and peroxidase activity are significant variables affecting fungal communities during cow manure-straw composting. Lignocellulose-degrading microbes favor high temperatures; those that produce C-degrading enzymes targeting hemicellulose (neutral xylanase), cellulose (cellulase), and lignin (laccase and Mn-dependent peroxidase) are active under high temperatures (Chefetz et al., 1998; Pérez et al., 2002). This phenomenon may be associated with enhanced activities of these C-compound-degrading enzymes under high temperatures (Chefetz et al., 1998; Pérez et al., 2002), because cellulase (cellulose-degrading), laccase and peroxidase (lignin-degrading) activity had significantly positive relationships with composting temperature in our study. Overall, changes in the above compost properties are also drivers of succession events in both the bacterial community and fungal composition (Guo et al., 2012; Neher et al., 2013; Antunes et al., 2016).

4.2. Variations in bacterial and fungal communities throughout the cow manure co-composting processes

Cow manure composting was dominated by widely-known types assigned to Proteobacteria, Bacteroidetes, Firmicutes. This was closely associated with their roles in organic matter mineralization (Li et al., 2020c) and compost humification (Steger et al., 2007a, 2007b; Zhao et al., 2016). Due to the differences in initial substrate C:N ratio (Straw *vs.* Sawdust), major bacterial genera varied significantly, but the N-fixing genus *Pseudomonas* remained dominant. This phenomenon may be linked to their beneficial roles in improving compost quality (Marcel et al., 2018; Wang et al., 2020). The co-composting of cow manure and agroforestry biowaste constructed in our study possessed a low C:N ratio which remained stable (~15) throughout composting. Fungal decomposers targeting recalcitrant lignocellulose content had a low abundance. Instead, we found that *Acaulium* strongly contributed to the degradation of easily-utilized total sugar and cellulose (Sandoval-Denis et al., 2016) and was abundant in all composts. Other abundant fungal taxa seem to play important roles in collaboration with *Acaulium*. For instance, *Luteimonas*, *Pseudallescheria, Thermobifida*, and *Trichoderma* produce cellulase and hemicellulase, accelerating cellulose decomposition (Nsereko et al., 2002; Zhang et al., 2016; Tian et al., 2017). Furthermore, the fungal community tended to vary substantially at various composting stages. We found *Pichia* and *Wickerhamomyces* were the dominant fungal genera in the initial stage, which was in line with other cow manure co-composting (Wang et al., 2014). The fungal genera shifted in the thermophilic stage to *Alternaria, Dipodascus*, *Remersonia*, *Talaromyces*, and *Thermoascus*, which were also detected in other studies (Ghaly et al., 2011; Neher et al., 2013; Sun et al., 2015; Xu et al., 2019). Fungi are resistant to stress, but very high humidity, excess ventilation, and artificial agitation are not conducive to fungal growth and reproduction (Ryckeboer et al., 2003). In general, most of the fungi in the composting system belong to mesophilic and thermophilic fungi, and their optimal growth temperatures ranged between 25 and 70 °C (Tuomela et al., 2000). Compared with the thermophilic stage, *Ascomycetes* proliferated significantly during the cooling and maturity stages (Fig. 4) (Neher et al., 2013; Sun et al., 2015; Meng et al., 2019). Our study demonstrates the characteristics of fungal and bacterial communities and their impact on low C:N ratio cow manure and agroforestry biowaste co-composting.

4.3. Microbial health and safety risks involved in the cow manure co-composting processes

Raw material types with varied C-additives structured distinct bacterial and fungal community taxa during the process of composting, driving us to seek more details in microbial risk to human health and safety during composting, especially for those involved in livestock manure (Yue et al., 2022; Chen et al., 2022). It is worth noting that pathogenic bacteria such as *Acinetobacter*, *Chryseobacterium*, and *Corynebacterium_1* gradually decreased during the composting process (Fig. 4, Fig. S4A). Consistent with these bacteria, fungal pathogens such as *Aspergillus*, *Candida*, *Fusarium*, *Ophiostoma*, and *Zopfiella* also diminished with composting time. The relative abundance of the main pathogens gradually decreased with the increase of compost temperature (Figs. 4 and 5, Figs. S4 and S7), indicating their reduction at high temperature during a thermophilic stage (Dumontet et al., 1999). Numerous studies have revealed that manure fertilizers are also one of the most abundant sources of microbial contamination, including antibiotic resistance genes (ARGs) (Yue et al., 2022; Chen et al., 2022). In this study, eight types of ARG were detected in two typical cow manure co-composts (Fig. S7). Among them, multidrug-resistant genes were the most abundant, and these genes are widely present in livestock feces due to the usage of antibiotics (Mackie and Yannarell, 2009; You and Silbergeld, 2014). This risk is greatly enhanced in certain other geographical settings, for example in complex pasturelands or agricultural ecosystems that are simultaneously exposed to other anthropogenic disturbances such as mine drainage, wastewater spills, and other hazardous industrial/municipal effluent-discharges, etc. (Hlava et al., 2017; Koley, 2022). The idea of safe co-composting of cow manure and contaminated Agri-byproducts would require additional study of the impact of heavy-metals and metalloids that may leach into groundwater or undergo biotic volatilizations, thereby proving to be hazardous to the general population of a region in a secondary manner (Koley, 2022; Mukherjee et al., 2022). In this context, assimilating further chemical stabilization methods, and subsequent analysis of the microbial communities, would be important as the future scope of this subject.

5. Conclusion

In summary, during this study the succession and response of key bacterial and fungal taxa to environmental variables during cow manure co-composting with C additives from agroforestry biowastes (straw/sawdust) were captured in detail. As a case study, we observed that the presence of pathogenic bacteria (*Acinetobacter*, *Chryseobacterium*, and *Corynebacterium_1*) and fungal pathogens (*Aspergillus*, *Candida*, *Fusarium*, *Ophiostoma*, and *Zopfiella*) gradually decreased, especially during the thermophilic stage. Nitrate N was identified as an important factor for structuring the bacterial and fungal communities, in conjunction with C-degrading enzymes. As an important method for harm and risk reduction treatment and for sustainable by-product resource use, co-composting of cow manure and agroforestry biowastes was shown to be beneficial for reutilization of cow manure.

Authors' contributions

FQY and DL designed the experiment, YH and DL wrote and revised the article, and contributed equally to the paper. YH completed all the chemical analysis. JWL, YLW, XFS helped with the composts sampling and paper discussion. With editing and co-writing from CC and XHH, all authors read and approved the final manuscript.

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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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2022.116377.

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