

Article

Influence of Effective Microbial Additives Inoculation on Indigenous Bacterial Community Dynamics and Co-Occurrence Patterns During the Composting of Mixed Food Waste and Livestock Manure

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Abstract: Sustainable management of organic waste, such as food waste (FW) and livestock manure (LS), is essential for reducing pollution and promoting resource recycling. This study investigated the effects of *Bacillus* sp. inoculation and biochar addition on composting efficiency, microbial dynamics, and physicochemical properties. *Bacillus* sp. accelerated the breakdown of cellulose and lignin, reduced moisture content, stabilized pH, and mitigated ammonia volatilization. Biochar reduced ammonia emissions by 17.04%, increasing to 28.89% with *Bacillus* sp. Next-generation sequencing revealed *Bacillus* sp. enhanced microbial diversity, suppressed pathogens, and promoted beneficial microbial interactions. LS treatments retained Firmicutes dominance (up to 95.17%), improving nitrogen retention, while FW treatments transitioned to Proteobacteria and Bacteroidota, driving plant material decomposition. By day 35, *Bacillus* sp. increased late-stage microbial taxa (Deinococcota, Myxococcota), linked to cellulose degradation and pathogen suppression. In FW biochar compost (FWBC), Planococcaceae and Bacillaceae synergistically decomposed complex organic matter. LS biochar compost (LSBC) reduced anaerobic families like Clostridiaceae and Peptostreptococcaceae, lowering methane and hydrogen sulfide emissions. Microbial network analysis highlighted improved cooperation under *Bacillus* sp., with LSBC sustaining positive interactions at higher dosages. These results demonstrate that microbial inoculants and biochar enhance composting efficiency, nutrient cycling, and environmental sustainability.

Keywords: microbial additives; biochar; indigenous bacterial community dynamics; food waste; livestock manure; composting; co-occurrence pattern



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1. Introduction

The global challenge of managing organic waste management, such as food waste and livestock manure, has become increasingly urgent because of rising agricultural production and consumption [1,2]. Each year, 1,000,000 tons of organic waste are generated worldwide. Much of this waste either ends up in landfills or is inadequately treated, leading to environmental pollution, water contamination [3], and the release of greenhouse gases. Therefore, finding a sustainable solution for managing the increasing volume of organic waste is critical for the sustainable development of human societies [4,5]. Composting has been proposed as a sustainable solution to address this problem. Composting is a biological process where organic waste undergoes microbial decomposition, transforming it into nutrient-rich humus, which enhances soil fertility. It plays a vital role in recycling nutrients back into the soil, reducing the environmental impact of organic waste, and generating high-quality soil amendments that boost crop productivity [6–8]. Traditional composting is simply the method of breaking down organic materials in a large container or heap through

natural microbial processes [9]. While this approach is cost-effective and easy to implement, it lacks the advanced control mechanisms required to optimize microbial activity, aeration, and temperature regulation. Traditional composting systems face significant limitations, particularly odor generation caused by incomplete organic matter decomposition. Food waste poses unique challenges due to its low pH and high moisture, leading to the production of malodorous compounds like volatile organic acids, VOCs, ammonia, and sulfides. These by-products contribute to air pollution, nitrogen loss, reduced compost quality, and environmental risks. Co-composting food waste with livestock manure, which has high nitrogen content and buffering capacity, effectively neutralizes acidity, stabilizes pH, enhances microbial activity, and promotes organic matter decomposition, mitigating these challenges and improving compost quality. However, the addition of livestock manure may also lead to increased emissions of ammonia (NH_3) and subsequent nitrogen loss, posing limitations that require careful management to optimize composting efficiency [10–12].

Various approaches have been suggested for minimizing nitrogen loss and improving the rate of organic matter degradation during composting. One of the primary strategies involves improving the aeration of compost piles to preserve nutrients and promote microbial growth and activity, and the application of compost additives has shown promising results [13–15]. Bulking agents such as sawdust and biochar are commonly used because of their high porosity and surface area, which facilitate oxygen diffusion. This creates a stable aerobic decomposition environment that reduces the production of VOCs and other malodorous compounds [16]. Moreover, adjusting the initial carbon-to-nitrogen (C/N) ratio of a compost pile can balance the nutrient content, which, in turn, enhances microbial metabolism, thus improving the efficiency of the decomposition process [16,17]. Recently, microbial inoculants have gained attention as a means of reducing odors and enhancing degradation efficiency during composting. These inoculants are composed of specific microorganisms that produce enzymes that facilitate the breakdown of recalcitrant compounds such as cellulose and lignin, thereby accelerating the composting process. Furthermore, microbial inoculants can inhibit pathogenic microorganisms by outcompeting them and promoting the growth of beneficial bacteria, thus balancing the microbial community, which, in turn, improves the overall composting process [18–20]. Furthermore, microbial inoculants provide significant economic and environmental benefits by reducing the operational demands of composting facilities. By mitigating odor emissions, the burden on odor control systems is reduced, leading to lower operational costs for such systems. Additionally, microbial inoculants contribute to reducing leachate treatment requirements, further lowering the cost and complexity of wastewater management processes. Enhancing degradation efficiency also allows for a shorter process duration, enabling composting facilities to operate more efficiently with reduced energy consumption and faster throughput. These combined effects demonstrate the potential for microbial inoculants to improve the sustainability and cost-effectiveness of composting operations, making them a valuable tool for modern waste management.

Composting is a highly dynamic process in which the microbial community undergoes successive changes over time. The composition of the composting materials, methods used, and environmental conditions can greatly influence microbial succession [21,22]. Moreover, microbial inoculants can affect the indigenous microbial communities in the composting systems, potentially altering the dynamics and interactions between microbial species [23]. Advanced sequencing techniques and network analysis have contributed to a better understanding of the roles and interactions of key microorganisms during the composting process. These insights are now being used to design more efficient composting systems, aimed at optimizing microbial activity for faster and more effective composting [20,24]. Recent composting strategies have focused on the core microbiome, which refers to the essential microbial species crucial for composting [25]. These core microbes are critical for nutrient cycling and organic matter degradation. New strategies involve expanding the core microbiome by introducing specific microbial inoculants during the early stages of composting that facilitate beneficial microbes to rapidly establish their dominance within

the microbial community. This approach not only enhances the composting rate but also suppresses the growth of odor-causing and harmful microorganisms. The adoption of core microbiome-based composting strategies extends beyond merely adding microbial inoculants; it involves comprehensive analysis and optimization of microbial community dynamics throughout the composting process [26,27]. Although research on microbial inoculants and their effects on composting is increasing, several questions remain unanswered. Specifically, the detailed impact of microbial inoculants on indigenous microbial communities and their interactions in composting systems is not completely understood [28]. Although co-occurrence network analysis has been employed to study microbial communities in composting, only limited studies exist on how microbial inoculants affect these networks, particularly in co-composting systems of food waste and livestock manure. Understanding how microbial inoculants influence microbial dynamics and co-occurrence patterns is crucial for optimizing composting processes and improving the quality of the final compost product.

This study aimed to address these knowledge gaps by investigating the effects of effective microbial additives on the dynamics of indigenous bacterial communities and co-occurrence patterns during the co-composting of food waste and livestock manure. Using next-generation sequencing and network analysis, we aim to identify key species that influence the microbial community structure and function during composting and examine the potential cooperative or antagonistic interactions between different microbial species. The findings of this study provide new insights into the microbial ecology of composting, offering practical recommendations for optimizing composting efficiency and improving compost quality. By understanding the impacts of microbial inoculants on microbial dynamics and interactions, composting methods can be refined to create more sustainable waste management strategies as well as generate high-quality compost for agricultural use.

2. Materials and Methods

2.1. Materials and Sample Collection

The biochar used in this experiment was procured from Yougi Industry Co. in Chuncheon-gangnam-do, Republic of Korea, and food waste was sourced from a public food waste treatment facility in Suwon, Republic of Korea. Swine manure was collected from Dong Il Livestock Industry, Yeosu, Republic of Korea. Chicken manure was obtained from Uhwa farm in Eumseong, Chuncheon-gangbuk-do, Republic of Korea. Sawdust derived from pine wood was used as a bulking agent in the composting process.

2.2. Microbial Additives Preparation

2.2.1. Sampling and Isolation of Effective Bacteria

The microbial strains used in this experiment were isolated from the rhizosphere soil surrounding the plant roots. Soil samples were collected from the root area soil, and processed by adding 5 g of soil to 50 mL of distilled water. The solution was cultured in a medium containing 5 g/L glucose and 5 g/L yeast extract, along with basic salts, and incubated at 30 °C for two weeks. Colonies were isolated from agar plates with the same medium composition and used for further analysis.

2.2.2. Characterization and Identification

Molecular identification was conducted using 16S rRNA sequencing. Universal primers amplified the 16S rRNA gene, and sequencing was performed using primers 518F and 800R. The sequence was analyzed with the EzBioCloud database for related strains. A phylogenetic tree was constructed using MEGA 7 software and the neighbor-joining method, with bootstrap values calculated from 1500 replicates. The identified *Bacillus* sp. *React3* sequence was submitted to GenBank with accession number OL440405. This strain is known to produce lignin peroxidase (LiP) enzymes, which facilitate the breakdown of lignin and other complex organic polymers. The production of LiP enzymes

was confirmed under mesophilic conditions and at neutral pH, where enzymatic activity was optimized [29,30]. The isolated colonies were then transferred onto fresh agar plates to obtain pure colonies and were subsequently used as inoculum for further analyses.

2.3. Experimental Design

The experiment was conducted in a room at a temperature of 25 ± 2 °C in Kyonggi University, Suwon, Gyeonggi-do, Republic of Korea in 2024. A vertical cylindrical reactor with a working volume of 2 L was manufactured to conduct the experiments. A steel support was installed at the bottom of the reactor to facilitate the smooth discharge of leachate during the composting process. To maintain aerobic conditions, air was injected at a rate of 4 L/min during the composting (Figure 1a). A vacuum pump and a silica gel reactor were installed at the air outlet to remove the generated water. Air was injected every 45 min/h. In addition, the compost was mixed once a week to improve nutrient availability and reduce incomplete decomposition. Each treatment was conducted in duplicate to ensure the reliability and reproducibility of the results.

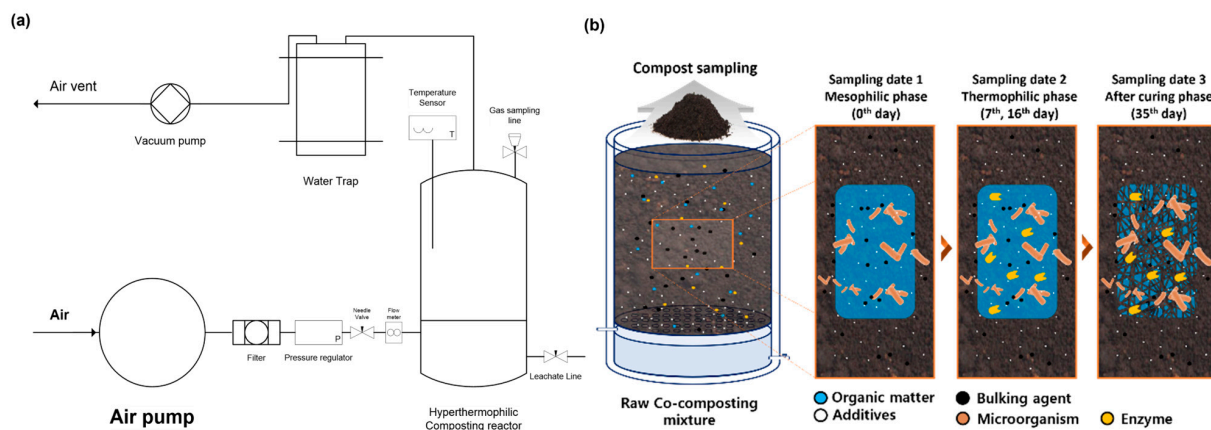


Figure 1. Composting equipment design (a) and process diagram (b).

This study was designed to evaluate the effects of microbial inoculants, specifically *Bacillus* sp. React3 with biochar as additives for odor reduction and assessing the physicochemical changes that occur during the composting process. The treatment conditions, as outlined in Table 1, were set to assess the efficiency of these additives in mitigating the emission of volatile compounds responsible for odor while ensuring the compost maintains a stable carbon-to-nitrogen (C/N) ratio, ranging from 19.6 to 20.9 across all treatments.

Table 1. Feedstock composition ratio of compost treatment.

Treatment	Food Waste	Swine Manure	Saw Dust	Additives	C/N Ratio
T1	600 g	200 g	100 g	-	19.9 ± 1.02
T2	600 g	200 g	100 g	Biochar 50 g	20.3 ± 1.58
T3	600 g	200 g	100 g	1.0×10^7 CFU (React3)	20.6 ± 1.39
T4	600 g	200 g	100 g	2.0×10^7 CFU (React3)	19.6 ± 1.32
T5	600 g	200 g	100 g	1.0×10^7 CFU (React3) + Biochar 50 g	20.7 ± 1.82
T6	600 g	200 g	100 g	2.0×10^7 CFU (React3) + Biochar 50 g	20.9 ± 1.89

To investigate the effects of biochar supplementation and varying dosages of microbial additives during the composting of livestock manure and food waste, we conducted microcosm experiments under controlled conditions to analyze microbial community structure

and potential interactions between species. The experiments were carried out at a temperature of 25 ± 2 °C, as outlined in Table 2, with the aim of analyzing differences between various substrates and their associated indigenous microbial communities. Samples for 16S rRNA amplicon sequencing were collected at specific time points (0 day, 7 day, 16 day, 35 day) during the experiment and preserved at -50 °C until further analysis (Figure 1b).

Table 2. Feedstock composition ratio for the microcosm experiment.

Treatment	Food Waste	Chicken Manure	Swine Manure	Saw Dust	Additives	
FW	FWBC	4.8 kg	0.8 kg	0.8 kg	1.36 kg	Biochar 240 g
	FWBC_R	4.8 kg	0.8 kg	0.8 kg	1.36 kg	Biochar 240 g, 2.0×10^8 CFU (React3)
	FWBC_2R	4.8 kg	0.8 kg	0.8 kg	1.36 kg	Biochar 240 g, 4.0×10^8 CFU (React3)
LS	LSBC	1.6 kg	2.4 kg	2.4 kg	1.36 kg	Biochar 240 g
	LSBC_R	1.6 kg	2.4 kg	2.4 kg	1.36 kg	Biochar 240 g, 2.0×10^8 CFU (React3)
	LSBC_R2	1.6 kg	2.4 kg	2.4 kg	1.36 kg	Biochar 240 g, 4.0×10^8 CFU (React3)

FW, food waste group; LS: livestock group; FWBC: food waste biochar compost; LSBC: livestock manure biochar compost; R, supplemented Bacillus React 3.

2.4. Physicochemical Analysis

For the physicochemical evaluation of the compost, common parameters such as temperature, pH, electric conductivity, moisture content, organic matter content, and generated CO₂ concentration were determined. The concentrations of NH₃, H₂S, acetaldehyde, and methyl mercaptan gas were measured to examine their odor reduction effects using a gas detector tube (Gastec Co., GV-100S, Ayase-Shi, Japan). The temperature of the compost was measured daily through the upper hole of the reactor using a probe-type thermometer (Hanna Instruments, HI98517, Woonsocket, RI, USA). The moisture content and organic matter were determined by measuring the weight change after drying during 24 h at 100 °C. To measure the pH and electric conductivity, a 1:10 (wt.%) dilution of the compost with distilled water was prepared, stirred for 30 min and pH was measured using a pH meter (Hanna Instruments, HI-2222, Woonsocket, RI, USA) and electric conductivity was measured using an electric conductivity meter (Ohaus Co., STARTER 300C, Parsippany, NJ, USA).

2.5. Microbial Analysis

2.5.1. DNA Extraction

The compost samples used for genomic DNA (gDNA) extraction, as detailed in Section 2.3, were immediately preserved at -50 °C after collection to maintain microbial DNA stability. Prior to gDNA extraction, the frozen samples were thawed at 25 °C and homogenized using a vortex mixer to ensure thorough mixing before aliquoting. A 400 µL aliquot from each sample was collected and centrifuged at $1118 \times g$, and the supernatant was carefully discarded. The resulting pellets were used for gDNA extraction using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The samples were subjected to bead homogenization with a Bead Ruptor Elite homogenizer (OMNI International, Kennesaw, GA, USA) at a speed of 6 m/s for 1 min, followed by a 1-min rest period. The homogenization and resting cycles were repeated three times, with the samples resting in a thermal bead bath maintained at 80 °C between cycles to dissipate the heat generated during the homogenization process. The quality of the extracted gDNA was verified using a Nanodrop Lite spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and the gDNA samples were stored at -80 °C until further use.

2.5.2. PCR Amplification and Sequence Data Processing

To enable multiplexing of the extracted samples, barcoding PCR was performed using the SQK-16S024 Kit (Oxford Nanopore Technologies, Oxford, UK) with the 27F forward primer (5'-ATCGCCTACCGTGAC-barcode-AGAGTTTGATCMTGGCTCAG-3') and the 1492R reverse primer (5'-ATCGCCTACCGTGAC-barcode-CGGTTACCTTGTTACGACTT-3'). The PCR conditions were as follows: initial denaturation at 98 °C for 30 s, followed by 25 cycles of 98 °C for 10 s for denaturation, 55 °C for 30 s for annealing, and 65 °C for 90 s for extension, followed by a final extension step at 65 °C for 5 min. The LongAmp® Taq 2X master mix (New England Biolabs, Ipswich, MA, USA) was used for all PCR reactions. Post-PCR, the amplicons were purified to match the target length of 1.5 kb using AMPure XP SPRI beads (Beckman Coulter, Brea, CA, USA), in accordance with the SQK-16S024 kit protocol. The purified amplicons were then quantified using the Invitrogen Qubit 4 Fluorometer (Thermo Fisher Scientific) for precise concentration analysis. The DNA library was subsequently pooled to a final concentration of 75 fmol for sequencing. The pooled library was sequenced using a MinION Flow Cell (R9.4.1) (Oxford Nanopore Technologies) on a MinKNOW software platform (Oxford Nanopore Technologies) with GPU support. Simultaneously, GPU base calling was conducted in a super-accurate mode to generate demultiplexed FASTQ files for each sample based on their barcodes. Sequencing depth was ensured by generating a minimum of 80,000 reads with a Phred Qscore ≥ 10 . The resulting sequencing data were processed using the QIIME2 bioinformatics platform, where operational taxonomic unit clustering was performed. Taxonomic classification was conducted using SILVA 138.1 database, with vsearch global alignment with a threshold of 90% sequence identity and 80% query coverage. The relative abundances at the phylum, genus, and species levels were calculated based on these results.

2.5.3. Statistical Analysis

All experimental indicators were analyzed in triplicate, and the results expressed as mean $\pm$ standard error. Statistical analyses of physicochemical characteristics and table preparation were performed using Microsoft Excel 2018. Data visualization and plotting were conducted using Sigma Plot 14.0. Bacterial community analysis was performed using R version 3.1.21, and subsequently visualized using Sigma Plot. These tools were applied to the Galaxy platform (www.freebioinfo.org). Microbiome Analyst 2.0 was used to analyze core bacteria and co-occurrence patterns between different microbial communities. Network analysis and centrality measurements were performed using Gephi version 0.9.2. Co-occurrence networks of bacterial genera were constructed based on correlation matrices generated from relative abundance data. A correlation threshold was applied to include only statistically significant associations ($p < 0.05$). The network layout was visualized using the Fruchterman–Reingold algorithm to enhance the spatial organization of co-occurring taxa. Centrality measures, including eigenvector, betweenness, closeness, and harmonic centralities, were calculated to analyze changes in the structure of the network. Additionally, network modularity was calculated to detect the community structures. Visualization of the network was customized for clarity and interpretation of key relationships within the microbial community.

3. Results and Discussion

3.1. Change in Physicochemical Characteristics

3.1.1. Effects of Treatments on the Physicochemical Properties of the Compost

Based on the results in Table 3, the moisture content is vital for maintaining microbial activity and enabling organic matter decomposition during composting. In this study, the moisture content decreased across all treatments, with the largest reduction in T4 (from 72.1% to 56.6%). This could be attributed to water evaporation driven by microbial metabolism, particularly during the thermophilic phase when temperatures exceed 40 °C [31,32]. The porous structure of the biochar in T2, T5, and T6 likely mitigated moisture loss, supporting microbial growth and enhancing water retention, as reported

in previous studies [33]. The decomposition of organic matter is vital in the composting process that is primarily facilitated by microbial enzymes. Organic matter content significantly declined across treatments, with T1 reducing from 90.77% to 60.99% and T4 showing the highest reduction (91.83% to 72.36%). This accelerated breakdown in T4 is attributed to *Bacillus* sp., which produces extracellular enzymes such as cellulases and proteases that facilitate decomposition of complex organic compounds [34]. These findings are consistent with previous research demonstrating the efficiency of *Bacillus* sp. in enhancing organic matter degradation during composting [34,35]. In addition, pH plays a crucial role in composting by influencing microbial activity, nutrient availability, and ammonia volatilization. Initially, the pH levels of the compost were mildly acidic (5.3–5.6), reflecting the early-stage production of organic acids. However, as composting progressed, pH increased, particularly in the biochar and *Bacillus* sp. treatments, with T4 and T6 reaching final pH levels of 8.13 and 8.52, respectively. The increase in pH was largely due to the microbial breakdown of organic acids and the accumulation of ammonium. The protease activity of *Bacillus* sp. converts proteins into ammonia (NH₃) and ammonium ions (NH₄⁺). Ammonium ions buffer the pH, preventing nitrogen loss as gaseous ammonia, especially in alkaline conditions [36,37]. This buffering effect stabilized the pH, creating a more favorable environment for microbial activity, as observed in T3, T4, T5, and T6. The control (T1) showed the highest EC increase, indicating significant soluble salt accumulation (Table 3). Treatments with biochar (T2, T5, T6) reduced EC levels, demonstrating biochar's ability to mitigate salt accumulation. *Bacillus* sp. treatments (T3, T4) moderately increased EC, promoting organic matter decomposition without excessive salt buildup. Combining biochar and microbial agents (T5, T6) effectively managed EC, enhancing compost quality and making it more suitable for agricultural use.

Table 3. Changes in physicochemical characteristics during the composting process.

Treatment	Moisture Content (%)		Organic Matter (%)		pH		Electrical Conductivity (mS/cm)		Weight Reduction Rate (%)
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	
T1	73.7 ± 3.24	58.2 ± 2.34	90.77 ± 3.44	60.99 ± 4.59	5.3 ± 0.31	7.72 ± 0.36	2.33 ± 0.25	2.72 ± 0.25	52.1 ± 2.34
T2	71.5 ± 2.45	60.1 ± 3.52	79.52 ± 3.01	57.45 ± 3.25	5.46 ± 0.25	8.28 ± 0.24	2.13 ± 0.13	2.35 ± 0.16	34.8 ± 1.52
T3	74.5 ± 3.41	61.9 ± 2.28	91.23 ± 5.22	68.82 ± 6.58	5.33 ± 0.32	8.07 ± 0.35	2.15 ± 0.14	2.34 ± 0.17	44.3 ± 1.89
T4	72.1 ± 3.12	56.6 ± 2.47	91.83 ± 3.33	72.36 ± 4.94	5.32 ± 0.27	8.13 ± 0.37	2.17 ± 0.18	2.35 ± 0.19	45.4 ± 2.21
T5	67.1 ± 2.34	60.7 ± 2.36	79.14 ± 3.06	60.58 ± 4.08	5.38 ± 0.28	8.42 ± 0.23	2.27 ± 0.24	2.15 ± 0.12	51.2 ± 2.89
T6	67.1 ± 2.21	63.2 ± 2.35	79.83 ± 5.63	56.08 ± 4.06	5.6 ± 0.23	8.52 ± 0.21	2.21 ± 0.17	2.14 ± 0.17	39.7 ± 2.31
<i>p</i> -Value (<i>p</i>)	0.722	0.224	0.161	0.464	0.800	0.574	0.104	0.124	0.471
F-statistic (<i>F</i>)	0.572	1.925	2.375	1.058	0.450	0.827	3.046	2.759	1.041

The ANOVA analysis showed no statistically significant differences among treatments for most of the composting variables, as indicated by high *p*-values (*p* > 0.05). The results indicate that the addition of biochar and *Bacillus* sp. inoculation exhibited similar patterns to the typical physicochemical changes observed during composting, confirming that there were no adverse effects caused by the application of these additives. However, numerical trends suggest that T4 (*Bacillus* sp. treatment) demonstrated slightly better performance in organic matter degradation. Similarly, biochar-treated groups (T2, T5, T6) appeared to enhance moisture retention and pH stabilization, supporting microbial activity and compost quality.

3.1.2. Effects of Treatments on Gas Emission Profiles

Temperature is a key indicator of microbial activity during composting. During the initial phase, temperatures across all treatments (T1–T6) increased significantly, with T4 reaching the highest peak at 39.55 °C by day 1, and T5 peaking slightly later at 38.5 °C

(Figure 2a). These temperature increases indicate a transition into the thermophilic phase, where high microbial activity leads to the rapid breakdown of organic matter. *Bacillus* sp. in T3 and T4, known for their thermotolerance and enzyme production, demonstrated enhanced organic matter degradation [38]. The biochar treatments (T2, T5, and T6) showed a moderate but sustained temperature increase, suggesting the role of biochar in stabilizing the microbial environment and supporting both heat retention and microbial diversity [39]. The temperature began to decrease after day 5 in all treatments, aligning with the exhaustion of readily degradable organic matter and a reduction in microbial activity. By day 21, the temperature had stabilized between 17–19 °C, reflecting the compost's maturation phase (Figure 2a). This cooling stage reflects the completion of most microbial degradation, leaving behind more recalcitrant organic matter [40]. Ammonia (NH_3) is an important nitrogenous compound released during the degradation of nitrogen-containing organic matter such as proteins. The initial ammonia emissions were low across all treatments; however, by day 5, significant ammonia production was observed in all *Bacillus* sp. and biochar treatments (T3–T6). T4 exhibited the highest ammonia concentration on day 5 (220 ppm), followed by a rapid decline and reaching zero by day 21 (Figure 2b). The reduction in ammonia emissions could be attributed to the microbial assimilation of nitrogen into the biomass and the adsorption capabilities of the biochar. In T3 and T4, the *Bacillus* sp. (Figure 2d) present actively converted ammonia into ammonium ions (NH_4^+), which are more stable and less prone to volatilization [41]. The porous structure of the biochar likely played a dual role by physically adsorbing ammonia as well as providing a surface for microbial colonization, thus further reducing ammonia loss. By day 21, all treatments showed no ammonia emissions, indicating effective nitrogen retention within the compost matrix [42]. CO_2 emissions provide a direct measure of microbial respiration and breakdown of organic carbon. As expected, CO_2 emissions peaked early, from day 2 to day 3, when microbial activity was the highest (Figure 2c). T5, which contained a combination of biochar and *Bacillus* sp., exhibited the highest initial CO_2 emission levels, peaking at 105,000 ppm on day 2, followed closely by T6 at 110,000 ppm (Figure 2c). This suggests that, in addition to enhancing ammonia adsorption, biochar facilitates greater microbial activity and organic matter decomposition [43]. CO_2 emissions gradually decreased across all treatments after day 5, consistent with the reduction in temperature and ammonia emissions. By day 21, CO_2 emissions decreased significantly, with T5 and T6 showing slightly higher residual emissions than the other treatments, potentially because of the ongoing decomposition of more recalcitrant organic matter.

The results of this study emphasize the complementary roles of *Bacillus* sp. inoculation and biochar addition in optimizing the composting process. While *Bacillus* sp. enhances organic matter degradation and ammonia stabilization through microbial activity, biochar improves heat retention, nitrogen retention, and microbial habitat stability. Together, these additives contribute to higher-quality compost with reduced environmental emissions. These findings offer valuable insights into sustainable composting practices and highlight biochar-*Bacillus* sp. synergy as an effective strategy for managing organic waste.

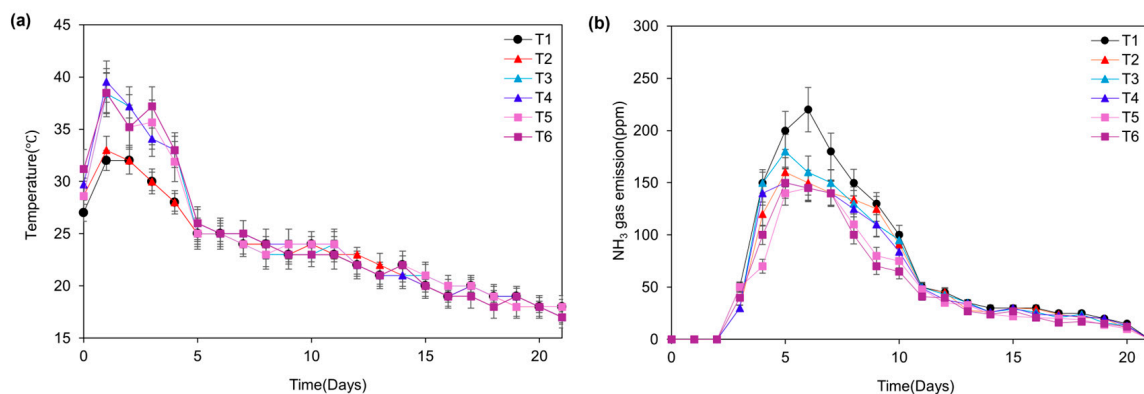


Figure 2. Cont.

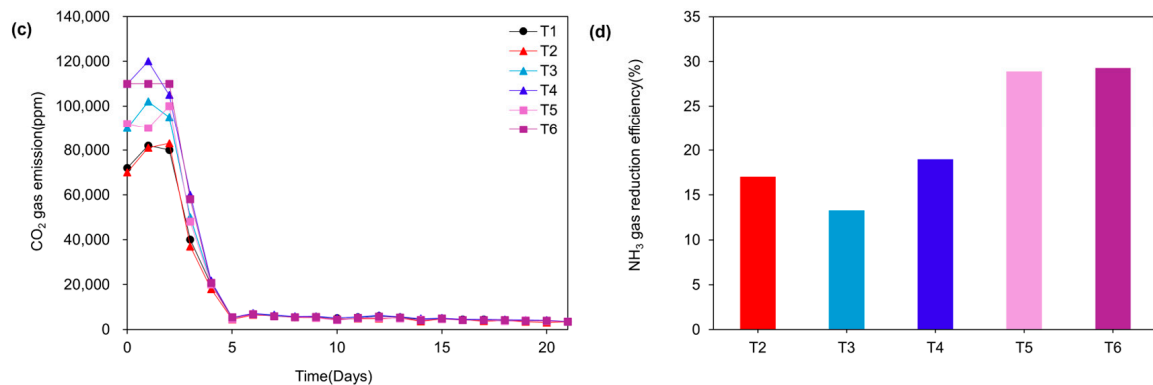


Figure 2. Effects of different treatments on gas emission profiles. (a) Changes in temperature (°C), (b) NH₃ (ppm), (c) CO₂ (ppm), gas emission profiles, and (d) NH₃ reduction efficiency during the composting process (%).

3.2. Comparative Dynamics of Microbial Communities in Compost

At the onset of the composting process, distinct differences in microbial community composition were observed between food waste (FW) and livestock manure (LS) substrates (Figure 3). The LS treatments were dominated by the phylum Firmicutes, with relative abundances of up to 95.17%, reflecting their adaptability to nutrient variability and their critical role in nitrogen retention and pathogen suppression—important functions for manure substrates [44]. In contrast, FW treatments exhibited a lower abundance of Firmicutes but showed higher levels of Proteobacteria and Bacteroidota, indicative of microbial communities better suited for rapid organic matter decomposition (Figure 3) [45]. These initial differences underscore the significant influence of substrate type on microbial community structure at the start of composting. During composting, a general decline in Firmicutes was observed across all treatments, with the decrease being more pronounced in FW substrates (Figures 3 and 4). This shift coincided with a marked increase in Proteobacteria and Bacteroidota, particularly after day 16. These changes corresponded to the degradation of complex polysaccharides and proteins, key stages of the composting process. Treatments supplemented with *Bacillus* sp. (T3 and T4) exhibited enhanced microbial diversity, particularly in taxa associated with nutrient cycling and the breakdown of recalcitrant organic compounds. By day 35, the *Bacillus* sp.-supplemented treatments exhibited increased abundances of Deinococcota and Myxococcota, which are associated with late-stage composting processes, including cellulose degradation and pathogen suppression (Figure 4) [46].

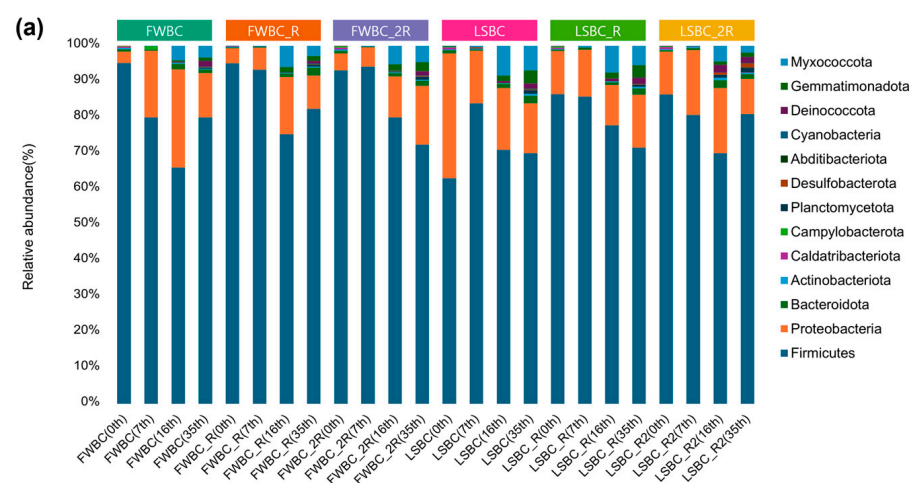


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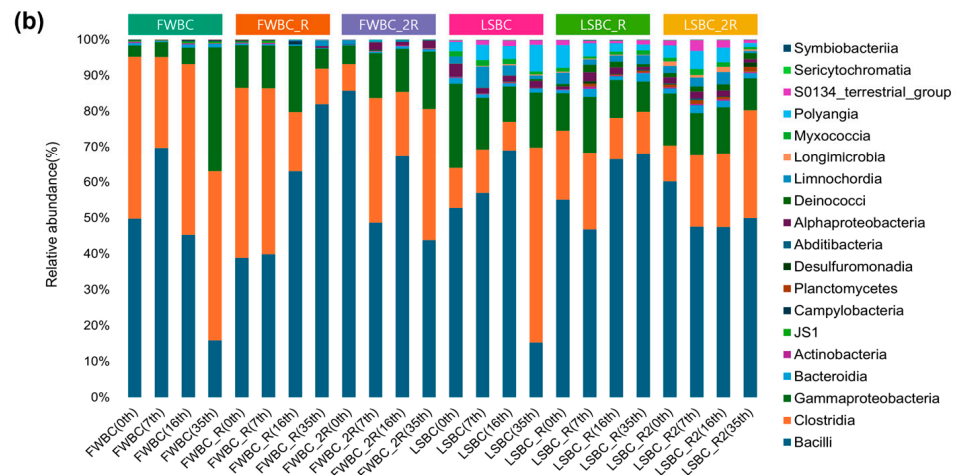


Figure 3. Taxonomic analysis of compost. (a) Phylum-level taxonomic analysis and (b) class-level taxonomic analysis.

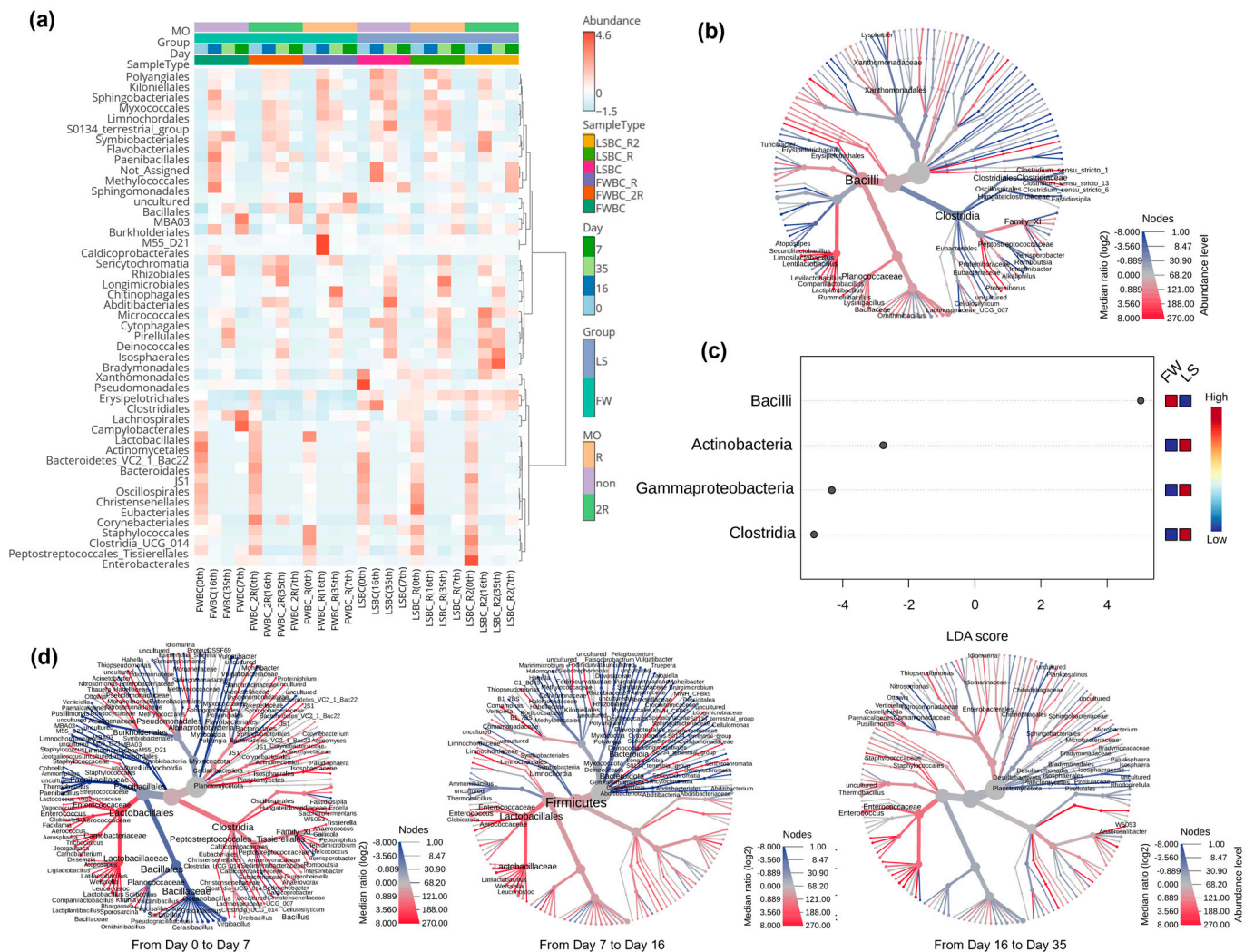


Figure 4. Dynamics of microbial communities in compost. (a) Heatmap comparison analysis of core bacteria in composts from different treatments and processes at the order-level, (c) linear discriminant analysis effect size (LEfSe) based on operational taxonomic units was used to differentiate between FW and LS group (p -value ≤ 0.1 , Log LDA score ≥ 2.0). Heat tree analysis based on bacterial taxonomic differences across composting feedstock composition (b) and duration (d).

The microbial change between FW and LS diverged significantly in response to *Bacillus* sp. supplementation. LS treatments retained Firmicutes dominance for a longer period, particularly Clostridia, which maintained stable abundances of 22–25% throughout the composting process. This stability highlights Clostridia's role in anaerobic or micro-aerobic decomposition, particularly for nitrogen-rich substrates (Figure 3). In contrast, FW treatments transitioned more rapidly to microbial communities dominated by Proteobacteria and Bacteroidota, favoring the degradation of plant-derived organic matter. By day 35, Firmicutes had lost dominance in FW treatments, particularly those supplemented with *Bacillus* sp. Proteobacteria, primarily represented by Pseudomonadales and Enterobacteriales, comprised up to 35% of the microbial community in *Bacillus* sp.-supplemented FW treatments, underscoring their importance in nitrogen cycling and the degradation of recalcitrant organic matter during the final composting stages. Similarly, Bacteroidota reached an abundance of 15% in non-supplemented FW treatments, contributing to the breakdown of complex carbohydrates. The supplementation of *Bacillus* sp., particularly at double dosages, significantly influenced microbial succession in FW treatments. These treatments exhibited higher microbial diversity and faster transitions from Firmicutes to Proteobacteria and Bacteroidota, resulting in a more balanced microbial community and more complete decomposition by day 35 compared to non-supplemented treatments (Figure 4). LEfSe analysis was conducted to identify microbial taxa significantly enriched in FW and LS groups, with thresholds set at $p < 0.01$ and LDA score > 2 . The results provide additional insights into the taxonomic differentiation between the two substrate groups. Bacilli were significantly enriched in FW ($p = 0.0012$, FDR = 0.022, LDA score = 5.02), with a relative abundance of 640,200 compared to 430,520 in LS. This highlights Bacilli's key role in organic matter decomposition and nutrient cycling in FW treatments. Clostridia were significantly enriched in LS ($p = 0.0056$, FDR = 0.050, LDA score = -4.87), with a relative abundance of 331,300 in LS compared to 182,480 in FW. The dominance of Clostridia underscores their involvement in lignocellulosic degradation and fermentation processes characteristic of manure substrates. Gammaproteobacteria (LDA score = -4.34) and Actinobacteria (LDA score = -2.77) were more abundant in LS, with relative abundances of 146,800 and 3015.7, respectively, compared to 103,340 and 1829 in FW. These taxa likely play specialized roles in lignocellulosic substrate degradation.

The findings emphasize the substantial influence of substrate type on the initial microbial community structure during composting. While LS substrates supported Firmicutes-dominated microbial profiles for nutrient variability and pathogen control, FW substrates promoted communities tailored for rapid organic matter decomposition. Throughout the composting process, microbial community dynamics shifted significantly, with Firmicutes declining and Proteobacteria and Bacteroidota increasing, reflecting the progression of organic matter degradation. The results of the LEfSe analysis further elucidate the taxonomic drivers underlying these compositional differences, highlighting Bacilli and Clostridia as key contributors to the distinct microbial processes in FW and LS composting systems. The supplementation of *Bacillus* sp. (T3 and T4) was particularly effective in enhancing microbial diversity and promoting the activity of taxa associated with late-stage composting, such as Deinococcota and Myxococcota. These shifts highlight the role of *Bacillus* sp. in accelerating compost maturation and improving nutrient cycling efficiency. These insights provide valuable implications for optimizing composting strategies based on substrate selection and microbial augmentation.

3.3. Core Bacteria Co-Occurrence Network and Interaction Patterns

The addition of *Bacillus* sp. significantly influenced microbial succession across both types of composting, leading to notable changes in the indigenous bacterial community, particularly affecting the balance between the aerobic and anaerobic microbial populations (Figures 5a and 6a). This shift fosters the growth of beneficial aerobic bacteria while reducing the prevalence of anaerobic species, thereby optimizing the overall microbial community for efficient composting. Specifically, the composition shifted toward Bacillaceae as

a central core family, with a marked reduction in the prevalence and abundance of anaerobic families such as Clostridiaceae and Family_XI. These changes were more pronounced with higher doses of *Bacillus* sp., indicating a dose-dependent effect on the microbial community structure and functionality. In the food waste biochar compost (FWBC), the addition of *Bacillus* sp. enhanced microbial interactions between Planococcaceae and Bacillaceae during the thermophilic composting phase (Figure 5b). Analysis of co-occurrence patterns involving the core bacteria Bacillaceae reveals significant correlations (0.74) with Planococcaceae and other thermophilic families. These two families are known for their ability to thrive in high-temperature environments, where they synergistically break down complex organic materials such as cellulose and lignin. Planococcaceae, a spore-forming and thermophilic family, plays a key role in decomposing organic matter and producing essential by-products such as CO₂ and water, which are crucial for the composting process [38,47]. Moreover, the interaction between *Bacillus* sp. and Planococcaceae enhanced pathogen suppression, as these bacteria produce antimicrobial compounds that are known to improve the hygiene and safety of the compost. This led to a more efficient composting process with accelerated maturation, rendering the final product more suitable for agricultural applications. Collaboration between Planococcaceae and *Bacillus* sp. is represented in Figure 7 [48].

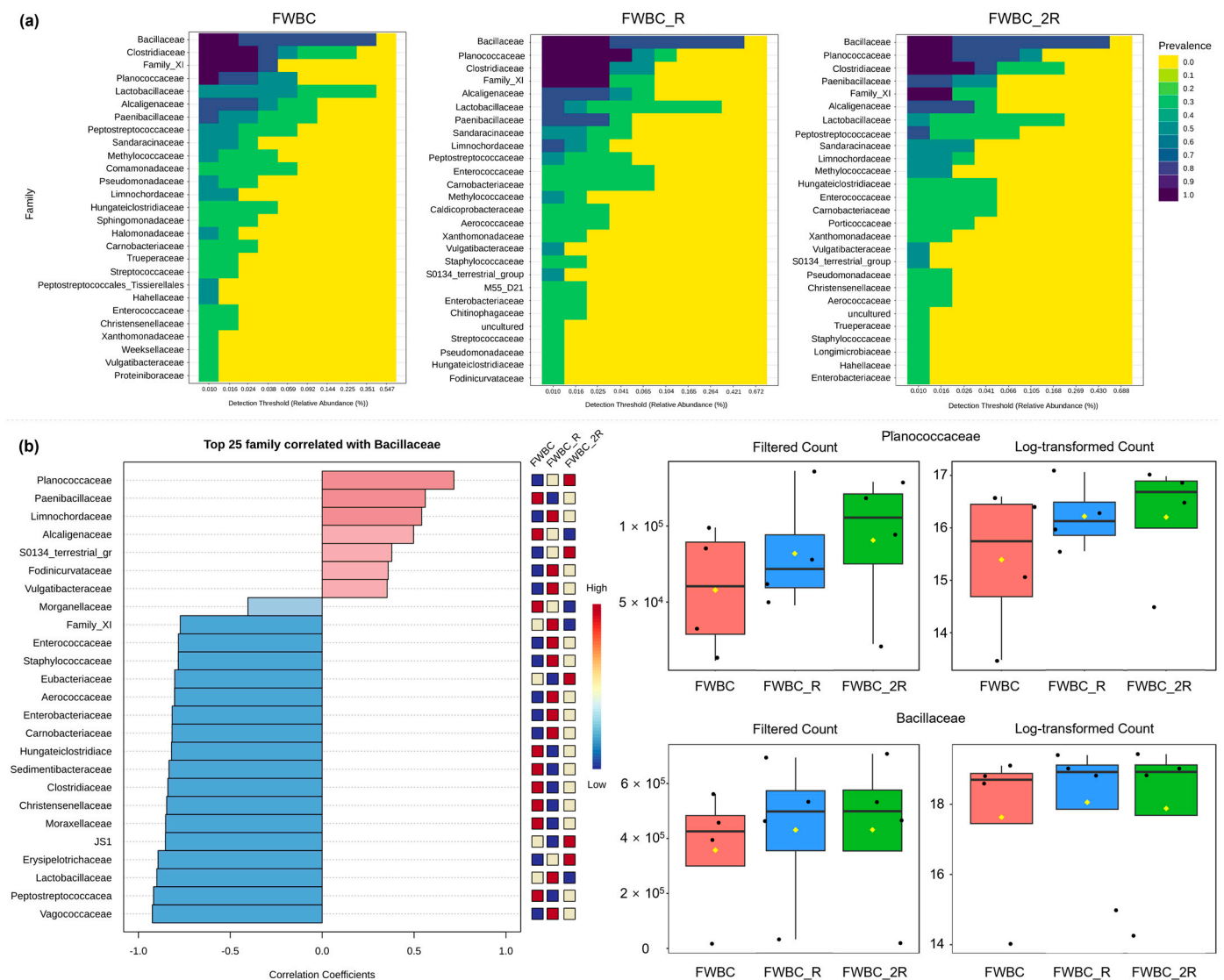


Figure 5. Meta-analysis to identify (a) core bacteria and (b) co-occurrence patterns with associated microbial communities in food waste treatment compost.

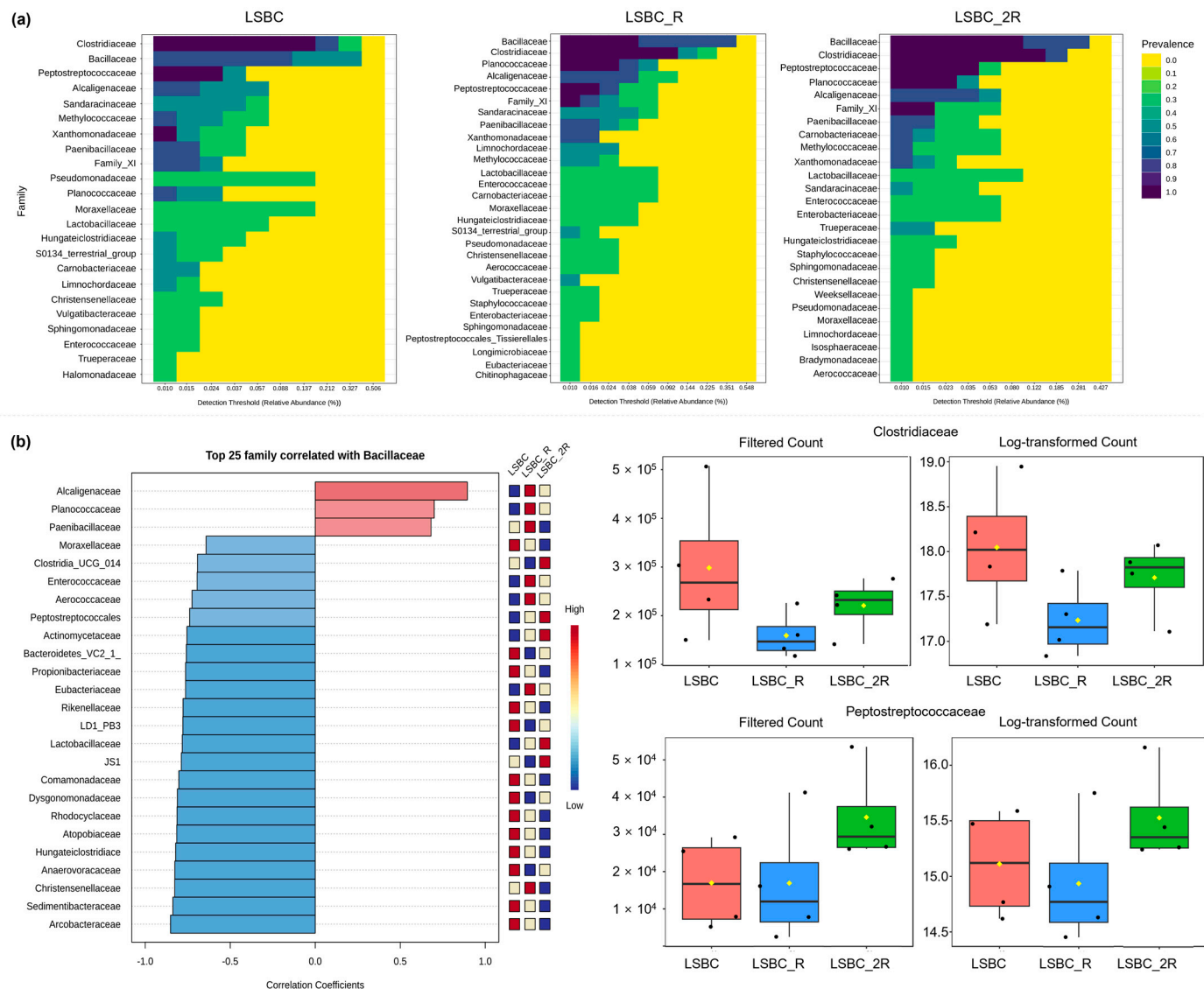


Figure 6. Meta-analysis to identify (a) core bacteria and (b) co-occurrence patterns with associated microbial communities in livestock treatment compost.

For livestock manure biochar compost (LSBC), the introduction of *Bacillus* sp. had a profound effect on the microbial balance by favoring aerobic over anaerobic bacterial families. Notably, the abundance of Clostridiaceae and Peptostreptococcaceae, both anaerobic families, was significantly reduced (Figure 6). Clostridiaceae typically thrive in low-oxygen environments and breakdown organic matter, but often produce undesirable by-products such as methane and hydrogen sulfide [49]. However, inoculation with *Bacillus* sp. fostered a more aerobic environment, suppressing Clostridiaceae activity and reducing harmful gas emissions. Similarly, the abundance of Peptostreptococcaceae, which is known for fermenting proteinaceous materials and producing ammonia, was also reduced under these conditions [50]. This reduction improves nitrogen cycling and prevents the accumulation of nitrogenous compounds, leading to faster compost stabilization and enhanced quality compost generated. The inoculation of *Bacillus* sp. in LSBC promoted a shift in the microbial community, favoring aerobic bacteria, such as Bacillaceae and Alcaligenaceae, which are instrumental in the thermophilic breakdown of complex organic compounds (Figures 6 and 7). This increased the efficiency of the composting process, with a faster reduction in the C/N ratio, indicating improved compost maturity. While both FWBC and LSBC benefited from the inoculation with *Bacillus* sp., there were notable differences

in how the microbial community structure was affected (Figures 7 and 8). In FWBC, the primary focus was on the synergistic activity of Planococcaceae and Bacillaceae, which accelerated the decomposition of complex organic materials, such as cellulose and lignin, while improving pathogen suppression. In contrast, LSBC showed a marked reduction in anaerobic bacterial families, such as Clostridiaceae and Peptostreptococcaceae. This improved nitrogen cycle and reduced emissions of harmful gases, such as methane and hydrogen sulfide. However, when the amount of microbial additive was doubled in the LSBC group, the relative abundance of Peptostreptococcaceae increased (Figure 3). This may be attributed to Peptostreptococcaceae's ability to thrive in specific niches formed in environments with intense microbial competition or increased organic nitrogen concentrations [50]. Such conditions could be temporarily favorable for certain anaerobic or facultative anaerobic bacteria, despite the overall shift towards aerobic conditions induced by *Bacillus* sp. inoculation. Another possible explanation is that certain species within Peptostreptococcaceae adapt to or survive fluctuating oxygen levels, particularly during the transition phases of composting [50].

Microbial community dynamics during composting are not limited to the interactions between individual microbial groups, but are often influenced by the complex network of relationships across the indigenous bacterial community. Therefore, to fully understand the effects of microbial inoculation during composting, it is necessary to analyze the network of microbial interactions rather than focusing solely on the behavior of specific groups [51]. This network analysis can reveal synergistic or competitive relationships between microorganisms and identify key species that drive the composting processes [52]. Such a detailed network analysis of microbial relationships would provide deeper insights into how *Bacillus* sp. and other inoculated microbes interact with the native microbial community, helping to optimize composting processes.

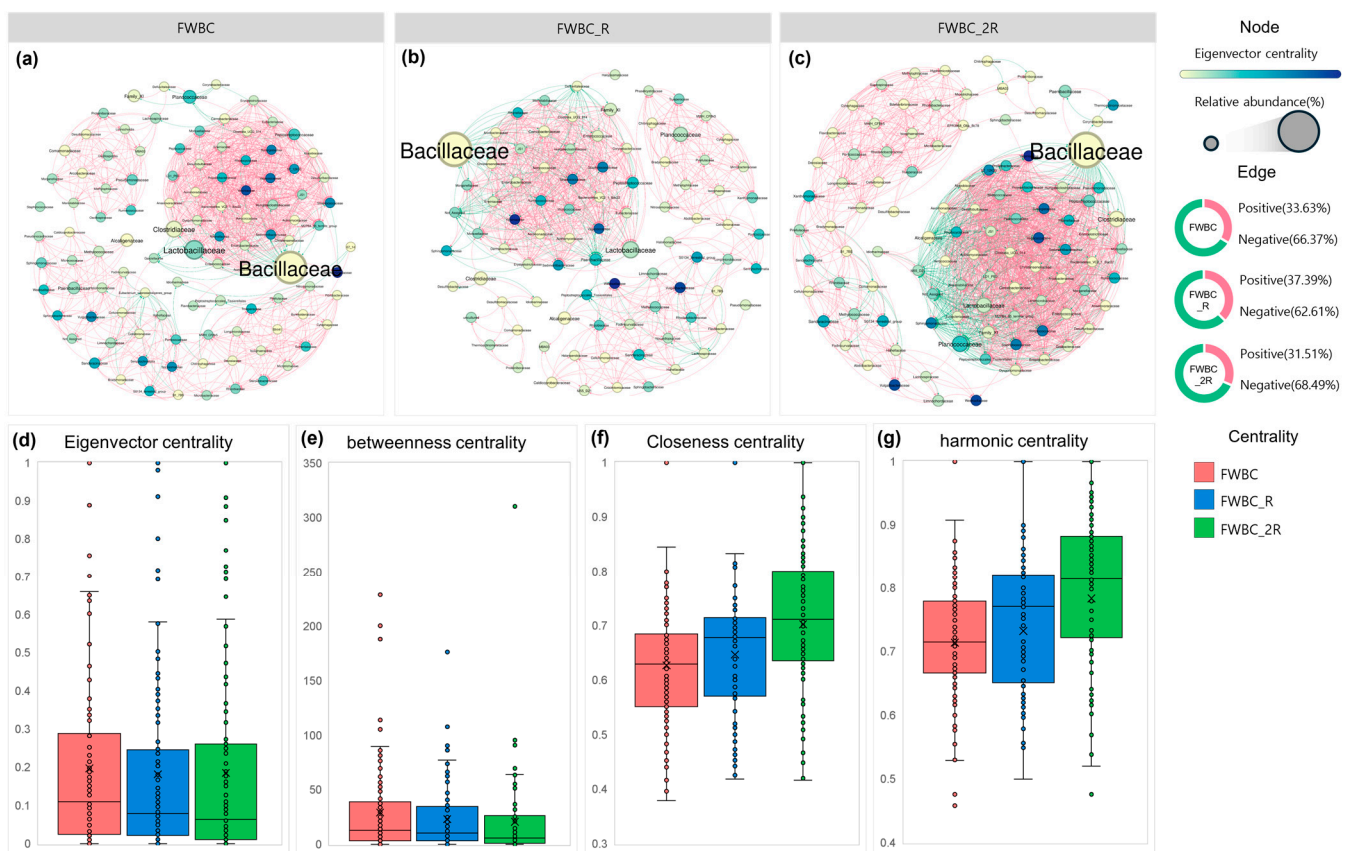


Figure 7. Microbial co-occurrence network of (a) FWBC, (b) FWBC_R, (c) FWBC_2R, and (d) network structure analysis with eigenvector centrality, (e) betweenness centrality, (f) closeness centrality, and (g) harmonic centrality metrics.

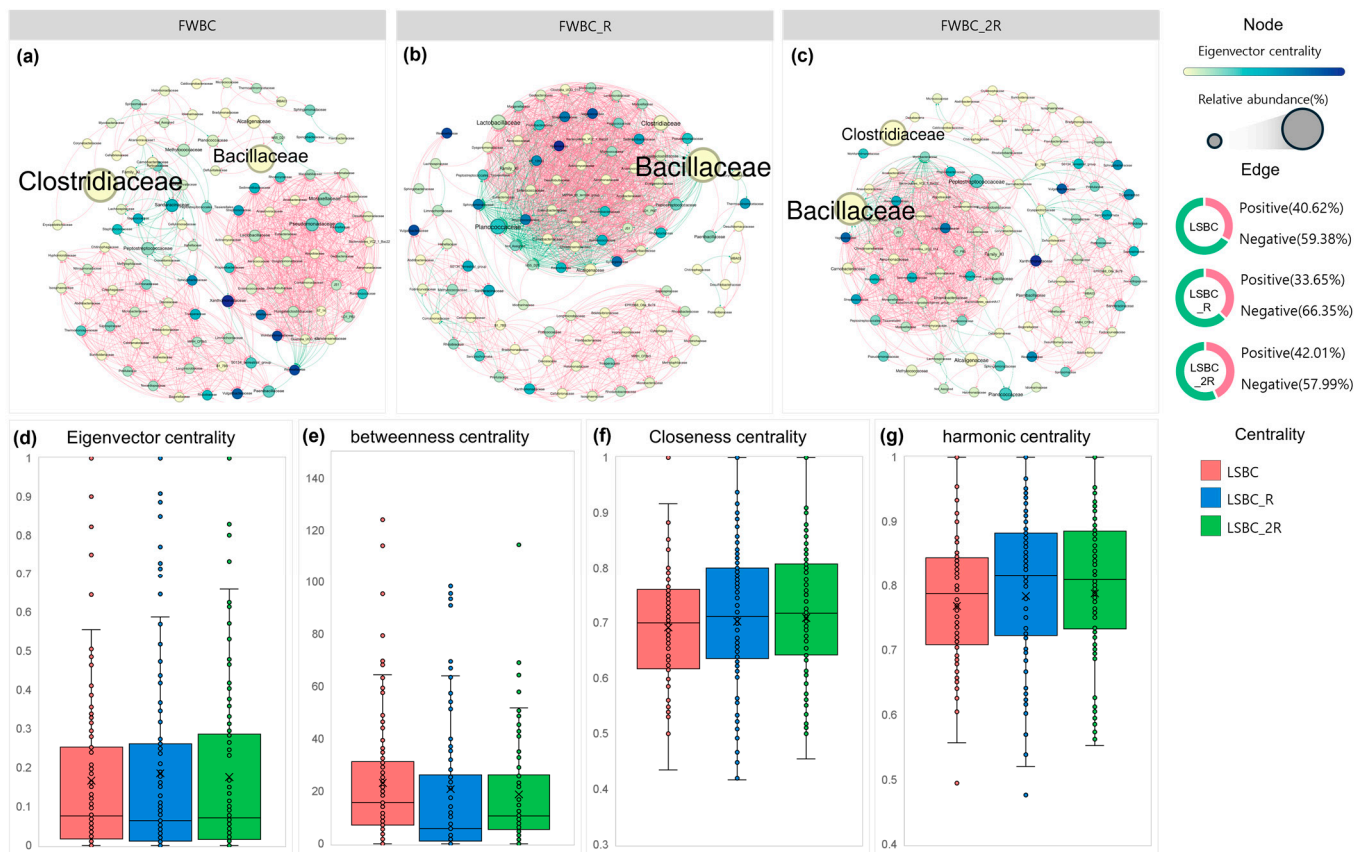


Figure 8. Microbial co-occurrence network of (a) LSBC, (b) LSBC_R, (c) LSBC_2R, and (d) network structure analysis with eigenvector centrality, (e) betweenness centrality, (f) closeness centrality, and (g) harmonic centrality metrics.

Microbial co-occurrence networks of the FWBC and LSBC groups were constructed to investigate the structural dynamics and interaction patterns of the microbial communities throughout the composting process. Figure 7 illustrates the co-occurrence network for the FWBC group, whereas Figure 8 presents the network for the LSBC group. These figures highlight the differences in the microbial interactions and structural properties between the two groups under various composting conditions. Additionally, various centrality metrics—eigenvector centrality, betweenness centrality, closeness centrality, and harmonic centrality—were used to assess the network properties and structural shifts arising due to the introduction of *Bacillus* sp. The analysis of edges, which represent pairwise microbial interactions, revealed distinct shifts in the balance between positive and negative correlations across both the FWBC and LSBC groups under different conditions.

In the FWBC group, positive correlations constituted 33.63% of the total number of edges, while negative correlations accounted for 66.37%. Supplementation of *Bacillus* sp. in the FWBC_R treatment increased the proportion of positive correlations slightly to 37.39%, whereas that of negative correlations decreased to 62.61%, indicating a slight shift towards more cooperative interactions. However, in the FWBC_2R treatment, positive correlations decreased again to 31.51%, while negative correlations increased to 68.49%, suggesting that higher doses of *Bacillus* sp. increased competitive or inhibitory interactions. In the LSBC group, the proportion of positive correlations was higher, accounting for 40.62%, while negative correlations accounted for 59.38%. After the addition of *Bacillus* sp., the FWBC_R treatment showed a decrease in positive correlations to 33.65%, whereas negative correlations increased to 66.35%, reflecting a shift towards more antagonistic interactions. Notably, in the FWBC_2R treatment, the network displayed a different trend, with positive correlations increasing to 42.01% and negative correlations decreasing to 57.99%, suggesting

that higher *Bacillus* sp. dosage might have re-established cooperation within the microbial community or alleviated certain competitive dynamics [53]. Network centrality analysis revealed significant structural shifts in microbial interactions. Eigenvector and betweenness centralities showed a decreasing trend with the addition of *Bacillus* sp. across both the FWBC and LSBC groups. A decrease in eigenvector centrality indicates a reduction in the influence of highly connected and influential microbial nodes within the network [54]. Nodes that were once central in terms of connectivity and association with other influential nodes lost their dominance as *Bacillus* sp. altered the microbial composition. Similarly, the decrease in betweenness centrality suggests that key nodes involved in bridging disparate communities or acting as intermediaries in the flow of biological interactions play a diminished role [54]. This could reflect a restructuring of the network, where specific microbial taxa were less critical for maintaining network integrity when *Bacillus* sp. were introduced. By contrast, closeness and harmonic centralities exhibited increasing trends, signifying a shift towards a more efficient and tightly connected network structure. The increase in closeness centrality indicated that the microbes, on average, were more closely connected within the network, leading to a reduction in the overall path length between the nodes [55]. The addition of *Bacillus* sp. may have facilitated direct and efficient microbial interactions, thereby enhancing communication across the community. Moreover, the increase in harmonic centrality supports this observation, as it reflects the improved overall accessibility of microbes within the network, even in cases of indirect connections. This pattern implies that *Bacillus* sp. contributed to a more cohesive and interactive microbial network, despite the presence of competing or antagonistic interactions (as indicated by the negative correlation increase in FWBC_2R).

The observed shifts in the edge correlations and centrality metrics highlight the dynamic and complex nature of microbial networks during composting. In the FWBC group, the initial increase in positive correlations following *Bacillus* sp. supplementation suggested a period of enhanced microbial cooperation or mutualistic interactions. However, as the *Bacillus* sp. population increased, the network became dominated by negative correlations, indicating intensified competition or inhibition among microbial taxa. A similar trend was observed in the LSBC group, although the FWBC_2R treatment within this group showed the recovery of positive interactions, indicating a more cooperative network at higher *Bacillus* sp. dosages.

4. Conclusions

This study evaluated the effects of *Bacillus* sp. inoculation on the physicochemical properties, microbial community dynamics, and co-occurrence networks during the composting of FW and LS manure.

Bacillus sp. inoculation accelerated the breakdown of complex compounds, such as cellulose and lignin, resulting in reductions in moisture content and organic matter. It also stabilized pH by converting proteins into ammonium, thereby reducing ammonia volatilization. Furthermore, the addition of biochar reduced ammonia emissions by 17.04%, and this reduction increased to 28.89% when combined with *Bacillus* sp. In terms of microbial community dynamics, *Bacillus* sp. influenced succession differently in FW and LS. LS treatments maintained Firmicutes dominance for an extended period, aiding nitrogen retention and pathogen suppression, whereas FW treatments shifted more quickly to Proteobacteria and Bacteroidota abundance, which efficiently decomposed the plant materials. Additionally, *Bacillus* sp. enhanced microbial diversity and accelerated the transition to late-stage communities, promoting faster compost maturation.

Microbial co-occurrence network analysis revealed that inoculation of *Bacillus* sp. promoted the growth of beneficial aerobic bacteria while suppressing the growth of anaerobic species, creating a more efficient microbial environment for composting. In the FWBC group, the interactions between Bacillaceae and Planococcaceae accelerated organic material decomposition and pathogen suppression. The LSBC group showed reduced anaerobic bacterial families, such as Clostridiaceae and Peptostreptococcaceae, improved nitrogen

cycling, and lowered methane and hydrogen sulfide emissions. However, higher doses of microbial additives in the LSBC group temporarily favored certain anaerobic species, indicating niche-specific adaptations. Network centrality metrics showed that *Bacillus* sp. inoculation altered the microbial network with decreasing eigenvector and betweenness centralities, suggesting less influence from highly connected nodes, whereas closeness and harmonic centralities increased, indicating highly efficient microbial interactions. This restructuring facilitates faster and more effective composting processes.

In conclusion, *Bacillus* sp. inoculation presents a transformative solution in organic waste management by accelerating microbial succession, enhancing nutrient cycling, and fostering efficient microbial interactions. These effects significantly improve composting efficiency and quality, supporting sustainable agriculture through enhanced soil fertility and crop productivity. Moreover, its role in reducing ammonia volatilization and greenhouse gas emissions aligns with environmental goals, contributing to circular waste management and a reduced carbon footprint. Future research should aim to optimize microbial inoculant combinations for specific waste substrates, evaluate their long-term impacts on soil health and agricultural productivity, and explore ecological sustainability. Additionally, investigating the scalability and cost-effectiveness of *Bacillus* sp. inoculation in commercial composting systems is crucial for broader adoption. These efforts will offer valuable insights into the potential of *Bacillus* sp. inoculation for large-scale applications. It serves as a key strategy for advancing sustainable waste management and agricultural practices, aligning with global goals to enhance environmental sustainability and resource efficiency.

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