Bioresource Technology 381 (2023) 129112

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Response characteristics of flax retting liquid addition during chicken manure composting: Focusing on core bacteria in organic carbon mineralization and humification

Yangcun Sun [a,1], Shanshan Sun [a,1], Fangyi Pei [a], Chi Zhang [a], Xinbo Cao [a], Jie Kang [a], Zhenchao Wu [a], Hongzhi Ling [a], Jingping Ge [a,b,*]

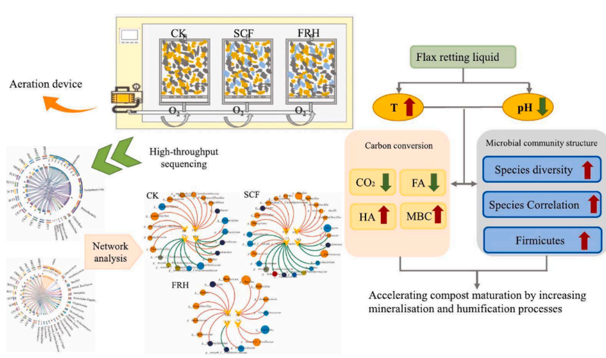
[a] *Engineering Research Center of Agricultural Microbiology Technology, Ministry of Education & Heilongjiang Provincial Key Laboratory of Ecological Restoration and Resource Utilization for Cold Region & Heilongjiang Provincial Key Laboratory of Plant Genetic Engineering and Biological Fermentation Engineering for Cold Region & Key Laboratory of Microbiology, College of Heilongjiang Province & School of Life Sciences, Heilongjiang University, Harbin 150080, China*

[b] *Hebei University of Environmental Engineering, Hebei Key Laboratory of Agroecological Safety, Qinhuangdao 066102, China*

HIGHLIGHTS

- Flax retting liquid (FRL) reduces the emission of CO_2 during mineralization process.
- FRL promotes the production of humic acid during humification process.
- FRL increases the degree of direct association between various bacterial genera.
- FRL alters the abundance of *Firmicutes*, *Actinomycetes*, *Proteobacteria*.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Chicken manure
Flax retting liquid
Microbial communities
Carbon conversion
Humification

ABSTRACT

To explore the applicability of flax retting liquid (FRL) addition, the physicochemical properties, microbial community structure and function, carbon conversion and humus (HS) formation were assessed during chicken manure (CM) aerobic composting. Compared with the control group, the addition of FRL increased the temperature at thermophilic phase, while the microbial mass carbon content (MBC) in SCF and FRH groups raised to 96.1 ± 0.25 g/Kg and 93.33 ± 0.27 g/Kg, respectively. Similarly, FRL also improved the concent of humic acid (HA) to 38.44 ± 0.85 g/Kg, 33.06 ± 0.8 g/Kg, respcetively. However, fulvic acid (FA) decreased to 30.02 ± 0.55 g/Kg, 31.4 ± 0.43 g/Kg, respectively and further reduced CO_2 emissions. FRL influenced the relative abundance of Firmicutes at thermophilic phase and *Ornithinimicrobium* at maturity phase. Additionally, FRL strengthen the association among flora and reduce the number of bacteria, which was negative correlated with HA and positive

* Corresponding author at: School of Life Sciences, Heilongjiang University, Harbin 150080, China.
E-mail address: gejingping@126.com (J. Ge).

[1] These authors contributed equally and share the first authorship: Yangcun Sun, Shanshan Sun.

https://doi.org/10.1016/j.biortech.2023.129112
Received 28 February 2023; Received in revised form 19 April 2023; Accepted 27 April 2023
Available online 1 May 2023

with CO_2 during composting. These findings offer powerful technological support for improving agricultural waste recycling.

1. Introduction

Flax is widely grown in France, Belgium, Russia and China as well as an important natural source of cellulose. About one million tons of flax fibers are produced annually (Melelli et al., 2022), and applied broadly in food, textile, construction, aerospace and medical fields. However, flax fibers were obtained via degumming process before application and producing retting liquid. Flax retting liquid (FRL) is degumming wastewater generated during the hemp fibers process using warm water. The major components of FRL involve pectin, hemicellulose, and its degradation products such as tannins and resin acids (Dey et al., 2021) with the characteristics of low pH, high nitrogen and phosphorus content, high chemical oxygen demand (COD) content and poor biochemical properties (Chen et al., 2015; Su et al., 2011). Without adding chemicals, the generated flax-degumming wastewater contained high levels of wastewater contaminants (Xun, 2011), The use and direct discharge of generated wastewater could inhibit seed germination and cause eutrophication in water bodies (Malik et al., 2019). Currently, the treatment of FRL is mainly carried out by sewage treatment, which meets the relevant discharge standards, however, it covers a large area, takes a long time to treat, consumes high energy, and has complex structure construction (Abou-Elela et al., 2016). Additionaly, wastewater treatment produces high carbon emissions which is contrary to the concept of carbon neutral and carbon peaking proposed in the fight against global climate change (Zhang et al., 2022). Therefore, the removal of FRL from the environment has aroused wide concern.

Aerobic composting is considered as a promising and effective strategy as it has the high efficiency of non-hazardousness and low biological risk to recycle waste and reduce carbon emissions. Composting not only effectively treat wastes and reduce toxic substances, but also produce nutrients rich in humus (HS) and available to plants, such as nitrogen (N), phosphorus (P) and potassium (K) compounds, which are important substances for improving soil fertility and quality. Moreover, studies had shown adding industrial wastewater could optimize the composting effect, for instance, the use of concentrated monosodium glutamate wastewater to treat livestock manure could reduce nitrogen losses (Liu et al., 2015), while the use of ammonium-rich wastewater as a moisture regulator could accelerated the hydrolysis and mineralization of organic matter during composting (Xie et al., 2021). Therefore, aerobic composting may be a new way to treat flax waste liquid, and realize the joint treatment of agricultural and industrial wastes (in other words, using industrial wastewater to treat agricultural wastes, IWTAW).

Microorganisms are an important factor in the composting process, and the essence of aerobic composting is the mineralization and humification of organic matter by microorganisms (Zhan et al., 2022). During the composting process, mineralization of organic matter leads to a large amount of carbon loss (Ba et al., 2020), while humification is considered a key step in carbon sequestration (Zhang et al., 2019), excessive mineralization not only causes to nutrient loss, but also air pollution problems (Yang et al., 2019). It had been shown that *Bacillus* sp. played a key role in the production of HS and fulvic acid (FA) during humification (Bian et al., 2019), and that *Actinomycetes* sp. were able to mineralize lignin and cellulose through carbohydrate metabolic pathways (Tuomela et al., 2000). Kang et al. found that penicillin was able to influence the humification process by altering the pentose phosphate pathway and the shikimate pathway of microbial flora (Kang et al., 2022). Therefore, exploring the influence of microbial community structure and function on organic matter mineralization and humification process will play a positive role in reducing carbon loss and strengthening carbon sequestration, including IWTAW method.

At present, the treatment of solid waste has produced a marked effect by aerobic composting, however, the researches on the management of organic waste liquids is still mainly limited to the field of wastewater treatment. With the proposal of carbon neutralization and carbon emissions peak wastewater treatment plants are under tremendous pressure. Therefore, in this study, from the perspective of treating waste with waste, the FRL was applied to adjust the moisture content, and the purpose of this study was (1) to investigate the effects of FRL on the physicochemical indexes and carbon conversion of agricultural waste aerobic composting; (2) to elaborate the changes of bacterial community structure during IWTAW, and (3) to clarify the regulation of the mineralization and humification process of carbon between the structure of the bacterial flora and the physicochemical indexes. This study provides an effective technical for the recycling and utilization of agricultural waste, improving the final quality of compost.

2. Materials and methods

2.1. Composting experimental design

The chicken manure (CM) and corn straw (CS) were collected from a farm in Harbin, Heilongjiang Province, China. The CM were air-dried before composting and the dried CM were broken into small pellets using a grinder. The CS were cut into small 1–2 cm pieces.

The mixed materials were adjusted C/N to approximately 25 and divided into three groups with the initial moisture of 60%. The moisture of the control group (CK) was adjusted with deionized water, while those of the experimental groups (adding flax retting in the initial stage and using water to adjust humidity, SCF; and adding flax retting in the initial stage and using flax retting liquid to adjust humidity, FRH) were regulated by FRL, and the physicochemical properties of FRL were shown (see Supplementary material). During the composting process, to ensure that the moisture content was maintained at 60%, compensated the moisture loss in the CK and SCF groups with deionised water and in the FRH group with FRL. The three groups were placed in a laboratory-scale adiabatic composting reactor system (ventilation rate of 0.45 L/min) for 35 d carrying out aerobic composting (Yin et al., 2022). The composting reactor is a 40 cm high, 22 cm diameter cylinder with an effective volume of 14 L. Samples were collected at 0, 3, 7, 22, 28 and 35 d for the further experiments and each sample was repeated three times, and the whole composting process was divided into four phases according to the changes of temperature: heating phase (0–3 d), thermophilic phase (4–12 d), cooling phase (13–24 d) and maturity phase (25–35 d).

2.2. Determination of physicochemical factors

The temperature was measured daily during the composting process using a thermometer (T-90, Tool Well, Chin). 4 g fresh samples of compost were mixed with 40 mL ionized water, macerated for 30 min with a shaking speed of 120 rpm and then filtered to detect pH value, electrical conductivity (EC) and redox potential (ORP) using pH meter (FE 20, Mettler Toledo, United States), electric conductometer (DDS-307, Shanghai Yidian Scientific Instruments Co., Ltd.) and redox potential meter (FE 20, Mettler Toledo, United States), respectively. Germination index (GI) was calculated using the method of Subhashini et al. (Subhashini and Kaushik, 1981). HS, humic acid (HA) and FA were analyzed according to the method in a previous study (Ye et al., 2021). In brief, HS was extracted by shaking samples at a speed of 200 rpm with a mixture of 0.1 M NaOH and $Na_4P_2O_7$ (1:10, w:v) at 25 °C for 24 h. The extracts were centrifuged at 12000 rpm for 25 min and passed through

0.45 μm fibre resin membrane to collect the filtrate. The separation of HA and FA was consulted the following steps. The pH of the filtrate was adjusted to 1.0 using 6 M HCl and left to stand for 12 h at 4 °C. HA and FA were separated by centrifugation at 12000 rpm for 20 min, the supernatant and precipitate were FA and HA, respectively. HA was dissolved using 0.05 M Na_2CO_3. The content of HA, FA and HS as well as TOC were determined by organic carbon analyzer (multi N/C 2100, Analytik Jena AG, Germany). Microbial biomass carbon (MBC) was determined using the method of vacuum chloroform fumigation incubation (Biswas and Narayanasamy, 2006), where the Kc value was 0.45. CO_2 content was determined using automated soil carbon flux measurement system (LI-8100A, LI-8150, USA). All experiments were replicated three times.

2.3. High-throughput sequencing

Samples were collected at 0, 3, 7, 22 and 35d during the composting process and DNA was extracted using the Power Soil DNA Isolation Kit (D5625-01, Omega Biotek, Inc, USA). All samples were examined by agarose gel electrophoresis for the required quality of DNA. The primers used for PCR were 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) using a miseq amplicon sequence targeting the 16S V3-V4 region. High-throughput sequencing of the 16S rDNA was carried out. The Operational Taxonomic Unit (OUT) (97% similarity) was compared using the Silva species taxonomy database for OTU clustering analysis and species taxonomic analysis.

2.4. Statistical analysis

The physical and chemical factors were analyzed and mapped using SPSS and Origin 2022b softwares. Microbial community structure was analyzed using the Meguiar's Biosign Cloud platform; and the network between microbial species was visualized using Gephi software. The network relationships between the microbial flora and environmental factors were visualized using Cytoscape and JAVA. The model of the structural equations for the relationship between microbial community structure and physicochemical factors was used by AMOS software.

3. Results and discussion

3.1. Influence of the FRL on the physicochemical factors during composting

The success of composting can be judged by measuring physical factors such as temperature, pH and GI (see Supplementary material), which can alter microbial metabolic activities and thus the quality of the composted products. CK, SCF and FRH entered the thermophilic phase (4–12 d) (>50 °C) at 4 d and the thermophilic phase was maintained for more than 7 d to effectively kill the pathogenic microorganisms in all groups (Chiarelotto et al., 2019). In addition, during the heating phase (0–3 d), the CK group showed a slower temperature increase trend than the SCF and FRH groups, and the highest temperature was lower than those of the SCF and FRH groups (62.5 °C, 64.7 °C and 65.3 °C, respectively) at the thermophilic phase, indicating that FRL was able to accelerate the entry into the thermophilic phase and increase the peak of the maximum temperature.

The initial pH of the SCF and FRH groups were lower than that of the CK group due to the addition of FRL. The pH increased rapidly during the heating phase, decreased after reaching the thermophilic phase, and gradually stabilized during the maturity phase. This trend was consistent with the findings of Liu et al. (2019) composting with pig manure and wheat straw. At the initial stage of composting, the massive ammonification of nitrogenous organic matter was the main reason for the rapid pH rising, while pH decreased gradually with the ammonia releasing, nitrification and organic acids formation. The pH of the SCF (8.72) and FRH (8.6) groups were higher than that of the CK group (8.26) at 35 d, the three groups met the criteria for compost maturity (8.0–9.0) (Wang et al., 2021).

EC reflects the changes in soluble salt content during the composting process. Soluble inorganic salts are released as organic matter is decomposed and utilized by microorganisms through the mineralization and humification process, which in turn causes an increase in EC values (Liu and Zhang, 2023). At the end of the composting period, the EC of the SCF and FRH groups were significantly ($p < 0.05$) higher than that of the CK group, indicating that the addition of FRL accelerated the decomposition of organic matter, which in turn accelerated the organic matter humification and mineralization process. The EC of all three groups were less than 4 ms/cm at the end of composting, meeting the requirements for safe agricultural production (Xu et al., 2020).

At the beginning of composting, the ORP of three groups were all at a high level, and rapidly decreased to the lowest during the heating phase, while gradually increased and stabilized at the later stage of composting. It has been shown that the increase in ORP during composting is mainly related to the oxidation of reducing compounds and chemical oxygen demand as well as the contact area with oxygen (Wang et al., 2018). In the present study, the ORP values of three groups remained above 0 mV and levelled off in the later stages of composting, indicating that the composting was aerobic and the organic matter was gradually stabilizing.

The determination of the GI value can evaluate the phytotoxicity of the compost products. The GI values gradually increased as the composting progressed. The GI values for the CK, SCF and FRH groups were 99.17%, 139.12% and 117.83% respectively at the end of composting, indicating that the compost products had no toxic to plants (GI > 80%) (Hu et al., 2022). During the initial period, the GI values increased, which demonstrated that the thermophilic phase could rapidly degrade toxic substances and reduce the toxic effect on plants (Xu et al., 2020). In addition, the GI values of the SCF and FRH groups were significantly higher than that of the CK group ($p < 0.01$), which illustrated that the use of FRL composting could promote the degradation of harmful substances and further improve the nutrient content. The above results declared that composting with FRL could influence compost maturation by changing environmental factors.

3.2. Effect of FRL on carbon indexes during composting

In the composting process, mineralization and humification are key steps in the carbon metabolism (Zhang et al., 2021; Xu et al., 2023). Organic matters are decomposed into CO_2 under microbial mineralization, and the mineralization process will affect carbon sequestration, causing some carbon loss in the process. Along with the composting process, the TOC concentration gradually decreased in three groups (Fig. 1a), which was caused by the continuous mineralization of organic carbon (Duan et al., 2020). The organic matter content of the CK group decreased rapidly in the early stage of composting, followed by FRH, which was consistent with the results of CO_2 emissions (Fig. 1b). The release of CO_2 mainly existed in the heating and thermophilic phases of composting as well as the mineralization of organic matter. The addition of FRL also changed the maximum amount of CO_2 releasing during the composting process. At the end of the composting process, the organic matter content of the CK, SCF and FRH groups decreased by 3.1%, 2.5% and 2.9%, respectively. This suggested that the addition of FRL could alter the flow of carbon during mineralization and reduce carbon losses in the composting process.

MBC is the driving force in the mineralization and humification of soil organic matter and is the most active component of soil organic matter (Bao et al., 2020). As shown in Fig. 1c, the MBC showed a trend of increasing, decreasing, and stabilizing. The MBC content was ranged from 3.82 to 4.49 mg/g at the beginning of composting, and then began to increase to peaks of 7.06, 8.25 and 8.66 mg/g in the CK, SCF and FRH groups, respectively at 7 d. Compared to the CK group, the addition of FRL significantly increased the MBC content of the SCF and FRH groups

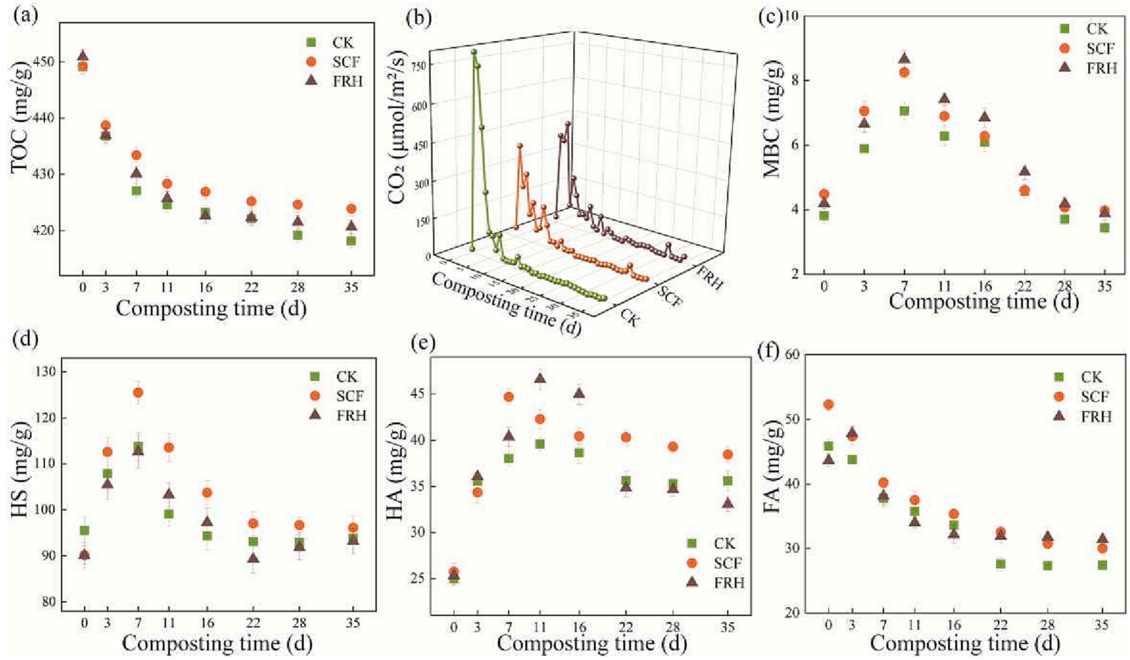


Fig. 1. Changes of TOC (a), CO_2 (b), MBC (c), HS (d), HA (e), FA (f) during composting under different treatment groups. All values represent means ± SD (n = 3).

($p < 0.01$), probably because the FRL contained simple nutrients which could be directly used by microorganisms. Besides, FRL introduced external microorganisms resulting in the FRH group had a higher microbial content and more active microorganisms. Adding FRL enhanced carbon sequestration of compost system. After 7 d of composting, with the arrival of the thermophilic phase and the depletion of nutrients, the MBC content decreased and stabilized after 28 d.

Organic substance will be converted into HS in the process of microbial humification, and the generation of HS is a key step in carbon sequestration (Yu et al., 2022). In the composting process, macromolecular complex organic components are hydrolyzed by hydrolytic enzymes released by microorganisms through the mineralization process, providing energy for cell growth and synthesizing precursors required for humus formation, which can be mineralized into CO_2 or converted into HS by microbial action (Wu et al., 2017). In the first 7 d of composting, the content of HS increased rapidly, which was due to the strong mineralization capacity of microorganisms, and a large amount of organic matter was decomposed into HS precursor substances, thus promoted the formation of HS. In addition, compared to the CK group, the HS in the SCF and FRH groups increased by 28.2% and 20.03%, respectively. Combined with the changes of CO_2 and MBC, addition of FRL was able to enhance the process of organic matter humification to generate HS and accelerate the carbon sequestration by increasing the MBC content and reducing the release of CO_2 during the initial mineralization process.

HA and FA are important components of HS, as FA has a simpler structure than HA, it is more readily available to microorganisms during the composting process including being consumed as a carbon source and converted to HS (Mei et al., 2021). Fig. 1e illustrated that HA content increased first and then decreased during composting. In the heating and thermophilic phases of composting, the mineralization of organic matter provided a large amount of precursor materials for HA synthesis, and the conversion of FA further contributed to the increase of HA content. In the later stages of composting, HA was further consumed as a simple carbon source in order to maintain normal microbial activities, which resulted in a decrease of HA content. The decreasing trend of FA content throughout the composting process was mainly the result of more rapid microbial utilization of simple organic matter (Fig. 1f), which was also confirmed by the findings of Huang et al (2019).

3.3. Correlation analysis of physicochemical factors and carbon conversion during composting

During the composting process, carbon conversion is affected by the changes of physicochemical factors. Redundancy analysis (RDA) can be used to explain the correlation between physicochemical factors and carbon conversion in composting (Ye et al. 2021). The results of the influence of physicochemical factors (time, temperature, EC, ORP, GI) on carbon conversion was analyzed (see Supplementary material). 81.62% and 16.39% of the variability could be explained by RDA1 and RDA2 respectively. GI and EC were significantly negatively correlated with TOC and FA ($p < 0.01$), which was related to the mineralization and humification of macromolecular organic matter (MOM). MOM can produce large amounts of small molecules of organic matter, further contribute to the increase of GI. It was worth noting that temperature and pH were the main factors on humification and mineralization. These two factors were positively correlated with MBC and CO_2 and negatively correlated with TOC, suggesting that temperature increasing could accelerate the microbial activities, promote microbial decomposition of organic matter such as cellulose and hemicellulose and increase the mineralization of organic matter. Moreover, temperature and pH were positively correlated with HS and HA, indicating that temperature and pH were the main driving factors during compost humification, which was consistent with the results of Yang et al. (2020). Therefore, strengthen the regulation of temperature and pH could effectively accelerate the degradation of organic matter and promote the conversion of HS and HA during composting.

3.4. Effect of FRL on microbial composition and diversity

To better understand the effect of FRL on the composting process, the structure of the microbial community was analyzed in this study. Fig. 2a demonstrated the changes in the levels of bacterial phyla where Firmicutes (10.29%–78.35%), Actinomycetes (11.89%–41.79%), Proteobacteria (0.8%–42.24%) and Chloroflexi (0%–13.97%) were the dominant phyla in the three groups, and the relative abundance was influenced by the composting time. In particular, the relative abundance of Firmicutes showed a temporal trend of increasing and then decreasing, which was due to the high survival rate under high temperature conditions, with

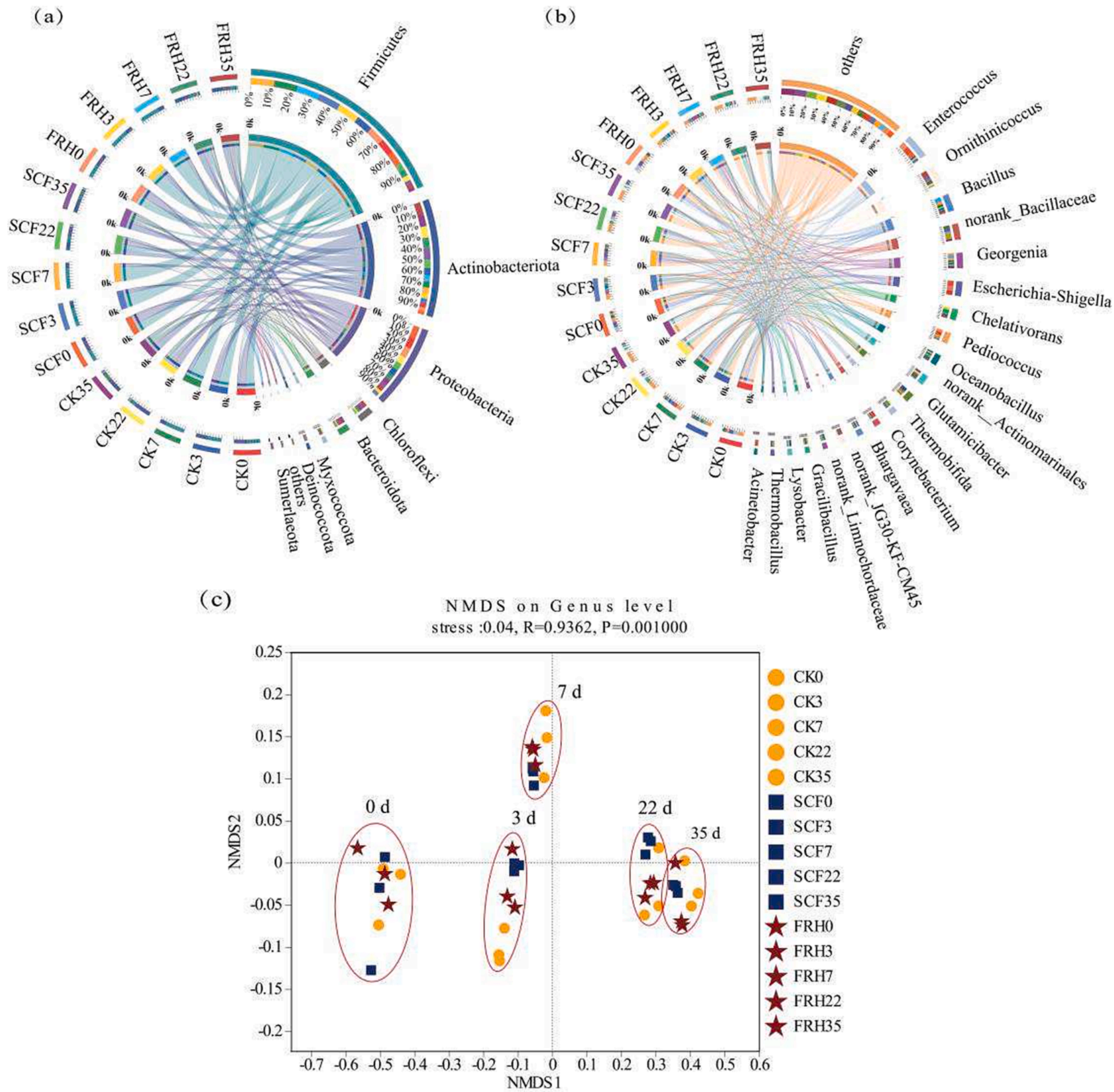


Fig. 2. Bacterial RAs from the top 10 bacterial phyla (a), the top 20 bacterial genera (b), and NMDS (c) analysisge of bacterial communities. The final numbers 0, 3, 7, 22 and 35 indicate sampling days.

the relative abundance of Firmicutes gradually increasing as the temperature gradually rose. Among them, the highest abundant of Firmicutes was found in the SCF group, probably because the addition of FRL significantly increased its relative abundance during the heating phase ($p < 0.01$). The bacteria in Firmicutes had the ability to decompose proteins and sugars, and also played an important role in the degradation of cellulose (Guo et al., 2021; Wang et al., 2022a,b). The abundance of Actinomycetes gradually increased throughout the composting process as they were a thermophilic group of bacteria (Zhou et al., 2019). Researches had shown that Actinomycetes played a dominant role in the degradation of antibiotics and the inhibition of pathogenic microorganisms (Tian et al., 2015), consequently, maintaining a high relative abundance of Actinomycetes during the composting process was beneficial to reducing the toxicity of the final compost products. The relative abundance of Proteobacteria in the CK, SCF, and FRH groups decreased from initial 35.47%, 42.24%, and 35.11% to 1.46%, 1.09%, and 1.06% during the thermophilic phase, respectively, which indicated the low survival rate of Proteobacteria under high temperature conditions. Furthermore, Proteobacteria was strongly participated in the carbon and nitrogen cycle, and their relative abundance increased during the cooling and maturity phase (25–35 d), which was important for the decay and stabilization of compost products.

At the genus level (Fig. 2b), the dominant genera varied between treatment groups and phases. At the beginning of composting, the genera *Enterococcus*, *Escherichia-Shigella*, *Pediococcus* and *Corynebacterium* were the dominant genera in CK, SCF and FRH, respectively. *Enterococcus* and *Pediococcus* belonged to Firmicutes, while *Escherichia-Shigella* and *Corynebacterium* belonged to Proteobacteria and Actinomycetes, respectively, which was corresponding to the results shown in Fig. 2a. The dominant flora in the CK group was *norank-f-Bacillaceae*, while, in the SCF and FRH groups was *Bacillus* at 7d. As *Bacillus* is a kind of thermotolerant bacterium, it could maintain high abundance during high temperature phases, which was conducive to the degradation of waste such as lignocellulosic (Akyol et al., 2019; Cai et al., 2016). At the end of composting, the bacterial community composition was essentially the same in three groups, with *Ornithinimicrobium* and *norank-f-norank-o-Actinomarinales.* The above results implied that the addition of FRL could alter the relative abundance of different genera and might further influence the carbon conversion during composting.

Alpha diversity such as Ace, Chao, Simpson and Shannon indices could reflect the abundance and diversity of microbial communities in compost (see Supplementary material). During the heating phase, the Ace and chao indices decreased in the CK group and increased in the SCF and FRH groups. During the thermophilic and the cooling phases, along with the degradation of organic matter by bacteria, bacteria entered a rapid growth phase and the Ace and chao indices increased in the three groups. Except for the maturity phase (25–35 d), the Simpson indices in the SCF and FRH groups were lower than that of the CK group, while the Shannon index was higher than that of the CK group, suggesting that the addition of FRL was able to increase the species diversity. The Shannon index of the three groups decreased at the maturity phase, and the species diversity decreased due to the decompose of easily degradable organic matter. The above results showed that the addition of FRL could induce rapid colonization of bacteria and increase the diversity of bacteria in the beginning composting process.

The similarities or differences in the composition of the three groups of bacterial communities were analyzed by NMDS. The bacterial communities were divided into five sections according to the phase of composting (Fig. 2c). At 0, 3 and 7 d of composting, the samples were distant within the group, which indicated that the community composition was more varied and microbial community succession occurred, possibly due to changes in temperature (Cai et al., 2016). The distances

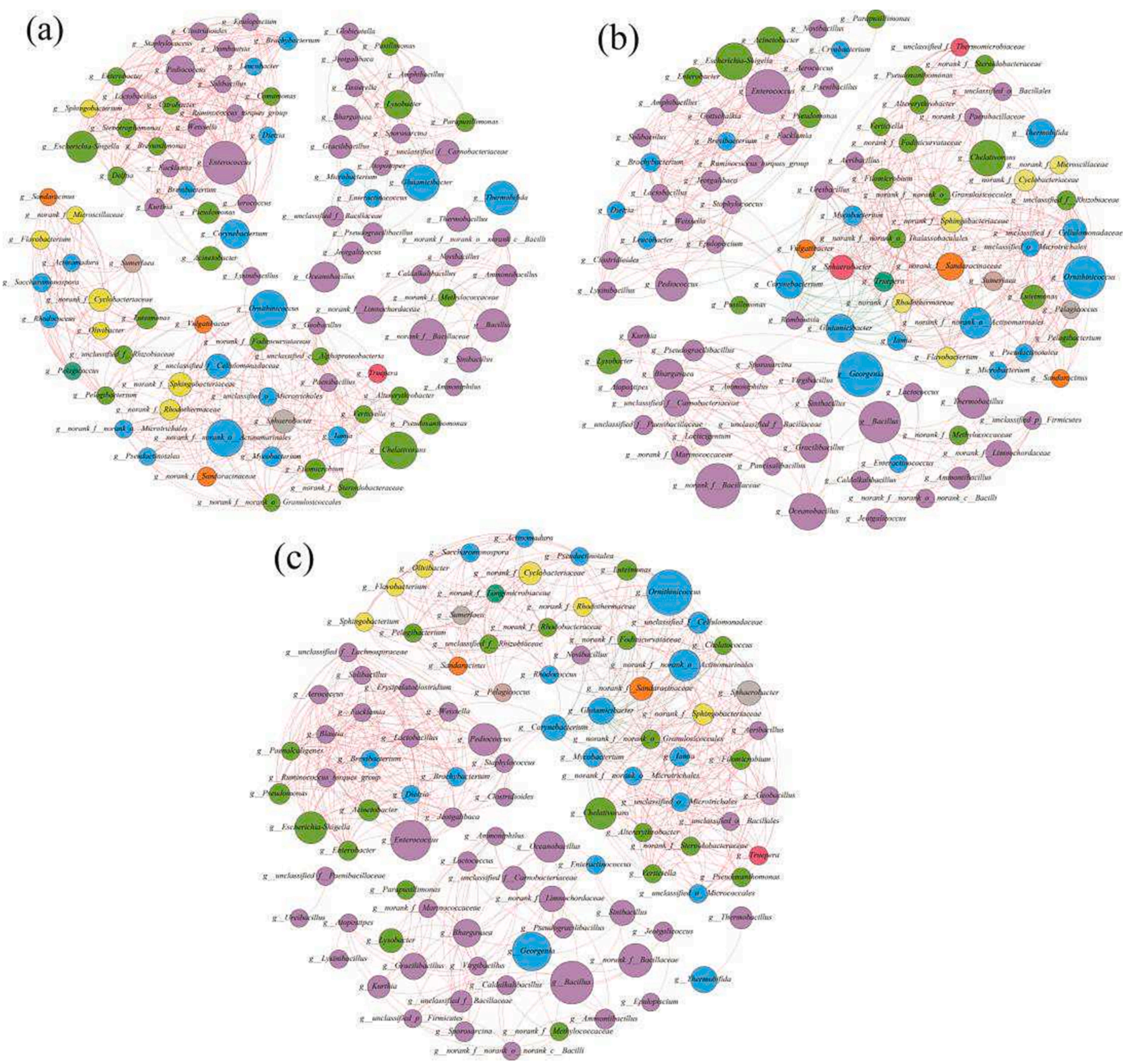


Fig. 3. Network analysis of bacterial genus levels in different treatment groups during composting. (a) CK, (b) SCF, (c) FRH, where the size of the dots represents the relative abundance, the colour of the dots represents the different bacterial phyla, and the red and green edges indicate positive and negative correlations, respectively.

between the sample groups at 22 and 35 d were closer together and the bacterial community composition was similar. Meanwhile, the addition of FRL affected the bacterial community structure resulting in greater variability in the bacterial communities of the three groups. As the composting progressed, the impact of increasing temperature on the bacteria in the compost increased, leading to a gradual similarity in the bacterial community structure of each group.

3.5. The impact of FRL on core bacteria community network

To further investigate the effect of FRL on core bacteria community complexity and stability, correlation network analysis was used to construct network profiles with significant correlations between bacteria ($r > 0.9$, $p < 0.05$) (Fig. 3). Compared with the CK and SCF groups, the number of microbial network edges increased in the FRH group with 654, 626 and 689, respectively. And modularity analysis yielded a lower modularity index in the SCF and FRH (0.621, 0.66) groups than in the CK group (0.7), implying that the SCF and FRH groups possessed a more active bacterial community, while the bacterial community in the CK group was relatively stable. Moreover, in the CK group, the bacterial genera within the modules had dense connections between them, however, the connections between the bacterial genera in different modules were sparse, whereas the SCF and FRH groups had more closely connected microbial networks between different modules than the CK group and a more complex microbial network within the modules. The analysis of the average degree of the network showed that the FRH (13.939) and SCF groups (12.667) had a higher average degree than the CK group (12.367). These results showed that the addition of FRL could increase the degree of direct association between the various bacterial genera, and that the relationships between the bacteria were more complex and closely linked resulting in a greater diversity of microbial functions.

Although each group showed different characteristics in the symbiotic network, the bacterial genera in the three groups were mainly distributed within four bacterial phyla, with nodes of Firmicutes, Proteobacteria and Actinobacteriota occupying the main positions (Awasthi et al., 2021). The number of microbial nodes of *Firmicutes* was increased in the SCF and FRH groups compared with the CK group, and the bacteria distributed in Firmicutes were an important source of a variety of extracellular enzymes such as cellulase, lipase, and protease production

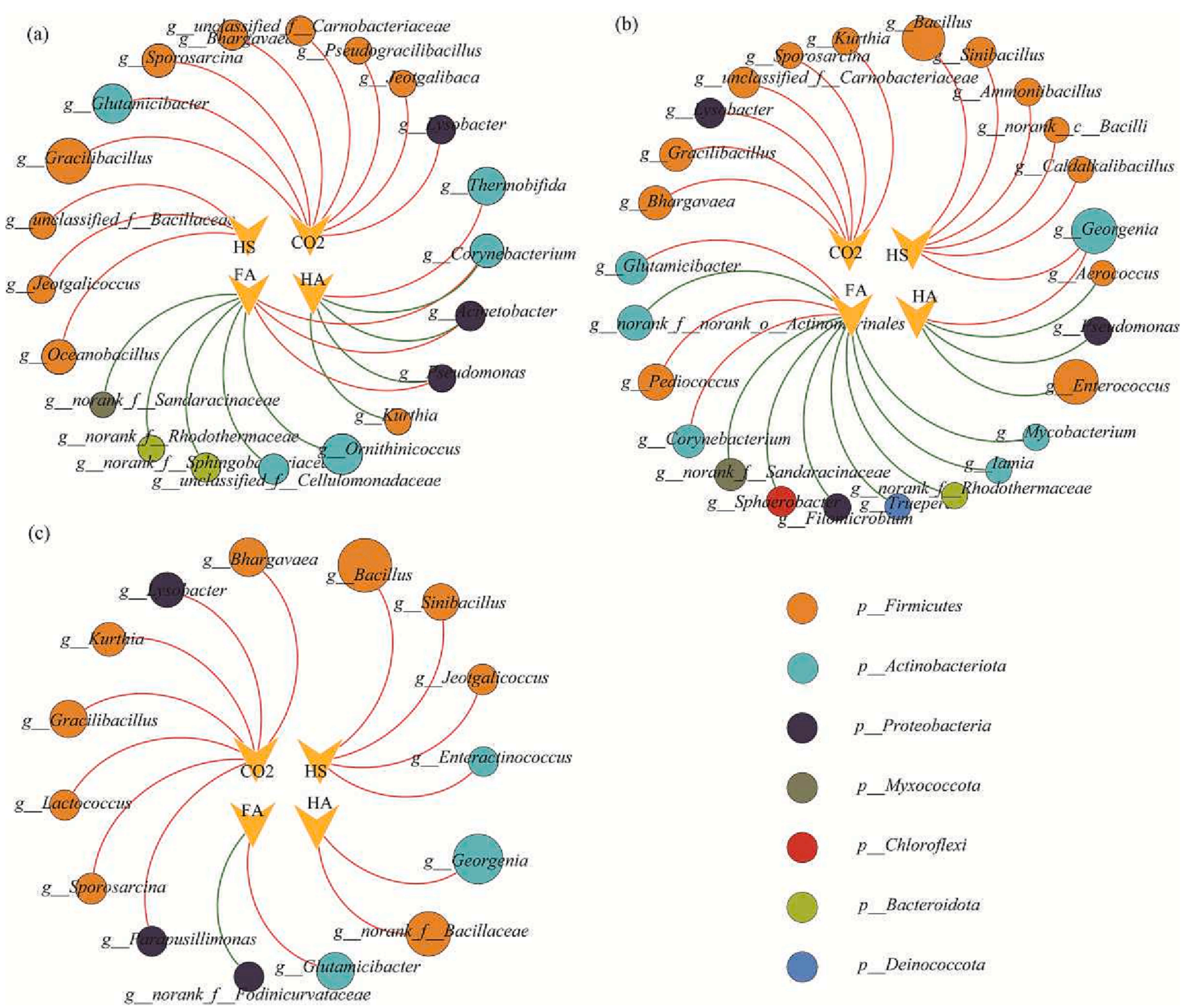


Fig. 4. Network analysis of different treatment groups of the composting process with HS, HA, FA, CO_2 and associated bacteria. (a) CK, (b) SCF, (c) FRH. where the size of the dots represents relative abundance and the colour of the dots represents the different bacterial phyla, with red and green indicating positive and negative correlations respectively.

(Arab et al., 2017). Therefore, the increased number of Firmicutes accelerated the decomposition of lignin, lipids, proteins and cellulose in the substrate and promoted the degradation of macromolecules in the composting process to further accelerate the composting process. Proteobacteria plays a dominant role in the catabolic transformation of small molecule organic matter (Xu et al., 2020), and the SCF group had an increased proportion of genera of Proteobacteria compared with CK, which had a positive effect on the synthesis of HA. Meanwhile, the number of genera negatively associated with Actinobacteriota was significantly increased in the SCF and FRH groups ($p < 0.01$), and the changes in microbial interrelationships had a significant impact on mineralization and humification ($p < 0.01$). The changes in the interrelationship of microbial groups caused by the addition of FRL may promote the rational use of resources by each group (Wang et al., 2022a, b) and increase the efficiency of microbial work, which in turn accelerate the degradation of organic matter.

3.6. Correlation analysis of core bacteria and carbon conversion

To reveal the effect of core bacteria on carbon transformation, core bacteria were screened with significant relationships between carbon and bacteria ($r > 0.85$, $p < 0.05$), and network analysis was performed. As shown in Fig. 4, the number of nodes and connections was significantly higher in SCF (26, 27) and significantly lower in FRH (15, 15) compared with CK (21, 24) ($p < 0.01$). This indicated that the addition of FRL could change the diversity of microorganisms in the composting process and that the effect on bacteria varies depending on the method of FRL addition. In the CK group, there were eight CO_2-positive core flora, with the addition of FRL, the number of CO_2-positive core flora decreased (SCF: 6, FRH: 7) and carbon emission-related genera such as *Glutamicibacter* and *Pseudogracilibacillus* disappeared from the SCF and FRH groups. Ma et al., found a significant ($p < 0.01$) correlation between CO_2 emission rates during *Pseudogracilibacillus* composting (Ma et al., 2020), resulting in protein and amino acids being retained and providing more precursors for HS formation (Shangguan et al., 2022). The increased number of core bacteria associated with HS formation in SCF(6) and FRH(4) compared with the CK(3) group suggested that FRL was able to increase the core flora associated with HS production. And the number of core bacteria negatively associated with FA increased significantly in the SCF group ($p < 0.01$) and those negatively associated with HA decreased, explaining that more core bacteria was activated in the SCF group, accelerating the conversion of FA to HA, and HA was accumulated. In contrast to the SCF group, the FRH group showed a decrease in the number of core bacteria associated with FA and HA conversion, while an increase in the number of core bacteria producing HA at the same time, which led to an increase in HA production as well. This suggested that FRL not only increases the number of humified core bacteria, but also reduced the activity of bacteria negatively associated with HA.

3.7. Mechanisms of FRL regulation of carbonaceous transformation

In order to assess the potential association between bacterial community composition, physicochemical factors and carbon indexes, and to investigate the regulation of carbon conversion by FRL in the composting process, a structural equation model was constructed (Fig. 5). In the CK group, positive correlations were found between bacteria and temperature ($\lambda = -0.808, p < 0.001$), temperature and pH ($\lambda = -0.811$, $p < 0.001$) as well as pH and CO_2 ($\lambda = -0.811$, $p < 0.05$). The results indicated that bacterial abundance and metabolism could effectively alter temperature and pH, affecting microbial mineralization processes of organic matter and disturbing emissions of CO_2. During carbon conversion, HS, bacteria and HA were positively correlated, while FA was negatively correlated with HA, indicating that microbial metabolic

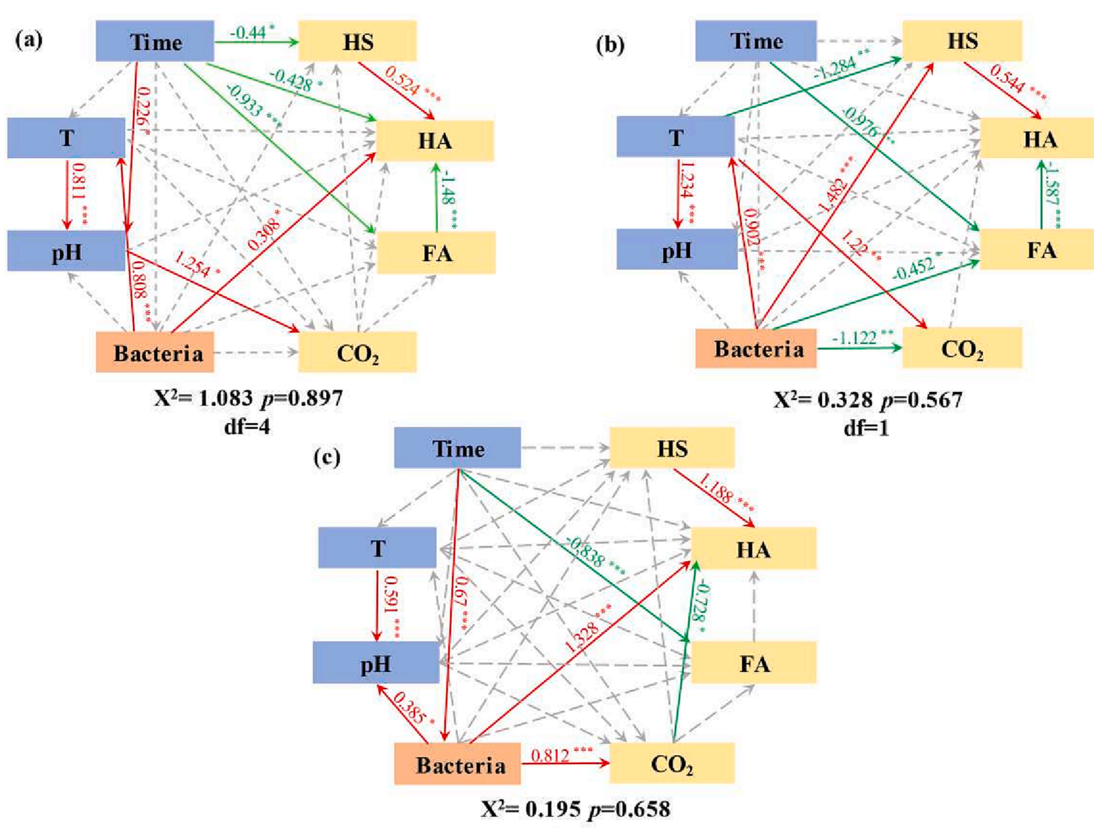


Fig. 5. SEM showing the regulation of carbon conversion during composting. (a) CK, (b) SCF, (c) FRH. Lines outlined in grey indicate no significance ($p > 0.05$). Red solid lines indicate positive correlation, green solid lines indicate negative correlation (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

activity during composting could facilitate the conversion of carbon to the stable state of HA (Ye et al., 2021). In addition, HS, HA and FA were negatively correlated with time, elucidating that the accumulation of HS, HA and FA was not favoured along with the increase of composting time. Compared with the CK group, temperature and bacteria were the two main factors affecting CO_2 emissions in the SCF group. Bacteria showed a significant negative correlation to CO_2 ($\lambda = -1.122$, $p < 0.01$) and they could be able to control CO_2 emissions through temperature. There was no significant correlation between bacteria and HA, whereas bacteria were able to increase the conversion of carbon to HA by promoting HA production, and HA synthesis by increasing FA decomposition. Unlike CK, the addition of FRL was able to modify the negative effects of time on HS, HA and FA in the FRH group, while there was a positive correlation between bacteria and CO_2 ($\lambda = 0.812$, $p < 0.001$), and the emission of CO_2 significantly affected HA production. These results suggested that the addition of FRL could reduce the negative effects of time on HS, HA and FA and enhanced the role of bacteria between carbon transformations.

4. Conclusions

Composting with organic waste liquids (FRL) altered physicochemical properties and bacterial communities to promote composting maturity compared with the non-FRL composting. By increasing the diversity of bacteria, the degree of association between bacteria was enhanced. The metabolic characteristics were changed by suppressing the number of CO_2 core bacteria, increasing the number of HS-positive core bacteria and decreasing the number of HA-negative core bacteria. As a consequence, FRL mended the flow of carbon ways during mineralization and reduced carbon loss, while also facilitated the production of HS and HA during humification.

CRediT authorship contribution statement

Yangcun Sun: Methodology, Data curation, Writing – original draft, Writing – review & editing. **Shanshan Sun:** Methodology, Data curation, Writing – review & editing. **Fangyi Pei:** Formal analysis. **Chi Zhang:** Methodology. **Xinbo Cao:** Investigation. **Jie Kang:** Conceptualization. **Zhenchao Wu:** Conceptualization. **Hongzhi Ling:** . **Jingping Ge:** Resources, Supervision.

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.

Acknowledgments

This work was supported by the Key Program of Heilongjiang Provincial Natural Science Foundation of China [No. ZD2020C008]; The National Natural Science Foundation of China [No. 32071519]; Heilongjiang University Graduate Innovation Research Project [YJSCX2022-091HLJU]; Heilongjiang University Graduate Innovation Research Project [YJSCX2022-031HLJU]; Scientific Research Project of Ecological Environment Protection of Heilongjiang Provincial Department of Ecological Environment (HST2022TR004). Development of Low-temperature Degradation Bacteria Position for Crop Straw of Collaborative, Innovation and Extension System of Modern Agricultural Industry Technology in Heilongjiang Province; Flax Dew Retting Position for Collaborative, Innovation and Extension System of Modern Agricultural Industry Technology in Heilongjiang Province.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2023.129112.

References

Abou-Elela, S.I., Ali, M.E.M., Ibrahim, H.S., 2016. Combined treatment of retting flax wastewater using Fenton oxidation and granular activated carbon. Arab. J. Chem. 9 (4), 511–517.
Akyol, Ç., Ince, O., Ince, B., 2019. Crop-based composting of lignocellulosic digestates: Focus on bacterial and fungal diversity. Bioresour. Technol. 288, 121549.
Arab, G., Razaviarani, V., Sheng, Z., Liu, Y., McCartney, D., 2017. Benefits to decomposition rates when using digestate as compost co-feedstock: Part II - Focus on microbial community dynamics. Waste Manag. 68, 85–95.
Awasthi, S.K., Duan, Y., Liu, T., Zhang, Z., Pandey, A., Varjani, S., Awasthi, M.K., Taherzadeh, M.J., 2021. Can biochar regulate the fate of heavy metals (Cu and Zn) resistant bacteria community during the poultry manure composting? J. Hazard. Mater. 406, 124593.
Ba, S., Qu, Q., Zhang, K., Groot, J.C.J., 2020. Meta-analysis of greenhouse gas and ammonia emissions from dairy manure composting. Biosyst. Eng. 193, 126–137.
Bao, H., Wang, J., Zhang, H.e., Li, J., Li, H., Wu, F., 2020. Effects of biochar and organic substrates on biodegradation of polycyclic aromatic hydrocarbons and microbial community structure in PAHs-contaminated soils. J. Hazard. Mater. 385, 121595.
Bian, B.o., Hu, X., Zhang, S., Lv, C., Yang, Z., Yang, W., Zhang, L., 2019. Pilot-scale composting of typical multiple agricultural wastes: Parameter optimization and mechanisms. Bioresour. Technol. 287, 121482.
Biswas, D.R., Narayanasamy, G., 2006. Rock phosphate enriched compost: an approach to improve low-grade Indian rock phosphate. Bioresour. Technol. 97 (18), 2243–2251.
Cai, L., Chen, T.B., Gao, D., Yu, J., 2016. Bacterial communities and their association with the bio-drying of sewage sludge. Water Res. 90, 44–51.
Chen, M., Ren, H., Ding, L., Gao, B., 2015. Effect of different carriers and operating parameters on degradation of flax wastewater by fluidized-bed Fenton process. Water Sci Technol. 71 (12), 1760–1767.
Chiarelotto, M., Damaceno, F.M., Lorin, H.E.F., Tonial, L.M.S., de Mendonca Costa, L.A., Bustamante, M.A., Moral, R., Marhuenda-Egea, F.C., Costa, M., 2019. Reducing the composting time of broiler agro-industrial wastes: The effect of process monitoring parameters and agronomic quality. Waste Manag. 96, 25–35.
Dey, P., Mahapatra, B.S., Pramanick, B., Kumar, A., Negi, M.S., Paul, J., Shukla, D.K., Singh, S.P., 2021. Quality optimization of flax fibre through durational management of water retting technology under sub-tropical climate. Ind. Crop. Prod. 162, 113277.
Duan, M., Zhang, Y., Zhou, B., Qin, Z., Wu, J., Wang, Q., Yin, Y., 2020. Effects of Bacillus subtilis on carbon components and microbial functional metabolism during cow manure-straw composting. Bioresour. Technol. 303, 122868.
Guo, Y.-X., Chen, Q.-J., Qin, Y., Yang, Y.-R., Yang, Q.-Z., Wang, Y.-X., Cheng, Z.-a., Cao, N.a., Zhang, G.-Q., 2021. Succession of the microbial communities and function prediction during short-term peach sawdust-based composting. Bioresour. Technol. 332, 125079.
Hu, X., Yang, Y.C., Zhou, K., Tian, G., Liu, B.o., He, H., Zhang, L., Cao, Y., Bian, B.o., 2022. Verification of agricultural cleaner production through rice-duck farming system and two-stage aerobic composting of typical organic waste. J. Clean. Prod. 337, 130576.
Huang, Y., L, D., Shah, G.M., Chen, W., Wang, W., Xu, Y., Huang, H., 2019. Hyperthermophilic pretreatment composting significantly accelerates humic substances formation by regulating precursors production and microbial communities. Waste Manag. 92, 89–96.
Kang, J., Yin, Z., Pei, F., Ye, Z., Song, G., Ling, H., Gao, D., Jiang, X., Zhang, C., Ge, J., 2022. Aerobic composting of chicken manure with penicillin G: Community classification and quorum sensing mediating its contribution to humification. Bioresour. Technol. 352, 127097.
Liu, N., Hou, T., Yin, H., Han, L., Huang, G., 2019. Effects of amoxicillin on nitrogen transformation and bacterial community succession during aerobic composting. J. Hazard. Mater. 362, 258–265.
Liu, L., Kong, H., Lu, B., Wang, J., Xie, Y., Fang, P., 2015. The use of concentrated monosodium glutamate wastewater as a conditioning agent for adjusting acidity and minimizing ammonia volatilization in livestock manure composting. J. Environ. Manage. 161, 131–136.
Liu, X., Zhang, L.u., 2023. Effects of additives on the co-composting of forest residues with cattle manure. Bioresour. Technol. 368, 128384.
Ma, S., Xiong, J., Cui, R., Sun, X., Han, L., Xu, Y., Kan, Z., Gong, X., Huang, G., 2020. Effects of intermittent aeration on greenhouse gas emissions and bacterial community succession during large-scale membrane-covered aerobic composting. J. Clean. Prod. 266, 121551.
Malik, S.N., Ghosh, P.C., Vaidya, A.N., Mudliar, S.N., 2019. Ozone pre-treatment of molasses-based biomethanated distillery wastewater for enhanced bio-composting. J. Environ. Manage. 246, 42–50.
Mei, J., Ji, K., Su, L., Wu, M., Zhou, X., Duan, E., 2021. Effects of FeSO(4) dosage on nitrogen loss and humification during the composting of cow dung and corn straw. Bioresour. Technol. 341, 125867.
Melelli, A., Jamme, F., Beaugrand, J., Bourmaud, A., 2022. Evolution of the ultrastructure and polysaccharide composition of flax fibres over time: When history meets science. Carbohydr. Polym. 291, 119584.

Shangguan, H., Fu, T., Shen, C., Mi, H., Wei, J., Tang, J., Zhou, S., 2022. In situ generated oxygen distribution causes maturity differentiation during electrolytic oxygen aerobic composting. Sci. Total Environ. 850, 157939.
Su, C.C., Pukdee-Asa, M., Ratanatamskul, C., Lu, M.C., 2011. Effect of operating parameters on the decolorization and oxidation of textile wastewater by the fluidized-bed Fenton process. Sep. Purif. Technol. 83, 100–105.
Subhashini, D., Kaushik, B.D., 1981. Amelioration of sodic soils with blue-green algae. Soil Res. 19 (3), 361–366.
Tian, W., Zhang, Z., Hu, X., Tian, R., Zhang, J., Xiao, X., Xi, Y., 2015. Short-term changes in total heavy metal concentration and bacterial community composition after replicated and heavy application of pig manure-based compost in an organic vegetable production system. Biol. Fertil. Soils 51 (5), 593–603.
Tuomela, M., Hatakka, A., Itavaara, M., Vikman, M., 2000. Biodegradation of lignin in a compost environment: a review. Bioresour. Technol. 72 (2), 169–183.
Wang, G., Li, G., Chang, J., Kong, Y., Jiang, T., Wang, J., Yuan, J., 2021. Enrichment of antibiotic resistance genes after sheep manure aerobic heap composting. Bioresour. Technol. 323, 124620.
Wang, K., Mao, H., Li, X., 2018. Functional characteristics and influence factors of microbial community in sewage sludge composting with inorganic bulking agent. Bioresour. Technol. 249, 527–535.
Wang, X., Tian, L., Li, Y., Zhong, C., Tian, C., 2022a. Effects of exogenous cellulose-degrading bacteria on humus formation and bacterial community stability during composting. Bioresour. Technol. 359, 127458.
Wang, Y., Wu, B., Ma, T., Mi, Y., Jiang, H., Yan, H., Zhao, P., Zhang, S., Wu, L., Chen, L., Zang, H., Li, C., 2022b. Efficient conversion of hemicellulose into 2, 3-butanediol by engineered psychrotrophic Raoultella terrigena: mechanism and efficiency. Bioresour. Technol. 359, 127453.
Wu, J., Zhao, Y., Qi, H., Zhao, X., Yang, T., Du, Y., Zhang, H., Wei, Z., 2017. Identifying the key factors that affect the formation of humic substance during different materials composting. Bioresour. Technol. 244 (Pt 1), 1193–1196.
Xie, D., Gao, M., Yang, M., Xu, M., Meng, J., Wu, C., Wang, Q., Liu, S., 2021. Re-using ammonium-rich wastewater as a moisture conditioning agent during composting thermophilic period improves composting performance. Bioresour. Technol. 332, 125084.
Xu, Z., Li, G., Huda, N., Zhang, B., Wang, M., Luo, W., 2020. Effects of moisture and carbon/nitrogen ratio on gaseous emissions and maturity during direct composting of cornstalks used for filtration of anaerobically digested manure centrate. Bioresour. Technol. 298, 122503.
Xu, C., Wu, B., Zhao, P., Wang, Y., Yang, H., Mi, Y., Zhou, Y., Ma, T., Zhang, S., Wu, L., Chen, L., Zang, H., Li, C., 2023. Biological saccharification coupled with anaerobic digestion using corn straw for sustainable methane production. Bioresour. Technol. 367, 128277.
Xun, Y., 2011. Study of Retting Flax Wastewater Treatment by Flotation/Flysoil Percolation Process. IEEE 211, 202–204.
Yang, Y., Awasthi, M.K., Bao, H., Bie, J., Lei, S., Lv, J., 2020. Exploring the microbial mechanisms of organic matter transformation during pig manure composting amended with bean dregs and biochar. Bioresour. Technol. 313, 123647.
Yang, F., Li, Y., Han, Y., Qian, W., Li, G., Luo, W., 2019. Performance of mature compost to control gaseous emissions in kitchen waste composting. Sci. Total Environ. 657, 262–269.
Ye, Z., Ding, H., Yin, Z., Ping, W., Ge, J., 2021. Evaluation of humic acid conversion during composting under amoxicillin stress: Emphasizes the driving role of core microbial communities. Bioresour. Technol. 337, 125483.
Yin, Z., Zhou, X., Kang, J., Pei, F., Du, R., Ye, Z., Ding, H., Ping, W., Ge, J., 2022. Intraspecific and interspecific quorum sensing of bacterial community affects the fate of antibiotic resistance genes during chicken manure composting under penicillin G stress. Bioresour. Technol. 347, 126372.
Yu, K., Sun, X., Li, S., Ding, H., Hao, D., Meng, T., Fu, B., Zou, R., Kang, Y., 2022. Promoting lignocellulose degradation during green waste composting by maintaining a specific temperature through heap size control. Environ. Technol. 43 (19), 2968–2980.
Zhan, Y., Chang, Y., Tao, Y., Zhang, H., Lin, Y., Deng, J., Ma, T., Ding, G., Wei, Y., Li, J.i., 2022. Insight into the dynamic microbial community and core bacteria in composting from different sources by advanced bioinformatics methods. Environ. Sci. Pollut. Res. Int. 30 (4), 8956–8966.
Zhang, Z., Li, X., Hu, X.i., Zhang, S., Li, A., Deng, Y., Wu, Y., Li, S., Che, R., Cui, X., 2021. Downward aeration promotes static composting by affecting mineralization and humification. Bioresour. Technol. 338, 125592.
Zhang, Q., Yang, Y., Zhang, X., Liu, F., Wang, G., 2022. Carbon neutral and techno-economic analysis for sewage treatment plants. Environ. Technol. Innov. 26, 102302.
Zhang, Z., Zhao, Y., Yang, T., Wei, Z., Li, Y., Wei, Y., Chen, X., Wang, L., 2019. Effects of exogenous protein-like precursors on humification process during lignocellulose-like biomass composting: Amino acids as the key linker to promote humification process. Bioresour. Technol. 291, 121882.
Zhou, G., Qiu, X., Zhang, J., Tao, C., 2019. Effects of seaweed fertilizer on enzyme activities, metabolic characteristics, and bacterial communities during maize straw composting. Bioresour. Technol. 286, 121375.