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Bamboo charcoal enhances cellulase and urease activities during chicken manure composting: Roles of the bacterial community and metabolic functions

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ABSTRACT

Microbial enzymes are crucial for material biotransformation during the composting process. In this study, we investigated the effects of adding bamboo charcoal (BC) (i.e., at 5%, 10%, and 20% corresponding to BC5, BC10, and BC20, respectively) on the enzyme activity levels during chicken manure composting. The results showed that BC10 could increase the cellulose and urease activities by 56% and 96%, respectively. The bacterial community structure in BC10 differed from those in the other treatments, and *Luteivirga, Lactobacillus, Paenalcaligenes, Ulvibacter, Bacillus, Facklamia, Pelagibacterium, Sporosarcina, Cellvibrio*, and *Corynebacterium* had the most important roles in composting. Compared with other treatments, BC10 significantly enhanced the average rates of degradation of carbohydrates (D-xylose (40%) and α-D-lactose (44%)) and amino acids (L-arginine (16%), L-asparagine (14%), and L-threonine (52%)). We also explored the associations among the bacterial community and their metabolic functions with the changes in the activities of enzymes. Network analysis demonstrated that BC10 altered the co-occurrence patterns of the bacterial communities, where *Ulvibacter* and class Bacilli were the keystone bacterial taxa with high capacities for degrading carbon source, and they were related to increases in the activities of cellulase and urease, respectively. The results obtained in this study may help to further enhance the efficiency of composting.

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Introduction

Chicken manure and straw are the most common types of agricultural waste in China, and studies have shown that the application of untreated chicken manure to agricultural farmland is a major source of environmental pollution (Hu et al., 2017; Ghirardini et al., 2020; Forsberg et al., 2012). However, the large amount of nutrients present in chicken manure and crop straw can be utilized by plants (Janczak et al., 2017), and these materials are important for the sustainable development of agriculture after suitable treatments.

Composting is an effective method for dealing with crop straw and chicken manure in order to obtain a harmless product that can be utilized in agriculture (Antić et al., 2020). The composting process relies on microorganisms, where the enzymes that they secrete biochemically convert organic matter into stable humus (Liu et al., 2017a). However, manure and straw contain large amounts of polymers (Mao et al., 2020) such as cellulose that need to be degraded by multiple microorganisms and extracellular enzymes (Hemati et al., 2021). Thus, microorganisms and their secreted enzymes are the limiting factors that affect the efficiency of composting and the quality of the product (Chen et al., 2021), and members of Bacilli and Flavobacteriales are the main microorganisms that secrete cellulase to degrade cellulose (Kim et al., 2012). Recent studies showed that directly regulating the composting conditions so they are suitable for microbial growth or adding microbial inocula may accelerate the degradation of organic matter during composting (Wu et al., 2019a; Zhang and Sun, 2014; Harindintwali et al., 2020).

Several recent studies found that the application of additives can improve the conversion of various materials (Zhou et al., 2018; Wu et al., 2019b; Yin et al., 2019; Zhang et al., 2017), where biochar was investigated most widely. Bamboo charcoal (BC) is a type of biochar with four times the amount of micropores and 10 times the specific surface area compared with standard charcoal. Studies have shown that BC can increase the amount of aromatic polymers (Guo et al., 2020), accelerate the humification process (Duan et al., 2019a), and promote the composting efficiency (Liu et al., 2017b). Enzymes play key roles in the conversion of organic matter during the composting (Chang et al., 2019), and thus these results may be related to increased enzyme activities (Mandpe et al., 2020). BC might affect the activities of enzymes but its effects and the potential proliferation of microorganisms with key roles in the conversion and degradation of organic matter are unclear.

The quantities of microorganisms as well as their metabolic relationships are important during composting (Li et al., 2021a). In recent years, the Biolog method has been used to investigate the degradation of organic substances and the synthesis of humus substances from the perspective of microbial metabolism (Zeng et al., 2018). The highly efficient degradation of amino acids by bacteria during composting is associated with increased organic mineralization and humus synthesis (Wu et al., 2019; Yao et al., 2021), as well as the bacterial degradation of D-cellobiose by strongly affecting the activity of the cellulase that they secrete (Yin et al., 2019). However, the effects of adding BC to compost on the enzyme activities and bacterial metabolism, as well as their relationships with the bacterial communities during composting are not clear.

Therefore, we investigated the effects of adding BC during chicken manure composting on the activities of specific enzymes, as well as exploring the roles of the bacterial communities and their metabolic functions. In particular, we examined: (1) how BC might influence the activities of cellulase and urease during composting; (2) the effects of the bacterial community structure, composition, and metabolic function under BC treatment; and (3) the changes in the cellulase and urease activities related to specific types of bacteria and their metabolism. The results obtained in this study enhance our knowledge of the effects of BC on the activities of enzymes as well as the roles of bacteria and their metabolism during chicken manure composting.

1. Materials and methods

2.1. *Composting experiment design*

Composting experiments were conducted in plastic composting reactors. Wheat straw and chicken manure were employed as the raw materials. The characteristics of the wheat straw and chicken manure were reported in a previous study (Yin et al., 2019). BC was made from bamboo decomposed at a temperature of 600°C and it was supplied by Shanghai Hainuo Charcoal Co. Ltd. (Shanghai, China). The BC had an organic carbon content of 32.03%, total nitrogen content of 0.33%, and the Brunauer–Emmett–Teller surface area was 300 m^2/g. The C/N ratio in the compost was adjusted to 25:1 (*m/m*) by mixing the chicken manure and wheat straw, and the water content was adjusted to 60%. BC was added to the compost materials at 5%, 10%, and 20% (dry weight) in the BC5, BC10, and BC20 treatments, respectively, where these percentages were selected based on the results reported by Li et al. (2017). BC was not added to the control treatment (CK). Each treatment was repeated in triplicate.

1.2. *Sampling and physicochemical parameters*

In this study, samples were collected after 2 (thermophilic phase), 7 (cooling phase), and 26 (maturation phase) days of composting, and each sample was divided into two parts. One part was used to extract DNA (stored at −80°C) for high-throughput sequencing and the other was employed to determine the chemical properties and for Biolog analyses (stored at 4°C). The process temperature was monitored at different locations in each compost reactor. The pH was determined with fresh samples suspended in water at 1:10 (W/W) using a Thermo Orion 3-star pH-meter (CA, USA) (Yin et al., 2017). The total organic carbon (TOC) content was measured after heating in a muffle furnace at 550 °C for 24 hr (Lu et al., 2009). Kjeldahl analysis was used to determine the total nitrogen (TN) content after digesting the compost sample with H_2SO_4 and H_2O_2 (Yin et al., 2017). In addition, each fresh compost sample was mixed with 2 mol/L KCl (Yin et al., 2020) before centrifugation, and the supernatant was then assayed to determine the NH_4^+ and NO_3^- concentrations by flow injection analysis (Systea, Italy).

1.3. *Urease and cellulase activities*

The cellulase activity was determined by adding carboxymethylcellulose to each compost sample and incubating at 37°C, where each 1 mg of glucose produced from carboxymethylcellulose by the enzyme was defined as the cellulase activity (Yin et al., 2019). The urease activity assay was based on the release of ammonia from a compost sample treated with urea and incubated at 37°C, and each 1 mg of NH_4^+-N produced from urea by the enzyme was defined as the urease activity (Yin et al., 2017).

1.4. *DNA extraction and sequencing analysis*

DNA was then extracted from the freeze-dried and sieved compost samples according to the instructions provided with an MP Fast DNA Spin Kit for Soil, before storing at −20°C.

The 16S rRNA gene V4 region was selected for amplification and sequencing using the primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTAC HVGGGTWTCTAAT). After quality control of the raw data and clustering, sequences with similarity > 97% were used to obtain the operational taxonomic units (OTUs) with USEARCH software (Caporaso et al., 2010). QIIME was used to analyze the OTUs, where a read was first extracted for each OTU as a representative sequence and the representative sequence was then compared with the RDP database to obtain the species annotation for each OTU (Zhang et al., 2019).

1.5. *Biolog EcoPlate analysis*

A suspension comprising 5 g of each compost sample and 45 mL sterile NaCl solution (0.85%) was shaken for 2 hr, before subjecting the supernatant to tenfold serial dilution. Next, a Biolog EcoPlate was inoculated with the 10^{-3} dilution (Liu et al., 2017a). The analysis was conducted according to a previously described method (Yin et al., 2019).

1.6. *Data analyses*

Cellulase and urease activity data were analyzed with SPSS 19.0 (SPSS Inc., USA), and the means and standard deviations were visualized using OrginPro 8.0 (OriginLab, Northampton, MA, USA). R (Version 3.3.5) was used to obtain Circos plots, for non-metric multidimensional scaling (NMDS) analysis, and to draw heatmaps. Redundancy analysis (RDA) was conducted with CANOCO 4.5. Network analysis based on the Spearman's rank correlation coefficients was performed with R (Version 3.3.5) and data were visualized using Gephi (Version 0.9.2).The most important bacteria were determined by random forest analysis (R Version 3.3.5).

2. Results and discussion

2.1. *Changes in enzyme activities during composting*

Composting involves biochemical processes that convert organic matter and many of these processes are related to changes in microbial enzymes (Yin et al., 2019). The cellulase activity can be used to indicate the degradation of cellulose in a material during composting (Zhang and Sun, 2014). Our results showed that the cellulase activity peaked on day 2 but it decreased by 86%–94% at the end of composting (Fig. 1a). All of the treatments reached the peak temperature (>60°C) on day 2 (thermophilic phase), thereby indicating that the activities of the microorganisms increased rapidly on day 2 (**Appendix A Table S1**). Indeed, increased enzyme activities are beneficial for the degradation of organic matter in raw materials and for raising the temperature during composting (Bernal et al., 2009). A previous study found that the cellulase activity was significantly higher during the thermophilic phase (>55°C) than the other phases (Liu et al., 2020). Similarly, the average cellulase activities were significantly higher in BC5 (27%), BC10 (56%), and BC20 (36%) on days 2 and 7, probably because the high specific surface area and microporosity of BC increased the possibility of microorganisms obtaining oxygen. Thus, BC10 could significantly increase the cellulase activity, and our results were similar to those obtained by adding rice straw biochar in a previous study (Du et al., 2019).

Urease catalyzes the conversion of urea into ammonia, and thus it plays important roles in the decomposition of agricultural waste. Similar to the cellulase activity, we found that the urease activity decreased in all of the treatments (Fig. 1b). During the thermophilic and cooling phases, we also found that the NH_4^+ concentrations were high in all treatments, but they decreased by 90%–97% at the end of composting (**Appendix A Table S1**). In addition, the urease activities were significantly higher in the BC treatments than CK during composting, and the NH_4^+ concentrations in the BC treatments were 17%–46% and 19%–43% higher compared with CK during the thermophilic and cooling phases, respectively (**Appendix A Table S1**). Compared with other treatments, the average urease activities in BC10 were 96% higher on days 2 and 7. Thus, we found that BC10 could enhance the activities of cellulase and urease, thereby indicating that BC10 might promote the degradation of humification and cellulose. Wu et al. (2016) found that BC may protect the proton exchange pathway, organic complexes with high molecular weights, or other interaction mechanisms. We hypothesize that these findings may be explained by the addition of 10% BC providing reasonable growth conditions for microorganisms, and the microorganisms attached to the surface of BC might have been the key species responsible for secreting cellulase and urease.

2.2. *Changes in bacterial community structure and composition*

The structures of the bacterial communities during chicken manure composting were determined by 16S rRNA gene sequencing. The similarities and differences in the community compositions were assessed by NMDS and hierarchical cluster analysis (Figs. 2 and 3a). The NMDS results showed that the compost samples could be separated into four different groups according to the composting phases (Fig. 2), i.e., samples from the raw materials, samples on day 2, samples on day 7, and samples on day 26. Thus, the bacterial communities were significantly separated in the different composting phases. Changes in environmental conditions may be responsible for changes in the bacterial community structure dur-

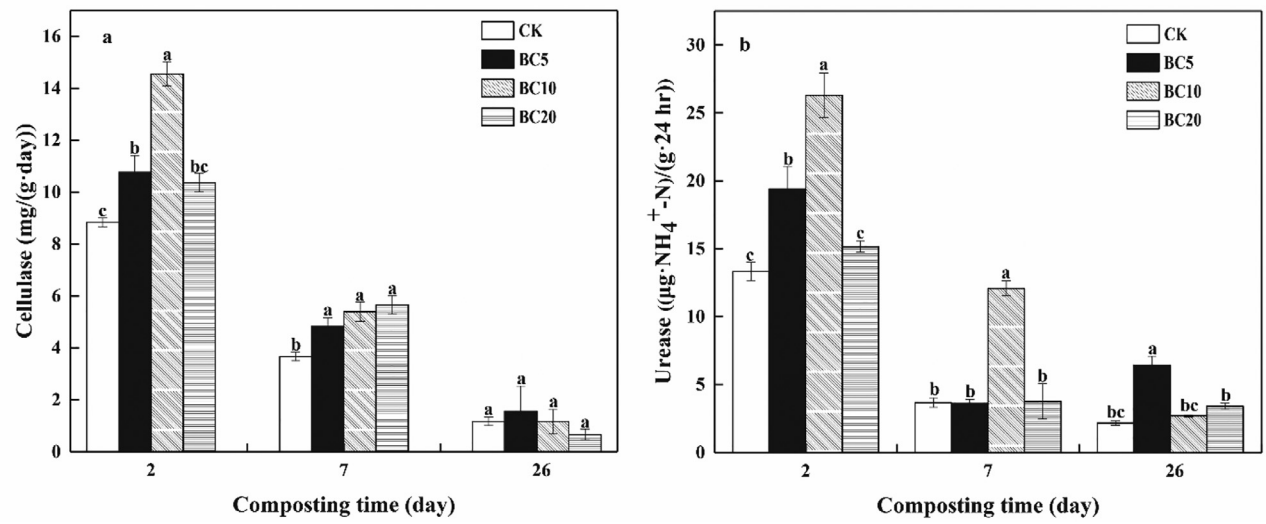

Fig. 1 – Changes in the cellulose (a) and urease (b) activities during composting. Significant difference level of enzyme activity between treated with 5% bamboo charcoal (BC5), 10% bamboo charcoal (BC10) and 20% bamboo charcoal (BC20) and a control with no added bamboo charcoal (CK) were indicated by a b and c with $P < 0.05$. Error bars represent standard error of the mean.

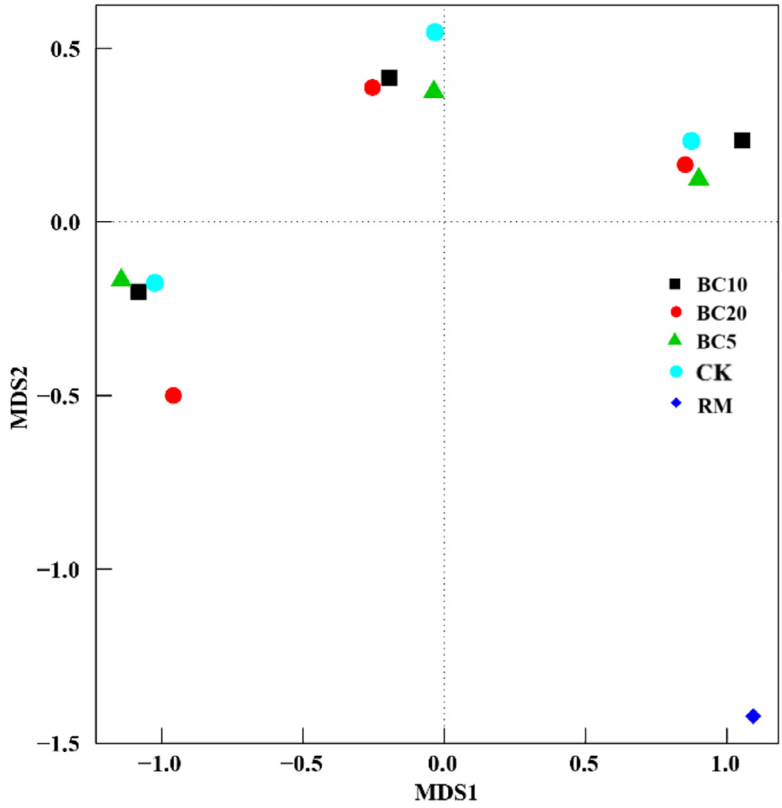

Fig. 2 – Non-metric multidimensional scaling (NMDS) analysis of the bacterial community compositions on days 2, 7 and 26 in compost treated with 5% bamboo charcoal (BC5), 10% bamboo charcoal (BC10) and 20% bamboo charcoal (BC20) and a control with no added bamboo charcoal (CK). The NMDS stress was 0.04. RM: raw materials.

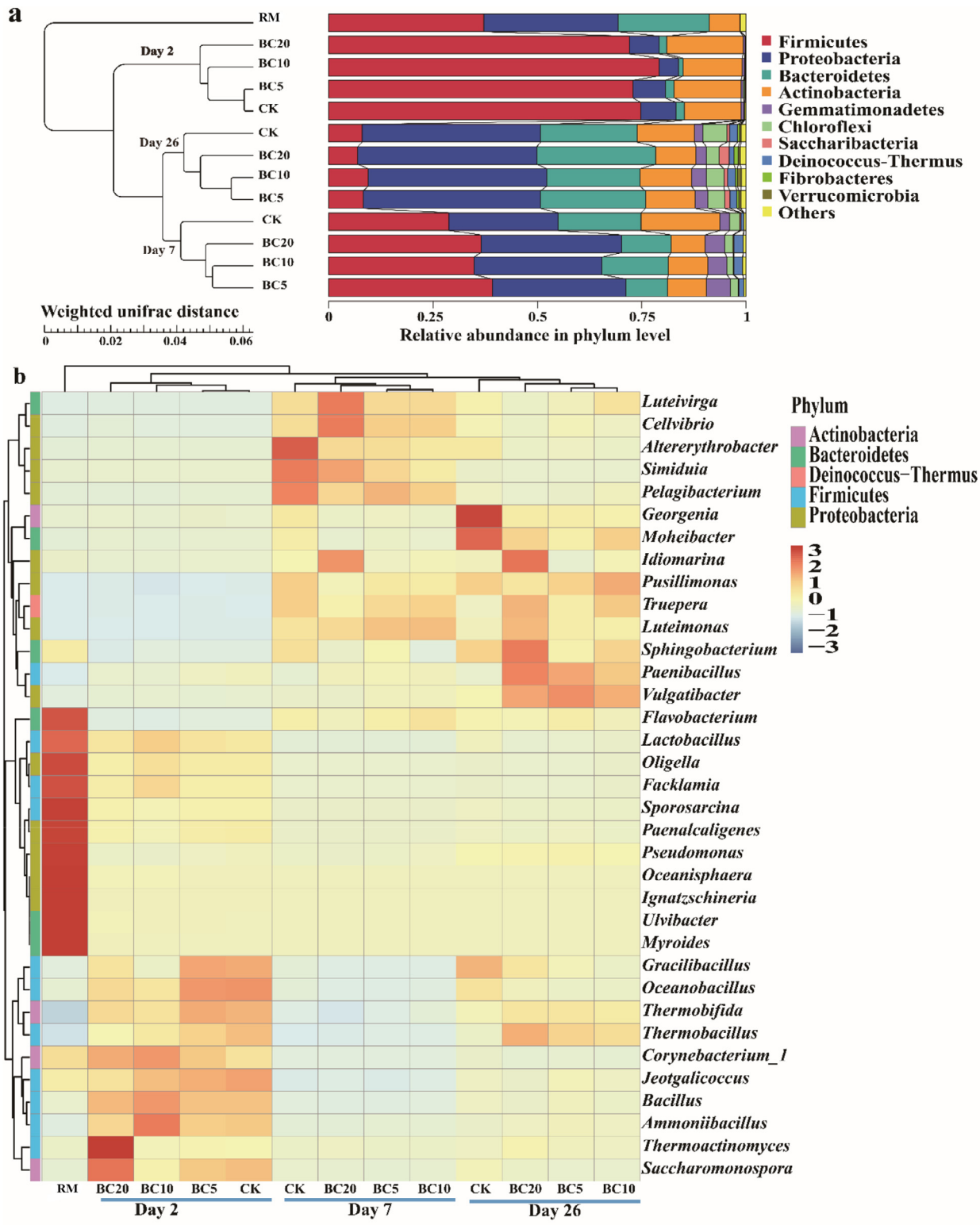

Fig. 3 – Relative abundances of bacterial communities at the (a) phylum and (b) genus taxonomic levels in compost treated with 5% bamboo charcoal (BC5), 10% bamboo charcoal (BC10) and 20% bamboo charcoal (BC20) and a control with no added bamboo charcoal (CK).

ing composting, such as the pH, temperature, and C/N ratio. In particular, the C/N ratio is a key factor that affects the growth of microbial communities. In the present study, the C/N ratios decreased in all treatments until the maturation phase, where the C/N ratios were 14.2, 15.7, 15.8, and 17.7 in CK, BC5, BC10, and BC20, respectively, at the end of composting. Previous studies have also shown that the succession of the overall bacterial community in a composting system is not shifted in the presence of additives (Yin et al., 2019) or residues from harmful substances (Yang et al., 2017). However, the BC10 samples were distant from CK and BC5 on days 2 and 7, thereby indicating that BC10 may have influenced the microbial communities during the thermophilic and cooling phases. Furthermore, the CK and BC5 samples were distant from BC20 after 2 days. We found that this situation was similar to the changes in the NO_3^- concentrations, where the NO_3^- concentration in CK increased by 21.2% on day 7 but decreased on day 26. By contrast, the NO_3^- concentrations decreased by 21% in BC5, 4% in BC10, and 20% in BC20 on day 7, but they increased on day 26. Therefore, these changes may have been related to nitrifying bacteria in the bacterial community, and biochar can create favorable conditions for the survival and activities of nitrifying bacteria (Godlewska et al., 2017).

In the raw materials, the sequencing reads (>98%) were mainly assigned to Firmicutes (37%), Proteobacteria (33%), and Bacteroidetes (22%) (Fig. 3a). During the thermophilic phase, the structure of the bacterial community changed dramatically, where Firmicutes was the most abundant phylum, with an increase of 94%–113%. *Bacillus* was the main taxon that changed in Firmicutes (Fig. 3b) and it increased by 313%–421% in all of the treatments. Compared with the other treatments, we found that the abundance of *Bacillus* in BC10 was 25%, 26%, and 18% higher compared with those in CK, BC5, and BC20, respectively. Duan et al. (2019b) also found that members of *Bacillus* were mainly responsible for the degradation of organic matter in the thermophilic phase (55–65°C). Thus, BC10 was more effective at increasing the abundance of *Bacillus*, which may have contributed to the greater degradation of organic matter during the thermophilic phase.

Proteobacteria was the most abundant phylum during the cooling phase (Fig. 3a), and compared with the thermophilic phase, its abundance increased by 210% in CK, 314% in BC5, 566% in BC10, and 371% in BC20. A previous study also found that the abundance of Proteobacteria decreased during the mesophilic or thermophilic phases but increased by 1.5–2.6 times in the subsequent phases (Zhang et al., 2016). During the cooling phase, *Luteimonas* was the dominant genus (Fig. 3b) and compared with CK, the abundance of this genus was 60% higher in BC5, 53% higher in BC10, and 189% higher in BC5. *Luteimonas* isolates from compost can effectively utilize simple sugars as well as some organic acids (acetate and propionate) and amino acids (Young et al., 2007). Thus, the dominant bacteria in the BC treatments during the cooling phase may have mediated the degradation of small organic molecules to maintain the compost temperature. We also found that the temperature was higher in the BC treatments than CK during the cooling phase (**Appendix A Table S1**).

Proteobacteria and Bacteroidetes were the dominant bacteria during the maturation phase (Fig. 3a), and their abundances increased by 27%–40% in BC5, 41%–152% in BC10, and 17%–31% in BC20 after the cooling phase. Under the BC treatments, the abundances of Proteobacteria and Bacteroidetes were higher than those in CK. *Luteimonas, Cellvibrio*, and *Pseudomonas* were the dominant genera from Proteobacteria, and the abundances of *Cellvibrio* were higher in the BC treatments than CK during the maturation phase (Fig. 3b). In particular, the abundance of *Cellvibrio* was 102% higher in BC20 than CK. In addition, the abundances of *Luteimonas* and *Pseudomonas* were 48% and 27% higher, respectively, in BC10 than CK. A previous study also found that *Luteimonas, Cellvibrio*, and *Pseudomonas* were the main bacterial genera in the maturation phase (Ince and Ince, 2019). Moreover, Wei et al. (2018) found that *Pseudomonas* can improve the composting quality because it was shown to be a plant disease-suppressing bacterial genus during the maturation phase. The nitrite-oxidizing bacterial genus *Luteivirga* was dominant among Bacteroidetes and its abundance was 94% higher in BC20 than CK (Li et al., 2021b). Indeed, compared with CK, we also found that the NO_3^- concentrations were 15% higher in BC5, 28% higher in BC10, and 28% higher in BC20 at the end of composting.

2.3. *Changes in carbon source metabolism by bacterial communities*

Biolog Ecoplate analysis was conducted to detect the utilization of various carbon sources by microorganisms in order to assess the metabolic activities (Fig. 4), where the average well color development (AWCD) indicated the activity of the microbial community during composting (Wang et al., 2018). During the thermophilic phase, the rates of degradation of carbohydrates by the bacterial communities were relatively high (Fig. 4). Some simple compounds (i.e., sugars) were rapidly utilized by microorganisms to increase the temperature during the early phases when the maximum degradation of organic matter occurred (Bernal et al., 2009), and the AWCD levels were relatively high in the thermophilic phases (Zhang and Sun, 2014; Sun et al., 2016). Furthermore, microorganisms exhibited higher metabolic capacities for utilizing D-xylose, β-methyl-D-glucoside, D-cellobiose, and α-D-lactose compared with other carbohydrates, and these sugars are units of lignocellulose. Bernal et al. (2009) reported that thermophilic microorganisms can degrade lignin, cellulose, fats, and hemicellulose. The AWCD values for the aforementioned carbon sources were much higher in BC10 than the other treatments, where the average AWCD values in BC10 were significantly higher for D-xylose (40%) and α-D-lactose (44%) compared with the other treatments. Thus, BC10 was more effective at enriching microorganisms that could degrade lignocellulose, possibly because the genetic and functional profiles of the microbial community exhibited short-term changes when BC was added (Guo et al., 2019).

Amino acids are produced throughout the entire composting process and they are sources of metabolic carbon and energy for bacteria (López-González et al., 2015). During the cooling phase, the amino acid metabolic activity peak occurred, but the average AWCD values for amino acids then decreased by 33% during the maturation phase. These changes may have been related to the succession of the microbial community, where mesophilic microorganisms dominated

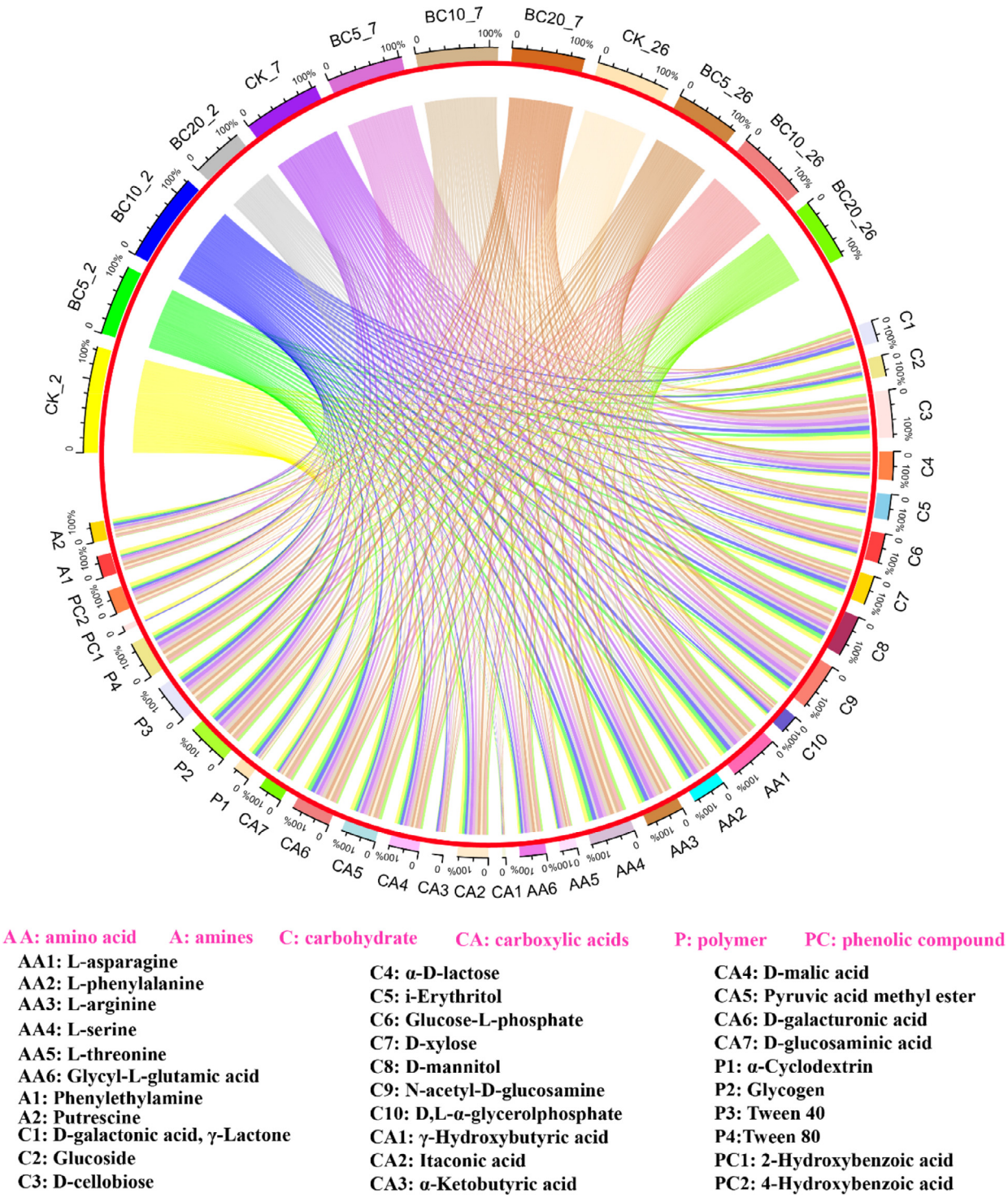

Fig. 4 – Average well color development (AWCD) values for carbon sources in Biolog Ecoplates in compost treated with 5% bamboo charcoal (BC5), 10% bamboo charcoal (BC10) and 20% bamboo charcoal (BC20) and a control with no added bamboo charcoal (CK).

thermophilic microorganisms after the thermophilic phase, and amino acids are essential for the activity of microbial communities (Chen et al., 2019). In the cooling and maturation phase, the changes in L-arginine, L-asparagine, and L-threonine were the most significant among the amino acid carbon sources, and their average AWCD values in BC10 were 16%, 14%, and 52% higher than those in CK, BC5, and BC20, respectively. Previous studies showed that amino acids were the main factors related to the activities of bacteria during the formation of humic substances (Yao et al., 2021). Thus, adding 10% BC may have promoted the synthesis of humic acids.

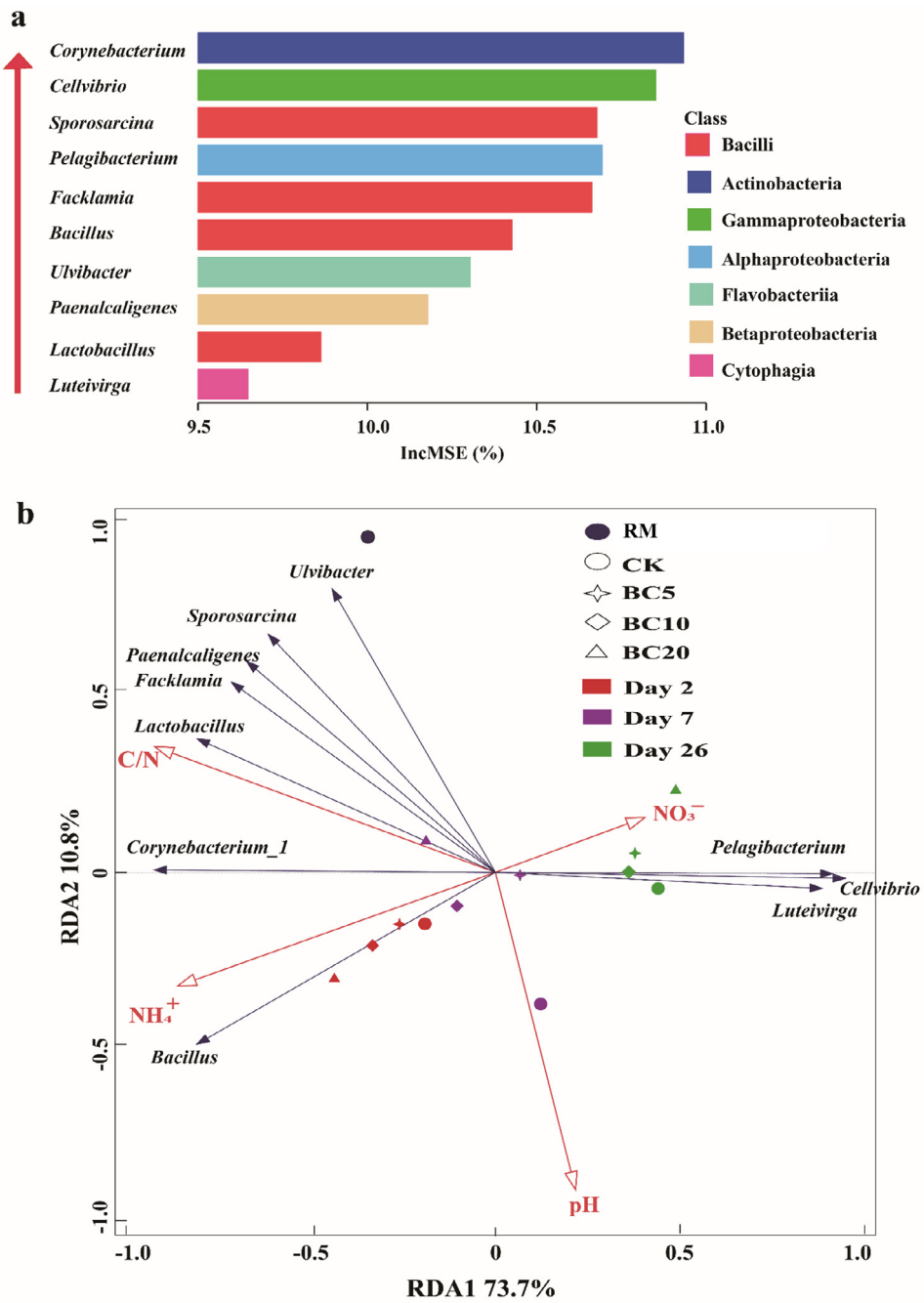

Fig. 5 – (a) Random forest analysis of the top 10 most important bacterial genera during composting; (b) Redundancy analysis based on the most important bacterial genera and the physical and chemical properties of compost.

The average AWCD values for carboxylic acids, polymers, phenolic compounds, and amines decreased by 12%–13%, 11%–24%, 65%–67%, and 29%–37% during the cooling and maturation phases, respectively. These carbon sources were utilized significantly more by microorganisms in CK during the thermophilic phase compared with the BC treatments, but the utilization of only some of these carbon sources differed between CK and the BC treatments during the cooling and maturation phases. For example, compared with other treatments, the AWCD values for D-malic acid were 2%–18% and 10%–26% higher in BC10 during the cooling and maturation phases, respectively. Therefore, BC10 may have reduced the accumulation of organic acids in the composting product, as well as decreasing the phytotoxicity of the compost and enhancing the activities of functional microorganisms.

2.4. *Relationships among enzyme activities, physicochemical properties, bacterial community compositions, and metabolic functions*

In order to explore the mechanisms that might allow BC to enhance the cellulase and urease activities in composting, we

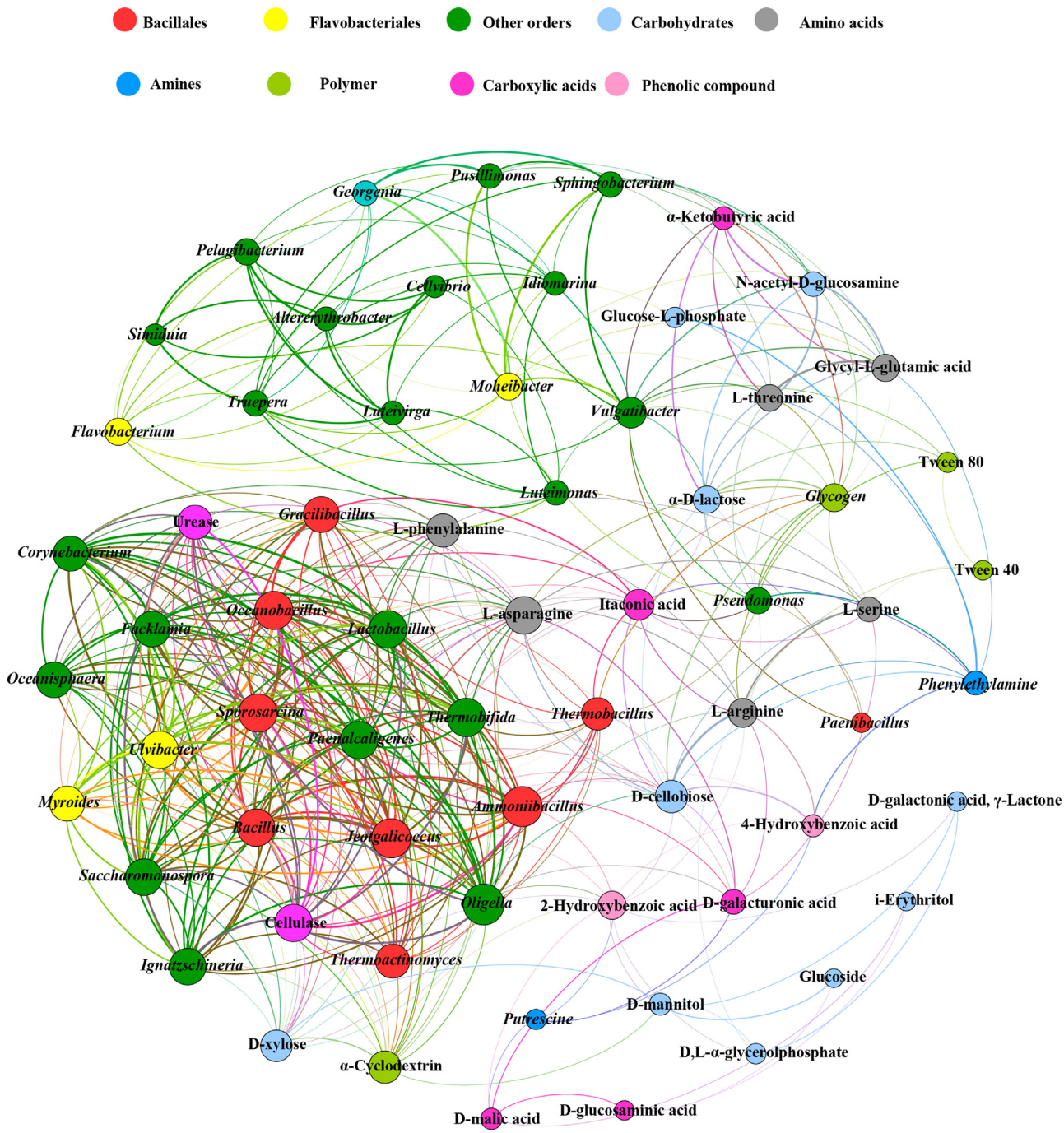

Fig. 6 – Relationships among the bacterial communities physicochemical parameters enzyme activities and utilization of carbon sources. Color lines represent significant strong positive linear relationships ($r > 0.90$).

analyzed the relationships among the enzyme activities, bacterial community compositions, and metabolism of carbon sources. Previous studies have shown that some important species in the bacterial community have crucial dominant functions. According to random forest analysis, *Luteivirga*, *Lactobacillus*, *Paenalcaligenes*, *Ulvibacter*, *Bacillus*, *Facklamia*, *Pelagibacterium*, *Sporosarcina*, *Cellvibrio*, and *Corynebacterium* were the most important bacteria (Fig. 5a). We found that C/N and NH_4^+ had significant positive correlations with 7/10 important bacteria ($P < 0.05$, Fig. 5b). For example, *Lactobacillus* and the C/N ratio had a positive relationship, thereby indicating that *Lactobacillus* made a significant contribution to the changes in C/N during composting. (Zhao et al. (2016) found that the concentration of water-soluble carbon increased with the abundance of *Lactobacillus*. Glucose and xylan are the main components of water-soluble carbon, and they are also degradation products of cellulose and hemicellulose (Nekliudov et al., 2006). We found that *Bacillus* had a positive correlation with NH_4^+, thereby indicating that the changes in NH_4^+ were closely related to this bacterial taxon. Cellulase mainly de-

grades cellulose or hemicellulose to generate simple carbohydrates (Panda et al., 2016), and urease can catalyze the conversion of urea into NH_4^+. Thus, we suggest that these microorganisms may have been closely related to the cellulase and urease activities.

Co-occurrence models were generated to visualize the complex relationships among the microbial, enzymatic, and physicochemical properties, and the results showed that the four treatments yielded different co-occurrence patterns (**Appendix A Fig. S1**). The model obtained for CK comprised 37 nodes, which was slightly less compared with those for BC5 (38), BC10 (40), and BC20 (40). The results showed that *Lactobacillus, Truepera*, and *Jeotgalicoccus* were the keystone genera in each treatment, and the BC treatments changed the keystone members of the bacterial community. In particular, *Sporosarcina* and *Facklamia* were unique keystone genera in BC5, while *Pelagibacterium* was shared by BC10 and BC20, and *Ulvibacter* was a unique keystone genus in BC10. The abundance of the latter is known to increase with the amount of organic matter available in the environment. *Ulvibacter* belongs to the family Flavobacteriaceae and a previous study found that some strains from this family can produce enzymes that degrade biopolymers during the composting process (Kim et al., 2012). Moreover, the cellulase activity was significantly higher in BC10 than the other treatments, which may have been related to specific microorganisms. For example, *Ulvibacter* was a unique bacterial genus associated with the cellulase activity in BC10 compared with the other treatments. Similarly, BC10 significantly enhanced the urease activity compared with the other treatments, but especially the CK treatment. Fourteen and five bacterial genera were related to the urease activity in the networks obtained for CK and BC10, respectively. Four bacterial genera in CK had negative interactions with the urease activity (including the keystone genus *Truepera*), whereas they all had positive interactions with the urease activity in BC10 (all belonged to the class Bacilli). Therefore, the microorganisms in CK may have detrimentally affected urease production and decreased the urease activity, possibly due to competition, predation, or amensalism among negatively interacting bacteria and urease-producing bacteria (Faust and Raes, 2012).

Furthermore, we used the network analysis determined the relationships between the enzyme activities, bacterial community structures, and capacities for utilizing carbon sources (Fig. 6). The cellulase activity and bacterial metabolism of D-xylose, D-cellobiose, and α-cyclodextrin interacted positively, and the urease activity and bacterial use of L-asparagine interacted positively. In addition, *Thermobacillus, Bacillus, Ammoniibacillus*, and *Ulvibacter* had high capacities for degrading D-xylose, D-cellobiose, L-phenylalanine, and L-asparagine. D-xylose and D-cellobiose are the basic units of lignocellulose, and the bacteria that utilized these two carbon sources dominated in the thermophilic and cooling phases under the BC treatments, but especially in BC10. Thus, the enhanced cellulase activity was related to the utilization of xylose and cellobiose by these microorganisms. The utilization rates of L-asparagine and L-phenylalanine by microorganisms were highest among the amino acid carbon sources, which may have led to increases in the urea contents during composting and the urease activity. In addition, the degradation products of these amino acids are required by microorganisms to synthesize enzymes.

3. Conclusions

In the present study, the results demonstrated that the cellulase and urease activities in BC10 increased by 56% and 96%, respectively. The bacterial community structure also changed in the BC10 treatment, and *Luteivirga, Lactobacillus, Paenalcaligenes, Ulvibacter, Bacillus, Facklamia, Pelagibacterium, Sporosarcina, Cellvibrio*, and *Corynebacterium* had the most important roles in composting. The degradation capacities of carbohydrates and amino acids in BC10 were enhanced by up to 44% and 52%, respectively. BC10 changed the bacterial community co-occurrence patterns and keystone bacteria. The genus *Ulvibacter* and class Bacilli were the keystone bacterial taxa, and their metabolic functions may have been responsible for the increased cellulase and urease activities in BC10, respectively.

Acknowledgments

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Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2021.02.007.

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