Effects of rhamnolipid and Tween-80 on cellulase activities and metabolic functions of the bacterial community during chicken manure composting

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\textbf{ABSTRACT}

Metabolism by microorganisms is the basis of composting. In this study, the dynamic changes in the enzyme activity levels, bacterial community structure, and metabolism functions were investigated during chicken manure composting with an added bio-surfactant (rhamnolipid) or chemical surfactant (Tween-80). The results showed that rhamnolipid and Tween-80 improved the quality of the finished compost in terms of the C/N ratio, water-soluble carbon content, germination index, E4/E6 ratio, and the cellulase activity, especially with Tween-80. Furthermore, the bacterial communities were determined by high-throughput sequencing, and their metabolism functions were evaluated using the PICRUSt and Biolog methods. Tween-80 greatly influenced the bacterial community structure, where it enhanced the abundances of bacteria that degrade cellulose and lignin (e.g., members of the order Bacillales) and the capacities for carbohydrate and amino acid metabolism. Network analysis also showed that the order Bacillales was closely related to the metabolism of characteristic carbon sources, especially carbohydrates.

\textbf{1. Introduction}

The large-scale livestock industry has developed rapidly in China and approximately 130 million tons of chicken manure are produced each year (Tian, 2012), which poses a potential threat to the environment. In addition, China is one of the main wheat-producing countries throughout the world, and thus it produces large amounts of wheat straw each year, most of which is disposed of by burning in the field (Zhou et al., 2018). These two forms of agricultural waste contain large amounts of the nutrients that are needed by plants and their reasonable disposal will be important for the sustainable development of agriculture. In general, composting is employed as an effective and economic approach for disposing of agricultural solid waste in China and other agricultural countries. The composting process is driven by microorganisms and their secreted enzymes during the biological and biochemical transformation of compost matrices, and thus bacteria play essential roles in composting systems (Wang et al., 2018a). The final compost product can be used as biofertilizer for application to land and soil amendment. However, the degradation of organic matter restricts the composting process and the quality of the compost products (Shi et al., 2006, 2018).

Most of the organic matter comprises polymers such as cellulose, which need to be decomposed into soluble organics by various microbial extracellular enzymes (Shi et al., 2006), thereby allowing them to enter microbial cells so they can be metabolized into the stable humus-like end product of composting (Shi et al., 2018). Previous studies have investigated the different composting conditions (such as the C/N ratio, particle size, and microbial inocula) that might accelerate aerobic composting and the degradation of organic matter (Nolan et al., 2011; Xi et al., 2015; Zhang and Sun, 2014), and several recent studies have also considered the effects of additives on this process (Shao et al., 2017; Zhou et al., 2018). Surfactants comprise a class of important additives, such as Tween-80 (TW) and rhamnolipid (RL), which could improve the efficiency of practical bioremediation. In particular, surfactants (including biosurfactants) can improve the activities of microorganisms by changing their surface properties to promote the interaction between the bacterial cell surface and hydrophobic substrates (Al-Tahhan et al., 2000; Van Hamme et al., 2006). Previous studies

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have shown that surfactants can accelerate the degradation of cellulose and hemicellulose during composting, as well as enhancing the activities of enzymes during the composting process. Shi et al. (2006) found that TW and RL could accelerate the breakdown of cellulose and hemicellulose during straw and bran composting. In addition, Zhang and Sun (2014) showed that RL enhanced the numbers of microorganisms as well as the enzyme yields and activities during the two-stage composting of green waste. These enhanced properties were mainly related to changes in some functional microorganisms and their metabolic activities (Zhang and Sun 2014), but few previous studies have investigated the characteristics of these functional microorganisms (Shao et al., 2017).

Composting is a dynamic process that involves the rapid succession of bacteria, fungi, and actinomycetes. In the early stage of composting, mesophilic bacteria are dominant, but thermophilic bacteria then dominate together with thermophilic fungi in the thermophilic composting stage. After the cooling phase, mesophilic bacteria and actinomycetes dominate (Tuomela et al., 2000). Bacteria are crucial for the decomposition and stabilization of organic matter in the composting system (Wang et al., 2018a), where the cellulose- and hemicellulose-degrading enzymes secreted by bacteria are mainly responsible for the degradation of lignocellulose. Previous studies investigated the functional roles of bacteria using culture techniques and showed that certain bacteria secrete enzymes to degrade lignocellulose during the composting process (Xi et al., 2015; Kim et al., 2012). However, the dynamic relationships among these bacteria, their metabolism, and the degradation of organic matter remain unclear. Both culturable and unculturable microorganisms need to be considered to address this problem. The Biolog method is an important technique for assessing the substrate utilization capacity by culturable bacteria in environmental samples (Insam et al., 1996; Liu et al., 2012; Wang et al., 2018a). Biolog microplates contain some of the typical substrates used by microorganisms, such as D-cellulbiose and D-xylose, which are important components of cellulose and lignin, respectively, and the results obtained can directly reflect the metabolic diversity of the bacteria involved in cellulose degradation (Wang et al., 2015). High-throughput sequencing analysis is a powerful tool for detecting culturable and unculturable microbial communities (Wei et al., 2018; Zhang et al., 2018). In addition, the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) method was developed to predict the functions of the microbial community based on data obtained by high-throughput sequencing (Langille et al., 2013). Some recent studies have employed the PICRUSt and Biolog techniques to investigate the changes in microbial metabolism during aerobic composting (Wang et al., 2018a,b). Thus, the combination of these two techniques may provide insights into the mechanisms that allow surfactants to affect enzymes and functional microorganisms during the composting process.

Therefore, in this study, the effects of a bio-surfactant (RL) and chemical surfactant (TW) on the cellulase enzyme activity and the succession of organic metabolic functions in the bacterial community were investigated during chicken manure composting. In particular, the present study examined: (i) how surfactants might influence various parameters during composting, including the temperature, pH, C/N ratio, water soluble carbon (WSC) contents, germination index (GI), E4/E6 ratio, and cellulase activity; (ii) how surfactants might influence the bacterial community diversity and metabolic characteristics during manure composting; and (iii) the relationships between parameters comprising the cellulase activity, bacterial community diversity, and metabolic functions. The results obtained enhance our understanding of the effects of surfactants on the metabolic functions of the bacterial community during the chicken manure composting process.

2. Materials and methods

2.1. Experimental design and sampling

Wheat straw was collected from an experimental field at Northwest A&F University, China, and fresh chicken manure was collected from a poultry farm in Yangling, Shaanxi, China. The chicken manure had a total nitrogen content of 34.22 g kg⁻¹ and a total carbon content of 463.82 g kg⁻¹. The wheat straw had a total nitrogen content of 6.28 g kg⁻¹ and a total carbon content of 556.06 g kg⁻¹. RL and TW were purchased from Zijin Biological Technology Co. (Huzhou, China) and Tianli Chemical Reagent Co. Ltd (Tianjin, China), respectively.

The manure composting experiment was performed using 43-L plastic boxes with dimensions of: length × width × height = 51.5 cm × 30.5 cm × 27.5 cm. Chicken manure and wheat straw were mixed to adjust the C/N ratio to 30:1, and the moisture content was adjusted to 60%. In addition, based on previous studies, the surfactant treatments were applied to the matrix by adding 0.15% RL or TW solutions (Shi et al., 2006; Zhang et al., 2016) as the RL and TW treatments, respectively. An equivalent amount of distilled water was added to the control (CK) without the addition of surfactant. Each treatment was repeated in triplicate. Samples were collected from the three treatments after 0, 2, 7, and 26 days. Each sample was divided into two parts, where the first was stored at 4°C for subsequent chemical and Biolog analyses, and the other was stored at −80°C for DNA extraction.

2.2. Physicochemical parameters and cellulase activities

The temperature of the compost was monitored at different locations (i.e., surface, core, and bottom). The moisture contents of the fresh samples were measured based on the weight lost after drying at 105°C for 24 h. Fresh samples were suspended in water at 1:10 (w/v) and mechanically shaken for 30 min at 200 rpm (SUKUN, Shanghai, China), before determining their pH values using a Thermo Orion 3-star pH-meter (CA, USA). The E4/E6 ratio was determined as the optical density or absorbance of the dilute, aqueous humic acid solution at 465 and 665 nm with an ultraviolet spectrophotometer. After determining the pH and E4/E6 ratios, the suspension was tested using a TOC Analyzer (Elementar, Germany) to measure the WSC, which was considered to represent the simple organic carbon contents after the degradation of lignocellulose. Total organic carbon was measured by incinerating the dried samples at 550°C for 24 h in a muffle furnace. The compost samples were digested using H₂SO₄ and H₂O₂, and the total nitrogen content was determined with a Kjeldahl analysis system (FOSS, Denmark). GI was determined as described previously (Gu et al., 2017). In particular, 5 mL of the compost sample extract and 20 radish seeds were evenly distributed in a Petri dish containing filter paper, and cultured in the dark at 30°C for 48 h. Distilled water was used as the control. GI (%) = (seed germination × root length per treatment × 100%)/(seed germination × root length per control). Each treatment was analyzed in three replicate dishes. The cellulase activity was assayed based on the amount of glucose formed after the incubation of compost samples with carboxymethylcellulose, where the cellulase activity was defined as the amount of enzyme required to produce 1 mg of glucose per 24 h.

2.3. Genomic DNA extraction and sequencing analysis

All of the compost samples were dried using a freeze dryer (Songyuan, Beijing, China) until the water content was at the same low level of 3–4%. The samples were then crushed using an ultracentrifugal mill (ZM200, Retsch, Germany) and sieved through 1-mm pore filters. Next, the total DNA was extracted from 0.1 g of each freeze-dried compost sample using a Fast DNA Spin Kit for Soil (MP Biomedicals LLC, Solon, OH, USA) according to the manufacturer's instructions. The concentration and quality of DNA were determined using an Epoch Multi-Volume Spectrophotometer System (BioTek, USA). The extracted DNA was stored at −20°C until subsequent analyses.

The V4 region of the 16s rDNA gene was analyzed by high-throughput sequencing using the Illumina HiSeq platform. The raw data were analyzed using QIIME software and the UPARSE pipeline
dilutions were prepared and the 10^{-3} dilution was used to inoculate the stand for 1 h so the organic matter contents could settle. Ten-fold serial 0.85% sterilized NaCl solution were shaken for 2 h and then allowed to settle.

2.4. Biolog EcoPlate inoculation and analyses

Sample suspensions prepared from 5 g compost matrix and 45 mL of 0.85% sterilized NaCl solution were shaken for 2 h and then allowed to stand for 1 h so the organic matter contents could settle. Ten-fold serial dilutions were prepared and the 10^{-3} dilution was used to inoculate the plates (Liu et al., 2012). Next, 150 μL of each 10^{-3} dilution was used to inoculate the micro-plates. Subsequently, the plates were incubated at 25°C in the darkness. The absorbance values for the plates were detected and recorded at 590 nm using an automatic microorganism identification instrument (Biolog, USA) every 12 h for 240 h. Optical density value obtained from each well was adjusted by subtracting the absorbance of the control (blank well) value. The optical density value obtained after incubation for 120 h represented the optimal range for the optical density readings, and thus the results obtained after incubation for 120 h were used for the statistical analyses. The average well color development (AWCD) values were calculated according to method described by Liu et al. (2012).

2.5. Data and statistical analyses

All of the statistical analyses were performed using SPSS 19.0 (SPSS Inc., USA), and the results were expressed as means and standard deviation (T ± SE). Principal component analysis (PCA) and the preparation of heatmaps and Circos graphs were conducted using R (Version 3.3.1). Network analysis was performed based on the Spearman’s rank correlation coefficients using the physicochemical parameters, cellulose activities, bacterial community, and the substrate utilization capacities with R (Version 3.3.1) and the Gephi (Version 0.9.2) platform.

3. Results and discussion

3.1. Physicochemical characteristics and cellulase enzyme activity of composting

The changes in the physicochemical properties and cellulase enzyme activities during chicken manure composting at different times are shown in Table 1. Temperature is the one of the most important indicators during composting, where it reflects the microbial activity and the different stages of the composting process (Bernal et al., 2009). The peak temperatures in all of the treatments (> 60°C) were reached on day 2 (thermophilic phase), and the peak temperatures in RL and TW were significantly higher than that in CK (Table 1), possibly because the surfactants improved the growth conditions for microorganisms and facilitated the interactions between the composting organic matter and microorganisms (Shao et al., 2017; Shi et al., 2006). The temperature gradually decreased until day 7 (cooling phase) and remained at ambient temperature until day 26 (maturing phase).

The pH values in all of the treatments increased during the first 2 days and then declined gradually in the maturing phase (Table 1), which was associated with the degradation of organic acids or the release of ammonia compounds (Yin et al., 2016). In addition, the pH value was slightly higher in RL compared with CK and TW on day 2, as found in previous studies (Shi et al., 2006). The C/N ratio decreased in all of the treatments throughout the composting process. The C/N ratio in TW was lower than those in RL and CK, which indicates that TW could enhance nitrification and carbon mineralization during the composting process (Khalil et al., 2008; Shi et al., 2006; Zhang and Sun, 2014). The WSC contents decreased gradually in all of the treatments during the first 2 days, possibly because WSC can be utilized directly by microorganisms in the initial composting process (Zhang and Sun, 2014), and the WSC contents then generally increased with the composting time. The WSC contents were significantly higher in TW than RL and CK during the thermophilic and cooling phases. The GI values for all treatments generally decreased until day 2, but then increased to 80.40% in CK, 125.02 in RL, and 113.73 in TW during the subsequent composting phases. The results showed that the addition of RL and TW generated the most effective compost in terms of seed germination, possibly because RL and TW led to the production of intermediates that are toxic to plants during the degradation of organic matter (Zhang and Sun 2014; Shao et al., 2017). The E4/E6 ratio increased in all treatments and reached a peak on day 2, before decreasing to < 3 by the end of the experiment. The E4/E6 ratios determined for RL and TW were significantly lower than those in CK throughout the entire composting process, thereby indicating that RL and TW could enhance the formation of humic acid during composting (Moharan and Biswas, 2016).

Cellulase is an important enzyme for carbon metabolism and its activity can be an indicator of organic matter degradation during the composting of agricultural waste (Guo et al., 2012). The cellulase enzyme activities increased and peaked on day 2, before decreasing in the subsequent composting phases (Table 1). Compared with CK, the cellulase activities were significantly higher in RL and TW during composting, i.e., 28.8–73.3% and 49.5–103.5% higher, respectively, which suggests that the cellulase activity was enhanced by the surfactants, especially by TW. Surfactants can promote the desorption of microbial extracellular enzymes from the surface of the compost medium and enhance the stability of enzymes to improve their activities (Shao et al., 2017).

3.2. Succession of the bacterial community

This study used 16S rRNA gene sequencing to investigate the responses of the bacterial community to surfactants during composting, and according to PCA, PC1 and PC2 together accounted for 41.77% of the total variation in the bacterial community (Fig. 1). The bacterial community structure was separated significantly during different composting periods, where TW was clearly separated from the control after the thermophilic phase, thereby demonstrating that TW may have influenced the microbial communities during the later composting phase (Shi et al., 2006).

Firmicutes (7.48–75.96%), Proteobacteria (5.62–47.37%), Bacteroidetes (1.07–22.24%), and Actinobacteria (6.02–19.63%) were the dominant bacterial communities throughout the composting process (Fig. 2a). Firmicutes was the dominant phylum in the thermophilic phase. Neher et al. (2013) also found that the abundance of Firmicutes was higher in the thermophilic phase than the other phases during dairy cow manure composting. The genus Bacillus was the main contributor to the changes in Firmicutes (Fig. 2b), where the members of this genus prefer warm environments (55–65°C) and they are responsible for degrading organic matter during composting (He et al., 2013). Thus, compared with raw material (RW), the abundance of Bacillus in all treatments increased by 213.92–321.36% in the thermophilic phase, where those in RL and TW were 34.22% and 21.75% higher than that in CK, respectively. Zeng et al. (2006) also found that the ability of some bacterial isolates from compost to decompose hemcelullose and cellulose could be enhanced by RL and TW. In addition, the present study showed that RL and TW enhanced the activities of cellulase enzymes and the WSC contents during composting (Table 1), and glucose and xylan, which are the chief constituents of cellulose and hemicellulose (Charest et al., 2004; Nekludov et al., 2006), are the main components of WSC. Thus, RL and TW may enhance the capacity...
to degrade organic matter during composting.

During the cooling phase, the abundances of Bacteroidetes increased in CK by 10.85 times, RL by 14.32 times, and TW by 19.29 times compared with the thermophilic phase (Fig. 2a). At the genus level, the dominant genus was *Flavobacterium* during the cooling phase. Compared with CK, the abundances of *Flavobacterium* were higher in RL and TW, i.e., by 25.54% and 56.98% (Fig. 2b), respectively. Kim et al. (2012) found that some *Flavobacterium* strains can produce enzymes that degrade various biopolymers (such as cellulose) during garden waste composting. Thus, TW might have improved the capacity to degrade cellulose by enhancing the abundance of *Flavobacterium* during the cooling phase. The WSC contents were also significantly higher in TW during the cooling phase.

The abundance of Proteobacteria increased significantly from 5.62 to 7.92% to 41.18–47.37% after the thermophilic phase in all treatments, and that of Actinobacteria increased by 176.87–208.04% in the maturing phase (Fig. 2a). Proteobacteria and Actinobacteria were the dominant phyla in the maturing phase.

*Pseudomonas* was the main genus in Proteobacteria (Fig. 2b) and previous studies have determined the various capacities of Pseudomonadaceae strains, where some are efficient at degrading cellulose and hemicellulose (Wang et al., 2013), as well as being involved in nitrogen transformations (Zhang et al., 2019). Thus, the occurrence of *Pseudomonas* spp. in the mature compost may have improved its quality (Ventorino et al., 2016). *Saccharomonospora* and *Corynebacterium I* were the main genera in Actinobacteria. *Saccharomonospora* strains may hydrolyze phenolic compounds into non-toxic forms and they possess specific phospholipids in the cell wall (Zhou et al., 2018). The abundances of *Pseudomonas* and *Saccharomonospora* were greater in TW than RL and CK during the maturing phase, which suggests that the rapid reduction in the C/N ratio in TW may have been related to these microorganisms at the end of composting. In addition, the abundance of *Corynebacterium I* decreased by 56.45–67.37% in the surfactant treatments compared RW, where those in RL and TW were 25.08% and 21.54% lower than that in CK (Fig. 2b), respectively. The genus *Corynebacterium* contains potential human pathogens (Mohammadi et al., 2013) and Shao et al. (2017) reported that RL may play a role in removing pathogenic bacteria during composting. TW may also reduce the abundances of pathogenic bacteria, and thus surfactants might decrease the risk of pathogenic bacteria entering the soil environment.

### Table 1
Changes in the physicochemical parameters and cellulase activities during the composting of chicken manure spiked with rhamnolipid (RL) or Tween-80 (TW), and a control with no added surfactant (CK).

<table>
<thead>
<tr>
<th>Compost Sample</th>
<th>Day-2</th>
<th>Day-7</th>
<th>Day-26</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CK</td>
<td>RL</td>
<td>TW</td>
</tr>
<tr>
<td>Temperature</td>
<td>63 ± 0.58</td>
<td>70 ± 0.58</td>
<td>68 ± 0.58</td>
</tr>
<tr>
<td>pH</td>
<td>8.70 ± 0.04</td>
<td>9.16 ± 0.01</td>
<td>8.89 ± 0.02</td>
</tr>
<tr>
<td>C/N</td>
<td>31.24 ± 4.64</td>
<td>24.25 ± 0.18</td>
<td>24.44 ± 0.03</td>
</tr>
<tr>
<td>WSC</td>
<td>6.47 ± 0.68</td>
<td>8.34 ± 0.62</td>
<td>10.39 ± 0.77</td>
</tr>
<tr>
<td>GI</td>
<td>26.90 ± 2.16</td>
<td>26.40 ± 3.84</td>
<td>25.28 ± 1.39</td>
</tr>
<tr>
<td>E4/E6</td>
<td>6.72 ± 0.10</td>
<td>6.01 ± 0.06</td>
<td>6.41 ± 0.17</td>
</tr>
<tr>
<td>Cellulase</td>
<td>6.47 ± 0.68</td>
<td>8.34 ± 0.62</td>
<td>10.39 ± 0.77</td>
</tr>
</tbody>
</table>

![Fig. 1. Principal component analysis of the bacterial community composition on days 2, 7, and 26 during the composting of chicken manure spiked with rhamnolipid (RL) or Tween-80 (TW), and a control with no added surfactant (CK).](image-url)
Fig. 2. Changes in the relative abundances of bacteria at the (a) phylum and (b) genus level during the composting of chicken manure spiked with rhamnolipid (RL) or Tween-80 (TW), and a control with no added surfactant (CK).
3.3. Predicted potential functions and substrate utilization patterns of the bacterial communities

The KEGG pathway database was employed in order to further analyze the effects of RL and TW on the functions of the bacterial community (Fig. 3a). The predicted protein sequences annotated based on KEGG pathways in 10 samples were related to functions such as metabolism (47.74–49.83%), genetic information processing (16.36–18.06%), environmental information processing (13.40–14.85%), and cellular processes (3.40–4.06%). The abundances of genes associated with metabolism increased throughout the whole composting process, whereas amino acid metabolism and carbohydrate metabolism were the main pathways in the metabolism cluster (Fig. 3b). The abundances of sequences related to carbohydrate metabolism were relatively high during the thermophilic phase, while the abundances of genes related to amino acid metabolism were relatively high in the maturation phase, which are consistent with the results reported by Wang et al. (2018b). Under aerobic composting conditions, carbohydrate metabolism can produce various compounds via the degradation of cellulose and hemicellulose (Toledo et al., 2017). In addition, the addition of RL and TW greatly increased the abundances of sequences related to carbohydrate metabolism during the thermophilic phase (Fig. 3b). The abundances of genes associated with amino acid metabolism increased slightly as the composting process progressed (Fig. 3b). For instance, the abundances of genes related to the biosynthesis of valine, leucine, isoleucine, phenylalanine, tyrosine, and tryptophan were higher in RL and TW than CK during composting. Previous studies have shown that the active metabolism of amino acids by bacteria can increase the production of amino acids and humic acids (Wei et al., 2018; Wu et al., 2017). Thus, RL and TW appeared to promote the formation of humic-like substances during composting, where RL and TW increased the formation of humic acid according to the E4/E6 ratio.

Biolog Ecopeleates were used to investigate the effects of surfactants on the capacities of the community of culturable bacteria to utilize various carbon sources in different phases during the chicken manure composting process (Fig. 4). The carbohydrate degradation rates of the bacterial community were relatively high level during the thermophilic phase, which are consistent with the results predicted by PICRUSt. D-cellobiose, D-mannitol, glucoside, and D-xylose were the main carbohydrates degraded by the bacterial community (Fig. 4), which suggests that the capacity of the bacterial community to degrade cellulose was high during the thermophilic phase (Loewenberg, 1984). In addition, the AWCD values for glucoside, D-cellobiose, and D-xylose were significantly higher with added surfactants, i.e., 24.56%, 10.26%, and 11.61% higher, respectively, with TW than CK during the thermophilic phase. However, the carbohydrate degradation rates of the bacterial community declined after the thermophilic phase, which could have been related to the rapid consumption of simple compounds as the composting process continued (Bernal et al., 2009). Remarkable reductions in the rates of carboxylic acid degradation by the bacterial community were detected during composting over time. The AWCD values determined for all of the carboxylic acids remained at high levels during the thermophilic and cooling phases, but the values for D-malic acid and D-glucosaminic acid increased in the maturing phase whereas those for γ-hydroxybutyric acid, itaconic acid, α-ketobutyric acid, pyruvic acid methyl ester, and D-galacturonic acid decreased.

The metabolism of amino acids by the bacterial community increased significantly during the cooling phase but decreased during the maturing phase (Fig. 4). The AWCD values for L-asparagine, L-phenylalanine, L-arginine, and L-serine were highest during the cooling phase, but their average AWCD values decreased by 25.90%, 13.88%, 16.74%, and 17.48%, respectively, during the maturing phase. In addition, the average AWCD values were higher with TW than the other treatments, which suggests that TW might have enhanced the synthesis of humic acid (Wu et al., 2017), which is consistent with the PICRUSt and E4/E6 results.

The changes in the AWCD values for polymers, amines, and phenolic compounds during composting are shown in Fig. 4. The AWCD values for 1-asparagine, 1-phenylalanine, 1-arginine, and 1-serine were highest during the cooling phase, but their average AWCD values decreased by 25.90%, 13.88%, 16.74%, and 17.48%, respectively, during the maturing phase. In addition, the average AWCD values were higher with TW than the other treatments, which suggests that TW might have enhanced the synthesis of humic acid (Wu et al., 2017), which is consistent with the PICRUSt and E4/E6 results.
hydroxybenzoic acid, phenylethylamine, putrescine, and glycogen were significantly higher in RL and TW during the cooling phase than CK, whereas those for Tween-80 and glycogen were higher in RL and TW during the maturing phase than CK. Albrecht et al. (2010) demonstrated the transformation of these macromolecular compounds in the later phase of composting, thereby indicating that surfactants could enhance the transformation of these macromolecular compounds during the later composting phase.

3.4. Relationships among microbial communities, physicochemical characteristics, and microbial metabolism

Network analysis was conducted to assess the relationships between the physicochemical characteristics and bacterial communities (Fig. 5a). The network comprised 47 nodes and 302 edges, with a modularity index of 0.463, which suggests that the network had a modular structure (Li et al., 2015). The entire network could be separated into four major modules according to the modularity classes. Module I comprised bacteria related to the C/N ratio, where Halomonas, Pseudomonas, Bacillus, and Sporosarcina had positive relationships with the C/N ratio. Wei et al. (2018) also found that Sporosarcina had a positive relationship with the C/N ratio, which was associated with the formation of humic acid during composting. Both Pseudomonas and Bacillus have the capacity to degrade organic matter such as cellulose, and Halomonas is related to the transformation of nitrogen (Miao et al., 2015). Module II comprising the cellulase activity, pH, temperature, and E4/E6 ratio had positive relationships with Bacillus and Thermobacillus, thereby indicating that the cellulase enzyme was secreted mainly by Bacillus and Thermobacillus during the composting process. These microorganisms may affect the temperature, pH, and E4/E6 ratio by degrading organic matter, and He et al. (2013) also found that the degradation of organic matter in compost was related to the pH and E4/E6 ratio. In addition, Saccharomonospora had a relationship with the E4/E6 ratio, where this genus has specific phospholipids in the cell wall that can participate in the aromatization of organic substances (Zhou et al., 2018). Module III comprising Cellvibrio and Luteimonas had relationships with the GI and WSC, and it is known that the members of Cellvibrio can degrade refractory organic matter by converting it into small molecular WSC components during the composting process. The GI reflects the phytotoxicity of toxic substances, such as ammonia and low molecular weight short chain volatile fatty acids (Chen et al., 2010), and thus Cellvibrio and Luteimonas might accelerate the degradation of harmful organic matter and the transformation of nitrogen (Severin et al., 2010).

The relationships between the bacterial community structure and the capacities for utilizing carbon sources were determined based on the network (Fig. 5b). The order Bacillales was closely related to the metabolism of six carbon sources, but especially the metabolism of carbohydrates. For example, Bacillus, Thermobacillus, Sporosarcina, and Jeotgalicoccus had high capacities for the degradation of D-cellobiose and D-mannitol, where both of these carbohydrates were detected mainly during the thermophilic and cooling phases of composting, as also shown by Yi et al. (2012). In addition, these microorganisms were more abundant in the RL and TW treatments than CK during the cooling phase.
thermophilic and cooling phases of composting, but especially in the TW treatment. \(\beta\)-Celllobiose is a unit of cellulose, thereby indicating that TW could increase the abundances of microorganisms with the capacity to utilize celllobiose to increase the cellulase activity. The capacities to utilize L-asparagine, L-phenylalanine, and L-arginine as carbon sources were higher than those of other amino acids, where \textit{Bacillus}, \textit{Pseudomonas}, and \textit{Facklamia} had higher capacities for degrading these carbon sources. This may have been related to the requirement of these microorganisms for synthesizing enzymes by degrading amino acids, which is consistent with the PICRUSt results.

4. Conclusion

In this study, RL and TW improved the quality of the finished compost in terms of its physical characteristics and cellulase activity, especially with TW. TW changed the bacterial community structure, possibly by enhancing the abundances of bacteria that degrade cellulose and lignin (e.g., \textit{Bacillales}) during composting. RL and TW also increased the abundance of genes related to carbohydrate and amino acid metabolism during the thermophilic phase. The bacterial community under TW had the highest capacity for degrading carbohydrates and amino acids, and it was closely related to metabolism by \textit{Bacillales}.

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