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# Effects of seaweed fertilizer on enzyme activities, metabolic characteristics, and bacterial communities during maize straw composting



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# ABSTRACT

The objective of this study was to investigate microbial responses when seaweed fertilizer was added to maize straw compost. The metabolic characteristics and bacterial communities were assessed using 16S rRNA sequencing and Biolog analysis. Results showed that the urease, dehydrogenase, and metabolic activities improved on day 21 after the addition of seaweed fertilizer. Firmicutes, Proteobacteria, and Actinobacteria were the dominant phyla in the compost. The relative abundances of Proteobacteria and Actinobacteria were increased by addition of seaweed fertilizer during the early composting stage. Temperature,  $NH_4^+$ ,  $NO_3^-$ . N concentrations, and carbon source metabolism had close relationships with bacterial community variation during composting. A network analysis revealed that  $NO_3^-$ . N content had a positive association with *Aeromicrobium*, and seaweed fertilizer application improved the growth of keystone species related to N cycling. These results suggested that seaweed fertilizer influenced bacterial community succession through its effects on N concentrations during the composting process.

### 1. Introduction

The world annual production of maize straw has been estimated to be approximately 1 billion tons (Kovacic et al., 2017). Large amounts of maize straw are traditionally burned in the field, which causes nutrient losses and serious environmental pollution. Therefore, it is necessary to look for an environmental-friendly method to deal with maize straw that avoids negative impacts on the environment. Composting, which can substantially reduce waste volumes (Zhang et al., 2016), is considered to be an effective and sustainable management method for the disposal of agricultural residues (Zhou et al., 2019). The final compost product can be used as an organic fertilizer and as a soil amendment in agroecosystems (Zorpas, 2014).

Maize straw contains a large number of recalcitrant substances (e.g., cellulose, hemicelluloses, and lignin) and has a high C/N ratio, which makes it difficult for microorganisms to decompose. Consequently, it requires a long time to reach the mature compost stage. Organic additives can optimize the C/N ratio, stimulate microbial activity, and accelerate decomposition during the composting process (Zhang and Sun, 2015). Organic additives have gained widespread attention because they can improve the composting process, and seaweed fertilizer is a natural organic additive that is harmless to the environment.

Furthermore, it is commonly used in agriculture and horticulture. Seaweed fertilizer contains various organic compounds, such as amino acids, proteins, and vitamins, and is rich in laminarin, alginate, and other polysaccharides that are not present in terrestrial plants (Khan et al., 2009). Previous studies have reported that seaweed fertilizer amendment increased nutrient uptake and plant growth rates (Wang et al., 2018b; Wang et al., 2016). In addition, seaweed fertilizer is particularly rich in a large number of enzymes (Heo et al., 2005) and contains abundant microorganisms (Egan et al., 2013). Tujula et al. (2010) reported that the epiphytic microbial community in seaweed was predominantly composed of Alphaproteobacteria (70%) and Bacteroidetes (13%). Therefore, seaweed fertilizer amendment could enhance microbial diversity, influence microbial community succession during composting, and ultimately affect the composting process.

Microorganisms play crucial roles in the maize straw composting process. Understanding microbial properties and activities in response to seaweed fertilizer amendment helps us to understand microbial functions and to identify reasonable management practices. The functions of microbial community regulated organics decomposition, humic-like substances formation, and nutrient transformations during the composting process are relatively well understood, but there is little available information about microbial functional characteristics,

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https://doi.org/10.1016/j.biortech.2019.121375 Received 18 March 2019; Received in revised form 19 April 2019; Accepted 20 April 2019 Available online 23 April 2019 0960-8524/ © 2019 Elsevier Ltd. All rights reserved. especially carbon utilization capacity. Biolog analysis could provide information about substrate utilization capacity and the metabolic characteristics of microbial communities during the composting process. This method has previously been used to evaluate metabolic diversity and bacterial community functions in soils (Gomez et al., 2006). Wang et al. (2018a) reported that metabolism of carbohydrates, lipids, and amino acids were higher during the thermophilic composting stage by using Biolog analysis. To date, few studies have investigated the effects of seaweed fertilizer addition on microbial communities and carbon utilization capacity in a maize straw composting system.

Microbial succession is caused by changes in nutrient levels and microbial substrate preferences. Some microbes may have an advantage in the community, and the microbial populations may be arranged in functionally distinct niches and trophic groups (Schimel and Schaeffer, 2012). Exploring these co-occurrences in composting can help identify complex microbial associations and modular patterns. Network analyses of co-occurrence focus on the correlations between microbial taxa and environmental parameters and can reveal inter-taxa associations in the shared niches (Qiu et al., 2019). Identifying the keystone species that play a more critical role may be important to improve our understanding of the decomposition of organic wastes during the composting process (Banerjee et al., 2016).

Therefore, the main objectives of this study were to reveal the effects of seaweed fertilizer on metabolic characteristics and the composition of the bacterial community, and to establish the relationships between nutrient properties and special bacterial taxa during maize straw composting. The bacterial community composition and carbon source utilization capacity of the microbial community were evaluated by high throughput sequencing and Biolog analysis, respectively. Additionally, the relationships between microbial communities and various environmental parameters were assessed in the composting system using network and redundancy analyses.

#### 2. Materials and methods

#### 2.1. Composting feedstock collection and process

The composting materials used in this study comprised a mixture of maize straw, which was cut into 2 mm sections, pig manure, and seaweed fertilizer. The pig manure and maize straw were obtained from a local farm in Jiujiang, China, and the granular seaweed fertilizer was purchased from the Beijing Leili Marine Biology New Industry Co. Ltd., China. The seaweed fertilizer was screened through a 2 mm sieve prior to application. The basic properties of these substances are shown in Table 1. A maize straw: pig manure ratio of 2:1 (w/w) was used in the control (CK) and the seaweed treatments, which contained 5% or 10% seaweed fertilizer (dry weight) and were labeled as S1 and S2, respectively. The composting mixtures were placed in aerated cuboidshaped containers ( $150 \times 100 \times 60$  cm) and each treatment was conducted in triplicate. The composting materials were mixed by manual turning every three days to provide aeration. The moisture in the piles was measured daily using a moisture meter (SK-100, Tokyo, Japan) and was maintained at 60% during the composting process by adding distilled water. Compost samples were collected on days 1, 3, 7, 14, 21, 28, 35, and 42. The collected samples were divided into two portions: one

Table 1	
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Material	Moisture (%)	рН	Total organic carbon (g kg $^{-1}$ )	Total nitrogen (g kg <sup>-1</sup> )
Pig manure Maize straw Seaweed fertilizer	$25.8 \pm 0.1$ $9.8 \pm 0.2$ $10.3 \pm 1.6$	$\begin{array}{r} 7.3 \ \pm \ 0.1 \\ 7.2 \ \pm \ 0.3 \\ 6.8 \ \pm \ 0.6 \end{array}$	$347.4 \pm 4.3$ $340.6 \pm 2.2$ $184.7 \pm 10.5$	$\begin{array}{r} 23.5 \ \pm \ 1.0 \\ 5.3 \ \pm \ 0.2 \\ 8.9 \ \pm \ 0.6 \end{array}$

The values are the mean and standard error of three subsamples.

was air-dried for physico-chemical analysis and the other was stored at -20 °C for further microbial analysis.

#### 2.2. Physico-chemical parameter analysis

The temperature of the composting pile and the environment was measured at 9:00 am and 16:00 pm every day using a thermometer. The pH was determined using a pH meter (Sartorius, Gottingen, Germany) after shaking the compost samples with deionized water (1:10 w/v). Total organic carbon (TOC), total nitrogen (TN), ammonia nitrogen ( $NH_4^+$ -N), and nitrate nitrogen ( $NO_3^-$ -N) were measured according to Zhang and Sun (2014).

The urease activity of the compost was determined according to Liu et al. (2014). Briefly, 1 g of compost was mixed with 0.1 M disodium tetraborate (pH = 10, 4 mL) and 0.4 M urea solution (0.5 mL). The mixture was incubated in an incubator (37 °C, 2 h), and then 6 mL of acidified 2 M KCl was added. The supernatant fraction was diluted after centrifugation for 5 min. A sodium salicylate/sodium nitroprussiate mixture, NaOH solution, and sodium dichloroisocyanurate were then added to the supernatant. The absorbance was recorded at 670 nm using a spectrophotometer (BloMate 3S, Thermo Fisher, USA).

The dehydrogenase activity was measured using 2,3,5-triphenyltetrazolium chloride (TTC). A fresh compost sample was mixed with TTC and buffer solution (0.1 M tris (hydroxymethyl) aminomethane, pH = 7.6) and incubated in an incubator (37 °C, 24 h). Then, acetone was added to stop the reaction and the absorbance value was determined at 546 nm using a spectrophotometer.

# 2.3. Biolog Ecoplate analysis

Biolog Ecoplate analysis was used to investigate the carbon source utilization capacity of the microbial community during the composting process. A 1 g fresh compost sample was added to 100 mL 0.85% (w/v) NaCl solution and shaken for 20 min. The collected suspension was diluted and then inoculated onto a 96 well Biolog Ecoplate. The plates were subsequently incubated at 25 °C in the dark. The absorbance of the plate was measured every 24 h until 144 h using an automatic microorganism identification instrument (Biolog, USA). Average well color development (AWCD) was calculated according to the manufacturer's instructions.

#### 2.4. High-throughput sequencing

The total DNA was extracted from a 0.5 g fresh sample using a Fast DNA SPIN Kit for Soil (MP Biomedicals, CA, USA) following the manufacturer's instructions. The extracted DNA concentration was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). After purification, the DNA was stored at -80 °C until needed for further PCR amplification. The primers used for the amplification were 338F (5'-ACTCCTACGGGAGGCA GCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR reactions were carried out according to Wei et al. (2018). The PCR products were purified using a PCR clean-up purification kit (MP Biomedicals, OH, USA). The quantified amplicons were sequenced on an Illumina Miseq platform (Illumina, San Diego, CA, USA) and the bacterial community was analyzed.

# 2.5. Data analysis

After the samples had been quality filtered, the high-throughput sequencing data were analyzed using the Quantitative Insights into Microbial Ecology (QIIME) package (http://qiime.org/). The sequences were clustered into operation taxonomy units (OTUs) at the 97% similarity level using the UPARSE pipeline. The Ribosomal Database Project (RDP) was used to assign the OTU sequences to a taxonomic annotation. The relationships among environmental factors, carbon



Fig. 1. Physico-chemical properties of compost samples at different composting periods. The values are the mean and standard error of three subsamples.

source utilization capacity, and bacterial community were characterized by a redundancy analysis (RDA) using the R Cluster package (https://www.r-project.org/). Network analysis was performed using the maximal information coefficient (MIC) in the MINE software. The MIC reveals the strength of the linear associations among variables. Only significantly positive (r > 0.8) and negative (r < -0.8) associations were chosen for network construction and visualization in Cytoscape (v.3.2.1). The modular structure of highly interconnected nodes was displayed. The display was based on the MCODE application with the default parameters. Additionally, OTUs with maximum betweenness centrality scores were identified as the keystone species.

#### 3. Results and discussion

#### 3.1. Physicochemical characterization during composting

Temperature is an important index that can influence the composting process and microbial activity during waste composting (Kulcu and Yaldiz, 2014). The temperature variations in the compost piles are shown in Fig. 1a. All three treatments had a consistent trend for temperature variation and reached the thermophilic stage (more than 50 °C) on day 3. The addition of seaweed fertilizer considerably increased the thermophilic temperature of the compost. The highest temperatures for S1 and S2 were 60.9 °C and 64.4 °C, respectively, which were much higher than in CK (54.3 °C). An explanation for this may be that seaweed fertilizer contains nutrients that facilitate microbial growth and accelerate the microbial degradation of organic matter, which results in the generation of substantial heat (Zhang and Sun, 2017). After the thermophilic stage, the compost temperature began to decrease for all treatments. This is probably due to the exhaustion of bio-available organic matter (Zhong et al., 2018). The thermophilic phase in CK, S1, and S2 lasted 6, 11, and 12 days, respectively. These results indicated that seaweed fertilizer amendment increased the pile temperature and the thermophilic period during maize straw composting.

Composting leads to the biological degradation of mixed organic matter. During composting, the percentage of total organic carbon (TOC) decreased in all treatments (Fig. 1b). The TOC contents were 49.8%, 50.8%, and 51.7% for CK, S1, and S2, respectively, after the first day of composting. The TOC contents further declined to 38.5%, 35.2%, and 32.0% for CK, S1, and S2, respectively, by day 42. The TOC loss can be attributed to microbial activity and mineralization of organic matter during the composting process (Belyaeva et al., 2012). The rich microbial community in seaweed fertilizer rapidly broke down the easily decomposable organic matter, which contributed to the greater TOC loss in S1 and S2. Furthermore, the increase in the duration of the thermophilic temperature stage caused by the addition of seaweed fertilizer may also have accelerated organic matter decomposition.

The NO<sub>3</sub><sup>-</sup>-N concentrations in all the treatments were low during the early stage (Fig. 1c). This was probably because of the low activity and growth of nitrifying bacteria at high temperatures (Rashad et al., 2010). The  $NO_3^{-}$ -N concentrations in all the treatments gradually rose with increasing time during composting and reached 280.1, 315.3 and  $331.8 \text{ mg kg}^{-1}$  for CK, S1, and S2, respectively, on day 42. Seaweed fertilizer contains high NO3<sup>-</sup>-N concentrations (Wang et al., 2016) and this could have accounted for the relatively high NO3<sup>-</sup>-N concentrations in the treatment compost piles. In this study, the NO3<sup>-</sup>-N concentrations in S1 and S2 were higher than in CK over the composting period. In contrast, S1 and S2 had lower NH4<sup>+</sup>-N concentrations than CK (Fig. 1d). The nutrients in seaweed fertilizer can improve the growth of the nitrifying bacteria that convert ammonia to nitrate, which efficiently increases nitrate nitrogen content. Overall, these results indicated that the addition of seaweed fertilizer could improve the activities of microorganisms involved in the nitrogen cycle during composting.

# 3.2. Changes in enzyme activity during composting

Enzymes are associated with nutrient cycling and their activities are indicators of the biochemical functioning of compost. Urease is closely related to the mineralization of nitrogen and plays important roles in the decomposition of agricultural wastes (Guo et al., 2012). The urease activities of CK, S1, and S2 were 9.1, 14.4, and 17.8  $\mu$ g NH<sub>4</sub><sup>+</sup>-N g h<sup>-1</sup> on day 21, respectively (Fig. 2a), which suggested that the addition of seaweed fertilizer enhanced urease activity during the early stage of composting. Lower enzyme activities were observed during the mature



Fig. 2. The enzyme activities (a, b) and average well-color developments (AWCD) of carbon sources in Biolog Ecoplate (c, d) of compost samples.

stage due to the decreased organic matter content, and there were no significant differences in urease activity between treatments on day 42 (Fig. 2a). Dehydrogenase is an important microbial enzyme that is related to carbon cycling (Sardans et al., 2008) and can catalyze the production of adenosine triphosphate (ATP) through the oxidation of organic matter (Alidadi et al., 2016). The dehydrogenase activities in S1 and S2 on day 21 were 14.6 and 15.3  $\mu$ g TPF N g h<sup>-1</sup>, respectively, which were significantly (P < 0.05) higher than in CK (Fig. 2b). Seaweed fertilizer contains macro- and micro-nutrients that contribute to the synthesis and secretion of enzymes by microorganisms (Baker et al., 2011). This, in turn, leads to higher enzyme activity in the compost. In addition, seaweed fertilizer is itself rich in enzymes that can also improve enzyme activity during composting (Leceta et al., 2014). In total, seaweed fertilizer addition, especially at 10% (S2), greatly stimulated the activities of microbial enzymes and influenced carbon and nitrogen cycling in the compost during the early stage of composting.

# 3.3. Carbon utilization capacity of the microbial community

Biolog Ecoplate analysis was used to evaluate the carbon sources utilization capacity of the microbial community during the composting process. Average well-color development (AWCD) reflects microbial community activity (Feng et al., 2011). The AWCDs of the carbohydrates, amino acids, esters, and carboxylic acids were higher in S1 and S2 than in CK on day 21 (Fig. 2c), which indicated that the addition of seaweed fertilizer enhanced the carbon sources utilization capacity of the microbial community. This enhancement could have been due to the seaweed fertilizer stimulating enzyme activities, which improved the AWCD values. The higher AWCD of the amino acids in the seaweed fertilizer treatments was associated with the increased mineralization of organic matter and humic substance synthesis (Lopez-Gonzalez et al., 2015; Wu et al., 2017). The main carbon sources were mostly metabolized during the thermophilic phase of composting, and the AWCD of the above four carbon sources showed no significant differences between treatments on day 42 (except for amino acids) (Fig. 2d). This was probably because the bio-available organic matter had been exhausted by the late stage of composting.

#### 3.4. Bacterial community composition during the composting process

Pyrosequencing was performed to improve understanding about the composition of the bacterial communities in composts with and without seaweed fertilizer addition. A total of 19 phyla were identified based on a taxonomic analysis of the 16S rRNA sequences. Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, and Proteobacteria were identified as the main phyla in composts. Together, they accounted for more than 92% of all sequences (Fig. 3). Our previous study demonstrated that Firmicutes predominate during the thermophilic phase of composting because members of the Firmicutes can form heat-resistant endospores and grow at the high temperatures (Zhou et al., 2019). Our results showed that Firmicutes was one of the most dominant bacterial phyla on day 21, with an abundance of 73.8%, 59.7%, and 37.2% for CK, S1, and S2, respectively. However, Firmicutes abundance declined as composting progressed. This progression was highly related to the decrease in temperature within the compost piles. Another dominant bacterial phylum was Proteobacteria, and it accounted for 16.8-45.8% of the microbes in all treatments on day 21. Proteobacteria play a crucial role in carbon and nitrogen cycling in compost. The addition of seaweed fertilizer considerably increased the abundance of Proteobacteria during the early stage of composting and its relative abundance increased with composting time. The relative abundance of Proteobacteria increased to 53.7% and 48.4% for S1 and S2 on day 42, respectively, which was significantly (P < 0.05) higher than for CK (44.5%). Members of Actinobacteria are able to degrade several complex organic compounds, such as lignocelluloses (Goodfellow et al., 2005). Some species of Actinobacteria are thermotolerant and can grow



Fig. 3. Relative abundance of bacterial communities during composting (a: at phylum level; b: top 20 at genus level).

when the temperature is more than 50 °C. On day 21, Actinobacteria abundance was increased by the addition of seaweed fertilizer, especially at 10% (S2). Therefore, the increased abundance of Actinobacteria in S1 and S2 during the early stage may indicate that seaweed fertilizer led to the more rapid degradation of organic wastes compared to CK.

There were 279 bacterial genera present during maize straw composting, including 142 unclassified groups. The dominant genera were *Virgibacillus, Devosia, Phyllobacterium*, and *Agrobacterium* in all treatments on day 21 (Fig. 3b). The relative abundance of *Virgibacillus* decreased, whereas *Devosia, Phyllobacterium*, and *Agrobacterium* abundances remained relatively high during the late stage of composting (day 42). *Virgibacillus* (Firmcutes) is an important bacterial genus and can produce extracellular hydrolytic enzymes, including proteinases (Phrommao et al., 2011). *Virgibacillus* abundance was highest in S1 during the early stage of composting. *Devosia* and *Agrobacterium*, which are in the class Alphaproteobacteria, have been recognized as possible biomarkers that are symbolic of the thermophilic phase during organic waste composting (Awasthi et al., 2017; de Gannes et al., 2013). It has been suggested that *Phyllobacterium*, which also belongs to the class Alphaproteobacteria, plays an important role in nitrogen fixation and helps promote plant growth (Rasolomampianina et al., 2005). *Microbacterium* and *Paracoccus* can accelerate the degradation of organic matter during composting, and the addition of seaweed fertilizer increased the relative abundance of *Microbacterium* and *Paracoccus* during the early stage of composting. These results indicated that the addition of seaweed fertilizer may contribute to the decomposition of organic matter and shift bacterial community composition during the composting process.

The similarity between the bacterial communities during the two composting periods was assessed by NMDS analysis (Fig. 4). The NMDS plot showed that there was a significant difference in microbial community composition between the composts on day 21 and day 42. This result indicated that bacterial community succession had occurred, which was probably due to nutrient variations during the composting process. In addition, the bacterial community composition of the



Fig. 4. NMDS plot based on Bray-Curtis distance metric.

composts with seaweed fertilizer was distinctly different from the compost without seaweed fertilizer on day 21, which indicated that seaweed fertilizer amendment leads to a clear variation in bacterial community structure. This result was supported by a previous study, which showed that the application of seaweed fertilizer increased bacterial diversity and richness, and shifted the soil bacterial community structure (Wang et al., 2018b).

# 3.5. Relationships between bacterial community, environmental factors, and carbon utilization capacity

The relationship between bacterial community structure and environmental factors was determined by RDA analysis (Fig. 5a). Axis 1 and axis 2 explained 64.76% and 20.32% of the total variance, respectively. The variation in bacterial community composition was mainly explained by temperature,  $NH_4^+$ -N,  $NO_3^-$ -N, and urease activity. *Virgibacillus* was closest to the arrow for  $NH_4^+$ -N content, which suggested that it played a crucial role in N transformation during maize straw composting. *Virgibacillus* are a group of thermophilic bacteria that can secrete a variety of extracellular hydrolytic enzymes, including

proteinases (Phrommao et al., 2011). Bacillus species have a positive relationship with dehydrogenase activity (Fig. 5a) and can secrete pyruvate dehydrogenase, which enables them to utilize pyruvate (Wang et al., 2007). The levels of Glycomyces, Promicromonospora, Jiangella, Zhouia, Olivibacter, Amaricoccus, Paracoccus, Sphingobium, Sphingomonas, Halomonas, and Luteimonas were related to temperature and urease activity. Seaweed fertilizer amendment increased the activity of urease and dehydrogenase during the early stage of composting, which indicated that seaweed fertilizer may benefit the populations of enzyme-producing microorganisms that are involved in N cycling (Wang et al., 2018b). The bacteria secrete a series of carbohydrate-degrading enzymes that degrade cellulose, hemicelluloses, and proteins. Amino acids were closely correlated with the synthesis of humic substances. which can be biologically utilized during the composting process. In this study, carbohydrates and amino acids had the greatest effects (longer arrows) on the top 20 genera (Fig. 5b). Paracoccus and Halomonas were closely related to the utilization of carbohydrates, whereas Bacillus had a close relationship with the utilization of carboxylic acids (Fig. 5b). These results suggested that the above taxa were likely to contain large numbers of genes involved in the transport of various molecules and can use them as energy source (Lasek et al., 2018; Leyn et al., 2017; Wang et al., 2007).

# 3.6. Network associations among OTUs, and physicochemical characterization

The network analysis was further used to assess the relationship between physico-chemical properties and the bacterial community. The network comprised of 978 significant associations (edges), all of which were positive associations (Fig. 6a, gray lines). This result suggested that potentially mutualistic interactions occurred among the bacterial assemblages during maize straw composting. The overall network consisted of 194 nodes with a clustering coefficient of 0.51. The top three clusters in this network had MCODE scores of 13.86, 7.15, and 5.88.

The dominant OTUs in the network were identified as members of the Proteobacteria, Actinobacteria, and Firmicutes (Fig. 6a). A co-occurrence based on network analysis revealed that the  $NO_3^{-}$ -N content of the compost showed a strong appositive association with *Aeromicrobium* (belonging to *Nocardioidaceae*, OTU14418). Members of the



Fig. 5. Redundancy analysis relating physico-chemical properties (a) and carbon source utilization (b) to bacterial community composition.



Fig. 6. Network analysis revealing the associations among bacterial OTUs and compost properties.

Aeromicrobium are Gram-positive bacteria that are isolated from seaweed and seawater and can utilize glucose, cellobiose, and D-xylose as carbon sources (Lee and Lee, 2008; Lee and Kim, 2007). Aeromicrobium showed higher relative abundances in the seaweed fertilizer treatments compared to CK, especially at the 10% dose level (Fig. 6e). In addition, the NO<sub>3</sub><sup>-</sup>-N contents of the composts in the seaweed fertilizer treatments were higher than in CK (Fig. 1c). These results indicated that the relative abundance of Aeromicrobium increased with increasing nitrogen content due to the application of seaweed fertilizer. Furthermore, NH<sub>4</sub><sup>+</sup>-N content exhibited a strong positive association with Thermomicrobia (OTU60594). Members of the class Thermomicrobia are widely distributed across a large range of habitats, such as composts and soils. Some strains of Thermomicrobia are able to use nitrate and ammonia as their sole nitrogen sources (Houghton et al., 2015). The results from this study indicated that the nitrogen concentration of the compost was highly associated with specific taxa that were related to nitrogen utilization.

The betweenness centrality score can be used to identify the keystone species because it discerns the most important modules that maintain connectivity in a network (Vick-Majors et al., 2014). Keystone populations show a greater capacity for nutrient exchange and a stronger resistance to environmental limiting factors. In this study, the *Aeromicrobium* (OTU14418), an unclassified *Rhizobiales* (OTU36598), and the *Microbacteriaceae* (OTU58873) were identified as the keystone

taxa (Fig. 6b, c, d). The relative abundances of Aeromicrobium, unclassified Rhizobiales, and Microbacteriaceae were higher in S1 and S2 than in CK (Fig. 6e, f, g), which indicated that seaweed fertilizer addition benefited the growth of keystone populations during composting. The Aeromicrobium and Rhizobiales, some of which play important roles in nitrogen cycling, showed positive associations with members of the Proteobacteria and Actinobacteria. In addition, Microbacteriaceae (OTU58873), which belong to Actinobacteria, had the most complex positive interactions with other nodes. They also had close positive associations with the Bacillaceae (OTU7354), which belong to the Firmicutes. Some members of the Microbacteriaceae have been isolated from seaweed and seawater (Kim et al., 2012). They are fast growing and aerobic nitrifying bacteria, and could be defined as a highly connected node that stabilizes the network. Therefore, the loss of Microbacteriaceae may significantly alter the network structure. This suggests that the addition of seaweed fertilizer may change bacterial community succession, especially the keystone species, by influencing the N concentrations of the compost during the maize straw composting process.

#### 4. Conclusion

This study demonstrated that the addition of seaweed fertilizer increased the thermophilic temperature and enzyme activities, as well as enhanced the utilization capacity of carbon sources of the microbial community during maize straw composting. Actinobacteria, Firmicutes, and Proteobacteria were the dominant phyla in the microbial community structure. Temperature,  $NH_4^{+}$ -N, and  $NO_3^{-}$ -N concentrations were the key factors affecting microbial community succession. Seaweed fertilizer changed the abundance of keystone species by influencing the N concentrations in the compost. Further research will be conducted to investigate the effects of seaweed fertilizer on the nitrogen-fixing bacterial community in composts.

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#### References

- Alidadi, H., Hosseinzadeh, A., Najafpoor, A.A., Esmaili, H., Zanganeh, J., Takabi, M.D., Piranloo, F.G., 2016. Waste recycling by vermicomposting: maturity and quality assessment via dehydrogenase enzyme activity, lignin, water soluble carbon, nitrogen, phosphorous and other indicators. J. Environ. Manage. 182, 134–140.
- Awasthi, M.K., Zhang, Z.Q., Wang, Q., Shen, F., Li, R.H., Li, D.S., Ren, X.N., Wang, M.J., Chen, H.Y., Zhao, J.C., 2017. New insight with the effects of biochar amendment on bacterial diversity as indicators of biomarkers support the thermophilic phase during sewage sludge composting. Bioresour. Technol. 238, 589–601.
- Baker, L.R., White, P.M., Pierzynski, G.M., 2011. Changes in microbial properties after manure, lime, and bentonite application to a heavy metal-contaminated mine waste. Appl. Soil Ecol. 48 (1), 1–10.
- Banerjee, S., Baah-Acheamfour, M., Carlyle, C.N., Bissett, A., Richardson, A.E., Siddique, T., Bork, E.W., Chang, S.X., 2016. Determinants of bacterial communities in Canadian agroforestry systems. Environ. Microbiol. 18 (6), 1805–1816.
- Belyaeva, O.N., Haynes, R.J., Sturm, E.C., 2012. Chemical, physical and microbial properties and microbial diversity in manufactured soils produced from co-composting green waste and biosolids. Waste Manage. 32 (12), 2248–2257.
- de Gannes, V., Eudoxie, G., Hickey, W.J., 2013. Prokaryotic successions and diversity in composts as revealed by 454-pyrosequencing. Bioresour. Technol. 133, 573–580.
- Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S., Thomas, T., 2013. The seaweed holobiont: understanding seaweed-bacteria interactions. FEMS Microbiol. Rev. 37 (3), 462–476.
- Feng, C.L., Zeng, G.M., Huang, D.L., Hu, S., Zhao, M.H., Lai, C., Huang, C., Wei, Z., Li, N.J., 2011. Effect of ligninolytic enzymes on lignin degradation and carbon utilization during lignocellulosic waste composting. Process Biochem. 46 (7), 1515–1520.

Gomez, E., Ferreras, L., Toresani, S., 2006. Soil bacterial functional diversity as influenced by organic amendment application. Bioresour. Technol. 97 (13), 1484–1489.

- Goodfellow, M., Maldonado, L.A., Quintana, E.T., 2005. Reclassification of Nonomuraea flexuosa (Meyer 1989) Zhang et al. 1998 as Thermopolyspora flexuosa gen. nov., comb. nov., nom. rev. (vol 55, pg 1979, 2005). Int. J. Syst. Evol. Micr. 55 2640–2640.
- Guo, X.L., Gu, J., Gao, H., Qin, Q.J., Chen, Z.X., Shao, L., Chen, L., Li, H.L., Zhang, W.J., Chen, S.N., Liu, J., 2012. Effects of Cu on metabolisms and enzyme activities of microbial communities in the process of composting. Bioresour. Technol. 108, 140–148.
- Heo, S.J., Park, E.J., Lee, K.W., Jeon, Y.J., 2005. Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresour. Technol. 96 (14), 1613–1623.
- Houghton, K.M., Morgan, X.C., Lagutin, K., MacKenzie, A.D., Vyssotskii, M., Mitchell, K.A., McDonald, I.R., Morgan, H.W., Power, J.F., Moreau, J.W., Hanssen, E., Stott, M.B., 2015. *Thermorudis pharmacophila* sp nov., a novel member of the class Thermomicrobia isolated from geothermal soil, and emended descriptions of *Thermomicrobium roseum*, *Thermomicrobium carboxidum*, *Thermorudis peleae* and *Sphaerobacter thermophilus*. Int. J. Syst. Evol. Micr. 65, 4479–4487.
- Khan, W., Rayirath, U.P., Subramanian, S., Jithesh, M.N., Rayorath, P., Hodges, D.M., Critchley, A.T., Craigie, J.S., Norrie, J., Prithiviraj, B., 2009. Seaweed extracts as biostimulants of plant growth and development. J. Plant Growth Regul. 28 (4), 386–399.
- Kim, S.J., Tamura, T., Hamada, M., Ahn, J.H., Weon, H.Y., Park, I.C., Suzuki, K., Kwon, S.W., 2012. *Compositimonas suwonensis* gen. nov., sp nov., isolated from spent mushroom compost. Int. J. Syst. Evol. Micr. 62, 2410–2416.
- Kovacic, D., Kralik, D., Rupcic, S., Jovicic, D., Spajic, R., Tisma, M., 2017. Soybean straw, corn stover and sunflower stalk as possible substrates for biogas production in Croatia: a review. Chem. Biochem. Eng. Q. 31 (3), 187–198.
- Kulcu, R., Yaldiz, O., 2014. The composing of agricultural wastes and the new parameter for the assessment of the process. Ecol. Eng. 69, 220–225.
- Lasek, R., Szuplewska, M., Mitura, M., Decewicz, P., Chmielowska, C., Pawlot, A., Sentkowska, D., Czarnecki, J., Bartosik, D., 2018. Genome structure of the opportunistic pathogen *Paracoccus yeei* (Alphaproteobacteria) and identification of putative virulence factors. Front. Microbiol. 9, 2553.
- Leceta, I., Etxabide, A., Cabezudo, S., de la Caba, K., Guerrero, P., 2014. Bio-based films prepared with by-products and wastes: environmental assessment. J. Clean. Prod. 64, 218–227.
- Lee, D.W., Lee, S.D., 2008. Aeromicrobium ponti sp nov., isolated from seawater. Int. J. Syst. Evol. Micr. 58, 987–991.
- Lee, S.D., Kim, S.J., 2007. Aeromicrobium tamlense sp nov., isolated from dried seaweed. Int. J. Syst. Evol. Micr. 57, 337–341.
- Leyn, S.A., Maezato, Y., Romine, M.F., Rodionov, D.A., 2017. Genomic reconstruction of carbohydrate utilization capacities in microbial-mat derived consortia. Front. Microbiol. 8, 1304
- Liu, B., Li, Y.X., Zhang, X.L., Wang, J., Gao, M., 2014. Combined effects of chlortetracycline and dissolved organic matter extracted from pig manure on the functional diversity of soil microbial community. Soil Biol. Biochem. 74, 148–155.
- Lopez-Gonzalez, J.A., Suarez-Estrella, F., Vargas-Garcia, M.C., Lopez, M.J., Jurado, M.M., Moreno, J., 2015. Dynamics of bacterial microbiota during lignocellulosic waste composting: Studies upon its structure, functionality and biodiversity. Bioresour. Technol. 175, 406–416.
- Phrommao, E., Rodtong, S., Yongsawatdigul, J., 2011. Identification of novel halotolerant bacillopeptidase F-like proteinases from a moderately halophilic bacterium, *Virgibacillus* sp. SK37. J. Appl. Microbiol. 110 (1), 191–201.
- Qiu, X.W., Zhou, G.X., Zhang, J.B., Wang, W., 2019. Microbial community responses to biochar addition when a green waste and manure mix are composted: A molecular ecological network analysis. Bioresour. Technol. 273, 666–671.
- Rashad, F.M., Saleh, W.D., Moselhy, M.A., 2010. Bioconversion of rice straw and certain agro-industrial wastes to amendments for organic farming systems: 1. Composting, quality, stability and maturity indices. Bioresour. Technol. 101 (15), 5952–5960.
- Rasolomampianina, R., Bailly, X., Fetiarison, R., Rabevohitra, R., Bena, G., Ramaroson, L., Raherimandimby, M., Moulin, L., De Lajudie, P., Dreyfus, B., Avarre, J.C., 2005. Nitrogen-fixing nodules from rose wood legume trees (*Dalbergia* spp.) endemic to Madagascar host seven different genera belonging to alpha- and beta-Proteobacteria. Mol. Ecol. 14 (13), 4135–4146.
- Sardans, J., Penuelas, J., Estiarte, M., 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. Appl. Soil Ecol. 39 (2), 223–235.
- Schimel, J.P., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Front. Microbiol. 3, 348.
- Tujula, N.A., Crocetti, G.R., Burke, C., Thomas, T., Holmstrom, C., Kjelleberg, S., 2010. Variability and abundance of the epiphytic bacterial community associated with a green marine Ulvacean alga. ISME J. 4 (2), 301–311.
- Vick-Majors, T.J., Priscu, J.C., Amaral-Zettler, L.A., 2014. Modular community structure suggests metabolic plasticity during the transition to polar night in ice-covered Antarctic lakes. ISME J. 8 (4), 778–789.
- Wang, C.M., Shyu, C.L., Ho, S.P., Chiou, S.H., 2007. Species diversity and substrate utilization patterns of thermophilic bacterial communities in hot aerobic poultry and cattle manure composts. Microb. Ecol. 54 (1), 1–9.
- Wang, K., Mao, H.L., Wang, Z., Tian, Y., 2018a. Succession of organics metabolic function of bacterial community in swine manure composting. J. Hazard. Mater. 360, 471–480.
- Wang, M.P., Chen, L., Li, Y.T., Chen, L., Liu, Z.Y., Wang, X.J., Yan, P.S., Qin, S., 2018b. Responses of soil microbial communities to a short-term application of seaweed fertilizer revealed by deep amplicon sequencing. Appl. Soil Ecol. 125, 288–296.
- Wang, Y.F., Fu, F.Y., Li, J.J., Wang, G.S., Wu, M.M., Zhan, J., Chen, X.S., Mao, Z.Q., 2016. Effects of seaweed fertilizer on the growth of *Malus hupehensis* Rehd. seedlings, soil

enzyme activities and fungal communities under replant condition. Eur. J. Soil Biol. 75, 1–7.

- Wei, H.W., Wang, L.H., Hassan, M., Xie, B., 2018. Succession of the functional microbial communities and the metabolic functions in maize straw composting process. Bioresour. Technol. 256, 333–341.
- Wu, J.Q., Zhao, Y., Qi, H.S., Zhao, X.Y., Yang, T.X., Du, Y.Q., Zhang, H., Wei, Z.M., 2017. Identifying the key factors that affect the formation of humic substance during different materials composting. Bioresour. Technol. 244, 1193–1196.
- Zhang, L., Sun, X.Y., 2017. Addition of seaweed and bentonite accelerates the two-stage composting of green waste. Bioresour. Technol. 243, 154–162.
- Zhang, L., Sun, X.Y., 2014. Changes in physical, chemical, and microbiological properties during the two-stage co-composting of green waste with spent mushroom compost and biochar. Bioresour. Technol. 171, 274–284.
- Zhang, L., Sun, X.Y., 2015. Effects of earthworm casts and zeolite on the two-stage

composting of green waste. Waste Manage. 39, 119-129.

- Zhang, L.L., Jia, Y.Y., Zhang, X.M., Feng, X.H., Wu, J.J., Wang, L.S., Chen, G.J., 2016. Wheat straw: an inefficient substrate for rapid natural lignocellulosic composting. Bioresour. Technol. 209, 402–406.
- Zhong, X.Z., Ma, S.C., Wang, S.P., Wang, T.T., Sun, Z.Y., Tang, Y.Q., Deng, Y., Kida, K.J., 2018. A comparative study of composting the solid fraction of dairy manure with or without bulking material: performance and microbial community dynamics. Bioresour. Technol. 247, 443–452.
- Zhou, G.X., Xu, X.F., Qiu, X.W., Zhang, J.B., 2019. Biochar influences the succession of microbial communities and the metabolic functions during rice straw composting with pig manure. Bioresour. Technol. 272, 10–18.
- Zorpas, A.A., 2014. Recycle and reuse of natural zeolites from composting process: a 7year project. Desalin. Water Treat. 52 (37–39), 6847–6857.