



# Succession of organics metabolic function of bacterial community in response to addition of earthworm casts and zeolite in maize straw composting



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## ABSTRACT

Succession and metabolism functions of bacterial communities were determined in maize straw composting with earthworm casts and zeolite addition by using high-throughput sequencing, Biolog and PICRUSt. Results showed that earthworm casts and zeolite addition increased the temperature, decreased  $\text{NH}_4^+$  contents and affected bacterial community structure. The relative abundances of *Firmicutes* and *Betaproteobacteria* increased with earthworm casts and zeolite addition in the late stage. The abundances of genes related to carbohydrate metabolism, amino acid metabolism, and energy metabolism were increased by these two additives in the early stage, but decreased in the late stage. Network analysis demonstrated that members of *Bacillaceae* were identified as the keystone taxa. Temperature showed negative relationship with *Georgenia*, while  $\text{NH}_4^+$  exhibited positive associations with *Georgenia*, *Devosia*, *Ruania* and *Mycobacterium*. These results indicated that earthworm casts and zeolite addition benefitted the keystone species and enhanced the metabolism capacity of bacterial community, thereby improved the quality of compost.

## 1. Introduction

The crop residues have dramatically increased because of significant population growth, and the annual production of maize straw was about 1 billion tons worldwide for the period 2014–2015 (Kovacic et al., 2017). The decomposition of maize straw exerts a vital effect on global carbon cycling. As important biomass resources, a large amount of maize straw is traditionally burned by farmers after harvest. Straw burning results in nutrient loss and a series of environmental pollution, including serious atmospheric pollution. It is therefore crucial to develop an effective and environment friendly management to dispose maize straw. Composting is one of the sustainable technologies for disposal of organic wastes. As a natural aerobic biological process, composting reduces environmental pollution and the final product is often used as a fertilizer and soil amendment (Zorpas, 2014).

In some cases, however, the properties of crop residues including high C/N ratio and lignocellulose contents may lead to insufficient decomposition and immaturity compost product. Organic or inorganic additives, such as earthworm casts and zeolite, can optimize composting conditions which benefit the microbial growth. Earthworm

casts are peat-like substances with high porosity and large surface area and can provide rich nutrient for microorganisms (Lalander et al., 2013). Previous studies have indicated that earthworm casts largely increased the decomposition of organic waste and improved the quality of the end compost product, the effects were likely due to the diverse microbial communities and enzymes in the earthworm casts (Sen and Chandra, 2009). Natural zeolite is a crystalline material with a special structure which adsorbs water, gases, heavy metals and nutrient (Wang et al., 2014a). Zeolite is considered as an effective additive used in composting as a result of its capacity in water and nutrient retention, as well as improving aeration and microbial activities (Zorpas and Loizidou, 2008).

Bacteria play an important role in aerobic composting system. The bacterial community composition differs owing to the various feedstock materials and bulking agents. Moreover, the function of microbial community dominates waste degradation, humic-like substances, nutrients transformation in composting process (Wang et al., 2014b). The dynamic of dominant functional microbial communities in composts need to be investigated in order to better understand the composting process. However, to date, information on the effects of earthworm

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casts and zeolite on functional characteristics of bacterial community composition during maize straw composting is very limited.

Fortunately, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) provides a way to predict the function of microbial communities based on the sequence provided by high-throughput sequencing. PICRUSt has already been used to evaluate the metabolic function of microbial community in the composting process and wastewater treatment (Mu et al., 2017; Wei et al., 2018). The metabolic activities of microbial community can also affect the microbial behavior. However, PICRUSt could not provide comprehensive information on metabolic activities in composting system. Biolog can be a supplementary method which focuses on the substrates utilization capacity of microbial community. Biolog Ecoplate contains six types of environmental applicable substrates, including carbohydrates, carboxylic acids, amino acids, esters, alcohols and amines. Therefore, the PICRUSt and Biolog could be considered as the complementary methods to comprehensively assess the function of microbial community in composting process.

The main aim of the present study was to reveal the influence of earthworm casts and zeolite on the dynamic of metabolic function of microbial community in maize straw composting with spent mushroom substrate. The predicted metabolic function and carbon sources utilization capacity of microbial community were assessed by PICRUSt and Biolog. Additionally, the relationships among microbial communities, metabolic function and physico-chemical characteristics were evaluated in composting system using redundancy analysis (RDA) and network analysis.

## 2. Materials and methods

### 2.1. Composting process and sample collection

The composting materials comprised a mixture of maize straw (2 mm mesh size) and spent mushroom substrate, with or without earthworm casts and zeolite. Maize straw and spent mushroom substrate were collected from a farmland in Jiujiang, China. The physico-chemical properties of these materials were shown in Table 1. Earthworm casts and zeolite (2 mm mesh size) were purchased from a factory from Hubei, China.

Three composting experiments with mixtures of maize straw and spent mushroom substrate at 2:1 (w/w) were carried out. A control composting experiment was prepared without addition of earthworm casts and zeolite (CK). Treatment contained 10% earthworm casts was labeled as EW, while that contained both 10% earthworm casts and 10% zeolite was labeled as EWZ, respectively. Each treatment was carried out in triplicates and the materials were mixed by manual operation. The composting mixtures were aerated by using natural ventilation in 150 × 100 × 60 cm cuboid-shaped plastic reactors and turned every three days by manual operation for ensuring oxygen supply. The moisture content of the composting mixture was detected daily with a moisture meter (SK-100, Tokyo, Japan) and maintained at 60% by adding ddH<sub>2</sub>O.

Samples were collected from five different portions of the compost (30 cm depth) and homogenized. Each replicate was sampled after 1, 3, 7, 14, 21, 28, 35, 42 days of composting. One portion of the collected

**Table 1**  
Physico-chemical properties of the raw materials.

Material	Moisture (%)	pH	Organic matter (g kg <sup>-1</sup> )	C/N
Earthworm casts	1.93 ± 0.02	6.25 ± 0.32	208.63 ± 4.01	15.12
Zeolite	3.75 ± 0.07	6.12 ± 0.12	1.03 ± 0.08	–
Maize straw	9.80 ± 0.22	7.16 ± 0.16	587.28 ± 6.38	64.11
Spent mushroom substrate	31.25 ± 0.42	6.32 ± 0.48	603.24 ± 10.35	35.31

samples was air-dried for analyzing the physico-chemical properties, and another portion was stored at –20 °C for DNA extraction.

### 2.2. Physical and chemical analyses

The temperature at the center of each pile was detected daily (9:00 am, 16:00 pm) using thermometer. The environment temperature was also measured daily at the same time. The organic matter contents of raw materials were measured with a muffle furnace at 550 °C (6 h). The pH value of a suspension of compost in water (1:10, w/v) was measured using a pH meter (Sartorius, Gottingen, Germany). TOC content was determined with a total organic carbon analyzer (TOC-VCPH, Shimadzu, Japan). Ammonia nitrogen (NH<sub>4</sub><sup>+</sup>) and nitrate nitrogen (NO<sub>3</sub><sup>-</sup>), TN and C/N ratio were determined according to the methods described by Zhang and Sun (2014).

### 2.3. Biolog Ecoplate analyses

The composting samples (1 g) were suspended into 100 mL sterilized saline solution, and shaken for 20 min at room temperature. The suspension was diluted and further inoculated to the Ecoplate which contained 96 plate wells. The plates were then cultivated at 25 °C in darkness for 6 days. The absorbance (at 590 nm and 750 nm) of plates was measured every day using automatic microorganism identification instrument (Biolog, USA). Average well color developments (AWCD) was calculated and used to assess the metabolic capacity of microbial community in the compost.

### 2.4. High-throughput sequencing

The genomic DNA was extracted from 0.5 g fresh samples by using a Fast DNA SPIN Kit for soil (MP Biomedicals, OH, USA). The DNA concentration was detected using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The extracted DNA was purified and stored at –80 °C until further used. The primers used for sequencing were 338F (5'-ACTCCTACGGGAGGCA GCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') primers. The library was sequenced on a Miseq platform.

### 2.5. Data analysis

The high-throughput sequencing data were analyzed using Quantitative Insights Into Microbial Ecology (QIIME) package (<http://qiime.org/>). After removing low-quality reads, the sequences were assigned according to the barcodes and clustered into operation taxonomy units (OTUs) at the 97% similarity level. Ribosomal Database Project (RDP) was used to assign each OTU to a taxonomic identity. PICRUSt was used to predict the microbial metabolic function based on the normalizing bacterial OTUs table according to Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Langille et al., 2013). Redundancy analysis (RDA) was conducted using R Cluster package (version 3.5.0 for windows). Network analysis was performed using MINE software. The OTUs with strong positive ( $r > 0.8$ ), negative ( $r < -0.8$ ) and strong non-linear ( $MIC-\rho^2 > 0.8$ ) associations were further used to construct network. The keystone species were identified according to the maximum betweenness centrality scores. The visualization of network was performed using Cytoscape (v.3.2.1).

## 3. Results and discussion

### 3.1. Physicochemical changes during composting

Temperature is a key indicator of microbiological activity during the composting process (Kulcu and Yaldiz, 2014). In this study, temperatures in all treatments sharply increased and reached the thermophilic phase (> 50 °C) in the first 3–4 days (Fig. 1a). The highest

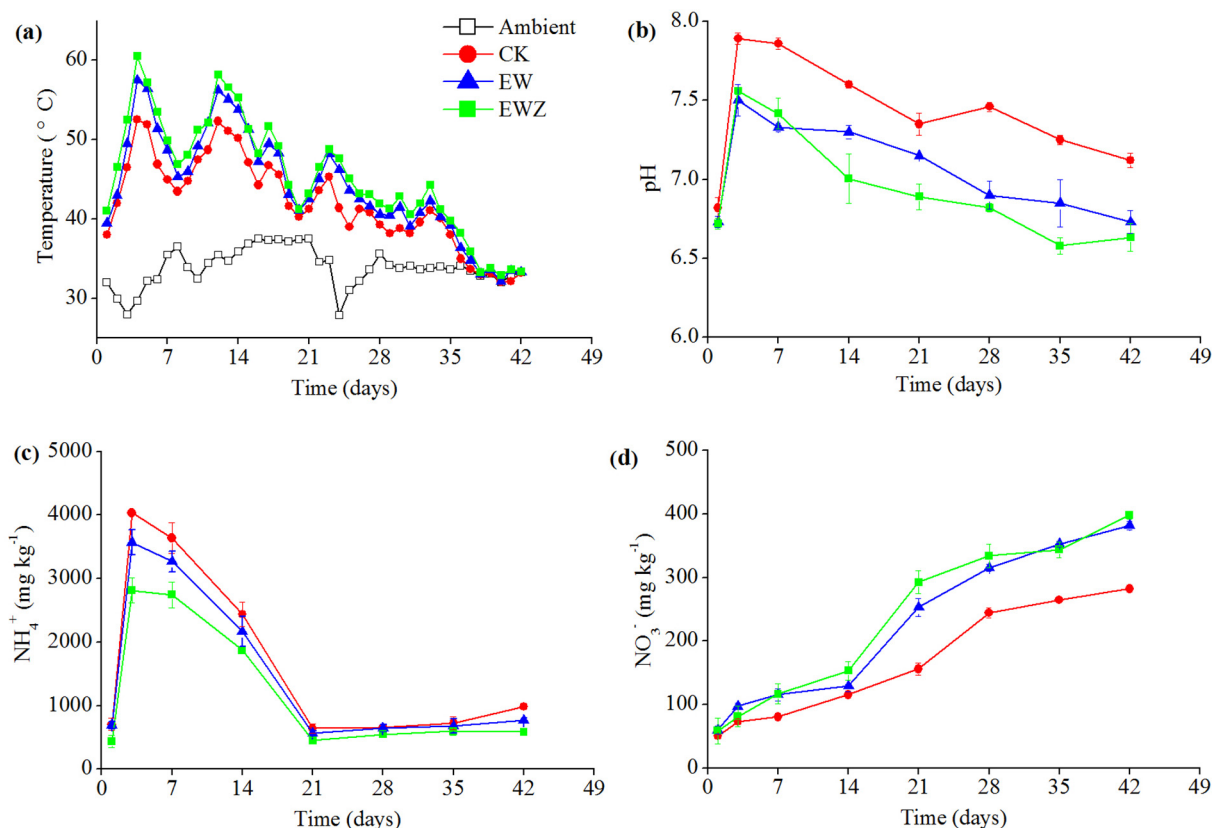


Fig. 1. Changes of temperature, pH,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  during the composting.

temperatures for CK, EW and EWZ during the thermophilic phase were 52.5, 57.5 and 60.5 °C, respectively. The rapid increase in temperature was resulted in the release of heat from the respiration of microorganisms and the rapid breakdown of degradable organic matter (Bernal et al., 2009; Jolanun and Towprayoon, 2010). Subsequently, all the temperatures declined until day 8 and began to increase thereafter to a second thermophilic phase. The temperatures of all the piles decreased and stabilized at 35 days during the maturation phase. For all the treatments, the duration of the thermophilic phase lasted 5, 8 and 11 days for CK, EW and EWZ, respectively. The higher temperature and longer duration of thermophilic phase in EW and EWZ indicated that the combined addition of earthworm casts and zeolite helped sustain the thermophilic temperature because these materials could improve the microbial metabolism activity and enhance the generation of heat (Gabhane et al., 2012). Zhang et al. (2015) also verified that cow dung amendment increased the temperature of maize straw compost piles, which was in line with our result. In addition, the temperature of EWZ was slightly higher than that of EW during the composting process, although not significant. Zeolite could enhance the amount of oxygen available and therefore result in the increase of compost temperature.

During the composting process, pH for all treatments increased to a maximum on day 3, with the maximum pH of 7.9, 7.5 and 7.6 for CK, EW and EWZ, respectively (Fig. 1b). Subsequently, pH values of all piles declined and the final pH were 7.1, 6.7 and 6.6 for CK, EW and EWZ, respectively. The increase of pH could be a result of ammonification in the early stage, while the decrease of pH was likely due to the nitrification in the later stage. Earthworm casts contained rich organic acids and affected the ammonia volatilization and therefore regulated the pH of compost piles (Raphael and Velmourougane, 2011). The lower pH values of zeolite amended treatment were due to the adsorption of ammonium onto zeolite (Venglovsky et al., 2005).

High  $\text{NH}_4^+$  concentrations were observed for all treatments in the first 7 days and sharply declined thereafter (Fig. 1c). The  $\text{NO}_3^-$

concentrations slightly changed in the first 14 days (Fig. 1d). In the thermophilic phase, the high temperatures and large amounts of ammonia could suppress the activity of nitrifying bacteria and inhibit nitrification (Rashad et al., 2010). The  $\text{NO}_3^-$  concentrations then rapidly increased when the temperatures declined to mesophilic values. The  $\text{NO}_3^-$  concentrations were higher in EW and EWZ compared to CK during the composting period. This indicated that earthworm casts and zeolite created a beneficial environment for the nitrifying bacteria, which can convert ammonia to nitrate. Additionally, the rich nutrient that earthworm casts contained can also promote the activity of N-cycle microorganisms (Curtin and Mullen, 2007). Zeolite with high sorption and ion exchange capacity would absorb  $\text{NH}_4^+$  ions. Therefore, EW and EWZ exhibited stronger nitrification and greater nutrient retention than CK, indicating that earthworm casts and zeolite played a role in N transformations during composting (Sen and Chandra, 2009).

The decline of TOC content was observed from day 21 to day 42, and TOC contents decreased 12%, 19% and 25% for CK, EW, EWZ, respectively (Fig. 2a). As shown in Fig. 2b, the TN content of all treatments increased with increasing composting time. The highest TN content in EWZ (Fig. 2b) further suggested that the combined addition of earthworm casts and zeolite effectively reduced N loss during composting.

### 3.2. Dynamic changes in the bacterial community during composting

The composition of bacterial community during composting was determined by high throughput sequencing. As shown in Fig. 3a, *Firmicutes*, *Proteobacteria*, *Actinobacteria* were the dominant phyla in all composting samples. The relative abundances of these bacteria varied in different composting stages. In the early stage (day 21), *Firmicutes* were the most abundant bacteria, the relative abundances of *Firmicutes* were 98%, 95% and 92% for CK, EW and EWZ, respectively. The dominance of *Firmicutes* at the thermophilic phase can probably be

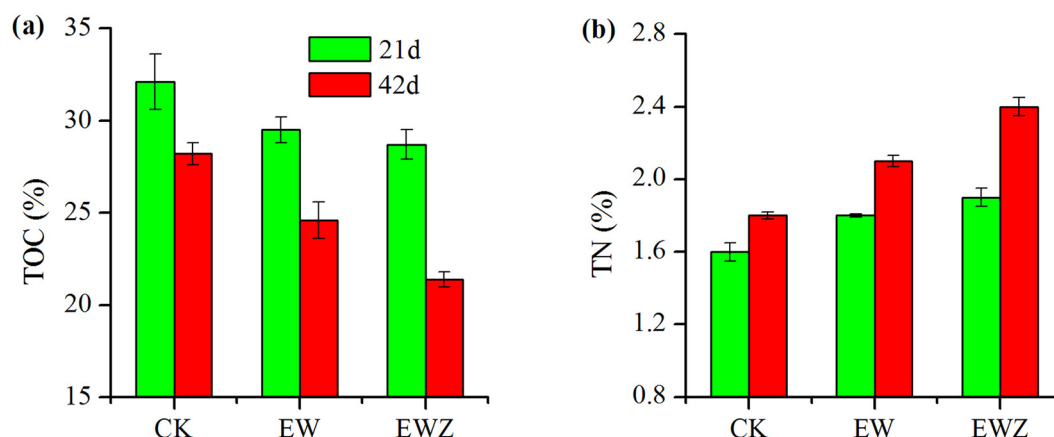


Fig. 2. Changes of total organic carbon and total nitrogen during composting in different treatments.

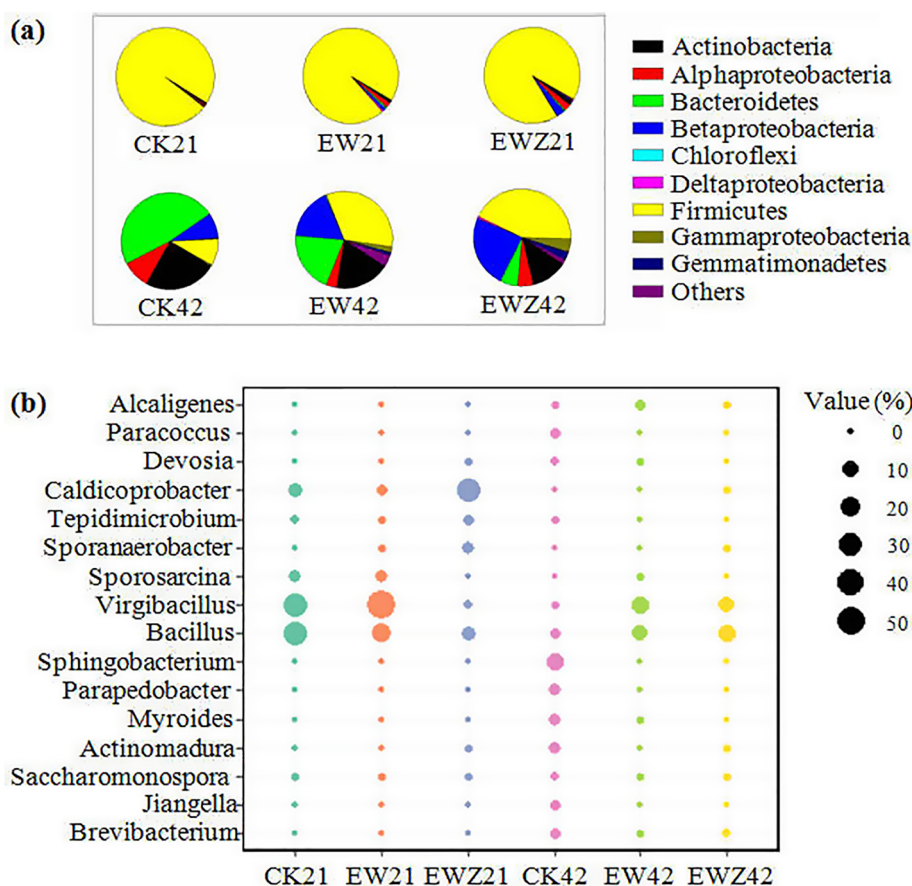


Fig. 3. The bacterial community composition at phylum-level (a) and top 16 on genus level (b) in the compost at different composting stages.

ascribed to its thermotolerance (Zhang et al., 2016a). As composting proceeded, the relative abundance of *Firmicutes* decreased, and accompanied the increased abundances of *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* for three treatments (day 42). Zhang et al. (2016a) reported that the succession of microbial community composition was possibly related to the temperature changes in different stages. In addition, *Actinobacteria* and *Proteobacteria* were considered as the key players in decomposition of organic materials, especially at curing stages (Jurado et al., 2014).

At the genus level, the dominant genera were mainly composed of *Bacillus*, *Virgibacillus* and *Caldicoprobacter* on day 21 (Fig. 3b). These genera accounted for 76%, 77% and 40% of the total bacteria for CK, EW, EWZ, respectively. As an ubiquity genus of *Firmicutes*, *Bacillus* is

wide-spread in lignocellulosic composting systems due to its thermotolerance and it is considered to have a great effect on waste degradation during composting (de Gannes et al., 2013). *Virgibacillus* is able to produce a variety of extracellular hydrolytic enzymes, such as proteinases (Phrommao et al., 2011). *Caldicoprobacter* shows the ability to produce thermophilic xylanase (Bouacem et al., 2014) and makes a contribution to hemicelluloses degradation. At the later stage (day 42), the dominant genera in EW and EWZ were *Bacillus*, *Virgibacillus*. These genera accounted for 21% and 28% of total bacteria in EW and EWZ, respectively (Fig. 3b). However, the dominant genera in CK included *Sphingobacterium*, *Actinomadura* and *Myroides*. *Sphingobacterium* belonging to the phylum *Bacteroidetes* became the most abundant genus (16%) in CK on day 42. Previous studies showed that *Sphingobacterium*

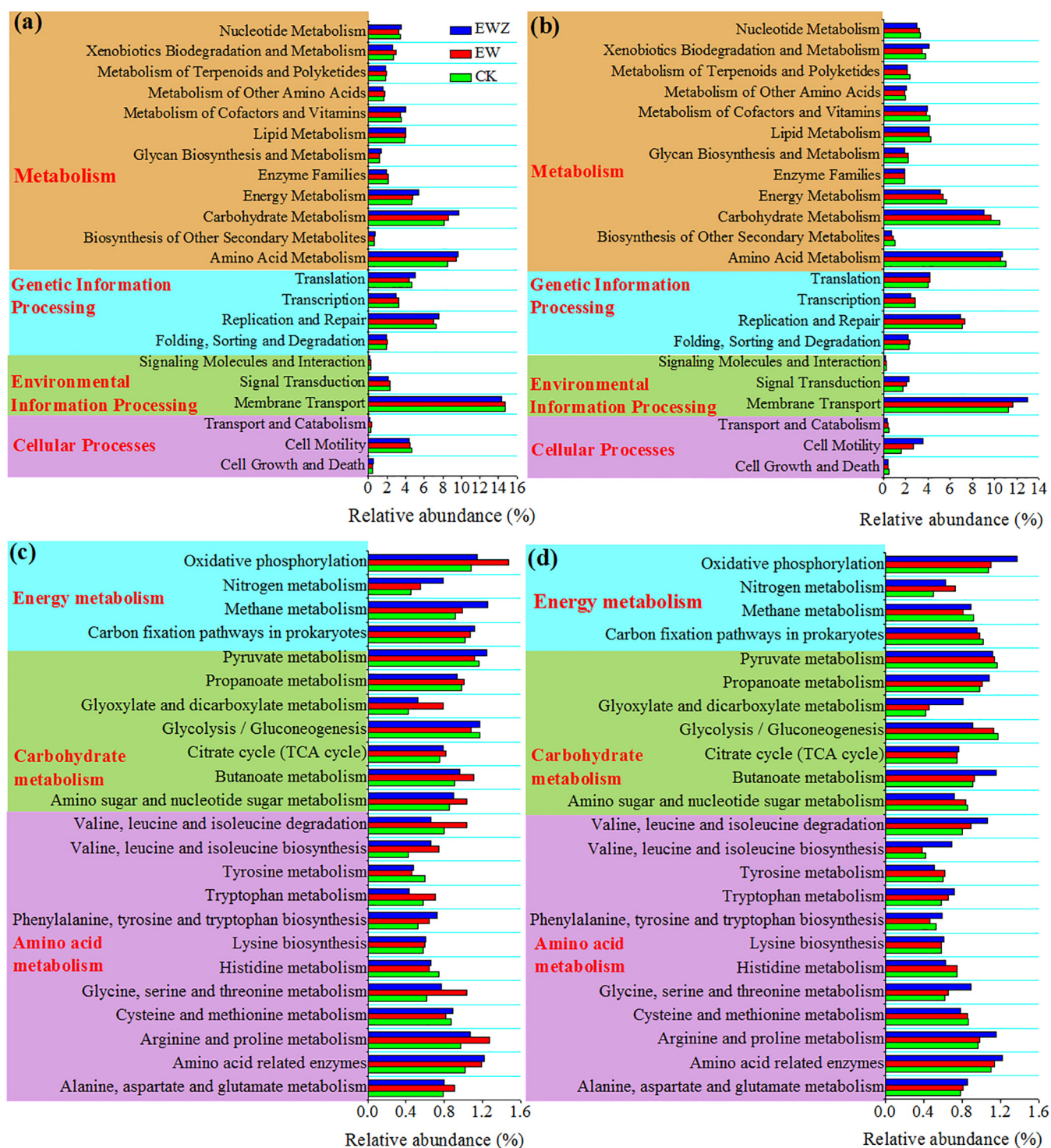


Fig. 4. Variation of bacterial function profiles during the composting process. (a) and (b) represent the level 2 KEGG ortholog function predictions analyzed by PICRUSt on day 21 and day 42, respectively. (c) and (d) represent the level 3 KEGG ortholog function predictions on day 21 and day 42, respectively.

could degrade herbicide quinclorac, alpha-ethynylestradiol and herbicide mefenacet to enhance the quality of composts (Ren et al., 2007; Wang et al., 2018a). *Actinomadura* can produce a variety of antibiotics (Euanorasetr et al., 2015), and a recent report also showed relative high abundance of *Actinomadura* in manure compost (Wang et al., 2018b). *Myroides* belonging to the phylum *Bacteroidetes* was involved in degrading macromolecules during composting (de Gannes et al., 2013). Other important sequenced genus included *Alcaligenes*, which belongs to *Betaproteobacteria*, has been reported to have heterotrophic

nitrification and aerobic denitrification abilities, and was used to remove ammonium (Shoda and Ishikawa, 2014). The abundance of *Alcaligenes* was higher in EW than CK on day 42, which may partly led to the lower  $\text{NH}_4^+$  in EW treatment compared to CK. These results showed that the composition of bacterial community varied significantly in different treatments of compost and a succession occurred in the bacterial community structure as time went on.

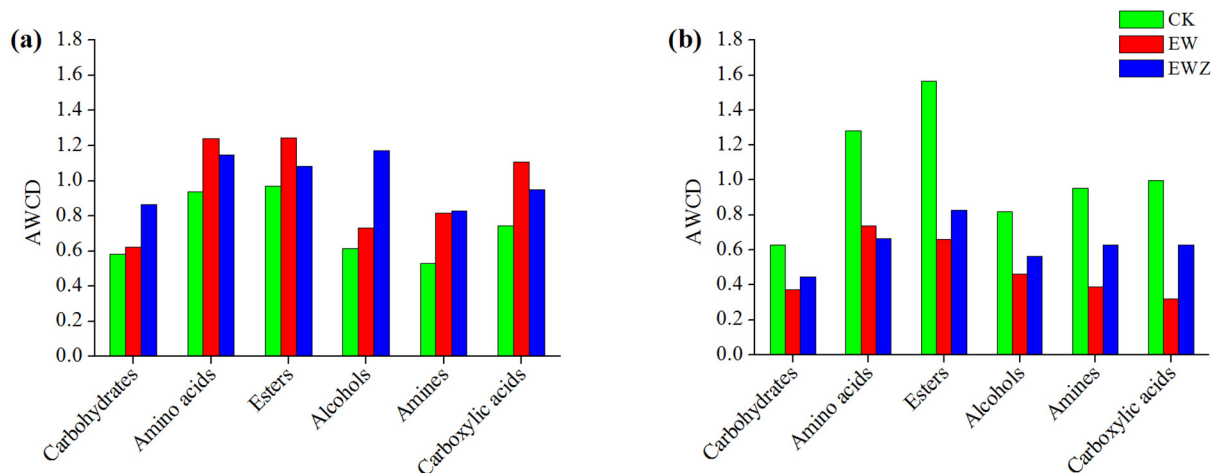


Fig. 5. The average well-color developments (AWCD) of carbon sources in Biolog Ecoplate on day 21 (a) and day 42 (b), respectively.

### 3.3. Microbial metabolism function analyzed by PICRUSt

The potential functional profiles of bacterial community in maize straw composting were predicted based on KEGG pathway. In this study, the majority of predicted protein sequences in compost samples were clustered into metabolism (42.08–52.30%), genetic information processing (15.82–17.59%), environmental information processing (13.29–17.28%) and cellular processes (2.52–5.35%). There were 12 pathways for metabolism, 4 pathways for genetic information processing and 3 pathways for environmental information processing and cellular processes (Fig. 4a, b). Carbohydrate metabolism, amino acid metabolism, energy metabolism and lipid metabolism were the main pathways in the cluster of metabolism during the composting process. Compared to CK, EW and EWZ increased the abundances of sequences related to carbohydrate metabolism, amino acid metabolism and energy metabolism on day 21, indicating that earthworm casts and zeolite increased the degradation of these complex compounds. On the contrary, the abundances of these sequences were lower in EW and EWZ than CK on day 42. Previous studies showed that bacteria secreted carbohydrate degrading enzymes to degrade cellulose, hemicelluloses and proteins in the thermophilic phase (Goh et al., 2013). Therefore, the biodegradable carbohydrate and amino acids rapidly declined in the late stage.

The addition of earthworm casts and zeolite improved the enzyme activity, as the abundances of amino acid related enzymes were 1.02%, 1.19% and 1.22% for CK, EW and EWZ on day 21, respectively (Fig. 4c). Similar pattern of amino acid related enzymes was also observed on day 42 (Fig. 4d). The sequences related to arginine and proline metabolism showed higher abundances in EW and EWZ than CK during the composting process. In addition, the sequences involved in amino sugar and nucleotide sugar metabolism also exhibited higher abundances in EW and EWZ compared to CK on day 21. Carbon fixation pathways in prokaryotes, methane metabolism, nitrogen metabolism and oxidative phosphorylation, which assigned with energy metabolism, were enhanced by addition of earthworm casts and zeolite on day 21. Hartman et al. (2017) found that more abundant carbohydrate degradation genes in mineral soils were related to higher rates of soil C turnover. The TOC contents in EW and EWZ were lower than that in CK (Fig. 2a), and the sequence abundances of carbohydrate were higher in EW and EWZ, indicating that addition of earthworm casts and zeolite increased the rates of C turnover in compost. However, the abundances of these four sequences exhibited a different pattern on day 42. Nutritional benefits may influence nitrogen fixation and the products of nitrogen metabolism during the transformation process (Morrow et al., 2015). Earthworm casts provided rich nutrient to the growth of microorganisms (Wang et al., 2011), and therefore, strongly influenced the abundance

of sequence related to nutrient metabolism, especially nitrogen metabolism. Higher TN contents were observed in EW and EWZ (Fig. 2b), which supported the predicted results from PICRUSt that the sequence abundance of nitrogen metabolism were higher in EW and EWZ. Furthermore, zeolite was demonstrated to have a positive effect on conservation of nitrogen in compost (Chan et al., 2016). Therefore, it is not surprise that addition of zeolite increased TN contents and the abundances of sequences related to nitrogen metabolism during composting.

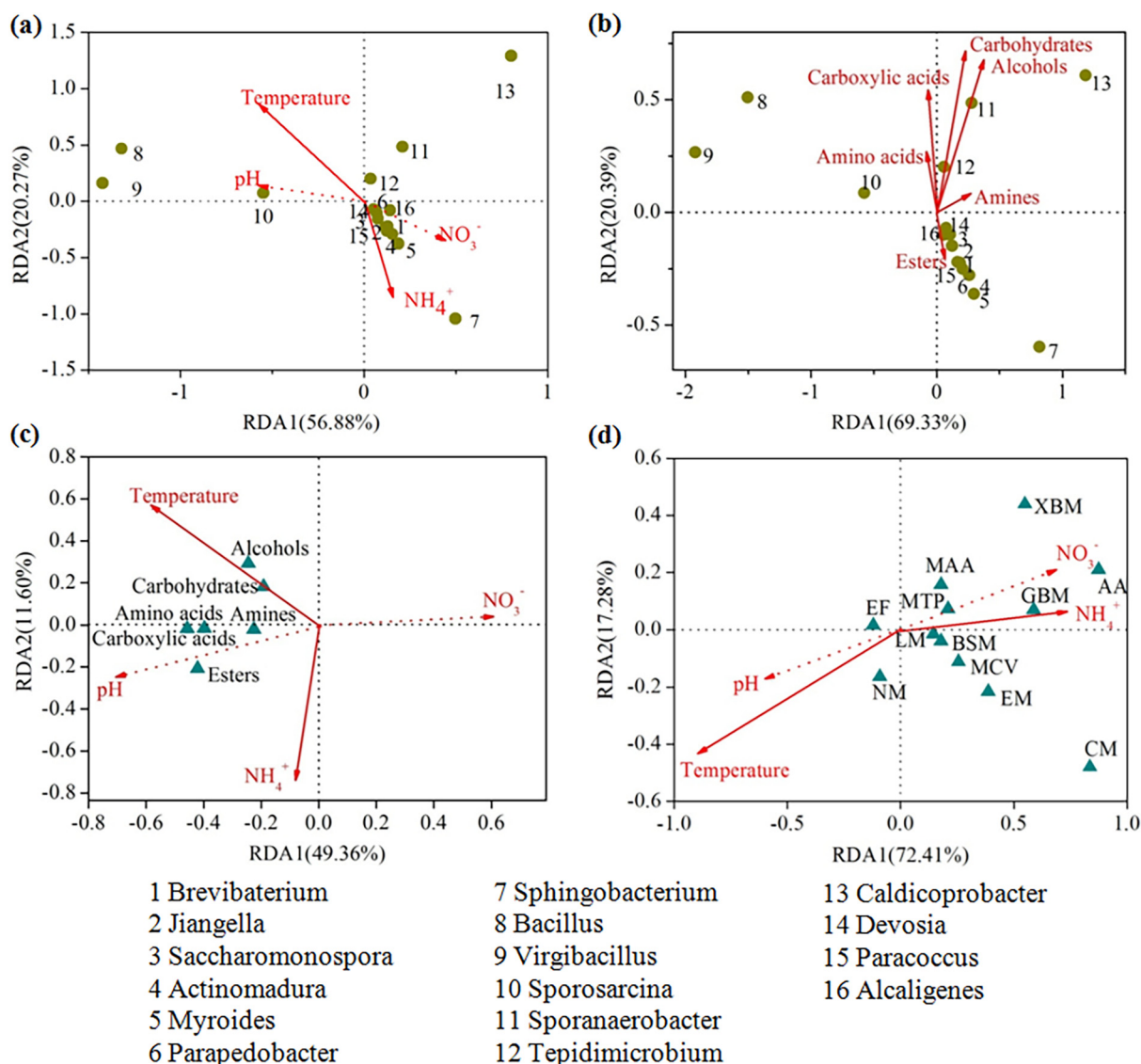
### 3.4. Carbon sources utilization capacity of bacterial community determined by Biolog

Biolog Ecoplate was used to verify the carbon sources utilization capacity of microbial community on day 21 and 42 during maize straw composting. The 31 carbon sources in the Biolog Ecoplate were classified into six biochemical categories. The average well-color developments (AWCD) of carbohydrates, amino acid and carboxylic acids were higher in EW and EWZ compared to CK on day 21 (Fig. 5a), suggesting that the carbon sources utilization capacity of bacterial community was improved by addition of earthworm casts and zeolite. An opposite pattern of carbon sources utilization capacity was observed on day 42 (Fig. 5b). Carbohydrates and carboxylic acids could be utilized rapidly at the early stage of composting (Albrecht et al., 2010). Amino acids are the energy and carbon source of bacterial metabolism and can be produced during the whole process of composting (Lopez-Gonzalez et al., 2015). Amino acids were closely associated with the synthesis of humic substance (Wu et al., 2017). The greater AWCD of amino acid in EW and EWZ on day 21 indicated the more amino acids production and humic substance synthesis. These results were consistent with the PICRUSt prediction that EW and EWZ strongly enhanced the abundances of sequences related to metabolism on day 21, especially the metabolism involved in carbohydrate, amino acids and energy during the composting.

### 3.5. Relationship among environmental factors, metabolism function and bacterial community

The effects of environmental factors on the main bacteria genera in the maize straw composting were assessed by RDA analysis (Fig. 6a). Axis 1 and axis 2 accounted for 56.88% and 20.27% of the total variance, respectively. Temperature and  $\text{NH}_4^+$  exhibited significant effects on the top 16 genera. Temperature was positively associated with *Bacillus*, *Virgibacillus* and *Sporosarcina*.  $\text{NH}_4^+$  showed positive relationships with *Brevibacterium*, *Jiangella*, *Saccharomonospora*, *Actinomadura*, *Myroides*, *Sphingobacterium*, *Devosia*, *Paracoccus* and *Alcaligenes*.

The impacts of carbon source utilization capacity on bacterial

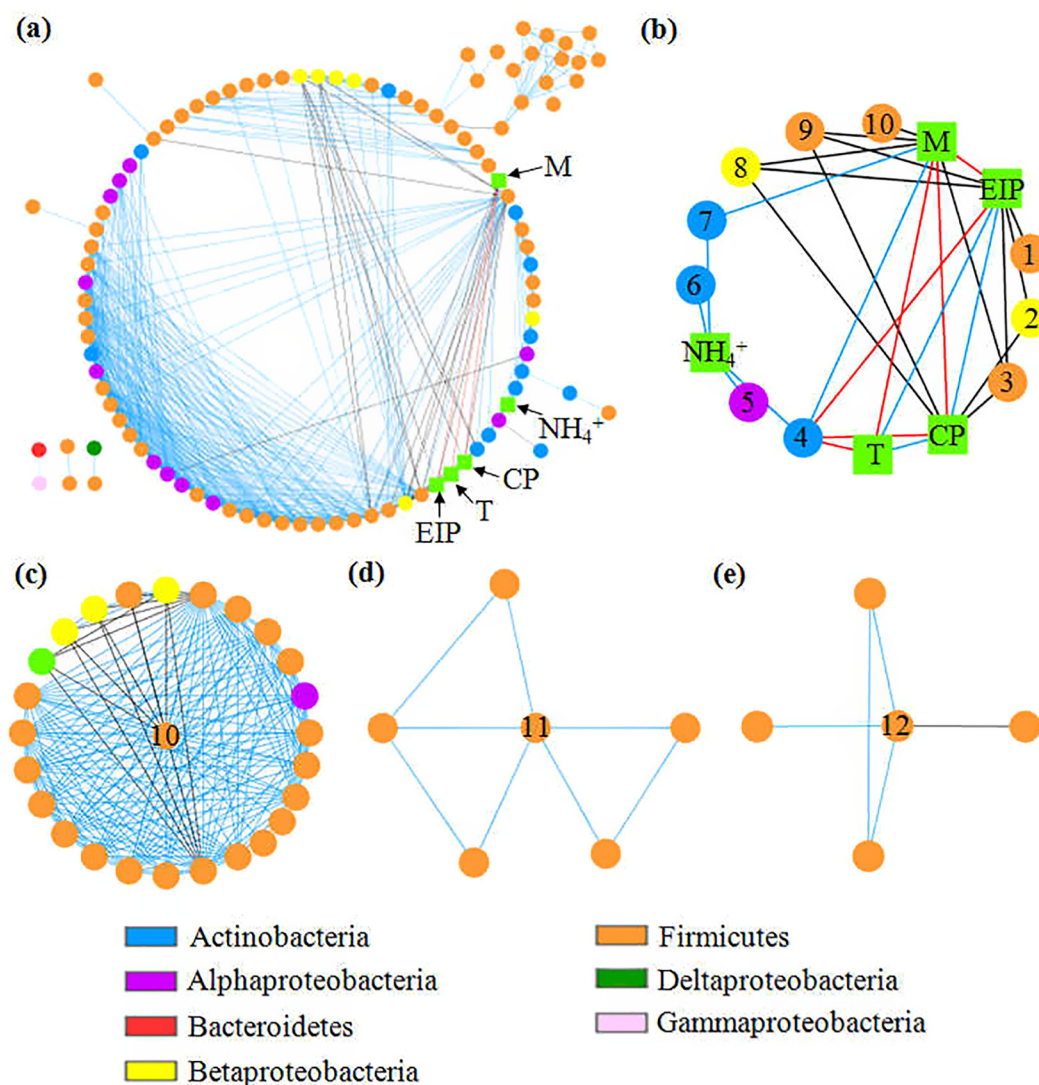


**Fig. 6.** Redundancy analysis on the influence of environmental properties on bacterial community composition (a); the relationship between carbon source utilization and bacterial community (b); the effect of environmental properties on carbon source utilization (c) and bacterial metabolism function (d). Red solid lines represent significant ( $P < 0.05$ ) factors and the dotted lines represent the rest factors. AA: amino acid metabolism; BSM: biosynthesis of other secondary metabolites; CM: carbohydrate metabolism; EF: enzyme families; EM: energy metabolism; GBM: glycan biosynthesis and metabolism; LM: lipid metabolism; MCV: metabolism of cofactors and vitamins; MTP: metabolism of terpenoids and polyketides; MAA: metabolism of other amino acids; NM: nucleotide metabolism; XBM: xenobiotics biodegradation and metabolism. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

community were presented in Fig. 6b. Axis 1 and axis 2 accounted for 69.33% and 20.39% of the total variance, respectively. *Bacillus*, *Virgibacillus* and *Sporosarcina* had high decomposition ability on amino acids and carboxylic acids. These three genera showed relative high abundances in the present study, and were known for the ability to utilize nitrogen sources of compost substances and facilitation of amino acid transport and metabolism (Zhang et al., 2018). Some species of *Bacillus* mainly use carbohydrates and amino acids for growth and produce lactic acid (Kim et al., 2010). The decomposition of carbohydrates and alcohols were positively involved in the genera of *Sporanaerobacter*, *Tepidimicrobium* and *Caldicoprobacter*. *Sporanaerobacter* was reported to have the ability to degrade proteins, carbohydrates and amino acids (Zhang et al., 2016b). As the gram-positive bacteria, *Tepidimicrobium* can utilize a variety of substrates from hydrolysis of cellulose and hemicellulose, such as glucose, cellobiose and xylan (Chen and Chang, 2017; Niu et al., 2009), indicating that this carbohydrates fermenting bacteria could play a vital role in decomposition of cellulose and hemicelluloses in maize straw composting. Several species of genus

*Caldicoprobacter* can ferment various sugars including glucose, cellobiose and sucrose as major energy sources, and thereby contribute to degradation of carbohydrates (Bouanane-Darenfed et al., 2015). Addition of earthworm casts and zeolite changed the function of the dominant microbial communities, especially these related to degradation of lignocellulose.

The relationships between environmental factors and carbon sources utilization capacity and metabolism function of bacterial community were evaluated by RDA analysis (Fig. 6c, d). The utilization capacities of carbohydrates and alcohols were positively associated with temperature. The organic nitrogen such as amino acids can be converted to ammonia during composting process. As a result, it is not surprise that amino acids had a positive relationship with the  $\text{NH}_4^+$  content. The nucleotide metabolism of bacterial community was positively related to temperature. Notably, the  $\text{NH}_4^+$  was also positively involved in the metabolism of terpenoids and polyketides, glycan biosynthesis and metabolism, xenobiotics biodegradation and metabolism, which indicated that the decomposition of these compounds was



**Fig. 7.** Bacterial co-occurrence of physico-chemical characteristics, microbial function and the bacterial communities during composting process (a, b) and the keystone species identified by network analysis (c, d, e). A connection means a significant positive correlation ( $P < 0.05$ ). The black, red and blue solid lines represent strong nonlinear ( $\text{MIC-p}2 > 0.8$ ), strong negative linear ( $r < -0.8$ ) and strong positive linear ( $r > 0.8$ ), respectively. Colored nodes signify corresponding OTUs assigned to major phyla and classes. T: temperature; M: metabolism; CP: cellular process; EIP: environmental information processing. 1: unclassified Bacillaceae; 2: unclassified Alcaligenaceae; 3: unclassified Bacillaceae; 4: *Georgenia*; 5: *Devosia*; 6: *Ruania*; 7: *Mycobacterium*; 8: unclassified Alcaligenaceae; 9: *Caldicoprobacter*; 10: unclassified Bacillaceae; 11. unclassified Bacillaceae; 12. unclassified Bacillaceae.

significantly influenced by  $\text{NH}_4^+$  contents.

### 3.6. Network associations among OTUs and compost properties

The relationships between physico-chemical properties, bacterial metabolism and bacterial community were evaluated by network analysis. The network comprised 448 significant associations (edges) and these edges were dominantly composed of positive associations (409), suggesting the potential for mutualistic interactions among bacterial assemblages in composting process. The main identifiable OTUs in the network belonged to *Firmicutes*, *Actinobacteria* and *Alphaproteobacteria* (Fig. 7a).

Temperature can affect the structure of compost microbial communities and thereby influence the species succession process in composts (Zhang et al., 2015). According to Fig. 7b, temperature was negatively related to *Georgenia*, suggested that temperature affected the growth of this genus to some extent. Notably, temperature showed strong influence on bacterial function, as negatively related to metabolism and positively related to cellular process and environmental

information processing. As an important nutrient substance,  $\text{NH}_4^+$  contents of compost affected the microbial community composition (Nie et al., 2018; Zhong et al., 2018). A previous study indicated that addition of nitrogen sources influenced the bacterial community composition during maize straw composting (Zhang et al., 2015), which was consistent with our results. *Georgenia*, *Devosia*, *Ruania* and *Mycobacterium* showed positive associations with  $\text{NH}_4^+$  contents.

There were positive relationships between metabolism and two genera (*Georgenia* and *Mycobacterium*), suggesting that metabolism strongly affected the evolutions of these two bacteria. The correlation between cellular process and *Georgenia* was negative, while one member of *Alcaligenaceae* and *Caldicoprobacter* were nonlinear correlated with cellular process. There were strong positive relationships among environmental information processing, cellular process and temperature, suggesting that temperature played an important role in environmental information processing and cellular process in the composting process.

As expected, the OTUs identified as keystone taxa were members of *Bacillaceae* based on betweenness centrality scores (Fig. 7c–e). The



family *Bacillaceae*, belonging to *Firmicutes*, was the dominant bacterium in three treatments. Some members of *Bacillaceae*, such as *Bacillus*, were reported to produce thermostable proteases (Anandharaj et al., 2016). *Bacillaceae* is prevalent in manure-based and lignocelluloses-based composts due to its formidable surviving ability in different conditions (Maeda et al., 2011). Therefore, *Bacillaceae* could play a key role in decomposition of nitrogen sources such as earthworm casts during composting.

#### 4. Conclusion

This study revealed that addition of earthworm casts and zeolite increased the temperature and the thermophilic period, decreased  $\text{NH}_4^+$  contents and significantly influenced the composition of bacterial community during maize straw composting. The microbial capacities of carbohydrate, amino acid and energy metabolism were enhanced by addition of earthworm casts and zeolite in the thermophilic phase. Network analysis indicated that metabolism, environmental information processing and cellular processes were the primary functions of bacterial community. Overall, the present work strengthens the understanding of metabolism function of bacterial community in response to organic or inorganic additives during composting process.

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