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Effect of pig manure on the chemical composition and microbial diversity during co-composting with spent mushroom substrate and rice husks

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1           **Effect of pig manure on the chemical composition and**  
2           **microbial diversity during co-composting with spent**  
3           **mushroom substrate and rice husks**

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13  
14   **Abstract**

15   In this study, the impact of pig manure on the maturity of compost consisting of spent  
16   mushroom substrate and rice husks was assessed. The results showed that the addition  
17   of pig manure (SMS-PM) reached 50°C 5 days earlier and lasted 15 days longer than  
18   without pig manure (SMS). Furthermore, the addition of pig manure improved  
19   nutrition and germination index. High-throughput 16S rRNA pyrosequencing was  
20   used to evaluate the bacterial and fungal composition during the composting process  
21   of SMS-PM compared to SMS alone. The SMS treatment showed a relatively higher  
22   abundance of carbon-degrading microbes (*Bacillaceae* and *Thermomyces*) and plant

23 pathogenic fungi (*Sordariomycetes\_unclassified*) at the end of the compost. In  
24 contrast, the SMS-PM showed an increased bacterial diversity with anti-pathogen  
25 (*Pseudomonas*). The results indicated that the addition of pig manure improved the  
26 decomposition of refractory carbon from the spent mushroom substrate and promoted  
27 the maturity and nutritional content of the compost product.

28

29 **Key words:** pig manure, microbial community, compost, spent mushroom substrate

30

### 31 **1. Introduction**

32 *Auricularia auricular* is a major edible fungus grown in northeast China, with a  
33 production of approximately 2.5 million tons in Heilongjiang Province alone. The  
34 consequent high volumes of waste, consisting of spent mushroom substrate, is more  
35 than 6 million tons a year, which has no means of efficient disposal due to the  
36 recalcitrant sawdust in the substrate (Tingting et al., 2014). In addition to *Auricularia*  
37 *auricular production*, Heilongjiang province is also one of the primary rice producing  
38 areas of China. During the rice-milling process the inedible rice husk is removed and  
39 is considered as a waste by-product of little economic value. Prior efforts to utilize  
40 rice husks have been hindered by its tough, woody and abrasive nature as well as its  
41 resistance to natural degradation, low nutritive properties and high ash content (Sun  
42 and Gong, 2001). In general, these wastes products have not been efficiently reused  
43 due to the high amount of recalcitrant carbon present in these materials.

44 Composting is supposed as sustainable and high-performance technology for

45 treatment and disposal of organic wastes, as it limits overall environmental pollution  
46 and the end product is suitable as a fertilizer and soil amendment (Rui Wang and  
47 Yuansong Wei, 2016; Ren et al., 2016). Compost is also able to reduce the incidence  
48 of plant diseases by suppressing soil-borne pathogens, providing essential nutrients  
49 for plant growth, improving soil properties (Lu Zhang, 2014; Zhou et al., 2016).

50 As such, composting could be a feasible way to dispose of spent mushroom substrate  
51 and rice husks; however, because of their chemical and physical properties, especially  
52 the presence of lignocellulose, these waste products are not suitable for composting  
53 by themselves. The term co-composting means the simultaneous composting of  
54 various waste materials, such as biogas residues and cow manure (ZHAO Hong-yan,  
55 2013), digested sludge mixed with mature compost and sawdust (Huang et al., 2017),  
56 green waste with spent mushroom compost and biochar (Lu Zhang, 2014), and cow  
57 manure and rice straw (Ren et al., 2016). Co-composting not only improves  
58 degradation of different organic materials, but it also simultaneously improves  
59 compost quality by increasing the nutritional content and shortening the composting  
60 time due to the diversification of the microbial population and comprehensive  
61 nutrition for microbial growth (Ren et al., 2016; Lu Zhang, 2014).

62 The current study investigates the composting of spent mushroom substrate and rice  
63 husks. Spent mushroom substrate, an organic waste of mushroom cultivation, contains  
64 mushroom mycelium and high levels of residual nutrients, including nitrogen (N),  
65 phosphorus (P), and potassium (K). Importantly, spent mushroom substrate from  
66 *Auricularia auricular* contains high levels of lignocellulose as well as a diversity of

67 microorganism. Some studies have reported that the addition of a bulking agent, such  
68 as rice husks, during composting helps to optimize properties such as air space,  
69 moisture content, carbon nitrogen (C:N) ratio C/N and mechanical structure,  
70 positively affecting the decomposition rate. According to this theory, lignocellulosic  
71 waste from agriculture was used as bulking agents in composting (Bernal et al., 2009).  
72 The composting of manures has been demonstrated to produce a stable and sanitized  
73 end product for use in plant growth. Pig manure in particular has high level of carbon  
74 (C), N, P, and K, and could provide additional microorganisms for improved  
75 composting. It is anticipated that the addition of pig manure would accelerate and  
76 lengthen the thermophilic period, which would help the decomposition of organic  
77 matter and help eradicate pathogens (Huang et al., 2006). While, the effect of pig  
78 manure on the spent mushroom substrate composting process was not reported. And  
79 the mechanism of the effect of pig manure on compost maturity was not illuminated.  
80 The process of composting primarily relies on interactions within highly diverse  
81 microbial communities. Microbial respiration and consumption of organic matter  
82 causes the accumulation of heat, resulting in an increase temperature during  
83 composting. Understandably, the composition and diversity of microbial communities  
84 are closely related to the duration of the composting process and the overall content of  
85 the compost (Ren et al., 2016). Microbes also have the capacity to mineralize  
86 nutrients through composting, and some microbes demonstrate powerful  
87 anti-pathogenic properties by competing for available nutrients or by producing  
88 chitinolytic enzymes to destroy the phytopathogens (Zhou et al., 2016). Traditionally,

89 assessment of microbial communities during the composting of organic waste is  
90 performed by way of cloning and sequencing (Zhao et al., 2016; ZHAO Hong-yan,  
91 2013). Consequently, detailed information of microbial community dynamics during  
92 co-composting especially the pig manure on the compost maturity is still lacking due  
93 to the limited taxonomic resolution of these traditional methods.

94 In this study, the composting of spent mushroom substrate with rice husk and spent  
95 mushroom substrate with rice husk with and without the addition of pig manure, was  
96 assessed in regards to composting time and the quality of the composting end product.  
97 The physical and chemical properties of the compost were measured during the  
98 co-composting process in order to assess maturity. Furthermore, the 16S  
99 high-throughput sequencing technology was used to obtain a detailed understanding  
100 of the microbial community at the initial and end stages of composting. The  
101 relationships between the composition of the microbial community and chemical  
102 composition of the compost were analyzed using redundancy analysis (RDA).

103

## 104 **2. Materials and Methods**

### 105 **2.1. Compost materials and preparations**

106 Spent mushroom substrate and rice husk composting was carried out in two windrow  
107 tests, including an experimental test with addition of pig manure (SMS-PM) and a  
108 control test (SMS) without pig manure from December 1, 2014 to January 20, 2015.  
109 Spent mushroom substrate was obtained from a commercial *Auricularia auricula*  
110 operation, located in Heilongjiang province, China, with the major component being

111 sawdust. Rice husk was obtained from Heilongjiang province, China. Fresh pig  
112 manure was obtained from Zhuozhou, Hebei province, China. Mixtures of spent  
113 mushroom substrate : rice husk : pig manure at 1:1:1 ratios (v/v/v) were used in  
114 SMS-PM, and a mixture of spent mushroom substrate : rice husk at a 1:1 ratio (v/v)  
115 was used in the SMS control.

## 116 **2.2. Compost process and compost samples**

117 The experiment was carried out in a concrete bunker (40 m×2 m×1.2 m) in the  
118 compost facility at Zhuozhou, Hebei province, China. Compost pile dimensions were  
119 as follows: 5 m bottom length×2 m top length×1.2 m width. When the compost  
120 temperature rose above 50°C, the compost piles were turned manually. Turning  
121 frequency and five-point sampling method at day 0, 5, 7, 9, 25, 76 was according to  
122 ZHAO (2013). A portion of the homogenized samples was stored at -20°C for  
123 microbiological analysis, and the remainder was air-dried.

124 Parameters of all composting materials, including total organic carbon (TOC), the  
125 total nitrogen (TN), total phosphorus (TP), and total potassium (TK) and nitrate as  
126 nitrogen (NO<sup>3</sup>-N) were determined according to the methods (Lu Zhang, 2014). The  
127 germination index (GI) assay was determined using lettuce seeds according to (ZHAO  
128 Hong-yan, 2013). Hemicellulose, cellulose, and lignin were determined according to  
129 validated methods described by Castillo (2013).

## 130 **2.3. DNA extraction and high-throughput 16S rRNA pyrosequencing**

131 The compost samples were centrifuged at 8000 rpm for 10 min, and the supernatant  
132 was decanted carefully to obtain sediment samples (0.3 g net weight) for DNA



133 extraction. Genomic DNA was extracted using the CTAB method for use as a PCR  
134 template (ZHAO Hong-yan, 2013).

135 After amplification, PCR products were purified using AMPure Beads (Beckman  
136 Coulter, USA). The application mixtures were used for pyrosequencing on a Miseq  
137 platform (Shanghai Majorbio Technology, China). All of the raw reads are archived at  
138 the NCBI Sequence Read Archive (SRA) database (accession number: SRR1765717).

### 139 **2.5. Data analysis**

140 Statistical calculations and data analysis were performed using the SPSS 23.0  
141 statistical software package (SPSS, USA) and Excel (Microsoft, USA). Redundancy  
142 analysis (RDA) was performed using the Canoco 4.5 software package  
143 (Microcomputer Power, USA).

## 145 **3. Results and discussion**

### 146 **3.1. Changes of pile temperature**

147 Temperature is one of the major parameters to monitor the composting process, since  
148 its variation is well correlated with the degradation of organic matter and the  
149 generation of microbes. Temperature changes in the two composts were recorded  
150 during decomposition, and both composts experienced the thermophilic and mature  
151 stages shown in Fig. 1. The ambient temperature ranged from 5°C to 15°C. The  
152 temperature in SMS-PM increased sharply and reached 50°C after 6 days of  
153 composting. The temperature in SMS treatment increased slowly than in SMS-PM  
154 and only reached 50°C after 11 days. This first thermophilic stage in SMS-PM lasted

155 15 days longer than in SMS. A similar finding was reported in compost with pig  
156 manure and sawdust, which had an initial thermophilic period greater than 35 days  
157 (Huang et al., 2006). At this stage, all mixtures were manually turned over every 2  
158 days. Following this first thermophilic stage, the average temperature values in the  
159 SMS compost dropped to 30°C-40°C suggesting limited biological activity.  
160 Subsequently, the temperature in both composts increased once again (secondary  
161 thermophilic stage) owing to secondary fermentation induced by the increased  
162 availability of organic components and increased microbial activity. Consequently, the  
163 frequency of turning was reduced to once every 5-10 days. After the secondary  
164 thermophilic stage, a stable mesophilic stage was maintained at 30-40°C until the end  
165 of the composing process (Huang et al., 2017). So, the compost needs a longer time to  
166 get the room temperature because of recalcitrant component in *Auricularia auricular*  
167 substrate.

168 The slower rise in temperature and shorter thermophilic stages in SMS compost  
169 indicated that the compost raw materials of spent mushroom substrate an impeded  
170 decomposition of this compost mixture. The higher temperature reached and longer  
171 thermophilic phases achieved in SMS-PM compost were likely made possibly by the  
172 pig manure substrates, which contained more easily decomposable components  
173 (ZHAO Hong-yan, 2013). Furthermore, the addition of pig manure likely functioned  
174 as inoculation to promote microbial activity and subsequent heat generation (Zhu et  
175 al., 2012). As the experimental continued, a decreased metabolic rate was observed,  
176 indicating the onset of the maturation period. If the thermophilic phase lasts longer

177 than 3 days, the compost will meet sanitation requirements, i.e. it will be free of weed  
178 seeds and pathogens (Zhang et al., 2013), and both of the composts fulfilled the  
179 sanitation standards. The raw materials were in light brown color with large particle  
180 size and bad smell. The final compost samples showed dark brown color, loose and  
181 granular product without odor and mosquitoes.

### 182 **3.2. Chemical composition**

183 The composted mixtures were sieved to obtain the 0, 5, 10, 25, 76-days compost  
184 samples. Sample characteristics are given in Table 1.

185 The TOC content increased gradually from their levels in the raw materials to those  
186 recorded on day 10, as follows: from 38.94% to 43.67% in SMS, and 35.66% to 43.51%  
187 in SMS-PM, only to fall at the end of day 76 to their corresponding levels of 38.69%  
188 in SMS and 34.92% in SMS-PM. These results, however, are different from those  
189 reported by (Lu Zhang, 2014). It should be noted, however, that the present study was  
190 based on sawdust substrate and rice husk, which is different from other spent  
191 mushroom compost containing easily degradable materials or biochar, as in the study  
192 by (Lu Zhang, 2014). The SMS and SMS-PM were both rich in sawdust and rice  
193 husks, which are difficult to degrade, resulting in a relatively immutable TOC content  
194 at the end of the composting period.

195 At first, TN levels declined at first, followed by a steady increase, as the rate of  
196 carbon loss was higher than the rate of N loss as  $\text{NH}_3$  and  $\text{N}_2$ . Subsequently, TN levels  
197 were observed to decrease again in both treatments at the end composting period. The  
198 SMS-PM showed a higher initial TN and an early increase at 5 days due to the

199 addition of pig manure, which enhanced the total nitrogen content and accelerated the  
200 composting process. A significant part of the initial TN loss was likely due to  $\text{NH}_3$   
201 volatilization promoted by high temperatures. As such, higher TN losses in SMS-PM  
202 may have been due to the sustained high temperature, as reported by several authors  
203 (Ogunwande et al., 2008). However, as N is one of the most valuable phytonutrients  
204 in fertilizer, the mass losses of N in SMS-PM reduced its value as fertilizer. In regard  
205 to  $\text{NO}_3\text{-N}$ , which is the major nitrogen source for most of plants, the levels of this  
206 compound showed a significant difference between the SMS-PM and SMS composts  
207 (Fig. 2). The content of  $\text{NO}_3\text{-N}$  was observed to slightly increase at the beginning of  
208 day 5 and then decreased from day 5-10, with the overall  $\text{NO}_3\text{-N}$  content substantially  
209 higher in SMS-PM than in SMS. Following this period, the  $\text{NO}_3\text{-N}$  content remained  
210 mostly stationary in both SMS-PM and SMS until day 25, after which the  $\text{NO}_3\text{-N}$   
211 content increased to their highest levels at the end of the composting period. This lag  
212 was expected, as nitrifying bacteria cannot grow at temperature above  $40^\circ\text{C}$  (Ren et al.,  
213 2016). As such, nitrification was anticipated to occur primarily during the cooling and  
214 maturation phases. The final  $\text{NO}_3\text{-N}$  content in SMS-PM was 3 times higher than that  
215 in SMS, indicating that the addition of pig manure substantially increased the  
216 fertilizer value of the compost.

217 Carbon and nitrogen are essential nutrients for microbial growth and energy  
218 production during composting, and this results in considerable changes in the C/N  
219 ratio. Thus, the C/N ratio can be used to assess the maturity of compost. Both SMS  
220 and SMS-PM provided a suitable physical-chemical environment for microorganisms.

221 At 10 days, the C/N ratio in the SMS compost demonstrated a sharp increase,  
222 indicating that the TN loss rate was faster than the organic matter loss rate. However,  
223 the C/N ratio in the SMS-PM compost at 10 days was lower than at day 0. The C/N  
224 ratio in SMS-PM was lower compared to SMS at the end of composting period. This  
225 is likely because the addition of pig manure can speed up the decomposition process  
226 and enhance the condensation and aromatization during the composting process (Zhu  
227 et al., 2010). Normally, C/N ratio is an important parameter in composting process  
228 and its normally recommended range is 20~35. Several reports manifested that  
229 additive of pig manure can improve the maturity of composting mainly because of the  
230 adjustment of C/N (Zhu et al., 2010; Huang et al., 2006; Zhu et al., 2010). However in  
231 our study, the C/N ratio in the mushroom was suitable for composting, but results  
232 showed that the composting of this mushroom was not very effective. So, the role of  
233 the additive of pig manure was not just balance the C/N.

234 Phosphorus and potassium are essential nutrients for plant growth, and the TP and TK  
235 content in SMS and SMS-PM at the end of the compost period were 3.00% and  
236 4.95%, 12.53% and 9.94%, respectively. This indicated that the addition of pig  
237 manure to the SMS-PM compost demonstrated a significant increase in TP and TK  
238 content compared with the SMS compost. The GI parameter is a sensitive indicator  
239 for accessing the maturity and phytotoxicity of compost, with a GI of more than 80%  
240 indicating phytotoxic-free and mature compost (Tiquia et al., 1996). The GI of the  
241 SMS was between 79%-84%, and there was no significant change during composting,  
242 suggesting that a longer time is required to form fully mature compost when a large

243 proportion of sawdust is present. In contrast, at the end of composting, the GI of  
244 SMS-PM was indicating a mature, phytotoxic-free compost. As for the degradation of  
245 lignocellulose that was shown in Fig 3, the additive of pig manure showed a higher  
246 degradation of cellulose, Hemicellulose and lignin than SMS.

### 247 **3.3. Bacterial community**

248 The composition of bacterial 16S rRNA sequences was determined by high  
249 throughput sequencing. The composition of the bacterial communities of the two  
250 composts demonstrated substantial differences. As shown in Fig. 4, Chloroflexi,  
251 Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria were the dominant phyla  
252 in all samples. However, the proportions of these bacteria were significantly different  
253 between the two composts. In the initial SMS compost, Proteobacteria (59%) were the  
254 most abundant bacteria, followed by Bacteroidetes (20%). Chloroflexi (2%),  
255 Firmicutes (6%) and Actinobacteria (6%) only represented a small proportion. In the  
256 initial SMS-PM compost, Proteobacteria (58%) were the primary bacteria, followed  
257 by Firmicutes (21%), Bacteroidetes (18%), and to a lesser degree of Actinobacteria  
258 (2%) and Chloroflexi (0.1%). However, as composting progressed, these populations  
259 changed substantially. At 76 days, after the thermophilic stage, the percentage of  
260 Proteobacteria decreased dramatically to 19% and 8% in SMS and SMS-PM,  
261 respectively. Bacteroidetes showed a similar decrease to 8% and 6% in SMS and  
262 SMS-PM, respectively. In contrast, Chloroflexi increased significantly to 44% and 57%  
263 in SMS and SMS-PM, respectively. The large differences in these bacterial  
264 percentages changes between the initial and end composts likely reflect the change in

265 the local environment from mushroom substrate to open air compost.

266 For a detailed analysis of the bacterial community structures, 48 abundant families  
267 (abundance >1% in any sample) were selected for comparison (Fig. 5). The family  
268 level was selected to avoid biasing the interpretation, as suggested in a previous study  
269 (Rui Wang and Yuansong Wei, 2016).

270 As noted earlier, the phyla Proteobacteria and Bacteroidetes both decreased over time  
271 in both compost. The family of Moraxellaceae was the primary bacteria in SMS-PM  
272 at the beginning of the experiment, and was observed to sharply from 47% to 0.06%  
273 over the course of the composting. Acinetobacter is the dominate genus in the family  
274 Moraxellaceae (phylum Proteobacteria) and can cause various infections and survive  
275 various common disinfectants, including those used in commercial pig farming where  
276 disinfectants are frequently used to avoid disease (Teixeira and Merquior, 2014).  
277 Therefore, it is not surprising that the addition of pig manure increased the abundance  
278 of Moraxellaceae compared with SMS. However, Moraxellaceae were nearly absent  
279 in both treatments after composting, indicating a safe final product in this regard. The  
280 phylum Proteobacteria was the dominant bacteria in SMS before composting and  
281 decreased dramatically from 59% to 19% after composting. The families  
282 Hyphomicrobiaceae (from 12% to 2%), Sphingobacteriaceae (from 5% to 0.1%),  
283 Caulobacteraceae (from 5% to 0.3%), Rhizobiaceae (from 6% to 0.2%),  
284 Comamonadaceae (from 2% to 0.03%), Oxalobacteraceae (from 4% to 0%) and  
285 Bradyrhizobiaceae (from 1% to 0.08%) belong to the phylum Proteobacteria and were  
286 all observed to decrease following composting. Hyphomicrobiaceae,

287 Sphingobacteriaceae, Rhizobiaceae, Comamonadaceae and Bradyrhizobiaceae are  
288 intimately involved in C and/or N cycling (Anderson et al., 2011). These bacterial  
289 families decreased following composting along with the decomposition of TOC and  
290 TN. The remaining bacteria Caulobacteraceae and Oxalobacteraceae, which belong to  
291 the phylum Proteobacteria, grow under mesophilic conditions and were observed to  
292 have decreased at the end of composting. Xanthomonadaceae, which are related to the  
293 plant pathogen group, were also identified, with a relative abundance in the SMS  
294 (0.6%) compost at least 6 times higher than in the SMS-PM (0.09%) compost at the  
295 end of composting (Anderson et al., 2011). Another family, Pseudomonadaceae  
296 (phylum Proteobacteria), decreased from 5% to 0.1% in the SMS compost, but was  
297 observed to increase from 1% to 3% in the SMS-PM compost. The majority of  
298 Pseudomonadaceae were *Pseudomonas* (0.1% in SMS and 3% in SMS-PM at the end  
299 of composting), and recent studies have proposed a role for *Pseudomonas* in disease  
300 suppression and in the promotion of plant growth (Mehta et al., 2014). Together, it is  
301 apparent that the addition of pig manure to the compost promoted decreased levels of  
302 pathogens, a higher propensity for suppression of disease bacteria, and an increased  
303 quality of compost.

304 The other bacteria that decreased significantly were the phylum Bacteroidetes. In the  
305 initial SMS and SMS-PM composts, a large microbial proportion belonged to the  
306 family Flavobacteriaceae (phylum Bacteroidetes), at 11% and 15%, respectively.  
307 However, these bacteria decreased considerably to 0.05% and 0.01% in SMS and  
308 SMS-PM after composting, respectively. Flavobacteriaceae are aerobic bacteria with



309 an optimum growth temperature ranging from 25°C to 35°C, but members of some  
310 species or genera are psychrophilic or psychrotolerant. Some species in the family  
311 Flavobacteriaceae degrade soluble cellulose derivatives such as  
312 carboxymethylcellulose or hydroxyethylcellulose (Bernardet et al., 2002). As such,  
313 the presence of Flavobacteriaceae in the mushroom substrate might aid in the  
314 decomposition of lignocellulose during mushroom cultivation at 25-35°C. However, as  
315 the compost environment changed from the mesophilic to the thermophilic stage their  
316 abundance decreased quickly. Another bacterial family, Sphingobacteriaceae  
317 belonging to the phylum Bacteroidetes, showed a decrease from 5% to 0.01% and 2%  
318 to 0.02% in SMS and SMS-PM, respectively. The Sphingobacteriaceae family is  
319 known for its ability to utilize a wide variety of C sources and demonstrated a  
320 decrease along with the decomposition of carbon resources (Anderson et al., 2011).  
321 However, the families Rhodothermaceae and Cytophagaceae, in the phylum  
322 Bacteroidetes, were increased. Rhodothermaceae can exhibit thermophilic or  
323 halophilic characteristics, while Cytophagaceae can digest macromolecules, such as  
324 polysaccharides or proteins and utilize cellulose (Park et al., 2014).  
325 Chloroflexi were the major bacterial phylum present at the end of composting, at 44%  
326 in the SMS and 57% in SMS-PM, respectively. Anaerolinaceae (phylum Chloroflexi)  
327 were the dominant bacterial family at the final compost, at 25% and 54% in the SMS  
328 and SMS-PM composts, respectively. Anaerolinaceae can inhabit thermophilic  
329 environments, digest carbohydrates and/or peptides, and might promote the  
330 degradation of carbohydrates or amino acids from dead cells (Yamada et al., 2006).

331 As the compost transformed to anaerobic conditions (76 days) and the proportion of  
332 living organism declined sharply. So Anaerolinaceae occupied the chief bacterial at  
333 the thermophilic period for compost quality promotion. As the Anaerolinaceae could  
334 not be identified in genus level, it was apparent that the mushroom substrate compost  
335 contained many novel organisms, consistent with previous reports (Ivors et al., 2000).  
336 The families Chloroflexaceae and Roseiflexaceae, which were subordinated to the  
337 phylum Chloroflexi, increased significantly in the SMS and SMS-PM composts from  
338 almost zero to 11% and 7%, respectively. The family Chloroflexaceae represents one  
339 of five major lineages of phototrophic bacteria that can inhabit heterotrophic  
340 environments and also grow in mineral salt medium with CO<sub>2</sub> as a sole carbon source.  
341 Moreover, Roseiflexaceae can also survive under thermophilic conditions. As such,  
342 the increasing abundance of the families Chloroflexaceae and Roseiflexaceae  
343 indicated the nutritional content of SMS was sufficient to sustain bacterial growth, but  
344 the temperature in the SMS compost remained hyperthermia at the 76 days which  
345 needs more time to cool down.

346 Many of Firmicutes produce endospores to resist dehydration and extreme  
347 environments. The families Clostridiaceae (0% in SMS and 8% in SMS-PM) and  
348 Bacillaceae (6% in SMS and 1% in SMS-PM) contain many microbes that can  
349 degrade cellulose during the composting (Xi et al., 2015; Antunes et al., 2016).The  
350 relative abundance of Streptosporangiaceae (from 0% to 0.6% in SMS and 0% to 3%  
351 in SMS-PM) and Thermomonosporaceae (from 0% to 0.4% in SMS and 0% to 3% in  
352 SMS-PM), which belong to the phylum Actinobacteria, were increased after

353 composting, but their relative abundance was higher in SMS-PM. Species within  
354 these bacterial families are chemo-organotrophs involved in the turnover of organic  
355 matter, and the degradation of cellulose and lignocellulose residues (Anderson et al.,  
356 2011). The addition of pig manure helped promote the degradation of lignocellulose  
357 and increase the quality of compost, same as previous study (Zhu et al., 2010). The  
358 family Micromonosporaceae (phylum Actinobacteria) is associated with secondary  
359 metabolite production, as some species are efficient solubilizers of rock phosphate  
360 (Hamdali et al., 2008). The relative abundance of Micromonosporaceae was 2% and  
361 0.3% in SMS and SMS-PM, respectively, indicating the incomplete continuing  
362 composting of SMS.

### 363 **3.4 Fungal community**

364 The compositions of the fungal communities were compared between the two  
365 composts by high throughput sequencing. In total, 18 abundant families  
366 (abundance >1% in any sample) were selected, and their abundances were compared  
367 (Table 2). As presented in Table 2, the phylum Ascomycota was the dominant fungi  
368 during composting, coincide with (Xi et al., 2015). The class Sordariomycetes  
369 (phylum Ascomycota) was the major fungi in both SMS (75.57%) and SMS-PM  
370 (72.19%) at the end of composting. The class Sordariomycetes are saprobic fungi and  
371 promote the decomposition and nutrient cycling of plant litter, including wood,  
372 herbaceous stems and dung. Important taxa of Sordariomycetes include the family  
373 Chaetomium which are often isolated from mushroom compost and are an important  
374 cellulolytic organism responsible for the degradation of paper and fabrics (Chefetz et

375 al., 1998; Zhang et al., 2006). *Chaetomium* demonstrated an increase from 6.30% to  
376 9.75% in SMS and an increase from 0.67% to 63.02% in SMS-PM. It is normal for  
377 fungi to increase during the maturation phase of composting as a result of the  
378 breakdown of substrates and the presence of favorable environmental factors (Villar  
379 et al., 2016). Lignocellulose is the major component in sawdust and is hard to degrade.  
380 The levels of *Chaetomium* increased steadily in SMS. In contrast, the levels of  
381 *Chaetomium* underwent a sudden increase in SMS-PM, indicating effective  
382 lignocellulose decomposition with the addition of pig manure. However, Most plant  
383 pathogens are distributed in the class Sordariomycetes. One of the Sordariomycetes  
384 member, Microascaceae, is often associated with opportunistic infections of plants,  
385 humans and other animals (Zhang et al., 2006). The abundance of Microascaceae and  
386 Sordariomycetes\_unclassified were higher in SMS relative to SMS-PM, indicating a  
387 higher levels of plant pathogens in SMS. The phylum Basidiomycota were decreased  
388 from 6.20% to 1.48% and 2.29% to 0.30% in SMS and SMS-PM, respectively.  
389 *Issatchenkia* (family Saccharomycetaceae) are often isolated from ethanol  
390 fermentation (Oguntoyinbo, 2011), and were predominantly found in the initial  
391 SMS-PM compost, indicating the more easily available components in pig manure  
392 that accumulated heat at the beginning of composting. *Galactomyces* (family  
393 Dipodascaceae) are principal colonizing fungus at 25°C. Though these fungi don't  
394 demonstrate any cellulolytic or hemicellulolytic activities (Fathallh Eida et al., 2013),  
395 *Galactomyces* do have an unknown role in composting. The *Thermomyces*, a genus of  
396 hemicellulose degraders, were present in both composts, 10.42% in the SMS and 1.65%

397 in SMS-PM (Zhang et al., 2015). Hemicellulose is easier to degrade than cellulose, so  
398 a relatively higher abundance of *Thermomyces* cooperated with other bacteria and  
399 fungi to degrade the lignocellulose in SMS and to complete decomposition of  
400 hemicellulose in SMS-PM. Pyronemataceae had levels of 10% in SMS before  
401 composting, but only 1%-2% in either compost at the maturation period. The major of  
402 taxa within the family Pyronemataceae have traditionally been considered  
403 saprotrophic, but the trophic strategies of most species are not well understood and  
404 remain undocumented (Perry et al., 2007). These findings indicated that many fungus  
405 have strong heat resistance, and may be difficult to culture based on traditional  
406 methods, limiting the development of high-efficiency inoculum.

407 The phylum Ciliophora showed a dramatic decrease from 51.64% to 3.13% in SMS  
408 and a significant increase from 0.95% to 19.52% in SMS-PM. Ciliophora primarily or  
409 exclusively feed on a wide variety of bacteria and are often found in moist soils.  
410 Because of their ability to readily enter protective cysts, they will quite frequently  
411 found in desiccated samples of soil and vegetation (Cochran-Stafira and von Ende,  
412 1998). The abundance of Ciliophora in SMS indicated an appropriately stable  
413 ecosystem in this compost, which was disrupted by increased temperature and  
414 reduced moisture in SMS-PM. The presence of *Auricularia* (phylum Basidiomycota)  
415 was the genus of mushroom originally grown from mushroom substrate, was absent  
416 by the end composting in both SMS and SMS-PM.

### 417 **3.5 Redundancy analysis**

418 The relationships between environmental factors (such as TOC, TN, TK, TP, C:N and

419 GI) and bacterial /fungal communities were evaluated via a redundancy analysis  
420 (RDA) (Fig. 6a and 6b). Analysis indicated that C:N, TN and GI were positively  
421 correlated with the first axis (explaining 70.3% of total variance in Fig. 6a and 53.1%  
422 of total variance in Fig. 6b), with TK, TP and TOC positively correlated with the  
423 second axis (explaining 23.6% of total variance in Fig. 6a and 30.3% of total variance  
424 in Fig. 6b).

425 According to Fig. 6a and 6b, the addition of pig manure increased the initial total N  
426 content, which could result in an increased nitrogen content of the organic product.  
427 The addition of pig manure also showed a positive correlation with the content of P  
428 and K. Thus, the addition of pig manure improved the nutrition of the compost  
429 product. While the SMS compost showed a positive correlation with TOC, these  
430 results were consistent with previous results that the content of TOC in SMS was  
431 higher than SMS-PM, indicating the maturity process for SMS was incomplete.  
432 Moreover, there was no difference in GI between day 0 and day 76 in SMS (Table 1),  
433 but a significant increase was apparent in SMS-PM over the same time period, as  
434 indicated by the RDA. SMS-PM at day 76 showed a positive correlation with GI.  
435 Composting can reduce various types of organic matter, possibly because of elevated  
436 temperatures and the partial aerobic conditions during the thermophilic phase, which  
437 improves the maturity of the compost. These observations suggested that pig manure  
438 promoted the efficiency of composting and accelerated the process of compost  
439 maturity.

440 There were close correlations between 12 families of bacteria and TOC, suggesting

441 that these bacteria cause or respond to changes in TOC (Fig. 6a). These bacteria were  
442 distributed across *Pseudomonadaceae*, *Xanthobacteraceae*, *Erythrobacteraceae*,  
443 *Hypomicrobiaceae*, *Rhizobiaceae*, *Methylobacteriaceae*, *Oxalobacteraceae*,  
444 *Candidate\_division\_TM7\_norank*, *Bradyrhizobiaceae*, *Microbacteriaceae*,  
445 *Caulobacteraceae* and *Enterobacteriaceae*. All of these are involved in C recycling  
446 (Anderson et al., 2011), which likely helped improve the stability of the compost.  
447 During the composting process, GI represented maturity and exerted an influences on  
448 bacterial community composition. As shown in Fig. 6a *Roseiflexaceae*,  
449 *Micromonosporaceae* and *Rhodothermaceae* were closely related with GI, and these  
450 bacteria exhibit thermophilic characteristics and were closely associated with the  
451 maturation period. Furthermore, the bacterial community, as opposed to the fungal  
452 community, influenced TP and TK levels during the composting process. As shown in  
453 Fig. 6a *Clostridiaceae* and *Peptostreptococcaceae* were strongly associated with TP  
454 and TK, suggesting that the growth of these bacteria were likely to influence or be  
455 influenced by high TP and TK content (Antunes et al., 2016). Moreover, the origin of  
456 the compost components, which characterize the chemical and physical properties of  
457 the compost, may influence the growth of the bacterial community during the  
458 thermophilic and maturation phases. As pig manure is an environment rich in bacteria  
459 with a high content of fermentative microbes (especially Gram + bacteria) during the  
460 initial phase of decomposition of manure due to the high availability of water and  
461 compounds that can be easily broken down (Villar et al., 2016).  
462 As for the relationship between the environment and the fungal community, the RDA

463 clearly indicated an interrelation., as shown in Fig. 6b Colpodea showed a positive  
464 correlation with GI, as Colpodea was dramatically decreased to 3.13% in SMS and  
465 significantly increased to 19.52% in SMS-PM. As such, the enrichment of this fungal  
466 community did not correlate with the maturity of compost. While Chaetomiaceae and  
467 Microascaceae also showed a positive relationship with GI (especially *Chaetomiaceae*,  
468 which was the dominant fungi in the end SMS-PM product), indicating a relatively  
469 stable compost. As shown in Fig. 6b, the family Sordariomycetes\_unclassified and the  
470 genus *Thermomyces* (Family Eurotiomycetes\_Incertae\_Sedis) were the dominate  
471 species in SMS and were greatly associated with TOC, suggesting that  
472 Sordariomycetes\_unclassified and *Thermomyces* were likely related to the  
473 decomposition of carbon or influenced by the rich TOC content. Importantly, the high  
474 abundance of these fungi indicated a high carbon content, demonstrating the need for  
475 a long maturation process to achieve a relatively stable compost.

476 Normally, C/N ratio is an important parameter in composting process and its normally  
477 recommended range is 20~35. Several reports manifested that additive of pig manure  
478 can improve the maturity of composting by adjustment of C/N. While according to the  
479 dates, the C/N ratio in the mushroom was suitable for composting, but results showed  
480 that the composting of this mushroom was not very effective. As showed in these  
481 results, the maturity of composting was improved as a results of additions of pig  
482 manure. Because the main function of pig manure was providing the indispensable  
483 microorganism that could degrade organic component at the beginning composting  
484 process rather than balancing the C/N ratio. And the pig manure provided diversiform



485 microorganism to decompose all kinds of organic matters and mineralize N, P and K  
486 to improve the maturity and fertilizer efficiency of the compost.

487

#### 488 **4. Conclusion**

489 Compared the compost with or without pig manure, the adding pig manure expedited  
490 and extended the high temperature period and effectively increased the temperature of  
491 the composting process. Meanwhile, the addition of pig manure improved the  
492 nutrition and GI parameter. The SMS demonstrated relative abundance of carbon  
493 decomposing microorganism and plant pathogens, while the microbial community  
494 about maturation and anti-pathogens were enriched in SMS-PM. Overall, the addition  
495 of pig manure provided essential microorganism as inoculum for heat accumulation at  
496 the initial composting process and promoted the stability, fertilizer efficiency and  
497 safety of the compost product.

498

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## References:

505

- 506 1. Anderson, C.R., Condon, L.M., Clough, T.J., Fiers, M., Stewart, A., Hill, R.A., Sherlock,  
507 R.R., 2011. Biochar induced soil microbial community change: Implications for  
508 biogeochemical cycling of carbon, nitrogen and phosphorus. *Pedobiologia*, 54, 309-320.
- 509 2. Antunes, L.P., Martins, L.F., Pereira, R.V., Thomas, A.M., Barbosa, D., Lemos, L.N.,  
510 Machado Silva, G.M., Silva Moura, L.M., Condomitti Epamino, G.W., Digiampietri, L.A.,  
511 Lombardi, K.C., Ramos, P.L., Quaggio, R.B., Franco De Oliveira, J.C., Pascon, R.C., Da  
512 Cruz, J.B., Da Silva, A.M., Setubal, J.C., 2016. Microbial community structure and dynamics  
513 in thermophilic composting viewed through metagenomics and metatranscriptomics.  
514 *Scientific Reports*, 6.
- 515 3. Bernal, M.P., Albuquerque, J.A., Moral, R., 2009. Composting of animal manures and  
516 chemical criteria for compost maturity assessment. A review. *Bioresource Technology*, 100,  
517 5444-5453.
- 518 4. Bernardet, J.F., Nakagawa, Y., Holmes, B., 2002. Proposed minimal standards for describing  
519 new taxa of the family Flavobacteriaceae and emended description of the family.  
520 *International Journal of Systematic and Evolutionary Microbiology*, 52, 1049-1070.
- 521 5. Chefetz, B., Chen, Y., Hadar, Y., 1998. Purification and characterization of laccase from  
522 *Chaetomium thermophilum* and its role in humification. *Applied and Environmental*  
523 *Microbiology*, 64, 3175-3179.
- 524 6. Cochran-Stafira, D.L., von Ende, C.N., 1998. Integrating bacteria into food webs: Studies  
525 with *Sarracenia purpurea* inquilines. *Ecology*, 79, 880-898.
- 526 7. Fathallah Eida, M., Nagaoka, T., Wasaki, J., Kouno, K., 2013. Phytate degradation by fungi

- 527 and bacteria that inhabit sawdust and coffee residue composts. *Microbes and environments*,  
528 28, 71-80.
- 529 8. Hamdali, H., Hafidi, M., Virolle, M.J., Ouhdouch, Y., 2008. Growth promotion and  
530 protection against damping-off of wheat by two rock phosphate solubilizing actinomycetes in  
531 a P-deficient soil under greenhouse conditions. *Applied Soil Ecology*, 40, 510-517.
- 532 9. Huang, G.F., Wu, Q.T., Wong, J.W.C., Nagar, B.B., 2006. Transformation of organic matter  
533 during co-composting of pig manure with sawdust. *Bioresource Technology*, 97, 1834-1842.
- 534 10. Huang, Y., Sun, Z., Zhong, X., Wang, T., Tan, L., Tang, Y., Kida, K., 2017. Aerobic  
535 composting of digested residue eluted from dry methane fermentation to develop a  
536 zero-emission process. *Waste management*.
- 537 11. Ivors, K.L., Collopy, P.D., Beyer, D.M., Kang, S., 2000. Identification of bacteria in  
538 mushroom compost using ribosomal RNA sequence. *Compost Science & Utilization*, 8,  
539 247-253.
- 540 12. Lu Zhang, X.S., 2014. Changes in physical, chemical, and microbiological properties during  
541 the two-stage co-composting of green waste with spent mushroom compost and biochar.  
542 *Bioresource Technology*.
- 543 13. Mehta, C.M., Palni, U., Franke-Whittle, I.H., Sharma, A.K., 2014. Compost: Its role,  
544 mechanism and impact on reducing soil-borne plant diseases. *Waste Management*, 34,  
545 607-622.
- 546 14. Oguntoyinbo, F.A., 2011. Culture-independent analysis for determination of yeast diversity  
547 during solid substrate fermentation of grated cassava for gari production. *World Journal of*  
548 *Microbiology & Biotechnology*, 27, 2461-2465.

- 549 15. Ogunwande, G.A., Osunade, J.A., Adekalu, K.O., Ogunjimi, L.A.O., 2008. Nitrogen loss in  
550 chicken litter compost as affected by carbon to nitrogen ratio and turning frequency.  
551 *Bioresource Technology*, 99, 7495-7503.
- 552 16. Park, S., Akira, Y., Kogure, K., 2014. The Family Rhodothermaceae; The Family  
553 Rhodothermaceae, pp. 849-856.
- 554 17. Perry, B.A., Hansen, K., Pfister, D.H., 2007. A phylogenetic overview of the family  
555 Pyronemataceae (Ascomycota, Pezizales). *Mycological Research*, 111, 549-571.
- 556 18. Ren, G., Xu, X., Qu, J., Zhu, L., Wang, T., 2016. Evaluation of microbial population  
557 dynamics in the co-composting of cow manure and rice straw using high throughput  
558 sequencing analysis. *World Journal of Microbiology and Biotechnology*, 32.
- 559 19. Rui Wang, J.Z.Q.S., Yuansong Wei, D.W., 2016. Effect of red mud addition on tetracycline  
560 and copper resistance genes and microbial community during the full scale swine manure  
561 composting. *Bioresource Technology*.
- 562 20. Sun, L.Y., Gong, K.C., 2001. Silicon-based materials from rice husks and their applications.  
563 *Industrial & Engineering Chemistry Research*, 40, 5861-5877.
- 564 21. Teixeira, L.M., Merquior, V.L.C., 2014. The Family Moraxellaceae; The Family  
565 Moraxellaceae, pp. 443-476.
- 566 22. Tingting, Z., Xiaojing, H.U., Haidong, G.U., Yu, J., Juanjuan, Q.U., 2014. Biosorption of  
567 Cu<sup>2+</sup> by *Auricularia auricula* spent substrate and its mechanism. *Acta Scientiae*  
568 *Circumstantiae*, 34, 1421-1428.
- 569 23. Tiquia, S.M., Tam, N., Hodgkiss, I.J., 1996. Effects of composting on phytotoxicity of spent  
570 pig-manure sawdust litter. *Environmental Pollution*, 93, 249-256.

- 571 24. Villar, I., Alves, D., Garrido, J., Mato, S., 2016. Evolution of microbial dynamics during the  
572 maturation phase of the composting of different types of waste. *Waste Management*, 54,  
573 83-92.
- 574 25. Xi, B., He, X., Dang, Q., Yang, T., Li, M., Wang, X., Li, D., Tang, J., 2015. Effect of  
575 multi-stage inoculation on the bacterial and fungal community structure during organic  
576 municipal solid wastes composting. *Bioresource Technology*, 196, 399-405.
- 577 26. Yamada, T., Sekiguchi, Y., Hanada, S., Imachi, H., Ohashi, A., Harada, H., Kamagata, Y.,  
578 2006. *Anaerolinea thermolimosa* sp nov., *Levilinea saccharolytica* gen. nov., sp nov and  
579 *Leptolinea tardivitalis* gen. nov., so. nov., novel filamentous anaerobes, and description of the  
580 new classes anaerolineae classis nov and Caldilineae classis nov in the bacterial phylum  
581 Chloroflexi. *International Journal Of Systematic And Evolutionary Microbiology*, 56,  
582 1331-1340.
- 583 27. Zhang, L., Ma, H., Zhang, H., Xun, L., Chen, G., Wang, L., 2015. *Thermomyces lanuginosus*  
584 is the dominant fungus in maize straw composts. *Bioresource Technology*, 197, 266-275.
- 585 28. Zhang, L., Sun, X., Tian, Y., Gong, X., 2013. Effects of brown sugar and calcium  
586 superphosphate on the secondary fermentation of green waste. *Bioresource Technology*, 131,  
587 68-75.
- 588 29. Zhang, N., Castlebury, L.A., Miller, A.N., Huhndorf, S.M., Schoch, C.L., Seifert, K.A.,  
589 Rossman, A.Y., Rogers, J.D., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Sung, G., 2006. An  
590 overview of the systematics of the Sordariomycetes based on a four-gene phylogeny.  
591 *Mycologia*, 98, 1076-1087.
- 592 30. ZHAO Hong-yan, L.J.L.J., 2013. Microbial Community Dynamics During Biogas Slurry and

- 593 Cow Manure Compost. *Journal of Integrative Agriculture*.
- 594 31. Zhao, X., He, X., Xi, B., Gao, R., Tan, W., Zhang, H., Li, D., 2016. The evolution of water  
595 extractable organic matter and its association with microbial community dynamics during  
596 municipal solid waste composting. *Waste Management*, 56, 79-87.
- 597 32. Zhou, Y., Selvam, A., Wong, J.W.C., 2016. Effect of Chinese medicinal herbal residues on  
598 microbial community succession and anti-pathogenic properties during co-composting with  
599 food waste. *Bioresource Technology*, 217, 190-199.
- 600 33. Zhu, H., Sun, L., Zhang, Y., Zhang, X., Qiao, J., 2012. Conversion of spent mushroom  
601 substrate to biofertilizer using a stress-tolerant phosphate-solubilizing *Pichia farinose* FL7.  
602 *Bioresource Technology*, 111, 410-416.
- 603 34. Zhu, J., Tang, L., Liu, X., Li, S., Gao, W., Li, H., 2010. Effects of pigs manure on high  
604 temperature compost maturity of tobacco fine waste. *Journal of Agro-Environment Science*,  
605 29, 779-784.
- 606 35. Castillo, J. M., Romero, E., & Nogales, R. 2013. Dynamics of microbial communities related  
607 to biochemical parameters during vermicomposting and maturation of agroindustrial  
608 lignocellulose wastes. *Bioresource Technology*, 146(10), 345-354.
- 609

610 Table 1 Characteristics of physical-chemical during composting

	TOC(%)	TN(%)	C/N	TP(%)	TK(%)	GI(%)
SMS						
0d	38.94±0.02bc	2.04±0.08e	19.10±0.78bc	2.38±0.02ab	2.46±0.03a	81±7.07abc
5d	37.07±1.40ab	2.07±0.01e	17.86±0.73b	2.65±0.08b	2.69±0.13a	79±1.41abc
10d	43.67±0.19d	1.07±0.08a	40.93±3.07e	2.33±0.03a	4.19±0.06b	84±2.83bc
25d	41.28±2.74cd	1.52±0.04bc	27.20±2.56d	2.49±0.06ab	4.05±0.06b	83±1.41abc
76d	38.69±0.59bc	1.46±0.02b	26.60±0.79d	3.00±0.05c	4.95±0.30c	80±2.83abc
SMS-PM						
0d	35.66±3.07ab	2.53±0.08f	14.09±1.64a	10.85±0.15e	9.43±0.80fg	73±1.41a
5d	39.22±1.11bc	1.87±0.01d	20.92±0.51bc	8.63±0.25d	8.14±0.06e	81±1.41abc
10d	43.51±0.82d	3.88±0.06g	11.22±0.38a	10.85±0.15e	9.09±0.10ef	75±1.41ab
25d	38.83±0.56bc	1.84±0.04d	21.16±0.10bc	11.31±0.18f	8.65±0.49de	77±9.90abc
76d	34.92±0.10a	1.62±0.10c	21.59±1.26c	12.53±0.09g	9.94±0.05g	87±1.41c

611 Mean values ± standard error. In columns, values followed by the same letter are not statistically  
612 different according to the Duncan test at P < 0.05.

613

Table 2 Fungal community evolution during composting (D0, day 0; D76, day 76)

Phylum	Class	Family	Gene	D0_SMS	D0_SMS-PM	D76_SMS	D76_SMS-PM
Ascomycota	Sordariomycetes	Sordariomycetes_unclassified	Sordariomycetes_unclassified	17.39%	2.37%	64.95%	2.81%
		Chaetomiaceae	Chaetomium	6.30%	0.67%	9.75%	63.02%
		Microascaceae	Microascaceae_unclassified	3.77%	0.97%	0.48%	6.24%
		Hypocreales_unclassified	Hypocreales_unclassified	1.13%	0.04%	0.34%	0.07%
	Saccharomycetes	Saccharomycetaceae	Issatchenkia	0.00%	11.92%	0.00%	0.00%
		Dipodascaceae	Galactomyces	0.05%	78.95%	0.03%	0.56%
	Eurotiomycetes	Incertae_Sedis	Thermomyces	0.67%	0.27%	10.42%	1.65%
	Pezizomycetes	Pyronemataceae	Pezizales_unclassified	10.11%	0.20%	0.26%	0.71%
			Pyronemataceae_unclassified	0.22%	0.04%	1.74%	0.29%
		Ascomycota_unclassified	Ascomycota_unclassified	Ascomycota_unclassified	0.55%	0.01%	0.11%
Ciliophora	Intramacronucleata	Colpodea	Colpodea_unclassified	51.50%	0.95%	3.13%	19.50%
Basidiomycota	Agaricomycetes	Auriculariaceae	Auricularia	4.10%	0.24%	0.80%	0.14%
		Agaricomycetes_unclassified	Agaricomycetes_unclassified	1.66%	0.56%	0.21%	0.04%
	Tremellomycetes	Tremellales_unclassified	Tremellales_unclassified	0.04%	1.46%	0.01%	0.08%
Schizoplasmodiida	Schizoplasmodiida_norank	Schizoplasmodiida_norank	Schizoplasmodium	0.02%	0.00%	3.92%	0.18%
Centrohelida	Centrohelida_norank	Centrohelida_norank	Centrohelida_norank	0.00%	0.00%	1.31%	0.00%
	Eukaryota_unclassified	Eukaryota_unclassified	Eukaryota_unclassified	0.16%	0.12%	0.31%	1.04%
			Others	2.34%	1.23%	2.22%	1.60%

This table is red-yellow-green color gradation table, red color means higher value and green color means lower value.



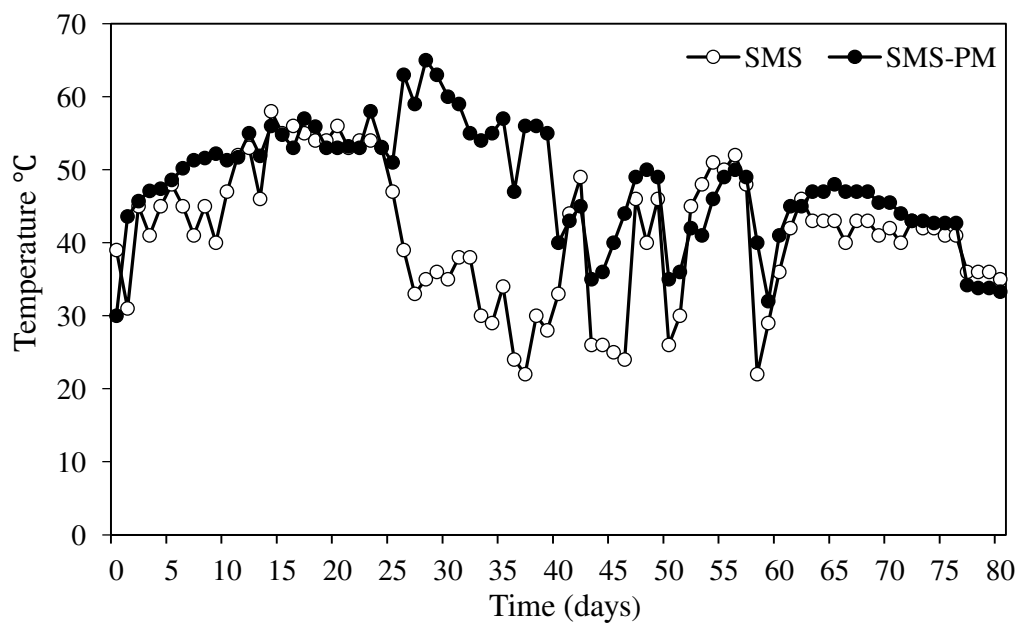


Fig. 1. Changes in temperature during the composting period

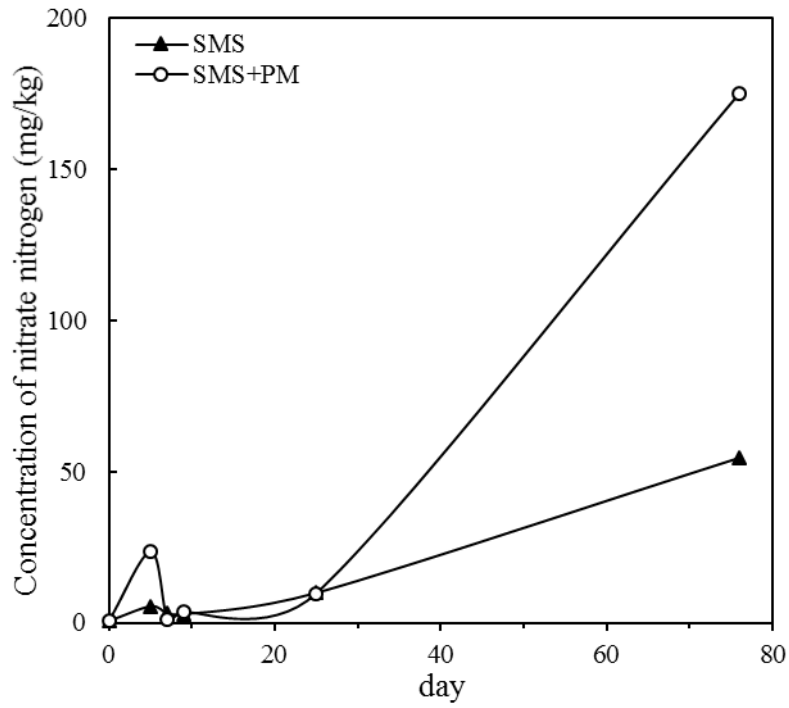


Fig. 2. Changes in the concentration of  $\text{NO}_3\text{-N}$  during the composting period.

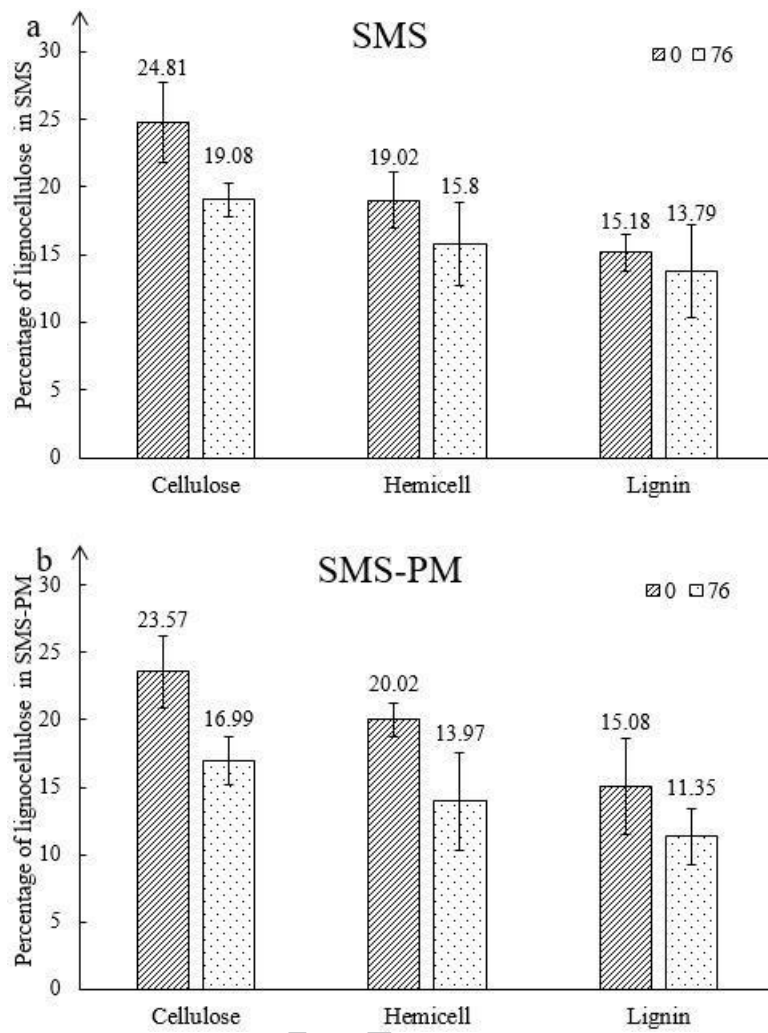


Fig. 3. Degradation rate of cellulose, hemicell and lignin. a: the percentage of cellulose, hemicell and lignin in SMS compost in day 0 and 76; b, the percentage of cellulose, hemicell and lignin in SMS-PM compost in day 0 and 76

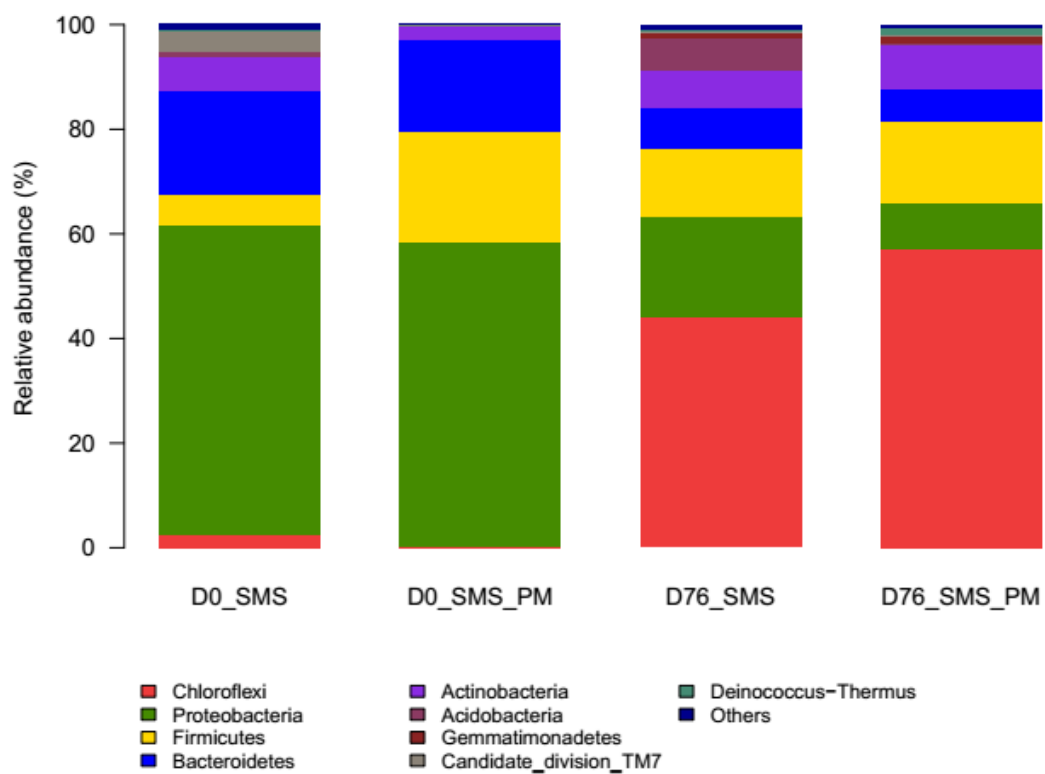


Fig. 4. Bacterial community evolution during composting (phylum level) (D0, day 0; D76, day 76)

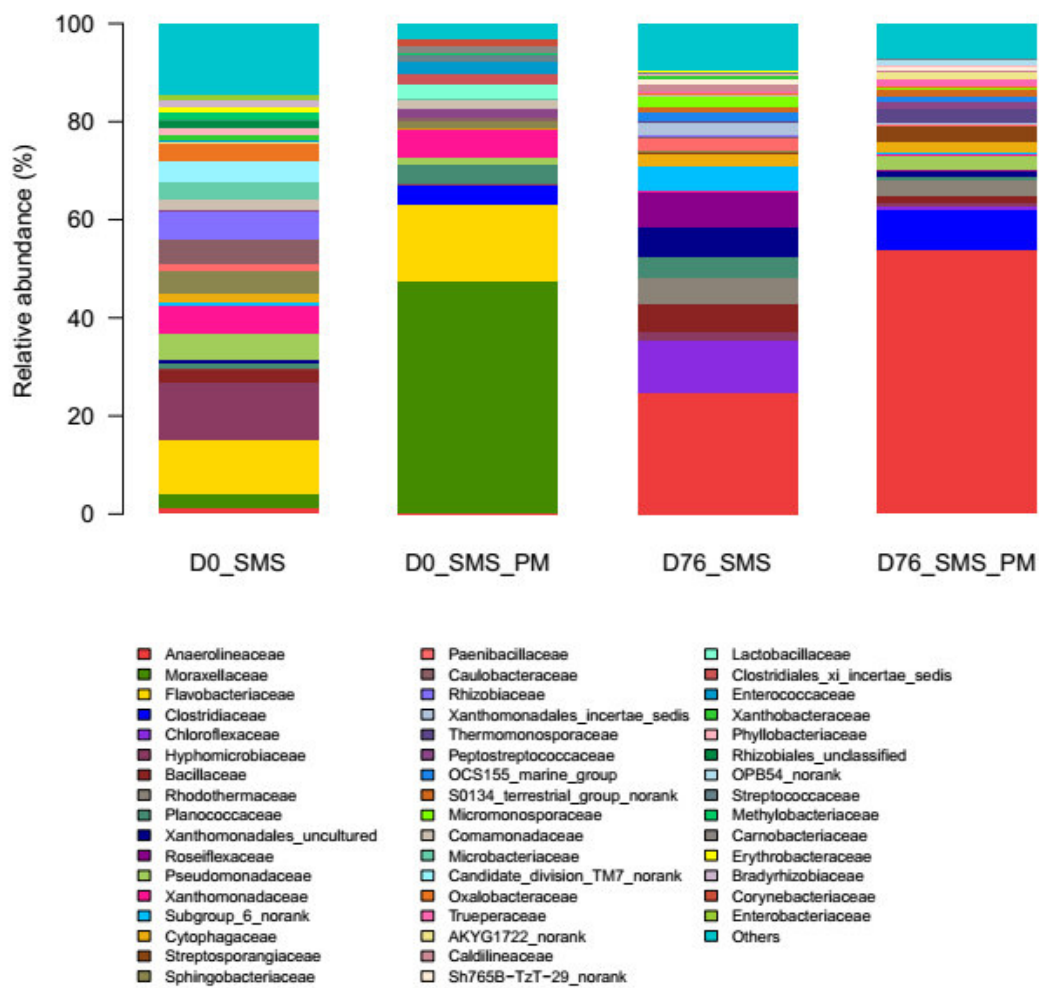


Fig. 5. Bacterial community evolution during composting (family level). (D0, day 0; D76, day 76)

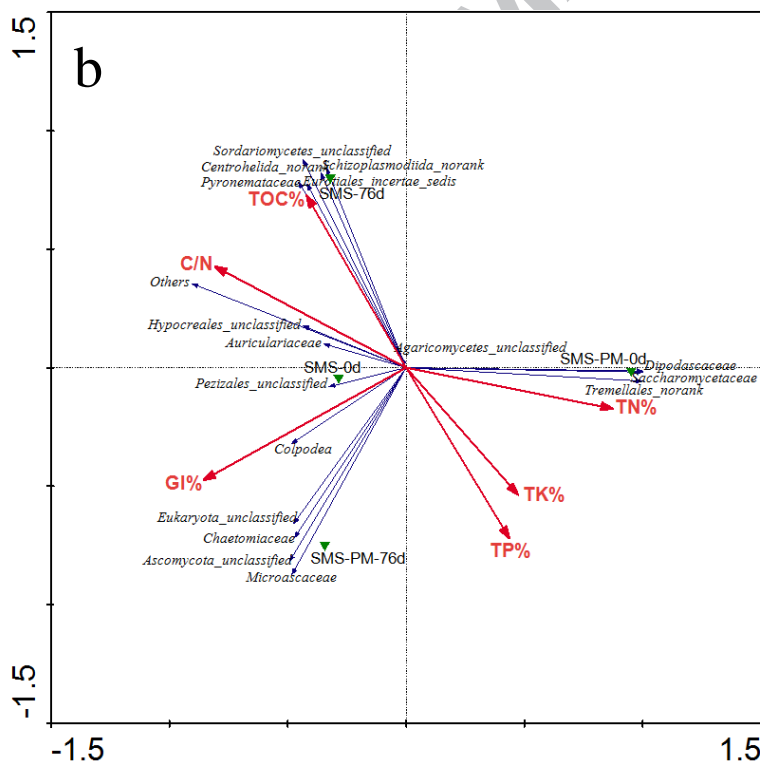
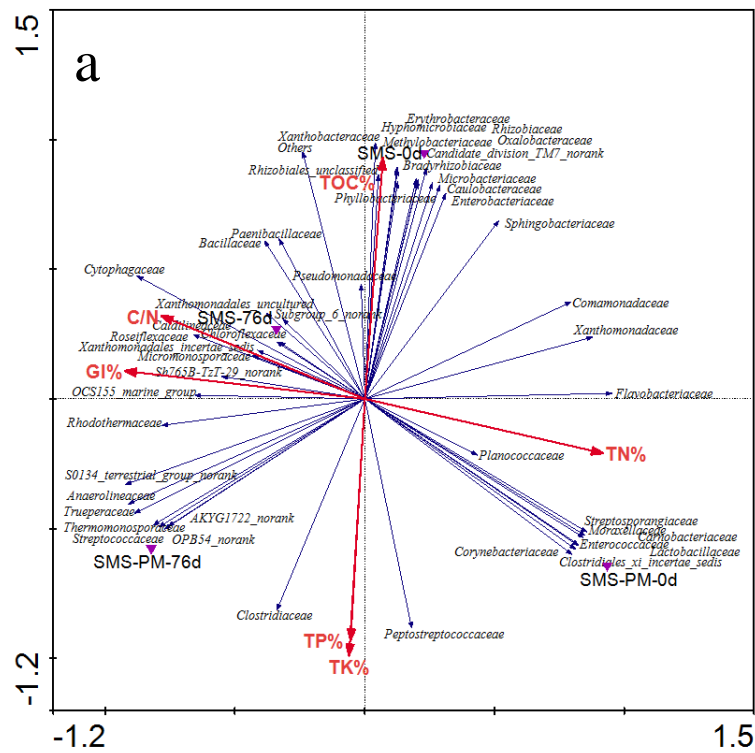


Fig. 6. Redundancy analysis assessing the relationship between environmental factors (red arrows) and microbial communities (blue arrows). Correlations between environmental variables and RDA axes were represented by the length and angle of arrows. (a, environmental factors and bacterial community; b, environmental factors and fungal community)

## Highlights

Pig manure expedited and extended the high temperature period during composting.

The addition of pig manure increased the nutrition content of N, P, K and Gl.

Pig manure provided the essential bacteria as inoculum.

Pig manure enriched anti-pathogens at the end of composting.

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