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1 **Changes and composition of microbial community during aerobic composting of**
2 **household food waste**
3
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ABSTRACT

In order to explore the effect of added bacteria on microbial community and determine the dominant bacteria in the aerobic composting process of household food waste (HFW), two groups of HFW composting experiments were conducted for 30 days. The final degradation rates of the two groups were 69.95% (group A, natural composting) and 73.52% (Group C, composting with added bacteria), respectively. 16S rRNA high-throughput sequencing was used to analyze the changes of microbial community in the composting process. As the result displays, at the classification of phylum level， the relatively abundant bacteria of two groups were *Firmicutes, Proteobacteria* and *Cyanobacteria*. At the classification of genus level, *Pediococcus* is the dominant bacteria of group A, which performed better in maintaining the microbial community stable in the later stage of composting, while *Weissella* accounted for a relatively large proportion of group C and behaved well in promoting the final degradation rate of composting. The proportion of *Ochrobactrum* in the early stage of group C is considerable and it is closely related to the removal of odour during composting. The relative abundance of added bacteria was always in a rather low level， suggested that the way they affect the composting process is to change the proportion of dominant bacteria in different stage of composting. This experiment provides an important reference for improving the microbial degradation efficiency of HFW .

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47 **IMPORTANCE**

48 In recent years, food waste has gradually become a global problem, the annual waste
49 of food is as high as 1.3 billion tons. FW, especially HFW, as a high content of
50 organic matter waste, has a pretty good recycling value. So how to deal with and
51 recycle it efficiently, quickly and conveniently becomes more and more important.
52 Among many treatment and recovery methods, microbial treatment (including
53 anaerobic digestion and aerobic composting) has gradually become a research hotspot
54 due to its advantages of low pollution and low energy consumption, and
55 microorganisms play a crucial role in these process.
56 In this study we use 16S rRNA high-throughput sequencing method to explore aerobic
57 composting of HFW. The purpose of this study is to find out the dominant bacteria
58 which can improve the degradation efficiency, remove the odor and prolong the
59 treatment cycle, and then provide further theoretical reference for future HFW related
60 research.

61 **INTRODUCTION**

62 With the improvement of people's living standards , the proportion of food waste
63 (FW) is also increasing year by year, being considered to be one of the largest groups
64 of organic solid waste in the world, and its generation rate is rising steadily (1,2). FW
65 mainly comes from household kitchens, restaurants, canteens, and food processing
66 industries (3). Among them, household food waste (HFW) is getting much attention
67 owing to its high organic content and excellent source of value added products (4).
68 About 1.3 billion tons of edible food are lost or wasted every year in the world (5), So
69 the effective and harmless treatment of FW has always been the focus of global

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70 attention (6,7).

71 At present, the treatment methods of FW mainly include direct crushing, dehydration,

72 chemical treatment, incineration and microbial treatment etc(8). Most of these

73 methods consume high energy and even cause secondary pollution to the environment

74 except microbial treatment (9). Therefore, microbial treatment of FW has become a

75 research hotspot at home and abroad. Microbial treatment methods include anaerobic

76 digestion and aerobic composting, both are the process of decomposing complex

77 organic matter into small molecule organic or inorganic matter by the metabolism of

78 microorganisms (10). This process can be realized not only by indigenous

79 microorganisms naturally growing in FW, but also by bacteria added during

80 composting (11). Anaerobic digestion and aerobic composting have good resource

81 recovery properties, and have the ability to produce high value-added products (12).

82 Thus, microbial treatment methods present a good impetus and vast potential for

83 future development (13). For instance, Quashie Frank Koblah, et al. use a continuous

84 stirred microbial electrolysis cell (CSMEC) with anaerobic digestion to deal with FW

85 and produce methanation and bioelectricity. The final COD removal of CSMEC

86 surpassed 92% with OLR (organic load rate) ranging from 0.4 to 21.31 kg

87 COD/m^3·d,and *Firmicutes*, *Proteobacteria*, and *Euryarchaeota* were the dominant

88 phyla observed (14). The natural composting method was implemented by Tran Huu

89 Tuan et al. to study the degradation effect of high concentration of dietyl terephthalate

90 (DOTP) FW by natural microorganisms proliferating during composting. The total

91 degradation efficiency of DOTP reached 98%,and *Firmicutes* was the most dominant

92 at the phylum level, followed by *Proteobacteria* and *Bacteroidetes* (15).Huang WY et

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93 al. determined *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Firmicutes* as the
94 main phyla during aerobic FW co-composting degradation of highly PCDD/F-
95 contaminated field soil (16). Wang et al. reported that the dominant phyla of the
96 community structure in fed-batch composting were *Firmicutes*, *Proteobacteria*,
97 *Bacteroidetes*, and *Actinobacteria* by high-throughput sequencing (17) .
98 Composting is an effective process for the valorisation of HFW into a stable and
99 nutrient-enrich biofertiliser (18), in which a variety of microorganisms play a critical
100 role (19). HFW is a kind of non-uniform material with high content of water, oil, salt
101 and cellulose, etc (20). Gaseous emissions, such as methane (CH_4), nitrous oxide
102 (N_2O), and ammonia (NH_3) which hinder composting application for food waste
103 treatment (21), are inherent by-products of the composting process regardless of the
104 initial organic material or process condition (22). These particular characteristics will
105 affect some aspects of the composting quality and process. As microorganisms play a
106 key role in the composting process, inoculating microorganisms with specific
107 functions may have a positive effect on the composting process and improve the
108 composting quality. So far, there are few studies on the changes of microbial
109 community in the process of HFW composting. In this study, 16SrRNA high-
110 throughput sequencing was used to analyze the microbial community changes and
111 composition in different stages of natural composting and composting with added
112 bacteria, aiming to find out the dominant bacteria which can remove odor, improve
113 the degradation rate and prolong the fermentation cycle in the composting process, so
114 as to provide a theoretical reference for the future aerobic composting process of
115 HFW.

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116 **RESULT AND DISCUSSION**

117 **Analysis of degradation rate and viable count.**

118 Table 1 shows the changes of related parameters in different stages of composting
119 process. The final degradation rates of group A and group C were 69.95% and 73.52%
120 respectively. From the perspective of degradation rate, there was no significant
121 difference between group A and group C. However, from the perspective of specific
122 composting process, during stage 1-3, the odor produced by group C was significantly
123 less than that by group A, and the composting residue of group C became looser and
124 drier, which is helpful for the follow-up treatment of HFW. In terms of the proportion
125 of composting residue in the machine volume, group C showed a good degradation
126 efficiency in each stage of composting process, being consistent with the result of
127 final degradation rate. From the change of pH in the composting, with the continuous
128 addition of HFW, the pH values in the machine of two groups decreased gradually,
129 while the pH value increased during 10 days naturally storage after the composting
130 process, indicating that the composting process of HFW was an acidic process, which
131 may be more suitable for the growth of some acidophilic strains. The total number of
132 viable bacteria in group A was larger than that in group C. During the process from
133 stage 1 to stage 2, the number of viable bacteria in group A decreased while that in
134 group C increased, which may be as a result of the inhibitory effect between the added
135 bacteria and the bacteria naturally growing during the composting process. In the later
136 stage, the microbial community gradually stabilized and the number of viable bacteria
137 began to increase, and then gradually decreased. The number of viable bacteria in the
138 two groups decreased slowly from stage 3 to stage 5. The reason for this phenomenon

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139 may be that the decline of pH value changed the composting environment of the
140 microbial community, at the same time, the increase of composting residue in the
141 machine volume leads to the decrease of oxygen content in the machine, leading to
142 the decrease of aerobic composting bacteria. These two factors may be the key points
143 of the decreasing of viable bacteria during stage 3-5.

144 **Analysis of sample sequencing data.**

145 As shown in Table 2, after obtaining the original sequence data of different samples
146 by Illumina high-throughput sequencing with optimization and statistics, the final
147 number of effective sequences of 10 samples was 1286075, and the range of effective
148 sequences of each sample was 121536-133111, of which 647227 were obtained from
149 5 samples in group A and 638848 were obtained from 5 samples in group C. The
150 average sequence length was 422.95 bp. Based on 97% sequence homology, a total of
151 6626 OTUs were obtained, including 4144 OTUs in group A, and 3282 OTUs in
152 group C. After filtering out the OTUs containing Chloroplast, 5949 OTUs were
153 obtained, including 3804 OTUs in group A and 2945 OTUs in group C. The results of
154 Venn diagram (Fig.1) showed that there were 800 OTUs shared by these two groups,
155 accounting for 19.31% of group A and 24.38% of group C, respectively. By
156 comparison, it was found that there was a significant difference in OTUs between the
157 two groups, which might be due to the effect of the added bacteria on the composition
158 and structure of microbial community.

159 **Analysis of alpha diversity index of samples.**

160 Single sample diversity analysis (alpha diversity) reflects the abundance and diversity
161 of microbial community. The alpha diversity index of each group is shown in Table 3.

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162 The results showed that the Chao1 index and Shannon index of group A increased
163 steadily, and reached the maximum value in A5. The Simpson index of group A
164 reached 0.7867, 0.8860 and 0.8823 in A3, A4 and A5, respectively, indicating that the
165 dominant bacteria of group A could be in a stable position for a long time in the
166 middle and late composting period. The Simpson index could still maintain a high
167 level in A5, indicating that the microbial community of group A has a good ability to
168 maintain self-stabilization, which is very important for the extension of composting
169 cycle. In group C, the Chao1 index and Shannon index of the microbial community
170 fluctuated greatly, decreased firstly and then increased, and finally reached the
171 maximum value of 1632 and 6.61 respectively in C3. The Chao1 index and Simpson
172 index decreased significantly in C4 stage, which indicated that the microbial
173 community abundance decreased greatly and the dominant strains maintained stable
174 only for a short time. The Chao1 index and Simpson index in C5 stage continued to
175 decline, indicating that the ability of group C to maintain community stable was not as
176 good as that of group A. The sequencing coverage of samples in each group was
177 above 0.99, indicating that the sequencing had high coverage of bacteria in samples
178 and suitable sequencing depth, which could meet the needs of bacterial diversity
179 analysis in each group. Fig. 2 A and B show the changes of coverage index and
180 Shannon index with the increase of sequencing depth, respectively. It can be seen
181 from the Fig. 2 that the coverage index and Shannon index in each group of samples
182 gradually increased and tend to be flat with the increasing of sequencing depth, which
183 further indicates that the sequencing results can more comprehensively reflect the
184 diversity information contained in these samples.

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185 **Analysis of bacteria composition during composting process at phylum level.**

186 Fig. 3 shows the bacteria composition in different stages at the level of phyla
187 classification. The main bacteria phyla in stage1 is: A1: *Proteobacteria* (98.81%),
188 *Firmicutes* (0.64%); C1: *Proteobacteria* (90.03%), *Firmicutes* (5.86%),
189 *Actinobacteria* (2.66%). The relative abundance of *Proteobacteria* in the two groups
190 of samples in stage1both exceeded 90%, indicating that *Proteobacteria* is in an
191 absolutely dominant position in both A1 and C1. Many bacteria of *Proteobacteria* are
192 pathogenic (23),such as *Cronobacter* (24), *Burkholderia* (25,26) and *Ochrobactrum*
193 (27). The main bacteria phyla in stage2 is: A2: *Firmicutes* (96.23%), *Proteobacteria*
194 (3.07%); C2: *Firmicutes* (96.60%), *Proteobacteria* (2.62%). In stage 2, the type and
195 proportion of bacteria phyla in A2 and C2 periods were almost the same and the
196 proportion of *Firmicutes* rose rapidly from stage 1 to stage 2 and replaced
197 *Proteobacteria* as the dominant bacteria phyla. The phylum *Firmicutes* includes
198 Gram-positive bacteria with rigid or semi-rigid cell walls that are predominantly from
199 the genera *Bacillus*, *Clostridium*, *Enterococcus*, *Lactobacillus* and *Ruminicoccus* (28).
200 Moreover, many *Firmicutes* produce spores that are resistant to dehydration and
201 extreme environments (29), which may be one of the reasons why group A had a
202 strong ability to maintain stable in the late stage, of which the proportion of
203 *Firmicutes* was more than 80%. The main bacteria phyla in stage3 is: A3: *Firmicutes*
204 (66.67%), *Cyanobacteria* (26.73%), *Proteobacteria* (4.89%); C3: *Firmicutes*
205 (49.09%), *Proteobacteria* (40.84%), *Actinobacteria* (2.70%), *Bacteroidetes* (2.70%),
206 *Acidobacteria* (1.19%). The emergence of *Cyanobacteria* in A3 led to a decrease in
207 the proportion of *Firmicutes*. Those bloom-forming *Cyanobacteria* may have a certain

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208 inhibitory effect on pathogenic bacteria of *Proteobacteria*, because some genera of it
209 possess the ability to produce various toxic or bioactive secondary metabolites, e.g.
210 toxic microcystins (MCs) and bioactive anabaenopeptins (APs) which is linked to
211 antibacterial, fungal or cytostatic effects (30). In contrast, the proportion of
212 *Proteobacteria* in C3 period increased greatly without *Cyanobacteria* existing, further
213 proving that the emergence of *cyanobacteria* in A3 could inhibit the proliferation of
214 the pathogenic bacteria of *Proteobacteria*. The main bacteria phyla in stage4 is: A4:
215 *Firmicutes* (93.66%), *Proteobacteria* (2.34%), *Cyanobacteria* (1.90%); C4:
216 *Firmicutes* (78.89%), *Cyanobacteria* (15.64%), *Proteobacteria* (4.64%). *Firmicutes*
217 still occupied the dominant position in A4, while the proportion of *Proteobacteria* in
218 C4 decreased significantly with a small amount of *Cyanobacteria* appeared, which
219 confirmed once again the inhibitory effect of *Cyanobacteria* on pathogenic bacteria of
220 *Proteobacteria*. The main bacteria phyla in stage 5 is: A5: *Firmicutes* (82.30%),
221 *Cyanobacteria* (6.87%), *Proteobacteria* (5.08%), *Bacteroidetes* (2.93%),
222 *Actinobacteria* (2.15%); C5: *Firmicutes* (54.40%), *Proteobacteria* (2.44%),
223 *Cyanobacteria* (42.61%). The proportion of *Firmicutes* in A5 was about 30% more
224 than that in C5, indicating that the relative abundance of dominant phyla in group A
225 could still maintain at a high level after ten days of storage, indicating that group A
226 had a good ability to maintain community stable.

227 **Analysis of bacteria composition during composting process at genus level.**

228 As shown in Fig. 4, at the level of genus classification, the relatively abundant
229 bacterial genera in the A1 are *Cronobacter* (57.44%),
230 unclassified_*Enterobacteriaceae* (24.75%), *Burkholderia-Caballeronia-*

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231 *ParaBurkholderia* (15.24%). These three genera belonging to *Proteobacteria* are all
232 opportunistic pathogens. Now, 10 individual species of *Cronobacter* have been
233 identified (31,32),which are associated with necrotizing enterocolitis, septicemia, and
234 meningitis (33). *Enterobacteriaceae* family can contaminate fresh products at any
235 stage of production either at pre-harvest or post-harvest stages (34). Many species of
236 *Burkholderia* are opportunistic pathogens, such as *Burkholderia pseudomallei* that
237 causes melioidosis (35) and *Burkholderia cepacia* that causes cystic fibrosis in human
238 lungs (36,37). The relatively abundant bacterial genera in C1 is *Ochrobactrum*
239 (84.61%), and the proportion of added bacteria is： *Bacillus* (1.89%), *Pseudomonas*
240 (0.24%),*Paenibacillus* (0.01%).Similarly, *Ochrobactrum* also belongs to the
241 *Proteobacteria*, and several *Ochrobactrum* spp. have been investigated for their
242 potential to degrade xenobiotic pollutants and for heavy metal detoxification under a
243 variety of environmental conditions (38). Moreover, the odour of group C in the early
244 stage of composting was significantly smaller than that of group A. Therefore,
245 *Ochrobactrum* may play a better role in degradation and deodorization of HFW in the
246 early stage of composting. However, in the early stage, the proportion of added
247 bacteria is less, which may be caused by the small initial addition or the inhibition of
248 the growth of pathogenic bacteria. It can be clearly seen that the types and proportions
249 of the dominant bacteria in the two groups in the early stage of composting are quite
250 different, but at the level of phylum classification they all belong to *Proteobacteria*.
251 The reason for this result may be that the presence of added bacteria affects the
252 proportion of the dominant bacteria during the process of composting. The relatively
253 abundant bacteria genera in the A2 are *Pediococcus* (93.12%), *Klebsiella* (1.40%),

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254 while that in the C2 are *Pediococcus* (94.82%), *Ochrobactrum* (1.29%), and the
255 proportion of added bacteria is: *Bacillus* (0.38%), *Pseudomonas* (0.021%),
256 *Paenibacillus*(0.02%). Obviously different from the first stage, the dominant bacteria
257 in both A2 and C2 are *Pediococcus* belonging to *Firmicutes* and the relative
258 abundance of them are both more than 90%. Literature review shows that
259 *Pediococcus* can secrete pediocins which are short peptides with strong antibacterial
260 activity, high temperature resistance, and acid resistance in the process of proliferation
261 (39). It is noteworthy that the proportion of pathogenic bacteria in A2 and C2 drops
262 dramatically. It is inferred that the pediocins secreted by *Pediococcus* inhibit and
263 control the opportunistic pathogens at a low level. Meanwhile,the proportion of added
264 bacteria is still maintained at a relatively low level, which may be due to the
265 inhibitory effect of the proliferation of *Pediococcus*. The relatively abundant bacterial
266 genera in A3 are *Pediococcus* (57.01%), *Bacillus* (2.53%), *Enterococcus* (1.33%), and
267 some Chloroplast (26.73%). The proportion of *Pediococcus* in A3 decreased to a
268 certain extent, and a small amount of Chloroplast appeared, which may be as a result
269 of incomplete degradation of vegetables in HFW. The main genera of C3 are
270 *Pediococcus* (20.73%), *Lactobacillus* (10.10%), *Proteus* (8.64%), *Acinetobacter*
271 (7.65%), *Klebsiella* (7.08%) and *Oceanobacillus* (6.74%). Among these genera,
272 *Proteus*, *Acinetobacter* and *Klebsiella* are all opportunistic pathogen. *Proteus* consists
273 of five species and three unnamed subspecies. Among these species, *P. vulgaris* and *P.*
274 *mirabilis* are most frequently linked with food contamination and food poisoning
275 (40,41).The non-fermentative bacteria of *Acinetobacter* are opportunistic
276 pathogens,for instance,*Acinetobacter Baumannii* has emerged as one of the most

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277 problematic common opportunistic nosocomial pathogens worldwide (42). The most
278 common species of *Klebsiella* is *Klebsiella Pneumoniae*, a conditional pathogen that
279 can settle in human skin and mucous membranes to cause urinary tract infections,
280 sepsis and pneumonia in individuals with weakened immunity (43,44).The
281 *Lactobacillus,* of which the relative abundance increased in C3, is a kind of probiotics
282 widely existing in human intestinal tract (45), and may play an important role in
283 inhibiting those opportunistic pathogens. The proportion of added bacteria in C3 is：
284 *Bacillus* (2.21%), *Pseudomonas* (0.68%), *Paenibacillus*(0.11%), increased slightly,
285 but still at a low level, which may be due to the low pH and high salt concentration. In
286 C3, the proportion of *Pediococcus* decreased significantly, and a large number of
287 miscellaneous bacteria appeared. The reason for this phenomenon may be that when
288 the proportion of *Pediococcus* decreased and *Weissella* did not occupy the dominant
289 position in the middle stage of composting, these pathogenic bacteria and
290 miscellaneous bacteria took this opportunity to proliferate and occupy a certain
291 proportion. As the proportion of *Weissella* increased in later stage, the relative
292 abundance of these miscellaneous bacteria decreased sharply to a very low level.
293 Members of the genus *Oceanobacillus* were aerobic, rod-shaped, endospore-forming,
294 halophilic bacteria (46). The increase of *Oceanobacillus* in C3 may be due to the
295 rising of salt concentration in the middle stage of composting. The relative abundance
296 of bacteria genera of Stage 4 was as follows, A4: *Pediococcus* (41.39%), *Weissella*
297 (34.97%), *Leuconostoc* (5.68%), *Bacillus* (2.08%), *Lactobacillus* (1.55%),
298 *Macrococcus* (1.55%); C4: *Weissella* (70.89%), *Leuconostoc* (3.07%), *Acinetobacter*
299 (1.16%), and added bacteria of *Bacillus* (0.46%), *Pseudomonas* (0.06%),

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300 *Paenibacillus* (0.05%). The proportion of *Weissella* in A4 and C4 increased greatly,
301 more than 70% in C4, indicating that *Weissella* played a greater role in the later stage
302 of composting. It should be noted that *Weissella* also belonged to *Firmicutes*. To this
303 day, 19 species of *Weissella* have been identified, such as *W. beninensis*, *W. ceti, W*
304 *cibaria*, *W. confuse* etc(47,48)]. Among them, *W. confuse* and *W. cibaria* have the
305 potential of anticancer, anti-inflammatory, antibacterial, antifungal and enhancing
306 immunity (49,50,51), which may be beneficial to the synergistic effect of
307 *Pediococcus* and *Weissella* in the middle and later stage of composting. The relative
308 abundance of bacteria genera of Stage 5 was as follows, A5: *Pediococcus* (47.51%),
309 *Weissella* (20.21%), *Bacillus* (2.60%), *Leuconostoc* (2.25%), *Bacteroides* (1.25%);
310 C5: *Weissella* (47.99%), *Leuconostoc* (3.86%), and added bacteria of *Bacillus*
311 (0.20%), *Pseudomonas* (0.11%), *Paenibacillus*(0.01%),and also some Chloroplast
312 (42.61%). Both A5 and C5 were the state of natural storage for 10 days after the end
313 of composting. In A5, *Pediococcus* still occupied the dominant position, followed by
314 *Weissella*. In C5 period, the proportion of *Weissella* decreased significantly, and a
315 large proportion of Chloroplast (42.61%) appeared, which may be caused by
316 incomplete degradation of vegetable wastes. Chloroplast has appeared in the
317 composting components since Stage 3, and always occupies a certain proportion
318 during stage3-stage5, indicating that the microbial bacteria is still relatively limited in
319 the degradation of vegetable waste. In the future composting process, strains with
320 strong cellulose degrading ability can be added and their comprehensive degradation
321 effect need to be determined. The proportion of added bacteria was maintained at a
322 low level during C1-C5, indicating that the added bacteria were not the dominant

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323 strains in the process of composting, and it is possible that the way they affect the
324 composting process is to change the relative abundance of dominant bacteria in
325 different stage of composting(Fig. S1).

326 **Analysis of Beta diversity.**

327 Through PCoA we can study the similarity and difference of data. Fig. 5 shows the
328 PCoA results of different samples in genus level. It can be seen from Fig. 5 that PC1
329 is the first principal coordinate component, and its contribution to the
330 representativeness of the total bacteria is 49.4%; PC2 is the second principal
331 component, and its contribution to the representativeness of the total bacteria is
332 26.6%; for the PC1-2 analysis of the principal component, A1 and C1 are farther apart
333 on the coordinate axis, indicating that the added bacteria may have a significant
334 impact on the composition of the microbial community at the early stage of the HFW
335 composting process. A2 and C2 are almost at the same position, indicating that the
336 microbial community composition of A2 and C2 at the genus level is almost the same
337 at this stage, and that the added bacteria species at this stage has little effect on the
338 microbial community. A4 and C4 are far apart on the coordinate axis, indicating that
339 the groups A and C have a large difference in the composting process in the later
340 stage. The added bacteria may affect the proportion of dominant bacteria in different
341 stages of the composting process to improve the composting rate and other critical
342 factors.

343 **CONCLUSION**

344 The above analysis shows that at phylum level, *Firmicutes*, *Proteobacteria* and
345 *Cyanobacteria* accounted for the largest proportion during the composting process of

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346 group A and C. At genus level, *Pediococcus* and *Weissella* were the dominant bacteria
347 of group A and C, and both of them belonged to *Firmicutes*. The abundance of the
348 added bacteria in the composting process of group C is always at a low level,
349 indicating that the added bacteria may affect the composting process by changing the
350 proportion of the dominant strains. The relative abundance of pathogenic bacteria
351 decreased to a very low level after *Pediococcus* and *Weissella* gradually increased and
352 occupied a dominant position in the middle stage of the two groups, indicating that
353 *Pediococcus* and *Weissella* might play a certain inhibitory role on pathogenic bacteria.
354 *Weissella* is the dominant bacteria in group C, and the final degradation rate of group
355 C is higher than that of group A, indicating that *Weissella* may play a positive role in
356 improving the degradation efficiency. In C1, the *Ochrobactrum* occupied a
357 considerable proportion and the smell during this stage is obviously better than that of
358 group A, indicating the *Ochrobactrum* may have an active impact on the removal of
359 peculiar smell. Group A performed better in stabilizing the microbial community in
360 the later stage and the *Pediococcus* took a rather high proportion during the
361 composting process of group A, indicating that *Pediococcus* have the ability of
362 maintaining system stable and prolonging the composting cycle.
363 In conclusion, the microbial communities of the two groups during the composting
364 process of HFW have a high degree of diversity. There are not only differences in
365 species distribution and relative abundance but also common core bacteria between
366 them. In this paper, the study of microbial community changes in the composting
367 process of HFW is helpful to explore the microbial resource pool, deepen the
368 understanding of the composting process of HFW, and is of great significance for the

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369 further harmless resource treatment of HFW.

370 MATERIALS AND METHODS

371 Materials.

372 The HFW was collected from the dining hall of Ningbo Tech University; The added
373 bacteria are: B2 (*Bacillus subtilis),* F4 (*Pseudomonas aeruginosa),* 303 (*Paenibacillus*
374 *lautus*), 792 (*Bacillus thuringiensis*), which are all preserved in our laboratory;
375 Sawdust; LB broth medium; 75510-019Agarose (Invitrogen); Non-fat milk powder;
376 NEB M0491L Q5® High-Fidelity DNA Polymerase (New England Biolabs (NEB);
377 P7598 Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen); DL2000 Marker (Takara);
378 AM9870 TAE (Invitrogen).

379 Equipment.

380 Small HFW treatment machine (Midea Group); Freeze dryer; pH meter; ABI 2720
381 PCR amplify instrument (ABI); FLX800T type enzyme labelling instrument (BioTek
382 Proton Instrument Co., Ltd.); DYY-6C electrophoresis apparatus (Beijing 61
383 Biological Technology Co., Ltd.); Microplate reader (BioTek, FLx800); BG-
384 gdsAUTO (130) gel imaging system (Beijing Bai Jing Biotechnology Co., Ltd.)

385 Preparation of bacteria freeze-dried powder.

386 These added bacteria which was preserved on slant culture medium were inoculated
387 200 ml LB medium (sterilized at 121 ° C for 20 min), and then cultured in a constant
388 shaker at 37 ° C, 200 rpm for 24 h. After culture, the broth was centrifugated at 4 ° C
389 for 15 min at 8000 rpm and the supernatant was discarded, and the bacteria precipitate
390 were thoroughly mixed with 5-10 ml of non-fat milk (sterilized at 100 ° C for 10 min)
391 and poured into the plate. After sealing the plastic wrap (poking several small holes)

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392 and pre freezing at - 4 ° C for 2 h, the plate was freeze-dried using a freeze dryer for
393 18 h, and then distributed into 10 ml EP tube (sterilized at 121 ° C for 20 min) and put
394 into 4 ° C freezer for further use (about 5 g of freeze-dried powder can be made for
395 every 200 ml of broth, the viable bacterial count of freeze-dried powder was about 10^9
396 CFU/ g).

397 **Experimental grouping and treatment.**

398 This experiment was divided into two groups: group A (natural composting) and
399 group C (composting with added bacteria), and the composting process was carried
400 out in the HFW treatment machine. 500g sawdust was added at the beginning of
401 composting of two groups. In group C, 5g freeze-dried powder with four kinds of
402 added bacteria was added, and 500g ~ 600g HFW was added at 1 pm every day. The
403 residue in the machine was sampled every two days to determine the number of viable
404 bacteria and the pH value, and 20g sample was stored in the refrigerator at - 70 ° C for
405 subsequent 16S rRNA sequencing analysis. The composting process sustained 20
406 days, and after that the composting residue was naturally stored for 10 days. The
407 composting samples on the 1st, 7th, 15th, 20th and 30th days were taken for 16S
408 rRNA sequencing analysis, named stage1-stage5. At the end of experiment, the weight
409 of composting residue was measured and the degradation rate was calculated. The
410 degradation rate can be calculated according to the following formula:

411 Degradation rate (%)= $(X_1-X_2+X_3)/X_1$

X_1—Total weight of HFW added (g)

X_2—Weight of composting residue in the machine (g)

X_3—Weight of sawdust added (g)

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412 **High throughput sequencing of 16S rRNA.**

413 According to the experience, the most suitable method of total DNA extraction was
414 utilized for different sources of microbiome samples. At the same time, DNA was
415 quantified by Nanodrop and the quality of DNA was detected by 1.2% agarose gel
416 electrophoresis.

417 Generally, the target sequences, such as ribosomal RNA or specific gene fragments,
418 which can reflect the composition and diversity of microbial community, are used to
419 design primers according to the V3 ~ V4 region of 16SrRNA gene, and the specific
420 barcode sequence was added to amplify the rRNA gene variable region (single or
421 continuous multiple) or specific gene fragment by PCR.

422 The PCR amplification adopts PFU high fidelity DNA polymerase (TransGen
423 Biotech), and the number of amplification cycles were strictly controlled, so as to
424 make the number of cycles as low as possible and ensure the same amplification
425 conditions of the same batch of samples. At the same time, a negative control was set,
426 which can detect environmental, reagent and other microbial contamination, and any
427 negative control amplification samples with bands can not be used for subsequent
428 experiments. The related information are shown in table 4.

429 **Fluorescence quantification of amplification products.**

430 The PCR products were quantified by fluorescent reagent quant (Quant-iT PicoGreen
431 dsDNA Assay Kit), and the quantitative instrument is Microplate reader (BioTek,
432 FLx800). According to the results of fluorescent quantitative analysis, the samples
433 were mixed according to the corresponding proportion according to the sequencing
434 requirements of each sample.

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435 **Construction and sequencing of Illumina Library.**

436 Illumina high-throughput sequencing and bioinformatics analysis were performed
437 with the assistance of Personalbio Biotechnology Co. Ltd. (Shanghai).

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444 We declare that we have no competing interests.

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609 **Tables**

610 Table 1: Changes of related parameters in different stages of composting process

Samples	Composting time (d)	Viable count (CFU/g)	pH	Percentage of composting residue in machine volume
A1	1	6.97×10^{11}	6.21	7.94%
A2	7	2.63×10^{8}	5.95	18.24%
A3	15	2.23×10^{6}	5.56	32.35%
A4	20	1.06×10^{6}	5.32	41.17%
A5	30	8.66×10^{5}	5.87	40.58%
C1	1	2.21×10^{6}	6.35	7.06%
C2	7	7.63×10^{7}	6.13	16.18%
C3	15	4.33×10^{5}	5.88	30.30%
C4	20	2.33×10^{5}	5.57	39.12%
C5	30	7.33×10^{4}	5.96	38.53%

611 Table 2: Statistics of optimized effective sequences and OTUs in each sample

Sample	optimized effective sequences	OTUs	OTUs (without Chloroplast)
A1	131796	388	387
A2	128788	460	459
A3	130073	1076	835
A4	132226	1118	1100
A5	124344	1349	1270
C1	133111	1300	1300

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C2	132463	565	565
C3	124660	1624	1620
C4	127078	824	717
C5	121536	622	396

612 Table 3: Alpha diversity index in each group

Sample	Chao1 index	Shannon index	Simpson index	Coverage index
A1	390	2.91	0.8172	0.999824
A2	481	2.22	0.6058	0.999341
A3	1119	3.68	0.7867	0.998544
A4	1146	5.01	0.8860	0.998834
A5	1380	5.31	0.8823	0.998801
C1	1346	2.64	0.3926	0.998469
C2	607	1.86	0.4941	0.999011
C3	1632	6.61	0.9585	0.999083
C4	848	4.11	0.7467	0.998987
C5	650	3.36	0.7235	0.999132

613 Table 4: Primer information of PCR amplification

Primer name	Pre-primer sequence	Post-primer sequence	length of Amplified fragment
V3 V4 region of standard bacteria	ACTCCTACGGGAGGCAGCA	GGACTACHVGGGTWTCTAAT	500bp

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614 Amplification system (25µL): 5×reaction buffer 5µL, 5×GC buffer 5µL, dNTP
615 (2.4mM) 2µL, Forwardprimer (10uM)1µL, Reverseprimer (10uM)1µL, DNA
616 Template 2µL, ddH_2O 8.75µL, Q5 DNA Polymerase 0.25µL
617 Amplification parameters：Initial denaturation 98℃ 2min，Denaturation 98℃ 15s，
618 Annealing 55℃ 30s，Extension 72℃ 30s，Final extension 72℃ 5min， 10℃ Hold,
619 25-30Cycles.
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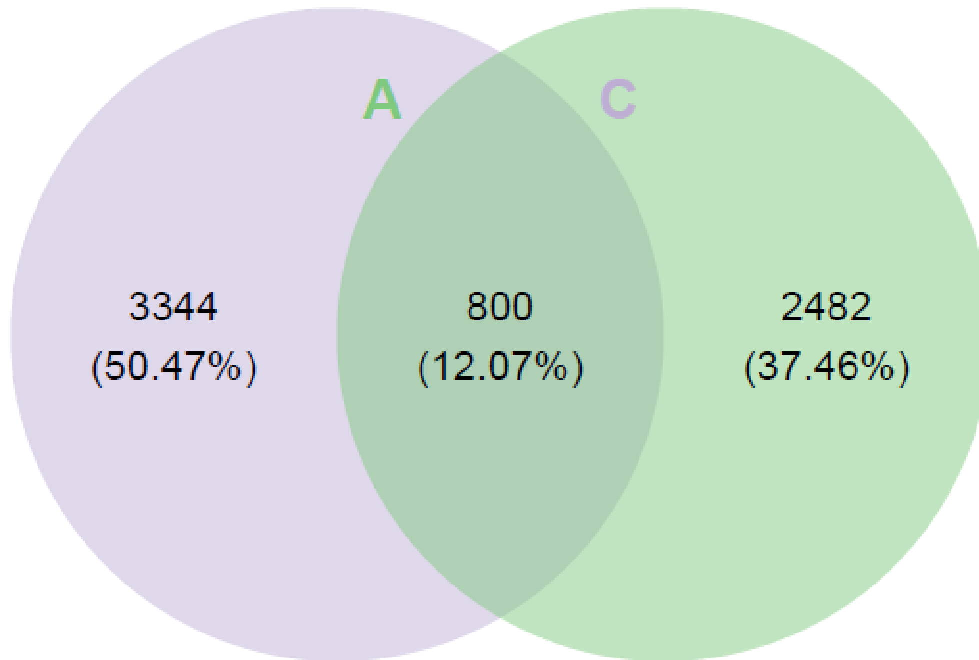
637

638 **Figures**



639

640 Figure 1 Venn graph analysis based on OTU number

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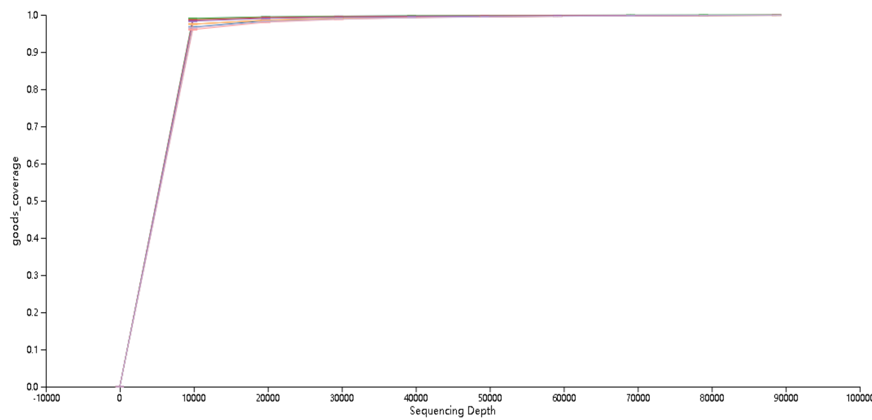

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643 Figure 2(A) Coverage-Sequencing Depth Curve

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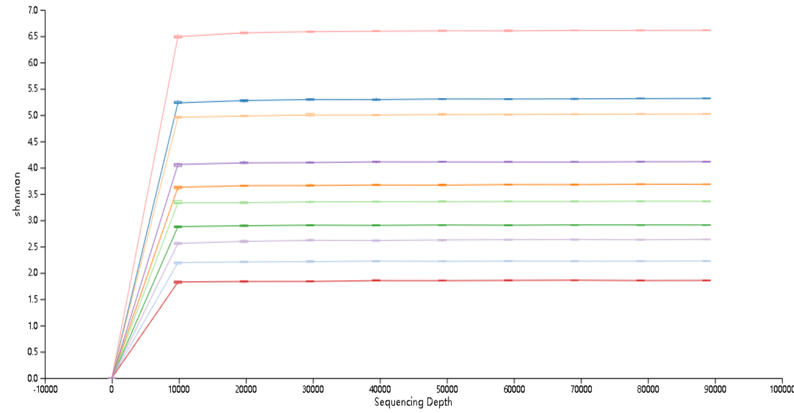
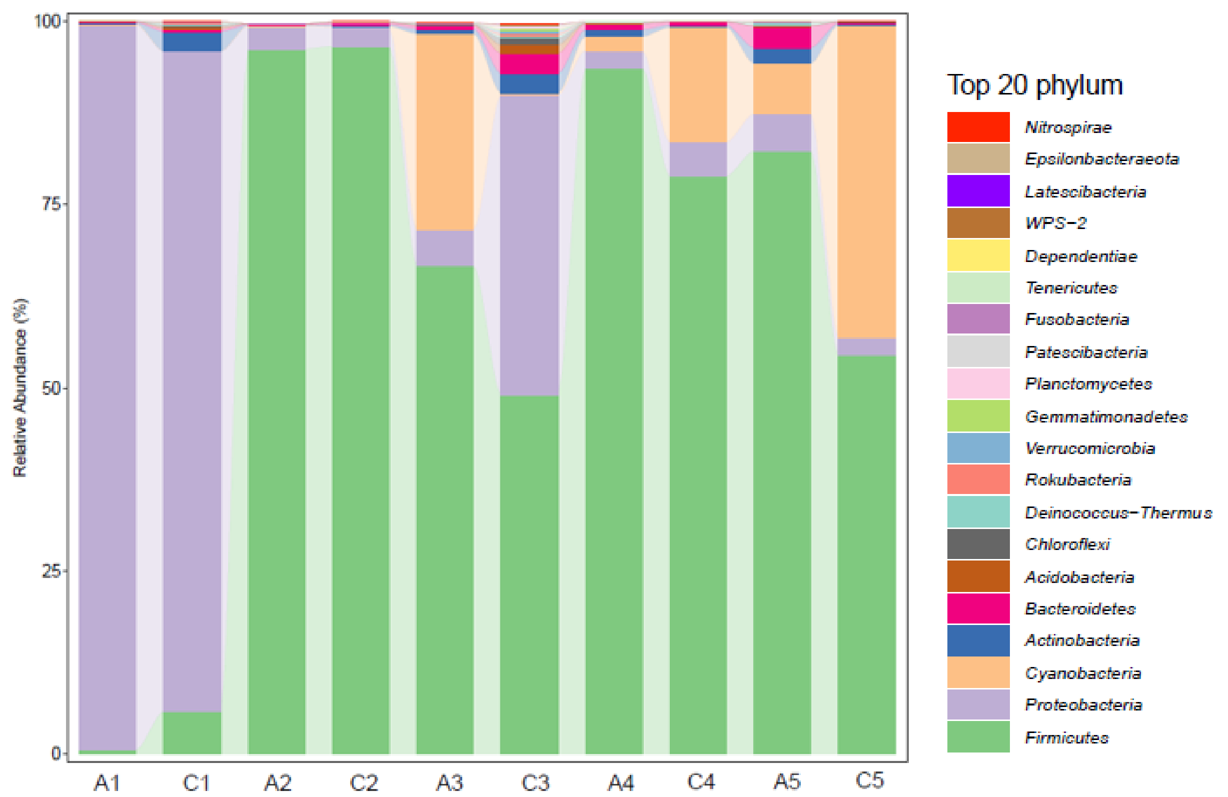
644 

645 Figure 2(B) Shannon-Sequencing Depth Curve



646
647 Figure 3 Bacteria composition at the level of phylum classification at different stages

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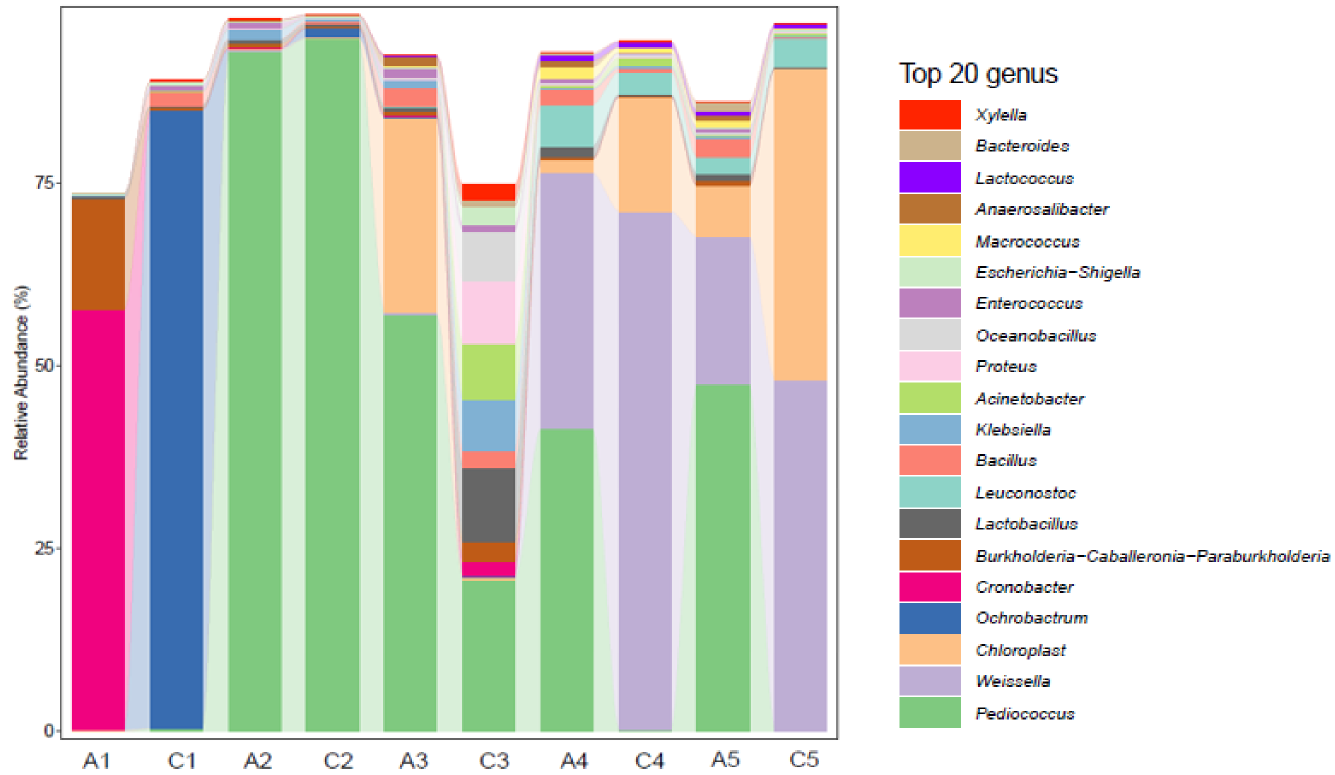

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649 Figure 4 Bacteria composition at different stages of genus classification

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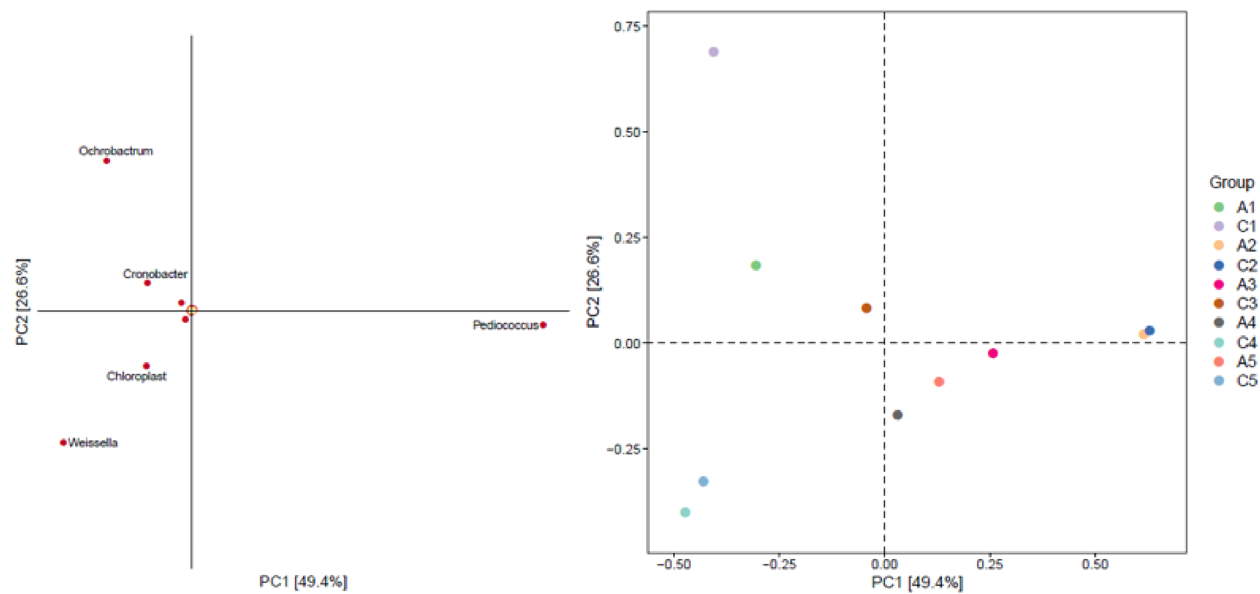

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652 Figure 5 Analysis of PCoA in different samples

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