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4 Effects of carbon-based additive and ventilation rate on nitrogen loss and microbial
5 community during chicken manure composting

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17 Abstract : Aerobic composting is a sustainable method for recycling of chicken manure, while its
18 unsuitable porosity and carbon to nitrogen ratio limit the oxygen supply, which must result in high
19 nitrogen loss because of anaerobic micro-zones in the materials. Treatments with five carbon-based
20 additives and two ventilation rates (0.18 and 0.36 L·min⁻¹·kg⁻¹ DM) were set in chicken manure
21 composting, to investigate their effects on biodegradation process, ammonia (NH₃) emission,
22 nitrogen loss, physiochemical properties and microbial community. The additives and ventilation
23 rates influenced the CO₂ production from the 2nd week, meanwhile varied the physiochemical
24 parameters all the process. No inhibitory effect on the maturity were observed in all treatments.
25 With woody peat as additive, the NH₃ emission amount and nitrogen loss rate were shown as 15.86
26 mg and 4.02 %, when compared with 31.08-80.13 mg and 24.26-34.24 % in other treatments. The
27 high aeration rate increased the NH₃ emission and nitrogen loss, which were varied with different
28 additives. The T-RFLP results showed that the additives and the ventilation rates changed the
29 microbial community, while the prominent microbial clones belonged to the class of *Bacilli* and
30 *Clostridia* (in the phylum of Firmicutes), and *Alphaproteobacteria*, *Deltaproteobacteria* and
31 *Gammaproteobacteria* (in the phylum of Proteobacteria). *Bacillus spp.* was observed to be the most
32 dominant bacteria in all the composting stages and treatments. We concluded that woody peat could
33 improve chicken manure composting more than other additives, especially on controlling nitrogen
34 loss. 0.18 L·min⁻¹·kg⁻¹ DM was suitable for chicken manure composting with different additives.

35 **Keywords** : Chicken manure; composting; carbon-based additive; ventilation rate; microbial
36 community

37 1. Introduction

38 Due to the rapid development of chicken farms in China, the output of chicken manure has risen
39 sharply in the past decades, which was nearly 102 million tons (dry weight) in 2016 (Jia et al., 2018).
40 The over production and accumulation of untreated chicken manure has caused a series of
41 environmental and social problems (Shi et al., 2018). Recycling the chicken manure to arable land
42 as fertilizers has been recognized as a sustainable utilization method, for chicken manure has high
43 concentration of macro and micro nutrients than other livestock manure. Aerobic composting could
44 effectively convert the livestock manure into fertilizer or amendments used to improve soil fertility
45 and promote plant growth (Hageman et al., 2018). While the low contents of lignocellulose and low
46 carbon to nitrogen ratio of chicken manure would limit the oxygen consumption and organic matter
47 degradation during chicken manure composting (Wang et al., 2015), which may contribute to more
48 nitrogen loss and anaerobic microdomains. Different additives and suitable rate of forced ventilation
49 used to improve the porosity and C/N ratio could make great significance to reduce the emission of
50 GHGs and NH₃, mitigate the mobility of heavy metals, and conserve other essential nutrients for
51 chicken manure composting (Awasthi et al., 2017; Mao et al., 2018). Many researches have
52 confirmed the effects of additives such as zeolite (Awasthi et al., 2016; Chan et al., 2016), bentonite
53 (Wang et al., 2016), medical stone (Wang et al., 2017), woody peat and biochar (Zhang et al., 2014;
54 Awasthi et al., 2017; Chang et al., 2019b), saw dust (Sharma et al., 2018), pine bark (Brito et al.,
55 2015) and peanut hull (Erickson et al., 2014), for various organic waste composting to mitigate the
56 emission of NH₃ and GHGs and conserve the nutrients. However, the varied biodegradable organic
57 matter content in these additives would significantly influence their improvement of composting
58 process and the temperature (Chang et al., 2019a). Forced ventilation is used to supply adequate O₂

59 during composting, which was found to result in lower GHG emissions when compared with
60 physical turning and passive ventilation systems (Hao et al., 2001; Park et al., 2011). Most previous
61 studies have reported that losses of NH₃ increase and CH₄ emissions decrease with increasing
62 ventilation rate (Osada et al., 2000; Jiang et al., 2011; Shen et al., 2011). However, the values of
63 N₂O emissions in composting could not follow any trend when the increasing ventilation rate
64 changes (Osada et al., 2000; Shen et al., 2011; Jiang et al., 2011). These inconsistent results may be
65 caused by the different C and N dynamics and the O₂ consumption in various raw materials because
66 of their different physicochemical characteristics and biodegradable organic matter content. In the
67 present study, considering the probable influence of carbon-based additives and ventilation rates on
68 composting and the nutrient loss, we carried out 2 series of 30-d chicken manure composting in a
69 lab-scale composting system, to explore the effects of carbon-based additives with different
70 biodegradable organic matter, and the effects of high and low ventilation rates with the same
71 additives on CO₂ and NH₃ emissions, composting process and the microbial community changes.

72 2 Material and methods

73 2.1 Set-up of experiment

74 Chicken manure, corn straw, saw dust, pine bark and peanut hull were collected from local
75 greenhouse and farmland in Beijing, China. The additives (corn straw, saw dust, pine bark, peanut
76 hull) were air dried and cut into 2-3 cm pieces to obtain a uniform particle size that enabled good
77 mixing. Woody peat was supplied by View Sino international Ltd., which was used in powder form.
78 Main characteristics of the raw materials were shown in Table 1.
79 The experiments were conducted in a bench-scale composting system (Fig. 1) in the lab of China

80 Agricultural University, designed to simulate the temperature changing without external effects (e.g.,
81 heat loss) (Michel and Reddy, 1998; Meng et al., 2016). The system details were described in our
82 previous study (Chang et al., 2019a). There were two series of treatments in our experiments, whose
83 materials ratios were shown in Table 2.

84 2.2 Samples collection and analysis

85 During the process, solid samples were collected on the days of 0, 3, 7, 14, 21, 28 and 35 after
86 mixing well. Each sample was thoroughly mixed and then divided into two parts: one part was air-
87 dried to analyze physicochemical characteristics, like total nitrogen (TN) and ash content; the other
88 part was stored in the freezer at -20 °C for determination of other parameters, like pH value, Electric
89 Conductivity (EC), Germination Index (GI), extractable ammonium and microbial community.

90 A 1:5 aqueous extract (w/v) of the fresh composts with 2N KCl solution was used for the analysis
91 of extractable ammonium (NH₄⁺-N), and NH₄⁺-N was analyzed by SEAL Analytical (BL-TECH).
92 Measurement of other parameters and calculation methods were followed the methods shown in
93 Chang et al. (2019a and 2019b).

94 2.3 Analysis of microbial community

95 Total community DNA was extracted from 0.5 g compost samples using the FastPrep DNA kit (MP
96 Biomedicals, Santa Ana, CA) according to the manufacturer's protocol (Feng et al, 2012). The
97 extracted DNA solutions were diluted in suitable times. The 16S rRNA genes were amplified using
98 universal bacterial primers: 27f forward (5'-AGAGTTTGATCCTGGCTCAG-3') and 907r (5'-
99 CCGTCAATTCMTTGTGAGTT -3') reverse. The 27f forward primer was labeled with 6-

100 carboxyfluorescein (FAM). Each PCR reaction mixture contained 50 μ l liquid: 37.5 μ l dd H₂O, 10*
101 PCR reaction buffer 5 μ l (Tiangen Biotech, Beijing), dTNPs 4 μ l, 27f-FAM 0.75 μ l, 907r 1.5 μ l, BSA
102 0.5 μ l, rTaq DNA polymerase 0.5 μ l (TakaRa), DNA template 2 μ l. The reaction mixture was
103 incubated at 94°C for 4 min, and then cycled 30 times through three steps: denaturing (94 °C; 45
104 s), annealing (52°C; 45 s), and primer extension (72°C; 60 s) in a PTC-100 thermal cycler. Then the
105 last step is 10 mins' primer extension. Amplification product sizes were verified by electrophoresis
106 in 2.0% agarose and ethidium bromide staining. To obtain sufficient DNA for T-RFLP analysis and
107 to minimize PCR bias, amplicons from three PCR runs for each root sample were combined
108 (Clement et al., 1998) and then purified using a PCR purification kit (PCR Clean-up Kit;
109 PROMEGA Inc., Wisconsin, USA).

110 To construct bacterial 16S rRNA gene-based clone libraries, we prepared DNA samples extracted
111 from five compost samples with the richest bacteria diversity from different additive treatments,
112 respectively. The PCR amplification used the same primers as those indicated above. PCR products
113 were purified and ligated into the pMD19-T Vector (TakaRa) according to the manufacturer's
114 instructions. 1 ml suction head was used to blow and absorb the bacteria at the bottom of the
115 centrifuge, and then 20-40 μ l of the cells were coated on LB AGAR plate medium containing X-
116 Gal, IPTG and Amp for overnight culture at 37°C, to form a single colony. White clones were
117 selected and underlined on LB-Amp plates, and cultured overnight at 37°C. The screened positive
118 clones were sent to the sequencing company for sequencing, which were screened with the primers
119 M13-47 (5'-CAGCAC TGA CCC TTT TGG GAC CGC-3') and RV-M (5'GAG CGG ATA ACA
120 ATT TCA CAC AGG-3'). Enter the results into NCBI GeneBank database and perform Blast search
121 to obtain similar gene sequences. Phylogenetic tree was constructed with MEGA software and NJ

122 method (neighbor-joining).

123 2.4 Statistical analysis

124 All the results were summarized and figured in Excel. Statistical comparisons were performed using
125 SPSS v.18.0 software with the two-way ANOVA analysis of variance test. A probability was
126 defined with a least significant difference at two sides of $P < 0.05$.

127 3. Results and discussion

128 3.1 Biodegradation estimated by accumulative amount of CO₂

129 Rapid decrease of total organic carbon and increase of cumulative CO₂ amount coincided with the
130 biodegradation of organic matter and the rise of temperature during composting (Wong and Fang,
131 2000). As shown in Fig. 2A, similar CO₂ emission amount were observed in the first 7 days when
132 the ventilation rate was 0.18 L·min⁻¹·kg⁻¹ DM, suggested the easily-degraded organic matter was
133 degraded and transferred to CO₂. Then the decreased rates in T2-T5 indicated that the easily-
134 degraded organic matter were less in these treatments than T1. For the concentrations of cellulose
135 and lignin were higher in saw dust, pine bark and peanut hull, when compared with wheat straw,
136 while cellulose and lignin were hard to be biodegraded directly (Chang et al., 2019b). Woody peat
137 is rich in carbon and humus but unavailable for microbes, so that the CO₂ amount was lower than
138 that in T1. Similar result was observed in Chang et al. (2019b), in which woody peat and corn stalk
139 were used in vegetable wastes or sewage sludge composting. When the ventilation rate was
140 increased to 0.36 L·min⁻¹·kg⁻¹ DM, less CO₂ emission amount were observed in S1, S3 and S5(Fig.
141 2B), suggested that the ventilation rate of 0.18 L·min⁻¹·kg⁻¹ corresponded to a higher biodegradation

142 than $0.36 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$ in the current study. Significant differences were observed after the
143 first 7 days. As shown in [Qasim et al. \(2019\)](#), in the ventilation range of $0.3\text{-}0.9 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$
144 when composting with poultry manure and sawdust, the low aeration rate ($0.3 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$)
145 corresponded to a higher and longer thermophilic phase than did the high aeration rate ($0.9 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$). The CO_2 volatilization was directly related to the temperature profile of the substrate,
146 shown as significant differences in the 2nd and 3rd weeks of composting but none in 1st week. What's
147 more, several previous studies have recommended the aeration methods and rates as, $0.44 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$
148 in the composting of maize stalks and cow feces ([Nada, 2015](#)), $0.62 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$ volatile
149 solids (VS) in the composting of vegetable and fruit wastes ([Arslan et al., 2011](#)), $0.5 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$
150 in the composting of chicken manure and sawdust ([Gao et al., 2010](#)), $0.25 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$ in
151 the composting of dairy manure with rice straw ([Li et al., 2008](#)), $0.43\text{-}0.86 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$ in the
152 composting of food waste ([Lu et al., 2001](#)), etc. All of these suggested that the aeration rate should
153 be set according to the compost material and composting process, based on the oxygen needed and
154 supplied during the process.

156 3.2 Physiochemical characteristics

157 The appropriate pH range for maintaining high microbial activity during composting is 7-8, which
158 would be changed along with the biodegradation of organic matter. For the complex components
159 were degraded to organic acids and then to CO_2 , meanwhile CO_2 , NH_3 , other gases and volatile
160 organic acids were emitted from the composting system ([Eklind and Kirchmann, 2000](#)). As shown
161 in Fig.3A, the pH values in all the treatments were in the range of 6.8~8.4, suggested the carbon
162 additives made no difference on the biodegradation process. Even the additives used in current

163 experiment changed pH value of the products, the final value were all in the range of 7.0~8.2, which
164 is good for agricultural utilization (Maso and Blasi., 2008). The pH value in T2 was lower than
165 others, indicated the potential advantage of woody peat, to reduce the NH₃ emission by decreasing
166 the material pH value. Increase of the aeration rate quickly increased the pH values (Fig. 3B), for
167 the gases and volatile organic acids were forced to emitted more frequently than in the low aeration
168 rate treatments. Then the reduction of biodegradation resulted in stable change of pH value, similar
169 as those shown in the low aeration rate treatments. The final pH values of products in S1-S3 were
170 higher than those in T1-T3.

171 For the compost products are always used as organic amendments or organic fertilizer in soil (Liu
172 et al., 2011), EC should be under 4 mS·cm⁻¹, which reflects no inhibitory effects on plant growth
173 from the compost products (Li et al., 2007). A slightly decrease was shown in the first several days
174 for almost every treatment, followed with a stable value till the end (Fig. 3C and 3D). The carbon
175 additives influenced the EC variation, while they were always in the safe range, except 4.37 mS·cm⁻¹
176 in T1. For more biodegradation happened in T1, which was indicated by the CO₂ production and
177 temperature. Woody peat used in T2 reduced the EC in the whole process, because of the absorption
178 caused by its rich humic acid. The rapid emission of gases and volatile organic acids in treatments
179 with high aeration rate also reduced the EC value caused.

180 To avoid the toxic effects on plant growth resulted from the toxic substances, such as short-chain
181 fatty acids, GI is always used as an important index to evaluate whether compost is mature enough.
182 A minimum value of 80% is considered to indicate the compost mature at an extraction ratio of 1:5
183 (compost: water wet w/v). As shown in Fig. 3E and Fig. 3F, the GIs increased with the
184 decomposition of toxic materials, especially in the 1st week. Nearly all the GIs were higher than 80%

185 in the treatments, except T4 in series I, and S1 and S3 in series II. Then the GIs keep slightly
186 increasing till the end of the process, with the GIs over 100%. The results indicated that the five
187 organic wastes chose in the current study all could be used to be composted with chicken manure, by
188 adjusting the free air space and C/N. While the higher aeration rate ($0.36 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$) decreased
189 the maturity process. However, it was opposite in a pig manure composting from [Guo et al. \(2012\)](#),
190 the suitable aeration was $0.48 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$ when considering the maturity ([Guo et al., 2012](#)),
191 even the lower aeration rate ($0.24 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$) had better performance on biodegradation. The
192 reasons should be complex, one is the aeration was intermittent in [Guo et al., \(2012\)](#) so that higher
193 aeration rate could supply enough O_2 than lower one. The other is that lower C/N of the mixed
194 materials (< 20) make high concentration of TAN (total ammonium nitrogen) in the materials, which
195 would inhibit the seedling.

196 3.3 NH_3 emission and nitrogen concentrations

197 The changes of accumulative NH_3 emission amount during the composting of chicken manure with
198 different additives and different ventilation rates were shown in Fig. 4A and Fig. 4B. Generally, the
199 accumulative amounts rapidly increased from the beginning to the 10th day in T1-T3, while to the
200 20th day in T4 and T5. Then the amount increased slowly till the end of the process. The significantly
201 lower cumulative NH_3 emission in T2 (15.86 mg) was related to the characteristics of woody peat,
202 than those with straw, which were widely used as additives during manure composting. Low pH
203 and rich of humic acid contributed to the absorption of NH_4^+ , which was proved by previous studies
204 ([Chang et al., 2019b](#)). More cumulative NH_3 amounts were observed in T3-T5 than that in T1, which
205 may be related with the lower biodegradable organic carbon in the mixed materials. For there are

206 high concentrations of lignocellulose in saw dust, pine bark and peanut hull, which may decrease
207 the biodegradable C/N and increase the NH₃ emission (Chang et al., 2019b). Comparing treatments
208 with the same compost materials, S1 had significantly higher cumulative NH₃ losses (by 117.70%)
209 compared with T1, S3 (by 25.55%) with T3, and S5 (by 38.14%) with T5. Which suggested the
210 increased aeration rate led to higher cumulative NH₃ losses.

211 During the composting, the TN always increase from the initial to the end, because of the
212 concentration effect caused by the significant organic decomposition (Chan et al., 2016). In current
213 study, most of the treatments were consistent with this theory, except T5 and S5, in which the peanut
214 hull was used as carbon additives (Table 3). For their NH₃ emission were really high when compared
215 with other treatments, shown as 69.79 mg and 96.41 mg (Table 3). What's more, less matter loss
216 resulted from less organic decomposition decreased the concentration effect, for a high ratio of
217 peanut hull was mixed with the chicken manure, which contained high concentrations of cellulose
218 and lignin. There are several forms of nitrogen in the compost, among which organic nitrogen (Nor)
219 and nitrate nitrogen (NO₃⁻-N) are normally stable in the materials and called stable nitrogen (Chang
220 et al., 2019b), while NH₄⁺-N may transformed to NO₃⁻-N or emitted as NH₃, when the temperature
221 was decreased or during the utilization of the compost in arable land. High NH₄⁺-N concentration
222 in the materials always contributes to high NH₃ emission rate, which was consistent in our study
223 except T2. The different results indicated the important function of woody peat to absorb and fix
224 the NH₄⁺-N, because of its porous structure and rich of humus acid (Chang et al., 2019b). Similar
225 results could be observed when biochar was used during composting (Qiu et al., 2019). More NH₄⁺-
226 N in treatments T3 and T5 than S3 and S5 (shown in Table 3), suggested high aeration rate helped
227 to transfer NH₄⁺-N into NH₃ ventilation, so that more NH₃ ventilation were observed in Fig.5 and

228 Table 2. Meanwhile the total nitrogen loss rates were higher in treatments with high aeration rate
229 resulted from high NH₃ ventilation.

230 3.4 Structure of microbial community

231 Compared with the normal additive corn straw, woody peat, saw dust and pine bark increased the
232 biodiversity at the beginning while peanut hull increased it at the 3rd day (Fig. 5(A)). At the
233 beginning, the most abundant T-RF was 85bp, with the ratio of 100%, 56%, 53%, 44% and 47%, in
234 T1-T5 respectively. The most abundant T-RFs were shown in the 3rd day of T2 and T4, while 7th
235 day of T3 and T5. After then, the numbers decreased significantly. In all the 60 T-RFs shown in the
236 figures, 139bp, 145bp, 147bp, 174bp, 176bp and 178bp were shown high relative abundance in two
237 or more treatments in different stages, indicated these T-RFs should be related with the
238 biodegradation of organic matter. While 158bp in T1, 295bp in T2, 155bp in T3, 140bp in T4 and
239 132bp in T5 were shown high relative abundance in only this treatment, which should be a signal
240 that this T-RF should be specific for this additive only. The results suggested the additives in the
241 current study influenced the microbial community and population, which would result in different
242 biodegradation process. The increase of the ventilation rate contributed to the higher biodiversity in
243 all the three treatments (Fig. 6(B)), even it occurred in the first 7 days in S1 and S5 while in later
244 two weeks in S3.

245 The species distribution of bacteria in composting system can be better understood by constructing
246 bacterial clone library. In this experiment, five of the compost samples in different treatments were
247 selected to construct the clone library. Library analysis showed that the 247 sequences belonged to
248 9 different phyla, among which Firmicutes, Proteobacteria, Bacteroides and Actinomycetes account

249 for 85.83% in the total sequences (Fig. 6(A)). Nearly 70 of the 247 sequences were *Bacillus* spp.,
250 which belongs to the phylum of Firmicutes. Their character of high-temperature resistance made it
251 important on organic matter biodegradation during composting (Koyama et al., 2018). The result of
252 the phylogenetic analysis affiliated with uncultured groups using the neighbor-joining method were
253 shown in Fig. 6(B). Prominent clones belonged to an uncultured group in the phylum Firmicutes
254 (class of *Bacilli* and *Clostridia*) and Proteobacteria (class of *Alphaproteobacteria*,
255 *Deltaproteobacteria* and *Gammaproteobacteria*). Carbon additives used in different treatments
256 influenced the clones, which were consistent in both Fig. 6(A) and Fig. 6(B). As Qiu et al. (2019)
257 indicated, the kind of manure and biochar addition would both influence the bacteria community,
258 especially when the materials were composted different duration. While it is similar that Firmicutes
259 (class of *Bacilli* and *Clostridia*) and Proteobacteria (class of *Alphaproteobacteria*,
260 *Deltaproteobacteria* and *Gammaproteobacteria*) were the main categories in the later time of
261 composting. High proportions of Actinobacteria in the samples of T1 and T3 suggested that the
262 addition of corn straw or saw dust may facilitate the growth of Actinobacteria and accelerate the
263 degradation of lignocelluloses during the maturity stage, similar results were observed and proved
264 in Qiu et al. (2019).

265 4. Conclusion

266 The biodegradation process and the pH, EC and GI values were influenced by the five carbon-based
267 additives used in our experiment, while no inhibitory on composting maturity were observed. The
268 aeration rate of $0.18 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$ was more suitable than $0.36 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$ for chicken
269 manure composting. Woody peat had shown better effect on reducing NH_3 emission and nitrogen

270 loss, while more NH₃ emission and nitrogen loss were observed when the aeration rate was higher.
271 The prominent clones of the compost samples belonged to the phylum Firmicutes (class of *Bacilli*
272 and *Clostridia*) and Proteobacteria (class of *Alphaproteobacteria*, *Deltaproteobacteria* and
273 *Gammaproteobacteria*). Carbon-based additives and ventilation rates set in our experiment had
274 made influences on the microbial community, while *Bacillus* spp. was always the most important
275 one. Therefore, woody peat could be used as carbon-based additives instead of corn straw, and the
276 suitable ventilation rates could reduce the NH₃ emission and nitrogen loss. Additives and ventilation
277 rates would not influence the prominent bacteria that used to promote the composting process.

278 Acknowledgement

279 Financial supported was provided by National Key R&D Program of China (2018YFC1901000),
280 China Agriculture Research System (CAR-23-B16) and Shandong Academy of Agricultural
281 Sciences “Science and Technology Innovation Project” (CXGC2018D06)

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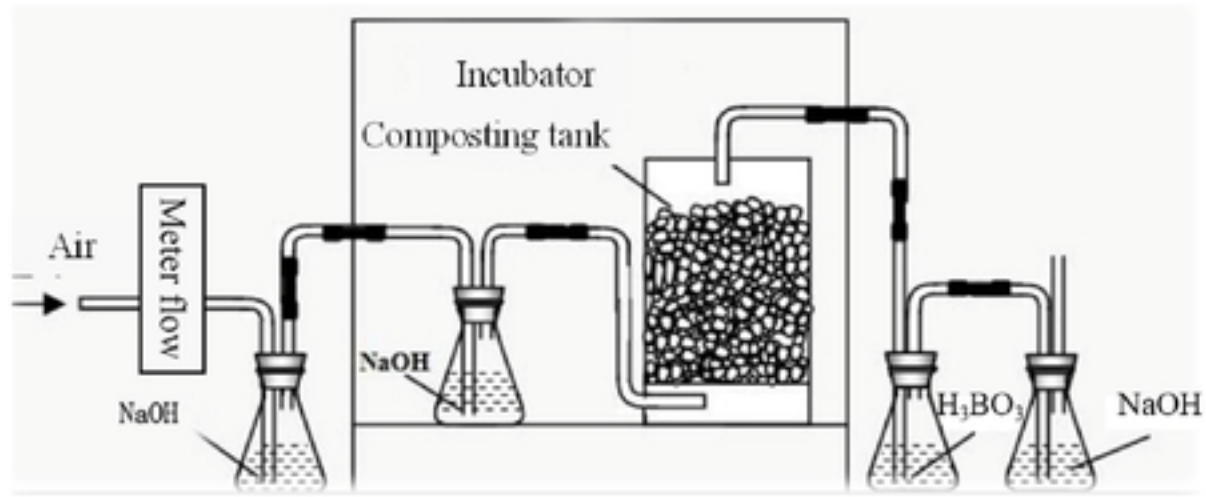


Fig 1. Diagram of composting system

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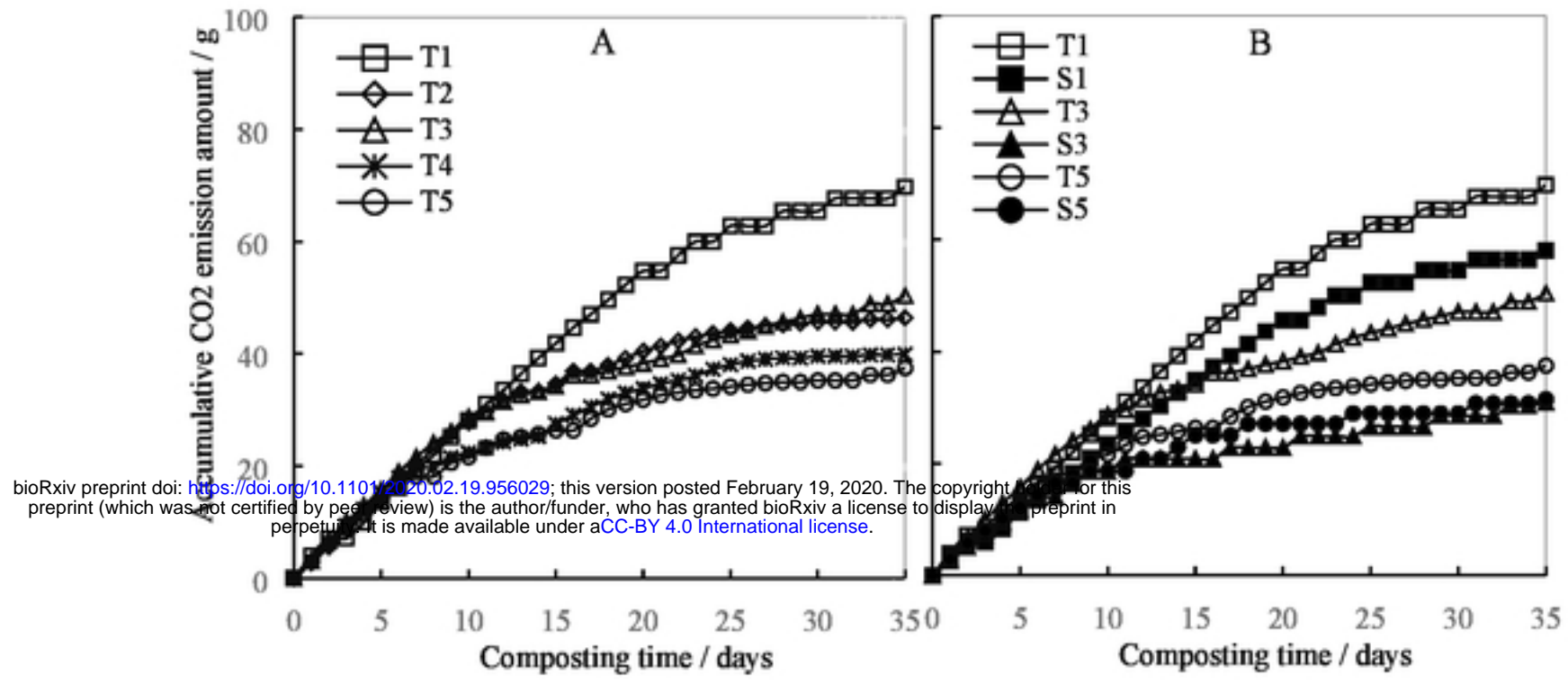


Fig. 2 Effects of carbon-based additives and ventilation rate on CO₂ emissions during chicken manure composting.

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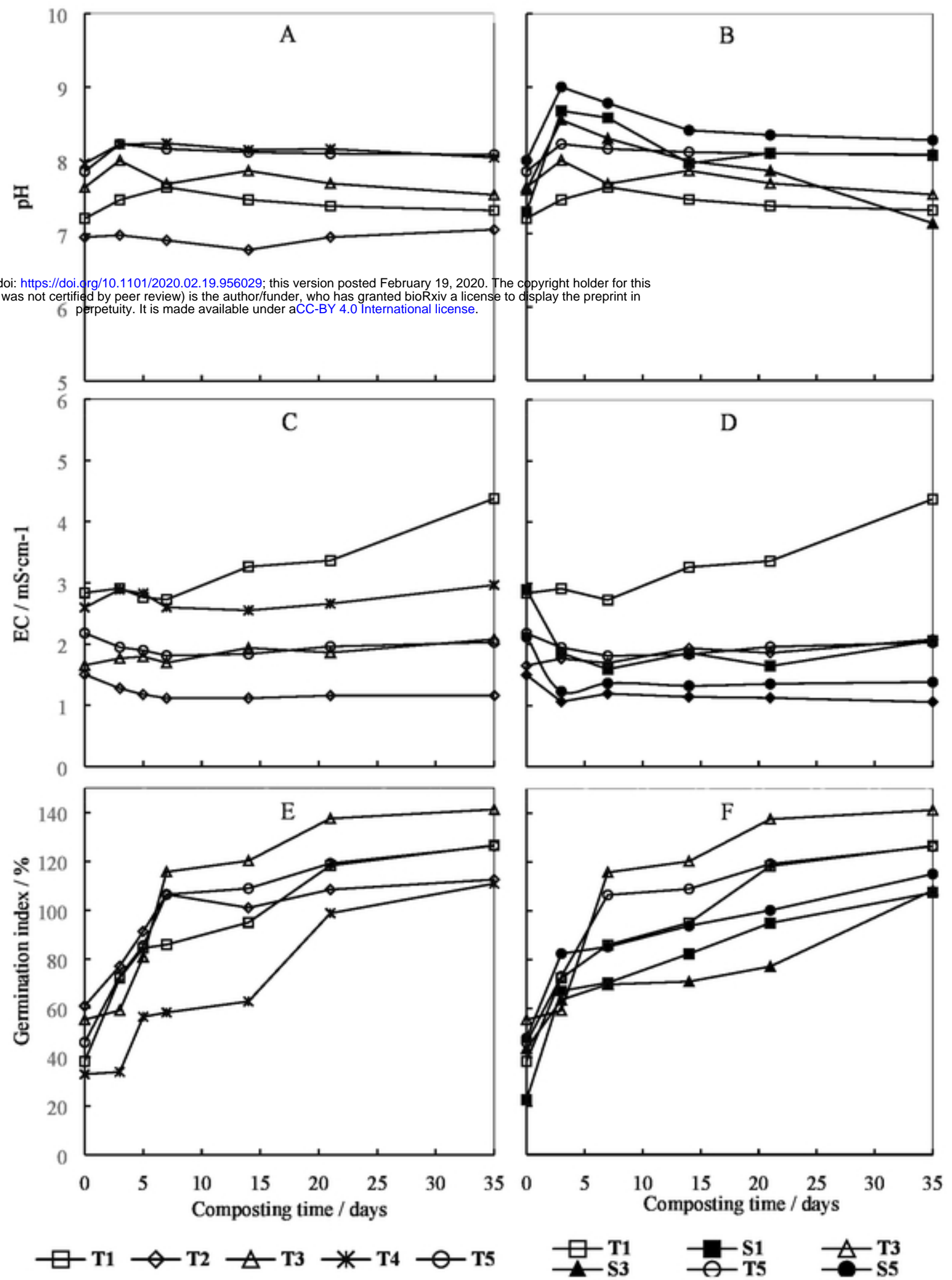


Fig. 3 Effects of carbon-based additives and ventilation rate on physiochemical characteristics during chicken

manure composting

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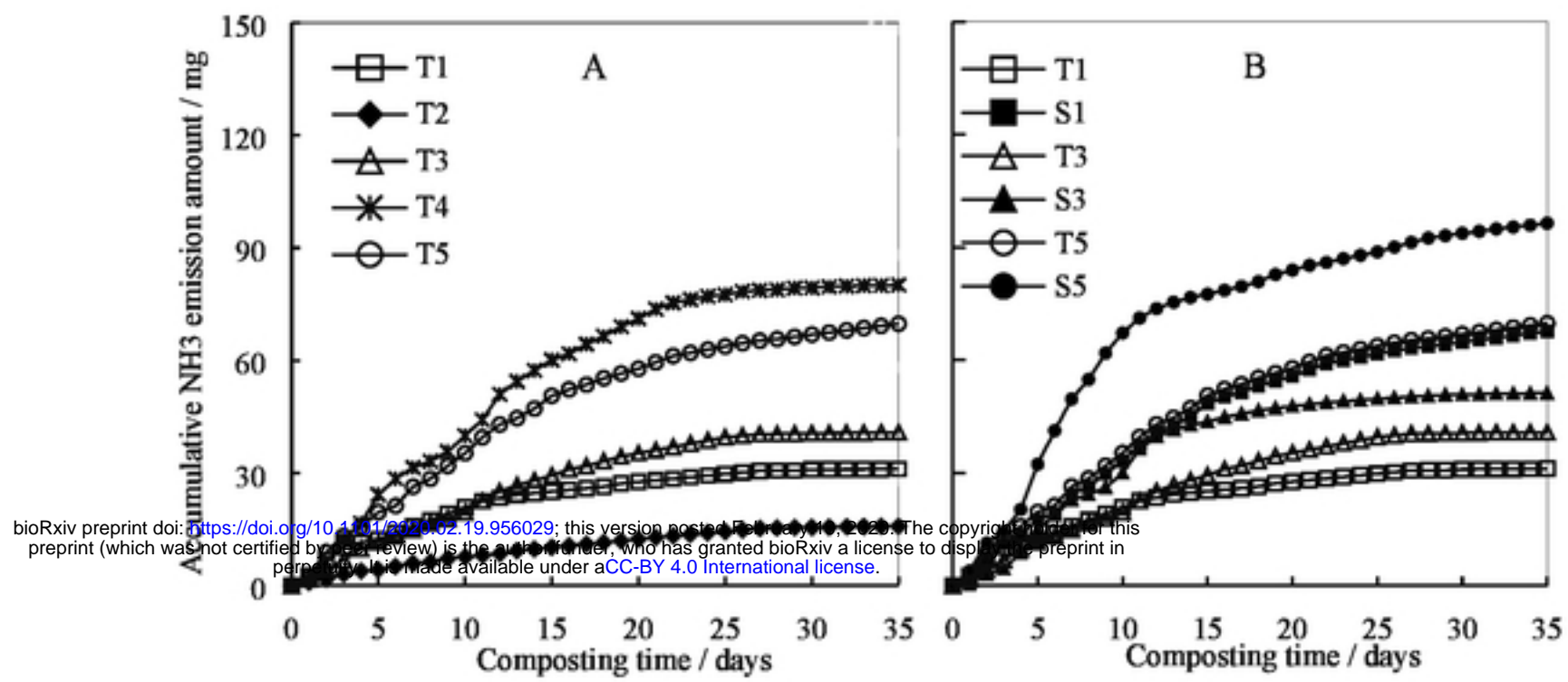


Fig. 4 Effects of carbon-based additives and ventilation rate on NH₃ emissions during chicken manure composting.

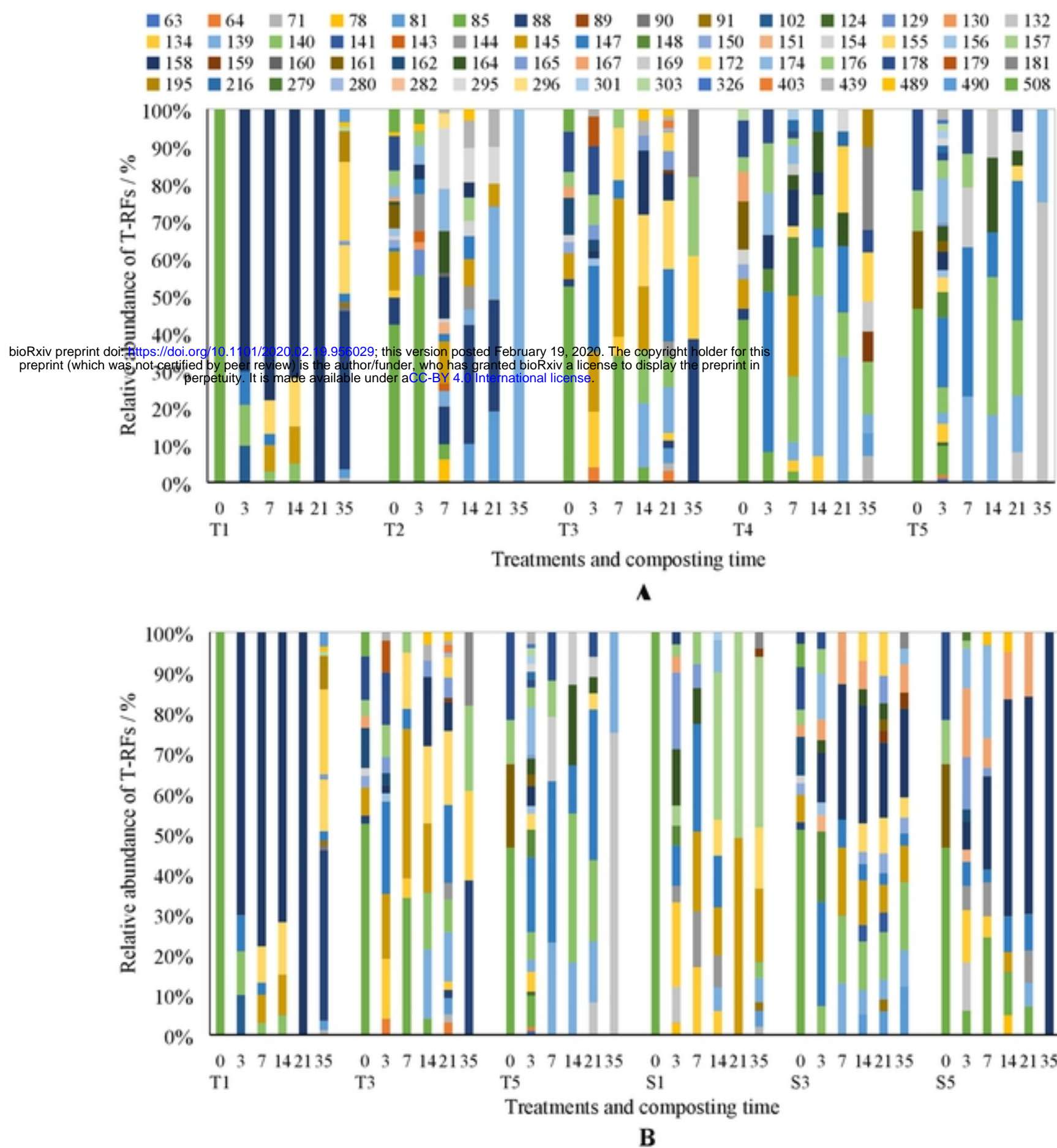
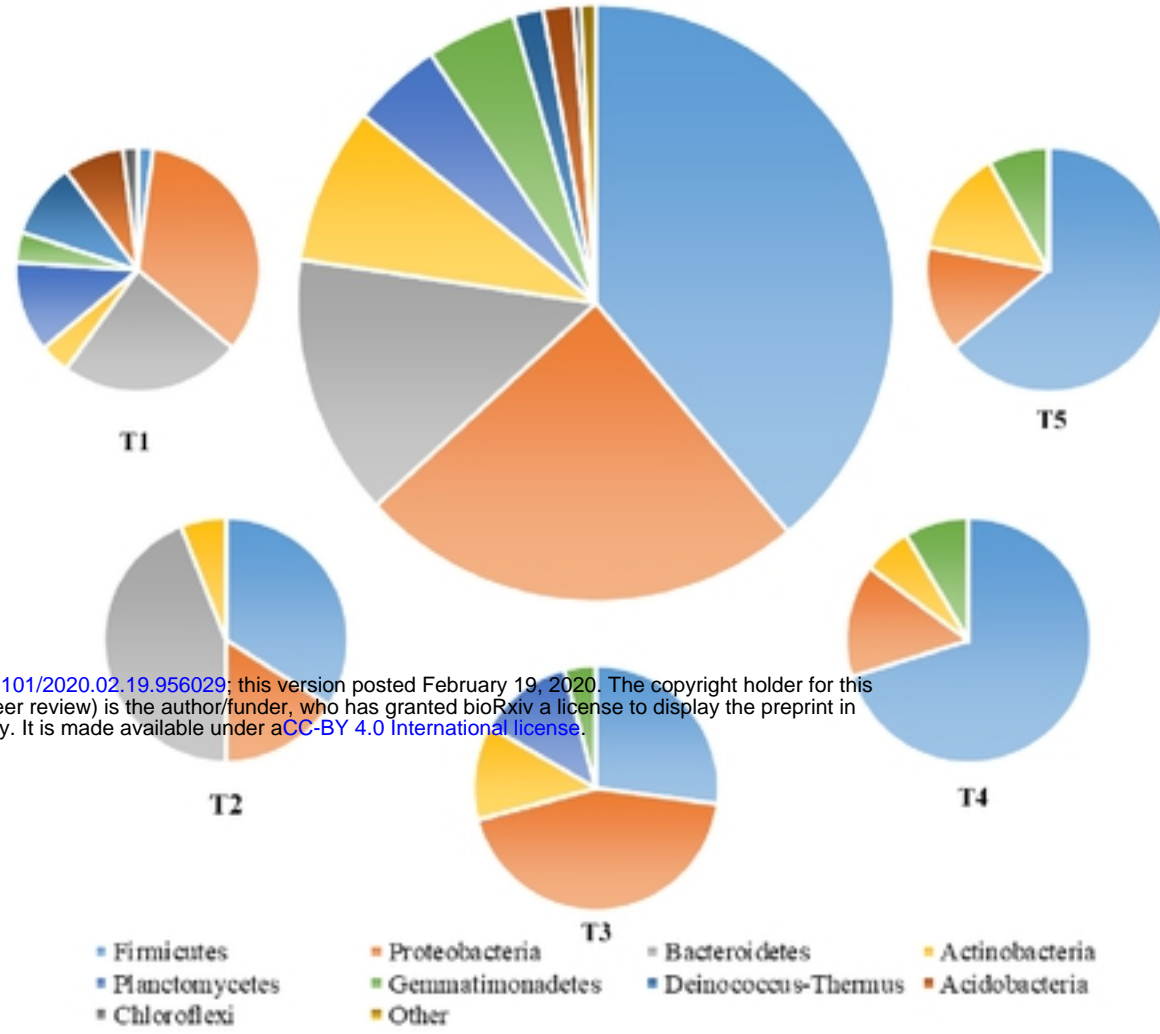


Fig. 5 Effects of carbon-based additives (A) and ventilation rate (B) on relative abundance of T-RFs during chicken manure composting.



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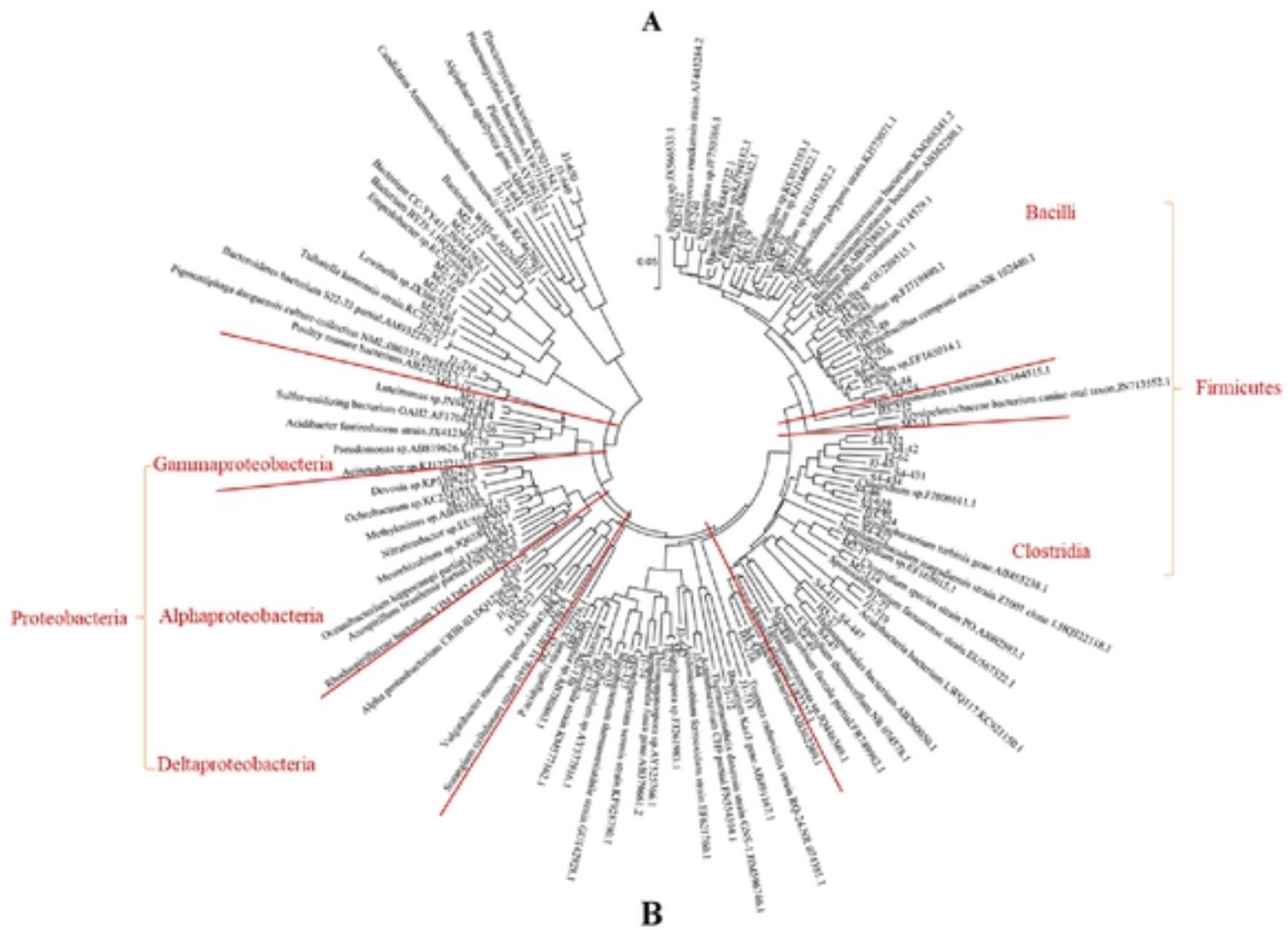


Fig. 6 Composition of 247 sequences in 5 compost samples based on the bacteria clone library analysis (A) and their phylogenetic analysis (shown as OUT and restriction enzyme cutting site) (B)

Table 1 Physical and chemical properties of the materials

Materials	Total carbon content / %	Total nitrogen content / %	C/N	Moisture / %	Cellulose / %	Lignin / %
Chicken manure	37.63	4.33	8.69	81.28	13.06	9.06
Wheat straw	62.35	0.73	85.41	7.14	42.03	8.86
Woody peat	62.84	0.59	196.51	14.04	2.92	28.91
Saw dust	67.70	0.41	165.12	8.33	50.85	17.39
Pine bark	73.52	0.44	167.09	8.79	61.62	31.22
Peanut hull	52.77	1.06	49.78	7.85	64.10	25.65

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Table 2 Experiment design of different resource carbon

Treatments		Raw materials mixed ratio (Fresh matter)	C/N	Ventilation rate / L·min ⁻¹ ·kg ⁻¹ DM
Series 1	T1	Chicken manure: wheat straw = 1:0.323	25:1	0.18
	T2	Chicken manure: woody peat = 1:0.321		
	T3	Chicken manure: saw dust = 1: 0.251		
	T4	Chicken manure: pine bark = 1:0.232		
	T5	Chicken manure: peanut hull = 1:0.548		
Series 2	T1	Chicken manure: wheat straw = 1:0.323	25:1	0.18
	S1			0.36
	T3	Chicken manure: saw dust = 1: 0.251		0.18
	S3			0.36
	T5	Chicken manure: peanut hull = 1:0.548		0.18
	S5		0.36	

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Table 3 Nitrogen transformation and nitrogen loss in all treatments

Treatments	TN / g·kg ⁻¹		Ammonium nitrogen / mg·kg ⁻¹		Total NH ₃ emission amount / mg	TN loss / %	
	Initial	End	Initial	End			
Series 1	T1	21.55	23.52	759.07	105.40	31.08	24.26
	T2	19.03	21.60	1310.33	840.73	15.86	4.02
	T3	23.89	26.44	963.93	212.07	40.79	28.45
	T4	19.85	20.65	1632.33	817.20	80.13	34.24
	T5	20.88	16.68	1236.73	877.80	69.79	32.74
Series 2	T1	21.55	23.52	759.07	105.40	31.08	24.26
	S1	21.55	22.19	759.07	138.80	67.66	35.75
	T3	23.89	26.44	963.93	212.07	40.79	28.45
	S3	23.89	27.08	963.93	149.80	51.21	29.51
	T5	20.88	16.68	1236.73	877.80	69.79	32.74
S5	20.88	16.73	1236.73	494.30	96.41	36.24	

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