

## RESEARCH ARTICLE

# Effects of carbon-based additives and ventilation rate on nitrogen loss and microbial community during chicken manure composting

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**Data Availability Statement:** The microbial gene sequences used in this study are available in GenBank. The full list of accession numbers is BioProject: PRJNA643343.

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## Abstract

Aerobic composting is a sustainable method for chicken manure recycling, while its unsuitable porosity and carbon to nitrogen ratio (C/N) may result in high nitrogen loss and incomplete composting. With the aim to investigate the effects of carbon-based additives and two ventilation rates on chicken manure composting and microbial community, two series of treatments were set up for chicken manure composting, in order to investigate their effects on the biodegradation process, ammonia (NH<sub>3</sub>) emission, nitrogen loss, physiochemical properties and microbial community. The results showed that additives and ventilation rates set in the current study influenced the carbon dioxide (CO<sub>2</sub>) production from the 2<sup>nd</sup> week and also the physiochemical parameters during the entire process, while no inhibitory effect on the maturity were observed. With woody peat as additive, the NH<sub>3</sub> emission amount and nitrogen loss rate were shown as 15.86 mg and 4.02%, less than those in other treatments, 31.08–80.13 mg and 24.26–34.24%, respectively. The high aeration rate increased the NH<sub>3</sub> emission and nitrogen loss, which were varied when the additives were different. The terminal restriction fragment length polymorphism (T-RFLP) results showed that the additives and the ventilation rates changed the microbial community, while the prominent microbial clones belonged to the class of *Bacilli* and *Clostridia* (in the phylum of *Firmicutes*), and *Alphaproteobacteria*, *Deltaproteobacteria* and *Gammaproteobacteria* (in the phylum of *Proteobacteria*). *Bacillus* spp. was observed to be the most dominant bacteria in all the composting stages and treatments. It was concluded that woody peat could improve chicken manure composting more than other additives, especially on reducing nitrogen loss, meanwhile 0.18 L·min<sup>-1</sup>·kg<sup>-1</sup> DM was suitable for various additives. Therefore, suitable additive and aeration rate could be used in practical application, which could significantly reduce nitrogen loss without influence on the compost maturity process.

## 1. Introduction

With the rapid development of intensive and large-scale chicken farms in China, nearly 102 million tons of chicken manure (dry weight) were produced in 2016 [1], which has become

one of the main causes of non-point source pollution threatening the environment (i.e. emissions to water and air) [2]. Aerobic composting shows potential for the conversion of the chicken manure into biofertilizers and reduce the potential pollution [3, 4]. Whereas the characteristics of poor porosity and low C/N ratio, limit the oxygen consumption and organic matter (OM) degradation, resulting in high nitrogen loss during chicken manure composting [5]. However, the high temperature ( $>45^{\circ}\text{C}$ ) reached during the composting process may help to shift the  $\text{NH}_4$  to  $\text{NH}_3$  [6, 7] and inhibit nitrification at the same time, thereby increasing  $\text{NH}_3$  volatilization [8]. Therefore, it is always of importance to improve the efficiency and product quality of chicken manure composting, by improving the selected materials and technique parameters.

Various composting studies have investigated emission ( $\text{N}_2\text{O}$  and  $\text{NH}_3$ ) abatement induced by additives such as, zeolite [9, 10], bentonite [11], medical stone [12], woody peat and biochar [7, 13, 14], saw dust [15], pine bark [16] and peanut hull [17] were studied in composting experiments of various feedstock materials to improve the porosity and C/N ratio, so that the emission of  $\text{N}_2\text{O}$  and  $\text{NH}_3$  could be reduced [14, 18]. Most of these studies gave detailed descriptions of the composting process, by analyzing temperature, gaseous emissions, microbial community dynamics, elements (C, N, P) and humic substance transformation, with the aim to find suitable composting additives. Few studies have compared the effect of varied biodegradable OM content in these additives, which may have a significantly positive impact on the composting process and the temperature [19, 20].

Meanwhile a suitable aeration rate in composting could provide sufficient oxygen, regulate reactor temperature, control water loss and reduce odorous matter emissions [21–23]. Suitable aeration rates vary in different studies with different feedstocks, reactors or additives [24–26]. Especially since different additives used in the materials may significantly influence the temperature and OM degradation, so that a great impact on nitrogen loss would be observed [7]. Based on this, the aeration rate would change the influence of additives on organic degradation, temperature variation and also on nitrogen loss.

In the present study, 2 series of 30-d chicken manure composting were carried out in a lab-scale composting system, with the aim to (1) explore the effects of carbon-based additives with different biodegradable OM, (2) investigate the effects of ventilation rates with the same or different additives on OM degradation, composting process and the microbial community changes.

## 2 Material and methods

### 2.1 Set-up of experiment

The feedstock (chicken manure) and additives (corn straw, saw dust, pine bark and peanut hull) used in the current study were collected from a greenhouse or farmland in Beijing, China. Powder woody peat was supplied by View Sino international Ltd. Prior to the composting process, the additives were air dried and cut into 2–3 cm to enable good mixing of raw materials. Main characteristics of the raw materials are shown in Table 1.

The experiments were conducted in the lab of China Agricultural University, Beijing, China. The bench-scale compost system (Fig 1) used in this study was designed to simulate the temperature ( $50^{\circ}\text{C}$ ), moisture (60% wet weight basis) and forced ventilation of the composting process, which could be vulnerable to external effects (e.g. heat loss) [19]. The reactor capacity is 5L, connected with solutions of NaOH and  $\text{H}_3\text{PO}_4$  before and after each reactor used to absorb the  $\text{NH}_3$  and  $\text{CO}_2$  in the air before and after the composting. There were two series of treatments sets in the current experiments (shown in Table 2), with different carbon additives and different aeration rates, respectively.

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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## 2.2 Samples collection and analysis

During the composting, CO<sub>2</sub> produced during composting was trapped by bubbling the exit gas through a solution of NaOH; the amount of CO<sub>2</sub> in the traps was determined by titration with standard H<sub>2</sub>SO<sub>4</sub> [27].

The solid samples were collected on the day 0, 3, 7, 14, 21, 28 and 35 after being mixing well. Each sample was thoroughly mixed and then divided into two parts: one part was air-dried to analyze physicochemical characteristics, like total nitrogen (TN) and ash content; the other part was stored in the freezer at -20°C for the determination of other parameters, like pH value, Electric Conductivity (EC), Germination Index (GI), extractable ammonium and microbial community. Measurement of these parameters and calculation methods were shown in previous studies [19].

A 1:5 aqueous extract (w/v) of the fresh composts with 2N KCl solution was used for the analysis of extractable ammonium (NH<sub>4</sub><sup>+</sup>-N), and NH<sub>4</sub><sup>+</sup>-N was analyzed by SEAL Analytical (BL-TECH).

## 2.3 Analysis of microbial community

Total community DNA was extracted from 0.5 g compost samples using the FastPrep DNA kit (MP Biomedicals, Santa Ana, CA) according to the manufacturer's protocol [28]. The extracted DNA solutions were diluted with suitable time. The 16S rRNA genes were amplified using universal bacterial primers: 27f forward (5' -AGAGTTTGATCCTGGCTCAG-3') and 907r (5' -CCGTCAATTCMTTGGAGTT -3') reverse. The 27f forward primer was labeled with 6-carboxyfluorescein (FAM). Each PCR reaction mixture contained 50 µl liquid: 37.5 µl

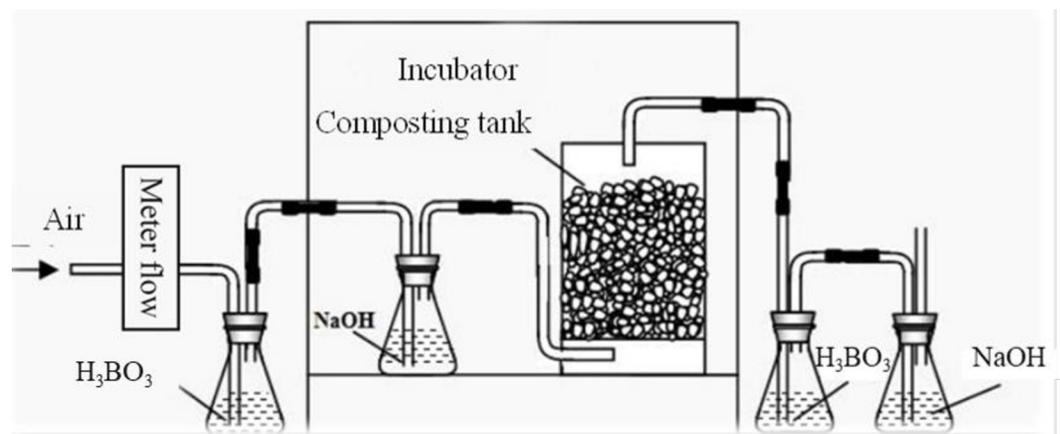


Fig 1. Diagram of the composting system.

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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 <sup>-1</sup> ·kg <sup>-1</sup> 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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dd H<sub>2</sub>O, 10\* PCR reaction buffer 5μl (Tiangen Biotech, Beijing), dTNPs 4μl, 27f-FAM 0.75μl, 907r 1.5 μl, BSA 0.5μl, rTaq DNA polymerase 0.5 μl (TakaRa), DNA template 2μl. The reaction mixture was incubated at 94°C for 4 min, and then cycled 30 times through three steps: denaturing (94°C; 45 s), annealing (52°C; 45 s), and primer extension (72°C; 60 s) in a PTC-100 thermal cycler. Then the last step was 10 mins primer extension. Amplification product sizes were verified by electrophoresis in 2.0% agarose and ethidium bromide staining. To obtain sufficient DNA for T-RFLP analysis and to minimize PCR bias, amplicons from three PCR runs for each root sample were combined [29] and then purified using a PCR purification kit (PCR Clean-up Kit; PROMEGA Inc., Wisconsin, USA).

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

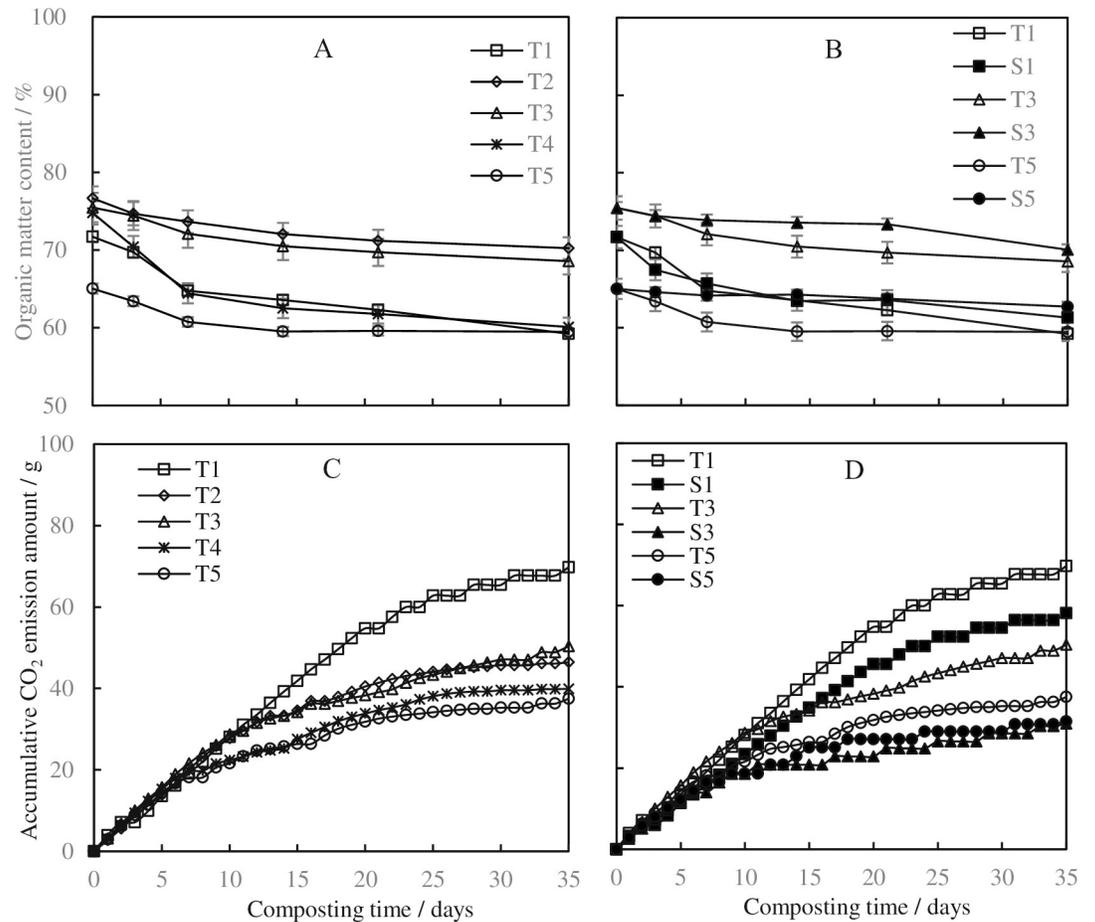
## 2.4 Statistical analysis

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

## 3. Results and discussion

### 3.1 OM biodegradation and CO<sub>2</sub> production

Rapid decrease of OM and increase of cumulative CO<sub>2</sub> amount coincided with the variation of temperature during composting [30]. As shown in Fig 2A, additives used in the current study



**Fig 2.** Effects of carbon-based additives (A, C) and ventilation rate (B, D) on organic matter degradation and CO<sub>2</sub> emissions during chicken manure composting.

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changed the OM contents in the initial materials, while all of them decreased along with the composting process. At the end of the process, the contents in T1, T4 and T5 were almost the same, while T2 and T3 were similar. The aeration rate of  $0.36 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$  decreased the OM degradation during the composting process (Fig 2B) in all the composting piles. Similar CO<sub>2</sub> emission trends were observed in T1-T5 in the first 7 days, suggesting the easily-degraded OM was quickly degraded and transferred to CO<sub>2</sub> (Fig 2C). Additives used in T1-T5 changed the CO<sub>2</sub> emission from Day 7. Less CO<sub>2</sub> emission amounts were observed when the ventilation rate was  $0.36 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$  in S1, S3 and S5 (Fig 2D), suggested that the  $0.18 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  was more suitable for the composting process in the current study. Based on the changes of OM and CO<sub>2</sub> emission, the easily-degraded OM in T2-T5 were less than that in T1. The concentration of cellulose and lignin was higher in saw dust, pine bark and peanut hull, when compared with wheat straw, while cellulose and lignin were hard to be biodegraded directly. Woody peat is rich in carbon and humus, but they were unavailable for microbial biodegradation, so that the CO<sub>2</sub> amount was lower than that in T1. Carbon additives, like biochar or woody peat, had shown similar results in vegetable wastes or sewage sludge composting [14].

In a previous study, the low aeration rate ( $0.3 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$ ) corresponded to a higher and longer thermophilic phase than the high aeration rate ( $0.9 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$ ) in the ventilation range of  $0.3\text{--}0.9 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \text{ DM}$  [31]. Some other studies recommended various

aeration methods and rates for different raw materials, like  $0.5 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  DM in the composting of chicken manure and sawdust [32],  $0.25 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  DM in the composting of dairy manure with rice straw [33],  $0.43\text{--}0.86 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  DM in the composting of food waste [34], etc. These suggested the aeration rate in composting should be set according to the compost material and composting process, based on the oxygen needed and supplied during the process.

### 3.2 Physiochemical characteristics

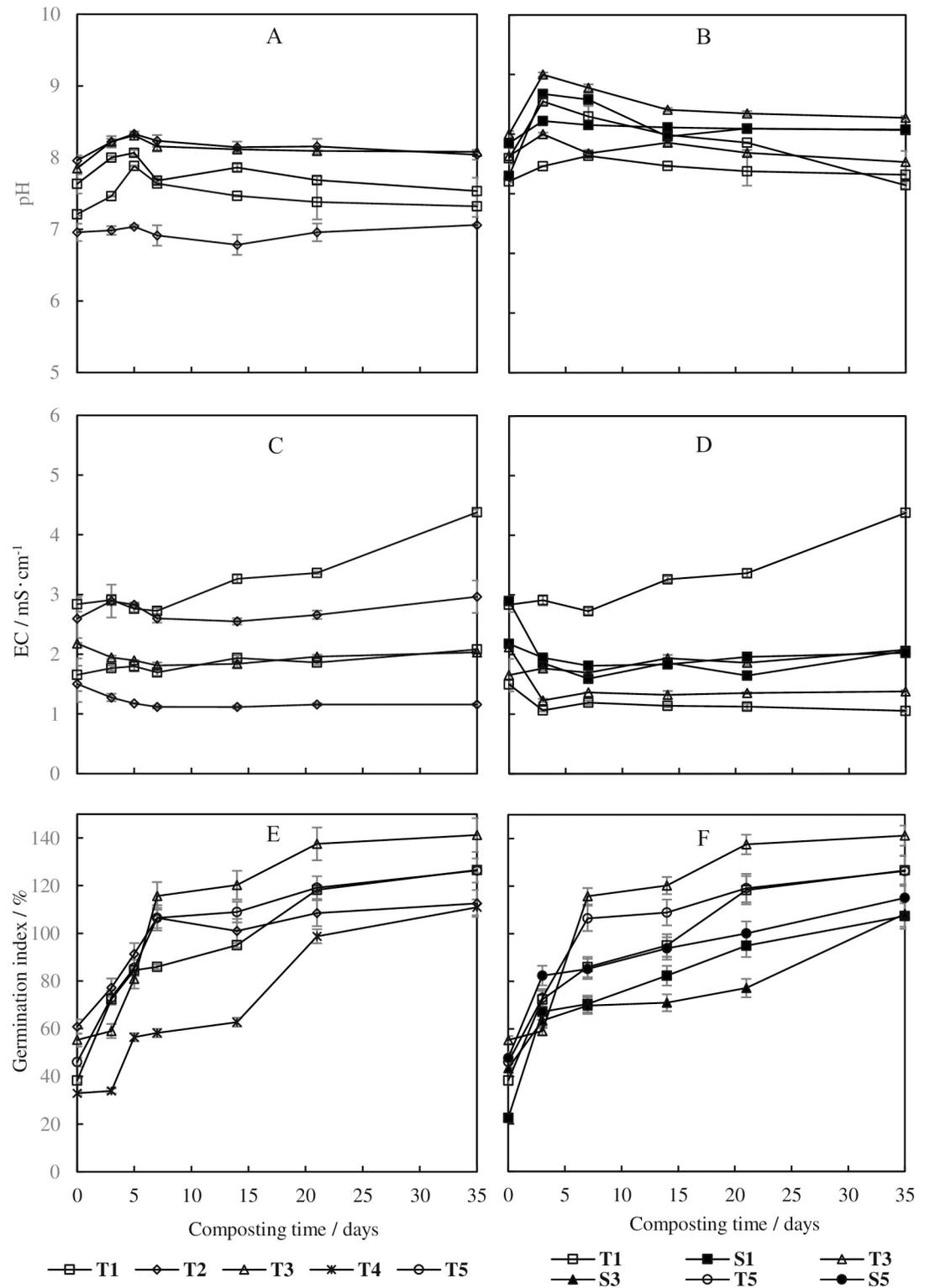
The appropriate pH range for maintaining high microbial activity during composting is 7–8, which would be changed along with the biodegradation of OM. The complex components were degraded to organic acids and then to  $\text{CO}_2$ , meanwhile  $\text{CO}_2$ ,  $\text{NH}_3$ , other gases and volatile organic acids were emitted from the composting system [35]. As shown in Fig 3A, the pH values in all the treatments were in the range of 6.8–8.4, suggesting the carbon additives changed the pH value and biodegradation process. The final values of the products were all in the range of 7.0–8.2, which were good for agricultural use [36]. The pH value in T2 was lower than others, indicating the potential advantage of woody peat, to reduce the  $\text{NH}_3$  emission by decreasing the material pH value. An increase of the aeration rate quickly increased the pH values (Fig 3B), as the gases and volatile organic acids were forced to be emitted more frequently than in the low aeration rate treatments. The final pH values of products in S1–S3 were higher than those in T1–T3.

A slight decrease was shown in the first several days for all treatments, followed by a stable value until the end (Fig 3C and 3D). The carbon additives influenced the EC variation, while they were always under  $4 \text{ mS}\cdot\text{cm}^{-1}$ , except  $4.37 \text{ mS}\cdot\text{cm}^{-1}$  in T1. The results reflected that the products could be used as organic amendments or organic fertilizer in soil [37], as they have no inhibitory effects on plant growth. Woody peat used in T2 reduced the EC in the whole process, because of the absorption caused by its rich humic acid. The rapid emission of gases and volatile organic acids in treatments with high aeration rate also reduced the EC of products.

To avoid the toxic effects on plant growth resulting from toxic substances, such as short-chain fatty acids, GI is always used as an important index to evaluate whether compost is mature enough. A minimum value of 80% is considered to indicate the compost maturity is at an extraction ratio of 1:5 (compost: water wet w/v). As shown in Fig 3E and 3F, the GI values increased with the decomposition of toxic materials, especially in the first 7 days. Nearly all the GI values were higher than 80% in the treatments, except T4 in series I, and S1 and S3 in series II. Then the GI values keep increasing slightly until the end of the process, with the GI values over 100%. The results indicated that the five additives chosen in the current study could adjust the free air space and C/N of chicken manure to improve the composting process. The higher aeration rate ( $0.36 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  DM) decreased the maturity process. However, it was opposite in a pig manure composting, in which the suitable aeration was  $0.48 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  DM even the lower aeration rate ( $0.24 \text{ L}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  DM) had better performance on biodegradation [38]. The reason may be complex: one is that higher aeration rate could supply enough  $\text{O}_2$  than a lower one when the aeration is intermittent; the other is that lower C/N of the mixed materials ( $< 20$ ) causes high concentration of TAN (total ammonium nitrogen) in the materials, which would inhibit the seedling.

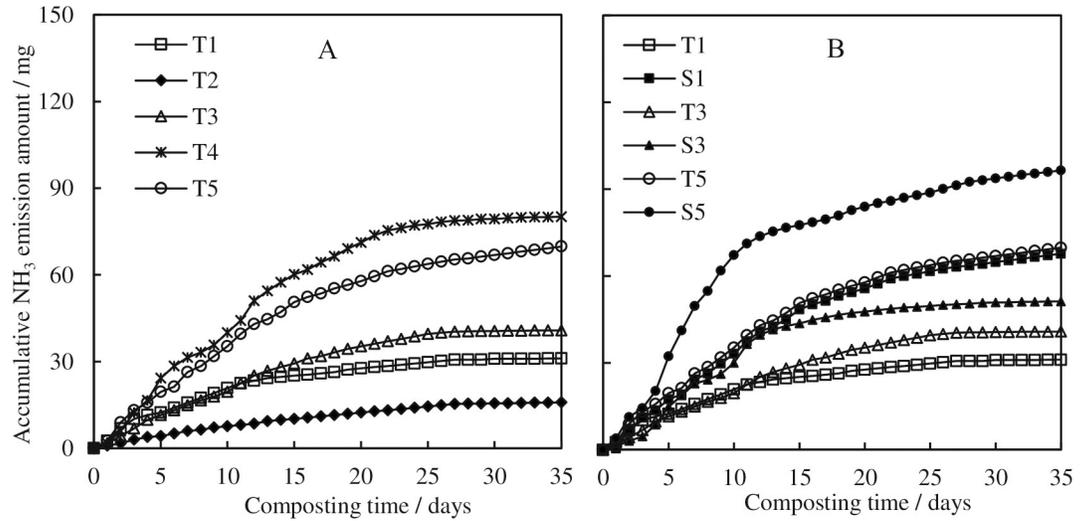
### 3.3 $\text{NH}_3$ emission and nitrogen concentrations

The changes of accumulative  $\text{NH}_3$  emission amount during the composting in the two series were shown in Fig 4. In all the treatments, the accumulative amounts rapidly increased and then the increasing rates slowed down. Additives used in series I changed the emission rate



**Fig 3.** Effects of carbon-based additives (A, C, E) and ventilation rate (B, D, F) on physiochemical characteristics during chicken manure composting.

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**Fig 4.** Effects of carbon-based additives (A) and ventilation rate (B) on NH<sub>3</sub> emissions during chicken manure composting.

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and the lasting time, as shown in Fig 4A. Significantly lower cumulative NH<sub>3</sub> emission in T2 (15.86 mg) was observed than those in other treatments. This should be related to the characteristics of woody peat, low pH and rich in humic acid, which contributed to the absorption of NH<sub>4</sub><sup>+</sup>. More cumulative NH<sub>3</sub> amounts were observed in T3-T5 than that in T1, which may be related to the lower biodegradable organic carbon in the mixed materials. There are high concentrations of lignocellulose in saw dust, pine bark and peanut hull, which may decrease the biodegradable C/N and increase the NH<sub>3</sub> emission. When it came to series II, S1-S3 had significantly higher cumulative NH<sub>3</sub> losses than T1-T3, respectively, suggesting the increased aeration rate in the current study would lead to more NH<sub>3</sub> emission.

TN concentrations of initial materials and products were shown in Table 3. Except T5 and S5, the TN concentrations were higher in the products of all treatments, because of the concentration effect caused by the significant organic decomposition [10]. Peanut hull, with high concentrations of cellulose and lignin, was used as carbon additive in T5 and S5, in which the NH<sub>3</sub> emission were higher than in other treatments, shown as 69.79 mg and 96.41 mg (Table 3).

**Table 3.** Nitrogen transformation and nitrogen loss in all treatments.

	Treatments	TN / g·kg <sup>-1</sup>		Ammonium nitrogen / mg·kg <sup>-1</sup>		Total NH <sub>3</sub> 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	23.68	25.06	963.93	154.80	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

<https://doi.org/10.1371/journal.pone.0229880.t003>

Meanwhile, less organic decomposition decreased the concentration effect of the mixed material. There are several forms of nitrogen in the compost, among which organic nitrogen (Nor) and nitrate nitrogen ( $\text{NO}_3^-$ -N) are normally stable in the materials and called stable nitrogen, while  $\text{NH}_4^+$ -N may transform to  $\text{NO}_3^-$ -N or be emitted as  $\text{NH}_3$ , when the temperature was decreased or during the utilization of the compost in arable land. High  $\text{NH}_4^+$ -N concentration in the materials always contributes to a high  $\text{NH}_3$  emission rate, which was consistent in our study except for T2. The different results indicated the important function of woody peat to absorb and fix the  $\text{NH}_4^+$ -N, because of its porous structure and richness of humus acid. Similar results could be observed when biochar was used during composting [39]. More  $\text{NH}_4^+$ -N in treatments T3 and T5 than S3 and S5 (shown in Table 3), suggested that the high aeration rate helped to transfer  $\text{NH}_4^+$ -N into  $\text{NH}_3$  ventilation, so that more  $\text{NH}_3$  ventilation was observed in Fig 5 and Table 2. Meanwhile the total nitrogen loss rates were higher in treatments with high aeration rate resulting from high  $\text{NH}_3$  ventilation.

### 3.4 Structure of microbial community

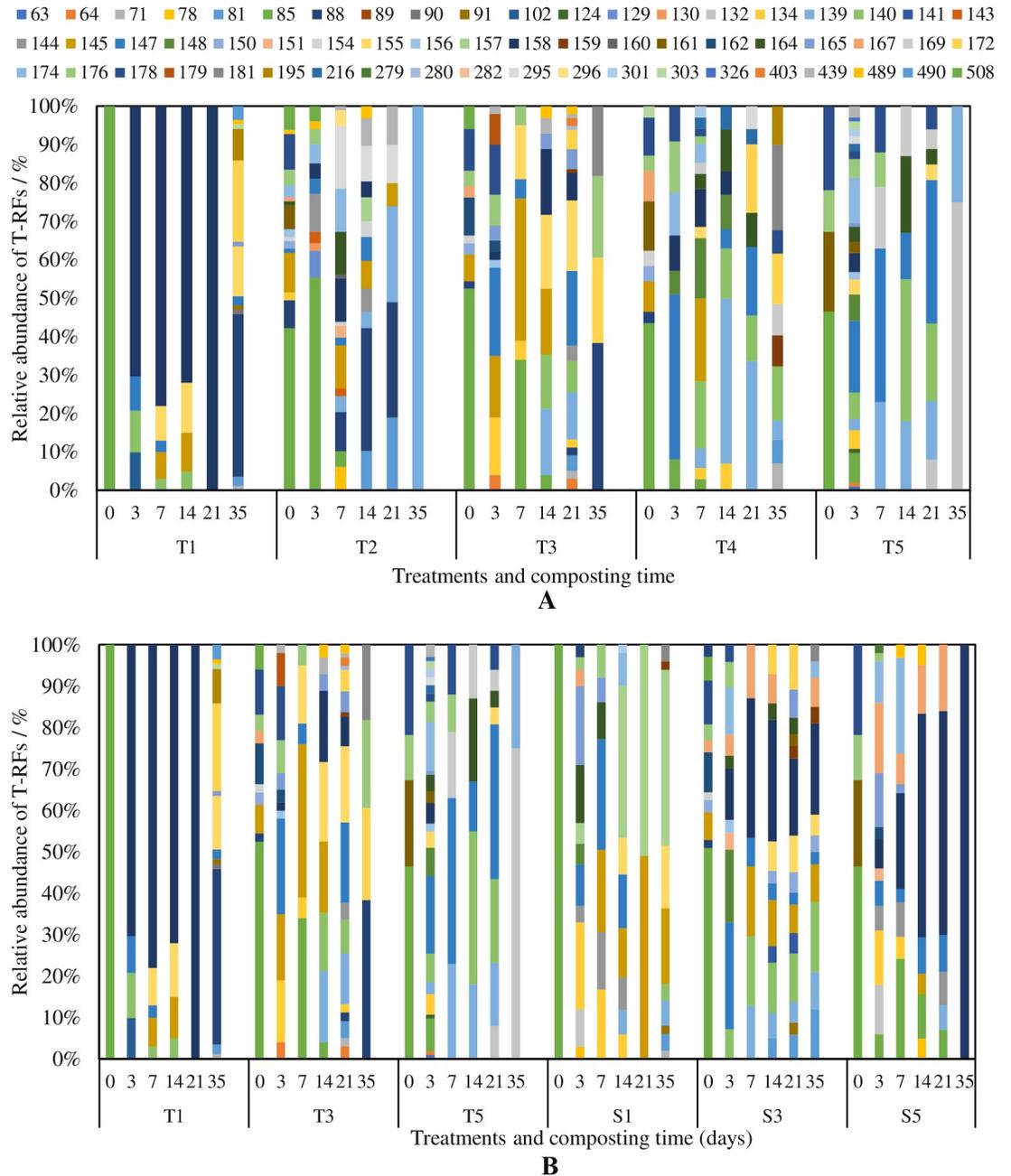
Different additives used in the current study changed the biodiversity during the composting process (Fig 5A). At the beginning, the most abundant T-RF was 85bp, with the ratio of 100%, 56%, 53%, 44% and 47%, in T1-T5 respectively. In all the 60 T-RFs shown in the figures, 139bp, 145bp, 147bp, 174bp, 176bp and 178bp had high relative abundances in two or more treatments in different stages, indicated these T-RFs should be related with the OM biodegradation. Meanwhile, 158bp (in T1), 295bp (in T2), 155bp (in T3), 140bp (in T4) and 132bp (in T5) had high relative abundance in specific treatment, which was related to this additive only. The increase of the ventilation rate contributed to higher biodiversity in all the treatments (Fig 5B). The results suggested the additives and aeration rates in the current study influenced the microbial population, resulting in a different biodegradation process.

Constructing a bacterial clone library could help to understand the species distribution in the composting system. Five of the compost samples in different treatments were selected to construct the clone library, in which *Firmicutes*, *Proteobacteria*, *Bacteroides* and *Actinomycetes* accounted for 85.83% in the total 247 sequences (Fig 6A). *Bacillus spp.*, belonging to the phylum of *Firmicutes*, was primary in all the sequences, because of its characteristics of high-temperature resistance and OM biodegradation ability [40]. The result of the phylogenetic analysis affiliated with uncultured groups using the neighbor-joining method is shown in Fig 6B. Carbon additives used in different treatments influenced the clones, similar as shown in Fig 6A. Prominent clones belonged to an uncultured group in the phylum *Firmicutes* (class of *Bacilli* and *Clostridia*) and *Proteobacteria* (class of *Alphaproteobacteria*, *Deltaproteobacteria* and *Gammaproteobacteria*), which were also observed in mature compost samples in previous studies [39–41]. High proportions of *Actinobacteria* in the samples of T1 and T3, suggested that the addition of corn straw or saw dust may facilitate the growth of *Actinobacteria* and accelerate the degradation of lignocelluloses during the maturity stage [39].

Based the results above, it can be concluded that the additives and aeration rates used in the current study changed the microbial community, which may be one of the main reasons for the change of biodegradation and the emission amounts of  $\text{CO}_2$  and  $\text{NH}_3$ . However, the composting process and the main functional microbes remained similar in different treatments even they were influenced by the additives and aeration rates.

## 4. Conclusion

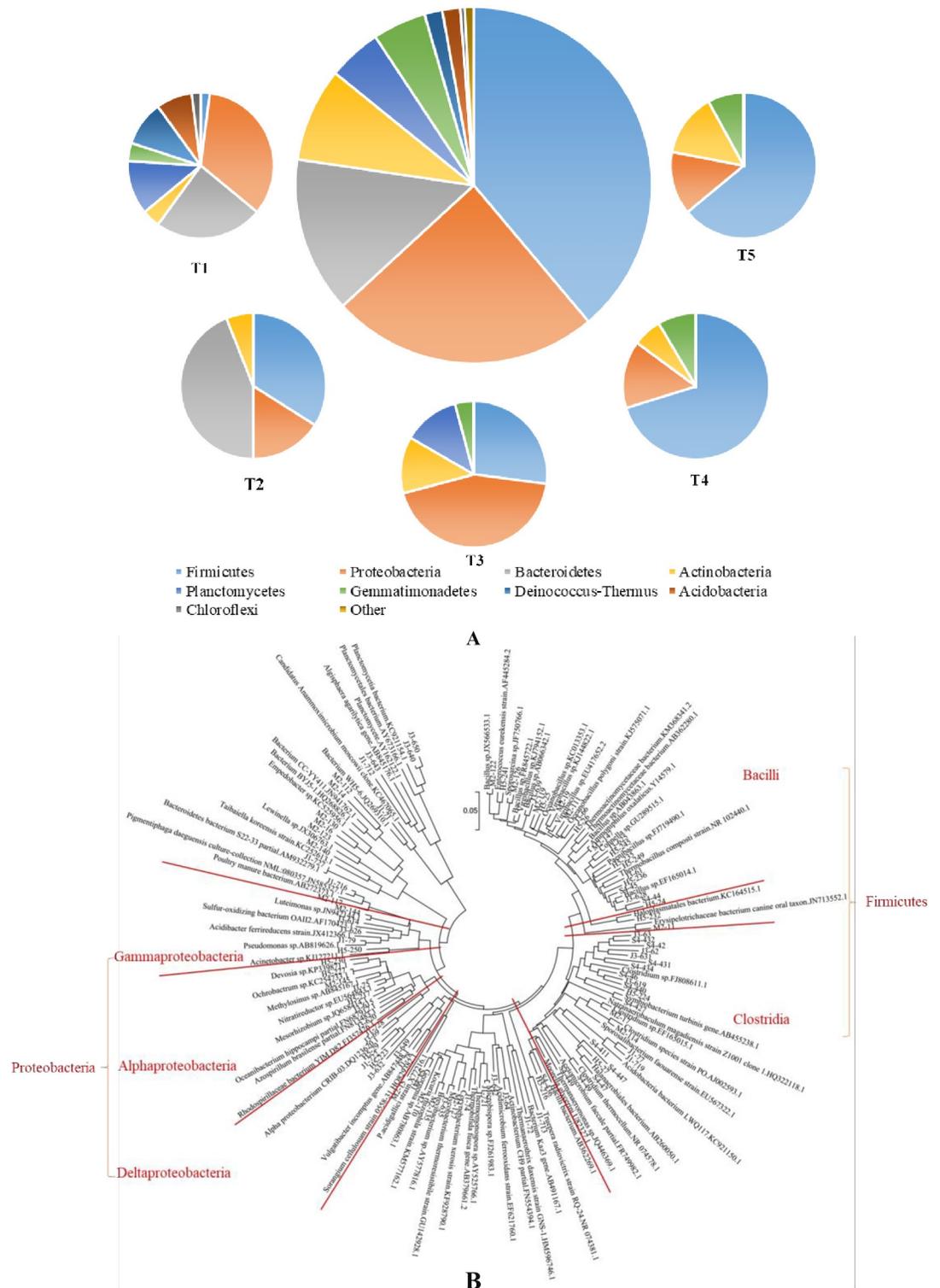
Composting using any of the additives in the current study improved the biodegradation process and influenced the pH, EC and GI values of the product. Among them, woody peat



**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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had the best effect on reducing NH<sub>3</sub> emission and nitrogen loss. The aeration rate of 0.18 L·min<sup>-1</sup>·kg<sup>-1</sup> DM was more suitable than 0.36 L·min<sup>-1</sup>·kg<sup>-1</sup> DM for chicken manure composting, which was also better for controlling NH<sub>3</sub> emission and nitrogen loss. The prominent clones of the compost samples belonged to the phylum Firmicutes (class of *Bacilli* and *Clostridia*) and Proteobacteria (class of *Alphaproteobacteria*, *Deltaproteobacteria* and *Gammaproteobacteria*). Among them, *Bacillus* spp. was always the most important one, even carbon-based additive and ventilation rate all made influences on the microbial community.



**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).

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Therefore, woody peat could be used as additive instead of corn straw, and suitable ventilation rates could reduce the NH<sub>3</sub> emission and nitrogen loss.

## Author Contributions

**Conceptualization:** Ruixue Chang.

**Data curation:** Ruixue Chang.

**Investigation:** Ruixue Chang, Xiaoyan Gong.

**Methodology:** Ruixue Chang, Yanming Li, Xiaoyan Gong.

**Project administration:** Yanming Li, Zicheng Qi.

**Software:** Xiaoyan Gong.

**Supervision:** Yanming Li, Qing Chen.

**Writing – original draft:** Ruixue Chang.

**Writing – review & editing:** Ruixue Chang, Yanming Li, Qing Chen.

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