



## Enhanced nitrogen retention by lignite during poultry litter composting

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### ABSTRACT

Composting is an effective method to transform organic wastes into soil amendments. However, significant nitrogen (N) is lost through ammonia (NH<sub>3</sub>) volatilization during composting. This work evaluated the effect of lignite amendment (0% (CK), 5% (T1), 10% (T2), 15% (T3)) on N transformation during composting. The results showed T3 increased total nitrogen content by 25% (33.0 g kg<sup>-1</sup> in T3 vs 21.0 g kg<sup>-1</sup> in CK), and increased mineral N by >60% compared with CK. The X-ray photo-electron spectroscopy and Boehm's titration analysis showed that composted T3 contained higher total acid groups than composted CK. Notably, the abundances of ammonium oxidizers decreased in all lignite treatments. High moisture content and low NH<sub>3</sub> availability could suppress ammonia oxidizers, therefore improved N retention. Partial least-squares path modeling suggested that physiochemical properties played a dominant role in N loss in composting with or without lignite, however, acid group content appeared to be able to strongly inhibited N loss by adsorption NH<sub>4</sub><sup>+</sup> under composting with lignite addition. The results of this study suggested lignite addition at 15% as a proper ratio for composting to achieve fast organic degradation and great N retention.

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### 1. Introduction

With an increasing number of modern intensive poultry farms, the excess of poultry manure in many areas has caused significant environmental challenges, in particular, the small areas with insufficient surrounding agricultural land for application as a fertilizer. Composting is a cost-effective way to convert poultry litter into stabilized compost. However, composting of poultry manure can cause high loss of nitrogen through ammonia volatilization. In poultry manure, organic N such as urea N and uric acid N can be mineralized by microorganisms and converted into ammonium (NH<sub>4</sub><sup>+</sup>) via ammonification, in which urease catalyzes the hydrolysis of urea to NH<sub>3</sub>. Reduced urease activity could be related to decreased N loss either in soil or composting systems (Cook et al.,

2011). Ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) have been discovered in composting and are significant contributors to NH<sub>3</sub> oxidation (Yamamoto et al., 2010). Any NH<sub>3</sub> not adsorbed by compost material is rapidly oxidized to NO<sub>2</sub><sup>-</sup> via hydroxylamine. Therefore, NH<sub>3</sub> oxidation may be restricted by the availability of NH<sub>4</sub><sup>+</sup> in composting.

To reduce nitrous emissions and retain N during poultry litter composting, diverse disposal strategies including bulking agents, bedding materials, microorganisms, chemicals and various amendments such as natural and mineral adsorbents have been widely used (Awasthi et al., 2019; Wu et al., 2019).

Lignite, also known as brown coal, has been used widely as a natural adsorbent and is globally abundant and mined in great quantities (Hughes, 2018). It has a similar structure and chemical composition in terms of C, H, N and O proportion to many biochars (Paramashivam et al., 2016). Due to its high cation exchange capacity derived from dissociation of carboxylic and phenolic groups, lignite has been used in removal of metal ions, dyes and ammonium from wastewater (Nazari et al., 2018). Granulation of urea with

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lignite delayed mineral N release and maintained higher N in soil than urea only (Dong et al., 2009; Saha et al., 2017).

More recently, Chen et al. (2015) and Sun et al. (2016a) demonstrated that lignite application on the surface of cattle pen decreased  $\text{NH}_3$  emissions by  $38 \text{ kg N head}^{-1} \text{ y}^{-1}$  and reduced approximately 30% of the excreted N lost via  $\text{NH}_3$  volatilization. Based on these studies, the increased N retention in soil and feedlot systems by lignite application was attributed to: 1) a part of the  $\text{NH}_4^+$ -N generated from organic N mineralization is brought into lignite due to the high CEC of lignite or direct binding of  $\text{NH}_3$  (Chen et al., 2015); and 2) lignite inhibits the activity of urease, which hydrolyzes urea to  $\text{NH}_3$ , resulting in a lower rate of  $\text{NH}_3$  emission (Shen et al., 2016). The decreased availability of  $\text{NH}_3$  could in turn affect the population size or even community structure of ammonia oxidizers in compost with consequences for the nitrification process. Lignite may also directly or indirectly influence microbial communities through increasing water retention and water holding capacity (Piccolo et al., 1996), decreasing bulk density and improving aeration, retaining nutrients and buffering pH (Dong et al., 2009). Most of the previous studies focused on the effects of lignite application on  $\text{NH}_3$  emission or N retention from soil or pure solution systems, few studies have been conducted to evaluate the potential use of lignite as an additive on N transformation during composting.

Therefore, three different ratios (5%, 10% and 15%) of lignite were co-composted with poultry litter in a lab-scale composting experiment. The objectives of the study were to evaluate the effects of lignite amendment on N retention, functional acid group concentration, the abundance of ammonia oxidizers and their correlation with physicochemical characteristics during poultry litter composting. The novelty of the study aimed to provide evidence for the potential use of lignite as an additive for the reduction of N loss during composting of agricultural wastes. Meanwhile, changes of functional acid group and evolution of ammonifiers during composting were analyzed to elucidate the mechanism of N retention by addition of lignite during composting.

## 2. Materials and methods

### 2.1. Description of composting feedstocks

Poultry litter was collected from a commercial broiler house near Victoria, Australia. The initial bedding material was composed of sawdust, straw and rice husk. The lignite was Bacchus Marsh brown coal obtained from Victoria, Australia.

### 2.2. Composting process

The composting was performed at the Animal House at the University of Melbourne, Australia. Poultry litter was sieved with dimensions from 1–2 cm. The lignite was screened with a 2-mm sieve. Previous study showed that lignite added at the rate of  $6 \text{ kg m}^{-2}$ , which was equivalent 10% to the total dry weight, could enhance mineral N retention in cattle manures in feedlots significantly (Shen et al., 2016). As a result, poultry litter was mixed with lignite at 0 (CK), 5% (T1), 10% (T2) and 15% (T3) to the total dry matter. Each treatment was adjusted to a final moisture content of 55–60% and had two replicates.

The raw materials of about 50 kg were transferred to commercial composting reactors. Each tumbling composter, insulated with 10-cm thick glasswool, had a volume of 160 L with a size of 725 mm (width)  $\times$  935 mm (height)  $\times$  660 mm (length). At 30 cm from the bottom of both sides of the reactor, there were twelve  $1 \times 1 \text{ mm}^2$  holes which allowed aeration from the left and right sides. The composting reactors were turned once every day by manually

rotating to provide fresh air to the composting mixtures. The mass of the materials in the composting reactors was weighted at the beginning and the end of the experiment. Compost samples from each reactor were collected on 1, 8, 15, 22, 28, 45 and 65 d and used for the analysis of moisture, pH, electric conductivity (EC), ash, ammonium and nitrate contents, total carbon (TC), total nitrogen (TN), seed germination index (GI), urease activity and DNA extraction. Temperatures were measured every day with a portable temperature monitoring probe.

### 2.3. Analytical methods

#### 2.3.1. Physicochemical analysis of the raw materials and compost products

Moisture content of fresh samples was measured by oven drying at  $105 \text{ }^\circ\text{C}$  for 24 h. The bulk density was determined using a container with which to apply vertical loads and a platform scale. The container had an approximate inside diameter of 60 mm and a fill mark of 50 mm from the bottom. The bulk density was calculated by dividing the dry mass of sample by its volume occupied (Iqbal et al., 2010). Particle size analyses for all samples were performed according to Schaub-Szabo and Leonard (1999). The dried compost was sieve-screened to different fractions and weighted.

The pH and EC were determined from 1:5 (w/v) water-soluble extract with a pH/EC meter. The water-soluble extract was also centrifuged to analyze the water-soluble carbon (DOC) and water-soluble nitrogen (DON) of the compost using a TOC/TN analyzer (multi N/C 3000, Analytik Jena AG, Germany). TC and TN were analyzed on a LECO Trumac CN at a furnace temperature of  $1350 \text{ }^\circ\text{C}$ . Dried samples were put into a muffle furnace at  $550 \text{ }^\circ\text{C}$  for 24 h for ash content calculation. Ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) was extracted with 2 M KCl (1:20) and analyzed on a Skalar SAN++ segment flow analyzer (TrACCESS Soil Node, University of Melbourne). Phytotoxicity of compost samples to the lettuce was carried out using the method provided by Zucconi et al. (1981). The cation exchange capacity (CEC) of composts was measured by the method of ammonium acetate (Ciarkowski et al., 2017). The oxygen-containing acidic surface functional groups of fresh and composted samples were determined by the method of Boehm's titration (Boehm, 1994).

#### 2.3.2. Urease activity measurement

Urease activity was determined using the Urease Activity Assay Kit (Sigma-Aldrich) according to the manufacturer's instruction. One unit of urease is the amount of enzyme that catalyzes the formation of  $1.0 \text{ } \mu\text{mol NH}_4^+ \text{ h}^{-1}$  at pH 7.0.

#### 2.3.3. DNA extraction and quantitative PCR in compost samples

Samples collected on 1, 8, 28 and 65 d representing the initial stage, thermophilic stage, cooling stage and maturity stage, respectively, were used for downstream molecular analysis. Genomic DNA was extracted from 0.25 g of frozen compost sample using FastDNA SPIN KIT for soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. After extraction, the DNA was purified by spin-bind cartridges after being precipitated by PEG 6000 (Yang et al., 2007). The abundances of bacterial 16S rRNA, AOA and AOB *amoA* genes were examined by quantitative PCR using the primer pairs and amplification conditions according to previous research (Shi et al., 2016).

#### 2.3.4. Spectroscopic characterization of composts by X-ray photoelectron spectroscopy (XPS)

XPS was performed on a Kratos Axis ULTRA X-ray Photoelectron Spectrometer using monochromatic Al  $K\alpha$  X-rays ( $1486.6 \text{ eV}$ ) at 150 W (15 kV, 15 ma) under base pressure of  $1.0 \times 10^{-8}$  torr. Pass

energy of 160 eV for survey (wide) scans and multiplex (narrow) scans at 20 eV was used. Scanned volume was ~2400 cubic micron with an area of  $0.8 \times 0.3 \text{ mm}^2$  and a depth of 10 nm. Survey scans and narrow high-resolution scans were carried out over 1200–0 eV binding energy range with 1.0 eV steps and 0.05 eV steps, respectively. Data analysis, curve fitting and calibration was carried out on CasaXPS software.

#### 2.4. Statistical analyses

Statistical analyses were carried out on the IBM SPSS Statistics 19 (IBM Corporation, Armonk, NY, USA). Redundancy analysis (RDA) was performed to analyze the relationships between the physicochemical characteristics and the population of AOA and AOB. Variance partitioning was used to assess the variations in the abundances of AOA and AOB explained by various physicochemical properties. RDA and variance partition were performed using the Canoco 5.0 (Microcomputer power, Ithaca, NY, USA). Partial least squares path modeling (PLS-PM) was used to examine the complex relationships among various variables on N loss using the R package *plspm* (v 0.4.7). The physicochemical properties (pH, EC, ash, DOC, CEC, moisture content, TC, TN,  $\text{NH}_4^+$ ), total bacterial abundance, ammonia oxidizers abundance and total acid group contents (-OH, COO, C=O) were included in the model.

### 3. Results and discussion

#### 3.1. Impact of lignite on the temperature variation during composting process

The temperature in all treatments followed the typical evolution of composting process, heating stage (d 1–d 2), thermophilic stage (d 3–d 12), cooling stage (d 13–d 28) and maturity stage (d 29–d 65) (Fig. 1). The temperatures increased quickly in all treatments and reached the maximum on day 2: 64.80 °C (CK), 67.05 °C (T1), 69.45 °C (T2), 69.45 °C (T3). The faster increase of temperature in the heating stage and longer thermophilic phase indicated that addition of lignite improved the performance the composting process (Fig. 1). Thermophilic phase (compost temperature >55 °C) in the study was extended by 3–7 d for the mixtures amended with lignite: i.e. 3, 9, 7 and 8 d for CK, T1, T2 and T3, respectively. Hence, the addition of lignite to the poultry litter resulted in the extension of the thermophilic stage of composting time. Higher temperatures observed at the beginning of the process and a longer thermophilic-active phase in the mixtures amended with lignite could accelerate the OM decomposition, and consequently shorten the time required for maturity. Lignite can be a habitat for microorganisms due to its large surface area and strong water retention capacity (Kolb et al., 2009). The results were similar to Georgacakis et al. (1996), where lignite amendment resulted in prolonged thermophilic stage and improved compost quality during solid swine manure composting.

#### 3.2. Impact of lignite on the evolution of physicochemical characteristics during composting

Physical properties (bulk density, particle sizes, and moisture content) significantly influence the performance of composting (Richard et al., 2002). At the beginning of composting, the addition of lignite at 10–15% significantly reduced the bulk density, which might be due to the high porosity of lignite (Yue et al., 1999). As composting progressed, the bulk density of all treatments increased and showed no significant difference among treatments (Table 1). The greatest increase in a percentage of bulk density and decrease in the volume of raw material was observed for T3 (42%).

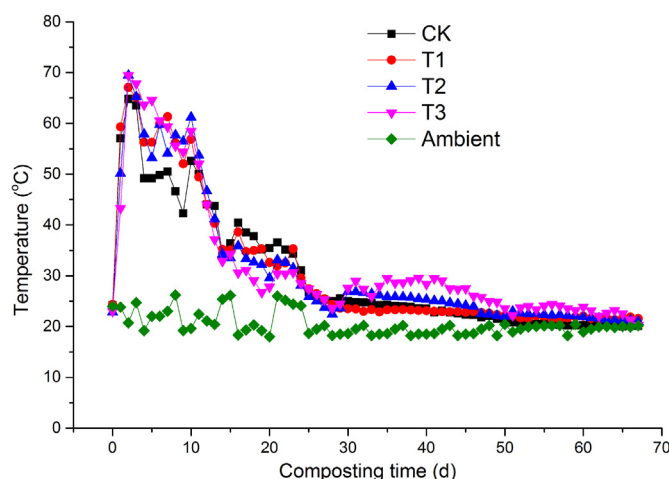


Fig. 1. Temperature profiles of four compost piles over the 65-d study. CK: poultry litter with no lignite, T1: poultry litter with 5% lignite; T2: poultry litter with 10% lignite; T3: poultry litter with 15% lignite.

By reducing the bulk density of compost material, the lignite addition may improve aeration in the feedstocks that promotes microbial activity and substantially improve the degradation of the OM (Jain et al., 2019).

Lignite addition changed moisture variation during composting. The moisture content of the mixtures decreased from 64.0% to 60.4% for CK and from 63.6% to 59.9% for T1, however, the moisture content for T2 and T3 showed an upward trend during composting (Table 1). The higher moisture content in T2 and T3 than that in CK could be attributed to higher microbial metabolic activity in OM degradation during composting. Furthermore, composting vessels without forced ventilation was used in the present study, so the water reduction would be reduced. The increase in the moisture content could be due to the water uptake and water holding capacity of lignite (Cihlár et al., 2014).

Particles of different sizes formed progressively in all treatments throughout composting regardless of turning. The average weight percentage corresponding to the fractions >3, 1–3 mm and <1 mm was 61.21%, 28.97% and 10.23% for CK and 83.76%, 12.63% and 3.61% for T3, respectively. Therefore, lignite addition at 15% level increased the generation of large particles, thus decreased the ratio of small (<1 mm) compared with CK. In fact, large clumps (>1 cm) accounted for 35.2% (dry weight) of the total particles (>3 mm) in T3 compared to 8.7% large clumps (>1 cm) in CK. The reduced bulk density and high moisture content of T3 treatment resulted in significant leachate formation, leading to the formation of large clumps at the curing stage. The formation of large clumps can facilitate the reduction in porosity and oxygen availability which would in turn inhibit the growth of microorganisms (Jain et al., 2019), and was supported by a decrease in copy numbers of the 16S rRNA gene, AOA *amoA* and AOB *amoA* genes recorded in T3 on day 65.

The initial CEC values were significantly higher in T2 and T3 than in CK and T1 due to the higher rates of lignite added. In all treatments, the CEC increased dramatically in the first week and then increased gradually until the end of the study (Table 1). The significantly higher CEC in T3 and T2 could be due to the higher contents of carboxyl and phenolic functional groups in the lignite (Benito et al., 2003). The initial pH in the compost mixtures was significantly decreased by the addition of lignite and the lowest pH was observed in T3 (pH 7.91) compared to CK (pH 8.19) (Fig. 2A). As the composting progressed, the pH in lignite added mixtures was

**Table 1**  
Variation in bulk density and moisture content of different composting materials during composting process.

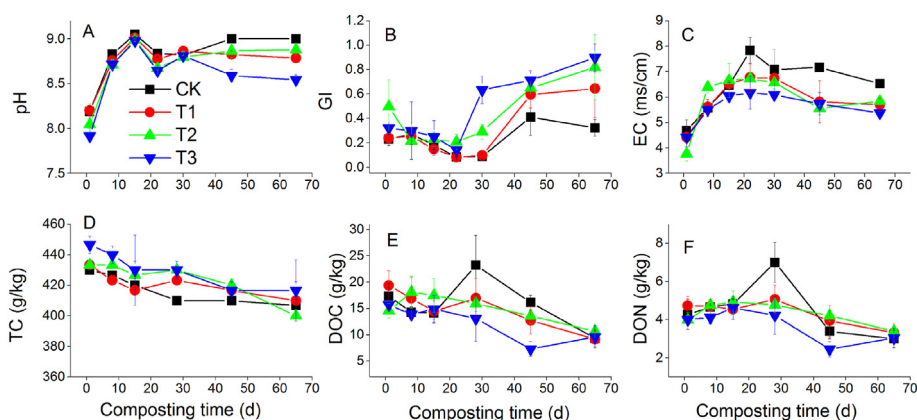
Treatment	Bulk density ( $\text{g cm}^{-3}$ )			Moisture content (%)			Cation exchange capacity (cmol/kg)		
	d1	d45	d65	d1	d45	d65	d1	d45	d65
CK	0.160(0.03) <sup>a</sup>	0.182(0.08) <sup>a</sup>	0.193(0.05) <sup>a</sup>	64.0(2.8) <sup>a</sup>	60.2(0.3) <sup>b</sup>	60.4(0.2) <sup>b</sup>	65.1(0.8) <sup>b</sup>	81.3(2.5) <sup>b</sup>	87.9(5.6) <sup>c</sup>
T1	0.151(0.04) <sup>a</sup>	0.183(0.04) <sup>a</sup>	0.196(0.04) <sup>a</sup>	63.6(2.5) <sup>a</sup>	60.1(0.2) <sup>b</sup>	59.9(2.8) <sup>b</sup>	66.6(1.3) <sup>ab</sup>	89.7(5.6) <sup>b</sup>	96.9(3.6) <sup>c</sup>
T2	0.132(0.02) <sup>b</sup>	0.138(0.02) <sup>b</sup>	0.188(0.05) <sup>a</sup>	59.1(2.6) <sup>ab</sup>	60.1(1.7) <sup>ab</sup>	63.1(1.2) <sup>ab</sup>	69.2(1.4) <sup>a</sup>	109.6(4.5) <sup>a</sup>	114.1(2.5) <sup>b</sup>
T3	0.123(0.04) <sup>b</sup>	0.128(0.01) <sup>b</sup>	0.182(0.01) <sup>a</sup>	58.1(2.4) <sup>b</sup>	62.8(3.3) <sup>ab</sup>	65.5(2.9) <sup>a</sup>	71.6(2.6) <sup>a</sup>	114.7(5.0) <sup>a</sup>	122.7(2.7) <sup>a</sup>

CK, poultry litter only; T1, poultry litter + 5% lignite; T2, poultry litter + 10% lignite; T3, poultry litter + 15% lignite. Means (standard error) (n = 3) within columns followed by different lowercase letters are significantly different at  $P = 0.05$  as determined by LSD test.

consistently lower than CK. In the initiation and thermophilic stages of composting, no significant difference was observed in EC between treatments. However, the EC was significantly lowered by lignite addition in the cooling and maturity stages (Fig. 2C). The reduction of pH and EC in the mixtures of T3 could be related to the low pH and high contents of carboxyl and phenolic groups providing the active sites for ion exchange (Qi et al., 2011). The cation exchange capability of lignite could contribute to the removal of cations from aqueous solution. However, DOC concentrations decreased gradually from 14.5–19.4  $\text{g kg}^{-1}$  at the beginning to 9.2–10.7  $\text{g kg}^{-1}$  at the end of the composting amended with different ratios of lignite (Fig. 2E). The conditions were favorable for microbes in T1, T2 and T3 to utilize DOC and proliferate, resulting in sustained decreasing of DOC during the composting process (Castaldi et al., 2008). The reduction of DOC could also be attributed to the absorption of DOC by lignite (Nazari et al., 2018). However, the DOC content in CK reached a peak on day 28 (23.2  $\text{g kg}^{-1}$ ) and decreased to 9.3  $\text{g kg}^{-1}$  by day 65. The increase of DOC in CK suggested that the degradation and utilization of DOC was much slower than the rate of synthesis. Although not quantified in this study, this result could be associated with the lower microbial activities in CK compared to lignite-amended treatments during the first 28 d (Liu et al., 2011). The variation in DON contents in CK and treatments showed a similar trend, increasing gradually during the first 28 d, reaching maximum value on day 28 and then decreasing slowly until the end of the experiment (Fig. 2F). DON and  $\text{NH}_4^+\text{-N}$  showed a similar trend and was inversely proportional to TN. It could be inferred that TN was quickly transformed into DON and  $\text{NH}_4^+\text{-N}$ . The GI values suggested that lignite could enhance the maturity of the compost (Fig. 2B). GI dropped from 0.23–0.49 on day 1 to 0.08–0.2 on day 22. However, the GI in T3 was increased to 0.63 on 28 d and kept rising afterwards, while it was only increased to 0.32 on 65 d. The TC content decreased with composting time in all the mixtures (Fig. 2D). Greater OM-loss of 51.92% and 49.17% was observed for T3 and T2 compared with 39.78% and 42.44% for T1 and CK, respectively. Therefore, the lignite addition at 15% level had the greatest impact on the overall OM degradation. The higher OM losses in T3 can be partly attributed to higher temperature and longer thermophilic stage compared to other treatments during composting. The C/N ratio increased rapidly from during the first 8 days, after which the value remained stable in CK, in lignite-added piles, however, the C/N decreased gradually from day 8 until the end of composting (Fig. 3A). The decrease of C/N was more rapid and pronounced in T3, which should be caused by the decrease in the content of TC and the increase in the TN.

### 3.3. Impact of lignite on N transformation

Lignite addition improved N retention in compost. The  $\text{NH}_4^+$  concentration in all treatments increased to a peak of 7.6, 7.7, 6.6 and 7.2  $\text{g NH}_4\text{-N kg}^{-1}$  for CK, T1, T2 and T3, respectively (Fig. 3B). This reflects higher organic N ammonification rate driven by intense microbial activity during the heating and thermophilic stage. The increase in  $\text{NH}_4^+$  concentration might be due to increased temperature which favored the mineralization of organic nitrogen compounds. In the curing stage,  $\text{NH}_4^+$  concentration was significantly higher in the mixtures with 10% (2.1  $\text{g kg}^{-1}$ ) and 15% (2.5  $\text{g kg}^{-1}$ ) than that of 5% (1.9  $\text{g kg}^{-1}$ ) and CK (1.5  $\text{g kg}^{-1}$ ). The higher  $\text{NH}_4^+$  concentration in T3 can be attributed to the strong adsorption capacity of  $\text{NH}_4^+$  (76.7  $\text{cmol kg}^{-1}$ ) by lignite. Nitrification was quite weak during the composting process. The initial nitrate concentration was below 4  $\text{mg kg}^{-1}$  for all mixtures and increased to 19, 15, 23 and 5  $\text{mg kg}^{-1}$  for mixtures with 0%, 5%, 10% and 15% lignite, suggesting that higher rates of lignite decreased the gross nitrification rate. The low rate of nitrification in T3 may be



**Fig. 2.** Changes in physico-chemical properties of four compost piles. CK: poultry litter with no lignite, T1: poultry litter with 5% lignite; T2: poultry litter with 10% lignite; T3: poultry litter with 15% lignite over the 65-d study.

attributed to unavailability of strongly adsorbed  $\text{NH}_4^+$  to ammonium oxidizers (Mahimairaja et al., 1994). These results were comparable with that reported by Patti et al. (1992) that brown coal when applied to soil and can act as nitrification inhibitors. The nitrification inhibition during composting could further reduce the potential for N loss through nitrate leaching.

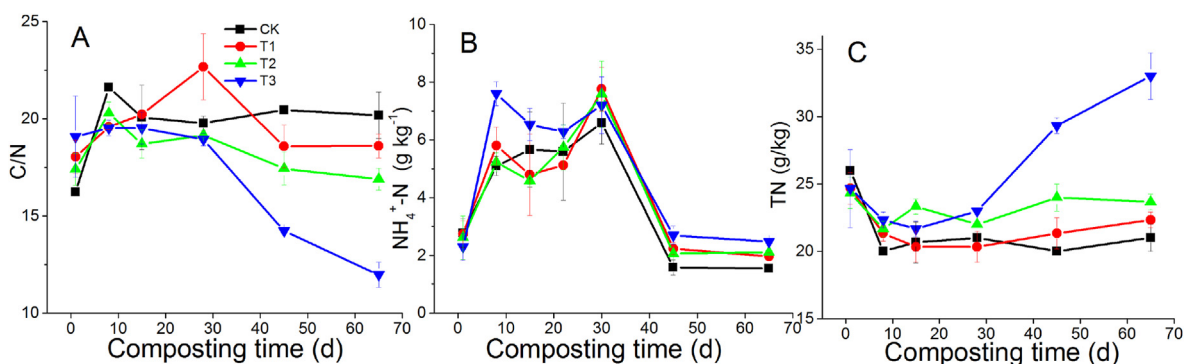
The TN decreased markedly from 26.0, 24.7, 24.3  $\text{g kg}^{-1}$  to 21.0, 22.3, 23.7  $\text{g kg}^{-1}$  for mixtures with 0, 5% and 10% lignite, respectively (Fig. 3E). However, TN in T3 treatment was increased significantly from 24.7 to 33.0  $\text{g kg}^{-1}$ . The significant increase of TN in T3 could be partly due to the concentration effect provoked by the OM biodegradation (weight loss). The minimum N loss was recorded in T3 and was 25% lower than CK (Table 2). The parameters of kinetic equations also suggested lower TN loss for mixture with 15% lignite during the composting process.

Higher temperature and pH of the mixtures favor  $\text{NH}_3$  volatilization. However, the temperature in lignite amended mixtures increased and the pH was not significantly changed by the addition of lignite during the thermophilic stage of composting. Therefore, higher N retention in lignite amended mixtures could not be explained by the temperature or pH increase. In fact, the  $\text{NH}_4^+$  produced from organic N mineralization during composting could be partially captured by lignite, since lignite amendment increased the CEC and the  $\text{NH}_4^+$ -N could be captured by the abundant functional groups such as  $-\text{COOH}$  and  $-\text{OH}$  (Paramashivam et al., 2016), which will be discussed later.

### 3.4. Characterization of functional groups of the fresh and composted mixtures

Table 3 shows the total acidity of three main functional groups (carboxyl/lactonic/phenolic groups) of lignite, fresh and composted samples. The acidity in lignite and compost samples was due to the carboxyl groups, lactones and phenols (Boehm, 1994). The results revealed that the concentrations of acidic functional groups increased gradually during composting, the total acidity increased from 1.74 to 2.17 and 1.86–2.46  $\text{mmol g}^{-1}$  in CK and T3, respectively. The increase in the total acidity might be due either to the oxidation of methoxyl and alcoholic groups of side chains of lignin or to the microbial degradation of carbohydrates to carboxyl, phenolic and ketone groups (Lou et al., 2015). The higher concentrations of acidic functional groups in T3 than CK should be attributed to the addition of lignite, which contain more lactones and phenols than poultry litter, indicating that it would be favorable for the degradation of OM and enhancing the microbial activity in lignite-poultry litter mixtures composting (Zhang et al., 2014).

As significant difference in N retention was only observed between CK and T3, which was discussed above, the oxygen-containing acidic functional groups of fresh and composted samples in CK and T3 were further characterized by XPS. The composted poultry litter (composted CK) had a higher relative concentration of C–O (alcoholic, phenolic, hydroxyl) and C=O (ketones and quinones) but lower concentrations of C–C and



**Fig. 3.** Changes in  $\text{NH}_4^+$ -N and TN of four compost piles. CK: poultry litter with no lignite, T1: poultry litter with 5% lignite; T2: poultry litter with 10% lignite; T3: poultry litter with 15% lignite over the 65-d study.

**Table 2**  
Nitrogen losses from different treatments during composting.

Treatment	TN before composting (kg/reactor)	TN retained in the end (kg/reactor)	TN loss (kg/reactor)	TN loss (%)
CK	0.54 (0.04) <sup>a</sup>	0.26 (0.02) <sup>b</sup>	0.28 (0.02) <sup>a</sup>	52.0 (2.8) <sup>a</sup>
T1	0.51 (0.02) <sup>b</sup>	0.26 (0.01) <sup>b</sup>	0.25 (0.02) <sup>b</sup>	49.0 (3.3) <sup>a</sup>
T2	0.49 (0.02) <sup>b</sup>	0.25 (0.01) <sup>b</sup>	0.24 (0.02) <sup>b</sup>	48.9 (3.4) <sup>a</sup>
T3	0.50 (0.02) <sup>a</sup>	0.29 (0.02) <sup>a</sup>	0.21 (0.03) <sup>b</sup>	42.4 (3.2) <sup>b</sup>

Values in parentheses are standard errors ( $n = 3$ ).

Values in a column with different letters indicate significant difference at  $P < 0.05$  according to LSD test.

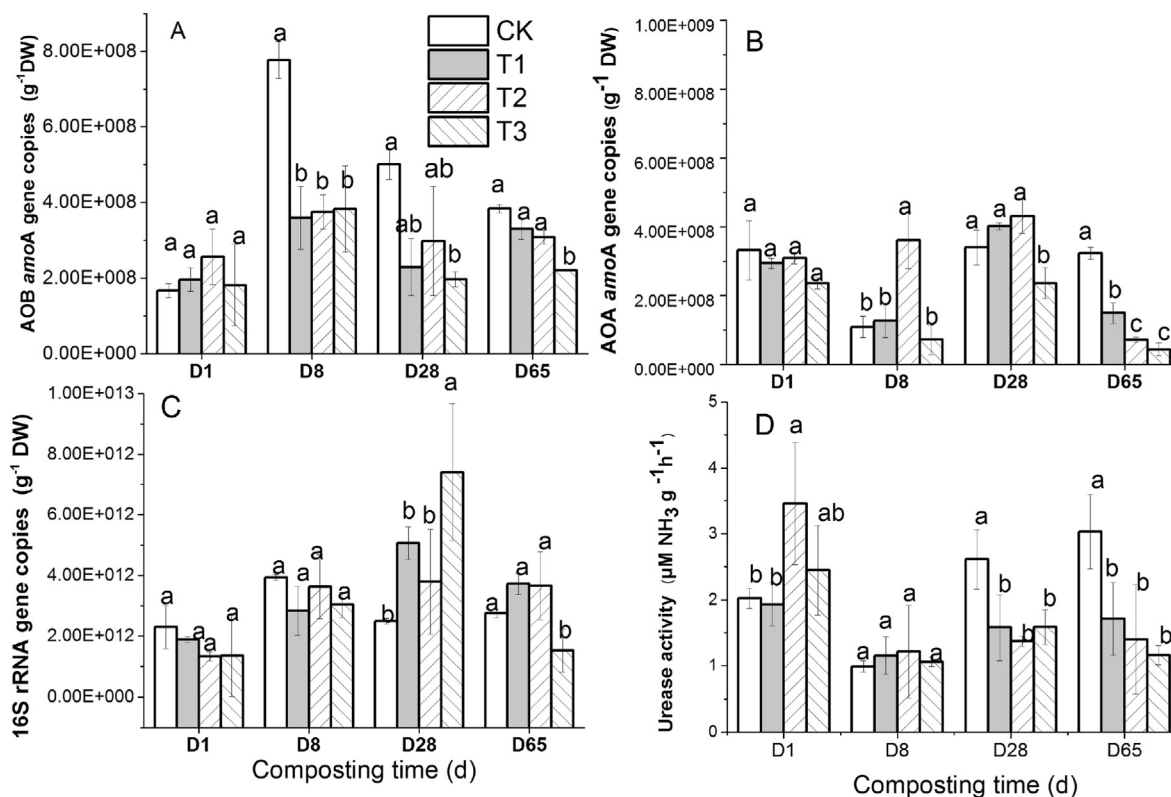
**Table 3**  
Acid functional groups of composts determined by Boehm's titration method.

Sample	Total acidity (mmol g <sup>-1</sup> )	Carboxylic groups (mmol g <sup>-1</sup> )	Lactonic groups (mmol g <sup>-1</sup> )	Phenolic groups (mmol g <sup>-1</sup> )
<b>Poultry litter</b>				
d1	1.74	0.90	0.21	0.63
d8	1.98	1.05	0.22	0.71
d28	2.07	1.08	0.24	0.75
d65	2.17	1.12	0.24	0.81
<b>Poultry litter + 15% lignite</b>				
d1	1.86	0.82	0.33	0.71
d8	2.07	0.86	0.37	0.89
d28	2.21	0.91	0.39	1.04
d65	2.46	0.92	0.41	1.13
<b>Lignite</b>	2.7	0.7	0.8	1.2

lower total C 1s compared to fresh poultry litter (fresh CK). Furthermore, the atomic concentrations of -OH and C=O increased by 74% and 132% in composted CK, and by 82% and 253% in composted T3, respectively. These results confirm the increasing density of acidic functional groups on the surface of composted materials.

### 3.5. Lignite effects on the abundance of 16S rRNA gene and ammonia oxidizers and urease activity

The 16S rRNA gene copy numbers of total bacteria and the *amoA* gene copy numbers of AOB and AOA in the mixtures are presented in Fig. 4. The 16S rRNA gene was the most abundant during the



**Fig. 4.** Abundances of A) AOB *amoA* gene, B) AOA *amoA* gene, C) 16S rRNA gene and D) urease activity in the composts without lignite (CK) or with 5% (T1), 10% (T2) and 15% (T3) lignite after 1 (D1), 8 (D8), 28 (D28) and 65 (D65) d composting.

whole composting process and ranged between  $1.6 \times 10^{12}$  to  $7.4 \times 10^{12}$  copies  $g^{-1}$  DW compost sample. It did not vary significantly between samples during the active fermentation phase. However, the 16S *rRNA* gene abundance increased sharply and was 1.2–1.8 times higher in the mixtures from T2 and T3 collected on day 8 compared to those collected on day 1. In the cooling stage (D28), the 16S *rRNA* gene abundance was significantly higher than CK and those with lower doses of lignite, indicating that lignite at particular levels could enhance the growth of microorganisms, probably due to greater water retention capacity creating a favorable micro-environment for bacteria (Richard et al., 2002). The AOA and AOB were abundant throughout the entire composting process. AOB abundances were generally higher than AOA in mixtures regardless of lignite addition. The abundances of AOB and AOA were in the range of  $1.67 \times 10^8$ – $7.77 \times 10^8$  and  $1.03 \times 10^7$ – $4.31 \times 10^8$  copies  $g^{-1}$  DW, respectively. The significantly lower AOB abundance in lignite added treatments in the thermophilic stage could be attributed to the higher temperature stress on the community. T2 had the highest copy of AOA, although significantly higher temperature in T2 (55.6 °C) was recorded than CK (46.6 °C) on day 8. The high density of AOA in the high temperature could be attributed to the reproduction thermophilic ammonia oxidizers (Awasthi et al., 2018). These results are consistent with previous studies, which demonstrated that AOA have tolerance to relatively high temperature (Zeng et al., 2011; Li et al., 2019). The application of lignite also inhibited AOB abundance in the cooling and maturing phase of composting. Mixtures with 15% lignite significantly inhibited AOA abundance by 30% on day 45 and the inhibition rate increased to 54%, 78% and 86% for mixtures with 5%, 10% and 15% lignite on day 65, respectively. The inhibition of lignite on the growth of AOA and AOB could be due to lower free substrate ( $NH_4^+$ ) concentration in T3, as a large proportion of  $NH_4^+$  could be captured by the acidic functional groups on the surface of lignite. Another possible reason for the growth inhibition of AOA and AOB might be related to the higher moisture content in compost materials from T3. Increased moisture content could decrease the  $O_2$  penetration and thus inhibit the nitrification (Guo et al., 2016; Sun et al., 2016b). The inhibition on the nitrification in the curing stage could be the main reason for the low nitrate content in mixture with 15% lignite.

Urease is responsible for the breakdown of urea into  $NH_4^+$  that can be easily lost as  $NH_3$  along with high pH and temperature (Castaldi et al., 2008). Low urease activity results in low  $NH_3$  volatilization during the process (Kong et al., 2018). The urease activity in all mixtures decreased significantly on day 8 compared to day 1. However, urease activity increased sharply from  $0.99 \mu\text{mol } NH_3 \text{ g}^{-1} \text{ h}^{-1}$  on day 8 to  $3.03 \mu\text{mol } NH_3 \text{ g}^{-1} \text{ h}^{-1}$  on day 65 in CK, while it changed slightly for mixtures with 5–15% lignite (Fig. 4D). The results are in line with those obtained by Liu et al. (2011), who reported that the increase in urease activity was attributed to the increased availability of water soluble  $NH_4^+$ , which could be absorbed by the acid functional groups of the lignite, resulting in suppressed urease activity.

### 3.6. Correlations between environmental factors and ammonia oxidizers

A total of 11 environmental factors (moisture, TC, TN, ash, DOC, DON, GI, pH, CEC and  $NH_4^+$ -N) were selected as independent parameters for RDA analysis (Fig. 5A). The first two axes of the RDA explained 87.2% of the cumulative variances. Based on the result from RDA, the variation of physicochemical properties was more closely related to the stage of composting rather than the type of raw materials for samples collected at day 1, 8 and 28. However, the end products were separated from each other. These results suggested that the addition of 15% lignite significantly changed the

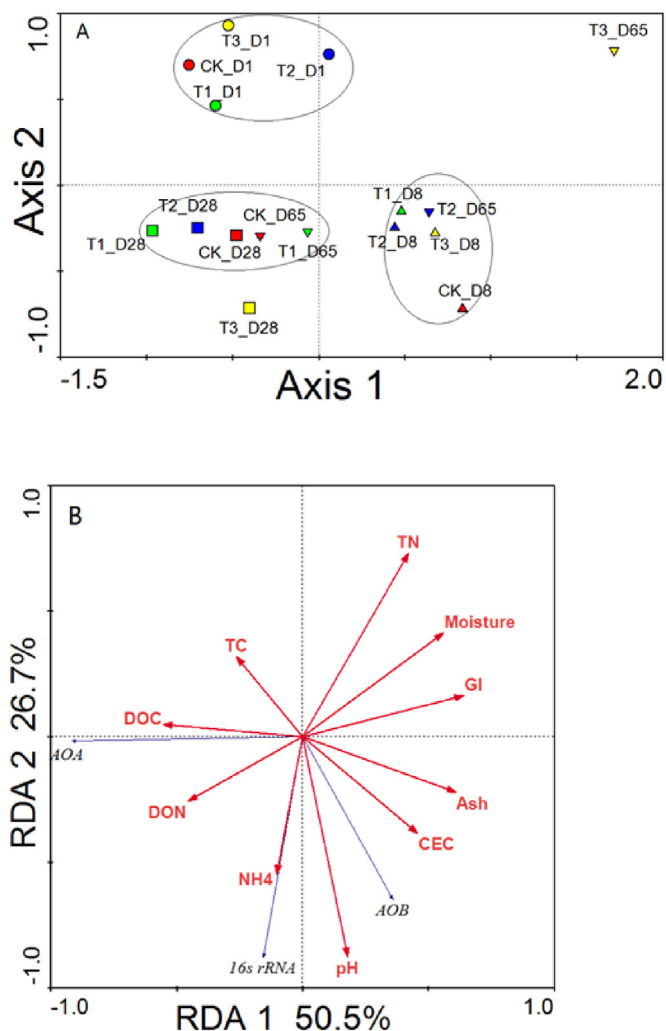
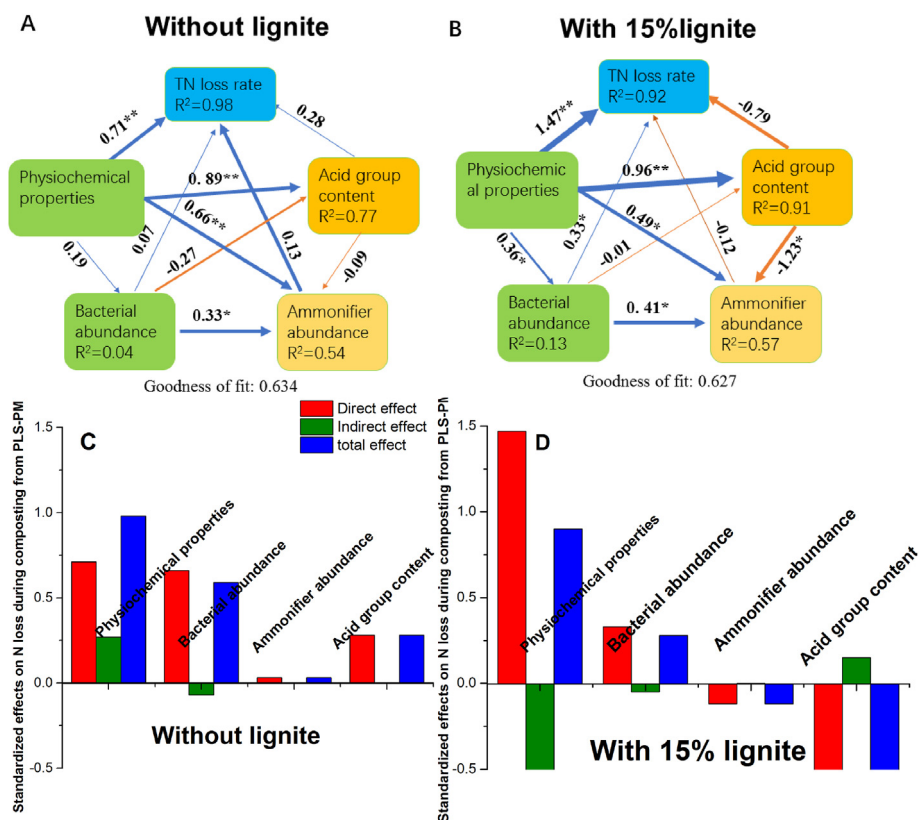


Fig. 5. Redundancy analysis (RDA) of physicochemical properties (TC, TN,  $NH_4^+$ -N, CEC, DOC, DON, ash, moisture, pH and GI) (a) and relationship between the relative abundances of the AOA and AOB and environmental parameters (b) in compost samples collected at d1, d8, d28 and d65 during composting. TC, total organic carbon; TN, total nitrogen; CEC, cation exchange capacity; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; GI, germination index.

physicochemical properties of poultry litter mixtures after 65-d composting.

Environmental factors may play a crucial role in the growth of AOB and AOA during composting. The first two canonical axes explained 57.5% and 26.7% of the variation in the abundances of total bacteria, AOA and AOB. TN, GI, pH, moisture, ash, and CEC contributed significantly ( $P < 0.05$ ) to the *amoA* gene abundance-environment relationship (Fig. 5B). Forward selection analysis showed that TN, moisture and CEC explained 28.4% ( $P < 0.01$ ), 23.7% ( $P < 0.05$ ) and 20.1% ( $P < 0.05$ ) of the total variation. DOC and  $NH_4^+$ -N were significantly positively related to AOA, while CEC was significantly negatively related to AOA. The increase in CEC during composting could be attributed to the accumulation of lignin-derived substances and the increased contents of carboxyl and/or phenolic hydroxyl groups (Satisha and Devarajan, 2007). Moisture was negatively related to AOA, AOB and 16S *rRNA*. The pH values had significantly positive correlation with AOB while TN was negatively correlated to the abundances of AOB. High pH in the active phase could enhance  $NH_3$  emission that would also remove the possible excessive amount of  $NH_4^+$  that would inhibit the



**Fig. 6.** Direct and indirect effects of different factors on total N loss during composting with or without lignite. A, B: PLS-PM showing the relationships among physicochemical properties (pH, EC, ash, DOC, CEC, moisture content, TC, TN, NH<sub>4</sub><sup>+</sup>), bacterial abundance (16s rRNA gene copy number), ammonifier abundance (AOA and AOB abundances) and functional acid group contents (-OH, COO, C=O) with respect to total N loss during composting without (A) and with 15% lignite (B). The positive and negative effects are indicated by blue and orange arrows, respectively. Larger path coefficients are indicated by wider arrows. Significance levels of Path coefficients are indicated by \* (P < 0.05) and \*\* (P < 0.01). C, standardized direct and indirect effects estimated by PLS-PM in composts without (C) or with 15% lignite (D).

activity and growth of AOB (Cáceres et al., 2018). High water content may influence gaseous exchange by limiting diffusion and this restricts oxygen utilization by microorganisms including ammonia oxidizers. This would in turn inhibit nitrification (Onwosi et al., 2017).

### 3.7. Relative contributions of selected factors to total N loss during composting

Physicochemical properties, microbial abundance and functional acid group contents determined by Boehm’s titration could directly or indirectly influence the total N loss during composting, a PLS-PM was constructed to explore how these factors contributed to total N loss (Cui et al., 2019). The physicochemical property was the key factor affecting the total N loss in composting with or without lignite (Fig. 6). The primary factor of physicochemical properties could directly influence the N loss and affect the abundances of total bacteria and ammonifiers, eventually impact the TN loss. These results could be related to the fact that the physicochemical can directly influence the microbial abundances and community structure (Zeng et al., 2011). In both treatments, physicochemical properties and total bacterial abundance had positive effect on TN losses, even though with different magnitude (Fig. 4). Although the ammonifier abundance and acid group content had a minor influence on TN loss compared to other factors, it had opposite roles in different treatments. These results suggested that the underlying mechanisms towards N loss during composting with or without lignite could be different (Liao et al., 2018). In compost with 15% lignite, acid group content appeared to be more

strongly inhibited N loss by more intensified absorption of NH<sub>4</sub><sup>+</sup>, which in turn had an inhibitory effect on the growth of ammonification oxidizers. In contrast, in compost without lignite, the ammonifiers abundance could be more strongly affected by physicochemical properties. These results further supported the idea that apart from the physicochemical properties, the N loss was strongly decreased by the adsorption of NH<sub>4</sub><sup>+</sup> by the functional acid groups of lignite and the associated decreased levels of ammonification oxidizers.

Given the market price for urea fertilizer (46% N) of \$600 AUD t<sup>-1</sup>, the increased N nutrient in the compost by lignite is equivalent to approximately \$1.8 AUD and the cost of 15% lignite application as compost additive to be \$1.2 AUD when composting of 1 t poultry litter. The addition of lignite is a cost-effective method for reducing N loss during composting and improve the quality of the final composts. However, further studies are needed to evaluate the environmental impacts of lignite as a compost additive by measuring the gaseous emissions of NH<sub>3</sub> and N<sub>2</sub>O directly during composting.

### 4. Conclusions

Adding 15% lignite (T3) to poultry litter significantly increased the temperature in the thermophilic stage of composting, leading to faster degradation of OM and accelerated detoxification than composting without lignite (CK). The results indicate that lignite amendment increased NH<sub>4</sub><sup>+</sup> content by 66% and decreased TN loss by 18%. The higher concentration of NH<sub>4</sub><sup>+</sup> corresponds with higher TN content (3.2% in T3 vs 2.2% in CK) and higher concentration of



total acid groups in T3 (2.46 mmol g<sup>-1</sup>) than those in CK (2.17 mmol g<sup>-1</sup>). Decreased urease activity and abundance of ammonia oxidizers caused by excessive NH<sub>4</sub><sup>+</sup>-N inhibition and moisture content were observed in T3. Although physicochemical properties were the key factor for N loss emission in all composting treatments, the N loss was strongly inhibited by NH<sub>4</sub><sup>+</sup> adsorption by functional acid groups in compost with lignite. This study provides new evidence for the potential use of lignite as a cost-effective compost additive to enhance N retention during composting. The hydrophilic property of lignite involved in reduced N loss and the effect of lignite on direct gaseous N emissions during composting needs to be further studied.

### CRediT authorship contribution statement

**Yun Cao:** Conceptualization, Data curation, Formal analysis, Writing - original draft. **Mei Bai:** Investigation, Methodology, Resources, Software. **Bing Han:** Methodology, Resources. **Robert Impraim:** Methodology, Software. **Clayton Butterly:** Writing - review & editing, Validation. **Hangwei Hu:** Methodology, Writing - review & editing. **Jizheng He:** Supervision, Project administration. **Deli Chen:** Funding acquisition, Supervision.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

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