Influence of FeONPs amendment on nitrogen conservation and microbial community succession during composting of agricultural waste: relative contributions of AOB and AOA to nitrogen conservation

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Abstract

Composting amended with iron oxide nanoparticles (FeONPs, α-Fe_2O_3 and Fe_3O_4 NPs) were conducted to study the impacts of FeONPs on nitrogen conservation and microbial community. It was found that amendment of FeONPs, especially α-Fe_2O_3 NPs, reduced total nitrogen (TN) loss, and reserved more NH_4^+-N and mineral N. Pearson correlation analysis revealed that decrease of ammonia-oxidizing bacteria (AOB) in FeONPs treatments played more important role than ammonia-oxidizing archaea (AOA) in reserving more NH_4^+-N and mineral N, and reducing TN loss. Bacterial community composition at phylum level did not shift with addition of FeONPs. *Firmicutes*, *Actinobacteria*, and *Proteobacteria* were the three most dominant phyla in all treatments. Germination index of final compost was also improved by FeONPs, especially α-Fe_2O_3 NPs. Overall, this study provides a method to reduce TN loss and improve mineral N reservation during composting, and gives a deep insight into the role of AOB and AOA in nitrogen transformation.

Keywords: Composting; FeONPs; AOB and AOA; Nitrogen conservation; Microbial community

1. Introduction

Composting is a biological process during which microorganisms convert unstable and complex organic matter into humus-like substance environmentally. Consequently, it has been widely used for recycling the agricultural waste, as the end-compost can be directly utilized as a valuable soil conditioner and organic fertilizer (Zeng et al., 2018; Ren et al., 2018a). The nitrogen dynamics during composting, including total nitrogen (TN) loss, emissions of NH_3 and N_2O, etc., have been extensively studied (Gong et al., 2009). It is well known that ammonia oxidizers can carry out the ammonia oxidation by transforming ammonia to hydroxylamine using ammonia monooxygenase (AMO) firstly, followed by further oxidation of hydroxylamine to nitrite with octahaem hydroxylamine oxidoreductase (HAO) (Kuypers et al., 2018; Tan et al., 2019).

Traditionally, ammonia-oxidizing bacteria (AOB) has been regarded as the dominance in ammonia oxidation in natural environments. While it was queried with the detection of archaeal *amoA* genes (Zhou et al., 2018). The isolation of archaea strains affiliated with *Crenarchaeota* clades suggested that ammonia-oxidizing archaea (AOA) also played an important role in ammonia oxidation (Liang et al., 2017). Previous studies reported that AOB and AOA co-existed diverse environments that have been detected to date (Zhang et al., 2016; Zhang et al., 2015). In some cases, the archaeal *amoA* gene copies were more abundant than AOB, even by 3000-fold (Leininger et al., 2006; Nicol et al., 2008). Ouyang et al. (2016) and Li et al. (2015) once found that AOB dominantly contributed to ammonia oxidation. While AOA was

also found to play more important role in nitrogen transformation in other previous studies (Chen et al., 2015; Deng et al., 2013; Lai et al., 2016; Wu et al., 2017). Their relative contributions to nitrogen transformation are related with complex factors (Cheng et al., 2016; Jiang et al., 2015).

Although the microbiological mechanisms of AOB and AOA during composting have been established (Zeng et al., 2011), as yet, little information is available about their dynamics and contributions to nitrogen transformation during composting in the presence of engineered nanoparticles (ENPs). As well known, the unique properties of ENPs have increased their production and application in wide fields, such as agricultural, commercial, industrial and medical products, etc. (Truong et al., 2014; Xu et al., 2012). Consequently, their impacts on crop, agroecosystem and microorganisms have been concerned and researched. Unlike CuO and Ag NPs, the iron oxide NPs (FeONPs) showed no or lower cytotoxicity, damage to DNA, and oxidative stress (Tang et al., 2014; He et al., 2016). Previous investigations suggested that Fe_2O_3 NPs changed microbial community composition and significantly increased species diversity (Long et al., 2011; Tang et al., 2017). He et al. (2016) found that nitrification and the abundance of AOB were affected by the presence of Fe_3O_4 NPs. Additionally, as most of existing studies about the impacts of NPs on composting focused on Ag or Ag based NPs (Gitipour et al., 2013; Stamou et al., 2016), it is necessary to further study more kinds of NPs.

Given the research background of NPs in composting systems and the potential effects of FeONPs on microbial populations, the impacts of FeONPs on nitrogen

conservation and functional microorganisms during composting are worthy of study. In the present study, two kinds of FeONPs (α-Fe_2O_3 NPs, Fe_3O_4 NPs) were added to investigate the impacts of FeONPs on composting from the following aspects: (i) TN loss and reservation of mineral N; (ii) relationships between AOB, AOA and nitrogen transformation; (iii) shifts in bacterial community diversity and composition; (iv) quality of the final compost. These results are expected to deepen the insights into the pathway of nitrogen transformation and ecological response which was reflected by bacterial community during composting amended with FeONPs.

2. Materials and methods

2.1. Preparation and characterization of FeONPs

α-Fe_2O_3 NPs was synthesized via forced hydrolysis of ferric nitrate salt solution ($Fe(NO_3)_3$ $9H_2O$) as described by a previous study (Sheng et al., 2016). Fe_3O_4 NPs was prepared using a chemical coprecipitation method modified from a previous study (Yang et al., 2012). More detailed synthetic methods were presented in Supplementary material.

Characterization of the two NPs was performed in terms of particle size distribution and morphology using transmission electron microscopy (TEM) (Tecnai G2 F20, FEI). The results showed that the average sizes of α-Fe_2O_3 and Fe_3O_4 NPs were about 8.7 and 15.6 nm (see Fig. S1 in Supplementary material), respectively. Both of the two NPs were spherical in shape.

2.2 Preparation of raw materials and composting set up

The raw materials composed of four components: rice straw, soil, vegetable, and

bran. The physico-chemical characteristics of raw materials were shown in Table 1, and the detailed preparation was presented in Supplementary material.

Composting experiments with three different treatments were conducted and lasted for 60 days. Original materials were blended at a weight ratio of 30:27:8:5 (rice straw : soil : vegetable : bran) to adjust the initial C/N to about 30. The initial moisture content were adjusted to about 55% (Zeng et al., 2018; Ren et al., 2018b). Treatment A was set up as the control (without α-Fe_2O_3 or Fe_3O_4 NPs), treatment B and C were treated with α-Fe_2O_3 and Fe_3O_4 NPs at a concentration of 10 mg/kg compost, respectively, as found by a previous study that FeONPs could enhance the microbial activity under a concentration of 10 mg/kg (He et al., 2016). Sufficient aeration was ensured by turning the composting piles periodically (Zeng et al., 2018).

2.3. Composting sampling and determination of physico-chemical properties

Three subsamples were collected from different points of composting piles on day 0, 1, 3, 5, 7, 17, 29, 43 and 60, and then homogenized. One part of the composite samples was saved at 4 °C for physicochemical analyses and the other part was stored at -20 °C for DNA extraction and subsequent analyses.

The temperatures of ambient air and three different positions in piles were recorded using a thermometer. pH was determined with a digital pH meter after the compost samples were shaken with ultrapure water at a weight/volume (w/v) ratio of 1:10 and filtered to collect the suspension. NH_4^+-N and NO_3^--N were extracted using 2 M KCl at a ratio of 1:50 (w/v) by shaking at 150 rpm for 1 h, and the concentrations were determined via flow injection analysis (AA3, Germany). Mineral N amounts to

125 the sum of NH_4^+-N and NO_3^--N. TN was determined using an elemental analyzer
126 (Elementar, Vario Max CN, Germany). TN losses were calculated according to our
127 previous literature (Zhang et al., 2017). The phytotoxicity of the final compost was
128 evaluated by the method of seed germination using radish seeds (Wu et al., 2019).

129 2.4. DNA extraction and quantitative PCR (qPCR)

130 Triplicate genomic DNA was extracted from 0.35 g compost samples using the
131 E.Z.N.A.® Soil DNA Kit (OMEGA Bio-Tek, Inc., Norcross, GA, USA) according to
132 the manufacturer's instructions. The DNA concentration and quality were determined
133 with a NanoDrop (Thermo Scientific, Wilmington, DE, USA).

134 Total bacteria, AOB, and AOA were enumerated via qPCR targeting 16S rRNA,
135 bacterial and archaeal *amoA* genes, respectively. Sequences of primers and thermal
136 cycling procedure were shown in Table 2. Detailed procedure was presented in
137 Supplementary material.

138 2.5. High-throughput sequencing of 16S rRNA gene and bioinformatic analyses

139 Total genomic DNA was extracted from the compost samples using FastDNA®
140 Kit for Soil (MP, USA) according to the manufacturer's instructions. The DNA purity
141 and concentration were determined by spectrophotometry using a NanoDrop 2000
142 (Thermo Scientific, Wilmington, USA). Detailed procedures were presented in
143 Supplementary material.

144 2.6. Statistical analyses

145 All parameters were determined in triplicate and expressed as mean ± a standard
146 deviation. Differences of physico-chemical parameters and gene abundances between

compost samples were evaluated by one-way analysis of variance (ANOVA) using SPSS 19.0 at a 95% confidence level. Pearson correlations between NH_4^+-N concentration and *amoA* genes abundance were also tested using SPSS 19.0. Nonparametric analysis of similarity (ANOSIM) was conducted using vegan package in R based on Bray-Curtis distance algorithm to compare the bacterial community of multiple groups (Mercier et al., 2017). The significance of different grouping factors was tested using 999 permutations.

3. Results and discussion

3.1. Changes of temperature and pH

As shown in Fig. 1A, the temperature changed following the typical three-phase pattern: mesophilic, thermophilic and cooling stage. The temperatures of all treatments rose rapidly in the early phase because of the heat release from the decomposition and metabolism of easily degradable organic matters (OM) by microbial communities. The highest temperatures in all treatments were recorded on day 6 with a little lower value in treatment C. The high temperature (≥50 ºC) was lasted for more than 5 days, which met the Chinese National Standard (GB7959-87), and it was capable to destroy pathogens since the temperatures of all treatments were maintained over 55 ºC for more than 3 days (Zhang et al., 2018b). The larger standard deviations during mesophilic and thermophilic stages were might due to the higher activities of microorganisms and the population succession of microorganisms which led to the temperature oscillations (Ge et al., 2014; Wan et al., 2017). The temperatures during day 10 ~ 12 in treatment B and C were significant higher than

169 treatment A (P < 0.05), suggesting that FeONPs might promote the microbial
170 metabolism to generate more heat during this phase. The high temperature and
171 depletion of easily degradable OM led the microorganisms to be less active,
172 consequently causing a drop of temperature. Interestingly, the temperature in
173 treatment A was higher than treatment B and C during day 17 to 43, and the possible
174 reason might be that more slowly degradation of OM in treatment A during early stage
175 slowed down the temperature drop.

176 The pH of all treatments presented similar trends during composting process (Fig.
177 1B). Before the first 17 days, the pH value fluctuated significantly as the degradation
178 of OM to organic acids caused a decrease of pH, while the mineralization of proteins
179 or organic nitrogen and the consumption of organic acids which led to pH increase
180 were also intensive during this stage (Jiang et al., 2016b). Afterwards, the pH changed
181 slowly until the end of composting. The ammonia emission and decomposition of
182 macromolecular organic compounds to organic acids caused the pH to decrease
183 slowly and then stabilize (Liu et al., 2017). At the end of composting, pH of all
184 treatments were in the range of 8~8.2, meeting the requirement for mature compost
185 (Zhang et al., 2018a).

186 3.2. Dynamics of nitrogen in different forms

187 From the beginning to the 3rd day, the NH_4^+-N decreased which might be related
188 to the nitrification during this mesophilic phase (Fig. 2A), as NO_3^--N increased
189 correspondingly at the same time (Fig. 2B). Then, the NH_4^+-N content showed an
190 increase and reached peak values on day 5. Previous study has suggested that the

191 increase of NH_4^+-N can be attributed to the mineralization and ammonification of
192 organic nitrogen, and the high temperature can inhibit the growth and activity of
193 nitrifying bacteria (Awasthi et al., 2017). Afterwards, NH_4^+-N showed a decreasing
194 trend till day 43. As shown in Fig. 2A and 2B, the NH_4^+-N contents in treatment B and
195 C were higher, especially that in treatment B was significant greater ($P < 0.05$) than
196 treatment A, while the NO_3^--N content in treatment A was inversely significantly
197 higher than that in the other two treatments, suggesting that the amendment of
198 FeONPs, especially α-Fe_2O_3 NPs, might weaken the oxidation of NH_4^+-N to NO_2^--N,
199 thus delayed the further conversion to NO_3^--N. At the end of composting, the NH_4^+-N
200 content was significantly higher ($P < 0.05$) in treatment B which was amended with
201 α-Fe_2O_3 NPs, and followed by treatment C and A orderly.

202 Mineral N which can be directly utilized by plant showed similar trend with
203 NH_4^+-N, as NH_4^+-N accounted for most of the mineral N (Fig. 2C). NO_3^--N is also an
204 important nutrient for plant, while it was once reported that NO_3^--N would be easily
205 lost through leaching, run-off, or denitrification before the plants utilize, causing
206 pollution to the groundwater, rivers, and estuaries (Leininger et al., 2006; Qiao et al.,
207 2015; Lam et al., 2017). Therefore, it might be better that FeONPs, especially α-Fe_2O_3
208 NPs, encouraged more N retention in compost mainly as NH_4^+-N, which could
209 subsequently improve the nitrogen use efficiency. Additionally, lower NO_3^--N reduced
210 substrate for denitrification, which could subsequently further reduce the TN loss
211 (Qiao et al., 2015). As shown in Fig. 2D, the TN loss in all treatments continuously
212 increased with the composting process proceeding, and at the end of composting, the

TN loss was 40.2%, 26.7 % and 32.1% in treatment A, B and C, respectively. The underlying reasons have been discussed above.

3.3. Quantification of AOB and AOA

The difference of nitrogen transformation among the three treatments implied that the oxidation of NH_4^+-N might be weakened by FeONPs amendment. Therefore, the copy numbers of bacterial and archaeal *amoA* genes were determined to mark the abundances of AOB and AOA in compost samples (Fig. 3). The qPCR efficiencies of bacterial and archaeal *amoA* gene were 103.7% and 110.7%, and the R^2 of standard curves were 0.998 and 0.995, respectively. Statistically significant differences in both AOB and AOA *amoA* gene abundances between different treatments indicated that FeONPs amendment could affect the ammonia-oxidizers number during composting. For AOB which was ranged from 3.23×10^7 to 1.90×10^{10}, the highest copy number was observed on day 3 in treatment A, while in treatment B and C, it was detected at the beginning of composting (Fig. 3A). During the first 5 days, the AOB abundance in the three treatments did not show a specific order. However, with the composting proceeding, it was found that the AOB abundances in treatment B and C were significantly lower ($P < 0.05$) than that in treatment A, and the lowest AOB abundance was detected in treatment B. These results indicated that the growth of AOB was inhibited by FeONPs, especially by α-Fe_2O_3 NPs.

Similar with AOB, the highest number of AOA was also found on day 3 in treatment A and at the beginning of composting in treatment B and C. While it started continuous decrease after the peak value in each treatment until the end of composting.

FeONPs, especially α-Fe_2O_3 NPs, also brought negative impacts on AOA abundance. The data showed that the average ratio of AOB/AOA in treatment A, B and C was 19.23, 2.47 and 3.33, respectively, indicating that the ratio was significantly lowered by FeONPs especially by α-Fe_2O_3 NPs. The dominance of AOB *amoA* gene over AOA *amoA* gene was sustained throughout the composting process. The alkaline pH in all treatments might be an important reason for the dominance of AOB over AOA, as a previous study demonstrated that AOB failed to grow in the soil below pH 7 where AOA was dominant (Nicol et al., 2008), while in alkaline soils, AOB was dominant over AOA (Jiang et al., 2015).

3.4. Pathway of the effect of FeONPs on nitrogen conservation during composting

Nitrogen is an essential nutrient for activities of all living organisms in environment and is demanded for the biosynthesis of some key cellular components, such as nucleic acids and proteins (Kuypers et al., 2018; Tang et al., 2019). The high availability efficiency of nitrogen in compost is an important indicator to evaluate the quality of compost for recycling it to agriculture (Zhang et al., 2017). Mineral N consisting of NH_4^+-N and NO_3^--N was directly available for plants. In this present study, the mineral N in treatment B was the highest, as well as NH_4^+-N. As shown in Fig. 2A and 2B, the contents of NH_4^+-N in all treatments were significantly higher than NO_3^--N, leading to the dominance of NH_4^+-N over NO_3^--N in mineral N. Therefore, higher NH_4^+-N resulted in more mineral N retention in treatment B. Lower NO_3^--N concentration in the compost will reduce the substrate availability for denitrification, thus decreasing the loss of TN as harmful gases, such as N_2O (Qiao et

al., 2015). In addition, the retention of compost N as NH_4^+-N rather than the easily leachable NO_3^--N form will reduce the nitrogen loss as NO_3^--N when the compost were reused for agriculture (Lam et al., 2017). It can been seen that the FeONPs in present study weakened the NH_4^+-N oxidation, and this effect was similar to previous study which used nitrification inhibitor to reduce TN loss and reserve more NH_4^+-N during composting (Jiang et al., 2016a).

According to the results of qPCR, the copy numbers of AOB in all treatments were generally higher than AOA during the whole composting process, and both AOB and AOA were reduced in the presence of FeONPs (Fig. 3). The average ratio of AOB/AOA was also significantly decreased from 19.3 in treatment A to 2.5 in treatment B and 3.3 in treatment C, implying that the reason for higher NH_4^+-N, higher mineral N, and less TN loss in treatments with FeONPs during composting might be the reduction of the copy numbers of AOB and AOA, especially AOB. Pearson correlation analysis (Table 3) between NH_4^+-N and *amoA* genes abundance in compost samples showed that NH_4^+-N was more negatively correlated with AOB *amoA* gene during the composting process, except for the first 5 days during which both NH_4^+-N and *amoA* gene changed irregularly in all treatments. While NH_4^+-N was not that regularly correlated with AOA *amoA* gene. This result further proved that AOB might be dominant in NH_4^+-N oxidation and the decrease of AOB played more important role than AOA in weakening of NH_4^+-N oxidation, similar to other previous studies that found AOB was dominant over AOA in NH_4^+-N dynamics (Di et al., 2009; Wang et al., 2014).

279 3.5. Effects of FeONPs on microbial community during composting

280 To study the influence of FeONPs on microbial community during composting,
281 16S rRNA gene in compost samples were qPCR analyzed and sequenced. The
282 samples of Day 1 and 60 from treatment B were analyzed in triplicate to verify the
283 reproducibility of high-throughput sequencing (Peng et al., 2014). After the
284 assembling and cleaning of raw reads, a total of 864350 high quality and effective
285 sequences were obtained from 19 samples, with sequences in each sample ranging
286 from 34879 to 59034. All these sequences were clustered into 566 OTUs based on ≥
287 3% dissimilarity cutoff.

288 The alpha diversity was investigated in terms of richness, diversity, evenness and
289 coverage of bacterial community, which were expressed as Chao 1 estimator, Shannon
290 diversity index, Shannon index-based evenness and Good's coverage, respectively
291 (see Fig. S2 in Supplementary material). The richness in all treatments increased with
292 the composting proceeding, but it was higher in treatment A than B and C during
293 some stages of composting, and contrary during some other stages. It was found that
294 the average richness in piles treated with FeONPs was a little higher than the control.
295 Nevertheless, the Shannon and Evenness index in treatment A were the highest
296 throughout the composting process, indicating that the bacterial community diversity
297 and evenness at OTU level were reduced in the presence of FeONPs especially Fe_3O_4
298 NPs, but it was not significant ($P > 0.05$). This was different from the abundance of
299 16S rRNA gene. The average abundance of 16S rRNA gene was significantly ($P <$
300 0.05) increased in the composting amended with α-Fe_2O_3 NPs, and the effect by

301 Fe_3O_4 NPs was not significant (see Fig. S3 in Supplementary material), similar with a
302 previous study that found iron oxide magnetic NPs had no significant impact on
303 bacterial abundance (He et al., 2011). The Good's coverage index of all composts
304 higher than 99% showed few differences, suggesting that the results of this
305 sequencing reflected the truth of bacterial community (see Fig. S2 in Supplementary
306 material). The little difference ($P > 0.05$) of all the tested indexes among different
307 treatments suggested FeONPs addition did not significantly drive bacterial
308 community alpha diversity at OTU level.

309 The community composition of bacteria at the phylum level in all treatments
310 displayed obviously temporal variations with composting proceeding (Fig. 4A). Eight
311 phyla with relative abundance more than 1% in at least one sample were presented.
312 *Firmicutes*, *Actinobacteria*, and *Proteobacteria*, arranged based on descending order,
313 were the three most dominant phyla, accounting for 74.2% ~ 99.5% of the total
314 representative 16S rRNA gene sequences in all samples. The other phyla were
315 *Gemmatimonadetes* (2.4% averaged from all samples), *Cyanobacteria* (0.7%),
316 *Chloroflexi* (1.9%), *Bacteroidetes* (1.4%), *Deinococcus*-Thermus (1.4%). As the most
317 abundant phylum, *Firmicutes* with about 37.7% average relative abundances in all
318 samples were mostly comprised of *Bacilli* (35.4% of total sequences averaged from
319 all samples) and the rest composed of *Clostridia*, *Limnochordia*, and
320 *A55-D21-H-B-C01* (Fig. 4B). *Actinobacteria* was the second dominant phylum
321 (35.6%) and was classified into class *Actinobacteria*. The *Proteobacteria* phylum
322 accounted for about 18.2% averagely and was comprised of *Gammaproteobacteria*,

323 *Alphaproteobacteria*, *Deltaproteobacteria*, and *Betaproteob- acteria* in order of
324 abundance. The variations of the three dominant phyla during composting were
325 similar among different treatments. Higher relative abundance of *Firmicutes* was
326 found during the first 5 days and then decreased until the end of composting (Fig. 4A),
327 and the most abundant class *Bacilli* contributed majority to this variation. The average
328 abundance of *Firmicutes* was lower in treatment A (36.4%) than other two treatments
329 with FeONPs (37.9% for α-Fe_2O_3 NPs, 38.8% for Fe_3O_4 NPs), similar with that of
330 *Actinobacteria* (29.8% in treatment A, 37.2% in treatment B, 39.7% in treatment C)
331 which showed continuous increase from the beginning to end of composting. In
332 contrast to *Firmicutes* and *Actinobacteria*, the average abundance of *Proteobacteria*
333 was higher in treatment A (23.5%) than treatment B (15.6%) and C (15.7%), and
334 showed a decrease during the first 5 days followed by a gradually increase until the
335 end of composting. It was found that the relative abundance of *Proteobacteria* was the
336 lowest on day 5 in all treatments, indicating that this phylum was unadapted to the
337 high temperature during this stage. At the beginning, *Gammaproteobacteria* class was
338 the most dominant class of *Proteobacteria*, while it was replaced by
339 *Alphaproteobacteria* since day 17 of the composting (Fig. 4B).

340 An non-metric multidimensional scaling (NMDS) and principal coordinate
341 analysis (PCoA) at phylum level using an algorithm of Bray-Curtis distance matrix
342 was conducted to investigate the similarities and differences of microbial
343 communities among the three treatments (Fig. 5A and Fig. 5B). The results showed
344 that the three replicates of compost samples from the beginning and end of treatment

B clustered closely, highlighting the robustness of molecular biology characterization of the microbial community and the high reproducibility of the bacterial community structures (Mercier et al., 2017). Moreover, three distinct clouds representing the microbial community composition of all samples were clustered, revealing that the microbial community structures shifted with the composting proceeding. Samples from different treatments but the same day clustered closely as the 1-day cloud, 5-day cloud, and 17~60-day cloud which were significantly distant from each other (Fig. 5A), as also revealed in Fig. 5B. This was also supported by analysis of ANOSIM based on groups of treatment A, B, and C (R = -0.0814; P = 0.833) and groups of 1-day samples, 5-day samples, and 17~60-day samples of all treatments (R = 0.98; P = 0.001). Overall, the bacterial community composition did not significantly change with the amendment of FeONPs but showed temporal difference, this might be contributed by the variations of physicochemical parameters at different stages of composting, similar results were also found in a previous literature that suggested the shift in bacterial community was mainly driven by changes in physico-chemical properties (Su et al., 2015). And it was also found in the study by He et al. (2011) that Fe_3O_4 NPs only caused a slight change in bacterial community structure compared with the control.

3.6. Germination test

Germination index (GI) is an important indicator related to compost maturity and phytotoxicity, and the mature compost should have a GI of > 80% (Wu et al., 2019). In this present study, the relative root length in treatment A, B and C was 125.2%,

133.7% and 127.1%, respectively. As all the seeds in three treatments germinated, the GI of final compost in treatment A, B and C was also 125.2%, 133.7% and 127.1%, respectively. Additionally, the average shoot length in treatment A, B and C was 3.72, 4.41 and 3.85 cm, respectively. These results suggested that the amendment of FeONPs, especially α-Fe_2O_3 NPs, improved the root growth, seed germination index and quality of the final compost, and this also eliminated the doubt that higher NH_4^+-N in treatment B and C might suppress the growth of plant when the end-product is used as a soil conditioner.

4. Conclusion

This study indicated that FeONPs especially α-Fe_2O_3 NPs weakened NH_4^+-N oxidation and encouraged mineral N retention as form of NH_4^+-N, thus improved nitrogen use efficiency of final compost. Less NO_3^--N might reduce substrate for denitrification, which would further reduce TN loss. Additionally, the decrease of AOB played more important role than AOA in weakening NH_4^+-N oxidation. Bacterial community composition at phylum level did not significantly change and quality of final compost was improved by the amendment of FeONPs. Therefore, composting amended with FeONPs especially α-Fe_2O_3 NPs was a useful method for reducing TN loss and conserving more mineral N.

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Appendix A. Supplementary data

The E-Supplementary data of this work can be found online.

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563

564 **Figure captions**

565 **Fig. 1.** Time courses of (A) temperature; (B) pH during composting (n = 3).

566 **Fig. 2.** Nitrogen transformations during composting (n = 3). (A) NH_4^+-N; (B) NO_3^--N;

567 (C) Mineral N; (D) TN loss. All data were shown on basis of dry-weight compost.

568 **Fig. 3.** Changes in copy numbers per kilogram of compost on basis of dry weight for

569 (A) bacterial *amoA* gene, (B) archaeal *amoA* gene. Error bars represented standard

570 deviation of the mean (n = 3).

571 **Fig. 4.** Phylogenetic composition of bacterial community (A) at phylum level; (B)

572 phyla with classes of the three dominant phyla *Firmicutes*, *Actinobacteria*, and

573 *Proteobacteria*. Different letters on x-axis indicated the three treatments: A for control,

574 B for treatment with α-Fe_2O_3 NPs at a concentration of 10 mg/kg compost, C for

575 treatment with Fe_3O_4 NPs at a concentration of 10 mg/kg compost, and the number

576 behind letter represented sampling day.

577 **Fig. 5.** (A) Non-metric multidimensional scaling (NMDS) analysis and (B) Principal

578 coordinate analysis (PCoA) of bacterial composition at phylum level. Different letters

579 A, B and C denoted control, treatment with α-Fe_2O_3 NPs, and with Fe_3O_4 NPs at a

580 concentration of 10 mg/kg compost, respectively, and the number behind letter

581 indicated sampling day.