Effects of earthworms on nitrification and ammonia oxidizers in vermicomposting systems for recycling of fruit and vegetable wastes

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Abstract

Although it is known that earthworms enrich the nitrate content in their final products, the detailed mechanisms behind this are not well understood, and this is important for determining the agricultural value of vermicomposting. Hence, this study aimed to investigate the effects of earthworms on ammonia oxidization and to clarify the functions of ammonia-oxidizing bacteria and archaea (AOB and AOA) during vermicomposting of fruit and vegetable wastes (FVWs). For this, two dry systems using dry FVWs and a fresh system using fresh FVWs were adopted and compared during 60 days of vermicomposting. Each system included two treatments, with earthworms and without earthworms. The results revealed that vermicomposting could facilitate the stabilization of FVWs, forming high value-added products. Based on the results of fluorescent excitation-emission matrix analysis, humification indices of the dry and fresh vermicomposts were 4.0 and 4.2, respectively. Moreover, compared to the minus net nitrification rates in groups without worm treatment, the net nitrification rates of 17.5 mg N/kg/d and 9.3 mg N/g/d, respectively, were found in dry and fresh vermicomposting systems, indicating that earthworms could significantly accelerate the nitrification process. Compost treated with earthworms exhibited elevated numbers of ammonia oxidizers (AOA and AOB) and greater community diversity in final products, compared to the counterparts without earthworms. Final vermicompost products were abundant in the AOB members of *Nitrosomonas* and *Nitrospira* along with AOA groups including Crenarchaeota and Thaumarchaeota. By contrast, AOA were the dominate members completing ammonia oxidization during vermicomposting of dry and fresh FVWs. This study suggests that earthworms facilitate the ammonia oxidization process by promoting both numbers and diversity of AOA and AOB during

vermicomposting of FVWs.

Keywords: *amoA* genes; Bio-fertilizer; *Eisenia fetida*; Nitrogen; PCR-DGGE

Introduction

In China, the total consumption of fruits and vegetables was ~ 500 million tons in 2016, based on a prospective study (Song and Wang, 2015). As a consequence, a large amount of fruit and vegetable wastes (FVWs) are generated, which are a potentially significant source of pollution in the environment. Taking into account that FVW is characterized by low toxicant risk and high water content, as well as easily degradable organic materials and micronutrients, it is suitable for recycling methods such as animal feed, anaerobic digestion, composting, and vermicomposting (Huang *et al.*, 2014).

Vermicomposting is a biochemical process for the degradation and mineralization of organic materials utilizing the combined action of earthworms and microorganisms.

During this process, organic matter content decreases and the forms changed, showing decreases in aromatic protein compounds and increases in humic acid-like and fulvic acid-like substances (Lv *et al.*, 2013; Yang *et al.*, 2014; Fernández-Gómez *et al.*, 2015).

Also, the inorganic material content is increased due to the mineralization and decomposition of organic material, with higher values of nitrate and phosphate in final vermicompost products (Fernández-Gómez *et al.*, 2010; Huang *et al.*, 2013). In addition, the microbial community structure in raw substrates is also modified after vermicomposting, displaying abundant and diverse microbial communities in end vermicompost production materials (Domínguez *et al.*, 2010).

As a bio-fertilizer, nitrogen availability is a limiting factor for the agricultural utilization of vermicomposts since elemental nitrogen is an essential nutrient for the plant growth and soil improvement (Leininger *et al.*, 2006). Nitrification, as a mechanism of maintaining nitrogen balance, regulates the transformation of ammonium into nitrate by ammonia-oxidizing microorganisms (Leininger *et al.*, 2006; Levy-Booth *et al.*, 2014), thus determining vermicompost quality. Thus far, it has been reported that a greater amount of nitrate is generated after vermicomposting of FVWs (Fernández-Gómez *et al.*, 2010; Hanc and Chadimova, 2014). Also, the inoculation of earthworms appeared to markedly elevate the nitrate content, compared to composting the same substrates without worms (Huang *et al.*, 2013). However, the mechanism behind this phenomenon of vermicomposting enhancing the nitrate content of compost products is still not understood. To better improve vermicompost quality for agricultural purposes, the ammonia oxidization involved and its relationship with earthworms in vermicomposting systems should be determined.

The ammonia-oxidizing process is believed to be a rate-limiting step in nitrification reactions that is driven by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Leininger *et al.*, 2006; Ke *et al.*, 2013; Levy-Booth *et al.*, 2014). Until now, most investigations focused on the distribution of oxidizers (AOA and AOB) and their contribution to different ecosystems (Leininger *et al.*, 2006; Chen *et al.*, 2008; Levy-Booth *et al.*, 2014). For example, increasing evidence suggests that AOA dominated in soil ecosystems, showing a higher number and more abundant community diversity than the AOB (Leininger *et al.*, 2006; Chen *et al.*, 2008; Zhang *et al.*, 2012). However, recent studies have determined that AOB members rather than AOA, were predominant in composting systems (Yamada *et al.*, 2013; Zeng *et al.*, 2013). Although

the detailed niches of AOA and AOB, and their functions in different ecosystems, have been disputed, the dominant status disparity of AOA and AOB, to some extent, differs in the ammonia oxidization process and efficiency, and thus affects the final quality of vermicompost. However, to date, few efforts have been made to investigate the ammonia oxidizers and their contributions in vermicomposting systems.

As mentioned above, the objectives of this study were (1) to investigate the effect of earthworms on ammonia oxidization and (2) to study the function of AOA and AOB species in vermicomposting systems. Given that some composting methods may affect ammonia oxidization performance, two common vermicomposting methods with both dry and fresh FVWs were separately adopted and compared in this study. In addition, AOA and AOB *amoA* gene copy numbers and community structures were determined using quantitative PCR (qPCR) and denaturing gradient gel electrophoresis (DGGE), respectively.

2. Methods

2.1 Materials

Young epigenic earthworms *Eisenia fetida* weighing approximately 0.3 g were chosen for vermicomposting. Prior to this experiment, earthworms were cultured in cow dung and fruit and vegetable wastes for 2 years. Fresh fruit and vegetable wastes were obtained from the supermarket of Kanesue, nearby Gifu University, Japan. After washing in tap water, the FVWs were chopped by knife and divided into duplicate groups. Then, one of the fresh FVWs was dried at 50 °C for 48 hours, to be used as the

dry substrate. Another duplicate was stored at 4 °C to be used as the fresh substrate. The bedding material of earthworms was produced from old vermicompost, which originated from the mixture of cow dung and fruit and vegetable wastes using the ratio of 1:2 (dry basis). The physicochemical properties of cow dung, fresh fruit and vegetable wastes and old vermicompost are given in Table S1. Plastic rectangle boxes pierced on the bottom were manufactured as vermicomposting reactors. All reactors were the same size at 255 mm × 175 mm × 200 mm (length × width × height).

2.2 Experimental setup

To determine the effect of operation modes on ammonia oxidation, a dry vermicomposting system using dry FVWs mixed with bedding material, and a fresh vermicomposting system using bedding material covered by fresh FVWs were designed and adopted simultaneously. For the dry system, vermicomposting was launched by adding 50 young earthworms into the reactor containing a mixture of 100 g dry FVWs (dry basis) and 200 g bedding material (dry basis). For the fresh system, 50 young earthworms were first released into the reactor with 200 g bedding material (dry basis) in the bottom. Then, 50 g of fresh FVWs (dry basis) were placed on the bedding material. To avoid the effect of leachate produced from fresh FVWs on earthworms, another 50 g fresh FVWs were added on the bedding material after 30 days. Meanwhile, the control treatments of both dry and fresh systems without earthworms inoculated were also built for comparison. All treatments were repeated three times. Hence, a total of 12 reactors were operated under a controlled temperature at 25 ± 2 °C. The initial moisture of the feeding material was approximate 70% as higher moisture could

accelerate the decomposition of FVWs. To maintain the moisture, a wet towel was used to cover the surface of the substrate and tap water was sprinkled every week. Moisture was determined using a humidometer inside in each reactor and kept at 70% - 75% throughout the experiment. In addition, a piece of shade cloth was used to cover each reactor for further maintaining moisture and providing a dark environment for earthworms. After 60 days, earthworms and their cocoons were removed by hand from each vermicomposting reactor. The collected samples were homogenized and divided into two subsamples. One was dried and stored into 4°C refrigerating chamber and the other was stored at -20°C until required.

2.3 Physicochemical analysis

Samples were collected and dried in an oven at 50 °C for 48 hours. Resulting samples were ground and sieved with mesh for physicochemical analysis. The pH and electrical conductivity were measured in a mixed water extract of sample prepared using Milli-Q water (sample: water = 1: 10, dry basis). The water content and organic matter content were analyzed using the oven method (Huang *et al.*, 2014). Total carbon and nitrogen in solid dry samples (dry basis) were simultaneously determined using an elemental analyzer (Yanaco CHN CORDER MT-6, Japan). The mixtures of samples and water (sample: water = 1: 50, dry basis) were shaken for 2 hours and filtered with a 0.45 µm membrane and then submitted for assays of nitrate, ammonia, and phosphate concentrations using an ion chromatograph system (SHIMADZU, Japan). The net nitrification rate was calculated by dividing the difference value of nitrate changes by the experimental days.

2.4 Three-dimensional fluorescence excitation-emission matrix spectroscopy

The mixture of solid samples and Milli-Q water (sample: water = 1:100, dry basis) were filtrated by a 0.45 μm membrane after shaking one hour in order to complete the analysis of three dimensional fluorescence excitation emission matrix spectroscopy (3D-EEM) by the RF-5300PC fluorescence spectrophotometers (SHIMADZU, Japan). The EEM spectra were recorded with emission wavelengths between 220 and 550 nm, while the excitation wavelength was increased sequentially from 220 to 550 nm. The emission and excitation slits were fixed at the 5 nm band width.

2.5 Real time quantitative PCR analysis

DNA extraction of each sample was conducted using the MoBio UltraClean Soil DNA Isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). On the basis of Chen *et al.* (2008), primers including amoA-1F and amoA-2R, as well as Arch-amoAF and Arch-amoAR, were separately chosen for the 16S rDNA gene fragment amplifications of ammonia-oxidizing bacteria and archaea. The characteristics of each primer are detailed in Table S2. According to the method of Huang *et al.* (2016), the standard for the 16S rDNA gene was established using 10-fold serial dilutions of DNA containing a full-length copy of the *Escherichia coli* 16S gene (NBRC, 13965). The bacterial and archaeal *amoA* gene densities were quantitated using SYBR[®] Premix Ex Taq[™] (TaKaRa, Japan) with the Thermal Cycler Dice (TP800, TAKARA). The same PCR amplification conditions were used for detecting gene copies of AOA and AOB with 3 min preheating

at 94°C, followed by 40 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 60 sec. The amplification procedure was checked with a heat dissociation protocol (from 60°C to 90°C) after the final cycle of the PCR.

2.6 PCR-DGGE and sequencing

The bacterial primers amoA-1F with GC clamp and amoA-2R, as well as archaeal primers Arch-amoAF with GC clamp and Arch-amoAR, were used for amplifying the *amoA* genes of AOB and AOA, respectively. The PCR amplification of AOB was conducted by 38 cycles of 95°C for 30 sec, 54°C for 40 sec and 72°C for 40 sec, and finally an extension step at 72°C for 10 min. For the AOA, PCR amplification was carried out with 35 cycles of 94°C for 40 sec, 51°C for 60 sec and then 72°C for 60 sec, and a final extension step at 72°C for 5 min. All PCR reactions were performed using a real time PCR system (Thermal Cycler Dice, TP800, TAKARA, Japan). The PCR products were detected using 1% agarose.

Next, DGGE was performed in 6% gel using the BIO-RAD Dcode™ system (Bio-Rad, USA). For AOB, the denaturant gradient was 35-60 % and electrophoresis was run at 100 V for 16 h. As for AOA, a gradient of 25-40 % was used at 120 V for 10 h. Then, the gels were stained with a solution of SYBR® Green Nucleic Acid Gel Stain (TAKARA) and visualized using a Gel Doc 2000 System (Bio-Rad, USA). After that, the bright bands were excised from the DGGE gels and then stored at 4°C in 80 µl Milli-Q water for 48 hours. Subsequently, the eluted DNA samples were re-amplified using the AOA and AOB primers without GC clamps. The final PCR products were purified using the EXOSAP IT kit (Affymetrix) and sequenced using the ABI 3100

Genetic Analyzer (Applied Biosystems, USA).

2.7 Data analysis

The significant differences between the mean values of each parameter of control and vermicomposting treatments were analyzed by t-test with a 95% confidence level using STATISTIC 8.0 software. The fluorescent humification index of an excitation wavelength of 254 nm was calculated by dividing the area from 435 to 480 nm by the area from 300 to 345 nm (Fernández-Gómez *et al.*, 2015). The DGGE fingerprints were digitalized using the Quantity One image analysis software v.4.2 (Bio-Rad Laboratories, Hercules, CA, USA). The sequencing results were assembled by the Geneious R 6 software and then submitted to the BLAST search option in the NCBI nucleotides sequence database. Phylogenetic trees were constructed using MEGA 6.0 software with the Neighbor joining method. The sequences were aligned using the positional tree server with a data set containing the nearest relative matches. Only unambiguously aligned positions from all sequences were used, and gaps in the both ends were cut before the match/mismatch count.

3. Results and discussion

3.1 Assessment of stability of final vermicomposting products

After vermicomposting, the growth rates of *E. fetida* reached 18.7 mg/d/worm and 21.2

mg/d/worm, respectively (shown in **Fig. S1**). This finding suggests that earthworms had a good growth rate in both reactors with dry and fresh FVWs. As displayed in **Table 1**, physicochemical properties of FVWs were remarkably modified after vermicomposting in both dry and fresh systems. It is apparent that the inoculation of earthworms could accelerate the increase of nitrate content and decrease of electrical conductivity, organic matter content, total carbon, total nitrogen, and ammonium content compared to the control treatments without earthworms. These findings are somewhat similar to earlier vermicomposting studies for FVWs (Huang *et al.*, 2013; Huang *et al.*, 2014; Hanc and Chadimova, 2014). However, compared to increased electrical conductivity and total nitrogen in previous vermicomposting studies for other substrates (Suthar, 2009; Yadav *et al.*, 2015), the opposite results of this study could be explained because higher moisture and water content were maintained during the vermicomposting process. Although higher moisture could accelerate the decomposition of FVWs, it also led to the greater loss of dissolved conductivity substances and total nitrogen, because earthworm activity increased the leachate production and enhanced the porosity of substrate.

Despite that a certain content of organic matter was lost in both vermicomposting systems of this study, it is still hard to understand the stable degree of final products because FVWs are mainly comprised of cellulose with high water content (Fernández-Gómez *et al.*, 2010). Thus, 3D-EEM was employed to assess the stability of final vermicomposting products, based on recent studies (Lv *et al.*, 2013; Fernández-Gómez *et al.*, 2015; Yang *et al.*, 2014). The fluorescence image of EEM and the peak position and intensity of both treated systems during the experiment are given in Fig. 1 and Table 2, respectively. Accordingly, peaks A and B are considered to locate

in the typical aromatic protein area and the soluble microbial byproduct-like area, respectively (Chen *et al.*, 2003). After the experiments were completed, the typical aromatic protein content was found to decline in all reactors, with a greater drop in the fresh system with earthworms. Moreover, a similar decrement trend of soluble microbial byproduct-like material was detected during the experimentation. Comparatively, the addition of earthworms could markedly facilitate the losses of those two protein-like materials. Such result is similar to the findings obtained from vermicomposting of cow dung (Lv *et al.*, 2013) and dewatered sludge (Yang *et al.*, 2014).

Peaks C and D are regarded as humic acid-like and fulvic acid-like materials, respectively (Chen *et al.*, 2003). On the basis of EEM, the fluorescent intensity of humic acid-like substances in controls and even the earthworms was found to decrease in both dry and fresh systems after treating for FVWs. In addition, the peak values of fulvic acid-like materials increased markedly in the vermicompost treatments while it decreased in the counterparts, relative to the initial substrates. These results indicate that earthworms could significantly enhance the content of fulvic acid-like substances rather than that of humic acid-like substances during vermicomposting of FVWs. This disagrees with earlier reports that earthworms could promote the humification process through converting large organic materials into humic acid-like and fulvic acid-like materials during vermicomposting of cow dung and dewatered sewage sludge (Lv *et al.*, 2013; Yang *et al.*, 2014).

Generally, the A435 –A480 nm / A300-A345 nm at an excitation wavelength of 254nm is considered as a fluorescence based humification index in vermicomposting systems (Fernández-Gómez *et al.*, 2015). As described in Table 2, the values of A435 –A480 nm / A300-A345 nm in the earthworms and the control treatments increased significantly

after treating for dry and fresh FVWs, showing higher values in vermicomposting treatments. Although different vermicomposting modes were used in this study, the values of humification index appeared to be close in two final products. Such results evinces that earthworms could promote the humification process during vermicomposting of FVWs, irrespective of the operating modes. This finding is in accordance with the report of Fernández-Gómez *et al.* (2015), who also found the similar humification indexes in two vermicomposting products obtained from two types of wastes with the different proportions. Consequently, the vermicomposts of FVWs containing lower organic matter contents and higher humification indexes indicates that the stable products were harvested in present study.

3.2 Effect of earthworms on the ammonia oxidization

3.2.1 Effect of earthworms on the net nitrification rate

As presented in Table 1, the nitrate contents in vermicomposting treatments were significantly higher than those in compared treatments after the experimentation. However, the opposite result of significantly declined ammonium contents in earthworm treated reactors was recorded. According to the net nitrification rate of both vermicomposting systems (Fig. 2), significantly high net nitrification rates were recorded in the vermicomposting treatments. By contrast, the net nitrification rate showed minus values in the controls without earthworms. This result further supports the idea that the presence of earthworms could accelerate the nitrification process during vermicomposting of FVWs, as corroborated with some studies (Parkin and Berry, 1999;

Wang *et al.*, 2011; Costello and Lamberti, 2009; Huang *et al.*, 2016). Compared to fresh systems, the net nitrification rate was relatively higher in the dry systems, showing the average value at 17.5 mg N/kg/day. This could be explained by a phenomenon that the greater volumes of leachate were produced in the fresh systems, carrying away a certain amount of inorganic nitrogen (Huang *et al.*, 2014; Huang *et al.*, 2016). Similarly, Chen *et al.* (2014) demonstrated that the contributions to the nitrification process by earthworms was significantly greater in the dry system with 33% soil moisture than in wet system with 97% moisture. Also, they found stronger denitrification enzyme activity in wet systems relative to in dry systems (Chen *et al.*, 2014). Another possibility may be linked with the differences of ammonia-oxidizing microorganisms inside both reactors. Hence, the features of ammonia oxidizers are given.

3.2.2 Effect of earthworms on numbers of AOA and AOB

Fig. 3 depicts the changes of numbers of ammonia oxidizers during vermicomposting for dry and fresh FVWs. After the experiment, the AOB numbers of vermicomposts significantly increased, showing greater values than those of controls in both dry and fresh systems. Such results imply that earthworm activity could significantly enrich the AOB numbers in vermicomposting system. Similarly, Gopal *et al.* (2009) observed that the AOB populations were dramatically elevated after vermicomposting of coconut leaf. Recently, Huang *et al.* (2016) reported that earthworms could significantly promote AOB densities in vermicomposts established from five different fresh FVWs. The higher density of AOB in vermicomposting systems may be ascribed to earthworms burrowing behavior that could promote the porosity and lower the anaerobic rate in the

inside of substrate, and thus improving the growth conditions for AOB (Parkin and Berry, 1999; Huang *et al.*, 2016). Additionally, in the burrows, earthworm feeding, excretion, and mucus production could drive the nitrogen cycling, as pointed out by Costello and Lamberti (2009). Consequently, although a greater amount of leachate is produced in fresh systems, no significant difference of AOB numbers in vermicomposting products was recorded between dry and fresh systems. In contrast, the lowest value of AOB numbers recorded in the control products of fresh system should be associated with the anaerobic environment resulted from production of leachate. Additionally, the AOA numbers were significantly augmented in final products of vermicomposting and even controls after these experiments. The highest numbers of AOA were found in the final vermicomposting products of a dry system, which reached $4.2\text{E}+9$ copies/g (dry basis). Moreover, the AOA numbers in vermicomposting products were significant higher than those in control products, which suggests that earthworms could also promote AOA numbers during vermicomposting of FVWs. The increased numbers of AOA in earthworm systems could be the result of the gut digestion process. Majeed *et al.* (2013) detected relatively higher AOA numbers in fresh castings of earthworms after excretion. For final vermicomposting products, the rates of AOA/AOB gene copies in dry and fresh systems were 28/1 and 18/1, respectively. Results that AOA outnumbered AOB in earthworm inoculation systems may suggest that the AOA appeared to play a more important role in nitrification during vermicomposting of FVWs. There is growing evidence that the AOA growth rate and *amoA* transcription were higher than the AOB in soil microcosms (Chen *et al.*, 2008; Tourna *et al.*, 2008; Zhang *et al.*, 2012), due to the higher tolerance degree of AOA to oxygen, pH, moisture, and NH_3 availability in environment (He and Zhang, 2009; Levy-Booth *et al.*, 2014).

3.2.3 Effect of earthworms on communities of AOA and AOB

The DGGE image of AOB community structure of vermicomposting for dry and fresh FVWs is presented in the Fig. 4. Compared to only one band in the initial substrate, the numbers of bands increased significantly after the experiment, showing greater numbers in the vermicomposting product (14 bands) than in the control (7 bands). As for the fresh system, unexpectedly, 13 bands were observed in the initial substrate. However, only one band was found in the control products after the experiment. Compared to the counterparts, numerous AOB bands were detected in final vermicomposting products which suggests that the abundance of the AOB community was significantly widened by earthworms during vermicomposting of FVWs. In addition, bands 2, 4, 6, 8, and 9 were only exhibited in the vermicompost products, indicating that the occurrence of these species likely has a connection with the earthworms.

The DGGE bands were sequenced for phylogenetic analysis, as shown in Fig. 5. The AOB belonging to the genera *Nitrosomonas* and *Nitrospira* were detected in both vermicomposting systems. It has been well understood that *Nitrosomonas* sp. and *Nitrospira* sp., a significant portion of known AOB, were abundant in final composting products of animal manures (Yamada *et al.*, 2013). For earthworm systems, Wang *et al.* (2011) and Wang *et al.* (2013) stated that the dominated AOB members were *Nitrosomonas* sp. and *Nitrospira* sp. in earthworms-filter wastewater treating reactors. Interestingly, bands 1-6 that dominated in fresh system were associated with the *Nitrosomonas*-like sequences. Bands 7-13 that predominated in dry systems were identified as the *Nitrospira*-like members. The differences of water content and

ammonia content in both reactors may be responsible for the dissimilar AOB ecological diversity (Ke *et al.*, 2013). Another explanation could be that the *Nitrosomonas* members could participate in the whole nitrification process and even inhabit the anoxic wastewater environment, compared to the *Nitrospira* members (Kotay *et al.*, 2013). The DGGE image in Fig. 4 also presents the AOA communities of initial substrate and final products of vermicomposting and their counterparts in both dry and fresh systems. The DGGE pattern of the AOA community was similar to that of AOB. For both dry and fresh systems, a significantly larger number of DGGE bands were recorded in the vermicomposting products than in the control products. Also, bands 2, 4, 5, 6, 7, 9, 12, 15, 16, 19, and 20 were only detected in the vermicomposting products, suggesting that earthworms also could widen the AOA community diversity. It is deduced that the effect of earthworms on AOA diversity may be explained by an affinity to their gut digestion process, because the universal archaeal community richness in the excreted castings of earthworms is significantly shaped and elevated, compared to the initial substrates (Koubová *et al.*, 2015). In contrast, vermicompost of the dry system (16 bands) displayed a greater number bands than these of fresh system (12 bands).

Sequencing results and phylogenetic analysis are given in Fig. 6. In present study, the AOA community component was clustered into three groups of Crenarchaeota, followed by Thaumarchaeota, and then Unclassified AOA. Normally, the Crenarchaeota group 1.1a and 1.1b are deemed to be numerically and transcriptionally important ammonium oxidizers in terrestrial ecosystems (Levy-Booth *et al.*, 2014). In composting systems, Yamamoto *et al.* (2011) found many archaea *amoA* genes like ‘*Candidatus Nitrososphaera gargensis*’ were involved. Xie *et al.* (2012) reported that the inoculation of AOA into a composting system of poultry feces could improve the quality

of the final product with a higher nitrogen content and a richer microbial community, compared to natural composting without inoculation. Recently, the autotrophic thermophilic AOA enriched from composting were successfully applied to hot wastewater treatment for nitrogen removal (Courtens *et al.*, 2016). Thaumarchaeota, as deep-branch members of mesophilic Crenarchaeota, have the capability to conduct ammonia oxidization with an autotrophic or heterotrophic lifestyle using diverse substrates (Pester *et al.*, 2011).

Given there was no significant difference in community richness and numbers of AOB between dry and fresh systems, final vermicomposting products containing higher net nitrification rates in dry systems should be matched with the abundant community diversity and with the greater number of AOA in the dry system. This finding indicates that the AOA is a dominant player for the ammonia-oxidizing process in vermicomposting systems, which is supported by multiple studies in soil environments (Leininger *et al.*, 2006; Chen *et al.*, 2008). However, the opposite results from Yamada *et al.* (2013) and Zeng *et al.* (2013) showed that the AOB, rather than the AOA, dominated in composting systems. They inferred that the AOA were abundant in natural oligotrophic environments and are supported by the addition of organic matter (Zeng *et al.*, 2013). This debate could be explained by a niche specialization in different temporal and spatial environments between the AOA and the AOB driven by NH_3 concentration, water content, oxygen content, organic matter component, and heavy metal content of the ecosystems (Ke *et al.*, 2013; Levy-Booth *et al.*, 2014). Undoubtedly, earthworms could significantly modify the ammonia-oxidizing process by regulating the numbers and diversity of AOA and AOB during vermicomposting of FVWs whatever different vermicomposting modes are used. However, because of the complexity of earthworm

behavior, it is still difficult to clearly define the relationship between ammonia oxidizers (AOA and AOB) and the contribution of earthworm behaviors in this study. Hence, a study of different earthworm behaviors affecting ammonia oxidizers should be considered in the future.

4. Conclusions

The present study revealed that vermicomposting could facilitate the stabilization of FVWs, forming high value-added products. During this process, earthworms significantly promoted the net nitrification rate, showing a higher contribution in dry systems. In addition, earthworms could elevate the numbers of ammonia-oxidizing bacteria and Archaea, and simultaneously broaden their community diversity during the nitrification process. Moreover, the AOA rather than the AOB dominated in the vermicomposting systems, notably in the dry one. It is suggested that earthworms could accelerate the ammonia oxidization process by promoting the numbers and diversity of AOA and AOB during vermicomposting of FVWs. In addition, the nitrification performance of dry systems was better than that of fresh systems.

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Caption of tables

Table 1 Physicochemical properties of initial substrates of fruit and vegetable wastes (FVWs) and final products of vermicomposting and control without earthworms in dry and fresh systems. Values are presented by means and standard deviation (n=3).

Table 2 Ex/Em maxima dissolved organic matter from initial substrates of fruit and vegetable wastes (FVWs) and final products of vermicomposting and control without earthworms in dry and fresh systems. Ex/Em means the excitation/emission wavelength pairs. FI represents the specific fluorescence intensity.

Captions of figures

Figure 1 Fluorescence excitation-emission matrix spectra of dissolved organic matter from initial substrate of fruit and vegetable wastes (FVWs) and final products of vermicomposting and control without earthworms in dry and fresh systems.

Figure 2 Net nitrification rate of vermicomposting and control without earthworms in dry and fresh systems for treating fruit and vegetable wastes.

Figure 3 Quantitative numbers of 16S rDNA *amoA* gene of ammonia-oxidizing bacteria and archaea (AOB and AOA) obtained from initial substrates of fruit and vegetable wastes (FVWs) and final products of vermicomposting and control without earthworms in dry and fresh systems. Letters 'IS', 'C', and 'V' under the horizontal axis represent the initial substrate, and final control product and vermicomposting product, respectively.

Figure 4 DGGE images of ammonia-oxidizing bacteria and archaea (AOB and AOA) obtained from initial substrates of fruit and vegetable wastes (FVWs) and final products of vermicomposting and control without earthworms in dry and fresh systems. Letters 'IS', 'C', and 'V' signed in the each lane represent the initial substrate, and final control product and vermicomposting product, respectively. Arabic numbers marked near the band denote that band was excised for sequencing analysis.

Figure 5 Phylogenetic analysis of ammonia-oxidizing bacterial *amoA* gene sequences from DGGE image. The numbers at the branch nodes are bootstrap values based on 1000 re-samplings for maximum likelihood. Only bootstrap values greater than 60% are shown.

Figure 6 Phylogenetic analysis of ammonia-oxidizing archaeal *amoA* gene sequences from DGGE image. The numbers at the branch nodes are bootstrap values based on 1000 re-samplings for maximum likelihood. Only bootstrap values greater than 60% are shown.

Table 1 Physicochemical properties of initial substrates of fruit and vegetable wastes (FVWs) and final products of vermicomposting and control without earthworms in dry and fresh systems. Values are presented by means and standard deviation (n=3).

	Dry system with dry FVWs			Fresh system with fresh FVWs		
	Initial substrates	Final products		Initial substrates	Final products	
		Without earthworms	With earthworms		Without earthworms	With earthworms
pH	7.1 ± 0.02	7.3 ± 0.02	7.5 ± 0.01	5.1 ± 0.02	7.5 ± 0.02	7.6 ± 0.01
Water content (%)	73.3 ± 1.7	70.6 ± 1.3	73.5 ± 1.9	93.5 ± 3.7	75.3 ± 2.7	77.9 ± 2.0
Electrical conductivity (mS/m)	432.7 ± 5.5	336.5 ± 4.2	323.6 ± 2.3	433.6 ± 5.5	504 ± 3.6	261.1 ± 1.7
Organic matter (%)	57.8 ± 1.3	51.6 ± 0.7	44.5 ± 1.7	90.3 ± 1.3	60.5 ± 1.7	41.9 ± 1.3
Total carbon (g/kg)	355.2 ± 3.3	339.4 ± 4.3	296.3 ± 2.0	391.6 ± 3.3	316.0 ± 4.7	284.5 ± 2.3
Total nitrogen (g/kg)	16.9 ± 0.1	16.5 ± 0.3	16.3 ± 0.4	19.5 ± 1.7	17.9 ± 1.3	16.0 ± 0.5
Ammonium (g/kg)	0.63 ± 0.05	0.47 ± 0.02	0.16 ± 0.02	0.18 ± 0.01	0.65 ± 0.05	0.35 ± 0.03
Nitrate (g/kg)	0.48 ± 0.02	0.31 ± 0.05	1.53 ± 0.1	0.66 ± 0.05	0.47 ± 0.1	1.22 ± 0.2

Table 2 Ex/Em matrix dissolved organic matter of initial substrates of FVWs and final products of vermicomposting and control without earthworms in dry and fresh systems. Ex/Em means the excitation/emission wavelength pairs. FI represents the specific fluorescence intensity.

Samples		Peak A		Peak B		Peak C		Peak D		A435-480nm/ A300-345nm
		Ex/Em	FI	Ex/Em	FI	Ex/Em	FI	Ex/Em	FI	
Dry system	Initial substrates	225/355	96.0	280/350	54.5	335/425	32.3	225/450	390	2.7
	Control	225/345	85.6	280/350	46.0	335/425	29.4	225/450	343.5	3.3
	Vermicomposting	225/340	82.9	280/355	40.1	325/435	32.0	225/450	763	4.0
Fresh system	Initial substrates	225/340	158.2	275/345	80.2	315/435	22.1	225/450	275.9	0.8
	Control	225/335	63.7	275/340	18.3	325/435	15.7	225/450	228.1	2.9
	Vermicomposting	220/365	29.6	275/340	5.4	352/435	16.1	225/450	380.3	4.2

Fig. 1

Fig. 1 Fluorescence excitation–emission matrix spectra of dissolved organic matter from initial substrate of FVWs and final products of vermicomposting and control without earthworms in dry and fresh systems.

Fig. 2

Fig. 2 Net nitrification rate of vermicomposting and control without earthworms in dry and fresh systems for treating fruit and vegetable wastes.

Fig. 3

Fig. 3 Quantitative numbers of 16S rDNA *amoA* gene of ammonia-oxidizing bacteria and archaea (AOB and AOA) obtained from initial substrates of fruit and vegetable wastes (FVWs) and final products of vermicomposting and control without earthworms in dry and fresh systems. Letters 'IS', 'C', and 'V' under the horizontal axis represent the initial substrate, and final control product and vermicomposting product, respectively.

Fig. 4

Fig. 4 DGGE images of ammonia-oxidizing bacteria and archaea (AOB and AOA) obtained from initial substrates of fruit and vegetable wastes (FWWs) and final products of vermicomposting and control without earthworms in dry and fresh systems. Letters 'IS', 'C', and 'V' signed in the each lane represent the initial substrate, and final control product and vermicomposting product, respectively. Arabic numbers marked near the band denote that band was excised for sequencing analysis.

Fig. 5

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Fig. 6

Fig. 6 Phylogenetic analysis of ammonia-oxidizing archaeal *amoA* gene sequences from DGGE image. The numbers at the branch nodes are bootstrap values based on 1000 re-samplings for maximum likelihood. Only bootstrap values greater than 60% are shown.

Promoting AOA and AOB gene numbers and diversity

Final stable vermicomposts