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

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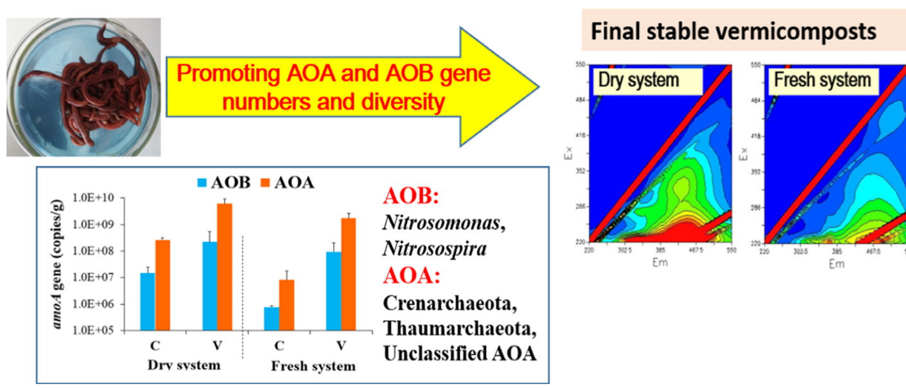
HIGHLIGHTS

- Mechanisms of earthworms promoting nitrification were investigated.
- Dry and fresh vermicomposting systems were used for recycling of FVWs.
- Earthworms promoted the numbers and community richness of ammonia oxidizers.
- AOA rather than AOB dominated in vermicomposting systems.
- Nitrification performance of dry system was better than that of fresh system.

GRAPHICAL ABSTRACT



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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 *Nitrosospira* 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.

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1. 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 (Chen et al., 2014) to investigate the effect of earthworms on ammonia oxidization and (Chen et al., 2003) 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 h, 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 proving 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 h. 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

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