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Changes in chemical and microbiological properties of rabbit manure in a continuous-feeding vermicomposting system

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HIGHLIGHTS

- ▶ *Eisenia fetida* had a great impact on microbial community phospholipid fatty acid (PLFA) profiles.
- ▶ Reduction in bacterial and fungal PLFA biomarkers occurred throughout the process of vermicomposting.
- ▶ High degree of stabilisation from a microbial viewpoint after maturation for 200 d.
- ▶ High levels of dissolved organic carbon were maintained until the end of the process.
- ▶ Continuous-feeding system is an environmentally sound management option.

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ABSTRACT

In the present study the potential of the earthworm *Eisenia fetida* to process large amounts of waste was evaluated through continuous feeding reactors in which new layers of rabbit manure were added sequentially to form an age gradient inside the reactors. An optimal moisture level, ranging from 66% to 76%, was maintained throughout the process using an automatic watering system. The pH was close to 8.3, but decreased to 7.6 after 200 d of vermicomposting. No changes in electrical conductivity through the profile of layers were detected. Based on comparisons of phospholipid fatty acid (PLFA) profiles and microbial activity measurements (basal respiration), a decrease in the levels of bacteria and fungi in layers corresponding to vermicomposting times of more than 200 d occurred. This points to a higher degree of stabilisation in the final product, which is of utmost importance for its safe use as an organic amendment.

1. Introduction

Appropriate management techniques can mitigate the health and environmental risks associated with the overproduction of animal manure by stabilising it prior to its use or disposal (Lazcano et al., 2008). Stabilisation involves the decomposition of an organic material to an extent that eliminates the hazards and is normally reflected in decreases in the microbial biomass and its activity and in the concentrations of labile compounds (Bernal et al., 2009). Vermicomposting, a process involving the bio-stabilisation of organic wastes under aerobic and mesophilic conditions through the joint action of earthworms and microorganisms, is a low-cost and rapid technique for the management of hazardous and worthless organic wastes of different natures, transforming them into safe and valuable products, called vermicomposts (Domínguez and Edwards, 2010a).

Vermicomposting systems sustain a complex food web (Sampedro and Domínguez, 2008), in which detritivore earthworms interact intensively with microorganisms and other fauna within the decomposer community, accelerating the stabilisation of organic matter and greatly modifying its physical and biochemical properties (Domínguez et al., 2010). The biochemical decomposition of the organic matter is primarily accomplished by microbes, but earthworms are crucial drivers of the process as they may affect microbial decomposer activity by grazing directly on microorganisms (Aira et al., 2009; Monroy et al., 2009; Gómez-Brandón et al., 2011a), and by increasing the surface area available for microbial attack after comminution of the organic matter (Domínguez et al., 2010). Recent studies related to the impact of

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epigeic earthworms on microorganisms through phospholipid fatty acid (PLFA) profiles has provided strong evidence for a bottleneck effect caused by worm digestion on the microbial populations of the originally consumed material (Gómez-Brandón et al., 2011a); such effects were species-specific (Gómez-Brandón et al., 2012). This fact points to the earthworm gut as a major shaper of microbial communities, acting as a selective filter for microorganisms contained in the substrate, thereby favouring the existence of a microbial community specialised in metabolising compounds produced or released by the earthworms, in the egested materials. In addition, the nutrient content of earthworm casts differs from that of the ingested material (Aira et al., 2008), which may enable a better exploitation of resources because of the presence of a pool of readily assimilable compounds in the casts (Domínguez et al., 2010). Indeed, Aira et al. (2008) found greater values of dissolved organic carbon (DOC) in the casts of *Eisenia fetida* fed with pig manure; such values were higher (DOC; 2174 ± 253 μg C g^{-1} dw) with the largest density of earthworms (100 earthworms per mesocosm) than that in the control (1146 ± 207 μg C g^{-1} dw). Broadly, the influence of epigeic earthworms on decomposition may be due to the gut associated processes (direct effects), the proximate effects of ingestion, digestion and assimilation of the organic matter and microorganisms in the gut (Gómez-Brandón et al., 2011a); and to cast associated processes (indirect effects) that are more closely associated with the presence of unworked material and to the physical modification of the egested material (Aira et al., 2007a; Gómez-Brandón et al., 2011b). Such indirect effects are derived from direct effects, and include processes such as the ageing of earthworm-inhabited material (weeks–months), and the mixing of such material with substrates that have yet to be processed by earthworms (Aira and Domínguez, 2011). According to this rationale, it is difficult to separate direct and indirect processes and their components, because they occur simultaneously in time and space. Therefore, the decaying organic matter in vermicomposting systems is a spatially and temporally heterogeneous matrix of organic resources with contrasting qualities that result from the different rates of degradation that occur during decomposition (Moore et al., 2004).

Overall, the vermicomposting process includes two different phases regarding the earthworm activity: (i) an active phase during which earthworms process the organic substrate, thereby modifying its physical state and microbial composition (Lores et al., 2006), and (ii) a maturation phase marked by the displacement of the earthworms towards fresher layers of undigested substrate, during which the microbes take over the decomposition of the earthworm-processed substrate (Aira et al., 2007b). The duration of the maturation phase is not fixed, and depends on the composition of the parent material and the efficiency with which the active phase of the process takes place, which in turn is determined by the rate at which the residue is applied (Aira and Domínguez, 2008), and the density and species of the earthworms (Domínguez et al., 2010). *E. fetida* is one of the most widely used earthworm species in vermicomposting systems (Garg et al., 2006; Aira et al., 2007a,b; Sangwan et al., 2008, 2010; Suthar and Singh, 2008; Khwairakpam and Bhargava, 2009; Vivas et al., 2009; Yadav and Garg, 2011), mainly due to its high rate of consumption, digestion and assimilation of organic matter, its tolerance to a wide range of environmental factors, short life cycle, high reproductive rate, and endurance and resistance to handling (Domínguez and Edwards, 2010b). *E. fetida* plays a key role in shaping the structure and activity of the microbial communities of animal manures in short- and long-term experiments (Aira et al., 2007a,b; Aira and Domínguez, 2008; Suthar and Singh, 2008; Gómez-Brandón et al., 2011b, 2012). For example, Aira et al. (2007a) detected an increase in the capabilities of the microbial populations of pig manure to use more diverse carbon pools in a long-term experiment (36 weeks) with the earthworm *E. fetida*, suggesting that microbial communities use the energy available more efficiently in the presence of earthworms. These authors also reported an up to 7.5 times higher fungal biomass, measured as ergosterol content in the presence of this earthworm species. This priming effect on the fungal populations was accompanied by a higher rate of cellulose decomposition with earthworm activity. However, on the whole, most of these previous studies have shown the efficiency of *E. fetida* to process animal manures in lab-scale systems. Therefore, the objective of the present study was to evaluate the potential of this earthworm species to process this type of substrate (i.e., rabbit manure) through a continuous feeding vermicomposting system that is designed to deal with larger amounts of waste. For this purpose, changes in the chemical parameters were monitored as well as those in the structure and activity of the microbial communities through a profile of layers of increasing age, with a gradient of fresh-to-processed manure, from the top to the bottom of the vermireactor. This study may have important implications for the large-scale optimisation of the vermicomposting process and can contribute in better understanding the relationships between epigeic earthworms and microorganisms during this biotransformation process.

2. Methods

2.1. Substrate and earthworm species

Rabbit manure was used as the food source for the earthworms and was collected from the facilities of the vermicomposting company Todo Verde in Ourense (Galicia, NW Spain). Specifically, the annual production of this type of manure is approximately 407×10^3 tonnes in Spain (Bernal and Gondar, 2008). As shown in Table 1, the elemental composition of rabbit manure (expressed on a dry weight basis) was: organic matter content of 69 ± 1%; pH and electrical conductivity of 7.75 ± 0.08 and 0.27 ± 0.01 ms cm^{-2}; total C and N content of 308 ± 27 and 22 ± 4 mg g^{-1}; and NH_4^+ and NO_3^- concentration of 4223 ± 134 and 397 ± 61 mg kg^{-1}. Specimens of the earthworm *E. fetida* (Savigny, 1986) were also provided by the company Todo Verde.

2.2. Vermireactor functioning and sampling method

The vermicomposting system consisted of polyethylene reactors (1.2 × 0.8 × 0.7 m; *n* = 5), initially comprised of a 10-cm layer of mature vermicompost (a stabilised non-toxic substrate that serves as a bed for earthworms), on which earthworms were placed, and a layer containing 5 kg of fresh rabbit manure, which was placed over a plastic mesh (5 mm pore size) to avoid sampling the earthworm bedding. New layers with the same amount of fresh rabbit manure were added to the vermireactor every fifty days according to the feeding activity of the earthworm population (i.e., as determined by the changes in the appearance of the rabbit manure as a result of the earthworm gut- and cast-associated processes; Gómez-Brandón et al., 2012). The initial earthworm biomass was approximately 2250 ± 640 g of earthworms (*E. fetida*) per reactor. This continuous feeding system allowed the addition of each layer to be dated within the reactors, permitting the evaluation of the role of the earthworms in the stabilisation of the manure from a chemical and microbiological viewpoint during the vermicomposting process. To prevent desiccation, the moisture content of the substrate in each reactor was kept approximately at 70% (Table 1) with an automatic watering system. The reactors were divided into four quadrants (0.60 × 0.35 m) and two samples were taken at random from each quadrant with a cylindrical corer (8 cm diameter), as shown by Aira et al. (2011). Each core sample