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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

Table 1
Changes in the chemical properties in the layers of reactors fed with rabbit manure throughout the process of vermicomposting. Different letters indicate significant differences between the layers based on post hoc test (Tukey HSD). Values are means ± SE (n = 5).

	Fresh manure	Worm-worked material				
	0 d	50 d	100 d	150 d	200 d	250 d
Humidity (%)	69 ± 1.95	76 ± 1.70a	75 ± 1.27a	74 ± 1.18a	71 ± 1.15ab	66 ± 2.06b
Organic matter content (%)	69 ± 2.67	67 ± 3.65a	69 ± 2.66a	62 ± 2.98a	60 ± 3.08a	57 ± 3.19a
pH	7.75 ± 0.12	8.31 ± 0.07a	8.35 ± 0.09a	7.89 ± 0.13ab	7.60 ± 0.08b	7.57 ± 0.07b
EC (ms cm^{-2})	0.27 ± 0.009	0.29 ± 0.007a	0.26 ± 0.01a	0.25 ± 0.01a	0.23 ± 0.01a	0.27 ± 0.02a
DOC (μg g^{-1} dw)	14214 ± 1997	14665 ± 795a	13138 ± 1554ab	8915 ± 349bc	5984 ± 598c	6748 ± 652c

EC: electrical conductivity.
DOC: dissolved organic carbon.

was divided into five layers of increasing age (each 7 cm deep), and the samples from the two corers and the same layer were thoroughly mixed for chemical and microbiological analyses. All samples were immediately stored at −20 °C for phospholipid fatty acid analysis or at 4 °C to determine the chemical parameters and microbial activity, assessed by basal respiration.

2.3. Analyses

Electrical conductivity and pH were measured in aqueous extracts (1:10, w/v) using a Crison conductometer CM35 and a Crison MicropH 2000 pH-metre, respectively. Total C and N contents were analysed in oven-dried (60 °C) samples, in a Carlo Erba (EA 1108 CHNS-O) 1500 C/N analyser. Inorganic N (NH_4^+ and NO_3^-) was determined in 0.5 M K_2SO_4 extracts (1:5, w/v) using the colorimetric modified indophenol blue technique (Sims et al., 1995) with a Bio-Rad Microplate Reader 550. Briefly, NH_4^+ is oxidised to monochloroamine by sodium dichloroisocyanuric acid and subsequently forms a green indophenol compound in the presence of phenolics with an unsubstituted paraposition in an alkaline medium. For the colour reaction 175 μL of the sample was pipetted into a microtiter plate, followed by 25 μL of citrate reagent, 50 μL of the colour reagent and 25 μL of sodium hypoclorite. The colour reagent was prepared by mixing equal volumes (1:1:1, v/v/v) of sodium salicylate and sodium nitroprusside solution with NaOH solution and deionized water. The mixtures were left at room temperature (21 °C) for 45 min for colour development, and the absorbance was measured at 650 nm on a Bio-Rad MicroPlate Reader 550. Standards were prepared fresh daily from an NH_4Cl stock solution (1000 mg N L^{-1}) and treated the same way as the samples. For determination of nitrate, nitrate was first reduced to NH_4^+ with Devarda alloy and its concentration determined as described for ammonia.

Soluble organic C was extracted from fumigated and unfumigated samples with 0.5 M K_2SO_4 (50 mL per sample) for 1.5 h on an end-over-end shaker. Extracts were filtered and dissolved organic C was determined colourimetrically in microplates by the acid dichromate oxidation method (Tate et al., 1988). Briefly, 2 mL of the filtrate were mixed with 1 mL of potassium dichromate ($K_2Cr_2O_7$) and 2 mL of sulphuric acid (H_2SO_4). After incubation for 30 min at 160 °C, the absorbance was measured at 590 nm on a Bio-Rad MicroPlate Reader 550. Standards were prepared fresh daily from a glucose stock solution and treated the same way as the samples.

Bacterial and fungal biomass was assessed by PLFA analysis. The sum of Gram-positive (i14:0, i15:0, a15:0, i16:0 and a17:0); and Gram-negative bacteria (16:1ω7c, 17:1ω7c, 18:1ω7c, cy17:0 and cy19:0) PLFAs was chosen to represent bacterial PLFAs; and the PLFA 18:2ω6c was taken to indicate the fungal biomass (Zelles, 1999). A total lipidic extract was obtained from 200 mg of freeze-dried samples with 60 mL of chloroform–methanol (2:1, v/v) in 100 mL sterilised plastic jars, following the method described by Gómez-Brandón et al. (2010) for highly organic samples. The jars were shaken vigorously for 30 min and the mixture was allowed to separate at room temperature for 24 h. The supernatant was filtered, collected in a glass test tube and evaporated to dryness under a stream of oxygen-free N_2 gas. The lipid extract was fractionated into neutral lipids, glycolipids and phospholipids with chloroform (5 mL), acetone (10 mL) and methanol (5 mL) on silicic acid columns (Strata SI-1 Silica (55 μm, 70 Å), 500 mg/6 mL). The fraction containing phospholipids was evaporated under an O_2-free N_2 stream and subjected to derivatisation with trimethylsulfonium hydroxide (TMSH) to obtain fatty acid methyl esters (FAMES), following the protocol of Batista et al. (2001). Briefly, phospholipid extracts were dissolved in 500 μL of methyl-*tert*-butyl ether. One hundred microliters of this solution was placed in a screw-cap vial with 50 μL of the derivatisating agent (TMSH), vortexed for 30 s and allowed to react for 30 min; 10 μL of the internal standard methyl nonadecanoate (19:0, 230 μg mL^{-1}) was added to the extract of FAMEs prior to gas chromatography–mass spectrometry (GC–MS) analysis. Detailed GC–MS experimental conditions have been described elsewhere (Gómez-Brandón et al., 2010). FAMEs were separated on a CP-SIL 88 Varian Select FAME FS 50 m × 0.25 mm × 0.2 μm capillary column (Varian Chromatography Systems). The GC oven temperature program was: 50 °C hold for 2 min, increase at a rate of 20° min^{-1} to 140 °C and 3° min^{-1} to 250 °C. Helium (purity 99.999%) was used as carrier gas at a constant column flow rate of 1 mL min^{-1}. The injector was operated in splitless mode and programmed to return to the split mode 2 min after the beginning of a run. The split ratio was 1:50. The injector temperature was 280 °C. The mass spectrometer was operated in the electron ionisation mode (70 eV). The mass range was scanned from 40 to 650 amu. Experimental conditions for ionisation were: multiplier voltage, 1650 V; filament emission current, 10 μA; axial modulation voltage, 4 V; trap, manifold and transfer line temperatures were 170, 70, and 280 °C, respectively. To identify the FAMEs, the retention times and the mass spectra were compared with those from known standard mixtures or pure PLFAs. FAMEs were quantified by an internal standard calibration procedure (Gómez-Brandón et al., 2010). The calibration levels of the FAMEs varied in the range 0.4–250 μg mL^{-1}. The coefficients of determination (R^2) were higher than 0.99 for all calibration curves. FAMEs were described by the standard ω-nomenclature A:BωC (IUPAC-IUC, 1977).

Total microbial activity was assessed as basal respiration, by measuring the rate of evolution of CO_2, as modified by Aira et al. (2007a) for solid organic samples. Briefly, fresh samples (5 g) were placed in 100-mL airtight glass vessels and incubated at 22 °C for 6 h. The CO_2 produced from the samples was trapped in 0.02 M NaOH, and measured by titration with HCl to a phenolphthalein end-point after adding excess $BaCl_2$.

2.4. Statistical analysis

Data were analysed by ANOVA, with depth of sampling (i.e. how processed the substrate was) as the main factor. Significant

differences in the main effects were analysed by paired comparisons with the Tukey HSD test. A principal component analysis was also used to analyse the PLFA data in order to assess overall differences in the microbial community structure of rabbit manure through the profile of the vermireactor layers. The normality and the variance homogeneity of the data were tested prior to the ANOVA and the principal component analysis. All the statistical analyses were performed with the Statistica software program v9.

3. Results and discussion

The earthworm *E. fetida* is relatively tolerant to the environmental conditions of organic wastes (Domínguez and Edwards, 2010a,b) and as such, this earthworm species has been found to be very competitive during the vermicomposting process (Domínguez et al., 2010). However, it has also been shown that epigeic earthworms have well-defined tolerance limits to several parameters, such as moisture and temperature and in general, wastes are processed much more efficiently within a relatively narrow range of favourable chemical and environmental conditions (Domínguez and Edwards, 2010a,b). For instance, in vermicomposting systems the optimum moisture contents for most species is between 50% and 90% (Edwards, 1988); specifically, the earthworm *E. fetida* can grow more rapidly when a value close to 80% is reached in the waste (Domínguez and Edwards, 1997). In our study, a moisture level, ranging from 66% to 76%, was maintained throughout the continuous feeding vermicomposting reactors. The moisture content decreased significantly with the level of processed manure from the upper to lower layers (ANOVA $F_{4,24} = 5.51$, $P = 0.002$), reaching 66 ± 2.06% after 250 d of vermicomposting (Table 1). In addition, most epigeic earthworms can tolerate pH levels of 5–9, but when given a choice in the pH gradient, they move toward the more acid material, with a pH preference of 5.0 (Domínguez, 2004). As for moisture content, a decreasing trend in pH was found with the depth of layers (ANOVA $F_{4,24} = 15.66$, $P < 0.0001$). The lowest pH was recorded after 250 d (7.57 ± 0.07; Table 1), close to that in the initial rabbit manure (7.75 ± 0.12; Table 1). The reduction in pH may be due to the mineralisation of nitrogen and phosphorus into nitrites/nitrates and orthophosphates, as well as to the bioconversion of the organic material into intermediate species of organic acids (Ndegwa and Thompson, 2000; Garg et al., 2006; Khwairakpam and Bhargava, 2009). The content of inorganic salts is also a crucial parameter with regards to the survival of earthworms (Domínguez and Edwards, 2010a,b), and levels lower than 0.5% are considered acceptable for vermicomposting systems (Edwards, 1988). No significant differences in the salt content (electrical conductivity) with depth of layer (ANOVA $F_{4,24} = 2.55$, $P = 0.06$; Table 1) were detected; overall, low values of electrical conductivity similar to those in the raw manure (0.27 ± 0.009 ms cm^{-2}; Table 1) were observed through the profile of layers in the vermireactors.

Epigeic earthworms are known to accelerate the rate of decomposition of organic matter during vermicomposting (Domínguez et al., 2010), thereby leading to important losses of total carbon throughout this biotransformation process (Garg et al., 2006; Aira et al., 2007a,b; Domínguez et al., 2010; Gómez-Brandón et al., 2011b, 2012). The total carbon mass balance was not calculated in the current study, but no significant differences were recorded over time regarding the organic matter content (ANOVA $F_{4,24} = 2.42$, $P = 0.07$), with a level close to 60% through the layers profile (Table 1). A reduction in the labile carbon pool (DOC) of rabbit manure was detected with depth of layer (ANOVA $F_{4,24} = 13.14$, $P = 0.0001$), with intermediate values after 150 d (8915 ± 1350 μg g^{-1} dw; Table 1) and the lowest values after vermicomposting for 200 d (5985 ± 598 μg g^{-1} dw; Table 1). Such values were lower compared to that observed in raw manure (14214 ± 1997 μg g^{-1} dw; Table 1). DOC generally contains organic compounds that have different susceptibilities to microbial degradation and different phytotoxic properties. More specifically, dissolved organic matter constitutes the organic fraction that contains organic materials utilised as an energy source, bio-originating macromolecules such as enzymes, polysaccharides and proteins, and breakdown products, as well as the repolymerised compounds that eventually impart stability to composted organic matter, which is crucial for its effective application to soil (Said-Pullicino et al., 2007). For this reason, DOC composition may have an important role in determining the stabilisation of the organic matter during biological processes, such as composting and vermicomposting; however, unlike for compost, where a limit value of 4000 mg kg^{-1} is suggested for a stable compost (Zmora-Nahum et al., 2005), there is still no threshold level of DOC at which vermicompost is considered stable. In the present study, a DOC value close to 6000 μg g^{-1} dw was reached after 200 d of vermicomposting. In contrast, Aira et al. (2007a) reported levels of DOC below 1500 μg g^{-1} dw in a long-term experiment (252 d) with *E. fetida*. Such differences could be due to the composition of the parent material (pig slurry *versus* rabbit manure) and/or to the experimental set-up.

In the present study, *E. fetida* greatly modified the structure of the microbial decomposer communities during vermicomposting, as revealed by the phospholipid fatty acid analysis. A clear

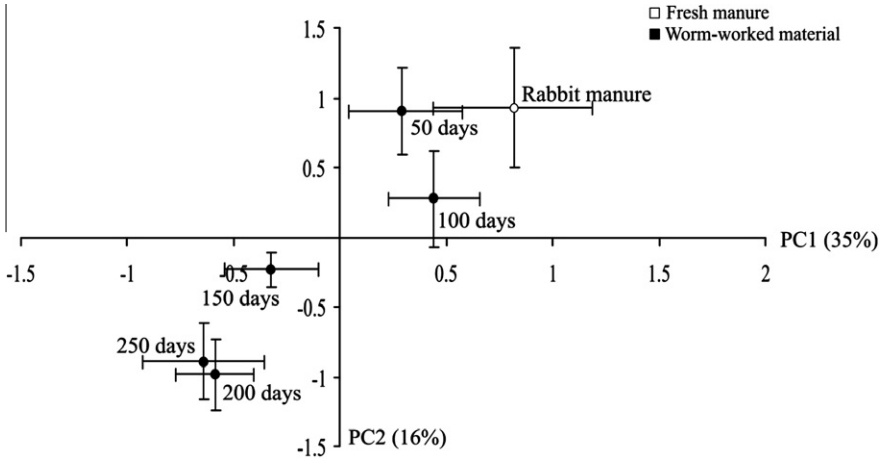

Fig. 1. Principal component analysis performed on the twenty-seven PLFAs identified in the layers of reactors fed with rabbit manure throughout the process of vermicomposting. Values are means ± SE ($n = 5$).

Table 2
Factor loadings of the twenty-seven identified PLFAs responsible for the changes along the first and second principal components (PC1 and PC2, respectively).

PLFAs	PC1	PC2
PLFA biomarkers		
G^+ bacteria		
i14:0	0.612	0.428
i15:0	0.730	0.468
a15:0	0.810	0.225
i16:0	0.834	0.158
a17:0	0.620	0.223
G^- bacteria		
16:1ω7c	−0.153	−0.654
17:1ω7c	0.133	−0.699
18:1ω7c	0.892	0.234
cy17:0	0.419	0.731
cy19:0	0.492	0.711
Fungi		
18:2ω6c	0.287	0.751
Other microbial PLFAs		
10:0	0.783	−0.197
12:0	0.867	−0.287
13:0	0.826	−0.178
14:0	0.889	−0.318
15:0	0.771	−0.358
16:0	0.735	−0.494
17:0	0.806	−0.328
18:0	0.614	−0.272
14:1ω5c	0.319	−0.195
15:1ω5c	−0.047	−0.196
18:2ω6t	−0.181	0.530
18:1ω9c	−0.077	0.277
18:1ω9t	−0.416	0.134
18:3ω6c	−0.063	−0.041
18:3ω3c	0.270	0.097
20:0	0.232	−0.234

separation between the samples was found along the first and second principal components (PC1 and PC2; accounting for 35% and 16% of the variance, respectively) as a function of the age of the layers (Fig. 1). Thus, the upper layers (50 and 100 d old) along with the fresh manure were clearly differentiated from the intermediate (150 d old) and lower layers (200 and 250 d old) mainly due to decreases in several bacterial PLFAs characteristic of Gram-positive (a15:0 and i16:0) and Gram-negative bacteria (18:1ω7c), which were strongly correlated with the positive side of PC1 (Table 2). Other PLFAs, such as 12:0, 13:0, 14:0 and 17:0 also contributed greatly to the separation along this axis (Table 2). The fungal PLFA 18:2ω6c was the most strongly correlated PLFA with PC2 (positively correlated; Table 2), which indicates that the abundance of this PLFA biomarker decreased throughout the layers. These findings are in accordance with those from studies with lab-scale reactors fed with doses of 1.5 and 3 kg of pig slurry. Such effects were more pronounced in the manure layers between 21 and 36 weeks old (Gómez-Brandón et al., 2011b). Similar results were obtained in an industrial-scale continuous-feeding vermireactor in the presence of the earthworm *Eisenia andrei* (Aira et al., 2011). In fact, the species *E. andrei* and *E. fetida* are closely related, but in mixed cultures *E. andrei* becomes dominant, especially when there is no substrate limitation (Domínguez et al., 2005).

Epigeic earthworms possess a diverse pool of digestive enzymes which enables them to digest bacteria, protozoa, fungi and partly decomposed plant debris (Zhang et al., 2000) and this ability can have a negative effect on microbial biomass (Aira et al., 2009; Monroy et al., 2009; Gómez-Brandón et al., 2011a). For instance, Gómez-Brandón et al. (2011a) found a reduction of up to a 40% reduction in the microbial biomass of cow, horse and pig manure after passage through the gut of *E. andrei*. In line with this, Fernández-Gómez et al. (2010) observed that the structure

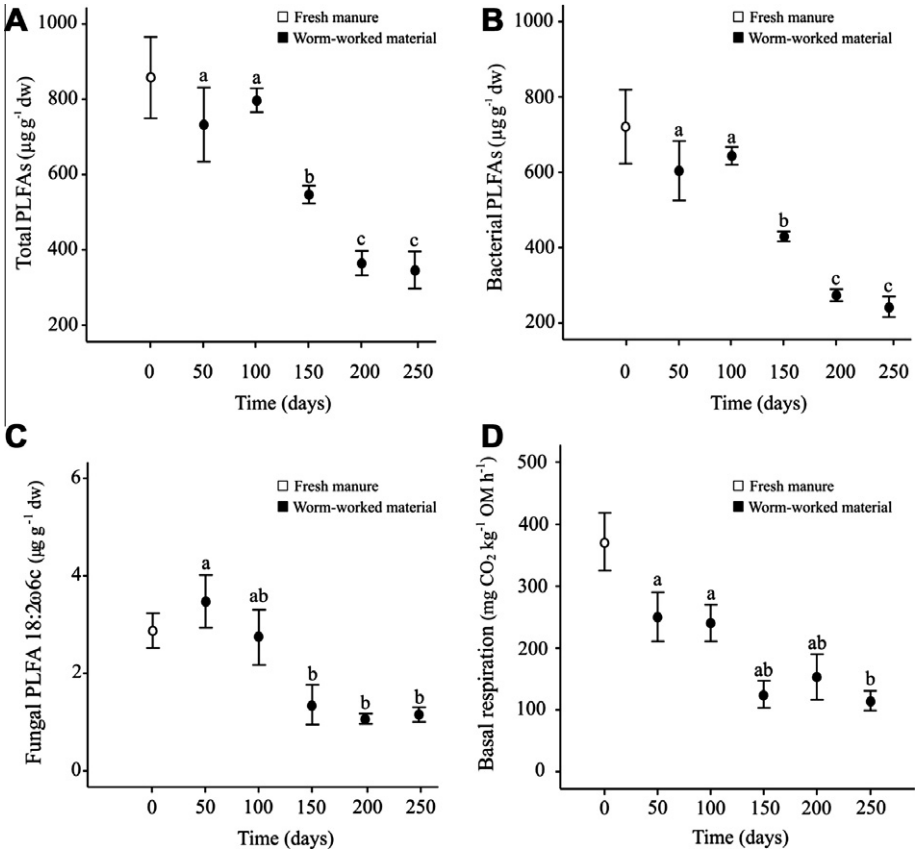

Fig. 2. Changes in (A) total, (B) bacterial PLFAs, (C) fungal PLFAs and (D) microbial activity assessed by basal respiration in the layers of reactors fed with rabbit manure throughout the process of vermicomposting. Different letters indicate significant differences between the layers based on post hoc test (Tukey HSD). Values are means ± SE ($n = 5$).

of fungal communities, assessed by denaturing gradient gel electrophoresis (DGGE) profiles, differed at the stage of maximum earthworm biomass the most, suggesting the existence of a strong gut passage effect. In accordance with this finding, the viable microbial biomass assessed by the total content of PLFAs, in the present study decreased greatly with the depth of layers (ANOVA $F_{4,24} = 7.45$, $P = 0.001$; Fig. 2A), i.e. from upper to intermediate and lower layers (1.4 and 1.9 decrease, respectively); and the total content of PLFAs was also lower than that in fresh rabbit manure (828.33 ± 147.78 μg g^{-1} dw; Fig. 2A) in the two last layers. A similar pattern was observed for bacterial (ANOVA $F_{4,24} = 12.64$, $P = 0.0001$; Fig. 2B) and fungal biomass (ANOVA $F_{4,24} = 6.64$, $P = 0.001$; Fig. 2C) as the corresponding dry weight contents in fresh rabbit manure were 683.12 ± 110.24 μg g^{-1} dw and 2.70 ± 0.26 μg g^{-1} dw, respectively (Fig. 2B and C). These results are in accordance with those previous studies based on PLFA profiles (Gómez-Brandón et al., 2011b; Aira et al., 2011) as the bacterial and fungal biomass reached 270 ± 45.12 μg g^{-1} dw and 1.3 ± 0.10 μg g^{-1} dw for bacterial and fungal populations, respectively. Decreases in microbial activity were also detected with depth of layer (ANOVA $F_{4,24} = 4.58$, $P = 0.009$; Fig. 2D) and, after a period of 250 d, basal respiration values dropped below 100 mg CO_2 kg^{-1} OM h^{-1} (76.96 ± 17.50 mg CO_2 kg^{-1} OM h^{-1}; Fig. 2D). This trend followed the typical pattern observed in vermicomposting (Gómez-Brandón et al., 2011b). Aira and Domínguez (2009) also found lower microbial activity, measured as basal respiration in the casts of *E. fetida* (510 μg CO_2 g^{-1} OM h^{-1}) than the initial cow manure (920 μg CO_2 g^{-1} OM h^{-1}).

4. Conclusions

The present study demonstrates the potential of *E. fetida* to process animal manures in a larger-scale vermicomposting system. Overall, a higher degree of stabilisation was reached in the organic substrate after 200–250 d, as indicated by the lower values of microbial biomass and activity compared to those in the fresh manure. These findings highlight the continuous-feeding vermicomposting system as an environmentally sound management option for recycling animal manures, as previously reported by Fernández-Gómez et al. (2010) regarding the use of this system for treating tomato-fruit waste from greenhouses. Such results must nonetheless be weighed against the fact that the functioning of this type of reactor leads to the gradual accumulation of layers and to the compaction of the substrate, thus minimizing earthworm-induced aeration, which can promote pathogen survival (Aira et al., 2011). Ultimately, there is a need for further studies to evaluate the efficiency of this type of reactor to process a wider range of residues from different sources, as well as to test the quality of the end products as fertilisers under field conditions.

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