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Changes in microbial community structure and function during vermicomposting of pig slurry

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ABSTRACT

Most studies investigating the effects of earthworms on microorganisms have focused on the changes before and after vermicomposting rather than those that occur throughout the process. In the present study, we designed continuous feeding reactors in which new layers of pig slurry (1.5 and 3 kg) were added sequentially to form an age gradient inside the reactors in order to evaluate the impact of the earthworm species *Eisenia fetida* on microbial community structure and function. The activity of earthworms greatly reduced the bacterial and fungal biomass and microbial diversity relative to the control values. However, the pronounced presence of earthworms in the younger layers stimulated microbial activity and as such increased carbon mineralization probably due to the fact that the microorganisms may have been less resource-limited as a result of earthworm activity, as indicated by the ratio of monounsaturated to saturated PLFAs.

1. Introduction

Vermicomposting is a bio-oxidative process in which detritivore earthworms interact intensively with microorganisms and other fauna within the decomposer community, accelerating the stabilization of organic matter and greatly modifying its physical and biochemical properties (Domínguez, 2004). The biochemical decomposition of organic matter is primarily accomplished by microorganisms, but earthworms are crucial drivers of the process as they are involved in the stimulation of microbial populations through ingestion and fragmentation of fresh organic matter, which results in a greater surface area available for microbial colonization, thereby drastically altering biological activity (Domínguez et al., 2010). Earthworms also modify microbial biomass and activity through stimulation, digestion and dispersion in casts (Brown and Doube, 2004; Aira and Domínguez, 2009; Monroy et al., 2009) and closely interact with other biological components of the vermicomposting system, thus affecting the structure of microflora and microfauna communities (Domínguez et al., 2003; Lores et al., 2006; Aira et al., 2007a; Monroy et al., 2009).

The impact of earthworms on the decomposition of organic waste during the vermicomposting process is initially due to gut associated processes (GAPs), i.e., modifications that the decaying organic matter and the microorganisms undergo in the intestinal tract (Aira et al., 2009; Monroy et al., 2009). Some of this material will be digested, but earthworms also excrete large amounts of casts that contain different nutrient and microbial populations than those contained in the material prior to ingestion (Haynes et al., 2003; Knapp et al., 2009). The resultant earthworm casts are mixed with material not ingested by the earthworms, which can enable the better exploitation of resources, either because of the appearance of microbial species in the fresh substrate or the pool of readily assimilable compounds in the casts (Brown and Doube, 2004). Upon completion of the GAPs, the egested materials undergo cast-associated processes that are more closely associated with aging processes, during which the effects of earthworms are mainly indirect and derived from the GAPs (Parthasarathi and Ranganathan, 2000; Aira et al., 2007b). Therefore, the vermicomposting process includes two different phases regarding the activity of earthworms: (i) an active phase during which earthworms process the organic waste, thereby modifying its physical state and microbial composition (Lores et al., 2006), and (ii) a maturation-like phase marked by the displacement of the earthworms towards fresher layers of undigested waste, during which the microbes take over the decomposition of the waste processed by the earthworms (Aira et al., 2007b). The duration of the maturation phase is not fixed, and depends on the composition of organic waste and the efficiency with which the active phase of the process takes place, which in turn is determined by the species and density of earthworms (Domínguez et al., 2010), and the rate at which the residue is applied (Aira and Domínguez, 2008).

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In vermicomposting systems, gut and cast-associated processes may play a key role in determining the characteristics of the microbial decomposer communities. Moreover, vermicomposting, and the decomposition of organic matter in general, are characterized by having a control donor dynamics (Pimm, 1982), in which the rate of detrital input is expected to be a major factor influencing the interactions within the decomposer community. However, little is known about the relative contribution of the above-mentioned processes to the changes in microbial populations during vermicomposting and how the availability of resources shapes the relationships between earthworms and microorganisms. Most studies have focused on the changes before and after vermicomposting (Anastasi et al., 2005; Fracchia et al., 2006; Lazcano et al., 2008; Vivas et al., 2009) rather than those that occur throughout the process.

In the present study we investigated the impact of the epigeic earthworm species *Eisenia fetida* on microbial community structure and function during the vermicomposting of pig slurry. We evaluated the effects of this earthworm species on the abundance of bacteria and fungi and on microbial diversity and total microbial activity. Moreover, we studied how such changes in microbial community structure and function affected organic matter decomposition, by analysing the concentration of total carbon. For this purpose, we designed a reactor that permitted us to monitor the age of the substrate and sample it without interfering with either the newer or older layers of the substrate within the reactor. At the end of the experiment we obtained a profile of layers in which we were able to observe the different phases of interaction between earthworms and microorganisms during the vermicomposting process.

2. Methods

2.1. Substrate and earthworm species

Fresh pig slurry was used as the food source for the earthworms and was obtained from a pig breeding farm near the University of Vigo (Galicia, NW Spain). The solid fraction (15% dry weight) of the slurry was selected in order to avoid any harmful effects that percolates may have had on the earthworms. The slurry was homogenized, and stored in sealed plastic containers at 5 °C until use. Specimens of the epigeic earthworm species *E. fetida* (Savigny, 1826) were obtained from a stock culture reared under laboratory conditions (20 ± 2 °C).

2.2. Reactor set-up and functioning

The reactors comprised PVC modules, resembling sieves, of an external diameter of 30 cm and a volume of 1413 cm^3. Each reactor was initially comprised of one module containing vermicompost from pig slurry, in which earthworms were placed, and another module containing a layer of fresh pig slurry (1.5 or 3 kg fresh weight, low and high rate, respectively). The bottom of each module consisted of a mesh (5 mm pore size), which allowed the mobility of earthworms between modules. New modules with the same amount of fresh pig slurry were added sequentially according to the feeding activity of the earthworm population. This procedure allowed the addition of each module to be dated within the reactors (Aira et al., 2007a).

2.3. Experimental design and sampling method

We set up twelve of the above mentioned reactors; six each for low (1.5 kg) and high doses (3 kg) of pig slurry. For each dose, three reactors were inoculated with 500 mature specimens of *E. fetida* and three were left without earthworms (control). After 36 weeks, the reactors consisted of modules of increasing age, resembling a time profile. Twelve modules, added after 2, 4, 7, 8, 11, 18, 21, 25, 27, 29, 33 and 36 weeks were dismantled and isolated to avoid earthworm escape. The earthworms were then removed manually from the substrate, counted and weighed. After 36 weeks, the mean population of *E. fetida* in the reactors was 2800 ± 200 individuals, with a mean biomass of 700 ± 30 g. This meant a 5- and 8-fold increase respectively over the initial number and the biomass of the inoculated population of *E. fetida*. There were no significant differences in the size of the earthworm population between reactors with low and high doses of manure. Seventy percent of the earthworms were located in the 2 and 4 week old manure layers, with a quantity of 1000 individuals per layer. The rest of the earthworms were distributed throughout the 7, 8, 11 and 18 week old layers, with no more than 200 individuals per layer. No earthworms were found in the remaining layers (21, 25, 27, 29, 33 and 36 weeks old). Five samples of substrate per module were taken at random and mixed gently in order to determine the following parameters, as detailed below.

2.4. Analyses

Microbial community structure was assessed by phospholipid fatty acid (PLFA) analysis. Briefly, the total lipidic extract was obtained from 200 mg of each freeze-dried sample with 60 mL of chloroform–methanol (2:1, v/v), following the method described by Gómez-Brandón et al. (2010a) for highly organic samples. The lipid extract was then fractionated into neutral lipids, glycolipids and phospholipids with chloroform (5 mL), acetone (10 mL) and methanol (5 mL), respectively, on silicic acid columns (Strata SI-1 Silica (55 μm, 70 Å), 500 mg/6 mL). The fraction containing phospholipids was subjected to alkaline methanolysis to obtain the fatty acid methyl esters (FAMEs), and analysed by gas chromatography-mass spectrometry (GC-MS). The detailed GC-MS experimental conditions have been described by the authors elsewhere (Gómez-Brandón et al., 2010a). In order to identify the FAMEs, the retention times and the mass spectra were compared with those obtained from the standards. FAMEs were quantified by an internal standard calibration procedure (see Gómez-Brandón et al., 2010a).

The sum of all identified PLFAs (total PLFAs) was used to estimate the viable microbial biomass (Zelles, 1999). Certain PLFAs were used as biomarkers to determine the presence and abundance of specific microbial groups (Joergensen and Wichern, 2008). The sum of PLFAs characteristic 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) was chosen to represent bacterial PLFAs, and the PLFA 18:2ω6c was used as a fungal biomarker. The ratio of monounsaturated to saturated (mono:sat) PLFAs was used as an indicator of physiological or nutritional stress in microbial communities (Bossio and Scow, 1998). This ratio is generally lower in microbial communities that inhabit environments where organic carbon and/or nutrients are limiting. The diversity of the microbial communities was measured by the Shannon index (H) for the PLFAs identified (H_{PLFA}) (Wardle et al., 2003).

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. Total C content was analyzed in dried samples, in a Carlo Erba NA 1500 C/N analyzer.

2.5. Statistical analysis

Data were analysed by a split-plot repeated measures analysis of variance (ANOVAR) in which single reactors were subjects, earthworm treatment (presence and absence) and the rate of

application of pig slurry (1.5 and 3 kg) were fixed as between- subject factors, and the age of layers (i.e., each single module) was fixed as a within-subject factor. This model assumes correlation between treatment levels within a block, i.e., the modules of each reactor (von Ende, 2001). A principal component analysis was also used to analyse the PLFA data in order to assess overall differences in the microbial community structure of pig slurry in function of earthworm presence, dose and time. This analysis enabled us to determine which PLFAs are primarily responsible for the overall differences in the first and second principal components (PC1 and PC2, respectively). These differences were analysed by ANOVAR, as above. The normality and the variance homogeneity of the data were tested prior to ANOVAR and principal component analysis. All statistical analyses were performed with the Statistica software program v7.

3. Results and discussion

The earthworm species *E. fetida* played a key role in the decomposition of organic matter during vermicomposting, greatly modifying the structure of microbial decomposer communities, as revealed by the phospholipid fatty acid analysis. The principal component analysis of the 25 identified PLFAs clearly distinguished between samples in function of earthworm presence and age of layers in both high and low dose reactors, which explained 63% of the variance in the data (Fig. 1a). The G+bacterial biomarkers (i15:0, a15:0, i16:0 and a17:0) and the fungal PLFA 18:2ω6c were strongly correlated with the positive side of PC1 (Fig. 1b). Earthworm activity thus led to a decrease in the abundance of these PLFA biomarkers relative to the control (ANOVAR $F_{1,8}$ = 56.64, $P < 0.0001$). This effect was more pronounced in the manure

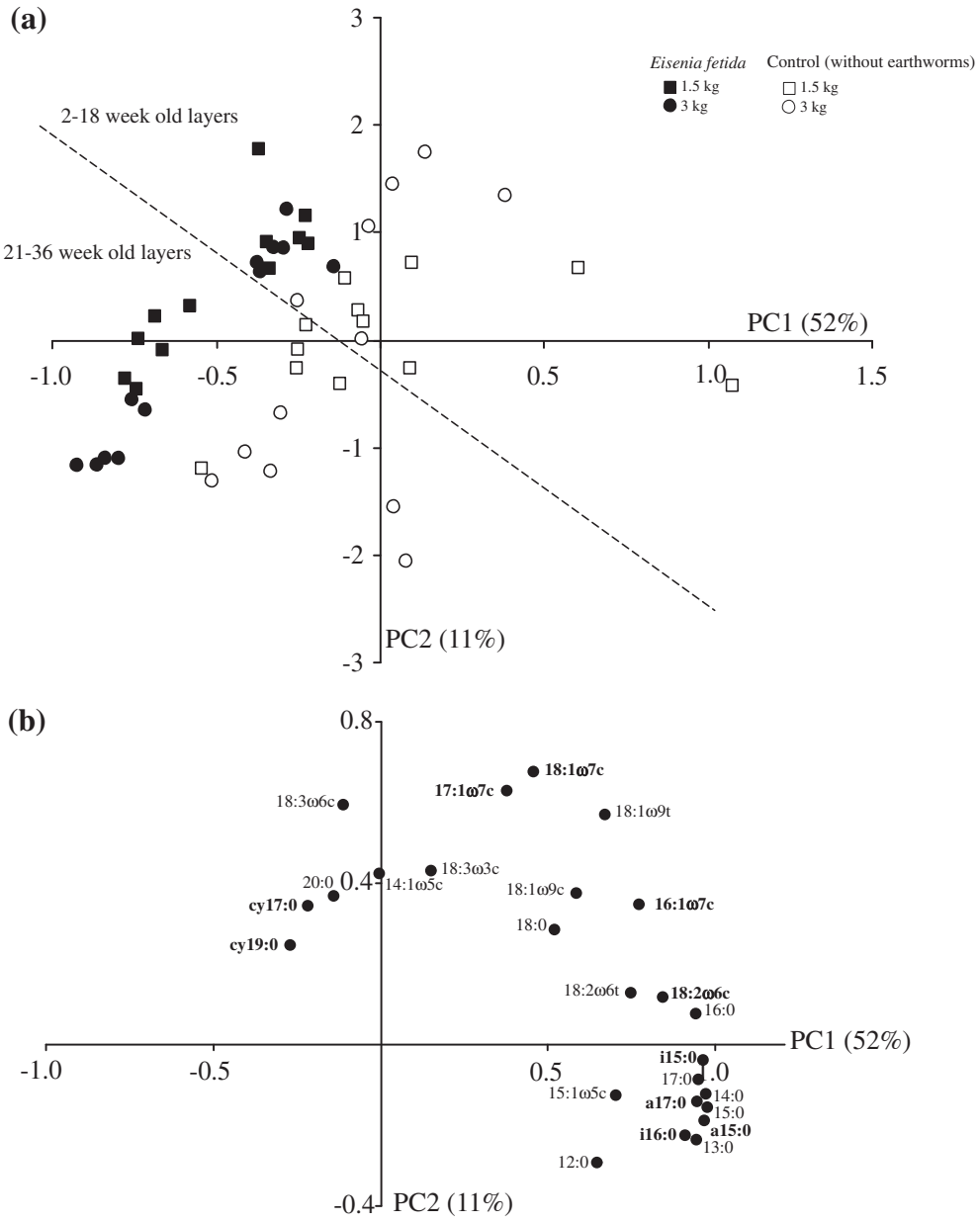

Fig. 1. Changes in the microbial community structure during vermicomposting of pig slurry: (a) Principal component analysis performed on the twenty-five PLFAs identified in layers of reactors fed with doses of 1.5 and 3 kg of pig slurry with *E. fetida* (filled symbols) and without *E. fetida* (open symbols); (b) Factor loadings of the twenty-five PLFAs identified. The PLFAs used as bacterial and fungal biomarkers are highlighted in bold.

layers of between 21 and 36 weeks old (Fig. 1a), resulting in a significant interaction between earthworm presence and age of layers (ANOVAR $F_{11,88} = 3.96$, $P < 0.001$). Some PLFAs characteristic of G−bacteria (17:1ω7c and 18:1ω7c) were the most strongly correlated with PC2 (positively correlated; Fig. 1b and, as such, were present at significantly higher concentrations in the younger layers (2–18 weeks old) than that in the older layers (21–36 weeks old), which shifted negatively along this component (Fig. 1a, ANOVAR $F_{11,88} = 31.72$, $P < 0.00001$). This is consistent with previous studies based on PLFA and total FAME profiles, which indicate that the activity of epigeic earthworms strongly modifies the structure of the microbial communities in animal manures of different origin (Lores et al., 2006) and in plant residues (Gómez-Brandón et al., 2010b).

There is recent evidence in the literature suggesting that the digestion of the organic material by these earthworm species has negative effects on microbial biomass. Indeed, Gómez-Brandón et al. (2010b) observed a reduction in the abundance of characteristic bacterial and fungal PLFAs in the casts of *Eisenia andrei*. Similarly, Aira et al. (2006) detected a decrease in microbial biomass C in the casts of *Eudrilus eugeniae* fed with pig slurry. In the present study, the activity of earthworms reduced the viable microbial biomass measured as total PLFAs by approximately 1.3 times relative to the control without earthworms during the vermicomposting of pig slurry (Fig. 2, ANOVAR $F_{1,8} = 51.75$, $P < 0.0001$), irrespective of the rate of application (ANOVAR $F_{1,8} = 1.41$, $P = 0.27$) and age of the layers (ANOVAR $F_{11,88} = 1.62$, $P = 0.11$). The high density of earthworms in the 2 and 4 week old layers led to an accelerated consumption of the pig slurry, and in turn, to the predominance of gut associated processes in the modifications that the residue undergo in these layers. Thus, the lower microbial biomass found in the 2 and 4 week old manure layers may be attributed to gut associated processes. Epigeic earthworms may also affect the microbial biomass by accelerating the depletion of resources for the microbes (Domínguez, 2004). However, we found that the effect of earthworms on the viable microbial biomass was independent of the dose of pig slurry applied.

Earthworm activity also greatly reduced the abundance of PLFAs characteristic of bacteria with respect to the control during the vermicomposting process of pig slurry (Fig. 3, ANOVAR $F_{1,8} = 39.42$, $P < 0.001$), although this effect was more pronounced in low dose reactors (1.4 times) than in high dose reactors (1.1 times), resulting in a significant interaction between earthworm presence and the rate of application (ANOVAR $F_{1,8} = 6.95$, $P < 0.05$). However, we found that the effect of earthworms on the abundance of the fungal PLFA biomarker 18:2ω6c was independent of the dose of pig slurry applied (Fig. 4, ANOVAR $F_{1,8} = 1.62$, $P = 0.24$). Animal manures are microbial-rich environments in which bacteria constitute the largest fraction, with fungi mainly present as spores (Garret, 1981); moreover, the first stages of decomposition in these organic wastes are mainly dominated by bacteria because of the availability of water and readily decomposable compounds. Fungi have been found to be more competitive with regard to the degradation of more slowly decomposable compounds such as cellulose, hemicellulose and lignin (de Boer et al., 2005). Hence, earthworm activity is expected to affect bacteria to a greater extent than fungi in the short-term. The impact of earthworms on bacterial populations became more apparent in the low dose reactors, in which the loss of carbon was greater than that in high dose reactors (Table 1).

As we found for microbial biomass, the values of microbial diversity assessed by the H_{PLFA} index were 1.4 times lower in the presence of earthworms than that in the control treatment (Table 1, ANOVAR $F_{1,8} = 226.29$, $P < 0.0001$), irrespective of the rate of application (ANOVAR $F_{1,8} = 5.53$, $P = 0.05$). However, the reduction in the H_{PLFA} index as a result of earthworm activity was more pronounced in the older layers of the reactors (1.6 times) than in the younger layers (1.2 times), thus producing a significant interaction between earthworm presence and age of layers (ANOVAR $F_{11,88} = 8.11$ $P < 0.0001$). Recently, Sen and Chandra (2009) and Vivas et al. (2009) used molecular tools to analyse the microbial communities in vermicompost and compost samples derived from sugar and olive industry wastes. These authors observed greater microbial diversity in vermicompost, relative to the initial substrate, than in compost.

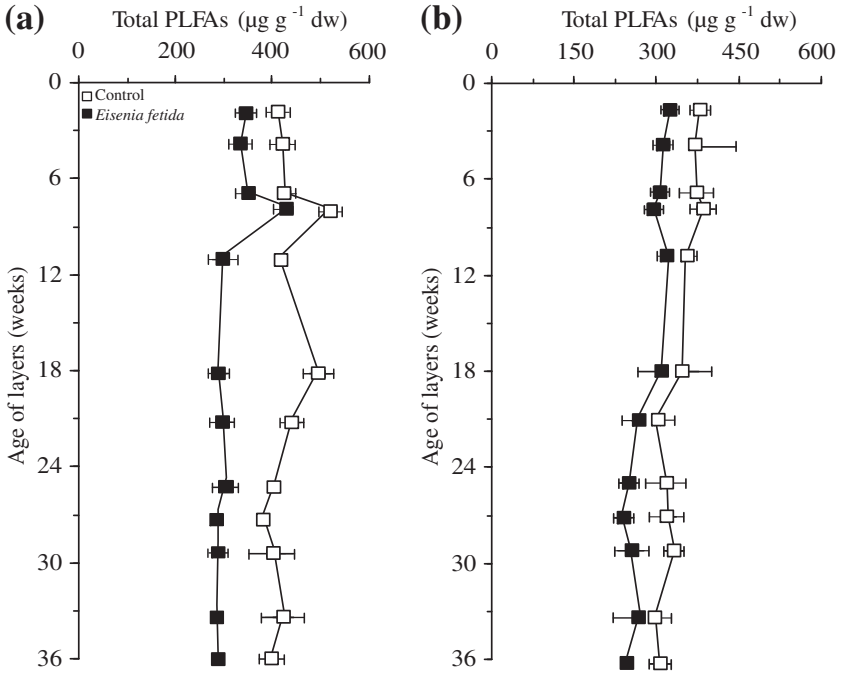

Fig. 2. Viable microbial biomass measured as total PLFA content in layers of reactors fed with doses of 1.5 kg (a) and 3 kg of pig slurry (b) with *E. fetida* (filled symbols) and without *E. fetida* (open symbols). Variable values (means ± SE) corresponding to the age of the layers of pig slurry are shown on the y axis.

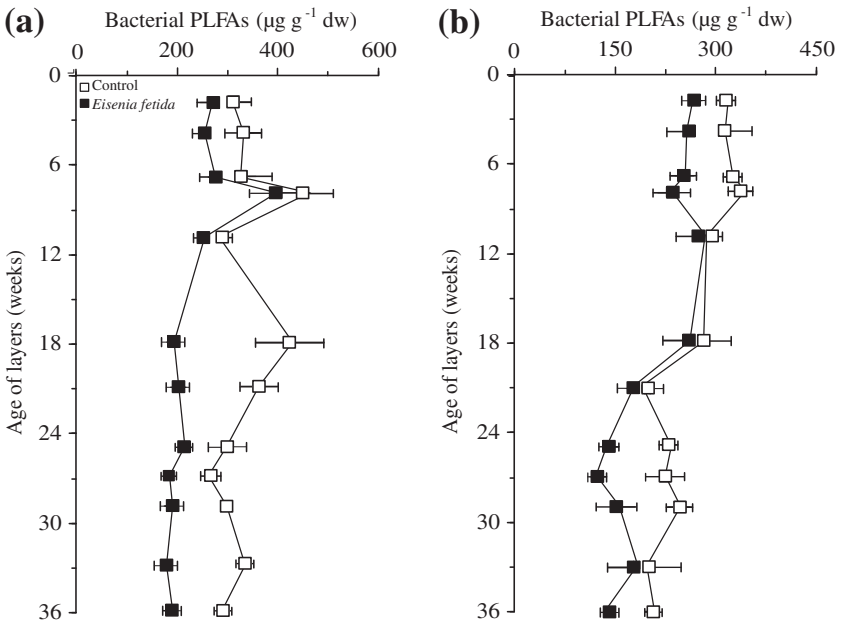

Fig. 3. Abundance of bacterial PLFA biomarkers in layers of reactors fed with doses of 1.5 kg (a) and 3 kg of pig slurry (b) with *E. fetida* (filled symbols) and without *E. fetida* (open symbols). Variable values (means ± SE) corresponding to the age of the layers of pig slurry are shown on the y axis.

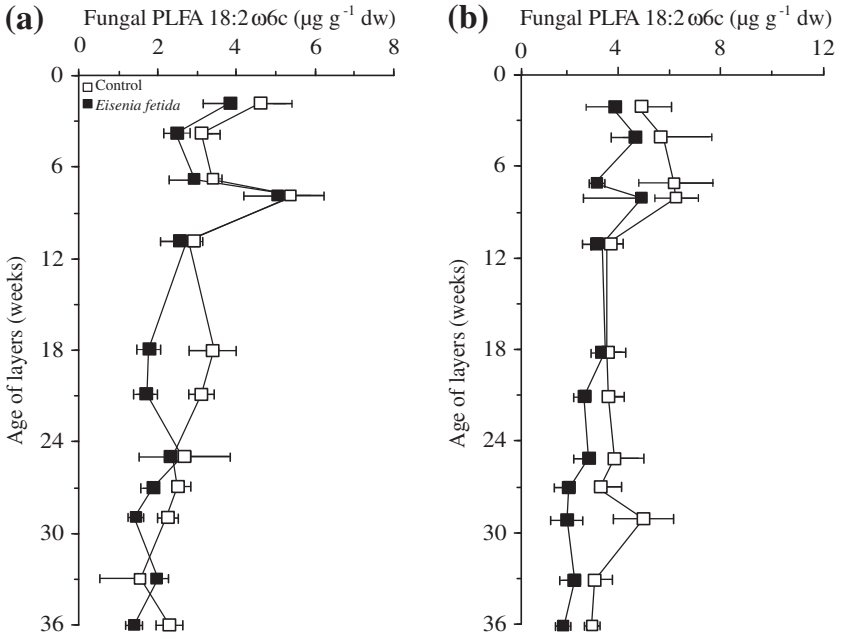

Fig. 4. Abundance of the fungal PLFA biomarker 18:2ω6c in layers of reactors fed with doses of 1.5 kg (a) and 3 kg of pig slurry (b) with *E. fetida* (filled symbols) and without *E. fetida* (open symbols). Variable values (means ± SE) corresponding to the age of the layers of pig slurry are shown on the y axis.

Despite having reduced the microbial biomass and diversity, the presence of earthworms increased the total microbial activity, measured as basal respiration, relative to the control for the 2 to 8 week old manure layers in the reactors fed with low and high doses of pig slurry (1.5 and 1.1 times; Fig. 5). However, the opposite effect was observed in the remaining layers, mainly those between 21 and 36 weeks old, in which basal respiration was much higher in the control treatment (Fig. 5), resulting in a significant interaction between earthworm presence and age of layers (ANOVAR $F_{11,88} = 13.43$, $P < 0.0001$). These results highlight the potential of vermicomposting for the biological stabilization of solid organic wastes because lower values of microbial activity that are indicative of stabilized materials (Benito et al., 2003), were achieved after eighteen and twenty-one weeks in low and high dose reactors respectively; whereas in those reactors without earthworms microbial activity was still high for both doses after thirty-six weeks of treatment. The high density of earthworms present in the younger layers and the relatively rapid gut transit time of the earthworm *E. fetida*, around 2.5–7 h (Hartenstein et al., 1981) have been found to play a key role in the time required for the stabilization of the residue. Similarly, Lazcano et al. (2008) reported lower values of microbial activity, relative to the control,

Table 1
Changes in the values of the Shannon diversity index for PLFAs (H_{PLFA}), monounsaturated to saturated (mono:sat) PLFA ratio and total C content through the layers of reactors with and without earthworms (Ew) fed with doses of 1.5 kg (low dose) and 3 kg (high dose) of pig slurry. Values are means ± standard error.

Age of layers	H_{PLFA}				Mono:sat PLFA ratio				Total C (mg g^{-1} dw)			
	Without Ew		With Ew		Without Ew		With Ew		Without Ew		With Ew	
	Low dose	High dose	Low dose	High dose	Low dose	High dose	Low dose	High dose	Low dose	High dose	Low dose	High dose
2	2.20 ± 0.01	2.42 ± 0.01	1.97 ± 0.06	2.20 ± 0.01	4.52 ± 0.05	3.95 ± 0.12	4.84 ± 0.10	4.50 ± 0.21	43.40 ± 0.72	40.94 ± 0.80	41.80 ± 0.36	39.73 ± 2.23
4	2.05 ± 0.02	2.36 ± 0.02	1.99 ± 0.01	2.05 ± 0.02	3.81 ± 1.23	3.64 ± 0.08	3.49 ± 0.37	6.30 ± 0.21	43.28 ± 0.36	39.31 ± 0.96	38.76 ± 0.79	40.62 ± 0.84
7	1.96 ± 0.02	2.28 ± 0.01	2.08 ± 0.02	1.96 ± 0.02	4.80 ± 0.42	3.98 ± 0.02	5.32 ± 0.08	4.86 ± 0.12	44.25 ± 0.03	40.10 ± 1.34	38.13 ± 0.49	39.11 ± 1.08
8	1.88 ± 0.00	2.25 ± 0.01	2.29 ± 0.02	1.88 ± 0.00	4.10 ± 0.29	1.48 ± 0.31	4.60 ± 0.51	2.97 ± 0.27	44.93 ± 0.18	39.98 ± 0.39	37.46 ± 0.40	37.68 ± 0.38
11	1.85 ± 0.03	2.18 ± 0.03	1.84 ± 0.02	1.85 ± 0.03	3.51 ± 0.09	2.97 ± 0.11	5.23 ± 0.37	5.34 ± 0.19	44.03 ± 0.22	42.50 ± 0.30	36.60 ± 0.83	36.28 ± 0.73
18	1.78 ± 0.02	2.11 ± 0.01	1.24 ± 0.02	1.78 ± 0.02	5.56 ± 1.89	3.59 ± 0.15	2.60 ± 0.26	7.36 ± 0.69	39.28 ± 0.19	41.57 ± 0.58	35.96 ± 0.54	36.89 ± 0.20
21	1.70 ± 0.02	2.04 ± 0.01	1.32 ± 0.03	1.70 ± 0.02	0.82 ± 0.17	3.66 ± 0.11	2.25 ± 1.06	7.65 ± 0.26	39.60 ± 0.18	42.78 ± 0.28	34.48 ± 0.60	37.27 ± 0.55
25	1.58 ± 0.03	2.00 ± 0.02	1.35 ± 0.01	1.58 ± 0.03	0.95 ± 0.11	4.28 ± 0.30	2.29 ± 1.03	6.94 ± 0.22	42.93 ± 0.37	40.85 ± 0.70	33.77 ± 0.45	37.32 ± 0.73
27	1.29 ± 0.09	1.96 ± 0.01	1.29 ± 0.00	1.29 ± 0.09	2.95 ± 0.85	5.55 ± 0.23	0.74 ± 0.08	6.58 ± 0.35	42.31 ± 0.45	41.65 ± 0.66	34.29 ± 0.24	36.39 ± 1.15
29	1.24 ± 0.02	1.92 ± 0.01	1.07 ± 0.02	1.24 ± 0.02	0.74 ± 0.07	2.46 ± 0.89	1.86 ± 0.94	7.52 ± 0.17	41.51 ± 0.33	39.26 ± 0.73	34.78 ± 0.31	35.86 ± 1.03
33	1.06 ± 0.11	1.79 ± 0.02	0.93 ± 0.17	1.06 ± 0.11	1.04 ± 0.08	4.08 ± 0.22	1.79 ± 0.94	5.01 ± 1.05	41.07 ± 0.71	41.72 ± 0.76	34.56 ± 0.47	35.08 ± 0.23
36	1.02 ± 0.02	1.54 ± 0.08	0.72 ± 0.04	1.02 ± 0.02	0.94 ± 0.06	4.63 ± 0.98	3.52 ± 0.10	9.98 ± 0.83	40.31 ± 2.23	39.32 ± 1.11	34.41 ± 0.38	35.37 ± 0.74

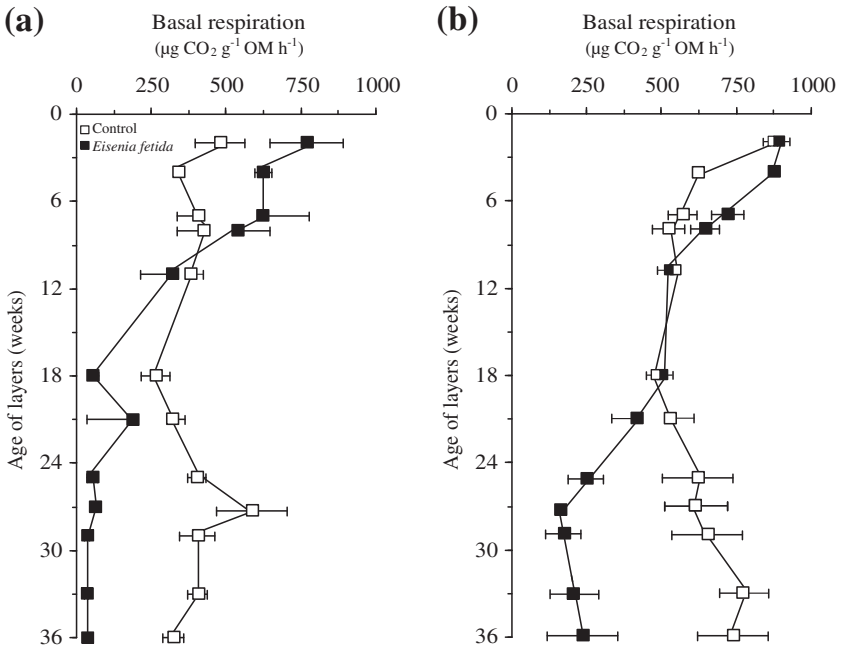

Fig. 5. Microbial activity measured as basal respiration in layers of reactors fed with doses of 1.5 kg (a) and 3 kg of pig slurry (b) with *E. fetida* (filled symbols) and without *E. fetida* (open symbols). Variable values (means ± SE) corresponding to the age of the layers of pig slurry are shown on the y axis.

after vermicomposting and composting with subsequent vermicomposting of cattle manure in the presence of the earthworm species *E. andrei*.

Previous studies of the effects of epigeic earthworms on microorganisms have shown that the passage of microorganisms through the earthworm gut may result in a smaller but metabolically more active microbial population (Zhang et al. 2000; Haynes et al. 2003; Pramanik et al., 2009). In this sense, we found that the activity of earthworms increased the ratio of monounsaturated to saturated PLFAs (with respect to the control) to a greater extent in the reactors fed with high dose of pig slurry (1.7 times; Table 1) than in those fed with the low dose (1.1 times; Table 1), resulting in a significant interaction between earthworm presence and rate of application (ANOVAR $F_{1,8} = 21.11$, $P < 0.01$). The higher values of monounsaturated to saturated PLFA ratio found in the younger layers of the reactors with earthworms indicate that microbial communities in these reactors may have been less resource-limited than the microorganisms in reactors without earthworms. This may be attributed to the mobilization of the labile carbon pool in the presence of earthworms, probably due to breakdown of cellulose and hemicellulose through the action of endosymbiotic microbes that reside in the earthworm gut (Domínguez et al., 2010) and/or to the production of mucus and excretory substances such as urea and ammonia, which constitute a readily assimilable pool of nutrients for microorganisms (Brown and Doube, 2004). Other physical modifications of the substrate caused by the digging activities of earthworms, such as aeration and homogenization of the substrate also favour microbial activity and further decomposition (Domínguez et al., 2004). As a consequence, the vermicomposting system functioned much better, as shown by the great loss of C as a result of earthworm activity, relative to the control (Table 1, ANOVAR $F_{1,8} = 317.75$, $P < 0.0001$). As we found with bacterial PLFA biomarkers, this effect was more pronounced in low dose reactors than in the high dose ones (1.2 and 0.9 times lower), resulting in a significant interaction between earthworm presence and the rate of application (ANOVAR $F_{1,8} = 21.18$, $P < 0.01$). In addition, the vermicomposting process was characterized by a significant loss of carbon through the

increasing age of layers of reactors with earthworms (ANOVAR $F_{11,88} = 10.96$, $P < 0.0001$), in which the total C content was 1.2 times lower in the older layers than in the younger layers (Table 1) .Similarly, Triphati and Bhardwaj (2004) also observed a decrease in the total C content in vermicomposting experiments with the earthworm species *E. fetida* and *Lampito mauritii* fed with slurries and mixtures of organic residues, respectively. These results are consistent with the general hypothesis that earthworms accelerate the rate of decomposition of organic matter during vermicomposting (Atiyeh et al., 2000; Domínguez et al., 2003; Aira et al., 2006; Aira and Domínguez, 2008; Domínguez et al., 2010). Moreover, the mineralization of C is generally enhanced in earthworm casts, and decreases with ageing (Aira et al., 2005). The present data are consistent with these findings, since in young layers more casting would occur than in the older layers where ageing of the casts is the predominant process. Contrary to the present findings, other authors have reported a decrease (Aira et al., 2006; Gómez-Brandón et al., 2010b) or no changes (Aira and Domínguez, 2009) in microbial activity measured as basal respiration in short-term experiments with epigeic earthworm species. Indeed, Aira et al. (2006) observed a reduction in microbial activity in the casts of *Eudrilus eugeniae* fed with pig manure, whereas in a later study, Aira and Domínguez (2009) did not detect any changes in this parameter in the presence of *E. fetida*. However, in the latter study, the authors observed a reduction in microbial activity when *E. fetida* was fed on cow manure rather than pig slurry. The contrasting results in microbial activity in the presence of earthworms may be related to the duration of the experiments, and to the quality and complexity of the food source ingested (Flegel and Schrader, 2000). Such differences in microbial activity may also be attributable to the earthworm species involved due to variations in the morphological and physiological characteristics of the intestinal tracts (Brown and Doube, 2004). Indeed, the gut transit time and the gut enzymatic array have been shown to differ depending on the earthworm species (McLean et al., 2006).

4. Conclusions

Although microbial biomass and diversity were reduced, there was an increase in microbial activity and, in turn in carbon mineralization with the presence of earthworms. These findings may have important implications for the optimization of the vermicomposting process because low values of microbial biomass that are indicative of stabilized materials were reached after two weeks of vermicomposting and were maintained until the end of the process. However, a period of between 18 and 21 weeks was needed to achieve a more stabilized substrate in relation to the microbial activity in low and high dose reactors respectively.

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