Original Article

wm&r

Waste Management & Research
31(8) 859–868
© The Author(s) 2013
Reprints and permissions:
sagepub.co.uk/journalsPermissions.nav
DOI: 10.1177/0734242X13497079
wmr.sagepub.com
SAGE

The influence of earthworms on nutrient dynamics during the process of vermicomposting

Jorge Domínguez[1] **and María Gómez-Brandón**[2]

Abstract

In the present study the potential of the earthworm *Eisenia andrei* to modify chemical and microbiological properties, with a special focus on the nutrient content of fresh organic matter, was evaluated during 16 weeks of vermicomposting of cattle manure and sewage sludge. Samples were periodically collected in order to determine the changes in inorganic nitrogen (N), in total microbial biomass and activity, as well as in the total and available content of macro- and micronutrients. An optimal moisture level, ranging from 75% to 88%, was maintained throughout the process. The content of organic matter decreased over time, but no changes were found in this parameter as a result of earthworm activity. The carbon/N ratio rapidly decreased, but only in the manure, reflecting rapid decomposition and mineralisation of the organic matter by the earthworms. An increase in N mineralisation was also attributable to the presence of earthworms, although in the manure this effect was hardly detectable before the eighth week of vermicomposting. Earthworm activity also enhanced the total content of potassium, calcium and iron together with an increase in the availability of phosphorus and zinc. We did not detect a significant earthworm effect on microbial respiration, but their activity increased greatly microbial biomass nitrogen in sewage sludge.

Keywords

Eisenia andrei, cattle manure, sewage sludge, nitrification, microbial activity, nutrient availability

Introduction

The European Waste Framework Directive (Directive 2008/98/EC, 2008) has highlighted several activities that include prevention, preparing for re-use, recycling and disposal so as to strengthen Europe's waste management policy, as well as to minimise the quantity of waste produced and landfilled. The use of appropriate management technologies could thus mitigate the health and environmental risks associated with the overproduction of organic wastes by stabilising them before their use or disposal. In this context, vermicomposting has progressed considerably in recent years, primarily owing to its low cost and the large amount of organic wastes that can be processed (Domínguez and Edwards, 2010a). Organic wastes from sewage sludge, paper industry waste, urban residues, food and animal waste, and horticultural residues from cultivars have been successfully managed by vermicomposting, producing vermicomposts that contain nutrients that are readily taken up by plants (Domínguez et al., 1997 Domínguez, 2004).

Vermicomposting is defined as a bio-oxidative process in which detritivorous earthworms interact with microorganisms and other fauna within the decomposer community, accelerating the stabilisation of organic matter (OM), and greatly modifying its physical and biochemical properties (Domínguez, 2004). The biochemical decomposition of OM is primarily accomplished by the microbes; however, earthworms are the crucial drivers of the process by fragmenting and conditioning the substrate, and by increasing the surface area of OM available for microbial attack after comminution (Domínguez et al., 2010). Essentially, epigeic earthworms directly affect the decomposition of OM through gut-associated processes, via the effects of ingestion, digestion and assimilation of the OM and microorganisms in the gut and casting (Aira et al., 2009; Gómez-Brandón et al., 2011a). Selective effects on microbial communities have been found as a result of gut transit. For instance, Monroy et al. (2009) observed a 98% reduction in the density of total coliforms after the passage of pig slurry through the gut of the epigeic earthworm *Eisenia fetida*. The reduction in total coliform numbers was not related to decreases in bacterial biomass carbon (C), which indicates a specific negative effect of the earthworms on this bacterial group. This fact may alter decomposition pathways during vermicomposting, probably by modifying the composition of the microbial communities involved in decomposition. Indeed, microbes from the gut are then released in faecal material in which the decomposition of the egested OM is continued by the microbes. The

---

[1] Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, Vigo, Spain
[2] Institute of Microbiology, University of Innsbruck, Innsbruck, Austria

Corresponding author:
María Gómez-Brandón, Institute of Microbiology, Technikerstrasse 25d, University of Innsbruck, 6020 Innsbruck, Austria.
Email: maria.gomez-brandon@uibk.ac.at

inputs of those communities to fresh OM is also expected to alter the activity levels of microbial communities and modify the functional diversity of microbial populations in vermicomposting systems (Aira and Domínguez, 2011). Moreover, the nutrient content of the egested material differs from the ingested material (Aira et al., 2008), and this fact may be used to better exploit resources because of the presence of a pool of bioavailable compounds in the earthworm casts. These newly-deposited casts are usually rich in ammonium-nitrogen (N) and partially-digested OM, providing a good substrate for microbial growth (Brown and Doube, 2004). Furthermore, cast-associated processes (indirect effects) that are more closely related to the presence of unworked material and to the physical modification of the egested material take place during the vermicomposting process (Gómez-Brandón and Domínguez, 2013). Such indirect effects include processes, such as the ageing of earthworm-inhabited material and the mixing of such material with substrates that have yet to be processed by the earthworms (Aira and Domínguez, 2011). As such, all of the above points out that gut- and cast-associated processes are expected to largely determine the characteristics of vermicomposts, influencing either their nutrient content or microbial community composition.

Eisenia andrei is one of the most widely used earthworm species in vermicomposting systems (Aira et al., 2011; Domínguez et al., 2010; Fernández-Gómez et al., 2010; Gómez-Brandón et al., 2012; Lazcano et al., 2008; Yadav and Garg, 2011), mainly owing to its high rate of consumption, digestion and assimilation of OM, 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). To date, most studies dealing with the role of this earthworm species in waste management have focused on the changes before and after vermicomposting (Gómez-Brandón et al., 2012; Lazcano et al., 2008; Vivas et al., 2009), rather than those that occur throughout the process. The objective of the present study was therefore to investigate whether, and to what extent, the epigeic earthworm species *E. andrei* is capable of altering the chemical and microbiological properties of fresh OM during the vermicomposting process. The increasing rate at which animal manures and sewage sludge are produced requires strategies for their disposal and/or management. For instance, more than 1500 million tonnes of manure are produced yearly in the 27 European Union countries (Faostat, 2003), as reported by Holm-Nielsen et al. (2009), which need to be efficiently recycled. As such, we evaluated the efficiency of the earthworm *E. andrei* in processing this type of waste in order to determine if a higher degree of stabilisation is reached throughout the process.

Materials and methods

Experimental material and set-up

Fresh cattle manure was collected from the Waterman Dairy Farm at the Ohio State University, OH, USA, and the sewage sludge was obtained from the Wastewater Treatment Plant of Columbus, OH, USA. The main properties of both organic wastes are shown in Table 1. Individuals of the earthworm species *E. andrei* were obtained from a stock culture reared in the laboratory.

Table 1. Main chemical properties of the organic waste used in the present study. All data are expressed as an oven-dried weight basis.

Parameters	Cattle manure	Sewage sludge
Moisture (%)	77 ± 1.6	85 ± 1.7
Organic matter (%)	85 ± 0.51	79 ± 0.49
pH	6.73	7.23
EC (mS cm^{-1})	0.65	0.80
Total C (%)	41 ± 1	38 ± 2
Total N (%)	2.3 ± 0.15	6.3 ± 0.04
C/N ratio	17.8 ± 0.09	5.98 ± 0.04
NH_4^+ (μg g^{-1})	360 ± 15	220 ± 18
Potassium (mg g^{-1})	22.1 ± 0.2	9.5 ± 0.2
Sodium (mg g^{-1})	11.4 ± 0.1	10.5 ± 0.2
Magnesium (mg g^{-1})	5.4 ± 0.2	3.2 ± 0.1
Calcium (mg g^{-1})	52.3 ± 4.2	39.7 ± 0.7
Phosphorus (mg g^{-1})	19.6 ± 0.8	65.3 ± 1.8
Iron (μg g^{-1})	6.4 ± 0.3	21.8 ± 0.8
Manganese (μg g^{-1})	363 ± 5	575 ± 8
Zinc (μg g^{-1})	592 ± 45	3230 ± 112

EC: electrical conductivity; C: carbon; N: nitrogen; NH_4^+: ammonium.

For the experimental set-up, five containers (2 l) were filled with 500 g cattle manure, and another five with the same amount of sewage sludge. Each of the containers was inoculated with 25 juvenile individuals of the earthworm species *E. andrei* [ca. 65 g fresh weight (fw)]. The control treatment consisted of each type of substrate incubated without earthworms (n = 5). All the containers were covered with perforated lids and maintained in the laboratory in an incubation chamber at 24 ± 2°C for 4 months. A sample (15 g fw) was collected from each container after 1, 3, 6, 9, 13 and 16 weeks. All the samples were gently mixed and several parameters were determined, as detailed below.

Analytical procedures

The moisture content of the samples was determined after drying at 60°C for 3 d, and the OM content after heating at 550°C for 4 h. Inorganic N [ammonium (NH_4^+) and nitrate (NO_3^-)] was determined in 0.5 M potassium sulphate extracts (1:5, w/v) using a modified indophenol blue technique (Sims et al., 1995) with a Bio-Rad Microplate Reader 550. Total extractable N was determined after oxidation with potassium persulphate, as described by Cabrera and Beare (1993), and the dissolved organic nitrogen (DON) content was calculated as the difference between the total extractable N reading and the combined NH_4^+ and NO_3^- reading. The total nutrient content, including sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P), iron (Fe), manganese (Mn) and zinc (Zn) was measured by atomic absorption spectrophotometry (Varian SpectrAA-250 Plus, Varian Inc. Walnut Creek, CA, USA). The content of available nutrients was determined in NH_4^+ bicarbonate-diethylenetriaminepentaacetic acid extracts of the samples (1:2, w/v) by atomic absorption spectrometry (Soltanpour and Schwab, 1977). The total content of carbohydrates was quantitated using the anthrone–sulphuric acid method with modifications as described by Laurentin and Edwards (2003). Microbial biomass-N (N_{mic}) was analysed from 1 g of sample (fw) by the chloroform fumigation-extraction method (Brookes et al., 1985). Microbial activity was determined by measuring the rate of carbon dioxide (CO_2) evolution from 2 g of sample (fw) after 24 h of incubation. The evolved CO_2 was trapped in 0.02 M sodium hydroxide and subsequently measured by titration with hydrochloric acid to a phenolphthalein endpoint, after adding excess barium chloride (Anderson, 1982).

Statistical analyses

Data were analysed by repeated measures analysis of variance in which type of waste (cattle manure and sewage sludge) and earthworm treatment (presence and absence) were fixed as the between-subject factors, and the sampling time (0–16 weeks) was fixed as a within-subject factor (Potvin et al., 1990). All variables fulfilled the sphericity assumption (Mauchly's test). The statistical analyses were performed using SPSS software (SPSS, Chicago, IL, USA).

Results and discussion

In our study, moisture content increased over time in the cattle manure reaching a final value close to 84% (Figure 1a), whereas it remained constant in the sewage sludge (around 87%; Figure 1b), irrespective of the activity of the earthworms, leading to a significant interaction between time and waste ($F_{4,64} = 31.82$, $P < 0.0001$). This indicates that the moisture content was not a limiting factor for *E. andrei* to process both the cattle manure and sewage sludge throughout the vermicomposting process. The earthworm *E. andrei* has been found to be very competitive during the vermicomposting process, mainly because this earthworm species is relatively tolerant to the environmental conditions of organic wastes (Domínguez and Edwards, 2010b). However, it should be noted that epigeic earthworms have well-defined tolerance limits to several parameters, such as moisture and temperature in general. As such, wastes are processed much more efficiently within a relatively narrow range of favourable chemical and environmental conditions (Domínguez and Edwards, 2010a, 2010b). Specifically, the earthworm *E. andrei* can grow more rapidly when a value of moisture close to 80% is reached in the waste (Domínguez and Edwards, 1997). The OM content in both residues decreased with time ($F_{1,16} = 88.21$, $P < 0.0001$), from 85% to 77% in the cattle manure, and from 79% to 72% in the sewage sludge (Figure 1c, d); however, earthworm activity did not significantly affect the percentage of OM content irrespective of the type of organic waste ($F = 1.53$, $P = 0.15$; Figure 1c, d).

The C/N ratio progressively decreased in the cattle manure from 17 to 13 (Figure 1e), while it remained hardly unaltered (around 6) in the sewage sludge (Figure 1f), thereby resulting in a significant interaction between time and type of waste ($F_{4,64} = 35.44$, $P < 0.0001$). In cattle manure, the total C content ranged from an initial value of 41.41% ± 0.29 to 39.18% ± 0.18 and 38.10% ± 0.37 in control and earthworm treatments after 16 weeks of vermicomposting. There was also a constant and quick depletion of total C in the sewage sludge from 37.92% ± 0.31 at the beginning of the process to 34.32% ± 0.38 and 32.81% ± 0.14 at the end of the process in the absence and presence of earthworms respectively. The N content followed a different trend in both wastes throughout the process. Sewage sludge had an initial N content higher (6.34% ± 0.19) than that in cattle manure (2.33% ± 0.15). A slight increase in this parameter was observed in cattle manure after 16 weeks of vermicomposting, reaching a value of 2.85% in both earthworm treatments, whereas in the sewage sludge a reduction in N content was recorded at the end of process (around 5.21% ± 0.07 and 5.65% ± 0.07 without and with earthworms respectively), although such differences were not statistically significant.

The levels of NH_4^+ throughout the vermicomposting process clearly differed between both wastes ($F_{1,16} = 13.61$, $P < 0.01$; Figure 2a, b). A drastic drop was recorded in the cattle manure (Figure 2a), whereas an increase was observed in the sewage sludge over time (Figure 2b), producing a significant interaction between type of waste and time ($F_{4,64} = 39.11$, $P < 0.0001$). The content of NH_4^+ present in the manure was lost throughout the process, most likely through nitrification; in fact, an increase in NO_3^- concentration was recorded in this waste after 8 weeks of vermicomposting (Figure 2c). This points out that a higher degree of stabilisation was recorded in the manure at the end of the process, as nitrification has been widely used as an indicator of stability in either composting or vermicomposting systems (Domínguez and Edwards, 2010a; Insam and de Bertoldi, 2007). However, no such a decrease in NH_4^+ content was recorded in the sludge throughout the process (Figure 2b), and, additionally, the levels of NH_4^+ were much higher in the presence of earthworms than in the control treatment during the whole process ($F_{4,64} = 13.65$, $P < 0.01$; Figure 2b). This could be owing to the fact that there was evidence of earthworm mortality on the mesocosms containing sewage sludge.

Overall, epigeic earthworms have a great impact on N transformations during vermicomposting by modifying the environmental conditions and/or through their interactions with microbial communities (Domínguez et al., 2010). They have been shown to enhance N mineralisation, thereby producing conditions in the organic wastes that favour nitrification, resulting in the rapid conversion of NH_4^+-N into NO_3^- (Aira and Domínguez, 2009; Aira et al., 2008; Atiyeh et al., 2000; Lazcano et al., 2008). The present data are consistent with these findings, as a higher NO_3^- content was found with the presence of earthworms (Figure 2c, d); this effect was more pronounced in the sewage sludge (Figure 2d), thereby producing a significant interaction between earthworms and type of waste ($F_{4,64} = 2.65$, $P < 0.0001$). More specifically, Aira et al. (2008) observed a strong and linear

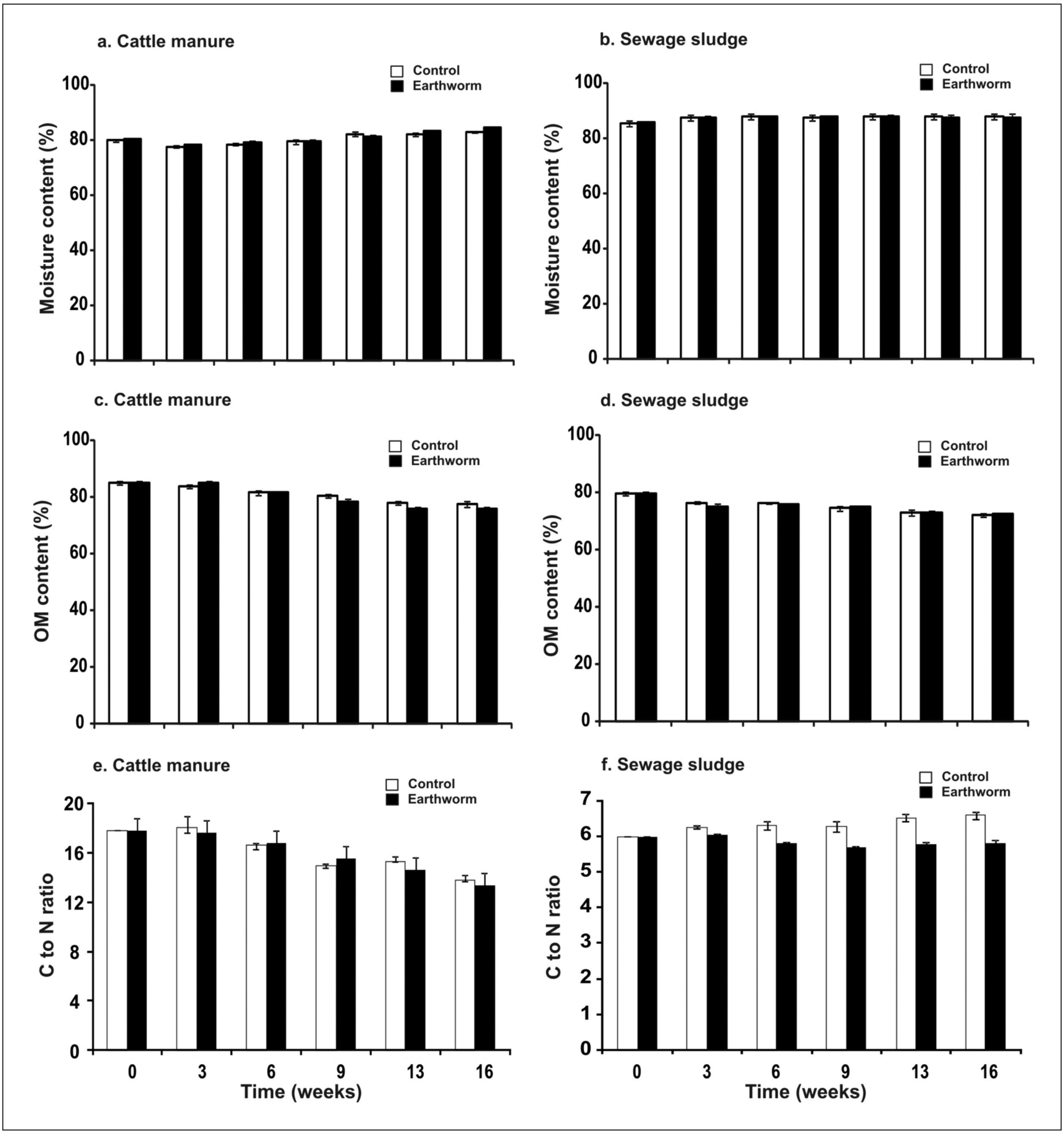

Figure 1. Changes in the moisture content, organic matter (OM) content and carbon (C) to nitrogen (N) ratio of cattle manure and sewage sludge throughout the process of vermicomposting in the presence of the earthworm *Eisenia andrei*. Controls are the organic wastes incubated without earthworms. Values are means ± SE (n = 5).

dependent response of the N mineralisation to the earthworm density in a short-term experiment (72 h) in the presence of *E. fetida*. They found that at the highest earthworm density (100 earthworms per mesocosm), the mineral N content was 1.15 times higher than the control. This indicates that the earthworm gut-associated processes, that is the ingestion, digestion and assimilation of the organic material in the gut and then casting, play a crucial role in substrate mineralisation. In fact, Aira and Domínguez (2009) detected greater levels of NO_3^- in fresh casts of *E. fetida* than in the parent material; this effect was dependent on the type of substrate. N mineralisation is regulated by the availability of DON and NH_4^+, the activity of microorganisms and their relative requirements for C and N (Bardgett, 2005). In the present study, the DON content was higher in the presence of earthworms than the control treatment (Figure 2e, f), although these effects were dependent on time ($F_{4,64} = 4.38$, $P < 0.01$).

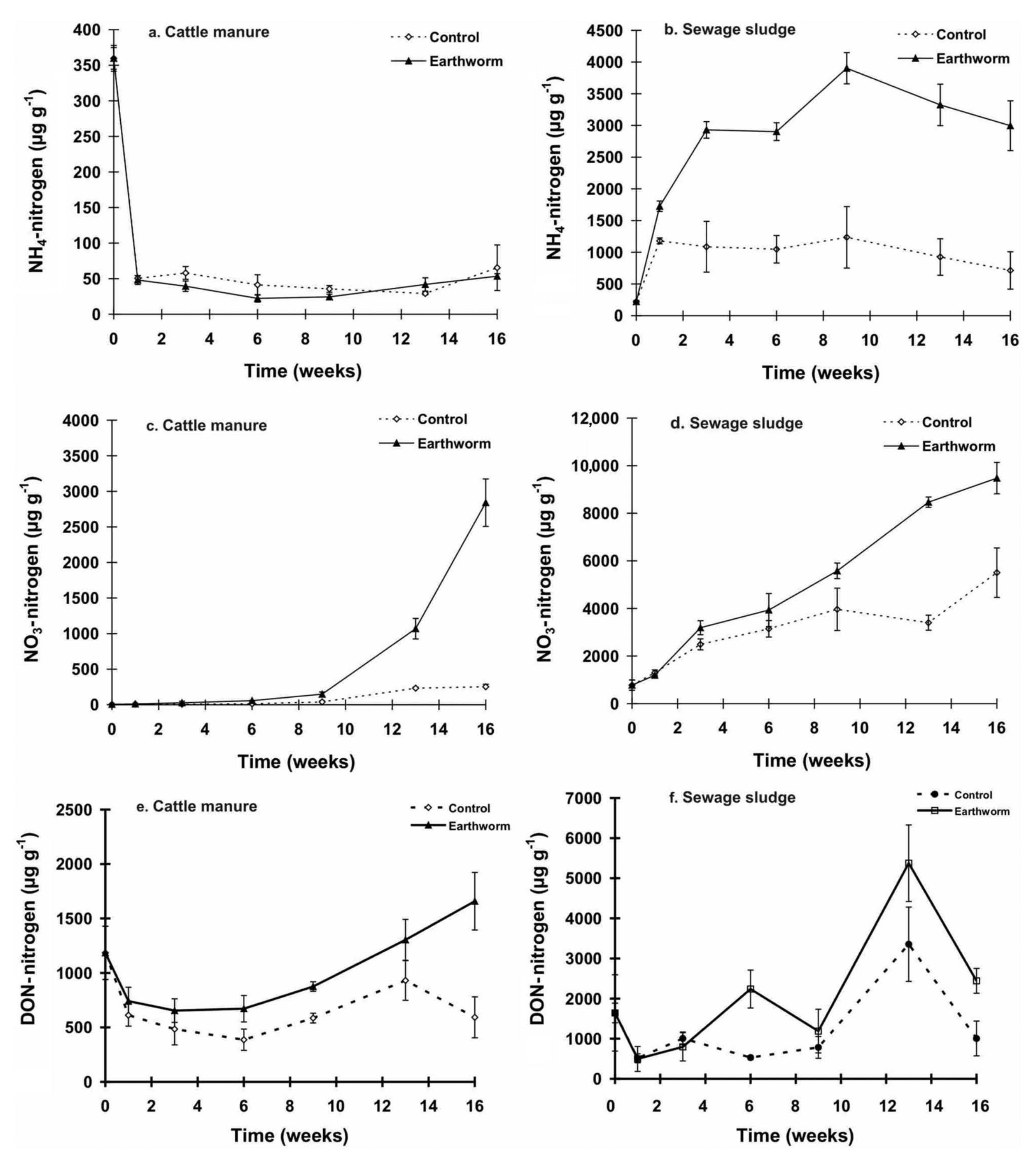

Figure 2. Changes in the ammonia-nitrogen (N), nitrate-N and dissolved organic nitrogen content of cattle manure and sewage sludge throughout the process of vermicomposting in the presence of the earthworm *Eisenia andrei*. Controls are the organic wastes incubated without earthworms. Values are means ± SE (n = 5). NH4+: ammonium; DON: dissolved organic nitrogen.

The total K content increased significantly with earthworm activity ($F_{1,16} = 444.06$, $P < 0.0001$), and its content was dependent on the type of waste ($F_{1,16} = 15.96$, $P < 0.01$) being up to 2.5 times higher in the cattle manure than in the sewage sludge (Table 2). No changes were found in this parameter over time (Table 2). Accordingly, previous studies have also shown a higher content of total K in the presence of earthworms in vermicomposting trials performed with either sewage sludge or cattle manure (Suthar, 2008; Yadav and Garg, 2009, 2011). The levels of available K varied greatly depending on the time (Table 3; $F_{4,64} = 22.77$, $P < 0.0001$). Moreover, these levels were slightly lower in the sewage sludge than in the manure ($F_{1,16} = 159.51$, $P < 0.0001$; Table 3). Earthworms significantly decreased the total Na content ($F_{1,16} = 7.19$, $P < 0.05$; Table 2), and its level was lower in the sewage sludge than in the manure ($F_{1,16} = 66.56$, $P<0.0001$; Table 2). As occurred with total K, no changes were

Table 2. Changes in the total nutrient content of cattle manure and sewage sludge throughout the process of vermicomposting in the presence of *Eisenia andrei*. Controls are the organic wastes incubated without earthworms. Values are means ± SE (n = 5). All data are expressed on an oven-dried weight basis.

		Time (weeks)					
		0	3	6	9	13	16
Manure							
Potassium (mg g^{-1})	Control	22.1 ± 0.2	20.2 ± 0.8	22.3 ± 0.3	23.7 ± 0.6	23.5 ± 0.8	23.0 ± 0.6
	Earthworm	22.1 ± 0.2	22.6 ± 0.3	22.8 ± 0.3	23.2 ± 0.5	23.7 ± 1.1	24.9 ± 0.9
Sodium (mg g^{-1})	Control	11.4 ± 0.1	12.2 ± 0.3	11.7 ± 0.2	11.8 ± 0.4	11.5 ± 0.4	13.9 ± 0.8
	Earthworm	11.4 ± 0.1	11.8 ± 0.1	11.9 ± 0.3	12.1 ± 0.3	11.1 ± 0.2	11.4 ± 0.1
Magnesium (mg g^{-1})	Control	5.4 ± 0.2	6.1 ± 1.1	5.7 ± 0.4	6.1 ± 0.2	5.2 ± 0.6	5.9 ± 0.5
	Earthworm	5.4 ± 0.2	4.7 ± 0.2	5.9 ± 0.2	7.1 ± 0.2	8.3 ± 0.1	8.3 ± 0.2
Calcium (mg g^{-1})	Control	52.3 ± 4.2	57.5 ± 5.1	57.8 ± 0.6	62.1 ± 2.5	57.2 ± 3.4	56.6 ± 1.7
	Earthworm	52.3 ± 4.2	51.8 ± 1.7	58.6 ± 0.9	63.8 ± 0.7	66.8 ± 1.2	68.5 ± 1.6
Phosphorus (mg g^{-1})	Control	19.6 ± 0.8	17.7 ± 1.1	21.1 ± 1.0	22.7 ± 0.7	21.8 ± 2.4	20.7 ± 0.6
	Earthworm	19.6 ± 0.8	18.3 ± 0.8	21.1 ± 0.5	23.5 ± 0.7	25.8 ± 0.6	26.9 ± 1.5
Iron (µg g^{-1})	Control	6.4 ± 0.3	6.7 ± 0.3	7.8 ± 0.2	7.6 ± 0.2	7.5 ± 0.7	7.6 ± 0.3
	Earthworm	6.4 ± 0.3	6.8 ± 0.3	7.7 ± 0.3	8.1 ± 0.2	8.6 ± 0.2	9.0 ± 0.1
Manganese (µg g^{-1})	Control	363 ± 5	362 ± 6	370 ± 9	360 ± 6	391 ± 13	387 ± 21
	Earthworm	363 ± 5	372 ± 5	364 ± 7	367 ± 5	368 ± 7	381 ± 2
Zinc (µg g^{-1})	Control	592 ± 45	568 ± 50	673 ± 33	703 ± 27	731 ± 48	716 ± 28
	Earthworm	592 ± 45	649 ± 33	651 ± 10	749 ± 28	766 ± 27	822 ± 22
Sewage sludge							
Potassium (mg g^{-1})	Control	9.5 ± 0.2	9.2 ± 0.5	9.1 ± 0.3	9.6 ± 0.4	9.9 ± 0.4	10.3 ± 0.4
	Earthworm	9.5 ± 0.2	9.8 ± 0.3	9.7 ± 0.3	10.1 ± 0.4	11.5 ± 0.5	10.9 ± 0.4
Sodium (mg g^{-1})	Control	10.5 ± 0.2	10.8 ± 0.3	11.3 ± 0.2	10.8 ± 0.1	10.6 ± 0.2	10.7 ± 0.2
	Earthworm	10.5 ± 0.2	11.0 ± 0.3	10.6 ± 0.2	10.6 ± 0.3	10.1 ± 0.1	10.3 ± 0.1
Magnesium (mg g^{-1})	Control	3.2 ± 0.1	3.6 ± 0.2	3.9 ± 0.6	3.8 ± 0.2	3.7 ± 0.1	3.5 ± 0.2
	Earthworm	3.2 ± 0.1	3.7 ± 0.1	3.5 ± 0.2	4.2 ± 0.3	4.1 ± 0.2	4.4 ± 0.2
Calcium (mg g^{-1})	Control	39.7 ± 0.7	43.6 ± 0.5	45.4 ± 3.8	40.8 ± 1.4	41.2 ± 1.4	40.0 ± 1.0
	Earthworm	39.6 ± 0.7	41.3 ± 0.4	39.6 ± 1.1	40.7 ± 2.0	42.1 ± 1.3	40.0 ± 0.7
Phosphorus (mg g^{-1})	Control	65.3 ± 1.8	67.9 ± 1.2	70.3 ± 0.4	70.7 ± 0.2	72.4 ± 0.6	69.6 ± 1.4
	Earthworm	65.3 ± 1.8	66.2 ± 1.5	68.5 ± 1.9	70.2 ± 1.4	70.9 ± 1.3	72.6 ± 0.6
Iron (µg g^{-1})	Control	21.8 ± 0.8	20.9 ± 0.6	18.7 ± 3.4	22.7 ± 0.1	23.7 ± 0.2	20.0 ± 1.3
	Earthworm	21.8 ± 0.8	22.6 ± 0.2	22.8 ± 0.2	23.6 ± 0.2	21.7 ± 0.2	22.9 ± 0.7
Manganese (µg g^{-1})	Control	575 ± 8	568 ± 6	529 ± 46	584 ± 1	596 ± 3	565 ± 11
	Earthworm	575 ± 8	583 ± 2	584 ± 3	592 ± 1	579 ± 5	591 ± 7
Zinc (µg g^{-1})	Control	3230 ± 112	3128 ± 188	3370 ± 122	3280 ± 180	3610 ± 280	2640 ± 230
	Earthworm	3230 ± 108	3100 ± 168	3050 ± 122	3172 ± 134	2970 ± 130	2770 ± 140

observed in the total Na content with the duration of vermicomposting (Table 2). Overall, available Na content decreased significantly over time ($F_{4,64} = 19.25$, $P < 0.0001$; Table 3), and its content was higher in the sewage sludge than in the manure ($F_{1,16} = 9.01$, $P < 0.01$; Table 3). Specifically, a reduction was found over time in the sewage sludge (Table 3), whereas in the manure there was first an increase, which was then followed by a decrease [time × waste interaction ($F_{4,64} = 3.74$, $P < 0.01$)]. Total Mg content was dependent on the type of waste ($F_{1,16} = 4.80$, $P < 0.01$). The level of Mg was higher in the manure than in the sewage sludge (Table 2). A similar pattern was recorded for total Ca content ($F_{1,16} = 112.38$, $P < 0.0001$). Specifically, the presence of earthworms increased its content over time (Table 2), but this effect was only observed in the manure, producing a significant interaction between time and waste ($F_{4,64} = 3.58$, $P < 0.05$). In line with this, Yadav and Garg (2011) observed that vermicomposts obtained from different feedstocks showed a higher total Ca content than the parent wastes (including cattle dung, poultry droppings and food industry sludge) in a laboratory-scale trial in the presence of *E. fetida*. Similar results were found by Garg and Kaushik (2005) during vermicomposting of textile mill sludge spiked with poultry droppings.

Sewage sludge had a higher P content than the cattle manure ($F_{1,16} = 5410.50$, $P < 0.0001$; Table 2). Overall, the total P content increased significantly throughout the process of vermicomposting ($F_{4,64} = 13.98$, $P < 0.0001$; Table 2). Moreover, earthworm activity led to an increase of the available P ($F_{1,16} = 12.65$, $P < 0.01$; Table 3), which was also higher in the sewage sludge than in the manure ($F_{1,16} = 668.56$, $P < 0.0001$). The available P showed an opposite trend in both wastes over time

Table 3. Changes in the available nutrient content of cattle manure and sewage sludge throughout the process of vermicomposting in the presence of *Eisenia andrei*. Controls are the organic wastes incubated without earthworms. Values are means ± SE (n = 5). All data are expressed on an oven-dried weight basis.

		Time (weeks)					
		0	3	6	9	13	16
Manure							
Potassium (mg l^{-1})	Control	335 ± 30	505 ± 48	611 ± 43	594 ± 66	315 ± 29	339 ± 35
	Earthworm	335 ± 30	418 ± 25	514 ± 40	473 ± 46	347 ± 29	263 ± 19
Sodium (mg l^{-1})	Control	63 ± 10	72 ± 10	82 ± 6	83 ± 8	58 ± 10	54 ± 7
	Earthworm	63 ± 10	54 ± 4	70 ± 9	60 ± 6	50 ± 5	35 ± 3
Phosphate (mg l^{-1})	Control	187 ± 12	132 ± 12	119 ± 13	104 ± 9	174 ± 08	111 ± 11
	Earthworm	187 ± 12	143 ± 9	116 ± 12	132 ± 7	183 ± 09	203 ± 10
Iron (mg l^{-1})	Control	8 ± 2	16 ± 2	15 ± 2	11 ± 1	12 ± 2	10 ± 1
	Earthworm	8 ± 2	13 ± 2	12 ± 1	12 ± 2	12 ± 1	10 ± 1
Manganese (mg l^{-1})	Control	11 ± 2	20 ± 3	23 ± 3	20 ± 2	12 ± 1	15 ± 1
	Earthworm	11 ± 2	16 ± 1	19 ± 2	18 ± 2	16 ± 2	10 ± 1
Zinc (mg l^{-1})	Control	4 ± 1	9 ± 1	11 ± 1	10 ± 1	7 ± 1	4 ± 1
	Earthworm	4 ± 1	8 ± 1	9 ± 1	9 ± 1	8 ± 1	4 ± 1
Sewage sludge							
Potassium (mg l^{-1})	Control	331 ± 18	153 ± 31	204 ± 13	255 ± 10	152 ± 19	124 ± 23
	Earthworm	331 ± 18	148 ±17	192 ± 19	267 ± 28	248 ± 28	218 ± 30
Sodium (mg l^{-1})	Control	150 ± 17	61 ± 11	86 ± 4	113 ± 2	62 ± 7	49 ± 2
	Earthworm	150 ± 17	83 ± 23	77 ± 8	111 ± 11	77 ± 6	72 ± 5
Phosphate (mg l^{-1})	Control	187 ± 12	230 ± 29	308 ± 12	264 ± 11	299 ± 6	323 ± 15
	Earthworm	187 ± 12	254 ± 24	294 ± 17	267 ± 21	307 ± 8	368 ± 11
Iron (mg l^{-1})	Control	44 ± 4	32 ± 3	40 ± 1	31 ± 1	34 ± 4	38 ± 2
	Earthworm	44 ± 4	32 ± 4	34 ± 3	34 ± 4	42 ± 2	42 ± 3
Manganese (mg l^{-1})	Control	11 ± 1	7 ± 1	10 ± 1	11 ± 1	7 ± 1	6 ± 1
	Earthworm	11 ± 1	7 ± 1	8 ± 1	11 ± 1	9 ± 1	7 ± 1
Zinc (mg l^{-1})	Control	45 ± 4	26 ± 5	37 ± 2	39 ± 2	26 ± 2	18 ± 2
	Earthworm	45 ± 4	23 ± 3	28 ± 3	41 ± 5	34 ± 4	30 ± 3

(Table 3), with a decrease in the cattle manure and an increase in the sewage sludge [time × waste interaction ($F_{4,64} = 6.41$, $P < 0.0001$)]. An increase in P content as a result of the earthworm activity was reported in earlier studies dealing with the vermicomposting of different wastes such as press mud (Sangwan et al., 2010), cattle dung (Yadav and Garg, 2011) and sludge from distillery industries (Suthar and Singh, 2008; Yadav and Garg, 2009), among others. Le Bayon and Binet (2006) found that earthworms had a great effect on the biogeochemical status of P, as a higher phosphatase activity was recorded in their casts and burrow-linings, ultimately leading to an increase in the availability of P in these two hot spots. However, it should be noted that this previous work was not conducted with epigeic earthworms and, specifically, it focused on the distribution and availability of P in the surface casts and the burrows-linings of the anecic earthworm *Lumbricus terrestris* and the endogeic *Aporrectodea caliginosa* in soil microcosms under laboratory conditions.

Total Fe content increased significantly with earthworm presence ($F_{1,16} = 12.26$, $P < 0.01$; Table 2) and over time ($F_{4,64} = 3.94$, $P < 0.01$; Table 2); moreover, its content was higher in the sewage sludge ($F_{1,16} = 5998.66$, $P < 0.0001$; Table 2). A higher content of available Fe was also observed in the sewage sludge than in the manure ($F_{1,16} = 490.12$, $P < 0.0001$; Table 3). In addition, a different trend in the available Fe was found in both wastes over time (Table 3), with a decrease in sewage sludge and a slight increase in the manure, resulting in a significant interaction between time and waste ($F_{4,64} = 4.40$, $P < 0.01$). Total Mn content was higher in the sewage sludge than in the manure ($F_{1,16} = 1037.50$, $P < 0.0001$; Table 2), and no significant changes were reported for this parameter, neither with time nor with the presence of earthworms (Table 2). Nevertheless, the available Mn content changed over time (Table 3), depending on the type of waste ($F_{4,64} = 3.87$, $P < 0.01$). Total Zn content was significantly higher in the sewage sludge than in the manure ($F_{1,16} = 1880.61$, $P < 0.0001$; Table 2). A reduction in this parameter was found over time in the sewage sludge (Table 2), whereas an increase was observed in the manure (interaction time × waste; $F_{4,64} = 4.71$, $P < 0.01$). Available Zn content was also higher in the sewage than in the manure ($F_{1,16} = 146.14$, $P < 0.0001$; Table 3). Earthworm activity greatly influenced the Zn content (Table 2), although this effect varied with time (time × earthworm interaction, $F_{4,64} = 6.73$, $P < 0.001$); more specifically, the presence of earthworms first led to a decrease that was later followed by an increase in the available Zn content (Table 3). Recent studies based on phospholipid fatty acid profiles

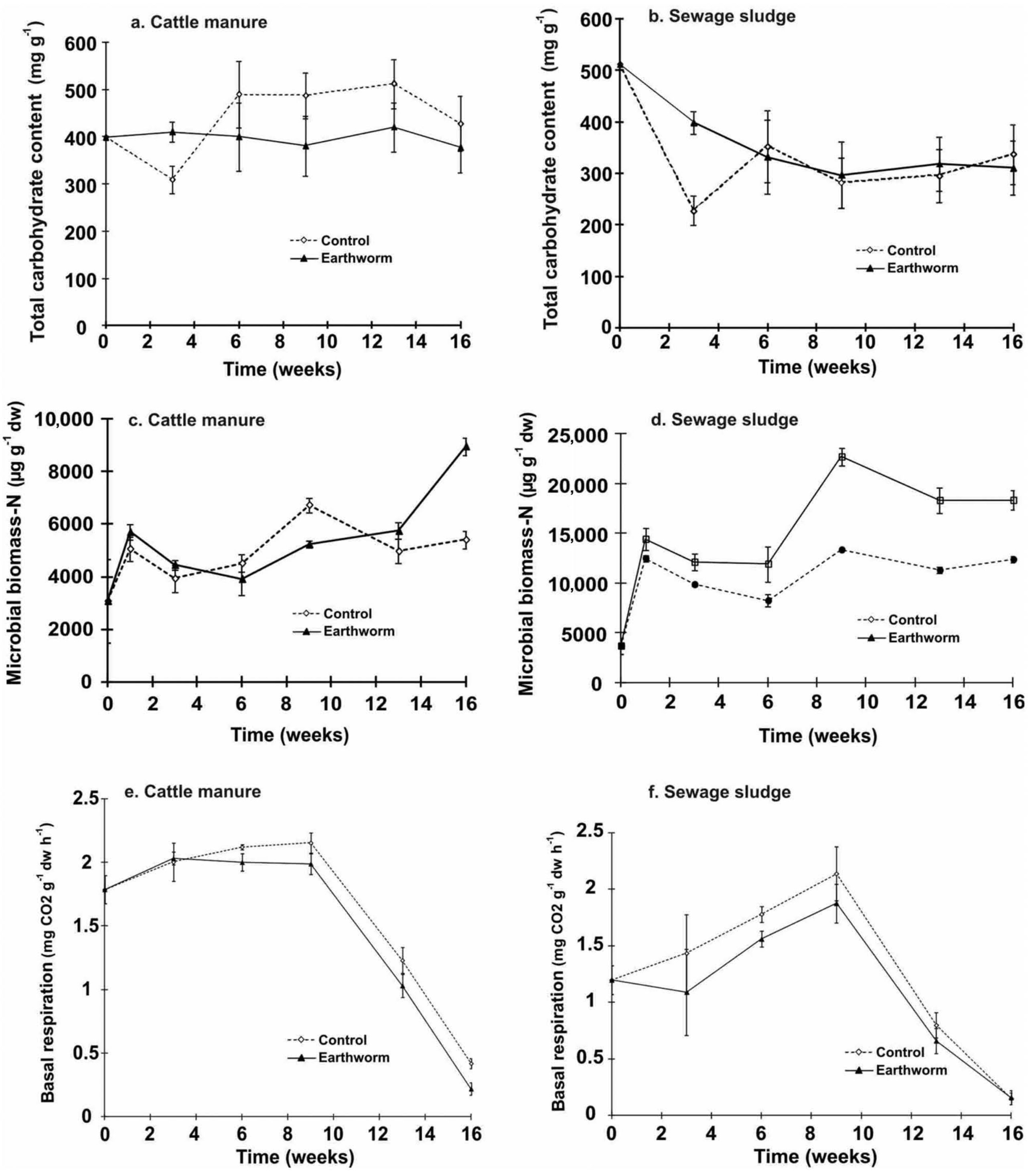

Figure 3. Changes in the total carbohydrate content, nitrogen- microbial biomass, and microbial activity assessed by basal respiration of cattle manure and sewage sludge throughout the process of vermicomposting in the presence of the earthworm Eisenia andrei. Controls are the organic wastes incubated without earthworms. Values are means ± SE (n = 5). CO2: carbon dioxide.

point to biotic interactions between epigeic earthworms and microorganisms through gut-associated processes as important drivers of nutrient cycling, by altering the levels of activity of the microbial communities (Gómez-Brandón et al., 2011a, 2012). These studies highlight the existence of a more active microbial community specialised in metabolising compounds produced or released by the earthworms in the egested materials.

The total carbohydrate content varied significantly over time (Figure 3a,b), and this effect was dependent on the type of organic waste (interaction time × waste; $F_{4,64} = 2.84$, $P < 0.01$). A rapid decrease in this parameter was recorded in cattle manure during the first 2 weeks, but only in the control without earthworms (Figure 3a). Such a reduction was followed by an increase in the carbohydrate content, reaching a value close to 500 mg g^{-1} after 6 weeks of vermicomposting (Figure 3a), whereas no significant changes were found from this point on until week 14, after which a reduction was observed. In contrast, hardly any changes were observed in the presence of *E. andrei* during the vermicomposting of cattle manure (Figure 3a). In sewage sludge, a significant decrease in the carbohydrate content was also detected at the beginning of the process (Figure 3b), and was more pronounced in the control treatment (Figure 3b). This was followed by an increase (Figure 3b), thereby reaching similar levels to those observed in the presence of earthworms (around 350 mg g^{-1}); from there on both treatments remained at a similar level (Figure 3b).

Microbial biomass-N changed substantially over time ($F_{4,64} = 13.71$; $P < 0.0001$; Figure 3c, d). A rapid increase in N_{mic} was observed in cattle manure, either with the presence or the absence of earthworms, during the first week (Figure 3c). The levels of N_{mic} remained more or less constant until week 13 in both treatments with and without earthworms (Figure 3c), whereas from this point on until the end of the experiment there was a pronounced increase in N_{mic} only with the presence of *E. andrei* (Figure 3c), probably owing to some earthworm mortality. However, in sewage sludge, N_{mic} rapidly increased in both earthworm treatments at the beginning of the process (Figure 3d), and then remained more or less constant until week 6. A second peak was then recorded at week 10, regardless of the earthworm treatment, while no major changes over time were detected from this point on (Figure 3d). Furthermore, the activity of the earthworms had a significant effect on N_{mic} during the vermicomposting of sewage sludge ($F_{1,16} = 30.14$, $P = 0.0001$) and, overall, higher levels of N_{mic} were found in the presence of *E. andrei* than in the control treatment until the end of the process (Figure 3c, d). The enhancement in microbial biomass may be partly due to the production of mucus by the earthworms and/or by increasing the surface area available for microbial attack after the comminution of OM (Domínguez et al., 2010). Similarly, Aira et al. (2007) found higher levels of microbial biomass-C with the presence of earthworms (*E. fetida*) than in the control without earthworms, from week 2 to week 18 of vermicomposting. However, from the 21- to 36-week-old layers, these authors reported a lower content of C_{mic} in the earthworm treatment relative to the control, which indicates that a period of between 18 and 21 weeks was needed to achieve a more stabilised substrate in relation to the microbial biomass. Nevertheless, Gómez-Brandón et al. (2011b) detected a reduced microbial biomass with earthworm presence, irrespective of the time of vermicomposting. One plausible explanation could be that these latter authors determined the living microbial biomass, assessed by the total content of phospholipid fatty acids.

Respirometric tests have also been shown to be adequate for assessing vermicompost stability because they are able to measure the extent of which readily biodegradable OM has decomposed during the process (Gómez-Brandón et al., 2008). In the present study, there was a rapid increase in the respiration rate of sewage sludge, measured as CO_2 evolution, from the beginning of the process until week 9, in both earthworm treatments (Figure 3f). This increase at the beginning in CO_2 evolution is probably tied to the consumption of easily biodegradable compounds by the microbes, as indicated by the rapid decrease in the total content of carbohydrates during the first 2 weeks of vermicomposting. Higher levels of microbial activity were found in the absence of earthworms than in the presence of *E. andrei* until week 9, although such differences were not statistically significant ($P = 0.08$). After 9 weeks a pronounced decrease was recorded in the respiration rate of sewage sludge until the end of the experiment, at which point the CO_2 evolution was similar in both earthworm treatments (Figure 3f). In cattle manure there was also a sharp decrease in microbial activity after week 9 (Figure 3e); however, in contrast to sewage sludge, there was no an initial increase in the respiration rate at the beginning of the process, as evidenced by a relatively stable respiration rate until week 9 (Figure 3e), thereby resulting in a significant interaction between type of waste and time ($F_{4,64} = 4.08$, $P = 0.005$).

As such, a higher degree of stabilisation was reached in both organic substrates over time, as indicated by the lower values of microbial activity recorded after 16 weeks compared with those observed at the beginning of the process. Nevertheless, it should be noted that the evaluation of compost stability based on CO_2 evolution, and the maturity based on seed germination, are two different parameters of compost quality. Indeed, Wu et al. (2000) reported that the low CO_2 evolution is not always an indicator of a non-phytotoxic compost. Thus, in the present study, although low rates of respiration were reported at the end of the process this does not imply that the final product can be considered phytotoxin-free.

Ultimately, these findings provide valuable information for the understanding of the transformations that OM undergoes during vermicomposting and, in addition, constitute a powerful tool for the development of strategies leading to a more efficient process for the disposal and/or management of organic wastes.

Acknowledgements

We acknowledge Paul Fraiz for his highly valuable help in language editing.

Conflict of interest

The authors have no potential conflict of interest to declare.

Funding

This research was financially supported by the Spanish Ministerio de Ciencia e Innovación (project CTM2009-08477).

References

Aira M and Domínguez J (2009) Microbial and nutrient stabilization of two animal manures after the transit through the gut of the earthworm *Eisenia fetida* (Savigny, 1826). *Journal of Hazardous Materials* 161: 1234–1238.

Aira M and Domínguez J (2011) Earthworm effects without earthworms: inoculation of raw organic matter with worm-worked substrates alters microbial community functioning. *Plos One* 6: 1–8.

Aira M, Monroy F and Domínguez J (2007) *Eisenia fetida* (Oligochaeta: Lumbricidae) modifies the structure and physiological capabilities of microbial communities improving carbon mineralization during vermicomposting of pig manure. *Microbial Ecology* 54: 662–671.

Aira M, Monroy F and Domínguez J (2008) Detritivorous earthworms directly modify the structure, thus altering the functioning of a microdecomposer food web. *Soil Biology and Biochemistry* 40: 2511–2516.

Aira M, Monroy F and Domínguez J (2009) Changes in bacterial numbers and microbial activity of pig slurry during gut transit of epigeic and anecic earthworms. *Journal of Hazardous Materials* 161: 1234–1238.

Aira M, Gómez-Brandón M, Gónzalez-Porto P and Domínguez J (2011) Selective reduction of the pathogenic load of cow manure in an industrial-scale continuous-feeding vermireactor. *Bioresource Technology* 102: 9633–9637.

Anderson JPE (1982) Soil respiration. In: Page AL (ed.) *Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. Agronomy Monograph No. 9, ASA-SSSA*. Madison, WI: American Society of Agronomy and Soil Science Society of America.

Atiyeh RM, Arancon NQ, Edwards CA and Metzger JD (2000) Influence of earthworm-processed pig manure on the growth and yield of greenhouse tomatoes. *Bioresource Technology* 75: 175–180.

Bardgett R (2005) *The Biology of Soil: A Community and Ecosystem Approach*. Oxford: Oxford University Press.

Brookes PC, Landman A, Pruden G and Jenkinson DS (1985) Chloroform fumigation and the release of soil ni- trogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry* 17: 837–842.

Brown G and Doube B (2004) Functional interactions between earthworms, microorganisms, organic matter and plants. In: Edwards CA (ed.) *Earthworm Ecology*. Boca Raton, FL: CRC Press, pp. 213–240.

Cabrera ML and Beare MH (1993) Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Science Society of America Journal* 57: 1007–1012.

Directive 2008/98/EC (Waste Framework Directive) (2008). European Parliament and Council. Available at: http://ec.europa.eu/environment/waste/framework/index.htm (accessed 17 February 2013).

Domínguez J. (2004) State of the art and new perspectives on vermicomposting research. In: Edwards, C.A. (ed.): Earthworm Ecology, pp. 401–424. CRC Press, Boca Raton, Florida.

Domínguez J and Edwards CA (2010a) Relationships between composting and vermicomposting. In: Edwards CA, Arancon NQ and Sherman RL (eds) *Vermiculture Technology: Earthworms, Organic Waste and Environmental Management*. Boca Raton, FL: CRC Press, pp. 11–25.

Domínguez J and Edwards CA (2010b) Biology and ecology of earthworm species used for vermicomposting. In: Edwards CA, Arancon NQ and Sherman RL (eds) *Vermiculture Technology: Earthworms, Organic Waste and Environmental Management*. Boca Raton, FL: CRC Press, pp. 25–37.

Domínguez J, Edwards CA and Subler S (1997) A comparison of vermicomposting and composting methods to process animal wastes. *Biocycle* 5: 57–59.

Domínguez J, Aira M and Gómez-Brandón M (2010) Vermicomposting: earthworms enhance the work of microbes. In: Insam H, Franke-Whittle H and Goberna M (eds) *Microbes at Work: from Wastes to Resources*. Berlin: Springer, pp. 93–114.

Faostat (Food and Agriculture Organization of the United Nations) (2003) FAO statistical databases. Available at: http://faostat.fao.org/ (accessed XX).

Fernández-Gómez MJ, Nogales R, Insam H, Romero E and Goberna M (2010) Continuous-feeding vermicomposting as a recycling management method to revalue tomato-fruit wastes from greenhouse crops. *Waste Management* 30: 2461–2468.

Garg VK and Kaushik P (2005) Vermistabilization of textile mill sludge spiked with poultry droppings by an epigeic earthworm *Eisenia foetida. Bioresource Technology* 96: 1063–1071.

Gómez-Brandón M and Domínguez J (2013) Solid waste management by vermicomposting: microbial community changes throughout the process and use of vermicompost as soil amendment. *Critical Reviews in Environmental Science and Technology*. DOI: 10.1080/10643389.2013.763588.

Gómez-Brandón M, Lazcano C and Domínguez J (2008) The evaluation of stability and maturity during the composting of cattle manure. *Chemosphere* 70: 436–444.

Gómez-Brandón M, Aira M, Lores M and Domínguez J (2011a) Epigeic earthworms exert a bottleneck effect on microbial communities through gut- associated processes. *Plos One* 6: 1–9.

Gómez-Brandón M, Aira M, Lores M and Domínguez J (2011b). Changes in microbial community structure and function during vermicomposting of pig slurry. *Bioresource Technology* 102: 4171–4178.

Gómez-Brandón M, Lores M and Domínguez J (2012) Species-specific effects of epigeic earthworms on microbial community structure during first stages of decomposition of organic matter. *Plos One* 7: 1–8.

Holm-Nielsen JB, Seado TA and Oleskowicz-Popiel P (2009) The future of anaerobic digestion and biogas utilization. *Bioresource Technology* 100: 5478–5484.

Insam H and de Bertoldi M (2007) Microbiology of the composting process. In: Diaz LF, de Bertoldi M and Bidlingmaier W (eds) *Compost Science and Technology*. Amsterdam: Elsevier Science, pp. 25–48.

Laurentin A and Edwards CA (2003) A microtiter modification of the anthrone sulfuric acid colorimetric assay for glucose-based carbohydrates. *Analytical Biochemistry* 315: 143–145.

Lazcano C, Gómez-Brandón M and Domínguez J (2008) Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere* 72: 1013–1019.

Le Bayon RC and Binet F (2006) Earthworms change the distribution and availability of phosphorous in organic substrates. *Soil Biology and Biochemistry* 38: 235–246.

Monroy F, Aira M and Domínguez J (2009) Reduction of total coliform numbers during vermicomposting is caused by short-term direct effects of earthworms on microorganisms and depend on the dose of application of pig slurry. *Science of the Total Environment* 407: 5411–5416.

Potvin C, Lechowicz MJ and Tardif S (1990) The statistical analyses of ecological response curves obtained from experiments involving repeated measures. *Ecology* 71: 1389–1400.

Sangwan P, Kaushik CP and Garg VK (2010) Vermicomposting of sugar industry waste (press mud) mixed with cow dung employing an epigeic earthworm *Eisenia fetida. Waste Management & Research* 28: 71–75.

Sims GK, Ellsworth TR and Mulvaney RL (1995) Microscale determination of inorganic nitrogen in water and soil extracts. *Communication in Soil Science and Plant Analysis* 26: 303–316.

Soltanpour PN and Schwab AP (1977) A new soil test for simultaneous extraction of macro- and micro-nutrients in alkaline soils. *Communication Soil Science and Plant Analysis* 8: 195–207.

Suthar S (2008) Bioremediation of aerobically treated distillery sludge mixed with cow dung by using an epigeic earthworm *Eisenia fetida. Environmentalist* 28: 76–84.

Suthar S and Singh S (2008) Feasibility of vermicomposting in biostabilization of sludge from a distillery industry. *Science of the Total Environment* 394: 237–243.

Vivas A, Moreno B, García-Rodríguez S and Benítez E (2009) Assessing the impact of composting and vermicomposting on bacterial community size and structure, and microbial functional diversity of an olive-mill waste. *Bioresource Technology* 100: 1319–1326.

Wu L, Ma LQ and Martinez GA (2000) Comparison of methods for evaluating stability and maturity of biosolids compost. *Journal of Environmental Quality* 29: 424–429.

Yadav A and Garg VK (2009) Feasibility of nutrient recovery from industrial sludge by vermicomposting technology. *Journal of Hazardous Materials* 168: 262–268.

Yadav A and Garg VK (2011) Industrial wastes and sledges management by vermicomposting. *Reviews in Environmental Science and Biotechnology* 10: 243–276.