Microbial and nutrient stabilization of two animal manures after the transit through the gut of the earthworm *Eisenia fetida* (Savigny, 1826)

Manuel Aira*, Jorge Domínguez

Departamento de Ecoloxía e Bioloxía Animal, Universidad de Vigo, Vigo E-36310, Spain

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ABSTRACT

Here we studied how the transit through the gut of the earthworm *Eisenia fetida* affects the microbial and nutrient stabilization of pig and cow manure, by analyzing fresh casts. Earthworms reduced the pools of dissolved organic C and N in casts from both types of manure, as wells as mineral N. Microbial biomass was enhanced only in casts from pig manure and did not change in casts from cow manure, and fungal populations only raised in casts from cow manure. Earthworms reduced microbial activity in casts from cow manure and did not modify in casts from pig slurry. Enzyme activities in casts also depended on the manure ingested; there were no changes in dehydrogenase and β-glucosidase activities, whereas acid and alkaline phosphatases increased. The results indicate that the first stage in vermicomposting of pig and cow manure by *E. fetida*, i.e. casting, produced a microbial stabilization decreasing the activity of microorganisms; this stabilization occurred despite of the increase in microbial biomass. The strong reduction in nutrient pools of manures may be the responsible of this contradiction. These changes will influence the dynamics of the organic matter degradation by reducing forms of C and N available to microorganisms and hence restricting their growth and multiplication. Nevertheless, casts were also characterized by an increased enzyme potential that might lead to a further thorough degradation of pig and cow manure.

1. Introduction

During recent years, release of unprocessed animal manures into agricultural fields has contaminated ground waters causing a strong public health risk, by means of nutrient and microbial contamination. In Spain the most common animal cultures are pig and cow breeding farms, which produce more than 40 million of tons of manure, which are processed in waste factories to produce methane and electricity. However, these facilities are an expensive option compared to the cheapest technology of vermicomposting.

Although microorganisms are largely responsible of organic matter decomposition, earthworms may also affect to rates of decomposition directly by feeding on and digesting organic matter and microorganisms, or indirectly affect them through their interactions with the microorganisms, basically involving stimulation or depression of the microbial populations [1,2]. Thus, relationships between earthworms and microorganisms are key to understand how these processes occur. Casting, the faecal products of earthworms, due to its significance in soil ecosystem processes, has been widely studied revealing changes in their nutrient status and microbial composition and activity with respect to the parent soil. Thus, casts are nutrient enriched structures [3–5]; microbial populations of ingested substrates are usually enhanced after transit through the earthworm's gut [4,6,7]. However, microorganisms may also constitute an important part of the diet of earthworms, which can feed on them selectively [8,9]. Earthworms can modify the diversity and abundance of the microflora directly, by selective feeding, or by stimulation of particular taxa of microorganisms [7,10–12]; further, earthworms exert other indirect effects on microbial communities, such as microbial dispersion and the release of additional food resources in their casts.

Vermicomposting involves biooxidation and stabilization of organic material through the interactions between earthworms and microorganisms. Although microorganisms are mainly responsible for the biochemical degradation of organic matter, earthworms play an important role in the process by fragmenting and conditioning the substrate, increasing surface area for growth of microorganisms and altering its biological activity [13,14]. The high population densities of earthworms in the vermicomposting systems result in a rapid turnover of fresh organic matter into earthworm casts. These casts can be deposited both inside and outside of the fresh organic matrix, thereby affecting the decomposition rates in their proximity because of their different nutrient and microbial composition. For all these reasons, a better knowledge of the first changes in the chemical and microbiological properties of the organic wastes once

* Corresponding author. Tel.: +34 986812593; fax: +34 986812556.
  E-mail address: aira@uvigo.es (M. Aira).

Table 1
Changes in biochemical and microbial parameters of pig and cow manure after transit through the gut of the earthworm *Eisenia fetida*

	Pig manure		Cow manure	
	Manure	Cast	Manure	Cast
N-NH$_4$$^+$ (μg g^{-1} dw)	13380 ± 320a	1170 ± 50b	1860 ± 50c	400 ± 10d
N-NO$_3$$^-$ (μg g^{-1} dw)	630 ± 603a	2130 ± 140b	2220 ± 190b	469 ± 20a
Dissolved organic carbon (μg g^{-1} dw)	2710 ± 150a	1650 ± 80b	5540 ± 700c	3220 ± 450d
Microbial biomass-N (μg g^{-1})	7280 ± 680a	9930 ± 280b	4200 ± 1780a	3500 ± 1700a
Ergosterol content (μg g^{-1})	0.50 ± 0.07a	0.60 ± 0.02a	1.90 ± 0.70a	5.70 ± 1.20b
Basal respiration (μg CO$_2$ g^{-1} OM h^{-1})	810 ± 15a	750 ± 15a	920 ± 30b	510 ± 20c
Dehydrogenase activity (μg TPF g^{-1} dw 24 h^{-1})	13110 ± 520a	12170 ± 530a	5500 ± 970b	4840 ± 1170b
β-glucosidase activity (μg PNP g^{-1} dw h^{-1})	1350 ± 60	930 ± 20	1230 ± 100	2750 ± 990
Acid phosphatase (μg PNP g^{-1} dw h^{-1})	1120 ± 130a	990 ± 10a	3460 ± 230b	5660 ± 160c
Alkaline phosphatase (μg PNP g^{-1} dw h^{-1})	2140 ± 210a	7220 ± 280b	5650 ± 490c	95350 ± 460d

Different letters (a, b, c, d) mean significant differences (Tukey HSD, $p < 0.05$) (mean ± S.E., $n = 15$, dw = dry weight, OM = organic matter, TPF = triphenylformazan, PNP = p-nitrophenol).

there are released as casts during the vermicomposting process are very important to better understand the effect of the earthworms on the processes of biodegradation.

We tested if the effects of earthworms on the chemical and microbiological characteristics depended on the type of substrate ingested (i.e. casts from different substrates produced by the same earthworm species have the same nutrient contents or microbiological properties). To do this, we choose two substrates differing in their chemical and microbiological characteristics like cow and pig manure [15]. The main objective of our study was to monitor the short-time changes (fresh manure to casts) of pig and cow manure, after passing through the gut of the epigeic earthworm *Eisenia fetida* under controlled environmental conditions. We monitored the changes in available pools of C and N of the manures; we analyzed microbial biomass and activity, very important parameters in regulating soil properties [16,17] and enzyme activities since they are reliable indicators of the response of microbial communities to variations in their environmental conditions [18].

2. Methods

2.1. Animal manures and experimental design

Fresh animal manures were obtained from a pig and a cow breeding farm facilities located near the University of Vigo, NW Spain. The manures were homogenized in the pit and then stored in sealed plastic containers and kept at 5 °C until use. Batches of 50 mature earthworms were each placed in plastic boxes ($n = 15$), which were filled with a layer of vermiculite (half of the box, to ensure that earthworms only feed the manure supplied) and 1 kg of fresh pig slurry and 1 kg of fresh cow manure, separated by a mesh (5 mm). We also have 15 boxes for the two manures without earthworms. The boxes, which were maintained at 20 °C, were reviewed daily to take fresh casts from the surface and the consumed manure replaced when required, removing the mesh with the consumed manure and substituting it by a new amount of fresh manure.

2.2. Analytical methods

The moisture content of the pig slurry and earthworm casts was determined after drying at 105 °C for 24 h, and the organic matter content after heating at 550 °C for 4 h. Inorganic N (N-NH$_4$$^+$ and N-NO$_3$$^-$) was determined in 0.5 M K$_2$SO$_4$ extracts (1:5 weight:volume) using a modified indophenol blue technique [19] with a Bio-Rad Microplate Reader 550. Dissolved organic C (DOC) of pig slurry and earthworm casts was determined colorimetri-

cally after moist digestion (K$_2$Cr$_2$O$_7$ and H$_2$SO$_4$) of aliquots of 0.5 M K$_2$SO$_4$ extracts of the samples [20–22].

Microbial biomass-N (N_{mic}) was analyzed by the chloroform fumigation–extraction method [23,24]. The ergosterol content of pig slurry was extracted by microwave-assisted extraction (MAE) and determined by HPLC analysis [25]. Microbial activity was assessed by measuring the rates of CO$_2$ evolution from samples after 6 h of incubation. The evolved CO$_2$ was trapped in 0.02 M NaOH and then measured by titration with HCl to a phenolphthalein endpoint, after adding excess BaCl$_2$ [26]. Dehydrogenase enzyme activity was measured by estimation of the rate of reduction of triphenyltetrazolium chloride (TTC) (1.5%) to triphenylformazan (TPF), after incubation at 30 °C for 24 h, in a Bio-Rad Microplate Reader 550 at 545 nm [27]. Acid and Alkaline phosphatase activity were estimated by determination of p-nitrophenol (PNP) released, after incubation of samples with p-nitrophenyl phosphate (0.025 M) for 1 h at 37 °C, in a Bio-Rad Microplate Reader 550 at 400 nm [28]. β-glucosidase activity was assessed by determination of the released p-nitrophenol, after the incubation of samples with p-nitrophenyl glucoside (0.025 M) for 1 h at 37 °C, in a Bio-Rad Microplate Reader at 400 nm [29].

2.3. Statistical analysis

Data were analyzed using a factorial design in which manure (pig slurry and cow manure) and earthworm (presence and absence) were fixed as factors. All variables fulfilled ANOVA assumptions. Post hoc comparisons (Tukey HSD) where carried out when ANOVA was significant at 0.05 level. All statistical analyses were performed using SPSS 11.5 software.

3. Results

The passage of pig and cow manure through the gut of *E. fetida* significantly reduced the moisture of the resulting casts. Moisture of casts from pig manure dropped from 81.5 ± 0.1 to 77.9 ± 0.1%, and casts from cow manure decreased from 82.7 ± 0.1 to 78.4 ± 0.2% (ANOVA, $F_{1,56} = 35.28$, $p < 0.01$). The same pattern occurred with the organic matter (88.1% initial organic matter in both manures) which strongly diminished after earthworm digestion; this reduction was higher in the digested cow manure (75.3 ± 0.2) than in the digested pig manure (80.7 ± 0.1) (ANOVA, $F_{1,56} = 47.96$, $p < 0.01$).

The transit through the earthworm gut strongly affected pools of assimilable C and N. The ammonium concentration of casts was always lower than in the corresponding manure (Table 1; ANOVA, $F_{1,56} = 1947.96$, $p < 0.0001$), with the strongest reduction in casts from pig manure. Nitrate concentration showed opposed trends depending on the type of manure (Table 1); thus, casts from pig

manure had 3.5 times more nitrate than the manure, whereas casts from cow manure had 4.3 times less nitrate concentration than the manure; this resulted in a significant interaction between manure and earthworm (ANOVA, $F_{1,56} = 27.97$, $p < 0.0001$). DOC contents showed also a marked decrease after the transit through the gut (Table 1; ANOVA, $F_{1,56} = 14.14$, $p < 0.001$) that did not depend on the type of manure.

The pass through the earthworm gut also modified the microbial biomass and activity and the enzymatic activities of pig and cow manure. The N_{mic} was only affected by the type of manure being higher in pig than in cow manure (Table 1; ANOVA, $F_{1,56} = 12.42$, $p < 0.001$); however, casts of pig manure showed a higher microbial biomass than the uningested pig manure (Table 1). Ergosterol content depended on the type of manure, since there were no changes in ergosterol content in casts from pig manure, whereas it increased 2.8 times in casts from cow manure, resulting in a significant interaction between manure and earthworm (Table 1; ANOVA, $F_{1,56} = 5.39$, $p < 0.05$). The same pattern occurred with the basal respiration. There were no changes in basal respiration in pig manure after transit through the earthworm gut, whereas it dropped markedly (1.8 times) in casts from cow manure, producing a significant interaction between manure and earthworm (Table 1; ANOVA, $F_{1,56} = 71.09$, $p < 0.001$). Dehydrogenase activity was only affected by the type of manure (Table 1; ANOVA, $F_{1,56} = 78.19$, $p < 0.0001$), with higher activities (almost double) in pig than in cow manure; the transit through the gut did not modify the activity of this enzyme. Activity of β-glucosidase did not depended on any of the factors studied (Table 1) and acid phosphatase activity depended on the type of manure (Table 1); there were no differences in this enzyme activity between casts and pig manure, whereas casts from cow manure showed higher values than the manure resulting in a significant interaction between manure and earthworm (ANOVA, $F_{1,56} = 45.13$, $p < 0.0001$). The activity of alkaline phosphatase increased strikingly after transit through the earthworm gut (Table 1; ANOVA, $F_{1,56} = 140.95$, $p < 0.0001$), and it was higher in cow than in pig manure (ANOVA, $F_{1,56} = 59.76$, $p < 0.0001$).

4. Discussion

The transit of pig and cow manure through the gut of the earthworm *E. fetida* produced a reduction in both available C and N forms (DOC and N-NH_4^+) in the casts. The lower contents of N-NH_4^+ found in the casts may be attributed to losses by volatilization before intake of the manure by the earthworms, more marked in the cow manure. Other alternative hypothesis for ammonium losses are nitrification, which is enhanced in casts [30] maybe responsible in casts from pig manure, and denitrification processes, typical from the anaerobic earthworm gut which use ammonium and also nitrate as sources to produce NO_2 [31], which may be acted in casts from cow manure. The large reduction in the DOC levels of casts may have been resulted from direct assimilation of DOC by *E. fetida* during digestion of the manures; this agrees with the strong reduction observed in the organic matter content in casts from both types of manure. This is consistent with the general assumption that labile C compounds are an important part of the diet of earthworms, at least in soils and for endogeic earthworms, and that C availability is a limiting factor for growth of soil earthworms [32,33]. Similar ammonium and DOC losses and nitrate content were reported in casts of *Eudrilus eugeniae* fed with pig manure [34].

Microbial biomass and activity of the two types of manure were affected differently by the earthworm digestion. Thus, it appears that *E. fetida* did not feed on the microorganisms present in the manure because there was no effect on cow manure, although tran-

sit through the gut increased the microbial biomass of pig manure. This result disagrees with the general findings that microorganisms, especially fungi, are an important part of the earthworm diet [8]. Furthermore, Moody et al. [9] showed that different earthworm species (*Lumbricus terrestris*, *Allolobophora longa* and *A. chlorotica*) preferentially fed on straw-decomposing fungi and rejected lignin-decomposing fungi. Contrary to expectations, fungal growth was then stimulated in casts from cow manure, and ageing cannot be responsible of this raise of fungal populations [5] because casts were sampled daily. In fact, fungal growth was also reported in early stages of vermicomposting of pig slurry with *E. fetida* [35]. The lower microbial activity observed in casts may have been caused by observed decreases in DOC due to C limitations for microorganisms similar to those in soil systems [36], rather than due to direct feeding by *E. fetida*. However, despite of comparable reductions of DOC observed in both manures (1.6 and 1.7 for cow and pig manure, respectively), microbial activity was only reduced in casts from cow manure; further, the increased microbial community of casts from pig manure was as active as the undigested microbial community of the manures. Conversely to our findings, microbial biomass and activity of pig manure were reduced after the transit through the gut of *E. eugeniae* [34]. However, Parthasarathi and Ranganathan [37] reported a fourfold increase in microbial populations (CFU) in casts of individuals of *E. eugeniae* fed with pressmud, indicating that effects of earthworms on microorganisms are clearly dependent on kind of food source and availability and the species of earthworm involved [38,39]. Similar reductions in microbial biomass were reported by Devliegher and Verstraete [11], Bohlen and Edwards [40] and Zhang et al. [41] in soils inhabited by different earthworm species; Scheu [3] and Aira et al. [4] reported increases in microbial biomass in casts of *Allolobophora caliginosa*, whereas there were not any changes in casts of *Lumbricus rubellus* [42].

There was also a significant increase in some of the enzyme activities analyzed. No effects of transit through the earthworm gut were found on dehydrogenase activity, which suggest that *E. fetida* did not affect aerobic microorganisms. Regarding to dehydrogenase assay it is necessary to point out that this technique only account for a limited percentage of respiration since oxygen is a better electron acceptor than the TTC used in our assay [16]. This lack of effect disagrees with the decreases in dehydrogenase activity reported in casts of *E. eugeniae* fed with pig manure [34], and during the vermicomposting of sewage sludge [43] and pig manure [44], with *Eisenia andrei* and *Eisenia fetida*, respectively. However, our results were not consistent with increased respiration rates and dehydrogenase activity in casts of *E. eugeniae* reported by Parthasarathi and Ranganathan [37] which were clearly related to higher CFU counts, or to the intense dehydrogenase activities observed during vermicomposting of cattle dung and cow dung with *E. fetida* [44,45]. This contradiction may be explained partly because pressmud is a richer (in nutrients and microflora) substrate than pig and cow manure, and digestion of *E. eugeniae* appears to increase the availability of these nutrients, thereby increasing the microbial populations. In the case of vermicomposting systems, the increase in dehydrogenase activity suggest the existence of more interactions between earthworms and microorganisms other than the simple dynamic of ingestion-casting, as also revealed by the increase in basal respiration in early stages of vermicomposting of pig manure with *E. fetida* [22]. We analyzed the enzyme activities (β-glucosidase, and acid and alkaline phosphatase) to monitor directly the functional responses of the microbial community of the manures to changes induced transit through the earthworm gut. This earthworm species is classified as epigeic (litter feeding) and therefore is characterized by the possession of diverse enzyme activities [2]. The increase in enzyme activities could depend on the increase of the relative substrates concentration during transit of the manures

through the gut. According to this hypothesis the greatest increase was in alkaline phosphatase activity, showing that during gut transit organic P pools, as it succeed with DOC, were also mobilized by *E. fetida*, enlarging substrate availability to the enzyme. Our results disagree with previous findings with the earthworm species *E. eugeniae* which reduced all the enzyme activities assayed [34]. The lack of effects in β-glucosidase may indicate that passage through the earthworm intestine did not affect cellulolytic compounds to the same extent. Nevertheless this hypothesis needs to be verified by analyzing how the concentration of enzyme substrates varies during the transit through the earthworm gut.

5. Conclusions

A high degree of microbial and nutrient stabilization was obtained with only the digestion of the two animal manures. Further, the present results are important because the microbial community in casts will initiate the vermicomposting process and the metabolic potential will determine the degree of organic matter degradation and stabilization. In this way, despite of the two substrates we used (cow and pig manure) differ in their microbial communities as revealed by FAMEs profiles [15], transit through the gut of *E. fetida* produced relevant differences in microbiological parameters, which resulted in shared effects in analyzed nutrient contents. Moreover, once vermicomposting ends microbial communities of both pig and cow manure processed by *E. fetida* are the same, as revealed by FAMEs profiles [15], suggesting that during organic matter decomposition in vermicomposting systems there should be more factors driving changes in microbial communities and hence in nutrient contents other than direct transformations produced in casts.

Acknowledgements

References

[1] C.A. Edwards, P.J. Bohlen, Biology and Ecology of Earthworms, 3rd ed., Chapman and Hill, London, New York, 1996.
[2] P. Lavelle, A.V. Spain, Soil Ecology, Kluwer Academic Publishers, London, 2001.
[3] S. Scheu, Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae), Biol. Fertil. Soils 5 (1987) 230–234.
[4] M. Aira, F. Monroy, J. Domínguez, Effects of two species of earthworms (*Allolobophora* sp.) on soil systems: a microfaunal and biochemical analysis, Pedobiologia 47 (2003) 877–881.
[5] M. Aira, F. Monroy, J. Domínguez, Ageing effects on nitrogen dynamics and enzyme activities in casts of *Aporrectodea caliginosa* (Lumbricidae), Pedobiologia 49 (2005) 467–473.
[6] Q.L. Zhang, P.F. Hendrix, Earthworm *Lumbricus rubellus* and *Aporrectodea caliginosa*) effects on carbon flux in soil, Soil Sci. Soc. Am. J. 59 (1995) 816–823.
[7] A.V. Tiunov, S. Scheu, Microfungal communities in soil, litter and casts of *Lumbricus terrestris* L. (Lumbricidae): a laboratory experiment, Appl. Soil Ecol. 14 (2000) 17–26.
[8] C.A. Edwards, Earthworm Ecology, 2nd ed., CRC Press, Boca Raton, 2004.
[9] S.A. Moody, M.J.I. Briones, T.G. Pierce, J. Dighton, Selective consumption of decomposing wheat straw by earthworms, Soil Biol. Biochem. 28 (1995) 533–537.
[10] J.C. Pedersen, N.B. Hendriksen, Effect of passage through the intestinal tract of detritivore earthworms (*Lumbricus* spp.) on the number of selected Gram-negative and total bacteria, Biol. Fertil. Soils 16 (1993) 227–232.
[11] W. Devliegher, W. Verstraete, *Lumbricus terrestris* in a soil core experiment: nutrient-enrichment processes (NEP) and gut-associated processes (GAP) and their effect on microbial biomass and microbial activity, Soil Biol. Biochem. 27 (1995) 1573–1580.
[12] C. Wolter, S. Scheu, Changes in bacterial numbers and hyphal lengths during the gut passage through *Lumbricus terrestris* (Lumbricidae, Oligochaeta), Pedobiologia 43 (1999) 891–900.
[13] J. Domínguez, State of the art and new perspectives on vermicomposting research, in: C.A. Edwards (Ed.), Earthworm Ecology, 2nd ed., CRC Press, Boca Raton, 2004, pp. 401–424.
[14] J. Domínguez, C.A. Edwards, Vermicomposting organic wastes: a review, in: S.H.S. Hanna, W.Z.A. Mikhail (Eds.), Soil Zoology for Sustainable Development in the 21st century, Cairo, 2004, pp. 369–395.
[15] M. Lores, M. Gomez-Brandon, D. Perez-Diaz, J. Domínguez, Using FAME profiles for the characterization of animal wastes and vermicomposts, Soil Biol. Biochem. 38 (2006) 2993–2996.
[16] P. Nannipieri, S. Grego, B. Ceccanti, Ecological significance of the biological activity in soil, in: J.M. Bollag, G. Stotzky (Eds.), Soil Biochemistry, vol. 6, Marcel Dekker, New York, 1990, pp. 293–355.
[17] R.P. Dick, A review: long-term effects of agricultural systems on soil biochemical and microbial parameters, Agric. Ecosyst. Environ. 40 (1992) 25–36.
[18] M.M. Carreiro, R.L. Sinsabaugh, D.A. Repert, D.F. Parkhurst, Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition, Ecology 81 (2000) 2359–2365.
[19] G.K. Sims, T.R. Ellsworth, R.L. Mulvaney, Microscale determination of inorganic nitrogen in water and soil extracts, Commun. Soil Sci. Plant Anal. 26 (1995) 303–316.
[20] M.L. Jackson, Soil Chemical Analysis, Constable & Co. Ltd., London, 1958.
[21] I.V. Tyurin, A new modification of the volumetric method of determining soil organic matter by means of chromic acid, Pochvovedenie 26 (1931) 36–47.
[22] M. Aira, F. Monroy, J. Domínguez, *Eisenia fetida* (Oligochaeta: Lumbricidae) modifies the structure and physiological capabilities of microbial communities improving carbon mineralization during vermicomposting of pig manure, Microb. Ecol. 54 (2007) 662–671.
[23] P.C. Brookes, A. Landman, G. Pruden, D.S. Jenkinson, Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil, Soil Biol. Biochem. 17 (1985) 837–842.
[24] J. Domínguez, R.W. Parmelee, C.A. Edwards, Interactions between *Eisenia andrei* (Oligochaeta) and nematode populations during vermicomposting, Pedobiologia 47 (2003) 53–60.
[25] J.C. Young, Microwave-assisted extraction of the fungal metabolite ergosterol and total fatty acids, J. Agric. Food Chem. 43 (1995) 2904–2910.
[26] J.P.E. Anderson, Soil respiration, in: A.L. Page, R.H. Miller (Eds.), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, Agronomy Monograph No. 9, 2nd ed., ASA-SSSA, Madison, WI, 1982, pp. 831–871.
[27] L.E. Casida, D.A. Klevin, T. Santero, Soil dehydrogenase activity, Soil Sci. 93 (1964) 371–376.
[28] F. Eivazi, M.N. Tabatabai, Phosphatases in soils, Soil Biol. Biochem. 9 (1977) 167–172.
[29] F. Eivazi, M.A. Tabatabai, Glucosidases and galactosidases in soils, Soil Biol. Biochem. 20 (1988) 601–606.
[30] T. Decaens, A.F. Rangel, N. Asakawa, R.J. Thomas, Carbon and nitrogen dynamics in ageing earthworm cast in grasslands of the eastern plains of Colombia, Biol. Fertil. Soils 30 (1999) 20–28.
[31] M.A. Horn, A. Schramm, H.L. Drake, The earthworm gut: an ideal habitat for ingested N_2O-producing microorganisms, Appl. Environ. Microbiol. 69 (2003) 1662–1669.
[32] S. Scheu, S. Schaefer, Bottom–up control of the soil macrofauna community in a beechwood on limestone: manipulation of food resources, Ecology 79 (1998) 1573–1585.
[33] A.V. Tiunov, S. Scheu, Carbon availability controls the growth of detritivores (Lumbricidae) and their effect on nitrogen mineralization, Oecologia 138 (2004) 83–90.
[34] M. Aira, F. Monroy, J. Domínguez, Changes in microbial biomass and microbial activity of pig slurry after the transit through the gut of the earthworm *Eudrilus eugeniae* (Kinberg, 1867), Biol. Fertil. Soils 42 (2006) 371–376.
[35] M. Aira, F. Monroy, J. Domínguez, *Eisenia fetida* (Oligochaeta, Lumbricidae) activates fungal growth, triggering cellulose decomposition during vermicomposting, Microb. Ecol. 52 (2006) 738–746.
[36] S. Scheu, Analysis of the microbial nutrient status in soil microcompartments: earthworm faeces from a basalt-limestone gradient, Geoderma 56 (1993) 575–586.
[37] K. Parthasarathi, L.S. Ranganathan, Longevity of microbial and enzyme activity and their influence on NPK content in pressmud vermicasts, Eur. J. Soil. Biol. 35 (1999) 107–113.
[38] A.V. Tiunov, S. Scheu, Microbial biomass, biovolume and respiration in *Lumbricus terrestris* L. cast material of different age, Soil Biol. Biochem. 32 (2000) 265–275.
[39] M. Flegel, S. Schrader, Importance of food quality on selected enzyme activities in earthworm casts (*Dendrobaena octaedra*, Lumbricidae), Soil Biol. Biochem. 32 (2000) 1191–1196.
[40] P.J. Bohlen, C.A. Edwards, Earthworms effects on N dynamics and soil respiration in microcosms receiving organic and inorganic nutrients, Soil Biol. Biochem. 27 (1995) 341–348.
[41] B. Zhang, G. Li, T. Shen, T. Wang, Z. Sun, Changes in microbial biomass C. N and P and enzyme activities in soil incubated with the earthworms *Metaphire guillelmi* or *Eisenia fetida*, Soil Biol. Biochem. 32 (2000) 2055–2062.
[42] O. Daniel, J.M. Anderson, Microbial biomass and activity in contrasting soil materials after passage through the gut of the earthworm *Lumbricus rubellus* Hoffmeister, Soil Biol. Biochem. 24 (1992) 465–470.

[43] E. Benítez, R. Nogales, C. Elvira, G. Masciandaro, B. Ceccanti, Enzyme activities as indicators of the stabilization of sewage sludge composting with *Eisenia fetida*, Biores. Technol. 67 (1999) 297–303.

[44] M. Aira, F. Monroy, J. Domínguez, S. Mato, How earthworm density affects microbial biomass and activity in pig manure, Eur. J. Soil Biol. 38 (2002) 7–10;

S. Bansal, K.K. Kapoor, Vermicomposting of crop residues and cattle dung with *Eisenia foetida*, Biores. Technol. 73 (2000) 95–98.

[45] P. Kaushik, V.K. Garg, Vermicomposting of solid textile mill sludge and cow dung with the epigeic earthworm *Eisenia foetida*, Biores. Technol. 90 (2003) 311–316.