Available online at www.sciencedirect.com
ScienceDirect

European Journal of Soil Biology 42 (2006) S127–S131

EUROPEAN JOURNAL OF SOIL BIOLOGY

http://france.elsevier.com/direct/ejsobi

Original article

C to N ratio strongly affects population structure of *Eisenia fetida* in vermicomposting systems

M. Aira*, F. Monroy, J. Domínguez

Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, Vigo 36310, Spain

Available online 10 August 2006

Abstract

Food quality influences not only the size of earthworm populations but also their growth and reproduction rates. Here we studied the effect of C to N ratio of pig slurry in microbial biomass and activity and in the growth and reproduction of the earthworm *Eisenia fetida*. We set up a batch of twelve vermireactors, six each for low (11) and high C to N ratio (19) of pig slurry applied; three of each without earthworms (control) and three containing 500 mature earthworms (*E. fetida*). After 36 weeks C to N ratio significantly affected earthworm numbers (sevenfold greater in high C to N ratio) and population structure. Thus, in the low C to N ratio treatment the population was composed mainly by mature earthworms (60%), with a higher mean weight than in the high C to N treatment. However, in the high C to N ratio treatment, the population was composed mainly by juvenile and hatchling earthworms (70%). A rapid depletion of dissolved organic C (DOC) content was observed in all treatments. Although earthworms produced an increase in microbial biomass and activity in young modules, finally a decrease in older modules was recorded. The decrease in available carbon did not seem to affect the relationships established between earthworms and microflora.

Keywords: Vermicomposting; Pig slurry; Earthworms; *Eisenia fetida*; C to N ratio; Microbial biomass and activity; Resource limitation

1. Introduction

Life history of an organism is the result of competition for limited resources in time and energy between growth and reproduction. It has been reported for some vermicomposting earthworms species that different food diets can affect their growth, reproduction or both [3,4], and moreover earthworm's growth is limited by carbon availability [11]. Therefore, it is possible that earthworms can allocate resources either to growth or reproduction depending on resource limitation and/or quality. This is important in vermicomposting, where high growth and reproduction rates are required in order to accelerate waste breakdown and stabilization.

In this work we tested the hypothesis whether C to N ratio of pig manures are affecting growth and reproduction of earthworms strongly; besides we questioned if the changes in earthworm population are also modifying the interactions that earthworms and microorganisms (microbial biomass and activity) establish during vermicomposting.

2. Materials and methods

We used two pig slurries obtained from two different pig breeding farms placed near the University of Vigo, that differed mainly in its C to N ratio, that were 11 and 19 (low and high C to N ratio, respectively). We set up

* Corresponding author. Tel.: +34 986 81 2593; fax: +34 986 81 2556.
E-mail address: aira@uvigo.es (M. Aira).

1164-5563/$ - see front matter
doi:10.1016/j.ejsobi.2006.07.039

a batch of twelve vermireactors, six each for low (11) and high (19) C to N ratio of pig slurry applied; three of each without earthworms (control) and three containing 500 mature earthworms (*Eisenia fetida*), with a mean biomass of 90 ± 10 g. Vermireactors were composed by modules that were sequentially added. The external diameter of each module was 30 cm with a height of 4 cm and the mesh size 5 mm, which allowed mobility of earthworms between modules. Each reactor was composed initially by a module with vermicompost, where earthworms were placed, and a first module with pig slurry (1.5 kg f.w.). New modules containing the same amount of fresh pig slurry were added over the other module when earthworms demanded it (i.e. changes in the appearance of pig slurry, becoming the coarse fraction, like seeds and straw, more evident). When the experiment finished after 36 weeks, the reactors had nine modules or layers with an increasing age gradient from upper to bottom of 0, 4, 8, 13, 21, 25, 27, 33 and 36 weeks. The vermireactors were kept in laboratory conditions at 20 °C.

At sampling time the vermireactors were dismantled and the modules isolated to avoid earthworm escape. The earthworms were then manually removed from the substrate of each layer across the entire vermireactor. We measured earthworm numbers and biomass of different age stages (clitellated, preclitellated, juvenile, and hatchlings).

For chemical and biochemical analyses five samples of substrate per module were taken randomly and gently mixed for analyses of total C and N, dissolved organic carbon (DOC), basal respiration (BR) and substrate induced respiration (SIR). Total C and N levels were determined on a Carlo Erba 1500 C/N analyzer on dried samples [2]. Extractable DOC was determined in 0.5 M K_2SO_4 extracts (1:6, w/v) by colorimetry in a Microplate Reader at 590 nm.

Basal and SIR were determined by measuring CO_2 evolution in NaOH traps (0.02 and 0.06 M, respectively). Samples (5 g f.w.) were incubated at 22 °C for 6 and 12 h (BR and SIR, respectively). For SIR respiration 0.75 ml glucose solution (equal to 100 mg g^{-1} pig slurry) was added to samples [1].

Earthworm population data were analyzed by fitting generalized linear models to the data [9]. The error distribution and link function were chosen according to presumed error of data in order to reduce deviance in the model [8]. Data from biochemical analyses were analyzed under repeated measures ANOVA design.

3. Results

Total number of earthworms was significantly higher in the high C to N ratio treatment (2800 ± 200) than in the low one (410 ± 20) (GLM $\chi^2 = 1680.68$, d.f. = 1, $P < 0.01$), which is a sevenfold increase. Earthworm biomass was also significantly higher (threefold increase) in high C to N ratio treatment (700 ± 30 g) than in low C to N ratio treatment (230 ± 20 g) (GLM $\chi^2 = 6.02$, d.f. = 1, $P < 0.05$) which are a eight and threefold increase compared with the initial biomass (high and low C to N ratio treatments, respectively). In both C to N ratio treatments the higher numbers of earthworm were found in the youngest modules (4, 8 and 13 weeks old). Earthworm population structure clearly differed between treatments with increased numbers of immature earthworms in the high C to N ratio treatment (Table 1). In the low C to N ratio treatment earthworm population was mainly composed by mature earthworms (59%) significantly larger (twofold) than those in the high C to N ratio treatment (Table 1).

In both C to N ratio treatments aging produced a significantly strong decrease in DOC levels (Fig. 1A and Table 2); these reductions were higher in the high C to N ratio treatment resulting in a significant interaction between age of layers and C to N ratio treatments (Table 2), this depletion of DOC was more intense in presence of earthworms.

Microbial biomass showed higher values in the high than in the low C to N ratio treatment, which dimin-

Table 1
Structure of earthworms population in vermireactors with high and low C to N ratio. Numbers and mean weights of mature, preclitellated, juvenile, hatchlings and cocoons are shown (mean ± S.E.). Values in bold mean significant effects of different C to N ratios (**$P < 0.01$)

	High C to N ratio	Low C to N ratio
Number of mature earthworms	259 ± 61	244 ± 37
Mean mature individual weight (g)	0.37 ± 0.05	*0.67 ± 0.02***
Number of preclitellated earthworms	284 ± 89	*37 ± 15***
Mean preclitellated individual weight (g)	0.37 ± 0.04	0.37 ± 0.03
Number of juvenile earthworms	847 ± 254	*98 ± 31***
Mean juvenile individual weight (g)	0.19 ± 0.02	0.15 ± 0.03
Number of hatchlings	472 ± 201	*33 ± 30***
Number of cocoons	750 ± 198	782 ± 105

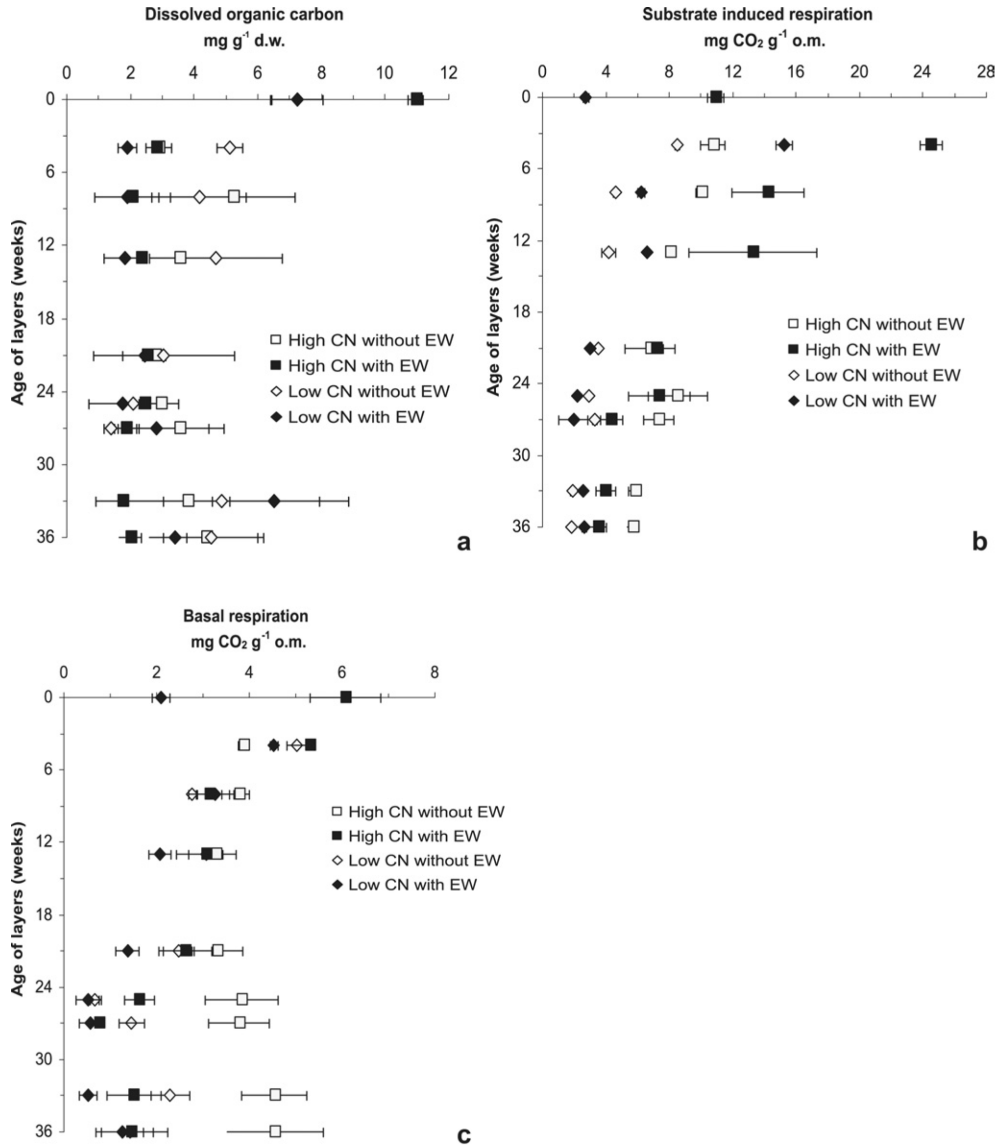

Fig. 1. Effects of the different C to N ratio of pig slurries (high and low), earthworm (presence and absence) and time (age of modules) treatments on A) dissolved organic carbon content, B) substrate induced respiration and C) basal respiration.

ished with aging (Fig. 1B and Table 2). Moreover, the presence of earthworms significantly enhanced these effects with increased and reduced values of SIR respiration in younger and older modules, respectively, resulting in a significantly interaction between age of layers and earthworm treatments (Table 2). Microbial activity was significantly affected by C to N ratio of pig slurry, showing a continuous fall with aging in both C to N ratio treatments (Fig. 1C and Table 2). Earthworms significantly affected microbial activity, increasing it in young modules and reducing it dramatically in older ones (significant interaction Age × Earthworm, Table 2), which it is indicative of the high degree of humification and stabilization of the organic matter.

4. Discussion

The application of different C to N ratio pig slurries in vermicomposting systems caused a dramatic effect on earthworm numbers and earthworm population structure. After 36 weeks the earthworm population was sevenfold greater in high than in low C to N ratio treatment, moreover population structure and weight of

Table 2
Repeated measures ANOVA Table on the response of DOC, BR and SIR to different C to N ratio pig slurries, earthworm presence and age of layers. Significances followed by * indicate that correction of Huynh–Feldt was used

	DOC			BR			SIR		
	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Between subjects									
CN ratio	1.8	0.03	ns	1.8	31.68	*< 0.001*	1.8	92.75	*< 0.0001*
Earthworm	1.8	2.06	ns	1.8	15.94	*< 0.01*	1.8	8.40	*< 0.05*
CN ratio × Earthworm	1.8	0.08	ns	1.8	2.81	ns	1.8	0.47	ns
Within subjects									
Age of layers	8.64	17.40	*< 0.0001**	8.64	26.40	*< 0.0001**	8.64	56.43	*< 0.0001**
Age × CN	8.64	3.26	*< 0.01**	8.64	7.71	*< 0.0001**	8.64	3.83	*< 0.01**
Age × Earthworm	8.64	1.02	ns	8.64	5.37	*< 0.001**	8.64	15.93	*< 0.0001**
Age × CN × Earthworm	8.64	1.15	ns	8.64	3.82	*< 0.01**	8.64	2.73	*< 0.05**

mature earthworms were also affected (Table 1) suggesting that earthworms in low C to N ratio treatment invested preferentially more energy in growth than in reproduction. In this way the mean individual weight of mature earthworms from high C to N ratio treatment was in the range of this earthworm species (0.30–0.40 g) as it was described by Watanabe and Sukamoto [14], although Venter and Reinecke [13] recorded a maximum weight of 1.6 g for a mature individual of *E. fetida*. However Gunadi et al. [7] found a relationship between increased growth and reproductive rates of *E. fetida* with low C to N ratios of cattle and pig manure, and Ndegwa and Thompson [10] reported a decrease in growth rates with increased C to N ratio of paper mulch. Domínguez et al. [3,4] found different growth and reproduction rates of *Eisenia andrei* in different diets and they also found that earthworms invested preferentially their energy either to growth or to reproduction depending of the food quality.

Despite the differences in the earthworm population numbers and biomass and the C to N ratio of the slurries, earthworms had similar effects in both high and low C to N ratios on microbial biomass and activity. In fact, the main effect of earthworms on microflora was the reduction of microbial biomass and activity in ageing modules; although in young modules, where higher numbers of earthworms were recorded, earthworms' activities like burrowing, casting and mucus production could be related with the increase in microbial biomass and activity observed. Earthworms can obtain their energy from organic residues and the microorganisms associated, which are supposed to be the main constituents of their diet, digesting them selectively [5,6]; nevertheless our results show the relative minor importance of microorganisms as food for *E. fetida*, since the higher biomass and activity were recorded in layers with the highest densities of earthworms. This could be due to enhanced enzymatic capabilities of epigeic earthworms [12] which allow them to obtain energy from alternative sources like labile organic C and N pools. Further researches are needed to clarify relationships established between earthworms and microorganisms during organic matter decomposition since it seem to depend on ecological category of earthworm and available food resources.

Acknowledgements

This research was supported by CICYT (AGL2003-01570) and Xunta de Galicia (PGIDIT03P-XIB30102PR) grants.

References

[1] J.P.E. Anderson, Soil respiration, in: A.L. Page, R.H. Miller (Eds.), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, second ed., Agronomy Monograph no. 9, ASA-SSSA, Madison, WI, 1986, pp. 831–871.

[2] AOAC (Association of Official Analytical Chemists), Official Methods of Analysis, 14th ed, Arlington, USA, 1984.

[3] J. Domínguez, M.J.I. Briones, S. Mato, Effect of the diet on growth and reproduction of *Eisenia andrei* (Oligochaeta, Lumbricidae), Pedobiologia (Jena) 41 (1997) 566–577.

[4] J. Domínguez, C.A. Edwards, M. Webster, Vermicomposting of sewage sludge: effect of bulking materials on the growth and reproduction of the earthworm *Eisenia andrei*, Pedobiologia (Jena) 44 (2000) 24–32.

[5] M.B. Doube, G.G. Brown, Life in a complex community: functional interactions between earthworms, organic matter, microorganisms, and plant growth, in: C.A. Edwards (Ed.), Earthworm Ecology, St. Lucie Press, Boca Raton, 1998, pp. 179–211.

[6] C.A. Edwards, Earthworm Ecology, St. Lucie Press, Boca Raton, 1998.

[7] B. Gunadi, C.A. Edwards, C. Blount, The influence of different moisture levels on the growth, fecundity and survival of *Eisenia foetida* (Savigny) in cattle and pig manure solids, Eur. J. Soil Biol. 39 (2003) 19–24.

[8] C.M. Herrera, Flower-to-seedling consequences of different pollination regimes in an insect-pollinated shrub, Ecology 81 (2000) 15–29.
[9] P. McCullagh, J.A. Nelder, Generalized Linear Models, second ed, Chapman and Hall, London, England, 1989.
[10] P.M. Ndegwa, S.A. Thompson, Effects of C-to-N ratio on vermicomposting of biosolids, Bioresour. Technol. 75 (2000) 7–12.
[11] A.V. Tiunov, S. Scheu, Carbon availability control the growth of detritivores (Lumbricidae) and their effect on nitrogen mineralization, Oecologia 138 (2004) 83–90.
[12] F. Urbasek, V. Pizl, Activity of digestive enzymes in the gut of five earthworm species (Oligochaeta: Lumbricidae, Rev. Ecol. Biol. Sol 28 (1991) 461–468.
[13] J.M. Venter, A.J. Reinecke, The life-cycle of the compost worm *Eisenia foetida* (Oligochaeta), S. Afr. J. Zool. 23 (1988) 161–165.
[14] H. Watanabe, J. Tsukamoto, Seasonal changes in size class and stage structure of Lumbricid *Eisenia foetida* population in a field compost and its practical application as the decomposer of organic waste matter, Rev. Ecol. Biol. Sol 13 (1976) 141–146.