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Optimizing vermicomposting of animal wastes: Effects of rate of manure application on carbon loss and microbial stabilization

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Abstract

Vermicomposting is the process whereby organic residues are broken down by earthworms and microorganisms. Addition of manure has been shown to be of critical importance and determines most of the changes that take place during vermicomposting. Here, we study how the rate of manure applied affects microbial biomass and activity and carbon losses. For this, we designed continuous feeding reactors in which new layers of manure were added sequentially to form an age gradient inside the reactors. We compared two application rates of pig slurry (1.5 and 3 kg) and set up six reactors for each one; half of the 12 reactors initially contained a population of 500 earthworms (*Eisenia fetida*). We found that earthworms increased microbial biomass and were more active in reactors fed with 3 kg of slurry. However, the differential rates of respiration were not reflected in C losses. The results thus showed that loss of C was not affected by the rate of pig slurry applied. We conclude that despite the strong effect that the rate of manure has on microbe–earthworm relationships, it did not affect carbon losses. We therefore recommend the use of low application rates of manure when the objective is the microbial stabilization of the residue.

Keywords: Decomposition; Pig slurry; Earthworms; *Eisenia fetida*; Microbial respiration; Microbial populations

1. Introduction

The increasing rate at which organic residues are generated has become a problem that requires strategies for disposal and/or management. Vermicomposting, i.e., the processing of organic residues by earthworms, has proved to be a suitable technique because of its low cost and the large amounts of organic wastes that can be processed. Vermicomposting involves the biooxidation and stabilization of organic material by the joint action of earthworms and microorganisms. Although microbes cause the biochemical degradation of organic matter, earthworms are the main drivers of the process, as they condition the substrate and alter its biological activity (Domínguez, 2004). It has been shown that earthworms can process sewage sludge and soils from waste water, materials from breweries, paper wastes, urban residues and animal wastes (review in Domínguez, 2004). Edwards and Arancon (2004) classified the different methods of processing substrates with earthworms (beds or windrows, container systems, wedge systems and continuous flow reactors), but stated that in all systems the critical step is how the waste is added to the system. They recommend that, independently of the system employed, substrate must be added frequently in thin layers to accelerate the process and to ensure the correct transformation of the organic materials.

It is known that the dynamics of decomposition systems (such as vermicomposting) depend on inputs of resources, that is, decomposer and detritivores do not control the rate of regeneration of their resources (Pimm, 1982). The rate at which the residue is applied and the rate of application of organic waste may therefore be of a major importance as it will modify the conditions under which these relationships take place, by increasing or decreasing the quantity of the resource and its availability. Moreover, it has been reported that earthworms and microorganisms may compete for C resources (Tiunov and Scheu, 2004), and thus differences in the rate of application of substrate will

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intensify the competition, by decreasing the rate of decomposition or even halting it.

Here, we studied the effect of dose of organic substrate on the vermicomposting of pig slurry. We monitored the changes in total C content and microbial biomass and activity to investigate how the presence of earthworms and dose of application interact during vermicomposting.

2. Material and methods

2.1. Slurries

Fresh pig slurry was obtained from a pig-breeding farm near the University of Vigo, NW Spain. Pig slurry was homogenized in a slurry pit, then stored in sealed plastic containers and maintained at 5 °C until use.

2.2. Vermireactor set-up and functioning

Vermireactors comprised modules that were added sequentially. The modules were made of PVC and resembled sieves. The external diameter of each was 30 cm and the mesh size 5 mm, which allowed mobility of earthworms between modules. Each vermireactor was initially composed of one module containing vermicompost, 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). New modules containing the same amount of fresh pig slurry were added when required; this procedure allowed us to date the addition of each module within the vermireactors.

2.3. Experimental design and sampling method

We set up a batch of twelve vermireactors, six for each low and high dose of pig slurry, and three without earthworms (control) and three containing 500 mature earthworms (*Eisenia fetida*) for each dose. At the end of the experiment (i.e., after 36 weeks), the vermireactors comprised 12 modules with an increasing gradient of age, resembling a soil profile, from upper to lower layers as follows: 2, 4, 7, 8, 11, 18, 21,25, 27, 29, 33 and 36 weeks.

At each sampling time, the vermireactors were dismantled and the modules isolated to avoid earthworm escape. The earthworms were then manually removed from the substrate, weighed and counted; we found earthworms only in layers of 2, 4, 7, 8, 11 and 18 weeks of age (Aira et al., 2006). In order to better understand the effects of rate, earthworms and time on the decomposition of pig slurry, the figures represent the values of the initial or raw pig slurry, the mean of the values corresponding to 2–18-week-old layers (layers in which earthworms were present at sampling time) and the mean of values corresponding to 21–36-week-old layers (oldest layers without earthworms at sampling time). Five samples of substrate per module were taken at random and gently mixed for biochemical analyses, i.e., organic matter content, total C, microbial biomass C and basal respiration.

2.4. Analytical procedures

Total C was measured on oven-dried (60 °C) samples with a Carlo Erba NA 1500 C/N analyzer. Microbial biomass C (C_{mic}) was determined by the chloroform fumigation–extraction method (Vance et al., 1987) with field-moist samples (5 g fresh weight) and was estimated as the difference between the organic C extracted from the fumigated and from the non-fumigated sample, multiplied by the K_2SO_4 extract efficiency factor for microbial C ($k_c = 2.64$; Vance et al., 1987). The filtered extracts (0.5 M K_2SO_4) of both fumigated and unfumigated samples were analyzed for soluble organic C by use of a Microplate Reader (Bio-Rad Microplate Reader 550, 590 nm). Microbial activity was assessed by measuring the rate of CO_2 evolution from the sample during 6 h of incubation. The evolved CO_2 was trapped in 0.02 M NaOH and subsequently measured by titration with HCl to a phenolphthalein endpoint, after adding excess $BaCl_2$ (Anderson, 1982).

2.5. Statistical analysis

Data were analyzed by split plot repeated measures ANOVA (RM-ANOVA) with single reactors as subjects, rate of application of pig slurry (1.5 and 3 kg) and earthworm treatment (presence and absence) fixed as between subject factors and week (i.e., each single module) fixed as within subject factor. This model assumes correlation between treatment levels within a block, i.e., the modules of each vermireactor (von Ende, 2001). When sphericity assumptions (Mauchly's test) could not be met we used Huynh–Feldt correction of P whenever values of ε were close to 1 (Potvin et al., 1990). All statistical analyses were performed with SPSS version 11.5 software.

3. Results

The mean number of earthworms per reactor at the end of the experiment (after 36 weeks) was 2800 ± 200, with a mean biomass of 700 ± 30 g, i.e., more than a 5- and 8-fold increase over the initial population of 500 mature earthworms (mean biomass 90 ± 10 g). Earthworms were mainly located in the younger layers and two groups of layers were distinguished on the basis of their density. The first group was formed by 2- and 4-week-old layers with over 1000 earthworms per layer, respectively. The second group was formed by 7-, 8-, 11- and 18-week-old layers, with decreasing numbers of earthworms, from 200 to 30, with increasing age of layers. No earthworms were found in the remaining layers.

The vermicomposting process was characterized by a continuous and significant loss of C over time (i.e., age of layers), and this loss did not depend on the rate of application of pig slurry (Fig. 1a, b; Table 1). Loss of C was higher in the reactors without earthworms and fed with 3 kg of pig slurry, a trend that was the opposite of that shown by reactors containing earthworms, in which the loss of carbon was higher in the reactors fed with 1.5 kg of pig slurry; this resulted in a significant interaction between dose of application of manure and presence of earthworms (Table 1). Earthworms significantly enhanced the loss of C, but this effect clearly depended on the age of layers, and was much higher in 21–36-week-old layers than in 2–18-week-old layers; this produced a significant interaction between age of layers and earthworms (Fig. 1a, b; Table 1).

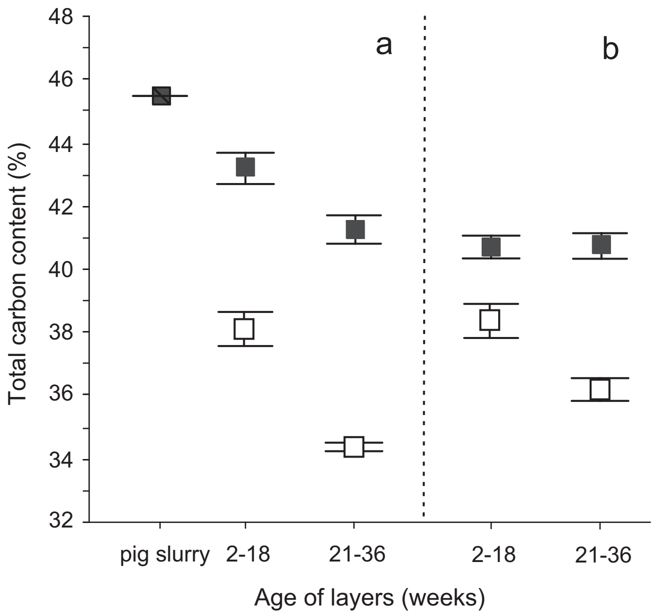

Fig. 1. Changes in total C content (mean ± S.E.) in layers of reactors fed with 1.5 kg (a) and 3 kg (b) of pig slurry with (white squares) and without earthworms (black squares). The vertical distributions of the variable values corresponding to the age of layers of pig slurry (from 2 to 36 weeks) are shown on the *y*-axis.

Earthworms enhanced the C_{mic} of the pig slurry during vermicomposting; thus, in layers with earthworms (2–18-week-old layers) C_{mic} reached values close to or higher than in the fresh pig slurry (1.5 and 3 kg dose, respectively) (Fig. 2a, b). However, in the older layers (21–36-weeks old), earthworms produced a decrease in C_{mic} values, resulting in a significant interaction between age of layers and earthworms (Table 1). The dose of pig slurry applied had no effect on C_{mic}.

Microbial activity (basal respiration) was higher in the system to which the highest doses of pig slurry were added, but decreased significantly over time during the vermicomposting process (Fig. 3a, b; Table 1). Earthworms significantly affected the changes in basal respiration of

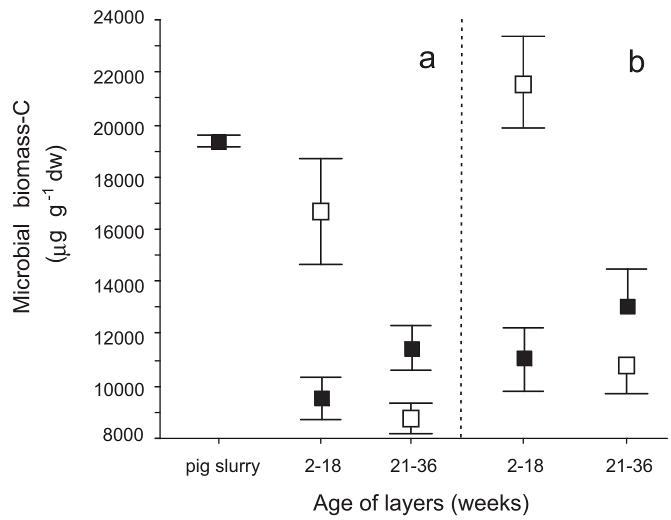

Fig. 2. Changes in microbial biomass C (mean ± S.E.) in layers of reactors fed with 1.5 kg (a) and 3 kg (b) of pig slurry with (white squares) and without earthworms (black squares). The vertical distributions of the variable values corresponding to age of layers of pig slurry (from 2 to 36 weeks) are represented on the *y*-axis.

Table 1
Repeated measures ANOVA for both microbial biomass and activity measurements

Source of variation	Rate of C loss			Microbial biomass C			Basal respiration		
	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Between subjects									
Rate of application	1.8	1.34	ns[a]	1.8	4.76	ns	1.8	48.39	<0.001
Earthworm presence	1.8	355.12	<0.0001	1.8	7.85	<0.05	1.8	23.18	<0.01
Dose × earthworm	1.8	29.21	<0.01	1.8	0.74	ns	1.8	1.15	ns
Within subjects									
Age of layers	11.88	9.24	<0.0001	11.88	5.73	<0.01[b]	11.88	14.56	<0.0001
Age × earthworm	11.88	2.97	<0.01	11.88	8.77	<0.001[b]	11.88	7.49	<0.0001
Age × dose	11.88	1.91	ns	11.88	1.55	ns[b]	11.88	1.70	ns

Rate of application of pig slurry (1.5 and 3 kg per layer), earthworm (presence/absence) and age of layers were fixed as factors and analyzed in a full factorial model.

[a]ns, not significant.

[b]Huynd and Feldt corrected values of *P*.

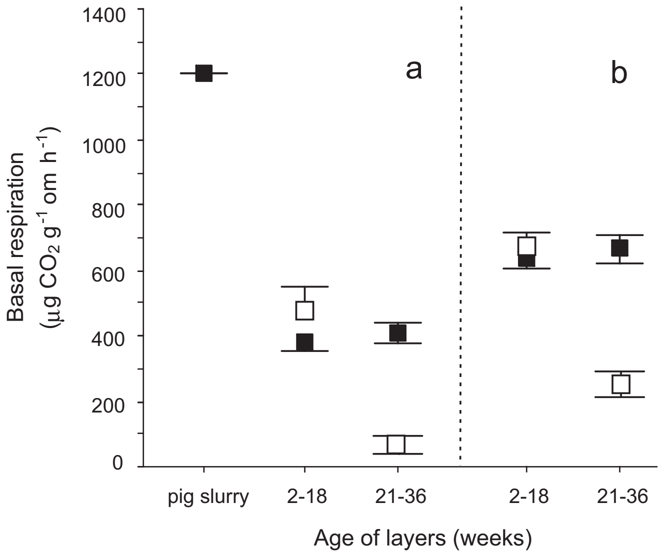

Fig. 3. Changes in basal respiration (mean ± S.E.) in layers of reactors fed with 1.5 kg (a) and 3 kg (b) of pig slurry with (white squares) and without earthworms (black squares). The vertical distributions of the variable values corresponding to the age of layers of pig slurry (from 2 to 36 weeks) are represented on the *y*-axis.

pig slurry during vermicomposting (Fig. 3a, b; Table 1); thus, values of basal respiration were higher in young layers (2–18-weeks old) than in old layers (21–36-weeks old), which resulted in a significant interaction between age of layers and presence of earthworms (Table 1).

4. Discussion

The results show that vermicomposting of pig slurry was characterized by a continuous loss of C, but there were no great differences in the rate at which the process occurred in terms of dose of manure or presence of earthworms. Thus, the presence of earthworms doubled the rate of C loss (440 and 400 $\mu g\, g^{-1}\, day^{-1}$ for reactors with the low and high doses of manure) with respect to reactors without earthworms (206 and 246 $\mu g\, g^{-1}\, day^{-1}$ for reactors with low and high doses of manure). Although earthworms had an overall strong effect on C loss during vermicomposting this effect only appeared in the oldest layers (21–36-weeks old) whereas in the layers with earthworms (2–18-week-old layers), with higher microbial biomass and respiration rates, there were no apparent differences in the total C contents.

It has been suggested that at earlier stages of decomposition earthworms only mobilize a limited fraction of labile C, which would thus limit microbial metabolism (Saetre, 1998), and it has also been reported that microorganisms and earthworms are limited for these C resources (Tiunov and Scheu, 2004). However, we observed much lower total C contents even in the youngest layers of reactors containing earthworms, than in fresh pig slurry, which suggests that in the youngest layers earthworms and microorganisms mineralized both the labile and recalcitrant fraction of C of pig slurry.

The high rates of respiration recorded in the reactors fed with the high dose of manure, 1.4 and 2.5 times higher (2–18- and 21–36-week-old layers, respectively) than in the reactors fed with the low doses of manure did not lead to a corresponding loss of C, since the greatest losses of C occurred in the reactors fed with the low doses of manure. Unlike in the reactors fed with the low doses of manure, in which there were layers in which the substrate almost disappeared completely, in the reactors fed with the high doses of manure, there appeared to be enough pig slurry to enable growth of earthworms and microorganisms, as revealed by the differences in microbial biomass and activity. Moreover the earthworms' contribution to respiration of the system is usually low, ranging between 6% and 30% at high densities of earthworms (Edwards and Arancon, 2004). Nevertheless, we did not find any differences in earthworm populations in reactors fed with the different application rates of manure, so this could not explain the observed differences in mineralization of C.

The results are in consistent with the general hypothesis that earthworms accelerate the rate of C mineralization (Atiyeh et al., 2000; Dominguez et al., 2003; Aira et al., 2006); more recently Triphati and Bhardwaj (2004) reported similar reductions in total C content in vermicomposting experiments with the earthworms *E. fetida* and *Lampito mauritii* fed with slurries and mixtures of organic residues, respectively. Furthermore, it has been demonstrated that mineralization of C is enhanced in earthworm casts and that it decreases with ageing (Aira et al., 2005), and similar results were found in other earthworm structures such as burrows and middens (Bohlen et al., 1997). The data are consistent with these findings, since in young layers casting would be higher than in older layers where ageing of the casts would be the predominant process.

5. Conclusions

We conclude that as regards mineralization of carbon, the rate of application of residue seems to be of little or no importance, because there were no great differences in C content during vermicomposting. However, we suggest addition of low doses of manure if the objective of vermicomposting is the microbiological stabilization of the organic residue.

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