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Reduction of total coliform numbers during vermicomposting is caused by short-term direct effects of earthworms on microorganisms and depends on the dose of application of pig slurry

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ABSTRACT

During vermicomposting of organic waste, the interactions between epigeic earthworms and the detrital microbial community lead to decreases in the abundance of some potentially pathogenic microorganisms. Despite its importance, little is known about the mechanisms involved and the factors that affect the intensity of this effect. In the present study, we carried out three experiments to test the effect of the earthworm *Eisenia fetida* on total coliform numbers in pig slurry. We firstly applied low and high doses (1.5 and 3 kg, respectively) of pig slurry to small scale vermireactors with and without earthworms. We found that *E. fetida* significantly reduced total coliform numbers after 2 weeks, but only in the low dose vermireactors. In a subsequent feeding experiment in mesocosms, we observed that the coliform population was reduced by 98% after passage through the earthworms' guts, which suggests that digestive processes in the gut of *E. fetida* are the main factors involved in the decrease in total coliforms observed in the low dose vermireactors. Decreases in total coliform numbers were not related to decreases in bacterial biomass, which indicates a specific negative effect of earthworms on the coliforms. In the third experiment, we tested the indirect effect of earthworms on total coliforms by inoculating pig slurry with either 2 or 10% vermicompost. The addition of vermicompost did not affect the number of coliforms either after 15, 30 or 60 days, which supports the idea that this bacterial group is more affected by the passage through the gut of *E. fetida* than by interactions with the earthworm-shaped microbial community.

1. Introduction

Vermicomposting is defined as an accelerated process of biooxidation and stabilization of organic wastes that involves interactions between earthworms and microorganisms (Domínguez, 2004). These interactions lead to changes in microbial biomass and activity related to changes in the structure of the microbial community (Lores et al., 2006; Aira et al., 2007a). The elimination of pathogenic microorganisms, particularly human ones, is an additional consequence of this process. Although vermicomposting has been shown to reduce the number of human pathogenic microorganisms in a variety of organic wastes (Eastman et al., 2001; Contreras-Ramos et al., 2005; Craig and Ankers, 2006), little is known about the factors involved in this.

Earthworms can interact with the soil microbial community either directly or indirectly through their feeding, burrowing and casting activities (Lavelle and Spain, 2001). Digestion of the ingested material, which occurs in a time scale of hours, is the first step in this interaction process. Specific microbial groups can respond differently to the gut environment (Schönholzer et al., 1999; Byzov et al., 2007) and selective effects on the presence and abundance of soil microorganisms have been found during the passage of material through the guts of earthworms (Pedersen and Hendriksen, 1993; Karsten and Drake, 1995). The interaction between the microorganisms delivered in the earthworm casts and the surrounding environment constitutes a further step in the earthworm-microorganism interaction (Domínguez, 2004). In the field, accumulation of casts and plant litter in small patches results in hot-spots of increased microbial activity and C assimilation (Bohlen et al., 2002). In vermicomposting systems, where the earthworm populations are raised at higher densities than in the field (Edwards and Bohlen, 1996), the gut and cast associated processes may play a key role in determining the characteristics of the microbial community (Domínguez, 2004). However, almost nothing is known about the relative contribution of these two types of processes to the observed changes in microbial populations during the decomposition of organic matter. In addition, vermicomposting, and decomposition of organic matter in general, are donor-controlled processes (Pimm, 1982), in which the rate of detrital input is expected to be a major factor influencing the interactions within the decomposer community. The availability of resources will shape the

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relationships between earthworms and microorganisms, but the outcome of these interactions may also depend on the microbial group considered (Brown and Doube, 2004).

In animal manures, potentially pathogenic microorganisms are a common fraction of the microbial community (Zhu, 2000; Sidhu and Toze, 2009). The group of bacteria known as total coliforms constitutes a good example of this. Total coliforms are all aerobes although many are facultative anaerobes, and are Gram-negative, non-spore-forming, rod-shaped bacteria that develop a red colony with a metallic sheen within 24 h at 35 °C on an Endo-type medium containing lactose (Clesceri et al., 1998). Coliforms are present in large numbers in the intestinal flora of most warm-blooded animals, and therefore their presence in the environment is associated with sources of fecal contamination. Because of this, they are used as an indicator of the potential presence of entero-pathogens, such as *Escherichia coli* O157:H7, in water and soil environments (Rompré et al., 2002; Sidhu and Toze, 2009). Although coliforms may also be found in the soil environment as part of the native microflora (Geldreich et al., 1962; Duncan and Razzell, 1972; Byappanahalli and Fujioka, 1998), their presence in agricultural amendments represents a potential threat and their screening is of special relevance in vermicomposts produced from animal manures (Smith, 2001; Contreras-Ramos et al., 2005).

In the present study, we performed three experiments to explore how the earthworm *Eisenia fetida* affects the abundance of total coliforms during the vermicomposting process. In the first experiment, we used small scale reactors to investigate the effect of the presence of earthworms, time and application rates of pig slurry on the number of total coliforms and bacterial biomass C. In the second experiment, we compared the density of total coliforms and the bacterial biomass C in the gut content of *E. fetida* and in the initial waste provided as a food source to the earthworms. Finally, in the third experiment we inoculated pig slurry with vermicompost to test for negative effects of worm-worked material on total coliforms. We discuss our results in relation to the relative contribution of direct and indirect effects of earthworms to the suppression of potentially pathogenic microorganisms.

2. Materials and methods

2.1. Earthworms, pig slurry and vermicompost

Specimens of the lumbricid earthworm *Eisenia fetida* (Savigny, 1826) were obtained from stock cultures reared under laboratory conditions (20 ± 2 ºC). Fresh pig slurry was used as food source for the earthworms and was obtained from a pig-breeding farm near the University of Vigo. The solid fraction (15% dry weight) of the slurry was selected in order to avoid any harmful effects that percolates may have on earthworms. The slurry was homogenized, and stored in sealed plastic containers at 5 ºC until use. Vermicompost, a stabilized peat-like material with low C:N ratio, was used as amendment in the inoculation experiment. The vermicompost was obtained from the *E. fetida* cultures reared in the laboratory with pig slurry as breeding medium. The main physicochemical characteristics of both the pig slurry and the vermicompost are shown in Table 1.

Table 1
Physicochemical characteristics of the pig slurry and the vermicompost used in the experiments.

	Pig slurry	Vermicompost
Moisture content (%)	85 ± 2	80 ± 1
Organic matter content (%)	86 ± 1	58 ± 2
pH	8.3 ± 1.0	6.2 ± 1.0
Electrical conductivity (mS cm^{-1})	0.25 ± 0.01	0.44 ± 0.08
Total nitrogen (mg g^{-1} dw)	24 ± 2	35 ± 2
$N\text{–}NH_4^+$ (μg g^{-1} dw)	2400 ± 100	56 ± 17
$N\text{–}NO_3^-$ (μg g^{-1} dw)	250 ± 50	271 ± 28
Total carbon (mg g^{-1} dw)	455 ± 60	363 ± 5
Dissolved organic carbon (μg g^{-1} dw)	11.1 ± 0.1	90 ± 11

dw = dry weight.

2.2. Experimental set up 1: Changes in total coliforms during vermicomposting as affected by time, presence of earthworms and the application rates of pig slurry

Temporal changes in the total coliform numbers in the pig slurry were studied by use of continuous feeding vermireactors (Aira et al., 2006). These small scale reactors were formed by PVC modules resembling sieves, with an external diameter of 30 cm. The mesh size was 5 cm, which allowed mobility of earthworms between modules. To set up the reactors, a module with fresh pig slurry was placed on top of another module containing vermicompost and earthworms. The pig slurry was applied as doses of 1.5 or 3 kg fresh weight (low and high doses, respectively). New modules with the same amount of pig slurry were added sequentially following the feeding activity of the earthworm population. This procedure allowed the addition of each module to be dated within the reactors.

The experimental set up consisted of twelve of the above-mentioned reactors. Six of them were provided with a low dose of pig slurry and the other six with a high dose. For each dose, three reactors were inoculated with 500 mature specimens of *E. fetida* and three remained without earthworms (control). By adding 500 earthworms to the vermireactors, we got an initial density ≈ 7000 ind. m^{-2}, which is within the range observed for *E. fetida* in the field (Monroy et al., 2006). After 36 weeks the reactors provided modules of increasing age, resembling a time profile. Twelve modules, added after 2, 4, 7, 8, 11, 18, 21, 25, 27, 29, 33 and 36 weeks were dismantled and isolated to avoid earthworm escape. The earthworms were then manually removed from the substrate, counted and weighed. Samples were taken from all the modules in order to quantify the bacterial biomass C. The total coliform numbers were determined in the modules added 2, 4, 7, 8, 25 and 36 weeks after the start of the experiment.

2.3. Experimental set up 2: changes in density of total coliforms after transit through the gut of E. fetida

Five mesocosms consisting of 3 L plastic containers were filled to three quarters of their capacity with sieved (>2 mm) and moistened (80% moisture content) vermiculite. Vermiculite is a hydrated silicate mineral resembling mica and does not contain any organic nutrients, which thus obliged the earthworms to ingest the pig slurry provided. Each of the mesocosms was inoculated with 50 mature specimens of *E. fetida*. A plastic mesh (1 cm pore size) was placed over the surface of the vermiculite and 200 g (fresh weight) of pig slurry were placed on top of the mesh, to avoid mixing the pig slurry with the vermiculite and to facilitate removal of the slurry. Mesocosms were checked every three days, the pig slurry was replaced and the vermiculite washed to prevent earthworms from ingesting casts. The mesocosms were maintained at a constant temperature (20 °C) in a scientific incubator and were covered with perforated aluminum foil to avoid desiccation of the pig slurry.

After 1 week, the earthworms were removed from the mesocosms, washed three times with sterile distilled water, and the gut contents released by gently pressing the bodies of intact worms with tweezers, from the last third to the posterior end (Bonkowski and Schaefer, 1997; Horn et al., 2003). The gut content corresponding to the final section of the intestine, the hindgut, was thus obtained. Several gut contents from earthworms from the same mesocosms were pooled to obtain samples that weighed approximately 50 mg (fresh weight). These samples were analyzed to estimate total coliform and total bacteria numbers.

2.4. Experimental set up 3: Effect of the inoculation of vermicompost on total coliform numbers in pig slurry

Portions of 100 g (fresh weight) of pig slurry inoculated with 0, 2.5 and 10% of vermicompost were incubated in 200 mL plastic containers at 20 ºC in a scientific incubator. The inoculation of vermicompost was carried out by replacing 2.5 or 10% of the pig slurry in the containers with the corresponding amount of vermicompost. Both the slurry-vermicompost mixtures and the portions of slurry with 0% of vermicompost were gently homogenized to control for handling effects. The containers were covered with perforated plastic lids to prevent the pig slurry from desiccating. The different mixtures were destructively sampled after 0, 15, 30 and 60 days. Five replicates were established for each combination of inoculum × time. Total coliform numbers and microbial biomass C were estimated for all treatment combinations. Due to an analytical eventuality, data for microbial biomass C are only provided for samples at 0, 30 and 60 days.

2.5. Microbial analysis

The total coliform numbers were estimated by the modified Membrane Filtration Technique (Clesceri et al., 1998; EPA, 2000). Samples of 1 g of pig slurry or 30–60 mg of gut content from the earthworms were suspended at 1:100 in sterile saline. Aliquots of 100 µL from each sample were diluted in 100 mL of sterile saline and then filtered through membrane filters. The filters were incubated in Petri dishes with Chromocult® Coliform Agar (Merk KGAA, Darmstad, Germany) for 24 h in an incubator chamber at 37 ºC. The total coliform numbers were then estimated by counting the number of colony forming units (CFU), at 140× under a dissecting microscope.

Microbial biomass C was determined by the chloroform fumigation-extraction method (Vance et al., 1987) with 5 g (fresh weight) samples. The filtered extracts (0.5 M K_2SO_4) of both fumigated and unfumigated samples were analyzed for soluble organic C with a Microplate Reader (Bio-Rad Microplate Reader 550, 590 nm). Microbial biomass C was estimated as the difference between the organic C extracted from the fumigated and that from the unfumigated sample, multiplied by the K_2SO_4 extract efficiency factor for microbial C ($k_c = 2.64$) (Vance et al., 1987).

The ergosterol present in the pig slurry was extracted by microwave-assisted extraction (Young, 1995) and quantified by high-performance liquid chromatography (HPLC). The detailed procedures of the analysis were as described by Aira et al. (2006). Values of ergosterol were converted to fungal biomass C by application of the conversion factor of 5.4 mg ergosterol g^{-1} microbial biomass C reported by Klamer and Bååth (2004) for compost samples. Bacterial biomass C was then calculated as the difference between overall microbial and fungal biomass C (Aira et al., 2006).

2.6. Statistical analysis

Data were analyzed by use of R environment (R Development Core Team, 2008). In experiment 1, a repeated-measures analysis of variance (ANOVAR) was performed on the bacterial biomass C and total coliform data. A mixed model was assumed, where the repeated subject (the vermireactor) was random and the remaining effects were fixed (Potvin et al., 1990). For bacterial biomass C, the model was analyzed including the Error( ) statement within the aov( ) function (Venables and Ripley, 2002), with the repeated subject (the vermireactor) mean square as the appropriate error term. Violation of the sphericity assumption (Maulchy's test) was amended by application of the Huynh-Feldt correction to the significance level (Potvin et al., 1990). For the total coliform numbers, a non-parametric split-plot analysis was carried out due to strong violation of the sphericity assumption even after data transformation. The between-subject effects, dose, earthworm and dose × earthworm, were tested by use of the Wilcoxon rank sum test. The within-subject effects, time, dose × time and earthworm × time, were tested by means of the Friedman test. The dose × earthworm × time effect could not be computed with this method. To allow the test of the different effects, data were summed in the several ways described in Potvin et al. (1990).

In experiment 2, coliform data were not normally distributed after transformation and the differences in both total coliforms and bacterial biomass C between the fresh pig slurry and the gut content of the earthworms were tested with the Wilcoxon rank sum test. In experiment 3, analysis of variance was carried out to test the effect of time and vermicompost inoculum on both the microbial biomass C and total coliforms. In this case, the values corresponding to microbial biomass C and total coliforms were, respectively, log- and $\log(x+1)$-transformed and the requirements of normal distribution and homogeneity of variance were achieved. Multiple comparisons were performed with the Tukey HSD test. The values shown throughout the text are means ± SE.

3. Results

3.1. Experiment 1

After 36 weeks, the mean population of *E. fetida* in the vermireactors with earthworms was 2800 ± 200 individuals, with a mean biomass of 700 ± 30 g. This meant a 5- and 8-fold increase respectively over the initial number and biomass of the inoculated population of *E. fetida*. There were no significant differences in the size of the earthworm population between reactors with low and high doses of manure. Seventy percent of the earthworms were located in the 2 and 4 week old manure layers, with ca. 1000 individuals per layer. The rest of the earthworms were distributed throughout the 7, 8, 11 and 18 week old layers, with no more than 200 individuals per layer. No earthworms were found in the remaining layers (21, 25, 27, 29, 33 and 36 weeks old).

The total coliform numbers were 5.7 times greater in the reactors with high dose of pig slurry than in those with low dose (Wilcoxon $W = 0$, $P = 0.002$). Total coliforms were more abundant in the young layers of the vermireactors (Friedman $\chi^2 = 18.89$, $P = 0.002$), especially in the reactors without earthworms (Fig. 1a,b). The presence of earthworms tended to decrease the number of coliforms, although there was no overall significant effect (Wilcoxon $W = 25$, $P = 0.31$). Nevertheless, when considered separately, the reactors with low dose

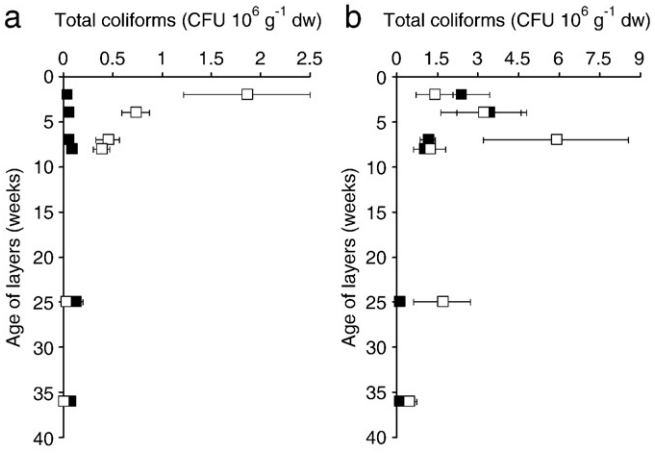

Fig. 1. Total coliform numbers in continuous feeding vermireactors ($n = 3$) fed with doses of 1.5 kg (a) and 3 kg of pig slurry (b) with *E. fetida* (filled symbols) and without *E. fetida* (open symbols). Variable values (means ± SE) corresponding to the age of the layers of pig slurry are shown on the *y* axis.

of pig slurry contained fewer coliforms when earthworms were present (Wilcoxon $W=9$, $P<0.05$; Fig. 1a).

In contrast to the observed differences in total coliforms, the dose of pig slurry had no effect on bacterial biomass C (ANOVAR $F_{1,8}=2.02$, $P=0.19$). Variation in bacterial biomass C depended on the presence of earthworms and the age of the layers (earthworm × time interaction, ANOVAR $F_{11,88}=4.23$, $P<0.001$). In the vermireactors containing earthworms, there was a 37% increase in bacterial biomass C in the layers where the earthworm population was located (2–18 weeks old). Nevertheless, the opposite effect was observed in the 21–36 weeks old layers, with 22% more bacteria in the reactors without earthworms (Fig. 2).

3.2. Experiment 2

In the fresh pig slurry, total coliforms constituted ≈0.1‰ of the bacterial population. The transit of the pig slurry through the gut of *E. fetida* reduced the total coliform numbers by 98% (Wilcoxon $W=25$, $P=0.008$), but had no effect on bacterial biomass C (Wilcoxon $W=9$, $P=0.89$; Fig. 3a,b).

3.3. Experiment 3

The initial number of total coliforms in the fresh pig slurry was 276 ± 29 10^3 CFU g^{-1} (dry weight). The presence of these microorganisms was significantly affected by incubation time (ANOVA $F_{3,46}=18.63$, $P<0.001$), with a decrease of 82% after the first 15 days (Fig. 4a). The population of coliforms recovered significantly after 60 days, although the numbers remained 2.5 times lower than the initial numbers ($P<0.001$). There was no effect of the inoculation of vermicompost on total coliform numbers (ANOVA $F_{2,46}=2.20$, $P=0.12$).

The microbial biomass C of the pig slurry was also affected by incubation time (ANOVA $F_{2,33}=16.09$, $P<0.001$). There was a peak in bacterial presence after 60 days, with an increase by 3.4 times with respect to the values at time 0 ($P<0.001$; Fig. 4b). As with total coliforms, the inoculation of vermicompost had no effect on the microbial biomass C (ANOVA $F_{2,33}=0.22$, $P=0.80$).

4. Discussion

In the first experiment, we found that the presence of *E. fetida* accelerated the decline in bacterial numbers during vermicomposting. The data indicate that there were two stages in this process, which were related to the presence of earthworms in the layers of the reactors. The first stage was characterized by an increase in bacterial biomass in the youngest layers, where the earthworm population was located. The second stage occurred in the oldest layers and was characterized by a decrease in bacterial biomass, due to the depletion

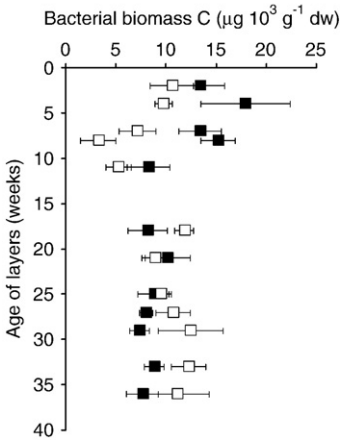

Fig. 2. Bacterial biomass C in continuous feeding vermireactors ($n=6$) with *E. fetida* (filled symbols) and without *E. fetida* (open symbols). Variable values (means ± SE) corresponding to the age of the layers of pig slurry are shown on the *y* axis.

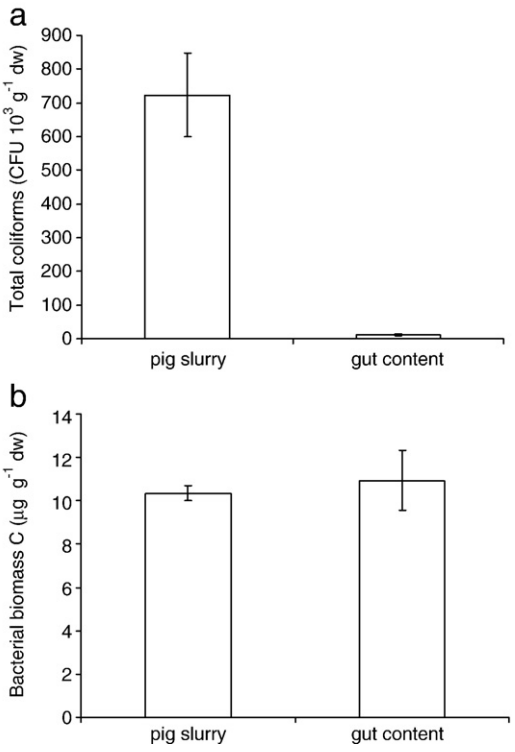

Fig. 3. Comparison of the content of (a) total coliforms ($n=5$) and (b) bacterial biomass C ($n=4$) in fresh pig slurry before and after digestion by the earthworm *E. fetida*. Values are means ± SE.

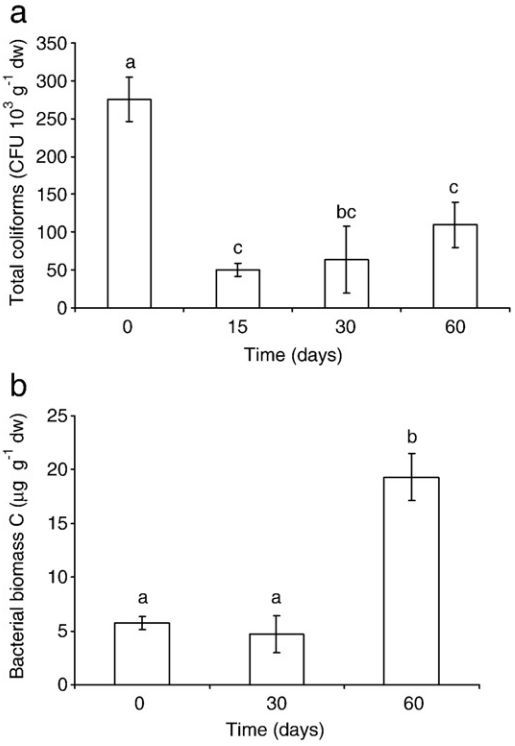

Fig. 4. Changes during incubation in the number of total coliforms (a) and microbial biomass C (b) in fresh pig slurry ($n=5$). Different letters indicate significant differences at $\alpha=0.05$ (Tukey HSD test). Values are means ± SE.

of nutrient pools (Domínguez, 2004; Aira et al., 2007a). The effect of earthworms on the whole bacterial population was independent of the dose of pig slurry applied. On the contrary, the interactions between *E. fetida* and specific bacterial groups such as total coliforms were dependent on the input of slurry. This suggests that in vermicomposting systems, changes in the structure and functioning of the microbial community may occur without affecting microbial biomass or decomposer activity (Aira et al., 2007b).

The activity of earthworms significantly reduced the total coliform numbers only with the low dose of slurry, suggesting a density dependent effect of earthworms on these bacteria. Such an effect is expected considering that there is a direct relationship between the ingestion of pig slurry by earthworms and the decrease in total coliforms (Monroy et al., 2008). After 2 weeks in the low dose reactors, the presence of *E. fetida* reduced the number of total coliforms and these microorganisms remained at low levels in the other layers, suggesting that the pig slurry processed by earthworms constitutes an unfavourable environment for the regrowth of coliforms. This may be explained by the decrease in the amount of assimilable C in the pig slurry caused by *E. fetida* (Aira and Domínguez, 2009), and also by competition between the remaining coliforms and other members of the microbial community (Byappanahalli and Fujioka, 2004).

Survival of total coliforms was greater in the high dose vermireactors, and their numbers were not consistently reduced by the presence of earthworms. The differences in survival may be due to differences in aeration in the reactors with different application rates of slurry. In the low dose reactors, the ratio between surface and volume of the pig slurry layer was greater than in the high dose reactors, and this would have favoured aeration of the substrate. Indeed, extended bacterial survival has been found in concentrated waste storages (Crane and Moore, 1986), and in wastewater sand filters, aeration negatively affects survival of fecal indicator microorganisms (Potts et al., 2004).

The inoculation of coliforms from uningested pig slurry into the fresh casts may be responsible for the lack of effect of earthworms on the number of total coliforms in the high rate reactors. Twice as much food was provided with the high dose of pig slurry than with the low dose, and casts produced by *E. fetida* in the layers of the high rate reactors were in contact with a larger amount of uningested pig slurry than casts produced in the layers of the low rate reactors. This may have favoured recolonization of total coliforms, since pathogenic microorganisms from fresh animal wastes may be able to recolonize biosolids with decreased levels of those microorganisms (Zaleski et al., 2005).

In the absence of earthworms, the rate of survival of total coliforms was lower in the reactors with the low dose of pig slurry. This reduction in viability may have enhanced the negative effect of *E. fetida* on this bacterial group. However, the decrease in the number of coliforms caused by the presence of earthworms was observed after 2 weeks, before the decline in the coliform population in the reactors without earthworms. These results indicate that the negative interaction between *E. fetida* and total coliforms may be a short term process. The passage through the gut of earthworms, which in *E. fetida* only takes between 2.5 and 7 h (Hartenstein et al., 1981), may explain the rapid decrease in total coliforms. The results of our experiment on gut transit further supported this hypothesis, since the decrease in coliforms caused by the presence of earthworms in the low dose reactors was entirely explained by the gut transit effect. In addition, the passage of the pig slurry through the gut of *E. fetida* did not reduce the total abundance of bacteria, providing evidence of a selective effect of *E. fetida* on total coliforms, which may be replaced by other bacterial groups. Accordingly, Pedersen and Hendriksen (1993) reported selective reduction of the coliform *E. coli* BJ18 in cattle dung during the passage through the gut of several species of the genus *Lumbricus*. This reduction did not affect other specific bacterial groups, resulting in shifts in the composition of the microbial community. The selective reduction of total coliforms observed in the present study after passage of the slurry through the gut of *E. fetida* may be caused by competitive interactions between coliforms and microorganisms that are specific to the earthworm gut (Brown and Mitchell, 1981). The presence of a specific microbial community in the digestive tract of earthworms is supported by recent analysis of the fatty acid composition of gut microorganisms from *Lumbricus terrestris* (Sampedro et al., 2006; Sampedro and Whalen, 2007). These gut microorganisms may outcompete ingested microbes because of better competitive abilities in the gut environment or selective suppressive activity of gut fluids against specific microbial groups (Byzov et al., 2007).

In the third experiment, the inoculation of vermicompost did not affect either the number of total coliforms or the microbial biomass in the pig slurry. The results further support the idea that the coliform die-offs that occur during the passage through the gut of earthworms constitute a major step in the interactions between earthworms and total coliforms (Monroy et al., 2008). We did not estimate the effect caused by the inoculation of vermicompost on the composition of the microbial community of the pig slurry. However, this effect, if present, had no effect on the number of coliforms. The initial decrease in total coliforms indicates that this bacterial group was sensitive to the experimental manipulation, but this initial decrease did not prevent subsequent regrowth of coliforms. Total coliforms increased independently of the inoculation treatment, suggesting that worm-worked material may only play a secondary role, if any, in the suppression of fecal indicator microorganisms.

Overall, we conclude that the passage of pig slurry through the gut of *E. fetida* can cause die-off of the total coliform population. This process is probably the main factor involved in the observed reduction in this bacterial group during vermicomposting of pig slurry in small scale reactors. However, removal of coliforms is only effective at low doses of slurry, suggesting that with high doses, both reinoculation from uningested pig slurry and regrowth on earthworm casts may allow coliform bacteria to overcome the negative effect of earthworms. Accordingly, there was no evidence of antagonistic interactions between the microorganisms inoculated with the vermicompost and the total coliforms in the pig slurry. These results may have important implications for optimization of the vermicomposting process and contribute to better understand the relationships between earthworms and specific microbial groups during the decomposition of organic matter.

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References

Aira M, Domínguez J. Microbial and nutrient stabilization of two animal manures after the transit through the gut of the earthworm *Eisenia fetida* (Savigny, 1826). J Hazard Mater 2009;161:1234–8.

Aira M, Monroy F, Domínguez J. *Eisenia fetida* (Oligochaeta, Lumbricidae) activates fungal growth, triggering cellulose decomposition during vermicomposting. Microb Ecol 2006;52:738–46.

Aira M, Monroy F, Domínguez J. *Eisenia fetida* (Oligochaeta: Lumbricidae) modifies the structure and physiological capabilities of microbial communities improving carbon mineralization during vermicomposting of pig manure. Microb Ecol 2007a;54:662–71.

Aira M, Monroy F, Domínguez J. Earthworms strongly modify microbial biomass and activity triggering enzymatic activities during vermicomposting independently of the application rates of pig slurry. Sci Total Environ 2007b;385:252–61.

Bohlen PJ, Edwards CA, Zhang Q, Parmelee RW, Allen M. Indirect effects of earthworms on microbial assimilation of labile carbon. Appl Soil Ecol 2002;20:255–61.

Bonkowski M, Schaefer M. Interactions between earthworms and soil protozoa: a trophic component in the soil food web. Soil Biol Biochem 1997;29:499–502.

Brown GG, Doube BM. Functional interactions between earthworms, microorganisms, organic matter and plants. In: Edwards CA, editor. Earthworm ecology. 2nd edition. Boca Raton: CRC Press; 2004. p. 213–39.
Brown BA, Mitchell MJ. Role of the earthworm, *Eisenia foetida*, in affecting survival of *Salmonella enteriditis* ser. *typhimurium*. Pedobiologia 1981;22:434–8.
Byappanahalli MN, Fujioka RS. Evidence that tropical soil environment can support the growth of *Escherichia coli*. Water Sci Technol 1998;38:171–4.
Byappanahalli MN, Fujioka RS. Indigenous soil bacteria and low moisture may limit but allow faecal bacteria to multiply and become a minor population in tropical soils. Water Sci Technol 2004;50:27–32.
Byzov BA, Khomyakov NV, Kharin SA, Kurakov AV. Fate of soil bacteria and fungi in the gut of earthworms. Eur J Soil Biol 2007;43:S149–56.
Clesceri LS, Greenberg AE, Eaton AD. Standard methods for the examination of water and wastewater, 20th Edition. Washington DC: American Public Health Association; 1998.
Contreras-Ramos SM, Escamilla-Silva EM, Dendooven L. Vermicomposting of biosolids with cow manure and oat straw. Biol Fertil Soils 2005;41:190–8.
Craig L, Ankers S. Vermiculture produces EQ class A biosolids at wastewater plant. BioCycle 2006;47:42–6.
Crane SR, Moore JA. Modeling enteric bacterial die-off: a review. Water Air Soil Poll 1986;27:411–39.
Domínguez J. State of the art and new perspectives in vermicomposting research. In: Edwards CA, editor. Earthworm ecology. 2nd edition. Boca Raton: CRC Press; 2004. p. 401–25.
Duncan DW, Razzell WE. *Klebsiella* biotypes among coliforms isolated from forest environments and farm produce. Appl Microbiol 1972;24:933–8.
Eastman BR, Kane PN, Edwards CA, Trytek L, Gunadi B, Stermer AL, Mobley JR. The effectiveness of vermiculture in human pathogen reduction for USEPA biosolids stabilization. Compost Sci Util 2001;9:38–49.
Edwards CA, Bohlen PJ. Biology and ecology of earthworms. London: Chapman & Hall; 1996.
EPA. Membrane Filter Method for the Simultaneous Detection of Total Coliforms and *Escherichia coli* in Drinking Water. Cincinnati: United States Environmental Protection Agency, Office of Research and Development; 2000.
Geldreich EE, Bordner RH, Huff CB, Clark HF, Kabler PW. Faecal coli-aerogenes flora of soils from various geographical areas. J Appl Bacteriol 1962;25:87–93.
Hartenstein F, Hartenstein E, Hartenstein R. Gut load and transit-time in the earthworm *Eisenia foetida*. Pedobiologia 1981;22:5-20.
Horn MA, Schramm A, Drake HL. The earthworm gut: an ideal habitat for ingested N_2O-producing microorganisms. Appl Environ Microbiol 2003;69:1662–9.
Karsten GR, Drake HL. Comparative assessment of the aerobic and anaerobic microfloras of earthworm guts and forest soils. Appl Environ Microb 1995;61:1039–44.
Klamer M, Bååth E. Estimation of conversion factors for fungal biomass determination in compost using ergosterol and PLFA 18:2ω6, 9. Soil Biol Biochem 2004;36:57–65.
Lavelle P, Spain AV. Soil ecology. Dordrecht: Kluwer Academic Publishers; 2001.
Lores M, Gómez Brandón M, Pérez Díaz D. Domínguez. J. Using FAME profiles for the characterization of animal wastes and vermicomposts. Soil Biol Biochem 2006;38: 2993–6.
Monroy F, Aira M, Domínguez J. Changes in density of nematodes, protozoa and total coliforms after transit through the gut of four epigeic earthworms (Oligochaeta). Appl Soil Ecol 2008;39:127–32.
Monroy F, Aira M, Domínguez J, Velando A. Seasonal population dynamics of *Eisenia fetida* (Savigny, 1826) (Oligochaeta, Lumbricidae) in the field. CR Biol 2006;329:912–5.
Pedersen JC, Hendriksen NB. 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 1993;16:227–32.
Pimm SL. Food webs. London: Chapman & Hall; 1982.
Potts DA, Gorres JH, Nicosia EL, Amador JA. Effects of aeration on water quality from septic system leachfields. J Environ Qual 2004;33:1828–38.
Potvin C, Lechowicz MJ, Tardif S. The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. Ecology 1990;71:1389–400.
R Development Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2008.
Rompré A, Servais P, Baudart J, M.R. de-Roubin, Laurent P. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. J Microbiol Meth 2002;49:31–54.
Sampedro L, Jeannotte R, Whalen JK. Trophic transfer of fatty acids from gut microbiota to the earthworm *Lumbricus terrestris* L. Soil Biol Biochem 2006;38:2188–98.
Sampedro L, Whalen JK. Changes in the fatty acid profiles through the digestive tract of the earthworm *Lumbricus terrestris* L. Appl Soil Ecol 2007;35:226–36.
Schönholzer F, Hahn D, Zeyer J. Origins and fate of fungi and bacteria in the gut of Lumbricus terrestris L. studied by image analysis. FEMS Microbiol Ecol 1999;28: 235–48.
Sidhu JPS, Toze SG. Human pathogens and their indicators in biosolids: a literature review. Environ Int 2009;35:187–201.
Smith L. The incidence of potentially pathogenic bacteria in liquor from selected wormfarms. Biol Fertil Soils 2001;34:215–7.
Vance ED, Brookes PC, Jenkinson DS. An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 1987;19:703–7.
Venables WN, Ripley BD. Modern applied statistics with S4th edition. New York: Springer-Verlag; 2002.
Young JC. Microwave-assisted extraction of the fungal metabolite ergosterol and total fatty acids. J Agric Food Chem 1995;43:2904–10.
Zaleski KJ, Josephson KL, Gerba CP, Pepper IL. Potential regrowth and recolonization of salmonellae and indicators in biosolids and biosolid-amended soil. Appl Environ Microb 2005;71:3701–8.
Zhu J. A review of microbiology in swine manure odor control. Agr Ecosyst Environ 2000;78:93-106.