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How earthworm density affects microbial biomas and activity in pig manure

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Abstract

We studied the influence of the earthworm, *Eisenia fetida*, on the microbial populations during the vermicomposting of pig manure. Fresh pig manure was placed in replicated boxes with (two densities, 25 and 50) and without earthworms for a period of 16 d. Samples were destructively collected periodically and analyzed for microbial biomass nitrogen, microbial respiration, substrate-induced respiration (SIR) and substrate dehydrogenase activity. Microbial biomass N, microbial respiration, SIR and substrate dehydrogenase activity were significantly lower in the earthworm treatments after 8 d.

Keywords: Dehydrogenase activity; Earthworms; Microorganisms; Pig manure; SIR

1. Introduction

Vermicomposting is a biooxidation and stabilization of organic material involving the joint action of earthworms and microorganisms. Although microbes are responsible for the biochemical degradation of organic matter, earthworms are the important drivers of the process, conditioning the substrate and altering biological activity. Invertebrates have indirect effects on the structure and activities of bacteria and fungal communities through inoculum dispersal, grazing, litter comminution, gut passage and aggregate formation [1]. Although the interactions between earthworms and microorganisms have received a considerable attention (see reviews by Brown [4]; Doube and Brown [5]), the fate of microorganisms during the gut transit through earthworms is still controversial [14]. There is evidence that earthworms digest bacteria [7,8] and also fungi [13]. Summarizing the existing knowledge, Doube and Brown [5] concluded that "earthworms have minimal capacity to digest organic residues and obtain nutrition by digestion of microorganisms associated with ingested organic matter". However, manipulating the food resources of the decomposer community of a beechwood on limestone, Scheu and Schaefer [11] concluded that earthworms are limited by the availability of labile carbon resources for which they appear to compete with soil microorganisms.

The main objective of this study was to monitor the prime short-time changes in the microbial populations of pig manure during processing by the epigeic earthworm, *Eisenia fetida* (Savigny, 1826) under controlled environmental conditions. By determining the indirect measurements of both bacteria and fungi in this microbial-rich substrate, we expected to better understand the effects of gut transit on the microbial populations. In addition to studying two different population densities, we tried to know whether these effects were in relationship to the earthworm density.

2. Materials and methods

Pig manure (100 g f m) with approximately 75% moisture content was placed in 200 ml plastic containers. The earthworms, adult specimens of *E. fetida*, were added to the containers in three densities: 0, 25 and 50 earthworms. All containers were covered with perforated lids, and main

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tained in the laboratory at 24 ± 2 °C for a period of 16 d. There were three replicate containers for each treatment, giving a total of 45 dishes. A subset of dishes was destructively sampled after 1, 2, 4, 8 and 16 d The pig manure from each plastic container was removed and the recovered earthworms were counted and weighed. The manure was mixed gently, then subsampled to determine the rate of biological activity (basal respiration), microbial biomass nitrogen (Bio-N), substrate-induced respiration (SIR) and substrate enzyme activity (dehydrogenase).

The moisture content of the manure was determined by drying it at 60 ºC for 3 d and the ash content by heating at 550 ºC for 4 h.

The microbial biomass N was measured using the chloroform fumigation–direct extraction method [3]. The rate of biological activity was determined by measuring the rate of CO_2 evolution from the sample during a 6 h 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 $BaCl_2$ [2]. Microbial activity was determined by measuring SIR and dehydrogenase activity. SIR was determined as the rate of CO_2 evolution during a 6 h incubation after adding 5 ml of glucose solution (32 mg ml^{-1}) to the manure. The CO_2 evolved was trapped by 0.02 N NaOH, precipitated with 3 N $BaCl_2$, and then titrated with 0.02 N HCl with phenolphthalein. Dehydrogenase activity was measured using a modified method of Tabatabai and Bremner [12]. Three per cent 2,3,5-triphenyl-tetrazolium chloride (TTC) was used as substrate. The reduced TTC (triphenyl formazan) was measured in a spectrophotometer at 490 nm.

One-way analysis of variance (ANOVA) and separation of means based on the least significant difference (LSD, $P \leq 0.05$) allowed the determination of significant differences between the control (no earthworms) and the earthworm treatments. All statistical tests were evaluated at the 95% confidence level.

3. Results

Earthworm biomass remained stable during the study. Mortality was relatively low (i.e. < 2% for all the microcosms) until day 8. After 16 d, mortality was higher (17.6% and 28% for the 25 and 50 earthworm microcosms, respectively).

Earthworms had a negative effect on microbial biomass on day 8. A reduction in the Bio-N also occurred on day 4 in the 25 earthworm microcosms and although it was lower in the 25 earthworm treatment than in the controls (0 earthworms), these differences were not significant. After day 8, Bio-N increased progressively in the 50 earthworm treatment and remained relatively stable in the 25 earthworm treatment and in the control (Fig. 1).

Similarly as microbial biomass nitrogen was significantly affected, there was a reduction of the basal and

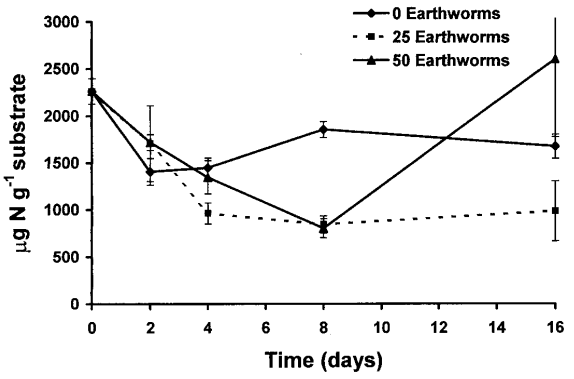

Fig. 1. Substrate microbial biomass N by earthworm-density treatment. Bars are ± SE.

substrate-induced respiration on day 8. The differences between the two earthworm treatments were not significant, except for the basal respiration on day 8 (Fig. 2). Basal and SIR increased after day 8 and was higher than it was initially in the earthworm treatments (Figs 2 and 3).

Dehydrogenase activity was significantly lower in the earthworm treatments than in the control from day 4 until

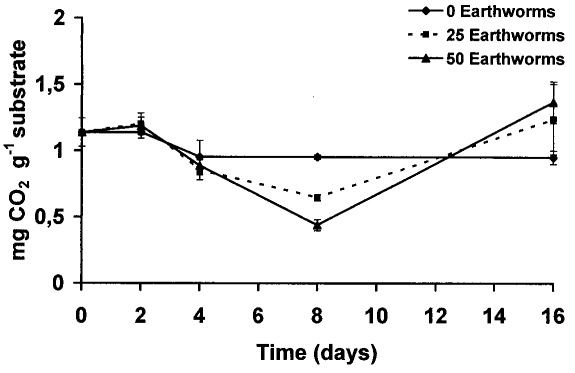

Fig. 2. The effect of the earthworm density on the total substrate respiration during the study period. Bars are ± SE.

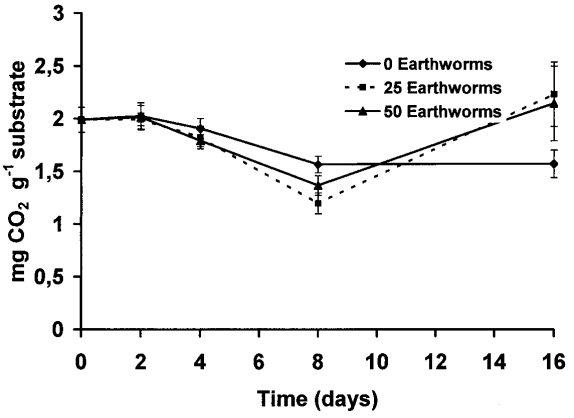

Fig. 3. Substrate-induced respiration by earthworm-density treatment. Bars are ± SE.

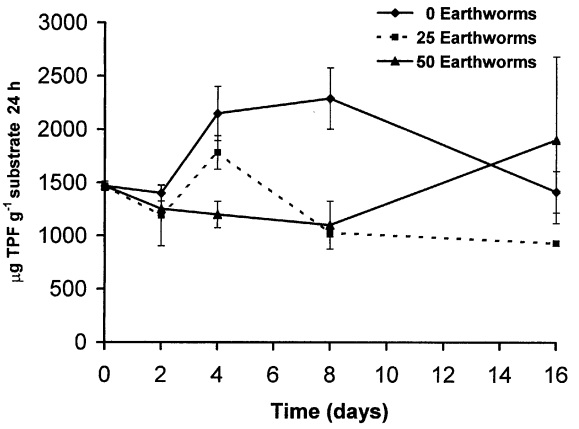

Fig. 4. Substrate dehydrogenase activity by earthworm-density treatment. Bars are ± SE.

the experiment ended on day 16. After 8 d, this enzymatic activity remained stabilized in the earthworm treatments and decreased in the control (Fig. 4).

4. Discussion

Our results indicate that earthworms modify the activity of the microorganisms during vermicomposting of pig manure in a short-time period. From a morphological point of view, the earthworm-worked material was totally processed into a heterogeneous mass of casts and fibre particles after 48 h of processing, whereas the material without earthworms remained in compact clumps. Within the first 8 d, earthworms and their associated microflora seemed to rapidly destroy most of the easily biodegradable substances, as may be indicated by the rapid reduction in the amount of CO_2 evolving from the manure and the stabilization of the dehydrogenase activity. Flushes of CO_2 production on day 16 in the presence of earthworms could be possibly attributed to the dead earthworms that might have been incorporated as fresh organic matter in the substrate. Other measurements such as Bio-N, SIR and dehydrogenase activity seem to indicate that earthworms were grazing on the microorganisms until day 8. Mortality occurring during the second week obscures the relationships between earthworm and microbial activities.

It is a well-known fact that earthworm activity in the soil enhances microbial population and biomass [6]. There is an increasing evidence that the microbial biomass changes little during the gut passage through various earthworm species [9,10,15]. This contradicts the assumption that microorganisms are a major constituent of the diet of earthworms as frequently assumed. However, in our study, we found important changes both in microbial biomass and in microbial activity caused by the action of the earthworms.

In summary, we have demonstrated that the first steps of vermicomposting, the passage of pig manure through the gut of the earthworm, *Eisenia fetida*, decrease microbial biomass and microbial activity. This has important implications in order to understand how this earthworm–microorganism interaction process works and it could help in the difficult task of learning about earthworm nutrition.

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