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Interactions between *Eisenia andrei* (Oligochaeta) and nematode populations during vermicomposting

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Summary

We studied the effect of the earthworm, *Eisenia andrei*, on the nematode community and on the microbial activity during the vermicomposting of two organic wastes, cow manure and sewage sludge. Fresh cow manure and sewage sludge was placed in five replicated boxes with and without earthworms for a period of 16 weeks. Samples were collected periodically and nematodes were extracted in Baermann funnels, counted and separated into different trophic groups. Samples were also analyzed for microbial respiration and microbial biomass nitrogen. Nematode communities were dominated by bacterivores, and their abundance was dramatically affected by earthworm activity. In both substrates, numbers of nematodes decreased in the presence of earthworms. Fungivore nematodes were observed after 6 weeks in the cow manure and in contrast to bacterial feeders, their number continuously increased in the treatment without earthworms but remained low in the presence of the earthworms. We did not detect a significant earthworm effect on microbial respiration but they significantly increased microbial biomass nitrogen in sewage sludge. Our results indicate that earthworms have a strong influence on nematode densities and nematode trophic structure, and that further investigations are necessary to determine the mechanisms of this influence.

Key words: Earthworms, *Eisenia andrei*, nematodes, vermicomposting, decomposition, cattle manure, sewage sludge

Introduction

Decomposition of organic matter under varying environmental conditions is a fundamental feature of terrestrial ecosystems. In vermicomposting, complex interactions between the organic waste, microorganisms, earthworms and other soil fauna animals result in the biooxidation and stabilization of wastes. A large variety of microorganisms and soil invertebrates proliferate and interact contributing to the "cycle of matter" in vermicomposting. The vermicomposting system sustains complex food webs, and at the same time, modifies different chemical forms of several nutrient elements into long-lived organic compounds, which are important for nutrient dynamics (Domínguez et al. 1997).

While microbes are responsible for the biochemical degradation of organic matter in the vermicomposting process, earthworms are important in conditioning the substrate and promoting microbial activity. Earthworms act as mechanical blenders since they break down organic material, increase the surface area ex-

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posed to microbes and move fragments and bacteria-rich excrements, thus homogenizing the organic material. Decomposition rates and soil biological activity may be affected by earthworm-induced physico-chemical changes to the resource base (e.g. litter, organic matter) (Lofty 1974; Mackay and Kladivko 1985; Scheu 1987; Tiwari et al. 1989). Specifically, earthworms can directly or indirectly exert influence on soil microflora and fauna populations via comminution, burrowing, casting activities, grazing and dispersal. Not only does the physico-chemical and biological status of the soil change through these activities but the characteristics of the drilosphere may also be dramatically altered (see reviews by Brown 1995; Doube and Brown 1998).

Yet, while both microbes and earthworms are essential to the vermicomposting process, it is the entire soil fauna community that plays an important role through its interactions with soil microbes. Thus, although previous studies have investigated the functional significance of microbial-earthworm interactions, little is known about the effects of earthworms on microfloral and faunal diversity. Therefore, a wholistic perspective of these interactions must be taken.

The aim of our experiments was to study the influence of the earthworm, *Eisenia andrei*, on the density and the trophic structure of the nematode community and on microbial activity during the vermicomposting of cow manure and sewage sludge.

Materials and Methods

Fresh cow manure was collected from the Waterman Dairy Farm at the Ohio State University, Ohio and sewage sludge was obtained from the Wastewater Treatment Plant of Columbus, Ohio. Five hundred g of both manure (77 % moisture; 414 mg g^{-1} C; 23 mg g^{-1} N) and sewage sludge (85 % moisture; 379 mg g^{-1} C; 63 mg g^{-1} N) were placed into each of 10 plastic boxes. Juvenile earthworms, *Eisenia andrei*, were added into five of the boxes and the other five containers remained without earthworms and served as controls. The initial number of earthworms was 25 per container (ca. 65 g). All containers were covered with perforated lids, and maintained in the laboratory at 24 ± 2 °C for a period of four months. After 1, 3, 6, 9, 13 and 16 weeks, approximately 15 g of manure were removed from each container, mixed gently, and sampled to determine moisture, ash content, total C and N content, microbial biomass and basal respiration.

Nematodes

Fifteen grams of substrate (fresh weight) were taken from each container for nematode extraction and placed on Baermann funnels for 48h at room temperature (McSorley and Walter 1991). Nematodes were counted live at a magnification of 140× under a dissecting microscope and sorted by trophic group using esophageal morphology as described by Parmelee and Alston (1986). The trophic groups only consisted of fungivores and bacterivores since no plant-parasitic, dorylaimid or mononchid nematodes were recovered.

Microbial biomass and activity

Microbial biomass N (BION) was measured from 1 g (fresh weight) subsamples using the chloroform fumigation-extraction method (Brookes et al. 1985). Biological activity was determined by measuring the rate of CO_2 evolution from 2 g (fresh weight) subsamples during a 24-hour 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).

Statistical analysis

Data was analyzed using repeated measures ANOVA in the general linear model (GLM) procedure using the SAS software (SAS Institute Inc. 1990).

Results

Nematodes

The density of bacterivore nematodes increased with time during the first 6 weeks in both cow manure and sewage sludge in the absence of earthworms. In their presence, the number of bacterivore nematodes presented the same pattern in sewage sludge but remained more or less stable in cow manure. Except for the earthworm + cow manure treatment, the density of bacterivore nematodes declined in both substrates after week 6 in all other treatments. However, it is remarkable that the number of bacterivore nematodes was always lower in presence of earthworms; a difference that was statistically significant after 10 weeks in both substrates (Fig. 1, Table 1). In cow manure, the density of bacterivore nematodes was as high as 1480 ± 240 nematodes g^{-1} dry weight at week 6 in the non earthworm treatment compared to 646 ± 276 nematodes g^{-1} dry weight at week 9 in the earthworm treatment. In contrast, nematode numbers were much higher in the sewage sludge

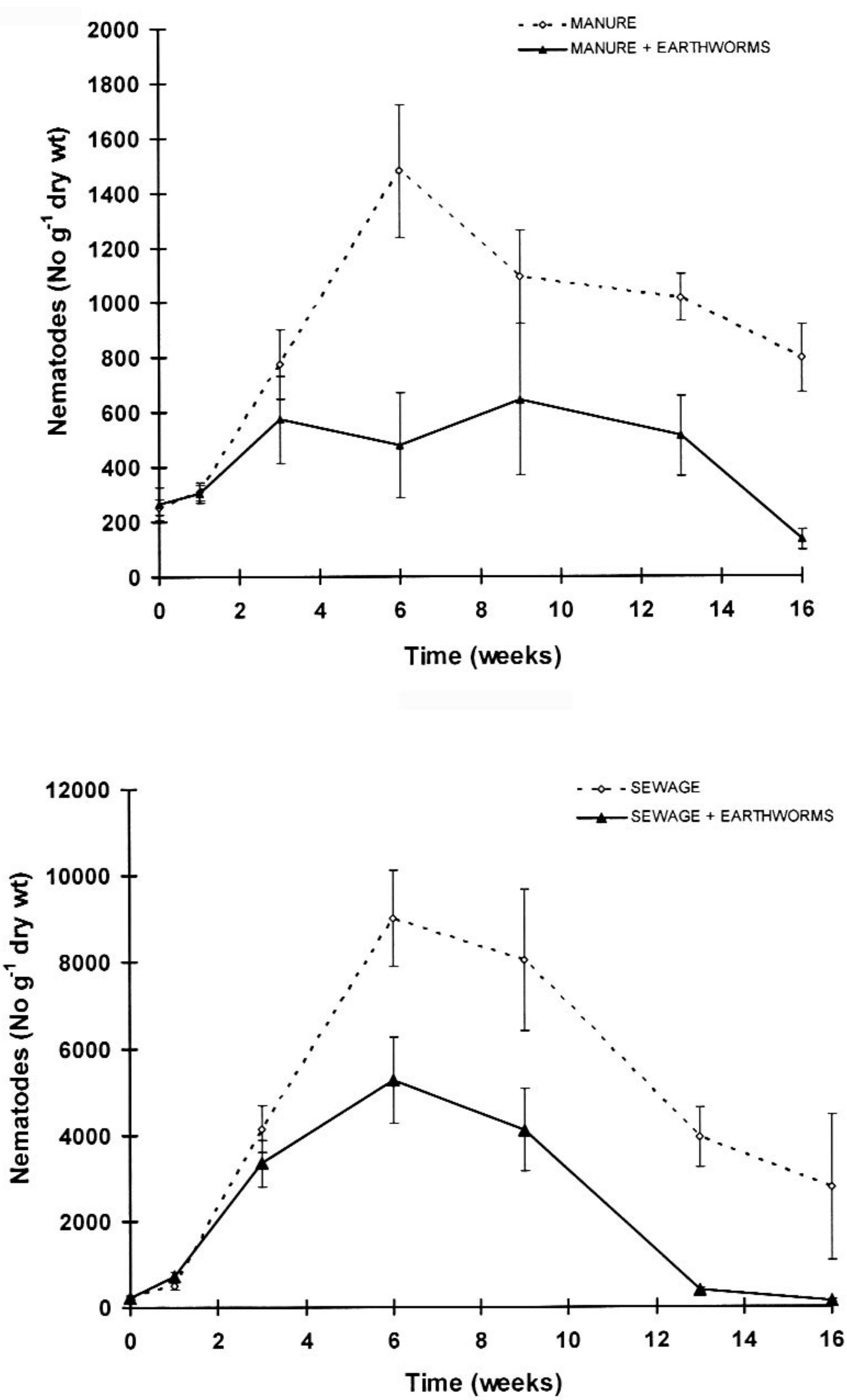

Fig. 1. Bacterivore nematode abundance (mean ± SE) in the presence and absence of the earthworm *Eisenia andrei* during vermicomposting of cow manure and sewage sludge. Note the different scales in the Y-axis

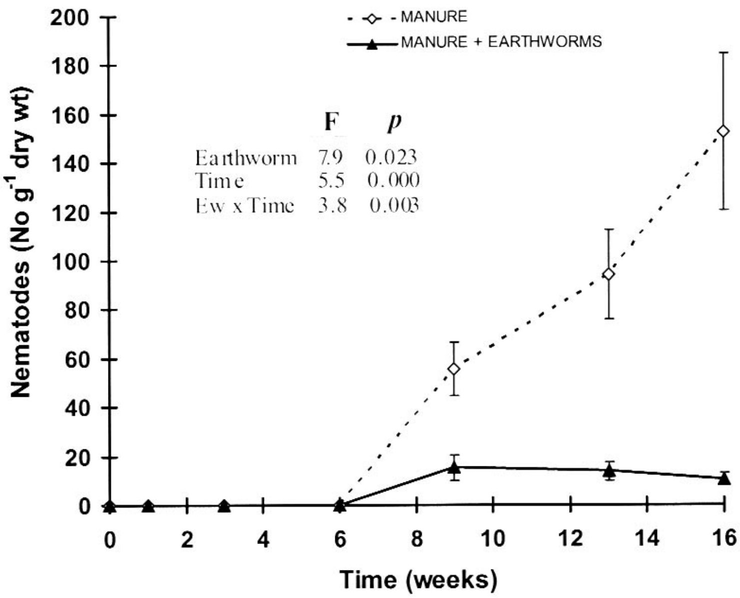

Fig. 2. Fungivore nematode abundance (mean ± SE) in the presence and absence of the earthworm *Eisenia andrei* during vermicomposting of cow manure. The figure includes the results of repeated-measures ANOVA for the presence of earthworms

Table 1. Repeated-measures analysis of variance for the effects of the earthworm *Eisenia andrei* on the density of bacterivore nematodes during vermicomposting of cow manure and sewage sludge

	Bacterivore nematodes		
Between subjects	df	F	p
Earthworm	1	25,74	0,0001
Waste	1	106,64	0,0000
Ew* x waste	1	12,62	0,0027
Error	16		
Within subjects			
Time	5	22,01	0,0000
Time x ew	5	2,68	0,0275
Time x waste	5	15,22	0,0000
Time x waste x ew	5	1,49	0,2017
Error	80		

*Ew: earthworm

with 9000 ± 1100 nematodes g^{-1} dry weight in the presence of earthworms and 5260 ± 990 nematodes g^{-1}dry weight in their absence (Fig. 1). Fungivore nematodes were not observed in sewage sludge during the entire 16 weeks of the experiment and were only observed in cow manure after week 6. At this point, their numbers increased strongly in the absence of earthworm but remained consistently low in their presence. After 16 weeks the density of fungivore nematodes in the cow manure was 150 ± 30 nematodes g^{-1} dry wt in the non-earthworm treatment and 10 ± 2 nematodes g^{-1}dry weight in the earthworm treatment (Fig. 2).

Overall the density of nematodes was significantly higher in the absence of earthworms and there was a significant interaction between time and earthworm treatment, indicating that earthworms had a larger effect on decreasing the number of nematodes over time (Table 1).

Microbial biomass and basal respiration

Heterotrophic activity, as determined by CO_2 evolution, revealed a rapid increase in the rate of respiration from the beginning until week 9 in the sewage sludge, both in the earthworm and in the non-earthworm treatments (Fig. 3) yet the respiration rate of the substrate was consistently higher in the absence of earthworms. After 9 weeks there was a sharp decrease in biological activity until the end of the experiment at which point the respiration rate was similar in both treatments. The latter dynamic was similar in the manure with a sharp decrease after week 9, although there was no initial increase in biological activity as evidenced by a relatively stable respiration rate until week 9. In the three-way repeated measures ANOVA including waste as a factor, there were no significant differences between the earthworm and the non-earthworm treatments ($p = 0.08$). Overall, time significantly decreased the rate of respiration; a trend that was affected by the type of waste (i.e. time × waste interaction) (Table 2).

BION was significantly higher in the earthworm treatment and it also changed significantly over time, however there were several significant interactions between time, type of waste and the earthworm treatment (Table 2). In the cow manure, microbial biomass nitrogen (BION) (Fig. 4) rapidly increased in both treatments during the first week, and then remained more

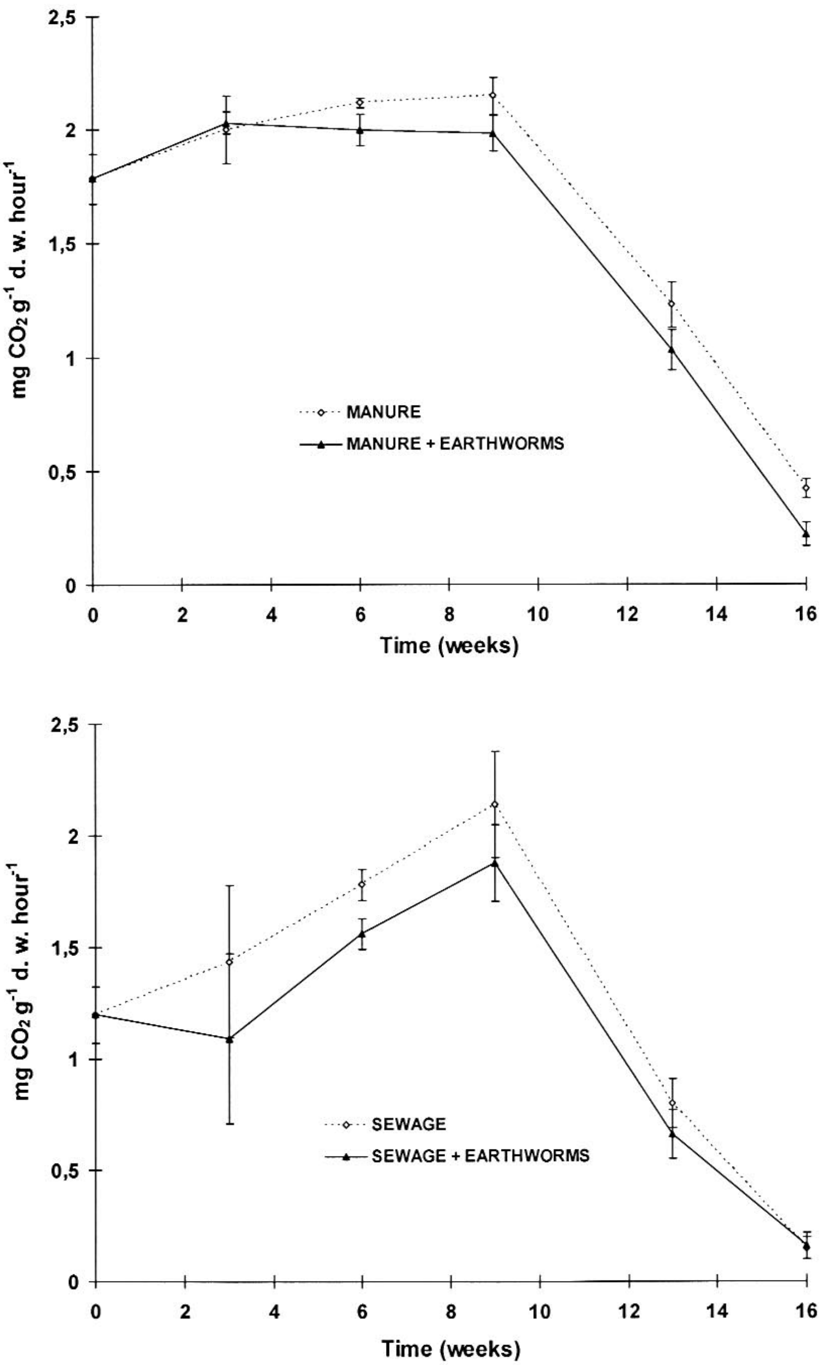

Fig. 3. CO_2 evolution (mean ± SE) in the presence and absence of the earthworm *Eisenia andrei* during vermicomposting of cow manure and sewage sludge

Table 2. Repeated-measures analysis of variance for the effects of the earthworm *Eisenia andrei* on basal respiration and microbial biomass nitrogen (BION) during vermicomposting of cow manure and sewage sludge

		Basal respiration		Bio-N	
Between subjects	**df**	**F**	**p**	**F**	**p**
Earthworm	1	3,41	0,0835	30,14	0,0001
Waste	1	21,56	0,0003	241,97	0,0000
Ew* x waste	1	0,01	0,9052	19,55	0,0006
Error	16				
Within subjects					
Time	5	108,29	0,0000	13,71	0,0000
Time x ew	5	0,40	0,8044	2,59	0,0331
Time x waste	5	4,08	0,0053	4,33	0,0017
Time x waste x ew	5	0,70	0,5933	3,55	0,0064
Error	80				

*Ew: earthworm

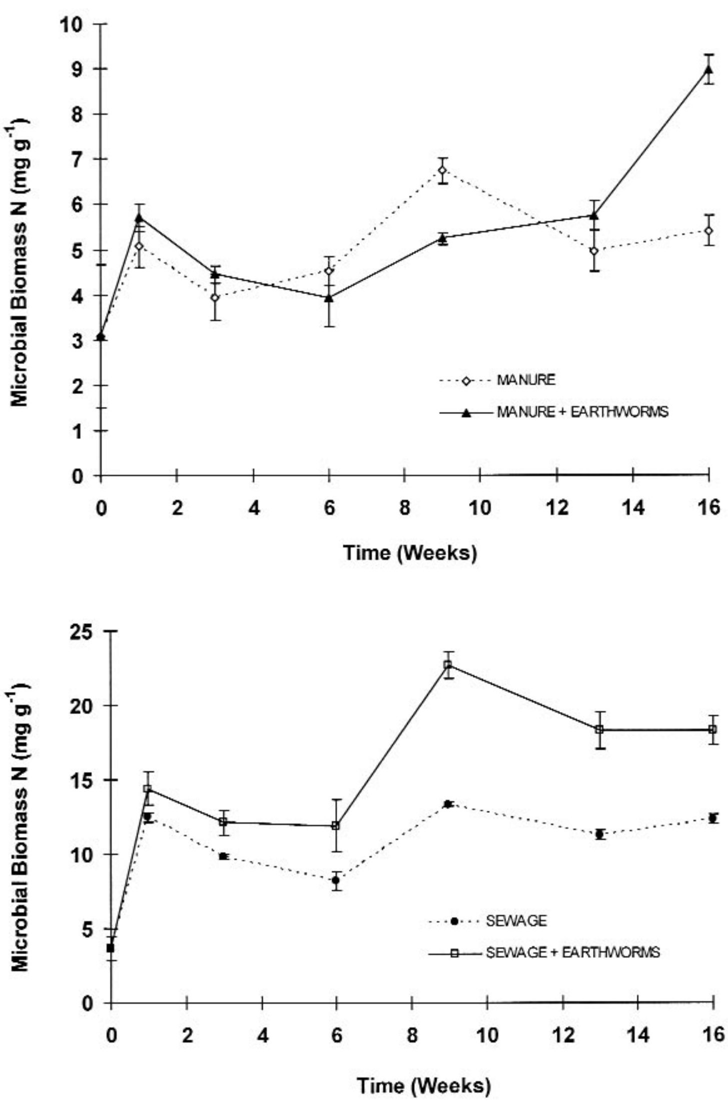

Fig. 4. Changes in microbial biomass nitrogen (chloroform labile) in the presence and absence of the earthworm *Eisenia andrei* during vermicomposting of cow manure and sewage sludge

or less constant until week 13. From week 13 to week 16, BION values were similar without earthworms but showed a sharp increase in the presence of earthworms, possibly due to some earthworm mortality. In sewage sludge there was also a rapid increase in BION during the first week after which time microbial biomass nitrogen was significantly higher in the earthworm treatment and at the end of the experiment BION was 12.36 ± 1.8 $mg.g^{-1}$ (without earthworms) and 18.32 ± 9.7 $mg.g^{-1}$ (with earthworms).

Discussion

In our study, nematode communities were dominated by bacterivores, and their abundance was strongly reduced (more than 50 %) by earthworm activity both in fresh cow manure and sewage sludge. Fungivore nematodes were observed after 6 weeks in the cow manure and in contrast to bacterial feeders, their number continuously increased in the treatment without earthworms but remained low in the presence of the earthworms, indicating an increase in fungal growth in the manure. The decrease of nematode numbers in the presence of earthworms suggests direct grazing and/or, in the case of fungivore nematodes, competition for fungi. As others have observed, the effect of earthworms on nematodes may be due to direct ingestion and digestion (Dash et al. 1980), and consequent effects on the fertility, viability and germination of cysts present in casts (Ellenby 1945; Roessner 1981), or the effect may be indirect, through modifications in soil structure, water regimes, and nutrient cycling processes (Yeates 1981). Perhaps earthworms also spread nematode-trapping fungi that are of major importance in controlling nematode populations (Edwards and Fletcher 1988).

Earthworms may decrease total free-living soil nematode populations by up to 66 % (Yeates 1981), and plant parasitic nematodes by up to 64 % (Dash et al. 1980). Hyvönen et al. (1994) found that the presence of active *Dendrobaena octaedra* decreased nematode numbers, probably as a result of predation rather than competition for the same food (bacteria and/or protozoans) in coniferous mor humus. Roessner (1981) found that *Eisenia fetida* reduced plant-parasitic nematode abundance more than 60 % in soil cultures, 98.8 % in casts, and 50 % in cultures with alfalfa root tissue. Further tests with eight other lumbricid earthworms (Roessner 1986) revealed lower plant parasitic nematode populations in all cases, but variable effects on bacterivores. *Lumbricus rubellus*, *Allolobophora longa* and *Lumbricus terrestris* greatly reduced phytoparasitic nematode species, while *Octolasion cyaneum* had a marked effect on increasing total bacterivore populations. Senapati (1992) likewise observed higher (4–24 %) bacterivore nematode populations and lower (20–50 %) number of plant parasites in the presence of the tropical earthworm *Lampito mauritii*.

It is well established that earthworms modify the biological activity of soils (Daniel and Anderson 1992; Edwards and Bohlen 1996) and, at least in soils, there is also agreement that earthworm activity reduces microbial biomass but increases the specific respiratory activity of microorganisms (Scheu 1987; Wolters and Joergensen 1992; Scheu and Parkinson 1994; Alphei et al. 1996). However, in our study earthworms did not significantly affect respiration but significantly increased microbial biomass nitrogen.

Our understanding of the resource requirements and the relationship between food concentration and population growth rate for bacterial-feeding nematodes is extremely limited (Venette and Ferris 1998). Despite this, our data strongly suggest an important earthworm grazing activity may have been responsible for the decrease in bacterivore nematodes.

The same argument could apply to the fungivore nematodes in the cow manure in which earthworms significantly decreased their populations. However, since we did not measure fungal biomass we can not know if this is a direct effect on fungivore nematodes or indirect, through fungal feeding which is an important part of earthworm diets (Dash et al. 1979, 1980; Edwards and Fletcher 1988; Morgan 1988) or through the modification of the fungal bacterial ratio.

Another important aspect to consider is earthworm diet and we should probably reconsider the hypothesis that epigeic earthworms feeds exclusively on litter and microbes; Hyvonen et al. (1994) came to the same conclusion studying the effects of *D. octaedra* on nematodes in forest soils.

Our results indicate that earthworms have a strong influence on nematode densities and trophic structure. While the mechanisms remain unclear, the effects of earthworms, particularly through grazing, could modify the balance between sequestration and mobilization of nutrients, especially nitrogen. Further investigations including fungal, bacterial and protozoan counts, and other more accurate analysis of microbial activity are necessary to determine if the effect of earthworms on nematodes is due to direct ingestion or indirect, through modifications in the soil microbial community.

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