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Detritivorous earthworms directly modify the structure, thus altering the functioning of a microdecomposer food web

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ABSTRACT

Epigeic earthworms are key organisms in fresh organic matter mounds and other hotspots of heterotrophic activity. They turn and ingest the substrate intensively interacting with microorganisms and other soil fauna. By ingesting, digesting and assimilating the surrounding substrate, earthworms could directly modify the microdecomposer community, yet little is known of such direct effects. Here we investigate the direct effects of detritivore epigeic earthworms on the structure and function of the decomposer community. We characterized changes in the microfauna, microflora and the biochemical properties of the organic substrate over a short time (72 h) exposure to 4 different densities of the earthworm *Eisenia fetida* using replicate mesocosms (500 ml). We observed a strong and linear density-dependent response of the C and N mineralization to the detritivore earthworm density. Earthworm density also linearly increased CO_2 efflux and pools of labile C and inorganic N. This effect on the function was likely a direct consequence of earthworm activity. Furthermore, earthworms affected the microbial metabolic activity, but this response was not linearly related to the earthworm density, possibly because of indirect effects through the microbial community. Earthworms also had strong effects on the structure of the two trophic levels examined; they enhanced the fungal populations and reduced the numbers of bacterivore nematodes. The effect on the fungi was clearly dependent on the earthworm density, and the reduction of bacterivorous nematodes was also related to the earthworm density, but only marginally. In contrast, earthworms did not have significant effects on microbial biomass carbon, flagellate protozoa or ciliate protozoa. A meaningful part of the short term changes in microflora and microfaunal communities after some hours might be attributable to the earthworm gut associated processes. Hence, detritivore earthworms can directly and quickly modulate the decomposer community altering the decomposition rates of organic matter.

1. Introduction

Earthworms are known to be key species in the soil system, where they modify microbial communities and nutrient dynamics (Lee, 1985; Edwards and Bohlen, 1996). Earthworm casts are usually enriched in nutrients such as C, N or P (Scheu, 1987; Aira et al., 2003). Further, microbial communities of casts and earthworm burrows differ from those of uningested soil (Sampedro and Whalen, 2007; Tiunov and Scheu, 1999, 2000), which would finally result in changes in decomposition rates (Swift et al., 1979; Saetre, 1998). Most of these studies have been focused on long-term or indirect effects of earthworms. Despite there is increasing understanding of the effect of earthworms in mineral nutrient cycling and fluxes, the relationships that earthworms establish with microorganisms and their proximate effects on microfauna and microbial communities are far to be fully understood.

Studies investigating the direct effect of earthworms on microorganisms are in need particularly for epigeic earthworm species living in organic matter-rich environments, because most such studies focus on soil-dwelling endogeic and anecic species. It is known that epigeic earthworms can modify the fungal composition of forest soils (McLean and Parkinson, 2000), that they posses a diverse digestive enzyme pool (Lavelle and Spain, 2001), and that their feeding habits rely on microorganisms or organic matter, depending on the species (Scheu and Schaefer, 1998). In nature, epigeic earthworms live in fresh organic matter of forest litter, in litter mounds, in herbivore dungs, and in anthropogenic environments such as manure heaps, vegetal debris and vermicomposting beds common in agricultural landscapes. These habitats are hotspots of heterotrophic activity, where epigeic earthworms intensively interact with microorganisms and other soil fauna within

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the decomposer community, strongly affecting decomposition processes (Swift et al., 1979; Monroy et al., 2006; Sampedro and Domínguez, 2008). Microorganisms are the main agents of biochemical decomposition, whereas earthworms are involved in the indirect stimulation of microbial populations through comminution of organic matter, e.g. by increasing the surface area available for attack by microbes. Earthworms also modify the microbial populations through digestion, stimulation and dispersion in casts (Edwards, 2004). Epigeic earthworms closely interact with other biological components of the soil system, and they can affect the structure of microfauna and microflora (Domínguez et al., 2003; Lores et al., 2006).

Detritivorous earthworms modulate the stabilization of organic matter and strongly modify the physical and biochemical properties of the substrates in which they live (e. g. Aira et al., 2007). Broadly, the influence of epigeic earthworms on decomposition might be due to their gut associated processes (GAPs), the proximate effects of ingestion, digestion and assimilation in the gut (hours); or to cast associated processes (CAP), which are more related to ageing processes and to the physical modification of the egested materials (days to weeks; Scheu, 1987; Parthasarathi and Ranganathan, 2000; Aira et al., 2005). Physical modification of the substrate by burrowing habits such as aeration and promotion of leaching (Domínguez et al., 2004), are expected to be more remarkable in soil dwelling than in epigeic earthworms.

Because the direct effects of earthworms should be essentially density-dependent (i.e. proportional to the number of earthworms and to the length of their gut and gut transit time), these effects could be separated from other indirect subsequent effects by exposing during a short time the microdecomposer community to high earthworm densities. In this study we investigate the direct, proximate, influence of detritivore earthworms on the structure and function of the decomposer community. Using mesocosms, we examine how the activity of an epigeic earthworm modifies the microfauna, microflora and biochemical properties of the organic substrate where they live during a very short time period (72 h).

2. Materials and methods

We performed a bifactorial experiment in order to study the effect of (i) earthworm density and (ii) time on the structure and function of a microdecomposer foodweb. We used pig manure as substrate, which is known to support a dense decomposer food web (Sampedro and Domínguez, 2008). Pig manure was obtained from a farm near the University of Vigo, NW Spain, homogenized, stored at 5 °C until use, and preincubated before the experiment for 2 d at 20 °C. Some physico-chemical characteristics are summarized in Table 1. The mesocosms consisted of 500 ml mason jars filled with 100 g (fresh weight, fw) of substrate. After filling the jars, we allowed the substrate to stabilize for 72 h before inoculating the earthworms. We used the epigeic earthworm species *Eisenia fetida* (Savigny, 1826), broadly distributed and easy to manage under lab conditions. We allowed mature individuals (303 ± 7 mg; mean individual fw ± SEM) of our culture bins to empty their guts on moistened tissue paper for 24 h at room temperature before the experiment. We considered four densities of earthworms: control, low, medium and high (0, 25, 50 and 100 earthworms per mesocosm, respectively). Fifteen replicate mesocosms were established for each density, with a mean weight of 7.3 ± 0.1, 14.4 ± 0.3 and 29.8 ± 0.4 g fw of earthworms (low, medium and high earthworm density respectively). We covered the jars (containing the substrate and the earthworms) with perforated lids, stored them at random in a dark cabinet, and maintained under laboratory conditions (20 ± 2 °C) for 72 h. In total we established 72 mesocosms corresponding to 6 levels of time, 4 levels of earthworm density and 3 replicates each. A random subset of mesocosms of each density ($n = 3$) was destructively sampled after 0, 12, 24, 36, 48 and 72 h.

Table 1
Physico-chemical characteristics of fresh pig manure used as substrate

Moisture content (%)	86 ± 10
pH	8.3 ± 1.0
Electrical conductivity ($mS\,cm^{-2}$)	0.25 ± 0.01
Organic matter ($mg\,g^{-1}$ dw)[a]	860 ± 10
Total carbon ($mg\,g^{-1}$ dw)[b]	455 ± 60
Dissolved organic carbon ($\mu g\,g^{-1}$ dw)	4100 ± 100
Microbial biomass carbon ($\mu g\,g^{-1}$ dw)	12361 ± 895
Total nitrogen ($mg\,g^{-1}$ dw)[b]	24 ± 2
$N–NH_4^+$ ($\mu g\,g^{-1}$ dw)	2400 ± 100
$N–NO_3^-$ ($\mu g\,g^{-1}$ dw)	70 ± 10

Mean ± SEM; $n = 3$.
[a] dw = Dry weight.
[b] Carlo Erba elemental analyzer.

The mineralization rate was immediately determined by measuring the production of CO_2 from the whole mesocosm (earthworms included) after 30 min 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). Earthworms were then removed from each jar, and the substrate was weighed, gently homogenized and immediately processed for chemical, biochemical and faunal analyses.

We determined the moisture content of the manure gravimetrically after drying at 105 °C for 24 h, and the organic matter content after ashing at 550 ± 50 °C for 4 h. Chemical and biological soil characteristics are expressed on the basis of the oven-dry weight (dw). The extractable mineral N ($N–NH_4^+$ and $N–NO_3^-$) content of samples was determined in 0.5 M K_2SO_4 extracts (1:5, fw:v) using the indophenol blue technique in a Bio-Rad Microplate Reader 550 (Sims et al., 1995). Microbial biomass C (C_{mic}) was analyzed by the chloroform fumigation–extraction method using a $K_{EC} = 2.64$ (Vance et al., 1987). Dehydrogenase enzyme activity was measured colorimetrically at 545 nm by estimation of the rate of reduction of triphenyltetrazolium chloride (TTC) (1.5%) to triphenylformazan (TPF) after incubation at 30 °C for 24 h (Casida et al., 1964). Dissolved organic carbon (DOC) was determined also colorimetrically in microplates after moist digestion ($K_2Cr_2O_7$ and H_2SO_4) of aliquots of 0.5 M K_2SO_4 extracts of the unfumigated samples. Microbial activity within the substrate was determined by measuring the CO_2 evolution from 5 g fw samples during 6 h incubation as above.

Fungal biomass was estimated analyzing the ergosterol content in the samples at the end of the experiment (72 h samples). Ergosterol was extracted by microwave-assisted extraction (MAE) and determined by HPLC analysis (Young, 1995; Aira et al., 2006). Briefly, for the MAE procedure, 500 mg fw samples were digested in a microwave oven into Teflon bottles with 2 ml of methanol and 0.5 ml of 2 M NaOH. The digested samples were extracted with pentane (3 × ca. 2 ml), then evaporated to dryness under a stream of N_2 and redissolved with methanol (1 ml). Subsequently, quantitative determination of ergosterol was performed by reverse-phase HPLC analysis using a C18 column of 12.5×4 mm 5 μm Hypersil with a mobile phase of methanol:water (95:5, v: v) and detection set at 282 nm.

Nematodes were extracted from 10 g fw samples in modified Baermann funnels, counted live at a magnification of 140× under a dissecting microscope and sorted into trophic groups (bacterivores, fungivores, omnivores and herbivores) using oesophageal morphology, as described by Parmelee and Alston (1986). Ciliate and flagellate protozoa were grown in NBAS medium (Griffiths, 1989) from 1 g fw samples of substrate in a 1:100 (fw:v) ratio. The estimation of the number of each category was done in serial dilutions of these original cultures in microplates, following a modification of the more probable number technique