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Effects of two species of earthworms (*Allolobophora* spp.) on soil systems: a microfaunal and biochemical analysis

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Summary

In this work we studied how earthworm digestion affects both soil biochemical and microfaunal characteristics. We sampled paired fresh casts and bulk soil of two earthworm species, *Allolobophora caliginosa* and *A. molleri*, inhabiting a pasture and a riverside, respectively. We focused on the differences in faunal composition and biochemical characteristics between cast and bulk soil.

Ammonium and carbohydrate contents were higher in casts than in the surrounding soil. DON content was lower in the casts than in the surrounding soil. Microbial biomass (measured as microbial biomass-N, arginine ammonification and SIR) and microbial activity (measured as basal respiration, β-glucosidase, alkaline phosphatase, dehydrogenase and protease) were also higher in casts in the surrounding soil than. Flagellate protozoa and nematode numbers were lower in *A. caliginosa* casts and higher in *A. molleri* casts than in the surrounding soil.

Key words: Earthworm digestion, microbial biomass, microbial activity, enzyme assays, flagellate protozoa, nematodes, casts

Introduction

There are contradictory data in the literature on how earthworms affect soil biochemical, microflora and microfauna characteristics. Previous studies have shown that earthworm activity promotes organic matter decomposition significantly (Parmelee et al. 1990) and enhances mineralization and humification of soil organic matter. Moreover, there is a positive relationship between earthworm activity, soil respiration and nutrient cycling (Haimi & Einbork 1992).

There is abundant evidence demonstrating the importance of earthworm-microorganism interactions on soil organic matter degradation and nutrient release (Lee 1985). Earthworm activity can stimulate microbial activity in casts (Lavelle & Martin 1992) and the presence of earthworms can modify soil microbial activity (Binet & Trehen 1992).

Microorganisms generally flourish in earthworm casts (Domsch & Banse 1972) and it has been suggested that they, especially fungi, constitute a nutrient pool for earthworms (Edwards & Fletcher 1988). Tiunov & Scheu (2000a) reported that the biomass of fungi is higher in *Lumbricus terrestris* casts than in the

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surrounding soil. However, contradictory results have also been found for the effect of earthworm digestion on nematodes; Russom et al. (1993) reported more nematodes in *Agrotoreutus nyongi* casts than in bulk soil whilst Yeates (1981) found that nematode populations were reduced by earthworms.

Our investigation focused on the effects of the digestion of *Allolobophora caliginosa* and *A. molleri* on soil microflora, microfauna and nutrient pools, studying the differences between casts and surrounding bulk soil in two different habitats.

Materials and Methods

We selected two locations with established populations of the earthworm species *Allolobophora caliginosa* and *A. molleri*, living in two different soil habitats, a pasture and a riverside, respectively. Paired fresh casts and their surrounding soil (n = 10) were sampled and sieved (2 mm) and subsamples were taken to determine soil moisture content (105 °C, 24 h), organic matter content (550 °C, 4 h), pH and conductivity (1 : 20 soil:deionized water). Bulk soil samples were taken at a distance of no more than 10 cm from the earthworm casts. In the pasture we only found *A. caliginosa* and in the riverside the only other earthworm species present was *Eiseniella tetraedra*, although in much lower numbers and with casts clearly smaller than those of *A. molleri*.

Fresh samples (fumigated and non-fumigated; Vance et al. 1987) were extracted with 0.5 M K_2SO_4 (1:10) for analyses of NH_4^+-N, NO_3^--N (Sims et al. 1995), DON and biomass-N (Cabrera & Beare 1993). Carbohydrates were determined by phenol-sulphuric digestion (Safarik & Santruckova 1992). Basal respiration, SIR and dehydrogenase, urease, protease, β-glucosidase, cellulase, alkaline phosphatase and arginine ammonification activities were also analyzed in the fresh samples. Other subsamples were microwave digested and analyzed for ergosterol content (Young 1995). Total C and N levels were determined on a Carlo Erba 1500 C/N analyzer on dried samples.

Nematodes were extracted from 10 g of casts and bulk soil (fresh weight) in modified Baermann funnels, counted live at a magnification of 140× under a dissecting microscope and sorted by trophic groups (bacterivores, fungivores, omnivores and herbivores) using œsophageal morphology as described by Parmelee and Alston (1986). Flagellate protozoa were estimated by the MPN method (Darbyshire et al. 1974). Means were separated using paired t-tests.

Results

Compared to surrounding soil, NH_4^+-N concentrations were significantly higher in the earthworms cast samples (10 and 3-fold for *A. caliginosa* and *A. molleri*, respectively; Table 1). DON content was significantly lower in both *A. caliginosa* and *A. molleri* casts than in the surrounding soil. Total C and carbohydrate content were significantly higher in *A. caliginosa* casts (Table 1).

Substrate-induced respiration (SIR), arginine ammonification, basal respiration and dehydrogenase activity were significantly higher in *A. caliginosa* casts compared to the surrounding soil. Basal respiration and SIR were significantly higher in *A. molleri* casts than in the surrounding soil. There were no significant differences in ergosterol content between casts and surrounding soil (Table 2).

Alkaline phosphatase, b-glucosidase and protease activity were higher in *A. caliginosa* casts than in the surrounding soil; urease and cellulase activity were lower in *A. caliginosa* casts than in the surrounding soil (Table 2).

Bacterivore, omnivore and herbivore nematode numbers were significantly lower in *A. caliginosa* casts than in the surrounding soil and there were no

Table 1. Chemical properties in cast of *Allolobophora caliginosa* and surrounding soil (paired samples n=10) and cast of *A. molleri* and surrounding soil. Significant differences (paired t test) * p<0.05, ** p<0.01

	A. caliginosa		*A. molleri*	
Chemical characteristics	**soil**	**cast**	**soil**	**cast**
Total C (%)	5.60±0.24	7.69±0.16*	6.32±0.65	7.38±0.44
Total N (%)	0.55±0.09	0.65±0.07	0.52±0.04	0.59±0.03
Total P (%)	0.13±0.00	0.13±0.00	0.01±0.00	0.01±0.00
NH_4^+-N (μg g^{-1})	6.52±0.66	70.41±4.14**	17.57±1.67	53.65±11.02**
NO_3^--N (μg g^{-1})	14.54±3.78	11.57±6.68	0	0
DON (μg g^{-1})	40.86±2.42	18.32±6.66*	62.99±3.42	53.14±3.14*
Carbohydrates (μg g^{-1})	16.49±1.14	26.12±0.94**	19.81±1.50	21.76±1.32

Table 2. Biochemical and microbial parameters in cast of *Allolobophora caliginosa* and surrounding soil (paired samples n=10) and cast of *A. molleri* and surrounding soil. Significant differences (paired t test) * p<0.05, ** p<0.01

	A. caliginosa		*A. molleri*	
Biochemical and microbial characteristics	**soil**	**cast**	**soil**	**cast**
Microbial biomass-N (µg g^{-1})	32±5	43±7	23±10	19±2
Ergosterol (pg g^{-1})	624±91	519±144	115±44	140±33
SIR respiration (mg CO_2 g^{-1} afdw[1])	315±27	544±42**	71±16	193±44*
Arginine (µg NH_4^+-N g^{-1})	6±2	7±4*	2±1	6±3
Basal respiration (µg CO_2 g^{-1} afdw)	274±22	117±13**	63±13	160±34
Dehydrogenase (µg TPF g^{-1})	217±22	389±50**	148±114	101±8*
b-glucosidase (µg (-nitrophenol g^{-1})	355±33	622±49**	640±227	556±135
Cellulase (µg glucose equivalent g^{-1})	1750±160	1449±157	1241±424	1524±292
Protease (µg tyrosine g^{-1})	116±12	230±21**	103±31	184±30*
Urease (µg NH_4^+-N g^{-1})	19.6±2.6	0**	12±3	11.2±2.3
Alkaline phosphatase (µg p-nitrophenol g^{-1})	1494±177	1617±145	615±145	1113±198

[1] asfw: ash free dry weight

Table 3. Microfauna composition in cast of *Allolobophora caliginosa* and surrounding soil (paired samples n=10) and cast of *A. molleri* and surrounding soil. All data are expressed as individual number g^{-1} d.w. Significant differences (paired t test) * p<0.05, ** p<0.01

	A. caliginosa		*A. molleri*	
Microfauna	**soil**	**cast**	**soil**	**cast**
Flagellate protozoa	55900±13100	45700±5400	10800±4000	25100±3500
Bdelloid rotifers	0.3±0.1	0.03±0.03	0	0.1±0.1
Bacterivorous nematodes	17.9±3.2	5.8±0.6**	1.9±0.9	7.9±2.9
Fungivorous nematodes	6.7±2.5	6.4±2.3	0.6±0.3	0.5±0.2
Omnivorous nematodes	1.7±0.4	0.7±0.2*	0.4±0.3	0.2±0.2
Herbivorous nematodes	14.2±4.5	2.3±0.9*	0.1±0.1	0.5±0.5

statistical differences in the microfaunal composition of casts and bulking surrounding soil of *A. molleri* (Table 3).

Discussion

Earthworm casts generally present higher concentrations of NH_4^+-N (Parkin & Berry 1994) which is in agreement with our results. The lower DON levels in the casts could be due to earthworm digestion or/and ingestion by the microflora, which can directly assimilate organic nitrogen (Jensen 1997). Contrary to Scheu (1987) for the same species, total N was significantly higher in *A. caliginosa* casts. Higher total carbon content in casts compared to surrounding soil had been also reported by McInerney & Bolger (2000) and probably resulted from concentrating of organic material.

Domsch & Banse (1972) found that microorganisms flourish in earthworm casts and Hirth et al. (1998) reported that earthworms ingest microorganisms with soil. It is generally accepted that microbial biomass and respiration are higher in casts than in soil (Zhang & Hendrix 1995; Tiunov & Scheu 2000a) and this agrees with our results, mainly in the case of *A. caliginosa*. It is also accepted that earthworms digest fungi (Doube & Brown 1998) and modify fungal diversity in the soil (Tiunov & Scheu 2000b) although opposite effects, with higher fungal biomass in the casts than in the soil, have also been reported (Tiunov & Scheu 2000a).

We used enzyme activity as a measure of different physiological capabilities, assuming that the performed assays are representative of microbial community activity (Waldrop et al. 2000). Carbon metabolism enzymes were higher (b-glucosidase) or not different (cellulase) in *A. caliginosa* casts compared to soil, probably due to the fact that cellulase is produced mainly by fungi and ergosterol content did not vary between casts and bulk soil. These results are in contrast to previous findings by Zhang et al. (2000) who found higher levels of cellulase activity in casts. We measured two nitrogen metabolism enzymes, protease and urease; protease is linked with ammonia release, so the significantly higher activities found in the casts could

be correlated with higher ammonia content and this agrees with the findings of Zhang et al. (2000). Urease is considered an important agent in N mineralization in terrestrial ecosystems. Both the low and no urease activity found respectively in the *A. caliginosa* and *A. molleri* casts could be due to the low DON content found in the casts. High activities found in phosphatase agree with findings by Jegou et al. (2001) on soil phosphatases.

According to Brown et al. (2000) gut passage may decrease protozoa and nematode populations, although these organisms probably supply a minor component of the earthworms' energy needs. It has been shown that earthworms can increase protozoa activity (Winding et al. 1997), increase their abundance in soil (Binet et al. 1998) or decrease it (Bonkowski & Schaefer 1997). Our results show that the two earthworms have different effects on soil flagellate protozoa: *A. caliginosa* reduced while *A. molleri* increased their numbers.

Earthworms can digest nematodes (Dash et al. 1980) so we expected a decrease in their populations in fresh casts. We found lower nematode numbers in the casts than in the soil, although some resources for nematodes were increased in the casts (greater and more active microbial populations available for bacterivore and omnivore nematodes) or unaffected (fungi for fungivores). However, bacterivore nematode numbers were higher and the other nematode groups were lower in *A. molleri* casts.

We conclude that *A. caliginosa* reduced soil microfauna and increased soil microflora. In the case of *A. molleri* such effects were not clear mainly due to the low number of microorganisms and soil animals in the bulk soil. More studies are necessary to find out if earthworm feeding is more or less directed to microfauna patches or if ingestion of microfauna predation is accidental.

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