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## Original article

## Is there food competition between *Hormogaster elisae* (Oligochaeta, Hormogastridae) and soil microarthropods at El Molar (Madrid)?

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## ABSTRACT

In a previous paper [M. Gutiérrez, M. Ramajo, J.B. Jesús, D.J. Díaz, The effect of *Hormogaster elisae* (Hormogastridae) on the abundance of soil Collembola and Acari in laboratory cultures, *Biol. Fertil. Soils* 37 (2003) 231–236] we reported the negative effect of the earthworm *Hormogaster elisae* on microarthropods at El Molar (Madrid, Spain). This paper examines the possibility of food competition existing between them in laboratory cultures.

Microcosms were constructed from plastic boxes and a cage made from 2 mm mesh, with two earthworms inside, was placed at the centre of each. The soil of the cage was enriched with defaunated soil sieved to 0.2 mm or with defaunated, homogenised topsoil (first 3 cm) to increase the quantity of organic matter. In the controls, earthworms were absent from both microcosm compartments. The microcosms were kept at 15 °C for 21 days before being dismantled and the microarthropods extracted using the Berlese–Tulgren method, identified and counted. The numbers inside and outside the cages were then compared, and the data obtained were subjected to analysis of variance (ANOVA).

In the microcosms enriched with the  $\leq 0.2$  mm soil fraction, nearly all the microarthropods decreased in number inside the cages, when earthworms were present despite containing extra organic matter. In the controls, no significant differences were seen between the compartments, suggesting that these particles are not used as a food source by microarthropods. In the microcosms enriched with homogenised topsoil, the microarthropods were just as numerous in both compartments or indeed even more numerous inside the cage. This shows that this material nullifies the negative effect of earthworms on microarthropod numbers. The soil horizons closest to the surface might provide food resources used by both groups, for which they compete.

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## 1. Introduction

A number of authors have reported negative relationships between earthworms and certain microarthropods (i.e., the more earthworms the fewer the microarthropods) [18,21,22,29], while others report the opposite [15,20,28]. At El Molar (near Madrid, central Spain), Gutiérrez et al. [9] reported the same kind of negative relationship between the earthworm *Hormogaster elisae* Álvarez, 1977, and microarthropods. There are several possible explanations for this relationship, such as interspecific competition, predation, or earthworm-generated physical and chemical disturbance of the microarthropod niches.

With respect to competition, both types of organism may compete for soil resources. In fact, the majority of soil communities concentrate around hot-spots, i.e., accumulations of organic matter. Usher et al. [31] suggested that the distribution of soil arthropods is not random but that accumulations occur in certain areas, a consequence of the distribution of trophic resources and environmental conditions forming favourable microhabitats. Stanton [30] indicates that the greater the amount of organic matter available, the greater the abundance of microarthropods and the greater their species richness. He also indicates that this is seen more clearly in habitats protected from extreme conditions. It is therefore not surprising that earthworms and microarthropods should be found in the same areas of soil, and that competition should occur between them for resources.

The literature contains several studies on the possible competition between earthworms and microarthropods. Dunger [8] indicates that collembolans and earthworms compete for plant resources in certain cultivated soils. McLean and Parkinson [21] suggest that earthworms that feed selectively on fungi and detritus might compete with mycophagous and detritivorous microarthropods, and given that earthworms are much larger, a reduction in microarthropod numbers might be expected in their presence. Migge [22] also reports negative effects of earthworms on microarthropod abundance due to competition, and points out that since earthworms are relatively larger and more mobile, they are probably the better competitor.

Scheu et al. [29] report that earthworms inhibit the growth of collembolan numbers and that both groups feed selectively on and compete for the same food resources. Some earthworms feed selectively on bacteria and fungi [5], and collembolans and oribatids feed selectively on very similar fungal species [16]. In fact, there is even evidence that earthworms and many other soil organisms, particularly mites and collembolans, frequently coincide in their preference for darkly pigmented fungi [19,28].

Many authors therefore define competition for food as the main reason for the negative impact of earthworms on microarthropods; it would therefore seem possible that such a relationship might also exist at El Molar between *H. elisae* and the latter.

The aim of the present work was to determine whether the negative relationship between *H. elisae* and the microarthropods of El Molar, reported by Gutiérrez et al. [9], is due to competition between these organisms for food. Food competition experiments were performed in the laboratory involving different sources of organic matter.

## 2. Materials and methods

The soil and the organisms used in all experiments came from a plot at El Molar (some 42 km to the northeast of Madrid; UTM30TVL525095; altitude 817 m). The area is one of transition between the mountains of Spain's Central System and the plain to the south. The climate of the area is temperate Mediterranean. The plot supports subnitrophilous pasture (Mediterranean grass communities on soils slightly enriched in nitrates) and a few woody, aromatic plants. The climatic and edaphic characteristics of the site are fully described in Valle et al. [32] and Gutiérrez et al. [10]. The earthworm *H. elisae*, which is found in the plot, belongs to the family Hormogastridae; the species is endogamic and endemic to the centre of the Iberian Peninsula [2].

Food competition experiments were performed in microcosms similar to those used by Gutiérrez et al. [9]. These consisted of plastic recipients (19 × 14 × 7 cm) at the centre of which a wire mesh (2 mm) cage (15 × 10 × 5 cm) was placed. This mesh size allows the passage of microarthropods, but not that of earthworms. Each microcosm compartment (i.e., inside and outside the cage) was provided with 500 g of 20% moist and non-sieved soil (The initial moisture was measured (a sample of soil was kept at 105 °C for 24 h) and then the soil was brought to 20% moisture (wet weight) adding water).

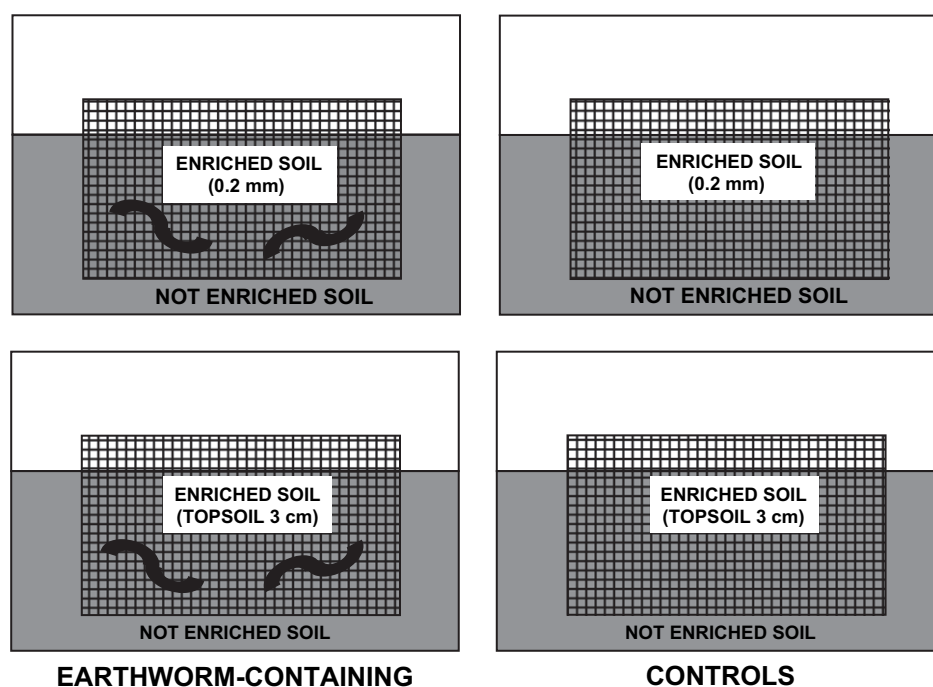
Two earthworms (body weight between 2 and 3 g) were then placed inside the cages (control experiments were performed without earthworms). To determine microarthropod numbers at time 0 (i.e., at the beginning of the experiment), six samples of 500 g of non-sieved soil were analysed.

Different sources of organic material were then added to the soil inside the cages. Glucose was not used, since Ruiz [26] found this to lead to a reduction in *H. elisae* body weight, and sometimes even the death of these earthworms. In addition, this practice increases the possibility of the growth of bacteria and fungi. Rather, two natural soil fractions from the plot soil itself were used; thus, some cages received 100 g of soil sieved to a particle size of ≤0.2 mm (apart from containing relatively more organic matter, *H. elisae* positively selects soil fractions with a size of 0–2 μm [27]), while others received 100 g of topsoil (the top 3 cm), the zone in which most microarthropods are found [11,33]. In fact some two-thirds of all collembolans are commonly found in the first 3 cm of soil [34], and the majority of oribatids in the top 5 cm, where they take part in the decomposition of plant material [13]. To quantify the extra provision of organic matter in these soils, carbon analysis was performed following the method of Anne [1].

To eliminate all microarthropods from the additional soils, and thus avoid the introduction of more of these organisms into the cages, these soils were defaunated following a method similar to that of Huhta et al. [12], Wright et al. [36] and Bruckner et al. [6] (cooling to –32 °C for 24 h, thawing for 24 h, and finally heating in an oven to 60 °C for 24 h).

Each microcosm type was replicated six times (including the controls). Fig. 1 shows an outline of the different experimental conditions.

During the experimental period the microcosms were kept in the dark in culture chamber at 15 °C (soil moisture 20%) for 21 days. This was time sufficient for the earthworms to have consumed all the soil available to them according to the rate



**Fig. 1 – Structure of the microcosms used. Upper panels: enrichment of the cages with the  $\leq 0.2$  mm soil fraction; bottom panels, enrichment of the cages with topsoil. Left (top and bottom panels), with earthworms; right (both cases), controls.**

of cast production of *H. elisae* ( $3.18 \text{ g cast per earthworm g}^{-1} \text{ day}^{-1}$  in natural soil) [7]. After this period the microcosms were dismantled and the microarthropods extracted from inside and outside the cages using the Berlese–Tulgren method [14]. They were then preserved in Scheerpeltz solution, identified using a stereomicroscope and a microscope to the lowest taxonomic level possible, and counted.

One-way ANOVA's were performed (after verifying the normal distribution of the results using the Shapiro–Wilks and Kolmogorov–Smirnov tests) followed by the Duncan test to examine the differences between the numbers of microarthropods at time 0 and at the end of the experiment, under each experimental condition. The analyses were made independently in both experiments: microcosms enriched with  $< 0.2$  mm soil fraction and with topsoil, as they were different experiments made in different moments. When the examined variables were not normally distributed they were compared using the non-parametric Kruskal–Wallis test. All calculations were undertaken using SPSS software v. 12.0.

### 3. Results

Table 1 shows that the two types of soil added to the non-sieved cage soil (i.e., the  $\leq 0.2$  mm sieved soil and the topsoil) contained higher percentages of carbon and therefore more organic material than the latter soil.

#### 3.1. Microcosms with cages enriched with the $\leq 0.2$ mm soil fraction

Table 2 shows the mean abundance of each group of arthropods at time 0 and at the end of the experiment under each

set of experimental conditions, as well as the results of the ANOVA and Duncan tests.

At the end of the experiment, the abundance of most groups (collembolans of the family Isotomidae and mites of the suborders Gamasida, Acaridida and Oribatida) in the earthworm-containing microcosms was significantly greater outside the cages than within them. No such differences were seen in the control microcosms, suggesting that these organisms are lower in the cages only when they contain earthworms.

For Oribatida Poronota, however, significant differences in abundance were also seen in the control microcosms; more of these organisms were found outside the cage than inside, even though there were no earthworms present.

At the end of the experiment a significant reduction was seen in the numbers of collembolans of the Poduromorpha group as well as those of the family Onychiuridae plus the arthropods recorded as 'others', both inside and outside the cage. However, the differences between the numbers of these taxa inside and outside were not significantly different.

Thus, in the microcosms with earthworm-containing cages, microarthropod abundance in general was significantly greater outside the cage. Despite the addition of the  $\leq 0.2$  mm soil fraction, nearly all the microarthropod groups decreased

**Table 1 – Mean percentages of carbon and organic matter in the three soils employed**

Type of soil	Natural soil	0.2 mm	Topsoil 3 cm
% C	0.80	2.90	1.62
% Organic matter	1.38	5.01	2.79

**Table 2 – Enrichment with the  $\leq 0.2$  mm soil fraction: mean abundance of the different groups of microarthropods and the beginning (time 0) and end of the experiment under the different experimental conditions, and the results of statistical analysis (\* $P < 0.05$ )**

Taxonomic group	Time 0	Earthworm-containing		Control		F	P
		Inside	Outside	Inside	Outside		
Isotomidae	7.33 <sup>a</sup>	13.33 <sup>a</sup>	24.17 <sup>b</sup>	9.67 <sup>a</sup>	15.33 <sup>ab</sup>	3.706	0.017*
Poduromorpha	22.33 <sup>c</sup>	5.17 <sup>ab</sup>	7.50 <sup>b</sup>	1.17 <sup>a</sup>	5.50 <sup>ab</sup>	23.760	0.000*
Sminthuridae	1.67	3.83	2.83	2.17	4.50	1.045	0.404
Onychiuridae	11.17 <sup>b</sup>	2.33 <sup>a</sup>	4.50 <sup>a</sup>	3.67 <sup>a</sup>	2.83 <sup>a</sup>	6.750	0.001*
Gamasida	3.33 <sup>a</sup>	2.17 <sup>a</sup>	6.67 <sup>b</sup>	2.00 <sup>a</sup>	2.67 <sup>a</sup>	4.276	0.009*
Acaridida	1.00 <sup>a</sup>	1.17 <sup>a</sup>	5.33 <sup>b</sup>	3.00 <sup>ab</sup>	2.67 <sup>ab</sup>	3.156	0.031*
Actinedida	6.17	3.33	3.50	3.00	3.00	0.828	0.520
Tarsonemidae	3.50	3.17	6.67	1.67	2.67	2.013	0.123
Oribatida Macropylina	13.67 <sup>a</sup>	13.50 <sup>a</sup>	23.00 <sup>b</sup>	13.00 <sup>a</sup>	17.50 <sup>ab</sup>	2.249	0.092*
Oribatida Brachypylina Gymnonota	42.00 <sup>b</sup>	16.00 <sup>a</sup>	55.50 <sup>c</sup>	18.83 <sup>a</sup>	28.17 <sup>a</sup>	16.384	0.000*
Oribatida Brachypylina Poronota	14.17 <sup>c</sup>	4.00 <sup>a</sup>	14.00 <sup>c</sup>	2.67 <sup>a</sup>	8.17 <sup>b</sup>	17.862	0.000*
Other	11.17 <sup>b</sup>	2.33 <sup>a</sup>	4.50 <sup>a</sup>	3.33 <sup>a</sup>	5.50 <sup>a</sup>	6.668	0.001*

Different letters indicate significant differences as determined by the Duncan test.

in number when earthworms were present, leaving the cage. Except for Oribatida Poronota, which left the cage even when there were no earthworms present, no significant differences were seen in terms of microarthropod abundance inside or outside in the control experiments.

### 3.2. Microcosms with cages enriched with topsoil

Table 3 shows the mean abundance of each group of microarthropods at time 0 and at the end of the experiment under each set of experimental conditions, as well as the results of all statistical comparisons.

At the end of the experiment, the collembolans of the Isotomidae, Sminthuridae and Poduromorpha, and the members of Acaridida, were significantly more abundant inside than outside the cages – both in the experimental and control microcosms. The overall numbers of the Isotomidae and Sminthuridae collembolans increased significantly by the

end of the experiment compared to time 0, both inside and outside the cage. This was also the case in both the experimental and control microcosms.

For the members of Acaridida, abundance at time 0 and by the end of the experiment outside the cages in both the earthworm-containing experiments and the controls was similar, but their numbers greatly increased inside the cages by the end of the experiment in both types of microcosms.

With respect to the members of Poduromorpha and Tarsonemidae, abundance at time 0 was significantly greater than at the end of the experiment under all conditions; this has been seen in other experiments performed by our group. However, for most microarthropod groups, abundance was significantly greater inside the cage than outside. Thus, the behaviour of the microarthropods was largely the reverse of that seen in the experiment with the  $\leq 0.2$  mm soil enrichment; they tended to stay inside the cage rather than try to escape it, even if earthworms were present.

**Table 3 – Enrichment with topsoil: mean abundance of the different groups of microarthropods and the beginning (time 0) and end of the experiment under the different experimental conditions, and the results of statistical analysis (\* $P < 0.05$ )**

Taxonomic group	Time 0	Earthworm-containing		Control		F	P
		Inside	Outside	Inside	Outside		
Isotomidae	95.50 <sup>a</sup>	549.67 <sup>c</sup>	264.83 <sup>ab</sup>	539.00 <sup>c</sup>	324.33 <sup>b</sup>	6.968	0.001*
Poduromorpha	6.00 <sup>c</sup>	2.33 <sup>b</sup>	0.67 <sup>a</sup>	2.50 <sup>b</sup>	0.67 <sup>a</sup>	18.017	0.000*
Sminthuridae	2.83 <sup>a</sup>	164.17 <sup>d</sup>	93.00 <sup>b</sup>	152.50 <sup>cd</sup>	102.83 <sup>bc</sup>	13.525	0.000*
Onychiuridae	4.50	3.17	6.00	1.83	4.33	1.981	0.128
Entomobryidae	1.00 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	7.500	0.000*
Gamasida	4.00	2.17	3.67	2.50	4.33	1.488	0.236
Acaridida	4.50 <sup>a</sup>	10.33 <sup>b</sup>	5.50 <sup>a</sup>	13.33 <sup>b</sup>	5.33 <sup>a</sup>	6.599	0.001*
Actinedida	18.83 <sup>a</sup>	34.00 <sup>ab</sup>	22.17 <sup>a</sup>	48.33 <sup>b</sup>	44.17 <sup>b</sup>	6.570	0.001*
Tarsonemidae	89.50 <sup>c</sup>	32.33 <sup>ab</sup>	21.83 <sup>a</sup>	40.67 <sup>ab</sup>	52.83 <sup>b</sup>	8.189	0.000*
Oribatida Macropylina	1.83	4.33	3.50	2.33	2.00	1.460	0.244
Oribatida Brachypylina Gymnonota	2.17	3.17	3.17	4.33	4.33	0.682	0.611
Oribatida Brachypylina Poronota	10.50	9.50	12.17	10.83	10.83	0.083	0.987
Other	6.33	2.17	3.50	8.00	12.17	10.171	0.038

Different letters indicate significant differences as determined by the Duncan test.

No significant differences were ever seen in Actinedida or Oribatida numbers inside and outside the cages; these taxa showed no tendency to move whether earthworms were present or not.

In summary, the results of this soil-enrichment experiment were different to those of the former. At the end of the experiment microarthropod numbers were similar inside and outside the cages both with and without earthworms. Some groups (Isotomidae, Sminthuridae, Poduromorpha and Acaridida) even became more abundant inside the cages, where the organic material was in greater supply, even though they contained earthworms.

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#### 4. Discussion

Gutiérrez et al. [9] reported a negative influence of *H. elisae* on the abundance of microarthropods at El Molar: generally, they were more abundant in the absence of earthworms. This effect had previously been noticed with other earthworm species [18,22,29] with results varying depending on the fauna present and the reigning environmental conditions.

Several mechanisms may be involved in this negative relationship, such as physical and chemical perturbations caused by the earthworms, predation, or competition. However, Gutiérrez et al. [10] indicate that the active predation of microarthropods by *H. elisae* is unlikely.

Competition is an everyday fact of the food webs of most ecosystems. In the soil, many of the relationships between organisms are based on competition, although this has been little studied. The literature indicates that earthworms and microarthropods may compete for some food resources [8,21,22,24,29]. It is therefore not surprising that earthworms and microarthropods should be found in the same areas of soil, as in the first centimetres of the soil or the drilosfera (a zone of soil some 1–2 mm thick surrounding earthworm galleries [4], generally richer in nutrients than the more distant soil, and where some 40% of the soil's nitrifying bacteria, 13% of its nitrogen fixers, and 16% of its denitrifying bacteria are found [3]). This could influence the distribution of some collembolans since these microorganisms form an important part of their diet [35]. Therefore both groups (earthworms and microarthropods) would be forced to live in places with the enough organic matter and competition should occur between them for resources.

The results of the present work suggest this may be the case between microarthropods and *H. elisae*, at least under the present experimental conditions.

In the experiments involving enrichment with the  $\leq 0.2$  mm soil fraction, nearly all the microarthropods seemed to try to escape the cage, fleeing the negative effect of the earthworms despite the enrichment in organic matter. No significant differences were seen in microarthropod numbers inside and outside the cage in nearly any of the control experiments suggesting that when no earthworms are present the microarthropods have sufficient food resources and do not need to seek them elsewhere. It might also indicate, however, that the  $\leq 0.2$  mm soil fraction might not have the food resources the microarthropods need. Although smaller fractions contain more organic material [25], this fraction contains more humus which they

might not find suitable. This, although it contains organic matter, it might not be of sufficient quality to merit their interest (whether earthworms are present or not).

The Oribatida Poronota mites also showed a tendency to escape the cage in the controls of the latter experiments. This might be because this group is very sensitive to perturbations in the environment [23]: simply adding the  $\leq 0.2$  mm soil fraction may have been enough to drive them out of the cage.

When the microcosms were enriched with topsoil, the results were quite different. Instead of escaping the earthworm-containing cages, the microarthropods remained inside; indeed, they even appeared to enter from outside. This suggests that this material cancels out the negative effect of the earthworms. Topsoil may therefore contain food resources used by both groups, and when in sufficient quantities it allows these organisms to coexist. This fraction of the soil had a relatively large amount of organic material, but also contains relatively more fresh matter (less humus) and has a high C/N ratio [25]; these conditions may attract microarthropods.

Competition for food, especially in the top few centimetres of the soil, may be one of the reasons for the negative relationship between *H. elisae* and microarthropods. This negative effect might directly affect only detritivorous, fungivorous and microbivorous microarthropods (basically collembolans and the mites of Oribatida and Acaridida), since, given their size, earthworms are almost certainly able to harvest these resources more efficiently. Predatory microarthropods such as Gamasida and Actinedida mites might be indirectly affected; these would tend to congregate in areas where their prey is found. Their greater numbers in the absence of earthworms is probably due to the greater numbers of collembolans.

The members of Oribatida are particularly sensitive to perturbations in the environment [23] and apart from competition for food with earthworms, they might suffer from the latter's activities causing unfavourable physico-chemical changes to the soil [17]; in fact, even the simple addition of the extra soil may have been enough to disturb them. Maraun et al. [18] performed experiments similar to those of the present work, adding different sources of carbon, nitrogen and phosphorus to see how these affected the abundance of different soil organisms. The numbers of the earthworms *Aporrectodea caliginosa*, *Octolasion tyraeum* and *Dendrodrilus rubidus* increased with the extra carbon, as did those of nematodes and protozoa, but mites and collembolan numbers fell, perhaps as an indirect effect of earthworm presence.

Thus, the relationship between earthworms and microarthropods might be more complex than at first glance, the product of multiple, interacting processes that lead to different results under different circumstances. This might explain why varying results have been obtained by different authors, according to the species investigated and the environmental conditions reigning. In the present work, the negative effect of earthworms on microarthropod numbers disappeared when the cages were enriched with topsoil, suggesting that competition occurs between these organisms, but that when food resources are abundant the microarthropods are not terribly affected. It cannot, however, be ruled out that other processes that were not studied are at work.



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