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Movement response of Collembola to the excreta of two earthworm species: Importance of ammonium content and nitrogen forms

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ABSTRACT

Several studies reported variable effects of earthworms on microarthropod density and variety. The present study tests the attraction of seven collembolan species belonging to four families, to the excreta of two earthworm species belonging to two families and two ecological categories, *Aporrectodea giardi* and *Hormogaster elisae*. Our objectives were (1) to better understand the impact of earthworms on the composition and density of Collembola communities, and (2) to dissect mechanisms involved in the attraction. Experiments were performed in Petri dishes containing two half-disks of filter paper, one with earthworm excreta, i.e. casts or a mix of mucus and urine, and the other with natural soil aggregates or water, respectively. Collembola were introduced half-way between the two half-disks and their number was counted on each half-disk and compared over 140 min. The content of ammonium in casts and mucus-urine of both earthworm species was analyzed to determine whether it altered the responses of Collembola faced with different types of earthworm excreta. The behaviour of Collembola varied strongly among the seven collembolan species, and with type of excreta and earthworm species. Six collembolan species were attracted to the mucus and urine of at least one earthworm species. The mucus-urine mixture of *A. giardi*, with low ammonium content, was generally more attractive than that of *H. elisae*, which was even repulsive in some cases, probably because of high levels of ammonium. The attraction to casts of the two earthworm species was less frequent and more variable. *Folsomia candida* was neither attracted to the casts nor to the mucus and urine of any earthworm species. Therefore, (1) earthworm species with different ecology, and different nitrogen excretion pathway impact differently the behaviour of collembolan species belonging to the same family or arising from the same habitat, and (2) variations in the sensitivity to ammonium among collembolan species partially explain the variable response of Collembola to earthworm excreta.

1. Introduction

There has been much discussion about the effect of earthworms on density, species assemblage and behaviour of soil microarthropods. Several studies have focused on this important relationship in the soil system, leading to variable results. Some of these studies showed that density and variety of microarthropods are greater in soils with higher numbers of earthworms (Marinissen and Bok, 1988; Hamilton and Sillman, 1989; Loranger et al., 1998; Salmon and Ponge, 1999, 2001; Tiunov, 2003; Salmon et al., 2005) while other observations reported a negative impact of earthworms on the density of microarthropods (Lagerlöf and Lofs-Holmin, 1987; McLean and Parkinson, 1998, 2000; Maraun et al., 2001; Migge, 2001; Gutiérrez et al., 2003).

To understand the impact of earthworms on the distribution of collembolan species and their population density, previous experimental studies dissecting the different impacts of earthworms were performed (Salmon, 2001, 2004; Salmon and Ponge, 2001; Salmon et al., 2005). They demonstrated that the anecic earthworm *Aporrectodea giardi* and its excreta (mixture of mucus and urine) were attractive to one collembolan species, *Heteromurus nitidus*. This springtail feeds on earthworm excreta and reaches higher population densities in the presence of *A. giardi* and *Lumbricus terrestris*, when submitted to predation pressure (being able to use earthworm galleries to escape from predators).

However, the endogeic earthworm *Hormogaster elisae* shows a negative effect on much of the microarthropod community, being repulsive to most taxa (Gutiérrez et al., 2003, 2008). Those results suggest that, in the experimental conditions employed, *H. elisae*

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reduces the abundance of microarthropods by changing environmental heterogeneity or through a possible competition with microarthropods. However, those studies were performed at the community level, not distinguishing between responses of each microarthropod species.

Therefore, it would be interesting to observe separately the behaviour of several collembolan species to determine repulsion or attraction to excreta of earthworms with the aim of investigate possible causes of positive or negative relationships between these two groups of soil fauna. Owing to their ecologic category, anecic and endogeic earthworms habits *sensu* Bouché (1972) could have different effects on the soil microarthropod community. The response of several collembolan species to earthworm excreta could show whether relationships observed with *A. giardi* at the population scale may be generalised at the community scale. Collembolan species used in our experiments belong to different families to examine the behaviour of taxa phylogenetically far distant from each other. Some of these species (*Folsomia candida*, *Pseudosinella alba*) are especially of interest because they have been frequently recorded in soils inhabited by earthworms or live directly in their galleries (Wickenbrock and Heisler, 1997; Tiunov and Kuznetsova, 2000).

The objectives of the present study were to assess whether (1) excreta of *A. giardi* may impact Collembola from the same habitat, not only at the species level (*H. nitidus*) but also at the scale of the community (many species), and (2) the negative impact of *H. elisae* on microarthropod densities may be explained by the response of Collembola to its excreta. We assessed the repulsive or attractive behaviour of seven collembolan species from four families to two forms of excreta (casts and mixture of mucus and urine) of the earthworms *A. giardi* and *H. elisae*, belonging to different families and ecological categories. The ammonium concentration was measured in the two types of excreta of both earthworm species with the aim of explaining the contrasting response of collembolan species to different earthworm species.

2. Materials and methods

2.1. Organisms used

All specimens of Collembola used in the experiments were obtained from batch cultures supplied with species originating from the calcic mull of the MNHN laboratory park (Brunoy, France) and from the Pyrenees Mountains. Collembolan cultures were kept on moist Fontainebleau sand at 15 °C in permanent darkness. They were fed with dried and ground cow manure and with lichens and epiphytic microalgae from various tree barks. Each experiment was performed with naive specimens previously starved for 3 days. The collembolan species used in the experiments were *Heteromurus nitidus* (Templeton, 1835) (Entomobryidae), *Pseudosinella alba* (Packard, 1873) (Entomobryidae), *Folsomia candida* Willem 1902 (Isotomidae), *Parisotoma notabilis* Schäffer 1896 (Isotomidae), *Onychiurus pseudogranulosus* (Sabatini and Innocenti 1995) (Onychiuridae), *Protaphorura prolata* (Gisin, 1956) (Onychiuridae), and *Arrhopalites caecus* (Tullberg, 1871) (Arrhopalitidae).

Those species differed from each other through their habitat and the area of their biogeographic distribution. *H. nitidus*, *P. alba*, *P. notabilis* and *O. pseudogranulosus* are hemiedaphic to euedaphic since they live in litter and deeper in the soil, while *F. candida* and *P. prolata* are preferentially soil-dwelling species (euedaphic), and *A. caecus* strictly a litter-dwelling species (hemiedaphic) (Deharveng and Lek, 1995; Ponge, 1993; Moore et al., 2005). *H. nitidus*, *F. candida* and *P. prolata* are also troglophilic, cave-dwelling species (Deharveng and Lek, 1995; Arbea and Baena, 2003). *H. nitidus*, *P. alba*, *O. pseudogranulosus* and *A. caecus* lives in neutral or sub-acidic soil, *A. caecus* being acid-intolerant (Ponge, 1993). All these species occur in Europe but *P. prolata* is endemic from the Pyrenees Mountains, while *P. alba* (European species), *F. candida* (cosmopolitan species except in Africa and India) and *P. notabilis* (cosmopolitan species) are more widely distributed (Hopkin, 1997).

The earthworm species used in the experiments were *Aporrectodea giardi* (Ribaucourt, 1900) (Lumbricidae), a large anecic earthworm (150 mm length) (Bouché, 1972), and *Hormogaster elisae* Álvarez, 1977 (Hormogastridae), a large endogeic earthworm (120–175 mm length) endemic to the centre of the Iberian Peninsula (Álvarez, 1977). *A. giardi* was sampled in a calcic mull from the laboratory park in Brunoy (France) and *H. elisae* in a plot from El Molar (Madrid, Spain) by handsorting several days before start of the experiments. They were kept in their original soil at 15 °C in darkness.

2.2. Experimental design

The experimental design was based on the method used by Salmon and Ponge (2001). The experiments were performed in six Petri dishes (8 cm diameter) containing two half-disks (5 cm diameter) of filter paper placed at 1.5 cm distance one from each other. Due to their smaller size, the behaviour of the collembolans *P. alba* and *P. notabilis* was studied in Petri dishes of 5 cm diameter with two half-disks of 3 cm diameter placed at 1 cm from each other. One half disk contained earthworm excreta (casts or mucus and urine) and the other was the reference substrate (filter paper covered with natural soil aggregates or impregnated with water, respectively). The two half-disks were equally moistened so that Collembola could choose between two substrates of similar color and consistency, i.e. between casts and calcic mull or between mucus-urine and water.

Casts were obtained from earthworms kept in large Petri dishes (14 cm diameter) on moistened filter paper during 3 days at 15 °C in darkness. For some collembolan species, experiments were also performed with casts obtained in the same conditions and kept during 10 days to assess the influence of aging of casts on the attraction of Collembola. Mucus and urine were obtained from earthworms that had previously voided their gut by keeping them for 3 days in large Petri dishes with moistened filter paper at 15 °C in darkness. Thereafter, they were placed in Petri dishes with six half disks of filter paper to saturate them with mucus and urine during 4 hours at 15 °C in darkness.

Ten adult Collembola were introduced half-way between the two half-disks in each Petri dish. Their number on each half-disk was counted every 10 min for 140 min. Specimens outside half-disk areas were ignored. The experiment was performed at ambient temperature (20 °C ± 1 °C) and under homogeneous light conditions measured with a light sensor and a LI 1000 Data Logger. Before experiments with earthworm excreta, five control experiments were performed with five collembolan species in Petri dishes containing two half-disks moistened with deionized water only. The purpose of these preliminary experiments was to ensure that other factors such as light, or temperature gradient did not influence their distribution.

Mean Collembola numbers on each half-disk for the 6 Petri dishes at each time-counting and global means of the 14 time-countings were calculated and compared by paired t-tests when data were normally distributed or a Wilcoxon test for paired samples when data were not normally distributed.

2.3. NH_4^+ content analysis

The NH_4^+ concentration was determined for fresh casts and for mucus-urine of both earthworm species following the distillation

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Kjeldahl method (IPLA, 1984; Ribó et al., 2003). To determine NH_4^+ concentration in mucus-urine, half-discs of filter paper impregnated with excreta of each earthworm species were analyzed. Earthworms had previously voided their gut by keeping them for three days in Petri dishes with moistened filter paper at 15 °C in darkness. They were then placed in Petri dishes with six half disks of filter paper to saturate them with mucus-urine during 4 h at 15 °C in darkness as done to obtain mucus-urine for attraction experiments. NH_4^+ concentration was referred to fresh weight of mucus-urine after calculating the amount of mucus-urine produced by increase in weight during these 4 h; the rate of mucus-urine production was calculated and referred to weight of earthworm and hour. Casts produced by earthworms during these three days were collected daily and maintained at −20 °C until analysis for NH_4^+. The rate of fresh cast production was referred to weight of earthworm and day. NH_4^+ concentration in casts was referred to dry weight of casts after measuring cast moisture in an oven at 105 °C for 24 h. Finally means of NH_4^+ concentration in casts and mucus-urine of both earthworm species were compared by *t*-tests for independent samples.

3. Results

Control experiments (deionized water in both half-disks), performed with *H. nitidus*, *F. candida*, *P. notabilis*, *O. pseudogranulosus* and *P. prolata* showed no preferences for any of the positions (Table 1) indicating that other factors such as light, moisture or temperature gradient did not influenced their distribution and that the experiment design appeared suitable for our objectives.

H. nitidus was more abundant on half-disks impregnated with mucus and urine of *A. giardi* and *H. elisae* than on half-disks saturated with deionized water (Tables 1 and 2, Fig. 1A and 2C), showing an attraction. However, it did not show any significant attraction neither to recent nor to old casts of *H. elisae*, although the mean abundance of *H. nitidus* was slightly higher on half-disks with recent casts than on half-disks with bulk soil (Table 2, Fig. 2A and B). *P. prolata* was also strongly attracted to mucus and urine of both earthworm species, nearly for all time-countings (Tables 1 and 2, Figs. 1H and 2J) and did not show any attraction to recent casts of both earthworm species (Tables 1 and 2). *P. alba* and *P. notabilis* were both more abundant on half-disks impregnated with mucus and urine of *A. giardi* than on half-disks impregnated with water, indicating an attraction (Table 1; Fig. 1B and D).

F. candida did not show any attraction to earthworm casts (recent or old) of *H. elisae* (Table 2). The attraction of this species to mucus and urine of both earthworm species was marginally significant, the mean number being significantly higher on half-disks impregnated with mucus and urine of *A. giardi* and *H. elisae*, respectively for two (30 and 120 min, Fig. 1C) and five time-countings (100 to 140 min, Fig. 2F).

O. pseudogranulosus was not attracted to any excreta (neither casts nor mucus and urine) of *A. giardi* (Table 1) although for two time-countings (40 and 50 min) their number was higher on half-disks with mucus and urine than on half-disks with water (Fig. 1F). In the case of excreta of *H. elisae*, *O. pseudogranulosus* was highly attracted to recent casts, but not to its mucus and urine, which tended to be repulsive (Table 2, Fig. 2G and H). The number of *O. pseudogranulosus* was even, for three time-countings, higher on half-disks with water than on half-disks with mucus and urine of *H. elisae*.

A. caecus was more abundant on half-disks with mucus and urine of *A. giardi* than on half-disks with water, but it did not show any attraction to recent casts (Table 1, Fig. 1I and J). In contrast, *A. caecus* was attracted to recent casts of *H. elisae,* but mucus and urine of *H. elisae* were repulsive for *A. caecus* (Table 2; Fig. 2K and L).

Table 3 shows the NH_4^+ content of casts and mucus-urine of both earthworm species and the results of t-tests. The content of NH_4^+ in casts of *A. giardi* was higher than in casts of *H. elisae*. The rate of cast production was 0.0755 g. fresh body $g^{-1}d^{-1}$ for *H. elisae* and 0.0425 g. fresh body $g^{-1}d^{-1}$ for *A. giardi*. Conversely, the NH_4^+ content in mucus-urine was lower in *A. giardi* than in *H. elisae* indicating differences in the nitrogen excretion path ways between

Table 1
Mean numbers of collembolan specimen on each half-disk and results of paired t-tests for attraction experiments performed with excreta of *A. giardi* (**p* < 0.05; ***p* < 0.01; ****p* < 0.001). Control A and Control B: in the control experiments, both half-disks were moistened only with deionized water and named “A” and “B” to distinguish half-discs.

Collembolan species	Analysis	Control		Mucus-urine		Recent casts	
				Aporrectodea giardi		*Aporrectodea giardi*	
		Control A	Control B	Mucus-urine	Water	Casts	Soil
Heteromurus nitidus	Mean	3.809	3.511	3.77	2.91	–	–
	T- test	0.146		5.916		–	
	p value	0.445		9.83E-04***		–	
Pseudosinella alba	Mean	–	–	5.51	0.68	–	–
	T- test	–		10.045		–	
	p value	–		8.36E-05***		–	
Folsomia candida	Mean	4.559	4.297	3.73	2.67	–	–
	T- test	0.281		1.577		–	
	p value	0.395		0.088		–	
Parisotoma notabilis	Mean	2.05	2.17	5.51	2.18	–	–
	T- test	-0.292		2.884		–	
	p value	0.391		0.017*		–	
Onychiurus pseudogranulosus	Mean	4.130	3.714	4.738	3.964	4.714	5.011
	T- test	0.922		0.823		−0.289	
	p value	0.199		0.224		0.392	
Protaphorura prolata	Mean	4.369	4.738	7.571	2.166	5.035	4.821
	T- test	−0.183		2.851		0.187	
	p value	0.431		0.018*		0.430	
Arrhopalites caecus	Mean	–	–	5.952	2.190	4.452	3.721
	T- test	–		3.770		0.526	
	p value	–		0.007**		0.311	

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Table 2
Mean numbers of collembolan specimen on each half-disk and results of paired t-tests for attraction experiments performed with excreta of *H. elisae* (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Collembolan species	Analysis	Mucus-urine		Recent casts		Old casts	
		Hormogaster elisae		*Hormogaster elisae*		*Hormogaster elisae*	
		Mucus-urine	Water	Casts	Soil	Casts	Soil
Heteromurus nitidus	Mean	6.392	2.059	5.583	3.654	4.678	4.857
	T- test	2.385		1.136		−0.108	
	p value	0.031*		0.154		0.459	
Folsomia candida	Mean	6.238	3.357	4.869	4.369	4.035	4.164
	T- test	1.830		0.355		−0.096	
	p value	0.063		0.369		0.464	
Onychiurus pseudogranulosus	Mean	3.630	4.488	6.702	2.547	–	–
	T- test	−1.940		3.867		–	
	p value	0.055		0.006**		–	
Protaphorura prolata	Mean	6.357	3.154	4.880	4.750	–	–
	T- test	3.144		0.086		–	
	p value	0.013*		0.467		–	
Arrhopalites caecus	Mean	2.952	4.166	4.488	2.964	–	–
	T- test	−3.343		2.602		–	
	p value	0.010*		0.024*		–	

these two species. The rate of mucus-urine production was 0.0007 g. fresh body $g^{-1}h^{-1}$ for *H. elisae* and 0.0015 g. fresh body $g^{-1}h^{-1}$ for *A. giardi*.

4. Discussion

The two studied earthworm species affected seven collembolan species in different ways, as shown by diverse attractive, neutral or repulsive reactions to earthworm excreta.

4.1. Responses to mucus-urine

Six collembolan species out of seven were attracted to mucus and urine of at least one earthworm species. Previous studies showed the important effects of earthworm mucus in varied invertebrate taxa: attraction of some species of Coleoptera (Digweed, 1994), repellence of ants by the mucus of litter-dwelling earthworms, suggesting a chemical defence against predation (Laakso and Setälä, 1997), stimulation of the oviposition behaviour in *Coenosia tigrina* (Diptera) females (Morris and Pivnick, 1991). The attraction of collembolan species to mucus and urine of earthworms could be due to molecules present in mucus (essentially glycoproteins, peptides and amino acids) and in urine (urea and ammonium) (Edwards and Bohlen, 1996). Those small molecules could be easily assimilable by Collembola among others, and are known to stimulate microflora (Brown, 1995; Trigo et al., 1999) that are grazed by Collembola. In addition ammonium may or may not be transformed in volatile ammonia according to concentration and affect the sensitivity of *H. nitidus* to the odour of earthworm excreta (Salmon, 2001; Salmon and Ponge, 2001).

The mucus-urine mixture of *A. giardi* was generally more attractive than that of *H. elisae*, since it strongly attracted five collembolan species versus two. The attraction of *H. nitidus* to the mucus and urine of both species supports observations of Salmon and Ponge (1999, 2001), that *H. nitidus* consumed mucus and urine of *A. giardi,* showing that the interaction was at least partly trophic. *P. alba*, and *P. notabilis* were also attracted to mucus and urine of *A. giardi*, which corroborates the observation of *P. alba* in earthworms middens by Maraun et al. (1999), and *P. notabilis* in earthworm galleries (Marinissen and Bok, 1988) that are lined with earthworm casts and mucus (Kretzschmar, 1987). *P. prolata* was also highly attracted to mucus and urine of both earthworm species while the other Onychiuridae species *O. pseudogranulosus* only tended to be slightly attracted to mucus and urine of *A. giardi*. This indicates that even taxonomically close collembolan species show different preferences.

Attraction was not the only reaction to earthworm mucus and urine we observed, as shown by the repulsion of *O. pseudogranulosus* and *A. caecus* to mucus and urine of *H. elisae,* providing evidence that different earthworm species (and even family) impact the surrounding microarthropod community in various ways. Differences in nitrogen cycling between earthworm species may explain the lower attraction or the repellence effect of the mucus and urine of *H. elisae*. Some collembolan species avoid substrates containing high amounts of ammonium but are attracted to very low levels of ammonium while they do not respond to intermediate amounts of ammonium (Salmon, 2001). The urine of *H. elisae* contains high levels of ammonium that are able to repel some collembolan species. The absence of attraction or the repulsion of *O. pseudogranulosus* and *A. caecus* to the mucus-urine of *H. elisae* may thus be attributed to higher levels of ammonium, and suggests that some collembolan species are more sensitive than others to high concentration levels of ammonium.

4.2. Responses to fresh casts

The attraction to casts was less frequent and more variable compared to attraction to mucus and urine. None of the tested species showed an attraction to fresh casts of *A. giardi*, and casts of *H. elisae* were attractive, only for *O. pseudogranulosus* and *A. caecus*. Earthworm casts were expected to attract Collembola, especially casts of anecic earthworms, which contain a wide variety of food materials consumed usually by several Collembola, such as fungi, lichens, decomposing carcasses, vegetation or detritus, microbial flora, and faecal pellets (Payne et al., 1968; Ponge, 1991; Hopkin, 1997). They also contain intestinal mucus and proteins, glycoproteins, urea, amino-acids, vitamins and glycosides (El Duweini and Ghabbour, 1971). However intestinal mucus is poorer than epidermal mucus, most nitrogenous compounds being reabsorbed in the foregut (Bernier, 1998). A lower content in organic nitrogenous molecules in casts than in epidermal mucus could partly

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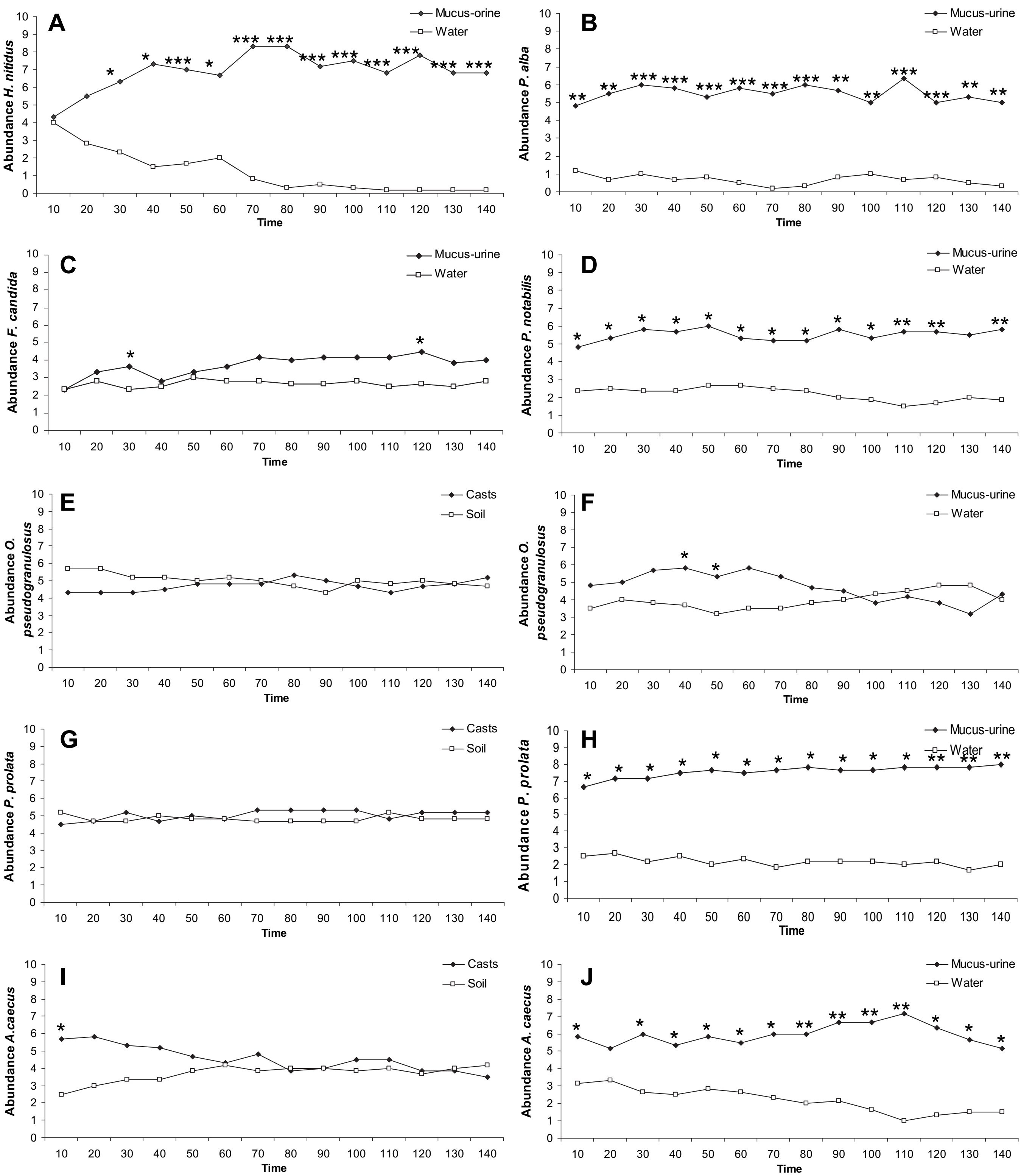

Fig. 1. Mean numbers of each Collembolan species on the two half-disks of filter paper in experiments performed with excreta of *Aporrectodea giardi*. A: *Heteromurus nitidus*; B: *Pseudosinella alba*; C: *Folsomia candida*; D: *Parisotoma notabilis*; E and F: *Onychiurus pseudogranulosus*; G and H: *Protaphorura prolata;* I and J: *Arrhopalites caecus*. Results of statistical analyses are also indicated (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

explain the lower attractiveness of casts compared to mucus and urine.

The indifference to recent casts of *A. giardi* supports the behaviour of *H. nitidus* to casts of *A. giardi* and *A. chlorotica* observed by Salmon and Ponge (2001). These authors attributed the absence of attraction to high concentrations of ammonium in fresh casts of *A. giardi* (0.37 g $NH_4^+ l^{-1}$), occurring in the range of ammonium contents avoided by *H. nitidus* (between 0.2 and 0.4 g $NH_4^+ l^{-1}$)

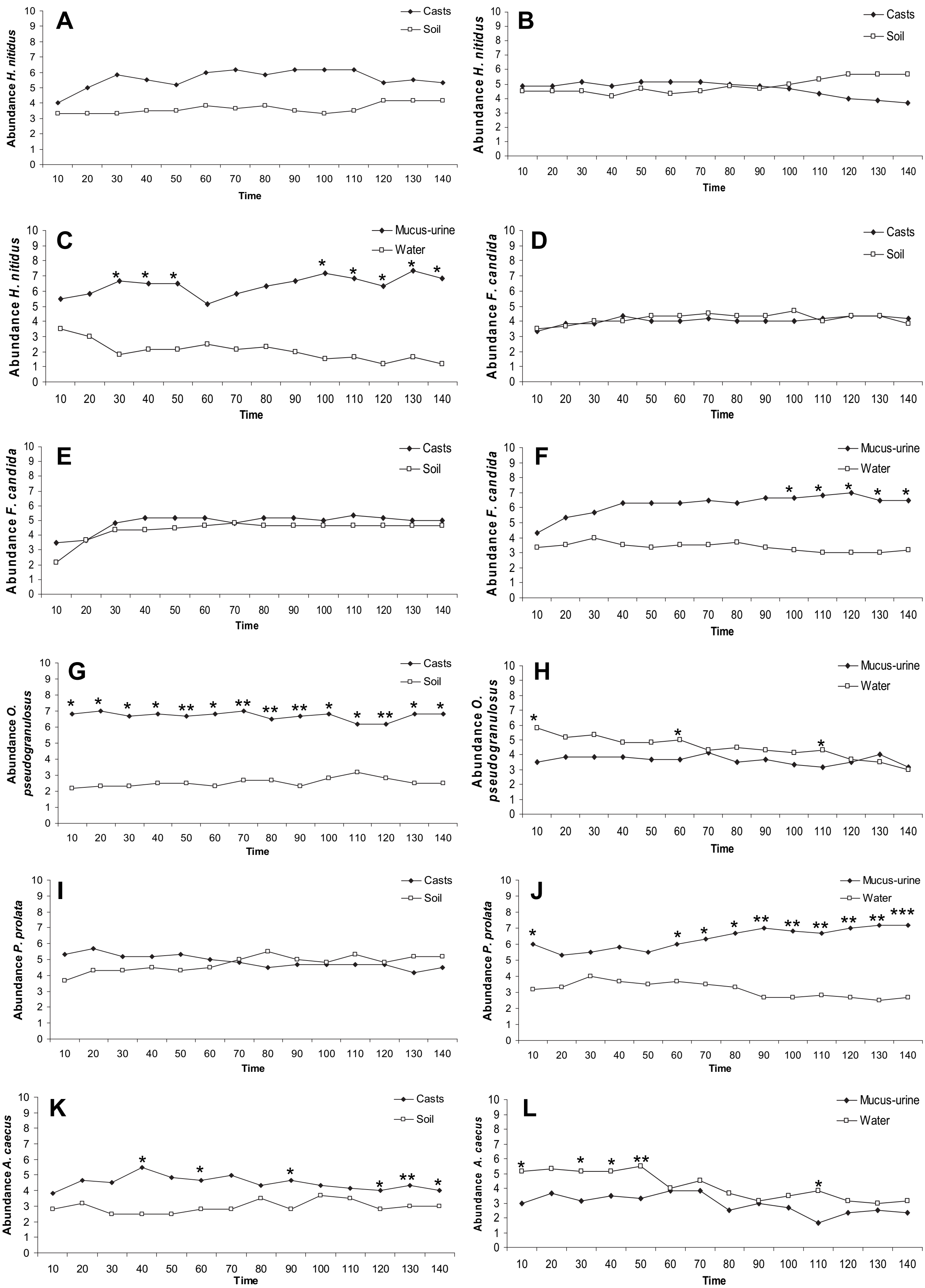

Fig. 2. Mean numbers of each Collembolan species on the two half-disks of filter paper in experiments performed with excreta of *Hormogaster elisae*. A (recent casts), B (old casts) and C: *Heteromurus nitidus*; D (old casts), E (recent casts) and F: *Folsomia candida*; G and H: *Onychiurus pseudogranulosus*; I and J: *Protaphorura prolata*; K and L: *Arrhopalites caecus*. Results of statistical analyses are also indicated (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Table 3
Mean weight of casts produced, mean weight of mucus-urine produced, % NH_4^+ in casts and mucus-urine of both earthworm species and results of t-tests ($^*p < 0.05$).

	Hormogaster elisae	*Aporrectodea giardi*	T-test	p-value
Casts g d^{-1}	0.0755	0.0425		
Mucus-Urine g h^{-1}	0.0007	0.0015		
% NH_4^+ (Casts)	0.0515	0.5164	−8.294	1.4E−07*
% NH_4^+ (Mucus-Urine)	0.2925	0.0474	3.445	0.006*

(Salmon, 2001, 2004). The excessive level of ammonium in casts of *A. giardi*, confirmed by the dosage of ammonium, would counterbalance the nutritional quality of casts and alter their attractiveness. For *H. elisae*, processes explaining the reactions of Collembola to casts are probably different. The attraction to casts of *H. elisae* of two collembolan species (*O. pseudogranulosus* and *A. caecus*), previously proved to be repelled by mucus and urine of *H. elisae,* could be explained by differences in the nitrogen excretion path way between *A. giardi* and *H. elisae*, resulting in lower levels of ammonium in casts than in the mucus-urine mix of *H. elisae*. In fact, most ammonium is excreted in casts in *A. giardi* while urine is the main pathway of ammonium excretion in *H. elisae*. The absence of attraction of other collembolan species (*H. nitidus*, *P. prolata*, *F. candida*) to casts of *H. elisae* could arise from the trophic regime of *H. elisae* leading to weaker nutritional quality of its casts (poorer in organic matter, (Bouché, 1972). Nevertheless casts of *H. elisae* may provide enough organic and overall mineral nutrients (Gutiérrez et al., 2006) for *O. pseudogranulosus* that is a soil-dwelling Collembola (Deharveng and Lek, 1995), feeding on a fraction of mineral particles (Saur and Ponge, 1988). The indifference of *F. candida* to casts of any earthworm species (of any age), may further be explained by its diet, consisting mainly of different fungal species and vesicular arbuscular mycorrhizae (Booth and Anderson, 1979; Moore et al., 1985), without cast and plant debris.

4.3. Responses to old casts

Respecting to the age of casts, although some studies showed variations in microbial biomass and nutrient content of casts with age (Migge, 2001), no differences in attraction between old (10–13 days) and recent casts (1–3 days) of *H. elisae* were found for the two collembolan species tested, *H. nitidus* and *F. candida*. Contrarily to 15-day-old casts of *A. giardi* that became more attractant to *H.nitidus* than fresh casts (Salmon, 2004) following upon the decline of ammonium (Salmon, 2001, 2004; see above), in the case of *H. elisae*, the ammonium content of casts is low (and thus not repellent) even in fresh casts, which could explain with a stable microbial biomass, the absence of differences in the response to fresh and old casts.

4.4. Conclusions

In conclusion, most of the collembolan species tested here were attracted to mucus and urine of earthworms, which could explain the favourable impact of earthworms on microarthropod communities observed by several authors (Marinissen and Bok, 1988; Hamilton and Sillman, 1989; Loranger et al., 1998; Salmon and Ponge, 1999, 2001; Tiunov, 2003; Salmon et al., 2005). However, the behaviour of Collembola (attraction or repulsion) varied with the quality of excreta (casts or mucus-urine) as well as with the physiology (nitrogen excretion path way) of the earthworm species considered, which supports contrasting observations about the influence of earthworms on microarthropod communities, usually explained by perturbation of habitat or even competition for resources (Lagerlöf and Lofs-Holmin, 1987; McLean and Parkinson, 1998, 2000; Maraun et al., 2001; Migge, 2001; Gutiérrez et al, 2003, 2008). Nutritional quality and ammonium concentration seem to be the most likely causes of response of Collembola to earthworm excreta. However other causes such as the improvement of the habitat quality by earthworms cannot be excluded, excreta signalling only the presence of earthworms. Choice experiments between artificial and natural galleries of earthworms would clarify this point, particularly for some species for which the attraction was not so clear, e.g. *F. candida*.

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References

Álvarez, J., 1977. El género *Hormogaster* en España. Publicaciones del Centro Pirenaico de Biología Experimental 9, 27–35.
Arbea, J.I., Baena, M., 2003. Colémbolos cavernícolas de Andalucía (Insecta: Collembola). Zoologica Baetica 13-14, 71–84.
Bernier, N., 1998. Earthworm feeding activity and development of the humus profile. Biology and Fertility of Soils 26, 215–223.
Booth, R.G., Anderson, J.M., 1979. The influence of fungal food quality on the growth and fertility of *Folsomia candida* (Collembola: Isotomidae). Oecologia 38, 317–323.
Bouché, M.B., 1972. Lombriciens de France. Ecologie et Systématique. I.N.R.A, Paris.
Brown, G.G., 1995. How do earthworms affect microfloral and faunal community diversity? Plant and Soil 170, 209–231.
Deharveng, L., Lek, S., 1995. High diversity and community permeability: the riparian Collembola (Insecta) of a Pyrenean massif. Hydrobiologia 312, 59–74.
Digweed, S.C., 1994. Detection of mucus-producing prey by *Carabus nemoralis* Mueller and *Scaphinotus marginatus* Fischer (Coleoptera: Carabidae). Coleopterists Bulletin 48, 361–369.
Edwards, C.A., Bohlen, P.J., 1996. Biology and Ecology of Earthworms. Chapman and Hall, London, UK.
El Duweini, A.K., Ghabbour, I., 1971. Nitrogen contribution by live earthwormsto the soil. IVth Colloquium Pedobiologiae, 1970, September, Dijon, France, pp. 495–501. INRA, Paris.
Gutiérrez, M., Ramajo, M., Jesús, J.B., Díaz Cosín, D.J., 2003. The effect of *Hormogaster elisae* (Hormogastridae) on the abundance of soil Collembola and Acari in laboratory cultures. Biology and Fertility of Soils 37, 231–236.
Gutiérrez, M., Jesús, J.B., Trigo, D., Díaz Cosín, D., 2006. Is *Hormogaster elisae* (Oligochaeta, Hormogastridae) a predator of mites and springtails? European Journal of Soil Biology 42, S186–S190.
Gutiérrez, M., Jesús, J.B., Trigo, D., Novo, M., Díaz Cosín, D.J., 2008. Is there food competition between *Hormogaster elisae* (Oligochaeta, Hormogastridae) and soil microarthropods at El Molar (Madrid)? European Journal of Soil Biology 44, 207–212.
Hamilton, W.E., Sillman, D.Y., 1989. Influence of earthworm middens on the distribution of soil microarthropods. Biology and Fertility of Soils 8, 279–284.
Hopkin, S.P., 1997. Biology of the Springtails (Insecta: Collembola). Oxford University Press, Oxford.
IPLA, 1984. Metodi analitici. Fertilizanti organici, compost, fanghi degli impianti di depurazione, rifiuti organici, substrati in fermentazione metanica, bioga. IPLA, Milan.
Kretzschmar, A., 1987. Caractérisation microscopique de l'activité des lombriciens endogés. In: Fedoroff, N., Bresson, L.M., Courty, M.A. (Eds.), Micromorphologie des Sols. Soil Micromorphology. AFES, Paris, pp. 325–330.
Laakso, J., Setälä, H., 1997. Nest mounds of red wood ants (*Formica aquilonia*): hot spots for litter-dwelling earthworms. Oecologia 111, 565–569.
Lagerlöf, J., Lofs-Holmin, A., 1987. Relationships between earthworms and soil mesofauna during decomposition of crop residues. In: Striganova, B.R. (Ed.), Soil Fauna and Soil Fertility. Nauka, Moscow, pp. 377–381.
Loranger, G., Ponge, J.F., Blanchart, E., Lavelle, P., 1998. Influence of agricultural practices on arthropod communities in a vertisol (Martinique). European Journal of Soil Biology 34, 157–165.
Maraun, M., Alphei, J., Bonkowski, M., Buryn, R., Migge, S., Peter, M., Schaefer, M., Scheu, S., 1999. Middens of the earthworm *Lumbricus terrestris* (Lumbricidae): microhabitats for micro- and mesofauna in forest soil. Pedobiologia 43, 276–287.
Maraun, M., Alphei, J., Beste, P., Bonkowski, M., Buryn, R., Migge, S., Peter, M., Schaefer, M., Scheu, S., 2001. Indirect effects of carbon and nutrient

amendments on the soil meso- and microfauna of a beechwood. Biology and Fertility of Soils 34, 222–229.
Marinissen, J.C.Y., Bok, J., 1988. Earthworm-amended soil structure: its influence on Collembola populations in grassland. Pedobiologia 32, 243–252.
McLean, M.A., Parkinson, D., 1998. Impacts of the epigeic earthworm *Dendrobaena octaedra* on oribatid mite community diversity and microarthropod abundances in pine forest floor: a mesocosm study. Applied Soil Ecology 7, 125–136.
McLean, M.A., Parkinson, D., 2000. Introduction of the epigeic earthworm *Dendrobaena octaedra* changes the oribatid community and microarthropod abundances in a pine forest. Soil Biology and Biochemistry 32, 1671–1681.
Migge, S., 2001. The effect of earthworm invasion on nutrient turnover, microorganisms and microarthropods in Canadian aspenforest soil (PhD dissertation). Darmstadt, Germany: Technische Universitat. Darmstadt.
Moore, J.C., St. John, T.V., Coleman, D.C., 1985. Ingestion of vesicular-arbuscular mycorrhizal hyphae and spores by soil microarthropods. Ecology 66, 1979–1981.
Moore, J.C., Saunders, P., Selby, G., Horton, H., Chelius, M.K., Chapman, A., Horrocks, R.D., 2005. The distribution and life history of *Arrhopalites caecus* (Tullberg): Order: Collembola, in Wind Cave, South Dakota, USA. Journal of Cave and Karst Studies 67, 110–119.
Morris, D.E., Pivnick, K.A., 1991. Earthworm mucus stimulates oviposition in a predatory fly (Diptera: Anthomyiidae). Journal of Chemical Ecology 17, 1573–1581.
Payne, J.A., King, E.W., Beinhart, G., 1968. Arthropod succession and decomposition of buried pigs. Nature 219, 1180–1181.
Ponge, J.F., 1991. Food resources and diets of soil animals in a small area of Scots pine litter. Geoderma 49, 33–62.
Ponge, J.F., 1993. Biocenoses of Collembola in atlantic temperate grass-woodland ecosystems. Pedobiologia 37, 223–244.
Ribó, M., Canet, R., Albiach, M.R., Pomares, F., 2003. Mineralización del nitrógeno del suelo. In: García, C., Gil, F., Hernández, T., Trasar, C. (Eds.), Técnicas de Análisis de Parámetros Bioquímicos en Suelos: Medida de Actividades Enzimáticas y Biomasa Microbiana. Mundi-Prensa, Madrid.
Salmon, S., 2001. Earthworm excreta (mucus and urine) affect the distribution of springtails in forest soils. Biology and Fertility of Soils 34, 304–310.
Salmon, S., 2004. The impact of earthworms on the abundance of collembola: improvement of food resources or of habitat? Biology and Fertility of Soils 34, 304–310.
Salmon, S., Ponge, J.F., 1999. Distribution of *Heteromurus nitidus* (Hexapoda, Collembola) according to soil acidity: interactions with earthworms and predator pressure. Soil Biology and Biochemistry 31, 1161–1170.
Salmon, S., Ponge, J.F., 2001. Earthworm excreta attract soil springtails: laboratory experiments on *Heteromurus nitidus* (Collembola: Entomobridae). Soil Biology and Biochemistry 33, 1959–1969.
Salmon, S., Geoffroy, J.J., Ponge, J.F., 2005. Earthworms and Collembola relationships: effects of predatory centipedes and humus forms. Soil Biology and Biochemistry 37, 487–495.
Saur, E., Ponge, J.-F., 1988. Alimentary studies on the Collembolan *Paratullbergia callipygos* using transmission electron microscopy. Pedobiologia 31, 355–379.
Tiunov, A.V., 2003. Effect of *Lumbricus terrestris* burrows on spatial and taxonomic Structure of soil communities. Entomological Review 83, S91–S96.
Tiunov, A.V., Kuznetsova, N.A., 2000. Environmental activity of anecic earthworms (*Lumbricus terrestris* L.) and spatial organization of soil communities (in Russian). Izvestiya Akademii Nauk Seriya Biologicheskaya 5, 607–616.
Trigo, D., Barois, I., Garvín, M.H., Huerta, E., Irisson, S., Lavelle, P., 1999. Mutualism between earthworms and soil microflora. Pedobiología 43, 866–873.
Wickenbrock, L., Heisler, C., 1997. Influence of earthworm activity on the abundance of Collembola in soil. Soil Biology and Biochemistry 29, 517–521.

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