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Earthworm excreta attract soil springtails: laboratory experiments on Heteromurus Nitidus (Collembola: Entomobryidae)

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Abstract

Microarthropods are often found more abundantly in soils with earthworms than in soils without. Earthworms probably create a favourable environment for microarthropods but few studies have aimed to explain this earthworm effect. The soil collembolan (Hexapoda) *Heteromurus nitidus*, living in soils at pH > 5 only and thus rich in earthworms, is particularly attracted by earthworms in humus cores. The effect of earthworms on the distribution of *H. nitidus* can be mediated either by direct contact or by odour perception. Two experimental designs were used to determine the pathway of attraction. The first set of experiments studied the effect of direct contact with earthworm excreta on the distribution of *H. nitidus*. The mixture of urine and mucus of the lumbricid earthworms *Aporrectodea giardi* and *Alollobophora chlorotica* signifcantly attracted *H. nitidus* as compared to deionized water while fresh earthworm casts were not preferred to calcic mull made of older casts. The same experiment involving direct contact with mucus and methyl blue showed that Collembola sucked on mucus/urine, indicating that the interaction of Collembola and earthworms was at least partly trophic. The second experiment demonstrated that *H. nitidus* was attracted by the odour of *Aporrectodea giardi* at short distance. The odour of excreta (mucus, urine and casts) of *Aporrectodea giardi* also attracted *H. nitidus* but this attraction was weaker and did not occur constantly, possibly due to interactions with light and aggregation pheromones. We conclude that the prominent pathway by which earthworms could attract *H. nitidus* in the field is through direct contact with earthworm mucus and urine. The acid-intolerant distribution of this species in the field could be partly explained by a trophic interaction with some earthworm species.

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1. Introduction

Collembolan communities vary in soils according to several factors, among them the presence of earthworms, high populations of which characterize mull humus forms with a moderately low to high pH (Satchell, 1967; Piearce, 1972). In fact, some studies have shown that the density and diversity of microarthropods increase in soils with earthworms (Bayoumi, 1978; Marinissen and Bok, 1988; Hamilton and Sillman, 1989; Loranger et al., 1998). Moreover, numerous Collembola have been observed directly wandering on giant earthworm bodies (Bouché personal communication) and in rearing beds of earthworms (Greenslade and Fletcher, 1986; Arbea and Jordana, 1988). However no attempt has been made to study direct positive effects of earthworms on the surrounding fauna.

Earthworms, through their action on the surrounding soil, improve water availability, aeration, and pore size (Wickenbrock and Heisler, 1997; Devliegher and Verstraete, 1997; Subler and Kirsch, 1998), which may attract microarthropods. In addition, earthworms increase the availability of a number of inorganic (Jeanson, 1972; Scheu, 1987; Robinson et al., 1992) and organic compounds (Dubash and Ganti, 1964; Martin et al., 1987), favour some bacteria (Kozlovskaja, 1969; Saetre, 1998) and increase or decrease fungal populations (Brown, 1995) on which Collembola may feed. These products may attract Collembola or favour their abundance and diversity.

The collembolan species *Heteromurus nitidus* is always found in soils at pH > 5 that generally bear mull humus (Ponge, 1993; Salmon and Ponge, 1999). This species seems to be particularly dependent on the presence of earthworms since mull humus is characterised by a high abundance of earthworms and particularly we demonstrated, from laboratory experiments, that *Heteromurus nitidus* preferred humus blocks with rather than without earthworms (Salmon and Ponge, 1999). However, we know neither the causes nor the pathway of this attraction. One or many of the substances and microorganisms excreted by earthworms (see above) may attract Collembola. In fact, fungal-feeding Collembola were able to discriminate between different fungal species and showed preferences among them (Shaw, 1988; Thimm and Larink, 1995; Kaneko et al., 1995). They recognized fungal odours and were attracted or repelled by them (Bengtsson et al., 1988; Hedlund et al., 1995;

Sadaka-Laulan et al., 1999). Some Collembola were also attracted by a mite species (Huber, 1979). In addition, earthworms are known to attract other invertebrate species (Halpern et al., 1984; Morris and Pivnick, 1991).

The aim of this study was to determine the causes and the pathway of attraction of the collembolan *H. nitidus* by two earthworm species. Is *H. nitidus* attracted at a distance by the odour of earthworms or does it remain in the vicinity of the earthworms after direct contact? Earthworms and their excreta (mucus, urine and casts) were tested in odour experiments. In direct contact experiments, the effects of mucus plus urine, and casts, were studied. One experiment using earthworm mucus and urine and methyl blue was performed to assess whether mucus was consumed by Collembola. The present paper focuses on two lumbricid species *Aporrectodea giardi* and *Allolobophora chlorotica* which attracted *H. nitidus* in humus blocks (Salmon and Ponge, 1999). They were extracted from a calcic mull, a humus form in which *H. nitidus* is commonly found. The mechanisms of attraction and the impact of earthworms on the distribution of *H. nitidus* are discussed.

2. Materials and methods

2.1. Test organisms

Specimens of *H. nitidus* were reared in laboratory cultures on moist Fontainebleau sand (pure fine quartz sand). They were fed with lichens and terrestrial microalgae (*Pleurococcus*) from bark scrapings. Cultures were kept at 15°C in permanent darkness. Each experimental run was performed with new specimens.

Two earthworm species were sampled from a calcic mull in the laboratory park. *Aporrectodea giardi* is a large earthworm (150 mm in length) belonging to the anecic category (Bouché, 1972). *Allolobophora chlorotica* is smaller (50 mm) and classified as endogeic. The extraction was performed with 4% formalin a few days before the start of each experiment, and in the mean time earthworms were kept in their original soil at 15°C in darkness.

2.2. Direct contact experiments

2.2.1. General protocol

Direct contact experiments were performed in six Petri dishes (∅ 8 cm) containing two half-disks (∅ 5 cm) of filter paper placed at 1.5 cm distance from each other. The dishes were placed on a laboratory table. Only one of the half-disks contained earthworm excreta (casts or mucus plus urine; see below), the other served as reference (calcic mull aggregates or water). Ten adult *H. nitidus* were taken randomly from the rearing boxes then introduced in each of the six Petri dishes (replicates), half-way between the two half-disks. Their abundance was counted on each half-disk every 10 min for 140 min. Animals outside the half-disk areas were ignored. All runs were established at ambient temperature (around 20°C), under homogeneous light conditions (checked with LI 1000 Data Logger and LI-COR Radiation sensors). The two half-disks were equally moistened. A control experiment was performed with both half-disks moistened with deionized water only, in order to verify that some factor other than earthworm excreta, especially light, did not influence the distribution of *H. nitidus*. This species is highly sensitive to light (Salmon and Ponge, 1998) and although light conditions were as homogenous as possible a control experiment was necessary to verify this point.

For each replicate, means of 14 time-measures of numbers of Collembola on each half-disks were calculated. The normality of the data was checked, and means of six replicates in 'earthworm excreta' were compared to means in 'reference substrate' by a paired t-test (Sokal and Rohlf, 1995).

2.2.2. Experiments with mucus and urine

Adult earthworms, eight *A. giardi* or 54 *A. chlorotica*, were rinsed under tap water. They were then placed on moistened filter paper to void their guts and kept at 15°C in darkness, in a water-tight plastic box, for 60 h. The filter paper was renewed every 20 h.

Three days after washing the worms, six half-disks of filter paper were saturated with mucus and urine by incubating them with the earthworms. For this purpose, half-disks and earthworms, the two species separately, were placed for 5 h in a water-tight enclosure at ambient temperature, in darkness. Mucus and urine were tested together because both are excreted from the wall of earthworms (Bouché, 1972). Six other half-disks were saturated with deionized water. *H. nitidus* could then choose between mucus plus urine or deionized water during 140 min as described above. As *H. nitidus* is known to be especially sensitive to desiccation (Bauer and Christian, 1987), the two half-disks were equally moistened (saturated) with deionized water or mucus plus

urine.

2.2.3. Experiments with casts

20 h before the start of the experiment eight adult *A. giardi* or 60 adult *A. chlorotica* were rinsed under tap water and added to Petri dishes (0 14 cm) containing moistened filter paper, to collect fresh casts. In order to condition reference substrates in the same manner, moistened filter paper lining the bottom of Petri dishes (0 14 cm) was covered by calcic mull aggregates collected at the same time as earthworms. Earthworms and calcic mull were then kept at 15°C in darkness, in a water-tight enclosure. On the day of the experiment, fresh casts and calcic mull aggregates were smeared separately on six half disks each by means of a scalpel. Both types of half disk were deposited in each Petri dish (replicate). Thereafter, choice experiments were run as described above. *H. nitidus* could then choose between casts and calcic mull material with similar colour and consistency, the hemorganic horizon of calcic mull being formed of aged earthworm casts.

2.2.4. Experiments with methyl blue

A similar experiment with mucus plus urine of eight *A. giardi* was carried out on half-disks previously stained with methyl blue dye. Methyl blue was used to see whether Collembola grazed and absorbed the mucus/urine secretion. In each box the two half-disks were equally stained to prevent any differential effect of colour upon the distribution of Collembola. Twelve half-disks were impregnated by a methyl blue water solution (25 g l^{-1}) and left to dry in darkness for 6 days until the choice experiments started. During the experiment *H. nitidus* could choose between two blue half-disks, the one impregnated with mucus plus urine, the other with deionized water.

A control experiment was performed in which each test box contained two blue half-disks saturated with deionized water. This control experiment allowed verification of the harmlessness of the dye and a contrast between the absorptive behaviour of *H. nitidus* in the presence of water only and water and mucus plus urine (test-experiment).

At the end of these choice experiments Collembola were transferred to ethanol (90%) and the colour of their gut contents was observed under a dissecting microscope.

2.3. Odour attraction experiments

To test the attraction by the odour of earthworm excreta, 22 adult *A. giardi* were rinsed in tap water and then placed in three Petri dishes (∅ 8 cm) containing three or four disks of filter paper 1 day before the experiments started. Petri dishes with earthworms were then placed in a watertight plastic box in darkness at ambient temperature, for 20 h, in order to impregnate the filter paper disks with earthworm excreta, i.e. mucus, urine and fresh casts. In the experiment where the odour of the earthworm itself (not its excreta) was studied, ten *A. giardi* were rinsed and used directly.

The experimental design allowed volatile compounds from earthworm excreta to diffuse without any contact or visual perception by Collembola (Fig. 1). All odour experiments comprised ten replicates (= ten experimental chambers). The experimental chambers consisted of rectangular plastic boxes 11.4 X 8.4 X 6.5 cm (l X l X h) divided into two compartments, each containing a plastic vessel filled with remoistened Fontainebleau sand. The top of one of the vessels was covered by a filter disk impregnated with *A. giardi* excreta. The other vessel was covered by a filter disk impregnated with deionized water only to obtain a similar moisture. The vessels were covered by a platform on which 12 *H. nitidus* individuals were allowed to wander. The platform varied according to the type of experiment (see below). Odour attraction experiments were accompanied by a control experiment, using the same apparatus but without earthworm excreta. This control experiment was designed to assess possible effects of other factors such as light on collembolan distribution.

In the first two sets of experiments, the platform for *H. nitidus* consisted of a moistened sheet of filter paper which was fixed to the box walls, 0.8-1 cm above the vessels (Fig. 1). The platform area was arbitrarily divided into two sectors by a drawn line. Above each vessel, the moist filter paper was perforated to allow the passage of lipophilic olfactory compounds through the suspended platform. Twelve adult or sub-adult *H. nitidus* (different for each replicate) were released over the suspended platform (six in each sector) and each test box was placed immediately in a dark enclosure. Light was present only during the short time spent for releasing (3 min) and counting (1 min) animals.

In the first series of experiments *H. nitidus* individuals were counted in both sectors every 10 min for 2.5 h. One experimental set assessed the effect of the odour of earthworm excreta, the other was a control experiment (two sectors 'no odour'). The second series of experiments, also comprising an odour and a control experimental set, was performed in the same device but Collembola were counted every 30 min over 7 h in order to have an overview of longer-term effects. Results were analysed as for direct contact experiments using paired t-test.

In the third series of experiments the apparatus was modified to allow for a better balance in the distribution of animals between the two sectors in the absence of an earthworm effect. Since the dispersal of Collembola is favoured by an increase in population density (Bengtsson et al., 1994), we reduced the size of the moist area by replacing the platform made of a filter paper sheet by two much smaller wet areas. The platform was made of a plastic sheet with two holes (∅ 3.2 cm) each covered with a perforated filter paper disk. Paper disks were moistened with an equal volume of deionized water to avoid the influence of varying humidity (Joosse and Verhoef, 1974). Twelve Collembola were released in the space between the two moist areas. Their number was counted on both filter paper disks each 30 min over 5.5 h. A control experiment (a) with two areas 'no odour', was performed to verify the homogeneity of the distribution in the two areas in the absence of an earthworm effect. An experiment (b) was carried out to assess the odour of earthworm excreta as in the previous device. A third experiment (c) was performed after replacing earthworm excreta by the earthworm itself: an individual of *A. giardi* was introduced in one of both vessels, then the corresponding vessel was locked up with a lid of perforated filter paper. *H. nitidus* individuals were counted in each area at each time. Mean differences between both areas in the ten boxes (replicates) were compared in the same way as in previous experiments.

3. Results

3.1. Direct contact with earthworm excreta

H. nitidus was significantly more abundant on half-disks impregnated with mucus and urine from *A. giardi* than on half-disks saturated with deionized water (Table 1). *H. nitidus* was also more attracted by mucus and urine from *A. chlorotica* than by water (Table 1) and the attraction was faster than for *A. giardi* (Fig. 2A). Preliminary experiments in the same conditions but using earthworms previously kept on moistened paper

during 0, 12 or 36 h instead of 60 h (thus the gut was incompletely voided), gave similar results (data not shown), confirming the trend of the choice exerted by animals. The control experiment indicated the absence of an uncontrolled preference within the dish (Table 1). Thus mucus and urine of both earthworm species attracted *H. nitidus* individuals. Observations between successive measurements showed that generally Collembola did not redistribute between half-disks when in contact with mucus and urine from earthworms. Numerical changes were only due to new individuals coming in from other places of the Petri dish.

When *H. nitidus* individuals were allowed to choose between calcic mull and casts of *A. giardi* or *A. chlorotica*, their density was slightly higher on casts but differences between both substrates were not significant (Table 2, Fig. 2B).

In the presence of stained half-disks, H. nitidus individuals did not show any preferences for a given area in the control experiment (Table 3). All animals survived and were distributed as in the case of undyed half-disks (Fig. 2C, compare to Fig. 2A). Thus the methyl blue dye did not affect the distribution and the behaviour of Collembola. In experiments with water only, neither the gut contents nor ventral tubes of Collembola showed any blue coloration, indicating that in the absence of mucus they did not suck on the half-disks. When one of the two half-disks was saturated with mucus and urine from *A. giardi* then the corresponding number of *H. nitidus* was significantly higher on it (Table 3). The abundance of animals on mucus and urine increased up to 60 min and thereafter remained stable (Fig. 2C). Methyl blue did not disturb the attraction of *H. nitidus* by mucus and urine (compare with Fig. 2A). Forty per cent of individuals exhibited a blue coloration in their gut, indicating that Collembola sucked on methyl blue stained half-disks impregnated with mucus and urine. Two individuals (3%) had only the end of their ventral tube blue stained.

3.2. Odour of earthworm and earthworm excreta (casts + mucus + urine)

The first odour experiment (Table 4) showed that *H. nitidus* was significantly more abundant in the sector above earthworm excreta than in the sector without the odour of earthworm excreta, especially from 80 min onwards (Fig. 3A). 80 min is probably the time needed for Collembola to explore their milieu and for the odour to reach them. The aggregation towards the side with odour of earthworm excreta increased with time up to the end of the experiment, i.e. 160 min (Fig. 3). The control experiment showed a little but not significant

clustering of animals which occurred indifferently in one of both sectors (Table 4 and Fig. 3A).

Because a threshold level was not reached at the end of the 160 min experiment, a longer-lasting experiment was performed over 420 min (Fig. 3B). *H. nitidus* was significantly more abundant in the 'earthworm excreta odour' sector (Table 4) particularly from 60 min up to the end of the experiment (Fig. 3B). Thus, the attraction of Collembola by the odour of earthworm excreta became effective at the same time as in the shorter experiment and was stable over time, maybe reinforced by aggregation pheromones. In the control experiment the individuals tended to aggregate in one or the other of both sectors, as indicated by the high level of standard errors (Fig. 3B). Collembola were slightly more abundant in one of both sectors, but this trend was not significant.

The experiment using the device with smaller moist areas gave results different from those obtained with a wider moist area. In the control experiment with water only the mean difference between both areas was not significant and smaller than in the previous experiment and the standard error was reduced thus indicating a better balanced distribution (Table 5 and Fig. 3C). The experiment using earthworm excreta indicated that the number of *H. nitidus* was always higher in the area 'earthworm excreta odour' (Fig. 3C) but that this preference was not significant (Table 5). However, the preference became significant ($P = 0.005$) when an outlier replicate (where an unexpected aggregation occurred in the area "no odour") was eliminated.

When earthworm excreta were replaced by earthworms, the mean abundance of Collembola was significantly higher in the area with the odour of earthworms than in the area without odour (Table 5 and Fig. 3C). Thus earthworms themselves, not defecating but rather excreting mucus and urine, produced an olfactory signal, which was attractive to *H. nitidus*. The attraction occurred more rapidly with the odour of earthworms than with that of their excreta.

4. Discussion

4.1. Direct contact with earthworm excreta

The mixture of mucus and urine excreted by the earthworms *A. giardi* and *A. chlorotica* strongly attracted *H. nitidus* individuals when direct contact was possible. Earthworm mucus is known to affect insect

behaviour, either as an attractant (Morris and Pivnick, 1991), or as a repellent (Laakso and Setailai, 1997). The earthworm *Lumbricus terrestris* has been shown to excrete a cutaneous snake-attracting compound that acts after contact through the vomeronasal system of the snake (Halpern et al., 1984; Kirschenbaum et al., 1985; Wang et al., 1988). Attraction could be mediated by various nitrogenous molecules which are contained in the epidermal earthworm mucus (glycoproteins, peptides, amino acids) and in urine (ammonia, urea) which mingles with mucus (El Duweini and Ghabbour, 1971; Cortez and Bouché, 1987).

The frst two experiments indicated that *H. nitidus* was attracted more rapidly by mucus and urine of *A. chlorotica* than by that of *A. giardi*. This suggests that this kind of attraction varies according to the earthworm species. However the attraction could vary according to experimental conditions and physiological state of earthworms, since in the experiment with methyl blue the attraction by excreta of *A. giardi* was more rapid than without staining. In addition the sensitivity of Collembola to signals, like their aggregational habit, could vary whether they are feeding or moulting (Joosse and Verhoef, 1974; Bengtsson et al., 1994; Eisenbeis, 1982).

Some of the components of earthworm mucus or urine not only constituted an attractant for *H. nitidus* but also induced sucking of impregnated filter paper. The fact that collembolan guts did not contain methyl blue in the absence of mucus and urine (control experiment with water only) gave indirect evidence that earthworm epidermal excreta were actually absorbed by *H. nitidus*. This result means that one of the reasons for the attraction is that *H. nitidus* may feed on earthworm mucus or urine. These excreta contain a number of nitrogen-rich molecules (see above) and epidermal mucus also contains easily assimilable carbohydrates (Cortez and Bouché, 1987) which may be consumed by Collembola.

Two individuals found with the apex of their ventral tube stained blue had probably tried to absorb mucus by this way, unless staining occurred during absorption of water only. Nevertheless this mode of absorption could be taken as negligible since the ventral tube is better known to allow uptake of diluted salt solutions (Eisenbeis, 1982) rather than that of high molecular weight compounds such as mucus and methyl blue. Earthworm urine could nonetheless be absorbed by this way.

A set of choice experiments, not described in this paper, has been performed in darkness on isolated individuals, thus preventing aggregation and interaction with light. Animals were repeatedly photographed under fash. A preference of *H. nitidus* for mucus plus urine was demonstrated with both *A. chlorotica* and *A. giardi*.

Although these results confirmed the above demonstrated preferences, data are not shown here because unexpected preferences for a given side were observed in the control experiment with water only. The flash was probably responsible for this bias as it affected the behaviour of the animals. Nevertheless these choice experiments using methyl blue stained filter paper supported the absorption of mucus and urine by *H. nitidus*, since a blue coloration of gut contents was observed only in the presence of *A. giardi* and *A. chlorotica* excreta.

Casts of *A. giardi* and *A. chlorotica* did not significantly attract *H. nitidus*. At first sight this result is surprising since earthworm faeces concentrate a number of nutrients like N (Scheu, 1987; Parkin and Berry, 1994), Ca and P (Heine and Larink, 1993; Sharpley and Syers, 1976; Lunt and Jacobson, 1944). Moreover *H. nitidus* ingest mainly invertebrate faeces (Arpin et al., 1980; Salmon, unpublished). In our experiments, the number of individuals was always greater, although not significantly, in fresh casts than in calcic mull made of aged casts. Earthworm faeces contain intestinal mucus but most of the nitrogenous compounds within it are reabsorbed in the foregut (Martin et al., 1987; Bernier, 1998). Bouché et al. (1997) estimated that N excretion by epidermal mucus of earthworms exceeded that by faeces. Thus our results reinforce the hypothesis that *H. nitidus* may be attracted by easily available nitrogen-rich compounds such as amino-acids, proteins, urea and ammonia, which can be used by hexapods in protein synthesis (Martin, 1979).

4.2. Odour of earthworm and earthworm excreta

Our experiments showed that *H. nitidus* was attracted by the odour of *A. giardi* and to a less extent by the odour of its excreta (mucus, urine and faeces) although this attraction did not occur systematically. Ammonia and amino acids are volatile attractants for a fruit fly (Morton and Bateman, 1981; Bateman and Morton, 1981). Thus, some nitrogenous compounds present in earthworm excreta (see above) might be volatile and attract Collembola at a distance.

The attraction was more efficient when *H. nitidus* was allowed to contact mucus and urine (attraction always significant) than when it perceived only the odour of excreta. In addition it was necessary to increase the number of replicates and individuals in order to detect the effect of odour. The attraction after direct contact is the common case in the interactions between earthworms and other animals (see above mentioned references). In our odour experiments this attraction occurred at 1 cm distance. This is a short distance compared to field

conditions, except if the olfactory signal can be transported by an air stream, like for instance in an earthworm burrow. Moreover the olfactory attraction of *H. nitidus* by *A. giardi* did not occur systematically in our experiments and some (but not significant) aggregation arose in one of both sectors even in the absence of earthworm odour. Thus in field conditions the attraction by the odour of earthworm excreta probably interacts with one or several other factors.

In another experiment (not presented here), Collembola exhibited no preference at all for the odour of earthworm excreta. We attributed this negative result to the fact that excreta were not moist enough (with regard to other experimental runs). Variations from a replicate to another in the same experiment and from an experiment to another could be due to the fact that the volatility of the chemicals involved in the attraction is weak as in the case for amino acids (Morton and Bateman, 1981). Another explanation, compatible with the former, is that light may interact with the odour of earthworm excreta. Although experiments were carried out in darkness, test-boxes were submitted to ambient light for a short time during counting. In fact, light has a strong effect on the distribution of *H. nitidus* (Salmon and Ponge, 1998). The effect of light may persist in darkness, probably following the deposition of aggregation pheromones (Krool and Bauer, 1987). Interactions between light and odour of earthworms or earthworm excreta could partially explain the absence of attraction by odour in some replicates as well as the aggregation observed in the control experiment over 430 min. Our experiments also revealed that clustering interacted with the attraction to earthworm odour. *H. nitidus* produces an aggregation pheromone which induces a decrease in its locomotory activity (Verhoef et al., 1977; Verhoef, 1984; Krool and Bauer, 1987). This effect depends on environmental factors like water and food availability, physiological state and population density (Usher and Hider, 1975; Verhoef and Nagelkerke, 1977; Leonard and Bradbury, 1984; Bengtsson et al., 1994). When individuals find favourable conditions, (e.g. in our moist experimental environment), together with the odour of earthworm excreta and darkness, they cluster and remain motionless. This probably lasts until they need to seek food (Verhoef and Nagelkerke, 1977). As in the present study, the pH preferenda of *Folsomia candida* (Van Straalen and Verhoef, 1997) could not be determined accurately because of interaction with a similar aggregative process.

5. Conclusion

We proved that the mixture of earthworm mucus and urine is a strong attractant for *H. nitidus* while the impact of the odour of earthworm excreta on the distribution of this species is not very pronounced, and the contact with earthworm faeces not efficient. We showed as well that *H. nitidus* absorbs earthworm mucus. The attraction to earthworm mucus, which lines burrow walls (Kretzschmar, 1987), could thus explain at least partly the field distribution of *H. nitidus* according to a trophic interaction. This conclusion corroborates the results of field studies from which *H. nitidus* had been found only in mull at pH > 5 (Ponge, 1993; Salmon and Ponge, 1999) that is a humus form characterized by a high number and diversity of earthworms (Ponge et al., 1997). In mull humus at pH < 5 this collembolan species is absent (Ponge, 1993). A choice experiment designed to assess the attraction of Collembola by mucus and urine from earthworms living at pH < 5 will enable a better understanding of the distribution of *H. nitidus* in the field.

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Legends of figures

Figure 1. Design of test-boxes used in odour experiments with one moistened area of filter paper as bottom substrate for Collembola.

Figure 2. Differences in the number of *Heteromurus nitidus* (means of six replicates ± standard errors) between two half-disks of filter paper ('earthworm excreta' versus 'reference substrate') in an experiment involving a direct contact over 140 min. (A) Experiment with mucus and urine versus water. (B) Experiment with fresh casts versus calcic mull. (C) Experiment with mucus and urine versus water in half-disks previously stained with methyl blue dye.

Figure 3. Differences in the number of *Heteromurus nitidus* (means of ten replicates ± standard errors) between two sectors ('earthworm excreta odour' or 'earthworm odour' versus 'no odour') in odour experiments. (A) Experiments over 160 and 130 min with two sectors in a moist area of filter paper. (B) Experiment over 420 min with two sectors in a moist area of filter paper. (C) Experiment over 330 min with two distinct moist areas of filter paper.

Table 1 Numbers of H. nitidus on each half-disk in three choice experiments involving a direct contact over 140 min (means of 14 times and six replicates). NS = not significant at 0.05 level; **, *** = significant at the 0.01 and levels, respectively

Content of half-discs	Number of H. nitidus (mean ± SEM)	Results of paired t test (probability)
Mucus and urine of *A. giardi*	6.04 ± 0.34	0.0001***
Water	1.32 ± 0.18	
Mucus and urine of *A. chlorotica*	8.23 ± 0.73	0.0063**
Water	1.58 ± 0.74	
Water (disk E1)	3.95 ± 0.53	0.7889 NS
Water (disk E2)	3.69 ± 0.40	

Table 2 Numbers of *H. nitidus* on each half-disk in two choice experiments involving a direct contact over 140 min (means of 14 times and six replicates). NS = not significant at 0.05 level

Content of half-discs	Number of *H. nitidus* (mean ± SEM)	Results of paired t test (probability
Fresh casts of *A. giardi*	5.61 ± 0.92	0.2049 NS
Calcic mull	2.99 ± 0.88	
Fresh casts of *A. chlorotica*	5.87 ± 1.01	0.2661 NS
Calcic mull	3.49 ± 0.90	

Table 3 Numbers of *H. nitidus* on each half-disk in two choice experiments involving a direct contact over 140 min (means of 14 times and six replicates). NS = not significant at 0.05 level; * = significant at 0.05 level

Content of half-discs	Number of *H.nitidus* (mean ± SEM)	Results of paired t test (probability)
Methyl blue + water (E1)	2.92 ± 0.44	0.5606 NS
Methyl blue + water (E2)	3.39 ± 0.65	
Methyl blue + mucus and urine of *A. giardi*	7.51 ± 1.08	0.0134*
Methyl blue + water	1.08 ± 0.66	

Table 4 Numbers of *H. nitidus* in each sector of a moist area of filter paper, in the first set of four odour experiments (means of respectively 16, 13 or 14 times and ten replicates). NS = not significant at 0.05 level; **, *** = significant at the 0.01 and 0.001 levels, respectively

Experiments	Odour occurrence in sectors	Number of *H. nitidus* (mean ± SEM)	Results of paired t test (probability)
Odour of earthworm excreta over 160 min	Odour of *A. giardi* excreta	8.44 ± 0.94	0.0078**
	No odour (water)	3.54 ± 0.92	
Control over 130 min	No odour (water)	6.12 ± 0.97	0.8703 NS
	No odour (water)	5.87 ± 0.98	
Odour of earthworm excreta over 420 min	Odour of *A. giardi* excreta	9.86 ± 0.66	0.0000***
	No odour (water)	2.12 ± 0.67	
Control over 420 min	No odour (water)	7.64 ± 1.58	0.2139 NS
	No odour (water)	4.37 ± 1.57	

Table 5 Numbers of H. nitidus in each of two distinct moist areas of filter paper, in three odour experiments over 330 min (means of 11 times and ten replicates). NS = not significant at 0.05 level; ** = significant at 0.01 level

Experiments	Odour occurrence in sectors	Number of *H. nitidus* (mean ± SEM)	Results of paired t test (probability)
Control over 330 min	No odour (water) No odour (water)	5.35 ± 0.76 4.93 ± 0.54	0.6613 NS
Odour of earthworm excreta over 330 min	Odour of *A. giardi* excreta No odour (water)	7.31 ± 1.14 4.27 ± 1.18	0.1252 NS
Odour of earthworm over 330 min	Odour of *A. giardi* No odour (water)	8.03 ± 0.70 3.38 ± 0.70	0.0016**

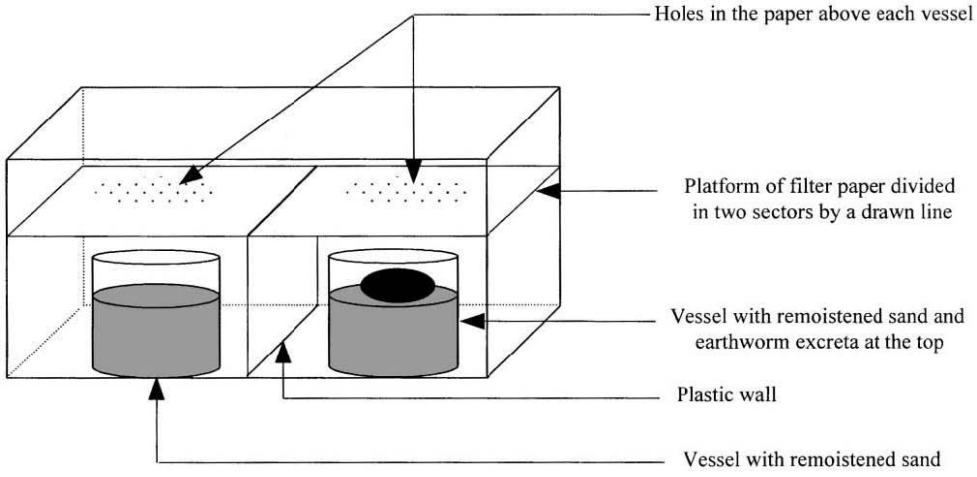
Holes in the paper above each vessel
Platform of filter paper divided in two sectors by a drawn line
Vessel with remoistened sand and earthworm excreta at the top
Plastic wall
Vessel with remoistened sand

Fig. 1

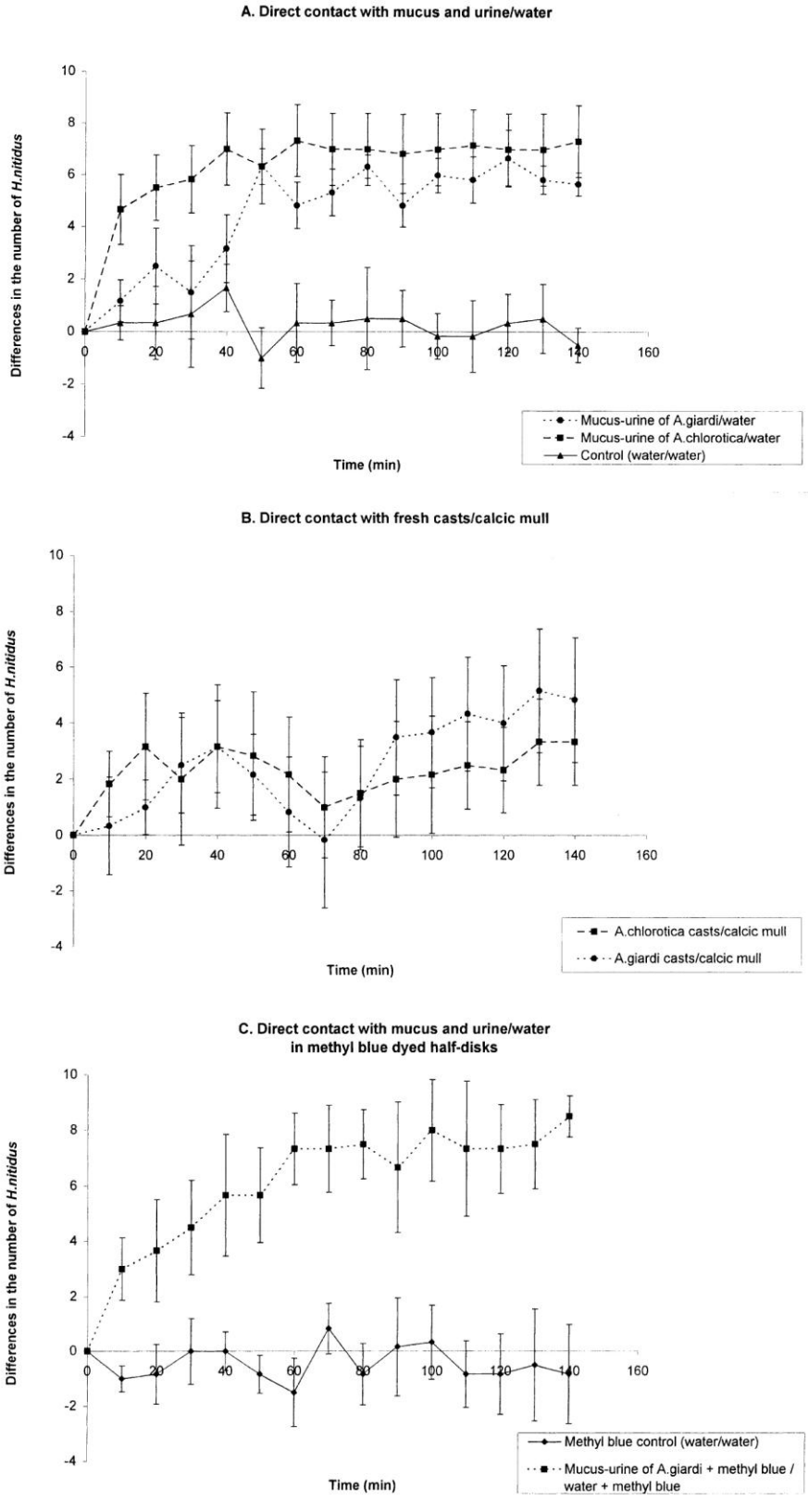
A. Direct contact with mucus and urine/water
Differences in the number of H.nitidus
Time (min)
Mucus-urine of A.giardi/water
Mucus-urine of A.chlorotica/water
Control (water/water)
B. Direct contact with fresh casts/calcic mull
Differences in the number of H.nitidus
Time (min)
A.chlorotica casts/calcic mull
A.giardi casts/calcic mull
C. Direct contact with mucus and urine/water
in methyl blue dyed half-disks
Differences in the number of H.nitidus
Time (min)
Methyl blue control (water/water)
Mucus-urine of A.giardi + methyl blue /
water + methyl blue

Fig. 2

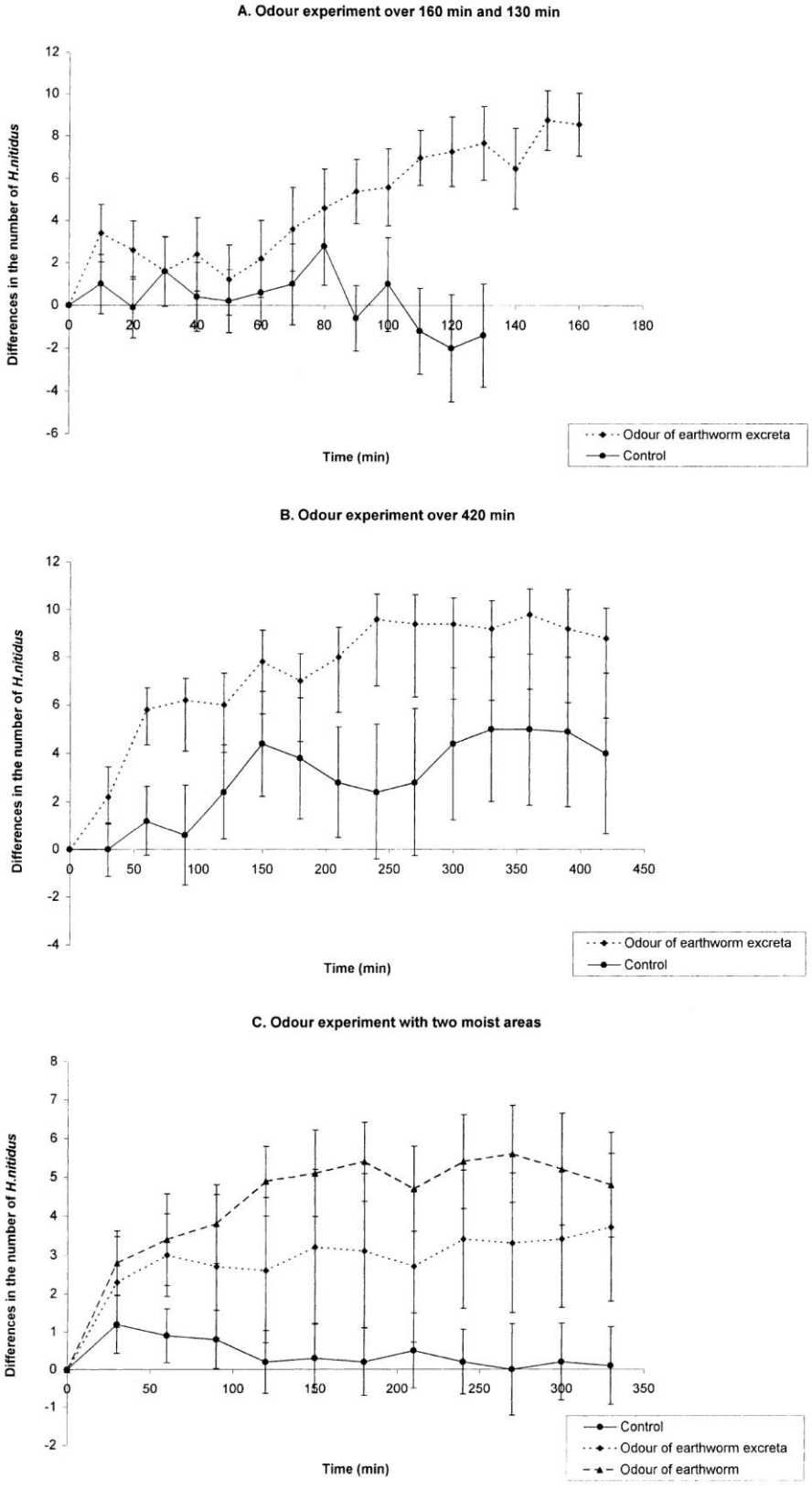
A. Odour experiment over 160 min and 130 min
Differences in the number of H.nitidus
Time (min)
Odour of earthworm excreta
Control
B. Odour experiment over 420 min
Differences in the number of H.nitidus
Time (min)
Odour of earthworm excreta
Control
C. Odour experiment with two moist areas
Differences in the number of H.nitidus
Time (min)
Control
Odour of earthworm excreta
Odour of earthworm

Fig. 3