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ORIGINAL PAPER

Sandrine Salmon

Earthworm excreta (mucus and urine) affect the distribution of springtails in forest soils

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Abstract The collembolan species *Heteromurus nitidus*, exclusively located in soils at pH>5, can be cultured in acidic humus. As this species is attracted to the excreta of earthworms from calcic mull, its distribution is supposed to be determined only indirectly by soil pH through the distribution of earthworms. Higher densities and biomasses of Lumbricidae were observed in a calcic mull (pH 7.8) than in an acidic mull (pH 4.8) and a moder humus (pH 4.2). Choice experiments were performed to compare the attraction of *H. nitidus* to the mucus-urine mix of five earthworm species from the calcic mull and the acidic mull. *H. nitidus* was attracted to the excreta of the five species, whatever their ecological category and the humus form from which they originated. The collembolan *Heteromurus major*, which was indifferent to soil pH, was not attracted to earthworm excreta, which emphasizes the significance of this phenomenon for the distribution of *H. nitidus* over a pH range. The attraction of *H. nitidus* to earthworm excreta tended to be weaker and more variable when earthworms originated from acidic mull compared to calcic mull, particularly in the case of *Lumbricus terrestris*. Increased earthworm density reinforced by better mucus quality and quantity could determine the distribution of *H. nitidus* according to soil acidity. The only urine compound capable of attracting *H. nitidus* was NH_3 at a low concentration (0.03 g l^{-1}). The NH_3 content of the mucus-urine mix which attracted *H. nitidus* was 0.037 g l^{-1}, and was therefore responsible, at least partly, for the attraction.

Keywords Ammonium · Attraction · Collembola · Earthworms · Mucus

S. Salmon (✉)
Muséum National d'Histoire Naturelle,
Laboratoire d'Écologie Générale, 4, Avenue du Petit-Château,
91800 Brunoy, France
e-mail: ssalmon@mnhn.fr
Fax: +33-1-60465719

Introduction

The distribution of collembolan species in relation to natural and artificial soil pH has been extensively studied (Hågvar and Kjøndal 1981; Hågvar 1984b; Ponge 1993; Geissen et al. 1997; Chagnon et al. 2000; Loranger et al. 2001). Collembola can be used as bioindicators for assessing soil acidification by acid rain and fertilisers (Kopeszki 1997; Van Straalen and Verhoef 1997). However, relationships between microarthropods and soil pH levels must be considered with caution since the latter variable depends on other factors. For example, humus forms that vary according to soil acidity may be more important, as habitats, than the pH itself, as well as the availability of mineral nutrients (Ca) that may be required by animals. Moreover, soil acidity may act indirectly through biological interactions, as different soil pH levels favour different species in competition or predation processes (Hågvar 1984a; Heungens and Van Daele 1984).

The collembolan species *Heteromurus nitidus* Templeton, 1835, is only found in mull humus at pH>5 (Ponge 1980, 1993; Salmon and Ponge 1999), but grows in cultures with sieved humus at pH<5 (Salmon and Ponge 1999). These contradictory results led to the conclusion that the effect of soil pH on the distribution of *H. nitidus* is indirect. This species turned out to be attracted to earthworms (Lumbricidae) and this effect was stronger than its preference for a given soil pH or humus form (Salmon and Ponge 1999). This attraction to earthworms corroborated several studies showing that some microarthropods are more abundant in soils with high earthworm populations (Marinissen and Bok 1988; Hamilton and Sillman 1989; Loranger et al. 1998), although some studies reported varying effects of earthworm activity on microarthropod densities (Brown 1995; McLean and Parkinson 2000). The attraction of *H. nitidus* to earthworms leads to the hypothesis that its distribution could be controlled, at least partly, by the distribution of earthworms.

Experiments have shown that a mix of mucus and urine from two earthworm species, *Aporrectodea giardi* and *Allolobophora chlorotica* (Lumbricidae), from a calcic mull (pH 7.5) was an attractant for *H. nitidus* (Salmon and Ponge, in press) and that this species grazes on mucus.

The hypothesis that the distribution of *H. nitidus* in soils at pH>5 is explained by its attraction to earthworm excreta implies that at least one of the two following conditions should be true: (1) the abundance of earthworms decreases in soils at pH<5 so that excreta are not produced in sufficient quantity to attract *H. nitidus*, (2) earthworm excreta differ in quality or quantity according to the environment of earthworms (soil pH, humus form) or according to the earthworm species, that varies in turn with soil pH.

It has been well established that the density of earthworms decreases in temperate acid soils (Piearce 1972; Petersen and Luxton 1982; Lavelle et al. 1995; Muys and Granval 1997). However, it was necessary to verify the first above-mentioned condition (1), namely to compare the density of earthworms in sites previously studied for the occurrence of *H. nitidus* (Ponge 1980, 1993; Salmon and Ponge 1999).

The second condition (2) was tested by assessing the attraction to excreta produced by different earthworm species originating from soils at varying pH and by studying the distribution of earthworm species in relation to soil pH. In fact, the amount or quality of the epidermal mucus may vary according to ecological earthworm categories and ingested substrates, as has been demonstrated for intestinal mucus (Trigo et al. 1999). Bouché (1972), who defined these ecological categories, showed that epigeic species (feeding on and inhabiting litter) are much more tolerant of acidity than endogeic (geophagous and inhabiting the soil) and anecic species (inhabiting soil burrows but feeding on litter) that prefer pH 6–7. In addition, the pattern of N output, a major component of mucus, differs according to earthworm species (Needham 1957). Consequently, the attraction of *H. nitidus* could vary according to earthworm species or ecological categories which are, in fact, controlled by soil pH. In addition, the attractive effect of excreta could be influenced directly by the characteristics of the soil in which earthworms are living, independently of their taxonomic status.

The initial aims of this paper were: (1) to compare the density of earthworms in three humus forms at different pH, a calcic mull (pH 7.8), an acidic mull (pH 4.8) and a eumoder (pH 4.2); and (2) to assess the attraction of *H. nitidus* to the mucus-urine mix from calcic mull and acidic mull earthworms in choice experiments. Data on *H. nitidus* were compared with those of another collembolan species belonging to the same genus, *H. major* Moniez, 1889, which is indifferent to soil pH (Ponge 1993, 2000); in this case, the attraction to earthworm mucus was expected not to occur. The further aim of the paper was to determine the role of urine from the mucus-urine mix in the attraction of *H. nitidus*. As both urine and mucus are excreted through earthworm body walls (Edwards and Bohlen 1996), it is difficult to assess their impact separately. Urea and NH_3 being considered as major components of earthworm urine (Edwards and Bohlen 1996), the effects of these two N compounds on the behaviour of *H. nitidus* were studied in choice experiments. The NH_4^+ concentrations in earthworm excreta were determined and compared to amounts of NH_4^+ offered to *H. nitidus* in choice experiments.

Materials and methods

Earthworm sampling

Earthworms were extracted by the formalin method (Raw 1959) from three sites with different pH values and humus forms. The humus form was determined by morphological features according to Brêthes et al. (1995). The calcic eumull (pH 7.8) was located in the Park of the Laboratory in Brunoy, near Paris (France). The oligomull (=acidic mull at pH 4.8) and the eumoder (pH 4.2) were situated in the Senart forest near Brunoy. Sampling was carried out on sites described by Arpin et al. (1984) and Bouché (1975), over a total surface area of 1.5 m² comprising six circular areas of 0.25 m² each. Litter and aerial parts of ground vegetation were examined for the presence of epigeic earthworms before being removed. All the areas were watered (5 l per area) every 15 min with 1‰, 2‰ and 3‰ diluted formalin, respectively. Expelled earthworms were recovered then killed and kept in 37% formalin. Endogeic earthworms that were not extracted by this method were collected by digging out the soil underneath. Earthworms were identified at the species level according to Bouché (1972) and Sims and Gerard (1985). Some immature individuals have been attributed either to *Lumbricus terrestris* or to the group *Aporrectodea giardi-A. longa* owing to their length and their clitellum when this organ was not completely regressed. Earthworm species were classified into ecological categories (epigeic, endogeic, anecic) according to Bouché (1972). The total number of adult and immature individuals was used to estimate earthworm densities per unit surface. The distribution of species in each ecological category was compared among the three humus forms with a χ^2 test (Sokal and Rohlf 1995).

Soil pH

Soil pH was determined for the three sites in which earthworms were sampled. Soil samples were air-dried and mixed with deionised water (soil:water 1:5 v/v) for 5 min (Anonymous 1999). The pH was measured after 4 h.

Earthworms used in choice experiments

Earthworms were extracted with 4‰ formalin from the calcic eumull and the acidic mull. The number of individuals used in each experiment varied according to the size of the species and the number of adult earthworms extracted (Table 1). When the number of individuals belonging to a given species was much too low to produce enough mucus for the experiments, this species was discarded. For this reason, the attraction to the excreta of earthworms from the eumoder could not be tested. Immediately after extraction, earthworms were rinsed in tap water and placed in Petri dishes (14 cm diameter) lined with water-moistened filter paper in order to void their guts. Petri dishes were kept for 60 h at 15°C in darkness in a water-tight enclosure. The paper was renewed 3 times. Four hours before the beginning of the experiment, worms (Table 1) and six half-discs of filter paper (5 cm diameter) were equally distributed between two clean Petri dishes (8 cm diameter), in order to fully impregnate the filter paper with earthworm mucus. The species *Lumbricus castaneus* and *Satchellius mammalis*, which could not be distinguished between in a living

Table 1 Numbers of Collembola on the different substrates: disc plus mucus from different earthworm species extracted from calcic mull or acidic mull, disc plus water and space beyond disc (means of 14 points in time and six replicates ±SE). *Within each row*, significant differences are indicated by *different letters* ($P<0.05$)

Collembola species	Humus form	Number of earthworms used	Earthworm mucus	Water	Space beyond disc
Heteromurus nitidus (starved)	Calcic mull	8 *Aporrectodea giardi*	7.33±0.31a	1.70±0.42b	0.96±0.22b
		45 *Allolobophora chlorotica*	7.61±0.36a	0.73±0.21b	1.66±0.36b
		12 *Lumbricus terrestris*	8.42±0.49a	0.80±0.36b	0.79±0.16b
	Acidic mull	8 *A. giardi*	7.26±0.99a	1.92±0.79b	0.82±0.25b
		45 *A. chlorotica*	6.29±0.60a	2.5±0.89b	1.21±0.47b
		9 *L. terrestris*	6.63±0.66a	2.89±0.63b	0.48±0.19c
		40 *Lumbricus castaneus-Satchellius mammalis*	6.73±0.56a	2.50±0.57b	0.77±0.30b
H. nitidus (unstarved)	Acidic mull	45 *A. chlorotica*	5.62±1.03a	3.89±1.15a	0.49±0.13b
		8 *A. giardi*	3.77±1.02a	4.2±1.17a	2.02±0.41a
H. major (starved)	Calcic mull	8 *A. giardi*	3.61±0.61b	0.57±0.21c	5.82±0.67a
		45 *A. chlorotica*	1.90±0.41b	0.71±0.10c	7.38±0.37a
		12 *L. terrestris*	1.94±0.21b	0.58±0.13c	7.48±0.25a
	Acidic mull	8 *A. giardi*	4.39±0.53a	0.94±0.31b	4.67±0.60a

state previous to the experiment, were used together. The number of adults used in each experiment and species of earthworms extracted from the two humus forms are listed in Table 1.

Collembola

H. nitidus and *H. major* were cultured on moistened Fontainebleau sand and a mix of plaster of Paris and charcoal, respectively. Both species were fed with lichen and terrestrial microalgae (*Desmococcus*) from bark scrapings. Cultures were kept in darkness at 15°C. All experiments were performed with na individuals which were starved for at least 3 days.

Urea and NH_3 solutions

Concentrations of urea and NH_3 used in these experiments were defined in relation to values given in the literature (Needham 1957; Tillinghast 1967; El Duweini and Ghabbour 1971). When these values were not expressed in mass per unit volume (excretion rate), they were extrapolated to the number and mean weight (5.52 g) of *A. giardi* used in choice experiments, accounting for the time (4 h) and the volume required to fully impregnate the half-discs. Two urea (Prolabo) concentrations of 0.4 g l^{-1} and 0.8 g l^{-1}, and four NH_4^+ (Prolabo) concentrations of 0.03 g l^{-1}, 0.08 g l^{-1}, 0.2 g l^{-1} and 0.4 g l^{-1}, were prepared using deionised water.

Choice experiments

Choice experiments were performed in six Petri dishes (8 cm diameter) placed on a laboratory table at right angles to the light gradient of a neon tube in order to avoid interactions with light (Salmon and Ponge 1998). In each of the six Petri dishes (replicates), two half-discs of filter paper were placed at 1.5 cm distance from each other. One half-disc had been previously saturated with a mix of earthworm urine and mucus, with urea or with NH_4^+, the other was soaked in deionised water. The experiment started by introducing ten adult or sub-adult Collembola mid way between the two half-discs. The number of individuals present on each substrate (i.e. the two half-discs and the space beyond the discs) was counted every 10 min over 140 min. For each Petri dish, mean numbers were calculated for 14 points in time. Means for each substrate were compared by one-way ANOVAs, the six Petri dishes being considered as blocks since numbers of Collembola present on different substrates were not independent. Data were log-transformed when variances within treatments (substrates) were not homogeneous. When a significant difference was detected, mean numbers of Collembola were scaled by Tukey's method (Sokal and Rohlf 1995). Results of experiments with *H. nitidus* and earthworm excreta were further analysed by using the mean difference of the number of animals on urine-mucus minus the number of animals on water, followed by one-way ANOVAs in order to analyse the effects of the different earthworm species on the one hand and of the different ecological categories (in acidic mull only) on the other hand. Petri dish were considered as replicates. Finally, the two humus forms from which earthworms originated (except for *L. castaneus* and *S. mammalis*) were compared by *t*-tests for equal or unequal variances (Scherrer 1984). Coefficients of variation were calculated for the experiments which used excreta from *A. giardi*, *L. terrestris* and *A. chlorotica* from the calcic mull and the acidic mull.

NH_4^+ excretion

The NH_4^+ concentration was determined for half-discs of filter paper impregnated with excreta of *A. giardi* extracted from the calcic eumull. Eighteen half-discs were weighed, impregnated with the urine-mucus mix of 24 *A. giardi*, as for choice experiments (see above), and weighed again. The mean increase in weight corresponded to 0.18 ml excreta. Half-discs were then separated into two batches of nine half-discs; 20 ml of 0.5 N sterile KCl was added to each. The suspension was homogenised for 2 min and centrifuged for 10 min at 3,600 r.p.m.. The supernatant was analysed for NH_4^+ by the indophenol blue method (Keeney and Nelson 1982) at the INRA Laboratory of Soil Analyses in Arras (France). The residual NH_4^+ concentration (nine half-discs without excreta suspended in 20 ml of 0.5 N KCl) was subtracted from that in the excreta suspension.

The rate of NH_4^+ excretion by *A. giardi* extracted from calcic mull was assessed. Twenty-four mature *A. giardi* were placed in dishes for 3 days to void their gut as described above. The earthworms were weighed and separated into two batches of 12 earthworms; each of these was incubated in 10 ml sterile distilled water for 4 h at 20°C in the dark. These solutions containing the earthworm excretory products were then mixed with 10 ml of 1 N sterile KCl, homogenised for 2 min, and analysed for NH_4^+ (see above). The residual NH_4^+ concentration in the control (20 ml of 0.5 N KCl) was subtracted from that in the excreta solution.

Table 2 Ecological categories (Bouché 1972), species and densities (no. m^{-2}) of adult and immature earthworms sampled in calcic mull, acidic mull and moder

Ecological category	Earthworm species	Density (no. m^{-2})		
		Calcic mull (pH 7.8)	Acidic mull (pH 4.8)	Moder (pH 4.2)
Anecic	*Aporrectodea giardi* (Savigny)	10.67	0.67	0.00
	Aporrectodea longa (Ude)	4.00	0.00	0.00
	Immature *A. giardi* and *A. longa*	26.00	3.33	0.00
	L. terrestris (L.)	20.67	2.67	0.00
	Total anecic	61.33	6.67	0.00
Endogeic	*Allolobophora chlorotica* (Savigny)	25.33	20.67	0.00
	Aporrectodea caliginosa (Savigny)	2.67	0.00	0.00
	Aporrectodea rosea (Savigny)	5.33	0.00	0.00
	Total endogeic	33.33	20.67	0.00
Epigeic	*Dendrobaena octaedra* (Savigny)	0.00	0.00	4.00
	Dendrodrilus rubidus (Savigny)	0.00	0.00	2.00
	L. castaneus (Savigny)	4.00	15.33	0.00
	S. mammalis (Savigny)	2.00	4.00	0.00
	Total epigeic	6.00	19.33	6.00
Immature individuals		26.00	17.33	12.67
Total density		126.67	64.00	18.67
Species richness		8	5	2

Results

Earthworm sampling

Species numbers and total densities of earthworms (Table 2) were ranked in the order: calcic mull (pH 7.8)> acidic mull (pH 4.8)>moder (pH 4.2). The densities of earthworms in the calcic mull were between two- and sixfold those in the acidic mull and the moder, respectively. Species occurring in the acidic mull also occurred in the calcic mull and belonged to anecic, endogeic and epigeic categories, whereas only epigeic earthworms were present in the moder (Table 2). The density of anecic earthworms in the calcic mull was 9 times higher than in the acidic mull. There was a significant relationship ($P<0.001$) between ecological categories of earthworms and the humus form. Anecic worms represented a larger proportion of the earthworm population in the calcic mull than in the acidic mull ($P<0.01$) and in the moder (not testable due to low densities), while epigeic worms were proportionally more abundant in the acidic mull ($P<0.01$) and in the moder (not testable due to low densities).

Choice experiments with earthworm excreta

Results of choice experiments indicated that significantly ($P<0.05$) larger numbers of *H. nitidus* occurred on half-discs saturated with excreta from *Aporrectodea giardi*, *Allolobophora chlorotica*, *L. terrestris* and the *L. castaneus*-*S. mammalis* group, than on water or in the outer area of the Petri dish, whether the earthworms originated from the calcic mull or the acidic mull (Table 1). *H. nitidus* was thus attracted to the urine-mucus mix of earthworms irrespective of their species and of their origin. The same experiments performed with unstarved Collembola did not show significant differences between earthworm excreta and water (Table 1).

When the earthworms originated from the calcic mull, *H. major* was significantly less abundant on the half-discs impregnated with the mucus and urine of *Aporrectodea giardi*, *L. terrestris* and *Allolobophora chlorotica* than in the outer area. No significant difference was observed between the abundance of *H. major* on the discs impregnated with the excreta of *A. giardi* originating from the acidic mull and in the outer area without excreta and water. Thus, *H. major* was not attracted to the mucus-urine mix from the three earthworm species considered. In every case, half-discs impregnated with water were avoided by *H. major*.

ANOVAs comparing the attraction of *H. nitidus* to the mucus-urine mix from different earthworm species or different ecological categories indicated no significant differences (Fig. 1). Conversely, the excreta from *Aporrectodea giardi*, *L. terrestris* and *Allolobophora chlorotica* tended to be more attractive when the earthworms originated from the calcic mull than when they originated from the acidic mull, but a significant increase was observed only in the case of *L. terrestris* (Fig. 1). Coefficients of variation in the experiments with *A. giardi*, *L. terrestris* and *A. chlorotica* extracted from the acidic mull were greater (73%, 71% and 127%, respectively) than in experiments with those extracted from the calcic mull (31%, 50% and 54%, respectively). This probably explains why the difference observed in the case of *A. cholorotica* was not significant, despite a considerable increase in the mean when earthworms originated from calcic mull.

Choice experiments with urea and NH_3

Numbers of *H. nitidus* on discs impregnated with 0.4 g urea l^{-1} and 0.8 g urea l^{-1} did not differ significantly

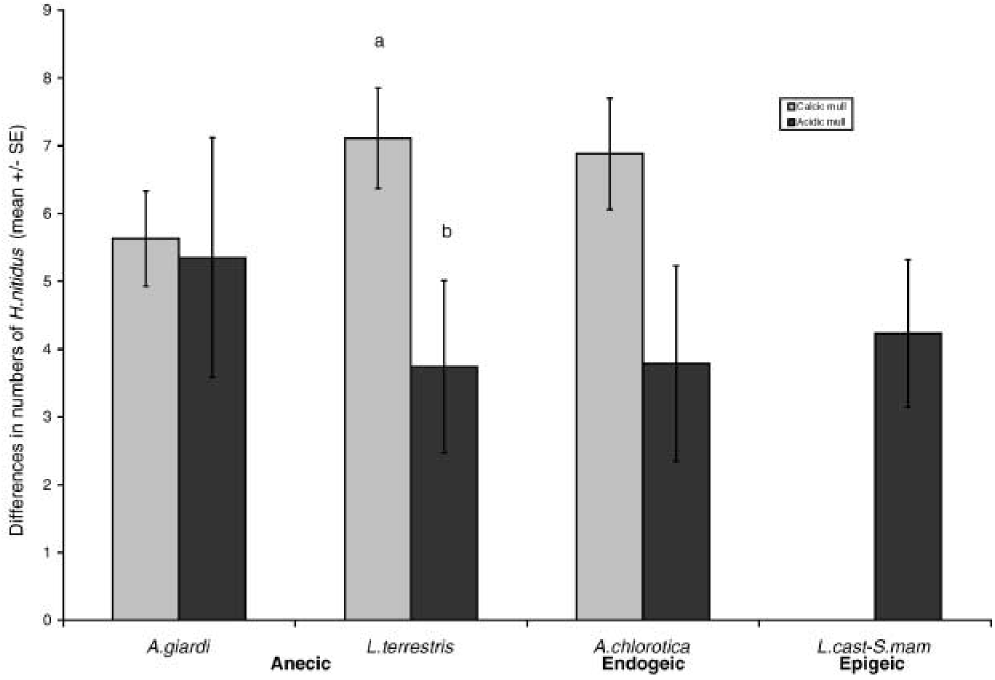

Fig. 1 Differences in numbers of *Heteromurus nitidus* between half-discs impregnated with earthworm excreta and half-discs soaked with water (means of 14 points in time and six replicates ±SE). All these differences were significant at $P<0.05$ (see text). Significant differences ($P<0.05$) between calcic mull and acidic mull have been indicated by *different letters*

Table 3 Numbers of *H. nitidus* on the different substrates: disc plus NH_3 or urea at different concentrations, disc plus water and space beyond disc (means of 14 points in time and six replicates ±SE). *Within each row*, significant differences are indicated by *different letters* ($P<0.05$)

	Concentration	Test compound	Water	Space beyond disc
Urea	0.4 g l^{-1}	4.40±1.09a	3.75±0.94a	1.85±0.47a
	0.8 g l^{-1}	4.00±1.25a	5.46±1.25ab	0.54±0.06b
NH_3	0.03 g l^{-1}	6.79±1.08a	2.38±0.88b	0.83±0.27b
	0.08 g l^{-1}	4.31±0.58a	2.83±0.27a	2.86±0.43a
	0.2 g l^{-1}	3.25±0.70b	5.54±0.58a	1.21±0.22b
	0.4 g l^{-1}	3.13±0.89b	6.21±0.91a	0.65±0.12b

from those on discs soaked in water (Table 3). Conversely, the effect of NH_4^+ varied with concentration. At the lowest concentration (0.03 g l^{-1}) *H. nitidus* was significantly more attracted to the NH_4^+ treatments than to the controls with water. There was no effect of NH_4^+ at 0.08 g l^{-1}. Deionised water was significantly preferred to NH_4^+ at 0.2 g l^{-1} and 0.4 g l^{-1}. In most cases *H. nitidus* avoided the space beyond the moistened half-discs.

NH_4^+ content in earthworm excreta

The NH_4^+ content of *A. giardi* excreta measured in saturated half-discs was 0.037 g l^{-1}. This value is comparable to the concentration of NH_4^+ (0.03 g l^{-1}) which was found to attract *H. nitidus* in choice experiments. The rate of NH_4^+ excretion for 4 h was 1.04 µg NH_4^+-N g^{-1} fresh body weight.

Discussion

Results from the earthworm sampling are in agreement with the fact that mull humus is characterised by a higher abundance of Lumbricidae than other humus forms (Bouché 1975; Römbke 1987; Schaefer and Schauermann 1990; Muys and Lust 1992; Bernier and Ponge 1998), and this may explain the distribution of *H. nitidus* in mull humus. Not only the total density of earthworms in the calcic mull (pH 7.8) was twice that found in the acidic mull (pH 4.8), but that of anecic worms (far longer in size than epigeic and endogeic species) was ninefold higher. A reduced density and biomass of Lumbricidae in mull humus at pH<5 has been already reported by Staaf (1987) and parallels the absence of *H. nitidus* in acidic mull.

Choice experiments demonstrated that *H. nitidus* was attracted to the mucus-urine mix from the five earthworm species tested. The attraction to excreta of *Aporrectodea giardi* and *Allolobophora chlorotica* taken from calcic mull confirmed previous results (Salmon and Ponge, in press). The mucus of earthworms is composed of 69% proteins and peptides and 31% carbohydrates (Cortez and Bouché 1987), which are nutrient-rich compounds on which *H. nitidus* may feed (Salmon and Ponge, in press). The fact that unstarved *H. nitidus* individuals were not attracted to earthworm excreta supports the trophic character of this attraction. Moreover, the cutaneous mucus of earthworms is capable of neutralising the pH of the immediate environment through its buffering capacity (Schrader 1994). *H. nitidus*, which avoids buffer solutions at pH<4 (S. Salmon, unpublished data), may be attracted to the higher pH of earthworm mucus, too. Finally, earthworm mucus contributes to a moistening of the milieu, which is favourable to *H. nitidus*, a soil-dwelling species intolerant of desiccation (Bauer and Christian 1987), as was shown by the low occurrence of individuals beyond the moistened half-discs.

H. nitidus was attracted to the excreta of earthworms taken from the acidic mull, too. However this attraction was less constant (particularly in the case of *A. chlorotica*) and tended to be lower than with earthworms originating from the calcic mull, especially in the case of of *L. terrestris*. The greater preference for the excreta of earthworms living in the calcic mull may be due to an increase in the quantity (concentration) or quality (chemical composition, pH) of mucus, that have been found to vary according to the nature or the acidity of the substrate (Günther and Greven 1990; Schrader 1994; Trigo et al. 1999).

In the present experiments neither the ecological category nor the species of Lumbricidae influenced the attraction of *H. nitidus* to earthworm excreta. According to Lee (1985), patterns of mucoprotein, NH_4^+, and urea outputs are similar in all earthworm species but vary with external conditions. Results of the present choice experiments are only qualitative since the number of earthworms used was chosen so that excess amounts of mucus and urine would be produced. In fact, 25 *A. chlorotica* specimens extracted from the calcic mull exerted the same attractive effect on *H. nitidus* (P=0.005) as 45 specimens of the same species (P<0.001). However, differences in field densities and biomasses of Lumbricidae obviously lead to variations in the total amount of mucus excreted in the soil. The decreased densities and biomasses of earthworms observed in soils at pH<5 (even of mull humus), associated with a lower quality of the mucus produced (perhaps of lower pH) would result in conditions that are not sufficiently attractive to *H. nitidus*.

In contrast to *H. nitidus*, *H. major* was not attracted to the excreta of earthworms originating either from calcic mull or from acidic mull. This result confirms the fact that the latter species is indifferent to soil pH (Ponge 1993, 2000) and highlights the particular character of the attraction of *H. nitidus* to earthworm excreta. Moreover, choice experiments revealed that *H. major* avoided moist substrates.

H. nitidus was neither attracted to urea nor to NH_4^+ at the three higher concentrations, in contrast to its attraction to NH_4^+ at a concentration as low as 0.03 g l^{-1}. The NH_4^+ content of 0.037 g l^{-1} found in the mucus-urine mix from *A. giardi*, which attracted *H. nitidus* in choice experiment, is thus at least partly responsible for the observed attraction.

In conclusion, the present results support, together with previously published reports (Salmon and Ponge 1999), the hypothesis according to which the field distribution of *H. nitidus* is, at least partly, controlled by earthworm density and particularly by the excretion of mucus and NH_4^+ of earthworms. The presence of *H. nitidus* is thus indirectly conditioned by soil pH through the abundance of earthworms. Burrow walls of earthworms that are lined with mucus and cast material (Kretzschmar 1987; Lavelle 1988) are thus enriched in NH_4^+ and soluble organic C, and have a higher pH and moisture content relative to non-burrow soil (Parkin and Berry 1994; Tiunov and Scheu 1999). Earthworm burrows that embrace all the conditions required by *H. nitidus* (enough moisture, high pH, nutrient-rich food, shelter against predation), could constitute a habitat favourable to this species.

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