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Changes in respiration rate and some physicochemical properties of soil during gut transit through *Allolobophora molleri* (Lumbricidae, Oligochaeta)

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Summary. Some physicochemical and biological measurements were carried out on the gut content and casts of *Allolobophora molleri,* an earthworm which lives in humid soils of Northern Spain. In the anterior part of the gut, water (22% of moist weight of soil) and soluble organic matter (27.4%) had been added to the ingested soil and pH had increased from 5.75 to 7.0. The amount of water-soluble compounds decreased sharply in the middle and posterior parts of the gut and were hardly detectable in control soil or casts. The average O_2 consumption, measured at 28° and 21°C, indicated respectively 2.75- to 12-fold increases in microbial respiration in the gut content compared to the non-ingested soil. These results extend the hypothesis of a mutualistic digestion in earthworms previously proposed for tropical endogeic species.

Key words: *Allolobophora molleri* – Earthworms – Physicochemical factors – Gut content – Mutualistic digestion – Respiration

Earthworm activities lead to important modifications in the physicochemical and biological properties of soils in their drilosphere, i.e., the whole soil is affected by earthworm activities (Lavelle 1988). The main processes affected are microbial activity (Lavelle et al. 1983, Barois 1987, Scheu 1990, Pashanasi et al. 1992), dynamics of organic matter (Martin et al. 1992, Lavelle and Martin 1992), nutrient release (Sharpley and Syers 1976, Bouché et al. 1987; Scheu 1987), and physical properties of soil such as aggregation or infiltration (Boström 1986, Blanchart and Spain 1989).

As a result of measuring several physicochemical and biological parameters in the gut content of two endogeic, mesohumic, tropical earthworm species, *Millsonia anomala* and *Pontoscolex corethrurus* (Barois and Lavelle 1986; Barois 1987), the existence of a mutualistic relationship between microflora and earthworms for the exploitation of soil organic matter was proposed. Similar observations carried out on the tropical oligohumic earthworm, *Dichogaster terrae-nigrae,* which feeds on deeper soil horizons and on two epigeic tropical species, *Amynthas corticis* and *Amynthas gracilis,* extended the hypothesis to tropical earthworms of different ecological categories (Martin 1988, Barois 1992). These authors hypothesized that digestion is largely performed by the ingested soil microflora; the addition of large amounts of water and water-soluble organic matter (intestinal mucus) in the anterior part of the gut results in a dramatic increase in microbial activity and further digestion of organic matter by these activated micro-organisms. This process is analogous to the "priming effect" described by Jenkinson (1966).

In the present study we sought evidence of a similar physiological process in earthworms living in temperate regions by measuring water content, pH, microbial respiration, and water-soluble organic matter content in non-ingested soil, in different parts of the gut, and in fresh casts of *A. molleri.* This species colonizes humid sites near running water with temporary flooding.

Materials and methods

Earthworms and soils from the 0–15 cm layer were collected from a riverbank, at Bembibre, La Coruña, Spain (U.T.M. 29TNH26). The soil contained 76.0% sand and 7.3% clay. The pH was 5.75, and the organic matter content 6.28%. The earthworms were cultured in plastic boxes containing the soil from which they were collected after it had been sieved through a 2-mm screen; the temperature was maintained at a constant 21° or 28°C.

Samples of the gut content were obtained after killing the earthworms by immersion in boiling water for 1 s. The gut was cut behind the gizzard and divided into three parts. The content of the anterior, middle, and posterior parts were carefully removed to prevent any contamination with coelomic fluid and blood. The water-soluble organic matter content was measured for each sample using the gut content of three or four specimens; pH was measured with a specially designed electrode, which allows *in situ* measurements of the gut content. Water-soluble organic matter was extracted after dilution in water at 60°C, fil-

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tered through a 0.2-µm filter, freeze-dried, weighed, and the result was expressed as a percentage of the dry gut content. Some samples of gut content were dried over silica gel for 24 h and used for water content determinations, expressed as a percentage of the moist gut content. O_2 consumption by the microflora contained in samples of the gut content (anterior, middle, posterior) was measured with a microrespirometer (Verdier 1983) over 3 h at 21° or 28 °C. Sample sizes ranged from 10 to 50 mg. Previous measurements of redox potential in the gut content of *P. corethrurus* revealed micro-aerophilic conditions in the gut content, decreasing from the anterior to the posterior (Barois and Lavelle 1986). These authors concluded that microbial respiration results in O_2 consumption and that suitable respirometric methods may be used to assess microbial activity.

The statistical analyses consisted of analysis of variance tests. The level of significance was defined as 95% and 99%.

Results

No water-soluble organic components were detected in the control soil. The concentration of these components (Table 1) increased dramatically in the anterior (27.4%), decreased in the median (6.0%) and posterior part of the gut (3.8%), and was very low in the casts (0.28%).

The pH values were slightly acidic in the control soil (5.8) and increased significantly in the gut towards neutral values; mean values varied from 7.0 in the anterior part to 7.4 in the middle and 7.2 in the posterior. However, a considerable decrease to 6.0 was observed in the casts, a value slightly higher than that observed in the control soil. All differences in pH between the different parts of the gut, soil, and casts were significant except for anterior versus posterior gut contents.

The water content was significantly higher in the gut than in the control soil and in the casts. This parameter tended to increase from the fore to the hind part of the gut, with respective values of 60.9% and 70.9%. A sharp decrease was observed in the casts (41.3%) compared to the hind part of the gut; the difference between casts and control soil was not significant.

Respiratory activity, measured as O_2 consumption at 28 °C, was lower in the soil and in casts than in the gut, with a progressive increase from the anterior to the middle part. The mean O_2 consumption was four times greater in the middle and posterior part of the gut than in the soil and casts (Table 1). At 21 °C, a temperature closer to that observed in the natural habitats of this species, the O_2 consumption in soil and casts was lower than at 28 °C; in the gut an inverse pattern was observed, with respiration being much higher at 21 °C than at 28 °C, although the differences were not statistically significant, due to the large dispersion of the data and the limited number of replications at 21 °C. A maximum value of 97.5 µl $O_2\,h^{-1}\,mg^{-1}$ dry soil was recorded in the fore part of the gut at 21 °C compared to 46.3 µl $O_2\,h^{-1}\,mg^{-1}$ dry soil at 28 °C. No significant differences were observed between the anterior, median, and posterior parts of the gut.

Discussion

Conditions for microbial activity were highly modified once the soil had been ingested by *A. molleri*. Large amounts of water (22%) and large amounts of water-soluble organic matter (27.4%) were added to the soil. The pH considerably increased during this first phase, from 5.8 to 7.0 in the anterior part of the gut. The addition of large amounts of water and the intense mixing in the gizzard results in homogenization of the soil, and improves contact between the soil microflora and organic substrates (Barois 1987). In the present study, due to this effect, the total metabolic activity of the soil microflora was considerably enhanced; at 28 °C, the O_2 consumption increased by a factor of 2.75 (from 16.8 in the soil to 46.3 µl $O_2\,h^{-1}\,mg^{-1}$ dry soil in the anterior gut), and at 21 °C by a factor of 12 (from 8.0 in the control soil to 97.5 µl $O_2\,h^{-1}\,mg^{-1}$ dry soil in the anterior gut).

Table 1. Physicochemical parameters measured in the gut content of *Allolobophora molleri*, in soil, and in casts

				Gut content											
	Control soil			Anterior			Middle			Posterior			Casts		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Water-soluble OM (%)	3	0.0	0.0	8	27.36	14.01	7	6.04	4.56	3	3.80	3.18	3	0.28	0.01
pH	19	5.75	0.03	19	7.01	0.36	18	7.36	0.30	17	7.16	0.31	20	5.94	0.01
Water content (%)	19	38.73	8.02	46	60.87	10.50	47	65.35	9.86	44	70.93	12.88	17	41.32	5.25
O_2 consumption, 21 °C	6	8.02	1.11	3	97.53	83.42	3	51.58	65.55	4	91.04	34.71	2	11.62	1.56
O_2 consumption, 28 °C	7	16.83	8.35	8	46.33	14.33	9	65.74	26.81	9	62.94	31.01	2	19.83	1.39

Analysis of variance

	Water soluble OM				pH				Water content				O_2 consumption, 21 °C				O_2 consumption, 28 °C			
	A	M	P	Cast	A	M	P	Cast	A	M	P	Cast	A	M	P	Cast	A	M	P	Cast
Soil	0	**	**	*	**	**	**	*	**	**	**	0	*	0	*	0	*	**	**	0
A		0	0	0		**	0	**		*	**	**		0	0	0		0	0	0
M			0	0			*	**			*	**			0	0			0	*
P				0				**				**				0				*

n, Number of replicates; OM, organic matter (% dry soil); O_2 consumption measured at given temperature as µl $O_2\,h^{-1}\,mg^{-1}$ dry soil; A, anterior gut; M, middle gut; P, posterior gut. $*P<0.05$, $**P<0.01$; 0, no significant difference

The amount of water-soluble organic matter was very high in the anterior part and oesophagus (Phillips Dales 1967; Michel and Devillez 1978). This soluble organic matter was clearly not the product of solubilization of ingested soil organic matter because the concentration of ingested soil organic matter (6.28%) was much lower than that of the water-soluble fraction (27.36%) in the anterior part of the gut. The concentration of water-soluble organic matter decreased very quickly as mucus was transformed or consumed during digestion, probably as a result of the intense metabolic activity of soil microorganisms (Martin et al. 1987); this process may be promoted by the addition of large amounts of water to the soil, which induces dispersion of the soil matrix and increases the accessibility of organic matter to soil microorganisms (Barois and Lavelle 1986). Mucus can also be a source of enzymes (Laverack 1963; Loquet and Vinceslas 1987; Martin et al. 1987). The origin of enzymes found in the gut of earthworms is still unclear; some may be produced by the worm itself, and some by soil microflora which has been activated during the gut transit. Intestinal mucus soon disappears in the gut as a result of microbial consumption. Part of this mucus may also be reabsorbed by the worm at the some time, as with the compounds produced by the degradation of soil organic matter. A rapid assessment of the amounts of energy required to produce such huge quantities of mucus shows that some of it must be reabsorbed and recycled within the earthworm.

The increase in soil pH during gut transit may be related to mucus production and to the activity of the Morren glands, which secrete large amounts of $CaCO_3$ (Prentø 1979). *A. molleri* has well developed Morren glands, with lateral evaginations in 10 and lamela in 11–13, the exchangeable Ca^{2+} of the soil being 5.5 $cmol_c\ kg^{-1}$, and that of the casts 6.5 $cmol_c\ kg^{-1}$.

Processes observed in the gut of *A. molleri* follow similar patterns to those described for the tropical endogeics *M. anomala* and *P. corethrurus* (Barois and Lavelle 1986; Barois 1987), *D. terrae-nigrae* (Martin 1988), and *Amynthas corticis* and *Amynthas gracilis* (Barois 1992). In these species, microflora ingested with the soil is highly stimulated in the anterior part of the gut by the addition of water and mucus, and intense mixing of the soil. In the median and posterior parts of the gut, this activated microflora is able to digest soil organic matter, in the micro-aerophilic conditions of the gut, to benefit both the microflora and the earthworm. In the casts, however, aerobic conditions decrease microflora activity (Barois 1987). This hypothesis is supported by the lack of correspondence generally observed between the peak of assimilable C (in the anterior part) and the peak of microbial respiration (in the medium and posterior parts). The high level of activity observed in the posterior part must be at the expense of the insoluble soil organic matter. Some questions, for example whether the microflora remain or are themselves digested further in the gut, have not yet been answered.

A. molleri has its own specific patterns, as the amount of water-soluble organic matter found in the anterior gut (27.4%) was much higher than that observed in peregrine and endemic tropical species (5%–16%; Martin et al. 1987; Barois 1992). Secretion of larger amounts of intestinal mucus by the temperate earthworm would allow rapid activation of microflora at comparatively low temperature. The trend towards a stronger response by gut microflora at 21 °C than at 28 °C, if confirmed, might be regarded as an adaptation of the ingested microflora to relatively low temperatures.

Thus, there is strong evidence that digestion of soil organic matter by endogeic earthworms takes place through a mutualistic relationship with ingested microflora. This digestive system, which was hypothesized for tropical earthworms feeding on poor soils, seems to occur in temperate earthworms, also. In the latter case, because of lower ambient temperatures, additional energy is invested by the worm in the form of intestinal mucus, to trigger a fast activation of microflora and allow the digestion of enough organic matter to balance the energy budget. However, the convergence of arguments as presented in this paper cannot be taken as positive proof. Identification of the exact origin of enzymes found in the gut (e.g., by identifying enzymes produced in axenic cultures of gut tissues) and *in vitro* simulations of the hypothesized digestive system should provide conclusive information.

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