

# THE STUDY OF RESPIRATORY AND ENZYMATIC ACTIVITIES OF EARTHWORMS-MADE PEDOLOGICAL STRUCTURES IN A GRASSLAND SOIL AT CITEAUX, FRANCE

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## SYNOPSIS

A multidisciplinary study concerning ecological effects of Lumbricids is now in progress. The study is being carried out by Professor Bouché and his co-workers. The study is particularly concerned with respiratory and enzymatic activity both in earthworm casts and in the "drilosphere" (the periphery of burrows affected by lumbricid activity). By their activity, Lumbricids modify soil properties, and among those are biological ones. When considering earthworm casts we have shown a simultaneous enrichment of organic matter and an increase in microbial populations with a stimulated and correlated respiratory and enzymatic activity throughout the year. Analysis of the drilospheric results is more difficult. Though activities develop in parallel in the different soil layers, in the drilosphere there might be positive or negative changes which might or might not be correlated with the results in the soil profile.

## INTRODUCTION

A multidisciplinary approach, concerning the ecological effects of Lumbricids, is now in progress by Professor Bouché and his co-workers. The study is particularly concerned with enzymatic activity both in earthworm casts and in the "drilosphere" (the periphery of burrows affected by lumbricid activity).

The research site is a permanent meadow (*Lolio-cynosuretum*) situated in Citeaux Monastery (30 km south of Dijon). The silty soil is leached and slightly acid. Earthworms represent *circa* 300 kg ha<sup>-1</sup> as dry weight. This stock comprises a typical mull soil fauna in which 81.2% of this biomass belongs to the two species *Nicodrilus longus longus* Ude and *N. nocturnus* Evans (Bouché 1972, 1976).

## SAMPLING AND METHODS

The times of sampling appear on the illustrations. Samples were taken in a soil pit where six layers could be recognised: earthworm casts, 0-6 cm, 6-20 cm, 20-40 cm, 40-60 cm, 60-100 cm. For the last five layers "drilospheric soil" (a ring 2 mm wide round burrows) was separated from "control soil", which was presumed to be devoid of burrows.

Estimates of the CO<sub>2</sub> release and of invertase, urease, and dehydrogenase activities were made on incubated soils in accordance with classical methods (Bauzon 1969).

Table 1. Organic matter (OM) and carbon (C) in % in the worm casts and in the five soil horizons.

Position in soil	Sampling date							
	August 1975		November 1975		April 1975		Nov. 1976	
	C	OM	C	OM	C	OM	C	OM
Worm casts	30.3	52.1	37.0	63.6	23.5	40.4	28.3	48.7
0-6 cm	24.6	42.3	28.9	49.7	17.8	30.6	25.0	43.0
6-20 cm	13.9	23.9	13.3	22.9	14.9	25.6	18.9	32.5
20-40 cm	6.5	11.2	9.0	15.5	5.8	10.0	6.4	11.0
40-60 cm	5.0	8.6	6.1	10.5	3.9	6.8	4.6	7.9
60-100 cm	1.7	2.9	4.1	7.1	2.3	3.9	--	--

Amylase and cellulolytic activities appeared, respectively, either very low and zero upon the first sampling, and so they were not determined thereafter. Results can be expressed either as a comparison of activities in soil, earthworm casts and drilosphere; or as the "drilospheric weight ratio" (R.D.P.), the ratio between the activity in the drilospheric and control soils.

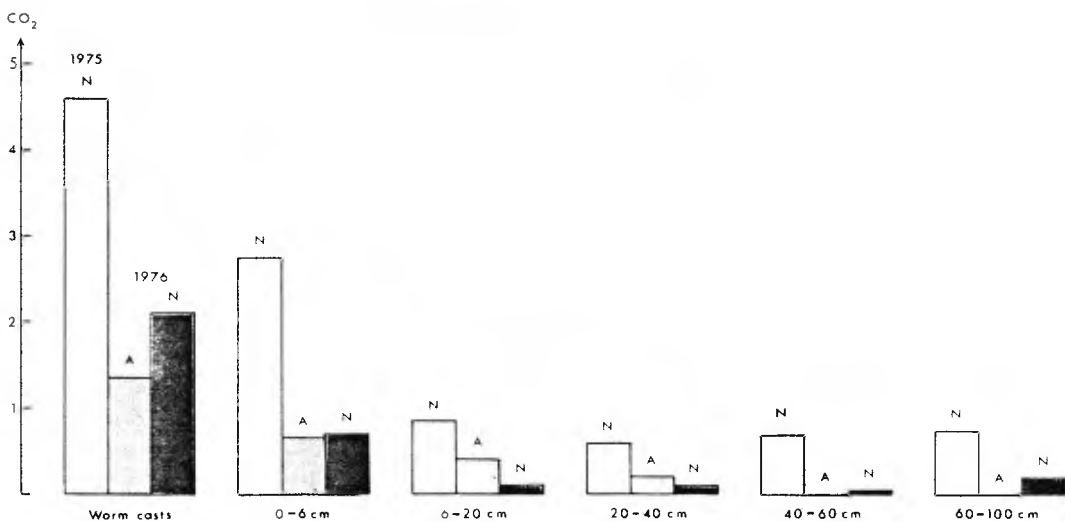


Fig. 1. Evolution of  $\text{CO}_2$  from earthworm casts and from different soil horizons. The first of each set of histograms (plain) represents the November 1975 data, and the second (lightly shaded) April 1976 data, and the third (strongly shaded) November 1976 data.  $\text{CO}_2$  evolution is given in mg per g dry soil per week.

The importance of this kind of study is demonstrated by the amount of soil moving through the lumbricid gut, which has been estimated as 100 to 1000 t ha<sup>-1</sup> yr<sup>-1</sup> by Kretzschmar (1976). It also relates to the importance of the burrow system developed in the profile, which has been quantified (Kretzschmar, unpubl.) at the dates of sampling. The values 1.6 to 12 m<sup>2</sup> per m<sup>2</sup> of soil surface show the importance of burrows in a meadow soil, their evolution all through the year, and their dissimilar distribution amongst the layers. These results warrant microbiological studies: the spatial distribution of microorganisms (Rouelle & Loquet, in progress) and the respiratory and enzymatic activities, in the particular structures depending upon worm activity.

#### RESULTS OF EXPERIMENTS

##### *Respiratory and invertase activities in earthworm casts*

It is interesting to estimate the respiratory and invertase activities and to relate them to the different layers of soil. It is well known that earthworm casts, resulting from mechanical and chemical effects on both the earth and plant remains, appear as "une substance plus fermentescible que le sol" (Margulis 1963).

Counting the whole microflora shows that populations in earthworm casts are about ten times higher than in the surrounding soil (Rouelle 1975). Also, amounts of carbon and of organic matter appear always higher in the casts (Table 1). These data explain why CO<sub>2</sub> release, indicating decay of carbon substrates, is regularly higher in the earthworm casts than in the soil below where values regularly decrease with depth (Fig.

1). Coefficients of mineralization 
$$\frac{\text{C degraded into CO}_2}{\text{Total C}} \times 100$$

following Dommergues (1968) are given in Table 2. Earthworm casts not only contain more organic matter, but mineralization of some of their components is easier.

Changes in endogeneous respiration in the earthworm casts follow the ones in the organic matter. The poor activity in November 1976 may be explained by the unusual summer drought whose impact on involved biological processes probably cannot be neglected.

Table 2. Coefficients of mineralization (see text for details).

Soil horizon	Sampling date		
	November 1975	April 1976	November 1976
earthworm casts	3.3	1.5	2.0
upper layer (0-6 cm)	2.5	0.9	0.6

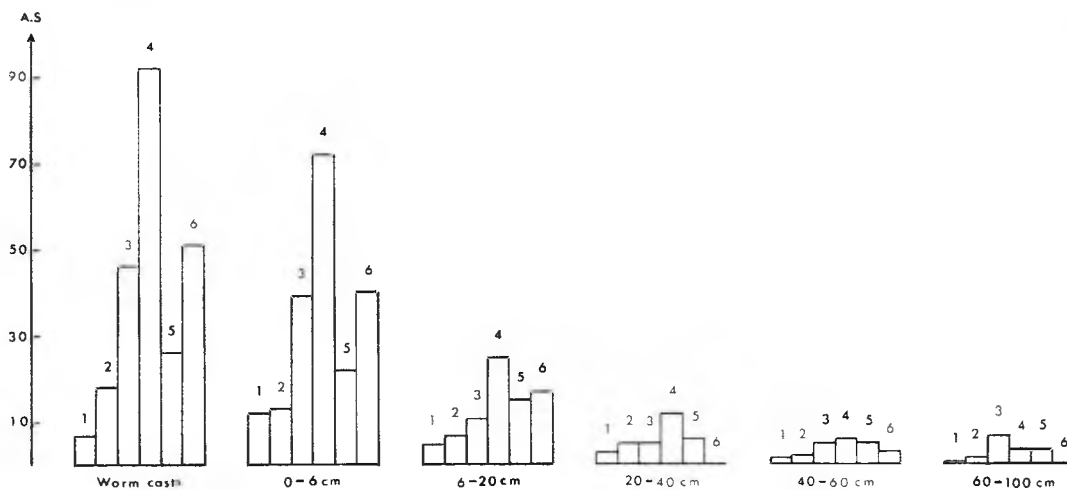


Fig. 2. Invertase activity (A.S.) in worm casts and in different soil horizons. The sampling dates are 1: March 1974, 2: May 1974, 3: June 1974, 4: November 1975, 5: April 1976, and 6: November 1976. Activity is given as mg of sugar reduced per g dry soil per day.

Except in March 1974, the invertase activity was always higher in earthworm casts. It should be noted that, when respiratory and invertase activities were simultaneously investigated, there were similar seasonal changes (November 1975, April and November 1976). This agreement indicates the relationship between populations of microbes and enzymatic activity in soils. The lumbricid influence gives rise to a layer, superficial casts, in which the activity always appears higher than in the most active layer of the soil (0-6 cm). Indeed, in the profile, the invertase activity decreases whilst depth increases (Fig. 2). It is a fairly general phenomenon (Durand 1965) which can also be observed with urease activity which similarly is higher in the earthworm casts.

#### *Respiratory and Enzymatic activity in the drilosphere.*

The interpretation of experimental results concerning the drilospheric soil is very difficult in the present state of knowledge, and thus only the localization and changes of these activities during the year will be considered.

Invertase activity regularly decreases from November until April, then increases from June. Such seasonal changes accord well with literature data (e.g. Durand 1965), where other fluctuations, often small ones, are superimposed on this regular variation. These are related to the invertase activity in the drilospheric microsite. Thus, the June and November 1975, as well as November 1976, invertase activities in the burrows are always higher (Table 3); the drilospheric ratio, more often than not, goes above unity. On the other hand, in April, invertase activity is lower, and in February the curve related to the drilosphere fluctuates with regard to the profile curve (Fig. 3). Owing to

Table 3. Data for the drilospheric weight ratio. The abbreviations relate to invertase activity (AS), dehydrogenase activity (AD), and evolution of CO<sub>2</sub>(CO<sub>2</sub>)

Position	Sampling date												
	June 1975		Nov. 1975		Feb. 1976		Apr. 1976		June 1976		Nov. 1976		
	AS	CO <sub>2</sub>	AS	CO <sub>2</sub>	AS	CO <sub>2</sub>	AS	CO <sub>2</sub>	AS	CO <sub>2</sub>	AD	AS	CO <sub>2</sub>
6-20 cm	1.12	1.1	2.16	0.6	0.7	0.26	1.0	—	—	1.2	1.0	1.46	8.0
20-40 cm	1.35	2.5	1.7	0.5	1.6	3.4	0	0.6	—	1.63	2.5	—	0.5
40-60 cm	2.7	0.6	1.1	0.7	0.4	2.15	0	0	—	3.4	8.3	1.3	4.5
60-100 cm	2.93	0.95	1.7	0.7	2.7	0.4	0.8	0	—	2.3	3.0	—	—

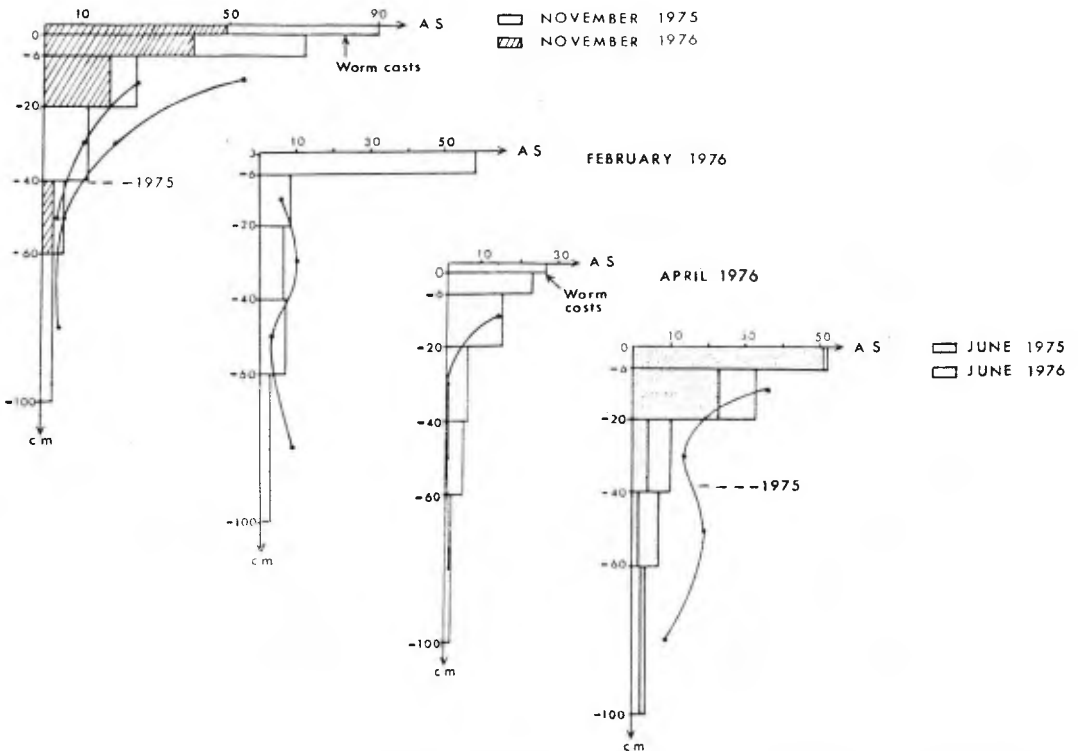


Fig. 3. Invertase activity in the soil and in the drilosphere at different depths and at different seasons. Histograms represent the soil, whilst the points joined by curved lines represent the drilosphere. Units are given in Fig. 2.

the fact that profiles of enzymatic activity in the soil are very regular, these changes probably mean that some unknown effect is present. However, Hoffman (in Durand 1965) was unable to detect any effect of the lumbricid activity upon enzyme rates. On the other hand, Kiss (in Durand 1965, and in Skujins 1967) estimates that invertase activity in *Lumbricus terrestris* L. burrows rises in value by 80% in the 0 to 2 cm layer of soil, and more than doubles at greater depths. The origin of such changes has still to be investigated.

Sometimes, either very important differences in the urease activity between control soil and drilosphere are seen (for instance in June 1975, 40-60 cm layer), or else very slight ones which are in accordance with those in the control soil profile (June and November 1976).

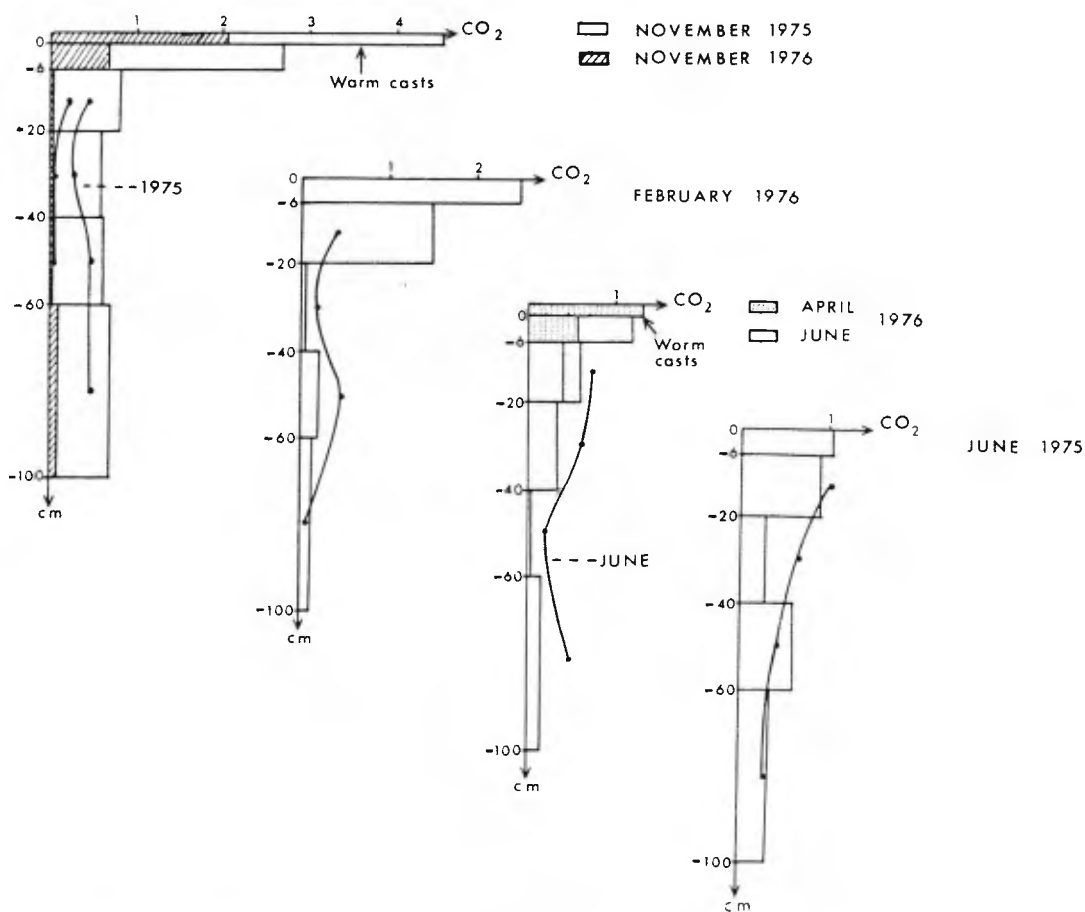


Fig. 4. Evolution of  $\text{CO}_2$  in the soil and in the drilosphere at different depths and at different seasons. The layout is as in Fig. 3, and the units are given in Fig. 1.

Both the CO<sub>2</sub> release and the dehydrogenase activity will be considered together because they are related to the total biological activity (Schaefer 1963). When both were studied (June 1976) there was a clear agreement for the curves and the drilospheric ratios (Table 3). During this period both features show a higher biological activity in burrows.

Considering CO<sub>2</sub> release as a whole there are similar seasonal changes as for invertase activity (Fig. 4). However, when considering detailed fluctuations in the drilospheric area, it becomes difficult to find a clear relation as was demonstrated for earthworm casts. It should be observed that in November 1975, at all depths, the drilospheric activity was lower than that in soil.

#### CONCLUSION — RESUMÉ

By their activity, lumbricids make pedological structures, casts and burrows, with modified soil properties. Among these properties are the biological ones, particularly the respiratory and enzymatic activities.

When considering earthworm casts we have shown a simultaneous enrichment of organic matter and an increase in microbial populations, with a stimulated and correlated respiratory and enzymatic activity throughout the year. Analysis of the drilospheric results is more difficult. Though activities develop in parallel in the different soil layers in the drilosphere, there might be positive or negative changes, which might or might not be correlated with the position in the soil profile.

#### REFERENCES

- Bauzon D. 1969. *Méthodes de mesure de l'activité respiratoire et de l'activité enzymatique dans les sols*. C.N.R.S., Centre de Pedologie-Biologie, p. 1-23.
- Bouché, M.B. 1972. Lombriciens de France. Ecologie et Systématique. *Ann. Zool. Ecol. Anim. I.N.R.A.*, n<sup>o</sup> spécial, 72, (2) p. 1-671.
- Bouché, M.B. 1976. Etude de l'activité des Invertébrés épigés prairiaux. *I. Rev. Ecol. Biol. Sol.*, 13, 261-281.
- Dommergues, Y. 1968. Dégagement tellurique de CO<sub>2</sub>. Mesure et signification. *Ann. Inst. Pasteur*, 115, p. 627-656.
- Durand, G. 1965. Les enzymes dans le sol. *Rev. Ecol. Biol. Sol.*, 2, 2, p. 141-205.
- Kretschmar, A. 1976. in Bouché, 1976. Ecologie et Paraécologie: peut-on apprécier le rôle de la faune dans les cycles biogéochimiques. In U. Lohm and T. Persson (Eds.), *Soil Organisms as Components of Ecosystems*. Proc. VI International Soil Zoology Colloquium. *Ecol. Bull.* (Stockholm) 25 (in press).

- Margulis M. 1963. *Pédologie générale*. Gautier-Villars éd., Paris, 1 vol. 116.
- Rouelle, J. 1975. *Quelques Aspects des Relations Existant entre Microorganismes et Lombriciens. Approches Techniques et Premiers Résultats*. Univ. Claude Bernard, Lyon I, pp. 1-19.
- Schaefer, R. 1963. L'activité déshydrogénasique comme mesure de l'activité biologique globale des sols. *Ann. Inst. Pasteur*, **105**, (2) p. 326-331.
- Skujins, J.J. 1967. Enzymes in soil. In A.D. McLaren and G.H. Peterson (Eds.), *Soil Biochemistry*. pp. 371-414. New York, M. Dekker Inc.