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Digestion of a vertisol by the endogeic earthworm *Polypheretima elongata*, megascolecidae, increases soil phosphate extractibility

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Abstract

The effect of the endogeic earthworm, *Polypheretima elongata*, on the phosphate status of vertisols (South East Martinique, French West Indies) has been evaluated in laboratory conditions. Fresh surface casts were collected and compared to the non-ingested soil. Phosphates were extracted by a sequencial procedure : anion resin desorption followed by sodium bicarbonate extraction. In some replicates chloroform fumigation followed the resin desorption to quantify bacterial immobilisation. Total phosphate contents were 43 % higher in casts than in soil, but the total phosphorus contents were not different. Fumigation had no effect on the phosphate contents as measured from bicarbonate extracts of casts. A particle size fractionation of casts and soil indicated that worms selectively ingest fine soil particles. However, the observed increase in phosphate extracted by anion resin was mainly attributable to digestive and microbial processes during the gut transit.

Keywords: *Polypheretima elongata*, earthworm, casts, phosphate, extractable and available phosphorus, vertisol, particle-size fractionation.

Augmentation de l'extractibilité du phosphore après digestion d'un vertisol par le ver de terre endogé Polypheretima elongata, *Megascolecidae.*

Résumé

L'effet du ver de terre endogé *Polypheretima elongata* sur l'état du phosphore dans des vertisols (Sud-Est de la Martinique, Antilles) a été étudié au laboratoire. Des turricules ont été récoltés en surface et comparés à du sol non ingéré. Les phosphates ont été extraits par un traitement séquentiel mettant en œuvre des résines anioniques suivies d'une extraction au bicarbonate de sodium. Pour certaines répétitions, une fumigation au chloroforme a suivi l'extraction par résine pour l'immobilisation microbienne. Les teneurs en phosphates extractibles sont plus élevées de 43 % dans les turricules que dans le sol mais il n'y a pas de différences de teneurs en phosphore total. La fumigation n'a aucun effet sur les teneurs des turricules en phosphates extractibles au bicarbonate. Un fractionnement granulométrique des turricules et du sol indique que les vers ingèrent préférentiellement des particules fines du sol. Cependant, l'augmentation des phosphates extraits par résine anionique est principalement attribuable à la digestion et aux processus microbiens qui ont lieu durant le transit intestinal.

Mots-clés : *Polypheretima elongata*, ver de terre, turricules, phosphate, phosphore extractible et disponible, vertisol, Antilles, fractionnement granulométrique.

INTRODUCTION

Endogeic earthworms have a manifold function in soil processes (Lavelle, 1988). They incorporate fresh organic materials to the soil, transport mineral and organic particles, and modify soil fabric and structure. Earthworms may affect phosphorus (P) cycling in soils by concentrating P in their casts due to the selective ingestion of P-rich particles, and modifying the relative proportions of different P forms and their stability. Several studies have shown that earthworm casts actually contain more "available" phosphorus than the bulk soil (James, 1991; Sharpley & Syers, 1976). In most cases, the increased phosphorus content was due to the addition of litter to the soil ingested by temperate anecic earthworms. In casts of the tropical geophageous species *Pontoscolex corethrurus*, concentrations of water extractable and exchangeable phosphate were increased during the two days following the egestion of soil (López-Hernández *et al.*, 1993).

This study addresses the effect of the endogeic earthworm *Polypheretima elongata* on short term P dynamics of a vertisol (South East of Martinique, French West Indies). These are either under pasture or long-term intensive market gardening management. In the later situation, biomass of *Polypheretima elongata*, a common endogeic earthworm in tropical clayey soils, is low compared to pastures, in which biomass may reach 3-4 t fresh weight per hectare (Barois *et al.*, 1988) and is expected to play a significant role in P dynamic, specially under pastures.

Different forms of P were analysed in casts of known origins and ages produced in laboratory cultures, at different times after their deposition, and compared to non-ingested soil. Soil phosphate was first desorbed with an anionic resin (Amer *et al.*, 1955) followed by a sodium bicarbonate extraction (Olsen & Sommers, 1982). These two methods are commonly used for the soils of the region (Wamsley and Cornfort, 1973). The sodium bicarbonate extraction made after the resin exchange allows to quantify biological effects on the available soil phosphate in short term studies (Hedley *et al.*, 1982). To examine the possibility that earthworms selectively ingest soil particles, a particle-size analysis of their casts and the non-ingested soil was carried out.

MATERIAL AND METHODS

Soil

The soil was a lithomorphic vertisol, according to the french classification it was a vertisol affected by run-off, with coarse angular peds, modal, developped on andesitic breccia (Colmet-Daage & Lagache, 1965; CPCS, 1967) from the South-East of Martinique island [French West Indies]. A representative soil sample was prepared by mixing 50 samples taken at random with an auger from the 0-20 cm layer in a *Digitaria decumbens* (Pangola grass) pasture (Brossard & Laurent, 1992). This sample was air dried, crushed and sieved at 2 mm, to provide an homogeneous medium for earthworm cultures. The main analytical properties of vertisols under different land management and the sample used in laboratory cultures are presented in *table* 1.

Table 1. – Selected physical and chemical characteristics of the 0-20 cm vertisol layers and of the sample used in earthworm cultures.

	Values in n = 8 sites			Worm cultures sample
	mean	*min.*	*max.*	
Bulk density (g cm^{-3})	1.0	0.7	1.2	1.0
Water content 33 kPA (g g^{-1})	0.55	0.44	0.71	0.6
0–20μm Fraction (g 100 g^{-1})	68	60	70	79.7
pH H_2O	6.3	5.5	7.0	6.0
C (mg g^{-1})	25.9	11.6	40.6	27.9
C/N	9.5	7	13	13.2
P (μg g^{-1})				
total	220	100	400	274
resin	16	11	21	14
$NaHCO_3$	14	10	19	10

Production of casts in the laboratory

Soil was moistened *per ascensum* (water content = 0.61 g g^{-1} soil) and filled into 10 boxes containing ca. 1 kg of compacted fresh soil. Due to the heavy clay content of the soil, casts produced inside the soil could not be separated and only fresh surface casts were collected twice a day. Two adult earthworms of ca. 2 g fresh weight were placed in each box. In such conditions, the average daily ingestion rate is estimated at 4 g g^{-1}, a significant proportion of which (20-25%) is egested at the surface (Lavelle *et al.*, 1993).

Representative samples of fresh casts and non-ingested soil were taken for the different analytical determinations.

Particle size fractionation

Fractionation was performed after total dispersion of 35 g fresh weight samples of soil (four replicates) and casts (two replicates) at neutral pH, in 300 ml distilled water with 100 ml of "Amberlite IRN 77" Na saturated resin (Feller *et al.*, 1991). Sands and coarse silts (20-2000 μm) were separated from the suspension by sieving at 20 μm. Sedimentation allowed the determination of the fine silt (2-20 μm) and clay (0-2 μm) fractions. The isolated fractions were further dried at 50°C and finely homogenised before analysis.

Extraction of phosphorus in soil and casts

Two extraction sequences derived from the first stages of Hedley *et al.* (1982), methodology were performed on fresh samples: (i) Anion resin (Cl^- saturated) desorption followed by extraction with $NaHCO_3$ pH 8.5; (ii) fumigation by $CHCl_3$ for 24 hours of soil and casts previously submitted to anion resin extraction and extraction of the phosphate released by $NaHCO_3$ pH 8.5. Resin and bicarbonate phosphate extractions desorb phosphates from mineral surfaces. The sum of the two extracts measures the potential exchangeable phosphates present in the sample at a given time. Fumigation was performed to quantify any immobilisation of phosphorus in the bacterial biomass since the transit through the earthworm gut considerably increases microbial activity (Trigo & Lavelle, 1993). Phosphate content in $NaHCO_3$ extracts was determined in supernatants after acidification and centrifugation. Total phosphorus contents in soil, casts and their respective particle size fractions were determined in sulphuric acid extracts after ignition and nitric oxidation of samples (Laurent & Brossard, 1991). Phosphate contents in the different extracts were determined by a colorimetric method (Duval, 1962).

Other analytical methods

Soil pH was measured in soil water suspension (1/2,5). Total carbon and nitrogen contents were measured with an elemental autoanalyser CNS Carlo Erba ANA 1500. All results except pH, were expressed on 105°C oven-dry basis.

RESULTS AND DISCUSSION

Total P content of fresh casts did not differ from that in the non-ingested soil. By contrast, extractable phosphate concentration was 43% higher with respective values of 31.6 (19.5 + 12.1) and 22.1 (13.0 + 9.1) µg P g^{-1} in the casts and in the control soil (*table* 2). Concentration of P-resin extracts from casts were still in the range of values observed in field conditions (*table* 1). This soil actually contains a high proportion of fresh casts at any time as a result of an intense earthworm activity.

Fumigation had a limited effect on the phosphate contents in soil bicarbonate extracts, and no significant effect on cast extracts. It can therefore be assumed that no significant bacterial immobilisation of P occurred during the gut transit.

The particle-size distribution in casts significantly differed from that in the non-ingested soil since casts contained a higher proportion of finer particles than the soil (*table* 3).

Although the organic carbon concentration (mg C g^{-1} fraction) in the coarser 20-2000 µm fraction was significantly higher in casts than in soil, the overall C content in the fraction was not different. Higher proportions of fine material (0-2 µm) in casts and greater C content in the coarse fraction may result from a selective ingestion of fine rather than coarse

Table 2. – Phosphate content (µg P g^{-1}) in resin and bicarbonate sequential extracts of non ingested soil and earthworm casts.

		Resin *	HCO_3Na	
			without $CHCL_3$ * pretreatment	after $CHCL_3$** pretreatment
Soil	mean	1.30	9.1 c	10.55 d
	σ	*0.5*	*0.2*	*0.4*
Casts	mean	19.5 b	12.1 e	12.1 e
	σ	*0.5*	*0.1*	*0.1*

Means followed by a common letter are not significantly different at the 5% level;

* 6 replicates; ** 3 replicates.

Table 3. – Mass, total phosphorus and carbon contents of particle-size fractions of non-ingested soil and earthworms casts (sandart deviation in parentheses).

Fraction (µm)	Soil	Casts	Soil vs. casts
Mass g 100 g^{-1}			
20-2000	24.57 (0.46)	20.69 (1.60)	S
2-20	15.34 (0.19)	14.37 (0.06)	S
0-2	59.00 (0.41)	63.91 (1.27)	S
Σ fractions	98.91 (0.22)	98.97 (0.27)	
P concentration µgP g^{-1} fraction			
20-2000	282.30 (14.0)	271.9 (14.0)	NS
2-20	206.60 (17.5)	232.7 (2.80)	NS
0-2	302.40 (9.2)	281.1 (13.0)	NS
P concent µgP g^{-1} soil			
20-2000	69.40 (4.3)	56.40 (7.3)	S
2-20	31.70 (3.1)	33.50 (0.2)	NS
0-2	178.40 (5.7)	179.60 (4.7)	NS
Σ fractions	279.50 (7.5)	269.40 (12.2)	NS
bulk sample	274.10	274.10	
C concentration mgC g^{-1} fraction			
20-2000	47.83 (2.01)	62.10 (2.63)	S
2-20	20.59 (0.76)	20.80 (0.09)	NS
0-2	19.31 (1.14)	19.09 (0.39)	NS
C content mgC g^{-1} soil			
20-2000	11.75 (0.63)	12.83 (0.45)	NS
2-20	3.17 (0.13)	2.99 (0.03)	NS
0-2	11.42 (0.69)	11.56 (0.48)	NS
Σ fractions	26.35 (1.20)	27.38 (0.06)	NS
bulk sample	27.94	27.95	

NS: Non significant $p > 0.05$.

S: Significant $p < 0.05$.

particles. Selection in feeding by the earthworms on the finer soil particles has been frequently reported for temperate (Sharpley & Syers, 1976) and tropical species (Hauser, 1993; Barois *et al.*, 1993).

However the differences in particle-size distribution have no effects on distribution of P in casts, except for the amount of P (μg P g^{-1} soil) in the coarser fraction of casts which is significantly lower than in the non-ingested soil (*table* 3). Overall P contents are similar in casts and control soil. As a result, changes in the inorganic pool of phosphate resulting from digestion processes account for the higher value of resin-extractable P (+9.5 μg g^{-1}) measured in casts.

During gut transit, comminution (fragmentation without digestion) of plant debris is observed in other endogeic tropical species (Lavelle *et al.*, 1992). This effect is not observed in this laboratory study, in which only roots fine debris were conserved after the crushing and sieving at 2 mm. An enhancement of microbial activity in the gut may have resulted in phosphate solubilisation. Low molecular weight organic acids produced by bacteria are known to release soil P by acidification, chelation and reduction (Tinker, 1983). Such compounds may have been released as a result of the increased microbial activity induced by the production of intestinal mucopolysaccharides by earthworms (Barois & Lavelle, 1986; Martin *et al.*, 1987; Trigo & Lavelle, 1993). The soil pH does not change significantly during gut transit and, therefore, desorption of P from soil particle surfaces is not expected. During gut transit the soil matrix is dispersed and reorganised (Barois *et al.*, 1993). This process may favour the mobilisation of phosphate by enzymatic hydrolysis of organic phosphorus compounds, since comminution and mixing by earthworms (Kretzschmar, 1987) increases the surface area of substrates that come in contact with appropriate organisms and enzymes. Microbial phosphatase activity may be strongly enhanced by such processes (Satchell *et al.*, 1984).

The results indicate a significant worms effect in laboratory conditions as it was showed for another tropical geophagous species. *Polypheretima elongata* may build up dense populations in vertisols under pastures in Martinique. Their effect on the short term dynamics of soil phosphate under field conditions will be studied in natural environments in the near Future to improve our knowledge of phosphorus behaviour and control.

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