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1 Burrowing activity of the geophagous earthworm *Pontoscolex corethrurus*

2 (Oligochaeta: Glossoscolecidae) in the presence of charcoal

3
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10
11 **Abstract**

12
13 The geophagous earthworm *Pontoscolex corethrurus* is frequently found in
14 burnt tropical soils where charcoal plays an important role in soil fertility. We studied
15 the burrowing activity of this species in two-dimensional microcosms with one half
16 filled with soil and the other with a 3:2 (w:w) mixture of charcoal and soil
17 (CHAR+soil). We measured the volume of empty burrows and those filled with black
18 or brown casts in both substrates, as well as the initial and final fresh weights of the
19 worms. The correlation between brown cast production and both initial and final fresh
20 weights of the worms, reinforced by the presence of feeding cavities in soil but not in
21 CHAR+soil, suggests that *P. corethrurus* would ingest soil to fulfill its nutrient
22 requirements, in contrast to charcoal which was ingested for other purposes. We
23 observed that at equal burrow volume created in the two substrates, *P. corethrurus*
24 produced smaller black casts than brown casts, suggesting that burrows were
25 created in CHAR+soil mainly by pushing aside the particles of this lighter substrate.

1 The observed transport of charcoal to soil points to the importance of *P. corethrurus*
2 in the incorporation of charcoal particles into organic-poor soil.

3

4 Key-words

5 Burrows; casts; charcoal; *Pontoscolex corethrurus*

6

7 **1. Introduction**

8

9 The geophagous earthworm *Pontoscolex corethrurus*, an endogeic species
10 feeding on soil with a low content of organic matter (Lavelle et al., 1987), exerts an
11 important effect on the soil structure in the upper 10 cm through its burrowing and
12 casting activity. While it has been reported to increase the porosity of compacted soil
13 (Zund et al., 1997), *P. corethrurus* is classified as a “soil compacting” earthworm
14 species (Lavelle et al., 1998) because it produces large coalescent aggregates
15 (Barois et al., 1993). The production of these macroaggregates (>1 cm) increases
16 bulk density and decreases water infiltration (Alegre et al., 1996), thus causing a
17 strong compaction of the soil surface. This detrimental effect of earthworm activity
18 occurs at a high density of *P. corethrurus* and in the absence of other earthworm
19 species reducing aggregate size (Barros et al., 2001; Chauvel et al., 1999).

20 Whereas the importance of charcoal in soil fertility has often been reported
21 (Tryon, 1948; Glaser et al., 2001; Lehmann et al., 2002; Topoliantz et al., 2002), no
22 published study has yet dealt with its possible incorporation into the soil by *P.*
23 *corethrurus* individuals living in burnt areas (Standen, 1988; personal observation) or
24 by other soil fauna. In the present study, we investigated the subterranean activity of
25 *P. corethrurus* and its growth in the presence of soil and charcoal.

1

2 **2. Material and methods**

3

4 We studied the burrowing activity of *P. corethrurus* in the presence of native
5 soil and charcoal in two-dimensional microcosms (Evans, 1947; Grant, 1956), made
6 of two parallel transparent plastic sheets each 20 cm high x 25 cm wide x 2 mm thick.
7 Bottom and side edges were sealed with 2 mm thick wooden strips, thus allowing
8 earthworm movement and observation of their burrow system and cast deposition.
9 The microcosms were filled with 80 g dry weight of soil on one side; the other half
10 side was filled with 40 g dry weight of a 3:2 (w:w) mixture of charcoal and soil
11 (CHAR+soil). The soil was taken from the upper 10 cm of an oxisol (65% sand, 12%
12 silt and 23% clay content) in a slash-and-burn field in maripasoula (French Guiana).
13 Charcoal was collected on the ground in a recently burnt field. Both substrates were
14 sieved at 2 mm mesh size. Physical and chemical properties of the substrates are
15 given in Table 1. Both substrates were moistened from the top edge by adding water
16 to 50-55 % substrate weight. Sub-adult *P. corethrurus* were obtained from a nearby
17 experimental area. In each microcosm, one individual (initial fresh weight from 209 to
18 591 mg) was inserted from the top edge at the border between the two substrates.
19 The top edges of the microcosms were closed with Parafilm® to avoid desiccation
20 and earthworm escape. Ten replicates were established and placed for two weeks in
21 a dark chamber with controlled temperature at 25°C. At the end of the experiment,
22 the surface of burrows and casts visible through the two transparent walls (planes 1
23 and 2) were drawn on a transparent film and measured with a surface integrator
24 (Numonics 1224®, resolution 0.1 mm). Casts were classified according to their
25 colour, as brown (soil) and black (mixture of charcoal and soil) casts. Very dark grey

1 casts were pooled with black ones because of their high content of charcoal. The
2 Mean surface area and the volume of empty and cast-filled burrows were calculated
3 from the following equations:

4

5 Mean surface area = (area on plane 1 + area on plane 2)/2

6

7 Volume = Mean surface area x substrate thickness (2 mm)

8

9 The burrow length was not measured because a high number of burrows
10 could not be considered as typical linear galleries (see Fig 1 as an example of the
11 burrow system). The final fresh weight of individuals was measured after rinsing
12 earthworms in water and gently blotting them with absorbent paper.

13 The volume of burrows, the volume of casts filling the burrow system and the
14 growth rate of earthworms were statistically analysed using only nine replicates, one
15 earthworm having died during the experiment. Initial and final fresh weights of worms
16 were compared using paired t-tests. The volumes of casts and burrows in soil,
17 CHAR+soil and both substrates pooled, were compared using t-tests or Mann-
18 Whitney rank tests when data were not normally distributed. Relationships between
19 growth rate, cast and burrow volume were tested by Bravais-Pearson correlation
20 coefficients. The coefficient of variation (SD/mean x 100) was calculated for each
21 variable (Sokal and Rohlf, 1995).

22

23 **3. Results**

24

1 During the experiment, the earthworm weight increased by 36 ± 17 % (mean \pm
2 S.D.) and the mean growth increment was 0.13 ± 0.06 g. The final fresh weight (0.49
3 ± 0.15 g, mean \pm S.D.) was significantly higher than the initial weight (0.36 ± 0.11 g,
4 mean \pm S.D.) at the 0.001 level ($t = -6.12$). No significant correlation was found
5 between the growth rate and other variables such as initial fresh weight or burrow
6 and cast volume, whether substrates were pooled or not. The initial fresh weight of
7 worms was positively correlated with the volume of total burrows calculated when
8 both substrates were pooled ($r = 0.736$, $P < 0.05$) and with the total volume of brown
9 casts deposited ($r = 0.82$, $P < 0.01$). The final fresh weight of worms was positively
10 correlated with the total brown cast volume ($r = 0.83$, $P < 0.01$) and with the total
11 brown cast / soil burrow volume ratio ($r = 0.695$, $P < 0.05$). Initial and final fresh
12 weights were not correlated with either black cast deposition or with burrowing
13 activity in CHAR+soil.

14 On average, in the total substrate, *P. corethrus* created burrows of $3.34 \pm$
15 0.74 cm³ (mean \pm S.D.) per g of earthworm biomass and per day. After 14 days, the
16 burrow system in the soil substrate reached 32.4 ± 8.8 % (mean \pm S.D.) of the total
17 soil volume and the burrow system in CHAR+soil 3.9 ± 2.4 % (mean \pm S.D.) of the
18 total CHAR+soil volume. Total brown casts deposited on both sides (soil and
19 CHAR+soil) and burrows in the soil half side were strongly correlated ($r = 0.92$,
20 $P < 0.001$), as were total black casts and burrows in CHAR+soil ($r = 0.95$, $P < 0.001$).
21 The total volume of brown casts amounted to 37.3 % of the burrow system on the soil
22 half side and the total volume of black casts 10.6 % of the burrow system in
23 CHAR+soil (Table 2).

24 Comparisons of burrow systems and cast deposition between soil and
25 CHAR+soil are summarised in Table 2. The initial volumes of soil and CHAR+soil

1 were not significantly different. The volume of the burrow system in the soil half side
2 was significantly higher than that in the CHAR+soil half side and the total volume of
3 brown casts deposited on both sides was significantly higher than that of black casts.
4 The ratio of total brown casts to burrows in soil was higher than that of black casts to
5 burrows in CHAR+soil. The percentage of burrows filled with casts (black and brown)
6 in soil was not significantly different to that in CHAR+soil. Coefficients of variation
7 were greater for black casts and CHAR+soil burrows than for brown casts and soil
8 burrows, indicating that burrowing and cast production were more variable in
9 CHAR+soil than in soil (Table 2). The ratio of black casts deposited in soil to total
10 black casts was not different of that of brown casts deposited in CHAR+soil to total
11 brown casts, both displaying a high coefficient of variation (Table 2).

12

13 **4. Discussion**

14

15 Heavier individuals of *P. corethrurus* constructed more channels, as Lavelle et
16 al. (1998) found for immature individuals only (weighing less than 0.6 g fresh weight).
17 Bigger immature earthworms ingested more soil but not more charcoal-soil mixture
18 than smaller ones, suggesting that, although no correlation between soil ingestion
19 and earthworm growth was found, the soil constituted a nutrient source in contrast to
20 charcoal. This result is reinforced by the presence of feeding cavities in soil only
21 (Fig.1), which are burrowed to exploit a food source (Martin, 1982). The mean growth
22 rate of *P. corethrurus* appears lower (2.5 % per day) than that found by Lavelle et al.
23 (1987) (5 to 6% per day) at the same soil moisture, despite a similar soil consumption
24 (5.4 g soil per g earthworm fresh weight). We can attribute this difference to the
25 estimating method of soil consumption, Lavelle et al. (1987) results being based on

1 the weight of casts produced and ours on burrow volume and soil bulk density. In our
2 2D microcosms, the soil was poorly compacted and allowed channelling activity
3 without necessarily ingestion of the substrate (Buck et al., 2000).

4 If all the burrow volume had been ingested by the worms, the ratio of black
5 cast/burrow volume in the charcoal/soil mixture (11%) and that of brown cast/burrow
6 volume in soil (37%) would represent a compaction of the ingested substrate of 9.4
7 and 2.7 for charcoal/soil and soil respectively. This result cannot totally be explained
8 by differences in bulk density (Table 1) and suggests that *P. corethrurus* may have
9 created channels in the charcoal/soil substrate mainly by pushing aside charcoal
10 particles and to a lesser extent by ingesting them. The microcosms being highly
11 artificial, the worms could ingest charcoal by accident as Fig 1 shows. However, *P.*
12 *corethrurus*, that selects particles before ingestion (Lavelle, 1997), could ingest
13 charcoal for its detoxifying and liming effects (Titoff, 1910;; Zackrisson et al., 1996)
14 and its enhancement of microbial communities (Pietikainen et al., 2000) which could
15 favour the production of earthworm's digestive enzymes of bacterial origin (Lattaud et
16 al., 1999).

17 The transport of ingested matter, here demonstrated through black and brown
18 cast deposition, underlines the importance of *P. corethrurus* for bioturbation (Garcia
19 and Fragoso, 2002). More especially, by ingesting charcoal and incorporating it to the
20 soil matrix, *P. corethrurus* could play an important role in burying this source of
21 fertility in burnt soils used for slash-and-burn agriculture (Topoliantz et al., 2002).

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3

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- 24

1 **Fig. 1.** Example of a reconstructed burrow system in soil and charcoal / soil
2 mixture made in two weeks by one individual *Pontoscolex corethrurus* (0.35 g initial
3 fresh weight) at 25°C. The image of the burrow system was obtained by
4 superimposing drawings done on the two walls of one microcosm. Black and brown
5 casts are represented by black and horizontal strips, respectively. Voids represent
6 ingested or pushed aside areas.

7

Table 1

Chemical and physical properties (pH, total C and N content) of substrates used in the microcosms

| Substrate | pH | C (%) | N (%) | C:N ratio | Mass:volume ratio (g.cm ⁻³) |
|-----------------------|------|-------|-------|-----------|---|
| Soil | 4.63 | 2.88 | 0.215 | 13.40 | 1.77 |
| Mixture charcoal+soil | 6.90 | 39.46 | 0.299 | 132.07 | 0.885 |

1

2

Table 2

Comparison of burrowing activity and cast deposition of *Pontoscolex corethrurus* after 2 weeks between soil and charcoal+soil (CHAR+soil), (n=9). Volumes and ratios (mean \pm S.D.) were compared by t-test (t) or Mann-Whitney rank test (T). Significant probability is indicated by *, **, *** for $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively and no significant differences are abbreviated NS. CV (%) is the coefficient of variation.

| Variables | Unity | Mean \pm S.D. | CV (%) | t or T |
|--|-----------------|------------------|--------|--------------|
| Initial soil volume | cm ³ | 45.12 \pm 1.34 | 2.97 | t= -0.118 NS |
| Initial CHAR+soil volume | cm ³ | 45.19 \pm 1.16 | 2.57 | |
| Burrow volume in soil | cm ³ | 14.60 \pm 3.95 | 27.03 | T=126 *** |
| Burrow volume in CHAR+soil | cm ³ | 1.76 \pm 1.16 | 65.95 | |
| Volume of total brown casts | cm ³ | 5.46 \pm 1.68 | 30.81 | T=126 *** |
| Volume of total black casts | cm ³ | 0.25 \pm 0.32 | 130.11 | |
| Total brown casts/burrows in soil | % | 37.3 \pm 4.9 | 13.27 | t=8.460*** |
| Total black casts/burrows in CHAR+soil | % | 10.6 \pm 8.1 | 75.94 | |
| Total casts in soil/burrows in soil | % | 34.7 \pm 4.8 | 13.9 | t=1.629 NS |
| Total casts in CHAR+soil/ burrows in CHAR+soil | % | 27.6 \pm 12.2 | 44.06 | |
| Brown casts in CHAR+soil/total brown casts | % | 7.36 \pm 8.04 | 109.34 | T=76 NS |
| Black casts in soil/total black casts ¹ | % | 33.2 \pm 29.8 | 89.97 | |

1 ¹Only seven replicates used, due to the absence of black casts in the other two.

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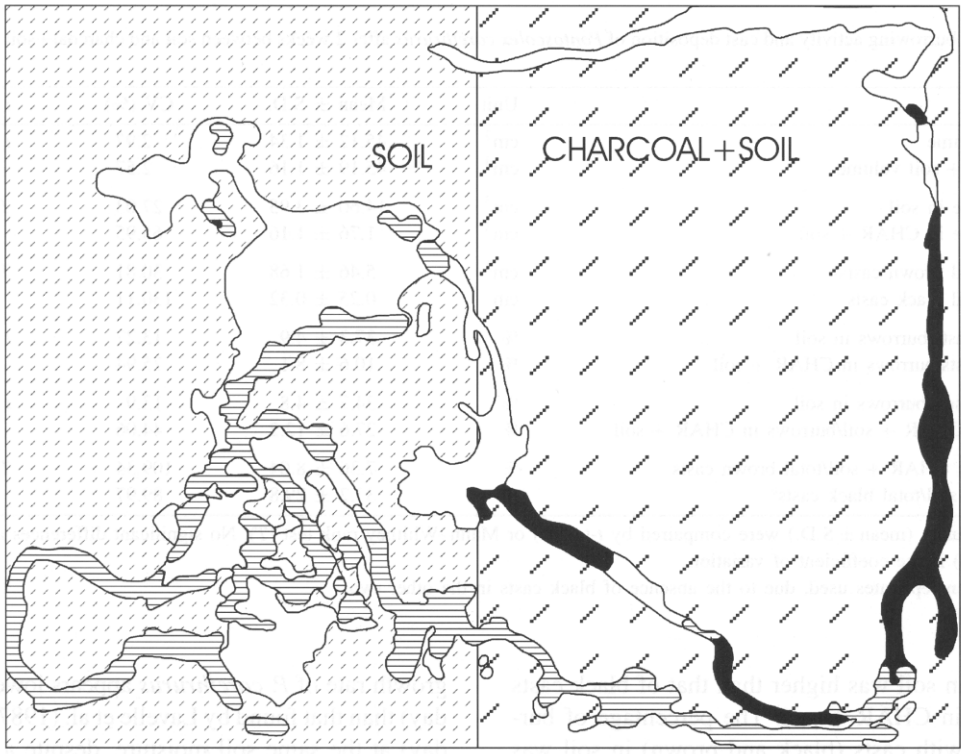


Fig. 1