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Nest construction is a common form of parental care in soil organisms. However, it is unknown whether the tropical earthworm Pontoscolex corethrurus selects sites for nest construction when the nutritional quality of the soil is irregular. Here we studied the reproductive behaviour and nest site selection of P. corethrurus. In tridimensional terrariums we evaluated the combined effect of the food quality (soil only = S, soil+grass = G, soil+legume = L) and soil depth (0-9 cm = Shallow, 10-18 cm = Intermediate, 19-27 cm = Deep) in a factorial 32 design. The number and biomass of cocoons, progeny and the production of internal and external excreta were evaluated. The nutritional quality and depth of soil and their interaction had a significant effect on nest site construction and the deposition of internal excreta. P. corethrurus built a higher amount of nests in the S-Intermediate and G-Intermediate treatments while more internal excreta were found in the L-Intermediate treatment. Offspring biomass was positively associated with internal excreta in the S (soil only) and G (soil + grass) treatments. We conclude that P. corethrurus shows parental care when selecting sites for its offspring in the form of nest construction and excreta deposition. Further research is needed on the ecological conditions that favour the evolution of parental care in earthworms according to their ecological category (anecic, endogeic and anecic).

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Abstract

Nest construction is a common form of parental care in soil organisms. However, it is unknown whether the tropical earthworm *Pontoscolex corethrurus* selects sites for nest construction when the nutritional quality of the soil is irregular. Here we studied the reproductive behaviour and nest site selection of *P. corethrurus*. In tridimensional terrariums we evaluated the combined effect of the food quality (soil only = S, soil+grass = G, soil+legume = L) and soil depth (0-9 cm = Shallow, 10-18 cm = Intermediate, 19-27 cm = Deep) in a factorial 3^2 design. The number and biomass of cocoons, progeny and the production of internal and external excreta were evaluated. The nutritional quality and depth of soil and their interaction had a significant effect on nest site construction and the deposition of internal excreta. *P. corethrurus* built a higher amount of nests in the S-Intermediate and G-Intermediate treatments while more internal excreta were found in the L-Intermediate treatment. Offspring biomass was positively associated with internal excreta in the S (soil only) and G (soil + grass) treatments. We conclude that *P. corethrurus* shows parental care when selecting sites for its offspring in the form of nest construction and excreta deposition. Further research is needed on the ecological conditions that favour the evolution of parental care in earthworms according to their ecological category (anecic, endogeic and anecic).

Keywords: Oligochaeta, endogeic earthworm, internal cast, feeding behaviour, nest building, parent-offspring, life history.

48

49 **Introduction**

50 Most animals, including the majority of invertebrates, do not provide any form of care for
51 their offspring (Smiseth *et al*. 2012). However, some animals make an effort to increase the
52 survival rate of their progeny by protecting them from predators, lack of food, desiccation and
53 other biotic and abiotic threats (Clutton-Brock, 1991; Smiseth *et al*. 2012; Furuichi & Kasuya,
54 2015). Mammals and birds provide elaborate forms of care by either one or both parents,
55 including: provision of gametes, oviposition-site selection, nest building and burrowing, egg
56 attendance, egg brooding, viviparity, offspring attendance, offspring brooding, food provision
57 and care even after nutritional independence (Gardner & Smiseth, 2011; Trumbo, 2012; Smiseth
58 *et al*. 2012).

59 Parental care has been widely studied in avian and mammal species but is also prevalent
60 in certain insects, such as water bugs and dung beetles (Jeanne, 1996; Munguía-Steyer &
61 Macías-Ordoñez, 2007; Trumbo, 2012; Smiseth *et al*. 2012). Rigorous, dangerous and
62 competitive environments are conducive to the incidence of parental care (Mori & Chiba 2009).
63 Some soil organisms develop parental care in order to increase the survival of their progeny; for
64 example, in at least 11 families of beetles, ants and termites, parental care seems to be a response
65 to severe environments (Currie, 2001; Muller *et al*., 2005; Mori & Chiba, 2009; Smiseth *et al*.
66 2012). However, despite the fact that earthworms are among the most ecologically important soil
67 organisms (Lee, 1985; Edwards & Bohlen 1996; Lavelle & Spain 2001), it is unknown whether
68 they exhibit parental care towards their progeny.

69 Edaphic (physical, chemical and biological), climatic (soil moisture and temperature) and
70 biological (symbiosis, competition, etc.) factors determine the life history of earthworms (Lee,

1985; Edwards & Bohlen 1996; Lavelle & Spain 2001). Based on their ecological niche, earthworms have been classified into functional groups (epigeic, endogeic and anecic) that develop different reproductive strategies (r and K) in order to more effectively exploit their edaphoclimatic environment (Lee, 1985; Edwards & Bohlen, 1996; Lavelle & Spain 2001). However, very little is known in terms of earthworm behaviour during the reproductive stage. Various earthworms provide cocoons with a small nutritious package that serves as a food source until the offspring are capable of feeding by themselves, thus increasing the survival of their progeny (Stephenson, 1930; Lee, 1985; Edwards & Bohlen, 1996; Lavelle & Spain, 2001).

Pontoscolex corethrurus is a tropical endogeic earthworm of extensive distribution in the tropical regions of the world (Hendrix *et al*. 2008). Within the different tropical soils it inhabits, its biological activity positively influences soil fertility and plant growth; thus providing environmental services in both agro and natural ecosystems alike (Scheu, 2003; van Groenigen *et al*., 2014), which has led to it being named the "ecosystem engineer" (Jones, 1994; Hastings *et al*., 2007). However, it is often considered an invasive species since it occupies environments disturbed by anthropogenic activities and can have a negative effect through promoting soil compaction (Chauvel *et al*., 1999). It has been suggested that the wide distribution of *P. corethrurus* is due to its parthenogenetic reproduction (Hendrix *et al*., 2008), but it may also be the result of parental behaviour that increases offspring survival.

Construction of nests and providing high quality food (for example, nitrogen in the excreta) are a form of parental care that is common among both vertebrates and invertebrates (Clutton-Brock, 1991; Mori & Chiba, 2009; Gardner & Smiseth, 2011). Previous studies have documented that *P. corethrurus* constructs incubation nests that contain one cocoon per nest and around these they build "feeding chambers" where excreta are deposited (Ortiz-Ceballos &

Fragoso, 2006; Ortiz-Ceballos *et al*., 2009; Buch *et al*., 2011), whereas the anecic earthworm *Lumbricus terrestris* covers its cocoons with its own excreta (Ramisch & Graff, 1985; Grigoropoulou *et al*., 2007). However, we do not know whether earthworms choose particular sites in which to construct these nests and place their cocoons, particularly when food and its quality is either ephemeral and/or distributed irregularly (Mori & Chiba, 2009). Here we determined whether *P. corethrurus* is capable of preferentially selecting a habitat for its progeny, thus indicating a form of parental care. This was achieved by examining the combined effect of soil depth and nutritional quality on the reproductive activity of this species.

Materials and methods

Terrariums

Fifteen tri-dimensional (45 × 35 × 5 cm) terrariums were utilized in the study. These were constructed of two panes of glass 5.3 mm thick, separated by thin balsa wood strips leaving an internal space of 0.5 cm (Evans, 1947; Capowiez, 2000; Ortiz-Ceballos *et al*., 2009). The glass was glued to the balsa wood on the two sides and bottom of the terrarium, leaving the top open. The sides of the terrariums were sealed with transparent adhesive tape. Four holes (2 mm wide) were made on the bottom in order to allow water to enter by capillary action.

Soil

Ten kg of soil were collected from a plot of maize under rotation with the tropical legume velvet bean [*Mucuna pruriens* var. *utilis* (Wall. ex Wight) Back. ex Burck] in the locality of village Tamulté de las Sabanas (18º08'N, 92º47'W), 30 km east of Villahermosa, Tabasco, Mexico. The silty clay loam soil (31.6 % silt, 26.8 % clay and 41.5 % sand) was air-dried in the shade at room

117 temperature, and sieved through a 5 mm mesh. The main chemical characteristics of this soil
118 were: 2.7 % organic matter, 0.14 % total N, 11.5 C/N and pH (H_2O) 6.3.
119
120 *Earthworms*
121 Thirty subadult *Pontoscolex corethrurus* earthworms were collected from a pasture of
122 *Brachiaria humidicola* (Rendle) Schweick located at Huimanguillo (17º48'N, 93º28'W), 79 km
123 southwest Villahermosa, Tabasco, Mexico. The earthworms were reared until reaching sexual
124 maturity in boxes (12 × 12 × 8 cm) with 300 g of the soil mixed with 3% legume (*Mucuna*
125 *pruriens* ssp. *utilis*) foliage. Prior to initiation of the experiment, the first 15 earthworms to
126 produce a cocoon were selected.
127
128 Food quality
129 The influence of the quality of food was evaluated using foliage from a legume (*M. pruriens* ssp.
130 *utilis*) or grass (*B. humidicola*) with 14.3 y 6.1 % crude protein, respectively. These were
131 collected from the same sites as the earthworms. 5 kg of legume and grass foliage were collected
132 and dried at 65 °C for 48 h. The dried materials were then sieved to 2 mm and 3.3 kg of the soil
133 was homogeneously mixed with 0.01 kg (3 %) of leguminous foliage, while another 3.3 kg of
134 soil was homogeneously mixed with grass (3 %).
135
136 *Experimental set-up*
137 To test preferences for nest location, an experiment was established utilizing a 3^2 factorial
138 design, i.e., two factors (food quality and soil depth) with three levels. Food consisted of either:
139 soil only (S), soil + grass (G), or soil + legume (L). The different soil depths tested were: 0-9 cm

(Shallow), 10-18 cm (Intermediate), or 19-27 cm (Deep). Each treatment had five replicates, utilizing a total of 15 terrariums. The terrariums were separated into three depths (layers), each containing 220 g of substrate with the following treatments: S-Shallow, L-Intermediate, G-Deep, L-Shallow, G-Intermediate, S-Deep, G-Shallow, S-Intermediate and L-Deep. The soil was then moistened with distilled water through capillary action to field capacity with 217 ml per terrarium. One adult *P. corethrurus* (with clitellum) of similar biomass (455 ± 25 mg) was introduced in each terrarium. The density was equivalent to the abundance and biomass recorded in the field (438 earthworms m^{-2} and 27 g m^{-2}, respectively). The terrariums were placed in an incubator at a temperature of 26 ± 1 °C. Water was added through capillary action every six days in order to maintain the soil moisture content at field capacity. Experiments were carried out at INBIOTECA, Universidad Veracruzana, Xalapa, Veracruz, Mexico.

Every third day, cocoon production and emergence of juveniles was recorded. The position of cocoons were marked. After 100 days, the number and biomass of cocoons, juveniles and adults were recorded. In addition, the external (excreta deposited on the soil surface) and internal (within each soil layer) excreta were separated, oven-dried (at 65 °C for 72 h) and weighed.

Data analysis

A one-way ANOVA was used to test for significant differences between the biomass parental earthworm (initial and final), total number cocoons, and biomass and number of juvenile earthworm per terrarium. The analysis was performed using Statistica software, ver 7 (StatSoft 1998).

To analyze the number of cocoons per treatment (y) we used a negative Binomial distribution as described in brief below:

$$P(Y=y)=\frac{\Gamma\left(k^{-1}+y\right)}{\Gamma\left(k^{-1}\right)y!}\left(\frac{k\mu}{1+k\mu}\right)^{y}\left(\frac{k\mu}{1+k\mu}\right)^{1/k}, y=0,1,2\ldots$$

where: k is a parameter to be estimated, $\log(\mu)=\boldsymbol{x}'\boldsymbol{\beta}$, which considers the effect of the factors considered in the experiment (soil depth, food quality, etc.). This model was fitted using the GENMOD routine included in the program SAS/STAT for Windows.

To analyze the weight of the excreta and its placement (internal or external), we used a linear model, with food type, soil depth and their interaction as independent factors. The model was fitted using the ANOVA routine of the software SAS 9.4 for windows. Means of the treatments were compared using a Tukey test.

To determine if the biomass of offspring was correlated to the amount of internal excreta we analysed the excreta depostited in the S, G and L treatments using a Pearson´s correlation test. Once a relationship was established we fitted a lineal model to find which type of excreta better predicted offspring biomass by comparing the estimated regression coefficients. Analysis were carried out using R software (R Core Team 20015).

Results

Nest construction and total number of cocoons laid

Pontoscolex corethrurus constructed one nest per cocoon (0.035 ± 0.006 g). There was no significant difference in the weight of the cocoon per treatment ($F_{2,\ 72}$ = 1.26, P = 0.29). Over the experimental period of 100 days, *P. corethrurus* produced an average of 14.6 ± 3.1 cocoons

185 per terrarium and 0.505 ± 0.148 g per cocoon. There was no significant difference in the number
186 of cocoons produced per terrarium ($F_{2, 12}$ = 0.368, P = 0.699). At the end of the experiment,
187 parental earthworms were found to share a similar biomass (average ± SD) 0.767 ± 0.13 g
188 between terrariums.
189
190 *Site selection for nest placement and deposition of cocoons*
191 Food quality, soil depth and their interaction had a significant effect on the construction of nests
192 ($F_{2, 36}$ = 7.29, P = 0.026 food quality, $F_{2, 36}$ = 51.42; P = 0.0001 soil depth, $F_{4, 36}$ = 14.00; P =
193 0.007, food quality x soil depth). More nests were found in the intermediate (10-18 cm) soil
194 depth layer and treatments S (soil only) and G (soil + grass) (Figure 1), while treatments L (soil
195 + legume) and G and the shallow layers (0-9 cm) presented a lower number of nests.
196
197 *External and internal deposition of excreta*
198 There was no significant difference in the production of superficial (external) excreta
199 across treatments ($F_{2, 12}$ = 0.186, P = 0.833), with an average of 10.8 ± 3.9 g of dry excreta. In
200 contrast, the production of internal excreta varied significantly with food type, soil depth and
201 their interaction ($F_{2, 36}$ = 21.96, P = 0.0001 food type; $F_{2, 36}$ = 4.94, P = 0.0127 soil depth; $F_{4, 36}$ =
202 3.81; P = 0.011, food type x soil depth). Diet L (soil + legume) and the intermediate soil depth
203 layer had the highest quantity of internal dry excreta (40.80 and 34.85 g), while treatments S and
204 the shallow and deep layers (0-9 and 19-27 cm, respectively) had lower quantities of internal dry
205 excreta (16.09, 25.39 and 24.11 g, respectively) (Figure 2).
206
207 *Offspring number and weight*

208 The number and biomass of juveniles per terrarium where similar, weighing on average 9.3 ±3.1
209 g per terrarium and 4.85 ± 1.42 g per offspring. The Correlation analysis found that the internal
210 excreta deposited in treatments S and G where positively associated with the biomass of
211 juveniles ($r_{15} = 0.68$, $P < 0.005$ y $r_{15} = 0.53$, $P < 0.043$, respectively), but was not associated to
212 the excreta in treatment L ($r_{15} = 0.240$, $P < 0.389$). The internal excreta placed in treatment S had
213 a strong association with the biomass of juveniles with an estimated regression coefficient of $\hat{\beta}$ =
214 0.012 ± 0.003 ($F_{1, 13} = 11.08$, $P = 0.005$, $R^2 = 0.678 \pm 0.203$). There was a weaker association
215 between juvenile biomass and the excreta deposited in treatment G ($\hat{\beta} = 0.006 \pm 0.0027$, $F_{1, 13}$ =
216 5.05, $P = 0.043$, $R^2 = 0.528 \pm 0.23$).
217
218 **Discussion**
219 Low quality of the environment can drive the evolution of parental care, and this can vary as a
220 function of the distribution, abundance, persistence and quality of different food resources
221 (Tallamy & Wood, 1986; Mori & Chiba, 2009; Smiseth *et al*. 2012). *Pontoscolex corethrurus*
222 constructed chambers or nests similar to those recorded in previous studies (Ortiz-Ceballos &
223 Fragoso 2006; Ortiz-Ceballos *et al*., 2009; Bunch *et al*., 2011). Here we show that *P. corethrurus*
224 selects and constructs nests in which to place its cocoons, a higher quantity of nests and a higher
225 quantity of internal excreta where deposited at an intermediate depth.
226 One simple form of parental care is to bury eggs in a substrate (Smiseth *et al*. 2012). For
227 example, *L. terrestris*, covers its eggs with its own excreta (Ramisch & Graff 1985;
228 Grigoropoulou *et al*., 2007). There are more elaborate forms of nest construction using materials
229 found in the environment (natural or processed), or the parents can use self-produced materials
230 such as mucus or silk, among others (Jeanne, 1996; Mori & Chiba, 2009; Smiseth *et al*. 2012,

231 Furuichi & Kasuya 2015). *Pontoscolex corethrurus* uses soil and mucus (Ortiz-Ceballos *et al*.,
232 2009) to construct nests with its mouth that are similar to those constructed in diapause (Jiménez
233 *et al*., 1999; Jiménez *et al*., 2000).

234 The inspection and selection of potential sites for oviposition is one of the most important
235 patterns of behaviour in animals (Lentfer *et al*., 2011; Smiseth *et al*. 2012). The selection of
236 adequate nest sites may increase offspring survival by choosing adequate abiotic factors such as
237 soil moisture and temperature. Our results indicate that the nesting behaviour of *P. corethrurus*
238 varied significantly with soil depth and food quality, a higher amount of nests were built at an
239 intermediate depth in the soil with lower and intermediate nutritional quality (S and G
240 treatments). This suggests that *P. corethrurus* selected the depth with the most favorable
241 environmental conditions for cocoon development and protection.

242 It has been suggested that nest architecture has evolved for multiple uses where the
243 exterior layer acts to hides the nest from predators and protect it from rain while the internal
244 layer isolates it from extremes of temperature, flooding, desiccation and hypoxia (Smiseth *et al*.
245 2012). This suggests that the nests constructed by *P. corethrurus* protect the cocoons from
246 abiotic and biotic threats, since the interior layer comprises a compacted wall formed by small
247 soil particles bound together by mucus produced by the earthworm, while the exterior layer acts
248 to disguise the presence of the nest. Furthermore, the cocoons within the nest are suspended from
249 a transparent layer of mucus (Ortiz-Ceballos *et al*., 2009). This is probably associated with
250 sanitation (antimicrobial properties), and can be found in epigeic earthworms (*Eisenia foetida*),
251 beetles (*Dendroctonus frontalis* and *Nicrophorus vespilloides*), hyperiid amphipods (genus
252 *Phronima*), the European beewolf (*Philanthus triangulum*), ants and termites (Currie, 2001;

253 Kaltenpoth *et al*., 2005; Muller *et al*., 2005; Hirose *et al*., 2005; Aruna *et al*., 2008; Rozen *et al*.,
254 2008; Scott *et al*., 2008; Smiseth *et al*. 2012).

255 The construction of nests and galleries provides a refuge for protecting the offspring from
256 the deleterious effects of their biotic and abiotic surroundings (Mori & Chiba, 2009; Smiseth *et*
257 *al*. 2012; Kingsbury et al., 2015). In earthworm studies, records of their natural enemies are rare
258 (Lee, 1985; Edwards & Bohlen, 1996; Lavelle & Spain, 2001); however, a previous study found
259 that 12% of cocoons had been parasitized by two acari species of the families Anoetidae (genus
260 *Histiostoma*) and Glycyphagidae (Pascacio *et al*., 2010). The acari infect the cocoons during
261 (external) fertilization, occupying the cocoon prior to its placement within the nest.

262 Another evolutionary characteristic of parental care is improvement of the food quality
263 (providing excreta of greater quality and of a particle size suitable for consumption) available to
264 the offspring in order to sustain quality growth and reduce mortality and the time necessary for
265 development (Mori & Chiba, 2009; Gardner & Smiseth, 2011). For example, larvae of the beetle
266 *Figulus binobulus* feed on excreta that are rich in nitrogen and sawdust (Mori & Chiba, 2009). In
267 xylophagous insects, the larvae feed on excreta rich in proteins produced by their parents (Ento
268 *et al*., 2008). Soil is a difficult environment in which it is hard to find plant material with
269 nutritional value (Bonkowski *et al*., 2000). Thus, providing highly nutritious excreta for
270 offspring may increase their biomass and survival.

271 Earthworms prefer leaf litter with high N content (Hendriksen, 1990; Bonkowski *et al*.,
272 2000). This may explain why in the soil with the highest nutritional quality (those mixed with the
273 legume) presented a lower number of nests. This leads us to suppose these sites are essential for
274 the reproductive activity (nutrition) of *P. corethrurus* (Lee, 1985; García & Fragoso, 2003; Ortiz-
275 Ceballos *et al*., 2005) and produced excreta as a source of food for their offspring. Excreta are

thought to contain nutritional resources for offspring with a high N content, water-soluble mixture of low molecular weight carbohydrates, aminoacids, glycosides and a glycoproteins, humic substances (endowed with hormone-like activity), low C:N content and can cause priming effects by stimulating microbial activity (Elliot *et al*., 1991; Tiwari & Mishra 1993; Nardi *et al*., 1994; Decaënset *et al*., 1999; Musculo *et al*., 1999; Trigo, 1999; Whalen *et al*., 2000; Salmon, 2001; Schönholzer *et al*., 2002; Ihssen *et al*., 2003; Egert *et al*., 2004; Furlong *et al*., 2002; Drake & Horn, 2007; Oleynik & Byzov, 2008; Bityutskii *et al*., 2012; Lipiec *et al*., 2015). Our results show that the interaction between food quality and soil depth had a significant influence on the production of internal excreta. *Pontoscolex corethrurus* deposited a higher quantity of internal excreta at an intermediate depth where there was also a higher quantity of nests. The excreta were deposited close to the nests in a similar manner to that reported in a previous study (Ortiz-Ceballos *et al*., 2009), in contrast to *L. terrestris*, which covers its cocoons with its own excreta (Ramisch & Graff, 1985; Grigoropoulou *et al*., 2008). After hatching, the offspring perhaps to survive, consume the internal excreta (Ortiz-Ceballos *et al*., 2009), characterized by its content of fine soil particles (the mouth of the offspring is not adapted to consume large soil particles), organic matter, nitrogen and microorganisms (Tiwari & Mishra, 1993; Devliegher & Verstraete, 1997; Trigo *et al*., 1999; Bonkowski, Griffiths, & Ritz, 2000; Lowe & Butt, 2003; Curry & Schmidt, 2007; Khomyakov et al., 2007; Mariani *et al*., 2007; Mori & Chiba, 2009). In this way, the offspring obtain nutrients suitable for their growth and development. This suggests that *P. corethrurus* as an additional form of parental care provides food (excreta with nutrients). Finally, all this shows as suggested by Drake & Horn (2007) “perhaps the most distinguishing feature of earthworms is their propensity to consume their house”.

Conclusions

As part of its reproductive activity, *P. corethrurus* selects nest sites based on the depth and nutritional quality of the soil. Cocoons are placed in these nests and additionally excreta are deposited as a source of food for the offspring. Further research is necessary to determine whether species of different ecological categories also provide parental care for their offspring.

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References

1. Aruna, S., Vijayalakshmi, K., Shashikanth, M., Rani, S., & Jyothi, K. (2008). First report of antimicrobial spectra of novel strain of *Streptomyces tritolerans* (Strain AS1) isolated from earthworm gut (*Eisenia foetida*) against plant pathogenic bacteria and fungi. *Current Research in Bacteriology*, *1*, 46-55.

2.

Bityutskii, N.P., Maiorov, E.I., & Orlova, N.E. (2012). The priming effects induced by earthworm mucus on mineralization and humification of plant residues. *European Journal of Soil Biology 50,* 1-6.

3. Bonkowski, M., Griffiths, B.S., & Ritz, K. (2000). Food preferences of earthworms for soil fungi. *Pedobiologia 44,* 666-676.

4. Buch, A.C., Brown, G.G., Niva, C.C., Sautter, K.D., & Lourençato, L.F. (2011). Life cycle of *Pontoscolex corethrurus* (Muller, 1857) in tropical artificial soil. *Pedobiologia, 54S,* S19-S25

5. Capowiez, Y. (2000). Difference in burrowing behaviour and spatial interaction between the two earthworm species *Aporrectodea nocturna* and *Allolobophora chlorotica*. *Biology and Fertility of Soils*, *30*, 341-346.

6. Chauvel, A., Grimaldi, M., Barros, E., Blanchart, E., Desjardins, T., Sarrazin, M., & Lavelle, P. (1999). Pasture damage by an Amazonian earthworm. *Nature*, *398*, 32-33.

7. Clutton-Brock, T.H. (1991). The evolution of parental care. Harvard, Princeton: University Press.

8. Currie, C. (2001). A community of ants, fungi, and bacteria: a multilateral approach to studying symbiosis. *Annual Review of Microbiology*, 55, 357-380.

344 9.

345 Curry, J.P., & Schmidt, O. (2007). The feeding ecology of earthworms-A review.
346 *Pedobiologia*, *50*, 463-477.

347 10.

348 Decaëns, T., Rangel, A.F., Asakawa, N., & Thomas, R.J. (1999). Carbon and nitrogen
349 dynamics in ageing earthworm casts in grasslands of the eastern plains of Colombia.
350 *Biology and Fertility of Soils*, 30, 20-28.

351 11.

352 Devliegher, W., & Verstraete, W. (1997). Microorganisms and soil physico-chemical
353 conditions in the drilosphere of *Lumbricus terrestris*. *Soil Biology and Biochemistry*, *29*,
354 1721-1729.

355 12.

356 Drake, H.L., & Horn, M.A. (2007). As the worm turns: the earthworm gut as a transient
357 habitat for soil microbial biomes. *Annual Review of Microbiology,* 61, 169-189.

358 13.

359 Edwards, C.A., & Bohlen, P.J. (1996). *Biology and Ecology of Earthworms*. London,
360 U.K.: Chapman & Hall.

361 14.

362 Egert, M., Marhan, S., Wagner, B., Scheu, S., & Friedrich, M.W. (2004). Molecular
363 profiling of 16S rRNA genes reveals diet-related differences of microbial communities in
364 soil, gut, and cast of *Lumbricus terrestris* L. (Oligochaeta: Lumbricidae). *FEMS*
365 *Microbiology Ecology,* 48, 187-197.

366 15.

Elliot, P.W., Knight, D., & Anderson, J.M. (1991). Variables controlling denitrification from earthworm casts and soil in permanent pastures. *Biology and Fertility of Soils*, 11, 24-29.

16. Ento, K., Araya, K., & Kudo, S.I. (2008). Trophic egg provisioning in a passalid beetle (Coleoptera). *European Journal of Entomology*, *105*, 99-104.

17. Evans, A.C. (1947). Method of studying the burrowing activity of earthworms. *Annals and Magazine of Natural History*, *11*, 643-650.

18. Furlong, M.A., Singleton, D.R., Coleman, D.C., & Whitman, W.B. (2002). Molecular and culture-based analyses of prokaryotic communities from an agricultural soil and the burrows and cast of the earthworm Lumbricus rubellus. *Applied and Environmental Microbiology, 68,* 1265-1279

19. Furuichi, S., & Kasuya, E. (2015). Construction of nest defensive structure according to offspring value and its effect on predator´s attack decisión in paper wasps. *Ethology*, *121*, 609-616.

20. Gardner, A., & Smiseth, P. (2011). Evolution of parental care driven by mutual reinforcement of parental food provisioning and sibling competition. *Proceeding of the Royal Society B*: *Biological Sciences, 278*, 196-203.

21.

Grigoropoulou, N., Butt, K.R., & Lowe, C.N. (2007). Effects of adult *Lumbricus terrestris* on cocoons and hatchlings in Evans' boxes. *Pedobiologia*, *51*, 343-349.

22.

Hastings A, Byers, J.E., Crooks, J.A., Cuddington, K., Jones, C.G., Lambrinos J.G., Talley, T.S., & Wilson, W.G. (2007). Ecosystem engineering in space and time. *Ecology Letters*, *10*, 153-164.

23.

Hendriksen, N.B. (1990). Leaf litter selection by detritivore and geophagous earthworms. *Biology and Fertility of Soils, 10,* 17-21.

24.

Hendrix, P.F., Callaham, Jr. M.A., Drake, J.M., Huang, Ch.Y., James, S.W., Snyder, B.A., & Zhang, W. (2008). Pandora's box contained bait: the global problem of introduced earthworm. *Annual Review of Ecology, Evolution, and Systematics, 39,* 593-613.

25.

Hirose, E., Aoki, M.N., & Nishikawa J. (2005). Still alive? Fine structure of the barrels made by *Phronima* (Crustacea: Amphipoda). *Journal of the Marine Biological Association of the United Kindon*, *85*, 1435-1439.

26.

Ihssen, J., Horn, M.A., Matthies, C., Gößner, A., Schramm, A., & Drake, H.L. (2003). N_2O-Producing microoganisms in the gut of the earthworm *Aporrectodea caliginosa* are indicative of ingested soil bacteria. *Applied and Environmental Microcbiology, 69,* 1655-1661.

413 27.

414 Jeanne, R.L. (1996). Regulation of nest construction behaviour in *Polybia occidentalis*.
415 *Animal Behaviour*, *52*, 473-488.

416 28.

417 Jiménez, J.J., Brown, G.G., Decaëns, T., Feijoo, A., & Lavelle, P. (2000). Differences in
418 the timing of diapause and patterns of aestivation in tropical earthworms. *Pedobiologia*,
419 *44*, 667-694.

420 29.

421 Jiménez, J.J., Moreno, A.G., & Lavelle, P. (1999). Reproductive strategies of three native
422 earthworm species from the savannas of Carimagua (Colombia). *Pedobiologia*, *43*, 851-
423 858.

424 30.

425 Jones, C.G., Lawton, J.H., & Shachak, M. (1994). Organisms as ecosystem engineers.
426 *Oikos*, *69*, 373-386.

427 31.

428 Kaltenpoth, M., Göttler, W., Herzner, G., & Strohm, E. (2005). Symbiotic bacteria
429 protect wasp larvae from fungal infestation. *Current Biology*, *15*, 475-479.

430 32.

431 Khomyakov, N.V., Kharin, S.A., Nechitailo, T.Y., Golyshin, P.N., Kurakov, A.V.,
432 Byzov, B.A., & Zvyagintsev, D.G. (2007). Reaction of microorganisms to the digestive
433 fluid of earthworms. *Microbiology*, *76*, 45-54.

434 33.

435 Kingsbury, M.A., Jan, M., Klatt, J.D., & Goodson J.L. (2015). Nesting behavior is
436 associated with VIP expression and VIP-Fos colocalization in a network-wide manner.
437 *Hormones and Behavior*, *69*, 68-61.
438 34.
439 Lattaud, C., Locati, S., Mora, P., Rouland, C., & Lavelle, P. (1998). The diversity of
440 digestive systems in tropical geophagous earthowrms. *Applied Soil Ecology, 9,* 189-195.
441 35.
442 Lavelle, P., & Spain, A.V. (2001). *Soil Ecology*. Dordrecht, Netherlands: Kluwer.
443 36.
444 Lee, K.E. (1985). *Earthworms: Their Ecology and Relationships With Soils and Land*
445 *Use*. Sydney, Australia: Academic Press.
446 37.
447 Lentfer, T.L., Gebhardt-Henrich, S.G., Fröhlich, E.K.F., & von Borell E. (2011).
448 Influence of nest site on the behaviour of laying hens. *Applied Animal Behaviour Science*,
449 *135*, 70-77.
450 38.
451 Lipiec, J., Brzezinska, M., Turski, M., Szarlip, P., & Frac, M. (2015). Wettability and
452 biogeochemical properties of the drilosphere and casts of endogeic earthworms in pear
453 orchard. *Soil & Tillage*, *145*, 55-61.
454 39.
455 Lowe, Ch.N., & Butt, K.R. (2002). Influence of food particle size on inter- and intra-
456 specific interactions of *Allolobophora chlorotica* (Savigny) and *Lumbricus terrestris*.
457 *Pedobiologia, 47,* 574-577.

458 40.

459 Mariani, L., Jiménez, J.J., Asakawa, N., Thomas, R.J., & Decaëns, T. (2007). What
460 happens to earthworm casts in the soil? A field study of carbon and nitrogen dynamics in
461 Neotropical savannahs. *Soil Biology and Biochemistry*, *39*, 757-767.

462 41.

463 Mori, H., & Chiba S. (2009). Sociality improves larval growth in the stag beetle *Figulus*
464 *binodulus* (Coleoptera: Lucanidae). *European Journal of Entomology*, *106*, 379-383.

465 42.

466 Muller, U.G., Gerardo, N.M., Aanen, D.K., Six, D.L., & Schultz, T.R. (2005). The
467 evolution of agriculture insects. *Annual Review of Ecology and Systematics, 36,* 563-595.

468 43.

469 Munguía-Steyer, R., & Macías-Ordoñez, R. (2007). Is it risky to be a father? Survival
470 assessment depending on sex and parental status in the water bug *Abedus breviceps* using
471 multistate modelling. *Canadian Journal of Zoology (Can j Zool), 85,* 49-55

472 44.

473 Muscolo, A., Bovalo, F., Gionfriddo, F., & Nardi, S. (1999). Earthworm humic matter
474 produces auxin-like effects on *Daucus carota* cell growth and nitrate metabolism. *Soil*
475 *Biology & Biochemestry, 31,* 1303-1311.

476 45.

477 Nardi, S., Panuccio, M.R., Abenavoli, M.R., & Muscolo, A. (1994). Auxin-like effect of
478 humic substances extracted from faeces of *Allolobophora caliginosa* and *A. rosea*. *Soil*
479 *Biology & Biochemestry, 10,* 1341-1346.

480 46.

Oleynik, A.S., & Byzov, B.A. (2008). Response of bacteria to earthworm surface excreta. *Microbiology, 77,* 854-862.

47. Ortiz-Ceballos, A.I., & Fragoso, C. (2006). Parental care of endogenic earthworm cocoons: Is cleaning, construction, and cast surrounding of chambers related to hatching and survival of juvenile worms? In *The 8th International Symposium on Earthworm Ecology* (Book Abstracts) (pp. 150). Kraków, Poland: Uniwersytet Jagiellonski.

48. Ortiz-Ceballos, A.I., Fragoso, C., M. Equihua, & G.G. Brown. (2005). Influence of food quality, soil moisture and the earthworm *Pontoscolex corethrurus* on growth and reproduction ofn the earthworm *Balanteodrilus pearsei*. *Pedobiologia*, *49*, 89-98.

49. Ortiz-Ceballos, A.I., Hernández-García, M.E.C., & Galindo-González, J. (2009). Nest and Feeding Chamber Construction for Cocoon Incubation in the Tropical Earthworm: *Pontoscolex corethrurus* In: Karmegam N (Ed) Vermitechnology I. *Dynamic Soil, Dynamic Plant 3*, (*Special Issue 2*), 15-18.

50. Pascacio-Velázquez, J.M., Ortiz-Ceballos, A.I., & Palacios-Vargas, J.G. (2010). Two parasitic mites in the cocoons of the pantropical earthworm *Pontoscolex corethrurus* (Anelida: Oligochaeta, Glossoscolecidae). In: 9th International Symposium on Earthworm Ecology (Book of Abstracts) (pp. 196). Xalapa, Veracruz, México: Instituto de Ecología A.C. and Universidad Veracruzana.

51.

504 Ramisch, H., & Graff, O. (1985). The cocoon chambers of some earthworms
505 (Lumbricidae: Oligocheta) from the Brunswick-area (Lower Saxony). *Braunschweiger*
506 *Naturkundliche Schriften*, *2*, 299-308.
507 52.
508 Rozen, D.E., Engelmoe, D.J.P., & Smiseth P.T. (2008). Antimicrobial strategies in
509 burying beetles breeding on carrion. *PNAS, 105,* 17890-17895.
510 53.
511 Salmon, S. (2001). Earthworm excreta (humus and urine) affect the distribution of
512 springtails in forest soils. *Biology and Fertility of Soils, 34,* 304-310.
513 54.
514 SAS Institute Inc. (2013). SAS/STAT 9.4 User's Guide. Cary, NC. SAS Institute Inc.
515 Cary, NC, USA.
516 55.
517 Scheu, S. (2003). Effects of earthworms on plant growth: patterns and perspectives.
518 *Pedobiologia*, *47*, 846-856.
519 56.
520 Schönholzer, F., Hahn, D., Zarda, B., Zeyer, J. (2002) Automated image analysis and in
521 situ hybridization as tolos to study bacterial populations in food resources, gut and cast of
522 *Lumbricus terrestris* L. *Journal of Microbiological Methods, 48,* 53-68.
523 57.
524 Scott, J.J., Oh, D.Ch., Yuceer, M.C., Klepzig, K.D., Clardy, J., & Currie, C.R. (2008).
525 Bacterial protection of beetle-fungus mutualism. *Science*, *322*, 63.
526 58.

527 Smiseth, P.T., Kölliker, M., & Royle, N.J. (2012). What is parental care?. In N.J. Royle,
528 P.T. Smiseth, M. Kölliker (Eds.), The evolution of parental care (pp. 1-17). Oxford, U.K.:
529 Oxford University Press.
530 59.
531 Stephenson, J. (1930). *The Oligochaeta*. Oxford, U.K.: Clarendon Press.
532 60.
533 Tallamy, D.W., & Wood, T.K. (1986). Convergence patterns in social insects. *Anual*
534 *Review of Entomology*, *31*, 369-390.
535 61.
536 Tiwari, S.C., & Mishra, R.R. (1993). Fungal abundance and diversity in earthowrm casts
537 and uningested soil. *Biology and Fertility of Soils, 16,* 131-134
538 62.
539 Trigo, D., Barois, I, Garvin, M.H., Huerta, E., Irisson, S., & Lavelle, P. (1999).
540 Mutualism between earthworms and soil microflora. *Pedobiologia*, *43*, 866-873.
541 63.
542 Trumbo, S.T. (2012). Patterns of parental care in invertebrates. In N.J. Royle, P.T.
543 Smiseth, M. Kölliker (Eds.), The evolution of parental care (pp. 81-100). Oxford, U.K.:
544 Oxford University Press.
545 64.
546 van Groenigen, J.W., Lubbers, I.M., Vos, M.J.V., Brown, G.G., De Deyn, G.B., & van
547 Groenigen, K.J. (2014). Earthworms increase plant production: a meta-analysis. *Scientific*
548 *Reports, 4,* 6365.
549 65.

550 Whalen, J.K., Parmelee, R.W., & Subler, S. (2000). Quantification of nitrogen excretion
551 rates for three lumbricid earthworms using ^{15}N. *Biology and Fertility of Soils, 32,* 347-
552 352
553

Figure 1(on next page)

Interaction between the depth and nutritional quality of the soil on nest construction in the tropical endogeic earthworm Pontoscolex corethrurus.

Soil depth: Shadow = 0-9 cm, Intermediate = 10-18 cm, Deep = 19-27 cm. Soil Quality: S = soil only, G = soil + grass, L = soil + legume. Vertical lines indicate 95 % confidence intervals.

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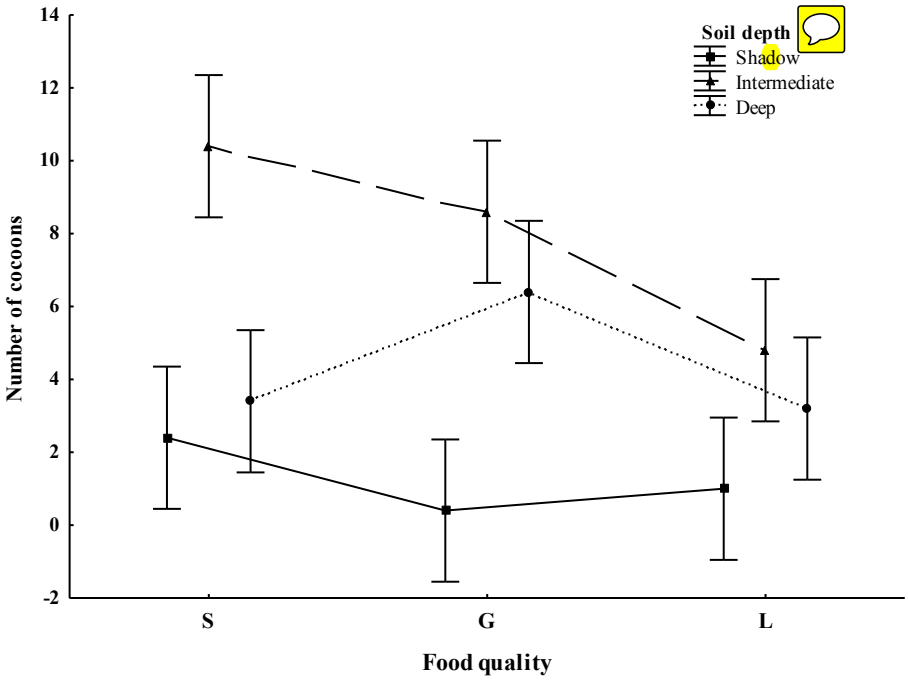
Soil depth
Shadow
Intermediate
Deep
Number of cocoons
14
12
10
8
6
4
2
0
-2
S
G
L
Food quality

8

Figure 2(on next page)

Interaction between the depth and nutritional quality of the soil on the production of internal excreta in the tropical endogeic earthworm Pontoscolex corethrurus.

Soil depth: Shadow = 0-9 cm, Intermediate = 10-18 cm, Deep = 19-27 cm. Soil Quality: S = soil only, G = soil + grass, L = soil + legume. Vertical lines indicate 95 % confidence intervals.

1

2

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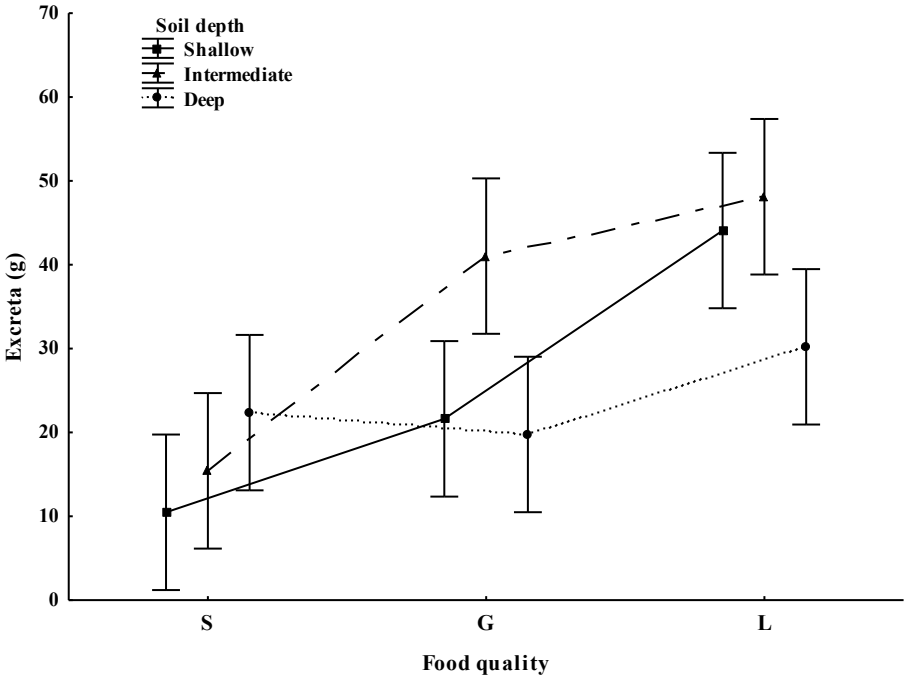
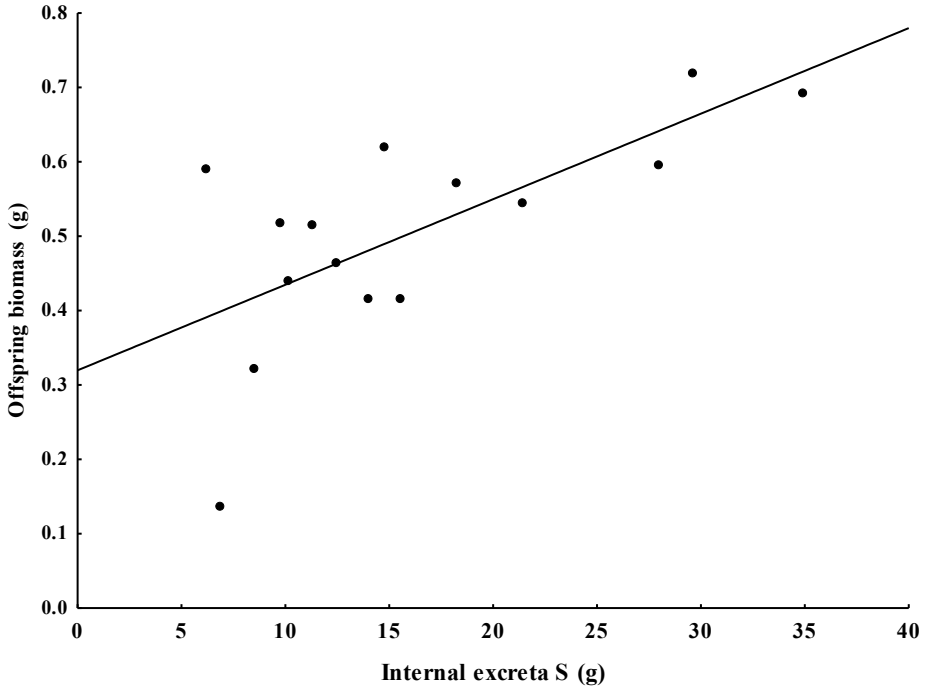

Soil depth
Shallow
Intermediate
Deep
Excreta (g)
70
60
50
40
30
20
10
0
S
G
L
Food quality

8

Figure 3(on next page)

Spatial association between biomass offspring and internal excretas (treatments S, N = 15) of the earthworm tropical endogeic Pontoscolex corethrurus.

S = only Soil. The line in fitted with a linear regression.


Offspring biomass (g)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
5
10
15
20
25
30
35
40
Internal excreta S (g)