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# **Nest site selection and nutritional provision through excreta: a form of parental care in a tropical endogeic earthworm**

Angel I. Ortiz-Ceballos, Diana Pérez-Staples, Paulino Pérez-Rodríguez

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).

**Nest site selection and nutritional provision through excreta: a form of parental care in a  
tropical endogeic earthworm**

Angel I. Ortiz-Ceballos<sup>1\*</sup>, Diana Pérez-Staples<sup>1</sup> and Paulino Pérez-Rodríguez<sup>2</sup>

<sup>1</sup>Instituto de Biotecnología y Ecología Aplicada (INBIOTECA), Universidad Veracruzana,  
Av. de las Culturas Veracruzananas No. 10, Col. Emiliano Zapata, 91090 Xalapa, Veracruz,  
México.

<sup>2</sup>Programa de Estadística, Campus Montecillo, Colegio de Postgraduados, 56230, Montecillo,  
Estado de México, México.

Address Corresponding Author: Angel I. Ortiz-Ceballos

Instituto de Biotecnología y Ecología Aplicada (INBIOTECA), Universidad Veracruzana,  
Av. de las Culturas Veracruzananas No. 10, Col. Emiliano Zapata, 91090 Xalapa, Veracruz,  
México. [angortiz@uv.mx](mailto:angortiz@uv.mx)

\* [angortiz@uv.mx](mailto:angortiz@uv.mx)

## 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<sup>2</sup> 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 (18°08'N, 92°47'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

temperature, and sieved through a 5 mm mesh. The main chemical characteristics of this soil were: 2.7 % organic matter, 0.14 % total N, 11.5 C/N and pH (H<sub>2</sub>O) 6.3.

#### *Earthworms*

Thirty subadult *Pontoscolex corethrurus* earthworms were collected from a pasture of *Brachiaria humidicola* (Rendle) Schweick located at Huimanguillo (17°48'N, 93°28'W), 79 km southwest Villahermosa, Tabasco, Mexico. The earthworms were reared until reaching sexual maturity in boxes (12 × 12 × 8 cm) with 300 g of the soil mixed with 3% legume (*Mucuna pruriens* ssp. *utilis*) foliage. Prior to initiation of the experiment, the first 15 earthworms to produce a cocoon were selected.

#### *Food quality*

The influence of the quality of food was evaluated using foliage from a legume (*M. pruriens* ssp. *utilis*) or grass (*B. humidicola*) with 14.3 y 6.1 % crude protein, respectively. These were collected from the same sites as the earthworms. 5 kg of legume and grass foliage were collected and dried at 65 °C for 48 h. The dried materials were then sieved to 2 mm and 3.3 kg of the soil was homogeneously mixed with 0.01 kg (3 %) of leguminous foliage, while another 3.3 kg of soil was homogeneously mixed with grass (3 %).

#### *Experimental set-up*

To test preferences for nest location, an experiment was established utilizing a 3<sup>2</sup> factorial design, i.e., two factors (food quality and soil depth) with three levels. Food consisted of either: 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 ( $455 \pm 25$  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  $26 \pm 1$  °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:

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

where:  $k$  is a parameter to be estimated,  $\log(\mu) = \mathbf{x}'\boldsymbol{\beta}$ , 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 deposited 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 ( $0.035 \pm 0.006$  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  $14.6 \pm 3.1$  cocoons

per terrarium and  $0.505 \pm 0.148$  g per cocoon. There was no significant difference in the number of cocoons produced per terrarium ( $F_{2, 12} = 0.368$ ,  $P = 0.699$ ). At the end of the experiment, parental earthworms were found to share a similar biomass (average  $\pm$  SD)  $0.767 \pm 0.13$  g between terrariums.

#### *Site selection for nest placement and deposition of cocoons*

Food quality, soil depth and their interaction had a significant effect on the construction of nests ( $F_{2, 36} = 7.29$ ,  $P = 0.026$  food quality,  $F_{2, 36} = 51.42$ ;  $P = 0.0001$  soil depth,  $F_{4, 36} = 14.00$ ;  $P = 0.007$ , food quality x soil depth). More nests were found in the intermediate (10-18 cm) soil depth layer and treatments S (soil only) and G (soil + grass) (Figure 1), while treatments L (soil + legume) and G and the shallow layers (0-9 cm) presented a lower number of nests.

#### *External and internal deposition of excreta*

There was no significant difference in the production of superficial (external) excreta across treatments ( $F_{2, 12} = 0.186$ ,  $P = 0.833$ ), with an average of  $10.8 \pm 3.9$  g of dry excreta. In contrast, the production of internal excreta varied significantly with food type, soil depth and their interaction ( $F_{2, 36} = 21.96$ ,  $P = 0.0001$  food type;  $F_{2, 36} = 4.94$ ,  $P = 0.0127$  soil depth;  $F_{4, 36} = 3.81$ ;  $P = 0.011$ , food type x soil depth). Diet L (soil + legume) and the intermediate soil depth layer had the highest quantity of internal dry excreta (40.80 and 34.85 g), while treatments S and the shallow and deep layers (0-9 and 19-27 cm, respectively) had lower quantities of internal dry excreta (16.09, 25.39 and 24.11 g, respectively) (Figure 2).

#### *Offspring number and weight*

208 The number and biomass of juveniles per terrarium were similar, weighing on average  $9.3 \pm 3.1$   
209 g per terrarium and  $4.85 \pm 1.42$  g per offspring. The Correlation analysis found that the internal  
210 excreta deposited in treatments S and G were 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  $0.012 \pm 0.003$  ( $F_{1, 13} = 11.08$ ,  $P = 0.005$ ,  $R^2 = 0.678 \pm 0.203$ ). 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 were 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,

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

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

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

9.

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

10.

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

11.

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

12.

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

13.

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

14.

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

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 decision 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<sub>2</sub>O-Producing microorganisms in the gut of the earthworm *Aporrectodea caliginosa* are indicative of ingested soil bacteria. *Applied and Environmental Microbiology*, 69, 1655-1661.

27.

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

28.

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

29.

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

30.

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

31.

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

32.

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

33.

Kingsbury, M.A., Jan, M., Klatt, J.D., & Goodson J.L. (2015). Nesting behavior is associated with VIP expression and VIP-Fos colocalization in a network-wide manner. *Hormones and Behavior*, 69, 68-61.

34.

Lattaud, C., Locati, S., Mora, P., Rouland, C., & Lavelle, P. (1998). The diversity of digestive systems in tropical geophagous earthworms. *Applied Soil Ecology*, 9, 189-195.

35.

Lavelle, P., & Spain, A.V. (2001). *Soil Ecology*. Dordrecht, Netherlands: Kluwer.

36.

Lee, K.E. (1985). *Earthworms: Their Ecology and Relationships With Soils and Land Use*. Sydney, Australia: Academic Press.

37.

Lentfer, T.L., Gebhardt-Henrich, S.G., Fröhlich, E.K.F., & von Borell E. (2011). Influence of nest site on the behaviour of laying hens. *Applied Animal Behaviour Science*, 135, 70-77.

38.

Lipiec, J., Brzezinska, M., Turski, M., Szarlip, P., & Frac, M. (2015). Wettability and biogeochemical properties of the drilosphere and casts of endogeic earthworms in pear orchard. *Soil & Tillage*, 145, 55-61.

39.

Lowe, Ch.N., & Butt, K.R. (2002). Influence of food particle size on inter- and intra-specific interactions of *Allolobophora chlorotica* (Savigny) and *Lumbricus terrestris*. *Pedobiologia*, 47, 574-577.

40.

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

41.

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

42.

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

43.

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

44.

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

45.

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

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 8<sup>th</sup> 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 of 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.

Ramisch, H., & Graff, O. (1985). The cocoon chambers of some earthworms (Lumbricidae: Oligocheta) from the Brunswick-area (Lower Saxony). *Braunschweiger Naturkundliche Schriften*, 2, 299-308.

52.

Rozen, D.E., Engelmoe, D.J.P., & Smiseth P.T. (2008). Antimicrobial strategies in burying beetles breeding on carrion. *PNAS*, 105, 17890-17895.

53.

Salmon, S. (2001). Earthworm excreta (humus and urine) affect the distribution of springtails in forest soils. *Biology and Fertility of Soils*, 34, 304-310.

54.

SAS Institute Inc. (2013). SAS/STAT 9.4 User's Guide. Cary, NC. SAS Institute Inc. Cary, NC, USA.

55.

Scheu, S. (2003). Effects of earthworms on plant growth: patterns and perspectives. *Pedobiologia*, 47, 846-856.

56.

Schönholzer, F., Hahn, D., Zarda, B., Zeyer, J. (2002) Automated image analysis and in situ hybridization as tools to study bacterial populations in food resources, gut and cast of *Lumbricus terrestris* L. *Journal of Microbiological Methods*, 48, 53-68.

57.

Scott, J.J., Oh, D.Ch., Yuceer, M.C., Klepzig, K.D., Clardy, J., & Currie, C.R. (2008). Bacterial protection of beetle-fungus mutualism. *Science*, 322, 63.

58.

Smiseth, P.T., Kölliker, M., & Royle, N.J. (2012). What is parental care?. In N.J. Royle, P.T. Smiseth, M. Kölliker (Eds.), *The evolution of parental care* (pp. 1-17). Oxford, U.K.: Oxford University Press.

59.

Stephenson, J. (1930). *The Oligochaeta*. Oxford, U.K.: Clarendon Press.

60.

Tallamy, D.W., & Wood, T.K. (1986). Convergence patterns in social insects. *Annual Review of Entomology*, 31, 369-390.

61.

Tiwari, S.C., & Mishra, R.R. (1993). Fungal abundance and diversity in earthworm casts and uningested soil. *Biology and Fertility of Soils*, 16, 131-134

62.

Trigo, D., Barois, I., Garvin, M.H., Huerta, E., Irisson, S., & Lavelle, P. (1999). Mutualism between earthworms and soil microflora. *Pedobiologia*, 43, 866-873.

63.

Trumbo, S.T. (2012). Patterns of parental care in invertebrates. In N.J. Royle, P.T. Smiseth, M. Kölliker (Eds.), *The evolution of parental care* (pp. 81-100). Oxford, U.K.: Oxford University Press.

64.

van Groenigen, J.W., Lubbers, I.M., Vos, M.J.V., Brown, G.G., De Deyn, G.B., & van Groenigen, K.J. (2014). Earthworms increase plant production: a meta-analysis. *Scientific Reports*, 4, 6365.

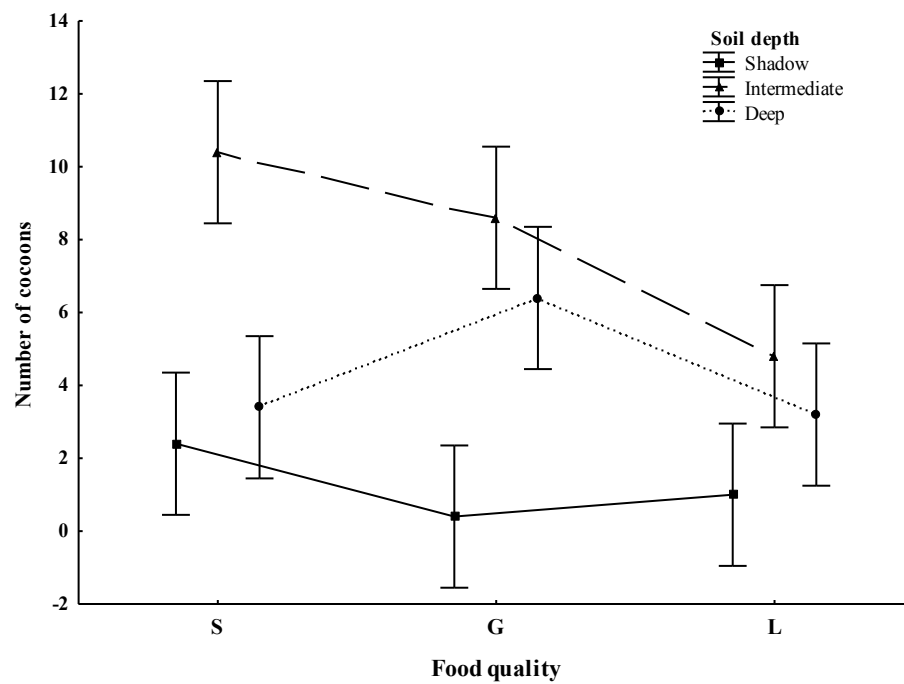
65.

550 Whalen, J.K., Parmelee, R.W., & Subler, S. (2000). Quantification of nitrogen excretion  
551 rates for three lumbricid earthworms using  $^{15}\text{N}$ . *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*.

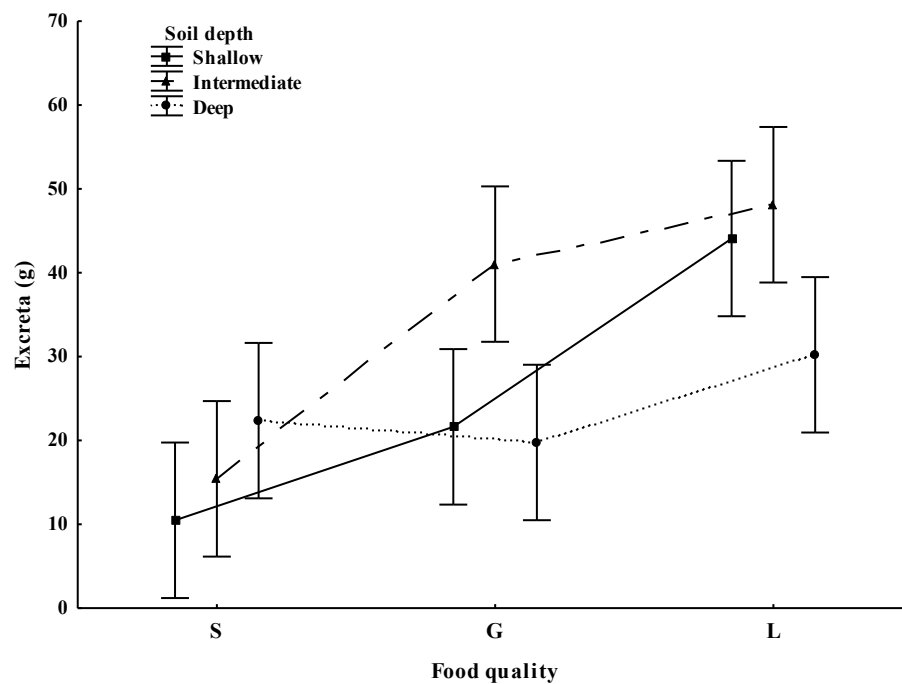
Soil depth: Shadow = 0-9 cm, Intermediate = 10-18 cm, Deep = 19-27 cm. Soil Quality: S = soil only, G = soil + grass, L = soil + legume. Vertical lines indicate 95 % confidence intervals.



## 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.



### 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 is fitted with a linear regression.

