Invasion of the tropical earthworm *Pontoscolex corethrurus* (Rhinodrilidae, Oligochaeta) in temperate grasslands

Diana Ortiz-Gamino[1], Paulino Pérez-Rodríguez[2] and Angel I. Ortiz-Ceballos[1]

[1] Instituto de Biotecnología y Ecología Aplicada (INBIOTECA), Universidad Veracruzana, Xalapa, Veracruz, México
[2] Programa de Estadística, Colegio de Postgraduados-Campus Montecillo, Texcoco, Estado de México, México

ABSTRACT

The tropical earthworm *Pontoscolex corethrurus* (Rhinodrilidae, Oligochaeta) presents a broad distribution (e.g., 56 countries from four continents). It is generally assumed that temperature appears to limit the success of tropical exotic species in temperate climates. However, the distribution range of this species could advance towards higher elevations (with lower temperatures) where no tropical species currently occur. The aim of this study was to evaluate the soil and climatic variables that could be closely associated with the distribution of *P. corethrurus* in four sites along an altitudinal gradient in central Veracruz, Mexico. We predicted that the distribution of *P. corethrurus* would be more related to climate variables than edaphic parameters. Five sampling points (in the grassland) were established at each of four sites along an altitudinal gradient: Laguna Verde (LV), La Concepción (LC), Naolinco (NA) and Acatlán (AC) at 11–55, 992–1,025, 1,550–1,619 y 1,772–1,800 masl, respectively. The climate ranged from tropical to temperate along the altitudinal gradient. Ten earthworm species (5 Neotropical, 4 Palearctic and 1 Nearctic) were found along the gradient, belonging to three families (Rhinodrilidae, Megascolecide and Lumbricidae). Soil properties showed a significant association (positive for Ngrass, pH, permanent wilting point, organic matter and P; and negative for Total N, K and water-holding capacity) with the abundance of the earthworm community. Also there seems to be a relationship between climate and earthworm distribution along the altitudinal gradient. *P. corethrurus* was recorded at tropical (LV and LC) and temperate sites (NA) along the altitudinal gradient. Our results reveal that soil fertility determines the abundance of earthworms and site (climate) can act as a barrier to their migration. Further research is needed to determine the genetic structure and lineages of *P. corethrurus* along altitudinal gradients.

INTRODUCTION

Within soil biodiversity, earthworms are key components of the guild of ecosystem engineers (*Jones, Lawton & Shachak, 1994*). They provide a considerable level of ecosystem services, such as contributing to biogeochemical cycling and crop productivity (*Turbé et al., 2010*; *Orgiazzi et al., 2016*). Depending on their ecological classification (epigeic, endogeic

or anecic), they can also modify the distribution and abundance of soil biodiversity, mainly by constructing structures and galleries within the soil profile and by producing casts and mucus (*Turbé et al., 2010*; *Orgiazzi et al., 2016*).

Most earthworm communities display an aggregated spatial distribution in response to soil environmental heterogeneity at a small scale (*Geissen, Peña-Peña & Huerta, 2009*; *Huerta & Van der Wal, 2012*; *Jiménez et al., 2012*). This is relevant because of the limited capabilities of earthworms for horizontal displacement, between 4 and 10 m per year (*Brown et al., 2006*; *King, Tibble & Symondson, 2008*), and may thus have had the opportunity to diversify allopatrically over relatively short distances (*Bickford et al., 2005*; *King, Tibble & Symondson, 2008*). Nevertheless, climate has a substantial influence on earthworms (physiology, development or activity) that is reflected in the seasonal dynamics of their life history (*Curry, 2004*; *Turbé et al., 2010*; *Orgiazzi et al., 2016*).

At the global level, apart from studies in North America where non-native earthworms are causing changes in soil biota and plant communities, little recognition has been given to invasions of soil organisms (*Gates, 1954*; *Bohlen et al., 2004*; *Fahey et al., 2013*). Among the 3,700 earthworm species described, approximately 3% (100–120) have been identified as invasive; for example, the ubiquitous *Pontoscolex corethrurus* (*Müller, 1857*), several temperate species from genera *Amynthas* and at least 10 species of Lumbricid (*Brown et al., 2006*; *Beddard, 1912*; *Hendrix et al., 2008*; *Dupont et al., 2012*). These earthworms have reached a broad distribution in many tropical and temperate agroecosystems and natural ecosystems. However, this has been facilitated by fishing bait, horticulture, waste management industries, road networks and vehicle transport which have contributed to surmount important biogeographic barriers (*Eisen, 1900*; *Beddard, 1912*; *Gates, 1954*; *Hendrix et al., 2008*).

P. corethrurus "Brush-tail" is native to the Guiana Shield area of the Amazon (*Müller, 1857*; *Brown et al., 2006*; *Dupont et al., 2012*). Due to a high level of genetic diversity in populations (*Dupont et al., 2012*; *Cunha et al., 2014*), its adaptive strategies include a high tolerance to soil conditions and climatic variables (precipitation) (*Lavelle et al., 1987*; *González et al., 2006*; *González et al., 2007*). For this reason, *P. corethrurus* is established throughout the tropical regions of over 56 countries (*González et al., 2006*). Given the wide distribution range of *P. corethrurus*, this species can be used as a model organism to investigate and compare the effects of native and introduced species on ecosystem processes. *P. corethrurus* generally plays a beneficial role in soils; that is, it has the potential to improve plant growth and productivity (*Senapati et al., 1999*; *Van Groenigen et al., 2014*; *Cunha et al., 2016*), and is used as a key indicator in toxicological research (*Zavala-Cruz et al., 2013*; *García-Pérez et al., 2014*). There are very few cases of negative or null effects of *P. corethrurus* (*Brown et al., 2006*); but soil compaction may be induced under particular situations (*Gates, 1954*; *Chauvel et al., 1999*; *Barros et al., 2004*).

In Mexico, since the early twentieth century, *P. corethrurus* has been the endogeic earthworm most commonly found in human-altered tropical ecosystems (*Eisen, 1900*; *Lavelle et al., 1987*; *Brown et al., 2004*; *Fragoso & Rojas, 2014*). However, the edge of the earthworm's distribution range could advance towards higher elevations where few or no tropical species currently occur (*Eisen, 1900*; *Beddard, 1912*; *Hendrix et al., 2008*). The
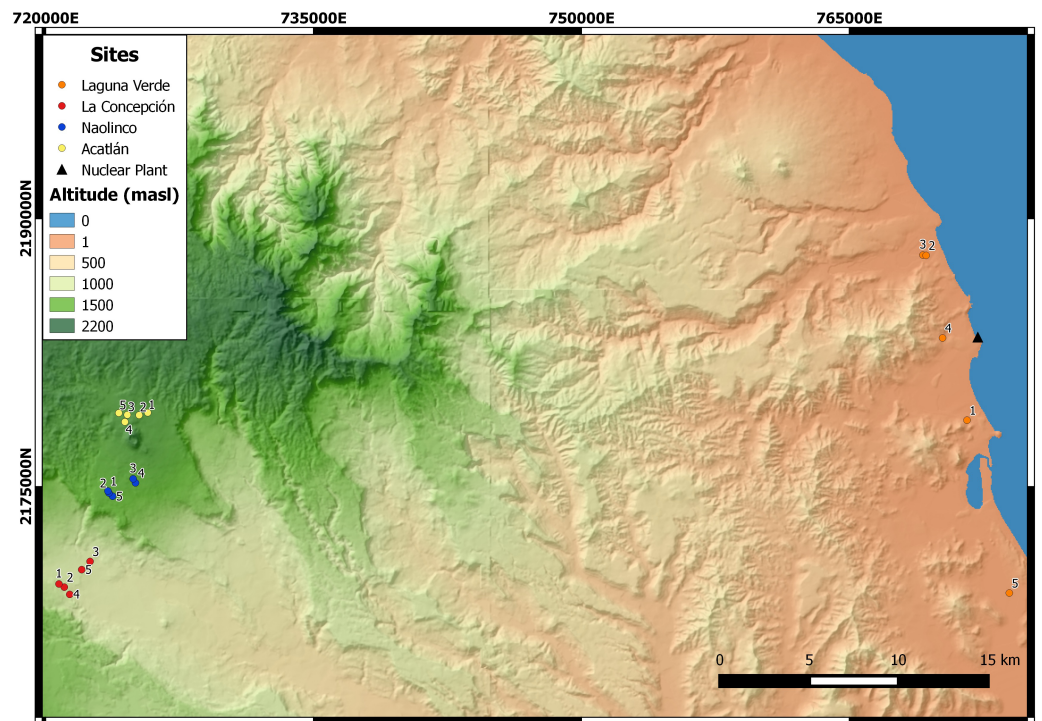
Figure 1 **Sampling sites of earthworms along an altitudinal gradient in central Veracruz, Mexico. For each site, the geographical coordinates (14N zone, Datum WGS84) are presented.** LV, Laguna Verde (11–55 masl); LC, La Concepción (992–1,025 masl); NA, Naolinco (1,550–1,619 masl); AC, Acatlán (1,772–1,800 masl). Digital elevation model created using the geographical data provided by Instituto Nacional de Estadística y Geografía, Mexico.

aim of this study was to evaluate the soil and climatic variables that could be closely associated with the distribution of *P. corethrurus*. We predicted that the distribution of *P. corethrurus* would be more related to climatic variables than edaphic parameters. We tested this hypothesis through a study of the earthworm community along an altitudinal gradient in central Veracruz, Mexico. In addition, we compared the occurrence of four possible situations in the altitudinal gradient (*Marichal et al., 2010*): (a) presence of *P. corethrurus* only, (b) coexistence of *P. corethrurus* and other species (native and invasive), (c) absence of *P. corethrurus* but presence of other species (native and exotic), and (d) absence of earthworms.

METHODS

Study area

An altitudinal transect, ranging from 11 to 1,800 masl, was established in the central region of the State of Veracruz, Mexico. Five sampling points were established at each of four sites along this altitudinal gradient (Fig. 1): Laguna Verde (LV), La Concepción (LC), Naolinco (NA) and Acatlán (AC) at 11–55, 992–1,025, 1,550–1,619 and 1,772–1,800 masl, respectively.

Climate information from the Mexican National Water Commission weather stations (http://www.conagua.gob.mx) was compiled for each site along the altitudinal transect. The monthly and annual 30-year averages were obtained for the following climate variables: average temperature (AT), average maximum temperature (AMT), average minimum temperature (AmT), total annual precipitation (TAP) and total evaporation (TE). With these data, climate types along the altitudinal gradient were determined using the Clima2 software (http://www.pablo-leautaud.com/home/proyectos/python/clima) and classified into one climate type according to the Köppen-Geiger system (*Kottek et al., 2006*; *Peel, Finlayson & McMahon, 2007*).

Earthworm sampling

For determining the distribution of earthworms, we choose the grasslands because they are a suitable habitat that fosters the growth of earthworms, and can be found along the altitudinal gradient. These grasslands are used as pasture for both an extensive (LV and LC) and semi-intensive cattle farming (NA and AC) under a rotational use of pasture (30–45 day grazing-rest cycle) and without the application of mineral fertilization (*Lavelle, Maury & Serrano, 1981*; *Brown et al., 2004*). The grasses species that grow in these grassland are: (a) native: sour paspalum *Paspalum conjugatum* P.J. Bergius (80% LV, 60% LC, 40% NA, and 40% AC), (b) introduced: bermudagrass or stargrass *Cynodon nlemfuensis* Vanderyst (20% LV, 40% LC, and 40% NA) and kikuyu *Pennisetum clandestinum* Hochst. Ex Chiov. (20% NA, and 60% AC). In the extensive production system (Dual-purpose cattle systems: milk and meat), cattle (*Bos indicus* × *Bos Taurus* cows: Zebu × Swiss or Holstein) was fed only on forage produced in grassland, ocassionaly suplemented with mineral salts. By contrast, under the semi-intensive system (milk), besides feeding on grassland forage, milk cows (Holstein) are given a dietary supplement made of carbohydrates (corn and barley stubble), protein (cane molasses, urea, dehydrated alfalfa and others) and mineral salts.

The quantitative sampling of earthworms was conducted along the altitudinal gradient (*International Organization for Standarization, 2011*). One monolith (25 × 25 × 30 cm deep) according to *Anderson & Ingram (1993)* was sampled at each of the five sampling points (in the grassland) established at least 200 m apart (*Marichal et al., 2010*), located at each of the four sites on the altitudinal transect, for a total of 20 monoliths along the altitudinal transect. Each monolith was separated into four strata: above-ground plant biomass, 0–10, 10–20 and 20–30 cm. Earthworms were then manually removed from each layer and preserved in 70% ethanol. In the laboratory, all specimens were fixed in 4% formaldehyde and then identified (to species or morphospecies), quantified and weighed. The sampling was conducted at the end of the rainy season (August–October 2011). Abundance and biomass data of the earthworms were converted into densities per square metre (ind. m^{-2} and g m^{-2}, respectively) for each site (*International Organization for Standarization, 2011*).

Soil and foliage sampling

Prior to removing the earthworms, the biomass of grass was harvested from each monolith. In the laboratory, this plant material was dried (60° C for 72 h) and weighed, and its total

Ortiz-Gamino et al. (2016), *PeerJ*, DOI 10.7717/peerj.2572

4/20

nitrogen content (N_grass) was determined using the Kjeldahl methods described in the Mexican Official Standard NOM-02-RECNAT-2000 (*SEMARNAT, 2002*).

Following the removal of earthworms, a 1-kg soil sample was taken from each stratum of each monolith. Soil samples were air-dried to constant weight and sieved (5 mm) to determine the texture (clay, silt and sand), water-holding capacity, permanent wilting point, pH, organic matter, Total C, Total N, P and K, using the methods described in the Mexican Official Standard NOM-02-RECNAT-2000 (*SEMARNAT, 2002*).

Statistical analysis

A one-way ANOVA was used to test for significant differences ($P < 0.05$) in soil properties between sites, using the Statistica software, ver. 7 (StatSoft, Tulsa, OK, USA).

We used a Linear Model (LM) to study earthworm abundance (ind. m^{-2}) using soil properties and climatic elements. The effect of the climatic elements was included in the model through the variable "site" because it was possible to clearly distinguish the sites in a scatter plot (figure not shown) of the scores for the first two principal components obtained from a PCA of the climatic variables. The dependent variable (earthworm abundance) was transformed using natural logarithms because the empirical distribution of earthworms was highly asymmetric. All the analyses were performed using R software (*R Core Development Team, 2015*). We also fitted Linear Mixed Models to take into account the sampling design consisting in clustered samples and the response variable measured at two different scales, i.e., soil properties at the sample scale and climatic conditions at the site scale. The results showed that the variance component associated with the site effect was misleading. Apart from the linear models and before log-transforming the data, we also fitted generalized linear models with different families (e.g., Poisson, Negative Binomial); however, some convergence issues arose when fitting the models. Consequently, only the results for the fitted Linear Model are reported here.

RESULTS

Site climate

The climate along the altitudinal gradient, from the lowest to the highest elevation sites, according to *Kottek et al. (2006)* and *Peel, Finlayson & McMahon (2007)* ranged from warm to humid tropical (Aw) to temperate (Cfb) (Table 1). There was a difference of approximately 11 °C in the average, minimum and maximum temperatures between the lowest (LV, 11–55 masl) and the highest elevation sites (AC, 1,772–1,800 masl) along the gradient. Rainfall was higher in site LC (1676.8 mm) than in site LV (1143.0 mm), whereas sites NA and AC (1,550–1,619 and 1,772–1,800 masl) had intermediate values of 1,461 mm (Table 1).

Soil properties and foliage

The physical and chemical variables of soil and nutritional quality of pasture along the altitudinal gradient displayed significant variations between tropical (LV and LC) and temperate (NA and AC) sites (Table 2). According to and the official Mexican standard soil fertility (*SEMARNAT, 2002*), soils from tropical sites had a heavy texture (clay loam;

Table 1 Climate variables at the four sampling sites along an altitudinal gradient in central Veracruz, Mexico.

Site	Altitude	Temperature (° C)			TAP	TE	Month most	
	(masl)	AT	AMT	AmT	(mm)		Warmer	Cooler
LV	11–55	26.0 ± 2.5	30.0 ± 2.5	21.0 ± 2.0	1143.0	1618.1	June	January
LC	992–1,025	20.0 ± 2.0	26.0 ± 2.2	14.0 ± 1.8	1676.8	1322.0	May	January
NA	1,550–1,619	17.0 ± 2.0	22.2 ± 2.0	12.0 ± 1.9	1462.0	1554.8	May	January
AC	1,772–1,800	15.0 ± 1.8	20.0 ± 1.9	10.0 ± 1.8	1461.0	1190.8	May	January

Notes.
LV, Laguna Verde; LC, La Concepción; NA, Naolinco; AC, Acatlán; AT, Average temperature; AMT, Average maximum temperature; AmT, Average minimum temperature; TAP, total annual precipitation; TE, total evapotranspiration.

Table 2 Soil properties and grass at the four sites along an elevation gradient in central Veracruz, Mexico.

Parameter	LV	LC	NA	AC
Clay, %	27.6 b	25.6 b	12.0 a	13.6 a
Silt, %	21.8 a	34.4 b	28.8 ab	35.8 b
Sand,%	50.6 ab	40.0 a	59.2 b	50.6 ab
pH, (H$_2$O)	6.6 b	6.6 b	5.5 a	5.6 a
Water-holding capacity, %	32.9 a	36.6 a	83.5 b	70.5 b
Permanent wilting point, %	20.0 a	20.2 a	53.0 b	40.4 b
Organic matter, %	8.0 a	8.4 a	43.7 b	34.2 b
Total N, %	0.31 a	0.31 a	1.13 c	0.83 b
C/N	15 a	16a	22 b	24 b
P, mg kg^{-1}	13.2 a	54.7 c	49.8 b	14.2 a
K, cmol$_c$ kg^{-1}	1.1 a	1.7 a	2.8 b	1.4 a
N$_{grass}$, %	1.1 a	0.71 a	1.46 b	1.45 b

Notes.
Within each row different letters indicate significant differences at $P < 0.05$ (Tukey's HSD test).
LV, Laguna Verde (11–55 masl); LC, La Concepción (992–1,025 masl); NA, Naolinco (1,550–1,619 masl); AC, Acatlán (1,772–1,800 masl).

Regosols, Phaeozems and Vertisols; (*Krasilnikov et al., 2013*); were mildly acidic; and displayed intermediate values for water-holding capacity and permanent wilting point; were very rich in organic matter, total N and P; and were extremely poor in K. In contrast, soils from temperate sites had a light texture (loam; Andosols); greater water-holding capacity and permanent wilting point; were slightly acidic; and were very rich in organic matter, total N and P; and were extremely poor in K. Quality grass (N$_{grass}$) in the temperate sites (NA and AC) was higher compared to tropical sites (LC an LC).

Earthworm communities

Ten earthworm species (Annelida: Oligochaeta: Crassiclitellata) were found in the whole sampling (Table 3). Seven of these are well-known, ubiquitous species, some of which are considered invasive, belonging to three different families (Rhinodrilidae, Megascolecidae and Lumbricidae). The remainder of the earthworms were native morphospecies

Table 3 Earthworm species recorded in four sampling sites along an altitudinal gradient in central Veracruz, Mexico.

Species	Family	Origin	Parthenogenetic	Ecological category	Sites and average density (ind./m²)			
					LV	LC	NA	AC
Pontoscolex corethrurus	Rhinodrilidae	South America	+	Endogeic	141	330	144	0
Onychochaeta windlei	Rhinodrilidae	South America	+	Endogeic	0	320	0	0
Morph 1	Morphospecies	Mexico	Uncertain	Endogeic	264	0	0	0
Morph 2	Morphospecies	Mexico	Uncertain	Endogeic	155	0	0	0
Morph 3	Morphospecies	Mexico	Uncertain	Endogeic	160	0	0	0
Amynthas gracilis	Megascolecidae	Asia	+	Epi-endogeic	0	240	53	80
Octolasion tyrtaeum	Lumbricidae	Europe	−	Endogeic	0	0	120	67
Aporrectodea trapezoides	Lumbricidae	Europe	+	Endogeic	0	0	0	128
Lumbricus rubellus	Lumbricidae	Europe	−	Epi-endogeic	0	0	0	72
Bimastos parvus	Lumbricidae	North America	+	Epigeic	0	0	0	21

Notes.
LV, Laguna Verde (11–55 masl); LC, La Concepción (992–1,025 masl); NA, Naolinco (1,550–1,619 masl); AC, Acatlán (1,772–1,800 masl).

(differentiated from others only by morphological features). The highest diversity was found at site AC, with five species. The total abundance of the earthworm community ranged from 0 to 864 ind. m^{-2} (Fig. 2), with an average of 332 ind. m^{-2}.

The LM analysis showed that the total abundance of the earthworm was significantly influenced by water-holding capacity ($P = 0.026$), permanent wilting point ($P = 0.019$), pH ($P = 0.045$), organic matter ($P = 0.029$), Total N ($P = 0.015$), P ($P = 0.031$), K ($P = 0.016$) and N$_{grass}$ ($P = 0.009$), while the climatic factors (sites) had no such effect ($F = 5.57$; $P = 0.0652$). That is, positive coefficients were associated with an increase in the number of earthworms, and negative coefficients were associated with a decrease in the number of earthworms (Table 4).

Pontoscolex corethrurus

Populations of *P. corethrurus* were found in 10 of the 20 samples from the gradient (Fig. 2A): LV (1/5), LC (5/5) and NA (4/5), but the species was absent in all samples of site AC (situated at 1,772–1,800 masl).

On average, the abundance of *P. corethrurus* accounted for 73% of the total earthworm density throughout the samples where the species was present. This percentage varied between sites LV, LC and NA at 92, 79 and 47%, respectively (Fig. 2B). In the sites where the species occurred, its average density was 273.5 ind. m^{-2}, ranging from 16 to 704 ind. m^{-2} (Fig. 2C).

Pontoscolex corethrurus coexisted with exotic (two and four of the five samples in LC and NA, respectively) and native (1/5 in LV) species (Table 5). In contrast, *P. corethrurus*
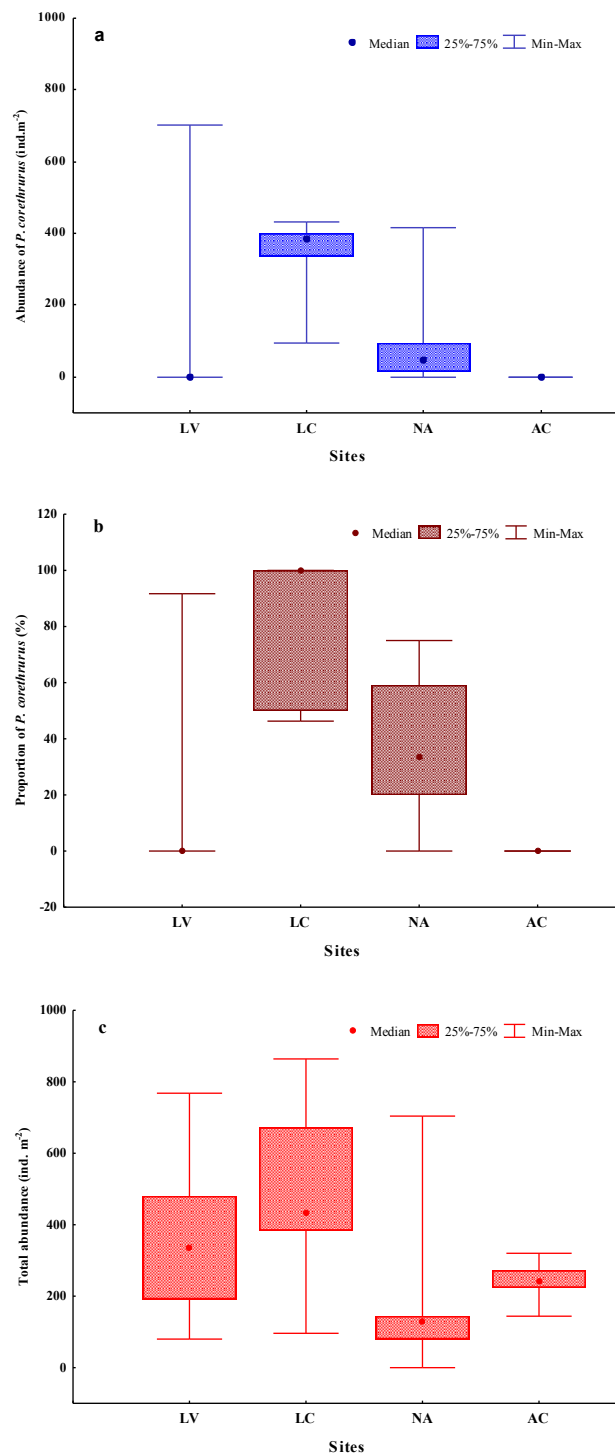
Ortiz-Gamino et al. (2016), *PeerJ*, DOI 10.7717/peerj.2572

7/20

Figure 2 **Abundance (A) and proportion (B) of *Pontoscolex corethrurus* and total earthworm community abundance (C) along an altitudinal gradient.** LV, Laguna Verde (11–55 masl); LC, La Concepción (992–1,025 masl); NA, Naolinco (1,550–1,619 masl); AC, Acatlán (1,772–1,800 masl).

Table 4 Estimated regression coefficients in the linear model that predict total earthworm abundance along an altitudinal gradient in central Veracruz, Mexico.

Factors	Estimate coefficients	Std. Error	t	P
Intercept	44.410	1882.0	0.024	0.982
Site AC[*]	−0.394	0.792	−0.497	0.645
Site LC[*]	0.626	0.554	1.130	0.322
Site NA[*]	−0.324	1.095	−0.296	0.782
Clay, %	−0.133	0.067	−1.98	0.118
Sand, %	0.001	0.034	0.06	0.957
Water-holding capacity, %	−0.208	0.061	−3.43	0.026
Permanent wilting point, %	0.484	0.128	3.77	0.019
pH, (H_2O)	0.920	0.322	2.86	0.045
Organic matter, %	0.156	0.044	3.32	0.029
Total N, %	−18.090	4.420	−4.01	0.015
P, $mg\,kg^{-1}$	0.018	0.006	3.26	0.031
K, $cmol_c\,kg^{-1}$	−1.053	0.263	−4.01	0.016
N_{grass}, %	3.559	0.770	4.62	0.009

Notes.

Sites:
LV, Laguna Verde (11–55 masl); LC, La Concepción (992–1,025 masl); NA, Naolinco (1,550–1,619 masl); AC, Acatlán (1,772–1,800 masl).

*The "site" was included in the linear model using Dummy variables with the reference cell method. The reference site was LV. We performed an analysis of variance for this linear model; the P-value associated to the site effect was 0.0652.

Table 5 Earthworm community composition in each of the five monoliths at the four sites along an altitudinal gradient in central Veracruz, Mexico. no earthworms, *Pontoscolex corethrurus* only, coexistence (exotic and native), others species (but no *P. corethrurus*).

Sites	No earthworm	*P. corethrurus* only	Coexistence		Others species only		Total
			Exotic	Native	Exotic	Native	
LV	0	0	0	1	0	4	5
LC	0	3	2	0	0	0	5
NA	1	0	4	0	0	0	5
AC	0	0	0	0	5	0	5
Total	1	3	6	1	5	4	20

Notes.

LV, Laguna Verde (11–55 masl); LC, La Concepción (992–1,025 masl); NA, Naolinco (1,550–1,619 masl); AC, Acatlán (1,772–1,800 masl).

was found alone in three of the five monoliths of site LC, while only native species were found alone in site LV.

DISCUSSION

Earthworm communities are determined by hierarchical organized factors: temperature operates at the highest hierarchical level, followed by soil nutrient and seasonality factors (*Gerard, 1967*; *Fragoso & Lavelle, 1992*; *Briones et al., 2009*; *Eisenhauer et al., 2014*). Compared to other types of terrestrial ecosystems, grasslands (which are the best carbon

storage systems) are structurally simple and appear to be relatively homogeneous in terms of richness and functional complexity, particularly belowground (*Stockdill, 1966*; *Stanton, 1988*; *Brown et al., 2004*). Here, we found that along an altitudinal gradient, site (climate) can act as a barrier to distribution of peregrine earthworms and their abundance was determined significantly by soil fertility and grass quality.

Earthworm community

Along the altitudinal gradient studied here, 10 species (seven exotic and three native morphospecies) were recorded in the grassland. The exotic species are among the 51 exotic species recorded in Mexico, and the three morphospecies are among the 40 native species that are already known but still undescribed (*Fragoso & Rojas, 2014*). Of the ten species that we found, five are Neotropical (*P. corethrurus*, *Onychochaeta windelei* and three morphspecies), three are Western Palearctic (*Lumbricus rubellus*, *Aporrectodea trapezoides* and *Octolasion tyrtaeum*), one is Nearctic (*Bimastos parvus*) and one more is Eastern Palearctic (*Amynthas gracilis*). The earthworm diversity was similar (five species) between the tropical (LV and LC) and temperate sites (NA and AC), similar to the diversity (4–14 species) that has been observed in tropical and temperate forests (*Fragoso & Lavelle, 1992*).

The current state of knowledge allows little generalization about the distribution patterns of invasive earthworms, as is the case of *P. corethrurus* (*Hendrix et al., 2008*). However, *Beddard (1912)* suggested that: (a) temperate species tend to invade temperate regions and montane areas of the tropical regions, and (b) tropical earthworms only tend to invade tropical regions; that is, low temperatures limit their colonization of temperate areas. Our results show a trend for the influence of climate (site) on the distribution of earthworm species throughout the altitudinal gradient: the Palearctic and Nearctic species where only found in the temperate sites (NA and AC) and the Neotropical species only in the tropical sites (LV and LC). However, *P. corethrurus* and *A. gracilis* was found in one temperate (NA), in one tropical (LC) site, respectively. Some characteristics of native species (morph 1 and 3) and their soil habitats (e.g., high content of expending clay) might be resistant to introduction of *P. corethrurus* in the site LV, that is, because their presence around this site was registered 35 years ago (*Lavelle, Maury & Serrano, 1981*). Also just as recorded by *Juárez-Ramón & Fragoso (2014)* and this study (site AC) *P. corethrurus* does not coexist with *L. rubellus* and *A. trapezoides*. In contrast, *P. corethrurus* coexists with Palearctic (*A. gracilis* and *O. tyrtaeum*) species as observed by *Huerta, Gaspar-Genico & Jarquin-Sanchez (2014)* and *Juárez-Ramón & Fragoso (2014)*, respectively. Furthermore, *P. corethrurus* coexist with Neotropical (morph 2 and *O. windelei*) species.

Grasslands have a positive effect on earthworm diversity and abundance, which is in line with known habitat preferences (*Stockdill, 1966*; *Stanton, 1988*; *Brown et al., 2004*; *Rutgers et al., 2016*). In this study the diversity and density of earthworms along the altitudinal gradient (ranging from 1–10 and 80–864 ind. m^{-2}, respectively) fall within the range reported (1–35 and 6–850 ind. m^{-2}, respectively) for grasslands, croplands and forests of different tropical and temperate regions of Mexico (*Fragoso & Lavelle, 1992*; *Ordaz, Barois & Aguilar, 1996*; *Brown et al., 2004*; *Huerta et al., 2007*; *Geissen, Peña-Peña & Huerta, 2009*; *Huerta & Van der Wal, 2012*; *Uribe et al., 2012*; *Lavelle et al., 1987*; *Fragoso, Coria-Martínez*

& Camarena, 2009; Juárez-Ramón & Fragoso, 2014), Puerto Rico (González et al., 2007), Colombia (Feijoo et al., 2010; Marichal et al., 2010; Gutiérrez-Sarmiento & Cardona, 2014), Brasil (Marichal et al., 2010), USA (Fahey et al., 2013; Eisenhauer et al., 2014), Canada (Eisenhauer et al., 2007) and Europe (Rutgers et al., 2016).

In grasslands, the net primary productivity and the secondary productivity take place in the soil: the former as rhizosphere (root and exudates), and the latter as soil biota (Stockdill, 1966; Stanton, 1988; Trujillo, Fisher & Lal, 2005). The higher organic matter, N and P content in soils across the altitudinal gradient derives from (Beetle, 1974; Trevaskis, Fulkerson & Nandra, 2004; Wright, Hons & Rouquette Jr, 2004; Trujillo, Fisher & Lal, 2005; Mislevy & Martin, 2006; Jones, Orton & Dalal, 2016): (a) the higher productivity and nutrient quality of introduced grasslands (C. nlemfuensis and P. clandestinum), (b) carbohydrate and protein supplementation (NA and AC), and (c) low cattle density (one animal per hectare). This allows to incorporate a higher amount of leaf and root litter, dung and urine into soil; that is, well-managed grasslands have the potential to sequestrate large amounts of carbon and nutrients (Lal, 2004; Wright, Hons & Rouquette Jr, 2004; Jones, Orton & Dalal, 2016). Also, our results, showed that the total abundance of earthworms, irrespective of the species is explained by foliage quality (nitrogen) in the pasture, and soil fertility; that is, we found that N_{grass}, organic matter, pH, P and permanent wilting point increase abundance, while total N, K and water-holding capacity apparently reduce it. This has also been documented in other field (Zou & González, 1997; Brown et al., 2004; González et al., 2007; Geissen, Peña-Peña & Huerta, 2009; Huerta & Van der Wal, 2012; Huerta & Van der Wal, 2012; Jiménez et al., 2012; Uribe et al., 2012) and laboratory studies (Patrón et al., 1999; Ganihar, 2003; Marichal et al., 2012). Soil organic matter is an important 'building block' for soil structure, to absorb water, to retain nutrients and for aeration (Turbé et al., 2010). Also, earthworms have higher abundance in soils with pH from 5 to 7 (LV, LC, NA and AC) and P is maximised when it is in this pH range (Lavelle et al., 1987; González et al., 2007; Marichal et al., 2010). Also, soil with high silt contents (LV, LC, NA and AC) is a favourable habitat for earthworms (Huerta et al., 2007; Geissen, Peña-Peña & Huerta, 2009; Marichal et al., 2010; Huerta & Van der Wal, 2012). However, abundance of earthworms is directly affected by the reduction of soil organic matter content and indirectly by the reduction in plant diversity and productivity (Brown et al., 2004; Brown et al., 2006; Marichal et al., 2010).

P. corethrurus

Our results showed that the most common species, with the exception of AC, along an altitudinal gradient was the endogeic P. corethrurus. Several field studies in different tropical regions of Mexico (Lavelle et al., 1987; Ordaz, Barois & Aguilar, 1996; Brown et al., 2004; Huerta et al., 2007; Fragoso, Coria-Martínez & Camarena, 2009; Geissen, Peña-Peña & Huerta, 2009; Huerta & Van der Wal, 2012; Uribe et al., 2012; Juárez-Ramón & Fragoso, 2014) and other tropical countries (Zou, 1993; Hallaire et al., 2000; Barros et al., 2004; Zou & González, 1997; González et al., 2007; Feijoo et al., 2010; Fonte & Six, 2010; Marichal et al., 2010) have documented that P. corethrurus populations (from 0 to 804 ind. m^{-2}) are only found in environments with an average annual temperature

of 24.1 ± 3.9 °C (range: 16–33 °C), similar to sites LV and LC that show average earthworm densities of 140 and 329 ind. m^{-2}, respectively. However, our findings reveal that the site LV is characterized by the absence of *P. corethrurus* in soils with a higher clay content (4:5 monoliths) and its presence associated with higher sand content (1:5 monoliths); similar findings were observed by *Lavelle, Maury & Serrano (1981)* and *Fragoso, Ángeles & Cruz (2006)* in LV: Palma Sola (clayey) and La Mancha (sandy), respectively. Contrary to the remarks by *Buch et al. (2011)*, our results suggests that soils with a heavy texture (predominance of expandable clays) restrains the survival, growth and movement (for instance, by its brush-tail) of *P. corethrurus,* since soil is characterized by: (a) humidity levels delimited between hydric stress (high temperature) and excess water (flooding), (b) adhesivity when wet, and (c) hardness when dry. Furthermore, our results suggest that the native morphospecies recorded prevent the establishment of *P. corethrurus*, similar to the findings reported by *Lavelle, Maury & Serrano (1981)* and *Fragoso (2011)* in clayey soils in LV (Palma Sola), where only native species were found: *Ramiellona* sp. Nov24, *Lavellodrilus parvus, Larsonidrilus microscolecinus, Diplotrema* sp. Nov9 y *Diplocardia* sp. Nov4. *Marichal et al. (2010)* suggest that *P. corethrurus* and the native species respond to different sets of conditions with variations that are independent between them. Thus, the biological resistance of native communities has been postulated as a key element to understand the presence of *P. corethrurus* in some habitats bot not in others (*Ortiz-Ceballos et al., 2005*; *Marichal et al., 2012*).

In addition, *Eisen (1900)* states that *P. corethrurus* "possesses a great vertical range. I do not think, however, that it occurs in localities subject to frost" We observed populations of *P. corethrurus* (with an average of 133 ind. m^{-2}) at 1550–1619 masl (site NA), where the average annual temperature is 17 °C. In San Jerónimo Tecoatl (Oaxaca, Mexico; *Juárez-Ramón & Fragoso, 2014*), Drakensberg (KwaZulu-Natal, South Africa; *Plisko, 2001*; *Janion-Scheepers et al., 2016*), Antsirabe region of Madagascar (*Chapuis-Lardy et al., 2010*; *Villenave et al., 2010*), Curitiba (Paraná State, Brazil; *Buch et al., 2011*), Zipacón (Cundinamarca, Colombia; *Gutiérrez-Sarmiento & Cardona, 2014*), Chatham (New Jersey, USA; Nearctic region; *Gates, 1954*) and São Miguel island (the Azores Archipelago; Palearctic region; *Cunha et al., 2014*), *P. corethrurus* has become established under similar temperatures (*Kottek et al., 2006*; *Peel, Finlayson & McMahon, 2007*; *Orgiazzi et al., 2016*). This suggests that the growth and reproduction of *P. corethrurus* may no longer be limited by temperature, as indicated by *Lavelle et al. (1987)* with ranges of 20–30 °C and 23–27 °C, respectively (*Zund, Pillai-McGarry & McGarry, 1997*; *Barois et al., 1999*; *Patrón et al., 1999*; *Ganihar, 2003*; *García & Fragoso, 2003*; *Topoliantz & Ponge, 2005*; *Lafont et al., 2007*; *Chapuis-Lardy et al., 2010*; *Hernández-Castellanos et al., 2010*; *Zhang et al., 2008*; *Villenave et al., 2010*; *Buch et al., 2011*; *Chaudhuri & Bhattacharjee, 2011*; *Marichal et al., 2012*; *Duarte et al., 2014*; *Kok et al., 2014*; *Nath & Chaudhuri, 2014*). In these studies, authors have found much faster or slower life cycles, mainly depending on the temperature of incubation and where they were collected (*Buch et al., 2011*).

Rapid adaptations or mutations in known invasive species should be considered as likely mechanisms that could facilitate their spread into new habitats (*Hendrix et al., 2008*). Genetic studies have shown a high level of genetic diversity in populations of *P. corethrurus*

and they are probably differentiated into cryptic species (*Dupont et al., 2012*; *Cunha et al., 2014*). Our findings suggest that *P. corethrurus* inhabiting temperate grasslands is a lineage different to sites LV and LC. The correct molecular identification of *P. corethrurus* is needed to comprehend their history of colonization and as a baseline for biology, ecology and ecotoxicology research on this species (*King, Tibble & Symondson, 2008*; *Dupont et al., 2012*; *Cunha et al., 2014*).

CONCLUSIONS

Our results showed that soil quality significantly determined the abundance of the earthworm community along an altitudinal gradient. In addition, climate was shown to be a barrier to distribution of peregrine earthworms as suggested by *Beddard (1912)*. *P. corethrurus* inhabiting tropical and temperate grasslands probably have 2–3 different lineages or ecotypes. Further studies will be needed to elucidate the genetic diversity of *P. corethrurus.*

ACKNOWLEDGEMENTS

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Diana Ortiz-Gamino and Angel I. Ortiz-Ceballos conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Paulino Pérez-Rodríguez analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

Data Availability

The following information was supplied regarding data availability:

The raw data has been supplied as Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.2572#supplemental-information.

REFERENCES

Anderson RV, Ingram JS. 1993. *Tropical biology and fertility: a handbook of methods.* Wallinford: CAB International.

Barois I, Lavelle P, Brossard M, Tondoh J, Martínez MR, Senapati BK, Angeles A, Fragoso C, Jiménez JJ, Decaens T, Lattaud C, Kanyonyo J, Blanchart E, Chapuis L, Brown GG, Moreno A. 1999. Ecology of earthworm species with large environmental tolerance and/or extended distributions. In: Lavelle P, Brussard L, Hendrix P, eds. *Earthworm management in tropical agroecosystems.* Wallingford: CAB International, 57–85.

Barros E, Grimaldi M, Sarrazin M, Chauvel A, Mitja D, Desjardins T, Lavelle P. 2004. Soil physical degradation and changes in macrofaunal communities in Central Amazon. *Applied Soil Ecology* **26**:157–168 DOI 10.1016/j.apsoil.2003.10.012.

Beddard FE. 1912. *Earthworms and their allies.* Cambridge: Cambridge University Press.

Beetle AA. 1974. Sour paspalum-tropical weed or forage? *Journal of Range Management* **27**:347–349 DOI 10.2307/3896488.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2005. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22**:149–155 DOI 10.1016/j.tree.2006.11.004.

Bohlen PJ, Groffman PM, Fahey TJ, Fisk MC, Suárez E, Pelletier DM, Fahey RT. 2004. Ecosystem consequences of exotic earthworm invasión of North temperate forest. *Ecosystems* **7**:1–12 DOI 10.1007/s10021-003-0126-z.

Briones M.JI, Ostle NJ, McNamara NP, Poskitt J. 2009. Funtional shifts of grassland soil communities in response to soil warming. *Soil Biology & Biochemistry* **47**:315–322 DOI 10.1016/j.soilbio.2008.11.003.

Brown GG, James SW, Pasini S, Nunes DH, Benito NP, Trigo MP, Sautter KD. 2006. Exotic, peregrine, and invasive earthworms in Brazil: diversity, distribution, and effects on soils and plants. *Caribbean Journal of Science* **42**:339–358.

Brown GG, Moreno A, Barois I, Fragoso C, Rojas P, Hernández B, Patrón JC. 2004. Soil macrofauna in SE Mexican pastures and the effect of conversion from native to introduced pastures. *Agriculture, Ecosystems & Environment* **103**:313–327 DOI 10.1016/j.agee.2003.12.006.

Buch AC, Brown GG, Niva CC, Sautter KD, Lourençato LF. 2011. Life cycle of *Pontoscolex corethrurus* (Muller, 1857) in tropical artificial soil. *Pedobiologia* **54S**:S19–S25 DOI 10.1016/j.pedobi.2011.07.007.

Chapuis-Lardy L, Brauman A, Bernard L, Pablo AL, Toucet J, Mano MJ, Weber L, Brunet D, Razafimbelo T, Chotte JL, Blanchart E. 2010. Effect of the endogeic earthworm *Pontoscolex corethrurus* on the microbial structure and activity related to CO_2 and N_2O fluxes from a tropical soil (Madagascar). *Applied Soil Ecology* **45**:201–208 DOI 10.1016/j.apsoil.2010.04.006.

Chaudhuri PS, Bhattacharjee SB. 2011. Reproductive biology of eight tropical earthworm species of rubber plantations in Tripura, India. *Tropical Ecology* **52**:49–60.

Chauvel A, Grimaldi M, Barros E, Blanchart E, Sarrazin M, Lavelle P. 1999. An amazonian earthworm compact more than a bulldozer. *Nature* **398**:32–33 DOI 10.1038/17946.

Cunha L, Brown G, Stanton DWG, Silva D, Hansel FA, Jorge G. 2016. Soil animals and pedogenesis: the role of earthworms in anthropogenic soils. *Soil Science* **181**:110–125 DOI 10.1097/SS.0000000000000144.

Cunha L, Montiel R, Novo M, Orozco-ter Wengel P, Rodrigues A, Morgan AJ, Kille P. 2014. Living on a volcano's edge: genetic isolation of an extremophile terrestrial metazoan. *Heredity* **112**:132–142 DOI 10.1038/hdy.2013.84.

Curry JP. 2004. Factors affecting the abundance of earthworms in soils. In: *Earthworm Ecology*. Boca Raton: CRC Press, pp. 91–113.

Duarte AP, Melo FV, Brown GG, Pauletti V. 2014. Earthworm (*Pontoscolex corethrurus*) survival and impacts on properties of soils from a lead mining site in Southern Brazil. *Biology and Fertility of Soils* **50**:851–860 DOI 10.1007/s00374-014-0906-y.

Dupont L, Decaëns T, Lapied E, Chassany V, Marichal R, Dubs F, Maillot M, Roy V. 2012. Genetic signature of accidental transfer of the peregrine earthworm *Pontoscolex corethrurus* (Clitellata, Glossoscolecidae) in French Guiana. *European Journal of Soil Biology* **53**:70–75 DOI 10.1016/j.ejsobi.2012.09.001.

Eisen G. 1900. Researches in the American Oligocheta, with special reference to those of the Pacific coast and adjacent islands. *Proceedings of California Academy of Science* **2**:85–276.

Eisenhauer N, Partsch S, Parkinson D, Scheu S. 2007. Invasion of a deciduous forest by eaarthworms: changes in soil chemistry, microflora, microarthropods and vegetation. *Soil Biology and Biochemistry* **39**:1099–1110 DOI 10.1016/j.soilbio.2006.12.019.

Eisenhauer N, Stefanski A, Fisichelli NA, Rice K, Rich R, Reich PB. 2014. Warming shifts 'worming: effects of experimental warming on invasive earthworms in northern North America. *Scientific Reports* **4**:6890 DOI 10.1038/srep06890.

Fahey TJ, Yavitt JB, Sherman RE, Maerz JC, Groffman PM, Fisk MC, Bohlen PJ. 2013. Earthworms, litter and soil carbon in a northern hardwood forest. *Biogeochemistry* **114**:269–280 DOI 10.1007/s10533-012-9808-y.

Feijoo MA, Zúñiga MC, Quintero H, Carvajal-Vanegas FC, Ortiz DP. 2010. Patrones de asociación entre variables del suelo y usos del terreno en la cuenca del río la Vieja, Colombia. *Acta Zoológica Mexicana (n.s.) Número Especial* **2**:151–164.

Fonte SJ, Six J. 2010. Earthworms and litter management contributions to ecosystem services in a tropical agroforestry system. *Ecological Applications* **20**:1061–1073 DOI 10.1890/09-0795.1.

Fragoso C. 2011. Lombrices de tierra (Annelida: Oligocheta). In: *La biodiversidad en Veracruz: estudio de estado. Vol. II. Diversidad de especies: conocimiento actual. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), Gobierno del Estado de Veracruz, Universidad Veracruzana.* Xalapa: Instituto de Ecología, A.C, 259–268.

Fragoso C, Ángeles JA, Cruz Y. 2006. Las lombrices de tierra. In: Moreno-Casasola P, ed. *Entornos veracruzanos: la costa de La Mancha.* Xalapa: Instituto de Ecología, A.C., 476–490.

Fragoso C, Coria-Martínez ML, Camarena LM. 2009. An update of the earthworm fauna of LosTuxtlas Ver. and adjacent regions: are native species in the risk of extinction? In: Barois I, Huising EJ, Okoth P, Trejo D, De los Santos M, eds. *Below-Ground Biodiversity in Sierra San Marta, Los Tuxtlas, Veracruz, México.* Xalapa: Instituto de Ecología, A.C., 219–228.

Fragoso C, Lavelle P. 1992. Earthworm communities of tropical rain forests. *Soil Biology & Biochemistry* **24**:1397–1408 DOI 10.1016/0038-0717(92)90124-G.

Fragoso C, Rojas P. 2014. Biodiversidad de lombrices de tierra (Annelida: Oligochaeta: Crassiclitellata). *Revista Mexicana de Biodiversidad* **85**:S197–S207 DOI 10.7550/rmb.33581.

Ganihar SR. 2003. Nutrient mineralization and leaf litter preference by the earthworm *Pontoscolex corethrurus* on iron ore mine wastes. *Restoration Ecology* **4**:475–482 DOI 10.1046/j.1526-100X.2003.rec0253.x.

García JA, Fragoso C. 2003. Influence of different food substrates on growth and reproduction of two tropical earthworms species (*Pontoscolex corethrurus* and *Amynthas corticis*). *Pedobiologia* **47**:754–763 DOI 10.1078/0031-4056-00255.

García-Pérez JA, Alarcón-Gutiérrez E, Perroni Y, Barois I. 2014. Earthworm communities and soil properties in shaded coffe plantations with and without application of glyphosate. *Applied Soil Ecology* **83**:230–237 DOI 10.1016/j.apsoil.2013.09.006.

Gates GE. 1954. Exotic earthworms of the United States. *Bulletin of the Museum of Comparative Zoology at Harvard College* **111**:217–258.

Geissen V, Peña-Peña K., Huerta E. 2009. Effects of different land use on soil chemical properties, decomposition rate and earthworm communities in tropical Mexico. *Pedobiologia* **53**:75–86 DOI 10.1016/j.pedobi.2009.03.004.

Gerard BM. 1967. Factors affecting earthworms in pastures. *Journal of Animal Ecology* **36**:235–252 DOI 10.2307/3024.

González G, Huang CY, Zou X, Rodríguez C. 2006. Earthworm invasions in the tropics. *Biological Invasions* **8**:1247–1256 DOI 10.1007/s10530-006-9023-7.

González G, García E, Cruz V, Borges S, Zalamea M, Rivera MM. 2007. Earthworm communities along an elevation gradient in Northeastern Puerto Rico. *European Journal of Soil Biology* **43**:S24–S32 DOI 10.1016/j.ejsobi.2007.08.044.

Gutiérrez-Sarmiento MC, Cardona CM. 2014. Caracterización ecológica de las lombrices de tierra (*Pontoscolex corethrurus*) como indicadoras de suelos compactados en condiciones de alta humedad del suelo con diferentes coberturas vegetales (Zipacón, Cundinamarca). *Revista Cientifica* **19**:41–55.

Hallaire V, Curmi P, Duboisset A, Lavelle P, Pashanasi B. 2000. Soil structure changes induced by the tropical earthworm *Pontoscolex corethrurus* and organic inputs in a Peruvian ultisol. *European Journal of Soil Biology* **36**:35–44 DOI 10.1016/S1164-5563(00)01048-7.

Hendrix PF, Callaham Jr MA, Drake JM, Huang CY, James SW, Snyder BA, Zhang W. 2008. Pandora's box contained bait: the global problem of introduced earthworm. *Annual Review of Ecology, Evolution, and Systematics* **39**:593–613 DOI 10.1146/annurev.ecolsys.39.110707.173426.

Hernández-Castellanos B, Barois I, Brown GG, García-Pérez A. 2010. Modificaciones químicas inducidas por dos especies de lombrices geófagas en suelos de Veracruz, México. *Acta Zoológica Mexicana (n.s.)* **2**:295–308.

Huerta E, Gaspar-Genico JA, Jarquin-Sanchez A. 2014. Biodiversity of Oligochaeta in Traditional banana plantations of *Musa acuminata* in Tabasco, Mexico (Oligochaeta). In: *Advances in earthworm taxonomy VI (Annelida: Oligochaeta). Proceedings of the 6th international Oligochaete taxonomy meeting (6th IOTM), Palmeira de Faro, Portugal, 22-25 April, 2013.* Heidelberg: Kasparek Verlag.

Huerta E, Rodríguez-Olán J, Evia-Castillo I, Montejo-Meneses E, Cruz-Mondragón M, García-Hernández R, Uribe S. 2007. Earthworm and soil properties in Tabasco, Mexico. *European Journal of Soil Science* **43**:S190–S195 DOI 10.1016/j.ejsobi.2007.08.024.

Huerta E, Van der Wal H. 2012. Soil macroinvertebrates' abundance and diversity in home gardens in Tabasco, Mexico, vary with soil texture, organic matter and vegetation cover. *European Journal of Soil Science* **50**:68–75 DOI 10.1016/j.ejsobi.2011.12.007.

International Organization for Standarization. 2011. Soil quality—sampling of soil invertebrates—part 5: sampling and extraction of soil macro-invertebrates. ISO 23611-5. Geneva: ISO. *Available at http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=50777*.

Janion-Scheepers C, Measey J, Braschler B, Chown SL, Coetzee L, Colville JF, Dames J, Davies AB, Davies SJ, Davis ALV, Dippenaar-Schoeman AS, Duffy GA, Fourie D, Griffiths C, Haddad CR, Hamer M, Herbert DG, Hugo-Coetzee EA, Jacobs A, Jacobs K, Van Rensburg CJ, Lamani S, Lotz LN, Louw SM, Lyle R, Malan AP, Marais M, Neethling JA, Nxele TC, Plisko DJ, Prendini L, Rink AN, Swart A, Theron P, Truter M, Ueckermann E, Uys VM, Villet MH, Willows-Munro S, Wilson JRU. 2016. Soil biota in megadiverse country: current knowledge and future research direction in South Africa. *Pedobiologia* **59**:129–174 DOI 10.1016/j.pedobi.2016.03.004.

Jiménez JJ, Decaëns T, Lavelle P, Rossi JP. 2012. Soil environmental heterogeneity allows spatial co-occurrence of competitor earthworm species in a gallery forest of the Colombian 'Llanos'. *Oikos* **121**:915–926 DOI 10.1111/j.1600-0706.2012.20428.x.

Jones AR, Orton TG, Dalal RC. 2016. The legacy of cropping history reduces the recovery of soil carbon and nitrogen after conversion from continuous cropping to permanent pasture. *Agriculture, Ecosystems and Environment* **216**:166–176 DOI 10.1016/j.agee.2015.09.029.

Jones CG, Lawton JH, Shachak M. 1994. Organisms as ecosystem engineers. *Oikos* **69**:373–386 DOI 10.2307/3545850.

Juárez-Ramón D, Fragoso C. 2014. Comunidades de lombrices de tierra en sistemas agroforestales intercalados, en dos regiones del centro de México. *Acta Zoológica Mexicana (n.s.)* **30**:637–654.

King RA, Tibble ML, Symondson OC. 2008. Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. *Molecular Ecology* **17**:4684–4698 DOI 10.1111/j.1365-294X.2008.03931.x.

Kok HY, Azwady AA, Loh KE, Muskhazli M, Zulkifli SZ. 2014. Optimal stoking density for culturing tropical soil-dwelling earthworm, *Pontoscolex corethrurus*. *Sains Malaysian* **43**:169–173.

Kottek M, Grieser JC, Beck C, Rudolf B, Rubel F. 2006. World map of koppen-geiger climate classification update. *Meteorologische Zeitschrift* **15**:259–263 DOI 10.1127/0941-2948/2006/0130.

Krasilnikov P, Gutiérrez-Castorena MC, Ahrens RJ, Cruz-Gaistardo CO, Sergey S, Solleiro-Rebolledo E. 2013. *The soils of Mexico. world soil series.* New York: Springer.

Lafont A, Risede JM, Loranger-Merciris G, Clermont-Dauphin C, Dorel M, Rhino B, Lavelle P. 2007. Effects of the earthworm *Pontoscolex corethrurus* on banana plants infected or not with the plant-parasitic nematode *Rodopholus similis*. *Pedobiologia* **51**:311–318 DOI 10.1016/j.pedobi.2007.05.004.

Lal R. 2004. Soil carbon sequestration impacts on global climate and food security. *Science* **304**:1623–1627 DOI 10.1126/science.1097396.

Lavelle P, Barois I, Cruz I, Fragoso C, Hernández A, Pineda A, Rangel P. 1987. Adaptive strategies of *Pontoscolex corethrurus* (Glossscolecidae, Oligochaeta), a peregrine geophagous earthworm of the humid tropics. *Biology and Fertility of Soils* **5**:188–194 DOI 10.1007/BF00256899.

Lavelle P, Maury ME, Serrano V. 1981. Estudio cuantitativo de la fauna del suelo en la región de Laguna Verde, Veracruz: Época de lluvias. *Instituto de Ecología de México Publicaciones* **6**:75–105.

Marichal R, Feijoo MA, Praxedes C, Ruiz D, Carvajal AF, Oszwald J, Hurtado MP, Brown GG, Grimaldi M, Desjardins T, Sarrazin M, Deaëns T, Velasquez E, Lavelle P. 2010. Invasion of *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta) in landscapes of the Amazonian deforestation arc. *Applied Soil Ecology* **46**:443–449 DOI 10.1016/j.apsoil.2010.09.001.

Marichal R, Grimaldi M, Mathieu J, Brown GG, Desjardins T, Lopes da Silva Jr M, Praxedes C, Martins MB, Velasquez E. 2012. Is invasion of deforested Amazonia by earthworm *Pontoscolex corethrurus* driven by soil texture and chemical properties? *Pedobiologia* **S5**:233–240 DOI 10.1016/j.pedobi.2012.03.006.

Mislevy P, Martin FG. 2006. Biomass yield and forage nutritive value of *Cynodon* grasses harvested monthly. *Soil and Crop Science Society of Florida proceedings* **65**:9–14.

Müller F. 1857. Description of a new species of earthworm (*Lumbricus corethrurus*). *The Annals and Magazine of Natural History* **20**:13–15 DOI 10.1080/00222935709487865.

Nath S, Chaudhuri PS. 2014. Growth and reproduction of *Pontoscolex corethrurus* (Müller) with different experimental diets. *Tropical Ecology* **55**:305–312.

Ordaz CM, Barois I, Aguilar AS. 1996. Fauna del suelo de la sabana de Huimanguillo alterada por cambios en el uso de la tierra. *Terra* **14**:387–393.

Orgiazzi A, Bardgett RD, Barrios E, Behan-Pelletier V, Briones MJI, Chotte J-L, De Deyn GB, Eggleton P, Fierer N, Fraser T, Hedlund K, Jeffery S, Johnson NC, Kandler E, Kaneko N, Lavelle P, Lemanceau P, Miko L, Montanarella L, Moreira FMS, Ramirez KS, Scheu S, Singh BK, Six J, Van der Putten WH, Wall DH. 2016. *Global Soil Biodiversity Atlas.* Luxembourg: European Commission publications, Office of the European Union.

Ortiz-Ceballos AI, Fragoso C, Equihua M, Brown GG. 2005. Influence of food quality, soil moisture and the earthworm *Pontoscolex corethrurus* on growth and reproduction of the tropical earthworm *Balanteodrilus pearsei*. *Pedobiologia* **49**:89–98 DOI 10.1016/j.pedobi.2004.08.006.

Patrón JC, Sánchez P, Brown GG, Brossard M, Barois I, Gutiérrez C. 1999. Phosphorus in soil and *Bracharia decumbens* plants as affected by the geophagous earthworm *Pontoscolex corethrurus* and P fertilization. *Pedobiologia* **43**:547–556.

Peel MC, Finlayson BL, McMahon TA. 2007. Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences* **11**:1633–1644 DOI 10.5194/hess-11-1633-2007.

Plisko JD. 2001. Notes on the occurrence of the introduced eathworm *Pontoscolex corethrurus* (Müller, 1857) in South Africa (Oligocheta: Glossoscolecidae). *African Invertebrates* **42**:323–334.

R Core Development Team. 2015. *R: a language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing, *Available at* http://www.R-project.org/.

Rutgers M, Orgiazzi A, Gardi C, Römbke J, Jänsch S, Keith AM, Neilson R, Boag B, Schmidt O, Murchie K, Blackshaw RP, Pérès G, Cluzeau D, Guernion M, Briones MJI, Rodeiro J, Piñeiro R, Díaz-Cosín DJ, Souza JP, Suhadolc M, Kos I, Krogh PH, Faber JH, Mulder C, Bogte JJ, Van Wijnen HJ, Schouten AJ, De Zwart D. 2016. Mapping earthworm communities in Europe. *Applied Soil Ecology* **97**:98–111 DOI 10.1016/j.apsoil.2015.08.015.

SEMARNAT. Official Mexican Norm NOM-021-SEMARNAT 2000. 2002. *Specifications of fertility, Salinity, Soil classification, survey, sampling and analysis.* Mexico: Official Journal of the Federation. *Available at* www.economia-noms.gob.mx/.

Senapati BK, Lavelle P, Pashanasi B, Alegre J, Decëns T, Jiménez JJ, Albrecht A, Blanchart E, Mahieux M, Rousseaus L, Thomas R, Panigrahi PK, Venkatachalam M. 1999. In-soil earthworm technologies for tropical agroecosystems. In: Lavelle P, Brussaard L, Hendrix P, eds. *Earthworm management in tropical agroecosystems.* Wallingford: CAB International, 199–237.

Stanton NL. 1988. The underground in grassland. *Annual Review of Ecology and Systematics* **19**:573–589 DOI 10.1146/annurev.es.19.110188.003041.

Stockdill SMJ. 1966. The effects of earthworms on pastures. *Proc NZ Ecol Soc* **13**:68–75.

Topoliantz S, Ponge JF. 2005. Charcoal consumption and casting activity by *Pontoscolex corethrurus* (Glossoscolecidae). *Applied Soil Ecology* **28**:217–224 DOI 10.1016/j.apsoil.2004.08.003.

Trevaskis LM, Fulkerson WJ, Nandra KS. 2004. Rice increases productivity comparaed to other carbohydrate supplements in dairy coes grazing kikuyu (Pennisetum clandestinum), but not ryegrass (Lolium multiflorum), pastures. *Livestock Production Science* **87**:197–206 DOI 10.1016/j.livprodsci.2003.08.004.

Trujillo W, Fisher MJ, Lal R. 2005. Root dynamics of native savanna and introduced pastures in the Eastern Plains of Colombia. *Soil and Tillage Research* **87**:28–38 DOI 10.1016/j.ejsobi.2011.12.007.

Turbé A, De Toni A, Benito P, Lavelle P, Lavelle P, Ruiz N, Van der Putten WH, Labouze E, Mudgal S. 2010. Soil biodiversity: functions, threats and tools for policy makes. Report for European Commission (DG Environment). Bio Intelligence Service, IRD., and NIOO, Paris.

Uribe S, Huerta E, Geissen V, Mendoza M, Godoy R, Jarquin A. 2012. *Pontoscolex corethrurus* (Annelida: Oligocheta) indicador de la calidad del suelo en sitios de *Eucalyptus grandis* (Myrtacea) con manejo tumba y quema. *Revista de Biología Tropical* **60**:1543–552 DOI 10.15517/rbt.v60i4.2072.

Van Groenigen JW, Lubbers IM, Vos HJV, Brown GG, De Deyn GB, Van Groenigen KJ. 2014. Earthworms increase plant production: a meta-analysis. *Scientific Reports* **4**:6365 DOI 10.1038/srep06365.

Villenave C, Rabary B, Kichenin E, Djigal D, Blanchart E. 2010. Earthworms and plant residues modify nematodes in tropical cropping soils (Madagascar): a mesocosm experiment. *Applied and Environmental Soil Science* **2010**:323–640 DOI 10.1155/2010/323640.

Wright AL, Hons FM, Rouquette Jr FM. 2004. Long-term management impacts on soil carbon and nitrogen dynamics of grazed bermudagrass pastures. *Soil Biology and Biochemistry* **36**:1809–1816 DOI 10.1016/j.soilbio.2004.05.004.

Zavala-Cruz J, Trujillo-Capistrán F, Ortiz-Ceballos G, Ortiz-Ceballos AI. 2013. Tropical endogeic earthworm population in a pollution gradient with weathered crude oil. *Research Journal of Environmental Sciences* **7**:15–26 DOI 10.3923/rjes.2013.15.26.

Zhang H, Yang XD, Du J, Wu YX. 2008. Influence of soil temperature and moisture on the cocoon production and hatching of the exotic earthworm *Pontoscolex corethrurus*. *Zoological Research* **29**:305–312 DOI 10.3724/SP.J.1141.2008.00305.

Zou X. 1993. Species effects on earthworm density in tropical tree plantations in Hawaii. *Biology and Fertility of Soils* **15**:35–38 DOI 10.1007/BF00336285.

Zou X, González G. 1997. Changes in earthworm density and community structure during secondary succession in abandoned tropical pastures. *Soil Biology and Biochemistry* **29**:627–629 DOI 10.1016/S0038-0717(96)00188-5.

Zund PR, Pillai-McGarry U, McGarry D. 1997. Repair of a compated Oxisol by the earthworm *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta). *Biology and Fertility of Soils* **25**:202–208 DOI 10.1007/s003740050304.