Zootaxa 5255 (1): 347–361
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5255.1.28
http://zoobank.org/urn:lsid:zoobank.org:pub:934C68DF-EE6E-4B12-BC5B-0A483BAD93DE

Earthworm communities in long-term no-tillage systems and secondary forest fragments in Paraná, Southern Brazil

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Abstract

The area destined for agricultural production in Paraná state in Brazil is ~6 million hectares, of which 79% are under no-tillage systems (NTS) that can positively affect earthworm populations. Furthermore, earthworm abundance and richness can be valuable soil quality. This study assessed earthworm communities in long-term no-tillage sites (NTS) and nearby secondary Atlantic Forest (SF) fragments. Sampling was performed in June 2018 and May 2019 using the quantitative Tropical Soil Biology and Fertility handsorting method (ISO-TSBF), complemented by qualitative sampling in three municipalities: Faxinal, Mauá da Serra and Palmeira, in NTS and SF. Eleven earthworm species, belonging to five families (Benhamiidae, Glossoscolecidae, Megascolecidae, Ocnerodrilidae, and Rhinodrilidae) were found along with seven native species (*Glossoscolex* sp.22, *Glossoscolex* sp.23, *Fimoscolex* sp.21, *Fimoscolex* sp.24, *Fimoscolex* sp.42, *Andiorrhinus duseni* and *Urobenus brasiliensis*) and four exotic or cosmopolitan species (*Dichogaster gracilis, Amynthas gracilis, Metaphire californica* and *Pontoscolex corethrurus*). The cosmopolitan *P. corethrurus* dominated Faxinal and Mauá da Serra, while in Palmeira *Fimoscolex* and *Glossoscolex* were the most abundant. Six species belonging to *Glossoscolex* and *Fimoscolex* were new to science and must be described. Overall, 239 individuals were found. In 2018 126 individuals were found (76 in NTS and 50 in SF) and in 2019, only 112 individuals were found (45 in NTS and 67 in SF). The highest earthworm abundance was in Faxinal (123 individuals), with 76 individuals in NTS and 47 in SF. In Mauá da Serra the same pattern was observed (SF>NTS), while in Palmeira in overall, fewer individuals (38) were collected (24 in NTS and 14 in SF). The NTS at Faxinal and SF Mauá da Serra in 2019 had only/more exotic/cosmopolitan species, while at the other dates and sites >50% of the specimens sampled were native species. Overall, forests had more native species than exotics: in 2018, natives represented > 75% of all specimens, but in 2019 both Faxinal and Mauá da Serra had more

Accepted by D. Marchán: 23 Dec. 2022; published: 15 Mar. 2023

exotic species (> 65%), while at Palmeira, they represented 50% of the individuals. Earthworm total biomass for forest sites was higher in Faxinal (3.2 g) and NTS in Mauá da Serra (1.7 g) in 2019. Overall, the total biomass was higher in SF sites than NTS. Using a classification available for earthworm populations in no-tillage systems, all three sites were considered to have poor quality in terms of abundance (ind m^{-2}), while for species richness, they were considered to have moderate (Faxinal and Mauá da Serra) and good quality (Palmeira).

Keywords: Oligochaeta, soil macrofauna, ecosystem engineer, conservation agriculture

Introduction

Earthworm communities are valuable indicators of soil quality, and these organisms are both easy to see and count and sensitive to environmental changes (Brown & Domínguez 2010). A high-quality soil system is when all its attributes (biological, chemical and physical) are working at full capacity, functioning to promote plant growth, water, carbon and nitrogen storage and nutrient cycling (Doran 1997).

The No-Tillage System (NTS) includes minimal soil disturbance, permanent soil cover, crop rotation and diversification with cover crops (Muzilli 2006; Bartz *et al*. 2010), and promotes high nutrient availability, increase in soil organic matter (OM) and biodiversity, improved soil structure and reduced soil erosion. In Brazil, NTS began in the states of Paraná and Rio Grande do Sul in the '70s as a no-tillage (NT) technique. This differs from NTS since NT only involves sowing over the remaining plant residues of the prior crop (Kochhann & Denardin, 2000), while NTS involves following the three principles described above. Nevertheless, NTS can be adapted for many crops, regions and farm sizes (Motter *et al*. 2015) and is an important soil management tool to enhance soil biota reproduction and development (Bartz *et al*. 2010).

In research carried out by Brown *et al*. (2011) to determine how NTS affects the soil fauna, it was possible to observe that due to improvements in the soil, such as litter and structure, the practice provides a favorable and ideal environment for organisms, including earthworms, to develop. Moreover, higher diversity of species, as well as the role in soil, benefits the heterogeneity of NTS biological activity. In a review by Demetrio *et al*. (2019), considering the NTS areas in Paraná, 33 species of earthworms were identified, of those 19 are native, most from Glossoscolecidae family, but found in lower abundance (<15 ind m^{-2}). The exotic species are mainly from Megascolecidae, Rhinodrilidae and Behanmiidae families (Demetrio *et al.* 2019). For forest areas, there are records for 71 species, most of them native (53), also from Glossoscolecidae (the majority of genera *Glossoscolex* and *Fimoscolex*) and some native species of Rhinodrilidae – *Urobenus* and *Andiorrhinus* – and exotic ones – *Pontoscolex corethrurus* (Dudas, 2020).

The state of Paraná has around 6 million ha in annual crops, of which close to 80% are under no-tillage (Fuentes-Llanillo *et al.* 2021). However, many of these farms practice NT and not NTS, and not enough information is available on earthworm populations in NTS vs NT, particularly in farms that have practiced NTS for long time periods. Hence, in the present study, we assessed earthworm communities in two sites with long-term NTS (>30 years) and one with long-term NT, as well as in nearby secondary Atlantic Forest (SF) fragments in Paraná state, Brazil.

Material and Methods

Study sites

Two sampling campaigns were performed in the municipalities of Faxinal (FX), Mauá da Serra (MS) and Palmeira (PL) counties, in Paraná state (Fig. 1). The municipalities of FX and MS were in the north-central part of the state, between the second and third plateau and part of the Paraná River Basin, a large sedimentary region of South America. The soils in the region are mainly Ferralsols and Cambisols (Santos *et al.* 2011; FAO 2014). The climate in the region is classified as Cfa, according to Köppen (1931), with hot, humid summer and annual precipitation of 1800 mm and 1600 mm in FX and MS, respectively. The mean monthly temperatures in FX in 2018 and 2019 ranged from 19.5ºC and 28.3ºC. In MS, the mean monthly temperature range was 17.8 ºC to 28 ºC. The precipitation for both locations was around 135 mm and 134.4 mm in the month of sampling of 2018 and 2019, respectively (SIMEPAR 2019; SIH 2019).

The county of PL is in the southeastern part of Paraná, belonging to the second plateau and where source materials are sedimentary rocks. The predominant soil types are Cambisols and Leptsols (Santos *et al.* 2011; FAO 2014). Köppen climate is Cfb, with a mean monthly temperature range from 14.2 ºC to 28.2 ºC, and annual precipitation of 1200 mm (SIMEPAR 2019). The precipitation in 2018 was 117 mm, and in 2019 261 mm in the month of sampling (SIH 2019).

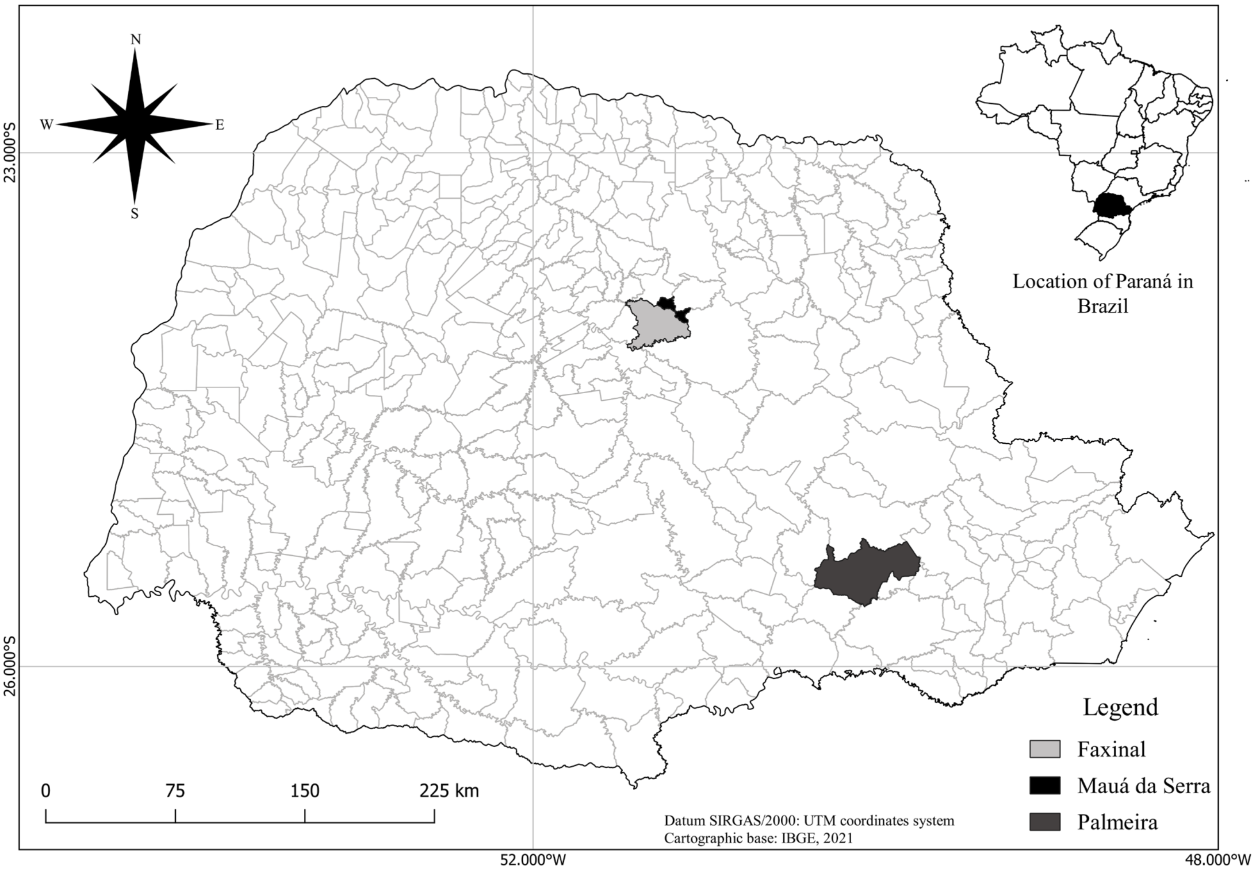

FIGURE 1. Location of the municipalities of the sampling sites in Paraná, Brazil. Light grey = Faxinal (FX), Black = Mauá da Serra (MS), Dark grey = Palmeira (PL)

In each county, two sites were selected, including one long-term NT or NTS (>30 years), and one nearby SF. At FX the farm had a total of 170 ha, and the sampling plot measured 27 ha, with 38 years under NTS. The crops planted in the last three years were soybean, maize, white oats and signalgrass (*Urochloa* sp.). At sampling, white oats were planted in 2018 with maize straw and dried *Urochloa* grass, while in 2019, it was under white oats with maize straw. In 2017 the site had been subsoiled, indicating that it was no longer under long-term NTS but in long-term NT.

At MS, the farm had a total of 750 ha and the sampling plot 23 ha, with 46 years under NTS. The crops planted in the last three years were soybean, maize, wheat, white oats and black oats. At sampling, white oats were growing with soy straw in 2018 and in 2019, the site was in the being prepared (sprayed and fertilized) for the winter crop (wheat) over maize straw.

At PL, the farm had a total of 1500 ha, and the sampling plot 42 ha, with 44 years under NTS. The crops planted in the last three years were soybean, maize, beans, wheat, white oats and black oats. At sampling, weeds were growing with white oat straw in 2018 and in 2019, the beans were in the maturation stage.

All sites received pesticide applications to control weeds (herbicides), diseases (fungicides) and pests (insecticides), according to technical recommendations for the agricultural crops sown (mainly soybean, maize and beans in the summer and white oats and wheat in the winter). In addition, they were fertilized with NPK, and the soybeans were inoculated with N_2-fixing bacteria (*Bradyrhizobium* sp.). The seeds used originated from commercial hybrids often treated with fungicides.

The secondary forest areas were all Mixed Ombrophilous Forest (MOF), belonging to the Atlantic Forest biome, with a dominance of the *Araucaria angustifolia* tree (evergreen gymnosperm), as well as several angiosperm trees and important undergrowth, including many vines in MS. The fragments were all in moderate to advanced regeneration stage (>15 yr in MS and >33 yrs in FX and PL), and measured 43 ha in FX, 5 ha in MS and 9 ha in PL. These areas were near (< 1.5 km) the cropping sites. Some of the main soil chemical and physical characteristics of each site are presented in Table 1.

Earthworm sampling

Earthworms were sampled using a modification of the *Tropical Soil Biology and Fertility (TSBF)* method (Anderson & Ingram 1993) in June 2018 and in May 2019. Soil monoliths of 25 x 25 cm width and 20 cm depth were removed, the soil was hand sorted, and the earthworms found were placed in 96% ethanol. The distance between samples was 15 to 20 m in both the NT/NTS fields and the SF. For the NT/NTS areas, the sample points were taken in three different transects with distances ranging from 150 to 200 m (see Silva *et al.* 2021 for further details).

In 2018, nine samples were taken from the three transects, placed along the catena, from higher to lower parts of the NT/NTS field, totalizing 27 monoliths per area. In 2019, five samples were taken in the same three transects, totaling 15 monoliths per area. In the SF sites, nine monoliths were taken in both years, following a straight-line transect of around 180 m in length.

In addition to the quantitative samples, nine qualitative samples were taken randomly at all sites, targeting more species (Bartz *et al*. 2014). These consisted of samples of similar dimensions, but also targeting earthworms in various other niches, such as inside and under logs, and in the litter.

Earthworms were identified at the family, genus and/or species level. The taxonomy follows mainly Righi (1990, 1995) and species descriptions in the literature. The species were classified as native and exotic/cosmopolitan species. The material is deposited at the Fritz Müller Oligochaeta Collection (Coleção de Oligoquetas Fritz Müller – COFM), and the codes refer to the COFM catalogue numbers at Embrapa Forestry in Colombo, Brazil. Species numbers refer to a listing of new *Glossoscolex* and *Fimoscolex* species from Brazil of co-authors MLCB and GGB.

The following earthworm data were obtained: abundance (total number of individuals, i.e. ind m^{-2}), biomass (g m^{-2} alcohol-preserved fresh weight including gut contents), number of individuals per family, genus or species and native and exotic species proportions. Total abundance was obtained by the sum of individuals found in both the quantitative (TSBF) and qualitative sampling. The results presented as abundance in individuals per square meter (ind m^{-2}) and biomass in grams per square meter (g m^{-2}) are the data from only the quantitative sampling (TSBF) method. Additionally, and when possible, we have generated gene barcodes for each species-representative individual. Briefly, tissue samples from the last two tail segments previously preserved in ethanol were taken from each representative individual and extracted for DNA (using Qiagen DNeasy kit according to the manufacturer's protocol). PCRs were performed targeting the cytochrome oxidase subunit II (COII), by using the primers LumbF1 – 5'CTTAAAGATTTTGGCGGTGTC'3, LumbR1 – 5'CCTTTGCACGGTTAGGATAC'3 (Perez-Lozada *et al.*, 2009). Additionally, a sequence for cytochrome oxidase subunit I (COI) using universal primers (Folmer *et al.*, 1994) was generated for *Andiorrhinus duseni* and *Fimoscolex sp.24*. The PCR was performed using the Biotaq Taq DNA polymerase (Bioline) kit. with samples being amplified through 35 cycles at the following parameters: 95°C for 3 min, 30 sec at 52°C, and one and 30 sec at 72°C, followed by a final extension step at 72°C for two minutes DNA was sequenced by Eurofins Scientific and sequence trace files were corrected and aligned with MEGA v.7 (Kumar *et al.* 2016). The individual sequence per species were deposited in GenBank (COII: OQ077048-OQ077070, and COI: OQ067381-OQ067384) GenBank accession numbers (GAN) are given in association with the collection voucher code. Whenever possible, juvenile individuals were assigned to species using genetic barcodes.

TABLE 1. Soil chemical and physical characterization of the secondary forest fragments (SF) and no-tillage sites (NT, NTS) in Faxinal (FX), Mauá da Serra (MS) and Palmeira (PL) counties, Paraná, Brazil, in 2018.

County	LUS	Coordinates		pH	Al^{3+}	H+Al	Ca^{2+}	Mg^{2+}	K^+	P	N	C	SB	CEC	Clay	Silt	Sand	Texture	Soil type
		Latitude	Longitude	$CaCl_2$	$cmol_c\ dm^{-3}$					$mg\ dm^{-3}$	%				$g\ kg^{-1}$				
FX	SF	-23,97425°	-51,39393°	5.1	0.2	5.4	5.6	2.4	0.04	9.0	0.7	6.3	8.1	8.3	712	242	47	Clay	Ferralsols, Cambisols
	NT	-23,99221°	-51,38992°	5.2	0.1	6.3	6.9	2.1	0.04	22.1	0.4	5.4	9.1	9.2	593	180	227	Clay	
MS	SF	-23,89658°	-51.20898°	4.0	1.4	9.2	1.2	1.0	0.02	5.7	0.4	4.8	2.3	3.7	699	171	130	Clay	Ferralsols, Cambisols
	NTS	-23.89199°	-51.21379°	4.7	0.3	6.2	3.3	1.5	0.04	28.1	0.3	3.4	4.9	5.2	695	126	178	Clay	
PL	SF	-25.39284°	-49.96545°	3.8	1.9	7.6	0.4	0.5	0.01	8.5	0.2	2.3	0.8	2.4	179	88	733	Sandy loam	Cambisols, Leptsols
	NTS	-25.38990°	-49.97696°	5.1	0.2	3.8	1.5	1.5	0.03	50.6	0.2	1.5	3.0	3.2	145	42	812	Loamy sand	

Materials examined

Family Glossoscolecidae

***Glossoscolex* sp.22**

BRPR1214, 1 individual with DNA analysis (GAN: OQ077055), **BRPR1215**, 1 individual with DNA analysis (GAN: OQ077054), **BRPR1216**, 1 individual with DNA analysis (GAN: OQ077051), **BRPR1218**, 1 individual juvenile with DNA analysis, **BRPR1958**, 1 individual juvenile with DNA analysis (GAN: OQ077066), **BRPR1959**, 1 individual with DNA analysis (GAN: OQ077059), **BRPR1960**, 1one individual with DNA analysis (GAN: OQ077057), **BRPR1961**, 1 individual juvenile with DNA analysis in Secondary Forest in Palmeira – PR, -25.39284°S, -49.96545W°, 885 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, A. Pasini, C.M.R. Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1223**, 4 individuals, in Secondary Forest in Palmeira – PR, -25.39284°S, -49.96545°W, 885 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N. O. Sátiro, G.G. Brown, M.L.C. Bartz colls.

***Glossoscolex* sp.23**

BRPR1185, 1 individual, in Secondary Forest in Faxinal – PR, -23.97425°S, -51.39393°W, 786 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, A. Pasini, C.M.R. Oliveira, G.G. Brown, M.L.C. Bartz colls.

***Fimoscolex* sp.**

BRPR1949, 1 individual (juvenile) with DNA analysis (GAN: OQ077053), in annual crop under No-Tillage System in Mauá da Serra – PR, -23.89199°S, -51.21379°W, 979 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1955**, 1 individual (juvenile) with DNA analysis (GAN: OQ077050), in Secondary Forest in Mauá da Serra – PR, -23.89658°S, -51.20898°W, 1022 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls.

***Fimoscolex* sp.21**

BRPR1200, 1 individual with DNA analysis (GAN: OQ077058), **BRPR1199**, 1 individual (juvenile) with DNA analysis (GAN: OQ077056), **BRPR1956**, 1 individual (juvenile) with DNA analysis (GAN: OQ077062), **BRPR1957**, 1 individual (juvenile) with DNA analysis (GAN: OQ077070), in Secondary Forest in Mauá da Serra – PR, -23.89658°S, -51.20898°W, 1022 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1212**, 1 individual, in annual crop under No-Tillage System in Palmeira – PR, -25.38990°S, -49.97696°W, 897 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls.

***Fimoscolex* sp.24**

BRPR1213, 1 individual, **BRPR1946,** 1 individual with DNA analysis (GAN: OQ067383), **BRPR1947,** 1 individual with DNA analysis (GAN: OQ067384), in annual crop under No-Tillage System in Palmeira – PR, -25.3899°S -49.97696°W, 897 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1222**, 11 individuals, in annual crop under No-Tillage System in Palmeira – PR, -25.38990°S -49.97696°W, 897 msnm, May 2019, R.T. Dudas, W.C.

Fimoscolex **sp.42**

BRPR1211, 2 individuals, in annual crop under No-Tillage System in Palmeira – PR, --25.38990°S -49.97696°W, 897 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls. **BRPR1221**, 1 individual, in Secondary Forest in Palmeira – PR, -25.39284°S, -49.96545°W, 885 msnm, May 2019, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls.

Glossoscolecidae (juveniles)

BRPR1186, 1 individual, in Secondary Forest in Faxinal – PR, -23.97425°S, -51.39393°W, 786 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1202**, 1 individual, in Secondary Forest in Mauá da Serra – PR, -23.89658°S -51.20898°W, 1022 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1219**, 2 individuals, in annual crop under No-Tillage System in Palmeira – PR, -25.3899°S, -49.97696°W, 897 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls.

Family Rhinodrilidae

Andiorrhinus duseni **Michaelsen**

BRPR1201, 1 individual**, BRPR1944**, 1 individual with DNA analysis (GAN: OQ067381), **BRPR1945**, 1 individual with DNA analysis (GAN: OQ067382), in annual crop under No-Tillage System in Mauá da Serra - PR -23.89199°S, -51.21379°W, 979 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1207**, 2 individuals, in annual crop under No-Tillage System in Mauá da Serra – PR, -23.89199°S, -51.21379°W, 979 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls.

Pontoscolex corethrurus **Müller**

BRPR1184, 47 individuals, in annual crop under No-Tillage in Faxinal – PR, -23.89199°S, -51.21379°W, 768 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1196**, 27 individuals, in Secondary Forest in Mauá da Serra - PR -23.89658°S, -51.20898°W, 1022 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1197**, 1 individual, in annual crop under No-Tillage System in Mauá da Serra – PR, -23.89199°S, -51.21379°W, 979 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1189**, 36 individuals, in Secondary Forest in Faxinal – PR, -23.97425°S, -51.39393°W, 786 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls. **BRPR1190**, 12 individuals, in annual crop under No-Tillage in Faxinal – PR, -23.89199°S, -51.21379°W, 768 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls. **BRPR1205**, 16 individuals, in Secondary Forest in Mauá da Serra – PR, -23.89658°S, -51.20898°W, 1022 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls. **BRPR1206**, 4 individuals, in annual crop under No-Tillage System in Mauá da Serra – PR, -23.89199°S, -51.21379°W, 979 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls.

Urobenus brasiliensis **Benham**

BRPR1198, 6 individuals, in Secondary Forest in Mauá da Serra – PR, -23.89658°S, -51.20898°W, 1022 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1191**, 1 individual, in Secondary Forest in Faxinal – PR, -23.97425°S, -51.39393°W, 786 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls.

Family Ocnerodrilidae

Ocnerodrilidae (juveniles)

BRPR1187, 1 individual, **BRPR1950**,1 individual with DNA analysis (GAN: OQ077048), **BRPR1951**, 1 individual with DNA analysis (GAN: OQ077052), **BRPR1952**, 1 individual with DNA analysis (GAN: OQ077067), **BRPR1953**, 1 individual with DNA analysis (GAN: OQ077068) in annual crop under No-Tillage in Faxinal – PR, -23.89199°S, -51.21379°W, 768 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1209**, 1 individual, in annual crop under No-Tillage System in Mauá da Serra – PR, -23.89199°S, -51.21379°W, 979 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls. **BRPR1204**, 1 individual with DNA analysis (GAN: OQ077049), **BRPR1948**, 1 individual with DNA analysis (GAN: OQ077061), in annual crop under No-Tillage System in Mauá da Serra – PR, -23.89199°S, -51.21379°W, 979 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls.

Family Benhamiidae

Dichogaster gracilis **Michaelsen**

BRPR1217, 1 individual with DNA analysis (GAN: OQ077069), in annual crop under No-Tillage System in Palmeira – PR, -25.3899°S -49.97696°W, 897 msnm, June 2018 R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1192**, 1 individual, in Secondary Forest in Faxinal - PR (-23.97425 -51.39393, 786 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls.

Benhamiidae (juveniles)

BRPR1194, 8 individuals, in annual crop under No-Tillage in Faxinal – PR, -23.89199°S -51.21379°W, 768 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls. **BRPR1193**, 7 individuals, in Secondary Forest in Faxinal – PR, -23.97425°S, -51.39393°W, 786 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls.

Family Megascolecidae

Amynthas gracilis **Kinberg**

BRPR1208, 1 individual, in Secondary Forest in Mauá da Serra – PR, -23.89658°S -51.20898°W, 1022 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls.

***Metaphire californica* Kinberg**

BRPR1224, 1 individual, in Secondary Forest in Palmeira – PR, -25.39284°S, -49.96545°W, 885 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls.

Megascolecidae (juveniles)

BRPR1220, 1 individual with DNA analysis (GAN: OQ077063), **BRPR1954**, 1 individual with DNA analysis (GAN: OQ077065), in annual crop under No-Tillage System in Palmeira – PR, -25.38990°S, -49.97696°W, 897 msnm, June 2018 R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, A. Pasini, C.M.R. Oliveira, G.G. Brown, M.L.C. Bartz colls.

Unidentified juveniles

BRPR1188, 2 individuals, in Secondary Forest in Faxinal – PR, -23.97425°S, -51.39393°W, 786 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, ML.C. Bartz colls. **BRPR1203**, 3 individuals, in annual crop under No-Tillage System in Mauá da Serra – PR, -23.89199°S, -51.21379°W, 979 msnm, June 2018, R.T. Dudas, K.A. da Silva, W.C. Demetrio, L.S. Maia, V.B. Nicola, A. Pasini, C.M.R. de Oliveira, G.G. Brown, M.L.C. Bartz colls. **BRPR1195**, 2 individuals, in Secondary Forest in Faxinal – PR, -23.97425°S, -51.39393°W, 786 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls. **BRPR1210**, 3 individuals, in annual crop under No-Tillage System in Mauá da Serra – PR, -23.89199°S, -51.21379°W, 979 msnm, May 2019, R.T. Dudas, W.C. Demetrio, J.N.O. Sátiro, G.G. Brown, M.L.C. Bartz colls.

Results and Discussion

A total of 238 individuals were found, 120 individuals collected with the quantitative TSBF method and 118 individuals in the qualitative samples. Eleven species of earthworms were identified, belonging to five families (Benhamiidae, Glossoscolecidae, Megascolecidae, Ocnerodrilidae, and Rhinodrilidae). Six of these species are native (*Glossoscolex* sp.22, *Fimoscolex* sp.21, *Fimoscolex* sp.24, *Fimoscolex* sp.42, *Andiorrhinus duseni* and *Urobenus brasiliensis*), while four are exotic widespread species in Brazil (*Dichogaster gracilis, Amynthas gracilis, Metaphire californica* and *Pontoscolex corethrurus*) (Table 2).

The peregrine species *P. corethrurus* was dominant in FX and MS (Table 2). This endogeic species is usually found in the first 30 cm of soil and has been found in different agricultural systems (Santos *et al.* 2018), pastures (Nunes *et al*. 2006; Bartz *et al*. 2014), urban areas (Ferreira *et al*. 2018), native forests and forest plantations (Silva *et al*. 2019) in Paraná state. It has wide tolerance to environmental disturbance and different soil conditions, and its parthenogenetic reproduction system makes it an excellent colonizer of new areas (Buch *et al.* 2011; Marichal *et al.* 2014).

In 2018, *U. brasiliensis* was found in secondary forests at FX and MS (Table 2). This epi-endogeic species has been found from Northern Amazonia to the Pampa biome (Righi 1984; Ferreira *et al.* 2023), in native vegetation, bromelias, pasture and NT sites (Bartz *et al.*, 2009; Steffen *et al.*, 2018; Santos *et al.*, 2019) usually in soils with higher organic matter levels, where soil revolving is not carried out and the organic matter remains on the soil surface (Bartz *et al*. 2009). Hence this species can be used as an indicator of the more conserved habitats (Bartz *et al*. 2011; Santos *et al*. 2018).

The large species *A. duseni*, found in the NTS in MS (Table 2), can reach up to 40 cm in length (Feijoo *et al.* 2017), and has been found in native forests and grasslands, pastures and forestry plantations (Cardoso *et al*. 2014; Feijoo *et al*. 2017; Silva *et al*. 2019), from Southern São Paulo state to Northern Rio Grande do Sul state (Ferreira *et al.* 2023). Although it is widely distributed, its biology and ecology are still poorly known.

Members of the Ocnerodrilidae family are found in South America, Africa, India and México in both tropical and subtropical climates (Fragoso & Rojas 2009, Misirlioğlu *et al.* 2023) and includes a large number of still undescribed species in Brazil (James *et al*. 2023), as well as several widespread peregrines, cosmopolitan species belonging to Megascolecidae, Ocnerodrilidae and Benhanmiidae families (Misirlioğlu *et al.* 2023). Individuals of this family were found in the NT only at FX, and their origin was not possible to determine as they were juveniles (Table 2). However, in Western Paraná, many individuals of this family have been observed in various annual crops under NT (Santos *et al.* 2018). Most of them are possible new species of Ocnerodrilidae, showing that they can adapt to those soil conditions.

TABLE 2. Total species abundance found by quantitative (TSBF) and qualitative methods, the proportion of native and exotic species in 2018 and 2019 in secondary forest (SF) and no-tillage sites (NT, NTS), with five and nine monoliths respectively, in the counties Faxinal (FX), Mauá da Serra (MS) and Palmeira (PL), in Paraná state, Brazil.

Earthworms	FX				MS				PL			
	SF		NT		SF		NTS		SF		NTS	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Native	-	-	-	-	-	-	-	-	-	-	-	-
Glossoscolecidae	-	-	-	-	-	-	-	-	-	-	-	-
Glossoscolex sp.22	-	-	-	-	-	-	-	-	3 / 5	3 / 1	-	-
Glossoscolex sp.23	-/1*	-	-	-	-	-	-	-	-	-	-	-
Fimoscolex sp.	-	-	-	-	- / 1	-	1/-	-	-	-	- / -	-
Fimoscolex sp.21	-	-	-	-	2 / 2	-	-	-	-	-	1 / -	-
Fimoscolex sp.24	-	-	-	-	-	-	-	-	-	-	1 / 2	3 / 8
Fimoscolex sp.42	-	-	-	-	-	-	-	-	- / 1	-	- / 2	-
Glossoscolecidae (juveniles)	- / 1	-	-	-	- / 1	-	-	-	-	-	- / 2	-
Rhinodrilidae	-	-	-	-	-	-	-	-	-	-	-	-
Andiorrhinus duseni	-	-	-	-	-	-	3 / -	2 / -	-	-	-	-
Urobenus brasiliensis	-	1 / -	-	-	4 / 2	-	-	-	-	-	-	-
Ocnerodrilidae	-	-	-	-	-	-	-	-	-	-	-	-
Ocnerodrilidae (juveniles)	-	-	3 / 2	-	-	-	2/-	1 / -	-	-	-	-
Exotic	-	-	-	-	-	-	-	-	-	-	-	-
Benhamiidae	-	-	-	-	-	-	-	-	-	-	-	-
Dichogaster gracilis	-	- / 1	-	-	-	-	-	-	-	-	1 / -	-
Benhamiidae (juveniles)	-	- / 7	-	8/-	-	-	-	-	-	-	-	-
Megascolecidae	-	-	-	-	-	-	-	-	-	-	-	-
Amynthas gracilis	-	-	-	-	-	- / 1	-	-	-	-	-	-
Metaphire californica	-	-	-	-	-	-	-	-	-	1 / -	-	-
Megascolecidae (juveniles)	-	-	-	-	-	-	-	-	-	-	- / 2	-
Rhinodrilidae	-	-	-	-	-	-	-	-	-	-	-	-
Pontoscolex corethrurus	-	27 / 9	16 / 31	12 / -	14 / 13	- / 16	1 / -	4 / -	-	-	-	-
Juveniles (unidentified)	-	-	2 / -	2 / -	-	-	1 / 3	1 / 2	-	-	-	-
Total abundance	-/2	28/17	21/33	22/-	20/19	-/17	8/3	8/2	3/6	4/1	3/8	3/8
Species Richness	1	3	2	1	4	2	4	3	2	2	4	1
Native(%)	100	33	50	0	75	0	75	67	100	50	75	100
Exotic (%)	0	67	50	100	25	100	25	33	0	50	25	0

*The first number before the forward slash is the sum of individuals sampled by the quantitative (TSBF) method, and the second is from the qualitative.

In PL, individuals of the family Glossoscolecidae were dominant in both years (Table 2), though they were found in low density. This family is exclusive to southern and Southeastern South America, and all species found in Brazil are native (Brown & Fragoso 2007). In Paraná until 2020, of the 91 species known, 31 belong to Glossoscolecidae family (Dudas 2021). *Glossoscolex* and *Fimoscolex* species have been found in several NT and NTS sites, indicating that this management can maintain native earthworm populations (Bartz *et al*. 2012). In comparison with native vegetation areas, NT/NTS sites, were the system where Glossoscolecidae species were mostly found in Paraná.

In areas of native vegetation, at least 30 different species were found and in areas of NT/NTS, between 10 and 15 species (Dudas, 2020). Furthermore, it indicates that these species have sufficient phenotypic plasticity to cope with the low level of soil disturbance present in these agricultural systems (Santos *et al.* 2018). In the case of NTS, other contributing factors are the higher availability of soil organic matter (e.g., surface litter/straw), of more diverse sources, and minimal soil disturbance, allowing the survival of the native species (Brown *et al*. 2003; Bartz *et al*. 2014).

The exotic species *A. gracilis* was found exclusively in qualitative samples, not TSBF, in the forest at MS in 2019. The presence of this species in native forests indicates a former disturbance in or around the area (Fernandes *et al.* 2010). This species is usually found in disturbed areas, and cosmopolitan species of both Megascolecidae and Benhanmiidae are found throughout Brazil (Brown *et al.* 2006). *Dichogaster gracilis* was found in the forest at FX in 2018, and the annual crops at FX and MS. These specimens are typically found in sites close to anthropic activities, such as home gardens (Sautter *et al.* 2007), and are generally quite resistant to environmental disturbances.
The areas with 100% exotic species were FX NT in 2019 and SF MS in 2019. Another area with a high proportion of exotics was the forest at FX in 2019 (67%). Furthermore, the forests in FX and PL in 2018 and the NTS at PL in 2019 had 100% native species. All other areas had a higher proportion of native species, mainly the forest and NTS at MS in 2018, and the NTS at PL in 2018 (both with 75% natives).

Several biotic and abiotic factors determine and influence earthworm abundance and richness, including soil management practices, land use and environmental conditions like precipitation, temperature and food (soil organic matter) availability (Lavelle *et al.* 1996; Brown & Domínguez 2010). Mean abundance and biomass found in all land uses were exceptionally low (Table 3) and were originally thought to be attributed to two very dry years prior to the first sampling and the relatively dry rainy season of 2018/2019. The highest mean abundance (2.77 ind. m^{-2}) and biomass (0.87 g, m^{-2}) were found in the forest at FX. However, values were lower than those reported by Brown *et al.* (2003) and Sautter *et al.* (2007) for other locations in Northern Paraná or other NT sites and forests in Brazil (Demetrio *et al*. 2020; Nadolny 2017).

TABLE 3. Abundance (ind m^{-2}) and biomass (g m^{-2}) with standard deviation (SD) of earthworms in 2018 and 2019 by quantitative (TSBF) methods, in secondary forest (SF) and no-tillage sites (NT, NTS) areas in the counties Faxinal (FX), Mauá da Serra (MS) and Palmeira (PL), in Paraná state, Brazil.

Variable		FX				MS				PL			
		SF		NT		SF		NTS		SF		NTS	
		2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Abundance	ind m^{-2}	0	2.77	0.70	1.30	1.98	0	0.30	0.50	0.30	0.40	0.10	0.20
	SD	0	±0.87	±0.25	±0.39	±0.51	0	±0.18	±0.23	±0.14	±0.25	±0.08	±0.14
Biomass	g m^{-2}	0	0.30	0.05	0.01	0.11	0	0.01	0.08	0.04	0.08	0.01	0.03
	SD	0	±0.11	±0.02	±0.05	±0.04	0	±0.01	±0.01	±0.02	±0.02	±0.01	±0.05

Using the no-tillage quality classification proposed by Bartz *et al*. (2013) for earthworm abundance (ind m^{-2}), i.e., <25 = Poor; ≥25 to <100 = Moderate; ≥100 to <200 = Good; and ≥200 = excellent, the three areas evaluated here had poor quality in both years (less than 25 ind. m^{-2}). However, considering species richness, the sites were classified as moderate in FX and MS, as both areas had three species, while PL was classified as of good quality with five species. Furthermore, the presence of native species in NTS is also an indication that the management practices adopted provided a favorable environment for these species (even though at a low abundance), several of which are new to science and should still be described.

The time of NT and NTS adoption is an important factor influencing earthworm abundance and biomass, as observed by Brown *et al*. (2003) and Dudas *et al*. (2020). Although two sites had been under NTS for over 35 years in this study, abundance and biomass were lower in relation to the nearby secondary forests. The reasons for the low numbers found are still not clear. However, non-compliance of NTS premises, such as the use of subsoiler (lack of minimal soil disturbance) in FX and the lack of a more diverse crop rotation and diversification in all sites, including leguminous cover crops, may be contributing to a poorer quality of the NTS (Demetrio *et al*., 2020). Furthermore, all sites have pesticide applications year-round, some with medium or high toxicity to earthworms (Demetrio *et al*., 2020; PDB pesticides Database). Further efforts are needed in order to evaluate if the pesticide residues found in these cropping systems (Silva *et al*. 2021) are limiting earthworm populations.

Still, both land uses had native species at low densities, highlighting the importance of NT systems in maintaining earthworm biodiversity. Nevertheless, considering the low abundance, efforts to encourage earthworm populations are needed, particularly considering the positive roles of these animals in soil fertility and crop production (van Groenigen *et al.* 2014, 2019), particularly in sandier and nutrient-poor soils (Brown *et al.* 1999).

Furthermore, as ecosystem engineers, earthworms can affect the soil as a habitat for other organisms, affecting especially soil organic matter distribution, nutrient availability and soil bioturbation, especially porosity and aggregation (Lavelle *et al.* 1997; Brown *et al*., 2015; Plaas *et al.* 2019). Therefore, increasing earthworm numbers could also benefit agricultural productivity, especially in NTS. Hence, further work is needed in order to find options that can increase earthworm populations in these systems in a partnership between farmers and research institutions, aiming to generate the required information for future decisions, always seeking the continuous improvement of soil and environmental quality and agricultural sustainability.

Conclusion

In total, 11 species were found, of which seven were native, four were exotic species and one with unknown origin. Nine species were found in the secondary forests, while the No-Tillage overall had only six species. Higher diversity in secondary forest sites indicates the importance of maintaining the environment as naturally as possible, with soil characteristics favorable to the organisms. The adoption of NT/NTS can be beneficial because of the non-revolving and preservation of the litter, which contributes to higher organic matter and microhabitats with temperatures, nutrients and humidity suitable to earthworm's development, despite the disturbances in these areas (pesticides and chemical fertilizers). The genera *Glossoscolex* and *Fimoscolex* were found in both systems, showing the resilience and adaptation of this family to different types of management, seeing that all species in this group are possibly new species.

Acknowledgements

The authors acknowledge financial support of the Higher Education Funding Council for Wales – HEFCW - through the Cardiff University Global Challenge Research Fund for sampling (in 2018), barcoding and soil parameters analyses. It was also supported by the Brazilian National Council for Scientific Research and Development (CNPq) for the 2018 and 2019 sampling campaigns (grant 461484/2014-5). To SENAR and Fundação Araucária for the scholarship, through the Centro de Pesquisa da Universidade Positivo (CPUP), to R. Dudas. Nuno G. C. Ferreira was supported by a MSCA COFUND Fellowship (H2020-COFUNDSIRCIW>MINT-512202) through Cardiff University, Welsh Government and the European Union. Luis Cunha was financially supported by FCT (CEECIND/01986/2017). Furthermore, we acknowledge all institutions that participated and contributed to the development of this work: University of Cardiff, Universidade Positivo, University of Coimbra, Federal University of Paraná and Embrapa Forestry and Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR). A special acknowledgement is also extended to the farmers who allowed sampling on their properties, provided logistical support and delivered the necessary information.

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