USING EARTHWORMS AS BIOINDICATORS OF QUALITY IN NO-TILL SYSTEMS*

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Resumo
No Brasil, o plantio direto é o sistema de manejo do solo mais praticado. Dentre os diversos benefícios, o plantio direto promove as funções dos organismos, especialmente as minhocas, sobre a fertilidade do solo. Portanto, o presente estudo teve como objetivo avaliar as populações de minhocas, visando uma classificação da densidade e diversidade de minhocas em áreas sob plantio direto. As coletadas foram realizadas em fevereiro/março de 2010 e 2011, num total de 34 e 25 propriedades respectivamente em seis municípios da Bacia do Paraná 3, parte da bacia do lago de Itaipu. As minhocas foram amostradas no final da estação chuvosa, utilizando uma adaptação do Método para Biologia e Fertilidade em Solos Tropicais (TSBF), consistindo na coleta de 5 monolitos de 20 x 20 por 20 cm de profundidade, espaçados por mínimo 20 m um do outro, em um transecto linear. Foi realizada triagem manual no campo e as minhocas foram estocadas em sacos plásticos contendo solução de formaldeído 5%. As minhocas foram contadas e identificadas em nível de família, gênero e espécies. A simplificação da metodologia de amostragem objetivou possibilitar que os próprios agricultores avaliem as populações de minhocas em suas propriedades. A abundância de minhocas variou de 5 a 600 ind m^{-2} e 5 a 1150 ind m^{-2}, respectivamente nas épocas de coletas em 2010 e 2011, enquanto o número de espécies variou de uma a seis em 2010 e de uma a sete em 2011 nas áreas sob plantio direto. Baseado neste estudo e em resultados de outros estudos anteriores, uma classificação para abundância e diversidade de minhocas em áreas sob plantio direto é proposta aqui, para Latossolos Vermelhos e regiões de clima quente do Paraná (Cfa Koeppen) : pobre >25 ind m^{-2} and 1 sp.; moderado ≥ 25 to <100 ind m^{-2} and 2 to 3 sp.; bom ≥ 100 to <200 ind m^{-2} and 4 to 5 sp.; excelente ≥ 200 ind m^{-2} and > 6 sp.

Palavras-chave: oligochaeta, bioindicadores do solo, qualidade no plantio direto, sustentabilidade.

Introduction

The no-till system is based on three principles: a permanent soil cover, minimum soil tillage and crop rotation including green manures (5). Complementarily, integrated pest management should also be associated with the production system. Brazil currently has more than 26 million hectares under no-tillage (21), being the most widely adopted conservation farming practice in the country. The use of no-till results in an agroecosystem with a lower degree of disturbance or disorder, when compared to other forms of management that employ intense soil mobilization. This is justified by the fact that this complex set of technologies requires less labor and fossil energy, stimulates soil aggregation processes, reduces the rates of organic matter mineralization, reduces erosion and promotes the biological control of pests, diseases and weeds, reducing the use of pesticides. In particular there is a significant stimulus and recolonization of soil life resulting from the lower human impact of the system (18, 17, 27, 35, 15).

Soil organisms promote a variety of ecosystem services, such as organic matter decomposition, nutrient mineralization, carbon sequestration, exchange and emission of gases, water infiltration, aggregation, protection against diseases and pests (biological control), bioremediation and restoration of degraded or contaminated soils (30). Earthworms in particular, play a predominant role in the formation and maintenance of soil structure (29) and production of biogenic structures (structures produced by the activity of organisms) (28), such as tunnels, galleries and casts. The physical and chemical attributes of these structures, as well as their spatial and temporal distribution define the adaptability of soil microhabitats for other organisms and modify topsoil quality,

facilitating the retention of nutrients and the entrance, storage and passage of water through the profile.
The abundance, distribution and activity of earthworms vary depending on weather (temperature and humidity), biological conditions (vegetation types and availability of food) and in particular on anthropogenic influences (soil management and inputs) (10). Among these, the factors that have most significant impact are vegetation cover (43), soil type and natural and human induced changes in this coverage (42), including soil and ecosystem management (Kang et al., 1994).
The use of earthworms as indicators of no-till soil quality is based on the perception of farmers in the development of this system in Brazil, since earthworm populations increased with the adoption of no-till and even became the symbol of no-till farmer associations. In fact, earthworms are generally considered by Brazilian farmers to be a sign or indication of healthy soil.
The present study was part of the Participatory Methods to Assess Quality in No-Till Systems in the Paraná River Basin 3, Brazil, a cooperation project between Itaipu Binacional and the Brazilian No-Till Federation (FEBRAPDP) and was undertaken to validate a proposal of no-till quality classification based on the abundance and diversity of earthworms in six watersheds in western Paraná,.

Material and Methods
The main soil type in the region is Rhodic Hapludox, according to FAO Soil Classification. The climate is typical subtropical Cfa, according to Koeppen's, characterized by having hot and humid summers and no defined dry season. The annual rainfall is <1800 mm and average annual temperature in the summer is around 27º C (23).
Samples were taken in February-March of 2010 and 2011 in a total of 34 and 25 farms respectively, in six watersheds (Ajuricaba, Buriti, Facão Torto, Pacuri, Mineira, Toledo) of six counties in western Paraná (Marechal Cândido Rondon, Itaipulândia, Entre Rios do Oeste, Santa Helena, Mercedes, Toledo). Earthworms were sampled at the end of wet season using an adaptation of the TSBF –(Tropical Soil Biology and Fertility) method (1), consisting in the removal of five monoliths of 20 x 20 cm square to 20 cm depth per farm, spaced at least 20 m apart (Figure 1). Worms were handsorted from the soil and stored in plastic bags (zip-lock type) containing 5% formaldehyde. All individuals collected were counted and identified (when possible) at family, genus and species levels, using identification keys and descriptions of Righi (38, 39) and Blakemore (9). Abundance was expressed as number of individuals per square meter (ind m^{-2}) but the classification system (ranking of no-till sites) also included the mean number of worms and species per sample. This simplification of the TSBF method aimed to eventually enable the farmers themselves to carry out the assessment of earthworm abundance on their farms.

Figure 1 Summary of the earthworm sampling procedure:
1. Plot a transect line with 5 sampling points spaced at least 20 m apart;
2. Select the sampling point and sift through the straw to collect surface-dwelling (epigeic) or surface-active earthworms;
3. Remove with straight spades the soil block (20 x 20 square, to 20 cm depth);
4. Place the soil in a tray to handsort for worms in the field;
5. Place the collected earthworms in plastic pots or bags (zip-lock type) containing 5% formaldehyde solution.

Results and Discussion

In the samples taken in 2010, earthworm abundance ranged from 5 to 600 ind m^{-2} while the number of species ranged from one to six (Table 1; 6). There were sites that had only juvenile earthworms, preventing the identification at species level, so these sites were considered as having only one species. In the 2011 samples, abundance ranged from 5 to 1150 ind m^{-2} and the number of species from one to seven in the no-till sites.

In northern Paraná, no-tillage sites with similar climatic and soil conditions to those in the present study had earthworm abundance ranging from 3 to 291 ind m^{-2}, with the lowest values obtained in the dry season (May-Sep.), while the number of species varied from one to more than four species (6).

According to Brown et al. (11), crops under NT and MT (minimum tillage) have higher populations of earthworms than under conventional tillage. In the region of Londrina - Paraná, these authors found 8 to 240 earthworms per m^{-2} in sites under NT and MT, while conventional tillage (CT) had 0 to 42 individuals per m^{-2}. Table 2 shows data available in the literature on earthworm abundance in no-till systems in Brazil, highlighting the sites (in bold) where climate and soil conditions are similar to those where the present study was conducted. Earthworm abundance in these sites varied from 3 to 510 individuals per m^{-2}. Compared with the results from northern Paraná, very few of the no-till farms studied here had higher abundance values; most were within the range reported previously.

Table 1 Mean earthworm abundance (Nº ind m^{-2}) and number of species in 34 no-till (NT) sites of six western Paraná watersheds, on two sample dates (Feb.-Mar. 2010 and 2011).

Watershed (County)	**Site**	**Abundance (ind m^{-2})**		**Nº species**	
		2010	**2011**	**2010**	**2011**
Ajuricaba (Marechal Cândido Rondon)	NT1	50	200	1	3
	NT2	605	1150	6	4
	NT3	65	350	3	4
	NT4	40	75	2	2
	NT5	305	-*	5	-
Buriti (Itaipulândia)	NT6	70	275	4	4
	NT7	60	385	2	6
	NT8	25	190	1	4
	NT9	85	-	6	-
	NT10	205	-	6	-
Facão Torto (Entre Rios do Oeste)	NT11	340	10	4	0
	NT12	190	175	3	7
	NT13	295	55	3	3
	NT14	10	25	1	2
Pacurí (Santa Helena)	NT15	110	75	4	3
	NT16	285	30	5	2
	NT17	80	30	4	3
	NT18	30	-	1	-
	NT19	125	-	2	-
Mineira (Mercedes)	NT20	5	220	1	5
	NT21	45	40	4	2
	NT22	55	15	1	1
	NT23	235	5	3	1
	NT24	105	-	4	-
Toledo (Toledo)	NT25	185	205	3	2
	NT26	50	5	2	0
	NT27	20	875	2	4
	NT28	50	90	1	2
	NT29	30	110	2	2
	NT30	20	110	1	3
	NT31	120	5	4	0
	NT32	55	-	1	-
	NT33	265	-	3	-
	NT34	95	-	4	-

* Sites not sampled.

Table 2 Earthworm abundance(Nº ind m^{-2}) and species diversity (Nº Ew sps) under no-till cropping in various Brazilian locations.

Location	**Abundance (Nº ind m^{-2})**	**Nº species**	**References**
Arapongas-PR	**18 to 37**	**nd****	Brown et al. (13)
Londrina-PR	**40* to 100**	**3**	Brown et al. (11, 14, 13), Derpsch et al. (17, 18)
Cornélio Procópio-PR	**176**	**nd**	Brown et al. (14)
Bela Vista do Paraíso-PR	**10* to 291**	**nd**	Brown et al. (11), Benito (7)
Lerroville-PR	**48 to 240**	**3**	Brown et al. (2004)
Rolândia – PR	**3* to 214**	**>4**	Derpsch (18), Guimarães et al. (22), Brown et al. (11), Benito et al. (8), Bartz et al. (6)
Cafeara-PR	**6 to 42**	**>3**	Brown et al. (14, 13)
Campo Mourão-PR	**12 to 144**	**>3**	Brown et al. (14)
São Jerônimo da Serra-PR	**142**	**1**	Brown et al. (14)
Nova Aurora-PR	**50 to 238**	**nd**	Brown et al. (13)
Cafelândia	**363**	**nd**	Brown et al. (13)
Cascavel-PR	**176**	**nd**	Brown et al. (13)
Palotina-PR	**18 to 510**	**nd**	Brown et al. (13)
Guarapuava-PR	3 to 12	nd	Mafra et al. (31)
Carambeí-PR	44 to 118	3	Tanck et al. (40), G. Brown and K. Sautter (observação pessoal, 2004)
Arapoti-PR	72 to 168	3	Peixoto e Marochi (34), G. Brown (personal observation, 2004)
Ponta Grossa-PR	44 to 117	2	Voss (41)
Castro-PR	123	nd	Ressetti (36)
Santo Antônio de Goiás-GO	25 to 250	3	G. Brown (personal observation, 2004)
Santa Helena-GO	288 to 340	nd	Minette (33), G. Brown (personal observation, 2004)
Planaltina-GO	164	nd	Marchão et al. (32)
Taciba-SP	138	>2	Brown et al. (unpublished data)
Dourados-MS	6 to 264	nd	Da Silva et al. (16), Aquino et al. (3)
Seropédica-RJ	67 to 320	nd	Rodrigues et al. (39), Aquino (2)
Teutônia-RS	28* to 299	2	Krabbe et al. (25, 26)
Chapecó - SC	150 to 625	nd	Baretta et al. (4)
Marechal Cândido Rondon	*40 to 1150*	*1 to 5*	*Present study*
Itaipulândia	*25 to 385*	*1 to 6*	*Present study*
Entre Rios do Oeste	*10 to 340*	*0 to 7*	*Present study*
Santa Helena	*30 to 285*	*1 to 5*	*Present study*
Mercedes	*5 a 235*	*1 to 5*	*Present study*
Toledo	*5 to 875*	*0 to 4*	*Present study*

(Source: Brown and James, 2007, taking a variety of sources). The municipalities in bold are considered with similar climate and soil conditions compared to the region W region of PR where this study was conducted. * Indicates samples taken during the dry season. ** Data not available.

As all the farms are on the same general soil type, the main reasons for the large variation in earthworm abundance observed between farms and sample years are more likely due to climatic differences between the sampling years, differences in the age and management of the NT systems, and the history of previous land uses on the farms. The application of certain pesticides on crops (insecticides and fungicides), can also affect negatively the populations of earthworms (20), although little is known of the effects of most pesticides used in maize, soybean, wheat and oats (the major crops grown in the region), on Brazilian earthworms.

Earthworm populations can recover rapidly after long-term conventional tillage practices (34), if provided the necessary soil protection and crop rotation practices, and reduced pesticide usage. However, the speed of recovery of these populations will also depend on the presence of inoculum sources (earthworms already present in the field or that can emigrate from nearby), and the natural richness of the habitat, particularly the presence of abundant soil organic matter, medium-to clayey texture and adequate soil depth. The soils of western Paraná have generally been cultivated for <50 yrs, as the colonization of that region boomed in the mid 1950's, with the cutting of native Atlantic Rainforest and the planting of coffee and subsistence crops. In the 1970's and especially after the 1980's soybean, maize, oat and wheat cropping occupied most of the area, and NT systems were adopted mainly in the 1990's and in the present century. Therefore, most of the soils were intensively tilled for only a few decades (generally <30 yrs) before they were transformed into NT systems, and in many of these farms native earthworms were encountered, indicating that they survived the period of intensive soil disturbance.

The presence or invasion of exotic or peregrine earthworms is another factor of importance determining the abundance of earthworm in a particular site. These earthworms are prolific breeders, generally parthenogenetic, and can rapidly colonize new no-tillage fields (34, 41). In general exotic earthworm species of the *Dichogaster* genus tended to predominate in the no-till sites evaluated in the present study, but these systems also allowed the survival of native species, although in low densities. Previous surveys (Table 2) also encountered mostly exotic species (mainly of *Dichogaster*, *Pontoscolex* and *Amynthas* genera), although in some cases native species of the genera *Andiorrhinus*, *Belladrilus*, *Glossoscolex* and *Fimoscolex* were also found but in low densities (12). However, many of the previous studies did not evaluate earthworm species diversity, and further efforts should be undertaken to adequately describe the species encountered, to achieve a better understanding of the requirements of these species, and how to better manage the NT system to promote earthworm abundance and diversity.

In the present study almost all the genera mentioned by Brown et al. (12) were found, in addition to a native *Glossoscolex* that had not been recorded in no-till fields. The earthworms of the genera *Dichogaster* and *Belladrilus* are small (about 3-5 cm long) and usually reddish. They inhabit the soil surface (between soil and straw) having an epi-endogeic behavior, although they can burrow deeper in the soil when conditions become adverse. Earthworms of the genera *Pontoscolex*, *Glossoscolex* and *Fimoscolex* have an endogeic behavior, living within and burrowing in the soil at greater depths (down to 30 cm). The *Amynthas* are highly dependent on soil organic matter and usually live in the surface litter and the first 10 cm of soil, but can also migrate to greater depths depending on soil water conditions.

The abundance values encountered in the present study were similar to what is often reported for temperate climate cropping systems (20), although the biomass values (data not shown) tended to be much lower, due to the predominance of very small acanthodriline earthworms (*Dichogaster* spp.) and small species of the Glossoscolecidae (*Fimoscolex* and *Glossoscolex* spp.) and Ocnerodrilidae (*Belladrilus* sp.) families.

Taking into account the results of the present study and the data available for northern and western Paraná from previous studies, we proposed a classification of the quality of the no-tillage farms evaluated according to abundance and number of earthworm species (Table 3). In 2010 eight of the 34 farms (25% of the total) fell into the excellent category for abundance and only three for diversity (9%), while four farms fell into the poor category for abundance (11%) and nine for diversity (26%). In 2011, eight of the 25 farms (32% of the total) fell into the excellent category for abundance and only two for diversity (8%), while five farms fell into the poor category for abundance and diversity (20%). For both years, most farms fell into the intermediate categories of good or moderate. The proposed classification using earthworms as bioindicators appears to be a useful way to classify soil quality in no-till farms in western and northern Paraná State, Brazil. However, the methodology must be further tested in other locations, soil types and agroecosystems, and compared with other soil chemical and physical soil quality classification schemes, in order to validate the present classification scheme.

Table 3 Proposed classification of no-till farms according to earthworm abundance and diversity in Rhodic Hapludox of the warmer climate region (Cfa Koeppen) of Paraná State, Brazil.

Classification	Earthworm abundance (No. ind m^{-2})	Number of farms in each abundance category		Number of earthworm species	Number of farms in each diversity category	
		2010	2011		2010	2011
Excelent	≥ 200	8	8	> 6	3	2
Good	≥ 100 to <200	7	4	4 - 5	9	6
Moderate	≥ 25 to <100	15	8	2 - 3	13	12
Poor	< 25	4	5	1	9	5

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