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Earthworms as soil quality indicators in Brazilian no-tillage systems

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ABSTRACT

It is well known that earthworm populations tend to increase under no-tillage (NT) practices, but abundances tend to be highly variable. In the present study, data from the literature together with those on earthworm populations sampled in six watersheds in SW Paraná State, Brazil, were used to build a classification of the biological soil quality of NT systems based on earthworm density and species richness. Earthworms were collected in 34 farms with NT aging from 3 to 27 yr, in February 2010, using an adaptation of the TSBF (Tropical Soil Biology and Fertility) Program method (hand sorting of five 20 cm × 20 cm holes to 20 cm depth). Six forest sites were also sampled in order to compare abundances and species richness with the NT systems. Species richness in the 34 NT sites and in the 6 forests ranged from 1 to 6 species. Most earthworms encountered were exotics belonging to the genus *Dichogaster* (*D. saliens*, *D. gracilis*, *D. bolaui* and *D. affinis*) and native Ocnerodrilidae (mainly *Belladrilus* sp.), all of small individual size. In a few sites, individuals of the Glossoscolecidae (*P. corethrurus*, *Glossoscolex* sp., *Fimoscolex* sp.) and Megascolecidae (*Amynthas gracilis*) families were also encountered, in low densities. *Urobenus brasiliensis* (Glossoscolecidae) were found only in the forest fragments. In the NT farms, earthworm abundance ranged from 5 to 605 ind m^{-2} and in the forest sites, from 10 to 285 ind m^{-2}. The ranking of the NT soil biological quality, based on earthworm abundance and species richness was: poor, with <25 individuals per m^{-2} and 1 sp.; moderate, with ≥25–100 individuals per m^{-2} and 2–3 sp.; good, with >100–200 individuals per m^{-2} and 4–5 sp.; excellent, with >200 individuals per m^{-2} and >6 sp. About 60% of the 34 farms fell into the poor to moderate categories based on this classification, so further improvements to the NT farm's management system are needed to enhance earthworm populations. Nevertheless, further validation of this ranking system is necessary to allow for its wider-spread use.

1. Introduction

No-tillage (NT) is the most widely adopted conservation farming practice in Brazil, where it currently covers more than 26 million hectares (Febrapdp, 2011). According to Brazilian farmers and researchers (e.g., Bolliger et al., 2006; Calegari, 2006; Bartz et al., 2010), this soil management system is based on three principles: (1) minimal soil movement, sufficient only for the placement of seeds and fertilizers in the soil; (2) maintenance of a permanent organic soil cover (usually crop residues), and (3) the adoption of crop rotations and green manures.

The use of NT results in an ecosystem with a lower degree of disturbance or disorder when compared to other management practices that include intense soil mobilization. This is because NT requires less labor and fossil energy, stimulates soil aggregation, reduces erosion and promotes biological control of pests, diseases and weeds, reducing pesticide use (Bartz et al., 2010, 2012). In particular there is a significant recovery of soil biodiversity, and improvement of the soil as a biotic environment, as a result of lower human impacts on the system (Derpsch et al., 1991; Derpsch and Florentín, 2000; Landers, 2001; Pieri et al., 2002; Casão Jr. et al., 2006).

Among the organisms most promoted by the adoption of NT are the earthworms (Brown et al., 2003). This fact led farmers in Paraná to adopt earthworms as the symbol of the "Earthworm Club", currently known as the 'Brazilian No-Till Federation'. The presence of large numbers of earthworms may promote a variety of biotically induced ecosystem services in NT systems, such as: organic matter decomposition, mineralization of nutrients, carbon sequestration, exchange and emission of gases, water infiltration, aggregation, protection of plants against diseases and pests (biological control), and restoration of degraded or contaminated soils (Lavelle et al., 2006). In fact, many farmers in Brazil associate the presence of earthworms with healthy soils, of good quality for cropping (Brown et al., 2003), although there are a few exceptions (Bartz et al., 2009).

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For this reason, and also because earthworm abundance is highly dependent on soil conditions, and their populations change with soil management practices, they have frequently been recognized and proposed as soil quality indicators, both by the scientific community (Paoletti, 1999; Huerta et al., 2009) and by Brazilian farmers (Lima and Brussaard, 2010). In several European countries earthworms are already part of monitoring programs of soil quality (Fründ et al., 2011; Pulleman et al., 2012). However, in Brazil, there is no program for monitoring soil quality at the national level and few initiatives have been developed to classify soils using bioindicators. Therefore, the present study was undertaken to classify the biological soil quality of NT systems, using earthworm abundance and species richness values obtained from the literature and from 34 NT farms belonging to six watersheds in six counties of SW Paraná state. The study was part of a cooperation project between Itaipu Binacional and the Brazilian No-Till Federation entitled "Participatory Methods to Assess Quality in No-Till Systems in the Paraná River Basin 3".

2. Material and methods

2.1. NT farming sites in SW Paraná

The region of this study is part of the third plateau of Paraná, formed mainly by basaltic rocks with some transitions of Caiuá sandstone. The main soil type is Red Latosol (Rhodic Hapludox), followed by Red Nitosol (Rhodic Kandiudox) (Embrapa, 1999a; Soil Survey Staff, 1994). The climate is typical subtropical Cfa, according to Koeppen's classification (1931), characterized by having typical hot, humid summers and no defined dry season. The annual rainfall is below 1250 mm and average annual temperatures in the summer are around 28 °C.

A total of 34 farms in six counties, belonging to six watersheds were sampled (Table 1): five farms in Mineira watershed in Mercedes county; five in Ajuricaba watershed in Marechal Cândido Rondon county; four in Facão Torto watershed in Entre Rios do Oeste county; five in Buriti watershed in Itaipulândia county; five in Pacurí watershed in Santa Helena county; and 10 farms in Toledo watershed in Toledo county. Five secondary forest fragments and an *Araucaria angustifolia* reforestation were used as references of native vegetation, one in each county/watershed (Table 1). In total 40 sites were sampled.

Some of the details of each farm, as well as their geographic coordinates are given in Table 1. All farms were family run and ranged from 5 ha (smallest, Toledo county) to 399 ha (largest, Santa Helena county), with a total average of around 52 ha. The time of NT adoption ranged from a minimum of 3 yr (just one farm) to 27 yr of age (average 14.8 yr), and all except one farm planted two or more crops yr^{-1} (average 2.9 yr^{-1}). Six years is considered to be the minimum age at which the system becomes consolidated (Franchini et al., 2007), but many farmers ($n = 12$) reported that they perform chiseling (surface soil disturbance to 10 cm depth) or subsoiling (to 30+ cm depth) in their NT sites every 2–6 yr mainly to avoid soil compaction (for some crops, such as cassava, this disturbance is necessary at every planting, i.e., every 2 yr).

2.2. Earthworm and soil sampling

Earthworms were sampled using an adaptation of the TSBF – Tropical Soil Biology and Fertility Programme – method (Anderson and Ingram, 1993), and consisted in taking five 20 cm × 20 cm × 20 cm deep monoliths in each site. The samples were spaced at least 20 m from each other and the earthworms hand sorted in the field. This simplification of the sampling methodology aimed to eventually enable farmers themselves to evaluate earthworm populations. Collected earthworms were placed in plastic bags containing 5% formaldehyde solution, taken to the laboratory, counted and identified to family, genus and species level, when possible, according to identification keys and descriptions of Righi (1990, 1995) and Blakemore (2002).

Bulk soil samples (5 samples per site, mixed thoroughly) were also collected for textural (% sand, silt, clay) and chemical analyses: pH $CaCl_2$ (pH), aluminum (Al^{3+}), exchangeable aluminum (Al + H), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), phosphorus (P), cation exchange capacity (CEC) and organic matter (OM) according to Embrapa (1997, 1999b).

2.3. Data analysis

All variables were subjected to normality tests (*Shapiro–Wilk*). The biological variables (abundance and average number of species per site) were submitted to analysis of variance (*Kruskal–Wallis*) and mean tests (*Dunn* and *Mann-Whitney U*), using the softwares BioStat 5.0 (Ayres et al., 2007) and Statistica 7.0 (Statsoft, 2004). Linear regressions were performed between all biological and soil variables measured both in the forests and the NT farms, together with the age of NT, the size of the farm and the number of crops grown on farm, to obtain Pearson's correlation coefficients and test their significance using Statistica 7.0.

The biological data (earthworm abundance – N Ew, total number of species per site – TN sps) were used to obtain the gradient length (DCA). Because this length was smaller than three (linear response), a principal component analysis (PCA) was performed using CANOCO version 4.5 (Ter Braak and Šmilauer, 2002), and the environmental variables (pH Al, Al + H, K, Ca, Mg, P, OM clay, silt and sand) used as explanatory variables.

Additional data on earthworm populations in NT fields throughout Brazil were obtained from the literature and these were used together with the results of the present study to perform a ranking of the biological soil quality of the NT farms according to earthworm populations, based on abundance and species richness values. This ranking included four category levels: excellent, good, moderate and poor.

3. Results

3.1. Soil properties

Chemical properties and particle size analysis results of the soils at all sample sites are shown in Table 2. All soils were slightly-to-highly acid with pH under 7, despite lime application on NT farms. CEC values were moderate, both in the forest and NT farm soils. All farms had clayey soil texture (with clay contents above 40%), except for farms 5 (sandy clay loam) and 3 (clay loam) in Mineira watershed and farms 2, 3 and 4 in Facão Torto watershed (clay loam). Four of the forest sites had clayey soils, but in Buriti watershed the soil was a clay loam and in Facão Torto watershed, a silty clay loam. Soil organic matter contents were generally high (above 3%) in most NT farms, except in Ajuricaba watershed where values tended to be lower. Potassium and phosphorus values showed high variation, probably due mainly to different fertilization regimes on farm, as well as to soil texture and OM contents. Overall, considering all farms and forest sites, both K and P values were significantly ($p < 0.01$) higher in the NT farms than in the forests. On the other hand, OM and Ca values were significantly ($p < 0.01$) higher in the forests than on farm soils.

A significant positive relationship between the age of NT of the farms and soil OM contents was observed ($r^2 = 0.35$, $p < 0.05$), indicating that older NT systems tended to have higher soil OM contents. This was the only significant relationship found with farm

Table 1

Selected characteristics of the study sites (size, no-till age, number of crops planted the last three years) and data earthworm abundance (no. ind. m^{-2}), average number of species per sample and total number of species per site.

Watershed	Site	Location		Size of the site	No-till age	Number of crops	Number of earthworms[b]		Average number of species per sample[b]		Total number of species in the site
		Latitude	Longitude	(ha)	(yr)[a]		ind. m^{-2}	SE[c]	No.	SE[c]	
Mineira	1	24° 27′ 8.28″ S	54° 8′ 53.19″W	68	6	2	5	11	0.2	0.45	1
	2	24° 26′ 17.79″ S	54° 9′ 17.74″W	12	22*	2	45	7	1.2	1.79	4
	3	24° 26′ 52.43″ S	54° 8′ 59.38″W	36	27*	4	55	37	0.8	0.45	1
	4	24° 24′ 48.01″ S	54° 9′ 41.79″W	12	10*	4	235	140	1.8	1.1	3
	5	24° 27′ 42.25″ S	54° 9′ 21.87″W	12	20*	2	105	91	1.6	1.14	4
	F	24° 27′ 15.99″ S	54° 8′ 43.64″W	–	–	–	55	37	0.8	0.45	1
Ajuricaba	1	24° 35′ 54.50″ S	54° 7′ 51.27″W	7	13*	4	50	53	0.8	0.84	1
	2	24° 33′ 51.48″ S	54° 6′ 40.20″W	12	12	4	605	460	2.6	1.67	6
	3	24° 35′ 21.47″ S	54° 8′ 2.83″W	133	12	4	65	63	1	1	3
	4	24° 36′ 42.40″ S	54° 9′ 22.95″W	7	7*	2	40	89	0.5	1	2
	5	24° 36′ 15.12″ S	54° 9′ 34.50″W	36	9	3	305	316	2.2	1.64	5
	F	24° 35′ 46.51″ S	54° 8′ 1.26″W	–	–	–	105	54	1.8	0.84	4
Facão Torto	1	24°42′29.47″S	54°13′30.89″W	165	15*	3	**340b**	398	**2bc**	1	4
	2	24° 43′ 40.64″ S	54° 11′ 43.30″W	12	17	1	**190ab**	144	**1.8ab**	0.84	3
	3	24° 42′ 29.42″ S	54° 13′ 58.38″W	?	3	2	**295ab**	185	**2bc**	0.71	3
	4	24° 43′ 21.17″ S	54° 12′ 37.71″W	5	20	3	**10a**	14	**0.4a**	0.55	1
	F	24° 42′ 50.39″ S	54° 13′ 31.73″W	–	–	–	**275ab**	127	**2.6c**	0.55	4
Buriti	1	25° 9′ 21.92″ S	54° 14′ 9.35″W	73	13*	4	**70ab**	57	**1.2ab**	1.1	4
	2	25° 9′ 49.78″ S	54° 15′ 7.35″W	17	14	3	**60ab**	76	**1ab**	0.71	2
	3	25° 9′ 10.73″ S	54° 14′ 51.09″W	48	13	2	**25ab**	31	**0.6ab**	0.55	1
	4	25° 9′ 21.53″ S	54° 14′ 45.05″W	12	12	4	**85ab**	123	**1.6ab**	1.52	6
	5	25° 9′ 45.24″ S	54° 14′ 55.82″W	17	18	4	**205b**	142	**2.4b**	1.34	6
	F	25° 9′ 3.50″ S	54° 15′ 19.60″W	–	**-**	**-**	**10a**	14	**0.4a**	0.55	2
Pacurí	1	24° 58′ 35.69″ S	54° 18′ 21.67″W	399	20*	4	110	163	1.6	1.34	4
	2	24° 57′ 3.16″ S	54° 17′ 36.21″W	121	18	3	285	270	1.8	0.45	5
	3	24° 56′ 58.19″ S	54° 18′ 10.74″W	31	15*	3	80	76	1.6	1.14	4
	4	24° 55′ 25.88″ S	54° 17′ 3.54″W	24	12	2	30	33	0.6	0.55	1
	5	24° 55′ 19.42″ S	54° 17′ 33.17″W	44	14	3	125	77	1.8	0.84	2
	F	24° 56′ 55.96″ S	54° 17′ 13.31″W	–	–	–	25	18	0.8	0.45	3
Toledo	1	24° 45′2.53″ S	53° 34′ 8.25″W	11	10	3	185	221	1.2	0.84	3
	2	24° 46′0.85″ S	53° 35′ 20.99″W	45	15	4	50	64	1	1	2
	3	24° 45′32.53″ S	53° 37′ 17.56″W	102	24	3	20	11	0.8	0.45	2
	4	24° 44′31.30″ S	53° 38′ 17.35″W	44	19	3	50	61	0.8	0.84	1
	5	24° 44′3.98″ S	53° 36′ 3.23″W	27	25	3	30	27	1	0.71	2
	6	24° 43′ 48.81″ S	53° 40′ 50.42″W	36	18	2	20	45	0.2	0.45	1
	7	24° 44′ 31.21″ S	53° 40′ 9.04″W	17	8*	2	120	187	1.8	1.48	4
	8	24° 44′ 51.06″ S	53° 45′ 3.77″W	5	10*	3	55	57	0.6	0.55	1
	9	24° 45′ 5.15″ S	53° 40′ 39.59″W	6	12	2	265	271	1.4	1.14	3
	10	24° 45′26.12″ S	53° 40′ 37.36″W	117	20	3	95	97	1.8	1.79	4
	RF	24° 45′11.03″ S	53° 34′ 17.45″W	–	–	–	285	541	2	2.35	6
Total means			No-till farms	51.9	14.8	2.9	127	22	1.3	0.11	2.9
			Forest sites	–	–	–	126	50	1.4	0.35	3.3

[a] An asterisk (*) indicates farmers who perform chiseling or subsoiling every 2–6 yr.

[b] Different letters in the same watershed mean significant differences at $P < 0.05$, using Kruskal–Wallis analysis of variance and Dunn mean test.

[c] SE = Standard error of the mean.

Table 2
Chemical attributes and particle size distribution of the soils at the sample sites in each watershed (F = forest, RF = reforestation).

Watershed, County	Site	pH $CaCl_2$	Al	H + Al	K	Ca	Mg	CEC	P	OM	Clay	Silt	Sand
			$cmol_c\ dm^{-3}$						$mg\ dm^{-3}$		$g\ kg^{-1}$		
Mineira, Mercedes	1	5.80	3.47	0.00	0.36	6.40	3.50	13.3	4.63	33.8	429	309	263
	2	5.60	4.44	0.00	0.25	8.20	3.30	16.2	2.28	30.0	282	307	412
	3	5.70	4.12	0.00	0.38	7.80	1.90	14.2	7.05	36.5	355	329	317
	4	5.60	4.51	0.00	0.57	9.50	2.40	16.7	11.28	31.2	405	292	304
	5	5.10	5.04	0.05	0.38	4.70	2.20	12.3	6.23	28.7	223	176	602
	F	5.00	4.96	0.00	0.29	5.50	2.20	13.8	1.51	37.9	638	139	224
Ajuricaba, Marechal Cândido Rondon	1	5.40	4.28	0.00	0.23	6.10	2.80	13.4	5.43	25.0	624	161	216
	2	5.30	4.37	0.00	0.42	9.00	4.90	18.7	7.88	26.2	514	221	266
	3	5.80	3.53	0.00	0.62	6.80	2.90	13.9	13.04	35.2	469	219	312
	4	5.70	3.35	0.00	0.36	5.20	3.10	12.0	19.54	28.7	632	125	244
	5	5.80	3.55	0.00	0.39	5.30	2.60	11.8	56.45	26.2	576	146	279
	F	5.30	5.11	0.00	0.12	10.00	5.50	20.7	3.05	39.3	464	187	350
Facão, Torto Entre Rios do Oestes	1	6.20	3.01	0.00	0.50	4.85	3.15	11.5	20.69	30.6	583	224	193
	2	5.30	5.23	0.00	0.58	6.00	3.40	15.2	9.56	32.5	266	492	243
	3	5.00	5.55	0.05	0.26	4.60	3.70	14.1	35.74	28.7	369	414	218
	4	5.60	3.97	0.00	0.60	5.60	2.90	13.1	5.43	30.0	322	411	268
	F	5.30	5.71	0.00	0.14	7.50	2.60	16.0	3.05	43.6	294	500	207
Buriti, Itaipulândia	1	5.30	5.42	0.00	0.32	5.80	2.10	13.6	27.74	39.3	404	398	198
	2	5.25	5.33	0.00	0.56	6.10	1.40	13.4	15.76	36.6	427	316	258
	3	5.40	4.06	0.00	0.28	5.60	1.30	11.2	3.05	43.6	434	344	223
	4	5.35	4.51	0.00	0.43	5.20	1.65	11.8	9.58	34.5	550	302	149
	5	6.20	3.15	0.00	0.57	5.75	3.40	12.9	34.20	32.0	547	279	175
	F	6.10	3.23	0.00	0.30	6.80	2.10	12.4	1.51	42.1	384	362	255
Pacurí, Santa Helena	1	5.80	3.13	0.00	0.55	5.80	3.60	13.1	10.41	35.2	497	286	218
	2	5.55	4.51	0.00	0.37	5.25	2.10	12.2	23.95	34.7	546	286	169
	3	5.80	3.53	0.00	0.72	5.90	3.50	13.7	34.55	32.5	523	292	186
	4	5.50	4.25	0.00	0.48	5.40	4.50	14.6	26.67	35.2	603	287	110
	5	4.90	5.26	0.05	0.33	5.40	2.90	13.9	4.63	30.0	544	221	236
	F	5.00	4.96	0.00	0.29	5.50	2.20	13.0	1.51	37.9	638	139	224
Toledo, Toledo	1	4.90	7.52	0.10	0.35	4.10	2.80	14.8	16.68	39.3	641	88	272
	2	5.10	5.93	0.00	0.50	4.20	2.30	12.9	9.56	37.9	639	119	243
	3	5.75	4.15	0.00	0.45	5.05	3.30	12.9	13.55	41.7	599	296	106
	4	5.40	4.85	0.00	0.41	5.60	2.70	13.6	17.62	35.2	619	270	112
	5	5.30	4.57	0.00	0.32	4.20	3.30	12.4	10.41	40.7	632	245	124
	6	4.50	6.93	0.25	0.22	3.90	2.20	13.3	13.93	35.2	735	152	113
	7	4.60	6.58	0.25	0.32	3.40	2.00	12.3	26.67	31.2	593	177	231
	8	5.30	4.28	0.00	0.35	4.70	2.20	11.5	0.75	21.5	524	170	307
	9	5.10	4.51	0.05	0.42	4.10	2.20	11.2	24.56	32.5	525	173	302
	10	4.90	5.26	0.10	0.22	4.80	1.20	11.5	5.43	37.9	627	170	204
	RF	4.90	5.80	0.10	0.09	4.40	2.50	12.8	0.75	46.6	502	205	293

characteristics (size, age and no. of crops) and the biological and soil variables analyzed.

3.2. *Earthworm abundance and species richness in forests and NT farms*

A total of 11 species belonging to four earthworm families and were recovered in the samples (all sites combined), of which a total of 755 ind. m^{-2} were from forest sites and 815 ind. m^{-2} from NT farms (Table 3). Mean abundance at each site ranged from 5 to 605 ind. m^{-2} in the NT sites and 5 to 285 ind. m^{-2} in the forest sites (F, RF); however, overall means in the forest and NT sites were practically identical, i.e., around 126 ind. m^{-2} (Table 1). Significant differences in the total abundance and in the average number of earthworms collected sample^{-1} at each site were only observed in two watersheds (Facão Torto and Buriti, Table 1). At Buriti, very few earthworms and low species richness were found in the forest, whereas in Facão Torto, the opposite was observed (high abundance and species richness sample^{-1}).

Species richness in all sites ranged from 1 to 6 species (Table 1), and total richness (10 sp.), number of species sample^{-1} and mean species richness in forests and NT farms were not significantly different (Tables 1 and 3).

Most individuals collected were of the Acanthodrilidae family, genus *Dichogaster*, belonging to four species: *D. saliens*, *D. gracilis*, *D. bolaui* and *D. affinis* (all exotic), although highest densities were of *Dichogaster* spp. juveniles (Table 3). Two species of Ocnerodrilidae family were not identifiable to species level: Ocnerodrilidae sp. (unknown origin) and *Belladrilus* sp. (native). The former species had the second highest abundance of all species collected, and the latter species was found only in two NT farms (Table 3). Only one species was of the Megascolecidae family: *Amynthas gracilis* (exotic), recovered only in the forests of Ajuricaba, Facão Torto and Toledo, and from NT farms ($n = 4$) in Toledo. Four species of the Glossoscolecidae family were found: *Urobenus brasiliensis*, *Glososcolex* sp. and *Fimoscolex* sp. (all three native) and a peregrine species, *Pontoscolex corethrurus* (Table 3). The first of these was found only in the forests sites, while the remaining were found in both ecosystems.

Significant differences in the number of individuals collected in forests vs. NT farms were encountered for a few species at a few sites (Table 3). In Mineira and Toledo, *U. brasiliensis* densities were higher in the forest than in NT, while the opposite was observed for *D. gracilis* and *Dichogaster* juvenile densities in Mineira. In Ajuricaba, the abundance of juvenile worms and of Oncerodrilidae sp. were higher in the NT sites, while in Facão Torto, densities of *D. gracilis*, *D. saliens* and *Dichogaster* juveniles were higher in NT and densities of *P. corethrurus* and *A. gracilis* were higher in the forest. In Buriti,

Table 3

Total species richness, average and total abundance (no. ind. m^{-2}) of the earthworm species identified in the 34 farms under no-till (NT), five forests (F) and one reforestation site (RF), in the 6 watersheds (Toledo, Pacurí, Buriti, Facão Torto, Ajuricada and Mineira) in the Paraná River Basin 3. Values represent means (first column) and standard errors (SE, second column).

Oligochaeta families and species	Origin	Mineira[a]				Ajuricaba[a]				Facão Torto[a]				Buriti[a]				Pacurí[a]				Toledo[a]				Total[a]			
		F	SE[b]	NT	SE[b]	F	SE[b]	NT	SE[b]	F	SE[b]	NT	SE[b]	F	SE[b]	NT	SE[b]	F	SE[b]	NT	SE[b]	RF	SE[b]	NT	SE[b]	F	SE[b]	NT	SE[b]
Glossoscolecidae																													
Pontoscolex corethrurus	Peregrine	0	0	0	0	25	16	1	1	**160b**	45	**0a**	0	0	0	0	0	0	0	0	0	0	0	7	4	185	143	8	12
Urobenus brasiliensis	Native	**55a**	17	**0a**	0	0	0	0	0	0	0	0	0	5	5	0	0	10	6	0	0	**25b**	16	**0a**	0	**95b**	48	**0a**	0
Glossoscolex sp.	Native	0	0	0	0	0	0	10	10	0	0	0	0	**0a**	0	**24b**	7	15	10	3	3	0	0	0	0	**15a**	14	**37b**	21
Fimoscolex sp.	Native	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	20	15	1	1	**20b**	18	**3a**	2
Megascolecidae																													
Amynthas gracilis	Exotic	0	0	0	0	30	18	0	0	**85b**	22	**0a**	0	0	0	0	0	0	0	0	0	30	24	4	3	145	74	4	6
Acanthodrilidae																													
Dichogaster saliens	Exotic	0	0	11	6	10	10	18	13	**0a**	0	**15b**	8	0	0	8	5	**0a**	0	**29b**	17	0	0	12	7	10	9	93	18
Dichogaster gracilis	Exotic	**0a**	0	**10b**	3	0	0	11	8	**0a**	0	**10b**	5	0	0	4	2	**0a**	0	**15b**	5	5	5	7	5	5	4	57	8
Dichogaster bolaui	Exotic	0	0	4	2	0	0	12	8	0	0	10	10	0	0	2	1	0	0	1	1	20	20	8	3	20	18	37	13
Dichogaster affinis	Exotic	0	0	0	0	25	8	15	13	5	5	0	0	**0a**	0	**4b**	1	0	0	2	2	0	0	0	0	30	22	21	13
Dichogaster spp.	Exotic	**0a**	0	**43b**	19	15	10	62	29	**10a**	10	**61b**	27	5	5	24	11	**0a**	0	**51b**	24	35	35	31	15	**65a**	30	**272b**	31
Ocnerodrolidade																													
Ocnerodrilidae sp.	?[c]	0	0	17	13	**0a**	0	**70b**	43	10	6	104	39	0	0	19	17	**0a**	0	**11b**	2	75	75	6	3	**85a**	67	**226b**	72
Belladrilus sp.	Native	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	3	1	0	0	5	5
Juveniles (general)		0	0	4	2	**0a**	0	**13b**	9	5	5	9	7	0	0	3	2	0	0	12	4	75	57	14	5	80	68	55	21
Total abundance		55	17	89	40	105	24	213	110	270	57	209	74	**10a**	6	**89a**	31	**16a**	8	**126b**	43	222	242	89	26	755	277	815	113
Species richness		1		4		4		8		4		3		2		7		2		7		7		8		10		10	

[a] Different letters in the same watershed mean significant differences between the forest site and the no-till site, using Mann-Whitney U test; lower case letters $P < 0.05$ and capital letters $P < 0.10$.

[b] SE = Standard error of the mean

[c] ? = Unknown origin.

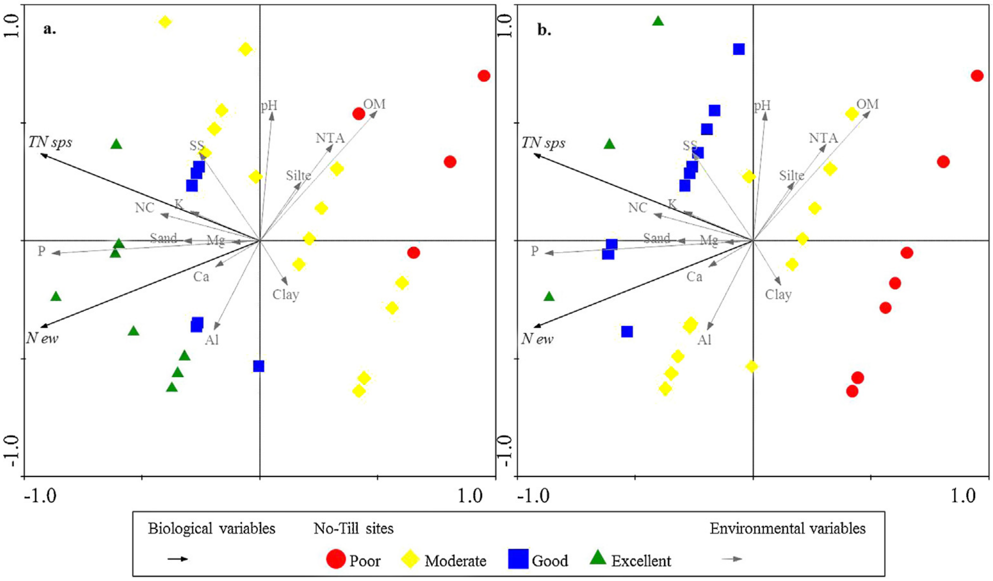

Fig. 1. Principal component analysis of the biological variables *N* sps (number of species per site) and *N* Ew (number of earthworms per site) of the 34 sites in the six watersheds of the Paraná River Basin, using chemical–physical variables* and site characteristics** as explanatory variables. (a) No-till sites arranged according the N Ew classification and (b) no-till sites arranged according de TN sps classification. (*Notes*: *Chemical–physical attributes: pH $CaCl_2$ (pH), aluminum (Al), exchangeable aluminum (Al + H), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), organic matter (OM) and soil texture (% clay, silt and sand). **Site characteristics: size of the farm (SS), no tillage age (NTA) and number of crops (NC)).

densities of both *Glossoscolex* sp. and *D. affinis* were higher in NT, while in Pacurí, densities of *D. saliens* and *D. gracilis* were all higher in NT. Combining all earthworm species, total densities were higher in NT farms than in forest sites both at Buriti and Pacurí (Table 3). Finally, combining all watersheds, densities of *U. brasiliensis* and *Fimoscolex* were higher in the forest sites while *Glossoscolex* sp., *Dichogaster* juveniles and Ocnerodrilidae sp. densities were higher in the NT farms.

Overall, combining all NT farms, earthworm abundance was positively correlated with both total species richness ($r^2 = 0.65$, $p < 0.05$) and no. of species collected sample^{-1} ($r^2 = 0.79$, $p < 0.05$), indicating that when more earthworms were collected, there was a higher probability of also collecting more species. Therefore, farms with higher earthworm abundance also tended to have higher species richness. Combining all forest sites, a significant positive relationship was also found only between earthworm abundance and no. of species sample^{-1} ($r^2 = 0.91$, $p < 0.05$).

3.3. *Earthworm relationships with soils and farm properties*

Combining all farms, significant relationships ($p < 0.05$) were found between earthworm abundance and soil OM content ($r^2 = -0.36$) and the sum of all bases (K + Ca + Mg + Na) ($r^2 = 0.35$), as well as between soil P contents and total species richness ($r^2 = 0.41$) and the no. of species collected sample^{-1} ($r^2 = 0.37$). Combining all forest sites, significant ($p < 0.05$) relationships were found between soil K content and earthworm abundance ($r^2 = -0.85$), total species richness and no. of species collected sample^{-1} ($r^2 = -0.9$ for both variables), as well as between exchangeable acidity (H + Al) and total species richness ($r^2 = 0.91$) and the no. of species collected sample^{-1} ($r^2 = 0.82$) and between soil pH and total species richness ($r^2 = -0.86$). Combining all farms, earthworm abundance and species richness were similar on farms that did ($n = 12$) vs. did not ($n = 22$) use subsoiling or chiseling every few years to avoid/reduce soil compaction (see Table 1).

The PCA shows the distribution of the 34 NT farm sites colored according earthworm abundance (number of earthworms, N Ew; Fig. 1a) and species richness (total number of earthworms species, TN sps; Fig. 1b), using the environmental variables [soil texture and chemical attributes and site characteristics, i.e., size of the site (SS), NT age (NTA) and number of crops (NC)] as explanatory variables. The dispersion of the points follows the no-till sites classification according the proposed classification (see Section 3.4). The first axis explained 86.4% of the variance and the second axis 13.6%. The environmental variables explained 49.3% of the biological data, and of these 92.6% were represented in the first axis.

Fig. 1a shows the NT sites colored according earthworm abundance. In general, sites with higher earthworm abundance (green and blue) were grouped near the N Ew variable, and associated mainly with higher soil P, but also higher Ca, Al, Mg and sand contents. Sites with lower densities (yellow and red) were opposed to N Ew, and associated mainly with higher soil OM, but also with higher pH and silt contents and older NT systems (NTA).

Fig. 1b shows the NT sites colored according the number of earthworm species per site. The sites with higher species richness (green and blue), near the TN sps variable, were basically associated with higher soil P, but also higher K content, number of crops (NC) and size of the site (SS).

3.4. *Biological soil quality of NT farms*

Earthworms populations have been assessed at 24 counties in the state of Paraná, as well as in at least eight other counties in the states of Goiás, São Paulo, Santa Catarina, Mato Grosso do Sul, Rio de Janeiro and Rio Grande do Sul (Table 4). Earthworm abundance ranged from a minimum of 3 ind. m^{-2} (measured in the dry season in Rolândia-PR) up to a maximum of 625 ind. m^{-2} (Chapecó-SC). Species richness ranged from a minimum of 1–6 species per site, with most sites having 2–3 species. The abundance values reported showed an approximately equal spread of ranges between 0–25, 25–100, 100–200 and >200 ind. m^{-2} ($n = 10$–13 each). Therefore, these four ranges of values of earthworm abundance were chosen to classify the biological soil quality of NT farms (Table 5), and the sample sites of the present study were ranked according to the following classes: <25 ind. m^{-2} = poor; ≥25–<100 ind. m^{-2} = moderate; ≥100–<200 ind. m^{-2} = good; and

Table 4
Average number of earthworms (ind. m^{-2}) found in various parts of Brazil in sites under no-tillage.

Site (county, state)[a]	Abundance (no. ind. m^{-2})[b]	No. of species	References
Arapongas, PR	**18–37**	**nd**[c]	Brown et al. (2008)
Londrina, PR	**40*–100**	**3**	Brown et al. (2003, 2004), Derpsch et al. (1984, 1991)
Cornélio Procópio, PR	**176**	**nd**	Brown et al. (2004)
Bela Vista do Paraíso, PR	**10*–291**	**nd**	Brown et al. (2003), Benito (2002)
Lerroville, PR	**48–240**	**3**	Brown et al. (2004)
Rolândia–PR	**3*–214**	**>4**	Derpsch et al. (1991), Guimarães et al. (2002), Brown et al. (2003), Benito et al. (2008), Bartz (2011)
Cafeara, PR	**6–42**	**>3**	Brown et al. (2004, 2008)
Campo Mourão, PR	**12–144**	**>3**	Brown et al. (2004)
São Jerônimo da Serra, PR	**142**	**1**	Brown et al. (2004)
Nova Aurora, PR	**50–238**	**nd**	Brown et al. (2008)
Cafelândia, PR	**363**	**nd**	Brown et al. (2008)
Cascavel, PR	**176**	**nd**	Brown et al. (2008)
Palotina, PR	**18–510**	**nd**	Brown et al. (2008)
Guarapuava, PR	3–12	nd	Mafra et al. (2002)
Carambeí, PR	44–118	3	Tanck et al. (2000), Brown and Sautter (unpublished data)
Arapoti, PR	72–168	3	Peixoto and Marochi (1996), Brown (unpublished data)
Ponta Grossa, PR	44–117	2	Voss (1986)
Castro, PR	123	nd	Ressetti (2004)
Santo Antônio de Goiás, GO	25–250	3	Brown (unpublished data)
Santa Helena, GO	288–340	nd	Minette (2000), Brown (unpublished data)
Planaltina, GO	164	nd	Marchão et al. (2009)
Taciba, SP	138	>2	Brown et al. (unpublished data)
Dourados, MS	6–264	nd	Da Silva et al. (2006), Aquino et al. (2000)
Seropédica, RJ	67–320	nd	Rodrigues et al. (2004), Aquino (2001)
Teutônia, RS	28*–299	2	Krabbe et al. (1993, 1994)
Chapecó, SC	150–625	nd	Baretta et al. (2003)
Mercedes, PR	*5–235*	*1–4*	*Present study*
Marechal Cândido Rondon, PR	*40–605*	*1–6*	*Present study*
Entre Rios do Oeste, PR	*10–340*	*1–4*	*Present study*
Itaipulândia, PR	*25–205*	*1–6*	*Present study*
Santa Helena, PR	*30–285*	*1–5*	*Present study*
Toledo, PR	*20–265*	*1–4*	*Present study*

Expanded from Brown and James (2007).

[a] Counties in bold have similar climate (Cfa) and soils found in the present study sites (SW Paraná). Counties in italics are those of the present study.

[b] Asterisk (*) indicates samples taken in the dry season.

[c] nd = not determined.

Table 5
Biological soil quality of NT farming systems based on the average number of earthworms (per sample and ind. m^{-2}) and species richness and the number of farms falling into each category in the present study.

Classification	Average number of earthworms (per sample)	Average number of earthworms (ind. m^{-2})	No. of farms	Total no. of earthworm species	No. of farms
Excellent	≥8	≥200	8	>6	3
Good	≥4–<8	≥100–<200	6	4–5	10
Moderate	≥1–<4	≥25–<100	16	2–3	12
Poor	<1	<25	4	1	9

≥200 ind. m^{-2} = excellent (Table 5). Of the 34 sample sites 4 were ranked as poor, 16 as moderate, 6 as good and 8 as excellent (Table 5).

Unfortunately, most of the sites studied in Table 5 did not measure earthworm species richness, so a classification scheme of the biological soil quality of the NT sites was proposed based on the results of the present study: one species = poor; 2–3 species = moderate; 4–5 species = good; and >6 species = excellent (Table 5). Of the 34 sample sites 9 were ranked as poor, 12 as moderate, 10 as good and only 3 as excellent (Table 5).

4. Discussion

It is well known from the literature that crops under NT and minimum tillage have higher populations of earthworms than those submitted to conventional tillage, mainly due to the negative effects of extensive and frequent soil disturbance on earthworm populations (Brown et al., 2003, 2008; Sautter et al., 2007), and the associated reduction in soil OM contents (food for the earthworms). The adoption of NT generally increases soil OM contents (Franchini et al., 2004), and positive correlations were found between the age of the NT farms and soil OM values in the present study. Soil OM and decomposing plant residues are the primary food source for earthworms (Brown et al., 2000), and their increase in NT systems generally leads to higher earthworm abundance (Brown et al., 2004; Hendrix et al., 1992). However, contrary to the expected, in the present study earthworm abundance was negatively related to soil OM contents on the NT farms. Nevertheless, other factors that affect earthworm populations on farm (and that were not measured here), both environmental and management-based may be partly responsible for these results (Edwards and Bohlen, 1996). Further sampling efforts are necessary to elucidate this phenomenon in the study region.

Chisel plowing or subsoiling in some of the NT fields in the present study (every 2–4 yr to avoid soil compaction) can affect crop residue decomposition and soil OM dynamics and may negatively affect earthworm populations, although no difference in abundance or diversity of worms were found comparing farms that did or did not use these techniques. Chiseling or subsoiling is usually applied to older NT sites when there is not enough crop rotation and green manuring to prevent soil compaction. Chisel plowing tends to be less harmful to earthworms than moldboard plowing (used in CT) or subsoiling due to a lower frequency of use and lower volume of soil plowed (Brown et al., 2003).

Crop rotations including a variety of commercial and cover crops are also important for earthworm populations, because they determine both richness and quality of food available as well as the rate of soil OM accumulation (Franchini et al., 2004). Differences between earthworm populations under rotations and simple double cropping have been observed in some cases in Paraná (Sautter et al., 2007), but the small number of samples and replicates does not yet permit generalizations. In the present study no relationships were found between the number of crops and earthworm populations.

With NT, soil P contents also tend to increase substantially, especially in the topsoil, mainly due to P fertilization (Gatiboni et al., 2007), although no relationship between age of NT farms and P contents were observed in the present study. Nevertheless, significant relationships were found between P contents and total earthworm species richness and the number of species collected in each sample. The multivariate analysis performed (PCA) also showed the important relationship between soil P and earthworm abundance and species richness in the present study. Higher soil P contents have been shown to be important determinants of *P. corethrurus* growth and biomass (Brown et al., 2007) and C/P ratios affected soil ingestion rates of this species (Marichal et al., 2012), but little is known of the relationships between earthworm communities and soil P and this topic warrants further attention, particularly in Brazilian NT systems.

Surveys of earthworms populations in agroecosystems of Paraná have mostly found exotic or invasive earthworms, mainly of the genera *Dichogaster* and *Pontoscolex*, although some native species of the genera *Andriorrhinus*, *Belladrilus*, *Glossoscolex* and *Fimoscolex* may also be present but in low densities (Brown et al., 2008). In forests of Northern Paraná, native endogeic and epigeic (saprophagic) species are dominant, mainly of the genera *Glossoscolex* and *Urobenus*, respectively. These genera are not normally found in cultivated systems, due to soil disturbance and/or absence of a dense and diverse straw layer, necessary for the survival of epigeic species. In the present survey most of the genera mentioned by Brown et al. (2008) were found, including *Glossoscolex*, a genus that had not yet been registered in NT fields.

Earthworms in the genera *Dichogaster* and of the Oncerodrilidae family (e.g., *Belladrilus*) are small (about 3–5 cm long) and usually reddish. They inhabit the soil surface (between soil and straw) and behave as epi-endogeics, but can burrow deeper into the soil under adverse soil moisture conditions. These earthworms were the most abundant found in NT fields, and tend to be much more resistant to soil disturbance, probably due to their small size, their feeding habit and reproductive strategy (*Dichogaster* are '*r*' strategists). The earthworms collected belonging to *Pontoscolex*, *Fimoscolex* and *Glossoscolex* are all endogeic and the former two were much more abundant in the forest system, while the latter was more abundant in the NT fields. Nevertheless, the three species were able to resist the farming practices applied to NT fields, although they survived only in low numbers. On the other hand, *Amynthas* and *Urobenus* were found mainly or exclusively in the forest sites in the present study. These earthworms are dependent on high soil OM levels and usually live in the residues and the first 10 cm of soil (*Amynthas* can also migrate to greater depths depending on soil water conditions).

The multivariate analysis showed clearly the importance of both soil OM (negative relationship) and P (positive relationship) contents in determining earthworm abundance and species richness. Sites with high earthworm abundance had lower soil OM and higher soil P and vice versa. The classes chosen for the ranking of the biological quality of the NT farms (Table 5) also revealed the influence of these two factors in their placement on the graphical planes of the PCA, for earthworm abundance (Fig. 1a) and especially for species richness (Fig. 1b), since the chosen classes were arranged mainly along the *x*-axis.

A comparison of the soil biological quality classes proposed in the present study with those proposed by the FAO mainly for temperate climate (Europe, New Zealand) agroecosystems (Shepherd et al., 2008) shows clearly the difference in abundance and diversity values typical of European and New Zealand vs. Southern Brazilian worm populations. In the FAO manual scores for good (>30 worms of preferably 3 or more species), moderate (15–30 and preferably 2 or more species) and poor (<15 and predominantly 1 species) involve much higher earthworm abundance and slightly lower earthworm diversity per sample than those proposed here. This comparison also highlights the importance of regionally obtained indexes that consider the typical earthworm abundance values in a region, that are governed by various biogeographical, edaphic and climatic factors, as well as human management of the ecosystem (Brown and Domínguez, 2010). Therefore, the use of abundance values obtained from temperate climate regions such as Europe or New Zealand in warm tropical climates is not reasonable, and may lead to erroneous interpretations of soil quality.

The present ranking system is probably most applicable for NT sites in W, SW and N regions of Paraná, where both climates (Cfb) and soils (Oxisols) are similar. However, further validation is necessary given that the results are based on only a single sampling, and because earthworm populations are known to vary from year to year. This validation should include expansion to other farms in the region and sampling in multiple years, together with analysis of soil properties and gathering of the information on farm management practices and crop yields. This should help solve the riddle of the inverse relationships between earthworm abundance and soil OM and reveal the adequacy (or need for adjustment) of the present NT soil biological quality classification system.

Of the 34 farms studied, only three were in the excellent class based on earthworm species richness, while a much higher number of farms were in the excellent category for abundance ($n = 8$). This is because most farms tend to have 4 species or less (with an average of close to 3 per farm in the present study), and indicates that the choice of 6 species as the cut-off value for excellent may be too high, particularly since only five farms had 5 or more species. Nevertheless, as earthworm abundance was related to earthworm species richness, and higher species richness in a field is a desirable trait for ecosystem resilience and ecosystem services (Coleman and Whitman, 2005), we advocate that NT farming practices should strive to increase both earthworm abundance and species richness. Furthermore, ecological strategy diversification is also desirable (epigeic, endogeic and anecic species) although most NT farms in Paraná have only endogeics. Anecic species are conspicuously absent from NT systems in Brazil and in fact very few anecic species are known from the country that could possibly function as vertical burrowers and incorporators of surface residues. The stimulation of epi-endogeics such as *Urobenus brasiliensis* and *Amynthas* sp. are an interesting alternative that merits further attention, but NT management must first provide adequate habitat for these earthworms to survive and thrive in the ecosystem. These species are already dominant in the colder climate (Cfb Köppen) region of Southern Brazil, where they perform important services to the soil and increase crop production (Peixoto and Marochi, 1996).

Analysis of the abundance and species richness values in Table 5 and Table 1 reveal that many sites are still below desirable levels (good and excellent scores) of earthworm abundance and species richness (Table 5). Therefore, in terms of soil biological quality, many of these NT systems could still benefit from significant improvement in management practices to enhance earthworm abundance and diversity. These should include greater crop diversification and use of legume cover-crops and cover-crops that help break compacted layers (e.g., turnip or *Crotalaria juncea*), which should also help reduce the need for corrective measures involving machinery (subsoiling or chisel plowing) and improve the soil as a habitat for earthworms and other beneficial soil animals.

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