



## SOIL SCIENCE

# Edaphic fauna in soil profile after three decades of different soil management and cover crops in a subtropical region

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**Abstract:** This research evaluated the effects of long-term (30 years) winter cover crops under conventional farming system and no-tillage system on edaphic fauna in a Rhodic Hapludox soil, from Paraná State, Brazil. We used three winter cover crops (black oat, hairy vetch and fallow), and as a reference a fragment of natural forest. Soil monoliths were collected at two times, one during the flowering of maize (April 2013) and the other during the flowering of soybean (January 2014). The extraction of the monoliths was carried out in three layers in the soil profile (0-10, 10-20 and 20-30 cm). Seventeen taxonomic groups were sampled. The density of the edaphic fauna is inversely related to soil depth. The winter crops associated with the no-tillage system in long-term resulted in fauna densities similar to the natural environment, with a higher density (density increase of 2.2x) at a depth of 10-20 cm in areas with black oat. At 0-10 cm depth, black oat and vetch under no-tillage systems resulted in an increase of 62% and 69% (April 2013) and 46 and 44% (January 2014), respectively, in the density of soil fauna, when compared to the same winter crops in conventional farming system.

**Key words:** biodiversity, cover crops, no-tillage system, soil biology, conventional system.

## INTRODUCTION

Compared to other habitats on Earth, soil biota is recognized for having the greatest richness and abundance of living organisms (Orgiazzi et al. 2016). The edaphic fauna represents the soil macrobiota, whose organisms directly and indirectly contribute to the chemical, physical and biogeochemical processes in the soil environment (Bardgett 2005, Thakur et al. 2020), being considered as “ecosystem engineers” (Lavelle et al. 2016). The superficial layers of the soil profile in natural and cultivated environments, especially in the first 10 cm of depth, commonly present a high number of taxa of the invertebrate edaphic fauna, mainly due to root activity, deposition of surface leaf litter and

nutrient availability (Balota 2017, Martins et al. 2019). In general, the patterns and the causes of the abundance of edaphic taxa in the soils are related to temporal and special changes in the abiotic and biotic components of this system (Bardgett 2002).

Some relationships that the taxa of the invertebrate edaphic fauna have with the soil are known. In the superficial layers of the soil profile, close to the soil- leaf litter interface, epigeic fauna organisms such as Collembola, Acari and Araneae respond directly to nutrient cycling, deposition of organic matter, destructuring, porosity, and water percolation in the soil profile (Machado et al. 2015). The Oligochaeta correlate with the physical-chemical alterations in the

edaphic microclimate in cultivation areas, such as soil temperature and humidity, carbon and organic nitrogen (Blouin et al. 2013). Still, the decomposition of dead organisms in the leaf litter is related to the Diptera order, while the groups Chilopoda, Coleoptera, Orthoptera and Hymenoptera play vital roles in various biological processes in the rhizosphere (Meitiyani & Dharma 2018). In addition to demonstrating that the edaphic fauna is an important component for soil fertility, these complex relationships indicate that it is influenced by the intrinsic factors of the soil and those provided by its use and management, such as resource availability, compaction, porosity and structure (Mueller et al. 2016, Balota 2017, Sauvadet et al. 2017).

In this context, pieces of evidence point that the change in land use and changes in vegetation cover are factors that influence density, diversity and abundance of soil fauna (Aquino et al. 2008). The available evidence indicates that the conventional farming system (CFS) decreases the overall biological activity of soils (Babujia et al. 2010). This phenomenon occurs because the turning of the soil alters its structure and destroys soil macroaggregates, it induces changes in the abiotic conditioning (humidity and temperature) capable of promoting alterations in the edaphic microclimate, it reduces the availability of resources due to the rapid degradation of leaf litter and increases abrasion, crushing and exposure of organisms (Balota et al. 2014, Jouquet et al. 2014). Unlike the CFS, evidence indicates that the no-tillage system (NT) favors soil macrofauna communities (Sithole et al. 2018, Melman et al. 2019). Thus, in a study performed in areas conducted under NT and CFS in continuous corn monoculture for 14 years, it was found that the adoption of NT favored the development of macrofauna communities, however, without favoring the diversity of orders (Sithole et al. 2018). Jiang et al. (2018), also in an

experiment with NT and CFS in continuous corn monoculture for 10 years, observed that the NT stimulates the assemblages of soil macrofauna, favoring the decomposing and predatory groups. Still, in a study conducted for seven years in the Brazilian Cerrado region, it was observed that the NT, in relation to the CFS, provided a greater abundance of predatory groups, such as Chilopoda and Arachnida (Aquino et al. 2008).

Furthermore, the adoption of NT with diversification of plant species and the use of individual cover crops or in consortium (mix) promotes improvements in the chemical, physical and biological properties of the soil, for example, it increases organic C and N, preserves soil moisture, minimizes temperature fluctuations on the soil surface and promotes phosphorus storage in microbial biomass (Rheinheimer et al. 2019), as well as, promotes increases in the diversity of invertebrate macrofauna groups in the soil (Silva et al. 2006, Bedano et al. 2016, Krolow et al. 2017, Melo et al. 2019). There is no doubt that the NT is only agroecologically viable if it contemplates high plant diversity and a large biomass production and, this way, it makes the agrosystem more ecologically complex than the conventional farming system, especially if pesticides are reduced or even eliminated. In this sense, the monitoring of changes in the edaphic fauna in a long-term experiment can elucidate the impacts of the system on organisms and, at the same time, its result in the chemical, physical and biological attributes of the soil (Tantachasatid et al. 2017). Given the above, this study aimed to evaluate the effects of a long-term experiment managed in different systems of soil use on soil biota and verify the influence of winter cover plants on the density of this fauna.

## MATERIALS AND METHODS

### Study site and experimental design

The experimental area is located at the Experimental Station of Paraná Agronomic Institute - IAPAR (52°41' W, 26°07' S), in Paraná state, Brazil. The relief in the area is smooth wavy with slope ranging from 4 to 7%. The soil of the region was formed from basaltic and is classified as very acid Oxisol (clayey Rhodic Hapludox) (70% clay, 15% silt and 15% sand). The soil mineralogy is composed of 130 g kg<sup>-1</sup> of 2:1 clay minerals, 680 g kg<sup>-1</sup> of 1:1 clay minerals (kaolinite and halloysite), 140 g kg<sup>-1</sup> of iron oxides (51% hematite, 36% goethite and 13% magnetite) and 50 g kg<sup>-1</sup> of gibbsite. According to the classification of Köppen, the climate of the region is Cfb subtropical humid with average annual precipitation ranging from 1,200 to 1,500 mm and minimal and maximal mean annual temperature of 15.6 and 25.7 °C.

In the summer of 1976, the native forest was cleared to grow maize (*Zea mays* L.) and beans (*Phaseolus vulgaris* L.). Both species were cultivated for 10 years under conventional farming system (CFS) with plowing that resulted intensive soil erosion, soil degradation and decrease of soil quality. In 1986, the experiment was set up with a randomized block design, in a factorial system with subdivided plots (bi-factorial), with three replications. The winter cover species were allocated as the main plots (20 × 12 m). Soil management (CFS and NT) were the subplots (20 × 6 m). The treatments of the main plots consisted of three winter cover crops: hairy vetch (*Vicia sativa* L.), black oat (*Avena strigosa* Schreb.) and fallow. During the summer, the whole area was cultivated with soybean (*Glycine max* L.) or maize (*Zea mays* L.). Soil under CFS continued to be prepared with one plowing and two disc-harrowing, before each cultivation, *i.e.* using the same management used in this area

as before the establishment of the experiment (between 1976 and 1986), therefore, totalizing 40 years of CFS in 2016.

All treatments received the same amounts of limestone, nitrogen (N), phosphorous (P) and potassium (K), according to the recommendation of IAPAR (2003). Chemical fertilizer was always performed in the summer crops (maize and soybean). The nutrients P and K and one third of N (for maize only) were applied in the planting row during the sowing, and the remaining N was applied 45 days after sowing. A total of 2,020 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, 995 kg ha<sup>-1</sup> of K<sub>2</sub>O and 668 kg ha<sup>-1</sup> of N were applied from 1986 to 2016. In addition, between 1976 and 1986, approximately 1,000 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> was added in the experimental area. The last two lime applications were performed in 2011 and 2015, using a rate of 2 Mg ha<sup>-1</sup> (Table I). The herbicides were used to control weeds in the fallow and, in some cases, to complement the thinning of the aerial part of the winter cover crops.

### Collections of macrofauna

The collections of macrofauna were carried out in the two soil management systems (NT and CFS) and in three soil cover plants (black oats - OB, hairy vetch - VH and fallow - F). For comparison, it was used a fragment area of Seasonal Semideciduous Forest (NF) located next to the experiment. The first collection occurred at corn flowering, 114 days after sowing the corn crop, the second, at soybean flowering, at 51 days after soybean sowing.

The collection method employed was of cutting and extraction of monoliths (Anderson & Ingram 1993) measuring 25 x 25 x 30 cm, subdivided into three layers in the soil profile that corresponded to 0-10, 10-20 and 20-30 cm. Two points were sampled in each plot, totaling 252 monoliths: 2 management systems x 3 cover plants x 3 soil layers x 6 repetitions

**Table I.** Physical and chemical properties of a clayey Rhodic Hapludox in the 0-10, 10-20 and 20-30 cm layer under no-tillage system (NT) and conventional tillage (CT) with three winter crops (BO = Black oat, HV – Hairy vetch and F - Fallow) during 30 years. NF = Native Forest.

Treatment	Layer (cm)	TN <sup>1</sup>	OC	pH	K	P	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	V
		.... (%) ....		H <sub>2</sub> O	(mg kg <sup>-1</sup> )		..... (cmol <sub>c</sub> dm <sup>-3</sup> ) .....		(%)	
NF	0-10	0.44	4.90	4.5	53	4.6	5.7	2.4	0.90	39
	10-20	0.23	3.80	4.3	35	3.1	3.3	1.0	1.20	27
	20-30	0.18	3.10	4.1	22	2.6	1.5	0.8	1.10	18
CT – BO	0-10	0.23	2.60	5.0	89	7.1	10.0	3.6	0.60	61
	10-20	0.20	2.41	5.0	62	6.1	9.5	3.4	0.50	61
	20-30	0.16	2.10	5.2	42	4.2	8.7	3.4	0.50	62
NT – BO	0-10	0.30	3.80	5.5	237	12.2	16.0	4.7	0.40	77
	10-20	0.20	2.47	5.4	148	5.7	10.2	2.9	0.50	64
	20-30	0.15	2.12	5.1	121	5.3	6.1	2.5	0.60	50
CT – HV	0-10	0.22	2.80	4.9	161	5.5	9.9	3.5	0.50	61
	10-20	0.16	2.30	5.0	121	4.9	9.2	3.4	0.50	61
	20-30	0.13	2.00	5.2	78	4.6	7.2	3.6	0.40	63
NT – HV	0-10	0.27	3.30	4.9	257	10.1	15.3	5.0	0.30	76
	10-20	0.16	2.60	5.1	138	2.1	9.1	4.1	0.40	64
	20-30	0.14	2.00	4.8	70	1.8	5.9	3.7	0.50	62
CT F	0-10	0.19	2.35	5.0	53	4.3	9.7	3.4	0.62	68
	10-20	0.14	2.10	5.1	42	4.1	9.5	3.3	0.60	67
	20-30	0.12	1.70	4.9	27	3.1	8.2	3.5	0.50	50
NT – F	0-10	0.20	2.76	5.3	107	10.4	15.2	4.5	0.43	82
	10-20	0.16	2.51	5.2	82	3.1	9.1	3.9	0.50	70
	20-30	0.21	2.21	5.0	40.2	3.0	5.1	3.2	0.60	46

<sup>1</sup>TN: Total Nitrogen; OC: Organic carbon; K: Potassium; P: phosphorus; Ca<sup>2+</sup>: Calcium; Mg<sup>2+</sup>: Magnesium; Al<sup>3+</sup>: Aluminum; V%: Base saturation.

+ 6 repetitions of 3 layers of native forest soil, in 2 sampling periods. Concomitantly to the extraction of monoliths, soil samples were taken to determine moisture content. Soil temperature was registered at all collection points and layers investigated using a digital thermometer with a sensitivity from 0 to 100 °C. Additionally, the values of air temperature and rainfall that occurred during the monitoring

period of the edaphic fauna were evaluated (IAPAR Agrometeorology Sector).

The monoliths were placed into hermetic bags and conducted to IAPAR's research support facilities, where it was performed the screening and collection of organisms (active and inactive) visible to the naked eye (> 2 mm). The organisms, after screening by naked eye, were placed in containers (500 mL) containing an

alcoholic solution (70%) and glycerin (1%), and then were sent to the Soil Biology Laboratory at the Federal University of Pelotas. Subsequently, it was performed the identification, separation by class and order, and counting, with the aid of a binocular magnifying glass (40 x) and bibliographic materials.

### Data analysis

The data were submitted to descriptive and multivariate analysis. The descriptive analysis was based on indicators of average, standard deviation ( $\sigma$ ) and standard error ( $\pm$ ). The values of the investigated variables were subjected to analysis with the Kolmogorov-Smirnov (KS) adherence test and Levene's variance homoscedasticity test ( $p$ -value  $< 0.05$ ). In addition, was it was performed the unidirectional analysis of variances (ANOVA one way,  $p$ -value  $< 0.05$ ) using the Statistical Package for the Social Sciences software (SPSS v. 20). The values of the means that met the ANOVA assumptions were summarized by Fischer LSD's pos-hoc test ( $p$ -value  $< 0.05$ ). Regarding multivariate statistics, the values obtained for the 31 investigated variables (chemical characterization of the soil, taxon and abiotic factors) were linearized, by applying the expression  $\ln(x + 1)$  since they have different quantities. Then, exploratory data analysis was carried out using the cluster analysis by the hierarchical method, based on the Euclidean distance measurement and Ward's agglomeration criterion (Minimum variance), aiming at obtaining Clusters of high internal homogeneity (dendrogram). Data processing was performed using the Statistica software, version 7.0 (Statsoft 2004). The capacity of the dendrogram to reproduce the dissimilarity matrices was assessed by the co-phenetic correlation coefficient (CCC), with the aid of the PAST 3.04 software (Hammer et al. 2001). The determination of the number of groups formed

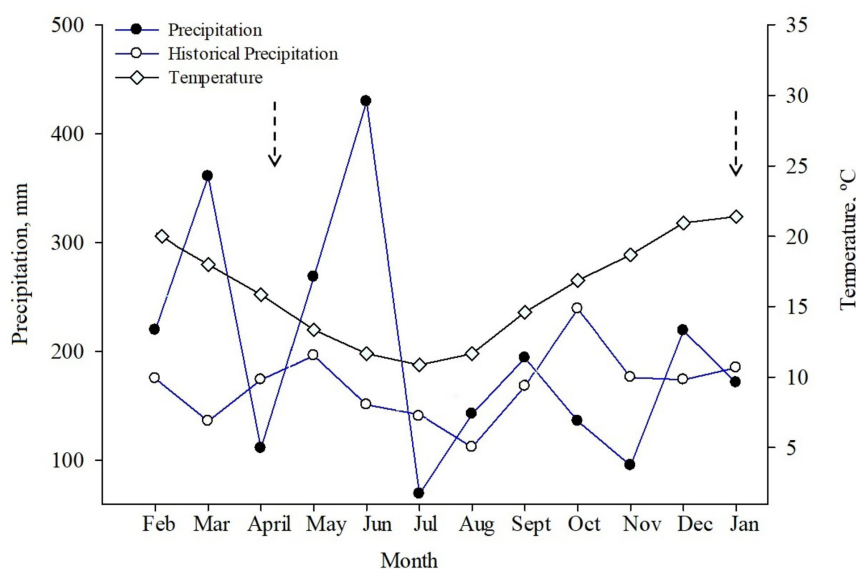
was in part by the criterion proposed by Mojena (1977).

The ecological behavior of the edaphic fauna was interpreted according to the calculated values of density (number of individuals per investigated layer), by relative frequency (% of sampling units in relation to the total number of samples), by the Constancy index, represented by the equation:  $C = p * 100 / N$ , where  $p$  = collections with the presence of the species surveyed and  $N$  = total number of samples and the result being classified into constant species ( $> 50\%$  of collections), accessory species ( $\geq 25$  and  $\leq 50\%$ ) and accidental species ( $< 25\%$ ) (Bodenheimer 1955).

## RESULTS AND DISCUSSION

The conditions of temperature and humidity of air and soil induce quantitative and qualitative changes in the edaphic fauna. These environmental conditions must be taken into account to explain the impact of agronomic practices, especially those that modify the soil surface coverage index, on soil organisms. In this sense, it is noteworthy that the rainfall volumes occurred during the monitoring period of the edaphic fauna were above historical levels: 62 and 20% higher for the collections carried out in April 2013 and January 2014, respectively (Figure 1).

Soil temperature and humidity showed significant differences ( $p < 0.05$ ) between the soil layers and between the monitored treatments (Table II). In general, soil from the 0-10 cm layer in the areas managed with the CFS showed higher temperature values compared to NT and NF. The maintenance of fallow soil in winter or cultivating with a legume crop with a high rate of decomposition combined with constant soil tillage (CFS) led to higher values of soil temperature than when the soil



**Figure 1.** Monthly average of temperature, precipitation and historical precipitation recorded by the Meteorological Station of Agronomic Institute of Paraná, Pato Branco, PR, from February to December 2013 and January 2014. The black arrows in the figure indicate the collection months. The black arrow on the left corresponds to corn flowering (April 2013). The black arrow to the right corresponds to the soybean flowering (January 2014).

was maintained without tillage and cultivated with a grass crop (NT with black oats). The hairy vetch, in comparison to black oats, presents a lower C/N ratio and lignin content, favoring its rapid decomposition (Balota et al. 2014) and, consequently, increased soil exposure to solar radiation (Table II).

The soil moisture content (determined by the gravimetric method) from the 0-10 cm layer was not significantly altered by soil management (Table II). However, the soil with native forest always maintained a higher moisture content in relation to the cultivated area. Again, the cultivation with hairy vetch, regardless of the management adopted, decreased the water supply available in April 2013 (corn flowering). Similarly, the maintenance of soil without coverage during winter also decreased the moisture content in the monitored soil in January 2014 (soybean flowering). Low rates of soil cover reduce the infiltration of water in the soil profile, enable the exposure of the soil to solar radiation and moisture loss (Calegari & Costa 2010).

### Density of edaphic fauna

The density values of the taxa were significantly altered ( $p < 0.05$ ) by the system of soil management, by the type of winter cover plant and by the sampled soil layer (Table III). During the full flowering of corn (April 2013), the soil contained 941 organisms (Average =  $44.81 \pm 11.55$ ,  $\sigma = 52.93$ ), while in the soybean flowering (January 2014) it contained 673 organisms (Average =  $48.07 \pm 18.25$ ,  $\sigma = 68.30$ ) (Table III). Krolow et al. (2017), using the Tullgren funnel as a fauna extractor, had already demonstrated that the soil from the 0-10 cm layer had more organisms during corn flowering (April 2013 - 60,138 individuals/m<sup>2</sup>) than during soybean flowering (January 2014 - 28,348 individuals/m<sup>2</sup>). In the two samples, the individuals were distributed in 17 taxonomic groups. It is shown that the adoption of NT associated with the cultivation of winter plants in the long-term provided fauna densities close to the natural environment, even exceeding it in the soil from the 10-20 cm layer (increase in density of ~2.2x) when cultivated with black oats. These results show the possibility of NT recovering the groups of edaphic fauna occurring in natural biomes



**Table II. Soil temperature and soil moisture content of a clayey Rhodic Hapludox in the 0-10, 10-20 and 20-30 cm layer under no-tillage system (NT) and conventional farming system (CFS) with three winter crops (black oat, hairy vetch and fallow) during 30 years.**

Sample site	Layer	Soil temperature (°C)		Soil moisture (%)	
	cm	April/2013	January/2014	April/2013	January/2014
Natural Forest	0-10	19.73±0.22 <sup>ghC</sup>	23.90±0.59 <sup>ed</sup>	53.02±2.37 <sup>ba</sup>	40.25±0.58 <sup>aa</sup>
	10-20	19.00±0.25 <sup>hiC</sup>	22.61±0.21 <sup>fc</sup>	50.52±1.27 <sup>ba</sup>	39.31±0.58 <sup>aba</sup>
	20-30	18.56±0.33 <sup>ib</sup>	-	58.66±6.73 <sup>aa</sup>	-
CFS Black oat	0-10	21.57±0.03 <sup>abcAB</sup>	27.34±0.18 <sup>abA</sup>	37.34±0.19 <sup>cdeB</sup>	35.09±0.16 <sup>eb</sup>
	10-20	20.60±0.36 <sup>efB</sup>	26.94±0.43 <sup>bcA</sup>	35.28±0.14 <sup>cdefgC</sup>	37.53±0.52 <sup>cb</sup>
	20-30	20.70±0.35 <sup>defA</sup>	-	36.90±0.47 <sup>cdeB</sup>	-
NT Black oats	0-10	21.09±0.24 <sup>cdeB</sup>	25.97±0.20 <sup>dc</sup>	35.43±0.64 <sup>cdefgB</sup>	35.96±0.80 <sup>eb</sup>
	10-20	21.11±0.26 <sup>cdeAB</sup>	25.85±0.36 <sup>db</sup>	34.16±0.77 <sup>efgC</sup>	33.37±0.38 <sup>fd</sup>
	20-30	20.37±0.19 <sup>efgA</sup>	-	39.75±0.52 <sup>cdB</sup>	-
CFS Hairy vetch	0-10	22.12±0.54 <sup>abA</sup>	28.01±0.21 <sup>aa</sup>	28.14±0.05 <sup>hc</sup>	36.00±0.13 <sup>deb</sup>
	10-20	20.43±0.07 <sup>efgB</sup>	27.69±0.22 <sup>abA</sup>	31.02±0.30 <sup>ghD</sup>	37.45±0.42 <sup>cdB</sup>
	20-30	20.40±0.10 <sup>efgA</sup>	-	31.36±0.26 <sup>fghC</sup>	-
NT Hairy vetch	0-10	21.41±0.20 <sup>bcdAB</sup>	27.10±0.23 <sup>abAB</sup>	31.33±0.10 <sup>fghC</sup>	39.39±0.72 <sup>abA</sup>
	10-20	20.50±0.10 <sup>efB</sup>	27.55±0.36 <sup>abA</sup>	35.25±0.27 <sup>cdefgC</sup>	35.35±0.24 <sup>ec</sup>
	20-30	20.48±0.41 <sup>efA</sup>	-	39.17±0.09 <sup>cdB</sup>	-
CFS Fallow	0-10	22.19±0.31 <sup>aa</sup>	27.70±0.12 <sup>abA</sup>	36.29±1.10 <sup>cdeB</sup>	33.01±0.49 <sup>fbC</sup>
	10-20	21.37±0.20 <sup>cdA</sup>	27.49±0.19 <sup>abA</sup>	35.06±0.18 <sup>defgC</sup>	33.47±0.38 <sup>fc</sup>
	20-30	20.35±0.16 <sup>fgA</sup>	-	34.33±1.28 <sup>efgB</sup>	-
NT Fallow	0-10	21.55±0.30 <sup>abcAB</sup>	26.11±0.62 <sup>cdBC</sup>	36.02±0.38 <sup>cdefB</sup>	32.65±0.72 <sup>fc</sup>
	10-20	20.93±0.03 <sup>cdefB</sup>	25.98±0.10 <sup>dB</sup>	39.77±1.03 <sup>cdB</sup>	38.55±0.33 <sup>bcA</sup>
	-	20.85±0.08 <sup>cdefA</sup>	-	40.04±0.95 <sup>cb</sup>	-

Averages followed by different lowercase letters in the column of each year, and averages followed by different capital letters in the same layers of soil differ from each other ( $p < 0.05$ ) by Fisher's LSD test.

if there is crop diversification, high biomass production and reduced pesticide input, as in the present experiment.

In general, the soil from the 0-10 cm layer under NT presents a higher density of fauna in comparison to the CFS. For example, the cultivation of black oats and vetch for a long period provided increases of 62.5% and 68.7%, respectively, in the density of soil fauna during the full flowering of corn (April 2013) and 45.9% and 44.3%, during the flowering of soybeans (January 2014). Regardless of the crop

present at the time of collection, the type of management and the type of winter cover crop, there was a decrease in fauna density with an increase in depth in the soil profile. In the full flowering of corn, the soil contained 554 and 292 individuals and in the flowering of soybeans 411 and 262, in the soil from layers 0-10 and 10-20 cm, respectively. These results reinforce the hypothesis that the no-tillage system and the adoption of winter cover plants favor the biodiversity of soil fauna, especially in the soil from more superficial layers (Krolow et al. 2017).

**Table III.** Density the individuals the soil fauna, in the 0-10, 10-20 and 20-30 cm layer, managed in a long-term experiment under No-tillage System (NT) and Conventional Farming System (CFS) with three winter crops (BO = Black oat, HV = Hairy vetch and F = Fallow) during 30 years. NF = Native Forest.

2013	<sup>1</sup> Ar	Ch	Col	Isop	Dip	HyF	Hy	O	E	Ort	Dipl	Is	D	Lc	Ler	Les	Lver	<sup>2</sup> Den	Aver
NF <sub>(0-10)</sub>	3	3	4	134	4	14	5	18	6	4	5	0	3	5	6	0	4	218	36.3 <sup>aA</sup>
NF <sub>(10-20)</sub>	3	0	3	22	3	12	3	5	0	1	1	0	2	3	2	0	2	62	10.3 <sup>cdeB</sup>
NF <sub>(20-30)</sub>	2	1	2	12	2	4	0	3	1	0	1	0	1	1	1	0	1	32	5.3 <sup>deB</sup>
CFS-BO <sub>(0-10)</sub>	0	0	1	0	2	2	0	20	6	0	0	0	2	1	2	0	0	36	6.0 <sup>deC</sup>
CFS-BO <sub>(10-20)</sub>	0	0	0	0	0	0	0	11	3	0	0	0	1	1	0	0	0	16	2.6 <sup>eC</sup>
CFS-BO <sub>(20-30)</sub>	0	0	1	1	0	10	0	4	1	0	0	0	0	0	0	0	0	17	2.8 <sup>eC</sup>
NT-BO <sub>(0-10)</sub>	1	0	1	0	0	15	0	66	3	0	3	0	3	0	2	1	1	96	16.0 <sup>bcdB</sup>
NT-BO <sub>(10-20)</sub>	1	0	3	20	1	6	0	86	13	0	2	0	0	2	1	0	0	135	22.5 <sup>bA</sup>
NT-BO <sub>(20-30)</sub>	0	0	0	0	0	0	0	2	5	0	0	0	0	0	0	0	0	7	1.2 <sup>eD</sup>
CFS-HV <sub>(0-10)</sub>	1	0	1	1	1	23	0	2	0	0	0	0	1	2	0	0	0	32	5.3 <sup>deC</sup>
CFS-HV <sub>(10-20)</sub>	2	0	3	0	0	10	0	7	0	0	0	0	0	2	0	2	0	26	4.3 <sup>eC</sup>
CFS-HV <sub>(20-30)</sub>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0.2 <sup>eE</sup>
NT-HV <sub>(0-10)</sub>	0	2	2	2	2	12	0	73	0	0	1	2	0	0	5	0	1	102	17.0 <sup>bcB</sup>
NT-HV <sub>(10-20)</sub>	1	0	1	0	0	2	0	17	4	0	0	0	0	0	0	0	0	25	4.2 <sup>eC</sup>
NT-HV <sub>(20-30)</sub>	0	0	3	0	1	0	0	30	10	0	0	0	0	0	0	0	1	45	7.5 <sup>cdeA</sup>
CFS-F <sub>(0-10)</sub>	0	0	2	0	3	3	1	14	3	0	0	0	0	1	1	0	0	28	4.7 <sup>eC</sup>
CFS-F <sub>(10-20)</sub>	0	0	0	0	0	1	0	10	3	0	0	0	0	0	0	1	0	15	2.5 <sup>eC</sup>
CFS-F <sub>(20-30)</sub>	0	0	0	0	0	0	0	5	1	0	0	0	0	0	1	0	0	7	1.2 <sup>eD</sup>
NT-F <sub>(0-10)</sub>	0	0	1	0	0	1	0	11	0	0	3	0	1	1	3	0	0	21	3.5 <sup>eCD</sup>
NT-F <sub>(10-20)</sub>	0	0	1	0	0	5	0	6	0	0	0	0	0	1	0	0	0	13	2.2 <sup>eD</sup>
NT-F <sub>(20-30)</sub>	0	0	1	1	0	5	0	0	0	0	0	0	0	0	0	0	0	7	1.2 <sup>eD</sup>
Σ	14	6	30	193	19	125	9	391	59	5	16	2	14	20	24	4	10	941 ± 11.55	
2014	Ar	Ch	Col	Isop	Dip	HyF	Hy	O	E	Ort	Dipl	Is	D	Lc	Ler	Les	Lver	Den	Aver
NF <sub>(0-10)</sub>	5	5	2	128	1	68	5	11	0	2	5	8	3	5	5	4	0	257	42.8 <sup>aA</sup>
NF <sub>(10-20)</sub>	1	1	3	26	1	57	2	9	2	2	3	5	5	9	4	2	4	136	22.6 <sup>bA</sup>
CFS-BO <sub>(0-10)</sub>	1	0	4	1	0	6	0	1	0	0	2	0	1	1	8	2	0	27	4.5 <sup>cdC</sup>
CFS-BO <sub>(10-20)</sub>	0	0	1	13	0	0	0	3	0	0	2	0	1	3	0	1	0	24	4.0 <sup>cdC</sup>
NT-BO <sub>(0-10)</sub>	1	0	5	0	0	33	0	3	0	0	2	0	2	0	4	0	0	50	8.3 <sup>bB</sup>
NT-BO <sub>(10-20)</sub>	0	0	5	10	0	16	0	5	0	0	1	0	1	3	2	1	0	44	7.3 <sup>cdB</sup>
CFS-HV <sub>(0-10)</sub>	1	0	1	1	0	2	0	2	0	0	0	0	0	0	3	0	0	10	1.6 <sup>cdD</sup>
CFS-HV <sub>(10-20)</sub>	0	0	4	2	0	5	0	3	0	0	0	0	0	0	4	0	0	18	3.0 <sup>cdC</sup>
NT-HV <sub>(0-10)</sub>	1	0	5	0	0	5	1	3	0	0	0	0	0	0	2	1	0	18	3.0 <sup>cdD</sup>
NT-HV <sub>(10-20)</sub>	0	0	3	0	0	0	0	5	0	0	0	0	0	0	5	0	0	13	2.1 <sup>cdC</sup>
CFS-F <sub>(0-10)</sub>	1	0	7	0	0	17	0	2	0	0	0	0	0	0	5	1	0	33	5.5 <sup>cdBC</sup>
CFS-F <sub>(10-20)</sub>	0	1	0	1	0	0	0	1	0	0	0	0	0	2	2	0	0	7	1.1 <sup>dD</sup>
NT-F <sub>(0-10)</sub>	2	0	5	2	0	0	0	1	0	0	0	0	0	0	5	1	0	16	2.7 <sup>cdD</sup>
NT-F <sub>(10-20)</sub>	0	1	0	5	0	0	1	4	0	0	0	0	0	3	4	2	0	20	3.3 <sup>cdC</sup>
Σ	13	8	45	189	2	209	9	53	2	4	15	13	13	26	53	15	4	673 ± 18.25	

<sup>1</sup>Ar = Arachnida, Ch = Chilopod, Col = Coleoptera, Isop = Isoptera, Dip = Diptera, HyF = Formicidae, Hy = Hymenoptera, O = Oligochaeta, E = Enchitreids, Ort = Orthoptera, Dipl = Diplopoda, Is = Isopoda, D = Dermaptera, Lc = curculioniform larvae, Ler = Ericiform larvae, Les = Scarabaeiform larvae and Lver = Vermiform larvae.

<sup>2</sup>Den - Density; Aver- Average. Means followed by different letters, lower case letters in the comparison between sites sampled each year, and upper case letters in the comparison between layers of the same thickness, differ by the (p < 0.05) by Fisher's LSD test.



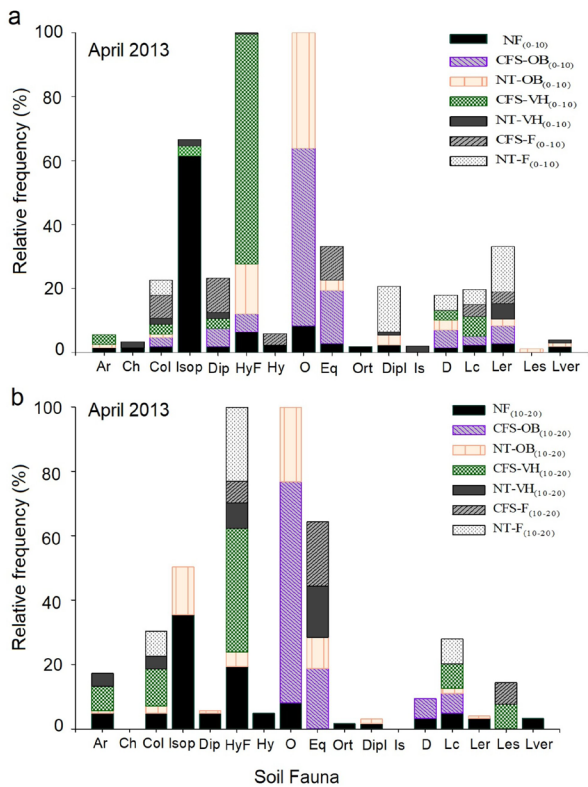
**Relative frequency and constancy**

The values of relative frequency (RF) of the captured taxa are shown in Figures 2, 3 and 4. In the soil from the 0-10 cm layer, Coleoptera, Formicidae and Oligochaeta were present in all sampled locations. Topsoil under native forest, sampled in April 2013, had a predominance of the Isoptera taxon (RF > 50%), while the Oligochaeta and Formicidae taxa were common (RF > 5 and < 25%). The other taxa were considered rare (RF < 5%, Figure 2a). In the soil from the 10-20 cm

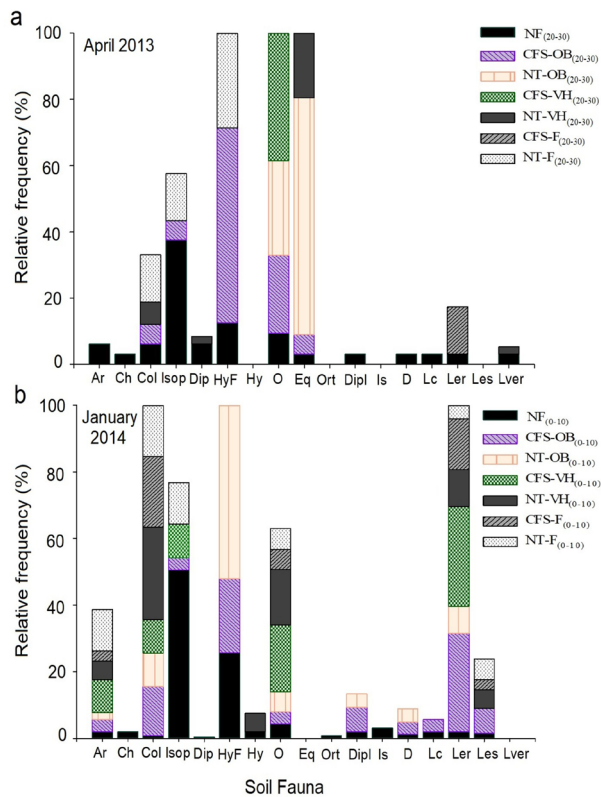
layer, only the Oligochaeta taxon was frequent in all locations (Figure 2b). In the soil from the 20-30 cm layer, when cultivated with black oats, it presented Formicidae and Enchytraeids under CFS and NT, respectively, as the dominant taxa (Figure 3a).

The dominance of the Oligochaeta taxon over the other taxonomic groups was less pronounced in areas with lower biomass input (hairy vetch and fallow) and intense soil tillage since 1976, due to higher temperature values and lower moisture contents of the soil from the 0-10 cm layer (Table II). This indicates that the Oligochaeta found a more favorable environment in the 0-10 cm layer of the sites managed in NT, either due to the constant supply of plant residues, greater availability of nutrients (Table I) or due to the milder temperatures and greater soil moisture (Table II). In addition, the cultivation of black oats and vetch associated with the no-tillage of the soil in the long-term increased by 266.67 and 305.56% and 1.620 and 240% the frequency of Oligochaeta in the soil from layers 0-10 and 10-20 cm, respectively, in comparison to soil tillage and fallow maintenance in winter since 1976. These results corroborate those obtained by Bartz et al. (2014) and Tiemann et al. (2015), whose higher frequencies of Oligochaeta are related to the maintenance of crop residues, lower exposure of the soil to sunlight and maintenance of humidity, which are only present with the adoption of NT.

The Formicidae taxon had a high relative frequency in some areas. In the full flowering of corn, a relative frequency of ~38% was found in the soil from the 10-20 cm layer kept in winter fallow without tillage and in the areas with hairy vetch with intense and frequent tillage. Similarly, it had a relative frequency of ~58% in the soil from the 20-30 cm layer when the soil was turned and cultivated with black



**Figure 2.** Relative frequency (%) the individuals the soil fauna, in the 0-10 (a) and 10-20 cm layer (b), managed in a long-term experiment under Conventional Farming System (CFS) and No-tillage (NT). Sampling held in April / 2013. Pato Branco, PR, Brazil. NF = Native forest, OB = Black oat, VH = Hairy vetch and F = Fallow. Ar = Arachnida, Ch = Chilopod, Col = Coleoptera, Isop = Isoptera, Dip = Diptera, HyF = Formicidae, Hy = Hymenoptera, O = Oligochaeta, Eq = Enchytraeids, Ort = Orthoptera, Dipl = Diplopoda, Is = Isopoda, D = Dermaptera, Lc = curculioniform larvae, Ler = Eruciform larvae, Les = Scarabaeiform larvae and Lver = Vermiform larvae.

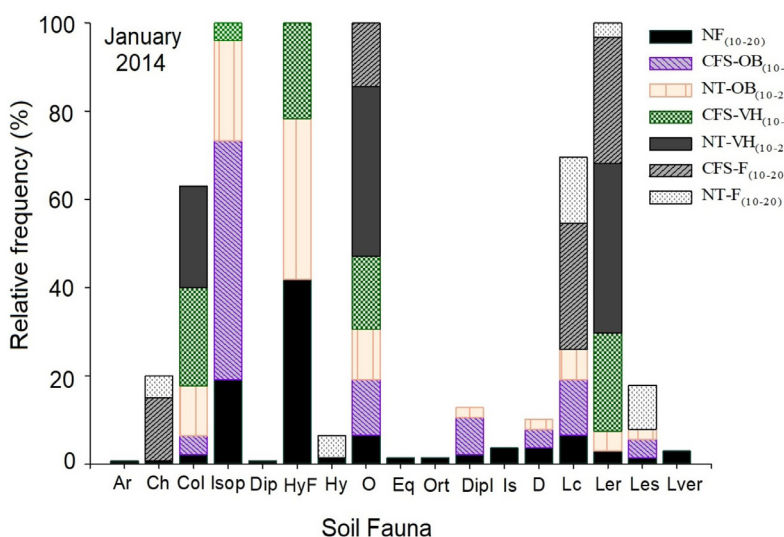


**Figure 3. Relative frequency (%) the individuals the soil fauna, in the 20-30 (a) and 0-10 cm layer (b), managed in a long-term experiment under Conventional Farming System (CFS) and No-tillage (NT). Sampling held in April / 2013 (20-30 cm layer) and January / 2014 (0-10 cm layer). Pato Branco, PR, Brazil. NF = Native forest, OB = Black oat, VH = Hairy vetch and F = Fallow. Ar = Arachnida, Ch = Chilopod, Col = Coleoptera, Isop = Isoptera, Dip = Diptera, HyF = Formicidae, Hy = Hymenoptera, O = Oligochaeta, Eq = Enchytraeids, Ort = Orthoptera, Dipl = Diplopoda, Is = Isopoda, D = Dermoptera, Lc = curculioniform larvae, Ler = Eruciform larvae, Les = Scarabaeiform larvae and Lver = Vermiform larvae.**

oats, ~71% in the soil from the 0-10 cm layer in the soil with tillage for four decades and cultivated with hairy vetch and in the soil from the 20-30 cm layer maintained for three decades without tillage and without cultivation in winter. The higher relative frequency of the Formicidae taxon is related to its capacity of occupying anthropized areas. They are social insects with high resilience, making it possible for them to survive and adapt to the adversities imputed

by anthropic action (Schmidt & Diehl 2008). For this reason, the richness and density of ants have been considered for the monitoring of the impacts of soil use and management (Hoffmann 2010, Schmidt & Diehl 2008).

In the soil collection (0-10 cm layer) performed in the full flowering of corn (April 2013), the Accidental groups (Isoptera, Oligochaeta and Formicidae) represented 39% of the total taxonomic groups identified in the soil, Accessories made up to 48% and only 2% belonged to the Constant groups (> 50% of representatives) (Supplementary Material - Table SI). The Isoptera taxon was classified as Abundant (> 25 and ≤ 50%) in the soil from the 10-20 cm layer with native forest (Figure 2b). For Santos et al. (2015), the Isoptera group was also frequent in areas of native forest, with a relative frequency above 35%. In the soil from the 10-20 cm layer, the values were 35%, 43% and 23%, respectively (Table II). The Formicidae taxon was considered dominant in the soil from layers 0-10 and 10-20 cm in areas with black oats cultivation in CFS and in fallow areas with NT. Finally, in the soil from the 20-30 cm layer, 41% were classified as Accidental, 50% as Accessories and 9% as Constant (Table SI). In the soil from this layer, the Oligochaeta taxon was dominant in the areas with fallow and hairy vetch in CFS and in the areas with hairy vetch in NT, while Enchytraeids were dominant in the areas occupied by black oats in NT. Also, the Coleoptera taxon was classified as common (> 5 and ≤ 25%) in the forest, in the areas of black oats in CFS and in the fallow and hairy vetch treatment in NT. The Oligochaeta taxon was common in areas managed with black oats, regardless of the type of soil management adopted, the Enchytraeid taxon was considered common in the fallow and black oat areas in CFS and in the areas with hairy vetch cultivation in NT, the Isoptera taxon was common in fallow areas managed in NT and in the areas with black



**Figure 4. Relative frequency (%) the individuals the soil fauna, in the 10-20 cm layer, managed in a long-term experiment under Conventional Farming System (CFS) and No-tillage (NT). Sampling held in January / 2014. Pato Branco, PR, Brazil. NF = Native forest, OB = Black oat, VH = Hairy vetch and F = Fallow. Ar = Arachnida, Ch = Chilopod, Col = Coleoptera, Isop = Isoptera, Dip = Diptera, HyF = Formicidae, Hy = Hymenoptera, O = Oligochaeta, Eq = Enchitreids, Ort = Orthoptera, Dipl = Diplopoda, Is = Isopoda, D = Dermaptera, Lc = curculioniform larvae, Ler = Eruciform larvae, Les = Scarabaeiform larvae and Lver = Vermiform larvae.**

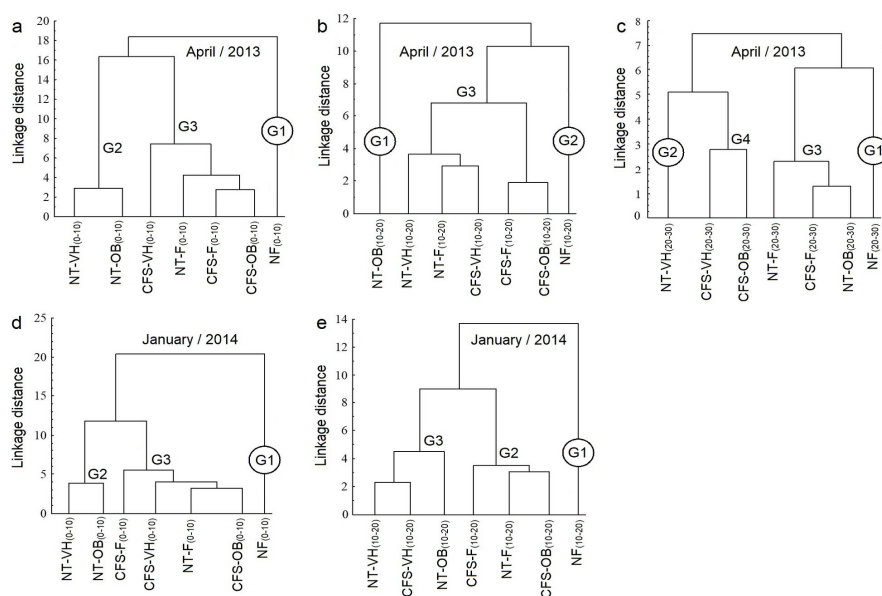
oats cultivation in CFS, the Eruciform larvae taxon was common in the fallow areas in CFS, all other taxa were considered as rare.

In the flowering of soybeans (January 2014), the soil from the 0-10 cm layer always contained individuals from the taxa Araneae, Coleoptera, Oligochaeta and Eruciform larvae (Figure 3b). The soil from this layer contained the largest number of taxa classified as Constant (Araneae, Chilopoda, Isoptera, Formicidae, Oligochaeta, Diplura, Isopoda and larvae of Coleoptera, Eruciform, Scarabiform), representing 29% of the individuals (Table III). The Isoptera taxon was classified as dominant in the native forest area, the Formicidae taxon was dominant in fallow areas associated with tillage and in areas with black oats cultivation in NT, the Coleoptera taxon was abundant in fallow areas and with hairy vetch cultivation in NT, the Oligochaeta taxon was common in fallow areas and with hairy vetch in CFS and in fallow and black oats areas in NT, the Araneae taxon was common in fallow areas in NT and in areas with hairy vetch for both management systems, the Formicidae taxon was common in areas with black oats and vetch in CFS. In the soil from the 10-20 cm layer (Figure 4), the Isoptera taxon presented the highest relative frequency (54%)

in the areas with black oats cultivation in CFS, being classified as dominant. Formicidae was abundant in areas with the cultivation of black oats in NT, in the areas with hairy vetch in CFS and in NT, Oligochaeta was abundant in hairy vetch cultivation areas in NT, Coleoptera larvae were abundant in fallow areas managed in CFS, Diplopoda was common in areas with black oats cultivation in CFS, Chilopoda was common in fallow areas for both soil management systems. Thirty, 47 and 23% of individuals were grouped into Accidental, Accessory and Constant, respectively (Table SI). In general, the Formicidae, Oligochaeta and Isoptera taxa were the most frequent, the Oligochaeta, in cultivated areas, and the Isoptera were not sensitive to soil use. These results show that the groups of edaphic fauna depend on the soil management system and on the cover crops present in the cultivation areas (Balota 2017, Sobucki et al. 2019).

### Cluster analysis

It can be seen (Figure 5) the formation of homogeneous groups as a result of the causes of variations tested (sampling time, cover plants, soil management and sampled layer) (Ward method) in the face of the interaction of chemical attributes and edaphic fauna representatives.



**Figure 5.** Dendrogram hierarchically formed between abiotic soil variables, chemical attributes and representatives of the soil fauna in the layers of 0-10, 10-20 and 20-30 cm (a, b and c) obtained in April / 2013 and in layers 0-10 and 10-20 cm (d and e) obtained in January / 2014 in a long-term experiment in Conventional Farming System (CFS) and No-tillage (NT). Pato Branco, PR, Brazil. NF = Native forest, OB = Black oat, VH = Hairy vetch and F = Fallow.

The co-phenetic correlation coefficients were greater than 90% (Figures 5a, 5d and 5e) and 70% (Figures 5b and 5c), well above the minimum limit recommended by Rohlf (1970). The results of the hierarchical grouping allowed identifying the formation of three groups (G1, G2 and G3) that were heterogeneous among themselves (Figures 5a, 5b, 5c and 5e). Only in Figure 5c it was considered the formation of a fourth group (G4) represented by the treatments with the cultivation of black oats and vetch in CFS (20-30 cm layer). The clusters obtained for the soil from the 0-10 cm layer, in both periods of collection (Figures 5a and 5d), were similar among themselves. G1, represented by the native forest (0-10 cm layer), resulted in dissimilarity in relation to the other locations, as it constituted an isolated branch in all performed evaluations. A similar behavior was observed when adopting the NT and cultivated with a grass crop in winter (10-20 cm layer, Figure 5b) and with a legume crop (20-30 cm layer, Figure 5c). The isolated branches were mainly influenced by the contribution of the edaphic fauna density, since the plotted performance is shown to be in line with the highest values of average density (Table III).

## CONCLUSIONS

The density of the edaphic fauna is influenced by the soil management system and winter cover plants. The adoption of the no-tillage system for a long period favors the biodiversity of soil fauna, especially when black oats are grown as a cover crop in winter. Black oats as a cover crop in winter favors an increase in the density of edaphic fauna in the subsurface layers, mainly in the no-tillage system. On the other hand, the conventional farming system in long-term favors mainly the increase in the ant populations.

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## SUPPLEMENTARY MATERIAL

### Table S1.

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## Author contributions

Rheinheimer DS and Antonioli ZI conceived the idea and supervised the experiments. Krolow IRC, Krolow DRV, Morselli TBGA performed the experiments. Calegari A and Krolow IRC performed the statistical analyses. Ramos RF, Rheinheimer DS and Andrade N discussed the results. All authors have read and approved the final manuscript.

