

Division - Soil Processes and Properties | Commission - Soil Biology

# Soil macrofauna correlations with soil chemical and physical properties and crop sequences under no-tillage

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**ABSTRACT:** Soil macrofauna is an important indicator of soil quality, as it is sensitive to changes in the environment as a result of soil management, which includes soil chemical and physical properties and the diversity of cultivated species. This study aimed to evaluate the composition and structure of soil macrofauna under a no-tillage system in different crop sequences, with and without crop rotation, over two growing seasons: a rainy summer and a dry winter. The crop sequences were soybean/corn rotation in the summer and corn in the winter; soybean/corn rotation in the summer and sunn hemp in the winter; soybean monoculture in the summer and sunn hemp in the winter; and corn monoculture in the summer and corn monoculture in the winter growing season. The nutrient content of the crop residues left on the soil surface, soil chemical and physical properties, and soil macrofauna were determined. Functional plant groups (grasses or legumes) individually influenced the composition of soil macrofauna more significantly than the effect of crop sequence, with or without rotation, and growing season. Grasses favored an increased density of groups such as Oligochaeta, Isoptera, and Formicidae. In contrast, legumes contributed to the variation in the total density of individuals and Diplura and Coleoptera groups. Furthermore, the influence of functional plant groups (grasses or legumes) on the composition and density of soil macrofauna were related to soil chemical (P and N content) and physical properties (particulate organic carbon and soil moisture), which determined the composition of soil macrofauna groups.

**Keywords:** rotation, organic matter, bottom-up effects, soil chemistry, ecosystem engineers.

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## INTRODUCTION

No-tillage system (NTS) is a concept developed in Brazil in 70's, which began with technique no-tillage (NT - the sowing of crops without soil preparation and with the presence of mulch or straw). The concept evolved, and its basic principles include crop diversification through rotation, maintenance of plant residues or cover crops, and minimal soil disturbance (Hernani and Salton, 1998; Fuentes-Llanillo et al., 2022; Possamai et al., 2022). Studies have shown that NTS positively alters soil chemical properties (Rodrigues et al., 2015; Marafon et al., 2020), organic matter (Six et al., 2000; Corbeels et al., 2016; Veloso et al., 2019), physical properties (Nascente et al., 2015; Veloso et al., 2019), and biological properties (Mathew et al., 2012; Rieff et al., 2020).

Among soil biological properties, soil fauna has been identified as an important indicator of soil conservation management (Coulis, 2021; Li et al., 2021), and soil macrofauna (organisms visible with the naked eye) (Lavelle et al., 1994). These organisms are prominent in key soil processes, such as nutrient cycling (Lal, 1988; Quadros and Zimmer, 2018); soil organic matter humification and mineralization (Frouz, 2018; Frouz et al., 2020); water infiltration rate due to the formation of channels and galleries (Lamoureux and O'Kane, 2012); changes in soil pH and N content (Sheehan et al., 2006; Frouz et al., 2020), and soil porosity (van Vliet et al., 1993; Bottinelli et al., 2010; Melo et al., 2019). Thus, these organisms modify their environment, facilitating plant growth (Coleman and Wall, 2014). Moreover, they are considered important indicators of soil quality (Brown et al., 2015), as they are sensitive to environmental changes in terms of soil management (Velásquez and Lavelle, 2019), chemistry (Vendrame et al., 2009; Errouissi et al., 2011), and physical properties (Brussaard and van Faassen, 1994). Furthermore, studies have pointed out the responses of soil organisms to plant community structure and composition (Bardgett and van der Putten, 2014).

Crop diversity favors the diversification of plant residues on the soil surface, with different qualities and decomposition stages, providing conditions and resources for the coexistence of diverse soil organisms (Hansen and Coleman, 1998). For instance, plant residue characteristics can influence the soil trophic chain because residues with a low C/N ratio can stimulate the activity of bacteria and their predators, whereas residues with a high C/N ratio can stimulate the activity of fungi and their predators (Bardgett, 2005; Ingham, 2000). In addition, plant residues can provide habitat heterogeneity (Hooper et al., 2000) and contribute to the likelihood of having species belonging to key faunal groups, which can increase primary production (Laossi et al., 2008). Thus, crop diversification can influence soil biology at the soil-litter/straw interface, as plant species diversity determines the patterns of spatial and temporal heterogeneity between herbivore populations and communities, known as the bottom-up effect (Hunter and Price, 1992).

In the same way that macrofauna can influence soil chemical and physical properties, these abiotic factors collaborate with the plant community to directly and indirectly affect the soil macrofauna community (Correia, 2002). Bardgett (2005) reported that individual plant species and functional group characteristics (e.g., grasses and legumes), which influence the quality and quantity of organic matter in the form of plant residues and exudates, seem to play a more important role than plant richness in soil biota structuring and ecosystem functioning. Some authors have emphasized that functional plant groups, such as legumes, increase the diversity of soil macrofauna because of their high organic matter production and lower C/N ratio (Laossi et al., 2008; Marchão et al., 2009; Velásquez et al., 2012; Batista et al., 2014). Other studies have demonstrated the effects of grasses on macrofaunal abundance due to the number of fine roots acting as a food source for decomposers (Salamon et al., 2011). Although the effects of crop diversification on fauna structure are known, researchers have yet to fully understand the mechanisms responsible for the benefits of plant diversification and functional plant groups to soil fauna. Soil invertebrates are the primary determinants of soil processes

in tropical ecosystems (Lavelle et al., 1994), and characterizing the biological activity and diversity of soil can help to understand soil dynamics, structure, and nutrient flux (Blanchart et al., 2006).

This study aimed to compare the soil macrofauna in crop sequences under no-tillage in different seasons. This study addressed the hypothesis that diversification of crop rotation under no-tillage is the dominant factor supporting the richness, diversity, and composition of soil macrofauna communities, rather than the individual effects of legume and grass species, by promoting changes in the quality and quantity of residuals and physical and chemical soil properties.

## MATERIALS AND METHODS

### Study area

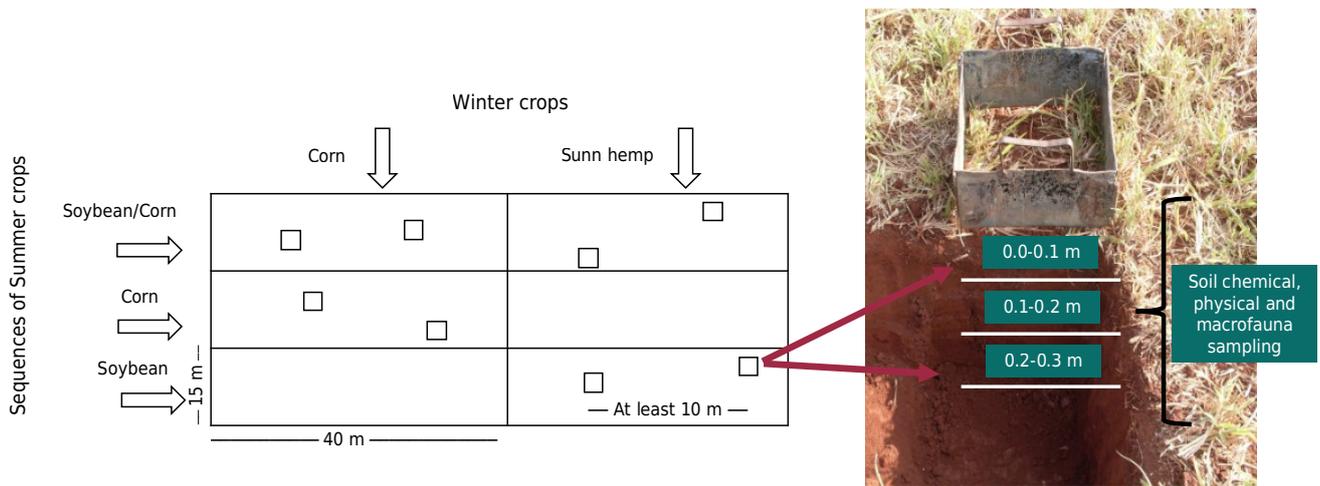
This research was conducted in the experimental field at São Paulo State University (UNESP), Jaboticabal (21° 15 02" S, 48° 16 07" W), São Paulo, Brazil. The experimental site's altitude is 595 m, and the climate is Aw, according to Köppen classification system (1936), that is, tropical with dry winters (Alvares et al., 2013). The average annual precipitation is 1.417 mm (1971–2020), concentrated from October to March, with an average annual temperature of 22 °C.

The soil in the experimental area was classified as *Latosolo Vermelho Eutrófico* (Santos et al., 2018). This type of soil corresponds to Ferralsol, according to the World Reference Base (IUSS Working Group WRB, 2015). Before starting the experiment, the soil properties at the layer of 0.00–0.20 m were: pH (0.01 mol L<sup>-1</sup> CaCl<sub>2</sub>) = 5.0; organic carbon = 11 g kg<sup>-1</sup>; P (resin) = 13 mg dm<sup>-3</sup>; K<sup>+</sup> = 4.1 mmol<sub>c</sub> dm<sup>-3</sup>; Ca<sup>2+</sup> = 15 mmol<sub>c</sub> dm<sup>-3</sup>; Mg<sup>2+</sup> = 9 mmol<sub>c</sub> dm<sup>-3</sup>; potential acidity = 34 mmol<sub>c</sub> dm<sup>-3</sup>; cation exchange capacity = 62.1 mmol<sub>c</sub> dm<sup>-3</sup>; base saturation = 45 %; and sand, silt, and clay contents of 370, 65, and 565 g kg<sup>-1</sup>, respectively.

### Experimental design

A research field under a no-tillage was established in September 2002 to evaluate the effect of crop sequences on soil properties and soybean (*Glycine max* L. Merrill) and corn (*Zea mays* L.) yields. The treatments consisted of a combination of summer crop sequences and winter crops, totaling four plots per experimental block, with each plot occupying an area of 600 m<sup>2</sup> (40 × 15 m). The three blocks were randomized to each other into strips in a randomized complete block design (Figure 1). Two samplings were performed in each plot to better represent it (soil properties and macrofauna); thus, the sample average was considered. All the properties were evaluated in three layers (0.00–0.10, 0.10–0.20, and 0.20–0.30 m) and in two seasonal periods, dry (August 2012) and rainy (March 2013). The total number of samples was 144, regarding four treatments, three blocks, two samplings, three depths and two seasons.

The summer crop sequences, sown in October, consisted of corn monoculture, soybean monoculture, and soybean–corn rotation, while the winter crops, sown in February–March, consisted of corn and sunn hemp (*Crotalaria juncea* L.) successively cultivated over 10 years. The same winter crop was cultivated in the same plot during each agricultural season. The following treatments were implemented: 1) soybean monoculture as a summer crop and sunn hemp as a winter crop (S-SH), corresponding to the growth of legumes only; 2) corn monoculture as a summer crop and corn as a winter crop (C-C), corresponding to the growth of grasses only; 3) soybean/corn rotation in the summer and corn as a winter crop (S/C-C), corresponding to legume/grass rotation in the summer and grass in the winter; and 4) soybean/corn rotation in the summer and sunn hemp as a winter crop (S/C-SH), corresponding to legume/grass rotation in the summer and legumes in the winter.



**Figure 1.** Schematic representation of the experimental block and sampling of soil properties.

Soybean/corn rotation under no-tillage treatments consisted of sowing corn and soybean in the summer growing seasons of 2011/2012 and 2012/2013, respectively. A corn hybrid was sown with an inter-row spacing of 0.90 m, targeting a population of 66 thousand plants per hectare. Sowing fertilization in both agricultural years consisted of 300 kg ha<sup>-1</sup> of 8-20-20 N-P-K formula + 1 % Ca + 5 % S + 0.3 % Zn, and a topdressing fertilization of 100 kg ha<sup>-1</sup> of N as ammonium sulfate was performed when the plants were in the vegetative stage (V6). Soybean was sown with a 0.45-m inter-row spacing, targeting 480 thousand plants ha<sup>-1</sup>. Sowing fertilizers consisted of 250 and 300 kg ha<sup>-1</sup> of a 2-20-20 N-P-K formula in the agricultural years 2011/2012 and 2012/2013, respectively. For the winter crops, corn was sown with a 0.90-m inter-row spacing, targeting 55 thousand plants ha<sup>-1</sup>, whereas sunn hemp was sown with a 0.45-m inter-row spacing, targeting 555 thousand plants ha<sup>-1</sup>; both of which did not receive sowing or topdressing fertilization. Winter corn was managed until grain harvest, whereas sunn hemp was chopped with a straw chopper at full flowering. After harvesting, the winter corn residue was chopped with a straw chopper to homogenize the residue distribution on the soil surface, aiming to replicate the sunn hemp conditions. Crop pest, disease and weed control were carried out when necessary, using products and doses recommended by the manufacturers.

### Soil macrofauna

Soil macrofauna was evaluated using the method recommended by the Tropical Soil Biology and Fertility (TSBF) program (Anderson and Ingram, 1993). Two trenches 0.3 m deep and 0.25 m wide were opened in each plot (i.e., two replicate samples per plot) and a block of soil (0.25 m wide × 0.25 m long × 0.10 m high) was collected from each trench wall at layers of 0.00-0.10, 0.10-0.20, and 0.20-0.30 m. The identification was performed at a higher taxa level, usually orders, according to criteria proposed by Costa et al. (1988), Csiro (1991), and Dindal (1990).

### Soil chemical and physical properties

After soil macrofauna handsorting, a portion of the soil from each layer was separated, air-dried, and passed through a 2 mm sieve. Subsequently, we determined soil pH(CaCl<sub>2</sub>), soil Al<sup>3+</sup>, H+Al, Ca<sup>2+</sup>, Mg<sup>2+</sup>, P, K<sup>+</sup>, total N, total carbon (TC), particulate organic carbon (POC), mineral-associated organic carbon (MAOC) content (Cambardella and Elliot, 1992), and particle size distribution (Claessen, 1997) for each sample. The TC and N contents were determined by the dry combustion method using an elemental carbon and nitrogen analyzer (AC350, LECO Corporation, St. Joseph, Michigan).

Additionally, soil samples using volumetric rings (0.05 m diameter, 0.06 m height) were collected from each trench wall at the layers of 0.00-0.10, 0.10-0.20, and 0.20-0.30 m for determining soil bulk density (BD), and percentages of total soil porosity (TP), macropores (MA) (>0.05 mm), and micropores (MI) (<0.05 mm) (Claessen, 1997).

### Plant residues

Plant residues dry matter ( $\text{kg ha}^{-1}$ ) was estimated by collecting residues in a  $0.25 \times 0.25$  m area in two locations per plot. The residues were cleaned, dried in a forced air circulation oven, weighed, ground, and chemically analyzed to determine the N, P, K, Ca, Mg, and S contents (Tedesco et al., 1985).

### Data analysis

The soil macrofauna community was analyzed, and correlations were calculated with the following parameters: density, standard error, total richness, mean richness, and Pielou's evenness index, the uniformity in the distribution of individuals among the existing species, with a range varying from zero (minimum uniformity) to 1 (maximum uniformity). In addition, individuals were standardized using the rarefaction technique (Gotelli and Cowel, 2001), as the total richness is dependent on the number of individuals sampled. Estimates of the mean rarefied richness were performed by block using the treatment with the lowest density.

Mixed effect models in the nlme statistical package for univariate statistical comparisons were used to test the effects of treatment and seasonality on the macrofaunal community and soil chemical and physical properties at the layer of 0.00-0.30 m and litter (Pinheiro et al., 2015). Soil macrofauna was calculated by the sum of total individuals at the soil layers (0.00-0.30 m), and chemical and physical properties were calculated as the mean of the soil layers. Blocks, treatments, sampling time/seasonality, and the interaction between treatment and seasonality were considered fixed factors. Seasonality nested in the plot was considered a random effect. The variables of macrofauna, soil, and plant residues were checked for normality and homogeneity of variances in the model and transformed into  $\log(x+1)$  when necessary. Tukey's test ( $p \leq 0.05$ ) was used for multiple comparisons of the means.

Redundancy analysis (RDA) was performed using the vegan package to evaluate the effects of soil chemical and physical properties on macrofaunal composition at the layers of 0.00-0.10, 0.10-0.20, and 0.20-0.30 m (Oksanen et al., 2015). Soil macrofauna data were transformed to the Hellinger distance, which is a Euclidean distance (Legendre and Gallagher, 2001). Transformations of relative abundance values reduce high abundance values. In this way, pre-transformations ensure that species data are treated according to their specificity, that is, without undue importance being given to zero values (Bocard et al., 2011). The sand content was added to the model as a co-variable as it presented a mean variation of 13 % in the experimental area; therefore, a partial RDA (RDAP) was performed. In addition, multivariate analysis of variance ( $p \leq 0.05$ ) was performed to test the influence of the fixed factor treatment, seasonality (dry and wet), and layers (0.00-0.10, 0.10-0.20, and 0.20-0.30 m) on macrofaunal composition. All analyses were completed using R Statistical Software (v3.1.2, R Development Core Team, 2014).

## RESULTS

### Plant residues

The quantity of plant residues left on the soil surface showed no variation between treatments for the same evaluation period ( $F = 0.46$ ,  $P = 0.72$ ), whereas differences were found between dry and rainy periods when soybean/corn rotation was the summer crop and corn was the winter crop (S/C-C) ( $F = 19.41$ ,  $P = 0.002$ ), with a 64 % reduction

in quantity from the dry season to the rainy season (Table 1). The N content in plant residues differed between treatments only during the dry season ( $F=4.50$ ,  $p=0.04$ ). Plant residues from soybean monoculture as a summer crop and sunn hemp as a winter crop (S-SH) had 46 % higher N content than those from corn monoculture as a summer and winter crop (C-C). No differences were observed in plant residue N content between the seasons ( $F=4.52$ ,  $p=0.07$ ) (Table 1).

### Soil chemical and physical

Overall, N and TC soil contents were higher in S-SH and lower in S/C-C in both the dry and rainy seasons (N:  $F=16.21$ ,  $p=0.003$ ; TC:  $F=8.23$ ,  $p=0.02$ ), but did not differ between the summer and winter seasons (N:  $F=4.41$ ,  $p=0.07$ ; TC:  $F=1.02$ ,  $p=0.34$ ) (Table 2). Soil moisture did not vary among crop sequences ( $F=1.94$ ,  $p=0.22$ ), but it was expectedly higher during the rainy season ( $F=132.79$ ,  $p<0.0001$ ), regardless of crop sequence.

### Structure and uniformity of soil macrofauna

The total macrofaunal density did not differ among crop sequences ( $F=1.06$ ,  $p=0.43$ ) or between the dry and rainy seasons ( $F=3.11$ ,  $p=0.12$ ) (Table 3). The results showed considerable variability in soil macrofauna density values (i.e., standard error of the mean) among replicates from treatments and between the dry and rainy seasons. The standard error from the dry to the rainy season increased by 295 % for S-SH, 4 % for C-C, 236 % for S/C-SH, and reduced 4 % for S/C-C.

Mean group richness, rarefied richness, and Pielou's index did not significantly differ among treatments ( $F=1.56$ ,  $p=0.29$ ;  $F=0.76$ ,  $p=0.56$ ;  $F=1.33$ ,  $p=0.35$ , respectively) or between the dry and rainy seasons ( $F=0.31$ ,  $p=0.59$ ;  $F=0.08$ ,  $p=0.78$ ;  $F=3.21$ ,  $p=0.11$ ) (Table 3).

**Table 1.** Mean values of litter/straw accumulated on the soil surface and nitrogen content of this material

Treatment	Dry matter		N	
	D	W	D	W
	Mg ha <sup>-1</sup>		g kg <sup>-1</sup>	
Soybean-Sunn hemp	1.95 Aa	0.98 Aa	11.8 Aa	9.96 Aa
Corn-Corn	2.02 Aa	1.40 Aa	6.35 Ba	8.59 Aa
Soybean/Corn-Corn	1.79 Aa	1.11 Aa	6.70 Ba	9.58 Aa
Soybean/Corn-Sunn hemp	2.42 Aa	0.86 Ab	8.82 ABa	9.74 Aa

Values followed by equal uppercase letters in the column and equal lowercase letters in the row do not differ studied by the Tukey's test ( $p\leq 0.05$ ). D: dry season; W: wet season.

**Table 2.** Average of chemical and physical properties of soil in the layer of 0.00-0.30 m

Crop	N		P		TC		POC		Moisture	
	D	W	D	W	D	W	D	W	D	W
	g kg <sup>-1</sup>		mg kg <sup>-1</sup>		g kg <sup>-1</sup>		g kg <sup>-1</sup>		%	
S-SH	1.3 Aa	1.2 Aa	44.8 Aa	27.45 Aa	15.7 Aa	15.7 Aa	1.03 Aa	1.05 Aa	16.1Ab	22.8 Aa
C-C	0.8 Ba	1.0 ABa	39.63 Aa	28.89 Aa	13.5ABa	13.6 ABa	0.87 Aa	0.80 ABa	15.1Ab	19.4 Aa
S/C-C	0.7 Ba	0.9 Ba	28.85 Aa	29.59 Aa	12.3 Ba	11.8 Ba	0.97 Aa	0.66 Ba	14.9Ab	19.9 Aa
S/C-SH	1.0 Ba	1.1 ABa	43.32 Aa	33.19 Aa	14.7 ABa	13.9 ABa	1.01 Aa	0.78 ABa	15.7Ab	22.4 Aa

Values followed by equal uppercase letters in the column and equal lowercase letters in the row do not differ studied by Tukey's test ( $p\leq 0.05$ ). D: dry season; W: wet season. S-SH: Soybean-Sunn hemp; C-C: Corn-Corn; S/C-C: Soybean/Corn-Corn; S/C-SH: Soybean/Corn- Sunn hemp; TC: total carbon; POC: particulate organic carbon.

**Table 3.** Density of the edaphic macrofauna (individuals m<sup>-2</sup> ± standard error), rarefied mean richness, observed mean richness, total richness and Pielou's Index at a depth of 0.00-0.30 m

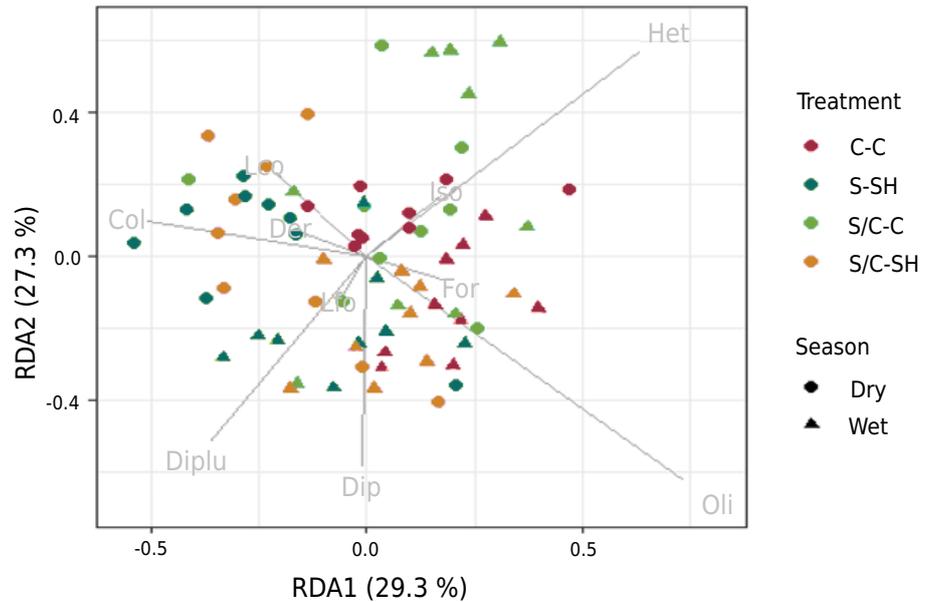
Ecological indices	Dry season			
	Soybean-Sunn hemp	Corn-Corn	Soybean/Corn-Corn	Soybean/Corn-Sunn hemp
Density* (ind.m <sup>-2</sup> ± standard error)	296 ± 139	803 ± 306	429 ± 110	243 ± 80
Mean rarefied richness*	7.0	4.9	6.2	7.1
Mean richness observed*	8.7	9.0	8.0	8.7
Total richness	16	18	13	15
Pielou's index*	0.82	0.60	0.80	0.84
Wet season				
Density* (ind.m <sup>-2</sup> ± standard error)	981 ± 550	859 ± 320	395 ± 106	709 ± 269
Mean rarefied richness*	6.1	6.8	5.2	8.1
Mean richness observed*	7.0	9.3	5.3	10.0
Total richness	12	13	8	17
Pielou's index*	0.71	0.63	0.64	0.71

\*The variables did not differ among crop sequences or between the dry and rainy seasons ( $p > 0.05$ ). Density among crop sequences ( $F=1.06$ ,  $p=0.43$ ) and between seasons ( $F=3.11$ ,  $p=0.12$ ); Mean rarefied richness among crop sequences ( $F=0.76$ ,  $p=0.56$ ) and between seasons ( $F=0.08$ ,  $p=0.78$ ); Mean richness observed among crop sequences ( $F=1.56$ ,  $p=0.29$ ) and between seasons ( $F=0.31$ ,  $p=0.59$ ); Pielou's index among crop sequences ( $F=1.33$ ,  $p=0.35$ ) and between seasons ( $F=3.21$ ,  $p=0.11$ ).

Conversely, the multivariate analysis showed pure and interactional effects of crop sequences, soil depth, and dry and rainy seasons on the soil macrofauna composition (Table 4). Crop sequences explained 8.2 %, dry and rainy seasons 3.9 %, soil depth 12.2 %, and the interaction between crop sequences and seasons explained 5.2 % of the macrofauna composition. Redundancy analysis clearly associated the groups Oligochaeta, Formicidae, and Diplopoda with the rainy season and Dermaptera, Coleoptera, and Coleoptera larvae with the dry season (Figure 2). Furthermore, redundancy analysis associated C-C with Oligochaeta (earthworms), Isoptera (termites), and Formicidae (ants), S-SH with Diplura and Coleoptera, and S/C-C negatively with Diplopoda.

**Table 4.** Multivariate ANOVA results of treatment effects, depth and seasonality on edaphic macrofauna density variation

Sources of variation	DF	SQTotal	F	p-value
Treatment (T)	3	0.05174	2.35	0.004
Seasonality (S)	1	0.02473	3.37	0.005
Depth (D)	2	0.07382	5.02	0.001
S × D	2	0.02846	1.99	0.019
D × T	3	0.07058	1.70	0.008
T × S	3	0.03257	1.51	0.067
T × S × D	17	0.17467	1.63	0.002
Residue	48	0.30279		
Total	71			



**Figure 2.** Correlation bi-plot based on a Redundancy Analysis (RDA) of soil macrofauna in relation to interaction between treatment and season, displaying 11.14 % of the variance in the density and 56.60 % of the variance in the fitted density. Eigenvalues of the first four axes are 0.05774, 0.05372, 0.04102 and 0.02281. The sum of all canonical eigenvalues is 17.37 %. S-SH: Soybean-Sunn hemp; C-C: Corn-Corn; S/C-C: Soybean/Corn-Corn; S/C-SH: Soybean/Corn-Sunn hemp; Col: Coleoptera; Der: Dermaptera; Dip: Diplopoda; Diplu: Diplura; For: Formicidae; Het: Heteroptera; Iso: Isoptera; Lco: Coleoptera larvae; Lfo: Formicidae larvae; Oli: Oligochaeta.

### Effects of soil properties on soil macrofauna

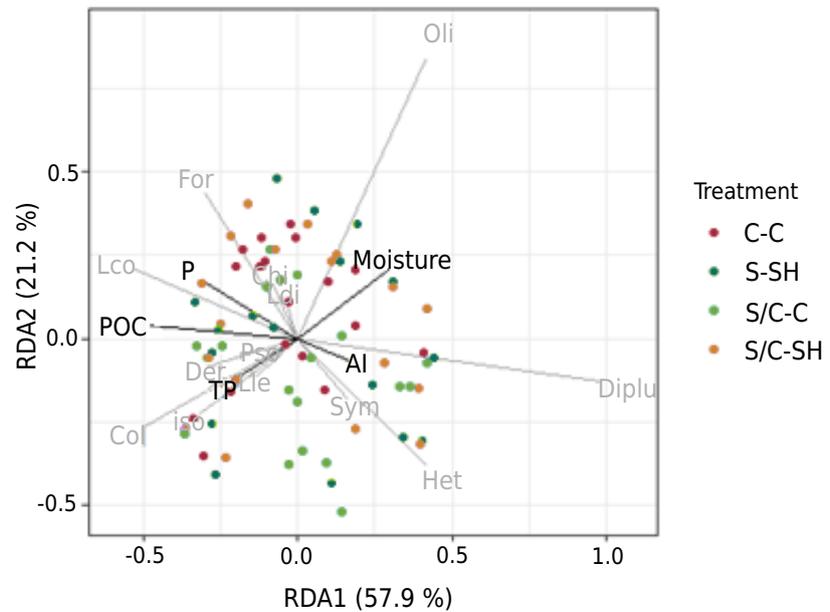
Partial redundancy analysis (RDAP) revealed the effects of soil chemical and physical properties on soil macrofaunal composition. The RDAP ( $F = 3.09$ ,  $p < 0.001$ ) and Axis 1 were significant ( $F = 8.95$ ,  $p < 0.001$ ), while Axis 2 was significant at 6 % ( $F = 3.28$ ,  $p = 0.06$ ). Soil macrofauna was affected by POC ( $F = 3.66$ ,  $p < 0.001$ ), P ( $F = 2.77$ ,  $p = 0.01$ ), Al content ( $F = 2.38$ ,  $p = 0.02$ ), TP ( $F = 0.40$ ,  $p = 0.93$ ), and soil moisture ( $F = 2.40$ ,  $p = 0.02$ ), which explained 62.8 % of the variation in the soil macrofaunal community, and the sum of all canonical axes explained 19.21 % of the total variation in soil macrofauna (Figure 3). The percentage of sand in the soil accounted for 2.1 % of the total soil macrofaunal variation.

Soil POC and P content were associated with Formicidae, Coleoptera larvae, Chilopoda, Diptera larvae, Dermaptera, and Psocoptera (Figure 3). Specifically, the soil P content was strongly associated with Formicidae and Coleoptera larvae. Soil total porosity was positively associated with Coleoptera, Dermaptera, Lepidoptera, and Isoptera larvae and negatively associated with Oligochaeta. Soil moisture was strongly associated with Oligochaeta density.

## DISCUSSION

### Structure and uniformity of soil macrofauna

The density of soil macrofauna did not vary among crop sequences or between the dry and rainy seasons. A possible explanation is that many of these species are generalists in terms of feeding and habitat preferences and do not respond to slight differences in environmental quality (Wardle et al., 2006), primarily in the absence of significant concomitant effects on plant development (Velásquez et al., 2012), such as soil disturbance and compaction. The non-variation in the density of soil macrofaunal species may have consequently influenced their richness of groups and uniformity.



**Figure 3.** Correlation bi-plot base on a Partial Redundancy Analysis (RDAP) of soil macrofauna in relation to chemical and physical soil properties, displaying 15.21 % of the variance in the density and 79.18 % of the variance in the fitted density. Eigenvalues of the first four axes are 0.1113, 0.04077, 0.02983 and 0.006210. The sum of all canonical axes is 9.21 %. S-SH: Soybean-Sunn hemp; C-C: Corn-Corn; S/C-C: Soybean/Corn-Corn; S/C-SH: Soybean/Corn-Sunn hemp; TP: total porosity; POC: particulate organic carbon; Al: aluminum; P: phosphorus; Chi: Chilopoda; Col: Coleoptera; Der: Dermaptera; Diplu: Diplura; For: Formicidae; Het: Heteroptera; Iso: Isoptera; Lco: Coleoptera larvae; Ldi: Diptera larvae; Lle: Lepidoptera larvae; Oli: Oligochaeta;; Pso: Psocoptera; Sym: Symphyla.

Although no differences were observed in the density, richness, and uniformity values, which are commonly used to understand the overall community structure, the standard error of soil macrofauna density was used as a measure of environmental heterogeneity (food resource and water availability) (Menezes et al., 2009). In crop sequences with higher corn contribution, such as C-C and S/C-C in the dry and rainy seasons, the density standard error values may indicate the effects of corn residues on the soil macrofauna by providing similar environmental conditions and food resources in the dry and wet seasons (Table 3). Conversely, the expressive variation in the standard error values observed in S-SH and S/C-SH from the dry to rainy season indicates a higher density of individuals in microsites/microenvironments with higher food resource availability and favorable environmental conditions, such as available water, plant residues, or both (Ettema and Wardle, 2002; Menezes et al., 2009). Thus, the effect of the previous crop on soil organic matter patterns (Ettema and Wardle, 2002) and, consequently, on the distribution of soil organisms should be considered. The quality of sunn hemp residues, with relatively higher N contents (Table 1), and low C/N ratio (Santos et al., 2008) may have contributed more significantly to the development and survival of soil organisms than the amount of residues alone, because the amount of plant residue dry matter did not differ among crop sequences. Therefore, residue quality is an important predictor of the bottom-up effect of crop sequences on the soil macrofauna community (Huang et al., 2020). In this context, the diversity of plant species can determine spatial and temporal heterogeneity patterns in herbivore populations (Hunter and Price, 1992).

The association of decomposers such as Oligochaeta, Isoptera, and Formicidae with C-C (Figure 2) may be related to the high number of fine roots, which allows the soil macrofauna to explore higher volumes of soil by increasing the richness of microhabitats below ground (Albers et al., 2006; Eisenhauer et al., 2011; Salamon et al., 2011). Therefore, fine root biomass can be considered a functional characteristic of plants that strongly influences the density of soil organisms (Ettema and Wardle, 2002; Salamon et al., 2011).

Lower N and TC soil contents (Table 2) appeared to restrict the density of Diplopoda organisms in S/C-C (Figure 2), which have detritivorous eating habits (Quadros and Zimmer, 2018). Therefore, low quality of plant residues (high C/N ratio) can provide low palatability and limit detritivorous groups from feeding on soil macrofauna (Li et al., 2016; Song et al., 2020). The results of the present study indicate that plant diversity can modify the structure of detritivorous soil organisms (Chen et al., 2017), such as Diplopoda, which proved to be sensitive to the quality of the plant residue.

### Effects of soil properties on soil macrofauna

Soil macrofauna was influenced by soil chemical and physical properties, which may directly affect crop sequences (Figure 3) (Rosa et al., 2015; Souza et al., 2016). The POC and P soil contents were positively associated with more macrofaunal groups and negatively associated with Diplura and Heteroptera (Figure 3). This may indicate the bottom-up effect of the crop sequence, in which the higher resource availability and diversity of environmental conditions in the soil promote positive effects on the density of macrofauna groups, which may reduce dominance and increase diversity (Pestana et al., 2020).

The Formicidae group was positively associated with soil P content (Figure 3), possibly because ants known as “ecosystem engineers” (Lavelle et al., 2001), have the ability to modify soil physical and chemical properties, by mixing the soil to build their nests and accumulating organic matter through excrement and construction material (Frouz et al., 2003, 2005). Thus, ants, which are detritivores and present high mobility, can connect mechanically and chemically above and below soil compartments (Quadros and Zimmer, 2018), which could explain the observed P soil content associated with the Formicidae group.

Isoptera was positively associated with soil TP (Figure 3), which correlates with the physical activity that Isoptera provides along the soil profile as bio-disturbers, and at the soil aggregate level as reorganizers of total soil porosity (Bottinelli et al., 2015; Jouquet et al., 2016, 2019). Isoptera organisms, also considered ecosystem engineers, provide soil biostructures such as galleries, channels, chambers, and stable biogenic aggregates (Lavelle et al., 2020), which operate as a network of horizontal and vertical macropores, where organic residues are often observed inside them (Jouquet et al., 2011). Thus, organic residues are directly related to soil micro-and macro-aggregation (Poffenbarger et al., 2020) and indirectly related to soil porosity (Six and Paustian, 2014).

Oligochaeta, another ecosystem engineer, was not associated with TP in the present study but was positively associated with soil moisture (Figure 3). In general, Oligochaeta, primarily earthworms, is negatively related to soil TP because organisms, when ingesting minerals and organic materials from soil, eliminate them as higher-density feces (Martin and Marinissen, 1993) than before the material is ingested (Lavelle et al., 2001). The low stability of newly formed feces can also decrease the total soil porosity after periods of rainfall owing to the loss of particles and, consequently, clogging of soil pores (Bottinelli et al., 2010). The positive association between Oligochaeta and soil moisture may be explained by the fact that water constitutes approximately 75-90 % of earthworm body weight (Kale and Karmegam, 2010) and their need for a humid environment due to physiological activities, such as skin respiration and ammonia and urea excretion. Therefore, the results concur that soil moisture is an important factor for the survival and development of Oligochaeta (Lavelle, 1983; Kale and Karmegam, 2010; Domínguez et al., 2018; Singh et al., 2020).

## CONCLUSIONS

Functional plants (grass and legumes) were the dominant factors affecting soil macrofauna variation when compared with crop rotation diversification. However, these functional plants affected the structure and composition of the soil macrofauna in different ways. The root biomass of corn increased the density of soil macrofauna engineers, such as earthworms, ants, and termites, and the quality of legume plants provided distinct microhabitats and food resources between seasons, resulting in differences in soil macrofauna community densities. In addition to the effects of functional plants, the findings showed that soil properties were directly affected by crop sequences and seasons, and particulate organic carbon, phosphorus, and moisture, determined the density of soil macrofaunal groups. These soil properties are related to the improvement of microhabitat to soil fauna in terms of basic food resources (e.g., P and POC) and physical structure (moisture). These results suggest that the increase in density of some macrofauna groups depended not only on the direct effects of functional plants, but also on the indirect effects of the soil physical and chemical properties, which can be affected by crop sequences (rotation and functional plants). For future studies, the assessment of soil properties and crop root quantification can assist in elucidating soil microhabitat characteristics and the spatial variation of organisms, especially ecosystem engineers.

## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at [https://www.rbcjournal.org/wp-content/uploads/articles\\_xml/1806-9657-rbcs-47-e0230006/1806-9657-rbcs-47-e0230006-suppl01.pdf](https://www.rbcjournal.org/wp-content/uploads/articles_xml/1806-9657-rbcs-47-e0230006/1806-9657-rbcs-47-e0230006-suppl01.pdf).

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