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Comparison of soil invertebrate communities in organic and conventional production systems in Southern Brazil

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Abstract

Invertebrates play important functional roles in soils, affecting several essential ecosystem services. However, their populations are sensitive to disturbance, and are therefore often used as bioindicators of soil quality. Conservation agriculture covers extensive areas in Brazil, and organic production techniques have been rapidly spreading, but little is known regarding their impacts on belowground invertebrate communities. Thus, the present study evaluated the effects of different land-use and management systems on macro- and mesofauna communities in rural areas near Quitandinha, Southern Brazil. Samples were taken in a native forest (NF), organic (OH) and conventional horticulture farms (CH) and a conventional reduced tillage field (RT). Soil macrofauna and earthworms were collected by hand sorting, using the Tropical Soil Biology and Fertility (TSBF) method, and mesofauna were collected using a modified Berlese funnel apparatus. Enchytraeids were sampled using the standard ISO 23611-3 method. Six earthworm species were found, in the genera *Glossoscolex* and *Amynthas* as well as Ocnerodrilidae juveniles and an unidentified species. Four genera of enchytraeids were found, two of them cosmopolitan (*Fridericia, Enchytraeus*) and two native (*Guaranidrilus, Hemienchytraeus*). Soil tillage practices (in CH and OH) were associated with lower earthworm populations, while ants, spiders, ecosystem engineers and enchytraeids were more associated with organic fertilization and no pesticide use. Conventional systems (RT and CH) had lower macrofauna, enchytraeid and ant populations than NF and OH, and CH had the lowest richness of both macro- and mesofauna, as well as the lowest abundance of earthworms, spiders, fly larvae and "other" macro and mesofauna. Reduced tillage had higher earthworm and mite populations, while NF had the highest macrofauna and earthworm taxonomic richness and termite abundance. Reducing tillage in OH and CH may improve conditions for soil fauna, but further work is still needed to determine the best suite of management practices that promote soil fauna and their contributions to soil function and ecosystem services in these systems.

Keywords macrofauna | mesofauna | earthworms | enchytraeids | soil management

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1. Introduction

Soils are essential to human life on the planet and provide important ecosystem services to human beings (Adhikari & Hartemink 2016). However, soils and their functions are threatened worldwide, due to unsustainable soil management practices (FAO/ITPS 2015). Soil erosion, compaction, salinization, contamination, land use change and organic matter decline, among a range of other drivers threaten soil biodiversity (Orgiazzi et al. 2016). In agricultural systems, soil tillage and large-scale pesticide use reduce soil biodiversity and affect ecosystem service provisioning (Bender et al. 2016). On the other hand, the adoption of sustainable soil management practices, such as reduced tillage and organic agriculture increase organic matter inputs into the soil, and can have important positive impacts on soil biodiversity and functioning (Mäder et al. 2002, Holland 2004, Bender et al. 2016, Ayuke et al. 2019).

Soil invertebrates have multiple feeding strategies and their activities in soils affect fundamental processes like decomposition, nutrient cycling, soil aggregation, water infiltration and holding capacity, pest control, seed dispersion and plant production, ultimately impacting ecosystem services important for human wellbeing on the planet (Lavelle et al. 2006). Hence, knowledge of the potential impacts of land use practices and management on soil fauna communities is important in order to guarantee proper functioning as well as biodiversity conservation in agricultural ecosystems (Brown et al. 2018).

Brazil has the largest area in no-tillage agriculture in the world (33 million ha), and most of the grain production in Brazil is done under reduced or no-tillage planting (IBGE 2018, Kassam et al. 2018). However, Brazil is also one of the largest pesticide consumers in the world (IBAMA 2018), although the area under organic cultivation in the country increased to over 1.1 million ha in the last decade (Lernoud & Willer 2019), mainly due to societal pressure (Lima et al. 2020). Paraná is the Brazilian state with the highest number of organic farms, representing 14% of all organic producers in the country in 2017 (Vilela et al. 2019).

Belowground invertebrate communities are highly sensitive to environmental disturbance, normally showing lower populations and species diversity in human-disturbed ecosystems compared to natural environments (Lavelle et al. 1989, Marichal et al. 2014, Kamau et al. 2017, Ratnadass et al. 2017). These losses in biodiversity may negatively impact soil functioning, reducing ecosystem service provisioning in agricultural lands, leading to soil degradation and reduced agricultural potential (Barros et al. 2004, Lavelle et al. 2006). Nevertheless, the response of soil fauna depends on the taxa, land-use system and biome evaluated. Some animals have low adaptive capacity, while others are more resilient to changes in both biotic and abiotic conditions (Fiera et al 2020), particularly the cosmopolitan and invasive species (Marichal et al. 2010). Hence, studies on soil invertebrate communities are fundamental to monitor the impacts of agricultural practices on environmental quality and soil functioning, and these animals are useful bioindicators to evaluate soil quality in human-altered systems (Velásquez et al. 2007, Rousseau et al. 2013). In Brazil, earthworms are a good example of invertebrates that have been proposed as indicators of soil quality in no-tillage systems (Bartz et al. 2013). However, further information is needed on other soil invertebrate taxa in order to better understand the effects of agricultural practices on soil biological quality.

Although the impacts of reduced-tillage practices, especially zero tillage on soil macroinvertebrates (particularly earthworms) have been relatively well studied in Brazil (Aquino et al. 2008, Zagatto et al. 2019, Demetrio et al. 2020), and the impacts of organic and conventional production systems on soil macro and/

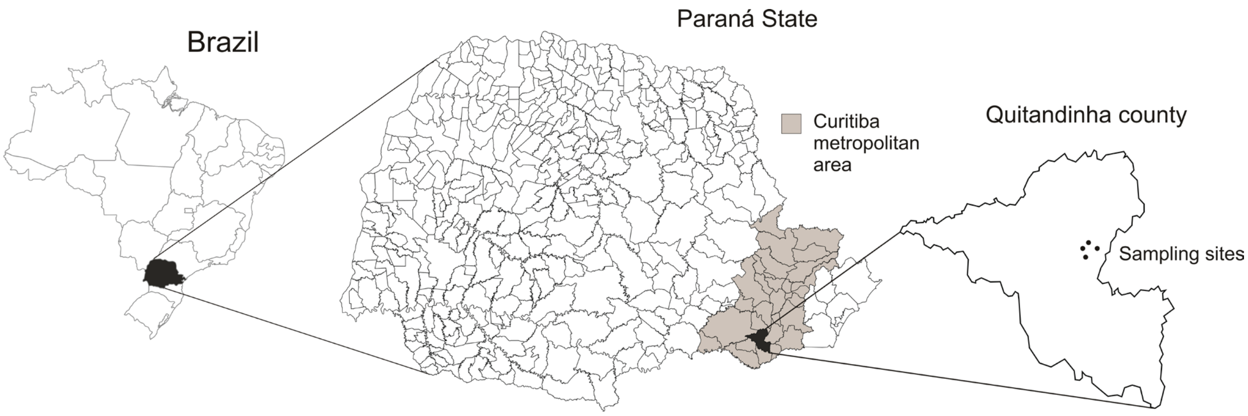

Figure 1. Location of sampling sites in Quitandinha county, part of the greater Curitiba metropolitan area, State of Paraná, Brazil.

or mesofauna communities have also been assessed by several authors, mainly in coffee or fruit production systems (Ricci et al. 1999, Aquino et al. 2000, Maluche et al. 2006, Uzêda et al. 2007, Bartz et al. 2009, Souza 2010, Silva et al. 2012), only a few of them involved annual crops (Lima et al. 2007, Trogello et al. 2008, Quadros et al. 2009) or horticultural production systems (Bettiol et al. 2002, Freitas 2007, Marchiori 2008).

The impacts of reduced tillage and organic production can be highly dependent on the taxa as well as the systems being compared (Bedano & Domínguez 2016), so the present study evaluated the effects of two conventional systems (with fertilizer and pesticide inputs and intense or reduced tillage) with an organic vegetable production system, on soil macro- and mesofauna communities in a rural area of Southern Brazil. Furthermore, we explored the relationships between the fauna and soil chemical and physical parameters in these systems, using a native forest fragment as a reference land-use.

2. Material and methods

2.1 Study sites

The study sites were located near Quitandinha, Paraná State (Figure 1), southern Brazil (25°53’22”S 49°27’28”W), part of the greater Curitiba metropolitan area, which includes 29 counties, covers an area of ca. 16,600 km^2 (the second largest in the country), and is home to around 3.5 million inhabitants (Comec, 2017). The climate in the region is classified as Cfb (temperate with mild summers and without a dry season) according to Köppen (Alvares et al. 2014), with mean annual precipitation and temperature of 1,755 mm and 16.5°C, respectively. The soils in all land use systems were classified as Ultisols (USDA 1999) or Acrisols (FAO/WRB 2015). In this rural area close to the state capital Curitiba, around 30,000 ha are used for cattle grazing and to produce tobacco, grain crops like maize, beans, soybeans and wheat, and vegetable crops such as cabbage, onions, beets, cauliflower, broccoli, parsley, arracacha, carrots and lettuce, among others (EMATER 2014).

Four land-use systems were selected: 1) a native vegetation ecosystem (NF) consisting of a secondary ombrophilous mixed forest (*Araucaria* forest) in advanced stage of regeneration (>80 years without human intervention); 2) an organic vegetable horticulture system (OH), with leveling disc harrowing, biofertilizers (2 L ha^{-1}), organic manure (1 T ha^{-1}) and ash (0.2 T ha^{-1}) application, and no chemical pesticide use (manual weeding and only organically certified products for insect and disease control); 3) a conventional crop production system (RT), managed using reduced tillage (chisel plow), chemical fertilizers (NPK) and pesticides (fungicides, insecticides and herbicides), including summer grain crops (maize or soybean) and winter cereal or cover crops (wheat or black oats) in rotation; and 4) a conventional vegetable horticulture system (CH), managed using conventional practices, including a rotary hoe (intense tillage), chemical fertilizers (NPK) and pesticides (herbicides, insecticides and fungicides) for weed, insect and fungal disease control. Sampling in 2013 was done during the horticultural crop cycle with potatoes in CH and cabbage and carrots in OH, and in fallow after maize harvest in RT. In 2014, sampling was done in squash in CH, potatoes in OH and maize in RT.

Although only one site per land-use type was sampled, internal replication was within that recommended by international standards (e.g., ISO 23611; Römbke et al. 2006), and all the agricultural systems (OH, CH, RT) have been conducted using these practices for more than 20 years. Hence, they were considered as representative of sites with long-term impacts of these land-use systems on soil fauna, as well as of the typical management practices performed in both conventional and organic farms in the Curitiba metropolitan area (Mazzoleni & Nogueira 2006).

2.2 Fauna sampling

Soil macrofauna were sampled in September 2013, using a modified version of the standard Tropical Soil Biology and Fertility (TSBF) method (Anderson & Ingram 1993). In each system, nine soil monoliths (25 x 25 cm to 20 cm depth) were collected, using three transects distanced 30 m from each other, and with 10 m between samples. All invertebrates visible to the naked eye (Ruiz et al. 2008) were hand sorted from the litter and two topsoil layers (0–10, 10–20 cm), and immediately fixed in alcohol (90% for earthworms and 70% for other fauna taxa). Earthworms were sampled again using a qualitative method for biodiversity estimation (Bartz et al. 2014), in January 2014. Soil mesofauna were sampled using metal cores (8 cm diameter, 5 cm depth) with attached funnels. Five samples were taken in each transect (10 m distance between samples), totaling 15 samples per land-use system. In the laboratory, the mesofauna were extracted using a modified Berlese-Tullgren extractor over 7 days, and fixed in alcohol (70%). In the laboratory, the animals were identified at higher taxonomic levels (Class, Order, Sub-order or Family; see Supplementary data file), and earthworms to genus or species-level, using keys of Ruiz et al. (2008) for macrofauna and Blakemore (2010) and Righi (1995) for earthworms.

Enchytraeids (pot worms) were sampled in September 2013 and January 2014, following a modified version of the standard ISO 23611-3 (ISO 2007) method, described in Niva et al. (2010). A total of ten samples were collected in each system using iron cylinders (5.8 cm diameter, 5 cm depth) divided into two (2014) or three (2013) transects. In 2013, samples were distanced 15 m between samples, with three samples in the side transects and four samples in the central transect, while in 2014, samples were 10 m apart and the transects 20 m from each other. In the laboratory, the soil was placed on a cloth submerged in bottled water and the enchytraeids extracted over 2.5 hours, using a heat gradient (created by a halogen lamp), and plastic funnels, following protocols detailed in Niva et al. (2010), with improvements (Niva et al. 2015). The potworms were identified to genus level, using keys and a manual prepared by Rüdiger Schmelz (unpublished), for Latin American enchytraeids. All invertebrates were identified using stereoscopic microscopes in the laboratories of Embrapa Forestry and the Federal University of Paraná.

2.3 Soil analysis

Samples for soil fertility characterization (0–20 cm depth) were taken from each TSBF monolith after sorting the macroinvertebrates. The soil samples were air dried (40ºC), homogenized and sieved (2 mm mesh) and the following chemical properties were obtained using standard soil analysis methods described in Marques & Motta (2003): pH ($CaCl_2$), phosphorous (Mehlich-1 extractor), exchangeable calcium and magnesium (KCl), exchangeable potassium ($CaCl_2$), organic carbon content (Walkley-Black method) and base saturation (V%). Soil particle size analysis (sand, clay and silt) was also performed using standard methods (Teixeira et al. 2017). Next to each monolith, a soil sample was collected to determine gravimetric moisture contents (soil dried at 105ºC for 24h).

2.4 Statistical Analysis

All fauna data were extrapolated to individuals per square meter. Invertebrates from macro- and mesofauna samples representing 2% or less of the overall abundance were considered ‘rare groups’ and were grouped as ‘Others’. Ecological indices of Shannon, Simpson, Equitability and richness (total number of taxa and mean no. taxa per sample) were calculated according to Magurran (2004). All data was submitted to normality tests (Kolmogorov-Smirnov), and whey they had non-normal probability distribution, General Linear Models were used to adjust the data to other distribution models. When the land use system showed significant effects on soil fauna and chemical variables, we

Table 1. Abundance (number of individuals m^{-2}) and selected diversity indices of soil macrofauna taxa and ecosystem engineers (earthworms, termites, ants) four land-use systems (NF = native forest, OH = organic horticulture, RT = reduced tillage, CH = conventional horticulture) in Quitandinha, Brazil.

Taxa or group	Land use system							
	NF		OH		RT		CH	
	Ind. m^{-2}	SE[1]	Ind. m^{-2}	SE	Ind. m^{-2}	SE	Ind. m^{-2}	SE
Earthworms[2]	**19b**	±5	**4c**	±3	**107a**	±36	**2c**	±2
Ants	**1,296a**	±334	**1,392a**	±324	**37b**	±18	**18b**	±8
Termites	**162a**	±77	**3b**	±3	**7b**	±7	**0b**	-
Ecosystem engineers	**1480a**	±358	**1399a**	±403	**151b**	±35	**19c**	±7
Beetles	219^{ns}	±38	119^{ns}	±41	130^{ns}	±31	99^{ns}	±43
Millipedes	**59a**	±24	**34ab**	±12	**71ab**	±61	**0b**	-
Centipedes	66^{ns}	±19	12^{ns}	±8	12^{ns}	±5	20^{ns}	±13
Spiders	**59a**	±15	**87a**	±51	**18ab**	±18	**0b**	-
Fly larvae	**75a**	±33	**9ab**	±6	**2b**	±2	**3b**	±2
Others	**94a**	±26	**32ab**	±4	**87a**	±4	**11b**	±2
Total	**2,049a**	±367	**1,692ab**	±414	**471b**	±93	**153c**	±45
Total richness	16		13		16		9	
Mean richness	**9.3a**	±0.7	**5.2b**	±0.6	**5.8b**	±0.7	**2.7c**	±0.3
Shannon (H’)	**1.28a**	±0.2	**0.66b**	±0.2	**1.33ab**	±0.2	**0.77ab**	±0.1
Simpson (D)	**0.55ab**	±0.07	**0.30b**	±0.08	**0.65a**	±0.07	**0.47ab**	±0.05
Equitability	**0.57ab**	±0.07	**0.40b**	±0.07	**0.78ab**	±0.07	**0.83a**	±0.06

[1] Standard Errors. [2] Different letter in the same line mean the statistical differences between the systems ($p < 0.05$). Variables with significant differences are shown in bold text. ^{ns} non-significant

tested the differences using post-hoc tests (HSD Tukey's test, $p < 0.05$). In some cases, GLM was unable to adjust the data, so we used non-parametric Kruskal-Wallis and Mann-Whitney U tests to identify differences between the land use systems studied. Additionally, to explore the correlations between the macrofauna community (density of earthworms, termites, ants, spiders, millipedes, centipedes, beetles, fly larvae and others; total abundance, richness, Shannon, Equitability and Simpson indexes) and soil chemical and textural variables (soil pH, Al^{3+}, H+Al, Ca^{2+}, Mg^{2+}, K^{+}, C, moisture, sand, clay and silt contents), we performed a non-metric multidimensional scaling analysis (NMDS) using Bray-Curtis distance with Vegan package for R (Oksanen et al. 2019).

2.5 Data availability

All data resulting from the soil chemical and physical analyses, as well as on the fauna taxa from each sample collected by soil hand sorting (macrofauna groups), Berlese extractions (mesofauna groups), and the liquid heat-gradient extraction (enchytraeids) are provided in a Supplementary data file that can be downloaded online.

3. Results

3.1 Macrofauna communities

Total macrofauna density ranged from 153 ± 45 to 2,049 ± 367 individuals per square meter (ind. m^{-2}) and was significantly lower in CH than all the other land-uses (Table 1). Abundance in NF was significantly higher than in RT but not different than in OH. Earthworm populations were higher in RT (107 ± 36 ind. m^{-2}) than all other land uses, and also higher in NF (19 ± 5 ind. m^{-2}) than OH and CH (<4 ind. m^{-2}), while termite abundance was higher in NF (162 ± 77 ind. m^{-2}) than the remaining land-uses (<7 ind. m^{-2} in RT and OH and absent in CH). Ants were more abundant in NF (ca. 1,300 ind. m^{-2}) and OH (ca. 1,400 ind. m^{-2}) compared with RT and CH (< 40 ind. m^{-2}) and represented 63 % and 82 % of total macrofauna in NF and OH, respectively (Figure 2A). Millipedes were more abundant in NF than CH, and spider abundance was higher in NF and OH than CH, where these invertebrates were not found. Fly larvae were more abundant in NF (75 ± 33 ind. m^{-2}) than RT and CH (< 3 ind. m^{-2}), while the 'others' group, consisting mainly of gastropods (mostly snails) and true bugs (Hemiptera), was significantly more abundant in RT and NF (ca. 90 ind. m^{-2}) than CH (ca. 10 ind. m^{-2}). Conversely, the abundance of beetles and centipedes was not significantly affected by the land-use systems. Nonetheless, beetles were dominant in CH and represented ca. 65 % of all individuals found in this system (Figure 2A). The abundance of ecosystem engineers was significantly higher in NF and OH (mainly due to ants), than RT and lowest abundance was observed in CH. Engineers represented the majority of the individuals collected in NF (72 % of total) and OH (83 % of total), compared with the other land-use systems (12 % in CH, 32 % in RT).

Total macrofauna group richness was highest in NF and RT (16 taxa), intermediate in OH (13) and lowest in CH (9). Diversity indices tended to be higher in NF compared to the other land use systems (Table 1): mean macrofauna

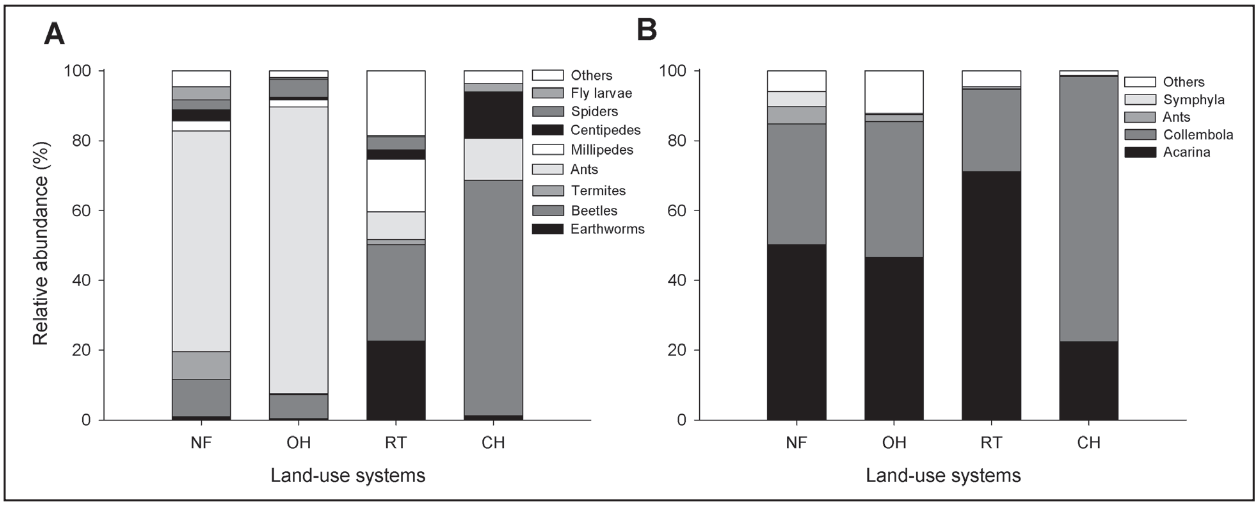

Figure 2. Relative abundance of the most representative soil invertebrate groups (> 2 %) and the ‚others' group of soil macrofauna (**A**) and soil mesofauna (**B**) in four land-use systems (NF = Native forest, OH = organic horticulture, RT = reduced tillage, and CH = conventional horticulture) in Quitandinha, Brazil.

richness (9.3 taxa sample^{-1}) was higher in NF than RT (5.8) and OH (5.2), and CH showed the lowest richness (2.7). The lower Simpson index and Equitability in OH suggests that a small number of taxa dominated the macrofauna community in this system.

A total of six earthworm species were found, belonging to three families, two of them native (Glossoscolecidae and Ocnerodrilidae) and one exotic (Megascolecidae) to Brazil, as well as one unidentifiable species of unknown origin (Table 2). Highest earthworm species richness (4 spp.) was found in NF, with both native *Glossoscolex* sp., one unidentified ocnerodrilid, and the unidentified species, followed by RT and OH with two species (one native and one exotic), and CH, with only the exotic *Amynthas gracilis* (Kinberg 1867). The tiny ocnerodrilids could not be identified to species level, because all individuals collected were juvenile.

The NMDS plot showed relatively good separation of the samples taken in NF, CH and RT, but a wide spread of OH samples indicating higher variability in this system (Figure 3). The variables most closely associated with both conventional production systems (CH and RT) were mainly soil-fertility related (i.e., higher contents of available K, Ca, Mg and pH), due to commercial fertilization and liming in these systems. In terms of the fauna, higher abundance of many taxa were more closely associated with NF samples, that also had higher overall abundance of macrofauna (Table 1). Ecosystem engineers were located at different ends of the plot, with ants more related with OH and NF, termites with NF and earthworms with RT.

Table 2. Terrestrial oligochaetes identified in four land-use systems (NF = native forest, OH = organic horticulture, RT = reduced tillage, CH = conventional horticulture) in Quitandinha, Brazil.

Taxa	**Land-use systems**			
	NF	OH	RT	CH
Native earthworms				
Glossoscolecidae family				
Glossoscolex n.sp. 46	x			
Glossoscolex n.sp. 47	x			
Glossoscolex n.sp. 48		x		
juveniles	x			
Ocnerodrilidae family				
juveniles	x		x	
Exotic earthworms				
Megascolecidae family				
Amynthas gracilis			x	x
juveniles		x		x
Unidentified sp. 1	x			
Total earthworm species	4	2	2	1
Enchytraeids				
Cosmopolitan genera				
Fridericia sp.	x	x	x	x
Enchytraeus sp.	x	x	x	x
Native genera				
Hemienchytraeus sp.	x	x		
Guaranidrilus sp.	x	x		
Total enchytraeid species	4	4	2	2

3.2 Mesofauna communities

Total mesofauna populations ranged from 8,585 ± 1,694 to 37,951 ± 13,185 ind. m^{-2}, and significant differences were detected between land use systems, with highest values in CH, and lowest in NF and OH (Table 3). Mites (Acari) and springtails (Collembola) dominated the mesofauna community in the four land-uses, representing more than 80 % of total abundance overall (Figure 2B). Mite abundance was higher in RT (ca. 14,500 ind. m^{-2}) compared to the other land-use systems. Ant abundance was significantly higher in NF (ca. 640 ind. m^{-2}) and OH (ca. 170 ind. m^{-2}) than CH and RT (ca. 40 ind. m^{-2}). The ‘others’ group, formed by less abundant taxa, including several predators (Aranae, Coleoptera, Diplura, Hemiptera, Dermaptera, Chilopoda), detritivores (Protura, Coleoptera, Thysanoptera, Diptera larvae, Diplopoda, Blattaria, Isopoda), and plant pests (Hemiptera, Coleoptera, Dermaptera) also showed significant differences between land-use systems, with higher density in RT (ca. 930 ind. m^{-2}) and OH (ca. 1,050 ind. m^{-2}) compared to CH (490 ind. m^{-2}). Total mesofauna group richness ranged from 11 (CH) to 13 (OH, NF, RT) taxa, while Shannon indices were higher in NF, OH and RT compared with CH. Simpson indices were higher in NF and OH compared to CH, and Equitability was higher in NF compared to RT and CH.

Enchytraeid populations were higher in NF (ca. 19,400 ind. m^{-2}) than all other land uses (Table 3) in the winter (Sept. 2013), while in the summer (Jan. 2014), they were higher in OH (> 26,000 ind. m^{-2}) than all the other sites. Abundance was significantly higher in the summer for all land uses except NF. Lowest abundance was found in CH, on both sampling dates. Overall, four genera, two of them cosmopolitan (*Fridericia, Enchytraeus*) and two native to South America (*Hemienchytraeus, Guaranidrilus*) were encountered (Table 2). Higher richness (4 genera) was found in OH and NF that had both native and exotic species, compared to CH and RT where only the cosmopolitan *Fridericia* and *Enchytraeus* were found.

Table 3. Soil mesofauna abundance (number of individuals m-2) and selected diversity indices in four land use systems (NF = native forest; OH = organic horticulture; RT = reduced tillage; CH = conventional horticulture) in Quitandinha, Brazil.

Taxa	Land use systems							
	NF		OH		RT		CH	
	Ind. m-2	SE	Ind. m-2	SE	Ind. m-2	SE	Ind. m-2	SE
Acarina[1]	**6,395b**	±2,206	**3,994b**	±946	**14,516a**	±3,170	**8,506b**	±2,853
Collembola ns	4,418	±853	3,343	±996	4,804	±921	28,848	±12,308
Symphyla ns	544	±445	27	±17	92	±36	67	±24.2
Ants	**636a**	±272	**172a**	±77	**39b**	±20	**40b**	±20.5
Others	**756ab**	±164	**1,048a**	±215	**928a**	±171	**490b**	±166
Total	**12,749b**	±2,802	**8,585b**	±1,694	**20,378ab**	±4,004	**37,951a**	±13,185
Enchytraeids Sept. 2013	**19,388Aa**	±4,878	**11,398Bb**	±3,908	**10,300Ab**	±2,821	**2,802Bc**	±675
Jan. 2014	**21,112Ab**	±6,386	**26,066Aa**	±10,874	**6,780Bc**	±1,086	**4,791Ad**	±1,143
Total richness	13		13		13		11	
Mean richness[2]	4.6	±0.4	4.5	±0.5	4.7	±0.2	3.5	±0.4
Shannon (H')	**1.01a**	±0.08	**1.06a**	±0.12	**0.87a**	±0.04	**0.64b**	±0.07
Simpson (D)	**0.54a**	±0.03	**0.54a**	±0.05	**0.47ab**	±0.02	**0.38b**	±0.04
Equitability	**0.69a**	±0.04	**0.67ab**	±0.06	**0.58b**	±0.03	**0.56b**	±0.07

[1] Different lower-case letters in the line means statistical differences between land use systems ($p < 0.05$), while upper-case letters mean significant differences between sampling dates for enchytraeids. Variables with significant differences are shown in bold text. [2] Average richness per land-use system (number of taxa per sample). ns Non-significant

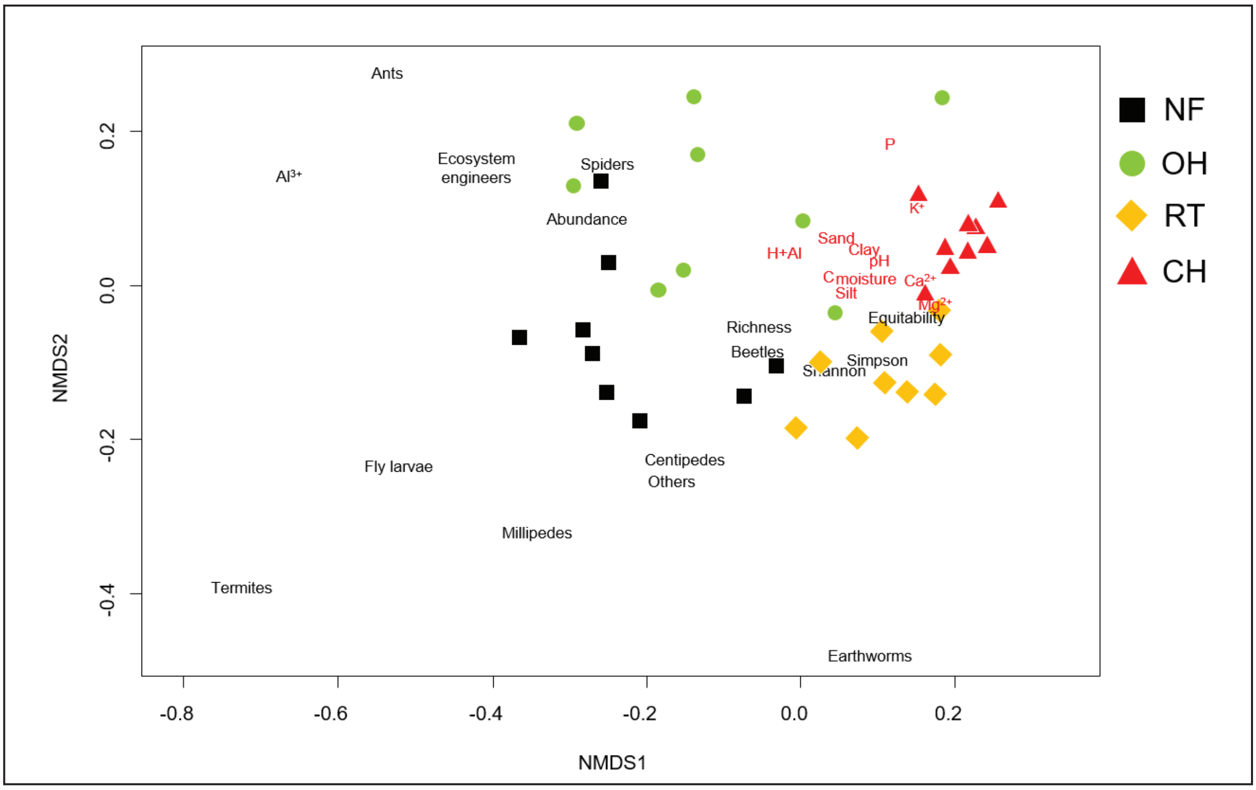

Figure 3. Non-metric multidimensional scaling (NMDS) plot showing the relationship between macrofauna taxa (black text) and soil chemical and physical properties (red text) of samples taken in four land-use system in Quitandinha, Brazil. NF = Native forest, OH = Organic horticulture, RT = Reduced tillage, CH = Conventional horticulture.

3.3 Soil properties

All soils had > 35% clay, but there were significant differences between the particle size distributions: CH had clayey texture, with significantly higher clay content than the remaining systems, all of which had a clay loam texture (Table 4). The silt contents were slightly higher in RT, but the difference was only significant compared to CH. Soil chemical attributes varied significantly among the land-use systems evaluated (Table 4). Higher soil pH values were found in CH (6.1) and RT (5.8), where base saturation (V) and Ca contents were also higher. Highest Mg contents were found in RT (2.8 $cmol_c$ dm^{-3}), followed by CH (2.2 $cmol_c$ dm^{-3}), NF (1.3 $cmol_c$ dm^{-3}) and OH (0.9 $cmol_c$ dm^{-3}). Soil moisture was very similar among the land-use systems, showing significant differences only between RT and OH (19 and 14.5%, respectively). Higher organic carbon contents were found in NF (20.9 g dm^{-3}), followed by RT (16.6 g dm^{-3}), while lowest values were found in OH (12.4 g dm^{-3}).

4. Discussion

Soil invertebrate communities are highly sensitive to soil disturbance, and more intensively managed systems, particularly with intensive soil tillage tend to be more detrimental (Wardle 1995, Kladivko 2001), by damaging larger soil animals directly (e.g., earthworms), destroying galleries and tunnels constructed by burrowing animals, modifying soil porosity and affecting soil temperature and moisture regimes, as well as reducing soil organic matter contents over time (Kladivko 2001). However, tillage impacts can be quite variable depending on the taxon in question, as well as on the soil type and texture, type and frequency of tillage and the use of other management practices (e.g., pesticides or organic fertilizers) in the agroecosystem (Wardle 1995, Holland 2004, Reeleder et al. 2006, Sheibani & Ahangar 2013, Ponge et al. 2013, Pelosi et al. 2014). Hence, it is not surprising that we found variable responses to soil disturbance, although the most disturbed system, with rotary hoe and pesticide application (CH) had the lowest soil macrofauna abundance and richness of all land use systems evaluated. Fewer taxa of both macro- and mesofauna, less earthworm species, and fewer earthworms, enchytraeids, ants, spiders, fly larvae and ‘other’ macro and mesofauna were found in this system compared with the others. With a dominance of only one macrofauna group (beetles) and two mesofauna taxa (mites and collembola) and a low abundance of predators and detritivores in general, this land use system’s biological functioning is bound to be impaired.

The widely reported positive impact of reduced tillage on soil fauna communities (Kladivko 2001, Holland 2004) was partially confirmed in the present case, since RT had higher macrofauna taxonomic richness than CH and OH (similar to NF), higher mesofauna taxonomic richness than CH, higher mite abundance than all the other land use systems, and higher earthworm abundance than the intensively cultivated systems (CH, OH), and NF. However, total abundance and that of ‘other’ mesofauna and enchytraeids (in 2013) was not different in RT and OH, and both ant and total macrofauna abundance as well as enchytraeid and earthworm richness were lower in RT than the intensively tilled OH. Most studies evaluating tillage impacts on soil fauna in Brazil compare no-tillage with conventional tillage (Sautter et al. 1999, Aquino et al. 2008, Demetrio et al. 2020), while few

Table 4. Soil chemical properties, moisture and particle size analysis results under native Atlantic rainforest (NF), organic horticultural production (OH), conventional grain crop production with reduced tillage (RT) and conventional horticulture production with intensive tillage (CH) in Quitandinha, Brazil.

Land-use systems	pH	H+Al	Ca	Mg	K	P	C	V[1]	Moisture	Sand	Clay	Silt
	$CaCl_2$		$cmol_c$ dm^{-3}			mg dm^{-3}	g dm^{-3}			%		
NF	4.4c[2]	6.6a	3.1b	1.3c	0.2b	13.3b	20.9a	41b	17.6ab	449	367b	184ab
OH	4.9bc	4.7b	2.6b	0.9d	0.3ab	36.0a	12.4c	44b	14.5b	444	371b	185ab
RT	5.8ab	3.3c	4.7a	2.8a	0.2b	12.7b	16.6b	70a	19a	379	354b	267a
CH	6.1a	2.9c	4.8a	2.2b	0.6a	35.9a	13.8bc	72a	19.9ab	410	454a	136b

[1]V= Base saturation; [2]Lower-case letters in the same column indicates statistical differences among the land-use systems ($p < 0.05$).

studies have compared RT with more intensively tilled systems (Pandolfo et al. 2005, Zagatto et al. 2019). For instance, the review of Brown et al. (2003) reported higher earthworm densities under RT (5 to 407 ind. m^{-2}) than conventional tillage (0 to 45 ind. m^{-2}) from eight sites in Northern Paraná, a trend that was confirmed in the present study.

In terms of mesofauna, Zagatto et al. (2019) also found high mite and collembola populations associated with reduced tillage systems in Southern Brazil (at Ponta Grossa, around 120 km from Quitandinha), while Pandolfo et al. (2005) found high enchytraeid populations in all tillage treatments (RT, zero-tillage and intense soil preparation), but a tendency for lower springtail numbers with increasing tillage intensity. Springtails are primarily fungivores and detritivores (Coleman et al. 2004), so are generally enhanced by organic or surface residue additions (House & Parmelee 1985). Hence, we expected their populations to be higher in RT and NF than OH and CH. However, abundance values were highly variable, and no differences between the land uses were detected, despite a trend for higher values in CH. Variable springtail responses to tillage intensity have been reported, supporting the notion that they might not be very useful soil disturbance indicators (Fiera et al. 2020). The higher springtail abundances found in conventionally tilled systems than under no-tillage (van Capelle et al. 2012) or RT (Fiera et al. 2020), may be related to abiotic factors such as changes in pore structure and connectivity, associated with RT, where compaction from repeated wheel traffic becomes a greater issue (Heisler & Kaiser 1995, Beylich et al. 2015). However, changes in pesticide use (particularly herbicides for weed control; Conti 2015) as well as biological interactions such as reduced competition or predation in more intensively tilled systems may also be important (Fiera et al. 2020).

The absence of disturbance and the presence of a thick and diversified leaf-litter layer in native forests reduces soil environmental (mainly temperature and moisture) variations and provides additional niches for the development of litter-dwelling invertebrate populations (Decaëns et al. 2004). Hence, it is not surprising that NF had high values of overall richness and diversity of soil macro and mesofauna, and the highest number of earthworm species and enchytraeid genera, confirming results found by other authors in the *Araucaria* forest region (Duarte 2000, Bartz et al. 2014, Niva et al. 2015, Pereira et al. 2015, Oliveira-Filho et al. 2018, Demetrio et al. 2018). It also had higher abundance of termites, spiders, fly larvae, 'other' soil invertebrates and earthworms than OH and CH, both high disturbance land-uses. Native forests in Southern and Southeastern Brazil, particularly *Araucaria* forests tend to have low earthworm abundance (Brown & James, 2007), ranging from 0 (Pompeo et al. 2016) to 93 individuals m^{-2} (Tanck et al. 2000), with an overall mean of 28 ± 7 individuals m^{-2} (mean ± SE), calculated from 17 sites and 11 studies (values from Nadolny et al. 2019), an abundance value close to that found at NF (19 ind. m^{-2}). The low soil chemical fertility in most *Araucaria* forests with highly acidic pH, may be limiting to the development of earthworm populations in these systems (Silva et al. 2019), affecting not only the invertebrates themselves, but also the quality of the organic material added to the soil surface (Ketterings et al. 1997).

All the oligochaete species, both of earthworms and enchytraeids were exotic and cosmopolitan in RT and CH, although their abundance was significantly higher in RT. These peregrine species may be more resistant to pesticide applications commonly used in high-input agroecosystems, and in Brazil *Amynthas* earthworms have been frequently found in agricultural fields (Brown et al. 2006). Their high abundance in fields with lower tillage may result in important improvements to soil structure and nutrient cycling, as well as plant production (Peixoto & Marochi 1996), phenomena which may be occurring in RT and which deserve further attention. Conversion of native vegetation, along with long-term soil disturbance in cropping systems tends to eliminate native earthworm species, and these empty niches are generally taken up by exotic peregrine or cosmopolitan species (Fragoso et al. 1997). However, under some circumstances, native species may survive (but generally in low abundance), such as in no-tillage agroecosystems (Bartz et al. 2014), although the reasons for this are still not well understood. In the present case, one *Glossoscolex* sp. was found in OH, despite intensive tillage, indicating that the organic inputs and absence of pesticide use over the long-term (> 20 yr) have allowed the survival of this species.

Enchytraeids are good indicators of soil disturbance (Pelosi & Römbke 2016), and in our study they were deeply affected by intensive tillage when combined with pesticide use, so that lowest abundance and richness were found in CH compared to all other systems. In OH high abundance of mostly the cosmopolitan *Fridericia* and *Enchytraeus* were observed, although native species were found in lower abundance. *Fridericia* and *Enchytraeus* are peregrine genera found worldwide (Schmelz et al., 2013), and their presence in temperate climate regions (such as Quitandinha), can be associated to disturbed soils (Jänsch et al. 2005). Several *Enchytraeus* and *Fridericia* found in Paraná State are fragmenting species common in open landscapes (Römbke et al. 2007). These species include mainly r-strategists, but also some cocoon-producing *Enchytraeus* (Graefe & Schmelz, 1999), making them especially good and rapid colonizers of anthropically disturbed areas. Only 62 species of enchytraeids were known from South America in 2013 (Schmelz et al. 2013), although they may even have originated in this continent (Coates, 1989), and their diversity is expected to be much higher (Schmelz et al. 2013). *Hemienchytraeus*, *Guaranidrilus* and *Achaeta* are the dominant genera in Atlantic Forests of Southern Brazil (Schmelz et al. 2009) and *Guaranidrilus* was the most abundant genus in NF.

Like earthworms, enchytraeids feed on soil organic matter and decomposing plant litter, creating biological aggregates that contribute to organic matter stabilization in soils (Coleman & Wall 2015). The abundance of these small oligochaetes in European regions can reach up to 50,000 ind. m^{-2} (Kapusta et al. 2003), but in Brazil few studies have focused on enchytraeids (Schmelz et al. 2009, 2013). Niva et al. (2015) reported populations around 12,667 ind. m^{-2} in *Araucaria* forests in Curitiba (45 km from Quitandinha), while Römbke et al. (2015) reported densities ranging from 124 to 5,194 ind. m^{-2} in regenerating Atlantic forests and pastures, on the coastal plain of Paraná (about 120 km from Quitandinha). Van Vliet et al. (1995) also reported that enchytraeids were important for soil structure, and more so in cultivated areas (with and without tillage) than areas with forests in Southeastern USA. Few studies have compared tillage system effects on enchytraeids in South America. Manetti et al. (2010) and Brown et al. (2001) reported higher abundance of enchytraeids in arable cropping than in no-tillage, but these studies counted enchytraeids from soil monoliths using hand sorting, which is much less effective for sampling enchytraeids (Niva et al. 2015). However, the present study somewhat confirmed this trend, although abundance was higher in the organically managed intensively tilled system (OH) only in the summer, compared with RT. It is likely that these high abundances are contributing to aggregate formation in OH, particularly by the larger-bodied *Fridericia* sp., although the role of enchytraeids in this process has been little explored outside the Northern Hemisphere (van Vliet & Hendrix 2012). Future research in this area will certainly provide novel results and help further understand the functional role of enchytraeids in South American soils under various land use systems.

Despite the intense disturbance (harrowing) performed in OH, the absence of pesticide use and the application of organic manures and ash resulted in much higher abundance and overall diversity of soil macro and mesofauna (although earthworm abundance was still very low). The abundance of ants, spiders, enchytraeids and 'other' mesofauna (including many predators and detritivores) were particularly enhanced in this land use system, resulting in a significantly different community composition than in CH, which will ultimately impact differently on soil functioning. Organic production systems are generally considered more environmentally-friendly, and the present study confirmed that several taxa of macro- and meso-fauna were benefitted from this management system compared to CH. However, the intensive tillage in this system preferentially benefitted enchytraeids over earthworms, and continuous soil disturbance, e.g., for seedbed preparation or weed control may become limiting factors over the long-term for the soil fauna (Nakamoto et al. 2006).

Reduced tillage generally increases the abundance of litter-dwelling macrofauna, particularly predators, saprotrophs/decomposers and ecosystem engineers such as earthworms, termites and burrowing scarab beetle larvae (Briones & Schmidt, 2018, House & Parmelee 1985, Brown et al. 2001, 2003, Holland 2004). Hence, reducing tillage in OH would likely improve conditions for soil fauna populations and their contribution to soil functioning in this system. Furthermore, organic agriculture can increase soil invertebrate populations, with the addition of organic manures and the lack of inorganic pesticide applications (Domínguez et al. 2014).

5. Conclusion

Conventionally managed agroecosystems with high level of soil disturbance and pesticide use can be catastrophic for soil macrofauna communities. For earthworms, soil tillage seems to be the main factor responsible for low density and diversity. On other hand, other taxa, such as ants, spiders and enchytraeids seem to be less dependent on tillage and more affected by pesticide use and organic matter additions. However, management practices in agroecosystems affect soil fauna in different ways, and few studies have addressed soil fauna communities in vegetable production systems in Brazil. Hence, further work is still needed in order to determine the impact of reduced tillage in both CH and OH and the best combination of management practices (e.g., cover crops, organic fertilizers, integrated pest management) that can promote beneficial taxa and fauna contributions to soil function and services in these systems.

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