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ORIGINAL PAPER

Community Structure of Soil Nematodes Associated with the Rhizosphere of *Solanum Lycopersicum* in a Major Production Area in Argentina: a Case Study Among Agroecosystem Types

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Abstract
The objective of this work was to describe the status of the assemblages of soil nematodes associated with tomato crops, in a major tomato production area in Argentina, by studying ecological indices of nematode food web and determining which indices describe alterations in horticultural soils. Three agroecosystem types were compared: agroecological, conventional with organic amendments, and conventional with methyl bromide (MeBr) application. A pristine site without anthropogenic intervention was also studied. Abundance, frequency, diversity indices, maturity indices, trophic groups, colonizer-persister values (cp), and guilds of nematodes were analyzed and compared statistically between the sampling sites. The most abundant genera in the agroecosystems were *Rhabditis*, *Helicotylenchus*, and *Filenchus* belonging to the guilds bacterivorous (Ba_1), plant feeders (Pl_3), and fungivorous (Fu_2). Within agroecosystems, the agroecological site showed the highest values of diversity and maturity, while the lowest values were found in the site treated with MeBr. Trophic groups, cp, guilds, and the indices provided useful information on the state of maturity and enrichment that effectively contrasted and differentiated the practices of horticultural management. This updated report of the nematode fauna constitutes one of the most important for the horticultural region of La Plata considering the scarcity of studies on this subject.

Keywords Soil nematodes · Agroecosystems · *Solanum lycopersicum* · Bioindicators · Argentina · Soil health

1 Introduction

Soil is the fundamental system in which agricultural production is maintained and where soil inhabitants provide vital ecosystem services (Bardgett and Van der Putten 2014). However, agroecosystems are generally characterized by having periodic perturbations due to management practices, thus altering the soil structure and influencing the faunal communities (Timper 2014). Agriculture worldwide uses a large percentage of the energy available on our planet, which is considered non-renewable energy and is directly related to greenhouse gas emissions (Platis et al. 2019). This issue is summarized in the use of fertilizers to increase crop yields, affecting the structure of communities of edaphic organisms; and pesticides: their use, continuously increasing, is considered a solution for pest control, without clearly determining the effect they have on accompanying organisms within agroecosystems (Yang et al. 2019, Zaller and Brühl 2019). The main objectives to face these problems must take into account the implementation of practices that are friendly to the environment and develop integral fertilization systems through organic amendments and share and integrate the concept of biological control within all the entities involved in the development of agricultural practices (Quiroz and Céspedes 2019, Wyckhuys et al. 2018). In order to measure and evaluate the impact that anthropic interventions have on soil ecosystems, the development of environmental indicators is necessary. Bioindicators are organisms whose responses to disturbances in the environment can be measured and evaluated using indices of diversity, similarity, and community comparison indices, indicating the biotic and abiotic state of the ecosystem (Gerhardt 2002).

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Nematodes are cosmopolitan organisms, found in large abundances and biodiversity in soil. They are involved in nutrient cycles through feeding on microorganisms, and the extent of their functioning is affected by environmental changes and by biological interactions and in the food web, they have specific roles classified into trophic groups and functional guilds (bacterivorous, herbivorous, fungivorous, omnivorous, and predatory nematodes) (Sánchez-Moreno and Talavera 2013). Considering these features, soil nematode abundance, diversity, and role in the trophic web can serve as bioindicators reflecting changes in agroecosystems (Bongers 1999; Neher 2001, 2010; Ferris 2010; Gnamkoulamba et al. 2018). The main parameters studied in order to characterize the soil nematode communities are species abundance and diversity (Sattler et al. 2010). Notwithstanding, such parameters themselves do not explain the effects of pollution in the environment. Therefore, some ecological indices have been proposed, as the maturity index (MI), structural index (SI), enrichment index (EI), and channel index (CI), diversity of Shannon-Weaver (H'), dominance of Simpson, and uniformity of Pielou (J'). These indices reveal descriptive and qualitative information on the soil nematode community and the conditions of the natural and/or agricultural systems (Bongers 1990; Ferris et al. 2001).

In the Province of Buenos Aires, Argentina, horticulture is one of the main agricultural activities, with the highest concentration of production in the La Plata area. This zone with about 5000 ha, cultivated mostly with conventional intensive agricultural systems, contributes 60% of the tomato production consumed at a national level (Ferratto et al. 2010). The agricultural practices alter the structure and characteristics of the community of nematodes in distinguishable patterns, forming networks of nematodes of different structures and composition. Using this framework, the objective of this work was to describe the status of the assemblages of soil nematodes associated with tomato crops, in a major tomato production area in Argentina, by studying ecological indices of nematode food webs to determine those that best describe the alterations suffered in horticultural soils.

2 Materials and Methods

2.1 Study Area

The study area was constituted by orchards associated mainly with tomato cultivation located in the horticultural area of La Plata, Buenos Aires, Argentina (Fig. 1). The sampling sites were characterized and defined according to the diversity and rotation of crops, the use of organic and inorganic fertilizers, and the application of biocides at the moment prior to the start of data collection (Table 1).

Agroecological site (S1): tomato crop (*Solanum lycopersicum* var. Platense) with infiltration irrigation (or channels), in which no type of agrochemical input is used. The crop rotation and the conservation of spontaneous weeds are part of the premises of these producers, who seek, increasing the diversity of species to reduce the appearance of organisms considered crop pest. At the beginning of the spring season, the land of the ridges is prepared by removing the weeds with a chisel plow. In the middle of the spring, the tomato seedlings are transplanted. During this season, the spontaneous weed (*Matricaria recutita* and *Silybum marianum*) is allowed to grow. In the summer, the crop is characterized by the presence of tomato plants (*Solanum lycopersicum* var. platense) and broccoli (*Brassica oleracea* var. *italica*). In the autumn, leeks (*Allium ampeloprasum* var. *Porrum*), and scallions (*Allium fistulosum* L.) are sown. During this season and until half-two of the winter season, the ridges were partially covered by weeds such as purslane (*Portulaca oleracea*) and thistles (Fam. Asteraceae). In late winter, the land was prepared to sow cabbage (*Brassica oleracea* var. *Capitata*), broccoli, and tomato. Soil sampling in this site was carried out for two seasons between spring 2015 and summer 2017.

Conventional site (S2): tomato crop (*Solanum lycopersicum* var. platense) under cover with drip irrigation. In this system, the use of organic amendments of the Brassicaceae family is applied two times per year as a method of fertilizing the soil based on organic nitrogen, in order to seek alternatives to the use of commercial inputs. Towards the end of the winter, the producers add the preparation of organic amendments (approximately 50 kg per crop ridge). At the beginning of the spring, the land is prepared by conventional plowing and the tomato transplant (*Solanum lycopersicum* var. platense) is carried out. At the end of summer, the second fertilization is applied after harvest, to prepare a second tomato crop during autumn and winter with crop rotation (*Solanum lycopersicum* var. elpida). Soil sampling in this site was carried out for two seasons between spring 2015 and summer 2017.

Intensive conventional site (S3): tomato crop (*Solanum lycopersicum*) under cover with drip irrigation and application of nematicides and nitrogen fertilization. Methyl bromide (MeBr) is applied as a soil disinfection method (nematicide) in order to control *Nacobbus aberrans*, the main genus of pest phytonematodes in the region. The application of MeBr (32 g/m^3) is made at the end of each winter season; then, the greenhouse is closed to prevent the access of people and animals. At the beginning of the spring of the same year, the soil is fertilized (100 kg ha^{-1} N-NO3$^-$) and tomato seedlings are transplanted (*Solanum lycopersicum* var. platense). At the end of the summer, the harvest is finished, the land is prepared, and in the autumn and winter season, tomato are transplanted again (*Solanum lycopersicum* var. elpida and var. cerasiforme). Soil sampling in this site was carried out for two seasons between the ends of spring 2016 and summer 2018.

Fig. 1 Location of the four sampling sites within the horticultural region of La Plata, Buenos Aires Province, Argentina, between spring 2014 and summer 2018 (S1 = agroecological site; S2 = conventional site with organic amendments; S3 = intensive conventional site with biocides application; S4 = pristine site without anthropic intervention). Map made with ArcGIS software by Esri

Pristine site (S4): forest located in the biosphere reserve of Pereyra Iraola Park without any anthropic intervention of any kind, which allowed to collect data from a stable system over time, without structural changes in the soil profile. The physiognomy of this site is characterized by the presence of trees of the genus *Quercus*, *Pinus*, *Cupressus*, and temperate grasslands (fam. Poaceae) which have a high degree of coverage (between 80 and 90%). This physiognomy did not change in the running of the samples. Sampling began during the winter of 2016 and culminated during the autumn season of 2018.

2.2 Obtaining Soil Samples

In order to monitor the edaphic nematode communities, soil sampling was carried out in eight instances every 3 months for each site between spring 2014 and summer 2018. In each of the four sites, a sampling plot of 15 × 30 m was delimited, taking two composite samples consisting of 20 cores (30 cm deep) with a soil auger in a Z-pattern, in the tomato plants' rhizosphere. Each composite sample was deposited in a bag protecting it from direct sunlight and labeled. In the laboratory, each composite sample was screened and homogenized before being stored in a refrigerator at 4 °C for further analysis.

2.3 Extraction, Identification, and Analysis of the Nematode Community

For the isolation of nematodes, 100 cm^3 of soil were taken from each sample and centrifuged, dissolved in water for 3 min at 3000 rpm to eliminate unwanted material that makes

Table 1 Main characteristics of the selected sampling sites (S1 = agroecological site; S2 = conventional site with organic amendments; S3 = intensive conventional site with biocide application; S4 = pristine site without anthropic intervention). In crops S2 and S3, the application of inputs was carried out 2 months before the start of data collection. S1 has the highest rotation and types of crops while conventional sites S2 and S3 have little rotation and application of agrochemical inputs. S4 presents a stable physiognomy throughout the year

Site	Localization	Applied inputs	Sampling timeline	Crop rotation	Present vegetation in the plot
S1	34° 48′ 21.632″ S 58° 7′ 27.005″ W Hudson, Berazategui	–	Spring 2015–summer 2017	High crop diversity, constant rotation among different horticultural species, development of seasonal crops.	*Solanum lycopersicum* var. platense, *Matricaria recutita*, Fam. Asteraceae, *Brassica oleracea* var. *italica*, *Allium ampeloprasum* var. *porrum*, *Allium fistulosum* L., *Portulaca oleracea*, *Brassica oleracea* var. *capitata*
S2	34° 59′ 6.104″ S 57° 59′ 49.708″ W Los Hornos, La Plata	Organic amendments of cruciferous (Brassicaceae) as fertilizer	Spring 2015–summer 2017	Low crop diversity, occasional crop rotation, intensive development of tomato cultivation.	*Solanum lycopersicum* var. platense, *Solanum lycopersicum* var. elpida
S3	34° 56′ 29.684″ S 58° 4′ 54.534″ W Melchor Romero, La Plata	MeBr (32 g/m^3) and nitrogen fertilizers (100 kg ha-1 NO3-)	Spring 2016–summer 2018	Low crop diversity, occasional crop rotation, intensive development of tomato cultivation.	*Solanum lycopersicum* var. platense, *Solanum lycopersicum* var. *cerasiforme*
S4	34° 50′ 56.062″ S 58° 7′ 12.256″ W Parque Pereyra Iraola, La Plata	–	Winter 2016–autumn 2018	–	*Quercus*, *Pinus*, *Cupressus* y grass (fam. Poaceae)

it difficult to observe specimens. The resulting supernatant containing residual organic matter was discarded. The decanted material was homogenized again in a sucrose solution (484 g sucrose/L H_2O) and centrifuged for 3 min at 3000 rpm (Caveness and Jensen 1955). The supernatant with nematodes in suspension was poured into screens with a 40-μm opening, collecting and depositing the specimens in a final solution of 25 ml: 22 ml of water and 3 ml of TAF (triethanolamine 2% (v/v) and formalin to 7% (v/v) in distilled water). From this final suspension, five random aliquots of 1 ml were taken for the nematode count. The final number of nematodes found was extrapolated to reach the volume of the initial solution (25 ml). Nematodes were identified to genus using the key of Heyns 1971, Manzanilla-López and Marbán-Mendoza 2012 and Chaves et al. 2019. The taxonomic determination was made at 40–100× using a stereoscopic magnifying glass (Hokenn Optik model ZTX E ZOOM) and a stereoscopic microscope (Leica model DM-500). In cases where it was not possible to identify individuals at the genus level, they were located at the family level.

2.4 Structure and Diversity Indices

In each sampling site, the abundance values of nematodes were used for the indices of structure and diversity of the ecosystem. For the calculation of the function indices, the nematodes had to be classified based on the diverse characteristics they present within an ecosystem: whether they are opportunistic colonizer or persistents sensitive to environmental disturbances (value of cp proposed by Bongers 1990); whether they feed on bacteria, fungi, plants, or other nematodes (trophic group, Yeates et al. 1993); and on the basis of what function they fulfill (ecological functional guilds, Ferris et al. 2001). The indices calculated were relative abundance (%) = $n_s/N_{\text{total}} \times 100$ (where n_s is the number of individuals of a given genus and N the total number of individuals in a sample); frequency (%) = 100 × (number of samples where a certain genus was present)/(number of total samples examined); genus richness (S): number of genera present in each sampling site; Margalef's richness index (D_{mg}) = $S{-}1/\ln N$ (where S indicates the number of genera and N the total number of individuals in a sample); the Shannon-Weaver diversity index (H') = $-\Sigma S_i = 1 p_i \cdot \log 2\ (pi)$ (where p_i is the total individuals' abundance of each genus i that contributes to total diversity); and Pielou Index (J') = $H'/H'_{\max}$ (where $H'_{\max} = \ln S$) (Norton 1978). The analysis of ecological index values was carried out using the PRIMER software (Plymouth Routines Multivariate Ecological Research, version 6) (Clarke and Gorley 2001). Nematodes can behave as colonizing or persistent organisms thus reflecting changes in the soil they inhabit. Based on these characteristics, Bongers (1990) assigned families of nematode values of 1–5 on a scale of colonizer-persister (cp). The cp 1 nematodes have short life cycles and are tolerant of environmental disturbances, they are considered colonizers and dominant in soil samples with anthropic intervention. The cp 2 nematodes have longer life cycles and less fertility than cp 1; they are very tolerant against polluting conditions indicating polluted soils. The cp 3, cp 4, and cp 5 nematodes have even longer life cycles than the previous categories and are considered persistent nematodes of great sensitivity to adverse conditions. The cp category for plant feeder nematodes (pp) takes values from 2 to 5 (pp 2–5). This differentiation is used to more adequately interpret the behavior of this group since its adaptive strategies against environmental changes depend to a greater extent on the availability of host plants and not on the state of the soil itself. Ferris et al. (2001) defined functional guild as an assembly of species with biological attributes and responses to similar environmental conditions and classified it as the most robust and informative category for the analysis of nematode communities. This category combines the trophic group of nematodes with their value on the cp scale, forming 16 known functional guilds: bacterivorous with cp 1–4 value (Ba1–4), plant feeders with cp 2–5 value (Pl 2–5), fungivorous with cp 2–4 (Fu 2–4), predators with cp 3–5 value (Pr 3–5), and omnivore nematodes with cp 4–5 value (Om 4–5). Using the NINJA (Nematode INdicator Joint Analysis) software (Sieriebriennikov et al. 2014), maturity indices were determined: maturity index (MI) $= \Sigma$ (vi × fi)/n (where vi = colonizer-persister value (cp) (assigned to the family, fi = frequency of family i in the sample, n = total number of individuals in a sample); plant-parasitic index (PPI); MI 2–5; ΣMI; and indices based on functional guilds, Enrichment Index (EI), Structure Index (SI), Channel Index (CI), and Basal Index (BI) (Neher, 2001; Neher and Darby 2009). Maturity indices were computed separately from free-living and plant-feeder nematodes.

To compare the different measurements between the sites, an analysis of variance (ANOVA) was used and the data was transformed to Log $(x+1)$. When the data did not meet the normality criterion, the Kruskal-Wallis test was used. If significant differences were found, post hoc analyses were performed with the HSD method of Tukey with a level of significance of 5%.

3 Results

A total of 47 taxa within 24 families of nematodes were found at the four sampling sites. Among them, 42 were determined at the genus level, while 5 taxa were identified at the family level. In S1, the relative abundances, expressed in percentage, were higher for the genus *Helicotylenchus*, doubling the value of abundance found for the second and third genus (*Tylenchus* and *Cruznema*, respectively). These three genera contributed to approximately 50% of the total nematodes. *Rhabditis* and *Filenchus* were the following genera surpassing 5% of relative abundance. *Tylenchus*, *Filenchus*, *Mesorhabditis*, and *Panagrolaimus* presented a frequency of 100% for this site (Table 2). In S2, the genera *Rhabditis*, *Helicotylenchus*, *Rhabditidae* (unknown genus), and *Mesorhabditis* were the most abundant, representing, overall, more than 60% of the relative abundance of the total genera in this community. In this site, the frequency analysis of these first three groups was 100% while for *Mesorhabditis*, it was 87.5% (Table 2). In S3, it was determined that the largest relative abundances were those presented by the genera *Rhabditis*, *Nacobbus*, *Rhabditidae* (unknown genus), and *Mesorhabditis*, the sum of their values reaching more than 70% of the relative abundance of the total genera analyzed for this site. Coinciding with the most abundant genus, *Rhabditis* was also the most frequent, reaching 100% in the total number of samples together with *Mesorhabditis* (Table 2). The genera that showed the greatest abundance in S4 were *Helicotylenchus*, *Filenchus*, and *Tylenchus*, adding up to a percentage of relative abundance exceeding 70% of the total; reaching the same genera a frequency of 100% (together with *Aphelenchus* and *Acrobeles*). The fact that these three genera have the highest values of abundance and frequency evidences an apparent dominance of these genera at the site without anthropic intervention (Table 2).

3.1 Trophic Groups

The morphological characterization of the main structures of the digestive system allowed to differentiate the five known trophic groups. The results of the study in this category showed that the highest average abundance of bacterivorous was found in conventional with MeBr and organic amendments (831.9 and 559.38 indv/100 cm^3, respectively). The plant parasites were found in a greater proportion in pristine than the agroecological site (1087.5 and 405 indv/100 cm^3, respectively). The fungivorous nematodes presented an average abundance of 702.5 indv/100 cm^3 in pristine site. Due to the few specimens of predators and omnivores found in each site, their respective abundances were added, finding the highest values of this category in the pristine site (42.5 indv/100 cm^3) (Fig. 2). The results of the analysis of variance (ANOVA) found significant differences for the trophic groups of bacterivorous and plant parasites (Fig. 2). Within the plant-feeder category, significant differences were found between those nematodes considered sedentary parasites represented by *Nacobbus* ($p = 0.0249$) between pairs of sites S3–S1 and S3–S4, and semi-endoparasites represented almost exclusively by *Helicotylenchus* between the pairs of sites S1–S2, S3–S4, and S2–S4.

3.2 Colonizer-Persister Scale

The result of the analysis of the adaptive characteristics of the edaphic nematodes determined representatives of the five categories of colonizer-persister proposed by Bongers (1990) to understand the life strategies that these organisms present in the different ecosystems. The result showed that the higher cp 1 average abundance was found in the S3 and S2 crops (805 indv/100 cm^3 and 500 indv/100 cm^3 respectively); in S4, the highest values of cp 2 (763.75 indv/100 cm^3) and cp 3–5 (46.25

Table 2 Relative abundance (r.a. %) and frequency (fr. %) of the determined genus in three agroecosystem and a pristine site in the horticultural region of La Plata, Buenos Aires Province, Argentina, between spring of 2014 and summer of 2018 (S1 = agroecological site; S2 = conventional site; S3 = intensive conventional site; S4 = pristine site without anthropic intervention)

	S1		S2		S3		S4	
	r.a. (%)	fr. (%)	r.a. (%)	fr. (%)	r.a. (%)	fr. (%)	r.a. (%)	fr. (%)
Acrobeles	3.06	100	1.43	62.5	0.28	37.5	1.34	100
Acrobeloides	1.32	37.5	1.19	50	0.56	50	–	–
Aglenchus	2.08	50	0.81	50	–	–	0.26	25
Aphelenchoides	0.63	50	0.31	37.5	–	–	–	–
Aphelenchus	4.03	50	4.18	75	3.58	75	1.98	100
Aporcelaimellus	0.35	25	–	–	–	–	0.26	25
Boleodorus	0.56	25	–	–	–	–	0.13	25
Cephalobidae	0.28	12.5	0.25	25	–	–	–	–
Cephalobus	2.36	37.5	0.56	25	0.28	25	1.09	75
Chiloplacus	0.49	37.5	1.75	87.5	1.17	50	0.06	25
Coomansus	–	–	–	–	–	–	0.06	25
Coslenchus	–	–	–	–	–	–	3.97	50
Criconema	0.28	12.5	0.37	25	–	–	0.83	50
Criconemella	–	–	0.56	25	–	–	2.11	75
Criconemoides	–	–	–	–	–	–	1.54	25
Cruznema	11.74	37.5	0.87	50	0.95	25	0.51	75
Diplogasteridae	0.21	12.5	–	–	1.4	37.5	–	–
Diploscapter	0.14	25	–	–	0.45	25	–	–
Distolabrellus	0.07	12.5	–	–	0.34	25	–	–
Ditylenchus	0.07	12.5	–	–	–	–	–	–
Dorylaimidae	0.69	50	0.56	50	1.45	50	0.58	50
Eucephalobus	0.69	75	0.75	37.5	0.06	12.5	0.7	50
Eudorylaimus	0.56	50	0.31	12.5	0.17	37.5	1.02	75
Filenchus	6.04	100	3.99	87.5	5.87	25	33.87	100
Helicotylenchus	24.58	62.5	24.64	100	0.5	37.5	34.7	100
Hoplolaimus	–	–	–	–	–	–	0.51	50
Hemicaloosia	–	–	0.62	12.5	–	–	–	–
Mesorhabditis	4.79	100	7.8	100	7.15	100	0.96	75
Mononchus	–	–	–	–	–	–	0.13	25
Mylonchulus	0.28	37.5	0.06	12.5	0.11	12.5	0.13	50
Nacobbus	0.07	12.5	1.5	62.5	12.85	62.5	–	–
Nothotylenchus	0.28	25	–	–	–	–	–	–
Panagrolaimus	4.51	100	1.19	50	0.39	37.5	0.06	25
Paratylenchus	0.07	12.5	–	–	–	–	2.24	75
Plectus	–	–	–	–	–	–	0.06	25
Pratylenchus	0.35	25	–	–	0.28	25	0.13	25
Prismatolaimus	–	–	–	–	0.06	12.5	0.06	25
Protorhabditis	–	–	–	–	0.28	12.5	–	–
Psilenchus	0.14	12.5	–	–	–	–	0.32	75
Qudsianematidae	0.07	12.5	0.12	12.5	–	–	–	–
Rhabditidae	5.56	75	13.97	75	9.94	87.5	0.06	25
Rhabditis	6.81	75	26.08	87.5	51.06	100	1.22	75
Rotylenchus	0.07	12.5	0.44	12.5	0.06	12.5	–	–
Sakia	–	–	0.12	12.5	–	–	–	–
Tylenchorhynchus	4.03	37.5	0.12	12.5	0.06	12.5	0.13	25
Tylenchus	12.78	100	5.43	100	0.73	62.5	8.96	100

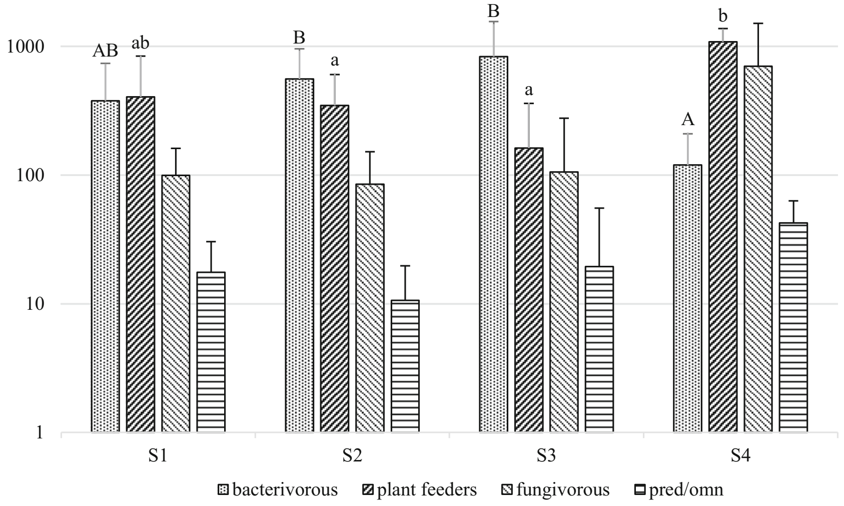

Fig. 2 Values of average abundance of nematodes belonging to the trophic groups (Yeates et al. 1993) in four sampling sites of the horticultural region of La Plata, Buenos Aires Province, Argentina, between spring 2014 and summer 2018 (S1 = agroecological site; S2 = conventional site with organic amendments; S3 = intensive conventional site with biocides application; S4 = pristine site without anthropic intervention). Different letters within a category (bacterivorous, plant feeders) indicate significant differences between sites with a probability $p < 0.05$ based on the Kruskal-Wallis method

indv/100 cm^3) were observed. For categories cp 1 and cp 2, significant differences were found between the sites corresponding to the agroecosystems vs the pristine site (Fig. 3). In the pp category of plant feeders, only pp 2 and pp 3 representatives were observed. The result of the global analysis of herbivorous nematodes revealed 74.85% of pp 3 and 25.15% of pp 2, and no significant differences were found for these groups among the different sites.

3.3 Functional Guilds

No significant differences were found for average abundance of Ba1 guild between the sites; however, the greatest values of this guild (805 indv/100 cm^3) was found in the crop treated with MeBr, being the dominant guild for this site and its lowest value was found in the pristine site (55 indv/100 cm^3) (Fig. 4). Between S4 and the three types of horticultural crops,

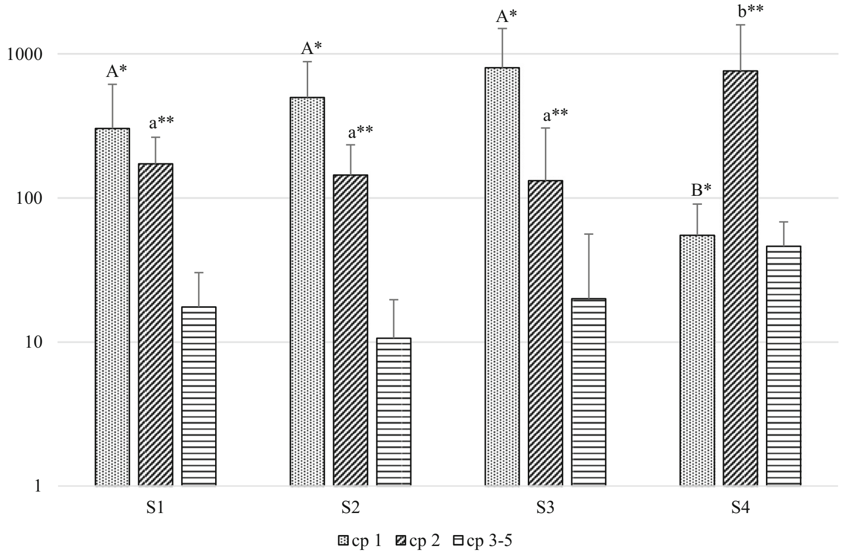

Fig. 3 Values of average abundance of nematodes in the colonizer-persistent scale (cp) (Bongers 1990) in four sampling sites of the horticultural region of La Plata, Buenos Aires Province, Argentina, between spring 2014 and summer 2018 (S1 = agroecological site; S2 = conventional site with organic amendments; S3 = intensive conventional site with biocides application; S4 = pristine site without anthropic intervention). Significant differences were found for cp1 nematodes between the three agroecosystems (S1, S2, and S3) and S4. Different letters within a category (cp 1, cp2) indicate significant differences between sites with a probability $p < 0.05$ based on Kruskal-Wallis* and ANOVA**

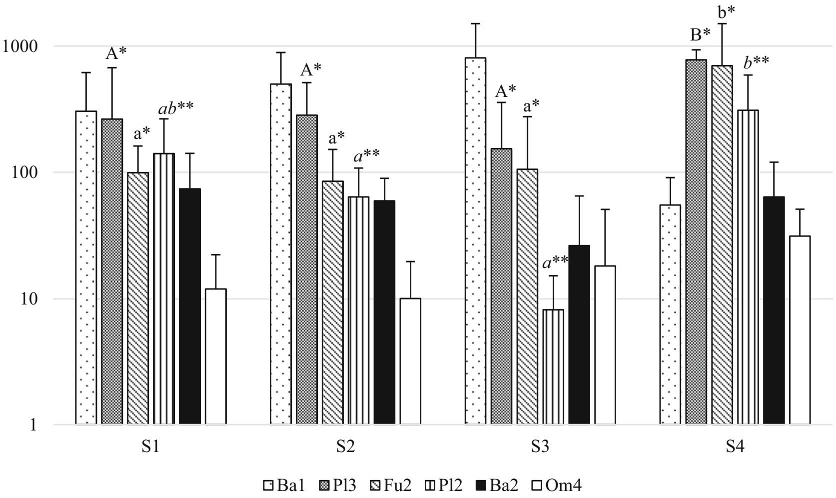

Fig. 4 Values of average abundance of nematodes in the functional guilds proposed by Ferris et al. (2001), in four sampling sites of the horticultural region of La Plata, Buenos Aires Province, Argentina, between spring of 2014 and summer of 2018 (S1 = agroecological site; S2 = conventional site with organic amendments; S3 = intensive conventional site with biocides application; S4 = pristine site without anthropic intervention). Different letters within a category (Pl3, Fu2, Pl2) indicate significant differences between sites with a probability $p < 0.05$ based on ANOVA* and Kruskal-Wallis** (Ba1 = bacterivorous with cp 1 value; Pl3 = plant feeders with cp 3 value; Fu2 = fungivorous with cp 2 value; Pl2 = plant feeders with cp 2 value; Ba 2 = bacterivorous with cp 2 value; Om4 = omnivorous with cp 4 value)

significant differences were found with respect to the abundances of the guilds Pl3 and Fu2 ($p = 0.0114$ and $p = 0.0124$ respectively). For the guild Pl2, significant differences were found ($p = 0.0003$) between S4–S2 and S3 (Fig. 4).

3.4 Structure Indices of the Community

The results of the analysis of nematode communities showed no significant differences in the number of individuals (N), with the highest values recorded in S4 and the lowest values in S1 (Table 3). Between the S3 and S4 sites, significant differences were found in the number of genera (S) ($p = 0.0301$), while Margalef's richness index (D_{mg}) and the Shannon-Weaver diversity index (H') showed significant differences ($p = 0.0352$ and $p = 0.0197$ respectively) between crops S1 and S3 (Table 3). The Pielou equitability index did not show significant differences between the sites, observing their highest average and maximum value in the agroecological crops (Table 3).

Table 3 Average, minimum, and maximum values (min-max) of the ecological indices analyzed in four sampling site in the horticultural region of La Plata, Buenos Aires Province, Argentina between spring of 2014 and summer of 2018 (S1 = agroecological site; S2 = conventional site; S3 = intensive conventional site; S4 = pristine site without anthropic intervention). Values within a row followed by a different letter (a–b) are significantly different with a probability $p < 0.05$ based on ANOVA* or Kruskal-Wallis**

	S1	S2	S3	S4
	Mean (min-max)	Mean (min-max)	Mean (min-max)	Mean (min-max)
S	15.125 (12–22) ab	13.5 (6–18) ab	10.5 (6–18) a	17.25 (14–20) b
N	900 (260–1510)	1001.88 (290–1710)	1118.75 (255–3055)	1952.5 (1075–3520)
D_{mf}	2.12 (1.62–3.34) b	1.81 (0.88–2.35) ab	1.4 (0.74–2.17) a	2.18 (1.84–2.44) ab
H'	1.99 (1.44–2.72) b	1.77 (1.39–2.07) ab	1.39 (0.93–2.25) a	1.61 (1.18–2.13) ab
J'	0.74 (0.55–0.88)	0.7 (0.58–0.84)	0.61 (0.39–0.88)	0.56 (0.45–0.75)
MI	1.6 (1.12–2.11) ab	1.29 (1.14–1.46) a	1.29 (1–2.24) a	2.11 (2.02–2.31) b
PPI	2.38 (2–2.88)	2.79 (2.68–2.92)	2.68 (2–3.0)	2.74 (2.54–2.97)
MI 2–5	2.22 (2.07–2.7)	2.13 (2–2.47)	2.26 (2–4.0)	2.22 (2.06–2.46)
ΣMI	1.95 (1.19–2.73) ab	1.87 (1.35–2.38) ab	1.46 (1.02–2.46) a	2.52 (2.23–2.88) b
EI	82.86 (60.95–97.62) a	92.38 (85.52–96.53) a	90.7 (57.72–100) a	56.87 (53.02–60) b
SI	30.32 (13.33–67.06)	19.60 (0–55.17)	30.81 (0–100)	30.23 (10.98–54.55)
CI	16.05 (0.76–56.25) a	4.48 (0–11.25) a	12.29 (0–71.83) a	67.79 (60–85.42) b
BI	15.15 (2.35–32.8) a	7.47 (3.32–14.29) a	7.83 (0–31.52) a	36.22 (27.03–44.41) b

3.5 Ecosystem Function Indices

The statistical analyses found no significant differences for the variables PPI and MI 2–5 (Table 3). The highest average and maximum values for the PPI were observed in the conventional treated crops (S2 and S3). Regarding MI 2–5, the highest average value was determined for S3 without observing large differences with respect to the other sites analyzed. With respect to the MI, the statistical analyses found significant differences between the S2–S4 and S3–S4 sites ($p = 0.0009$). ΣMI also showed significant differences between S3 and S4 ($p = 0.0064$). For the indices of the food web EI, CI, and BI, significant differences were determined between S4 and the rest of the horticultural crops (Table 3). The graphic representation of the EI and SI data (Fig. 5) shows that the communities studied are mostly in quadrant A, representing disturbed environments, conductive soils, enriched in nitrogen (N), and present bacterial communities. Only in three sites (S1, S2, and S4), there were samplings where the food web indices showed values within quadrant B, characterizing mature environments, with regulated soils, enriched in N and abundant bacteria communities (Fig. 5).

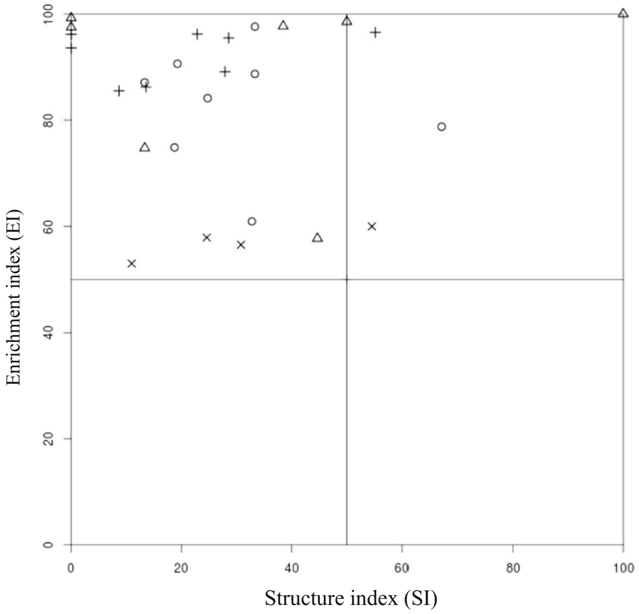

Fig. 5 Nematode faunal profiles representing the structure and enrichment conditions of soil food web, indicated as structure index (SI) and enrichment index (EI), respectively, in four sampling sites of the horticultural region of La Plata, Buenos Aires Province, Argentina, between spring of 2014 and summer of 2018 (agroecological site (circle), conventional site with organic soil amendments as fertilizer (plus sign), intensive conventional site with biocides application (triangle) and pristine site without anthropic intervention (multiplication sign). Graphic made with NINJA software (Sieriebriennikov et al. 2014)

4 Discussion

The analysis of nematode communities in this study showed the presence of 42 genera of edaphic nematodes, both free-living and plant-parasitic. In the agroecological site (S1), *Helicotylenchus* was the most abundant genus with a frequency higher than 60%. This genus was also found by other authors in areas with high crop diversity of the agroecological type (Gnamkoulamba et al. 2018). When observing the crop where organic amendments were applied as fertilizers (S2), it was determined that *Helicotylenchus* also showed a high frequency in the samplings and the highest abundances for this site next to the genus *Rhabditis* coinciding these results with those obtained by Daneel et al. 2018, where *Helicotylenchus* was the most abundant nematode within plant feeders in sites where organic fertilizers were applied with plants of the Brassicaceae family. In horticultural systems where inorganic fertilizers are not applied, the presence of bacterivorous nematodes could be a useful way to improve the release of minerals, such as phosphate, from bacterial biomass, improving the absorption by plants (Rehman et al. 2018).

The presence of *Rhabditis*, as well as other bacterivorous nematodes such as *Mesorhabditis* in S2, could be due to the effect that the organic amendments have on the opportunists of enrichment of bacterial-feeding, which can rapidly increase their populations as do populations of nitrifying bacteria. This type of amendments based on the addition of O.M. increase the availability of nutrients, such as nitrogen, microbial biomass, and therefore the abundance of bacterivorous (Briar et al. 2007). In this sense, Lavallén and Mondino (2015) determined that the addition of nitrogen in the soil through the addition of organic fertilizers raised bacterivorous populations in crops in the horticultural region of General Pueyrredón, Buenos Aires, as well as (Azpilicueta et al. 2008) in soils with nitrogen fertilization where the community of bacterivorous nematodes reached 100% frequencies in all the samplings carried out.

In S3, dominance of *Rhabditis* could be related to environmental disturbances due to application of MeBr. According to Zhang et al. (2019), these nematodes are common in soils in early succession stages. *Nacobbu*s presented the second highest value of relative abundance and a frequency of 60% in this site. Regarding *Helicotylenchus*, these nematodes showed a very low abundance and frequency, demonstrating a possible sensitivity of this genus to settle in soils treated with MeBr (Webster et al. 2001).

In the pristine forest (S4), a clear dominance of *Helicotylenchus* and *Filenchus* was observed, agreeing with data obtained by other authors in temperate forests (Sun et al. 2013). The fungal feeder genus *Filenchus* has been cited in forests as a persistent nematode in structured or late successional environments (Hánel 2001), which feed not only on mycorrhizal fungi but also on abundant saprophytic fungi in environments with high O.M. rich in carbon (Zhang et al. 2015; Mateille et al. 2016). In the S1 and S3 sites, the presence of *Diploscapter coronata* was the first record of the genus and species in Argentina (Salas et al. 2016). This nematode feeds on soil bacteria and is found in compost, sewage, or agricultural soil. Its importance lies in the facultative ability of parasite insects, vertebrates, and even humans (Athari and Mahmoudi 2008). Clinical symptoms include epigastric sensitivity, diarrhea, abdominal pain, and nausea. In addition, they have been considered potential carriers of pathogenic bacteria on the surface of fruits and vegetables in contact with the soil (Gibbs et al. 2005). The presence of *D. coronata* in S3 was found in association with root galls, caused by the parasitic nematode of *Nacobbus aberrans*. The detection of this nematode in greenhouses where dogs, cats, and poultry live together without any health control stresses the importance of applying adequate hygiene measures during agricultural practices to avoid contamination of fruits and vegetables and prevent infections in domestic animals and humans.

Soil fumigation with MeBr has been a common practice for the management of PPNs (Desaeger et al. 2017); but because MeBr was listed as an ozone-depleting substance by the Montreal Protocol in 1992, the production of that agent was largely discontinued by 1995. Nevertheless, in the years 2017 and 2018, countries such as Argentina, Australia, Canada, China, and South Africa have requested exemptions citing the critical use of MeBr in the treatment of strawberry, tomato, and ginger sprouts (UNEP 2016). The use of MeBr, as was observed in the present work, would not constitute a definitive means of eradicating PPNs dangerous to the region, as indicated by the presence of *Nacobbus* in S3. The persistence of *Nacobbus* and other PPNs of economic importance could lead to a frequency increase in the application of pesticides, thus altering communities of soil organisms and generating the rapid appearance of pest organisms in the absence of competitors for food. The results obtained for *Hemicaloosia* and *Hoplolaimus* (found exclusively at sites S2 and S3) were inconclusive to make inferences about their role as bioindicators. *Hoplolaimus* in S4 could be related to the presence of grass (dominant in the physiognomy of this site), since species belonging to this genus are cited as grass parasites (Subbotin and Chitambar 2018).

The omnivorous/predatory trophic groups did not show significant differences between the different sites indicating low abundance values; although, in the agroecological crop (S1), the highest abundance was found for this category. The nematodes cp 1 and cp 2 differed significantly between the pristine forest vs agroecosystems, showing a potential sensitivity of these variables as bioindicators of edaphic disturbances. Although the cp 3–5 did not show significant differences, the highest values were found in the pristine forest and in the agroecological crop, which could indicate more complex trophic networks than in the other sampling sites.

The analysis of guilds showed significant differences for guilds of plant feeders and fungivorous. Pl2, represented mainly by the genus *Tylenchus*, differed between the pristine forest and the crops treated with amendments (S2) and MeBr (S3). This species has been cited as a persistent nematode in conventional horticultural systems, especially in those that maintain monocultures. However, the feeding habits of *Tylenchus* have not yet been properly understood, requiring a more detailed analysis of the species of this genus and its relationship with the host plants in order to discuss their capacity as an indicator. Within the fungivorous guilds, Fu2 showed significant differences between S4 and the cultivated sites (S1, S2, and S3). Although Ferris et al. (2001) consider the guild Fu2 as an indicator of soils in basal conditions for being general opportunists, in this work, *Filenchus* has been found in greater abundance in forests and pastures without anthropic interventions that exhibit the fungal-based energy channel as dominant over bacteria-mediated, thus favoring the population growth of fungivorous guilds (Neher et al. 2017).

When considering the structure indices of the nematode community, the results of their values allowed establishing the agroecological crop (S1) as the most equitable and with the highest number of genera found. The significant differences found in genera richness (*S*), Margalef (D_{mg}), and Shannon-Weaver (H') indices showed that the conventional crop treated with MeBr (S3) presented the lowest diversity of nematodes due the permanence of few genera such as *Rhabditis* and *Nacobbus*. EI allowed to recognize enriched soils, observing significant differences between the pristine forest (S4) and the three horticultural crops, being most of the samplings located in quadrant A proposed by Ferris et al. (2001) in their classification of the soil profile. The enrichment with nitrogen and the tillage favors the energy channel of decomposition dominated by bacteria which would justify these results that are consistent with those obtained for the analysis of Ba1 guilds and the values of cp 1 genera of opportunistic nematodes. CI values were significantly higher in the pristine forest (S4) due to the high abundance of Fu2, which can be explained by the large amounts of terrigenous particulate organic matter in the soil profile (Fabian et al. 2016). In the three horticultural crops, the main energy channel of decomposition would be mediated by bacteria due to the presence of the Ba1 guild. The soils belonging to the three agroecosystems studied registered disturbance, so the implementation of practices that allow for the development of the highest trophic levels in the trophic network (promoting synergies between the components of biodiversity and thus increasing the ecosystem services that nematodes provide to natural environments, such as the regulation of plant-feeder nematodes by predators, nutrient recycling, and productivity) should be considered. Monitoring of the structure and diversity of nematodes communities, as carried out in the present work, could constitute a practice for preventing the appearance of diverse pests and pathogens along with developing more adequate control strategies that would enhance the sustainability of the environment and protect human health.

5 Conclusion

This updated survey of the nematode fauna constitutes one of the most important for the horticultural region of La Plata, Buenos Aires Province, Argentina, considering the few works carried out so far in this subject. Through the monitoring realized in this work, alterations in the structure of nematode communities were observed in the analyzed sites. As was indicated by other authors, this could be a consequence of environmental disturbance such as horticultural production practices. In the context of this case study, the agroecological crop was considered the least disturbed agroecosystem, presenting the highest value of predatory-omnivorous trophic groups and diversity and equitability indices, not finding sedentary plant-parasitic nematodes of economic importance.

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Compliance with Ethical Standards

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