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Responses of free-living and plant-parasitic nematodes to sugarcane crop in two soils

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ABSTRACT

Nematodes play different ecological roles in soil, interfering with feed rates by regulating microbiota and the development of plant diseases. The natural characteristics of an environment as well as the agricultural process alter the distribution of these organisms. The objective of this study is to assess the effect of continuous sugarcane crop time on free-living and plant-parasitic nematodes in soils with different textures. Soil samples were collected from areas of native vegetation and agricultural plots cropped with sugarcane in two municipalities in the state of Parana, Brazil (soil with medium-textured and clay-textured), with subsequent extraction and identification of trophic groups and genus of plant-parasitic nematodes. The nematodes structure was most severely affected by sugarcane crop time, with an increase in the plant-parasitic nematodes abundance over time. Agricultural plots were dominated by *Pratylenchus* sp. and *Helicotylenchus* sp., and communities in areas of native vegetation were dominated by free-living nematodes, especially bacterial-feeding, with an incidence of carnivorous/omnivorous nematodes and *Mesocriconema* sp.

Key words: bioindicators, *Helicotylenchus*, *Mesocriconema*, monoculture, *Pratylenchus*

Resposta de nematoides de vida livre e fitoparasitas ao cultivo de cana-de-açúcar em dois solos

RESUMO

Os nematoides desempenham diversas funções ecológicas no solo interferindo na taxa de rações, regulando a microbiota e o desenvolvimento de doenças em plantas. As características naturais de um ambiente, tal como também o processo agrícola, alteram a distribuição desses organismos. Assim, o objetivo deste trabalho foi avaliar o efeito do tempo de cultivo contínuo com cana-de-açúcar sobre nematoides fitoparasitas e de vida livre em solos com diferentes texturas. Foram coletadas amostras de solo em áreas de vegetação nativa e parcelas agrícolas cultivadas com cana-de-açúcar em dois municípios no estado do Paraná, Brasil (solo com textura média e textura argilosa), com posterior extração e identificação dos grupos tróficos e gêneros de nematoides fitoparasitas. O tempo de cultivo com cana-de-açúcar afetou mais severamente a estrutura das comunidades, ocorrendo aumento na abundância de nematoides fitoparasitas com o tempo. As parcelas agrícolas foram dominadas por *Pratylenchus* sp. e *Helicotylenchus* sp. e as comunidades nas áreas de vegetação nativa dominadas por nematoides de vida livre, principalmente bacteriófagos, com incidência de nematoides canívoros/onívoros e *Mesocriconema* sp.

Palavras-chave: bioindicadores, *Helicotylenchus*, *Mesocriconema*, monocultivo, *Pratylenchus*

Introduction

Grown in more than 100 countries, sugarcane (*Sacharum* sp. L.) represents one of the main global agricultural activities, taking up an area over 8 million hectares, just in 2010/2011 in Brazil (CONAB, 2011). The intensive crop management practices leads to changes in soil structure, which can directly and indirectly affect the composition, structure, abundance and distribution of nematode communities (Yeates & Bongers, 1999; Kandji et al., 2001), especially some groups of free-living nematodes, that are more sensitive to disturbances (Mondino et al., 2010).

Monoculture for a long time may favor plant-parasitic nematodes, leading to reduction in productivity and longevity in the areas (Dinardo-Miranda, 2006) and, although more than 300 nematode species could be associated with sugarcane (Cadet & Spaul, 2005), highest losses have been reported by *Meloidogyne incognita*, *M. javanica* and *Pratylenchus zaei* (Dinardo-Miranda & Fracasso, 2010), with frequent occurrence of *Helicotylenchus* and *Mesocriconema* (Bond et al., 2000).

According to feeding habits, nematodes play different ecological roles in soil, such as microbiota regulation, influence on mineralization and nutrient cycling (Bongers & Ferris, 1999; Coleman et al., 2004). They may also cause plant diseases (Agrios, 2005).

Once moving freely through water film they are able to feed and complete their life cycle. Soil texture and moisture as well as food availability are critical factors in nematode diversity (Yeates & Bongers, 1999). Thus, changes in land use impact and alter the density and diversity of these organisms (Araujo & Monteiro, 2007).

Changes in nematode communities in areas of continuous crop have been examined by monitoring the same area over time. However, seasonal changes can cause short-lived effects on community structure (McSorley & Frederick, 2000), mainly by changes in temperature and soil moisture (Bakonyi et al., 2007), leading to errors in results interpretation.

Thus, this study aims to assess changes in plant-parasitic and free-living nematodes populations according to sugarcane crop time in soil with different textures.

Material and Methods

The work was conducted in field areas with sugarcane in the municipalities of Rolândia (23°15'01" S; 51°28'36" W) and Jaguapitã (23°03'49" S; 51°27'52" W), both in the state of Paraná, Brazil, corresponding respectively to a Rhodic Eutrodox (Re) with loamy texture and a Rhodic Hapludox (Rh), with medium texture, according to Soil Taxonomy (Soil Survey Staff, 1999). In both municipalities, the relief is undulating and the climate is classified as Cfa (humid mesothermal), according to Köppen, and the typical native vegetation is part of the Atlantic Forest Domain, characterized as a Semi Deciduous Forest (IBGE, 2012).

In each soil, native vegetation was assessed (as an ecological reference) and five plots corresponding to different periods of continuous land use with sugarcane crop (1, 3, 8, 10

and 16 years of cultivation for Re and 1, 2, 3, 6 and 7 years of cultivation for Rh). These plots occupied the same topographic position in the middle third down.

The prior system production was crop rotation with maize, soybean and wheat, in Re and pasture, in Rh. Soil till was consisted of grid areas with heavy disk, sub soiling to 0.50 m deep and harrowing. Ridging was carried out 0.40 m depth, and 1.40 m between rows. The areas physical-chemical characterization and fertilizer management are shown in Table 1.

Table 1. Characterization of some physical and chemical attributes of soil and fertilizer management in areas under native vegetation and continuous sugarcane crop

Soil	Crop age	Clay (g kg ⁻¹)	OC ¹ (g dm ⁻³)	SD ² (Mg m ⁻³)	Mineral fertilization
Re ³	1	654	15,1	1,40	250 kg ha ⁻¹ (46% N)
	3	656	16,5	1,42	
	8	762	12,8	1,44	
	10	699	15,6	1,42	
	16	708	14,2	1,51	
	Forest	730	39,4	1,13	None
Rh ⁴	1	225	7,8	1,49	500 kg ha ⁻¹ (05-25-25*), and 413 kg ha ⁻¹ (25-00-25*)
	2	200	5,5	1,49	
	3	237	7,3	1,52	
	6	169	8,7	1,50	
	7	210	10,1	1,54	
	Forest	214	11,4	1,24	None

¹CO: organic carbon; ²SD: Soil density; ³Re: Rhodic Eutrodox Soil; ⁴Rh: Rhodic Hapludox Soil; *N-P-K.

Sampling was carried out according to changes in Anderson & Ingram (1993) methodology, along ten transects (50 m length) per treatment. In each transect, three monoliths of 25 x 25 cm at a depth of 00-30 cm were sampled and mixed to compose one sample. To proceed nematode extraction, soil samples were processed by suspending 300g of soil (approximate 300 cm³) in 2L of water, shaking and pouring the supernatant into consecutive sieves with openings of 850µm, 75µm and 25µm. The supernatant was clarified by centrifugal flotation in sucrose solution (Jenkins, 1964) and, subsequently, nematodes were counted under optical microscope. The plant-parasitic nematodes (PI) were identified to genus level while the free-living nematodes in following trophic groups: bacterial-feeding (Ba), fungal-feeding (Fu) and carnivores/omnivores (Ca/Om).

After nematode identification and counting, the communities were characterized by the following parameters: total abundance (number of nematodes), relative abundance (percentage of each trophic group) and relative abundance of plant-parasitic genus.

Data was subject to variance analysis (ANOVA) and means were compared by Tukey's test at 5% probability for each soil separately. Moreover, relative abundance of nematodes was associated to treatment using the Principal Component Analysis (PCA) for two soils together.

Results

Although there was lower total number of nematodes in the areas of native vegetation, communities were dominated by free-living nematodes, which made up over 60% of the organisms (Table 2). Bacterial-feeding had a ratio above 40%

Table 2. Trophic groups of soilborne nematodes abundance in areas under native vegetation and exposed to different continuous sugarcane crop time

Crop age	Ba ¹		Fu ²		Ca/Om ³		Pl ⁴		TA ⁵	
Rhodic Eutrodox Soil (Re)										
1 year	233,59 ⁶ (20,69) ⁷	a ⁸	319,05 (28,26)	a	0 (0)	a	576,35 (51,05)	ab	1129,12	a
3 years	265,24 (31,69)	a	173,92 (20,78)	ab	11,048 (1,32)	a	386,61 (46,19)	bc	837,34	ab
8 years	12,19 (3,86)	a	76,91 (24,34)	ab	0 (0)	a	226,88 (71,80)	a	316,54	b
10 years	284,85 (53,95)	a	25,13 (4,76)	b	2,53 (0,48)	a	215,47 (40,81)	bc	528,12	ab
16 years	152,12 (27,46)	a	206,14 (37,21)	a	0 (0)	a	195,78 (35,34)	c	554,02	ab
Forest	88,24 (46,94)	a	30,92 (16,45)	b	9,45 (5,03)	a	59,37 (31,58)	c	188,04	b
Rhodic Haplus Soil (Rh)										
1 year	198,80 (53,3)	a	90,82 (24,35)	a	0 (0)	a	83,36 (22,35)	c	373	abc
2 years	236,67 (22,14)	a	229,30 (21,45)	a	0 (0)	a	603,02 (56,41)	ab	1069,05	a
3 years	346,62 (50,75)	a	123,14 (18,03)	ab	13,66 (2,00)	a	199,57 (29,22)	bc	683,18	ab
6 years	113,82 (18,97)	a	65,10 (10,85)	b	3,66 (0,61)	a	417,42 (69,57)	a	600,71	ab
7 years	305,74 (37,7)	a	127,32 (15,70)	ab	3,24 (0,400)	a	374,68 (46,20)	ab	811,32	ab
Forest	29,90 (41,54)	a	14,56 (20,23)	ab	4,8744 (6,770)	a	9,00 (12,50)	c	72,02	c

¹Ba: Bacterial-feeding nematodes; ²Fu: Fungal-feeding nematodes; ³Ca/Om: Carnivores and omnivores nematodes; ⁴Pl: Plant-parasitic nematodes; ⁵TA: Total abundance (total number of nematodes in 300 cm³ of soil). Mean of 10 replicates; ⁶Absolute abundance (number of each trophic group in 300 cm³ of soil). Mean of 10 replicates; ⁷Relative abundance (% of each trophic group in the community). Mean of 10 replicates; ⁸Means followed by different letters, in column, indicate differences (at P ≤ 0.05, Tukey's test) among crop ages for each soil type separately.

in these communities, with a smaller proportion of nematode fungal-feeding and carnivores/omnivores.

On the other hand, the agricultural plots were dominated by plant-parasitic nematodes, with the exception of the area with 16 years of soil cultivation Re, which had a more homogeneous distribution than all the trophic groups. The maximum plant-parasitic nematodes dominance in the community was observed

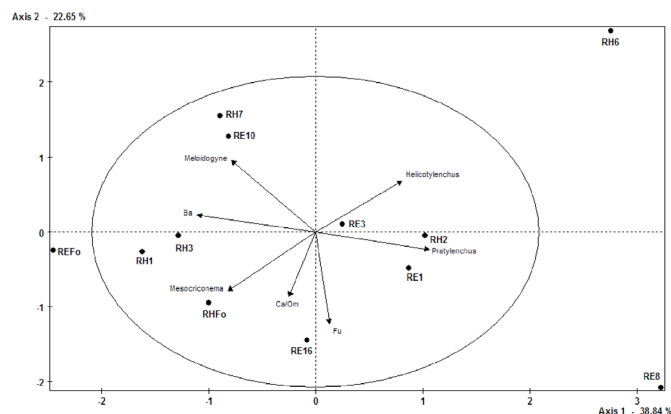
in the sixth year in Rh and in the eighth year in Re, when they reached about 70% of the organisms, basically represented by the genera *Pratylenchus* and *Helicotylenchus*, found in all plots, regardless of cultivation time (Table 3).

The principal component analysis (Figure 1) explains 61.49% of the variation, being 38.84% explained by the first axis and 22.65% explained by the second axis, with greater

Table 3. Absolute and relative abundances of plant-parasitic nematode genera in areas of native vegetation and exposed to different continuous sugarcane crop time

Crop age	<i>Pratylenchus</i>		<i>Helicotylenchus</i>		<i>Mesocriconema</i>		<i>Meloidogyne</i>	
Rhodic Eutrodox Soil (Re)								
1 year	417,20 ¹ (72,38) ²	bc ³	159,20 (27,619)	a	0 (0)	b	0 (0)	a
3 years	306,46 (79,23)	bc	80,30 (20,76)	ab	0 (0)	b	0 (0)	a
8 years	195,30 (85,92)	a	24,37 (10,72)	bc	0 (0)	b	7,62 (3,35)	a
10 years	205,91 (95,54)	ab	9,611 (4,45)	c	0 (0)	b	0 (0)	a
16 years	168,64 (86,1)	bc	27,14 (13,86)	bc	0 (0)	b	0 (0)	a
Forest	39,69 (57,6)	c	11,48 (16,68)	bc	10,75 (15,62)	a	6,90 (10,02)	a
Rhodic Haplus Soil (Rh)								
1 year	69,82 (83,75)	bc	13,53 (16,24)	b	0 (0)	a	0 (0)	a
2 years	456,91 (75,76)	a	146,13 (24,23)	b	0 (0)	a	0 (0)	a
3 years	192,79 (96,57)	bc	6,83 (3,42)	b	0 (0)	a	0 (0)	a
6 years	240,82 (57,62)	ab	170,42 (40,77)	a	6,66 (1,59)	a	0 (0)	a
7 years	289,31 (77,16)	bc	51,76 (13,80)	b	0 (0)	a	33,83 (9,02)	a
Forest	0 (0)	c	9,00 (100)	b	0 (0)	a	0 (0)	a

¹Absolute abundance (number of each genus in 300 cm³ of soil). Mean of 10 replicates; ²Relative abundance (% of each genus in the total of plant-parasitic nematodes). Mean of 10 replicates; ³Means followed by different letters, in column, indicate differences (at P ≤ 0.05, Tukey's test) among crop ages for each soil type separately.



RE: Rhodic Eutrodox Soil. RH: Rhodic Haplux Soil. Fo: Forest. Ba: Bacterial-feeding nematodes. Fu: Fungal-feeding nematodes. Ca/Om: Carnivores or omnivores nematodes. Pl: Plant-parasitic nematodes. The numbers represent the crop age.

Figure 1. Principal Component Analysis (PCA) of trophic groups of nematodes and plant-parasitic nematode genus in areas of native vegetation and exposed to different continuous sugarcane crop time, in two soils

cultivation time influence on soil texture on distribution of nematode groups. *Pratylenchus* and *Helicotylenchus* sp. correlate positively and were favored by the younger ages (up to 6 years in Rh and up to 8 years in Re), while *Meloidogyne* was related to growing longer (seven years in Rh and 10 years in Re). *Pratylenchus* and *Meloidogyne* negatively correlated.

Mesocricconema showed a similar trend to that of the nematodes Ca/Om, relating to areas of native vegetation and 16 years of cultivation in Re. The same trend was presented by microbial-feeding (bacterial and fungal-feeding) nematodes which also related to the first year of cultivation in both soils.

Discussion

Greater abundance of nematodes in the agricultural plots in relation to areas of native vegetation have been demonstrated in several studies (Yeates & King, 1997; Valocká et al., 2001; Goulart et al., 2003; Mattos et al., 2006), although differences are not always observed (Cardoso et al., 2012). This is because certain groups of nematodes are favored by agricultural management adopted (Tomazini et al., 2008).

The areas of native vegetation were dominated by free-living nematodes, especially bacterial-feeding, which were also observed by Cardoso et al. (2012), because the area of native vegetation has higher levels of organic matter and favors microbial fauna. The same authors found that nematodes carnivores/omnivores, even with a low abundance, were more representative in forests to sugarcane, which was also observed in this study. Although omnivorous nematodes generally represent only a small fraction of the communities of nematodes, they are good indicators because are more sensitive to disturbances (Stirling et al., 2003).

The monoculture of sugarcane favors various plant-parasitic nematodes, increasing the population levels compared with populations soon after the implementation of the culture (Cadet & Boer, 1990), so that in the present work, *Pratylenchus* and *Helicotylenchus*, typical parasites of sugarcane (Cadet & Spaul, 2003) were dominant in agricultural plots where they had been cultivated for up to ten years. On the other hand, populations decreased after a longer period.

The monoculture effects over long periods of time without tilling the soil may be similar to fallowing (Hornby & Bateman, 1997), with recovery of microfauna in the rhizosphere and the establishment of nematode suppressive mechanisms (Cadet et al., 2007). The same dynamic may also explain the fact that the cultivation for the 16th year in Re is similar to areas of native vegetation, favoring nematodes sensitivity to disturbance (Ca/Om) and *Mesocricconema* which were found at a low frequency in the first year.

Nematodes from Criconematidea are sensitive to disturbance and have been identified as key groups in several studies in Brazil (Goulart et al. 2003; Goulart & Ferraz et al, 2003; Cares & Huang, 2008; Mattos et al. 2008; Tomazini et al., 2008). Bond et al. (2000) had already observed these nematodes in areas of sugarcane, and smaller populations of *Mesocricconema* in the first year of cultivating.

Despite reports of reduced productivity and high incidence of *Meloidogyne* in areas of sugarcane (Cadet & Spaul, 2003; Severino et al., 2010), in this study populations of *Meloidogyne* remained low in all areas. However, several studies have found low densities of root-knot nematode, with dominance of *Pratylenchus*, *Helicotylenchus* (Rimé et al, 2003; Mondino et al., 2010; Cardoso et al., 2012) and even *Mesocricconema* (Rossi et al., 1996, Bond et al., 2000).

An inverse behavior between *Meloidogyne* and *Pratylenchus* had already been observed in studies performed by Berry et al. (2009) and Cardoso et al. (2012) in soils of sandy texture, which found a negative correlation between these two genera. Considering the fact that both are endoparasites, they can compete for food site, since the parasitism of one genus can be inhibited after the other (Umesh & Ferris, 1994) has established in the roots. Moreover, the fact that *Pratylenchus* (migratory nematode) can be associated with the occurrence of fungi and bacteria with degradation of roots (Agrios, 2005), this may reduce the establishment of *Meloidogyne* (sedentary nematode).

Similarly, *Helicotylenchus*, an ectoparasite, did not show an inverse relationship with these genera, because it does not occupy the same food site, only being influenced by the management. Sundararaj & Mehta (1993) observed that *P. zeae* showed an inverse association with *H. indicus*, but positive relationship with *H. dihystra*. Since *H. indicus* and *P. zeae* are endoparasite nematodes, both depend on roots for feeding and complete their life cycles, while, in other hand, *H. dihystra* feeds on cortical cells from outside root.

Conclusions

Soilborne nematode communities were a good parameter for assessing changes due to sugarcane crop over time, without presenting, however, a definite pattern. Forest plots presented communities with a better distribution by trophic groups, with higher proportion of free-living nematodes. Bacterial-feeding nematodes dominated free-living communities, even in forest plots. Plant-parasitic nematodes were found in greater amounts in soils cropped with sugarcane, indicating relation with monoculture, with predomination by the genera *Pratylenchus* and *Helicotylenchus*.

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