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Full Length Research Paper

Effects of continuous cropping duration on population dynamics of second-stage juvenile *Meloidogyne* spp. and free-living soil nematodes

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Due to continuous cropping over many years, the root-knot nematode (*Meloidogyne* spp.) has become a severe pest in plastic film greenhouses in China. This study investigated the populations of root-knot nematode second-stage juveniles (J2) in comparison to other free-living soil nematodes in soils continuous cropped for different durations. Overall, the J2 population increased with the duration of continuous cropping. The number of J2 at different soil depths in soils cropped for 0 and 5 years was significantly lower than in soils cropped for 8 and 12 years ($P < 0.05$). In soils that had been continuously cropped for 8 and 12 years, the average number of J2 was 154.9 and 861.8 nematodes per 100 g dry soil, respectively. The J2 numbers increased with soil depth, being predominantly distributed at 20 - 30 cm soil depths. Free-living nematode populations also increased with soil depth. The percentage of J2 nematodes based on total nematode numbers was significantly higher in soils continuously cropped for 8 and 12 years than for plots cropped for 0 or 5 years ($P < 0.05$).

Key words: Distribution, soil depth, soil nematode, tomato.

INTRODUCTION

Root-knot disease caused by Meloidogyne spp. nematodes, characterized by stunted growth and root galling, is the most serious below ground disease of tomato in China. Yield losses in tomato due to root-knot nematode in China are 20 to 30%, and most tomato seedlings growing in severely infected soil do not survive (Peng, 1998; Wang et al., 1998). In recent years, the populations of root-knot nematode have shown rapid increases, with great economic consequences. In some plastic film greenhouses, the incidence rate may exceed 90%, with these severely infected soils yielding no fruit (Duan and Wu, 2002). Root-knot nematode is therefore a major problem in greenhouse vegetable production in China.

The primary goal of agricultural nematologists is to prevent harmful nematode populations from reaching economically damaging threshold levels that cause noticeable losses in crop yields. However, this must be done without harming beneficial soil nematodes which, as ubiquitous members of the soil faunal community, often improve nutrient cycling and primary productivity in diverse ecosystems. Both beneficial and harmful nematode populations can respond to ecosystem disturbances (Freckman and Ettema, 1993) and in plastic film greenhouses, agricultural practices such as soil cultivation, monoculture and the application of chemicals such as fertilizers, herbicides, insecticides, and nematicides all can alter nematode populations. These disturbances change the original micro-ecology balance of the greenhouse soil, thus influencing the diversity of soil nematodes that are important in vital ecological processes such as soil organic matter decomposition and nutrient mineralization (Griffiths, 1990). Other practices, such as continuous cropping of the same plant, may provide preferential food resources for specific plant-parasitic nematodes, thus increasing the competitive advantage of disease-causing plant-parasitic nematodes over beneficial free-living forms, with resulting economic losses.

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The present study examined nematode populations in four different continuously cropped soils in a tomato-growing suburban area of Taian city. The objectives of this study were: to (1) assess the distribution in space and time of Meloidogyne spp. second-stage juveniles (J2) in soils continuously cropped with tomatoes for different durations in plastic greenhouses, (2) to evaluate the population dynamics of free-living soil nematodes in these soils, and (3) to assess the relationship between free-living nematodes and root-knot nematode J2s with increasing durations of continuous tomato-cropping.

MATERIALS AND METHODS

This work was conducted in Zoujiazhang village in Fangcun town, which is a tomato production area (35º54´N, 117º10´E) in the suburbs of Taian city, Shandong Province, in northern China. Four greenhouses were selected that had been continuously cropped at the same management level with tomatoes and for 0, 5, 8 and 12 years. In each greenhouse, a 30 m2 plot was selected and soil physical and chemical properties were measured using method of Bao (2000) (Table 1). Management of fertilizer and irrigation was uniform.

Tomato (*Solanum lycopersicum* L.) cv. Maofen 802 seedlings were transplanted into raised soil beds on August 30, 2007, with the final fruit harvest on June 28, 2008, giving a growing period of 304 days. Soil samples were collected at 38, 76, 114, 152, 190, 228, 266 and 304 days after transplanting (DAT). Ten soil sample cores were collected from the rhizosphere in a zigzag pattern from each plot. A 3.5 cm diameter soil corer was inserted to depth of 30 cm, and collected of 0 - 10, 10 - 20 and 20 - 30 cm depths respectively. The soils from each depth were combined for each greenhouse, mixed fully, and then divided into three subsamples for different depths per plot, so that a total of 9 subsamples were produced for each greenhouse each time. Nematodes were extracted from 100 g soil by a washing-sieving followed by a sugar flotation and centrifugation procedure and were preserved in TAF (triethanolamine formalin) (Liu, 2000). Soil moisture content was determined by drying at 105oC. Free-living soil nematodes and root-knot nematodes were counted and recorded, using a stereomicroscope. Nematode populations were expressed as numbers of nematodes per 100 g dry soil (100 g DS) (Li et al., 2007). All data were subjected to statistical analysis of variance using SPSS 12.0. Differences with $P < 0.05$ were considered significant.

RESULTS

Frequency of J2 root-knot nematode in soils continuously cropped for different durations

The numbers of J2 in the four different continuously cropped soils showed significant differences ($P < 0.05$). J2 populations in 0-year and 5-year cropped soils were significantly lower than in soils cropped for 8 or 12 years; J2 frequency was 6.9% (0-year), 9.7% (5-year), 59.7% (8-year) and 91.7% (12-year). The numbers of J2 ranged from 0 to 3.7 and from 0 to 4.8 J2 100 g^{-1} DS in 0 - and 5-year soils, respectively. The maximum numbers of J2 were found in 12-year soil at 20 - 30 cm soil depth, at 1,204.3 J2 100 g^{-1} DS. The numbers of J2 at the three soil depths were also significantly different ($P < 0.05$), with the highest populations seen at the 20 - 30 cm depth and the lowest in the 0 -10 cm soil layer.

Dynamics of root-knot nematode J2s during tomato growth

As shown in Figure 1, the J2 population in continuously cropped 0, 5 and 8 year plots was lower at 0 - 10 cm soil depths than at deeper soil depths. In the 0 year continuously cropped soil, J2 numbers were lowest, with an average of 0.8 and 0.4 J2 100 g^{-1} DS in samples taken at 76 and 190 DAT, and no root knot nematodes found in other samples. In the 5 year plot, the root-knot nematode J2s were found primarily distributed at 0 - 20 cm soil depth, while they were present mostly at 20 - 30 cm soil depth in the 8 year plot. The numbers of J2 in the 12 year plot were higher than in any other plots, and showed 3 peaks during the tomato growing period, at 76, 152, and 304 DAT. The numbers of J2 in samples from the continuously cropped 12 year plot were significantly higher than those of the other plots at 152, 266, and 304 DAT ($P < 0.05$).

At 10 - 20 cm soil depth, the J2 numbers ranged from 0 to 386.7 J2 100 g^{-1} DS (Figure 1). The maximum numbers of J2 were found in the 12 year plot at the last sampling time, at 386.7 J2 100 g-1 DS, which was significantly higher than the numbers in the other plots ($P < 0.05$). No significant differences were found among the other three continuously cropped plots. At 20 - 30 cm soil depth, the numbers of J2 showed significant differences among the different plots ($P < 0.05$). J2 were not found in these samples from the 0 and 5 year plots. In the 8 year plot, the J2 population was highest from 38 to 190 DAT, ranging from 66.9 to 96.7 J2 100 g^{-1} DS. The numbers of J2 in 12 year plot were always higher than those in the other plots, ranging from 36.5 to 1006.7 J2 100 g-1 DS. A first peak at 237.5 J2 100 g^{-1} DS was seen at 114 DAT, followed by a second at 266 DAT of 232.1 J2 100 g^{-1} DS and a third peak at 304 DAT of 1006.7 J2 100 g^{-1} DS ($P < 0.05$).

Dynamics of free-living nematodes during tomato growth

Figure 2 shows the dynamic changes in numbers of free-living nematodes in the different continuously cropped plots. The populations of free-living nematodes decreased with the soil depth in the 0- year plot, with the maximum and minimum appearing at 0 - 10 cm (152 DAT) and at 20 - 30 cm (190 DAT), respectively, and averages of 82.0 and 11.6 nematodes 100 g^{-1} DS, respectively. In the 5-year plot, the population increased with soil depth, with the maximum and minimum appearing at 20 - 30 cm (304 DAT) and at 0 - 10 cm (304 DAT), respectively, with an average of 442.7 and 13.3 individuals 100 g^{-1} DS,

Table 1. Main chemical properties of the soil.

Continuous time	Total N (g kg^{-1})	Available N (mg kg^{-1})	Available K (mg kg^{-1})	Available P (mg kg^{-1})	Organic matter (g kg^{-1})	pH
0–year	0.89	63.28	446.59	63.39	10.73	6.5
5–year	1.522	100.77	715.70	237.96	25.80	6.1
8–year	1.897	120.70	993.49	277.17	27.55	5.9
12–year	1.616	97.26	767.78	237.55	22.75	6.0

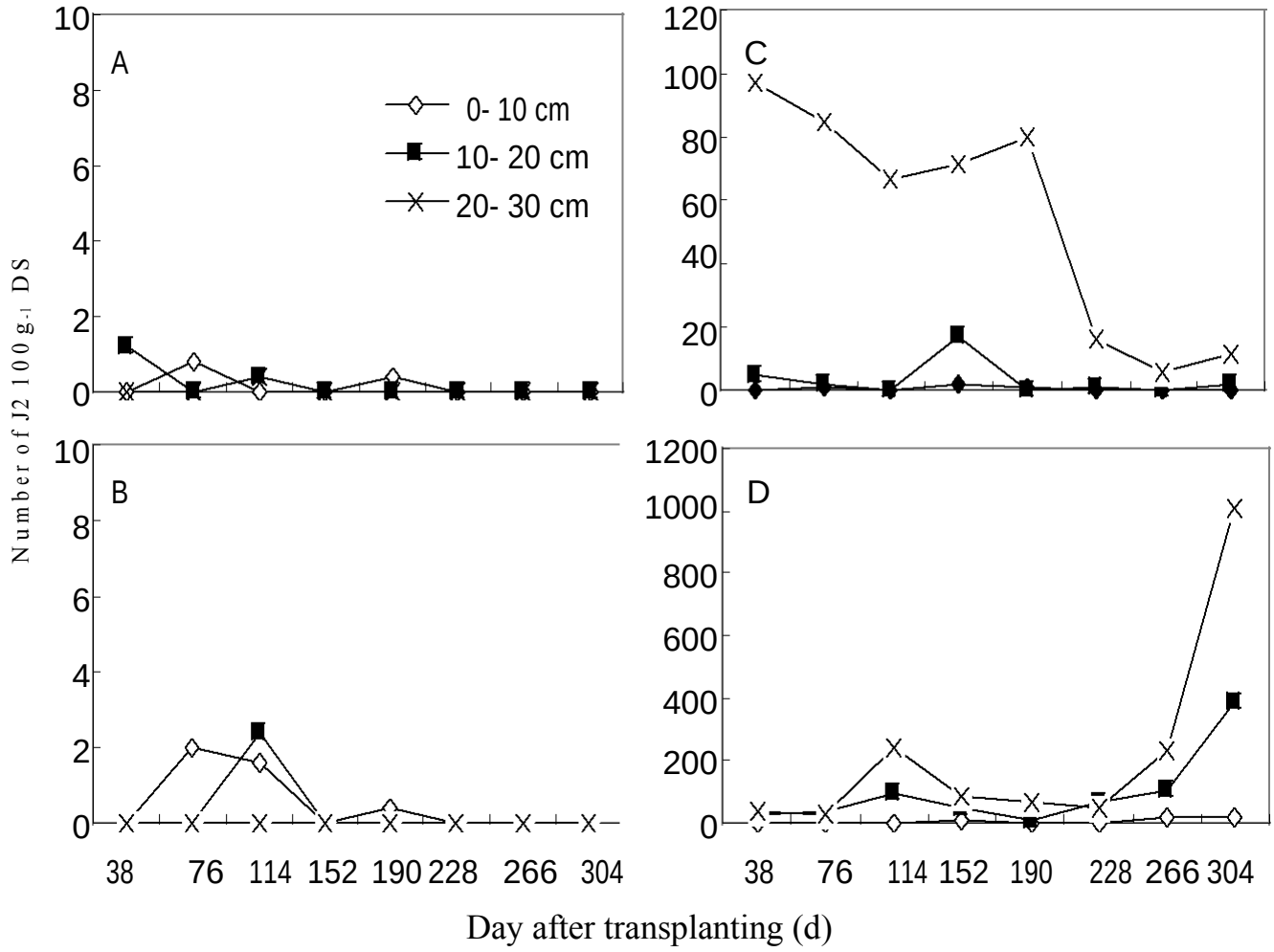

Figure 1. Root-knot nematode J2 in different soil depths of different continuous time with tomato growing A, 0 year; B, 5 year; C, 8 year; D, 12 year.

respectively. No obvious fluctuation in population size of free-living nematodes was noted in the 8-year plot at the three soil depths during tomato growth. In the 12-year plot, the free-living nematode population increased with the soil depth, and there was no fluctuation during the experimental period.

The number of free-living soil nematodes was different in the three soil layers, although they had a similar dynamic pattern. At 0 - 10 cm soil depth, free-living nematode numbers were higher than at the other two soil depths at 38 DAT. From 76 DAT to the last sampling, the numbers of free-living soil nematodes from four plots were different, decreasing in the order of 5- > 0- > 12- > 8-year plots (Figure 2a). At the 10-20 cm soil depth, the numbers of free-living soil nematodes in different plots ranged from 6.1 to 433.5 individual 100 g^{-1} DS. The minimum numbers were found in the 8-year plot at 266 DAT (average value 6.1 individuals 100 g^{-1} DS) and the maximum numbers

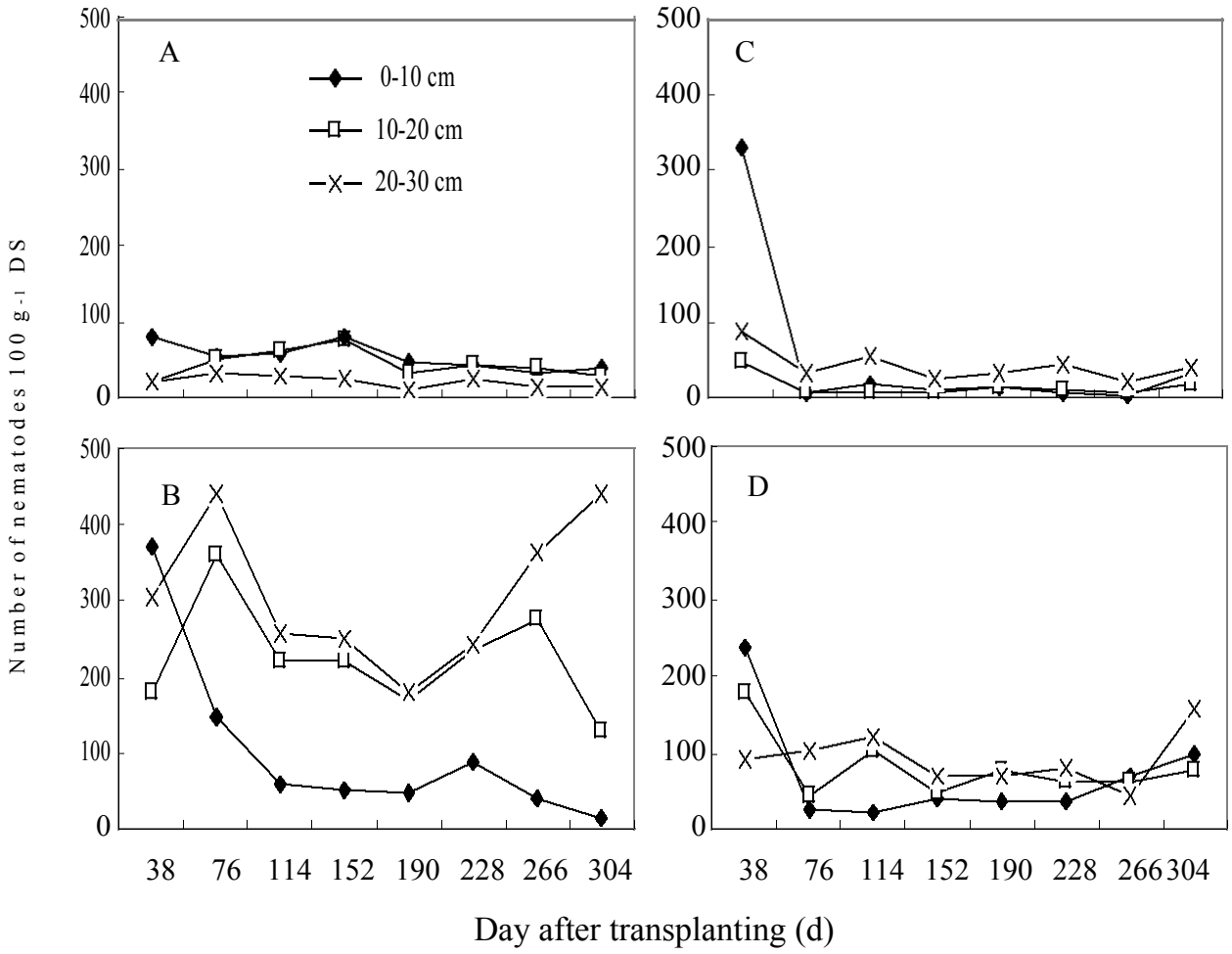

Figure 2. Soil free-living nematodes in different soil depths of different continuous time with tomato growing A, 0 year; B, 5 year; C, 8 year; D, 12 year.

were found in the 5-year plot at 76 DAT (average value 359.3 individuals 100 g^{-1} DS); again, the nematode numbers decreased in the same order as seen in the 0 - 10 cm depths: 5- > 0- > 12- > 8-year plots (Figure 2b). At 20 - 30 cm soil depths, the number of soil nematodes in the 5-year plot was significantly higher than that of the other three plots ($P < 0.05$). The average number of soil nematode from all four plots ranged from 11.6 to 442.8 individual 100 g^{-1} DS, with the maximum occurring in the 5-year plot at last sampling. The total numbers of free-living soil nematodes in the 5-year plot were higher, and those in the 8- and 12-year plots were lower than other tested soils, and these differences were significant ($P < 0.05$) (Figure 2c).

Proportional analysis of root-knot nematode J2 to free-living soil nematodes

The percentages of root-knot nematode J2 and free-living soil nematode with respect to total nematode numbers are shown in Table 2. In general, the ratio of J2 to free-living soil nematodes increased with increasing duration of cropping time. The ratio of J2 to total nematodes in the 12–year plot, with an average of 45.4%, was significantly higher than the ratio in the other plots ($P < 0.05$). The percentage of J2 in the 8 year plot was higher than that in the 0 and 5 year plots ($P < 0.05$); no significant difference was found between the 0– and 5–year plots. The percentage of J2 in the 0 and 5 year plots was less than 1.0%, and was significantly lower than in the other plots ($P < 0.05$).

Changes in the ratio of free-living soil nematodes to root-knot nematode J2 in the different continuously cropped plots at different DAT are shown in Table 3. The ratios of the 0– and 5–year plots were greater than 100. However, the ratios in the other plots were low during the experiment period, ranging from 0.48 to 6.63 (8–year) and from 0.23 to 7.64 (12–year). Therefore, the percentage of root-knot nematode J2 to total nematode (PJ2) and the

Table 2. The percentage of root-knot nematode in total soil nematodes (%).

Continuous time	Day after transplanting (d)								Average	Dominance
	38	76	114	152	190	228	266	304		
0-year	0.8	0.6	0.5	0.0	0.4	0.0	0.0	0.0	0.3c	+
5-year	0.0	0.2	0.8	0.0	0.1	0.0	0.0	0.0	0.1c	+
8-year	18.0	62.3	45.8	67.1	54.5	21.2	12.8	13.1	36.9b	+++
12-year	11.5	27.6	58.2	47.6	32.4	38.4	67.0	80.8	45.4a	+++

Dominance, "+" indicates the individual amounts less than 1% in the total quantity, "++" indicates the individual amounts more than 1% and less than 10% in the total quantity, "+++" indicates the individual amounts more than 10% in the total quantity. Mean values with different superscript letters in the same column differ significantly (LSD multiple range test) *P < 0.05.

Table 3. Ratio of free-living nematodes to root-knot nematode J2 at different days after transplanting.

Cropping time	Day after transplanting (d)							
	38	76	114	152	190	228	266	304
0–year	101.69	168.72	187.14	∞[X]	229.11	∞	∞	∞
5–year	∞	481.55	133.80	∞	1003.37	∞	∞	∞
8–year	4.59	0.56	1.17	0.48	0.76	3.54	5.65	6.63
12–year	7.64	2.62	0.71	1.05	2.09	1.57	0.49	0.23

[X] indicated the value was infinity (the number of root-knot nematode J2 was 0 individual 100 g^{-1} DS).

ratio of free-living soil nematode to root-knot nematode J2 (R) may be considered as useful biological indicator parameters. We characterized four patterns to express the severity of root-knot nematode disease:1) PJ2 < 1%, and R ≥ 100, soil was healthy; 2) 1% ≤ PJ2 < 10%, R value ranging from 50 to 100, there was potential risk of disease and this value could be used as an economic threshold parameter; 3) PJ2 > 10%, 10 ≤ R < 50, the soil was infected by root nematodes and would cause yield loss; 4) PJ2 > 10%, R < 10, infection would cause serious damage to the plants.

DISCUSSION

In agricultural systems, many management practices that affect the soil environment can also change the soil nematode community (Neher, 2001; Siddiqui and Akhtar, 2007). Therefore, it is not surprising that populations of *Meloidogyne* spp. increased rapidly in greenhouse conditions where there was little change in soil habitat, a same planting pattern for a long time, and the same tillage and cultivation, irrigation and other management measures. This disrupted the original nematode native biodiversity and community structure, resulting in an increase in *Meloidogyne* spp. populations. In natural ecosystems, the abundance of root-feeding nematodes can be controlled by natural enemies (Piśkiewicz et al., 2009), but our results showed that the population of root-knot nematode J2 from tomato rhizosphere soils increased with increases in the duration of continuous cropping. In the early years of planting, the population structure of *Meloidogyne* spp. and free-living soil nematodes were able to maintain a balance for 0 and 5 year, where the percentage of J2 with respect to the total nematode population was lower than in later years (values were 1.1, 2.1, 154.9 and 861.8 J2 100 g^{-1} DS in 0, 5, 8 and 12 year soils, respectively, P < 0.05). Based on our findings, the economic threshold population of root-knot nematode J2 can be controlled within a 5 year period of continuous cropping, however, further continuous plantings will increase the risk of overabundance of root-knot nematodes.

In our study, on a vertical scale, root-knot nematode J2 abundance increased with the soil depth except in the 0 year plot soil. Free-living soil nematodes increased in abundance with increasing soil depth, which contrasts with previous findings, where a gradual decrease in abundance was seen with increasing soil depth (Lazarova et al., 2004; OU et al., 2005; Li et al., 2007), due to long term disturbance as result of the same cultivation methods used year-round in plastic greenhouses. The root system of the tomato plant is mainly distributed at a 0

- 40 cm soil depth (Li et al., 2006). Root biomass, root activity, soil temperature and moisture content are also important factors that affect the vertical distribution of plant-feeding nematodes in the soil, and vertical migration of nematodes during the season was probably largely controlled by physical-chemical soil factors such as moisture content, temperature, texture and season

(Yeates, 1982; Norton and Niblack, 1991; Wang et al., 2007). Our result suggested that vertical distribution of soil nematodes in plastic greenhouses soils can be a response to the degree of human disturbance.

Nematodes are among the most diverse of soil animals and are usually the most abundant of the soil metazoans and the most important secondary consumers within the soil mesofauna (Mulder et al., 2005). Nematodes have been used extensively as indicators of soil diversity and functioning (Ekschmitt et al., 2001, 2003; Neher, 2001; Mulder et al., 2005), and there is abundant literature dealing with the nematode fauna as soil health indicators in different farming and natural systems. As the duration of continuous cropping increases, the physical and chemical characteristics of soil and soil habitats will change, becoming more sensitive to soil disturbance, so that nematode taxonomic diversity and the soil food web conditions will be influenced (Sánchez-Moreno, 2008). Consistent with previous studies, the soil nematode balance observed in the present study was a good indicator of soil micro-ecological health (Neher, 2001). In conclusion, this work has shown that the ratio of free-living nematodes to root-knot nematode J2 in a given soil could respond to the potential severity of the disease. Although the analyses lacked multiyear data, they indicated that the ratio of *Meloidogyne* spp. J2 to soil nematode should be considered as indicator parameter, as it reflects the potential severity of root-knot nematode disease. This viewpoint can be confirmed through further research.

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